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Effect of forest fire on soil microbial biomass and enzymatic activity in oak and pine forests of Uttarakhand Himalaya, India

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Abstract

Background: Forest fire incidences in the Himalayan region of Uttarakhand, India are very common in summers. Pine and oak are the principal and dominant species of Himalayan subtropical forest and Himalayan temperate forest, respectively. Forest vegetation influences the physicochemical and biological properties of soil and forest fire in pine and oak forests may have a different effect on the physicochemical and biological properties of soil. Therefore, the present study was carried out to assess the impact of forest fire on soil microbial properties, enzymatic activity, and their relationship with soil physicochemical properties in the advent of forest fire in the pine and oak forests of the Garhwal region of Uttarakhand Himalaya, India.

Results: The soil microbial biomass carbon and nitrogen, soil basal respiration, and acid phosphatase activity decreased, whereas dehydrogenase activity increased at burnt sites of both forest types. The overall change in soil microbial biomass carbon was 63 and 40% at the burnt oak forest and burnt pine forest, respectively. Dehydrogenase activity and acid phosphatase activity showed a strong positive correlation with soil organic matter ($r = 0.8$) and microbial indices, respectively. The ratio of soil microbial biomass carbon/nitrogen was reduced at burnt sites of both forest types. Factor analysis results showed that fire had a significant impact on soil characteristics. The soil basal respiration was linked with macro- and micronutrients at burnt sites, whereas at control sites, it was linked with physicochemical properties of soil along with nutrients.

Conclusion: Forest fire had a significant impact on soil properties of both forest types. The impact of forest fire on soil microbial biomass carbon was stronger in the oak forest than in the pine forest. Forest type influenced soil enzymatic activity at burnt sites. The bacterial community was dominated over fungi in burnt sites of both forests. Soil microbial indices can be used as a selective measure to assess the impact of fire. Furthermore, forest type plays an important role in regulating the impact of forest fire on soil properties.

Keywords: Microbial indices, Soil enzymes, Microbial biomass, Forest fire, Pine forest, Oak forest

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Background

Microbial biomass reflects the microbial status of soil responsible for maintaining the nutrients and fertility of the soil and therefore, contributes to the biological properties of the soil (Mataix-Solera et al. 2009; Manral et al. 2020). Microbes are solely responsible for nutrient cycling and play a major role in the transformation of nutrients and therefore, act as the soil health indicators. Microbial biomass is a sensitive indicator of soil and can be used to measure the impact of forest fire on soil (Sadeghifar et al. 2020). The loss in microbial biomass during a fire depends upon the intensity and duration of the fire (Girona-García et al. 2018; Lucas-Borja et al. 2019). Soil biological properties like microbial biomass carbon (C_{mic}), microbial biomass nitrogen (N_{mic}), soil basal respiration (SBR), and enzymatic activities also alter post-forest fire. The impact of forest fire on the soil is of two types: direct, because of the combustion of organic matter, and indirect, because of changes in other components of the ecosystem (Neary et al. 1999; Dooley et al. 2012). The change in soil properties due to fire has an indirect effect on soil microbes present in the soil as the physicochemical properties of soil also influence the behavior and nutrition requirements of microbes (Thirukkumaran and Parkinson 2000). The effect of fire on soil properties, in turn, depends upon the vegetation cover, fuel load, intensity, and duration of the fire. Soil properties like bulk density, soil moisture, cation exchange capacity, organic carbon, and electrical conductivity are reported to be changed after the fire (Busse et al. 1996; Neary et al. 1999; Boerner et al. 2009; Heydari et al. 2017). Soil biological properties also change due to alteration in these properties. Forest fire causes high temperature during burning, and most of the microbes are unable to tolerate high temperature and do not survive under such conditions.

The fire of high severity causes a greater reduction in microbial biomass and respiration than the fire of low severity (Holden et al. 2016; Girona-García et al. 2018). Unavailability of soil carbon and nutrients after fire is responsible for the reduction in microbial biomass (Zhou et al. 2018). The impact of forest fire on microbial biomass and diversity also depends on the topographic positions (ridge, middle slope, and valley bottom), and the most affected topography was reported to be ridge (Mabuhay et al. 2006).

The microbes have intracellular and extracellular enzymes present in them that are involved in the process of nutrient transformation. Soil enzymes are sensitive indicators of ecological change and these enzymes are involved in releasing the nutrients in the soil and made them available to plants (Arunachalam et al. 1999; Sadeghifar et al. 2020). Among the enzymatic activities, the dehydrogenases are exclusively intracellular enzymes, which play an important role in the initial stages of oxidation of soil organic matter by transferring electrons or hydrogen from substrates through co-enzymes to acceptors. Acid phosphatase activities (ACP) were reported to be reduced after the fire and had a positive correlation with microbial biomass (Nannipieri et al. 1983; Kandeler and Eder 1993; Sadeghifar et al. 2020). These enzymes function as a measurement of the metabolic state of soil microbes (Wolińska and Stępniewska 2012). Different ratios in soil microbial biomass such as C_{mic}/N_{mic}, C_{mic}/SOC, etc. serve as one of the important parameters in assessing the productivity of soil (Li et al. 2016).

In the Himalayan mountainous region of Uttarakhand, India, forest fire occurs every year due to which soil composition of the forest is prone to change. In general, most of the fire incidences start in the pine forest region because of its dry needle leaves and later spread over to the oak forest region. Pine is the principal species of Himalayan subtropical forest, and oak is the dominant species of Himalayan temperate forest (Zhou et al. 2018). Both the forests have different soil characteristics because of the differences in species composition of these forests. The vegetation of the forest is a determinant of the soil quality and influences the physicochemical and biological properties of soil (Ilorker and Totey 2001; Kumar et al. 2004). Forest fires usually occur when the temperature is high and humidity is low (Smith et al. 2008). Although the incidences of forest fires have been widely reported from this region (Mittal et al. 2019; Manral et al. 2020), the studies on the impact of fire on soil biological properties and soil quality are limited. The present study was carried out to evaluate the impact of forest fire on soil microbial properties and its interrelation with soil nutrients in oak- and pine-dominated forests of Uttarakhand, Himalayas. To the best of our knowledge, such comparative study has not been reported in the literature so far. Therefore, this study aimed to address the following questions: (1) Are soil properties of oak and pine forests affected in the same way following a surface fire? (2) Do microbial properties change after the fire due to changes in physicochemical properties? (3) What will be the impact of fire on the different ratios in soil microbial biomass? The study hypothesized that (1) soil properties change following forest fire, (2) impact of fire varies by forest types, (3) soil microbial properties respond to changes in soil physicochemical properties.

Materials and methods

Study area and site selection

The study was carried out on the control and the burnt sites for two different forest types (oak and pine) of the Garhwal division of Uttarakhand to evaluate the impact of fires on different physicochemical and soil enzymatic

activities. The study sites are located in Pauri Garhwal (29.8688° N, 78.8383° E) and Tehri Garhwal (30.3012° N, 78.5661° E) districts of Uttarakhand, India (Indian Himalayan Region). The mean annual values of temperature, rainfall, and relative humidity of the study sites are 23.97 and 24.22 °C, 144.10 and 128.4 mm, and 71.22 and 70.46%, respectively. The climate of Uttarakhand is characterized by hot dry summers and cool rainy winters, and the forest is dominated by oak and pine species. Two dominant forest types—i.e., oak (*Quercus leucotrichophora*) and pine species (*Pinus roxburghii*)—were selected for the study at an altitude of 1500–2000 m asl. The sites were selected based on frequent fire incidences reported during the summer season of 2018 (Uttarakhand Forest Database 2018). Sixteen burnt forest stands and four control forest stands were selected in pine- and oak-dominated forests in both Pauri and Tehri Garhwal districts.

Soil samples were collected in the month of May–June, 2018. Thus, the identified sampling sites to collect soil samples in burnt and control (fire-unaffected site) sites in oak and pine forests in Tehri and Pauri Garhwal districts are referred to as CSO (control site oak forest), BSO (burnt site oak forest), CSP (control site pine forest), and BSP (burnt site pine forest) (Fig. 1). Some of the associated species with pine-dominated forests are reported to be *Rhus parviflora*, *Lantana camara, Carissa spinarum, Anogeissus latifolia, Buchanania latifolia, Phyllanthus emblica, Pyrus pashia, Syzygium cumini, Bombax ceiba, Lannea coromandelica, Myrica esculenta, Rhododendron arboreum, Lyonia ovalifolia, Benthamidia capitata,* and *Carpinus viminea*. Associated species with oak-dominated forest are reported to be *Myrica esculenta, Pyrus pashia, Symplocos chinensis, Fraxinus micrantha, Juglans regia,* and *Cornus capitata* (Sheikh and Kumar 2010).

Collection of soil samples

Soil samples were collected from control sites and burnt sites (surface fire) of the forests having similar forest stand/community. After removing litter and debris in the burnt and control plots, soil samples were collected from the upper soil surface (0–15 cm) at randomly located points at each plot. Thus, a total of 80 samples were collected as burnt soil and 20 samples as control soil (a site where no forest fire incidences occurred for more than 6 years) in oak and pine forests. In each oak and pine forests, four burnt forest stands and one control forest stand (unburnt) were selected in each district. In each forest stand, five random points were selected to collect soil samples. The collected soil samples were air-dried, crushed, and passed through a 2-mm sieve. Soil samples were divided into two parts for the physico-chemical and nutrient analysis, and microbial properties. The soil samples were stored at 4 °C until microbial and enzymatic activities were analyzed.

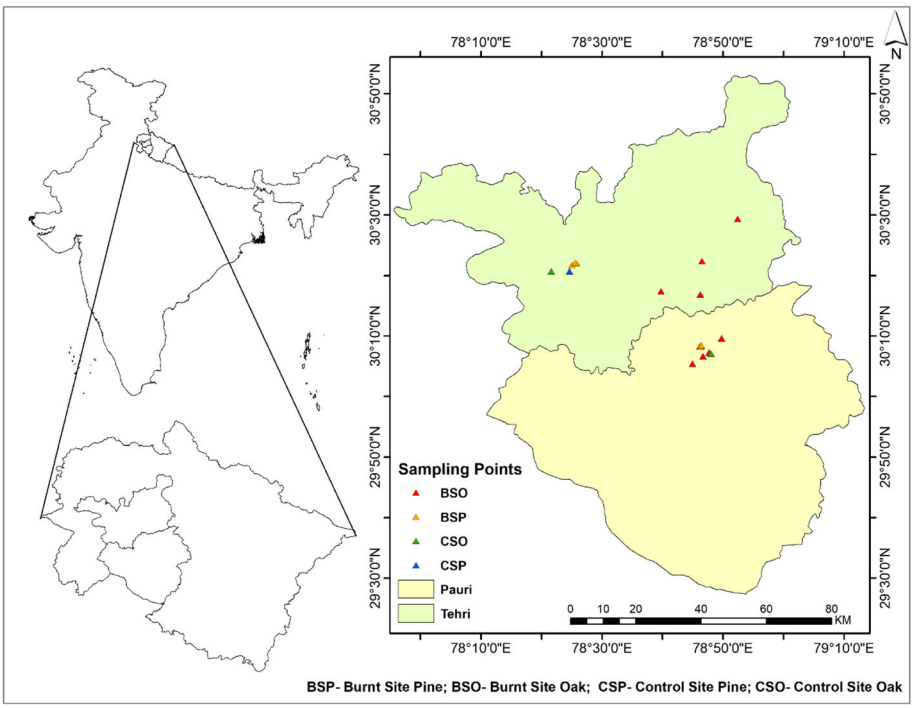

Fig. 1 Soil sampling locations in oak and pine forests of Tehri and Pauri Garhwal districts in Uttarakhand, Himalayas

Soil analysis

Soil physicochemical analysis

Soil pH was determined in 1:2.5 soil:water ratio using the pH meter with glass electrodes (Jackson 1973); electrical conductivity was determined by the method of Jackson (1973). The organic carbon in the soil was estimated by Walkley and Black (1934). The organic carbon values were then multiplied by a factor of 1.724 to estimate soil organic matter content. Total nitrogen (TN) was determined by alkaline potassium permanganate (Subbaiah 1956).

Soil microbial analysis

For estimation of microbial biomass and soil basal respiration, the soil moisture content was adjusted to 60% and incubated for a week to estimate microbial biomass. The microbial biomass carbon (C_{mic}) was then estimated by using the chloroform fumigation method as reported by Vance et al. (1987). The microbial biomass nitrogen (N_{mic}) was estimated by using the chloroform fumigation method (Brookes et al. 1985; Joergensen and Brookes 2005).

Briefly, for each soil sample, three of six fresh subsamples (10 g dry weight equivalent) were fumigated with ethanol-free chloroform for 24 h in vacuum desiccators, another three were not fumigated as the control. The soil samples were extracted with 0.5 M K_2SO_4 (the ratio of soil/extractant was 1:4) for 30 min (300 rpm) in an oscillator. The resulting extracts were filtered. C_{mic} in the filtered samples was determined by the potassium dichromate method. Organic C in the extracts was oxidized by dichromate digestion. The amount of dichromate left was determined after redox titration by the change in color from violet to dark green. In the presence of a strong acid and dichromate, organic matter is oxidized and Cr(+VI) is reduced to Cr(+III). The amount of dichromate left was back-titrated with an iron II ammonium sulfate complex solution (Kalembasa and Jenkinson 1973), and the amount of C oxidized was calculated. The N was estimated by the Kjeldahl method. C_{mic} and N_{mic} were computed by differences between fumigated and un-fumigated samples with a conversion factor of 0.38 for C_{mic} (Vance et al. 1987) and 0.54 for N_{mic} (Brookes et al. 1985). Soil basal respiration (SBR) was calculated by the alkali absorption method. Briefly, moist soils (25 g dry weight equivalent) were adjusted to 60% of the field holding capacity and preincubated at 25 °C for 20 days. The incubated soils were spread on the bottom of 500-ml glass jars in which an absorption bottle with 10 ml NaOH (0.1 M) solution was hanged. After incubation at 25 °C for 24 h, 2 ml of 0.5 M $BaCl_2$ (barium chloride) and 2 drops of phenolphthalein indicator were added into the bottles and then titrated with 0.1 M HCl. The jars without soil served as the controls. The difference of consumed volume of HCl (hydrochloric acid) between the treatment and the control in titration was used to calculate the quantity of CO_2 evolution from soil microbes, 1 ml of 0.1 M consumed NaOH (sodium hydroxide) was equivalent to 2.2 mg CO_2.

Soil enzymatic activity

The activity of dehydrogenase was estimated by using the protocol given by Casida et al. (1964). Briefly, 2 g of moist soil was taken in a test tube, and 2 ml of the substrate (1% TTC, Triphenyl tetrazolium chloride in 0.1 M Tris buffer) was added to the test tube. The blank sample contained 2 ml buffer instead of substrate. The mixture was then incubated in dark at 25 °C for 24 h. The product of the hydrolytic reaction (i.e., formazan) was extracted using 10 ml 1:1 DMF-ethanol (Dimethylformamide ethanol) by shaking the tubes. The tubes were kept in dark for 10 min. The liquid phase was carefully pipetted into the centrifuge tubes and centrifuged at 4 °C and 4500 rpm for 10 min before taking its absorbance at 485 nm. The dehydrogenase activity was expressed as microgram per gram per hour (μg/g/h).

The activity of acid phosphatase was measured following the standard protocol given by Tabatabai and Bremner (1969). *p*-nitrophenyl phosphate (*p*-NPP) was used as a substrate. Briefly, in 1 g of soil, 4 ml of tris (hydroxymethyl) aminomethane buffer (pH = 5.8), 0.25 ml of toluene, and 1.0 ml of *p*-nitrophenyl phosphate was added. The reaction mixture was mixed and incubated at 37 °C for 1 h without shaking. After 1-h incubation, 4 ml of 0.5 M NaOH and 1.0 ml of 0.5 M $CaCl_2$ (calcium chloride) were added to terminate the reaction. The mixture was shaken and transferred to a 10-ml centrifuge tube and centrifuged at 2500 rpm for 10 min. The supernatant liquid was then drawn off and transferred to a colorimeter tube; the optical density of the liquid was determined at 420 nm. The acid phosphatase activity was expressed as μg/g/h.

Statistical analysis

Paired *t* test and factor analysis were performed to evaluate the impact of forest fire on physicochemical, microbial, and enzymatic properties of soil. Correlation analysis was performed to find out the relationship between physicochemical and microbial properties. The analysis was carried out using SPSS 23.0 software.

Factor analysis was performed to find out the differences in soil characteristics of control and burnt sites depending upon the soil variables showing soil physical, chemical, and microbial properties. Thirteen variables (C_{mic}, N_{mic}, SBR, DHA, ACP, pH, moisture, OC, CEC, AN, AP, AK, Cu) were analyzed based on their

significance (i.e., that shows a significant change in control and burnt situation), tests for normality, and strong collinearity. These variables were subjected to a normality test (Shapiro–Wilk normality test) and suitably log-transformed before applying factor analysis. Bartlett's test of sphericity is applied to check that the correlation matrix is different than the Identity matrix (i.e., whether data are correlated too high). The test rejects the null hypothesis, and hence, no too high collinearity exists. Based on eigenvalues of the factors (scree plot) and the values of factor loadings concerning factors and variables, two factors were extracted both for the control as well as burnt sites.

Results

Soil physicochemical properties

Soil physical and chemical properties of the same study sites were reported by Sharma (2018), who studied the effect of forest fire on nutrient dynamics. The percentage composition of soil from the burnt and control sites of pine and oak forests are presented in Table 1. The pine forest soil is classified as clayey, while the oak forest is classified as sand loamy (Table 1). Soil pH of burnt pine and oak forests was 0.41 and 0.78 units higher than that of the control site of pine forest and oak forest, respectively. The mean value of electrical conductivity of pine and oak forests at control sites and burnt sites were 1.95 and 1.96 mS/m and 3.12 and 2.98 mS/m, respectively. Average values of percentage of soil organic carbon in pine forest varied from 1.4 to 1.5 at the control site and 1.8 to 2.7 at burnt sites. The percentage of soil organic carbon (C_{org}) in oak forests varied from 1.79 to 1.85 at control sites and 2.18 to 2.87 at burnt sites. The level of total nitrogen (TN) varied from 0.10 to 0.16 at control sites and 0.22 to 0.23 at burnt sites of pine forests. In the oak forest, the level of percentage of nitrogen varied from 0.12 to 0.18 at the control and 0.18 to 0.24 at burnt sites. In a pine forest, the percentage of C_{org} varied from 2.4 to 2.6 at the control site and 3.1 to 4.7 at burnt sites, whereas in the oak forest, its values varied from 3.1 to 3.2 at control sites and 3.7 to 4.9 at burnt sites. The average values (± standard error) of the percentage of soil moisture of pine forest were 27.56 ± 0.35 and 20.59 ± 0.53 at burnt sites, whereas these values in the oak forest were 39.20 ± 0.35 and 16.82 ± 0.53 at the control and burnt site, respectively. All the soil properties were found to be significantly changed at burnt and control sites.

Soil microbial properties

A *t* test was used to compare the mean values of control and burnt soil samples of two sites and the two sites showed significant differences in properties ($p \leq 0.05$) (Table 2). C_{mic} at the burnt site of both oak and pine forests showed greater significant changes while N_{mic} of the pine forest at Tehri district was found to be significant after burning than the oak forest. SBR of the pine forest of Pauri district showed significant changes when compared with the oak forest of the same district while a burnt oak forest of Tehri district showed greater changes than the pine forest in Tehri. Figures 2a–3e depict box plots of soil microbial properties in oak and pine forests at the control and burnt sites of Tehri and Pauri. The mean values of C_{mic} at control sites of the pine forest ranged from 276.1 ± 1.3 (Tehri) to 634.8 ± 3.4 μg/g (Pauri) and at burnt site 213.6 ± 14.5 (Tehri) to 243.2 ± 27.2 μg/g (Pauri). In the oak forest, C_{mic} varied from 418.5 ± 6.8 (Tehri) to 785.1 ± 4.4 μg/g (Pauri) at control sites, whereas C_{mic} varied from 189.61 ± 8.3 (Pauri) to 211.1 ± 12.9 (Tehri) (Fig. 2a). N_{mic} of the pine forest at the control site varied from 58.83 ± 1.37 (Tehri) to 65.07 ± 2.25 μg/g (Pauri), whereas at the burnt site, its values varied from 51.51 ± 0.51 (Tehri) to 59.97 ± 1.63 μg/g (Pauri). In the oak-dominated forest, N_{mic} was found to be 79.58 ± 2.1 (Pauri) and 87.22 ± 2 μg/g (Tehri) at control sites. N_{mic} at burnt sites was found to be 52.68 ± 2.3 (Tehri) and 64.46 ± 2.9 μg/g (Pauri) (Fig. 2b). The mean values of SBR in control sites of pine forests were 16.27 ± 0.2 (Tehri) to 17.18 ± 0.07 μg/g/h (Pauri). The values of SBR at burnt sites of pine forest were 8.03 ± 0.7 (Pauri) and 14.96 ± 0.4 μg/g/h (Tehri). In the oak forest, the values of SBR in control sites were found to be 18.81 ± 0.41 (Tehri) and 21.5 ± 0.5 μg/g/h (Pauri), and at burnt sites, their values were 12.83 ± 0.94 (Tehri) and 17.71 ± 1.3 μg/g/h (Pauri) (Fig. 2c). Overall, the results indicate that the effect of fire was different on C_{mic} and SBR.

Table 1 Soil texture compositions at depth of 0–15 cm in oak and pine forests of burnt and control sites at Pauri and Tehri (%)

Soil texture	Site 1 (Pauri)				Site 2 (Tehri)			
	Pine		Oak		Pine		Oak	
	Burnt	Control	Burnt	Control	Burnt	Control	Burnt	Control
Sand	43.4 ± 0.4	40.4 ± 0.4	74.7 ± 0.6	72.7 ± 0.8	46.6 ± 0.3	44.7 ± 0.7	71.2 ± 1.9	68.8 ± 1.8
Silt	15.6 ± 0.2	15.3 ± 0.6	22.9 ± 0.3	24.2 ± 0.5	20.6 ± 0.4	20.4 ± 0.3	14.6 ± 0.3	15.4 ± 0.7
Clay	41.3 ± 0.4	44.3 ± 0.6	2.4 ± 0.1	3.1 ± 0.4	32.8 ± 0.9	34.9 ± 0.6	14.2 ± 0.6	15.8 ± 0.8

Table 2 Comparison of physicochemical and biochemical properties of soil in oak and pine forests of Tehri and Pauri districts using *t* test. Entries in the table correspond to *p* values for the same soil parameters in control (C) and burnt (B) conditions

Parameters[a]	Pine forest		Oak forest	
	Pauri	Tehri	Pauri	Tehri
	C vs B	C vs B	C vs B	C vs B
C_{mic}	0.000^{***}	0.02^{**}	0.000^{***}	0.001^{***}
N_{mic}	0.06^{*}	0.008^{***}	0.01^{**}	0.19
SBR	0.001^{***}	0.01^{**}	0.03^{**}	0.005^{***}
DHA	0.04^{**}	0.03^{**}	0.008^{***}	0.009^{***}
ACP	0.007^{***}	0.006^{***}	0.007^{***}	0.0003^{***}
C_{mic}/N_{mic}	0.002^{***}	0.05^{*}	0.0001^{***}	0.002^{***}
C_{mic}/C_{org}	0.001^{***}	0.000^{***}	0.000^{***}	0.002^{***}
N_{mic}/TN	0.001^{***}	0.01^{**}	0.000^{***}	0.05^{*}

[a]C_{mic} soil microbial biomass carbon, N_{mic} soil microbial biomass nitrogen, *SBR* soil basal respiration, *DHA* dehydrogenase activity, *ACP* acid phosphatase activity, *TN* total nitrogen;
*** $p \leq 0.001$ (highly significant), ** $p \leq 0.05$ (medium significant), * $p \leq 0.1$ (less significant)

Soil enzymatic properties

The DHA activities in both forest types (pine and oak) and at both regions (Pauri and Tehri) were increased after fire. The oak forest showed greater significant differences in this enzyme activity as compared with the pine forest, while an increase in ACP activities was observed after fire, but the changes are similar. DHA activity varied 0.24 ± 0.02 (Pauri) to 0.81 ± 0.02 μg/g/h (Tehri) at control sites and 0.68 ± 0.09 (Pauri) to 3.71 ± 0.25 μg/g/h (Tehri) at burnt sites. In the oak forest, DHA activity varied from 0.96 ± 0.02 (Pauri) to 2.30 ± 0.16 μg/g/h (Tehri) at control sites and 1.44 ± 0.2 (Pauri) to 3.6 ± 0.46 μg/g/h (Tehri) at burnt sites (Fig. 2d). ACP activity of the pine forest at control sites varied from 253.74 ± 1.6 (Pauri) to 320.49 ± 8.21 μg/g/h (Tehri) and from 167.94 ± 11.6 (Pauri) to 213.74 ± 13.02 μg/g/h (Tehri) at the burnt site. ACP activity of the oak forest varied from 252.93 ± 2.3 (Pauri) to 290.18 ± 2.9 μg/g/h (Tehri) at control sites and from 155.32 ± 14.95 (Pauri) to 177.27 ± 3.3 μg/g/h (Tehri) at the burnt sites (Fig. 3e). The control and burnt sites of oak and pine forests showed significant differences in enzymatic activities.

Factor analysis for soil physicochemical and microbial properties at the control and burnt sites of oak and pine forests

Table 3 presents the factor loadings and communality for each of the soil parameters for both the control and burnt sites. For the control sites, factor 1 predominantly had a strong correlation with soil physicochemical properties [moisture (0.8), OC (0.53), AN (0.98), AP (0.73), AK (0.615), Cu (− 0.978)], while factor 2 predominantly had a strong correlation with microbial properties of the soil [C_{mic} (0.99), N_{mic} (0.96), SBR (0.71), ACP (− 0.95)]. For the control sites, the communality column reveals that most of the variances of these soil parameters can be explained based on these two factors, e.g., 99.5% variance in C_{mic}, 96.3% variance in N_{mic}, 71.5% variance in SBR, 94.6% variance in ACP, 79.1% variance in pH, 80.4% variance in moisture, 97.8% variance in AN, 73.4% variance in AP, 61.5% variance in AK, and 97.8% variance in Cu can be explained by these two factors only. It

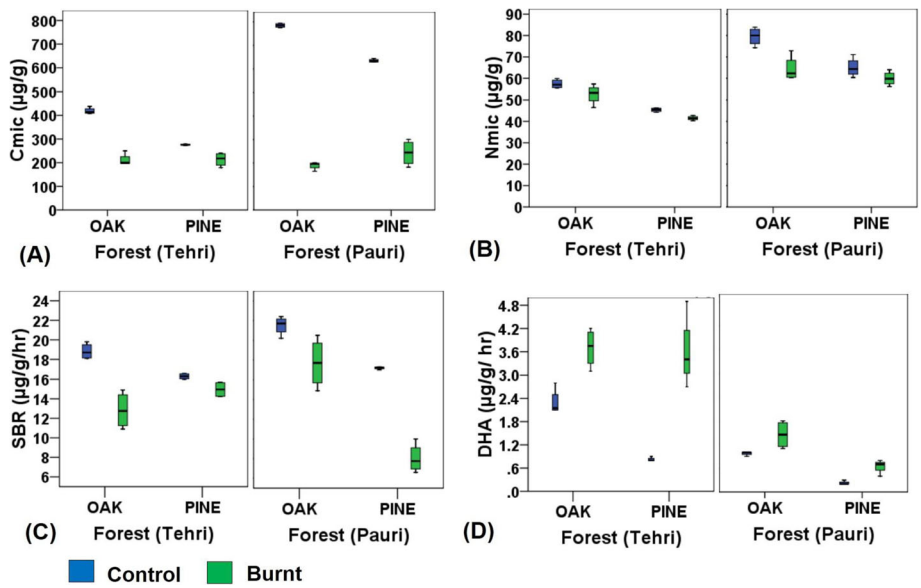

Fig. 2 Mean values of soil microbial properties at the control and burnt sites in two different forests (C_{mic}, soil microbial biomass carbon; N_{mic}, soil microbial biomass nitrogen; *SBR*, soil basal respiration; *DHA*, dehydrogenase activity)

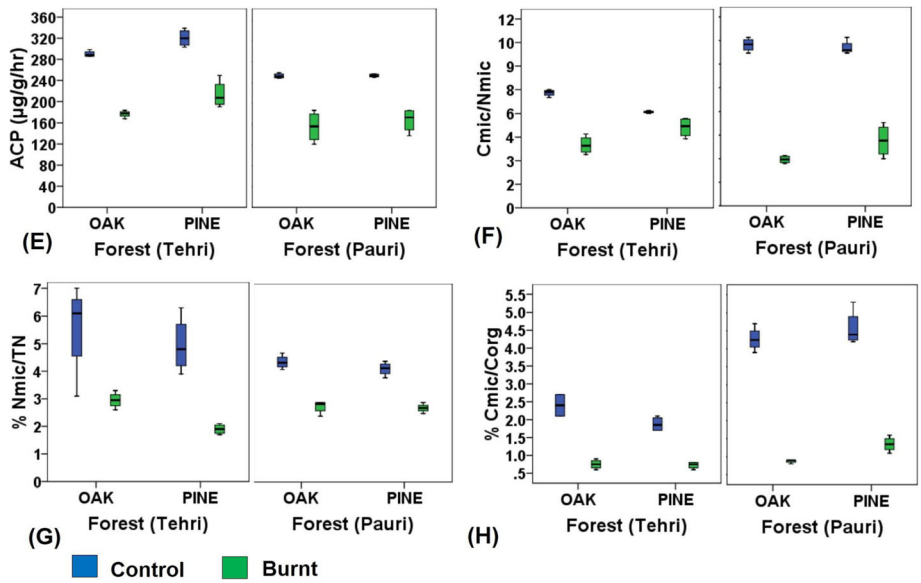

Fig. 3 e and **f**–**h** represent mean values of acid phosphatase activity (ACP) and soil microbial indices (C_{mic}/N_{mic}, $\%N_{mic}/TN$, $\%C_{mic}/C_{org}$) respectively at the control and burnt site in two different forests (C_{mic}, soil microbial biomass carbon; N_{mic}, soil microbial biomass nitrogen; C_{org}, organic carbon; *TN*, total nitrogen)

is also evident from the commonalities that for most of the soil parameters, these variances can be explained based on a single factor, either factor 1 or 2.

However, all these characteristics changed dramatically when the factor loadings and communality for the same soil parameters were examined for the burnt site. Microbial biomass carbon (C_{mic}) had no significant correlation with factor 1 and had a weak correlation (– 0.316) with factor 2. Microbial biomass nitrogen (N_{mic}) strongly negatively correlated (– 0.832) with factor 1 as against factor 2 at the control site. At the control site, SBR had a strong correlation with factor 1, while at the burnt sites, it changed and showed a weak correlation with both factor 1 and factor 2. ACP also had a strong correlation with factor 2 at the control site, but at the burnt site, it majorly associated (0.66) with factor 1. Available nitrogen (AN) at burnt site majorly associated itself with factor 2 as against factor 1 at the control site. The

Table 3 Factor analysis for the control and burnt sites for the measured soil parameters

Parameter[a]	Control sites			Burnt sites		
	Factor loadings		Communality	Factor loadings		Communality
	Factor 1	Factor 2		Factor 1	Factor 2	
C_{mic}		0.991	0.995		– 0.316	0.103
N_{mic}		0.962	0.963	– 0.832		0.995
SBR		0.715	0.940	0.501	0.464	0.466
DHA	0.958		0.954	0.923		0.855
ACP		– 0.946	0.941	0.666	– 0.501	0.695
pH	– 0.791		0.948		– 0.959	0.921
Moisture	0.804		0.898		0.255	0.066
OC	0.524		0.672	0.866		0.783
CEC		0.844	0.965	-0.539	0.502	0.543
AN	0.978		0.978		0.908	0.829
AP	0.734		0.642	0.590	0.310	0.444
AK	0.615		0.558	0.806		0.656
Cu	– 0.978		0.993	– 0.364	– 0.368	0.268

[a]C_{mic} soil microbial biomass carbon, N_{mic} soil microbial biomass nitrogen, *SBR* soil basal respiration, *DHA* dehydrogenase activity, *ACP* acid phosphatase activity, *OC* organic carbon, *CEC* cation exchange capacity, *AN* available nitrogen, *AP* available phosphorus, *AK* available potassium, *Cu* copper

association of Cu with factors also changed significantly at the burnt site. The variances explained by these two factorsalso changed mainly got reduced for most soil parameters. Only 10.3% of variances in C_{mic}, 46.6% variances in SBR, 69.5% variances in ACP, 6.6% variances in moisture, and 26.6% variances in Cu were explained by these two factors. For microbial biomass nitrogen (N_{mic}), DHA, pH, OC, AN, and AK values were 99.5, 85.5, 92.1, 78.3, 82.9, and 65.6%, respectively. Figures 4 and 5 present these changes on how the soil parameters differently correlate with factor 1 and factor 2 at the control and burnt sites.

Discussion

Soil microbial biomass

Similar to our findings, reduction in microbial biomass after the fire has been reported in many studies (Girona-García et al. 2018; Holden and Treseder 2013; Strand 2011). It has been suggested that burning kills microbes which result in the reduction of microbial biomass. The changes in the nutrient supply due to the loss of plant residues could also be a reason for the reduction in microbial biomass after fire (Mabuhay et al. 2003; Smith et al. 2008). Soil basal respiration (SBR) was also found to be lower at burnt sites, which is obvious as microbial biomass gets reduced after the fire. Mabuhay et al. (2003) and Smith et al. (2008) also reported a similar decrease in SBR after the fire. However, Sadeghifar et al. (2020) in their study did not find a significant decrease in SBR after 1 year of the fire, though it decreased significantly after 3 and 10 years of post-fire.

Reduction in C_{mic} of pine forests in Pauri and Tehri district were 61.7 and 17.4%, respectively, whereas in the oak forest, the percentage reductions of C_{mic} were much higher (75.8% in Pauri and 49.6% in Tehri district). Microbial biomass at the control and burnt sites of the oak forest was found to be greater as compared with the pine-dominated forest. This might be because the oak forest has higher C_{mic} than the pine forest due to the greater litter input, which provides a greater carbon source pool for microbial utilization.

Microbial biomass nitrogen (N_{mic}) of pine forest was reduced by 37% at Pauri and 22.2% at Tehri, whereas in the oak forest its values were 16.3% at Pauri and 8.8% at Tehri. SBR in the pine forest was reduced by 53.3 and 61.3% at Pauri and Tehri, whereas in the oak forest, reductions of SBR were 17.6 and 31.8% at Pauri and Tehri, respectively. These results suggest that forest types and location (altitude) significantly affect N_{mic} and SBR.

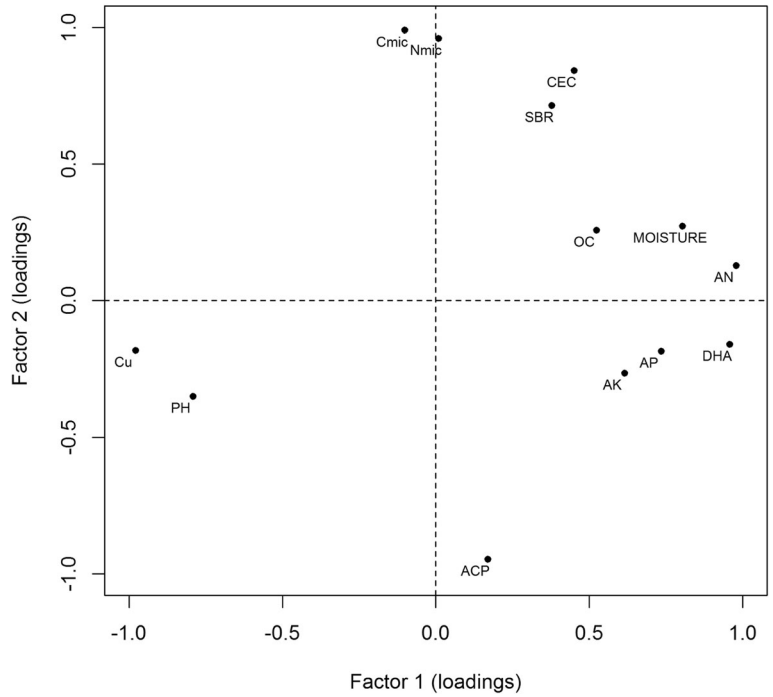

Fig. 4 Two factor components extracted from the variables for physicochemical and microbial properties of control sites of pine and oak forests (C_{mic}, soil microbial biomass carbon; N_{mic}, soil microbial biomass nitrogen; *SBR*, soil basal respiration; *DHA*, dehydrogenase activity; *ACP*, acid phosphatase activity; *OC*, organic carbon; *CEC*, cation exchange capacity; *AN*, available nitrogen; *AP*, available phosphorus; *AK*, available potassium; *Cu*, copper)

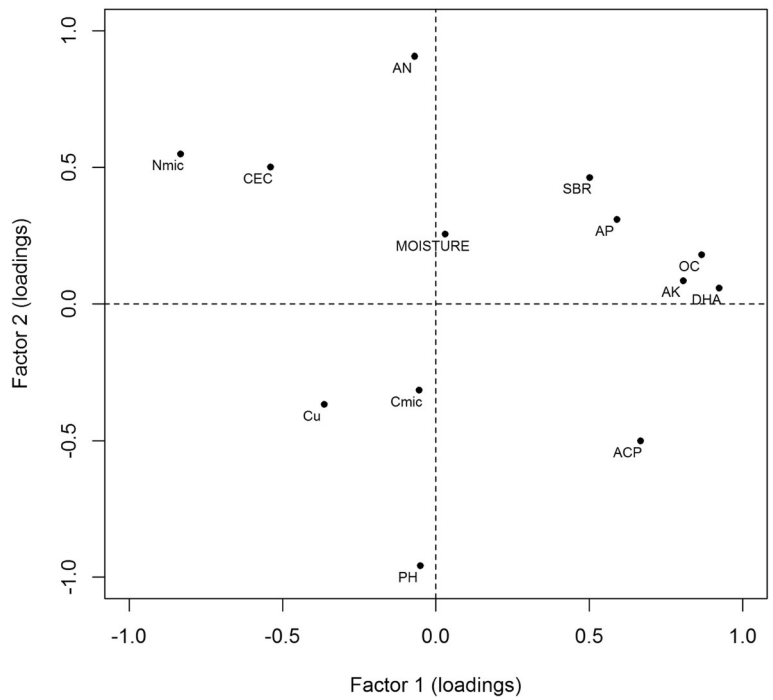

Fig. 5 Two factor components extracted from the variables for physicochemical and microbial properties of burnt sites of pine and oak forests (C_{mic}, soil microbial biomass carbon; N_{mic}, soil microbial biomass nitrogen; *SBR*, soil basal respiration; *DHA*, dehydrogenase activity; *ACP*, acid phosphatase activity; *OC*, organic carbon; *CEC*, cation exchange capacity; *AN*, available nitrogen; *AP*, available phosphorus; *AK*, available potassium; *Cu*, copper)

Soil microbial indices were also measured to estimate the impact of forest fire on soil quality. The result indicates that C_{mic}/N_{mic}, C_{mic}/C_{org}, and N_{mic}/TN significantly decreased after burning (Table 2, Fig. 3f–h). The C_{mic}/C_{org} percentage can be a useful indicator to predict the quality of soils having differences in their organic matter content (Balota et al. 2003). The percentage of C_{mic}/C_{org} will decrease at a faster rate when the soil is degraded, and this decrease is due to a decline in the microbial C pool (Kara and Bolat 2009). C_{mic}/N_{mic} percentage can be used to predict whether the soils were accumulating or losing C. In this study, the percentage of C_{mic}/C_{org} and N_{mic}/TN was found to be significantly lower as compared with control sites. The decrease in these indices at the burnt site can be attributed to the decrease in microbial biomass carbon and nitrogen and the increase in organic carbon and total nitrogen. The lower C_{mic}/C_{org} percentage reflects the inefficiency of microbial biomass to consume organic substrates (Anderson and Domsch 1989; Kara and Bolat 2009). Zhong and Makeschin (2006) suggested that C_{mic}/N_{mic} ratio can be used to describe the structure and state of a microbial community. The lower C_{mic}/N_{mic} ratio in the range 3–5 indicates the dominance of bacteria in the microbial community, while a higher C_{mic}/N_{mic} ratio in the range 4–5 is an indication of the dominance of fungi in the community. In our study, C_{mic}/N_{mic} ratio at burnt sites significantly lower than the control site. Similar to our results, Hamman (2007) and Smith et al. (2008) reported a decrease in C_{mic}/N_{mic} ratio at the burnt site. The decrease in this ratio at the burnt site indicates that the bacterial population tends to increase after the fire because of the more available carbon sources. The higher ratio at the control site indicates more dominance of fungi over bacteria; however, this ratio was found to be higher in the oak forest as compared with the pine forest in both control and burnt sites. The higher ratio signifies that as compared with the pine forest, the oak forest has high fungi population than the bacterial population. The correlation matrix shows a positive correlation among C_{mic}, SBR, and soil moisture. SBR indicates the microbial activity in the soil, and it reflects the quality and quantity of carbon source present in the soil. This suggested rapid decomposition of organic matter by the microbes to make nutrients available for the stimulation of heterotrophic microorganisms. Since microbes required water for their proper functioning, therefore, a positive correlation exists between the

biomass and soil moisture (Nannipieri et al. 1983; Kandeler and Eder 1993).

Soil enzymatic properties

The activity of DHA increased at burnt sites (Fig. 2d). The increase in activity of this enzyme can be the result of an increase in the organic carbon content of the soil at the burnt site and an increase in the metabolic activity of microbes as this enzyme is involved in carrying out the redox reaction of the cell. A strong positive correlation of DHA activity with organic carbon (r = 0.80) was found in our study. A similar positive correlation between DHA activity and soil organic carbon is reported in the literature by other authors (Leirós et al. 1999; Zhang et al. 2010). Zhang et al. (2010) reported that an increase in the amount of organic carbon improves soil oxidative activity, which increases the DHA activity. Therefore, as the soil organic matter content of the oak forest is higher than that in the pine forest, DHA activity of the oak forest was also higher at the burnt site of the oak forest.

On the contrary, the ACP activity decreased after the fire (Fig. 3e). ACP activity was found to be reduced by ~33% and ~39% in pine and oak forests, respectively at Pauri as well as the Tehri region. Similar observations were reported in the literature by many authors (Girona-García et al. 2018; Knelman et al. 2015; Nannipieri et al. 1983; Kandeler and Eder 1993). The decrease in soil microbial biomass and increase in soil phosphorus content after fire leads to the decreased activity of ACP (Fernández-García et al. 2019). The increase in soil available phosphorus resulted in the reduction of acid phosphatase activity due to the lesser need for these enzymes by microbes (Hamman et al. 2008).

Correlation among soil microbial biomass, enzymatic activities, and nutrients

The correlation matrix (Table 4) shows that soil C_{mic} was positively correlated with N_{mic}, soil moisture content, C_{mic}/N_{mic}, C_{mic}/C_{org}, and ACP activity. N_{mic} had a significant positive correlation with C_{mic}, C_{mic}/C_{org}, and C_{mic}/N_{mic} while a significant negative correlation with DHA and pH. Microbial biomass carbon (C_{mic}) was found to have a negative correlation with soil pH, and soil organic carbon (SOC). Arunachalam (2002) also reported a negative correlation of C_{mic} with soil pH, BD, and SOC after burning in agricultural fields. The reason for this contradictory negative correlation of C_{mic} with SOC showed that after fire C_{mic} is independent of SOC. The abrupt increase in the level of SOC immediately after burning might have affected the normally expected positive relationship. SBR had a positive correlation with soil moisture, ACP activity and a negative correlation with soil pH.

ACP activity was positively correlated with C_{mic} because these enzymes are produced by microbes to maintain the phosphorus concentration in soil. Nannipieri et al. (1983) also reported a positive correlation of ACP activity with microbial biomass. Recently, Fernández-García et al. (2019) also observed similar observations and reported a decrease in both ACP activity and microbial biomass after the fire. A strong positive correlation has been found between DHA activity and SOC, SOM, and EC while a negative correlation with microbial biomass and microbial indices. ACP activity was found to have a positive correlation with soil microbial indices and soil moisture while a negative relation with SOC, OM, CEC, and TN.

Micronutrients showed a strong correlation with the properties of soil (Table 5). Microbial biomass carbon, microbial biomass nitrogen, and SBR showed a strong correlation with Zn, Fe, Mn and Cu, and ACP activity. Microbial biomass was found to be negatively correlated with available potassium, sodium, and phosphorus. Among enzymatic activity, DHA was found to be positively correlated with available potassium (AK) and phosphorus (AP) and negative correlation with Cu and Zn. ACP activity showed a positive correlation with Fe, Zn, and Cu and a negative correlation with Mg, Na, AK, and AP. Microbial indices showed a positive correlation with Zn, Fe, and Cu and a strong negative correlation with Mg, Na, AK, and AP.

The microbes for the various metabolic activity required micronutrients (Fe, Zn, and Cu) in a certain concentration. Above the required concentration, they work as a toxicant and can inhibit or even kill the microbes. Cu is required in many enzymatic reactions by the enzyme produced by microbes. The positive correlation between Fe, Cu, and Zn with microbes shows that microbial population is influenced by the changes in these micronutrients. Since the concentration of Fe and Zn decreased after the fire, a similar pattern of decrease in microbial biomass was also observed. García-Marco and González-Prieto (2008) reported that the availability of Fe decreased after a forest fire, and a decrease in Fe could be due to losses and conversion into insoluble forms due to changes in soil pH and other nutrient contents. Soil C/N ratio was found to have a negative correlation with soil pH and total nitrogen, while it had a positive correlation with SOC and SOM. SBR is negatively correlated with soil pH. Similar to our findings, the lower C/N ratio after the fire has been reported by Rodríguez et al. (2017). The authors suggested that this reduction in the ratio was due to the formation and accumulation of new forms of recalcitrant heterocyclic nitrogen and to the volatilization of organic carbon compounds (Rodríguez et al. 2017).

Table 4 Correlation between microbial and physicochemical properties of soil in oak and pine forests of Tehri and Pauri Garhwal

Parameter[a]	C_{mic}	N_{mic}	SBR	DHA	ACP	pH	EC	BD	CEC	TN	OM	Moisture	SOC	N_{mic}/TN	C_{mic}/C_{org}	C_{mic}/N_{mic}
C_{mic}	1	0.703**	0.621**	− 0.450**	0.442*	− 0.406*	− 0.255	− 0.382*	− 0.433*	− 0.231	− 0.504**	0.633**	− 0.499**	0.454**	0.952**	0.948**
N_{mic}	0.703**	1	0.386*	− 0.513**	− 0.136	− 0.507**	− 0.185	− 0.057	0.137	0.217	− 0.347	0.372*	− 0.339	0.202	0.625**	0.480**
SBR	0.621**	0.386*	1	− 0.125	0.511**	− 0.663**	− 0.107	− 0.629**	− 0.375*	− 0.309	− 0.211	0.656**	− 0.209	0.471**	0.547**	0.599**
DHA	− 0.450**	− 0.513**	− 0.125	1	− 0.207	− 0.012	0.760**	− 0.228	0.340	0.172	0.807**	− 0.175	0.802**	− 0.369	− 0.583**	− 0.393*
ACP	0.442*	− 0.136	0.511**	− 0.207	1	− 0.245	− 0.286	− 0.638**	− 0.891**	− 0.803**	− 0.533**	0.628**	0.535**	0.703**	0.498**	0.625**
pH	− 0.406*	− 0.507**	− 0.663**	− 0.012	− 0.245	1	− 0.087	0.634**	0.101	0.305	0.096	− 0.682**	0.095	− 0.535**	− 0.313	− 0.292
EC	− 0.255	− 0.185	− 0.107	0.760**	− 0.286	− 0.087	1	− 0.367*	0.483**	0.275	0.728**	0.014	0.731**	− 0.319	− 0.473**	− 0.327
BD	− 0.382*	− 0.057	− 0.629**	− 0.228	− 0.638**	0.634**	− 0.367*	1	0.428*	0.522**	0.016	− 0.720**	0.018	− 0.555**	− 0.242	− 0.407*
CEC	− 0.433*	0.137	− 0.375*	0.340	− 0.891**	0.101	0.483**	0.428*	1	0.793**	0.601**	− 0.472**	0.605**	− 0.665**	− 0.539**	− 0.640**
TN	− 0.231	0.217	− 0.309	0.172	− 0.803**	0.305	0.275	0.522**	0.793**	1	0.413*	− 0.559**	0.419*	–.868**	− 0.313	− 0.394*
OM	− 0.504**	− 0.347	− 0.211	0.807**	− 0.533**	0.096	0.728**	0.016	0.601**	0.413*	1	− 0.361*	0.999**	− 0.550**	− 0.680**	− 0.538**
Moisture	0.633**	0.372*	0.656**	− 0.175	0.628**	− 0.682**	0.014	− 0.720**	− 0.472**	− 0.559**	− 0.361*	1	− 0.360*	0.715**	0.564**	0.634**
SOC	− 0.499**	− 0.339	− 0.209	0.802**	0.535**	0.095	0.731**	0.018	0.605**	0.419*	0.999**	− 0.360*	1	− 0.522**	− 0.677**	− 0.535**
N_{mic}/TN	0.454**	0.202	0.471**	− 0.369	0.703**	− 0.535**	− 0.319	− 0.555**	− 0.665**	− 0.868**	− 0.550**	0.715**	− 0.522**	1	0.494**	0.520**
C_{mic}/C_{org}	0.952**	0.625**	0.547**	− 0.583**	0.498**	− 0.313	− 0.473**	− 0.242	− -0.539**	− 0.313	− 0.680**	0.564**	− 0.677**	0.494**	1	0.935**
C_{mic}/N_{mic}	0.948**	0.480**	0.599**	− 0.393*	0.625**	− 0.292	− 0.327	− 0.407*	− 0.640**	− 0.394*	− 0.538**	0.634**	− 0.535**	0.520**	0.935**	1
C/N	− 0.210	− 0.468**	0.121	0.389*	0.401*	− 0.273	0.253	− 0.537**	− 0.317	− 0.713**	0.287	0.336	0.281	0.550**	− 0.263	− 0.75

***$p \leq 0.001$ (highly significant), ** $p \leq 0.05$ (medium significant), *$p \leq 0.1$ (less significant)

[a]C_{mic} soil microbial biomass carbon, N_{mic} soil microbial biomass nitrogen, *SBR* soil basal respiration, *DHA*, dehydrogenase activity, *ACP* acid phosphatase activity, *EC* electrical conductivity, *BD* bulk density, *CEC* cation exchange capacity, *TN* total nitrogen, *OM* organic matter

Table 5 Correlation between microbial properties and nutrients of soil in oak and pine forests of Tehri and Pauri Garhwal

Nutrients	Microbial properties[a]						
	C_{mic}	SBR	DHA	ACP	C_{mic}/N_{mic}	C_{mic}/C_{org}	N_{mic}/TN
Fe	0.73**	0.81**	-	-	0.70**	0.71**	0.76**
Na	− 0.74**	-	-	-	− 0.73**	− 0.72**	-
Available K	− 0.63**	-	0.77**	-	-	− 0.77**	-
Zn	-	0.71**	-	0.71**	-	-	0.76**
Cu	-	-	-	-	-	0.70**	-
Mg	-	-	-	− 0.71**	-	-	-
Mn	-	0.73**	-	-	-	-	-
Available P	-	-	0.70**	-	− 0.71**	− 0.73**	-

***$p \leq 0.001$ (highly significant), ** $p \leq 0.05$ (medium significant), *$p \leq 0.1$ (less significant)

[a]C_{mic} soil microbial carbon, *SBR* soil basal respiration, *DHA* dehydrogenase activity, *ACP* acid phosphatase activity, N_{mic} soil microbial nitrogen, C_{org} organic carbon, *TN* total nitrogen

Factor analysis

At the control site, factor 1 predominantly correlates with physicochemical properties, while factor 2 predominantly correlates with microbial properties of the soil. The variances (> 90%) of most of the soil parameters are also explained by these two factors. The change in these relations for the burnt site can be explained for each soil parameter based on their characteristics in the soil. Microbial biomass carbon (C_{mic}) no longer significantly associates with these factors at the burnt site neither its variance can be significantly explained by these factors at the burnt site, while at the control site, C_{mic} strongly correlates with factor 2, and 99.5% variance can be explained based on these two factors. At the burnt site, microbial activity is significantly reduced; hence, microbial biomass carbon also significantly drops and no longer associates with factor 2. Microbial biomass nitrogen (N_{mic}) also no longer significantly correlates with factor 2 at the burnt site, most likely due to reduced microbial activity in the nitrogen cycle; however, at the burnt site, it negatively correlates with factor 2 as total nitrogen has increased at burnt sites because left nitrogen at burning sites is degraded less (and nitrogen-fixing by oxidizing species has reduced significantly). SBR strongly correlates with factor 2 at control sites, but it no longer does so at burnt sites as soil basal respiration was reduced at burnt sites due to reduction/absence of microbes and therefore factor 1 and factor 2 no longer explains variances in SBR considerably at burnt sites. DHA strongly correlates with factor 1 at burnt sites, while it strongly correlates with factor 2 at control sites, mainly because DHA increased at burnt sites in absence of other microbial activities. ACP shows a strong correlation with factor 2 at control sites but no longer shows so at burnt sites, which is clear, as microbial activity has reduced at burnt sites. Moisture is expectedly absent at burnt sites and therefore no longer associates with factors at burnt sites. Organic carbon (OC) increased and showed a further stronger correlation with factor 1.

Thus, factor analysis also clearly brought out the impact of forest fire on soil physicochemical and microbial properties and their inter-relationship.

Conclusion

Our results indicated that forest fire has a significant impact on microbial properties and soil enzymatic activity along soil physical and chemical properties. The impact has similar pattern of changes in soil microbial biomass and enzymatic activity irrespective of the forest type. The changes in physical and chemical properties of soil were found to be correlated with soil microbial activity. Soil microbial biomass (C_{mic} and N_{mic}) and SBR were decreased following fire irrespective of forest type. Microbial biomass carbon (C_{mic}) of the oak forest was found to be higher than that of the pine forest. The impact of fire on C_{mic} was greater in the oak forest than in the pine forest as indicated by a greater percentage of reduction after the fire. The overall change in microbial biomass carbon was 63% in the oak forest and 40% in the pine forest. Significant differences in microbial indices indicate that microbial indices reflect the impact of fire. The decrease of C_{mic}/C_{org} percentage and N_{mic}/TN percentage after the fire showed the inability of the microbes to utilize the organic substrates and the inefficiency of the substrate conversion into biomass. Among enzymatic activity, DHA activity was enhanced with the increase in soil organic matter as an impact of fire. This enhanced DHA activity implies an increased rate of oxidation and reduction processes. ACP activity is linked with microbial indices that are associated with the soil quality after the fire. This enzyme is known to play a key role in the release of phosphorus whenever required in

the soil. Its activity was reduced due to the presence of an ample amount of phosphorus in the soil after the fire and was positively correlated with microbial indices because of the killing of microbes due to fire. The overall decrease in C_{mic}/N_{mic} ratio at burnt sites suggests the dominance of the bacterial community over fungi. This implies that the bacteria population tends to increase after the fire because of the more available carbon sources. At the control site, this ratio was higher, indicating more dominance of fungi over bacteria. The oak forest has higher C_{mic}/N_{mic} ratio as compared with the pine forest in both control and burnt sites. The higher ratio signifies that as compared with the pine forest, the oak forest has higher fungi population than the bacterial population. The impact of forest fire on soil physicochemical and microbial properties and their inter-relationship was also revealed by the factor analysis.

Abbreviations

C_{mic}: Microbial biomass carbon; N_{mic}: Microbial biomass nitrogen; SBR: Soil basal respiration; DHA: Dehydrogenase activity; ACP: Acid phosphatase activity; C_{mic}/N_{mic}: Microbial biomass carbon/microbial biomass nitrogen; C_{mic}/SOC: Microbial biomass carbon/soil organic carbon; N_{mic}/TN: Microbial biomass nitrogen/total nitrogen; EC: Electrical conductivity; BD: Bulk density; TN: Total nitrogen; CEC: Cation exchange capacity; SOC: Soil organic carbon; SOM: Soil organic matter; AN: Available nitrogen; AP: Available phosphorus; AK: Available potassium; Mn: Manganese; Mg: Magnesium; Na: Sodium; Fe: Iron; Zn: Zinc; Cu: Copper; Ca: Calcium

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Authors' contributions

DS conducted experiments and collected data, analyzed samples and data, and contributed in writing draft manuscript. PS conducted experiments and collected data, analyzed samples and data, and contributed in writing draft manuscript; UK did supervision, writing – review and editing, and was responsible for funding resources, project administration and funding acquisition; AD did supervision, writing – review and editing, and was responsible for funding resources, project administration, and funding acquisition; KA did supervision, writing – review and editing, and was responsible for funding resources, project administration, and funding acquisition. The authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

None.

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