



Effects of wildfire on soil properties and ecotoxicity: A short-term, site-specific remediation experiment (Cinigiano, Tuscany, Italy)

Serena Anselmi^{a,d}, Sara Pignattelli^b, Leja Goljat^c, Massimo Fanti^e, Karin Schlappa^{d,f},
Monia Renzi^{d,f,*}

^a Bioscience Research Center (BsRC), Via Aurelia Vecchia 32, Orbetello, 58015, Italy

^b Institute of Biosciences and BioResources (IBBR) - National Research Council, Madonna del Piano 10, Sesto Fiorentino, 50019, Firenze, Italy

^c Research and Innovation Department, Plastika Skaza d.o.o., Selo 22, SI-3320, Velenje, Slovenia

^d Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), Piazzale Flaminio 9, 00196, Roma, Italy

^e HERA Soluzioni Territoriali, Via Siria 102, 58100, Grosseto, Italy

^f Department of Life Science, Via Licio Giorgieri 10, University of Trieste, 34127, Trieste, Italy

ARTICLE INFO

Keywords:

Wildfire
Soil properties
Ecotoxicity
Remediation
Effective microorganisms
Hazard

ABSTRACT

Global change is increasing the occurrence and extent of wildfires, with growing impacts on the environment and human health. As wildfires can alter soil microbial diversity and increase contaminant presence, bioremediation may support soil recovery. This study investigated the short-term direct and indirect effects of wildfire that occurred on July 24, 2022, in the village of Cinigiano (Tuscany, Italy). Three soil types were analyzed based on their fire exposure: a control soil not affected by the fire (CS), a soil located near the burned area but affected by ash and heat (NBS), and a soil within the burned area (BS). Soils were tested both in the solid phase and as elutriate, either untreated or treated for 15 days with effective microorganisms (EM), under short-term experimental conditions. Ecotoxicity assays were performed considering *Lepidium sativum*, *Aliivibrio fischeri*, *Raphidocelis subcapitata* and *Daphnia magna*; the quality index (TBI) was calculated for each condition (CS, NBS, BS, -EM/+EM). Biochemical stress responses in *L. sativum* were also assessed. Under the tested experimental conditions, the 15-day EM treatment was generally accompanied by a decrease in pH, while increased ecotoxicological hazard was observed in +EM-treated soils. Although long-term EM applications may support remediation over long time scales, these findings indicate that short-term EM application should also be carefully evaluated, given the potential increase in ecotoxicological hazard and the possible repercussions for organisms from different environmental compartments.

1. Introduction

In the Anthropocene era, wildfires are becoming more frequent and severe, as global change promotes warmer conditions and enhanced biomass drying (Westerling et al., 2006). They affect the microclimate (Wolf et al., 2021), increase greenhouse gas emissions and produce chemical and toxic substances such as carbon dioxide, ozone, ultrafine particulate matter (PM_x), nitrogen dioxide and polycyclic aromatic hydrocarbons (Naeher et al., 2007). Consequently, impacts on ecosystems and human health are expected to intensify (Keywood et al., 2013; Reid et al., 2016; Rovithakis et al., 2022).

Fires affect the ecology and functioning of a forest, altering nutrient

turnover, hydrophobicity, species composition, biodiversity, as well as the biological, chemical, and physical properties of soil (Agbeshie et al., 2022). For example, studies conducted in Alaska, on chemicals that accumulate in burned soils, have shown that fires cause significant short-term changes in soil chemistry at a depth of 5 cm, depending on the forest type (Dyrness et al., 1989). However, fire temperature is critical in regulating nutrient availability because volatility changes with nutrients and fire intensity. In general, low-intensity fires tend to increase the availability of nutrients through ash deposition while high-intensity fires lead to complete combustion of organic matter and volatilization (Agbeshie et al., 2022).

Furthermore, extinguishing wildfires requires the use of hazardous chemicals or water, which further affects soil structure and microbial

* Corresponding author. Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), Piazzale Flaminio 9, 00196, Roma, Italy.

E-mail addresses: serena.anselmi@bsrc.it (S. Anselmi), sara.pignattelli@ibbr.cnr.it (S. Pignattelli), leja.goljat@ung.si (L. Goljat), massimo.fanti@herasrl.it (M. Fanti), karin.schlappa@phd.units.it (K. Schlappa), mrenzi@units.it (M. Renzi).

<https://doi.org/10.1016/j.envpol.2026.128239>

Received 29 December 2025; Received in revised form 1 April 2026; Accepted 28 April 2026

Available online 29 April 2026

0269-7491/© 2026 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Abbreviations:

BS –	burned soil
CS –	control soil
EM –	effective microorganisms
NBS –	near burned soil

populations (Ponomarenko et al., 2019). Contaminants persisting in burned areas can be an obstacle to restoring biodiversity because of their toxicity to plant and animal organisms (Andreolli et al., 2015). In addition, fires can lead to a loss of microbial diversity, either through direct or indirect effects (Köster et al., 2021). This aspect is particularly important, as soil microorganisms contribute to key ecosystem functions, including the degradation of recalcitrant substances, such as polycyclic aromatic hydrocarbons. Therefore, alterations in microbial communities may further delay ecosystem recovery. In this context, bioremediation interventions may support pollutant biodegradation and accelerate restoration processes, reducing recovery time (Andreolli et al., 2015).

Bioremediation encompasses a set of techniques aimed at removing or converting contaminants (such as in the case of heavy metals), or toxic elements, into less harmful substances, or degrading organic substances, promoting their mineralization (Kapahi & Sachdeva, 2019). Recently, an integrated bioremediation approach using effective microorganisms (EM), i.e. mixtures of naturally occurring organisms that can be used to increase and remediate microbial diversity in soil, was applied; the results showed that the combination of EM and sediment aeration could be feasible and effective for the remediation of nitrogen- and phosphorus-polluted aquaculture sediments (Wang et al., 2019). Biodegradation of chemical pollutants such as petroleum hydrocarbons (PHC) also occurs through the action of exogenous EM and the indigenous microbiome; the correlation between microbes and the degradation of PHC-based pollutants during the bioremediation process has been demonstrated in the literature (Li et al., 2023).

In a study conducted in a temperate climate, EM were not found to be effective in improving yield and soil quality during a 4-year application (Mayer et al., 2010). However, a more recent study shows that effective microorganisms can influence growth, yield, quality, and vegetable production. Olle and Williams (2013) reported that 70% of the published studies showed positive effects on vegetable growth, while the remaining 30% reported no significant results. The main effect evaluated by the authors was the protective activity of EM, which reduced the incidence of pests and diseases and protected against weeds.

In light of this general background, and given the potential of EM for remediation purpose, this study investigated the short-term environmental effects of a wildfire that occurred on July 24, 2022, in Cinigiano (Grosseto, Tuscany, Italy), (Cherubini, 2022; Coldiretti Livorno, 2022), also evaluating EM application to soils during an early post-fire phase, unlike previous studies focused on longer-term treatments. The socio-economic dimension of wildfire was also considered by quantifying the affected area and estimating related impacts, including those associated with ecosystem services. In this study, the term wildfire is used according to Art. 2 of Italian Law No. 353/2000 (ISPRA, n.d.).

Three soil types were analyzed based on their fire exposure: control soil not affected by the fire (CS), near-burned soil exposed to ash and heat but not directly burned (NBS), and burned soil collected within the burned area (BS). Wildfire effects were studied in terms of physico-chemical properties, ecotoxicological responses, and the related hazard index using species belonging to different trophic levels in both terrestrial and freshwater environments. The latter may experience indirect consequences of wildfires due to the runoff of toxic substances, which may interfere with freshwater organisms and their ecological functions (Carvalho et al., 2019). The tested organisms included bacteria

(*Aliivibrio fischeri*), algae (*Raphidocelis subcapitata*), plants (*Lepidium sativum*), and crustaceans (*Daphnia magna*). The same parameters were further evaluated under experimental controlled conditions following the short-term application of EM (15 days).

Based on this experimental framework, the following hypotheses were investigated.

- (i) wildfire altered the physicochemical characteristics of soils;
- (ii) these changes affected ecotoxicological responses, hazard index values, and the biochemical response of *L. sativum*;
- (iii) EM application further modified soil conditions and, consequently, all the measured parameters.

Ideally, EM application is expected to induce measurable changes in soil properties that can be detected through the considered variables.

Finally, although different fire suppression methods may influence post-fire soil and water conditions as well as ecological effects (Backer et al., 2004), the present study considered the overall post-fire status of the soils, as it was not possible to provide direct evidence of the contribution of the different natural and/or chemical extinguishing agents potentially involved during the event.

2. Materials and methods

The logic model of the study is reported in S1.1 section.

2.1. Sampling stations and study area

Samples were collected on August 4, 2022, immediately after fire suppression and prior to rainfall, from three sites located in proximity within a rural area near an unpaved secondary road. Three types of topsoil (0–5 cm) were considered (CS, NBS, BS), as wildfire effects are usually limited to the forest floor (Akburak et al., 2018). Sampling coordinates are reported in Table S1 and locations are shown in Fig. S1. The georeferenced localization of the sampling stations was carried out using the Google Earth® application (version 7.3.4 8642). The geographical coordinates were recorded in real time (accuracy of ± 2 m). AutoCAD® software (version 2023) was used to quantify the area affected by the fire and to evaluate the lithofacies. The burned-area polygon was delineated by digitizing raster data provided by the municipality and georeferencing them to the regional technical map (CTR F. 320090 and 320100; EPSG: 3003), overlaid with the geological map of the inter-municipal structural plan (municipalities of Cinigiano, Civitella Paganico, and Campagnatico). Land cover information was derived from the Copernicus Corine Land Cover (CLC) dataset 2018 (CORINE Land Cover, n.d.) and further refined using the Tuscany Regional Land Use Map (Regione Toscana—SIPT, nd). According to CLC 2018, all sampling sites fall within class 242 (“Complex cultivation patterns”). The Tuscany Regional Geoportal (Geoscopio) identifies the study area as a mosaic of broad-leaved forest (class 311) and olive groves (class 223). Although the control soil falls within class 223 (olive groves), sampling was conducted at the field margin, which is also characterized by broad-leaved vegetation, similarly to the BS and NBS stations (class 311). The permalink to the study area is reported in S1.2.1 with Fig. S2 (Land use map of the study area). Pre- and post-fire conditions (Sentinel-Hub EO-Browser3, n.d.) of the study area are shown in Fig. S3.

2.2. Solid phase: the soil

2.2.1. Application of effective microorganisms to soils

An aliquot of each collected and homogenized soil sample (CS, NBS, BS) was treated with effective microorganisms (+EM) to evaluate the short-term effects (15 days). The product Septogen (S-EM), which contains a carbon substrate-residues of mill products inoculated with EM, water, and natural sugars, was purchased from Em Tehnologija d.o.o

(Slovenske Konjice, Slovenia). 4 g of S-EM was applied to each 30 g of dried soil and mixed with 20 mL of MilliQ water. This mixture was then stored under micro-aerobic conditions in the dark, at $20 \pm 1^\circ\text{C}$ (Bosch et al., 2017; Footer, 2014), for 15 days, corresponding to the minimum treatment period indicated by the manufacturer. Subsequently, the mixture was dried in an oven (Peltier Memmert, UN55) at $105 \pm 1^\circ\text{C}$ for 5 days, or until completely dry, also, to eliminate EM completely. Although thermal treatment may have affected certain chemical properties, it was deliberately applied to halt microbial activity at a defined time point, accepting a possible partial loss of chemical profile.

2.2.2. Determination of soil properties

All the determinations described in this section were performed on both untreated (-EM) and treated (+EM) soils. A full description of the methods used in this study can be found in S1.3.

Color was determined with a colorimeter (EOPTIS, CLM-194) according to the CIEL*a*b* method (CIE, 1931). The water content of the soil was determined according to ICRAM (2001) by wet and dry weight (Sartotius Entris, 1241-1S) until constant weight was achieved; the results were expressed as % of dry weight (d.w.). The particle size of the tested soils was determined by laser granulometry (Malvern Instrument, Mastersize, 2000), the measurement range is 0.2–2000 μm and the result was expressed as % of equivalent volume (Callesen et al., 2018). pH and redox potential were measured potentiometrically. The pH was determined in a soil dispersion prepared under standard conditions in distilled water at a soil to water weight ratio of 1:2.5 (EDGE probe, Hanna Instrument, HI10480). The redox potential (Eh, mV) was measured with a platinum electrode (Hanna Instrument, A0049215). The contents of the most important macronutrients such as total organic carbon (TOC%), total nitrogen (TN%), total phosphorus (TP mg/kg) and ammoniacal nitrogen ($\text{NH}_4^+\text{-N}$ mg/kg) were determined in the soils. In addition, some plant micronutrients, and environmental pollutants such as metals and metalloids (As, Cd, Co, Cr, Cr-VI, Hg, Ni, Pb, Cu, Zn), trace elements, linear aliphatic and polycyclic aromatic hydrocarbons, and persistent organic pollutants (PCBs, pesticides) were determined. The methods are listed in Table S2(a). All samples were analyzed in triplicate. The results of chemical analyses (except for TOC, TN, TP, $\text{NH}_4^+\text{-N}$) were compared with the contamination threshold concentrations established by Italian legislation for sites intended for public and private green and residential use (Legislative Decree 152/2006).

2.2.3. Solid phase ecotoxicological tests

Solid phase ecotoxicity tests were performed at T_0 (-EM) and after +EM treatment (15 days). The species tested included.

- *Aliivibrio fischeri*, strain NRRL-B-11177, method ISPRA Quaderni 4/2021. This standardized marine bacterium is widely used for the ecotoxicological assessment of environmental matrices (including sediments, elutriates etc.) and following appropriate osmotic adjustment can also be applied to non-marine samples. The measured endpoint was the inhibition of the natural bioluminescence of bacteria (after 10 min of exposure), which is directly related to their metabolic state, and was evaluated relative to the negative control. The test was conducted duplicate; the results were reported as TU_{50} (TU = Toxic Unit), calculated as $\text{TU}_{50} = 1/\text{EC}_{50}$ along with confidence range (EC_{50} = concentration causing 50% inhibition of bacterial bioluminescence). TU_{50} values were then normalized using the ISPRA table (2016) considering the water content and particle size distribution of each sample, particularly the pelitic fraction, to account for the natural background toxicity associated with sediment characteristics. EC_{50} were calculated using Modern Water MicrotoxOmni software, version 4.3.
- *Lepidium sativum*, method UNI 11357:2010 (UNI, 2010); after 72 h of incubation, the test results were expressed as the mean number of germinated seeds (n) and the germination index % (reported as mean $\pm$ SD, along with its confidence range). Biometric parameters

recorded were seed germination (root elongation ≥ 1 mm), root growth (cm), and produced biomass (g). The percentage inhibition of seed germination was estimated as mean $\pm$ SD and calculated as $\text{I\%} = [(C - T)/C] \times 100$, where C represents the number of germinated seeds in the control and T (treatment) represents the number of germinated seeds in the soils (CS, NBS, BS, -/+EM). At the end of the ecotoxicological experiment, plants exposed to the tested soil were processed to determine stress biomarkers in the roots (see section 2.4). The ecotoxicological test was conducted in quadruplicate, whereas the biometric and biochemical analyses were performed in triplicate.

The procedures followed for the setup of the ecotoxicological tests are presented in full in section S1.4.1.

2.3. Biomarkers in *L. sativum*

Different biochemical stress markers (hydrogen peroxide and anti-oxidants) were measured in plants (*L. sativum*) exposed to +EM and -EM soils for 72 h, to determine the effects of wildfire and the bioremediation. Hydrogen peroxide (H_2O_2) was measured according to the method proposed by Alexieva et al. (2001). Ascorbic acid (AsA) was determined according to the method proposed by Okamura (1980) and modified by Law et al. (1983). Glutathione (GSH) was measured using a modification of the method of Sedlak and Lindsay (1968). All the results were expressed in $\mu\text{g} \cdot \text{g}^{-1}$ fresh tissue weight (f.t.w.). See section S1.4.2 for the extended procedures.

2.4. Water extract from soil (elutriate)

2.4.1. Determination of elutriate properties

Water extracts from the tested soils (elutriates) were prepared to evaluate the effects of soluble and extractable substances on ecosystems and to simulate their mobilization through rainfall-driven leaching and runoff. The elutriates were prepared in a 1:4 soil/water ratio (according to the guideline ISPRA 16/2021) with a natural synthetic freshwater solution, UNI EN ISO 6341:2013 (UNI, 2013). After 1 h of soil/water contact, the liquid phase was separated from the solid phase by centrifugation at 1200 rpm for 20 min. Physicochemical parameters such as pH, salinity (mg/L) and conductivity ($\mu\text{S}/\text{cm}$) were measured with field probes (respectively Water Tester JPB-607a; Hanna Instrument HI83141; Hanna Instrument HI763100). The chemical content of the substances listed in Table S2(b), ($\text{NH}_4^+\text{-N}$, Al, As, Ba, B, Cd, Cr, Cr-VI, Fe, Mn, Hg, Ni, Pb, Cu, Se, Sn, Zn) were compared with environmental quality limits for inland surface waters established by Italian legislation (Legislative Decree 172/2015), except for $\text{NH}_4^+\text{-N}$.

2.4.2. Ecotoxicological tests on elutriates

Ecotoxicity tests with the elutriates were performed at T_0 (-EM) and after +EM treatment. The species exposed to elutriates included.

- *A. fischeri*, strain NRRL-B-11177, method UNI EN ISO 11348-1:2019 (UNI, 2019); the measured endpoint was the inhibition of the natural bioluminescence (expressed as mean % and $\pm$ SD) of the bacteria after 15 and 30 min of exposure. The test was conducted in duplicate.
- *Raphidocelis subcapitata*, taken from a sterile maintenance culture obtained from Ecotox® LDS (Milano, Italy), method UNI EN ISO 8692:2012 (UNI, 2012); the measured endpoint was the growth inhibition (expressed as mean inhibition I% and $\pm$ SD) at the end of 72 h of incubation. The test was conducted in triplicate.
- *Daphnia magna*, (Ecotox® LDS) method UNI EN ISO 6341:2013 (UNI, 2013); the measured endpoint was the percentage of immobile organisms (expressed as mean % and $\pm$ SD) after 24 and 48 h of exposure. The test was conducted in quadruplicate.

The procedures followed for the setup of the ecotoxicological tests

are presented in full in [section S1.4.3](#).

2.5. Ecotoxicological hazard

To evaluate the biological responses by applying a weighted method that takes into account the ecotoxicological evidence for the whole battery of tested species, both in soils (excluding *A. fischeri* in solid phase) and in elutriates, the Weight-of-Evidence (WoE) approach proposed by [ISPRA \(2013\)](#) was followed. This approach allows for weighting the integrated environmental effect based on the collected experimental evidence and calculating the integrated battery index (TBI); a full version of the method is provided in [S1.6](#).

2.6. Statistical analysis

Two-tailed Student's *t*-tests assuming equal variances were performed in Excel® to identify statistically significant differences between -EM and +EM treatments for physicochemical parameters and nutrients. Considering *L. sativum*, descriptive statistics (means and standard errors) were performed for all measured parameters by using SigmaPlot 12.5 (SPSS Inc., Chicago, IL) scientific data analysis and graphing software. All data were assessed for normality by means of Shapiro-Wilk statistic ($p > 0.01$); then Two-way ANOVA was applied to determine significant differences between treatments. The statistical significance of rejection was set at $p < 0.05$ according to Holm-Sidak multiple comparison. Multivariate statistics were performed by Primer v7.0 (Primer-E Ltd., Plymouth Marine Laboratory, UK) on Euclidean matrices of distance calculated on standardized (square root) and normalized biometrical and biochemical responses to evaluate the significance of observed segregations according to the factors of the treatment (two levels, fixed; -EM, +EM).

2.7. Quality assurance

The analyses, including ecotoxicity tests and stress biomarkers, were performed in the accredited analytical laboratory BsRC – Bioscience Research Center (UNI CEI EN ISO/IEC 17025:2018, LAB n. 01403). Chemical analyses (nutrients and chemical contaminants) were performed by the accredited laboratory Biochimie Lab (LAB n. 0195 L). The validity of the ecotoxicological assays was assessed according to the criteria established by each method, based on both the respective negative and positive controls.

3. Results

Full results are reported in [section S2](#).

3.1. General features of the study area

The geology of the area affected by the fire is reported in [Fig. S4](#). The fire impacted an area primarily characterized by outcrops of Messinian deposits, of a lacustrine-lagoonal environment, both pre- and post-evaporitic event. The area is characterized by fine to coarse deposits with lithic clasts coming from the formations of the Tuscan spring water and previous fine deposits with gypsum lenses (Messinian evaporitic event). The surface impacted by the wildfire was 773 ha, corresponding to 4.8% of the whole surface of the municipality.

3.2. Solid phase

3.2.1. Soil properties

Based on soil analyses, the samples were characterized by a high percentage of particles $< 63 \mu\text{m}$ (silt and clay) in CS (silty loam), whereas BS and NBS were classified as loamy soils ([Table S4](#)). BS and NBS showed similar particle size distributions, with $d(0.9)$ and $d(0.5)$ values of $947 \mu\text{m}$ and $260 \mu\text{m}$ for BS, and $815 \mu\text{m}$ and $217 \mu\text{m}$ for NBS,

respectively. However, finer ash deposition was higher in NBS than in BS, as indicated by the lower $d(0.1)$ value in NBS ($14 \mu\text{m}$) compared with BS ($42 \mu\text{m}$). Soil texture was classified according to the Udden-Wentworth grain size scale. From the control to the burned soil, color darkening was observed together with a decrease in lightness (L^*), as reported in [Table S5](#).

The water content of the analyzed samples, expressed as % d.w. $\pm$ SD, is reported in [Table S6](#). BS showed a lower water content ($0.45 \pm 0.21\%$) compared to CS ($1.75 \pm 0.15\%$) and NBS ($0.93 \pm 0.09\%$). In contrast, soils treated with EM exhibited substantially and significantly higher water content ($p = 0.00004$), with values ranging from $23.97 \pm 0.62\%$ (CS+EM) to $27.64 \pm 0.57\%$ (BS+EM).

pH. Soil samples showed basic pH values ([Table S7](#)), ranging from 7.58 ± 0.04 in CS to 7.82 ± 0.1 in BS. Compared to CS, the wildfire slightly increased the pH values by about $+0.24$ units. The addition of EM was associated with a decrease in soil pH ($p = 0.047$), with values ranging from 6.58 ± 0.04 in NBS+EM to 6.80 ± 0.02 in CS+EM. The highest percentage decrease was observed for NBS+EM (-15%). After exposure to EM, all tested soils showed a significant increase in redox potential (Eh, mV) ($p = 0.045$), with relative increases ranging from 44% (BS+EM) to 136% (NBS+EM), [Table S7](#).

The results described below are shown in [Table S8](#).

TOC% generally increased from CS to BS, with the lowest value in CS ($1.94 \pm 0.14\%$) and the highest in BS ($19.6 \pm 1.0\%$). EM addition increased TOC in all soils (CS+EM: $4.18 \pm 0.27\%$; NBS+EM: $7.01 \pm 0.42\%$; BS+EM: $27.5 \pm 1.3\%$), but the -EM vs +EM comparison was not statistically significant ($p > 0.05$).

TN% content varied between $0.17 \pm 0.02\%$ in CS and $0.33 \pm 0.03\%$ in NBS; the addition of EM resulted in a significant ($p = 0.031$) increase in the TN content, with BS+EM being particularly affected ($0.68 \pm 0.06\%$).

TP (mg/kg) was not detected in any of the -EM soil samples ($< \text{LOQ}$, Limit Of Quantification). In soils +EM the highest value was recorded in the NBS sample ($1,100 \pm 317 \text{ mg/kg d.w.}$), while the lowest value was found in the BS sample ($671 \pm 193 \text{ mg/kg d.w.}$). A statistically significant difference was observed between -EM and +EM ($p = 0.010$).

$\text{NH}_4^+\text{-N}$ (mg/kg). BS showed an increase in ammoniacal nitrogen levels ($110.8 \pm 17.4 \text{ mg/kg d.w.}$) compared to CS and NBS, where this substance was not detected ($< \text{LOQ}$). Treatment with +EM resulted in a significant increase ($p = 0.031$) in all soils, with values ranging from $129.6 \pm 19.9 \text{ mg/kg d.w.}$ in CS+EM to $220 \pm 31.3 \text{ mg/kg d.w.}$ in NBS+EM.

Overall, metal and metalloid showed variable behavior among the tested soils. In general, lower concentrations were observed in NBS compared with CS and BS. Following EM treatment, some elements showed slight increases, particularly in CS+EM and NBS+EM. Cr-VI and Hg concentrations were below the limit of quantification (LOQ) in all soil samples. Cd was $< \text{LOQ}$ only in NBS. LOQ values are provided in [Table S2](#). All values remained below the regulatory threshold (L.D. 152/2006).

Organic chemicals. Almost all chemicals of organic origin were below the LOQ including those associated with the combustion of organic material such as PAHs. Linear hydrocarbons $C > 12$ showed highest concentrations in BS ($150 \pm 24 \text{ mg/kg d.w.}$), and BS+EM ($140 \pm 22 \text{ mg/kg}$) soils. In contrast, CS+EM showed a higher value compared to CS, increasing from $16 \pm 6 \text{ mg/kg d.w.}$ in CS to $70 \pm 11 \text{ mg/kg d.w.}$ in CS+EM. Linear hydrocarbons (C12–C40) exceeded the regulatory limit (50 mg/kg , L.D. 150/2006) in BS (150 mg/kg). After EM treatment, BS remained above the limit (BS = 140 mg/kg), while CS+EM increased to 70 mg/kg . CS, NBS and NBS+EM remained below the regulatory threshold.

3.2.2. Solid phase ecotoxicological tests

Aliivibrio fischeri. The results of the test are presented in [Table S9](#). The -EM soil samples showed no effect on the tested bacteria, although BS exposure showed the highest effect on *A. fischeri* (87.0 TU , range

68.8–110.0 TU). After treatment with +EM, only CS showed no toxicity (79.6 TU, range 50.3126.0 TU), although a percentage increase of about 197% was recorded.

Lepidium sativum. The results of the test are reported in Table S10. CS (83.7 ± 1.8%; negligible toxicity) and NBS (71.9 ± 1.4%; low toxicity) showed high germination indices %, whereas BS showed the lowest value (13.3 ± 1.8%; high toxicity). After EM treatment, CS+EM (76.5 ± 6.2%; low toxicity) and NBS+EM (69.5 ± 8.2%; low toxicity) showed slightly lower germination indices, while BS+EM increased to 30.7 ± 1.9%. The negative control was within the acceptable range defined by the method (10/10 seed germinated).

3.3. Biometrics and biochemical effects on plants

3.3.1. Biometric effects

The biometric and physical parameters of *L. sativum* exposed to the different soils under -EM/+EM conditions are shown in Fig. 1. Germination inhibition was significantly affected by soil type ($p < 0.001$) and the soil × EM interaction ($p = 0.004$), with higher inhibition generally observed in +EM soils (except for BS). Root length was significantly affected only by soil ($p < 0.001$), with the highest values in the standard control medium (C) and the lowest in BS, especially under +EM conditions. Biomass was significantly influenced by soil type, EM treatment, and their interaction ($p < 0.001$, $p < 0.001$, and $p = 0.014$, respectively), and was generally higher in -EM soils. Soil pH was also significantly affected by all factors considered ($p < 0.001$), with higher values generally recorded in soils not treated with EM, except for the control soil (C).

Hydrogen peroxide and antioxidant responses in *L. sativum* are

shown in Fig. 2. H_2O_2 was significantly affected by soil type ($p = 0.005$) and soil type × EM interaction ($p < 0.001$), with higher levels generally observed in NBS and BS under -EM conditions and in CS under +EM condition. GSH and AsA were significantly influenced by soils, EM, and their interaction (GSH: all $p < 0.001$; AsA: soil type $p < 0.001$; EM $p = 0.037$; soil type × EM $p < 0.001$). Overall, the highest responses were observed in NBS and BS, whereas lower values were mainly recorded in C and CS.

3.3.2. Integrated response

Multivariate analyses highlighted the integrated effects of fire intensity and EM-based bioremediation on plant responses (Fig. S5). PCA of biometric and physicochemical traits explained 95.5% of the variance and suggested a clear separation between EM-treated and untreated soils, with samples associated with differences in soil pH and biometric parameters, including root length and biomass. PERMANOVA confirmed significant differences among treatments in relation to EM application (Pseudo-F = 25.586, $p = 0.001$). PCA of biochemical markers explained 99.4% of the variance and indicated that EM application was associated with antioxidant responses, particularly AsA, whereas untreated soils were more closely associated with H_2O_2 and GSH. PERMANOVA also confirmed significant differences for the biochemical variables (Pseudo-F = 35.523, $p = 0.001$).

3.4. Elutriates

3.4.1. Properties

Across elutriates, pH (Table S11) was alkaline in the -EM samples and generally increased from CS to BS under both -EM and +EM

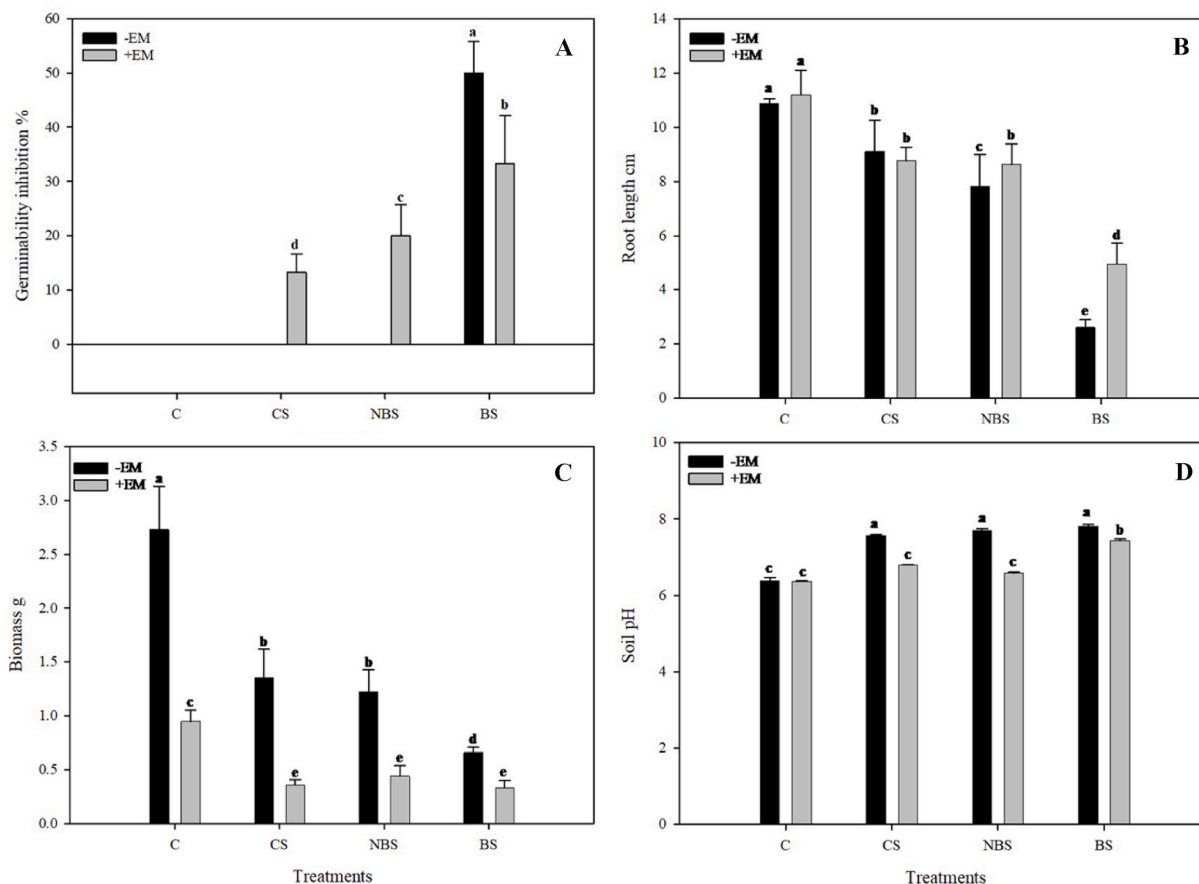


Fig. 1. Biometric and physical parameters recorded in *L. sativum*. C = reference medium. A) % germinability inhibition, B) root length, C) biomass and D) pH soils values. The data are expressed as a mean ± standard error (SE, n = 3). Bidirectional ANOVA was applied to determine significant differences between treatments. Different letters represent statistical differences between treatments (Holm-Sidak multiple comparison, $p < \text{level } 0.05$).

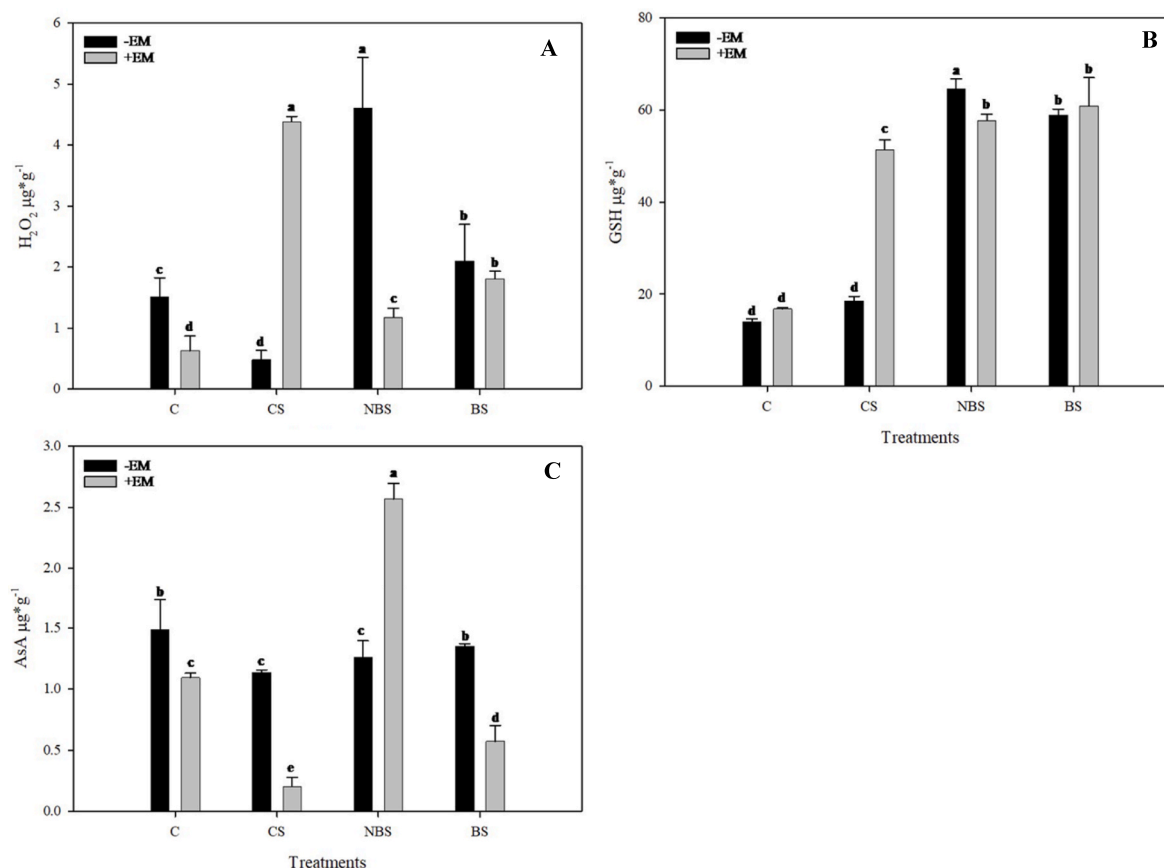


Fig. 2. Biochemical responses recorded in root of *L. sativum* plants. C = reference medium. A) Concentration of hydrogen peroxide; B) Glutathione concentration and C) Ascorbic acid concentration. The data are expressed as a mean $\pm$ standard error (SE, $n = 3$). Bidirectional ANOVA was applied to determine significant differences between treatments. Different letters represent statistical differences between treatments (Holm-Sidak multiple comparison, $p < 0.05$).

conditions. The elutriate of +EM soils showed a pH decrease ($p > 0.05$) while remaining alkaline. The largest percentage decrease was observed in NBS +EM compared with NBS (−8%).

BS showed the highest salinity under both -EM (597 ± 3 mg/L) and +EM (921 ± 7 mg/L) conditions, although all samples exhibited increased salinity after EM addition (+104% in CS, +108% in NBS, and +54% in BS), despite the differences not being statistically significant. CS and NBS showed similar salinity values under -EM conditions (325 ± 0.71 mg/L and 324 ± 3 mg/L, respectively), and this similarity was maintained after EM treatment (663 ± 5 mg/L in CS+EM and 676 ± 7 mg/L in NBS+EM), Table S11.

$\text{NH}_4^+\text{-N}$ levels (mg/L) were < LOQ in CS and NBS, whereas a value of 57.82 ± 3.43 mg/L was measured in BS. Higher concentrations were observed under +EM conditions, with 54.09 ± 0.08 mg/L in CS+EM, 89.22 ± 0.11 mg/L in NBS+EM and 80.21 ± 0.54 mg/L in BS+EM.

Metals and metalloids content (Table S12). In -EM elutriates, a fraction of the analyzed metals/metalloids was already detected above the LOQ (Table S2b), whereas Cd, Cr (VI) and Hg were below the LOQ. In +EM elutriates, the proportion of elements detected above the LOQ increased (compared to -EM) by +50% in CS+EM, +44% in NBS+EM and +9% in BS+EM, indicating an overall enhancement of metal/metalloid in the elutriates. Consistently, several dissolved concentrations tended to be higher under +EM conditions (e.g., Al and Cr), while Mn was the element that showed the greatest increase in the +EM treatments, with CS+EM characterized by the highest value (7.28 ± 1.72 mg/L), followed by NBS+EM (5.94 ± 1.40 mg/L) and BS+EM (1.11 ± 0.26 mg/L).

With respect to Italian L. D. No. 172 (2015), comparison was possible only for Cd, Hg, Ni, and Pb, as the other analyzed elements were not included among the priority substances for inland surface waters.

Among the comparable elements, only nickel showed concentrations exceeding the regulatory limit of $34 \mu\text{g/L}$ (equivalent to 0.034 mg/L) in the +EM treatments, with values of 0.200 mg/L in CS+EM, 0.0909 mg/L in NBS+EM, and 0.0403 mg/L in BS+EM. Cd, Hg, and Pb (except for Pb in NBS+EM) were all below the LOQ.

3.4.2. Ecotoxicological tests on elutriates

A. fischeri. Elutriates from -EM soils induced an increasing toxic response from CS to BS, measured as bioluminescence inhibition after 30 min. The lowest toxicity was observed in CS ($9.3 \pm 1.3\%$), followed by NBS ($22.8 \pm 2.9\%$), whereas BS showed the highest toxicity ($69.2 \pm 0.2\%$). Elutriates from +EM soils further increased toxicity in CS+EM ($66.1 \pm 0.3\%$) and NBS+EM ($78.1 \pm 0.1\%$), corresponding to an increase of 611% and 243%, respectively. In contrast, toxicity slightly decreased in BS+EM (−9%; $66.3 \pm 0.19\%$), Table S13.

Raphidocelis subcapitata. The highest toxicity (% growth inhibition) was observed for BS ($15.5 \pm 1.0\%$), whereas CS showed a slight stimulation ($-1.4 \pm 3.6\%$). Elutriates from +EM soils reduced toxicity across all samples, with inhibition values close to zero (ranging from $-1.5 \pm 4.2\%$ in CS+EM to $1.6 \pm 4.7\%$ in BS+EM). The largest relative change was observed for NBS+EM, Table S14. The validity criterion of the test was met, as the mean cell density in the negative control after 72 h increased by at least 16-fold compared with the initial concentration (10^3 cells/mL).

Daphnia magna. For crustaceans, the elutriates from -EM soils had no effect (0% immobilization). In contrast, the elutriates from +EM soils resulted in high toxicity (100% immobility after just 24 h exposure for CS+EM and NBS+EM; $95 \pm 10\%$ for BS+EM), Table S15. The negative control was within the acceptable range defined by the method (mortality was 0 % at 24 and 48 h).

3.5. Ecotoxicological hazard

The ecotoxicological hazard (Table 1), integrating the responses recorded in the considered organisms from the soil and aquatic compartments, is reported on a scale from A to D, where A = absent/relatively negligible hazard, B = moderate hazard, C = high hazard, and D = severe hazard. Under -EM conditions, an absent/relatively negligible hazard for CS and NBS were observed, whereas a moderate hazard was recorded for BS. After 15 days of +EM treatment, the hazard level increased in all conditions, reaching the highest hazard category (D).

4. Discussion

4.1. Socio-economic impact of the wildfire

On July 24, 2022, a wildfire affected the municipality of Cinigiano (Tuscany, Italy), impacting agricultural areas including vineyards, olive groves, forage crops, pastures etc., as well as local communities and related activities (Coldiretti Livorno, 2022). This event represents only one of the many wildfires that occur each year in southern Europe and across Italy, with varying intensity, seasonality, and frequency depending on the region (ISPRA, 2022). Despite their effects on public health (Ye et al., 2026) flora, fauna, and the physical, chemical, and biological properties of soils (Jhariya & Raj, 2014), wildfires can also generate regional economic impacts (Meier et al., 2023) and affect ecosystem services (Peñuelas et al., 2017). The economic impact of the wildfire that occurred in Cinigiano can be estimated using the specific values per hectare of land defined by the Italian Revenue Agency (AE, n. d.). In the municipality of Cinigiano, the value per hectare is €1203.00, corresponding to a total amount of €929,919.00 for the 773 ha burned. The economic value of a hectare of soil can also be estimated through the ecosystem services it provides, including ecological, functional, cultural, and recreational benefits. Costanza et al. (1997) reported a value of €279.7 ha/year, for a temperate forest. Based on this unit value, the total amount of soil burned is estimated at €216,208.00 (23% of the Italian Revenue Agency estimate). Following this approach, the estimate assumes a significantly lower value for ecosystem services compared with the land value provided by the Italian Revenue Agency. Although fire is often associated with negative consequences, it has historically been used as landscape management tool, for example to improve soil fertility or remove unwanted vegetation, while more recently prescribed fires have been used to reduce fires themselves (by reducing “fuel”) or for restoration activities. Overall, fires, including prescribed fires, can have short- and long-term impacts on ecosystem services (Pereira et al., 2021).

4.2. Effects of the wildfire on the soil properties (-EM)

Despite the loss of economic and environmental value, another key aspect to consider is the potential increase in soil pollution. Soil is a resource of fundamental importance due to its role in nutrient recycling, carbon sequestration and primary production, and it is also considered a non-renewable resource (Cunningham et al., 2004). Fire is a significant driver of soil degradation and biodiversity loss. Ash and

combustion-derived chemicals produced during the burning of organic matter can be transported by wind and may impact biodiversity by mixing with adjacent soil layers. In addition, they may be leached by rainfall, reaching deeper soil horizons, groundwater, and freshwater ecosystems (Agbeshie et al., 2022).

Fire temperature, together with environmental conditions and residence time, is a key factor controlling post-fire soil responses (Agbeshie et al., 2022).

According to Santín & Doerr (2016), fire-induced biological impacts may affect soil biodiversity within a soil temperature range of approximately 50–150 °C (generally below 200 °C), leading to the loss of fine roots, fungi, and bacteria, as well as the depletion of the soil seed bank. Chemical alterations may begin at relatively low temperatures (~50 °C) due to water evaporation and may include the combustion of soil organic matter and the formation of pyrogenic organic matter (~200–800 °C), together with an increase in pH at temperatures above approximately 250 °C. Changes in physical properties and soil structure may range from increased aggregate stability (approximately from 50 to < 250 °C) and the development of soil water repellency (~100–200/250 °C) to its breakdown (approximately 200–400 °C) and the fusion and structural collapse of clays at temperatures exceeding ~400 °C.

Low-temperature fires are generally associated with ash deposition and increases in soil surface pH and nutrient levels, whereas high-temperature fires may lead to complete mineralization of organic matter and substantial nutrient losses, particularly of nitrogen and phosphorus (Raison et al., 1986).

In light of these considerations and the obtained results, the overall pattern observed in the Cinigiano soils suggests a low-to moderate-intensity fire. The increase in fine particles, TOC, TN, and $\text{NH}_4^+\text{-N}$, together with the slight pH rise from CS to BS, is consistent with this interpretation. The fire appeared to exert both direct (BS) and indirect (NBS) effects on soil properties: although NBS and BS showed a similar grain structure, ash deposition was more evident in NBS, whereas BS showed increased aggregate stability, in agreement with Santín and Doerr (2016). Metals and metalloids were generally lower in NBS and BS than in CS, except for Pb. Wildfires may lead to temporary enrichment of some metals, as certain elements are relatively resistant to volatilization (Agbeshie et al., 2022) and may be released from burned vegetation, soil organic matter, or through the thermal alteration of soil minerals (Minos et al., 2026). Moreover, the decreasing trend in NBS and BS compared with CS suggests that the observed pattern is more likely related to pre-existing differences in soil composition rather than to wildfire effects. In the study area, no contamination was detected according to the thresholds established by Italian legislation (L.D. 152/2006). Finally, wildfires release hydrocarbons into the environment through the combustion of plant biomass (Andreolli et al., 2015). As expected, linear hydrocarbons (C12–C40) were detected in the burned soil (BS) at concentrations exceeding the regulatory limit of 50 mg/kg established by the Legislative Decree (152/2006), reaching 150 mg/kg in BS. These compounds may persist in soils for long periods, potentially affecting soil organisms and overall biodiversity, with recovery times that may extend up one year (Andreolli et al., 2015).

Table 1

Weight-of-Evidence (WoE) method applied to tested species and ecosystem; ecotoxicological hazard.

Sample	Eb	Σpsi	Fn	TBI	Ecotoxicological hazard	Classification of Hazard
CS	11.8	3.52	0.003	0.03	Absent/relatively negligible	A
NBS	51.4	6.40	0.002	0.08	Absent/relatively negligible	A
BS	155.8	6.59	0.002	0.24	Moderate	B
CS+EM	468.5	7.84	0.001	0.60	Severe	D
NBS+EM	528.4	7.84	0.001	0.67	Severe	D
BS+EM	524.5	7.84	0.001	0.67	Severe	D
t-test	0.001	0.078	0.183	0.001		

4.2.1. Bioremediation (+EM)

The +EM treatment (15-day bioremediation) further modified soil conditions by increasing soil water content and promoting higher nutrient levels in the treated soils. Li et al. (2024), showed that, over three years, EM application improved soil water-holding capacity, microbial activity, and nutrients levels. Although obtained under long-term conditions in a non-Mediterranean alpine context, these findings suggest that EM may begin to promote improvements in soil structure and nutrient cycling even within the first 15 days of treatment.

Following EM application, a slight increase in some metals and metalloids were observed, particularly in CS+EM and NBS+EM, although all values remained within the limits established by L.D. (152/2006). In acidic soils ($\text{pH} < 6.7$), the solubilization of elements is favored, allowing them to be more easily leached into deeper soil horizons, potentially reducing their availability for plant nutrition or, conversely, leading to toxicity (ARPAV, 2007). In the present study, EM addition appeared to be associated with a short-term decrease in soil pH. This effect may be linked to microbial metabolic activity, including the release of acidic metabolites such as carboxylic acids and amino acids which can increase H^+ concentration and lower soil pH. Such metabolites may also enhance metal chelation, potentially contributing to the restoration of soil chemical conditions (Kapahi & Sachdeva, 2019). Accordingly, the pH decrease observed in EM-treated soils may represent an indirect indicator of microbial metabolic activity, potentially reflecting a short-term response that could reverse over longer time scales (Li et al., 2024).

Regarding linear hydrocarbons ($\text{C} > 12$), EM treatment resulted in only minor changes after 15 days, with slight decreases in BS+EM (−7%) and NBS+EM (−4%), whereas the increase observed in CS+EM (+338%) remains difficult to explain. However, the effectiveness of remediation processes depends on several factors, including the applied technique, treatment duration, and the type of microorganisms involved, which may differ in their degradative capacities. In addition, the remediation of this class of contaminants often requires longer application periods, also extending up to one year, in order to observe significant degradation (Andreolli et al., 2015). Therefore, the lack of observable improvement or remediation of these compounds may be related to the short duration of EM application.

4.3. Effects of fire on the properties of elutriates (-EM/+EM)

Processes occurring in burned soils may also affect adjacent freshwater environments. Following wildfire, changes in soil properties, vegetation loss, and meteorological events can enhance erosion and runoff, generating a sediment cascade that transfers ash, nutrients, contaminants, and metals from terrestrial sources to freshwater systems, with potential consequences for water quality and aquatic organisms (Ducruet et al., 2026). The elutriate experiments, simulating the transfer of soil-derived substances to aquatic environments, showed an increase in electrical conductivity and salinity from CS to BS. These parameters are commonly associated with higher concentrations of dissolved ions and nutrients derived from ash deposition and post-fire runoff processes (Morales et al., 2022). Although no significant differences were detected (-EM vs. +EM), elutriates from EM-treated soils tended to show higher salinity. Since EM have been associated with enhanced organic matter decomposition and nutrient availability (Li et al., 2024), this trend may suggest that EM treatment indirectly promoted the release of nutrients into the elutriate. In addition, the highest Mn concentrations were observed in samples derived from soils with the lowest pH values, measured in the solid phase (CS+EM = 6.80 ± 0.1 pH; Mn = 7.28 ± 1.72 mg/L; NBS+EM = 6.58 ± 0.2 pH; Mn = 5.94 ± 1.40 mg/L). Since acidic conditions ($\text{pH} < 6.7$) can favor the mobilization and leaching of manganese and other elements (ARPAV, 2007), this may explain the increase observed in CS EM and NBS+EM compared with untreated soils. In burned environments, Mn availability may also be linked to vegetation

sources, as some forest species, particularly resinous ones, can accumulate concentrations of manganese in dead leaves (>1000 $\mu\text{g/g}$). During fire, the combustion of plant biomass may release this element, increasing its availability in ash and potentially in soil elutriates (Parra et al., 1996). Mn detected in the control soil +EM may reflect its initial presence (Sánchez-García et al., 2023) and could have become more available at the observed soil pH, potentially enhancing its mobilization into the elutriate.

4.4. Ecotoxicity and hazard level

4.4.1. Soil -EM/+EM

Wildfires can significantly affect plant productivity by destroying vegetation and altering substrate properties, potentially transforming soils into degraded and contaminated matrices (Barroso et al., 2021).

In this study, the response of *Lepidium sativum*, a terrestrial primary producer sensitive to organic and inorganic contaminants (Barroso et al., 2021), was used to assess the effects of wildfire on the soils and the short-term application of EM (15 days). The exposure to soil from the burned area was associated with reduced biomass production, lower root elongation, and increased germination inhibition, suggesting an indirect effect of the fire on plant responses. PCA of biometric and physicochemical traits showed a clear separation between EM-treated and untreated soils, mainly associated with variation in soil pH and biometric parameters (e.g. root length and biomass). Since *L. sativum* is known to prefer slightly acidic to neutral conditions ($\text{pH} 6\text{--}7$ (Barroso & Vavrková, 2020)), the values recorded in the standard control soil (C, $\text{pH} 6.4 \pm 0.1$), together with the highest biomass, germination, and root elongation, confirm that moderately acidic conditions may favor plant performance. However, the decrease in pH observed with EM application did not result in improved response, indicating that additional factors, including changes in element mobility and the presence of linear hydrocarbons, may also have played a role. This interpretation is also supported by the biochemical response of *L. sativum*, as multivariate analyses suggested differences in the distribution of measured stress biomarkers between EM-treated and untreated samples.

The response observed in *Aliivibrio fischeri*, widely used and known for its sensitivity to several classes of contaminants, particularly metals and hydrocarbons (Abbas et al., 2018) showed increased toxicity in EM-treated soils. This result is consistent with the effects observed in *L. sativum* and may be related to changes in soil physicochemical conditions, possibly associated with EM application.

4.4.2. Elutriates -EM/+EM

Wildfires can impact on all components of the ecosystem. Freshwater environments may act as final receiving systems, where direct and indirect effects can alter water properties and affect the survival of aquatic organisms, with multiple factors potentially acting synergistically (Carvalho et al., 2019; Ducruet et al., 2026).

Raphidocelis subcapitata, a freshwater primary producer, showed slight growth inhibition when exposed to elutriates from burned and near-burned soils, while in +EM treatments (except BS+EM), it showed a tendency toward growth stimulation. This response may be related to the potential release of nutrients and $\text{NH}_4\text{-N}$ promoted by EM activity, which can stimulate algal growth but may also increase the risk of eutrophication and algal bloom (Carvalho et al., 2019). Similarly, *A. fischeri* showed a gradual inhibition even in -EM treatments, with increasing responses from CS to BS, indicating sensitivity to the conditions of the elutriates. However, inhibition was more pronounced in EM-treated soils, which may be consistent with changes associated with EM application.

Interestingly, *Daphnia magna*, a freshwater crustacean widely recognized for its sensitivity and commonly used as an indicator of water quality (Harper et al., 2019) did not show immobilization under -EM conditions described when considering the maximum exposure time of 48 h. However, after 15 days, the elutriates derived from EM-treated

soils caused the highest mortality, indicating a stronger impact independent of soil type. The study by Harper et al. (2019) highlights that the effects of wildfire-derived materials on *D. magna* may vary depending on the ecosystem affected by fire. For instance, ashes derived from Australian eucalyptus forest, U.S. chaparral vegetation, and Canadian spruce forest showed significant effects on immobilization, whereas ashes from UK grassland and Spanish forest or heathland did not produce significant effects, despite the presence of heavy metals and hydrocarbons, suggesting that toxicity may depend on the type of vegetation affected by wildfire, fire dynamics, and chemical parameters such as pH and high conductivity. Similarly, in the present study, EM treatment may have contributed to short-term changes in soil physico-chemical properties and elutriate composition, with detectable effects on *D. magna*. In contrast, the type of burned material did not appear to influence the response (observable in -EM conditions). Among the tested organisms, *D. magna* appeared to be the most sensitive under +EM treatments.

4.4.3. Ecotoxicological hazard -EM/+EM

To obtain an overall assessment of the environmental impact of the event, the TBI environmental quality index was applied within a Weight of Evidence (WoE) framework, integrating the responses of the tested species under the experimental conditions considered within a short-term, site-specific context. This approach helps represent ecotoxicological responses and supports a more comprehensive environmental assessment (Linkov et al., 2009). Burned soils generally produced moderate ecological effects and no condition of hazard emerged when potential indirect impacts were considered. In contrast, after the first 15 days of EM treatment, all EM-treated soils were classified as posing a severe hazard according to the TBI index. Although several remediation approaches have been shown to be effective over the long term, and although soil acidification may reflect microbial metabolic activity, these findings indicate that this bioremediation strategy should be carefully evaluated with respect to its potential short-term effects on organisms, while not excluding possible long-term benefits, particularly in view of the hydrocarbon load detected in burned soils under both -EM and +EM conditions.

4.5. Conclusion

Wildfire is a complex phenomenon to analyze, as multiple factors may contribute to the observed outcomes, including the pre-existing properties of soils and the characteristics of the fire itself. The properties of the analyzed soils highlighted the effects resulting from the wildfire that occurred in the study area (24 July 2022, Cinigiano, Tuscany, Italy). Following wildfire, plausibly classified as low-medium intensity, the observed changes in soil had measurable effects on organisms exposed to the burned soil, with a maximum ecological hazard identified as moderate. However, no relevant indirect effects were recorded. Considering the experimental conditions, the short-term treatment (15 days) with effective microorganisms generally resulted in modifications of soil properties, with pH suggested as a key driver of these changes. Overall, EM application led to a measurable increase in ecotoxicological hazard in all analyzed samples (+EM = severe hazard). These findings suggest that the short-term application of effective microorganisms should be carefully evaluated when used to support soil functional recovery after fire, particularly considering the potential ecotoxicological effects. However, combining short-term observations with longer-term studies would provide a deeper understanding of how these changes evolve over time.

CRedit authorship contribution statement

Serena Anselmi: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sara Pignatelli:** Validation, Methodology, Investigation, Data curation. **Leja**

Goljat: Investigation. **Massimo Fanti:** Validation, Software, Data curation. **Karin Schlappa:** Writing – original draft, Data curation, Conceptualization. **Monia Renzi:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used DeepL and ChatGPT 5.3 to support language editing, grammar and text improvement. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Funding sources

This research was supported by the Bioscience Research Center under the internal grant No. RG_31_2025 (Principal Investigator: Dr. M. Renzi).

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.

Acknowledgements

Graphic abstract was created with BioRender (Academic subscription). The authors are grateful to Guido Anselmi for his support of our research activity during sampling activities carried out under emergency conditions. They also wish to express their sincere solidarity with the population of Cinigiano, who were deeply affected by the severe material damage caused by the wildfire, which had a profound impact on the local community and territory.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2026.128239>.

Data availability

Data will be made available on request.

References

- Abbas, M., Adil, M., Ehtisham-ul-Haque, S., Munir, B., Yameen, M., Ghaffar, A., Shar, G. A., Asif Tahir, M., Iqbal, M., 2018. *Vibrio fischeri* bioluminescence inhibition assay for ecotoxicity assessment: a review. *Sci. Total Environ.* 626, 1295–1309. <https://doi.org/10.1016/j.scitotenv.2018.01.066>.
- AE. (n.d.). Valori agricoli medi. Portale Agenzia Entrate. Retrieved February 20, 2025, from <https://www.agenziaentrate.gov.it/portale/schede/fabbricattiterreni/omi/banche-dati/valori-agricoli-medi>.
- Agbeshie, A.A., Abugre, S., Atta-Darkwa, T., Awuah, R., 2022. A review of the effects of forest fire on soil properties. *J. For. Res.* 33 (5), 1419–1441. <https://doi.org/10.1007/s11676-022-01475-4>.
- Akburak, S., Son, Y., Makineci, E., Çakir, M., 2018. Impacts of low-intensity prescribed fire on microbial and chemical soil properties in a Quercus frainetto forest. *J. For. Res.* 29 (3), 687–696. <https://doi.org/10.1007/s11676-017-0486-4>.
- Alexieva, V., Sergiev, I., Mapelli, S., Karanov, E., 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environ.* 24 (12), 1337–1344. <https://doi.org/10.1046/j.1365-3040.2001.00778.x>.
- Andreolli, M., Lampis, S., Brignoli, P., Vallini, G., 2015. Bioaugmentation and biostimulation as strategies for the bioremediation of a burned woodland soil contaminated by toxic hydrocarbons: a comparative study. *J. Environ. Manag.* 153, 121–131. <https://doi.org/10.1016/j.jenvman.2015.02.007>.
- ARPAV, 2007. L'interpretazione delle analisi del terreno. Strumento per la sostenibilità ambientale. Agenzia Regionale per la Prevenzione e Protezione Ambientale. <https://>

- www.arpa.veneto.it/arpavinforma/pubblicazioni/linterpretazione-delle-analisi-del-terreno.
- Backer, D.M., Jensen, S.E., McPHERSON, G.R., 2004. Impacts of fire-suppression activities on natural communities. *Conserv. Biol.* 18 (4), 937–946. <https://doi.org/10.1111/j.1523-1739.2004.494.1.x>.
- Barroso, P.M., Vavřková, M.D., 2020. Fire effects on soils – a pilot Scale Study on the soils affected by wildfires in the Czech Republic. *Journal of Ecological Engineering* 21 (nr 6). <https://doi.org/10.12911/22998993/123471>.
- Barroso, P.M., Vavřková, M.D., Elbl, J., 2021. Assessing the ecotoxicity of soil affected by wildfire. *Environments* 8 (1), 3. <https://doi.org/10.3390/environments8010003>.
- Bosch, M., Hitman, A., Hoekstra, J.F., 2017. Fermentation (Bokashi) Versus Composting of Organic Waste Materials: Consequences for Nutrient Losses and CO₂-footprint, vols. 1–3.
- Callesen, I., Keck, H., Andersen, T.J., 2018. Particle size distribution in soils and marine sediments by laser diffraction using Malvern Mastersizer 2000—Method uncertainty including the effect of hydrogen peroxide pretreatment. *J. Soils Sediments* 18 (7), 2500–2510. <https://doi.org/10.1007/s11368-018-1965-8>.
- Carvalho, F., Pradhan, A., Abrantes, N., Campos, I., Keizer, J.J., Cassio, F., Pascoal, C., 2019. Wildfire impacts on freshwater detrital food webs depend on runoff load, exposure time and burnt forest type. *Sci. Total Environ.* 692, 691–700. <https://doi.org/10.1016/j.scitotenv.2019.07.265>.
- Cherubini, J.D., 2022. Incendio in Maremma, fiamme vicino ad agriturismi e poderi: Evacuato il paese di Cinigiano. *Corr. Fiorent.* https://corrierefiorentino.corriere.it/firenze/notizie/cronaca/22_luglio_24/incendio-maremma-tre-elicotteri-azione-cinigiato-2576c554-0b5a-11ed-8edd-496886fe0721.shtml.
- CIE, 1931. *Commission Internationale De L'Éclairage*.
- Coldiretti Livorno, 2022. Incendi: A Cinigiano Bruciati Fienili, Vigne, Olivi, Foraggi E Pascoli. La Lotta Degli Agricoltori Contro Le Fiamme. Toscana Coldiretti. <https://livorno.coldiretti.it/news/incendi-a-cinigiano-bruciati-fienili-vigne-olivi-foraggi-e-pascoli-la-lotta-degli-agricoltori-contro-le-fiamme/>.
- CORINE Land Cover. (n.d.). Retrieved February 24, 2026, from <https://land.copernicus.eu/en/products/corine-land-cover>.
- Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R.V., Paruelo, J., Raskin, R.G., Sutton, P., Van Den Belt, M., 1997. The value of the world's ecosystem services and natural capital. *Nature* 387 (6630), 253–260. <https://doi.org/10.1038/387253a0>.
- Cunningham, W.P., Cunningham, M.A., Woodworth Saigo, B., 2004. *Environmental Science: a Global Concern*, seventh ed. The McGraw-Hill Companies, Inc. ISBN 88-386-6133-2.
- Ducruet, R., Foucher, A., Bardelle, A., Evrard, O., 2026. Investigating post-wildfire effects in contrasted Mediterranean ecosystems. *J. Soils Sediments* 26 (2), 21. <https://doi.org/10.1007/s11368-025-04216-2>.
- Dyrness, C.T., Cleve, K.V., Levison, J.D., 1989. The effect of wildfire on soil chemistry in four forest types in interior Alaska. *Can. J. For. Res.* 19 (11), 1389–1396. <https://doi.org/10.1139/x89-213>.
- Footer, A., 2014. *Bokashi Composting: Scraps to Soil in Weeks*. New Society Publishers.
- Harper, A.R., Santin, C., Doerr, S.H., Froyd, C.A., Albini, D., Otero, X.L., Vinas, L., Pérez-Fernández, B., 2019. Chemical composition of wildfire ash produced in contrasting ecosystems and its toxicity to *Daphnia magna*. *Int. J. Wildland Fire* 28 (10), 726–737. <https://doi.org/10.1071/WF18200>.
- ICRAM, 2001. *Programma Di Monitoraggio per il Controllo Dell'Ambiente marino-costiero (Triennio 2001-2003)*. Metodologie Analitiche Di Riferimento. Ministero dell'Ambiente e della Tutela del Territorio Servizio Difesa Mare. <https://www.isprambiente.gov.it/it/pubblicazioni/manuali-e-linee-guida/metodologie-analitiche-di-riferimento-del>.
- ISPRA. (n.d.). Definizioni. ISPRA Istituto Superiore per la Protezione e la Ricerca Ambientale. Retrieved February 20, 2025, from <https://www.isprambiente.gov.it/it/attivita/Crisi-Emergenze-ambientali-e-Danno/centro-operativo-per-la-sorveglianza-ambientale/ecosistemi-ed-incendi-boschivi-in-italia/definizioni-di-incendio-boschivo>.
- ISPRA, 2013. *Batterie Di Saggi Ecotossicologici per Sedimenti Di Acque Interne*. Istituto Superiore per la Protezione e la Ricerca Ambientale (No. 88/2013; I manuali di ecotossicologia). https://www.isprambiente.gov.it/files/pubblicazioni/manua-li-lineeguida/MLG_88_2013.pdf.
- ISPRA, 2016. *Documentazione E Software Di Supporto per L'Applicazione Del Decreto 15 Luglio 2016, N. 173*. ISPRA Istituto Superiore per la Protezione e la Ricerca Ambientale (ex art 109, comma 2 del D.lgs 152/06). <https://www.isprambiente.gov.it/it/servizi/documentazione-e-software-di-supporto-per-l2019applicazione-del-decreto-15-luglio-2016-n-173>.
- ISPRA, 2021. *Aspetti metodologici finalizzati all'applicazione dei saggi biologici previsti dall'allegato tecnico al D.M. 173/16: protocollo per la preparazione dell'elutriato*. Quaderni di ecotossicologia. ISBN 978-88-448-1079-5.
- ISPRA, 2021. *Procedura operativa del saggio in fase solida mediante Aliivibrio fischeri*. Quaderni Laboratorio 4/2021, 978-88-448-1076-4.
- ISPRA, 2022. *Ecosistemi Terrestri Ed Incendi Boschivi in Italia: Rapporto 2022 (Rapporto CSA IB1/2022)*. ISPRA.
- Jhariya, M.K., Raj, A., 2014. Effects of wildfires on flora, fauna and physico-chemical properties of soil-An overview. *Journal of Applied and Natural Science* 6 (2), 887–897. <https://doi.org/10.31018/jans.v6i2.550>.
- Kapahi, M., Sachdeva, S., 2019. Bioremediation options for heavy metal pollution. *Journal of Health and Pollution* 9 (24), 191203. <https://doi.org/10.5696/2156-9614-9-24.191203>.
- Keywood, M., Kanakidou, M., Stohl, A., Dentener, F., Grassi, G., Meyer, C.P., Torseth, K., Edwards, D., Thompson, A.M., Lohmann, U., Burrows, J., 2013. Fire in the air: biomass burning impacts in a changing climate. *Crit. Rev. Environ. Sci. Technol.* 43 (1), 40–83. <https://doi.org/10.1080/10643389.2011.604248>.
- Köster, K., Aaltonen, H., Berninger, F., Heinonsalo, J., Köster, E., Ribeiro-Kumara, C., Sun, H., Tedersoo, L., Zhou, X., Pumpanen, J., 2021. Impacts of wildfire on soil microbiome in Boreal environments. *Curr. Opin. Environ. Sci. Health* 22, 100258. <https://doi.org/10.1016/j.coesh.2021.100258>.
- Law, M.Y., Charles, S.A., Halliwell, B., 1983. Glutathione and ascorbic acid in spinach (*Spinacia oleracea*) chloroplasts. The effect of hydrogen peroxide and of Paraquat. *Biochem. J.* 210 (3), 899–903. <https://doi.org/10.1042/bj2100899>.
- Legislative Decree 152/2006, No. 152 § Part IV, Title V, Annex 5, table 1. <https://www.normattiva.it/uri-res/N2Ls?urn:nir:stato:decreto.legislativo:2006-04-03;152>, 2006.
- Legislative decree 172/2015, no. 172. <https://www.normattiva.it/uri-res/N2Ls?urn:nir:stato:decreto.legislativo:2015-10-13;172vig->, 2015.
- Li, C., Cui, C., Zhang, J., Shen, J., He, B., Long, Y., Ye, J., 2023. Biodegradation of petroleum hydrocarbons based pollutants in contaminated soil by exogenous effective microorganisms and indigenous microbiome. *Ecotoxicol. Environ. Saf.* 253, 114673. <https://doi.org/10.1016/j.ecoenv.2023.114673>.
- Li, J., Wei, J., Shao, X., Yan, X., Liu, K., 2024. Effective microorganisms input efficiently improves the vegetation and microbial community of degraded alpine grassland. *Front. Microbiol.* 14, 1330149. <https://doi.org/10.3389/fmicb.2023.1330149>.
- Linkov, I., Loney, D., Cormier, S., Satterstrom, F.K., Bridges, T., 2009. Weight-of-evidence evaluation in environmental assessment: review of qualitative and quantitative approaches. *Sci. Total Environ.* 407 (19), 5199–5205. <https://doi.org/10.1016/j.scitotenv.2009.05.004>.
- Mayer, J., Scheid, S., Widmer, F., Fließbach, A., Oberholzer, H.-R., 2010. How effective are 'effective microorganisms® (EM)'? Results from a field study in temperate climate. *Appl. Soil Ecol.* 46 (2), 230–239. <https://doi.org/10.1016/j.apsoil.2010.08.007>.
- Meier, S., Elliott, R.J.R., Strobl, E., 2023. The regional economic impact of wildfires: evidence from Southern Europe. *J. Environ. Econ. Manag.* 118, 102787. <https://doi.org/10.1016/j.jeem.2023.102787>.
- Minos, T., Stamatakis, A., Golia, E.E., Adamantidou, C., Tziourrou, P., Spiliotopoulos, M.-E., Barbieri, E., 2026. The impacts of extreme weather events on soil contamination by heavy metals and polycyclic aromatic hydrocarbons: an integrative review. *Land* 15 (1), 165. <https://doi.org/10.3390/land15010165>.
- Morales, J.J., Paes, N.D.S., Silva, A.C.M., Teixido, A.L., 2022. Fire and Water: Global Fire Impacts on Physicochemical Properties of Freshwater Ecosystems. <https://doi.org/10.21203/rs.3.rs-1233463/v1> submitted for publication.
- Naeher, L.P., Brauer, M., Lipsett, M., Zelikoff, J.T., Simpson, C.D., Koenig, J.Q., Smith, K. R., 2007. Woodsmoke health effects: a review. *Inhal. Toxicol.* 19 (1), 67–106. <https://doi.org/10.1080/08958370600985875>.
- Okamura, M., 1980. An improved method for determination of L-ascorbic acid and L-dehydroascorbic acid in blood plasma. *Clinica Chimica Acta* 103, 259–268. [https://doi.org/10.1016/0009-8981\(80\)90144-8](https://doi.org/10.1016/0009-8981(80)90144-8).
- Olle, M., Williams, I.H., 2013. Effective microorganisms and their influence on vegetable production – a review. *J. Hortic. Sci. Biotechnol.* 88 (4), 380–386. <https://doi.org/10.1080/14620316.2013.11512979>.
- Parra, J.G., Rivero, V.C., Lopez, T.L., 1996. Forms of Mn in soils affected by a forest fire. *Sci. Total Environ.* 181 (3), 231–236. [https://doi.org/10.1016/0048-9697\(95\)05022-1](https://doi.org/10.1016/0048-9697(95)05022-1).
- Peñuelas, J., Sardans, J., Filella, I., Estiarte, M., Llusià, J., Ogaya, R., Carnicer, J., Bartrons, M., Rivas-Ubach, A., Grau, O., Peguero, G., Margalef, O., Pla-Rabés, S., Stefanescu, C., Aensio, D., Preece, C., Liu, L., Verger, A., Barbeta, A., et al., 2017. Impacts of global change on Mediterranean forests and their services. *Forests* 8 (12), 463. <https://doi.org/10.3390/f8120463>.
- Pereira, P., Bogunovic, I., Zhao, W., Barcelo, D., 2021. Short-term effect of wildfires and prescribed fires on ecosystem services. *Curr. Opin. Environ. Sci. Health* 22, 100266. <https://doi.org/10.1016/j.coesh.2021.100266>.
- Ponomarenko, R., Loboichenko, V., Strelets, V., Gurbanova, M., Morozov, A., Kovalov, P., Shevchenko, R., Kovalova, T., 2019. Review of the environmental characteristics of fire extinguishing substances of different composition used for fires extinguishing of various classes. *J. Eng. Appl. Sci.* 14 (16), 5925–5941. <http://repositsc.nuczu.edu.ua/handle/123456789/9925>.
- Raison, R.J., Woods, P.V., Jakobsen, B.F., Bary, G.A.V., 1986. Soil temperatures during and following low-intensity prescribed burning in a *Eucalyptus pauciflora* forest. *Soil Res.* 24 (1), 33–47. <https://doi.org/10.1071/sr9860033>.
- Regione Toscana—SIPT: Uso E Copertura Del Suolo. (n.d.). Retrieved February 24, 2026, from <https://www502.regione.toscana.it/geoscopio/usocoperturasuolo.html>.
- Reid, C.E., Brauer, M., Johnston, F.H., Jerrett, M., Balmes, J.R., Elliott, C.T., 2016. Critical review of health impacts of wildfire smoke exposure. *Environ. Health Perspect.* 124 (9), 1334–1343. <https://doi.org/10.1289/ehp.1409277>.
- Rovithakis, A., Grillakis, M.G., Seiradakis, K.D., Giannakopoulos, C., Karali, A., Field, R., Lazaridis, M., Voulgarakis, A., 2022. Future climate change impact on wildfire danger over the Mediterranean: the case of Greece. *Environ. Res. Lett.* 17 (4), 045022. <https://doi.org/10.1088/1748-9326/ac5f94>.
- Sánchez-García, C., Santin, C., Neris, J., Sigmund, G., Otero, X.L., Manley, J., González-Rodríguez, G., Belcher, C.M., Cerdá, A., Marcotte, A.L., Murphy, S.F., Rhoades, C.C., Sheridan, G., Strydom, T., Robichaud, P.R., Doerr, S.H., 2023. Chemical characteristics of wildfire ash across the globe and their environmental and socio-economic implications. *Environ. Int.* 178, 108065. <https://doi.org/10.1016/j.envint.2023.108065>.
- Santin, C., Doerr, S.H., 2016. Fire effects on soils: the human dimension. *Phil. Trans. Biol. Sci.* 371 (1696), 20150171. <https://doi.org/10.1098/rstb.2015.0171>.
- Sedlak, J., Lindsay, R.H., 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.* 25, 192–205. [https://doi.org/10.1016/0003-2697\(68\)90092-4](https://doi.org/10.1016/0003-2697(68)90092-4).
- Sentinel-hub EO-Browser3. (n.d.). Retrieved February 26, 2026, from <https://apps.sentinel-hub.com/eo-browser/?zoom=15&lat=42.86645&lng=11.36673&them>

- eId=DEFAULT-THEME&visualizationUrl=U2FsdGVkX1%2FjZlA51OQbn9TrG66Esou27BaAI8%2BW7vTXjQrtWYH2ulJxqrUONEU6T%2BPG6B4yMwEka%2FivDQmSsxN85X8r%2F%2BaSYQRm8IGZ5A2fpGtmbEJKmJsc%2Fb2lAbc5&datasetId=S2L2A&fromTime=2022-07-22T00%3A00%3A00.000Z&toTime=2022-07-22T23%3A59%3A59.999Z&layerId=1_TRUE_COLOR&minQa=null&demSource3D=%22MAPZEN%22.
- UNI, 2010. UNI 11357:2010. Qualità dell'acqua—Determinazione dell'inibizione della germinazione e allungamento radicale in *Cucumis sativus* L. (Cetriolo), *Lepidium sativum* L. (Crescione), *Sorghum saccharatum* Moench (Sorgo)—Saggio di tossicità cronica breve. <https://conto.uni.com/uni-11357-2010>.
- UNI, 2012. UNI EN ISO 8692:2012. Qualità dell'acqua—Prova Di Inibizione Della Crescita Di Alghe D'Acqua Dolce per Mezzo Di Alghe Verdi. <https://store.uni.com/uni-en-iso-8692-2012>.
- UNI, 2013. UNI EN ISO 6341:2013. Qualità dell'acqua—Determinazione Dell'Inibizione Della Mobilità Di *Daphnia magna* Straus (Cladocera, Crustacea)—Prova Di Tossicità Acuta. <https://store.uni.com/uni-en-iso-6341-2013>.
- UNI, 2019. UNI EN ISO 11348-1:2019. Qualità dell'acqua—Determinazione Dell'Effetto Inibitorio Di Campioni Acquosi Sull'Emissione Di Luce Di *Vibrio Fischeri* (Prova Su Batteri luminescenti)—Parte 1: Metodo Con Batteri Preparati Di Fresco. <https://conto.uni.com/uni-en-iso-11348-1-2019>.
- Wang, L., Shao, X., Xu, M., Chen, S., 2019. Bioremediation of nitrogen- and phosphorus-polluted aquaculture sediment by utilizing combined immobilized effective microorganisms and. *Int. J. Agric. Biol. Eng.* 12 (6). <https://doi.org/10.25165/ijabe.v12i6.4904>. Article 6. (water environment).
- Westerling, A.L., Hidalgo, H.G., Cayan, D.R., Swetnam, T.W., 2006. Warming and earlier Spring increase Western U.S. Forest wildfire activity. *Science* 313 (5789), 940–943. <https://doi.org/10.1126/science.1128834>.
- Wolf, K.D., Higuera, P.E., Davis, K.T., Dobrowski, S.Z., 2021. Wildfire impacts on forest microclimate vary with biophysical context. *Ecosphere* 12 (5), e03467. <https://doi.org/10.1002/ecs2.3467>.
- Ye, X., Ye, Y., Huang, X., Onega, T., 2026. Wildfires and public health: a comprehensive review of human-centric studies. *GeoHealth* 10 (2). <https://doi.org/10.1029/2025GH001534>.