Science of the Total Environment 802 (2022) 149924

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Previous fire occurrence, but not fire recurrence, modulates the effect of charcoal and ash on soil C and N dynamics in *Pinus pinaster* Aiton forests

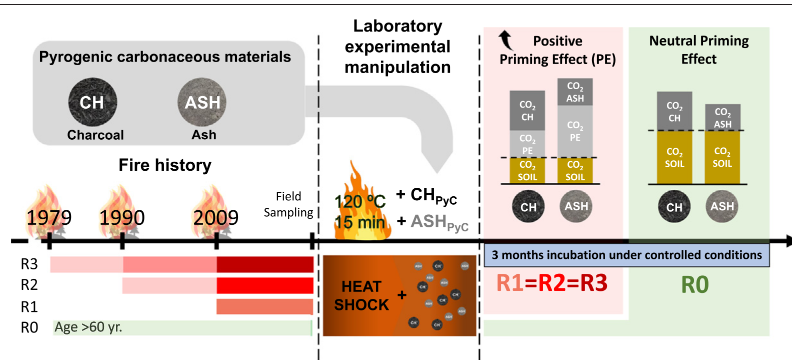
Enrique Albert-Belda [a,*], M. Belén Hinojosa [a,*], Vito Armando Laudicina [b], Roberto García-Ruiz [c], Beatriz Pérez [a], José M. Moreno [a]

[a] Departamento de Ciencias Ambientales, Universidad de Castilla–La Mancha, Campus Fábrica de Armas, E-45071 Toledo, Spain
[b] Dipartamento de Scienze Agrarie, Alimentari e Forestali, Università degli Studi di Palermo, Viale delle Scienze - Edificio 4 Ingr. B, I-90128 Palermo, Italy
[c] Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus Las Lagunillas, E-23071 Jaén, Spain

HIGHLIGHTS

- Charcoal and ash amendments increased CO_2-production only in formerly burned soils.
- Charcoal and ash produce positive priming effects only in formerly burned soils.
- Contrasting responses due to prior fires could be driven by different microbiota.
- Priming effects after ash addition could be driven by high P availability and pH.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 May 2021
Received in revised form 20 August 2021
Accepted 22 August 2021
Available online 26 August 2021

Editor: Paulo Pereira

Keywords:
Fire history
Pyrogenic carbonaceous materials
Priming effect
C mineralization
N mineralization
Microbial community

ABSTRACT

Understanding the effects of fire history on soil processes is key to characterise their resistance and resilience under future fire events. Wildfires produce pyrogenic carbonaceous material (PCM) that is incorporated into the soil, playing a critical role in the global carbon (C) cycle, but its interactions with soil processes are poorly understood. We evaluated if the previous occurrence of wildfires modulates the dynamic of soil C and nitrogen (N) and microbial community by soil ester linked fatty acids, after a new simulated low-medium intensity fire. Soils with a different fire history (none, one, two or three fires) were heat-shocked and amended with charcoal and/or ash derived from *Pinus pinaster*. Soil C and N mineralization rates were measured under controlled conditions, with burned soils showing lower values than unburned (without fire for more than sixty years). In general, no effects of fire recurrence were observed for any of the studied variables. Microbial biomass was lower in burned, with a clear dominance of Gram-positive bacteria in these soils. PCM amendments increased cumulative carbon dioxide (CO_2) production only in previously burned soils, especially when ash was added. This contrasted response to PCM between burned and unburned soils in CO_2 production could be related to the effect of the previous wildfire history on soil microorganisms. In burned soils some microorganisms might have been adapted to the resulting conditions after a new fire event. Burned soils showed a significant positive priming effect after PCM amendment, mainly ash, probably due to an increased pH and phosphorous availability. Our

Abbreviations: C, carbon; CO_2, carbon dioxide; C_0, potentially mineralizable carbon; CEC, cation exchange capacity; CH, charcoal; ELFAs, esther linked fatty acids; H, heat treatment; k, potentially mineralizable carbon decay rate; MRT, mean residence time; N, nitrogen; NH_4^+-N, ammonium; NO_3^-N, nitrate; PCM, pyrogenic carbonaceous material; PO_4^{3-}-P, labile phosphate; PyC, pyrogenic carbon; $t_{1/2}$, half life time; TC, total carbon; TH, total hydrogen; TIN, total inorganic nitrogen; TN, total nitrogen; UH, unheated treatment.

* Corresponding authors.
E-mail addresses: enrique.albert@uclm.es (E. Albert-Belda), mariabelen.hinojosa@uclm.es (M.B. Hinojosa).

https://doi.org/10.1016/j.scitotenv.2021.149924

results reveal the role of different PCMs as drivers of C and N mineralization processes in burned soils when a new fire occurs. This is relevant for improving models that evaluate the net impact of fire in C cycling and to reduce uncertainties under future changing fire regimes scenarios.

1. Introduction

The legacy of a disturbance might modulate current ecosystems functionality (Foster et al., 1998; Mori, 2011). It has been long recognized that fire is a major environmental disturbance and a driver of biogeochemical processes, notably those related to the carbon (C) cycle, across diverse ecosystems worldwide (Raison et al., 2009). Wildfires convert the majority of the C of the burned vegetation and soil organic C into carbon dioxide (CO_2) and pyrogenic C (hereinafter, PyC). Globally, each year, wildfires emit around 1.6–2.8 Tg of C (mainly as CO_2) to the atmosphere, and produce 114–383 Tg of PyC that are incorporated to the soil (Santín et al., 2016). Pyrogenic carbonaceous materials (hereinafter, PCM) comprise a wide range of chemical compounds that are produced both, *in situ* from the thermochemical rearrangement of precursor organic compounds and from gas-phase condensation of compounds volatilized during pyrolysis and/or combustion (Bird et al., 2015). Different forms of PCM are described as a "pyrogenic continuum", since they result from a thermal degradation continuum in which aromaticity, condensation and hydrogen to carbon (H:C) and hydrogen to carbon (O:C) ratios decrease (Cao et al., 2013; Hedges et al., 2000). Thus, PCMs range from charcoal, less thermally degraded and sometimes of recognized origin, to lighter PCM, much more thermally homogenized, like ash (Bird et al., 2015; Bodí et al., 2014). PyC is considered one of the most stable C components in the soil, and represent between 0 and 60% of the total soil organic C (Reisser et al., 2016). After fire, PyC, and PCM in general, end up interacting with the soil components in different ways, depending on the characteristics of both of them (Knicker, 2011). Although PCM incorporation into the soil plays a critical role in the global C cycle, its dynamic and interactions with other soil processes such as water erosion and redeposition, decomposition and leaching are still poorly understood (Lasslop et al., 2019; Santín et al., 2016).

Previous works refer to positive or negative priming effects when the addition of PyC increases or decreases, respectively, the mineralization rate of native soil organic C (Maestrini et al., 2015; Wang et al., 2016a). These contrasted results could be since different directions and magnitudes of the priming effect promoted by PyC seem to be modulated by multiple factors. Thus, it has been reported that the priming effect induced by PCM incorporation into soils could be modulated by changes in the soil microbial community structure, the nature of the C added (labile C *vs* less decomposable fractions) or by soil pH (Drake et al., 2013; Fang et al., 2019; Luo et al., 2011; Santos et al., 2012).

Many studies also have associated priming effect with changes in N dynamics (Chen et al., 2014; Fontaine et al., 2003; Kuzyakov et al., 2000). Clough et al. (2013) described that PCM can absorb N through ion-exchange, can remove ammonium (NH_4^+-N) by adsorption and can stimulate immobilization of nitrate (NO_3N). DeLuca et al. (2006) observed a short-term increase of nitrification rate promoted by the addition of PyC. In addition, Craine et al. (2007) found that a low soil N availability increases soil organic C mineralization rate. Based on these findings, Fontaine et al. (2011) and Chen et al. (2014) suggested that shortage of available N could favour microorganisms able to use C from recalcitrant organic matter (K-strategists), such as PCM, over microbial species that only feed on fresh-C and immobilize nutrients from the soil solution (r-strategists).

An increasing fire risk due to climate change (Bedia et al., 2014), as well as to continued land abandonment, could lead to increased fire severities or frequencies. The increasing number of fires occurring in areas in recovery from previous fires might results in shifts in the trajectories of the ecosystems in comparison with those expected after fires that occur after a long period since the previous one (Malkisnon et al., 2011). Under these new scenarios of changing fire regimes, a better understanding of the role PyC on soil organic matter mineralization is needed.

Wildfires or prescribed fires does not allow us to control and record the temperatures reached in the soil and the amount and type of PCM (from charcoal to ash) incorporated to the soil. In contrast, soil heating and PCM amendments under laboratory conditions provides the opportunity to isolate the effect of these environmental factors. Many examples about the utility of this methodology are reviewed in Pereira et al. (2019a). Until now, however, no study has evaluated the effects of thermal shock and PCM incorporation on C and N dynamics of soils with different fire history.

The main objective of this study was to assess the effect of fire on C and N mineralization rates and main microbial groups of soil with different fire history. We hypothesized that an increase in fire frequency would lead to a slower recovery of the soil microbial functionality, leading to cumulative imbalances in C and N cycling.

In order to test this hypothesis soils with different fire history were experimentally subjected to a heat-shock treatment and amended with different PCMs, derived from burning *Pinus pinaster* biomass. Soils were incubated under laboratory conditions and C and N mineralization rates were measured over a three-month period. In addition, the effect of these treatments in the main microbial groups was evaluated.

2. Material and methods

2.1. Soil sampling and pyrogenic carbonaceous material production

Soils were collected from forest ecosystems affected by different fire history. The sites were located in the south face of Sierra de Gredos, in the Sistema Central Mountain Chain (central Spain), at elevation between 750 and 1250 m.a.s.l. (Fig. 1; Supplementary material, Table S1). In this area, repeated fires have promoted a transition from *Pinus pinaster* Ait. forests to shrublands, dominated by *Cytisus striatus* (Hill) Rothm. and *Cytisus multiflorus* (L'Hér.) Sweet. Soils are Humic Cambisols (IUSS Working Group WRB, 2015) on Hercynian granites bed-rocks (GEODE, 2004). The soils have a loamy sand texture. The climate in the area is Mediterranean (*Csa-Csb*, as defined by the Köppen-Geiger), with dry and hot summers, and wet and mild winters (Kriticos et al., 2012). Average annual temperature is 10.9 ± 0.7 °C and with an average annual rainfall of 524 ± 99 mm (Climatic Research Unit TS V4.01) (Harris et al., 2014).

The perimeters of the three fires, occurred in 1979, 1990 and 2009, were chosen to delimit areas that were burned 1, 2 or 3 times, with moderate to high severity. In addition, a nearby area without fires, for at least 60 years since this study was carried out, was selected as an unburned reference (Fig. 1, Table S1). For each fire history, three sampling sites were selected with similar orientation, slope, parent material, soil type (400 m^2 size). They were more than 200 m apart. All areas were covered by *P. pinaster* forests when the first fire occurred. The sampling sites were selected so that they would not have any obvious signs of having been managed, except for salvage logging, which is regularly practiced in the area. In each sampling area, three sub-samples were collected from the top 5 cm of mineral soil within a 0.25 × 0.25 m still-frame. Soil sub-samples were transported in isothermal bag (4 °C) to the laboratory, where they were air dried and stored until further treatments or analysis. For each soil sub-sample, roots and coarse

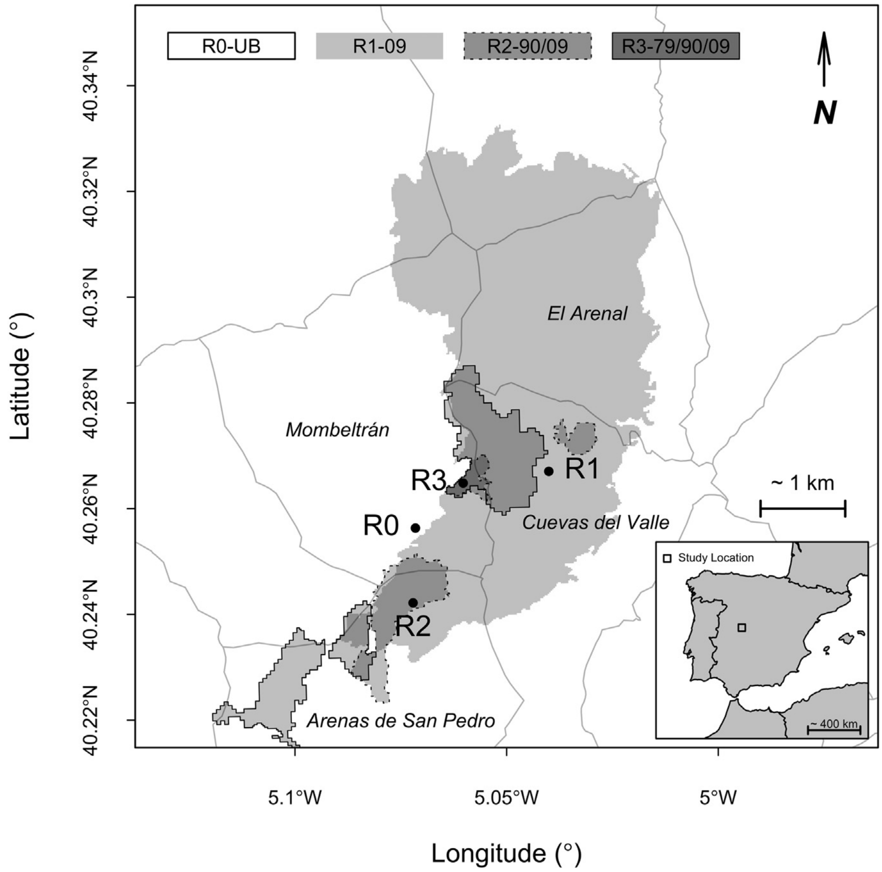


Fig. 1. Location of the study sites of unburned areas (R0-UB), areas burned once (2009; R1-09), twice (1990, 2009; R2-90/09) and thrice (1979, 1990 and 2009; R3-79/90/09).

litter fragments were removed, prior to sieving (<2 mm), after which they were mixed together to form a composite sample per sampling area.

The methods used to obtain experimental charcoal (CH hereafter) and ash (ASH) intended to simulate PCMs formed in different microsites of a low- to moderate-intensity surface wildfire under field conditions (Pereira et al., 2019b). Pyrogenic carbonaceous materials (PCM) were produced from litter collected from a *P. pinaster* forest from an area with similar conditions to the soil samples. Soil and litter sampling were carried out in February 2018. The collected litter was air dried and stored until further use. Pine twigs were separated from an aliquot of litter collected in the field and dried at 65 °C for 48 h. The mean diameter of twigs was 4.71 mm (ranging from 1.26 mm to 17.98 mm). CH was obtained from pine twigs by ignition in a muffle furnace at 450 °C during 15 min. Subsequently, the resulting CH was grounded and sieved (<2 mm), obtaining the following size distribution: 36% <500 μm, 27% 500–1000 μm, 23% 1000–1600 μm and 14% 1600–2000 μm. ASH, defined as the particulate residue remaining from the burning of wildland fuels and consisting of mineral materials and charred organic components (Bodí et al., 2014), was obtained by burning litter in a metallic container, reaching a maximum instantaneous temperature of 900 °C (recorded with a thermo-laser gun). The resulting material was homogenized at 550 °C for 1 h in a muffle furnace and sieved (<1 mm).

2.2. Characterization of soils and pyrogenic carbonaceous materials

Total carbon and nitrogen were quantified in soils by using an autoanalyzer CNHS (Leco TruSpec Micro). Water-soluble C was estimated following the methodology proposed by Ghani et al. (2003). Soil pH was determined in a slurry with 0.01 M $CaCl_2$ (1:1, w:w ratio), following McLean (1982). Soil organic matter was obtained by measuring weight lost on ignition (550 °C 2 h) (Nelson and Sommers, 1996). Soil cation exchange capacity (CEC) was analysed by Na saturation with NaOAc and displacement with $AcONH_4$, according to Rhoades (1982). Soil NH_4^+-N and NO_3^--N were analysed by spectrophotometry after 2 M KCl extraction, following Mulvaney (1996) and Keeney and Nelson (1982), respectively. The sum of both was considered as total inorganic N (TIN). Labile phosphate (PO_4^{-3}-P) was extracted in 0.5 M $NaHCO_3$ (pH: 8.5) (Olsen and Sommers, 1982), and quantified by the colorimetric method of John (1970). Soil texture was determined by pipette method (Gee and Bauder, 1986), after dispersion of 10 g of soil with a sodium hexametaphosphate and sodium carbonate solution.

Total C, (TC), total N (TN) and total hydrogen (TH) were quantified in PCM (CH and ASH) by using an autoanalyzer CNHS (Leco TruSpec Micro). Total oxygen of PCM was determined by difference, assuming that PCM is composed of C, N, H, O and residual-ash only. The elemental ratios C:N, H:C and O:C were calculated with the concentrations data of the elements and their atomic ratios were calculated by correcting them for their atomic masses. Volatile matter and residual-ash content in PCM (CH and ASH) were determined using a slightly modified version of the ASTM method (D-1762-84) described by Zimmerman (2010), involving measurement of weight loss following combustion. One gram PCM samples – in triplicate – were weighed in porcelain crucibles. For water content estimation, samples were dried at 104 °C for 16 h. Samples were heated to 750 °C for 6 h (uncovered crucible) for residual-ash content and to 950 °C for 6 min (covered crucible) for volatile matter quantification. The percentage of fixed carbon was determined, as described by Ronsse et al. (2013). Briefly, water, residual-ash and volatile matter content were subtracted from the weight of the original sample. Water-soluble C, pH, NH_4^+-N, NO_3^--N and PO_4^{-3}-P were also analysed in PCM, following the methodology described previously for soil samples.

2.3. Mesocosm experiment setup

The laboratory mesocosm experiment was established using a design to simulate the effects of fire (heat shock and PCM amendment) on C and N mineralization of soils that had been previously subjected to a different fire history. The general properties of soils and PCM used in this experiment are summarized in Tables 1 and 2. Aliquots of soil samples (150 g dry weight) representative of the different fire histories (R0, R1, R2 and R3) were weighted in jars and further subjected to a heat shock in a muffle furnace (120 °C during 15 min). This will be hereafter referred as a heat (H) treatment. As a control treatment, additional soil aliquots of the same fire histories where not treated (unheated, UH). These experimental conditions pretend to simulate the conditions described in the literature in low- to moderate-intensity wildfires or prescribed fires of *P. pinaster* forests, in which the maximum temperatures at 2–3 cm depth of mineral soil ranged about 40–125 °C (Maia et al., 2012; Merino et al., 2019; Plaza-Álvarez et al., 2019; Vega et al., 2005).

Subsequently, i) one gram of charcoal (CH), ii) one gram ash (ASH) or iii) one gram of charcoal plus one gram of ash (CH + ASH) were added into heated (H) soils, and mixed to simulate different PCM effects. Thus, the amount of PyC added into the soils was 4.66 mg C g^{-1} soil for CH, 0.62 mg C g^{-1} soil for ASH, 5.28 mg C g^{-1} soil for CH + ASH. These concentrations are equivalent to approximately 2.21 Mg C ha^{-1} for CH, 0.29 Mg C ha^{-1} for ASH and 2.51 Mg C ha^{-1} for CH + ASH, considering a soil bulk density of 0.95 g cm^{-3}. These values of PyC added to the soil are in the range of figures reported in the literature for field conditions (Bodí et al., 2014; Santín et al., 2016). A set of heated soil samples was left without any additional source of PCM. In addition, samples of the different PCMs used in this study were incubated alone (*i.e.*, without soil). Fig. S1 (supplementary material), shows an outline of the whole experimental design in which three replicates were considered per treatment. All soil samples were pre-incubated in the dark at 25 °C and 60% of their water holding capacity during fifteen days for their stabilization, prior to the beginning of the 98 days of incubation period (but after thermal shock). Water content of the soil samples was periodically adjusted, and its fluctuations were always lower than 0.5%.

2.4. C and N mineralization

In soil and PCM samples the CO_2-C production was continuously measured using the alkali trap method on days 14, 28, 42, 56, 70, 84 and 98 of incubation, following the methodology of Anderson (1982). One and two-pool exponential decay models were tested. However, the first order exponential decay model ($Q(t) = C_0 \cdot e^{-kt}$) was selected due to its best-fit, showing a higher R^2. C_0 represents the size of the potentially mineralizable C fraction (mg CO_2-C g^{-1}), k is the potentially mineralizable C decay rate (d^{-1}) and t is time of incubation (days). The mean residence time (*MRT*) of C_0 was estimated as the inverse of k ($MRT = 1/k$) and expressed in days. Half-life time ($t_{1/2}$) was calculated as $t_{1/2} = \ln(2) \cdot MRT$.

In order to evaluate the dynamic of available inorganic N, soil subsamples were taken from each experimental unit after 0, 14, 28, 42, 56, 70, 84 and 98 days of incubation. The content of ammonium and nitrate was analysed as previously described, following Mulvaney (1996) and Keeney and Nelson (1982), respectively. Net N mineralization was calculated as the net increase in NH_4^+-N and NO_3^--N over the incubation periods; the net increase in NO_3^--N was used to calculate the net nitrification rate (Hart et al., 1994).

2.5. Estimation of the priming effect

The priming effect is considered as the extra soil organic matter mineralization, quantified as CO_2-C production, caused by the addition of PCM to the soil (Kuzyakov et al., 2000). Thus, the priming effect magnitude was calculated for each sample as the difference between the observed CO_2-C (R_{obs}) produced by heated soils in which PCM had been added ($Q_{(H/PCM)}$), and the sum of CO_2-C (R_{exp}) produced by PCM ($Q_{(PCM)}$) plus that

Table 2
General chemical properties of pyrogenic carbonaceous materials (charcoal and ash). Different letters denote significant differences between them ($\alpha = 0.05$); mean ± standard deviation.

	Pyrogenic carbonaceous materials	
	Charcoal (n = 3)	Ash (n = 3)
pH	8.1 ± 0.1 b	12.0 ± 0.1 a
Total C (%)	67.1 ± 0.1 a	8.6 ± 0.2 b
WSC (mg g^{-1})	0.69 ± 0.0 a	0.33 ± 0.0 b
Total N (%)§	0.62 ± 0.0 a	0.15 ± 0.0 b
NO_3^--N (μg g^{-1})	3.5 ± 3.7 a	2.1 ± 1.7 a
NH_4^+-N (μg g^{-1})	2.4 ± 1.5 a	3.3 ± 3.0 a
TIN (μg g^{-1})	5.9 ± 5.1 a	5.4 ± 4.6 a
Total H (%)	3.6 ± 0.0 a	0.2 ± 0.1 b
Total O (%)§	23.1 ± 0.0 a	8.9 ± 0.5 b
PO_4^--P (μg g^{-1})	0.7 ± 0.0 b	4.3 ± 0.3 a
Residual ash (%)	5.6 ± 0.0 b	82.2 ± 0.2 a
Volatile matter (VM) (%)	30.9 ± 0.1 a	5.0 ± 0.2 b
Fixed C (FC) (%)	63.5 ± 0.1 a	12.9 ± 0.2 b
VM:FC§	0.49 ± 0.0 a	0.39 ± 0.0 b
Elemental ratio C:N	108.3 ± 0.7 a	59.5 ± 4.2 b
Elemental ratio O:C§	0.34 ± 0.0 b	1.03 ± 0.1 a
Elemental ratio H:C§	0.05 ± 0.0 a	0.03 ± 0.0 b
Atomic ratio C:N	126.3 ± 0.9 a	69.3 ± 4.9 b
Atomic ratio O:C§	0.26 ± 0.0 b	0.77 ± 0.1 a
Atomic ratio H:C§	0.65 ± 0.0 a	0.30 ± 0.1 b

§ Welch's Heteroscedastic F-test ($\alpha = 0.05$).

Table 1
General soil properties of the study areas. Different letters denote significant differences among fire histories ($\alpha = 0.05$); mean ± standard deviation.

	Fire history			
	R0	R1	R2	R3
pH	4.1 ± 0.0 c	4.4 ± 0.1 b	5.2 ± 0.2 a	4.6 ± 0.1 b
Soil organic matter (mg g^{-1})	162 ± 20.1 a	132 ± 9.9 ab	122 ± 18.1 ab	101 ± 12.8 b
Total C (mg g^{-1})	93 ± 6.8 a	58 ± 8.9 b	51 ± 10.3 b	41 ± 9.0 b
Water-soluble C (WSC) (mg g^{-1})	1.0 ± 0.1 a	0.6 ± 0.0 b	0.8 ± 0.1 b	0.7 ± 0.1 b
Total N (mg g^{-1})	3.4 ± 0.3	3.5 ± 0.6	2.8 ± 0.4	2.9 ± 0.8
NO_3^--N (μg g^{-1})	4.0 ± 2.1 c	36.4 ± 10.9 b	57.5 ± 10.6 ab	70.5 ± 15.1 a
NH_4^+-N (μg g^{-1})§	3.1 ± 0.6 a	1.0 ± 0.1 ab	0.9 ± 0.1 bc	0.9 ± 0.0 c
Total inorganic N (μg g^{-1})	7.1 ± 2.7 c	37.4 ± 11.0 b	58.4 ± 10.6 ab	71.3 ± 15.1 a
WSC:TOC†	1.1 ± 0.2 a	0.8 ± 0.0 b	1.1 ± 0.2 ab	1.1 ± 0.1 a
C:N	28 ± 2.8 a	22 ± 2.4 ab	26 ± 3.2 ab	21 ± 3.1 b
PO_4^{-3}-P (μg g^{-1})	8.5 ± 2.1 c	36.4 ± 9.5 b	27.2 ± 8.4 b	68.7 ± 4.5 a

§ Welch's Heteroscedastic F-test ($\alpha = 0.05$).
† 1.724 as conversion factor was applied to estimate the total organic C (TOC) from the SOM.

produced by the heated soils without PCM additions ($Q_{(H)}$), according to (Eq. (1)):

$$PE = R_{obs} - R_{exp} \quad (1)$$

The R_{exp} values were estimated by bootstrapping according to (Eq. (2)):

$$Q_{(H)} + Q_{(PCM)} = Q_{(Hij\cdot)} + (f/3) \times \sum Q_{(PCM\cdot jk)} \quad (2)$$

where f is the proportion of material added to the sample, $Q_{(Hij\cdot)}$ is the CO_2-C produced by the heated soils (H) incubated separately, $Q_{(PCM\cdot jk)}$ is the CO_2-C produced by PCM incubated separately (CH, ASH and CH + ASH) and k is the number of samples of PCM incubated in the time j. The relative intensity of the priming effect (PE %) was estimated as a percentage of change relative to the control CO_2-C production, as follow (Eq. (3)).

$$PE\ (\%) = 100 \times [[Q_{H+PCM} - (Q_H + Q_{PCM})]/(Q_H + Q_{PCM})] \quad (3)$$

2.6. Microbial community structure

The abundance of the main microbial groups and an estimate of microbial biomass were determined by the direct extraction of ester-linked fatty acids (ELFAs) from an aliquot of soil samples collected at the day 14 of incubation, following Schutter and Dick (2000). Briefly, 3 g of soil (fresh weight) were mixed with 15 mL 0.2 M KOH in methanol and 2.5 μg of internal standard (C19:0), then shaken at 100 rpm at 37 °C for 1 h, thus allowing the release and subsequent methylation of ELFAs. Soil pH was then neutralized by the addition of 3 mL 1.0 M acetic acid, and fatty acid methyl esters were extracted with 10 mL hexane. After centrifugation (5 min, 5000 rpm), the upper hexane layer was transferred to clean tubes and evaporated by using a rotavapor equipped with a vacuum pump. Dried fatty acids were re-suspended in 180 μL hexane to be analysed by a gas chromatograph (Thermo Scientific FOCUS™), equipped with a flame ionization detector and a fused-silica capillary column Mega-10 (50 m × 0.32 mm I.D.; film thickness 0.25 μm). The gas chromatograph temperature progression was as follows: initial isotherm at 115 °C for 5 min, increase at a rate of 1.5 °C per minute from 115 to 230 °C, and final isotherm at 230 °C for 2 min. The identification of the fatty acid peaks was carried out by the comparison of the retention time of each one with known standard compounds (Supelco Bacterial Acid Methyl Esters mix cat no. 47080-U and Supelco 37 Component FAME mix cat no. 47885-U). Fatty acids were quantified relative to nonadecanoic acid methyl ester (C19:0; N-5377, Sigma Chemical Inc.), used as an internal standard assuming equimolar responses by all fatty acids. Concentrations of fatty acids were expressed as micromole per gram of dry soil (μmol of FA g^{-1}). The total ELFAs was assumed as a measure of the total soil microbial biomass. Peaks in the region between tetradecanoic methyl ester (C14:0) and arachidonic acid (C20:4ω6c) were included in this analysis, unless otherwise stated, as of microbial origin (Laudicina et al., 2012). The fatty acid nomenclature was as described by Hinojosa et al. (2005). Fatty acid methyl esters C14:0i, C15:0a, C15:0i, C16:0i, C17:0a and C17:0i were reported as marker of Gram-positive bacteria. C16:1ω7c, C16:1ω9c, C18:1ω7c, C17:0cy and C19:0cy were used as Gram-negative bacteria markers. The sum of both Gram-positive and Gram-negative was considered as total bacterial (B) marker (Frostegård and Bååth, 1996). C16:1ω5c was used as arbuscular mycorrhizal marker in soils (Madan et al., 2002), polyunsaturated fatty acids 18:3ω6,9,12c and 18:2ω6,9c as fungal biomarkers (Bossio and Scow, 1998) and 10Me16:0, 10Me17:0 and 10Me18:0 for actinobacteria (Lechevalier and Moss, 1977).

2.7. Statistical analysis

The impossibility to replicate disturbance events such as wildfires, makes experimental replication challenging (Oksanen, 2001; Van Mantgem et al., 2001). To take advantage of the different fire histories registered in our study area, we considered sampling sites within each fire perimeter as independent, acknowledging that doing that constrains the scope of our inference, as it often occurs with wildfire field experiments (Driscoll et al., 2010; Mayor et al., 2016; Van Mantgem et al., 2001).

One-way ANOVA was used to assess significant differences among soils with different fire history, using Fisher LSD as *post hoc* analysis. Previously, normality and homogeneity of variance were tested in the data with Shapiro-Wilk and "Levene" Barlett tests. When ANOVA assumptions were violated, Welch's heteroscedastic F-test was applied. A general lineal model (GLM) was performed for each soil variable, using "fire history" (R0, R1, R2 and R3) and "fire simulation treatments" (UH, H, H/CH, H/ASH, H/CH + ASH) as main factors and considering "sampling sites" (nested in "fire history") as a random factor. When there was not "fire history × fire simulation treatments" interaction effect but "fire history" effect was significant, a Tukey multiple comparison test was used to identify significant differences among specific means.

Additionally, for each "fire history" level (R0, R1, R2 and R3) the significance of fire simulation treatments effects was tested using one-way ANOVA. Two-way repeated measures ANOVA was applied to defined temporal processes interactions by factors. For repeated measures ANOVA, Mauchly's Test was used to evaluate sphericity assumption. The Greenhouse–Geisser correction was used for adjusting for lack of sphericity when it was needed. One-way ANOVA tests were used to assess significant differences among treatments for each sampling time. Paired t-test was applied to compare expected and observed CO_2-C production rate at each time.

The effect of study factors (fire history and a new fire simulation treatments) on the main soil microbial groups was tested using PERMANOVA (permutational multivariate analysis of variance), employing the relative abundance of the full set of fatty acids present in the soil samples. This analysis was carried out with PERMANOVA, based on 9999 permutations, and the Bray-Curtis distance measure of dissimilarity for untransformed and unstandardized data. To aid the interpretation of the PERMANOVA analyses, a non-metric multidimensional scaling (NMDS) analysis was performed. NMDS ordination was based on Bray-Curtis distance and carried out in R with the *vegan* and *MASS* package. The final stability of each run was evaluated by examining plots of stress *vs* the number of iterations. The percentage of variance represented by each Axis were calculated (McCune et al., 2002). In addition, the correlation between NMDS coordinates and the main fatty acids-biomarkers were tested using Spearman correlation coefficients ($\alpha = 0.05$). The Pearson correlations coefficients were also used to determine the relationship between the microbial community structure (coordinates in NMDS axes 1 and 2) and the studied soil variables ($\alpha = 0.05$). The vectors, the determination coefficients (R^2) and their significances (*p-value*) were fitted in the NMDS ordination using the *envfit* function, implemented with 9999 random permutations, for microbiological properties. The statistical analysis and graphical representation were carried out with the R Core Team version 3.6.1 (R Core Team, 2019).

3. Results

3.1. General properties of pyrogenic carbonaceous materials

The properties of PCM used in this experiment are summarized in Table 2. The PCM was characterized by a high C:N ratio, twice higher in CH than in ASH. The CH produced in our study contained 671 ± 1 mg g^{-1} of C and 6.20 ± 0.04 mg g^{-1} of N, with a mean C:N ratio of

108 ± 1. The ASH produced in our study contained 86 ± 2 mg g^{-1} of C and 1.45 ± 0.01 mg g^{-1} of N, and a mean C:N ratio of 60 ± 4. The H:C and O:C atomic ratios of CH, generally used as an indirect measure of the aromaticity of a substance, were 0.65 ± 0.02 and 0.26 ± 0.01, respectively. However, in the ASH produced in our study the H:C and O:C atomic ratios were 0.30 ± 0.08 and 0.77 ± 0.07, respectively (Table 2).

3.2. Soil carbon mineralization

Independently of the treatments, the cumulative amount and the rate of CO_2-C produced by unburnt soils (R0) were about twice higher than those observed in soils previously affected by wildfires (R1, R2 and R3) (Fig. 2). Soil CO_2-C production rate significantly decreased over incubation time in all the treatments ($p < 0.001$). For unburned soils (R0), the heat-shock and PCM addition treatments did not significantly affect soil CO_2-C production compared to the UH control (Fig. 2a). On the other hand, CO_2-C production in previously burned soils (R1, R2 and R3) was significantly affected by the interaction between fire simulation treatments (heat-shock and PCM additions) and time (Fig. 2b–d). In the short-term (first 15 days of incubation), heated and previously burned soils (R1, R2 and R3) amended with any PCM showed a CO_2-C production rate significantly higher than the controls (H and UH). Soil amended with ASH and CH + ASH showed the highest initial CO_2-C production rates. Differences in the CO_2-C production rate among heated soils with PCM amendment and the controls (H and UH) decreased over the incubation time, and were not significant at 98 days in R1 and R3, although still significant in R2. However, the significantly higher cumulated total CO_2-C production on soils (R1, R2 and R3) heated and amended with ash (ASH and CH + ASH) was maintained at the end of the experiment. In all cases, CO_2-C production rate fitted a single exponential decay model ($R^2 > 0.66$; $p < 0.05$) (the parameters of the models are shown in Fig. 3). Potentially mineralizable C (C_0) ranged from 1.3 to 4.1 mg of C g^{-1}, and it was significantly affected by the interaction between fire simulation treatments (heat-shock and PCM additions) and the previous wildfire history (Fig. 3a). In general, C_0 in unburned soils (R0) was about twice (2.1–2.5) higher than that of soils previously affected by wildfires (R1, R2 and R3). Among burned soils (mainly R1 and R3), those heated and amended with ash (ASH or CH + ASH) had values of C_0 0.69–0.86 times higher than the controls (UH and H). A similar pattern was observed also for the combined kC_0 parameter (Fig. 3c). For the study soils, the half-life time ($t_{1/2}$) of 1 g of C_0 ranged between 101 and 328 years (Fig. 3d) but for burned soils (mainly R1 and R3) the addition of ash (ASH or CH + ASH) significantly decreased the $t_{1/2}$ of C_0.

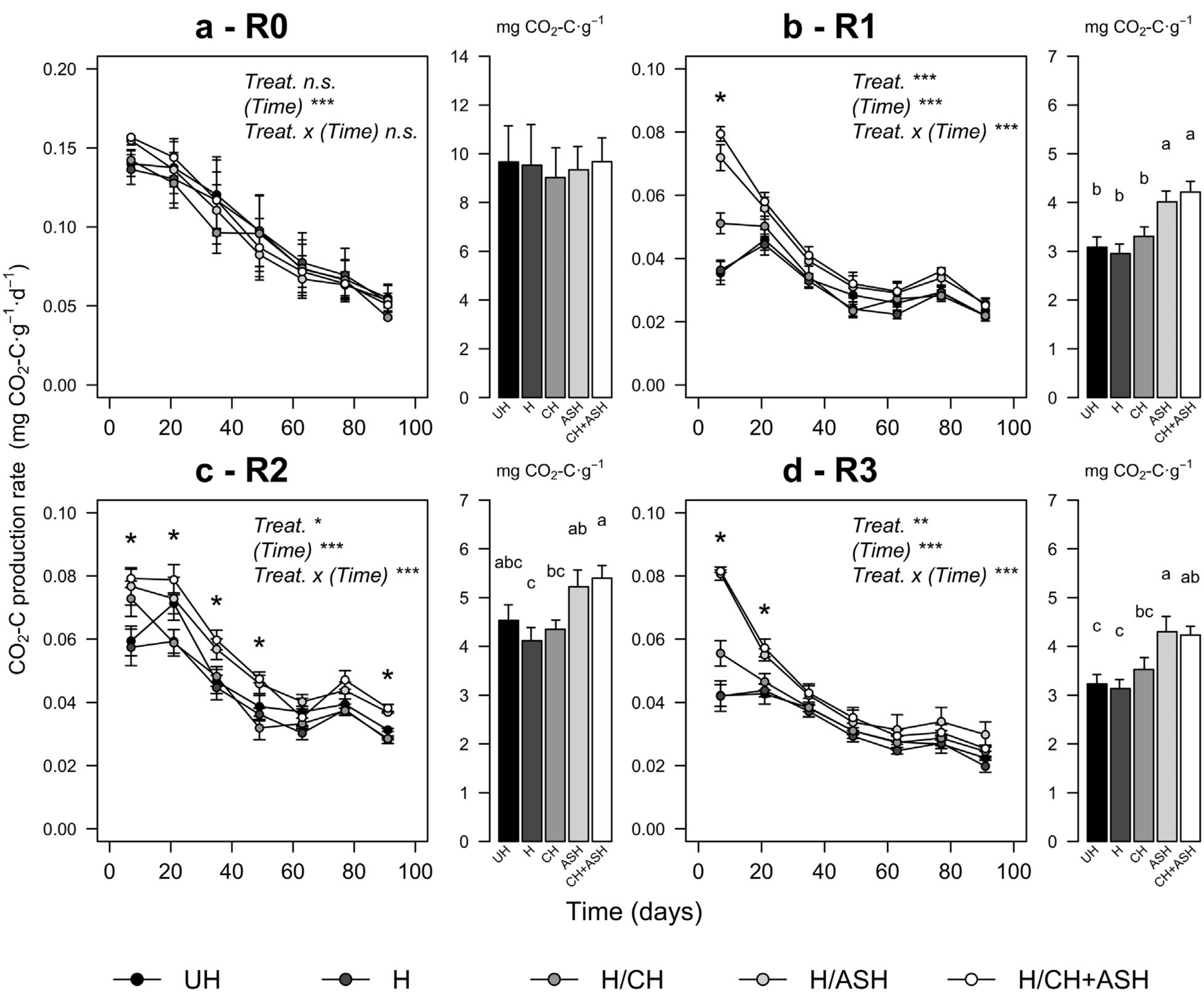


Fig. 2. CO_2-C production rate (mg CO_2-C g^{-1} d^{-1}) and accumulate CO_2-C production (mg CO_2-C g^{-1}) at the day 98 of incubation in soils with different fire history (R0, R1, R2 and R3) with and without heat treatment (H and UH) and with differential PCM amendments: charcoal (CH), ash (ASH) or both (CH + ASH); mean ± standard error. For CO_2-C production rate data points are allocated between the two adjacent sampling dates. Significant effect of a two-way ANOVA repeated measures is noted with * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$). *n.s.* denote no significant effect. Significant differences among treatments for each sampling time are noted with * ($p < 0.05$). For accumulate CO_2-C production lower-case letters denote significant differences (Fisher-LSD) among treatments within each fire history scenario ($p < 0.05$).

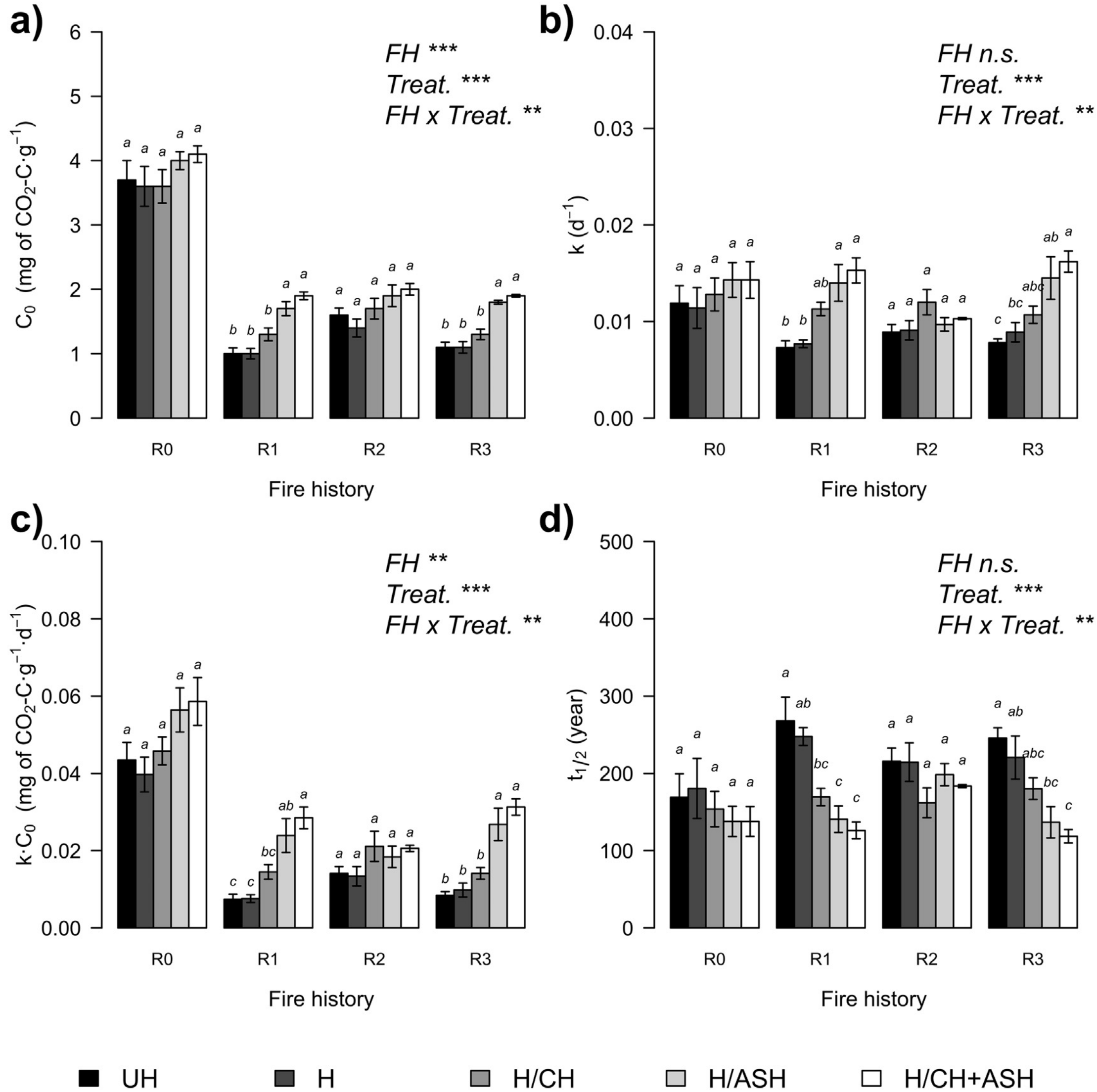


Fig. 3. Parameters estimated according to the first order exponential decay model ($Q(t) = C_0 \cdot e^{-kt}$) for soil CO_2-C production rate: a) C_0 - potentially mineralizable C; b) potentially mineralizable C decay rate; c) their combination k·C0 and d) the half-life time. All parameters were estimated in soils with different fire history (R0, R1, R2 and R3) with and without heat treatment (H and UH) and with different PCM amendment: charcoal (CH), ash (ASH) or both (CH + ASH); mean ± standard error. Significant effects of fire history [FH], treatment [Treat], and their interaction [FH × Treat] are shown within the figures (*ns*, no significant effect; *, $p < 0.05$; ***, $p < 0.001$). Different lower-case letters denote significant differences (Fisher-LSD) among treatments within each fire history scenario ($p < 0.05$).

3.3. Net N mineralization and nitrification rates

Burned soils had about 4–7 times more TIN (mainly in the form of NO_3^--N) than the unburned R0 control (Table 1; Figs. S2–S3). For all burned soils, TIN increased along the incubation period (98 days), with no differences among the heat shock and PCM addition treatments applied (Fig. 4). As an exception, in R3, at the end of the incubation period all heated soils (H), with and without PCM additions, had highe TIN values than the unburned control (UH). For the net N mineralization and nitrification rates a significant interaction between fire simulation treatments (heat-shock and PCM additions) and the previous wildfire history was observed (Fig. S4). In general, unburned soils (R0) showed higher net N mineralization and nitrification rates than burned soils (R1, R2 and R3). Except for R3 soil, none of the study soils (R0, R1 and R2) had significant effects on net N mineralization and nitrification rates after the heat-shock and PCM amendments additions. For R3 soil, net N mineralization and nitrification rates significantly increased after heat-shock and PCM amendments.

3.4. Magnitude of the priming effect

The priming effect of the studied soils was significantly affected by the interaction between fire simulation treatments (heat-shock and PCM additions) and time (Fig. 5; Figs. S5–S6). In all cases, the highest priming effect was observed in the short-term (14 days after the start of the incubation), as it decreased with the incubation time ($p < 0.05$). In unburned soil (R0), no priming effect was detected when comparing the observed and the expected levels of cumulative CO_2-C productions of soil heated and amended with PCM. However, a positive priming effect was observed in soils previously affected by wildfires (R1, R2 and R3) when they were heated and amended with PCM. In all previously burned soils (R1, R2 and R3), ash amendments (ASH and CH + ASH) resulted in the highest magnitude of priming effect. In soil amended with CH, the priming effect was positive but lower than when it was amended with ASH.

In our study, PO_4^{-3}-P was higher in burned than in unburned soils and at 98 days of incubation it was significantly correlated with the magnitude of priming effect ($r = 0.443$; $p = 0.007$) (Table 1, Fig. S7).

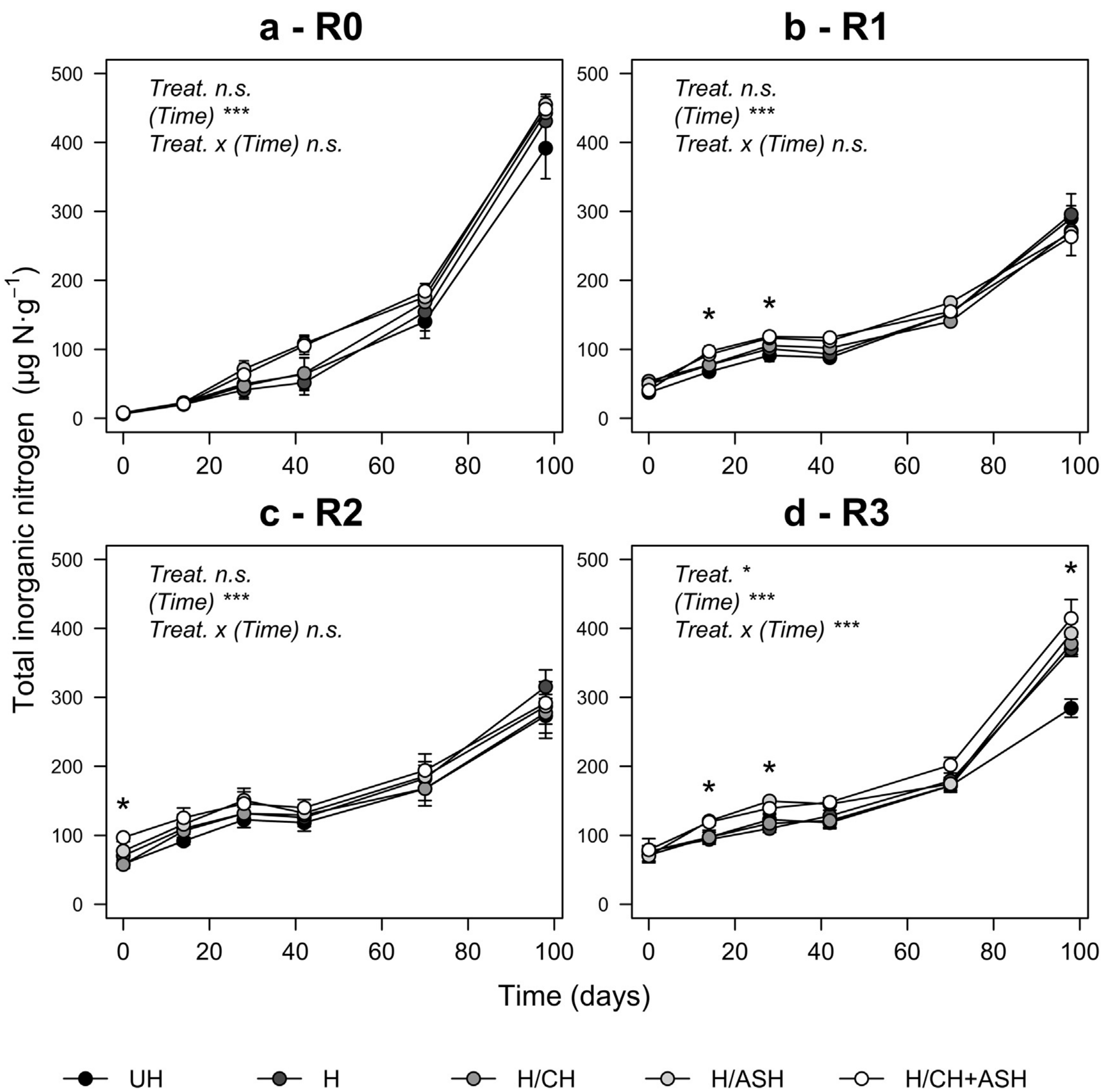


Fig. 4. Total inorganic nitrogen along the 98 days of incubation for soils with different fire history (R0, R1, R2 and R3) with and without heat treatment (H and UH) and with different PCM amendment: charcoal (CH), ash (ASH) or both (CH + ASH); mean ± standard error. Significant effects of a two-way ANOVA repeated measures are noted with * ($p < 0.05$) or *** ($p < 0.001$). *n.s.* denote no significant effect. Significant differences among treatments for each sampling time are noted with * ($p < 0.05$).

Soil pH was significantly higher in burned than in unburned soils (Table 1) at the end of the incubation period (98 days). After PCM amendment soil pH remarkably increased in soils amended with ASH (Fig. S8).

3.5. Microbial community structure by ELFAs

In general, the total amount of ELFAs and the relative abundance of Gram-positive bacteria of unburned soils (R0) was significantly higher than those of the soils previously affected by wildfires (R1, R2 and R3) (Table S2). No significant differences in the total amount of ELFAs were observed due to heat-shock and PCM addition treatments in any of the studied soils, at least at the sampling time. Similarly, the heat-shock and PCM treatments did not have significant effects in most of the cases, except for fungi, which decreased with heat treatment in unburned soils. The PERMANOVA analysis of the whole profile of ELFAs extracted from soil revealed significant differences in the initial microbial community structure among soils, with strong differences between the unburned (R0) and burned soils (R1, R2 and R3) ($p < 0.001$) (Table S3). The results from NMDS, using the whole set of ELFAs, confirmed the PERMANOVA results and discriminated between the microbial communities in burned and unburned soils in NMDS Axis 1, which explained most of the variance (79.76%) (Fig. 6). Axis 1 was positively correlated with Gram-positive bacteria markers and the Gram-positive to Gram-negative ratio and negatively correlated with total amount of ELFAs (Table S4). On the other hand, no significant effect was observed due to heat shock and PCM amendment treatments for most of the studied soils ELFAs profiles (Fig. S9). Furthermore, a significant correlation was found between the positive priming effect magnitude and the relative abundance of Gram-positive bacteria ($r = 0.352$; $p = 0.035$).

4. Discussion

4.1. The effect of fire history on soil C and N mineralization rates and microbial community structure

Our results demonstrate that wildfire is a disturbance that can have long-lasting effects on the C and N mineralization rates and on the microbial community of soils, even nine years after the last fire, in *P. pinaster* forests of Central Spain.

The CO_2-C production rate was lower in burned soils, with no differences due to the previous fire history (R1, R2 and R3). This agrees with Wang et al. (2012), who described a significant reduction in CO_2-C production rate of burned soil up to 3 years after the fire and suggested that

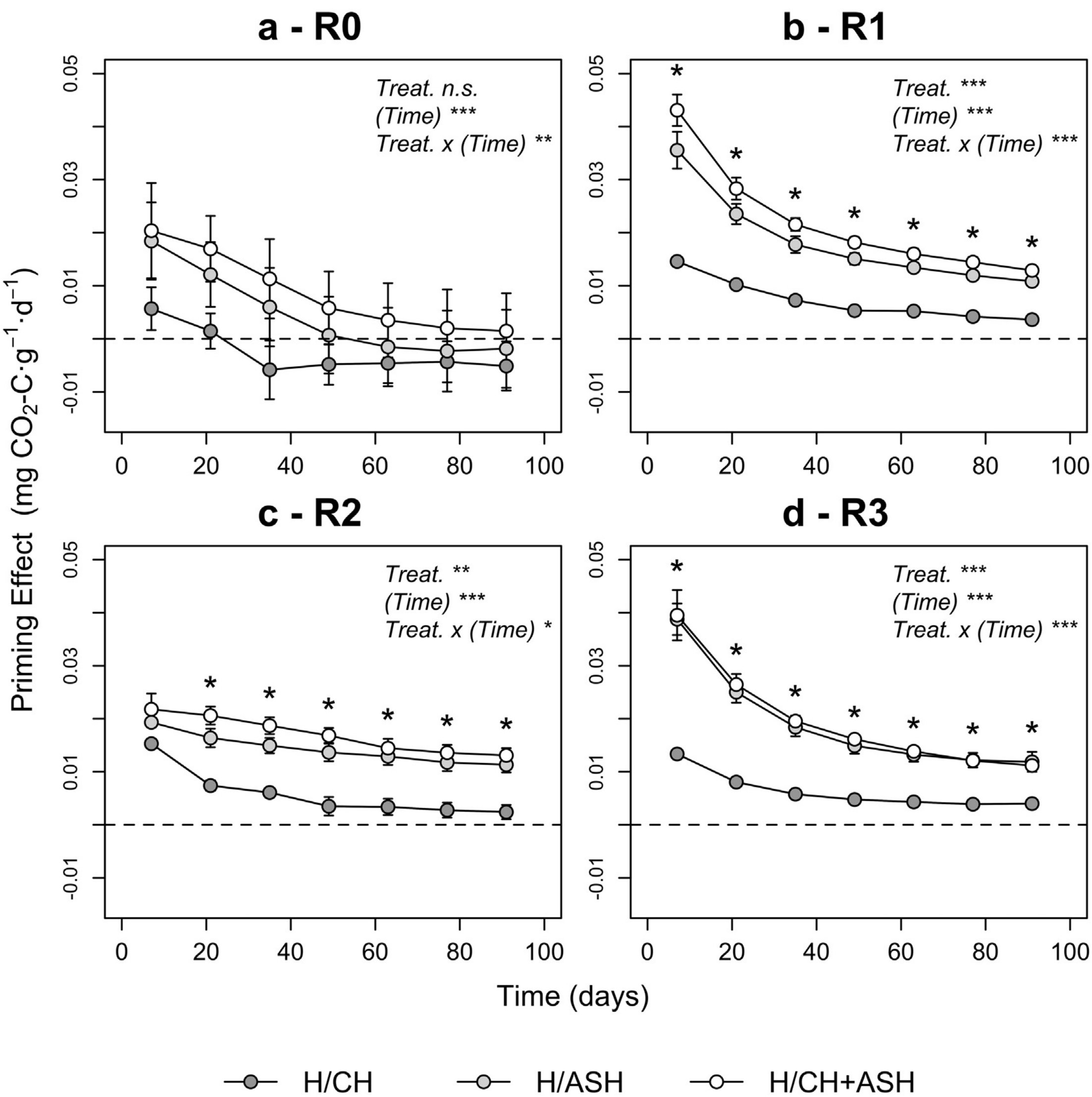


Fig. 5. Changes in the magnitude of priming effect in soils with different fire history (R0, R1, R2 and R3), when they were heated and amended with different PCM: charcoal (CH), ash (ASH) or both (CH/ASH); mean ± standard error. Dotted line indicate neutral priming effects. Significant effects of a two-way ANOVA repeated measures are noted with * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$). *n.s.* denote no significant effect. Significant differences among treatments for each sampling time are noted above with * ($p < 0.05$).

this might be largely driven by a reduction of soil organic matter and microbial biomass. At longer-term, other factors, such as a reduction of the amount and quality of soil organic matter (Vargas et al., 2012) and/or changes in the soil microbial community structure (Goberna et al., 2012; Hinojosa et al., 2019), could also affect soil CO_2-C production rate.

In this study, fire promoted changes from a *P. pinaster* woodlands to shrublands dominated by *Cytisus striatus* and *Cytisus multiflorus*. Despite a rapid post-fire recovery of plant cover of these ecosystems (Moreno et al., 1996), the input of plant organic C to soil was limited, due to the non-leafy nature of these shrubs and their tendency to accumulate the dead branches on the shrub for long time. This is confirmed by the low soil organic matter and water-soluble C observed in the burned soils. In addition, lower values of both C_0 and C_0k were observed in burned soils than in unburned ones, with similar values to those obtained by Fernández et al. (1999) under *P. pinaster* forest stands two years after fire. Overall, such changes suggest that a lower availability of C substrates for microbial respiration could explain, at least partially, the lowest soil CO_2-C production rate in burned soils (Tessler et al., 2013).

In our study, there were not effects of fire frequency in soil CO_2-C production rate. It could be due to the fact that all burned soils had similar values of total C, total N, water-soluble C and soil organic matter.

An increase of N mineralization rate immediately after fire has been widely described in literature (Caon et al., 2014; Pellegrini and Jackson, 2020). In our study, the N mineralization rate was lower in burned than in unburned soils still nine years post-fire. This agrees with previous medium- and long-term post-fire studies which have shown a decrease of N mineralization rate after frequent and infrequent fires up to 17 years later (Guénon et al., 2013; Koyama et al., 2010). The lower net N mineralization and nitrification rates in burned soils than in unburned ones could be explained by their lower total C and N content, and their lower microbial biomass (estimated as total ELFAs amount) (Guénon et al., 2013).

As previously mentioned, the lower C and N mineralization rates observed in burned soils compared with unburned ones could be also partially attributed to the legacy effect of fire in soil microbiota (Guénon et al., 2013; Hinojosa et al., 2019). In our study, burned soils showed lower soil microbial biomass than unburned ones, with no remarkable differences among fire histories. This negative effect of fire on soil microbial biomass, that even persist nine years post-fire, aligns with results reported by Pressler et al. (2019) and Dooley and Treseder (2012), who found limited evidence of recovery of microbial biomass 10 years after fire. This lower microbial biomass in burned soils can be explained by several potential mechanisms. During the fire, the short-

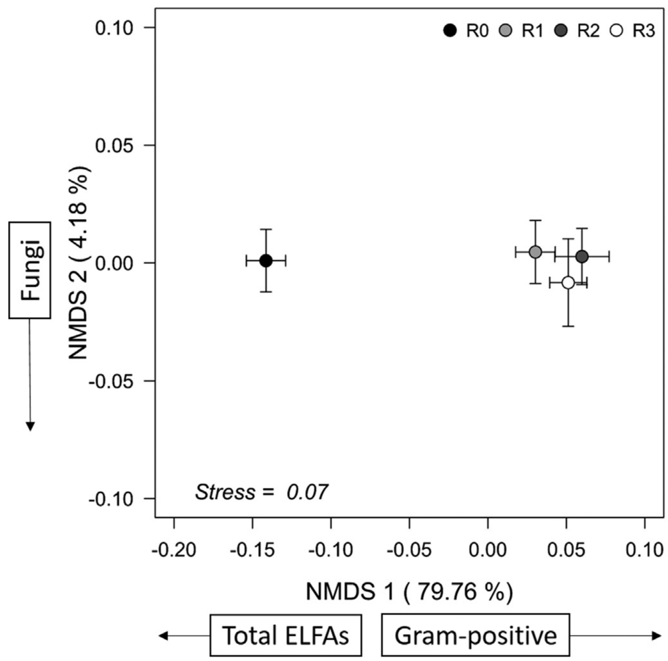


Fig. 6. Non-metric multidimensional scaling (NMDS) ordination plot of the relationship between soil microbial community composition under different wildfire history; mean ± standard deviation. NMDS solution was rotated to match NMDS-Axis 1 to fire history. Significant correlations between soil microbial variables and the ordination axes are shown next to them (see Table S4), $p < 0.05$. G+:G- refers to Gram-positive to Gram-negative ratio.

term heat-transfer to soil may lead to an increase in the microbial mortality (Hart et al., 2005). Afterward, the reduced microbial biomass following fires may be attributable to the reduced labile C input into soil due a lower aboveground net primary productivity in burned soils (Goberna et al., 2007; Hart et al., 2005); but also be driven by changes in soil nutrients and soil water content (O'Donnell et al., 2009). In our study, Gram-positive bacteria were more abundant in burned soils than in unburned ones. This could be due to the fact that Gram-positive bacteria can survive during fire forming heat resistant forms, as spores, able of withstanding high temperatures (Russell, 2003; Wang et al., 2016b). Additionally, Gram-negative bacteria use to be associated with simple C compounds whereas Gram-positive bacteria are associated with more complex C forms (Fanin et al., 2019).

4.2. Response of C and N mineralization rates in soil with different fire history after a new fire event

Fire not only had residual effects on soil C and N mineralization rates and microbial biomass and community structure, as previously described, but also modified the responsiveness of these soil properties and functions after a new simulated fire.

No significant effects were observed in C and N mineralization rates of soils with different fire history when only heat treatment was applied. This lack of heat-shock effect is probably due to the fact that we used thermo-temporal conditions that simulated a low-medium-intensity wildfire or a prescribed fire (120 °C during 15 min), without severe changes in soil properties (Neary et al., 1999). Our results agree with Raison and McGarity (1980) and Guerrero et al. (2005) who also observed no effect of similar heat-shock conditions on soil mineralization rates.

The amendments of PCM after the heat treatment did not affect C and N mineralization rates of unburned soils. Nevertheless, PCM amendment (CH, ASH and CH + ASH) to previously burned soils increased CO_2-C production rates, without differences among fire histories (R1, R2 and R3). In addition, only burned soils also showed a significant positive priming effect, mainly those treated with ASH alone or in combination to CH. Different CO_2-C production rate after PCM incorporation between unburned and previous burned soils could be associated, at least partially, to the fire legacy on soil microbial community structure. In our study, as previously described, soil ELFAs profiles showed different microbial community structure in burned and unburned soils. Thus, some of the microorganisms present in previously burned soils might be adapted to the resulting conditions after the new fire event simulated here (with heat-shock and PCM incorporation), according to previous studies (Santos et al., 2012; Vázquez et al., 1993). Thus, our results showed a significant correlation between the positive priming effect magnitude and the relative abundance of Gram-positive bacteria. Gram-positive bacteria are considered well-adapted to fire for using aromatic-C and slowly decomposing sources of organic C (Santos et al., 2012), such as lignin and aromatic/alkenes-C that are found in high concentrations in PCM (Chapman and Koch, 2007).

Chen et al. (2014) suggested that the interactions between C and N availability might control the soil priming effect. However, our results did not show any correlation between priming effect and soil TIN concentration. This could be mainly due to the low TN and TIN added to the soils with PCM amendment, at least compared with the mineralized N. Our results are consistent with those reported by Wild et al. (2019), that decouple priming effect and N-mining in the short-term, suggesting that the addition of C promotes the mobilization of other soil organic C sources associated with microbial food-web, releasing the chemically protected fractions (mineral bonds).

On the other hand, the observation of a higher P availability in burned than unburned soil and the correlation between P availability and the magnitude of the priming effects suggests that the highest P availability in burned soils might enhance the microbial turnover (Gross and Angert, 2017). According to Chen et al. (2019), in soil with high P availability the growth/death mode of microbial turnover predominates, whereas the maintenance mode is more important in soil with low P concentration.

ASH alone or in combination with charcoal (CH + ASH) stimulated soil CO_2-C production rate more than CH alone. The CO_2-C exponential decay model showed that the ASH amendment significantly increased C_0, and k compared with soil without any PCM amendments. This agrees with previous studies, in which ash addition resulted in a rapid increase of the CO_2-C released by soils with (Raison and McGarity, 1980) or without previous heat treatment (Zimmermann and Frey, 2002). Additionally, immediately after a wildfire, Bauhus et al. (1993) found positive effects on CO_2-C production at short-term, in the ash-bed of a Podzolic soil.

Noyce et al. (2016) suggested that the effects of wood-ash on microbial activity may be related to limitation by nutrients availability. However, in our case the incorporation of available P and TIN to soils resulting from ASH amendment was very slow, suggests that in our case the stimulating effect of ASH on CO_2-C emission was not related to nutrient availability limitation. Increased pH as a consequence of ashes incorporation into soils is considered another key driver of soil microbial activity (Pereira et al., 2019a). Thus, ASH amendment could generate micro-changes in soil pH and a raise of dissolved soil organic matter, increasing the CO_2-C production rate. Curtin et al. (2016) indicate that the response of dissolved organic matter to base addition depends, to a large extent, on how cation and anion solubility change. The input of a base cation with a weak affinity for the newly created (pH-dependent) exchange sites could result in a large increase in cation solubility, which must be balanced by inorganic and organic anions. The relative amounts of organic and inorganic anions released by added base (OH^-) will vary depending on the quantity of adsorbed anions in the soil and their bonding strength. This agrees with previous studies which reported that PCM incorporated to soils generates particular conditions (*e.g.* higher pH and adsorption capacity) than enhance microbial activity in its closest area of influence, the "charosphere" (Pingree and DeLuca, 2017). Despite increasing microbial activity, ASH amendment did not significantly modify soil microbial biomass or community structure. It is probably because the ASH amendment had not enough time to alter soil microbial composition; however, larger effects could be seen in the longer term. Noyce et al. (2016) found only minimal changes in the native soil microbial community 12 months after wood-ash addition on top of soil. However, Bang-Andreasen et al. (2017) reported that wood-ash application to forest soil caused significant changes to bacterial numbers, richness, diversity and community composition after 42 days of incubation on a microcosms experiment. These apparently contradictory results could be due to the different nature and amount of ash incorporated in the different studies, and also to different approaches used to evaluate changes in microbial community structure (for example, DNA-based methods or based on soil fatty acid profile).

The positive correlation between the priming effect magnitude and soil pH found at 98 days of incubation ($r = 0.622$; $p < 0.001$) suggests that changes in soil pH could also drive the higher positive priming effect observed in burned soils amended with ASH.

The priming effect found in burned soil after PCM amendment (mainly ASH) should be distinguished in "real" (soil organic matter decomposition as a result of co-metabolism) and "apparent" priming effect (changes in microbial biomass turnover without effects on soil organic matter decomposition) (Blagodatskaya and Kuzyakov, 2008). Since we determined CO_2-C production rates after PCM amendment, but not directly the soil organic matter turnover, the origin of the extra CO_2-C (primed-C) cannot be directly evaluated, and thus the "real" priming effect cannot be directly assessed. Nevertheless, our results suggest that when ASH was supplied to soils the positive priming effect was "real" because the balance between CO_2-C produced *vs* PyC added was positive (Fig. S5), implying that the CO_2-C produced was derived from native soil organic matter. Previous studies (*e.g.*, Kuzyakov et al., 2009) have suggested that in general the decomposition of PyC in the soils is very slow and its mineralization decreases with time due to physical protection. In the same way, in our study most of the CO_2-C produced when CH was added might also derived from the mineralization of the native soil organic matter.

5. Conclusions

This study demonstrates that fire occurrence can leave a legacy on soil C and N mineralization processes; likely due to changes in the soil microbial community and soil C and N pools. In addition, this study shows that the legacy of previous fires can change the rate of these soil processes after a new fire event. After a simulated new fire event, CO_2-C production rates increased only on soils previously burned, independently of their previous fire history. The results show that fire-derived carbonaceous material can produce a positive priming effect only in previously burned soils, enhancing the CO_2-C emissions from them, at least in the short-term. This positive priming effect produced by pyrogenic carbonaceous material could be due to an increase in the activity of soil microorganisms more adapted to fire (probably Gram-positive bacteria) and to the higher P availability. In addition, the higher CO_2-C production rate and priming effect observed when ash was amended to burned soils might be also due to the increased soil pH.

Notwithstanding, the knowledge gained from this study can help to improve the models that represent the net impact of fire within the Earth system carbon cycling, to decrease the uncertainties in future projections and to understand the potential role of fire management for climate change mitigation. This is especially relevant due to the increased fire risk associated with changes in climate and land-use in many fire-prone regions of the world. Nevertheless, further studies are needed to more fully understand the mechanisms underlying these processes, including the use of approaches based on stable isotope labelling and the identification of both soil organic C fractions of different protection levels and microorganism responsible of the increase in CO_2 production. Since the observed effects here may in part respond to the nature of the fire treatment, further studies are also needed to evaluate the effects of high intensity fires.

CRediT authorship contribution statement

Enrique Albert-Belda: Conceptualization, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **M. Belén Hinojosa:** Conceptualization, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Vito Armando Laudicina:** Investigation, Writing – original draft, Writing – review & editing. **Roberto García-Ruiz:** Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Beatriz Pérez:** Conceptualization, Writing – review & editing. **José M. Moreno:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition.

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

Funding was provided by the Spanish Ministry of Economy, Industry and Competitiveness (FOCCLIM, CGL2016-78357-R). E.A.B. is supported by a pre-doctoral grant co-funded by the Regional Castilla-La Mancha Government and the European Social Fund (SBPLY/16/180501/000145). The authors would like to thank A. Jouini, A. Iopolo, T. Lorenzo and G. Cano for their assistance in the laboratory.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.149924.

References

Anderson, J.P.E., 1982. Soil respiration. In: Page, A.L., Miller, R., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy-Soil Science Society of America, Madison, WI (USA), pp. 831–871. https://doi.org/10.2134/agronmonogr9.2.2ed.c41.

Bang-Andreasen, T., Nielsen, J.T., Voriskova, J., Heise, J., Rønn, R., Kjøller, R., Hansen, H.C.B., Jacobsen, C.S., 2017. Wood ash induced pH changes strongly affect soil bacterial numbers and community composition. Front. Microbiol. 8, 1–14. https://doi.org/10.3389/fmicb.2017.01400.

Bauhus, J., Khanna, P.K., Raison, R.J., 1993. The effect of fire on carbon and nitrogen mineralization and nitrification in an australian forest soil. Aust. J. Soil Res. 31, 621–639. https://doi.org/10.1071/SR9930621.

Bedia, J., Herrera, S., Camia, A., Moreno, J.M., Gutiérrez, J.M., 2014. Forest fire danger projections in the Mediterranean using ENSEMBLES regional climate change scenarios. Clim. Chang. 122, 185–199. https://doi.org/10.1007/s10584-013-1005-z.

Bird, M.I., Wynn, J.G., Saiz, G., Wurster, C.M., McBeath, A., 2015. The pyrogenic carbon cycle. Annu. Rev. Earth Planet. Sci. 43, 273–298. https://doi.org/10.1146/annurev-earth-060614-105038.

Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol. Fertil. Soils 45, 115–131. https://doi.org/10.1007/s00374-008-0334-y.

Bodí, M.B., Martin, D.A., Balfour, V.N., Santín, C., Doerr, S.H., Pereira, P., Cerdà, A., Mataix-Solera, J., 2014. Wildland fire ash: production, composition and eco-hydro-geomorphic effects. Earth Sci. Rev. 130, 103–127. https://doi.org/10.1016/j.earscirev.2013.12.007.

Bossio, D.A., Scow, K.M., 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microb. Ecol. 35, 265–278. https://doi.org/10.1007/s002489900082.

Cao, X., Ro, K.S., Libra, J.A., Kammann, C.I., Lima, I., Berge, N., Li, A., Li, Y., Chen, N., Yang, J., Deng, B., Mao, J., 2013. Effects of biomass types and carbonization conditions on the chemical characteristics of hydrochars. J. Agric. Food Chem. 61, 9401–9411. https://doi.org/10.1021/jf402345k.

Caon, L., Vallejo, V.R., Ritsema, C.J., Geissen, V., 2014. Effects of wildfire on soil nutrients in Mediterranean ecosystems. Earth Sci. Rev. 139, 47–58. https://doi.org/10.1016/j.earscirev.2014.09.001.

Chapman, S.K., Koch, G.W., 2007. What type of diversity yields synergy during mixed litter decomposition in a natural forest ecosystem? Plant Soil 299, 153–162. https://doi.org/10.1007/s11104-007-9372-8.
Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya, E., Kuzyakov, Y., 2014. Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. Glob. Chang. Biol. 20, 2356–2367. https://doi.org/10.1111/gcb.12475.
Chen, J., Seven, J., Zilla, T., Dippold, M.A., Blagodatskaya, E., Kuzyakov, Y., 2019. Microbial C:N: P stoichiometry and turnover depend on nutrients availability in soil: a 14C, 15N and 33P triple labelling study. Soil Biol. Biochem. 131, 206–216. https://doi.org/10.1016/j.soilbio.2019.01.017.
Clough, T., Condron, L., Kammann, C., Müller, C., 2013. A review of biochar and soil nitrogen dynamics. Agronomy 3, 275–293. https://doi.org/10.3390/agronomy3020275.
Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105–2113. https://doi.org/10.1890/06-1847.1.
Curtin, D., Peterson, M.E., Anderson, C.R., 2016. pH-dependence of organic matter solubility: base type effects on dissolved organic C, N, P, and S in soils with contrasting mineralogy. Geoderma 271, 161–172. https://doi.org/10.1016/j.geoderma.2016.02.009.
DeLuca, T.H., MacKenzie, M.D., Gundale, M.J., Holben, W.E., 2006. Wildfire-produced charcoal directly influences nitrogen cycling in ponderosa pine forests. Soil Sci. Soc. Am. J. 70, 448–453. https://doi.org/10.2136/sssaj2005.0096.
Dooley, S.R., Treseder, K.K., 2012. The effect of fire on microbial biomass: a meta-analysis of field studies. Biogeochemistry 109, 49–61. https://doi.org/10.1007/s10533-011-9633-8.
Drake, J.E., Darby, B.A., Giasson, M.A., Kramer, M.A., Phillips, R.P., Finzi, A.C., 2013. Stoichiometry constrains microbial response to root exudation-insights from a model and a field experiment in a temperate forest. Biogeosciences 10, 821–838. https://doi.org/10.5194/bg-10-821-2013.
Driscoll, D.A., Lindenmayer, D.B., Bennett, A.F., Bode, M., Bradstock, R.A., Cary, G.J., Clarke, M.F., Dexter, N., Fensham, R., Friend, G., Gill, M., James, S., Kay, G., Keith, D.A., MacGregor, C., Russell-Smith, J., Salt, D., Watson James, J.E.M., Williams Richard, J.R.J., York, A., 2010. Fire management for biodiversity conservation: Key research questions and our capacity to answer them. Biol. Conserv. https://doi.org/10.1016/j.biocon.2010.05.026.
Fang, Y., Singh, B.P., Nazaries, L., Keith, A., Tavakkoli, E., Wilson, N., Singh, B., 2019. Interactive carbon priming, microbial response and biochar persistence in a vertisol with varied inputs of biochar and labile organic matter. Eur. J. Soil Sci. 70, 960–974. https://doi.org/10.1111/ejss.12808.
Fanin, N., Kardol, P., Farrell, M., Nilsson, M.C., Gundale, M.J., Wardle, D.A., 2019. The ratio of gram-positive to gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. Soil Biol. Biochem. 128, 111–114. https://doi.org/10.1016/j.soilbio.2018.10.010.
Fernández, I., Cabaneiro, A., Carballas, T., 1999. Carbon mineralization dynamics in soils after wildfires in two galician forests. Soil Biol. Biochem. 31, 1853–1865. https://doi.org/10.1016/S0038-0717(99)00116-9.
Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil biol. Biochemist 35, 837–843. https://doi.org/10.1016/S0038-0717(03)00123-8.
Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J.M.G., Maire, V., Mary, B., Revaillot, S., Maron, P.A., 2011. Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. Soil Biol. Biochem. 43, 86–96. https://doi.org/10.1016/j.soilbio.2010.09.017.
Foster, D.R., Knight, D.H., Franklin, J.F., 1998. Landscape patterns and legacies resulting from large, infrequent forest disturbances. Ecosystems 1, 497–510. https://doi.org/10.1007/s100219900046.
Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fertil. Soils 22, 59–65. https://doi.org/10.1007/s003740050076.
Gee, G.W., Bauder, J.W., 1986. Particle-size analysis. In: Klute, A. (Ed.), Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods. American Society of Agronomy-Soil Science Society of America, Madison, WI (USA), pp. 383–411. https://doi.org/10.2136/sssabookser5.1.2ed.c15.
GEODE, 2004. MapasIGME - Portal de cartografía del IGME: GEODE - Cartografía geológica digital continua a escala 1:50.000. http://info.igme.es/cartografiadigital/geologica/Geode.aspx.
Ghani, A., Dexter, M., Perrott, K.W., 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biol. Biochem. 35, 1231–1243. https://doi.org/10.1016/S0038-0717(03)00186-X.
Goberna, M., Sánchez, J., Pascual, J.A., García, C., 2007. Pinus halepensis mill. plantations did not restore organic carbon, microbial biomass and activity levels in a semi-arid Mediterranean soil. Appl. Soil Ecol. 36 (2–3), 107–115. https://doi.org/10.1016/j.apsoil.2006.12.003.
Goberna, M., García, C., Insam, H., Hernández, M.T., Verdú, M., 2012. Burning fire-prone Mediterranean shrublands: immediate changes in soil microbial community structure and ecosystem functions. Microb. Ecol. 64, 242–255. https://doi.org/10.1007/s00248-011-9995-4.
Gross, A., Angert, A., 2017. Use of 13C- and phosphate 18O-labeled substrate for studying phosphorus and carbon cycling in soils: a proof of concept. Rapid Commun. Mass Spectrom. 31, 969–977. https://doi.org/10.1002/rcm.7863.
Guénon, R., Vennetier, M., Dupuy, N., Roussos, S., Pailler, A., Gros, R., 2013. Trends in recovery of mediterranean soil chemical properties and microbial activities after infrequent and frequent wildfires. L. Degrad. Dev. 24, 115–128. https://doi.org/10.1002/ldr.1109.
Guerrero, C., Mataix-Solera, J., Gómez, I., García-Orenes, F., Jordán, M.M., 2005. Microbial recolonization and chemical changes in a soil heated at different temperatures. Int. J. Wildl. Fire 14, 385–400. https://doi.org/10.1071/WF05039.
Harris, I., Jones, P.D., Osborn, T.J., Lister, D.H., 2014. Updated high-resolution grids of monthly climatic observations - the CRU TS3.10 dataset. Int. J. Climatol. 34, 623–642. https://doi.org/10.1002/joc.3711.
Hart, S.C., Stark, J.M., Davidson, E.A., Firestone, M.K., 1994. Nitrogen mineralization, immobilization, and nitrification. In: Weaver, R.W., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., Tabatabai, A., Wollum, A. (Eds.), Methods of Soil Analysis: Part 2. Microbiological and Biochemical Properties. American Society of Agronomy-Soil Science Society of America, Madison, WI (USA), pp. 985–1018. https://doi.org/10.2136/sssabookser5.2.c42.
Hart, S.C., DeLuca, T.H., Newman, G.S., MacKenzie, M.D., Boyle, S.I., 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. For. Ecol. Manag. 220, 166–184. https://doi.org/10.1016/j.foreco.2005.08.012.
Hedges, J.I., Eglinton, G., Hatcher, P.G., Kirchman, D.L., Arnosti, C., Derenne, S., Evershed, R.P., Kögel-Knabner, I., De Leeuw, J.W., Littke, R., Michaelis, W., Rullkötter, J., 2000. The molecularly-uncharacterized component of nonliving organic matter in natural environments. Org. Geochem. 31, 945–958. https://doi.org/10.1016/S0146-6380(00)00096-6.
Hinojosa, M.B., Carreira, J.A., García-Ruíz, R., Dick, R.P., 2005. Microbial response to heavy metal-polluted soils. J. Environ. Qual. 34, 1789–1800. https://doi.org/10.2134/jeq2004.0470.
Hinojosa, M.B., Laudicina, V.A., Parra, A., Albert-Belda, E., Moreno, J.M., 2019. Drought and its legacy modulate the post-fire recovery of soil functionality and microbial community structure in a Mediterranean shrubland. Glob. Chang. Biol. 25, 1409–1427. https://doi.org/10.1111/gcb.14575.
IUSS Working Group WRB, 2015. World Reference Base for Soil Resources 2014, update 2015 International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome.
John, M.K., 1970. Colorimetric determination of phosphorus in soil and plant material with ascorbic acid. Soil Sci. 109, 214–220. https://doi.org/10.1097/00010694-197004000-00002.
Keeney, D.R., Nelson, D.W., 1982. Nitrogen-inorganic forms. In: Page, A.L., Miller, R., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy-Soil Science Society of America, Madison, WI (USA), pp. 643–698. https://doi.org/10.2134/agronmonogr9.2.2ed.c33.
Knicker, H., 2011. Pyrogenic organic matter in soil: its origin and occurrence, its chemistry and survival in soil environments. Quat. Int. 243, 251–263. https://doi.org/10.1016/j.quaint.2011.02.037.
Koyama, A., Kavanagh, K.L., Stephan, K., 2010. Wildfire effects on soil gross nitrogen transformation rates in coniferous forests of Central Idaho, USA. Ecosystems 13, 1112–1126. https://doi.org/10.1007/s10021-010-9377-7.
Kriticos, D.J., Webber, B.L., Leriche, A., Ota, N., Macadam, I., Bathols, J., Scott, J.K., 2012. CliMond: global high-resolution historical and future scenario climate surfaces for bioclimatic modelling. Methods Ecol. Evol. 3, 53–64. https://doi.org/10.1111/j.2041-210X.2011.00134.x.
Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biol. Biochem. 32, 1485–1498. https://doi.org/10.1016/S0038-0717(00)00084-5.
Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labeling. Soil Biol. Biochem. 41, 210–219. https://doi.org/10.1016/j.soilbio.2008.10.016.
Lasslop, G., Coppola, A.I., Voulgarakis, A., Yue, C., Veraverbeke, S., 2019. Influence of fire on the carbon cycle and climate. Curr. Clim. Chang. Rep. 5, 112–123. https://doi.org/10.1007/s40641-019-00128-9.
Laudicina, V.A., Dennis, P.G., Palazzolo, E., Badalucco, L., 2012. Key biochemical attributes to assess soil ecosystem sustainability. In: Malik, A., Grohmann, E. (Eds.), Environmental Protection Strategies for Sustainable Development. Springer, Netherlands, Dordrecht, pp. 193–227. https://doi.org/10.1007/978-94-007-1591-2_6.
Lechevalier, M.P., Moss, C.W., 1977. Lipids in bacterial taxonomy - a taxonomist's view. CRC Crit. Rev. Microbiol. 5, 109–210. https://doi.org/10.3109/10408417709102311.
Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Brookes, P.C., 2011. Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. Soil Biol. Biochem. 43, 2304–2314. https://doi.org/10.1016/j.soilbio.2011.07.020.
Madan, R., Pankhurst, C., Hawke, B., Smith, S., 2002. Use of fatty acids for identification of AM fungi and estimation of the biomass of AM spores in soil. Soil Biol. Biochem. 34, 125–128. https://doi.org/10.1016/S0038-0717(01)00151-1.
Maestrini, B., Nannipieri, P., Abiven, S., 2015. A meta-analysis on pyrogenic organic matter induced priming effect. GCB Bioenergy 7, 577–590. https://doi.org/10.1111/gcbb.12194.
Maia, P., Pausas, J.G., Arcenegui, V., Guerrero, C., Pérez-Bejarano, A., Mataix-Solera, J., Varela, M.E.T., Fernandes, I., Pedrosa, E.T., Keizer, J.J., 2012. Wildfire effects on the soil seed bank of a maritime pine stand—the importance of fire severity. Geoderma 191, 80–88. https://doi.org/10.1016/j.geoderma.2012.02.001.
Malkisnon, D., Wittenberg, L., Beeri, O., Barzilai, R., 2011. Effects of repeated fires on the structure, composition, and dynamics of Mediterranean maquis: short-and long-term perspectives. Ecosystems 14, 478–488. https://doi.org/10.1007/s10021-011-9424-z.
Mayor, A.G., Valdecantos, A., Vallejo, V.R., Keizer, J.J., Bloem, J., Baeza, J., González-Pelayo, O., Machado, A.I., de Ruiter, P.C., 2016. Fire-induced pine woodland to shrubland transitions in southern Europe may promote shifts in soil fertility. Sci. Total Environ. 573, 1232–1241. https://doi.org/10.1016/j.scitotenv.2016.03.243.
McCune, B., Grace, J.B., Urban, D.L., 2002. Analysis of Ecological Communities. MjM software design, Gleneden Beach, OR.
McLean, E.O., 1982. Soil pH and lime requirement. In: Page, A.L., Miller, R., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties.

American Society of Agronomy-Soil Science Society of America, Madison, WI (USA), pp. 199–224. https://doi.org/10.2134/agronmonogr9.2.2ed.c12.
Merino, A., Jiménez, E., Fernández, C., Fontúrbel, M.T., Campo, J., Vega, J.A., 2019. Soil organic matter and phosphorus dynamics after low intensity prescribed burning in forests and shrubland. J. Environ. Manage. 234, 214–225. https://doi.org/10.1016/j.jenvman.2018.12.055.
Moreno, J.M., Vázquez, A., Pérez, B., Faraco, A.M., Fernández-González, F., Quintana, J.R., Cruz, A., 1996. Los incendios forestales en España y su impacto sobre los ecosistemas: lecciones del estudio de los montes de gredos. In: Loidi, J.J. (Ed.), Avances en Fitosolociología. Universidad del País Vasco, Bilbao, pp. 23–42.
Mori, A.S., 2011. Ecosystem management based on natural disturbances: hierarchical context and non-equilibrium paradigm. J. Appl. Ecol. 48, 280–292. https://doi.org/10.1111/j.1365-2664.2010.01956.x.
Mulvaney, R.L., 1996. Nitrogen-inorganic forms. In: Sumner, D.L., Sparks, A.L., Page, P.A., Helmke, R.H., Loeppert, P.N., Soltanpour, M.A., Tabatabai, C.T., Johnston, M.E. (Eds.), Methods of Soil Analysis. Part 3. Chemical Methods. American Society of Agronomy-Soil Science Society of America, Madison, WI (USA), pp. 1123–1184. https://doi.org/10.2136/sssabookser5.3.c38.
Neary, D.G., Klopatek, C.C., DeBano, L.F., Ffolliott, P.F., 1999. Fire effects on belowground sustainability: a review and synthesis. For. Ecol. Manag. 122, 51–71. https://doi.org/10.1016/S0378-1127(99)00032-8.
Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter. In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Nelson, D.W., Sommers, L.E. (Eds.), Methods of Soil Analysis Part 3. Chemical Methods-SSSA. Soil Science Society of America and American Society of Agronomy, 677 S. Segoe Rd., Madison, WI 53711, USA, pp. 961–1010. https://doi.org/10.2136/sssabookser5.3.c34.
Noyce, G.L., Fulthorpe, R., Gorgolewski, A., Hazlett, P., Tran, H., Basiliko, N., 2016. Soil microbial responses to wood ash addition and forest fire in managed Ontario forests. Appl. Soil Ecol. 107, 368–380. https://doi.org/10.1016/j.apsoil.2016.07.006.
O'Donnell, J.A., Turetsky, M.R., Harden, J.W., Manies, K.L., Pruett, L.E., Shetler, G., Neff, J.C., 2009. Interactive effects of fire, soil climate, and moss on CO2 fluxes in black spruce ecosystems of interior Alaska. Ecosystems 12, 57–72. https://doi.org/10.1007/s10021-008-9206-4.
Oksanen, L., 2001. Logic of experiments in ecology: is pseudoreplication a pseudoissue? Oikos, 27–38. https://doi.org/10.1034/j.1600-0706.2001.11311.x.
Olsen, S.R., Sommers, L.E., 1982. Phosphorus. In: Page, A.L., Keeney, D.R., Miller, R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy-Soil Science Society of America, Madison, WI (USA), pp. 403–430. https://doi.org/10.2134/agronmonogr9.2.2ed.c24.
Pellegrini, A.F., Jackson, R.B., 2020. The long and short of it: a review of the timescales of how fire affects soils using the pulse-press framework. Adv. Ecol. Res. 62, 147–171. https://doi.org/10.1016/bs.aecr.2020.01.010.
Pereira, P., Brevik, E., Bogunovic, I., Estebaranz-Sánchez, F., 2019a. Ash and soils: a close relationship in fire-affected areas. In: Pereira, P., Mataix-Solera, J., Úbeda, X., Rein, G. (Eds.), Fire Effects on Soil Properties. CSIRO Publishing, pp. 39–67.
Pereira, P., Úbeda, X., Francos, M., 2019b. Laboratory fire simulations: plant litter and soils. In: Pereira, P., Mataix-Solera, J., Úbeda, X., Rein, G. (Eds.), Fire Effects on Soil Properties. CSIRO Publishing, pp. 15–38.
Pingree, M.R.A., DeLuca, T.H., 2017. Function of wildfire-deposited pyrogenic carbon in terrestrial ecosystems. Front. Environ. Sci. 5, 1–7. https://doi.org/10.3389/fenvs.2017.00053.
Plaza-Álvarez, P.A., Lucas-Borja, M.E., Sagra, J., Zema, D.A., González-Romero, J., Moya, D., De las Heras, J., 2019. Changes in soil hydraulic conductivity after prescribed fires in Mediterranean pine forests. J. Environ. Manage. 232, 1021–1027. https://doi.org/10.1016/j.jenvman.2018.12.012.
Pressler, Y., Moore, J.C., Cotrufo, M.F., 2019. Belowground community responses to fire: meta-analysis reveals contrasting responses of soil microorganisms and mesofauna. Oikos 128, 309–327. https://doi.org/10.1111/oik.05738.
R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Raison, R.J., McGarity, J.W., 1980. Effects of ash, heat, and the ash-heat interaction on biological activities in two contrasting soils - I. Respiration rate. Plant Soil 55, 363–376. https://doi.org/10.1007/BF02182697.
Raison, R.J., Khanna, P.K., Jacobsen, K.L.S., Romanya, J., Serrasolses, I., 2009. Effects of fire on forest nutrient cycles, fire effects on soils and restoration. Strategies, 225–256. https://doi.org/10.1201/9781439843338-c8.
Reisser, M., Purves, R.S., Schmidt, M.W.I., Abiven, S., 2016. Pyrogenic carbon in soils: a literature-based inventory and a global estimation of its content in soil organic carbon and stocks. Front. Earth Sci. 4, 1–14. https://doi.org/10.3389/feart.2016.00080.
Rhoades, J.D., 1982. Cation exchange capacity. In: Page, A.L., Miller, R., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy-Soil Science Society of America, Madison, WI (USA), pp. 149–157. https://doi.org/10.2134/agronmonogr9.2.2ed.c8.
Ronsse, F., van Hecke, S., Dickinson, D., Prins, W., 2013. Production and characterization of slow pyrolysis biochar: influence of feedstock type and pyrolysis conditions. GCB Bioenergy 5, 104–115. https://doi.org/10.1111/gcbb.12018.
Russell, A.D., 2003. Lethal effects of heat on bacterial physiology and structure. Sci. Prog. 86, 115–137. https://doi.org/10.3184/003685003783238699.
Santín, C., Doerr, S.H., Kane, E.S., Masiello, C.A., Ohlson, M., de la Rosa, J.M., Preston, C.M., Dittmar, T., 2016. Towards a global assessment of pyrogenic carbon from vegetation fires. Glob. Chang. Biol. 22, 76–91. https://doi.org/10.1111/gcb.12985.
Santos, F., Torn, M.S., Bird, J.A., 2012. Biological degradation of pyrogenic organic matter in temperate forest soils. Soil Biol. Biochem. 51, 115–124. https://doi.org/10.1016/j.soilbio.2012.04.005.
Schutter, M.E., Dick, R.P., 2000. Comparison of fatty acid methyl Ester (FAME) methods for characterizing microbial communities. Soil Sci. Soc. Am. J. 64, 1659–1668. https://doi.org/10.2136/sssaj2000.6451659x.
Tessler, N., Wittenberg, L., Greenbaum, N., 2013. Soil water repellency persistence after recurrent forest fires on Mount Carmel, Israel. Int. J. Wildl. Fire 22, 515–526. https://doi.org/10.1071/WF12063.
Van Mantgem, P., Schwartz, M., Keifer, M., 2001. Monitoring fire effects for managed burns and wildfires: coming to terms with pseudoreplication. Nat. Areas J. 21, 266–273.
Vargas, R., Collins, S.L., Thomey, M.L., Johnson, J.E., Brown, R.F., Natvig, D.O., Friggens, M.T., 2012. Precipitation variability and fire influence the temporal dynamics of soil CO 2 efflux in an arid grassland. Glob. Chang. Biol. 18, 1401–1411. https://doi.org/10.1111/j.1365-2486.2011.02628.x.
Vázquez, F.J., Acea, M.J., Carballas, T., 1993. Soil microbial populations after wildfire. FEMS Microbiol. Ecol. 13, 93–103. https://doi.org/10.1016/0168-6496(93)90027-5.
ZaragozaVega, J.A., Alonso, M., Fonturbel, T., Pérez-Gorostiaga, P., Cuiñas, P., Fernández, C., 2005. Efectos Inmediatos de la Severidad del Fuego Sobre Algunas características químicas de Un Suelo de Pinus Pinaster. Proceedings of the fourth Congreso Forestal Español. Proceedings of the fourth Congreso Forestal Español.
Wang, Q., Zhong, M., Wang, S., 2012. A meta-analysis on the response of microbial biomass, dissolved organic matter, respiration, and N mineralization in mineral soil to fire in forest ecosystems. For. Ecol. Manag. 271, 91–97. https://doi.org/10.1016/j.foreco.2012.02.006.
Wang, J., Xiong, Z., Kuzyakov, Y., 2016a. Biochar stability in soil: meta-analysis of decomposition and priming effects. GCB Bioenergy 8, 512–523. https://doi.org/10.1111/gcbb.12266.
Wang, C., Wang, G., Wang, Y., Rafique, R., Ma, L., Hu, L., Luo, Y., 2016b. Fire alters vegetation and soil microbial Community in Alpine Meadow. L. Degrad. Dev. 27, 1379–1390. https://doi.org/10.1002/ldr.2367.
Wild, B., Li, J., Pihlblad, J., Bengtson, P., Rütting, T., 2019. Decoupling of priming and microbial N mining during a short-term soil incubation. Soil Biol. Biochem. 129, 71–79. https://doi.org/10.1016/j.soilbio.2018.11.014.
Zimmerman, A.R., 2010. Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). Environ. Sci. Technol. 44, 1295–1301. https://doi.org/10.1021/es903140c.
Zimmermann, S., Frey, B., 2002. Soil respiration and microbial properties in an acid forest soil: effects of wood ash. Soil Biol. Biochem. 34, 1727–1737. https://doi.org/10.1016/S0038-0717(02)00160-8.