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RESEARCH

Long-term impact of wildfire on soil physical, chemical and biological properties within a pine forest

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Abstract

Anthropogenic fires pose a serious threat to many terrestrial ecosystems because they can cause loss of biodiversity and carbon stocks in the biosphere. Specifically, wildfires impacting natural conservation areas such as European Natura 2000 sites (N2K) are of particular concern. The main study objective was to evaluate the long-term effects of wildfires on the organic layer and some physical, chemical and biological properties of the underlying soil mineral layer, linked to soil quality. Here, we studied two coastal Mediterranean Aleppo pine stands within an N2K site differing for the fires' years of occurrence, the time between fires (TBF) and the time since last fire (TSLF) throughout 24 years. Furthermore, in each stand, differences in fire frequency (FF) were considered by selecting three sites—double-fire, single-fire and control (unburnt). Our results show the absence of the O-layer in double-fire sites, indicating a loss of this organic carbon (if compared to control) pool of 204 g m^{-2} in R2F and 139 g m^{-2} in M2F. Despite this loss being offset by the C_{org} increase in soil mineral layer, the disappearance of O-layer may compromise the ecosystem services provided by soil. In each stand, long-term fire effects were evident at both single-fire and double-fire sites for some chemical as well as biological soil properties and depended on TSLF. Increased rates of nitrogen mineralization and nitrification were found at all burned sites, persisting up to 24 years post-fire. Soil quality indicators data highlighted the recovery handicap of the microbial community within the considered period. Since our outcomes showed wildfires enduring consequences, mainly relating to TSLF and FF, on different organic and mineral soil properties, we advocate employing prompt strategies to mitigate recurring fires.

Keywords Fire history · *Pinus halepensis* forest · Topsoil property recovery · Carbon pools · Microbial biomass · Microbial activities

Introduction

Fire acts as a key ecological factor in several terrestrial ecosystems worldwide (Bowman et al. 2009; Pausas and Keeley 2019; Xu et al. 2021), such as in the Mediterranean basin, Australia and in the coastal and sub-coastal areas of California, Chile, or southern Africa (Blasi et al. 2004; Enright and Thomas 2008; Úbeda and Sarricolea 2016). Temperate and boreal forests have also experienced a long coexistence with wildfires (Hoecker et al. 2020; Krikken et al. 2021), but with return intervals between natural fires much longer than the Mediterranean biome (Buma et al. 2022; Hoy et al. 2016; Kasischke 2000). Globally, natural wildfires, mainly caused by lightning (Bowman et al. 2011; Leone et al. 2009), are responsible for less than 5% (Hirschberger 2016; Moreira et al. 2012), whereas the majority of fires are caused by anthropogenic activity coupled with the effect of climate change (IPCC 2022; Martínez et al. 2009; Senf et al. 2021). Wildfire frequency, size and severity, as well as the duration of fire season, are increasing in many regions of the Earth (OECD 2023), damaging ecosystems and species, human health, as well as built assets and economic activity (IPCC 2022). Fire regime trends are not uniform around the Earth

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(IPCC 2022). In the Mediterranean European countries (Portugal, Spain, France, Italy and Greece), more affected by wildfire, the average number of fires per year increased by 20% from the period 1980–1990 to 2010–2019. In the last decade (2010–2019) a reverse trend was observed compared to 2000–2010 (31% decrease in average fire number year^{-1}) due to the improvements in fire control/prevention strategy (San-Miguel-Ayanz et al. 2021). However, the extreme wildfire events that occurred in these same countries, between 2016 and 2018, have highlighted the limits of wildfire suppression capabilities under exceptional fire-weather conditions, leading to this biome being endangered (Ruffault et al. 2020).

In addition to the direct impact of greenhouse gas emissions into the atmosphere (Andela et al. 2019; Castaldi et al. 2010; Jhariya and Raj 2014), a wildfire generally can impact several ecosystem components, including flora and fauna, as well as biotic and abiotic soil characteristics (Cunillera-Montcusí et al. 2019; Niccoli et al. 2023a,b; Stinca et al. 2020). From an ecological perspective, natural areas of conservation interest are being increasingly impacted by wildfire events. In Europe in 2022 ~ 365,308 hectares of surface within Nature 2000 Network (N2K) were burnt (highest value in the last decade; San-Miguel-Ayanz et al. 2023). These sites, hosting habitats protected by the Habitats Directive 92/43/EEC (HD), in addition to ensuring high biodiversity, also provide other ecosystem services such as water regulation, carbon sequestration, nutrient cycling, etc. (Pereira et al. 2018), in which soils play crucial roles (Adhikari and Hartemink 2016).

An increase in the fire frequency (number of fire events that occur within an area over a specified period) can lead to a shift in the plant species composition (Li et al. 2013), especially if return intervals exceed the recovery time of soil characteristics and species' resilience (Syphard et al. 2007). According to Notario del Pino and Ruiz-Gallardo (2015), fire frequency can be considered high or low depending on the different ecosystem types and species-specific. Pausas et al. (2004) and Climent et al. (2008) state that wildfire return intervals of < 15 years could lead to the local loss of the *Pinus halepensis* Mill. (Aleppo pine), one of the most widespread conifers in the Mediterranean basin (EL Khayati et al. 2023; Nahal 1962; Rojas-Briales et al. 2023), given the time needed to produce fertile seeds (10 ± 5 years; Tapias et al. 2001; Trabaud et al. 1985b). According to Flannigan et al. (2000) the regularity of fires recurrence favours organisms that better tolerate changes in selection pressure frequency. For example, fires could trigger an invasion of alien species or sustain an invasion already underway (Keeley 2001; Wells et al. 2021; Saulino et al. 2023), which added to the direct fire effect could lead to the substitution of a habitat as well as inducing changes in the fire regime itself (Brooks et al. 2004). Also, more frequent wildfires in the long-term (> 5 years) are able to trigger degradation processes in the soil, influencing the functioning of the ecosystem as well as determining a loss of its resilience, through (i) the reduction/elimination of vegetation cover and litter (Caon et al. 2014; Cerdà and Doerr 2008), (ii) the progressive depletion of soil organic matter (SOM) and nutrients (Bowd et al. 2019; Pellegrini et al. 2018), (iii) the alteration of microbial communities (Dangi et al. 2010; Guénon et al. 2011), (iv) exposure to erosion by water and wind (Cerdà and Lasanta 2005; Santana et al. 2016); which mainly in the Mediterranean environment can lead to desertification (Rutigliano et al. 2023; Vallejo et al. 2012).

Under current global climate and environmental changes, during which fires are expected to increase (Moritz et al. 2012), it is crucial to intensify studies to better understand the long-term relationships between fire history and soil quality through the quantification of impacts to help facilitate new land management and planning tools.

The present work aims to evaluate the long-term effects of wildfires with different fire histories (i.e. fire frequency, time between fires and time since last fire) on the physical, chemical and biological properties of the topsoil in two Aleppo pine stands within the Special Area of Conservation (SAC) IT9130006—Pinewoods of the Ionian Arch (Apulia region, Southern Italy) which hosts the Habitat of Priority Interest 2270*. We hypothesized that the longer time passes since the last fire, the more likely it is that soil properties will return to pre-fire conditions, regardless of the number and time elapsed between fires.

In sites with differing fire histories (in terms of fire frequency, time since last fire and time between fires), the stated objectives were pursued through the analysis of the organic layer (O-layer) as well as physical, chemical and microbial properties of mineral soil. These are commonly used as indicators of disturbance and also serve as proxies for understanding ecosystem functioning linked to the ecosystem services provision (Rutigliano et al. 2023). The outcomes of this research could provide knowledge to conserve soil quality, which in turn supports the biodiversity of the entire ecosystem. Here, this is essential to identify timely management actions for high conservation value habitats at risk of fire, both in this and other N2K sites.

Material and methods

Study area and sampling methodology

The study was conducted in two Aleppo pine stands within the SAC-IT9130006 (Fig. 1). Here, the dominant tree layer is characterised by the presence of *Pinus halepensis* Mill. subsp. *halepensis* and the understory vegetation consists of typical Mediterranean shrub formations including *Cistus*

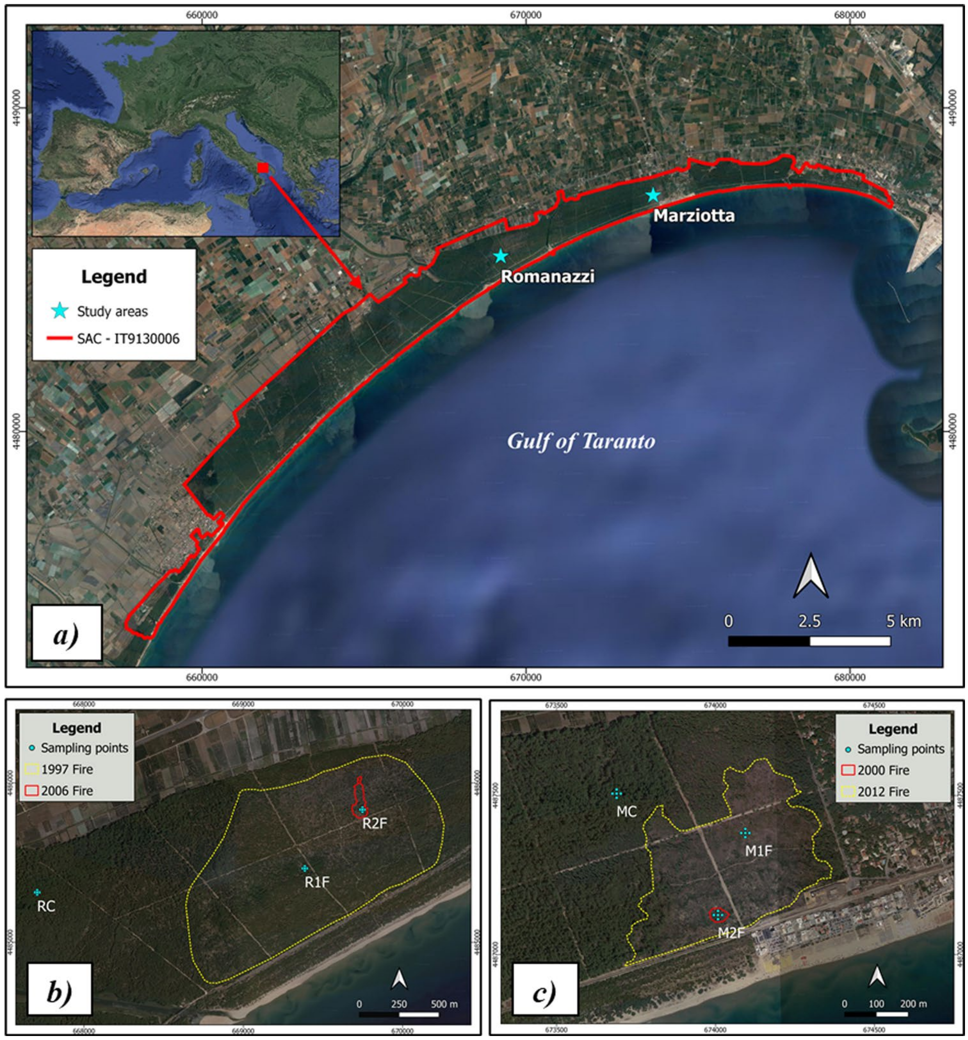


Fig. 1 **a** Location map of the SAC-IT9130006 (red boundary) with the cyan stars indicating the study areas, Romanazzi and Marziotta Aleppo pine stands; **b** Romanazzi stand map (1:10,000) with the yellow and red boundaries indicating the extent of the 1997 and 2006 wildfires, respectively, and the five cyan circles indicating the positions of the field replicates sampled in each of the three sampling sites (RC, control; R1F, single fire; R2F, double fire); **c** Marziotta stand map (1:5000) with the yellow and red boundaries indicating the extent of the 2000 and 2012 wildfire and the five cyan circles indicating the positions of the field replicates in each of the three sampling sites (MC, control; M1F, single fire; M2F, double fire)

salviifolius L., *Myrtus communis* L., *Pistacia lentiscus* L., *Phillyrea angustifolia* L., *Rhamnus alaternus* L., *Rosmarinus officinalis* L. (Pazienza G., personal observations). Additionally, within the SAC according to the N2K site's data form, the following Habitats are reported: 1210—Annual vegetation of drift lines; 1420—Mediterranean and thermo-Atlantic halophilous scrubs (*Sarcocornetiea fruticosi*); 2230—*Malcolmietalia* dune grasslands; 2240—*Brachypodietalia* dune grasslands with annuals; 2250*—Coastal dunes with *Juniperus* spp., 2270*—Wooded dunes with *Pinus pinea* and/or *Pinus pinaster* (data form: https://natura2000.eea.europa.eu/Natura2000/SDF.aspx?site=IT9130006, last accessed January 2023; the * symbol indicates the Habitats of Priority Interest).

Based on the climatic data collected by Ginosa Marina Meteorological Station within the SAC (data sourced from the Civil Protection Section of the Apulia Region, last accessed January 2023), a thermo-pluviometric diagram illustrating a typical Mediterranean climate was constructed (Fig. S1). Furthermore, the three reported time frames (1941–1970, 1971–2000 and 2001–2021) reporting an unchanged trend over time, also highlighted a greater intensity of summer aridity in 1941–1970 and 2001–2021 as well as a slight temperature increase in the last twenty years (Fig. S1). According to the FAO Digital Soil World Map (DSMW; FAO 2007) classification system, the soil of the study area is *Gleyic Luvisol*.

Throughout the SAC, one of the most important disturbances are wildfires in terms of both severity and recurrence (Leone et al. 2000; Moya et al. 2008; Saracino et al. 1997). Due to these disturbances, the invasive alien species *Acacia saligna* (Labill.) H.L. Wendl is locally colonising the south-western sector, named "Galaso", approximately 20 km away from the two stands here studied (Marfella et al. 2023a). Within the SAC, two Aleppo pine stands (Romanazzi and Marziotta) were selected where wildfires had occurred twice within a 24-year period (1997–2021, i.e., from the year of the first wildfire to the year of the sampling; Figs. 1, S2).

In each of the two stands, sites were chosen with comparable physiographic characteristics as possible. The stands differed for (i) years of fires occurrence, (ii) time between fires and (iii) time since last fire (within the 24-year period). Accordingly, inside each stand, three sites—double-fire, single-fire and control (unburnt)—were selected based on differences in fire frequency (FF), time between fires (TBF) and time since last fire (TSLF) related to the same time interval (24 years; Table 1). The control sites (RC and MC) in both stands, based on available information and local knowledge, have been fire-free for the last 40 years. The burnt surfaces in 1997 and 2006 in Romanazzi (R1F and R2F) were respectively 134 and 1.38 ha, whereas in 2000 and 2012 in Marziotta (M2F and M1F) 0.19 and 21.4 ha were burned, respectively (Fig. S3). The 1997 and 2012 fires (in Romanazzi and Marziotta, respectively) were stand-replacing fires, but no further specific data on severity, intensity, or duration of the four fire events were available.

In each of the three sites for both stands, the sampling was carried out in March 2021 considering five field replicates, at the centre and at the four cardinal points in a circular sampling unit (13 m radius, Fig. S4a). Samples of the organic layer (O-layer; comprising litter and fermentation layers) and the underlying 10 cm of soil (S-Layer) were collected from 40 × 40 cm squares. Within each square, the O-layer (where present and after thickness measurement) was gathered in paper bags, while five soil samples (Fig. S4b) were cored using a cylindrical auger (10 cm height, 6 cm diameter). These soil samples were then homogenized in polyethylene bags to create composite samples, which were stored in a thermal bag with ice for subsequent analyses (Soil Survey Staff, 2014a). Although several studies explore the effects of fire in the top 5 cm (Certini et al. 2011; Moya et al. 2018; Muñoz-Rojas et al. 2016), our interest had laid in the deeper investigation to evaluate the microbial transformative activity and their signal within 10 cm, as also shown elsewhere (Lucas-Borja et al. 2020; Memoli et al. 2021; Pellegrini et al. 2021). Specifically, this soil depth corresponds to the depth at which most microbial biomass and activity are expected (Jandl et al. 2014) as well as at which the partially burned organic matter could be translocated (Knicker 2011; Velasco-Molina et al. 2016). Thus, twenty O-layer bags (this layer was missing in R2F and M2F) and one hundred and fifty soil mineral cores were collected in total from both stands. Moreover, a soil core of the same size as above was sampled near each 40 × 40 cm square and brought undisturbed to the laboratory to determine the soil's water holding capacity, bulk density and porosity (30 undisturbed cores). Subsequently, the O-layers samples were air-dried to measure weight (OW) and organic carbon ($O–C_{org}$); the soil samples were sieved (2 mm mesh size) to exclude soil coarse fragments and larger plant roots (Soil Survey Staff, 2014b). Finally, two soil aliquots were identified: one air-dried to chemical analyses (pH, electrical conductivity, cation exchange capacity, organic carbon, extractable carbon, mineralizable carbon, total nitrogen, C/N ratio, ammonium and nitrate content) and the other stored at 4 °C for biological analyses (microbial biomass carbon, total microbial activity, nitrogen mineralization and nitrification).

Soil physical and chemical characterization

Water content (WC) and water holding capacity (WHC) were measured by the gravimetric method according to Allen (1989). Bulk density (BD) was assayed on undisturbed soil cores (of known volume) dried for 48 h at 105 °C, and subsequently porosity (Po) was calculated from BD (Soil Survey Staff 2014a). Soil pH was determined by potentiometric method with a specific probe (HANNA Instruments, HI8424) on a soil/water suspension (1:2.5 ratio). Electrical conductivity (EC) was assessed on aqueous soil extracts (1:2 soil/water ratio) by using a digital conductivity meter (COND 51^+). Cation exchange capacity (CEC) was measured by complexometric titration after soil treatment with barium chloride ($BaCl_2$) and triethanolamine (TEA) solution at pH 8.2 (Soil Survey Staff, 2014a). Organic carbon content (C_{org}) of dried O- and S- layers samples was determined by Springer and Klee (1954) method, which included an oxidation in acid environment (H_2SO_4) with potassium dichromate (0.33 M $K_2Cr_2O_7$), during a controlled heating step (160 °C for 10 min; for complete recovery of the organic carbon after Sleutel et al. 2007), followed by back titration with 0.2 M $Fe(II)SO_4$ solution. Total nitrogen (N_{tot}) was measured using LECO TruSpec® Micro CHN Analyser on 0.03 g dry

Table 1 Stand and site identification codes, altitude above sea level, slope percentage and wildfire history in terms of time of fires occurrence (FO), fire frequency (FF), time between fires (TBF), time since last fire (TSLF) and fires extent are reported

Stand	Site	Altitude (m)	Slope (%)	FO (year)		FF	TBF (year)	TSLF (year)	Fire extent (ha)
Romanazzi	RC	7	< 5	–	–	0	–	≥ 40	–
	R1F	5	< 5	1997	–	0.042	–	24	134
	R2F	9	< 5	1997	2006	0.083	9	15	1.38
Marziotta	MC	9	< 5	–	–	0	–	≥ 40	–
	M1F	5	< 5	2012	–	0.042	–	9	21.4
	M2F	9	< 5	2000	2012	0.083	12	9	0.19

soil, and then the C/N ratio was calculated as the ratio of C_{org} to N_{tot} both expressed in g kg^{-1} d.w. Ammonium and nitrate contents were assayed by potentiometric method using ion-selective electrodes specific for ammonia (NH_4^+–N; ORION Model 95–12 ionplus) and nitrate (NO_3^-–N, ORION Model 97-07 ionplus), after extraction with 0.5 M potassium sulphate (1:5 soil/water ratio; Castaldi et al. 2011).

Soil biological characterization

Microbial biomass carbon (C_{mic}), which expresses the quantity of microbial carbon present in the soil in mg C kg^{-1} d.w., was determined according to the fumigation-extraction method with chloroform (Vance et al. 1987). From fumigated and non-fumigated samples, the organic material was extracted by means of a solution of 0.5 M K_2SO_4 (1:4 ratio) and the total organic carbon (C_{org}) was determined by oxidation with potassium dichromate in an acid environment. After, from the C_{org} content difference between the fumigated and non-fumigated samples (the latter corresponding to the extractable organic carbon (C_{ext})), the microbial biomass carbon (C_{mic}) was calculated after Vance et al. (1987). The total microbial activity was measured as soil potential respiration (R), i.e. the evolved CO_2 from the samples was measured by the alkali trapping method and then quantified by titrating according to ISO 16072 (2002). Before performing the analysis, the soil was preincubated (1 week), to allow the initial carbon flush to diminish (Pell et al. 2006). Then, soil samples (5 g), inside glass containers, were placed in airtight jars containing 10 ml of 0.1 N NaOH and incubated for 13 days in standard conditions (25 °C, 55% of WHC, in the dark). The CO_2 developed by soil was monitored about every 3 days (on the 3rd, 6th, 9th and 13th day of incubation), by titrating the excess of NaOH in the jars with 0.05 M HCl and re-incubating soil samples after the addition of a new NaOH solution. Respiration was calculated as the average value in the considered period and expressed in mg CO_2–C kg^{-1} d.w. d^{-1}. The respiration of the last incubation period (from the 9th to the 13th day) corresponds to basal respiration (R_{bas}). Lastly, the fast mineralizable carbon (C_{min}) was calculated fitting cumulated CO_2–C evolved from soil samples *vs*. incubation time, using a first-order pool kinetics model (Riffaldi et al. 1996), with the following equation:

$$C_{cum} = C_0 \times \left(1 - e^{-k \times t}\right) \tag{1}$$

where C_{cum} is the cumulated mineralized carbon; C_0 is the asymptotic maximum quantity of CO_2–C evolved from the samples (from now on called C_{min}; g CO_2–C kg^{-1} d.w.); k is the mineralization rate constant in $days^{-1}$; and t is the incubation time in days.

Using the values of NH_4^+–N and NO_3^-–N measured on the soil samples at the beginning (t_0) and after a 14-day period (t_{14}) of aerobic incubation (55% of field capacity, at 25 °C, in the dark), nitrogen mineralization (Min) and nitrification (Nit) were calculated according to Castaldi et al. (2011), following the Eqs. (2) and (3) respectively:

$$\text{Min} = \frac{\left(NH_4^+{-}N + NO_3^-{-}N\right)_{t14} - \left(NH_4^+{-}N + NO_3^-{-}N\right)_{t0}}{d} \tag{2}$$

$$\text{Nit} = \frac{\left(NO_3^-{-}N\right)_{t14} - \left(NO_3^-{-}N\right)_{t0}}{d} \tag{3}$$

where $(NH_4^+{-}N + NO_3^-{-}N)_{t14}$ is the mineral N content post incubation (t_{14}); $(NH_4^+{-}N + NO_3^-{-}N)_{t0}$ is the starting mineral N content before the incubation (t_0); $(NO_3^-{-}N)_{t14}$ is the NO_3^- content after incubation (t_{14}); $(NO_3^-{-}N)_{t0}$ is the starting NO_3^- content (t_0) and d is the incubation timing expresses in days (14 days).

Soil microbial indices

Three microbial indices (Mataix-Solera et al. 2009; Moya et al. 2018), all expressed as percentage, were calculated as described in Eqs. (4–6): (i) quotient of mineralization (qM) derived from C_{min} and C_{org} (Dommergues 1960); (ii) microbial percentage of total organic carbon content (C_{mic}/C_{org}; Anderson and Domsch 1993) and (iii) metabolic quotient (qCO_2) calculated according to Anderson and Domsch (1993) from basal respiration (R_{bas}) and microbial biomass (C_{mic}).

$$qM = \frac{C_{min}}{C_{org}} \tag{4}$$

$$C_{mic}/C_{org} = \frac{C_{mic}}{C_{org}} \tag{5}$$

$$qCO_2 = \frac{R_{bas}}{C_{mic}} \tag{6}$$

Data analysis

Comparative data analysis between sites in the two stands assumed that the properties assessed at the control sites (RC and MC) are representative of the pre-fire condition. Mean (± standard deviations) and coefficient of variation (reported in Table S1) for all variables were calculated. To test for normal distribution of the data before performing parametric tests, the Shapiro–Wilk normality and the Levene homogeneity variance tests were run; when not normally distributed, data were transformed according to Sokal and Rohlf (2011) by using $\log_{10}$ (except for pH). For each variable, to determine the significance of differences ($p < 0.05$) among the three sites in each stand, one-way ANOVA (followed, if required, by Student–Newman–Keuls post-hoc test) was applied. Multivariate analysis was performed using principal component analysis (PCA) for each stand on a matrix of 15 sites and 19 variables (excluding the derivate ones to avoid collinearity, i.e. the three calculated microbial indexes and C/N ratio). Then, by means of Euclidean distance and Ward's method, the Cluster analysis to verify the similarity between sites was also run. Pearson correlation coefficient was used to test further relationships among all the 23 properties evaluated from the O- and S- layers, the two PCA axes and the different wildfire history conditions. The term fire frequency refers to the number of fires occurring within a designated area over a specified time unit (i.e. spatiotemporal scale-dependent Johnson, 1996) and accordingly was calculated as follows:

$$FF = \frac{F_n}{T} \Rightarrow \frac{1}{24} = 0.042 \text{ and } \frac{2}{24} = 0.083 \tag{7}$$

where F_n represents the number of fires (1 or 2) recurring in the two stands and T the time period considered (24 year).

For time between fires (TBF) and time since the last fire (TSLF) the absolute number of years was used (after Guénon et al. 2015; Albert-Belda et al. 2023).

Furthermore, for all soil properties that resulted in significant differences between the control and the burned sites, their percentage variation was calculated according to Eq. (8).

$$\frac{\rho_n - \overline{\rho_c}}{\overline{\rho_c}} \times 100 \tag{8}$$

where ρ_n is the replica value for a specific parameter and $\overline{\rho_c}$ is the mean value of the control for the same parameter. Subsequently, it has been possible to compare the two stands with equal FF (R1F *vs* M1F and R2F *vs* M2F) using Student's *t*-test, in order to further explore the effect of time since last fire (TSLF). Pearson correlation, PCA and Cluster Analysis were run by XLSTAT, all other statistical analyses were performed using the software program SigmaPlot 12.5 (Sigma Stat, Jandel Scientific). The mapping of the study areas and the calculation of the burned area were performed through the open-source software QGIS Desktop 3.12.1. using the official *shape files* reporting the different extensions of the wildfires provided by Comando Unità Forestali, Ambientali e Agroalimentari (CUFAA).

Results

Fire effect on soil organic layer

In both stands, the O-layer was entirely absent in the sites affected by the double fire event (R2F and M2F; Table 2). In Romanazzi stand, the O-layer of the R1F site had recovered (~0.3 cm) 24 years after the disturbance, showing no change in weight and C_{org} content expressed in g m^{-2} compared to the control site (RC), but there was a significant decrease ($p < 0.001$) in C_{org} concentration (mg g^{-1} d.w). On the contrary, no differences were found between the M1F, burnt in 2012, and MC sites regarding the three considered properties. However, both R1F and M1F showed higher coefficient

Table 2 Mean values (± standard deviations, n = 5) of weight (g m^{-2}) of the organic layer (O-layer = litter + fermentation layers) and organic carbon content (expressed as both mg g^{-1} d.w. and g m^{-2}) for the two stands (Romanazzi and Marziotta) in the sites affected by single (R1F, M1F), double (R2F, M2F) fire and unburnt sites (RC, MC). In the last column is reported the calculated C loss, expressed in g m^{-2} for the sites which lost O-layer (R2F, M2F)

	O-layer			Calculated C loss[#]
	Weight (g m^{-2})	C_{org} (mg g^{-1}d.w.)	C_{org} (g m^{-2})	g m^{-2}
RC	472 (± 220)^a	428 (± 25.7)^a	204 (± 96.7)^a	
R1F	447 (± 241)^a	267 (± 38.9)^b	123 (± 79.3)^a	
R2F	0^b	0^c	0^b	204
One-way ANOVA[§]	**	***	***	
MC	397 (± 125)^a	346 (± 56.0)^a	139 (± 55.7)^a	
M1F	625 (± 260)^a	307 (± 23.0)^a	188 (± 65.2)^a	
M2F	0^b	0^b	0^b	139
One-way ANOVA[§]	***	***	***	

[§]Significant differences between the burnt and control sites, in each stand, evaluated by the one-way ANOVA (** = $p < 0.01$, *** = $p < 0.001$) are reported in the row below sites of each stand. Different letters in superscripts (a, b, c) indicate significant ($p \leq 0.05$) differences among sites assayed by Student–Newman–Keuls test

[#]Calculated by assuming that burnt sites should show the same C_{org} content of control in O-layers if wildfires had not occurred of variation of O-layer weight and, limited to R1F, also for the O-layer C_{org} ($g\ m^{-2}$) (Table S1).

Under the assumption that the O-layer at the burnt sites should have had a C_{org} content equivalent to the sites unaffected by the wildfire if wildfires had not occurred, the estimated organic carbon losses of 204 $g\ m^{-2}$ in R2F and 139 $g\ m^{-2}$ in M2F were reported in Table 2.

Fire effect on soil mineral layer

At Romanazzi, no variations were evident in the topsoil (10 cm) for WC, WHC, BD and Po ($p > 0.05$) between the three sites (Table 3). Compared to the control (RC), both burnt sites (R1F and R2F) showed higher ($p < 0.001$) pH and EC, with the highest value (pH: 7.66 ± 0.26; EC: 210 ± 19.6 $\mu S\ cm^{-1}$) measured in the R1F site (burnt once 24 years prior to sampling) and intermediate values in R2F (burnt for the second time 15 years prior to sampling). Compared to RC, a significant increase ($p < 0.05$) in C_{org} was only found in the R1F site (41.3 ± 2.58 $g\ kg^{-1}$ d.w.), while in R2F the value of the organic carbon content did not differ from those found at both the RC and R1F sites. Likewise, the extractable organic C (C_{ext}) was higher ($p < 0.01$) at R1F and R2F sites by 46 and 38%, respectively, compared to RC. No significant differences ($p > 0.05$) were detected for CEC, C_{min}, N_{tot} and C/N ratio across the three sites (Table 3). A significant rise ($p < 0.001$) in NH_4^+–N and NO_3^-–N content was found in both burnt sites compared to RC; with the highest values of 28.1 ± 10.5 and 191 ± 43.6 $mg\ kg^{-1}$ d.w., respectively, found at the R2F site.

Microbial biomass (C_{mic}, Fig. 2a), was significantly lower ($p < 0.001$) at both burnt sites compared to RC, showing the lowest value (269 ± 50 $mg\ kg^{-1}$ d.w.) in R1F. Total microbial activity, here evaluated as soil respiration (R; Fig. 2b), showed significant change ($p < 0.05$) only between R1F and R2F, but both burnt sites were like RC. Specific microbial activities related to the nitrogen cycle were greatly affected by fire (Fig. 2c, d), with significant increases ($p < 0.001$) in burnt soils (R1F and R2F) compared to RC for both nitrogen mineralization (Min) and nitrification (Nit). Regarding index of microbial metabolism, changes due to fire were observed for C_{mic}/C_{org}, showing a decrease ($p < 0.001$) in R1F and R2F by approximately 70 and 50%, respectively, compared to RC (Fig. 2e), and for qCO_2 (Fig. 2f) that was higher ($p < 0.05$) in R1F site (4.37 ± 1.47 mg CO_2–C % C_{mic} d^{-1}) than RC (2.21 ± 0.95 mg CO_2–C % C_{mic} d^{-1}) and R2F (2.51 ± 1.15 mg CO_2–C% C_{mic} d^{-1}). Lastly, no changes were highlighted for the qM (Fig. 2g).

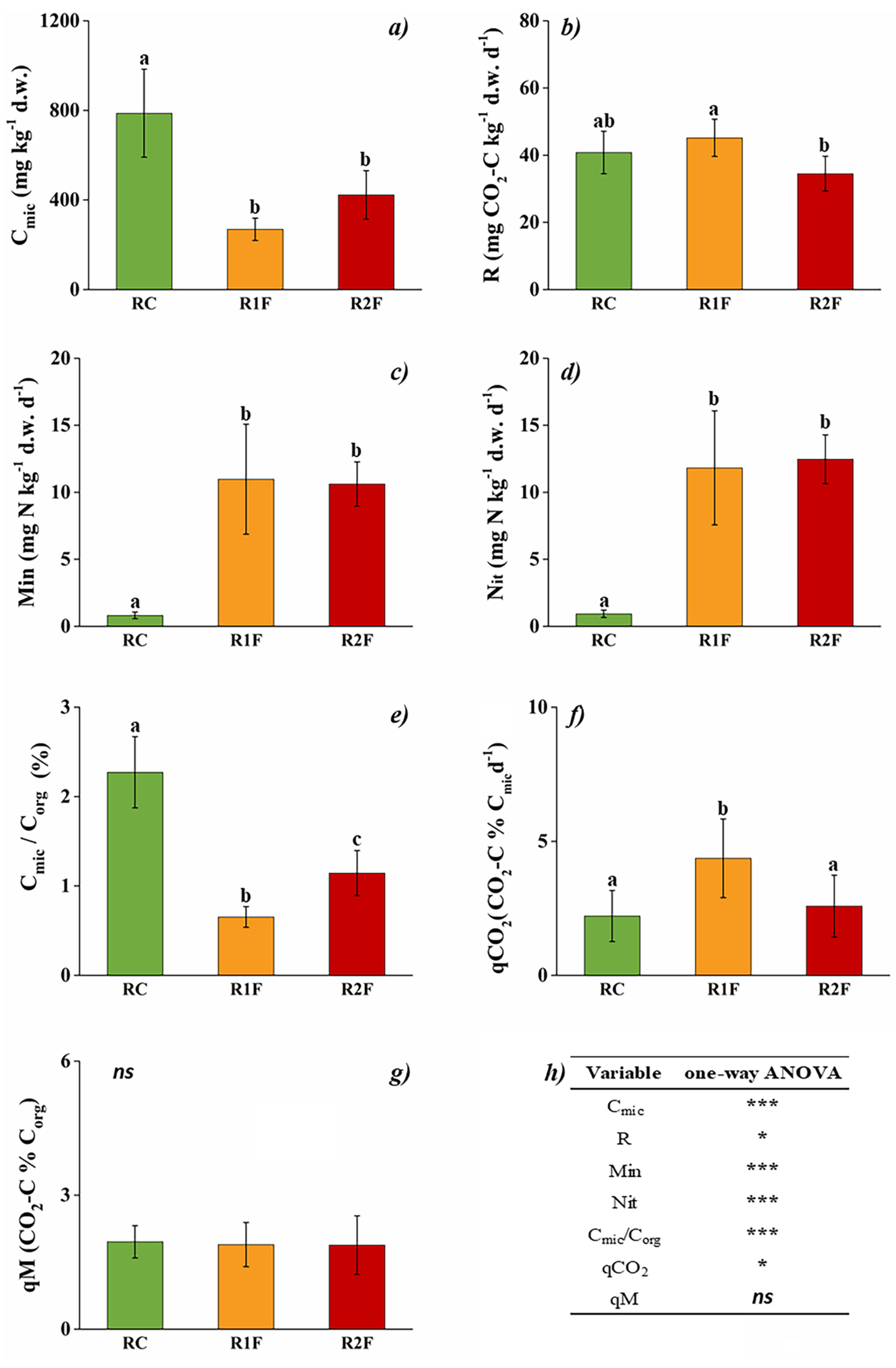
The three sites in the Marziotta stand (MC, M1F and M2F) did not significantly differ for WC, WHC, CEC, N_{tot} and NH_4^+–N (Table 4). All other considered properties showed significant differences among sites. Po, BD and EC exhibited significant differences ($p < 0.05$) between M1F and M2F, but no difference was observed with MC. On the other hand, burnt sites showed a significant pH increase ($p < 0.001$) compared to MC, with the maximum values of 7.59 (± 0.15) in M1F also higher ($p < 0.05$) than that measured in M2F (7.41 ± 0.14). A significant increase ($p < 0.01$) in C_{org} was found in M2F compared to both MC and M1F sites, which showed no difference (Table 4). On the contrary, labile organic C fractions (C_{ext} and C_{min}) decreased in M1F and M2F sites, compared to MC (respectively, $p < 0.05$ and $p < 0.001$). The C/N ratio showed an increasing trend (M2F > M1F > MC) with the highest value found in M2F

Table 3 Mean (± standard deviations; n = 5) values of soil properties at Romanazzi stand in control (RC) and sites affected by single (R1F) or double (R2F) fire events: water content (WC), water holding capacity (WHC), bulk density (BD), porosity (Po), pH, electrical conductivity (EC), cation exchange capacity (CEC), contents of total organic C (C_{org}), extractable C (C_{ext}), mineralizable C (C_{min}), total N, C/N ratio and mineral N (NH_4^+–N and NO_3–N)

Variable	RC	R1F	R2F	one-way ANOVA
WC (%)	13.6 (± 1.10)	16.4 (± 4.72)	12.3 (± 4.42)	*ns*
WHC (%)	33.7 (± 3.17)	40.7 (± 8.27)	32.8 (± 6.18)	*ns*
BD ($g\ cm^{-3}$)	1.06 (± 0.06)	0.89 (± 0.25)	1.11 (± 0.19)	*ns*
Po (%)	60.0 (± 2.3)	66.5 (± 9.5)	58.1 (± 7.0)	*ns*
pH	6.29 (± 0.18)^{a}	7.66 (± 0.26)^{b}	7.22 (± 0.06)^{c}	***
EC ($\mu S\ cm^{-1}$)	103 (± 19.6)^{a}	210 (± 19.6)^{b}	166 (± 20.8)^{c}	***
CEC ($cmol\ kg^{-1}$d.w.)	10.9 (± 2.59)	8.89 (± 2.02)	10.4 (± 2.96)	*ns*
C_{org} ($g\ kg^{-1}$d.w.)	34.6 (± 5.11)^{a}	41.3 (± 2.58)^{b}	36.6 (± 2.76)^{ab}	*
C_{ext} ($g\ kg^{-1}$d.w.)	0.24 (± 0.02)^{a}	0.35 (± 0.03)^{b}	0.33 (± 0.08)^{b}	**
C_{min} ($g\ CO_2$–C kg^{-1}d.w.)	0.67 (± 0.13)	0.77 (± 0.17)	0.69 (± 0.27)	*ns*
N_{tot} ($g\ kg^{-1}$d.w.)	1.60 (± 0.52)	1.48 (± 0.35)	1.71 (± 0.48)	*ns*
C/N	23.0 (± 5.87)	29.4 (± 9.06)	23.0 (± 7.48)	*ns*
NH_4^+–N ($mg\ kg^{-1}$d.w.)	3.42 (± 1.04)^{a}	13.6 (± 3.59)^{b}	28.1 (± 10.5)^{c}	***
NO_3^-–N ($mg\ kg^{-1}$d.w.)	5.17 (± 2.34)^{a}	122 (± 46.3)^{b}	191 (± 43.6)^{c}	***

Results of the one-way ANOVA (*ns* = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$) between the sites are reported in the last column. Different letters in superscripts indicate statistically significant ($p \leq 0.05$) differences among plots assayed by Student–Newman–Keuls test

Fig. 2 Mean (± standard deviation) values of soil microbial variables in control (RC) and sites affected by single (R1F) or double (R2F) fire in Romanazzi (R) stand: **a** microbial biomass C (C_{mic}), **b** respiration (R), **c** nitrogen mineralization (Min), **d** nitrification (Nit), **e** C_{mic}/C_{org} ratio, **f** metabolic quotient (qCO_2), **g** quotient of mineralization (qM) and **h** results of one-way ANOVA (*ns* = not significant, * = $p < 0.05$, *** = $p < 0.001$). For each variable, significant ($p \leq 0.05$) differences among experimental conditions (assayed by Student–Newman–Keuls test) are reported as different letters on bars



differing significantly ($p < 0.05$) only from MC. Similarly, the values measured for nitrate followed the same trend, increasing by 56 and 204% in M1F and M2F, respectively, compared to MC.

A significant decrease ($p < 0.001$) was found for C_{mic} at the burnt sites compared to MC with the lowest value detected at the M1F site (Fig. 3a). Soil respiration exhibited a significant decrease ($p < 0.01$) in both burnt sites compared to MC, with no differences between M1F and M2F (Fig. 3b). The nitrogen cycle was also affected by the fires in this stand (Fig. 3c, d), compared to MC with a significant increase ($p < 0.001$) detected in M1F and M2F for both Min and Nit. Microbial percentage of total organic carbon content (C_{mic}/C_{org}) showed a decrease ($p < 0.001$) in M1F and M2F (which were also different each other) compared to MC (Fig. 3e). A significant decrease of about 65% in qCO_2 was found in M2F

Table 4 Mean (± standard deviations; n = 5) values of soil properties at Marziotta stand in control (MC) and sites affected by single (M1F) or double (M2F) fire events: water content (WC), water holding capacity (WHC), bulk density (BD), porosity (Po), pH, electrical conductivity (EC), cation exchange capacity (CEC), contents of total organic C (C_{org}), extractable C (C_{ext}), mineralizable C (C_{min}), total N, C/N ratio and mineral N ($N–NH_4^+$ and $N–NO_3^-$)

Variable	MC	M1F	M2F	one-way ANOVA
WC (%)	16.5 (±3.53)	16.1 (±5.30)	24.8 (±8.82)	*ns*
WHC (%)	42.8 (±6.97)	36.8 (±5.90)	45.6 (±9.01)	*ns*
BD (g cm^{-3})	0.81 (±0.09)^{ab}	0.91 (±0.08)^{a}	0.72 (±0.11)^{b}	*
Po (%)	69.6 (±3.34)^{ab}	65.7 (±3.13)^{a}	72.9 (±4.10)^{b}	*
pH	7.18 (±0.06)^{a}	7.59 (±0.15)^{b}	7.41 (±0.14)^{c}	***
EC (µS cm^{-1})	227 (±16.7)^{ab}	186 (±40.9)^{a}	250 (±36.4)^{b}	*
CEC (cmol kg^{-1}d.w.)	12.0 (±2.90)	9.29 (±2.46)	10.3 (±5.28)	*ns*
C_{org} (g kg^{-1}d.w.)	33.3 (±2.94)^{a}	30.2 (±4.39)^{a}	39.7 (±1.78)^{b}	**
C_{ext} (g kg^{-1}d.w.)	0.42 (±0.05)^{a}	0.35 (±0.05)^{b}	0.33 (±0.03)^{b}	*
C_{min} (g CO_2–C kg^{-1}d.w.)	1.40 (±0.41)^{a}	0.72 (±0.13)^{b}	0.56 (±0.14)^{b}	***
N_{tot} (g kg^{-1}d.w.)	2.60 (±0.39)	2.01 (±0.78)	2.00 (±0.56)	*ns*
C/N	12.9 (±1.14)^{a}	16.3 (±4.72)^{ab}	21.0 (±5.44)^{b}	*
NH_4^+–N (mg kg^{-1}d.w.)	3.78 (±1.82)	4.79 (±2.58)	4.74 (±2.69)	*ns*
NO_3^-–N (mg kg^{-1}d.w.)	34.4 (±4.12)^{a}	53.5 (±13.2)^{b}	104.5 (±34.0)^{c}	***

Results of the one-way ANOVA (ns = not significant, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$) between the sites are reported in the last column. Different letters in superscripts indicate statistically significant ($p \leq 0.05$) differences among plots assayed by Student–Newman–Keuls test

compared to both MC and M1F ($p<0.01$; Fig. 3f) and qM exhibited changes in both burnt sites compared to the MC ($p<0.01$), showing the lowest values of 1.4 (±0.4) CO_2–C % C_{org} in M2F (Fig. 3g). Finally, it is worth noting that most soil properties showed higher variability in burned sites compared to the relative control (Table S1).

Overall relationship across sites, stands and variables

The overall fire effect on soil, by considering all soil variables together, was clearly shown by Principal Component Analysis (PCA) and Cluster Analysis (Figs. 4, 5). The first two axes of the Romanazzi PCA biplot explained 65% of the variance (Fig. 4a). Along axis 1 (explaining 36% of variance) cluster I, comprising the control site, was distinctly separated by cluster II, which was constituted by burnt sites. Along axis 2 (explaining 29% of variance) clusters IIA (including most R2F sites) and IIB (including most R1F sites) were separated from each other and placed in opposite positions at the bottom and the top of the biplot, respectively. The Cluster Analysis (Fig. 4b) shows the sites distribution just described above.

The soil properties influencing the separation between the control (cluster I) and burnt sites (cluster II) were pH, EC, C_{ext}, NH_4^+–N, NO_3^-–N, Min and Nit, all positively correlated with axis 1, as well as OW, O–C_{org}, and C_{mic}, which were negatively correlated with the same axis (Table S2). On the contrary, soil properties that affected the separation between clusters IIA and IIB were OW, WC, WHC, Po, C_{org}, C_{min}, and R, all positively and BD negatively correlated with axes 2. Axis 1 was correlated positively (r = 0.86, $p<0.001$) with fire frequency (FF) and negatively (r = -0.92, $p<0.001$) with time since last fire (TSLF), while axis 2 was not correlated with either the latter parameters or TBS as well (Table S2). Several soil properties (OW, O–C_{org}, pH, EC, C_{ext}, NH_4^+–N, NO_3^-–N, C_{mic}, Min, and Nit) were correlated with both FF and TSLF, which in turn were negatively (r = − 0.99, $p<0.001$) correlated each other. OW, O–C_{org}, NH_4^+–N, NO_3^-–N, and R, were also correlated to TBS, which was positively (r = 0.86, $p<0.001$) and negatively (r = − 0.76, $p<0.001$) correlated with FF and TLSF, respectively. As expected, the microbial biomass (C_{mic}) and activity (R, Min and Nit) were affected by chemical changes as confirmed by correlations of (i) C_{mic} with pH, EC, C_{ext}, NH_4^+–N, NO_3^-–N (− 0.84 < r < − 0.53; $p<0.05$), (ii) R with C_{min} and qM (0.52 < r < 0.64; $p<0.05$) and (iii) Min and Nit with pH, EC, C_{ext}, NH_4^+–N, NO_3^-–N (0.60 < r < 0.87; $p<0.05$) as well as with O–C_{org} and C_{mic} (− 0.76 < r < − 0.68; $p<0.01$; Table S2). Changes in C_{org}, which were positively correlated with Po, EC, and negatively with BD, did not affect microbial biomass (C_{mic}) and activity (R, Min and Nit), similarly, N_{tot} content, which was positively correlated with CEC (r = 0.80, $p<0.001$) did not influence C_{mic}, R, Min and Nit.

The two principal axes of the PCA (Fig. 5a) explained 68% of the variance for Marziotta sites. Along axis 1 (explaining 36% of variance) cluster I, comprising M2F site, was separated from cluster II, which was constituted from MC and M1F sites (cluster IIA and cluster IIB, respectively). Along axis 2 (explaining 32% of variance) clusters IIA and IIB were separated from each other and placed in opposite positions at the top and bottom of the biplot, respectively. Sites separation is shown in the Cluster Analysis (Fig. 5b).

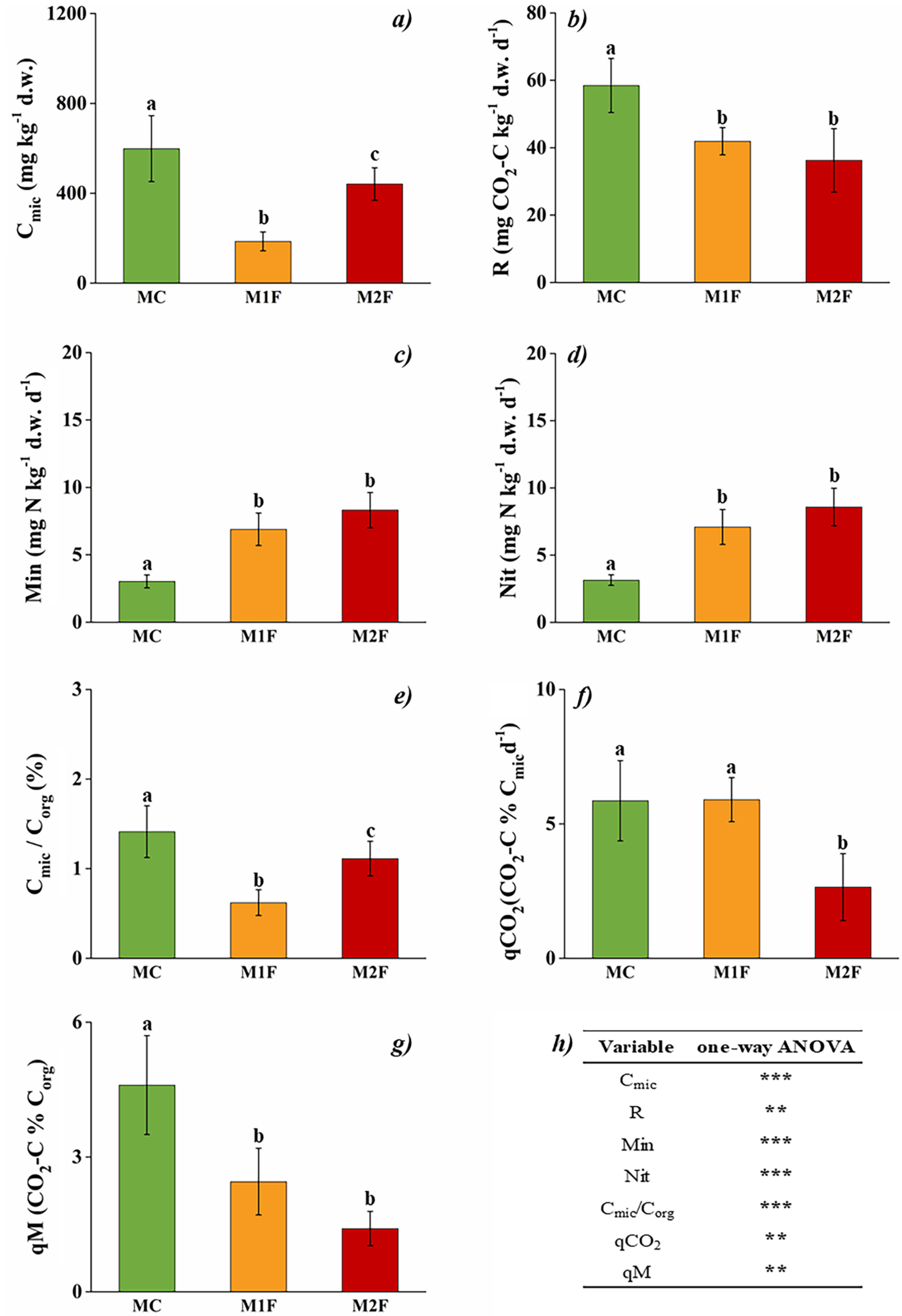


Fig. 3 Mean (±standard deviation) values of soil microbial variables in control (MC) and sites affected by single (M1F) or double (M2F) fire in Marziotta (M) stand: **a** microbial biomass C (C_{mic}), **b** respiration (R), **c** nitrogen mineralization (Min), **d** nitrification (Nit), **e** C_{mic}/C_{org} ratio, **f** metabolic quotient (qCO_2), **g** quotient of mineralization (qM) and **h** results of one-way ANOVA (** = $p < 0.01$ and *** = $p < 0.001$). For each variable, significant ($p \leq 0.05$) differences among experimental conditions (assayed by Student–Newman–Keuls test) are reported as different letters on bars

The soil properties determining separation between cluster I (M2F) and cluster II (MC and M1F) were BD, OW and O–C_{org}, positively correlated with axis I, as well as WC, WHC, Po, EC, C_{org} and NO_3^-–N, all negatively correlated with the same axis (Table S3). In contrast, along axis 2, the separation between MC and M1F (cluster IIA and IIB, respectively) was linked to the positive correlations between CEC, C_{ext}, C_{min}, N_{tot}, C_{mic} and R and to the negative correlations of the pH, NO_3^-–N, Min, Nit with the same axis. Axis 1 was negatively correlated (r = –0.58, $p < 0.05$ and r = –0.81, $p < 0.001$) with both FF and TBF, while axis 2 was positively (0.89, $p < 0.001$) correlated with TSLF and negatively (r = –0.77, $p < 0.001$) with FF. Different properties (OW, O–C_{org}, C_{min}, NO_3^-–N, R, Min, Nit, qM, qCO_2 and C/N; Table S3) were generally correlated with FF, TLSF and TBF, which in turn resulted in correlation only between FF with TSLF and TBF (r = –0.87, $p < 0.001$ and r = 0.86, $p < 0.001$). Furthermore, C_{ext} was correlated

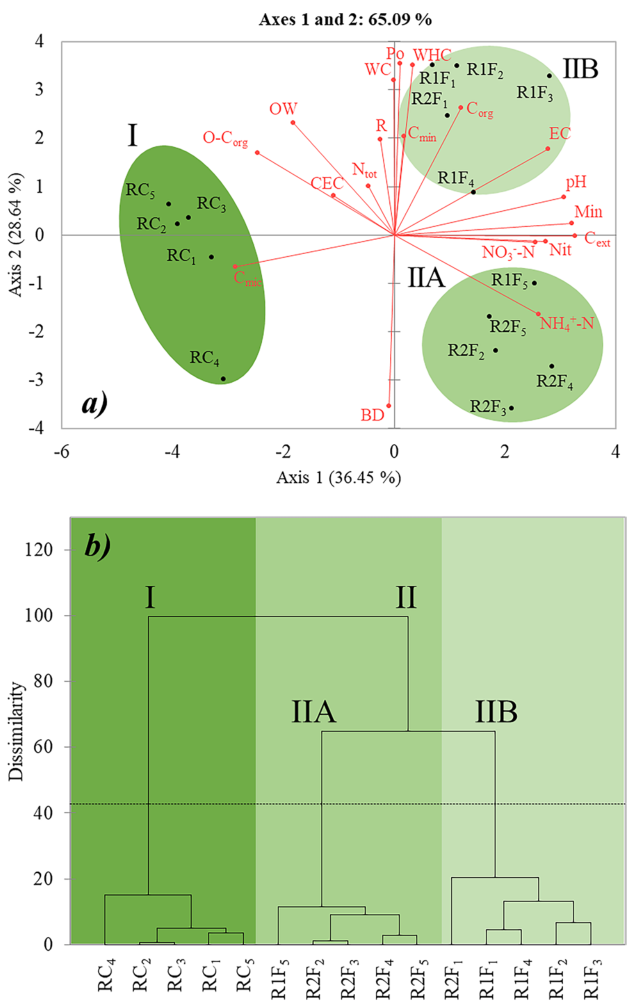


Fig. 4 **a** Biplot deriving from the Principal Component Analysis (PCA) and **b** dendrogram deriving from Cluster Analysis referring to 15 sampling points, corresponding to 5 field replicates for each sampling site (RC, control; R1F, single fire; R2F, double fire) and 19 variables. In the PCA biplot **a** the 15 sampling points are indicated in black and the following 19 variables are in red (with vector length reflecting the strength of each driving factor): weight of the organic layer (OW), total organic C (g m^{-2}) in organic layer (O–C_{org}), soil water content (WC), water holding capacity (WHC), bulk density (BD), porosity (Po), pH, electrical conductivity (EC), cation exchange capacity (CEC), organic C (C_{org}), extractable C (C_{ext}), mineralizable C (C_{min}), total nitrogen (N_{tot}), mineral nitrogen (NH_4^+–N and NO_3^-–N), microbial C (C_{mic}), respiration (R), N mineralization (Min), and nitrification (Nit). The lines in the dendrogram **b** represent the automatic truncations, leading to three main clusters (I, IIA and IIB)

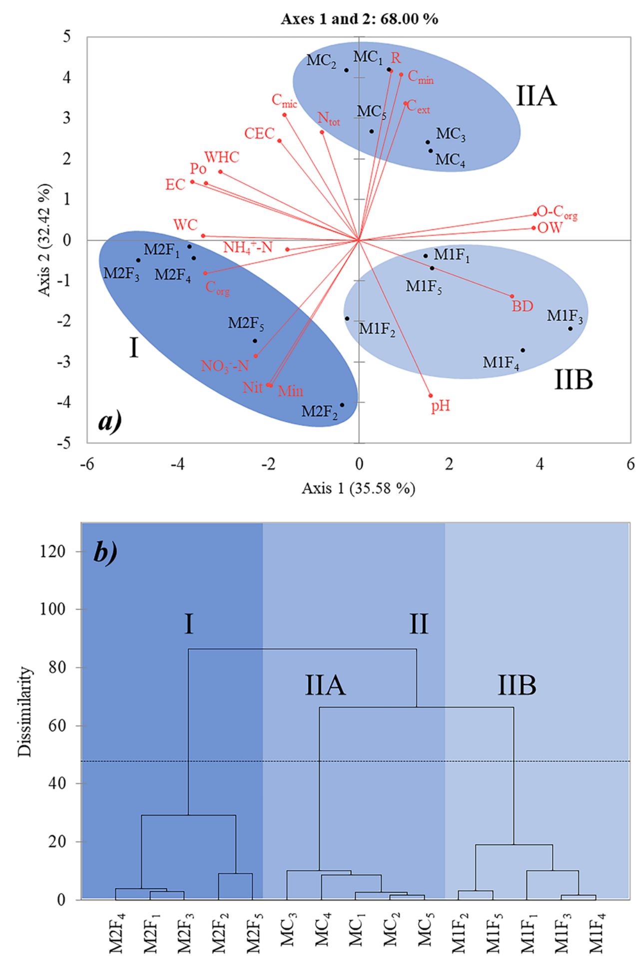


Fig. 5 **a** Biplot deriving from the Principal Component Analysis (PCA) and **b** dendrogram deriving from Cluster Analysis referring to 15 sampling points, corresponding to 5 field replicates for each sampling site (MC, control; M1F, single fire; M2F, double fire) and 19 variables. In the PCA biplot **a** the 15 sampling points are indicated in black and the following 19 variables are in red (with vector length reflecting the strength of each driving factor): weight of the organic layer (OW), total organic C (g m^{-2}) in organic layer (O–C_{org}), soil water content (WC), water holding capacity (WHC), bulk density (BD), porosity (Po), pH, electrical conductivity (EC), cation exchange capacity (CEC), organic C (C_{org}), extractable C (C_{ext}), mineralizable C (C_{min}), total nitrogen (N_{tot}), mineral nitrogen (NH_4^+–N and NO_3^-–N), microbial C (C_{mic}), respiration (R), N mineralization (Min), and nitrification (Nit). The lines in the dendrogram **b** represent the automatic truncations, leading to three main clusters (I, IIA and IIB)

with FF and TSLF ($r = -0.66$, and $r = 0.69$, respectively, $p < 0.01$); OW, O–C_{org}, with FF ($r = -0.54$, and $r = -0.63$, respectively, $p < 0.01$) and TBF ($r = -0.81$ and $r = -0,85$, respectively, $p < 0.001$); pH and C_{mic} with TLSF ($r = -0.75$, $p < 0.01$ and $r = 0.70$, $p < 0.01$, respectively); C_{org} with FF ($r = 0.53$, $p < 0.05$). No correlation was finally highlighted between C_{org} and N_{tot} contents with soil biological properties, however, some changes in other chemical parameters influenced microbial biomass and activity (i.e. C_{mic} was correlated negatively with pH and positively with EC; soil respiration (R) was positively correlated with C_{ext}, C_{min}, and negatively with pH and C/N ratio; Min and Nit were positively correlated to C/N ratio and negatively with C_{ext}, C_{min}, R, qCO_2, and qM).

The comparison between the two stand sites with the same fire frequency (expressed as the percentage variation between the respective control; Fig. 6) allows to quantify the effect of time since last fire (TSLF) i.e. recovery period. Indeed, in the sites affected by a single fire (R1F *vs* M1F; Fig. 6a), a significant increase 24 years after the fire, at the R1F site, compared to the 9 years after fire, at M1F site, was found for the following properties: pH, EC, C_{org}, C_{ext}, C_{min}, NH_4^+–N, NO_3^-–N, R, Min, Nit ($p < 0.001$). In contrast, O–C_{org} at the M1F site emerged significantly higher after 9 years ($p < 0.05$) than in R1F and no differences were found for OW, C/N, C_{mic}, C_{mic}/C_{org}, and qCO_2. Likewise, in the sites burned twice (Fig. 6b), the percentage increase in pH, EC, C_{ext}, C_{min}, NH_4^+–N, NO_3^-–N, Min, Nit and qCO_2, compared to the respective control, was raised as a function of the greater TLSF (15 vs. 9 years, respectively at R2F and M2F sites). On the contrary, changes in C_{org} and C/N were higher ($p < 0.05$) in M2F versus R2F. As regards C_{mic}, the percentage decrease, compared to the control, was lower in M2F, the reverse was observed for R and qM, showing significant lower decreased in R2F. No difference in C_{mic}/C_{org} variation, compared to the control, was evidenced between R2F and M2F and no organic layers were detected at both twice burnt sites.

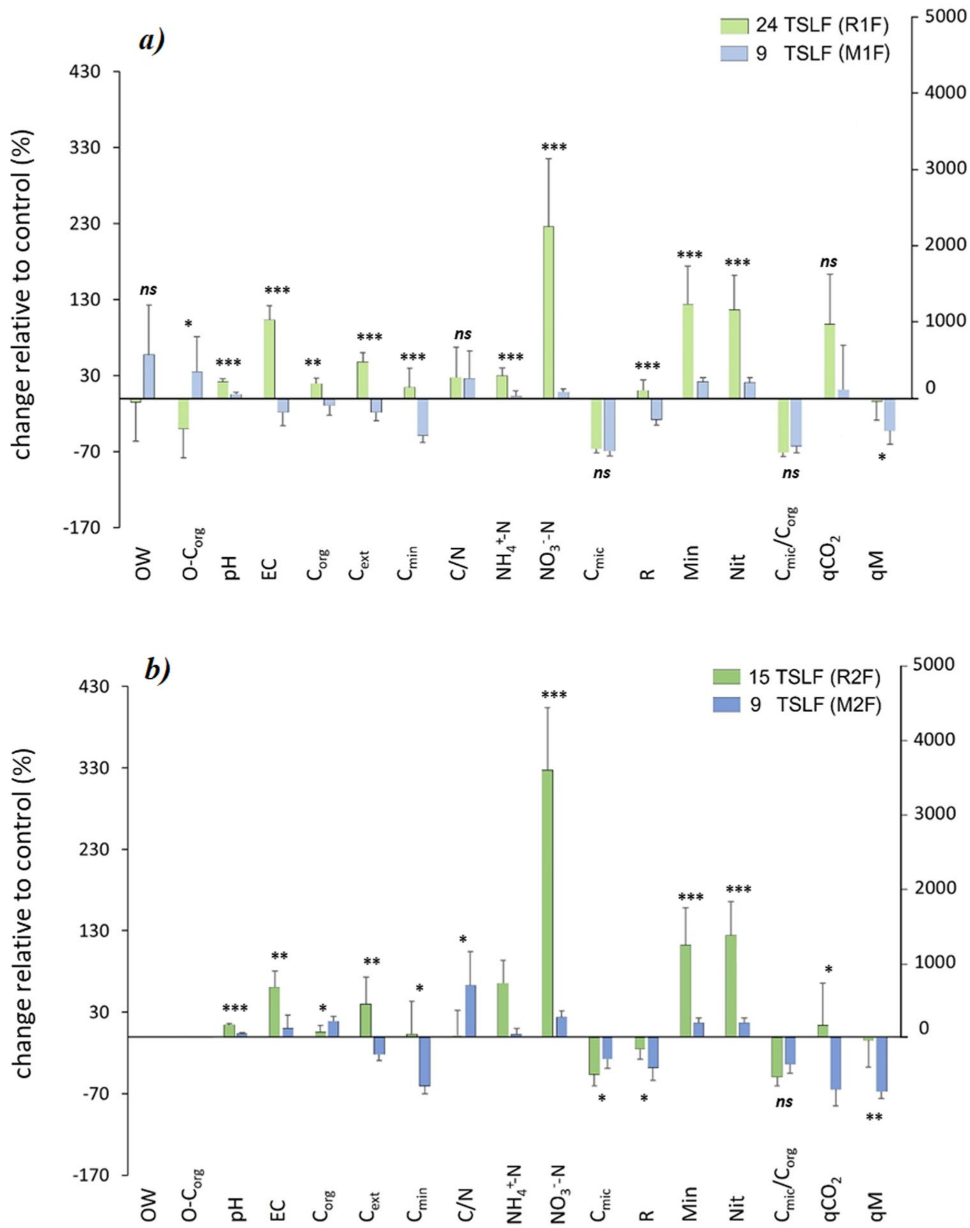


Fig. 6 Percentage variations, compared to the relative control, of O-layer and soil variables in single **a** and double **b** fire sites of Romanazzi (R1F, R2F, green bars) and Marziotta (M1F, M2F, blue bars) stands. TSLF refers to the time since the last fire for each site (see Table 1). The following properties NH_4^+–N, NO_3^-–N, Min and Nit refer to the right axis, all the other properties refer to the left axis (both the axes indicate the percentage change relative to control). Results of the t-test (*ns* = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$) between the sites are shown above or below the bars. For parameter labels see Fig. 5

Discussion

Fire effect on soil organic layer

At Marziotta stand, the total loss of the O-layer in the double-fire site (M2F) was accompanied by a significant increase in C_{org} in the underlying mineral layer of soil, likely as a result of the transfer of partially mineralized organic layer materials to the mineral soil, as observed during low severity fires (Rutigliano et al. 2007). In contrast, this did not occur in R2F (where the C_{org} increase was not significant) most likely due to higher fire severity that completely burnt the organic layer, however in R1F we observed a significant increase in C_{org} compared to the control. By calculating the increase of soil C_{org} stock (according to Kirby and Potvin 2007) in R2F and M2F sites, compared to control, values of 395 g m^{-2} and 161 g m^{-2} were obtained, respectively. By comparing these data with the C_{org} lost from the O-layer (204 and 139 g m^{-2}, respectively) within Romanazzi and Marziotta stands (Table 2), we can conclude that at both stands C_{org} stock increase in the soil outweighed the loss in the O-layer suggesting no net transfer to the atmosphere occurred. On the contrary, in other pine stand within the same SAC (near the Galaso river), affected by a large wildfire in 2017, the observed C_{org} loss of about 200 g m^{-2} from the organic layers observed four years after the wildfire was not compensated by accumulation in the soil (Marfella et al. 2023a). This suggests the highly variable severity of wildfires occurring in the same area. Certini et al. 2011 also found, in two forests respectively dominated by *P. pinaster* and *P. pinea* growing along the Tuscan coast (central Italy), that the C loss relative to the pre-fire stock was almost totally ascribable to the organic horizon burning. Although the disappearance of O-layer, in some burnt sites, was balanced by C_{org} accumulation in soil mineral, the loss of this layer (including litter and fermentation layer) compromises habitat complexity (Moghli et al. 2022) with serious negative effects on the soil-related ecosystem services such as increased erosion and runoff (Gehring et al. 2019; Rulli and Rosso 2007) and alteration of the nutrients and water cycle (Giuditta et al. 2018, 2019; Moya et al. 2020). Furthermore, the loss of O-layer in both double-fire sites and higher variability of O-layer weight in single fire sites, compared to control, caused higher variability also in the underlying soil layer, probably determining instability in soil processes. Accordingly, Choreño-Parra et al. (2022) reported that increased heterogeneity in the tree forest layer due to disturbances (such as fire and reforestation with non-native species) can result in instability in litter decomposition process. Our findings also showed different recovery dynamics for the O-layer in the single-fire sites. In fact, while all the O-layer properties in M1F were comparable to control, in R1F, the concentration of organic carbon (mg g^{-1} d.w.) was still lower than in the control. However, in terms of absolute quantity (g m^{-2}), the recovery of the organic layer in R1F found 24 years after fire is consistent with Trabaud et al. (1985a) who reported that the complete litter restoration in a *P. halepensis* forest is potentially achievable after about 25–30 years. Consequently, we suggest most likely the 2012 fire in M1F only partially affected the O-layer. Eugenio et al. (2006) also observed, comparing *P. halepensis* stands hit by fire once and twice within a span of 19 years, that the organic layers were thicker in areas affected by a single fire. Moreover, they did not find significant distinctions between areas that had experienced one and two fire events regarding the other properties assessed within the organic layers as well as mineral soil.

Fire effect on soil mineral layer

Physical and chemical changes

After the last fires occurred 15 year ago in Romanazzi and 9 year ago in Marziotta, regardless of the frequency and time between fires, the physical properties of the soil have been restored. Likewise, Xue et al. (2014) found, in a *Pinus massoniana* Lamb. forest, soil physical variables were comparable to the control within 7 years. Generally, very evident shifts in the soil physicochemical properties are most often found immediately after fires, especially when the fire intensity (temperatures reached at the soil level) and the residence time are elevated (Capogna et al. 2009; Giovannini et al. 1988; Giovannini and Lucchesi 1997). In our case, the lack of this information during the last fires makes it difficult to depict firm inferences on the unchanged physical properties. Soil pH increased following the same trend at all burnt sites, becoming slightly alkaline, within the two pine stands. This is not surprising, as it has been widely observed in different ecosystems and under controlled conditions (heating or prescribed burning) that the conversion of organic matter to ash can lead to an increase in soil pH (Catalanotti et al. 2018; Certini 2005; Moya et al. 2018; Stinca et al. 2020). Although this alkalizing effect is transient because it is influenced by water or wind transport (Certini et al. 2011; Granged et al. 2011a; Úbeda et al. 2009), in the absence of these processes it can be observed even several years after the disturbance (Granged et al. 2011b; Khanna and Raison 1986). Post-fire electrical conductivity (EC) also increases mainly due to the release of ions (Certini 2005; Notario del Pino et al. 2008; Úbeda et al. 2009) and this effect is still observable in Romanazzi rather than in Marziotta, where EC measurements returned comparable values than control.

Muñoz-Rojas et al. (2016) found for both pH and EC by analysing a chronosequence of fires (1, 5, 7 and 14 years since the last fire), that after an initial increase, they returned to control values within 5 years, or even lower in the electrical conductivity case. However, our Aleppo pine forest is rather particular from this point of view because it is affected by saline intrusion phenomena (Leone et al. 2000).

Thus, depending on the season, an alteration in the salinity values of the shallow water table can impact the salinity of the entire system (as already shown elsewhere Antonellini et al. 2010; Buscaroli et al. 2017; Marfella et al. 2023b; Teobaldelli et al. 2004). This may have influenced the pH and also explain the non-linear variations in EC between the two stands, but above all the 30% higher average values measured in the Marziotta stand, which could be more prone to salt intrusion being closer to the coastline than to the Romanazzi stand.

As mentioned earlier, the accumulation of partially burnt material may explain the C_{org} rise in some burnt sites (R1F and M2F) found over the decades (Liu et al. 2023; Knicker 2007). Similarly, Li et al. 2021 noted after performing a global meta-analysis, that C_{org}, after reaching pre-fire levels ~ 10 years post fire, then further increased significantly for up to five decades after the fire. Different C pool dynamics were observed in the two stands. In Romanazzi burnt sites, we found a higher rise, respect to its control, in C_{org} and C_{ext} values in site affected by one wildfire (R1F) than in site affected by two wildfires (R2F) as a consequence of more time since the last fire (24 year in R1F, and 15 year in R2F), as also reported by Mayor et al. (2016), as well as of lower fire frequency. According to DeLuca and Sala (2006) and Knicker (2007), an increase in fire frequency as well as a shorter time between fires could reduce SOM in the long-term, as repeated fires leads to a decrease in soil organic carbon and nutrients. Compared to Romanazzi burnt sites, Marziotta sites showed only an increase in total C_{org} limited to site affected by two wildfires (M2F), the last occurring 9 year before our sampling. If of low-medium intensity (from 100 to 400 °C; Garcia-Pausas et al. 2022; Ulery et al. 1996), more recurrent fires could also enrich the soil through a fertilizing effect (Pyne 2019; Rodríguez et al. 2017), thus explaining organic carbon content increase in M2F than M1F. In addition, Pellegrini et al. (2018) following a global meta-analysis of 48 sites covering 65 years, demonstrated that a high fire frequency corresponded to a reduction in carbon and nitrogen concentrations in the soil, but mainly in deciduous forests and in the savannah grasslands. In contrast, they observed that the same parameters in coniferous forests increased by 26% and 21%, respectively, in burned sites compared to controls. Despite C_{org} shifts, no changes in CEC as well as N_{tot} content were found in our Aleppo pine stands. The unchanged CEC is consistent with findings by Muñoz-Rojas et al. (2016) and Hatten et al. (2008), wherein 14 and 15 years post fire and multiple fire events had not evidenced changes in CEC. Also, N_{tot} could be lost during fires through volatilization, but this effect is generally temporary (Barcenas-Moreno and Bååth 2009). The accumulation of C_{org} together with no alteration in N_{tot} resulted in an increased C/N ratio in M2F. Conversely, Rodríguez et al. (2017) found, in a Mediterranean forest hit by multiple fires at different times, an increased N percentage in the burnt soils compared to the control ones and consequently lower values in the C/N ratio. In our stands, the lack of formation and accumulation of newly-formed recalcitrant heterocyclic N forms due to the incorporation of semi-pyrolyzed materials could explain the unchanged N_{tot} as well as the C/N variations only as a function of changes in C_{org} (Almendros et al. 2003; De la Rosa et al. 2008).

Several studies report on the effects of fires on the mineral nitrogen components, and there is therefore ample evidence that after a wildfire there is a surge of NH_4^+–N that is subsequently oxidized into NO_3^-–N, in the short to medium-term, by nitrifying bacteria (Alcañiz et al. 2018; Guénon et al. 2013; Kong et al. 2019; Wang et al. 2012). According to Prieto-Fernández et al. (2004), the NH_4^+content increased both after the fire and after heating at different temperatures, persisting up to more than 1 year after combustion. In fact, 2, 5 and 10 years post fire, NH_4^+–N was equivalent to or slightly higher than that of undisturbed soil, as was the case for Marziotta burnt sites. In contrast, in the Romanazzi stand this did not happen showing consistently high NH_4^+values and NO_3^- as well. The high amount of NO_3^-–N may be attributed to high activity of nitrifying microorganisms, which can be favoured by the increased pH (Hanan et al. 2016; Raison 1979). The nitrate availability found in all our burnt sites may lead to a rapid recovery of the underwood, as it is absorbable by plants, but at the same time subject to leaching (Padilla et al. 2018). However, the high nitrate content measured 24 and 15 years post fires in Romanazzi and 9 in Marziotta could also be attributed both to low uptake by post-fire vegetation and/or low immobilization by microbial communities (Guénon et al. 2013).

Biological changes

The response of the microbial community to the different fire conditions examined highlighted a long-term impact. The negative fire effect on C_{mic} in our burnt sites, which in R1F even persists 24 years post-fire, complies with the results of a meta-analysis reported by Pressler et al. (2019), who discovered little proof of long-term microbial biomass recovery. Frequently in pinewoods, a reduction both in microbial biomass and activity has been documented because of the fire direct impacts (Hernández et al. 1997; Mataix-Solera et al. 2009). However, a few weeks after a wildfire, the wide availability of nutrients released by the ash and partially burnt organic matter may stimulate the growth of the microorganisms determining a short-term increase in the microbial biomass and activity (Dumontet et al. 1996; Rutigliano et al. 2007). Thereafter, from 2 to > 5 years, there is a decrease in C_{mic} due to indirect impacts from fires influencing plant cover and soil properties, which in turn affect the microbial community (Fierro et al. 2007; Snyman 2004). Then, in the long-term, other environmental components greatly influence soil microorganisms such as temperature and water availability in burnt areas (Dooley and Treseder 2012; Ross 1987). These dynamics could explain the unrecovered microbial biomass independently of fire frequency in our burned soils and suggest that, regardless of the extent of the disturbance in terms of severity or intensity, the recurrence of more than one fire in 24 years can lead to land degradation in arid Mediterranean ecosystems, compromising their functions. On the other hand, Moya et al. (2018) studied a chronosequence of wildfires (3, 15, and 21 years) of different severities in *P. halepensis* forests in semi-arid areas of southeastern Spain, and observed that, irrespective of the fire severity, soil quality indicators (C_{org}, C_{mic}, C_{mic}/C_{org}, etc.) recovered 21 years after the fire. Indeed, the longer the time between fires, mainly after highly severe fires, the greater the likelihood (under optimal conditions) that the ecosystem recovers (Buma et al. 2022; Dooley and Treseder 2012). On the contrary, in our study at Romanazzi stand, 24 and 15 years post-fire was enough to find microbial respiration comparable to control, but not for C_{mic} and C_{mic}/C_{org} still showing a clear disturbance evidence, mainly at R1F site where this was also supported by the highest qCO_2 value, indicating stress conditions for microorganisms (Anderson and Domsch 1993; Marzaioli et al. 2010), and corroborating the expert knowledge that the 1997 wildfire has been a stand-replacing fire. Accordingly, Moya et al. (2019) stated that in Mediterranean fire-prone Aleppo pine stands, decreases in C_{mic} and C_{mic}/C_{org} ratio can be found in soil after five years in soil affected by fire. Similarly, 9 years after the last fire in both Marziotta burnt sites the same dynamics for the same soil microbial indices were observed, in addition to unrecovered R as well as low qM values indicating that the C_{org} increase found in M2F was poor mineralizable (Marfella et al. 2023a).

Microbial indices provided clues about our soils stress conditions, particularly low C_{mic}/C_{org} and high qCO_2 values which identifed a weak efficiency of microorganisms to convert the C_{org} into microbial biomass and consequently low growth rates (Vittori Antisari et al. 2021). In agreement with our results, Liu et al. (2023) reported that microbial biomass and metabolic quotient can significantly decrease and increase respectively due to fires, stating that both parameters are particularly sensitive to this disturbance especially in forest ecosystems. In addition, considering that C_{mic}/C_{org} ratios lower than 2% are critical for microbial growth (Anderson 2003; Paz-Ferreiro and Fu 2016), our results suggest a decrease in the organic matter quality in the R1F and M1F, where the C_{mic}/C_{org} values were less than 1%.

Regardless of different fire conditions, nitrogen mineralization and nitrification increased in all burnt sites. The same trend was also observed in Galaso stand four years after the 2017 wildfire with no dependency on fire severity (Marfella et al. 2023a). Increased Min and Nit have usually been reported after one or more fire events, both the short-medium to long-term, in several environments such as chaparral and Mediterranean maquis (Hanan et al. 2016; Pellegrini and Jackson 2020), as well as temperate and boreal forests (Choromanska and DeLuca 2002; DeLuca et al. 2002; DeLuca and Sala 2006). These processes strictly depend on specific conditions, such as pH, substrate availability and competition with plants and heterotrophic microbes, which can be substantially altered due to fires through direct and indirect effects as observed in our study sites and also shown elsewhere (Hanan et al. 2016; Knicker 2007). Decades may pass before the heterotrophic biomass recovers (Choromanska and DeLuca 2002; Grasso et al. 1996) allowing microorganisms engaged in nitrogen mineralization and nitrification to increase their activity in the long-term. The increase in pH at all our burnt sites, as well as the supply of NH_4^+–N rich ash rather than partially burnt organic matter, may also explain this increase. In agreement with DeLuca and Sala (2006) charcoal plays a key role in stimulating nitrification because substances that could inhibit nitrification (phenolic compounds, etc.) can be adsorbed on its surface, inducing the immobilization of NH_4^+–N and NO_3^-–N. This overstated increase (especially in R1F and R2F up to 15 times compared to the control) could also be explained because of the accumulation of pyrogenic organic matter as a nitrogen source in line with Xu et al. (2022). However, it is not necessarily a positive effect as it may stimulate the invasion of nitrophilous and/or exotic invasive species that with shorter life cycles may have an advantage on the native flora in frequently disturbed ecosystems. Nevertheless, in order to avoid long-term N losses in this forest ecosystem, the role of recovering plants is necessary to limit leaching N losses.

Overall soil response to fire history variables

The PCA analyses, highlighting the main soil properties mostly affected and responsible for the separation of the clusters, allowed us to assess the long-term impacts of the wildfires. The study of fire impact on soil are complex because the results depend on the effects of the different components of fire characteristics, such as fire and fuel type, fire intensity and severity, residence time, size of burnt area, fire frequency and interval, fire season (Mataix-Solera et al. 2011a, b; Galizia et al. 2022) and their interactions. In the Romanazzi stand, the separation between control and burnt sites first and foremost shows a clear fire effect with R2F experiencing an additive effect due to the second fire that broke out 15 years before sampling. Therefore, in R2F the worst soil conditions, compared to R1F (one fire, 24 year before sampling) may depend on both higher fire frequency (FF) and shorter time since last fire (TSLF). On the other hand, in the Marziotta stand, the TSLF is the same for sites affected by one (M1F) or two (M2F) fires, so the observed difference between these sites, which were separated in PCA biplot and dendrogram deriving from Cluster Analysis, mainly depended on FF. To better evidence the effect of TSLF, we compared, for each FF, the variation of soil variables in two stands, with respect to the relative control. This analysis show that most variables increased with increase of TSLF in both FF. The effect of time between fires (TBF) also appeared a weak regulating factor considering that dissimilarity between R1F and R2F (Fig. 4b) was lower than that between M1F and M2F (Fig. 5b) notwithstanding lower TBF at Romanazzi than at Marziotta (9 vs. 12 year). The effect of TBF appeared less relevant than TSLF because only few variables appeared improved in Marziotta than in Romanazzi when double fire sites were compared (Fig. 6b). Therefore, the following order of importance between fire history variables TSLF = FF > TBF would be recognized. According to this, Albert-Belda et al. (2023) showed no implication of TBF over TSLF and fire recurrence leading to changes in soil mineral properties. These latter two fire history variables strongly interact with each other and are highly dependent on the spatiotemporal scale at which they are considered, making it complex to discern between them (Fernández-Guisuraga et al. 2023; Moghli et al. 2022). In fact, in our correlations, in Romanazzi the same soil properties correlated with both TSLF and FF (in opposite ways), while TBF, completely insignificant in Romanazzi, was more relevant in Marziotta probably because it was longer than in Romanazzi.

Conclusions

Notably, our findings illustrate that fires represent a disturbance that yields enduring consequences on C_{org} pools and soil chemical properties, as well as impacting soil microbial communities. Rejecting our initial hypothesis, these effects are still observed from 9 to 24 years since the last fire in *P. halepensis* coastal forests of southern Italy. The accumulation of partially burnt material in mineral soil may explain the C_{org} rise over the decades found in our sites that could have had a long-term effect on the microbial community processes (i.e. C or N mineralization). Increased nitrogen mineralization and nitrification may lead to a change in vegetation successional pathways in the medium to long term. At this N2K site, we suggest an effort to monitor vegetation in the surrounding areas and within the fire perimeter to prevent the establishment of invasive species.

Both fire frequency (FF) and times since the last fire (TSLF) affected soil properties. FF was the main factor influencing O-layer which was completely missing in double-fire sites i.e. still not recovered 9 and 15 years since the last fire. This caused a C_{org} loss of 204 g m^{-2} in R2F and 139 g m^{-2} in M2F over 24 years, which was more than compensated by organic C accumulation in the mineral soil. However, the loss of O-layer compromises habitat complexity, which could lead to a severe decline in crucial ecosystem services such as protection from erosion and runoff as well as regulation of water cycle. Moreover, regardless of frequency, the longer the time since the last fire (TSLF), the greater the soil recovery. Therefore, in arid ecosystems subject to recurring fires, strategies that reduce the frequency of fires to allow the system to recover are strongly suggested.

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