



Impact of prescribed burning, mowing and abandonment on a Mediterranean grassland: A 5-year multi-kingdom comparison



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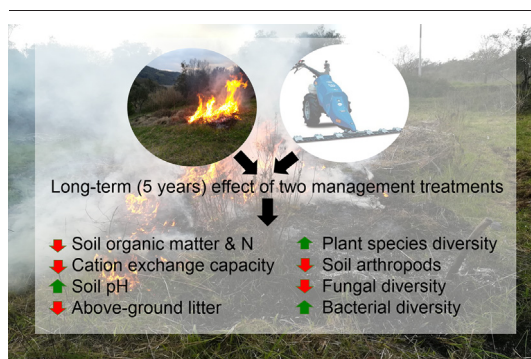
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HIGHLIGHTS

- 5-Year experiment testing prescribed burning, mowing and abandonment on grassland.
- We used vegetation analysis, soil chemistry and next-generation sequencing of the soil microbiota.
- Burning significantly increased pH, reduced soil organic carbon and nitrogen.
- Burning and mowing significantly increased plant species richness.
- Soil microbiota was shaped by burning and mowing, but was unaffected by land abandonment.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Wei Shi

Keywords:

Long-term experiment
Biodiversity conservation
Soil microbiota
Arthropods
Brachyodinium

ABSTRACT

Mediterranean grasslands are semi-natural, fire-prone, species-rich ecosystems that have been maintained for centuries through a combination of fire, grazing, and mowing. Over the past half century, however, grasslands have faced numerous threats, including the abandonment of traditional agro-pastoral practices. Our hypothesis was that mowing and prescribed burning are management practices potentially effective in counteracting the reduction of plant diversity triggered by land abandonment. However, the long-term effects of such management practices on plant communities and soil microbiota in Mediterranean grassland remain poorly studied. Here, we conducted a 5-year field experiment comparing prescribed fire, vegetation mowing, and abandonment in a fire-prone Mediterranean grassland in southern Italy in order to evaluate the capability of such management strategies to counteract the detrimental impacts of land abandonment on plant diversity and the associated increase of wildfire. We combined vegetation analysis and soil chemical characterization and several microbiota analyses, including microbial biomass and respiration, arthropod community, and high-throughput sequencing of bacterial and eukaryotic rRNA gene markers. Burning and mowing significantly increased plant species richness and diversity compared to abandonment plots, reducing the abundance of perennial tall grasses in favour of short-lived species. Standing litter followed the same trend, being 3.8-fold greater and largely composed of grass remains in the abandoned compared to burnt and mowed plots. In the soil, prescribed burning caused significant increase in pH, a reduction in organic carbon, total N, and cation exchange capacity. Diversity and taxonomic composition of bacterial and fungal microbiota was affected by burning

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<http://dx.doi.org/10.1016/j.scitotenv.2022.155442>

Received 11 December 2021; Received in revised form 20 March 2022; Accepted 18 April 2022

Available online xxx

and mowing treatments. Abandonment caused shifts of microbiota towards a fungal-dominated system, composed of late successional fungi of the Basidiomycota. Fast-growing and putative fungal pathogens were more abundant under burnt and mowed treatments. Soil arthropods were influenced by vegetation and microbiota changes, being strongly reduced in mowed plots. Our study demonstrated that grassland abandonment promotes the spread of tall grasses, reducing plant diversity and increasing the risk of wildfire, while prescribed burning and mowing are effective in counteracting such negative effects.

1. Introduction

Mediterranean grasslands are semi-natural, fire-prone, and species-rich ecosystems (Verdú et al., 2000; Harrison et al., 2003). In Europe, since the Neolithic age, these plant communities have been generated by opening gaps in the forest and maintained for centuries by a combination of fire, grazing, mowing, and farming (Bugalho et al., 2011). However, in the last half-century, ecosystems like grasslands are subject to multiple threats including climatic changes (Nogueira et al., 2018), invasion by non-native species (Funk et al., 2016), and land abandonment (Plieninger et al., 2014).

In the Mediterranean basin, in the last decades, traditional agro-pastoral practices have been subject to sudden changes caused by the dramatic reduction of the active rural human population (FAOSTAT, 2012; Fayet et al., 2022). As a consequence, grasslands lost their economic functions and were progressively abandoned all over Europe (Poschod and WallisDeVries, 2002). Notably, land abandonment has mostly affected the less productive, marginal areas, where semi-natural grasslands often harbour rare and endemic species. Land abandonment of semi-natural grassland leads to changes in the plant communities with a progressive increase of few competitive, tall-grass species that suppress small, short-lived species with a consequent reduction in species diversity and, in Mediterranean climate, increase the risk of wildfire. Notable examples are the diffusion in abandoned sites by the grasses like *Brachypodium pinnatum* (L.) P. Beauv in north Europe (Bobbink and Willems, 1987), or *B. genuense* (DC.) Roem. & Schult. and *B. rupestre* (Host) Roem. & Schult. in the mountains of central Apennines (Catorci et al., 2011; Bonanomi et al., 2006). Intense competition for light and above-ground accumulation of litter causes the reduction of plant species richness. A dense litter layer could inhibit seedling establishment by releasing phytotoxic compounds during decomposition (Loydi et al., 2015), physical obstruction (Wedin and Tilman, 1993), or the reduction or alteration of light available at ground level (Facelli and Pickett, 1991). Moreover, in the long-term litter accumulation, it could also buffer soil conditions in terms of temperature and moisture regimes that could trigger shrub and tree encroachment, especially in arid and semi-arid grasslands (Loydi et al., 2013). In this regards several studies demonstrated that mowing is effective management practice to contract tall-grass invasion and woody species encroachment by reducing above-ground competition (Bobbink and Willems, 1991; Bonanomi et al., 2013; Tardella et al., 2020). Unfortunately, mowing as environmental management is time-consuming and costly, with obvious practical limitations especially in economically depressed areas. In this context, a potential alternative is the use of prescribed burning.

In the Mediterranean region grasslands co-evolved with fire for millennia. Despite this, the application of prescribed fire is a poorly accepted practice by politicians, farmers, and citizens, with systematic fire suppression and exclusion that become routine in the twentieth century in European countries and, to a lesser extent, in North America (Ryan et al., 2013). The concerns about economic losses caused by fire promoted the systematic planning of fire suppression. Although fire suppression is capable to control most of the wildfires, the widespread accumulation of leaf litter and wood debris dramatically increases the risk of catastrophic fire as recently observed in the USA and European Mediterranean countries (Ferreira-Leite et al., 2016). In the last decades, scientists learned the negative consequences of systematic fire exclusion for grassland ecosystems, with many studies that demonstrated the possibility to safely conduct prescribed fire (Valkó et al., 2014). In Europe, most of the studies concerning prescribed fire focused on fire-prone forests like *Pinus* spp. and *Eucalyptus*

spp. plantations or natural stands (Catalanotti et al., 2018; Espinosa et al., 2018; Fernandes, 2018), with far less attention paid to grasslands.

Previous studies focused their attention on the impact of prescribed fire on soil chemical and hydraulic properties including water repellency and infiltration (Hubbert et al., 2006), organic carbon stock and pH, nitrogen cycle, and the dynamics of phosphorus and major cations (Alcañiz et al., 2018). Concerning the biotic soil components, most of the available studies are short-term and used only aggregated parameters like microbial biomass, fungal biomass, and soil respiration (Wang et al., 2012; Catalanotti et al., 2018; Pérez-Valera et al., 2020). The few studies that used next generations sequencing approach to investigate fire impact on soil microbiota were carried-out in areas subject to wildfire (Fernández-González et al., 2017; Dove et al., 2021) or after prescribed burning in North American grasslands (Qin et al., 2020). Nonetheless, none of the previous studies about prescribed fire used a holistic approach that includes the response of the most important ecosystem compartments, i.e. higher plants, arthropods, and soil microbiota.

Here, our general goal is to evaluate the capability of different management strategies i.e. mowing and prescribed burning, to counteract the detrimental impacts of land abandonment on plant diversity and the associated increase of wildfire. Intending to overcome the methodological limitations of previous studies, we carried out a 5-years experiment that compared prescribed fire applied twice, yearly vegetation mowing, and abandonment in a fire-prone Mediterranean grassland located in Southern Italy. Our hypothesis was that land abandonment would cause a reduction of plant species diversity and change the trajectories of microbiome development, while prescribed fire would have a stronger impact on counteracting these effects compared to vegetation mowing. In order to provide a complete view of the grassland response, we combined vegetation analysis with extensive soil chemical characterization i.e. pH, electrical conductivity, particle-size distribution, organic carbon, total nitrogen, C/N ratio, total carbonates, available phosphorus, cation exchange capacity, exchangeable cations (Ca^{2+} , Mg^{2+} , K^+ , Na^+), bioavailable micronutrients (Fe, Zn, Mn, Cu). Soil microbiota was studied using both integrated parameters, i.e. microbial biomass, fungal biomass and respiration, and high-throughput sequencing of bacterial and eukaryotic rRNA gene markers. Finally, the arthropod community response was also studied. The specific hypotheses of our study were:

- (i) prescribed-fire and mowing restore higher plants diversity compared to land abandonment by reducing the dominance of perennial grasses;
- (ii) prescribed-fire increases soil pH and the availability of major cations but reduces the stock of organic carbon and nitrogen compared to mowing and land abandonment;
- (iii) prescribed-fire, by removing plant litter with periodical burning, reduces microbial and fungal biomass as well as arthropod abundance compared to mowing and land abandonment.

2. Materials and methods

2.1. Study site description

This study was conducted in Southern Italy (Cicerale; 40°19'41.76"N, 15°07'23.55"E) in Mediterranean grassland currently managed by annual mowing and grazed at low intensity by sheep and goats. The altitude of the field site is 183 m a.s.l., with a south-facing slope of about 10° slope. The underlying soil is a clay-loam with underlying sedimentary flysch

rocks (Cambisol according to World Reference Base for Soil Resources). The total mean annual rainfall is high at 1328 mm, but with a pronounced summer drought. The mean annual temperature is 14.8 °C, with monthly temperatures that range between 24.4 °C (August) and 10.8 °C (January). The study site lies in the private property of one of the Authors (G. Bonanomi), hence no specific permission was required.

2.2. Long-term field experiment

In January 2013, nine plots (20 × 10 m) were established. Plots were arranged in parallel columns, each separated by a 2 m buffer strip and protected from grazing by caging. Three plots were selected for each of the following treatments: (1) untreated control subject to abandonment (abandoned); (2) yearly mowing in March at the beginning of the growing season (mowed); (3) prescribed burning made in March at the beginning of the growing season (burnt). Mowing was carried out each year with professional mechanical haymaking (BCS 615 L Max, BCS, Milan, Italy) at ~2 cm above the soil surface. Prescribed burning was carried out by setting fire at the lower, right corner of the plots in the afternoon during a clear and calm day (wind speed < 5 m s⁻¹). Prescribed burning was carried out twice, in March 2013 and in March 2017 with a standing litter of 456 ± 85 g m⁻² and 304 ± 31 g m⁻², respectively. The presence of rather homogeneous litter layers mainly composed of grass remains allowed a low-intensity fire that spread rapidly over the entire plot (Supplementary Fig. S1). The experiment is still running and here we present data collected in June 2019, 5 years after the beginning of the experiment.

2.3. Vegetation survey

In mid-June 2019, aboveground living plant biomass was cut by scissors and litter harvested from each plot within five sampling squares (25 × 25 cm). The aboveground biomass collected from each plot was accurately sorted into living plant material separated for each species and standing litter.

The dry weight of each of the species was measured after drying at 30 °C for 10 days in a ventilated chamber. Species diversity (Shannon index = H) was calculated based on the biomass data. One-way ANOVA was used to test the effects of treatment (abandoned, mowed, and burnt) on the total aboveground living plant biomass, standing litter, and species diversity (H) using Statistica 10 software, and the means were pairwise separated using the post hoc Tukey test. Vegetation biomass was analyzed based on plant functional types, i.e. grass, forb, nitrogen-fixing forb, sedge, and parasite. To individuate plant species associated with specific management practice we calculated the association index between different plant species (Clarke and Gorley, 2015). The resulting contingency matrix was used to produce Hierarchical clustering based on the complete linkage method. Clusters were individuated for a threshold of 60 for the association index. For each species, abundance was recalculated as a percentage of variation (i.e. association values) between the different management practices. Percentage variations were then compared for each cluster individuated in the Hierarchical clustering. The vascular plants observed in the study site were identified in the field except for dubious cases, which were collected and later identified at the Herbarium Porticense (PORUN, MUSA Museum, University of Naples Federico II, Italy), according to Pignatti et al. (2019). The nomenclature follows the WFO (2021). Families are organized according to APG IV system for angiosperms (Stevens, 2015). Finally, in mid-May 2019 during the growing season, photosynthetic active radiation (PAR, wavelength between 400 and 700 nm) was quantified using ADC-L2A light sensor at a height of 0, 50, and 100 cm above the ground in each plot.

2.4. Soil sampling

The soil was sampled during the plant growing season (May and mid-June 2019) by a plastic core and soil samples (~1000 g) were collected from the topsoil (0–10 cm), after removal of the litter layer, in all sampling plots. In each plot, five soil sub-samples were collected following a W

scheme (four sampling plots near corners and one sampling plot in the center of the plot) and pooled to have nine composite soil samples (three treatments with three replicates each).

Samples were immediately packed in polyethylene bags and transferred to the laboratory for further analyses. The biochemical and biological analyses and bioassays were carried out on fresh soil stored at +4 °C until the time of measurements (within 7 days). Chemical analyses were carried out on soil dried at room temperature. Similarly, three soil samples per area with different management practices were sampled for metagenomic analysis. Between each sampling, soil tools were sterilized to avoid sample contamination. For metagenomic analysis sub-samples were stored in sterile plastic bags and frozen at -80 °C until DNA extraction.

2.5. Soil physical and chemical analyses

Soil samples (2 mm sieved air-dried soil) were analyzed for 19 physico-chemical parameters according to the Italian official methods of soil analysis (MIPAF, 1999). In details: pH was determined potentiometrically by applying a 1:2.5 soil:1 M KCl ratio; electrical conductivity was determined on a 1:5 soil:water ratio extract; organic carbon, total nitrogen, available phosphorus and bioavailable micronutrients (Cu, Mn, Fe, Zn) were determined according to Walkley and Black, Kjeldahl, Olsen and Lindsay and Norwell methods, respectively; cation exchange capacity was determined in BaCl₂ + TEA solution at pH 8.1; exchangeable cations (Ca, Mg, K, Na) were determined in the BaCl₂ + TEA extract by atomic absorption spectroscopy; total carbonates were determined by pressure Dietrich-Fruhling calcimeter; particle size analysis (sand, silt and clay) was determined by Andreasen's pipette method after wet sieving by sedimentation in aqueous medium; C/N ratio was calculated from organic carbon and total nitrogen data.

2.6. Soil microbiota and bioinformatics analysis

Microbiota response was performed by a multi-technique approach combining conventional and molecular methods. The microbial biomass was evaluated as microbial carbon, according to Anderson and Domsch (1978), by the method of substrate induced respiration (SIR). SIR was determined using glucose (75 mM), as the easily mineralisable substrate and the evolved CO₂ in 72 h incubation at 25 °C in the dark (Degens et al., 2000) was adsorbed in NaOH and measured by two-phase titration with HCl (Froment, 1972). The magnitude of the respiratory response was converted to mg of microbial biomass carbon using the conversion factor introduced by Sparling (1995). The potential respiration of soil samples was estimated as the CO₂ evolution after adding 3 mL distilled water to 3 g of sample. The obtained results were used to calculate the metabolic quotient (qCO₂), i.e. the degree of activity of the microbial biomass (Anderson and Domsch, 1993); and the coefficient of endogenous mineralisation (CEM), i.e. the rate of organic carbon mineralisation (Rutigliano et al., 2002). qCO₂ was calculated as the ratio between Resp (C-CO₂) and Cmic, whereas CEM was calculated as the ratio between Resp (C-CO₂) and Corg. The fungal biomass (FB) was assayed by membrane filter technique (Sundman and Sivelä, 1978), after staining with Aniline Blue, determining hypha length by the intersection method (Olson, 1950) with an optical microscope (Optika, B-252). Data were statistically analyzed by analysis of variance (One-way ANOVA) followed by post hoc Duncan tests.

Total microbial DNA extraction was carried out on a quota of 200 mg for each sample using the DNeasy Power Soil kit (Qiagen). The diversity and composition of soil microbial communities were analyzed by high-throughput sequencing with the Illumina MiSeq platform. Bacterial and fungal diversity was assessed by sequencing of the amplified V3-V4 regions of the 16S rRNA gene (~460 bp) and ITS1-2 (~300 bp), respectively. PCR was carried out with the primers S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 (Berni Canani et al., 2017; Idbella et al., 2022) and BITS1fw/B58S3-ITS2rev (Bokulich and Mills, 2013) using conditions reported in the original studies. Raw reads obtained were filtered and analyzed by using QIIME 1.9.0 software. Reads shorter than 300 bp or 150 bp (for bacteria and fungi, respectively), with more than one primer mismatch and with

an average quality score (Phred) lower than 30 were discarded. Operational taxonomic units (OTUs) were picked through a de novo approach and the “ucrust” method with a similarity threshold of 97%, and the taxonomic assignment was obtained by using the RDP classifier against the Greengenes and the UNITE v.8 databases (Nilsson et al., 2019) for both bacterial and fungal communities, respectively. *Chloroplast* and *Streptophyta* contamination, as well as singleton OTUs, were removed and the relative abundance recalculated. To avoid biases due to the different sequencing depths, OTUs tables were rarefied to the lowest number of sequences per sample. Raw sequences are available at the Sequence Read Archive (SRA) of the National Centre for Biotechnology Information (NCBI), under accession number PRJNA781120.

Diversity metrics identified as OTUs number, Shannon diversity index, and the number of reads of the microbial communities in the zones with different management methods was calculated. A resemblance matrix calculated on Bray-Curtis similarity distance was used to perform Non-Metric Multidimensional Scaling (NMDS). Significant changes in beta diversity data were tested through PERMANOVA (999 permutations), using management practice as a fixed factor. Bonferroni correction was applied to avoid biases of multiple comparisons. For bacteria and fungi, PERMANOVA were made both at OTUs and phylum levels. Instead, PERMANOVA for plant community data were run at species level and according to plant life forms.

To obtain a better assessment of the changes in the studied biological communities, simpler analysis was made for community data organized by phylum for bacteria and fungi, and life form for plants. Data obtained were then used to compare community structure in the managed plots (Mowed and Burnt) against the unmanaged plots (Abandoned).

To observe the specific association of the group of taxa and management practice, heatmaps were generated using the 50 most frequent species for both bacterial and fungal communities. The taxa were ordered by hierarchical clustering according to an index of association similarity. Multivariate Data Analysis and multivariate significance tests were carried on Hellinger transformed matrices using the Primer 7 software (Clarke and Gorley, 2015). Univariate tests of significance based on ANOVA were performed using Statistica 10 (statsoft®). Furthermore, we analyzed functional group variation for the fungal community, identifying putative fungal functional groups using FUNGuild (Nguyen et al., 2016). The core microbiome based on a presence/absence dataset was identified by constructing Venn diagrams for 3 sets i.e., 3 treatments for each microbial community using R with the VennDiagram package (Chen and Boutros, 2011). Co-occurrence networks incorporating communities containing bacteria and fungi were based on single OTUs and generated to assess co-occurrence or potential interactions between species. To reveal microbiome complexity and potential interrelationships among microbial community members, network analyses were conducted for communities at the three different sampled regions. For the purpose of focusing on the most commonly occurring OTUs and decreasing the effects of rare OTUs, only the most 50 frequent OTUs were analyzed for each bacteria and fungi. The pairwise correlations between OTUs were calculated using the Spearman correlation in R (version 3.3.2 and Hmisc package 4.0–1). Based on the statistical analysis, only strong and significant (Spearman's $r > 0.6$ or $r < -0.6$ and $p < 0.05$) correlations were considered. The network visualization was made using Gephi (version 0.9.2). An edge-weighted and spring-embedded network was applied to display the co-occurrence patterns of the OTUs. Each edge represents a robust and significant correlation and each node represents an OUT. A set of integrative metrics were calculated and compared to describe the network topology. The modular structural analysis of each network was conducted according to the Louvain algorithm within Gephi.

2.7. Arthropods community composition and diversity

In mid-June 2019, eight core samples were taken from the surface soil (0–5 cm deep, 5 cm diameter) within each experimental plot, following a W scheme. The arthropods were then extracted using the MacFadyen method over a one-week period (Cortet et al., 2007). The arthropods

were collected in jars containing a 70% ethanol solution and then identified using a dissecting stereomicroscope, according to the major taxonomic groups. The results of the arthropod community analyses are reported as density (i.e., organism number m^{-2}) and taxa richness (i.e., mean taxa number in each plot). For each site, data on the abundance and diversity of soil arthropod taxon were integrated to calculate the diversity (Shannon, 1948) and Evenness (Pielou, 1969) indices. In addition, the soil biological quality index (QBS) was evaluated as reported by Parisi et al. (2005). This QBS index classifies soil arthropods on the basis of 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. (2005). The QBS is calculated as the sum of EMI values in each soil (Santorufu et al., 2015). Data were statistically analyzed by analysis of variance (One-way ANOVA), followed by post hoc Duncan tests.

3. Results

3.1. Plant diversity, biomass, and standing litter

The above-ground living plant biomass and standing litter were significantly affected by the treatments (ANOVA, $p < 0.01$, in both cases). The living biomass was highest in abandoned plots, intermediate in mowed, and lowest in burnt plots (Fig. 1a). Standing litter has followed the same trend, being 3.8-fold greater in the abandoned compared to burnt plots where it was mainly composed of grass remains (Fig. 1b). On the contrary, species richness was lowest in abandoned plots, with no differences between mowed and burnt treatment (Fig. 1c).

Grasses dominated in abandoned (62.7%) and mowed (76.2%) plots, while were far less abundant after prescribed burning (15.7%). On the contrary, prescribed burning has increased forbs abundance that becomes the most abundant functional type (Fig. 1d). As a functional group, nitrogen-fixing species showed no significant response, with sedges slightly more abundant in burnt plots and the parasitic plant *Orobanche crenata* that were present, at low relative abundance, only in abandoned and mowed plots. The application of the treatments affected the biomass of plant species in specific ways as evidence by the species association index (Fig. 2). The perennial grass *Brachypodium retusum* (Pers.) P. Beauv., the most abundant species in abandoned plots, was associated with several rare species at the study site such as *Lysimachia arvensis* (L.) U. Manns & Anderb., *Daucus carota* L., *Erigeron sumatrensis* Retz., *Lathyrus sphaericus* Retz., *Picris hieracioides* L., and the parasite *Orobanche crenata*. Different grasses were associated with mowed, i.e. *Aegilops geniculata* Roth, *Avena barbata* Pott ex Link and *Briza media* L., with the forb *Foeniculum vulgare* Mill. Instead, the perennial grass *Dactylis glomerata* L. was associated with both abandoned and mowed, but not with burnt plots. Several short-lived species were associated with burnt plots, including some nitrogen-fixing species (i.e. *Medicago* sp. *Trifolium campestre* Schreb., *T. angustifolium* L.), the grass *Hordeum murinum* subsp. *leporinum* (Link) Arcang., and several species belonging to the Asteraceae family. Notably, the perennial forb *Dittrichia viscosa* (L.) Greuter was associated with mowed and burnt plots (Fig. 2).

Significant PAR attenuation occurred in all plots compared to full light conditions (mean and s.e.m. $1234 \pm 45 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Fig. S2). In detail, PAR attenuation was similar in the three treatments at 100 and 50 cm above-ground level, while a stronger attenuation was found in the abandoned plot at ground level.

3.2. Soil properties

In all plots, soil had a clay-loam texture, subacid-neutral pH (6.19–6.70 in KCl), absence of salinity (EC of 0.1 dS/m), traces of carbonates, total nitrogen ranging from 1.85 to 2.17 g/kg, organic carbon from 21.6 to 24.6, low C/N ratio ranging from 11.2 to 11.7, low P bioavailability (8.2–11.1 mg/kg P_2O_5), medium cation exchange capacity (20.8–24.0 cmol^+/kg) mostly

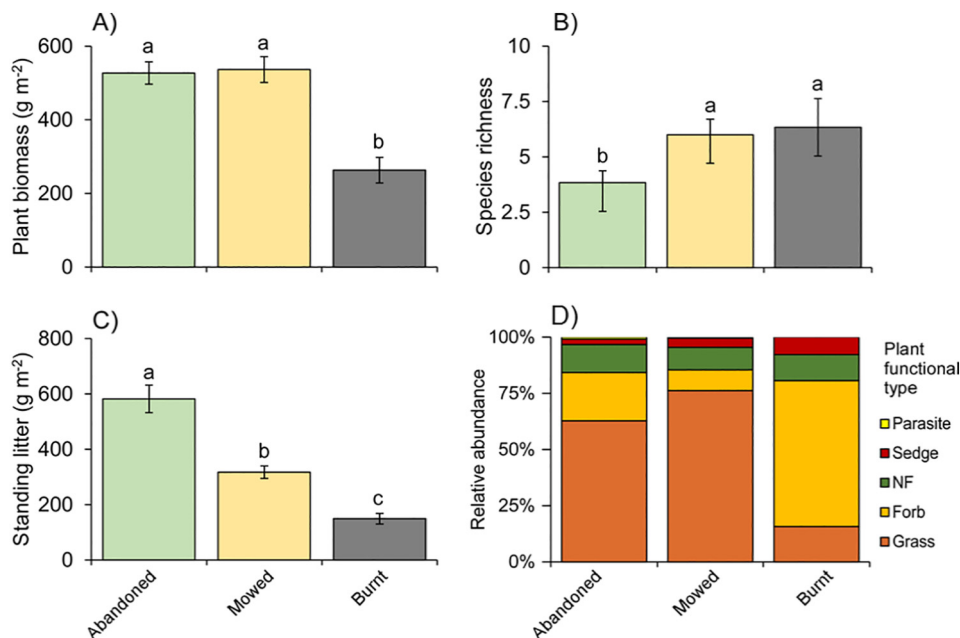


Fig. 1. Effect of abandonment, mowing and prescribed fire on plant biomass (A), species richness (B), standing litter (C) and relative abundance of plant functional type (D). The data are given as mean \pm SE, different letters per each graph indicate significant differences (Duncan test, $P < 0.05$).

saturated by Ca^{2+} (on average 83%), poor micronutrients bioavailability (Table 1).

Texture, C/N ratio, exchangeable Mg^{2+} , K^+ and Na^+ , bioavailable phosphorous, Zn, Mn and Fe, were properties not significantly affected by experimental treatments (Tables 1, S3, S4). By contrast and in comparison with abandoned plots, prescribed burning increased soil pH, and reduced the stock of organic carbon, total nitrogen, total carbonates, cation exchange capacity, and bioavailable Cu. In comparison with abandoned plots, soil under mowed had similar values of pH, electrical conductivity, organic carbon, total nitrogen, total carbonates, cation exchange capacity, but lower Cu bioavailability.

3.3. Bacterial and fungal microbiota

Microbial biomass was significantly higher for mowed compared to burnt and abandoned plots (Table 1). The opposite was found for fungal biomass, being highest in abandoned plots followed by burnt and mowed. Soil respiration rate was similar for burnt and mowed, with the lowest value for abandoned (Table 1).

Comparing diversity indices for the bacterial and fungal community we observed opposite trends in response to abandoned, mowed, and prescribed burning. Lower species richness (S) and values for Shannon index (H') were observed in abandoned compared to mowed and burnt plots for the bacterial community (Fig. 3). Oppositely, the fungal community showed higher values for S and H' in the abandoned plots compared to those that were burnt or mowed (Fig. 3).

At the phylum level, Proteobacteria were the dominant bacterial taxa in abandoned plots, comprising 51% of total relative abundance. With management, Proteobacteria was the only bacterial group whose relative abundance is decreasing, although it remained the dominant group. Taxa favoured by mowing and prescribed burning included Actinobacteria, Acidobacteria, and to a lesser extent Planctomyces (Fig. 4a). PERMANOVA (Table S1) showed that the change in bacterial community at the phylum level was significant only when plots were managed with prescribed fire (Abandoned vs Burnt, p -value = 0.019). For the fungal community at the phylum level, we observed higher relative abundance of Basidiomycota and Zygomycota in abandoned plots. A shift in dominance in favour of Ascomycota and unidentified fungi was observed with management (Fig. 4c). Of the two management methods, prescribed fire most

strongly altered fungal community composition when compared to abandoned plots (p -value = 0.047).

Non-metric multidimensional scaling (NMDS) showed similar pattern of differentiation between management practice for both bacterial and fungal communities. Sharp separation was observed between communities belonging to abandoned and burnt plots. Instead, configuration in the multivariate space of mowed plots indicated community composition intermediate between abandoned and burnt plots (Fig. S3).

Detailed representation of the changes in community composition was observed in heatmaps of the bacterial community (Fig. 5a). The abandoned plots were characterized by the higher abundance of *Kaistobacter*, *Lysobacter*, and the families of Rhodospirillaceae. The burnt plots showed a higher presence of *Chloracidobacteria* genus, the class of Acidobacteriales, and the family of Phycisphaerae. No specific bacterial taxa were associated with mowed in exception of MC47 belonging to Acidobacteria phylum. Concerning fungi (Fig. 5b), OTUs related to the Agaricomycetes class, including Sebaciales, *Omphalotus*, *Resupinatus*, *Armillaria*, and *Agrocybe*, as well as an OTU of unidentified Mucorales, were observed with higher frequency in abandoned plots. With the disturbance effect of management practices, an increase of *Hygrocybe* was observed in both mowed and burnt plots. In the burnt plot, we found increase contribution of fungi belonging to *Scleroderma*, *Entoloma*, *Debariomyces* and an OTU of the family of the Marasmiaceae.

As revealed by FUNGuild analysis (Fig. S4), most of the fungi detected were pathotrophs and saprotrophs associated with the groups of endophytes, epiphytes, animal and plant pathogens, parasites, and saprophytes of various plant parts. Symbiotrophic fungi represented by ecto- and endomycorrhizal were relative less abundant as functional types. The distribution patterns of dominant ecological guild functions were mostly similar between treatments with some differences. In detail, plant saprotrophs were absent in the burnt soil compared to other treatments, the very low abundance of the lichen parasites in the burnt soil compared to others. Moreover, the fungal parasites were more abundant in the abandoned soil compared to others. Finally, the burnt plot enclosed the highest amount of the animal pathogens, and the soil subjected to the cutting has enclosed exclusively a small amount of lichenized.

The Venn diagram demonstrated that microbial OTUs differed among the three soil types (Fig. S5). The three soils have shared more fungal OTUs than the bacterial ones, i.e. 79 shared bacterial OTUs against 138

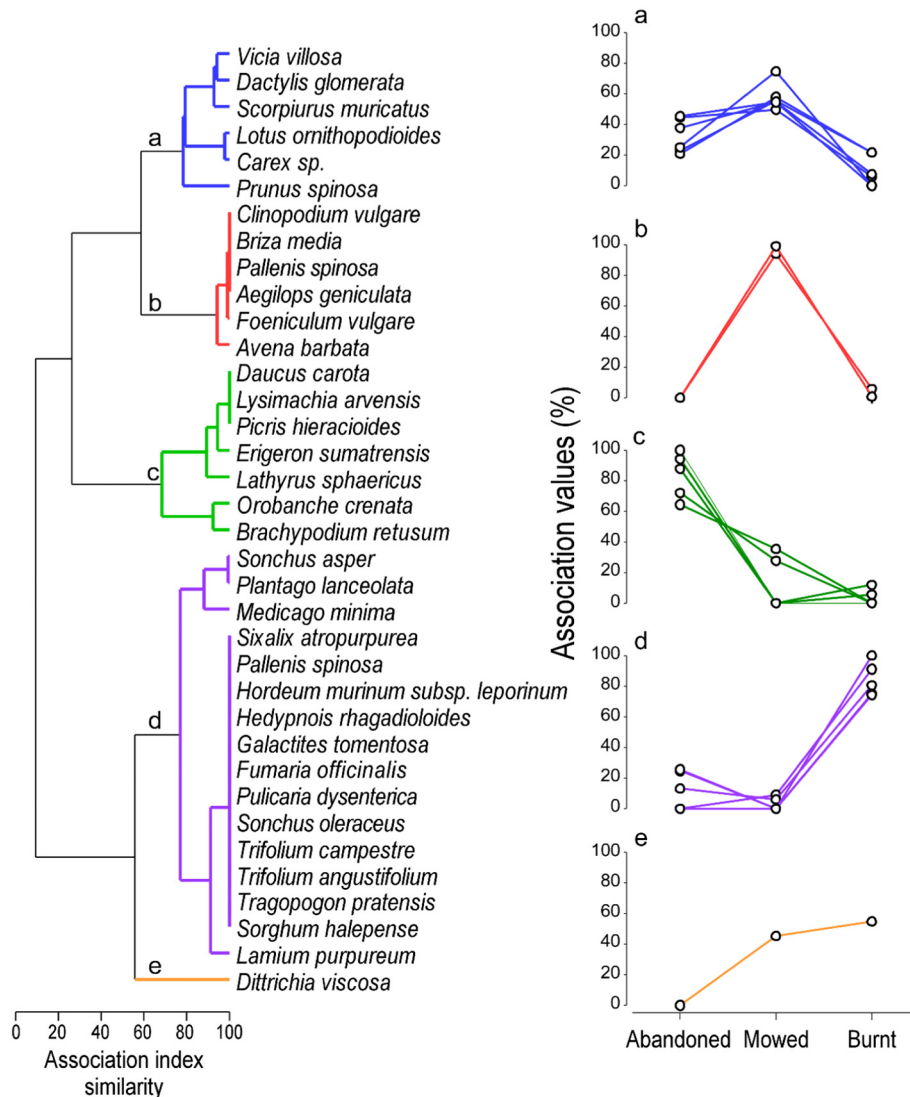


Fig. 2. Hierarchical clustering dendrogram (left) based on Association index similarity on the plant species present in the study sites. Different colours and letters associated to dendrogram represent different group of species. Corresponding letters and colours are represented in the line plots (right) showing the association values based on recalculated relative abundance of the single species within the cluster in the three different management practice.

fungal ones. For bacteria, the mowed soil had only 2 exclusive OTUs, 11 shared with the burnt soil, and none shared with the abandoned soil. On the other hand, burnt soil had 90 exclusive OTUs, 83 ones shared with the abandoned soil, while only 11 OTUs were shared with the mowed soil. Abandoned soil, similarly, has enclosed a high amount of exclusive OTUs (i.e., 61). For fungi, the distribution of shared and exclusive OTUs was more or less similar between the three soil types. All the soil types have enclosed a high amount of exclusive OTUs ranging from 57 OTUs in the abandoned soil to 52 in the mowed and 41 in the burnt.

We constructed three networks based on samples from the abandoned, burnt, and mowed areas (Fig. 6) and calculated six topological parameters to assess the interactions between the OTUs in the three networks (Table S2). The microbial network for the abandoned and mowed areas contained 92 nodes for each, while the network for the burnt area contained 88 nodes. The proportions of negative correlations in the microbial networks of the abandoned, mowed, and burnt areas were 33.27, 39.15 and 41.71%, respectively. Network centralization and heterogeneity values were significantly higher in the abandoned area followed by the burnt area, while highly low in the mowed area, indicating the shift from highly complex, heterogenic, and centralized networks for abandoned area mainly followed by burnt, to low complexity for the mowed area. Compared with the abandoned and burnt, the clustering coefficient value was higher in the mowed area,

indicating a less structured, weakly correlated, and widely dispersed network with less centralized links. Nodes with high degrees (bacteria > 44; fungi > 32), high closeness centrality (>0.5), and low betweenness centrality (<2.0) were considered keystone taxa. In the three areas, the most abundant taxa among the top 14 keystone taxa were *Ascomycota* with a percentage of 34%, 32%, and 36% in the abandoned, burnt, and mowed areas, respectively; followed by *Proteobacteria* and *Actinobacteria*. In the abandoned and burnt areas, the center of the network was formed mainly by the interactions between both bacteria and fungi equally, while in the mowed area, two centers were formed, one main dominated by fungal interactions and another one dominated by bacterial interactions.

3.4. Arthropods community

Soil arthropod densities ranged from 45 to 1195 individuals m^{-2} of soil (Table 1). Soil arthropods were significantly affected by experimental treatments with higher density in burnt plots, whereas taxa richness, Shannon and Pielou indices and the QBS-ar index were significantly higher both in burnt and abandoned plots (Table 1). Altogether, 13 arthropod taxa were found in the experimental plots, with Acarina as the most abundant and ubiquitous taxon present in all the treatment plots (Fig. 7). The relative abundances of each arthropod group revealed that in mowed plots the

Table 1

Physical, chemical, and biological parameters in the soil of abandoned, mowed, and burnt plots. Different letters within each row indicate significant differences (Duncan test, $p < 0.05$).

Soil parameters	Treatments		
	Abandoned	Mowed	Burnt
Physical & chemical			
Sand (g/kg)	610 ± 32 a	569 ± 45 a	570 ± 54 a
Silt (g/kg)	200 ± 12 a	197 ± 21 a	182 ± 17 a
Clay (g/kg)	190 ± 35 a	234 ± 19 a	248 ± 22 a
pH _{KCl}	6.19 ± 0.17 b	6.20 ± 0.15 b	6.70 ± 0.27 a
Electrical conductivity (ds/m)	0.11 ± 0.01 a	0.11 ± 0.01 a	0.13 ± 0.02 a
Total carbonates (g/kg)	3.18 ± 1.16 a	3.80 ± 3.26 a	1.90 ± 0.05 b
Total nitrogen (g/kg)	2.17 ± 0.24 a	2.09 ± 0.24 a	1.85 ± 0.01 b
Bioavailable phosphorus (P ₂ O ₅ , mg/kg)	11.1 ± 1.3 a	8.2 ± 0.7 a	10.8 ± 2.4 a
Organic carbon (g/kg)	24.6 ± 1.5 a	23.4 ± 1.7 a	21.6 ± 0.7 b
C/N	11.4 ± 0.7 a	11.2 ± 0.5 a	11.7 ± 0.3 a
Cations exchange capacity (cmol(+)/kg)	22.0 ± 0.5 ab	24.0 ± 1.3 a	20.8 ± 1.6 b
Exchangeable Ca (cmol(+)/kg)	18.4 ± 0.5 a	20.5 ± 1.7 a	17.6 ± 1.8 a
Exchangeable Mg (cmol(+)/kg)	2.29 ± 0.26 a	2.57 ± 0.22 a	2.22 ± 0.40 a
Exchangeable K (cmol(+)/kg)	0.91 ± 0.13 a	0.77 ± 0.11 a	0.85 ± 0.20 a
Exchangeable Na (cmol(+)/kg)	0.14 ± 0.06 a	0.19 ± 0.05 a	0.12 ± 0.04 a
Bioavailable Cu (mg/kg)	5.04 ± 0.40 a	4.02 ± 0.31 b	3.16 ± 0.34 c
Bioavailable Zn (mg/kg)	1.07 ± 0.15 a	0.83 ± 0.09 a	1.11 ± 0.31 a
Bioavailable Mn (mg/kg)	22.6 ± 0.2 a	19.8 ± 1.8 a	21.8 ± 2.2 a
Bioavailable Fe (mg/kg)	24.8 ± 5.7 a	17.3 ± 2.1 a	15.3 ± 3.9 a
Biochemical & microbiological			
Microbial biomass (mg Cmic/g d.w.)	1.54 ± 0.63 b	2.31 ± 0.69 a	1.91 ± 0.89 b
Fungal biomass (mg/g d.w.)	1.29 ± 0.43 a	0.54 ± 0.12 b	0.82 ± 0.14 b
Respiration (mg CO ₂ /g d.w./d)	0.29 ± 0.21 b	0.36 ± 0.14 a	0.37 ± 0.15 a
qCO ₂ (mg C-CO ₂ /mg Cmic)	0.07 ± 0.08 a	0.04 ± 0.03 a	0.06 ± 0.03 a
CEM (mg C-CO ₂ /g Corg)	3.09 ± 1.72 a	3.22 ± 1.18 a	3.90 ± 1.11 a
Arthropod community			
Density (n.org m ⁻²)	330 ± 170 b	45.0 ± 14.0 b	1195 ± 641 a
Taxa Richness (mean n. of taxa)	3.00 ± 0.77 a	0.87 ± 0.23 b	5.00 ± 1.12 a
Shannon index (H')	0.83 ± 0.17 a	0.11 ± 0.09 b	1.12 ± 0.22 a
Pielou index (E)	0.81 ± 0.12 a	0.15 ± 0.13 b	0.75 ± 0.11 ab
Biological soil quality index (QBS)	37.6 ± 6.37 a	13.0 ± 3.23 b	54.0 ± 11.6 a

community was constituted only by Acarina and Isopoda. Acarina, Collembola, Isopoda, Hymenoptera, Coleoptera and Thysanoptera were present both in abandoned and burnt plots, whereas Diplopoda and Diptera larvae were present only in abandoned plots and Coleoptera larvae, Symphyla, Chilopoda and Pauropoda were present only in burnt plots.

4. Discussion

4.1. Vegetation and soil responses to prescribed burning, mowing, and abandonment

Five years of abandonment have driven to the dominance of perennial grasses, in particular the late-successional *B. ramosum*, leading to a heavy litter accumulation and a reduction in plant species diversity. *B. ramosum* in few years accumulates large amount of standing litter, a condition that dramatically increases the risk of wildfire during the summer season. In this regards, both mowing and prescribed burning were effective practices in contrasting these rapid successional dynamics and the dominance of perennial grasses. A positive effect of repeated mowing on species diversity was previously reported in northern Europe for a grassland dominated by *B. pinnatum* (Bobbink and Willems, 1991) and for a species-rich grassland dominated by *B. rupestre* in central Italy (Bonanomi et al., 2009; Tardella et al., 2020). Here, however, mowing caused a shift in grasses composition, while prescribed burning reduced grass dominance in favour of forbs and nitrogen-fixing species. The ability of repeated disturbances to limit the spread of perennial grasses could be explained by the reduction of the stored energy in rhizomes, i.e., non-structural carbohydrate content, which may lead to a vigour reduction of the shoots in subsequent seasons (Bobbink and Willems, 1991; Klimeš and Klimešová, 2002).

The maintenance of high plant species diversity by mowing and prescribed burning has probably occurred due to a combination of reduced

competition from perennial grasses, i.e. *B. ramosum*, and the positive responses of small, short-lived species to increased light availability at the soil surface. In the abandoned plots, we recorded a substantial litter accumulation reaching $582 \pm 49 \text{ g/m}^2$, which is quite a high amount compared to other abandoned grasslands and old fields (Foster and Gross, 1998; Bonanomi et al., 2009; Deák et al., 2011). The formation of a dense and deep litter layer can limit the germination and establishment of species with small seeds through a combination of shading, mechanical impediment, and release of allelochemicals during the decomposition process (Xiong and Nilsson, 1999; Loydi et al., 2013). In this regard, the utility of prescribed fire is two-fold, by periodically removing standing litter, thus reducing fuel loads and the associated risk of wildfire during summer drought, and by promoting the maintenance of plant diversity throughout opening vegetation gaps for small, short-lived species. Although prescribed fire was applied only twice in five years, it was more effective than annual mowing in litter removal. Unlike most previous prescribed burning experiments, we attempted to apply a natural fire frequency by burning twice in 5 years. This approach allows the regeneration of vegetation between fires and maintains the course of post-fire recovery, as post-fire regeneration and the beneficial post-fire effects are often visible by the second year (Valkó and Deák, 2021). In addition, prescribed fire caused more profound changes in vegetation compared to mowing, with a shift from a grass- to a forbs-dominated community, and the appearance of several short-lived species belonging to Fabaceae and Asteraceae families. These changes could be related not only to litter removal, but also to soil chemical changes induced by fire. Mowing caused no changes in pH, organic C stock, and availability of major and minor nutrients compared to the abandoned plots. Instead, prescribed burning caused small but significant differences with an increase in soil pH, affecting nutrients availability (Rieuwerts et al., 1998), a reduction in organic C and total N stocks, and consequently a decrease in cation exchange capacity (Parfitt et al., 1995).

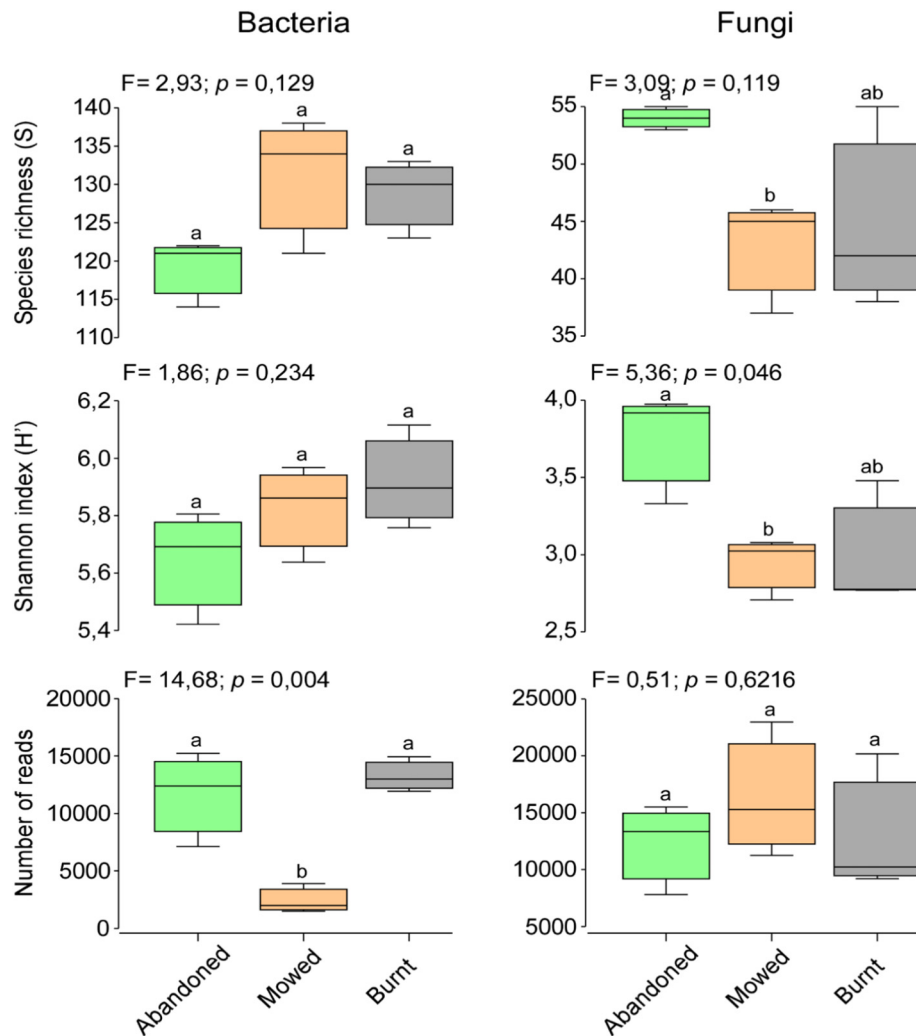


Fig. 3. Box and whisker plots showing the distribution of diversity indices for each management practice in Cicerale study site. S, Number of species, H' , Shannon Index; and number of reads for bacterial and fungal communities. Letters indicate significant ($P < 0.05$) differences in diversity indices across the management practice. Significance was assigned according to univariate ANOVA and post hoc Duncan test. The lower and upper bounds of the boxplots show the first and third quartiles (the 25th and 75th percentiles), the middle line shows the median, whiskers above and below the boxplot indicate inter-quartile range.

and in the availability of copper, which has a great affinity for the organic matter (Sims, 1986). An increase in soil pH in the upper soil layers has been reported previously, especially in short-term after the fire, and is caused by the local accumulation of ash and charcoal residues (Certini, 2005). Both prescribed burning and wildfire are known to reduce soil organic carbon stock and, in some cases total N, especially where the fire is intense, and in forest ecosystems where the upper organic layers could be consumed by flames (Alcañiz et al., 2018; Panico et al., 2020). Here, the reduction in organic C and N, as well as the increase in pH, although not dramatic quantitatively, suggest that in the long term repeated burning, even at low intensity as in our case, could drive to a progressive depletion of soil organic carbon and N, and consequently, a reduction in nutrients availability, thus negatively impacting soil functioning and vegetation health.

4.2. Microbiome response to abandonment and repeated disturbances

The work represented here gives an example of how grassland management practices, especially burning and mowing, shape the soil microbial community, both in terms of composition and diversity. From a broader perspective, we observed a global increase in bacterial species richness at disturbed sites. Specifically, in abandoned areas, we observed a decrease in Proteobacteria, mainly due to *Kaistobacter*, *Lysobacter*, and Rhodospirillaceae. These genera are bacteria with a well-known role in

suppressing plant pathogenic fungi (Liu et al., 2013; Postma et al., 2005). In the case of abandoned areas, the prevailing presence of *Kaistobacter* and *Lysobacter* could be interpreted as consequential to the increased basidiomycetes biomass, which in turn could represent an advantageous ecological interaction for the two bacterial genera (Zotti et al., 2021). Conversely, the Rodospirillaceae family is typically associated with aquatic environments (Biebl and Pfennig, 1981), leading to the speculation that the higher amount of litter in abandoned areas increases water retention in these soil spaces and provides refuge for some bacteria related to wetter environment. With vegetation disturbance, the presence of Proteobacteria decreased favouring the rise in the relative abundance of other bacterial phyla. In the case of mowed area, a significant decrease in bacterial reads number is observed, indicating the instauration of oligotrophic conditions in these areas. After mowing, no specific bacterial genera were found associated with these areas, although the change in the bacterial community due to the decrease in Proteobacteria was similar to the burnt areas. However, the prescribed fire significantly increased the extent to which bacterial taxa reacted to the disturbance, as indicated by the recorded high number of reads. Specifically, the major change after prescribed fire was due to the presence of *Chloracidobacterium*, a thermophilic and photosynthetic member of Acidobacteria (Bryant et al., 2007). It is likely that these bacteria were favoured by the moderate burning but were also promoted by the increased solar radiation on the soil surfaces in the burnt areas. The

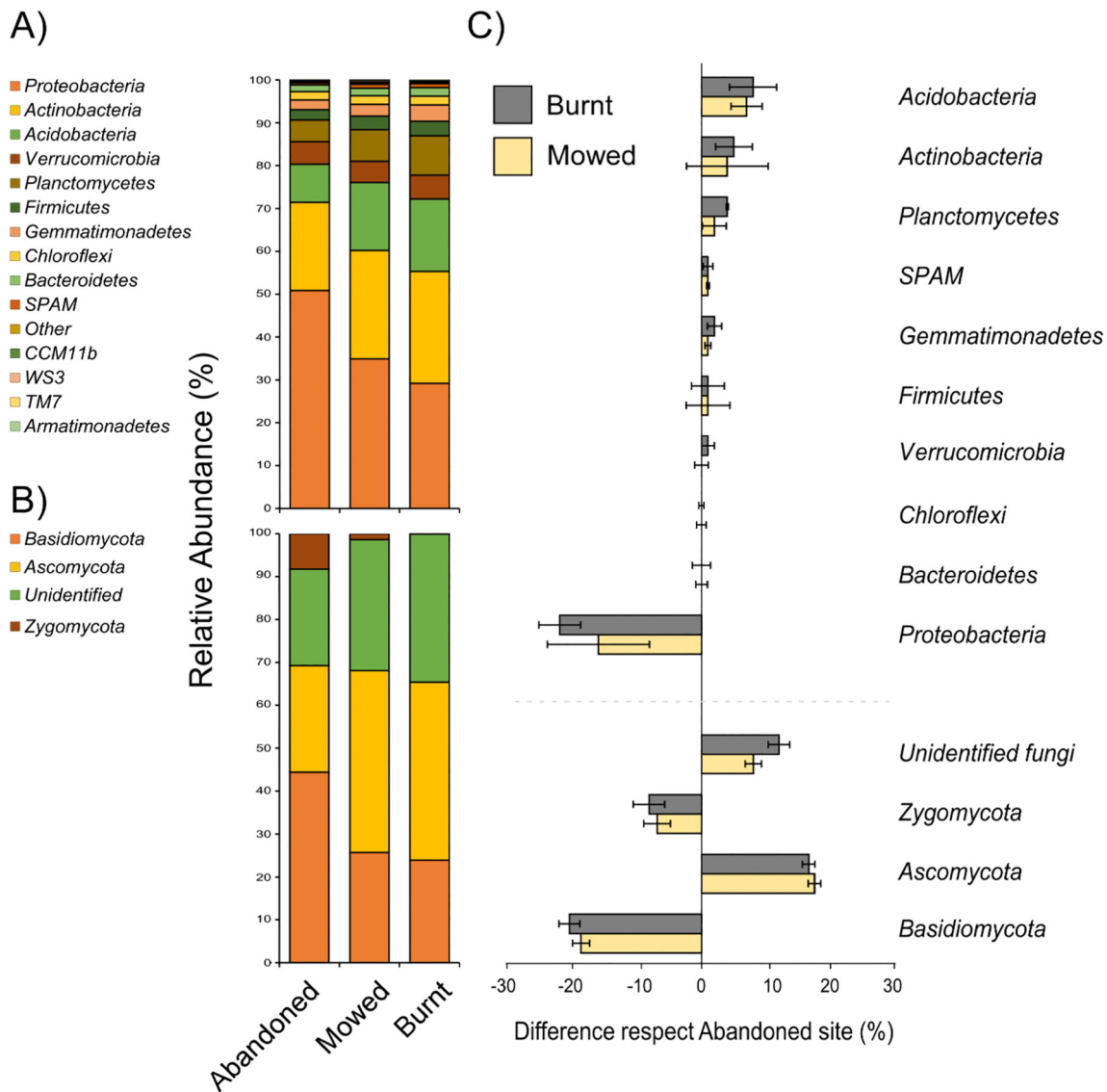


Fig. 4. Stacked bar chart of relative abundance of bacterial (A) and fungal (B) phyla for each management practice in Cicerale study site. Bar plot (C) showing differences in relative abundance of microbial phyla respect to the abandoned sites.

Phycisphaerae family has also been associated with moderate thermophilic conditions (Kovaleva et al., 2015), suggesting that prescribed fire management of grasslands may have a legacy effect on bacterial community composition, although it is still unclear how this effect persists after fire. To date, this is the first record of Phycisphaerae associated with burnt grasslands.

Related to the fungal community, disturbance caused by management reduced the relative abundance of Basidiomycetes that predominate in abandoned soils. Typically, low levels of disturbance result in Basidiomycota-dominated communities, as this phylum is characterized by a slow growth rate and a combative strategy for resources favoured in late succession processes of organic matter degradation (Bonanomi et al., 2019; Frankland et al., 1982; Purahong et al., 2016). Fungal community structure in the abandoned plot showed an increase in basidiomycetous taxa. In detail, *Omphalotus*, *Armillaria*, *Resupinatus*, and *Agrocybe* form several groups dominating the abandoned plots. Comprehensively, all of these genera are capable of degrading recalcitrant compounds such as lignin-rich litter and are known as wood decomposers or pathogens of woody species with a certain degree of specificity to the host tree, i.e., *Omphalotus olearius* is associated with *Olea europea* and/or *Agrocybe aegerita* (*Cyclocybe cilindracea*). It is possible that, in the absence of specific woody matrices in grasslands, these fungi shifted their trophic target to the

accumulated litter in abandoned plots, which in this case served as refugia. More commonly, OTUs belonging to the saprotrophic genus *Agaricus* and OTUs of the family Sebacinaceae were the distinguishing taxa of abandoned areas. The former is a well-known genus of soil-dwelling saprotrophs capable of becoming dominant fungi in the soil (Zotti et al., 2020), which is likely also related to the higher amount of litter that accumulates on abandoned sites as they use it as a growth substrate. Instead, OTUs of the Sebacinaceae family include several fungal genera with the ability to form different symbiotic types. Most of the Sebacinaceae form both ecto-, ericoid, and orchid mycorrhizae (Weiß et al., 2016), but also as endophytes (Selosse et al., 2009), suggesting a strong relationship with the plant species. Our vegetation surveys did not find any plant that has been shown to form mycorrhizal or endophytic associations with members of the Sebacinaceae family, leaving little room for conclusions on their function in ecosystems. Nevertheless, in a successional framework, we suspect that these fungi live in abandoned grassland as free-living saprotrophs and have the potential to act as symbionts for woody species such as *Quercus* sp. and hence implying a possible successional shift from grassland to ectomycorrhizal woodland. However, this hypothesis remains to be tested, as no study has documented the specific role of Sebacinaceae in successional processes.

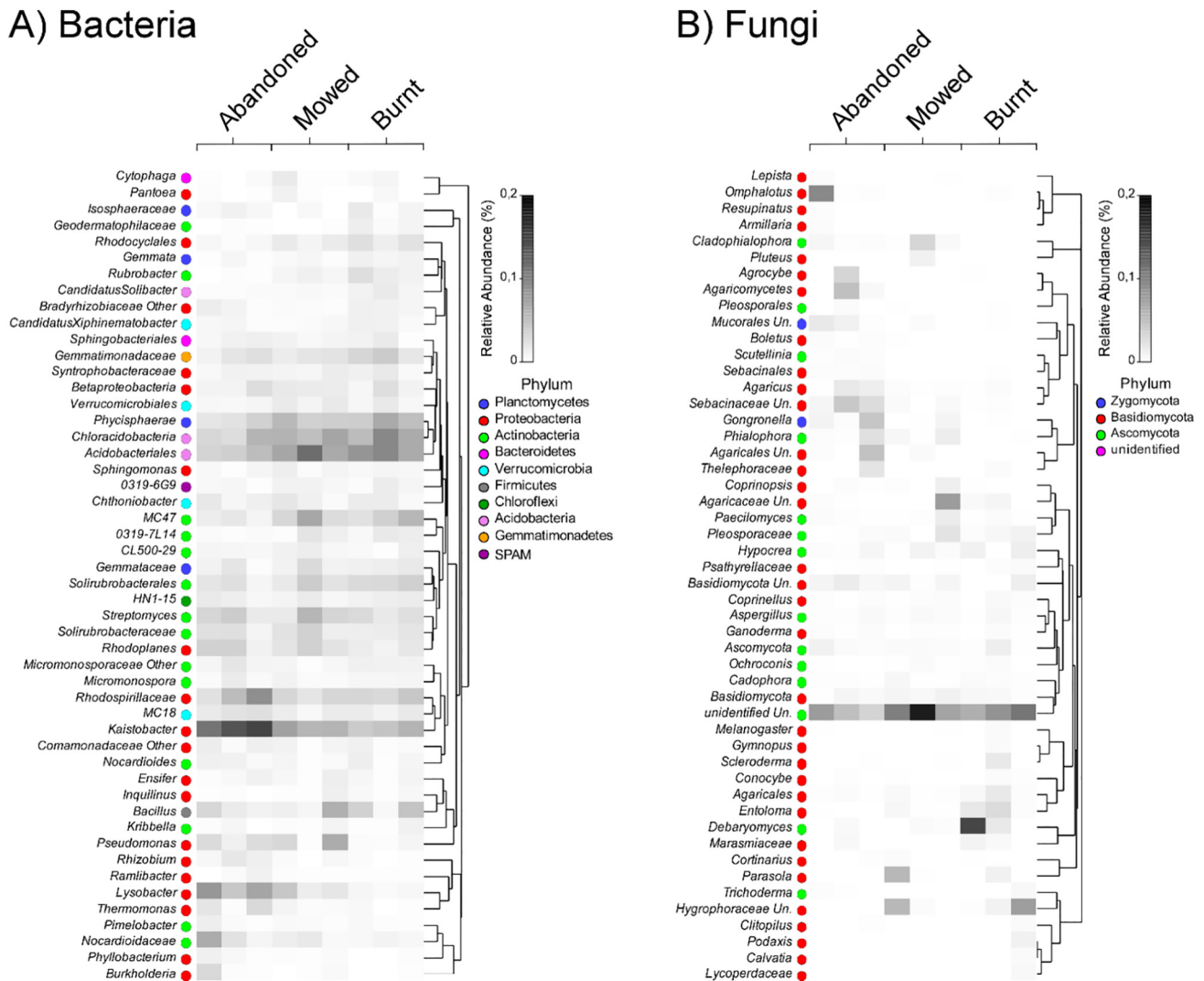


Fig. 5. Heatmap showing relative abundance of the 50 most frequent operational taxonomic units (OTUs) in the bacterial (A) and fungal (B) community. Hierarchical clustering of variables is based on an association index similarity.

Our results indicate that management by burning and mowing has the potential to alter the relative abundance of important fungal taxa that serve as indicators of habitat stability. For example, an increase in fungi of the genus *Hygrocybe* was observed in both burnt and mowed areas. The waxcap fungus *Hygrocybe* is considered one of the most distinctive members of the mycota in mowed or grazed grasslands (Griffith et al., 2013) and serves as a biological indicator of grassland stability (Griffith et al., 2002). Specific to the case reported here, the presence in mowed plot is well documented (Newton et al., 2003) and disappearance in abandoned areas is consistent with literature describing detrimental effects of successional changes in the plant community on *Hygrocybe* (Keizer, 1993). Surprisingly, we also observed *Hygrocybe* in plots managed with prescribe fire, along with OTUs of the genus *Scleroderma*, *Entoloma*, and *Debaryomyces*. To our knowledge, this is the first record of *Hygrocybe* associated with burnt soil. In this case, the effect of repeated and moderate fire may have excluded the previous mycobiota, allowing opportunistic colonization of empty niches in the soil. Particularly for *Hygrocybe*, the soil-induced disturbance may have created more favourable conditions due to enhanced denitrification and solar radiation after fire. The genus *Hygrocybe* is also known to occur under oligotrophic conditions and to exhibit high levels of protective colourful pigments for protection against damaging soil radiation.

In addition to altering the relative abundance of important taxa, our management practices have also shown the potential to disrupt the co-

occurrence state of the soil microbial communities. In details, the microbial co-occurrence network was more complex in the abandoned area, which could have been caused by the temporal and spatial continuity of the connections between the microbes, in contrast to the burnt and mowed areas, where the connections could have been interrupted by the process of burning and cutting. The reason for this result may be the fact that a large number of herbaceous plants are destroyed and many soil microbiota die after the fire and cutting processes, leading to the collapse of the whole ecosystem (Taş et al., 2014). In our study, all networks had a high proportion of positive correlations among nodes. However, the proportion of positive correlations was higher in the abandoned area than in the rest, indicating a high degree of coexistence among microbial taxa and thus a lower ability to differentiate into different sub-communities in the abandoned site. This result could be explained by the fact that there is a strong competition between microorganisms after burning and cutting, resulting in a higher amount of negative correlations compared to the abandoned area. In fact, previous studies have shown that microorganisms compete with each other for limited resources in low nutritional conditions (Zhu et al., 2021). Conversely, microorganisms are more likely to coexist under high nutrient conditions (Birkhofer et al., 2008). Topological parameters provide important information for understanding microbial community structure (Newman, 2006; Ren et al., 2017). Higher values of network centralization and heterogeneity were observed in the abandoned site compared to the other areas, suggesting that the microbiota tends to connect

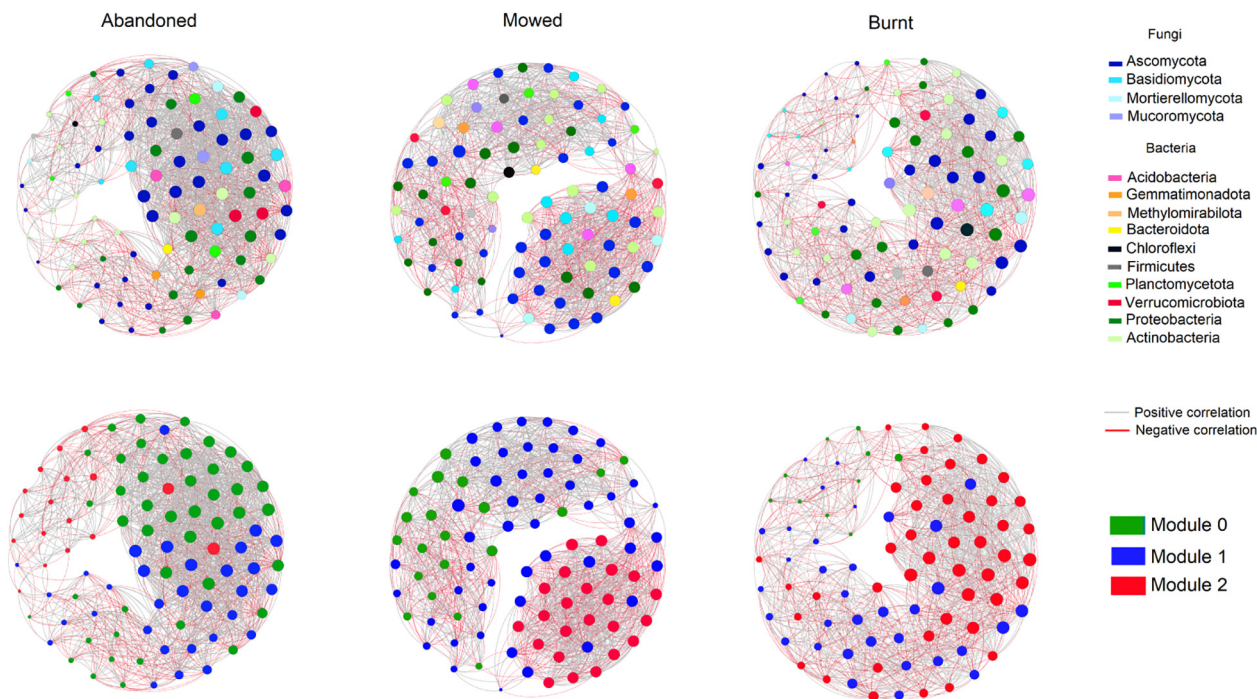


Fig. 6. Correlation base network analysis showing potential interactions between bacterial and fungal families for each treatment in the three experimental sites. The lines connecting nodes (edges) represent positive (grey) or negative (red) co-occurrence relationship. The length of the edges represents the strength of correlation. The connection stands for a strong (Spearman's $\rho > 0.6$ and $\rho < -0.6$) and significant ($P < 0.05$) correlation. The size of each node is proportional to the ASV relative abundance, only the top 50 ASVs were kept. The nodes were coloured by phylum level.

closely with each other rather than forming many dispersed sub-communities as it is the case for the burnt and mowed areas.

4.3. Arthropod community response to prescribed burning, mowing, and abandonment

The experimental management practices investigated in the present work showed contrasting effects on the arthropod community structure. In particular, arthropods appeared to be favoured by fire and abandonment and disadvantaged by mowing. This result is consistent with other studies that have found negative effects of mowing and positive effects of fire on arthropods (Noordijk et al., 2010; Rutigliano et al., 2013; Kaynas, 2016). Although the effects of fire are disruptive to soil fauna, some organisms may exhibit considerable mobility and morpho-physiological adaptations to fire (Certini et al., 2021). In addition, post-fire colonization by arthropods can begin very quickly and is strictly tied to vegetation. In fact, in our burnt plots, the change in vegetation from grass species to nitrogen-fixing

species may have increased resource diversification, attracting a more diverse community of soil arthropods. Similarly, abandoned plots had high arthropod abundance and diversity. Land abandonment from agricultural practices leads to successional changes towards semi-natural habitats that provide more favourable conditions for macro-arthropods (Tóth et al., 2016). Indeed, the stable environmental conditions and deep litter layer found in the abandoned plots studied may have harboured a large number and diversity of organisms. In contrast, the sharp decline in arthropod abundance and diversity in mowed plots may have been due to the homogenisation of vegetation observed during this practice (Dover et al., 2011; Noordijk et al., 2010), with the loss of many microhabitats and resources.

The different management practices also influenced the taxonomic structure of the arthropod community. With the exception of Acarina, which were abundant in all treatments as they are ubiquitous and among the most numerically dominant arthropod groups (Santorufu et al., 2014). In particular, the burnt plot was characterized by a high number of taxa of arthropod groups, with Diplura, Chilopoda, Symphyla, Pauropoda, and Thysanoptera present only in this treatment. This result confirms that post-fire soil can host biologically and functionally different organisms (Malmström et al., 2008; Catry et al., 2017). In fact, decomposers, predators, and herbivorous organisms were present in the burnt plots. In the abandoned field, the presence of Acarina, Collembola, Diplopoda, and Diptera larvae suggests that most of the community constituted of decomposers, herbivorous, and omnivorous organisms (Stoev et al., 2010; Nartshuk, 2014), probably due to the great amount of undecomposed litter creating a specific environment. In contrast, only Acarina and Isopoda were present in the mowed treatment, belonging mainly to decomposers and herbivorous trophic groups (Montesanto and Cividini, 2017). This could be due to the presence of cut herbs on the soil surface that constituted the main food resource in this treatment.

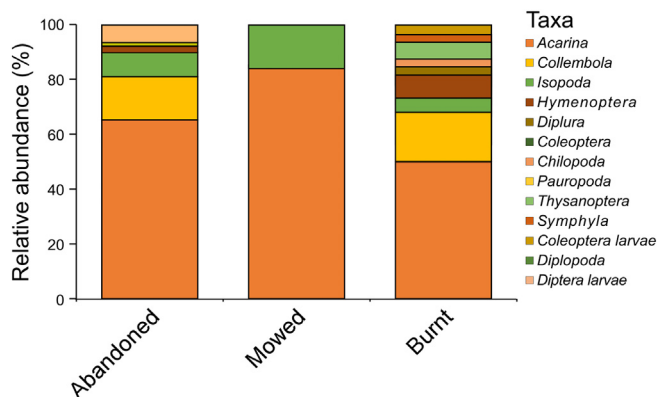


Fig. 7. Relative abundances of different arthropod groups recorded in plots abandonment, mowed or subjected to prescribed fire.

5. Conclusion

In our Mediterranean grassland, the repeated application of prescribed burning and vegetation mowing influenced, over a five years period, soil

chemical properties, vegetation composition, and diversity, with an impact on soil microbiome and fauna. Both burning and mowing significantly increased plant species richness and diversity compared to abandonment plots, reducing the abundance of perennial grasses in favour of short-lived species. Soil microbiota was shaped by above-ground treatments, with both bacterial and fungal communities that were associated with both changes in plant communities and soil chemistry. Abandonment results in improved soil fertility, i.e. increase of organic carbon, total N and cation exchange capacity, that likely contribute to the shifts of microbiota towards fungal-dominated communities. The taxonomic composition of the fungal community changed from one dominated by a fast-growing and large presence of pathogenic species to one consisting of slower-growing, late-successional fungi belonging to Basidiomycota. Soil arthropods were influenced by vegetation and microbiota changes, being strongly reduced in mowed plots. In addition, the arthropod community in mowed plots was dominated by few ubiquitous taxa. Overall, abandonment or management by burning and mowing cause multiple changes in grassland compartments and processes (i.e. soil fertility, microbiome, arthropod and plant community diversity) with contrasting impacts on ecosystem functions. Policy maker and stakeholders would decide for the more appropriate management based on the balance between ecosystem services (i.e. carbon stock, reducing wildfire risk, biodiversity conservation) and the direct and indirect costs of the different practices.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Giuliano Bonanomi: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Supervision, Project administration. **Mohamed Idbella:** Methodology, Formal analysis, Investigation, Data curation, Writing - review & editing, Visualization. **Ahmed M. Abd-ElGawad:** Validation, Writing - review & editing, Funding acquisition. **Riccardo Motti:** Validation, Resources, Writing - review & editing. **Francesca Ippolito:** Formal analysis, Investigation, Data curation, Writing - review & editing. **Lucia Santorufo:** Formal analysis, Investigation, Writing - review & editing. **Paola Adamo:** Validation, Resources, Writing - review & editing. **Diana Agrelli:** Formal analysis, Investigation, Data curation. **Anna De Marco:** Formal analysis, Investigation, Writing - review & editing. **Giulia Maisto:** Validation, Resources, Writing - review & editing. **Maurizio Zotti:** Methodology, Formal analysis, Investigation, Data curation, Writing - review & editing, Visualization.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.155442>.

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