

## Wildfire and harvesting effects on carbon dynamics in an oak-pine mixed forest

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CO<sub>2</sub> emission to the atmosphere is the main cause of global warming. The impacts of land-use changes for agriculture and urbanisation, deforestation, and fire disturbance are attributed to the increase in CO<sub>2</sub> emissions. Soil respiration, largely due to microbial activity, is one of the CO<sub>2</sub> sources being released to the atmosphere. In this regard, several soil parameters related with carbon cycle, including organic matter, total N, C/N ratio, CO<sub>2</sub> efflux, microbial biomass C ( $C_{mic}$ ), the  $C_{mic}/C_{org}$  ratio, the metabolic quotient  $qCO_2$ , and  $\beta$ -D glucosidase activity, were determined in a burned (harvested, H; non-harvested, NH), and its adjacent unburned (UB), mixed oak-pine forest to estimate the effects of burning and removal of residual woods. The  $C_{mic}$  increased gradually with burning and harvesting after Month 9, and sharp increases were observed in all areas, likely due to the abundant rainfall after Month 12. CO<sub>2</sub> efflux decreased in the burned areas at Months 4 and 6; however, this reversed in Month 9. In spite of non-significant differences, we detected higher CO<sub>2</sub> efflux values in the unburned areas compared to the burned ones, probably as a result of the drought effect observed in the burned areas up to Months 9 and 12 due to the increased soil heat. There was no significant difference between the H and NH burned areas, while both areas were different from the unburned areas in all soil parameters, except CO<sub>2</sub> efflux and  $qCO_2$ . The harvesting effect was not significant compared to the fire effect with regard to the considered soil variables, likely due to the management and protection of the burned area which allowed a fast vegetation recover. The abundance of the microbial biomass was independent of the changes in CO<sub>2</sub> efflux and showed a negative correlation with  $\beta$ -D glucosidase activity. This might be related to the variation in substrate quality, microbial composition and abundance after burning and harvesting.

**Keywords:** CO<sub>2</sub> Evolution,  $\beta$ -D Glucosidase Activity,  $qCO_2$ , Soil Microbial Biomass Carbon, Wildfire

### Introduction

The increase in C stocks in forest ecosystems depends on the preservation of C in tree biomass and soil against degradation. Wildfire and harvesting both affect the soil organic carbon storage by combusting organic matter, volatilization, changing soil structure, vegetation canopy, microclimatic conditions, erosion, litter layer composition, microbial population and their activity, and decomposition (Smith et al. 2008, Kara & Bolat 2009, Mataix-Solera et al. 2009, Poirier et al. 2014, Marañón-Jiménez

& Castro 2013, Akburak et al. 2017). Fire may accelerate the decomposition of organic matter (OM), promoting CO<sub>2</sub> emission. Logging and removal of burned trees increase the CO<sub>2</sub> emissions (about 120 gr C m<sup>-2</sup> in burned pine forests) and reduce the renovation capacity of Mediterranean ecosystems after the wildfire (Serrano-Ortiz et al. 2011). On the other hand, though no significant effect on total ecosystem carbon stocks was observed, a decrease was seen in carbon stored in snags and down woody material after the salvage logging in

burned sub-boreal jack pine forests (Bradford et al. 2012). The soil microflora contributes to soil C storage, soil respiration and ecosystem productivity, despite representing only a small portion of the soil (Bauhus et al. 1997). Microbial biomass C ( $C_{mic}$ ) has been suggested as an indicator of changes in the soil (Bauhus et al. 1997) as a consequence of forest management (Bauhus & Barthel 1995, Pietikäinen & Fritze 1995, Oh-tonen et al. 1992). Fire may alter the microbial abundance both directly by killing microbes soil surface due to heating and indirectly by modifying the community composition and environmental conditions (Docherty et al. 2011, Hart et al. 2005, Yang et al. 2020). Additionally, harvesting may affect these conditions by compacting the soil and causing increases in runoff rates and erosion (Mataix-Solera et al. 2016, García-Orenes et al. 2017). Decreases were found in microbial biomass carbon after harvest (18%), burn (74%), and burn-salvage (53%) treatments (Smith et al. 2008).

The metabolic quotient ( $qCO_2$ ), which is the microbial respiration/microbial biomass C ratio, decreases with increasing the quality of substrate, and increases under un-

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favourable conditions (Anderson & Domisch 1993, Wardle & Ghani 1995, Bauhus et al. 1997, Mahía et al. 2006). Microbial parameters, including  $C_{mic}$ ,  $qCO_2$  and  $qC$ , have been used in several studies (Nannipieri 1994) in order to understand the impacts of soil management or disturbance.

Soil microorganisms apply different strategies to use available C, such as biotic (e.g., release of extracellular enzymes) and abiotic (e.g., redox and metal complexation) mechanisms (Hibbing et al. 2010).  $\beta$ -D glucosidase which takes part in the degradation of cellulose, decreased in burned soils, as reported by Hernandez et al. (1997), but little is known about the harvesting effect of burned residues on this enzyme activity.

The purpose of this study was to understand the relationship between the C dynamics and the microbial activity after a wildfire, and after harvesting of the residues, by using several microbial parameters, including  $CO_2$  evolution,  $C_{mic}$ ,  $qCO_2$ , and  $\beta$ -D glucosidase enzyme activity. It was hypothesized that burning alters the microbial activity related to C dynamics, and harvesting contributes to these changes.

## Materials and methods

### Study area and soil sampling

We conducted this study in a mixed pine (*Pinus nigra* Arnld. subsp. *pallasiana* Lamb.) and oak (*Quercus pubescens* Wild.) forest in Safranbolu, Turkey (41° 16' 11" N, 32° 37' 41" E) following a wildfire occurred on the 9<sup>th</sup> and 10<sup>th</sup> of October, 2011. The fire was caused by a picnic fire, and affected an area of 5 hectares. Fire severity was estimated based on the litter consumption (Ryan & Noste 1985) and whether the ashes whiten or not (Kara & Bolat 2009). The severity of the fire ranged from low to medium due to black ash and bare soil surface dominated the majority of the site. Additionally, this

determination also complies with the criteria of Ryan & Noste (1985) modified from Ryan (2002) and Turner et al. (1994) (Kealey 2009). We did not measure the percentage of vegetation cover, but based on our observation the understory was mainly composed of *Phillyrea latifolia* L., *Cistus creticus* L., *Juniperus oxycedrus* L., *Colutea ciliata* Boiss. & Balansa, and *Rubus* sp. The annual rainfall was between 630 and 650 mm in the region during the study period. The elevation of the area was 720-760 m a.s.l., and the slope 30-45%. The soil with typical A-C horizons belongs to the order intrazonal and suborder calsimorphic and was classified as a rendzina, according to FAO/UNESCO (IUSS-WRB 2014, Soil Survey Staff 2014). The main characteristics of the soils in the study area are given in Tab. 1. The area was covered with residues, consisting of twigs, for the protection of the soil and the new generation. In several parts of the burnt area the residual trees were removed (harvested).

Soil sampling was conducted in Months 4, 6, 9, 12 and 24 after the fire. We selected 22 plots (10 × 10 m<sup>2</sup>) including eight burned-non harvested (NH), eight burned-harvested (H) and three adjacent, unburned areas for each species. Three soil samples per plot were collected at depths of 0-7.5 cm, following removal of the surface residues, including oak leaves and pine needles. We collected the samples from low- and medium-severity burned areas in order to achieve homogeneity. Soil samples were transferred to the laboratory, sieved through a 2-mm mesh and stored at +4 °C.

### Laboratory analyses

The  $CO_2$  evolution was determined according to the method of Isermeyer (1952), modified by Alef (1995). Following incubation of the samples with NaOH traps for 24 hours at 25 °C in sealed containers, the re-

maining NaOH was titrated with HCl after adding BaCl<sub>2</sub> solution and phenolphthalein. The  $CO_2$  flux was calculated from the amount of consumption of HCl through titration given by the following formula (eqn. 1):

$$CO_2(mg)/SW/t = \frac{(V_0 - V) \cdot 1.1}{dwt} \quad (1)$$

where SW is the amount of soil dry weight, t is the incubation time,  $V_0$  is the HCl used for titration, V is the HCl used for soil sample, dwt is the dry weight of 1 g moist soil, and 1.1 is the conversion factor (i.e., 1 ml 0.05 M NaOH equals 1.1 mg  $CO_2$ ).

The  $C_{mic}$  was determined according to the chloroform extraction method described by Vance et al. (1987). Half of each sample was fumigated with  $CHCl_3$  and then incubated at 25 °C for 24 h. The fumigated and non-fumigated samples were extracted using 0.5 M  $K_2SO_4$  at a soil/extract ratio of 1:4, and then kept at -20 °C until they were analysed using a TOC-L<sup>®</sup> analyser (Shimadzu Corp., Kyoto, Japan). The  $C_{mic}$  was calculated following Wu et al. (1990) using the following equation (eqn. 2):

$$Biomass\ C = 2.22 \cdot Ec \quad (2)$$

where Ec is the difference between the amount of  $C_{org}$  extracted from the fumigated and non-fumigated soils.

The  $qCO_2$  (metabolic quotient) is a specific respiration rate of  $CO_2$  C evolved per unit of  $C_{mic}$ , which is calculated from  $CO_2$  evolution and  $C_{mic}$ .

The  $\beta$ -D glucosidase activity was measured according to the method of Naseby & Lynch (1997). A 1.5 g aliquot of soil was extracted using an acetate buffer at pH 5.5, agitated on an orbital rotary shaker with a slope angle of 60° to the horizontal for 1 h, and then centrifuged at 4000 rpm for 15 min. Following incubation at 37 °C for 24 h with buffered substrate p-nitrophenyl- $\beta$ -D glucoside, 1 ml NaOHCO<sub>3</sub> was added to the extracts, which were then quantified at 400 nm in a spectrophotometer.

### Statistical analyses

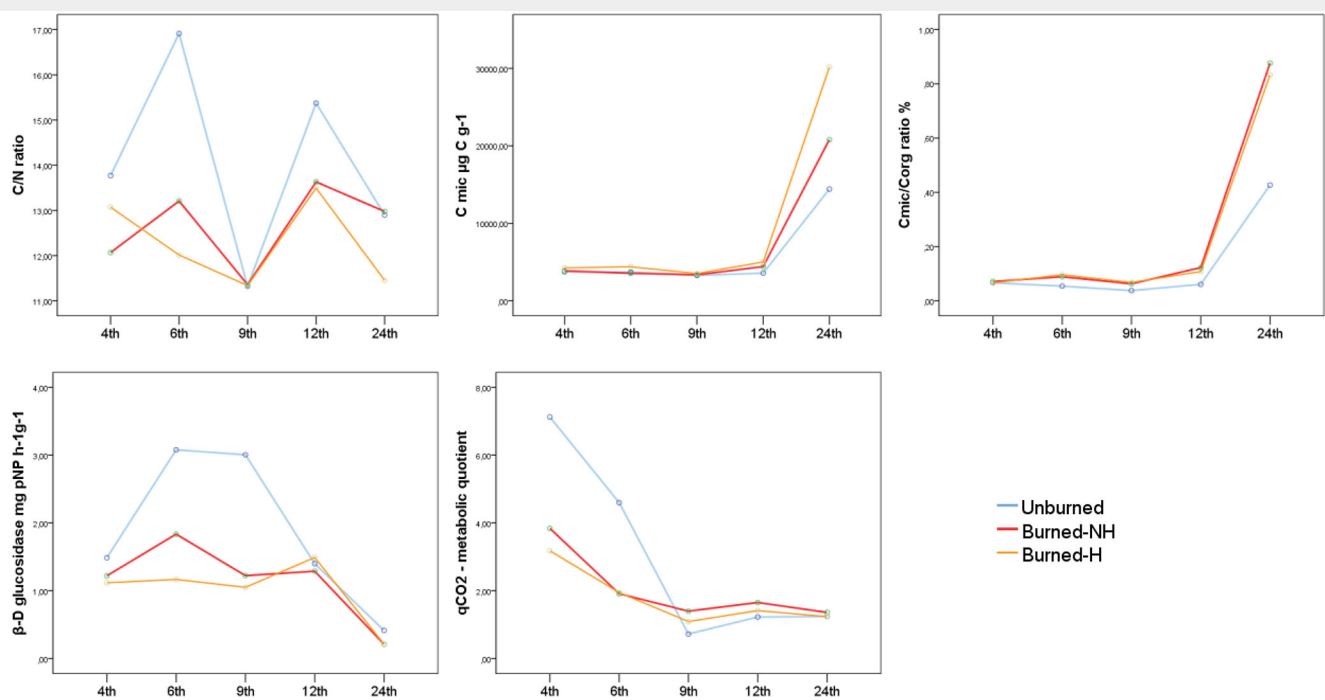
Differences between the burned (H and NH) and unburned soil samples were determined by ANOVA for each period and in total. Data was transformed when it was not normally distributed as  $\log(1 + \sqrt{qCO_2})$ , or alternatively a non-parametric test (Mann-Whitney U) was used. Correlations between the parameters were estimated. All statistical analyses were performed using the software SPSS<sup>®</sup> v. 21 (IBM, Armonk, NY, USA).

## Results

We observed significant differences in most of the soil samples in terms of burning, while the harvested and non-harvested samples were similar for most of the variables analysed. A significant decrease was determined in  $C_{org}$  in burned samples but the results were relatively higher in

Tab. 1 - Main soil characteristics in the study area. (EC): Electrical conductivity.

Variable	Profile horizons			
	Ah	C1	C2	C3
Depth (cm)	0-2	2-36	36-46	46-83
Total weight (g)	1614.48	1946.15	1740.84	2080.9
Gravel weight (g)	211.48	389.51	377.71	546.70
Gravel volume (ml)	87.50	158.33	155.00	240.00
Sand (%)	29.41	37.07	36.97	42.36
Silt (%)	29.63	26.95	21.47	26.42
Clay (%)	40.97	35.99	41.56	31.22
Soil texture	Silty clay loam	Silty clay loam	Clay loam	Clay loam
pH 1:2.5	7.2	7.5	7.6	7.7
Total lime (%)	2.915	14.84	28.09	24.11
Organic Matter, OM (%)	6.88	1.65	1.07	0.87
Total Nitrogen, N <sub>t</sub> (%)	0.39	0.09	0.06	0.04
P <sub>2</sub> O <sub>5</sub> (mg kg <sup>-1</sup> )	9.73	16.8	11.2	9.05
EC (×10 <sup>3</sup> , mS cm <sup>-1</sup> )	0.70	0.59	0.41	0.71



**Fig. 1** - (a) C/N ratio, (b)  $C_{mic}$  values, (c)  $C_{mic}/C_{org}$  ratio, (d)  $\beta$ -D glucosidase activity, and (e)  $qCO_2$  ratios in burned (H: harvested; NH: non-harvested) and unburned areas.

**Tab. 2** - Means ( $\pm$  standard error) of the soil parameters analysed for different periods after fire (Month) and level of disturbance (Dist). Different letters indicate significant differences ( $p < 0.05$ ) between unburned (UB), burned non-harvested (B-NH) and burned harvested (B-H) areas. (OM): organic matter (%); ( $N_t$ ): total nitrogen; ( $C_{mic}$ ): microbial carbon ( $\mu g C g^{-1}$ ); ( $C_{org}$ ): organic carbon; ( $\beta$ -DG):  $\beta$ -D glucosidase ( $mg pNP h^{-1} g^{-1}$ ); (N): sample size.

Month	Dist	OM (%)	$N_t$ (%)	C/N ratio	$CO_2$ efflux ( $mg CO_2 g^{-1} h^{-1}$ )	$C_{mic}$ ( $\mu g C g^{-1}$ )	$C_{mic}/C_{org}$ ratio	$qCO_2$ ( $mg CO_2 g^{-1} C_{mic} h^{-1}$ )	$\beta$ -DG ( $mg pNP h^{-1} g^{-1}$ )	N
4	UB	6.09 $\pm$ 1.48 <sup>a</sup>	0.26 $\pm$ 0.07 <sup>a</sup>	14 $\pm$ 1 <sup>b</sup>	19.81 $\pm$ 6.11 <sup>a</sup>	3760 $\pm$ 2239 <sup>a</sup>	0.07 $\pm$ 0.04 <sup>a</sup>	7.12 $\pm$ 5.23 <sup>b</sup>	1.49 $\pm$ 0.32 <sup>a</sup>	6
	B-NH	5.49 $\pm$ 1.72 <sup>a</sup>	0.27 $\pm$ 0.09 <sup>a</sup>	12 $\pm$ 1 <sup>a</sup>	13.45 $\pm$ 2.88 <sup>a</sup>	3862 $\pm$ 1200 <sup>a</sup>	0.07 $\pm$ 0.02 <sup>a</sup>	3.84 $\pm$ 1.52 <sup>a</sup>	1.22 $\pm$ 0.97 <sup>a</sup>	8
	B-H	6.50 $\pm$ 1.60 <sup>a</sup>	0.29 $\pm$ 0.09 <sup>a</sup>	13 $\pm$ 2 <sup>ab</sup>	14.36 $\pm$ 9.89 <sup>a</sup>	4251 $\pm$ 1489 <sup>a</sup>	0.07 $\pm$ 0.02 <sup>a</sup>	3.18 $\pm$ 0.96 <sup>a</sup>	1.12 $\pm$ 0.84 <sup>a</sup>	8
	Total	6.02 $\pm$ 1.60	0.28 $\pm$ 0.08	13 $\pm$ 1	15.51 $\pm$ 7.19	3976 $\pm$ 1568	0.07 $\pm$ 0.02	4.50 $\pm$ 3.22	1.26 $\pm$ 0.77	22
6	UB	6.57 $\pm$ 1.22 <sup>b</sup>	0.25 $\pm$ 0.08 <sup>a</sup>	17 $\pm$ 8 <sup>a</sup>	8.88 $\pm$ 2.15 <sup>b</sup>	3703 $\pm$ 2533 <sup>a</sup>	0.05 $\pm$ 0.03 <sup>a</sup>	4.60 $\pm$ 4.45 <sup>a</sup>	3.08 $\pm$ 3.32 <sup>a</sup>	6
	B-NH	4.14 $\pm$ 0.84 <sup>a</sup>	0.19 $\pm$ 0.04 <sup>a</sup>	13 $\pm$ 4 <sup>a</sup>	5.52 $\pm$ 1.06 <sup>a</sup>	3555 $\pm$ 1777 <sup>a</sup>	0.09 $\pm$ 0.04 <sup>a</sup>	1.91 $\pm$ 1.05 <sup>a</sup>	1.84 $\pm$ 1.94 <sup>a</sup>	8
	B-H	5.15 $\pm$ 2.18 <sup>ab</sup>	0.24 $\pm$ 0.06 <sup>a</sup>	12 $\pm$ 3 <sup>a</sup>	6.53 $\pm$ 2.20 <sup>ab</sup>	4393 $\pm$ 3056 <sup>a</sup>	0.10 $\pm$ 0.07 <sup>a</sup>	1.95 $\pm$ 0.85 <sup>a</sup>	1.17 $\pm$ 0.85 <sup>a</sup>	8
	Total	5.17 $\pm$ 1.77	0.23 $\pm$ 0.06	14 $\pm$ 5	6.80 $\pm$ 2.23	3900 $\pm$ 2417	0.08 $\pm$ 0.05	2.66 $\pm$ 2.61	1.93 $\pm$ 2.17	22
9	UB	8.37 $\pm$ 3.16 <sup>b</sup>	0.43 $\pm$ 0.17 <sup>b</sup>	11 $\pm$ 0 <sup>a</sup>	1.96 $\pm$ 0.93 <sup>a</sup>	3276 $\pm$ 1872 <sup>a</sup>	0.04 $\pm$ 0.01 <sup>a</sup>	0.73 $\pm$ 0.41 <sup>a</sup>	3.01 $\pm$ 1.36 <sup>b</sup>	6
	B-NH	5.37 $\pm$ 1.40 <sup>a</sup>	0.27 $\pm$ 0.07 <sup>a</sup>	11 $\pm$ 0 <sup>a</sup>	3.70 $\pm$ 1.58 <sup>a</sup>	3357 $\pm$ 1428 <sup>a</sup>	0.06 $\pm$ 0.02 <sup>a</sup>	1.40 $\pm$ 1.10 <sup>a</sup>	1.22 $\pm$ 1.06 <sup>ab</sup>	8
	B-H	5.73 $\pm$ 2.55 <sup>ab</sup>	0.29 $\pm$ 0.13 <sup>ab</sup>	11 $\pm$ 0 <sup>a</sup>	3.25 $\pm$ 2.03 <sup>a</sup>	3522 $\pm$ 1590 <sup>a</sup>	0.07 $\pm$ 0.04 <sup>a</sup>	1.09 $\pm$ 0.82 <sup>a</sup>	1.05 $\pm$ 0.64 <sup>a</sup>	8
	Total	6.32 $\pm$ 2.62	0.32 $\pm$ 0.14	11 $\pm$ 0	3.06 $\pm$ 1.71	3395 $\pm$ 1539	0.06 $\pm$ 0.03	1.11 $\pm$ 0.86	1.65 $\pm$ 1.30	22
12	UB	6.18 $\pm$ 1.11 <sup>a</sup>	0.24 $\pm$ 0.05 <sup>a</sup>	15 $\pm$ 2 <sup>a</sup>	4.21 $\pm$ 1.30 <sup>a</sup>	3575 $\pm$ 7880 <sup>a</sup>	0.06 $\pm$ 0.02 <sup>a</sup>	1.23 $\pm$ 0.51 <sup>a</sup>	1.40 $\pm$ 0.36 <sup>a</sup>	6
	B-NH	4.40 $\pm$ 1.99 <sup>a</sup>	0.19 $\pm$ 0.06 <sup>a</sup>	14 $\pm$ 5 <sup>a</sup>	6.95 $\pm$ 2.56 <sup>a</sup>	4415 $\pm$ 1023 <sup>ab</sup>	0.12 $\pm$ 0.06 <sup>b</sup>	1.66 $\pm$ 0.66 <sup>a</sup>	1.29 $\pm$ 1.34 <sup>a</sup>	8
	B-H	4.88 $\pm$ 1.28 <sup>a</sup>	0.22 $\pm$ 0.06 <sup>a</sup>	13 $\pm$ 3 <sup>a</sup>	6.59 $\pm$ 2.86 <sup>a</sup>	5009 $\pm$ 1359 <sup>b</sup>	0.11 $\pm$ 0.03 <sup>ab</sup>	1.42 $\pm$ 0.75 <sup>a</sup>	1.49 $\pm$ 1.19 <sup>a</sup>	8
	Total	5.06 $\pm$ 1.64	0.21 $\pm$ 0.06	14 $\pm$ 4	6.07 $\pm$ 2.59	4402 $\pm$ 1203	0.10 $\pm$ 0.05	1.45 $\pm$ 0.65	1.39 $\pm$ 1.05	22
24	UB	4.52 $\pm$ 2.08 <sup>a</sup>	0.21 $\pm$ 0.12 <sup>a</sup>	13 $\pm$ 2 <sup>a</sup>	9.47 $\pm$ 2.57 <sup>a</sup>	14417 $\pm$ 15842 <sup>a</sup>	0.43 $\pm$ 0.52 <sup>a</sup>	1.24 $\pm$ 0.87 <sup>a</sup>	0.42 $\pm$ 0.24 <sup>a</sup>	6
	B-NH	3.57 $\pm$ 1.43 <sup>a</sup>	0.16 $\pm$ 0.05 <sup>a</sup>	13 $\pm$ 3 <sup>a</sup>	8.86 $\pm$ 1.92 <sup>a</sup>	20805 $\pm$ 20733 <sup>a</sup>	0.88 $\pm$ 1.07 <sup>a</sup>	1.36 $\pm$ 1.25 <sup>a</sup>	0.21 $\pm$ 0.17 <sup>a</sup>	8
	B-H	4.00 $\pm$ 0.84 <sup>a</sup>	0.21 $\pm$ 0.05 <sup>a</sup>	11 $\pm$ 3 <sup>a</sup>	9.13 $\pm$ 1.99 <sup>a</sup>	30156 $\pm$ 28705 <sup>a</sup>	0.83 $\pm$ 0.82 <sup>a</sup>	1.24 $\pm$ 1.39 <sup>a</sup>	0.22 $\pm$ 0.15 <sup>a</sup>	8
	Total	3.99 $\pm$ 1.45	0.19 $\pm$ 0.08	12 $\pm$ 3	9.12 $\pm$ 2.04	22463 $\pm$ 22799	0.74 $\pm$ 0.84	1.29 $\pm$ 1.16	0.27 $\pm$ 0.20	22
Total	UB	6.35 $\pm$ 2.21 <sup>b</sup>	0.28 $\pm$ 0.13 <sup>a</sup>	14 $\pm$ 4 <sup>b</sup>	8.87 $\pm$ 6.93 <sup>a</sup>	5746 $\pm$ 8088 <sup>a</sup>	0.13 $\pm$ 0.26 <sup>a</sup>	2.98 $\pm$ 3.84 <sup>a</sup>	1.88 $\pm$ 1.83 <sup>b</sup>	30
	B-NH	4.59 $\pm$ 1.62 <sup>a</sup>	0.22 $\pm$ 0.08 <sup>a</sup>	13 $\pm$ 3 <sup>a</sup>	7.69 $\pm$ 3.93 <sup>a</sup>	7199 $\pm$ 11231 <sup>b</sup>	0.24 $\pm$ 0.56 <sup>b</sup>	2.03 $\pm$ 1.44 <sup>a</sup>	1.16 $\pm$ 1.29 <sup>a</sup>	40
	B-H	5.25 $\pm$ 1.90 <sup>a</sup>	0.25 $\pm$ 0.09 <sup>a</sup>	12 $\pm$ 2 <sup>a</sup>	7.97 $\pm$ 5.95 <sup>a</sup>	9466 $\pm$ 16148 <sup>b</sup>	0.23 $\pm$ 0.46 <sup>b</sup>	1.78 $\pm$ 1.20 <sup>a</sup>	1.01 $\pm$ 0.88 <sup>a</sup>	40
	Total	5.31 $\pm$ 2.01	0.25 $\pm$ 0.1	13 $\pm$ 3	8.11 $\pm$ 5.58	7627 $\pm$ 12574	0.21 $\pm$ 0.46	2.20 $\pm$ 2.33	1.30 $\pm$ 1.38	110

**Tab. 3** - Correlation coefficients among the analyzed soil parameters (n=110). ( $N_t$ ): total nitrogen; ( $C_{mic}$ ): microbial carbon; ( $C_{org}$ ): organic carbon; ( $\beta$ -DG):  $\beta$ -D glucosidase; (\*):  $p < 0.05$ ; (\*\*):  $p < 0.001$ ; (ns): non-significant.

	$C_{org}$	$N_t$	C/N	$CO_2$ efflux	$C_{mic}$	$C_{mic}/C_{org}$	$qCO_2$	$\beta$ -DG
$C_{org}$	1	0.873**	0.207*	0.128 <sup>ns</sup>	-0.277**	-0.384**	0.135 <sup>ns</sup>	0.181 <sup>ns</sup>
$N_t$	-	1	-0.274**	0.078 <sup>ns</sup>	-0.266**	-0.341**	0.106 <sup>ns</sup>	0.143 <sup>ns</sup>
C/N	-	-	1	0.055 <sup>ns</sup>	-0.001 <sup>ns</sup>	-0.076 <sup>ns</sup>	0.000 <sup>ns</sup>	0.097 <sup>ns</sup>
$CO_2$ efflux	-	-	-	1	0.046 <sup>ns</sup>	0.011 <sup>ns</sup>	0.592**	-0.154 <sup>ns</sup>
$C_{mic}$	-	-	-	-	1	0.925**	-0.298**	-0.241*
$C_{mic}/C_{org}$	-	-	-	-	-	1	-0.274**	-0.241*
$qCO_2$	-	-	-	-	-	-	1	-0.024 <sup>ns</sup>
$\beta$ -DG	-	-	-	-	-	-	-	1

$C_{mic}$  and  $qCO_2$  that is inconsistent with the results from previous studies (Wardle & Ghani 1995, Bolat & Oztürk 2017), whereas a positive correlation between  $C_{mic}$  and  $CO_2$  evolution was detected. A high  $qCO_2$  indicates the need for restoring the C demand as a result of the microbial biomass depending on the renewal of C lost through respiration (Anderson & Domsch 2010, Bolat & Oztürk 2017). After a low-intensity prescribed fire in a *Picea abies* forest, basal respiration diminished while there was a disproportionate decrease in  $C_{mic}$  (Pietikäinen & Fritze 1995), which clearly led to higher values of  $qCO_2$  in the burned areas compared to unburned ones (Certini 2005). This indicator predicts soil fertility (Anderson & Domsch 1993, Wardle & Ghani 1995, Bauhus et al. 1997, Mahía et al. 2006, Bolat 2014). The lower levels of  $qCO_2$  in the burned soils in the first and second sampling dates indicate an improvement in soil conditions for the new vegetation due to the warmer soils in burned areas. Similar values in the burned and unburned areas after Month 9 may have resulted from vegetation succession (Insam & Haselwandter 1989).

$\beta$ -D glucosidase enzyme activity plays an important role in energy availability in the soil, as it is directly related to the labile C content and its ability to stabilise the soil organic matter independently of seasonal variability (Martinez-Salgado et al. 2010). Our results indicate that this enzyme activity decreased with burning up to the last sampling date, two years after the wildfire, and was significantly different among H, NH, and UB plots at Month 9. The decrease in enzyme activity related with the lack of C entry to the system via plant inputs of  $C_{org}$  and  $N_t$  (Sinsabaugh & Moorhead 1994, Sinsabaugh et al. 2008) is consistent with our results. There was a negative correlation between enzyme activity and  $C_{mic}$ . The decrease in enzyme activity compared to  $C_{mic}$  likely resulted from the enzymes being retained in the stabilised fraction of the soil rather than that part associated with the viable microbial population (Knight & Dick 2004).

Decomposition increased due to harvesting, possibly resulting in changes to the C budget (Noormets et al. 2015). The harvesting of burned tree residues did not affect the measured soil parameters significantly in this study, probably because of the sensitive protection precautions used after the wildfire (such as covering the soil surface with residual brushwood material) and the rapid regeneration of forest (Gömöryová et al. 2017, Gómez-Sánchez et al. 2019), which is characteristic of oak forests.

## Conclusions

Fire affected the microbial biomass likely due to changes in the substrate quality, microbial composition, and abundance. However, this increase was not significant one year after burning. The correlation between  $qCO_2$  and  $\beta$ -D glucosidase activity

burned-H samples. No difference in total nitrogen ( $N_t$ ) was found between the unburned and burned soils but the Month 9. The C/N ratios were slightly different in favor of the unburned samples ( $p = 0.05$  – Fig. 1a, Tab. 2). There was no significant difference in  $CO_2$  evolution ( $p = 0.956$ ); however, the maximum values were observed in the unburned soils, and the minimum values were found in the burned-NH soils (Tab. 2). The  $C_{mic}$  values were different at Month 12 ( $p = 0.036$ ) while it was comparatively high in the burned areas at all months (Fig. 1b, Tab. 2). We observed significant differences ( $p = 0.029$ ) in  $C_{mic}/C_{org}$  at Month 12 similarly (Fig. 1c, Tab. 2). An increase in the total amount of  $C_{mic}$  in all areas was observed through the time, but the increase was higher in the burned areas between Months 12 and 24.

Significant differences were observed between the burned and unburned areas ( $p = 0.023$ ) in favor of unburned areas in regard to  $\beta$ -D glucosidase enzyme activity (Fig. 1d, Tab. 2). The  $qCO_2$  values did not vary significantly with burning ( $p = 0.786$ ) except for Month 4 (Fig. 1e, Tab. 2).

There were significant differences between the different periods, in terms of the soil properties, with  $p < 0.05$  for C/N and  $\beta$ -D glucosidase, and  $p < 0.001$  for the others. The interaction effect of period and burning for  $qCO_2$  was significant ( $p < 0.05$ ), while it was unclear for  $CO_2$  evolution ( $p = 0.052$ ).

$C_{mic}$  and  $C_{mic}/C_{org}$  were well correlated with each other, while negative relations between  $C_{org}$ , TN,  $qCO_2$  and  $\beta$ -D glucosidase were recorded (Tab. 3).

## Discussion

Although the  $C_{org}$ , C/N,  $C_{mic}$ ,  $C_{mic}/C_{org}$  and  $\beta$ -D glucosidase values were different between the burned and unburned plots, there were no significant differences between the H (burned, harvested) and NH (burned, non-harvested) areas, in terms of the soil parameters.

Organic carbon content decreases as a result of fire as observed in this study, and changes may occur in organic matter fractions. As a matter of fact, loss of  $C_{org}$  and  $N_t$  has been reported due to the fire severity

reaching 220-460 °C (Giovannini et al. 1990, DeBano et al. 1998). Similarly, the lower levels of OM were significant at Month 6 and 9 according to our results. Relatively high OM content in burned-harvested samples was likely due to mixing of the forest floor with the mineral soil during harvesting (Poirier et al. 2014).  $N_t$  content in burned samples were low as well as non-significant in general. Higher  $N_t$  concentration was observed at Month 9 likely due to the warmer conditions in more moist unburned samples (Jaeger et al. 1999, Kaiser et al. 2011, Yokobe et al. 2018). Slight changes in C/N ratio may be related with the formation of new recalcitrant N and volatilisation of C compounds, as suggested by previous studies conducted in burned pine forests (Rodríguez et al. 2017, Gómez-Sánchez et al. 2019).

A general increase in  $C_{mic}$  was probably due to the increase in the concentration of oxidisable C and nutrients in burned soils as previously reported (Gómez-Sánchez et al. 2019, Giuditta et al. 2020); immediate increases have been found in soils after fire (Turgay et al. 2002, Dooley & Treseder 2012). Likely because of the relatively late sampling in our study, we did not detect significant changes in the above variables in burned (especially burned and harvested) areas in Month 4 and Month 6. Bárcenas-Moreno et al. (2011) categorised the process of microbial recovery after a wildfire as initial depletion of microorganisms in soil and the proliferation of fast-growing bacteria after a few months due to rapid and short increase of available nutrients.  $C_{mic}$  increase was reported after 8 months in a burned pine (*Pinus halepensis*) and oak (*Quercus coccifera*) mixed forest by Bárcenas-Moreno et al. (2011) which was maintained for the rest of their study and reversed after 32 months. We assessed a significant decrease at Month 12 and non-significant decrease at Month 24 in  $C_{mic}$  values of the unburned areas, in comparison with those at Months 4 and 6. An increase in the total amount of  $C_{mic}$  was observed in all areas, more in the burned areas, likely due to the higher amount of rainfall between Months 12 and 24.

We found a negative correlation between

showed that the former might be a more coherent indicator of microbial functionality than  $C_{mic}$  and  $CO_2$  evolution. In fact, enzymes might be independent of microbial proliferation, as it can attach to dead cells or cellular fragments instead of viable microorganisms.

The decrease in  $qCO_2$  after burning at low severity may indicate to the forest managers that the soil has relatively ideal conditions, simplifying the preparation of the area for new vegetation. The harvesting of burned trees in post-fire areas provides many advantages, in terms of economic benefits or protection of the forest against pests. Proper precautions for the soil, such as covering it after the fire with residues, comprising branches and small, felled burned trees, may be advantageous by allowing soil and nutrient loss to be avoided.

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