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Post-fire recovery of soil microbial functions is promoted by plant growth

Jordi Garcia-Pausas[1,2] | **Joan Romanyà**[2,3] | **Pere Casals**[1]

[1]Multifunctional Forest Management Programme, Joint Research Unit CTFC - AGROTECNIO, Solsona, Catalonia, Spain

[2]Department de Biologia, Sanitat i Medi Ambient, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Barcelona, Catalonia, Spain

[3]INSA-UB, Nutrition and Food Safety Research Institute, University of Barcelona, Barcelona, Catalonia, Spain

Correspondence
Jordi Garcia-Pausas, Joint Research Unit CTFC – AGROTECNIO, Ctra. St. Llorenç de Morunys, Km 2, 25280 Solsona, Catalonia, Spain.
Email: jordi.gpausas@gmail.com

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Abstract

Forest fires can alter the biological properties of soils. There is increasing evidence that fires cause a shift in soil microbial communities, which play a central role in forest carbon and nutrient cycling. In this study, we evaluate the effect of soil heating on soil microbial functions. We hypothesised that fire reduces the catabolic functional diversity of soil, and that post-fire plant growth enhances its recovery. To test this, we experimentally heated a forest soil at 200°C (T200) or 450°C (T450). Heated and unheated soils were then incubated in tubs with or without live grass (*Lolium perenne* L.). We determined the functional profiles by measuring the substrate-induced respiration (SIR) using the Microresp™ technique and analysed nutrient availability at the end of the incubation. At both temperatures, soil heating altered the respiration responses to substrate additions and the catabolic functional diversity of soils. Functional diversity was initially reduced in T200 soils but recovered at the end of the incubation. In contrast, T450 soils initially maintained the catabolic functional diversity, but decreased at the end of the incubation. Heating-induced nutrient availability stimulated the growth of grass, which in turn increased the response to several substrates and increased the functional diversity to values similar to the unheated controls. Our results suggest that fire-driven alteration of soil microbial communities has consequences at a functional level, and that the recovery of plant communities enhances the recovery of soil microbial functions.

Highlights

- Soil experimental heating altered microbial functions and reduced soil functional diversity.
- Soil heating also increased nutrient availability, enhancing plant growth.
- Growth of plants promoted the recovery of soil functional diversity.
- Post-fire recovery of functional diversity may be related to the recovery of photosynthetic tissues.

KEYWORDS

aboveground biomass, catabolic functional diversity, forest fires, soil heating, soil microbial functions, substrate-induced respiration

1 | INTRODUCTION

Wildfires are natural disturbances common in Mediterranean ecosystems (Keeley et al., 2012) that modify ecosystem functions through changes in both biotic and abiotic components. Surface temperature during a forest fire depends on fire intensity, but usually ranges between 200°C and 300°C (Neary et al., 1999), with intense heating affecting only the first few centimetres. However, in sites with high fuel accumulation or under wood piles, soil temperature can reach >200°C at 10 cm depth (Busse et al., 2013; Neary et al., 1999). These temperatures can alter biological, physical, and chemical soil properties, with effects that can persist for decades after the event (Dove et al., 2020).

The immediate effect of fire on soil microbiology is the reduction of microbial biomass (e.g., Banning & Murphy, 2008), by direct effect of high temperatures. Even though different biological temperature thresholds of death in soil biota may differentially affect roots and microbial communities (Pingree & Kobziar, 2019), peak temperatures in surface soil during a fire event usually exceeds the killing temperature for many microorganisms (Dunn et al., 1985; Guerrero et al., 2005; Neary et al., 1999), which causes a release of nutrients and soluble organic compounds (Guerrero et al., 2005). This nutrient release may reduce microbial biomass and activity (Treseder, 2008) as well as microbial diversity (Liang et al., 2019); however, organic compounds are readily metabolisable, allowing a rapid regrowth of soil microbiota. The combination of these factors may affect soil microorganisms depending on whether they are more resistant and/or resilient than others, thus resulting in changes in the microbial community. For instance, it has been shown that bacteria are both less sensitive and more resilient to a fire disturbance than fungi (Bárcenas-Moreno et al., 2011; Dunn et al., 1985). In spite of their post-fire bacterial dominance, fire may also reduce bacterial diversity (Sáenz de Miera et al., 2020). As such, depending on the temperature reached, the duration of the heating event, and the moisture content, soil heating caused by wildfires can significantly alter the size and the structure of soil microbial communities (Bárcenas-Moreno et al., 2011; Pérez-Valera et al., 2019; Prendergast-Miller et al., 2017).

Soil microbiota have key roles in ecosystem function (Soong et al., 2020; Turner et al., 2013), but the effect of changes in the diversity and structure of microbial communities, like those caused by disturbances, may depend on the community characteristics or the intensity of alterations among other factors. Experimental manipulations of soil microbial diversity do not always lead to changes in soil functions (e.g., Wertz et al., 2006), probably because there is high functional redundancy in soil communities. But, in general, carbon (C) and nitrogen (N) cycling processes are better predicted by including measures of microbial community structure in models (De Vries et al., 2013; Graham et al., 2016). Pérez-Varela et al. (2020), for example, have shown that microbial diversity promotes the restoration of soil functions after fire, showing links between community structure and functions. In general, common functions such as the mineralisation of labile organic compounds are considered to be carried out by most microorganisms and may therefore be insensitive to changes in community structure and diversity. But some specialised functions such as denitrification (Philippot et al., 2013), degradation of recalcitrant organic matter (Singh et al., 2014; Trivedi et al., 2019), or priming effect of soil organic matter (SOM) mineralisation (Garcia-Pausas & Paterson, 2011) may be carried out by specific groups of microorganisms and therefore may be particularly affected by changes in the microbial community.

Many plant species in fire-prone ecosystems have developed strategies to recover after fire (Pausas et al., 2004), contributing to the recovery of ecosystem functions. Post-fire increase of available nutrients (Romanyà et al., 2001) may stimulate plant regrowth (Johnson et al., 2011), facilitating such recovery, and subsequently improve soil properties and functions (Moya et al., 2019). Plant roots release a substantial amount of organic C into the soil, mostly in form of carbohydrates, amino acids, and organic acids (Hütsch et al., 2002). These compounds fuel microbial activity and shape their community structure in both the rhizosphere and bulk soil (Koranda et al., 2011; Paterson et al., 2007). This, in turn, may have consequences for soil function. For instance, the absence of roots in the soil may reduce the capacity of microorganisms to degrade some recalcitrant substrates (Paterson et al., 2011), emphasising the importance of plant roots for maintaining soil microbial functions.

We hypothesise that wildfire heat alters the capacity of microbial communities to use some organic substrates,

reducing the catabolic functional diversity of soil communities. Moreover, we also hypothesise that post-fire plant activity stimulates the recovery of these functions. To test these hypotheses, we experimentally heated forest soil at two temperatures to simulate different fire severities and studied the impact on soil carbon, nutrients, and soil microbial functions. The latter was assessed by determining the functional profiles and catabolic functional diversity of heated and unheated forest soils during a 142 day incubation. To test the effect of the presence of plants (i.e., root activity and exudates) on the post-heating changes of microbial functions, we incubated soils with and without ryegrass (*Lolium perenne* L.), a species native to the study area.

2 | MATERIAL AND METHODS

2.1 | Soil collection

Mineral soil was collected from a sub-Mediterranean forest (41°57′8.4″ N, 1°27′54.6″ E, 820 m a.s.l.) located in Llobera, at the southern foothills of the eastern Pre-Pyrenees, NE Iberian Peninsula. The dominant tree species at this site was *Pinus nigra* Arnold subsp. *salzmannii* (Dunal) Franco, with some *Quercus pubescens* Willd. also present. *Buxus sempervirens* L. dominated the understory. According to the Digital Climatic Atlas of the Iberian Peninsula (Ninyerola et al., 2005), mean annual temperature of the site is 11°C and mean annual precipitation is 662 mm. The soil developed from a calcareous limestone bedrock and is classified as a Lithic or Typic Xerorthent (Soil Survey Staff, 2014). The collected soil (5–15 cm depth) had a silt loam texture, with pH_w of 8.3 ± 0.03 ($n = 3$), organic C content of 44.2 ± 0.08 mg g^{-1} ($n = 4$), and $CaCO_3$ content of 31.5 ± 0.29% ($n = 4$). To collect the soil, we selected two areas of about 0.5 m^2 separated at least 1 m from any tree and removed the organic layers. Then, using a shovel, we collected about 150–160 kg of soil from 0 to 10 cm depth, which were transported in buckets to the laboratory.

2.2 | Treatment application and incubation

Collected soil was sieved to 5 mm to remove most large roots and gravel and to reduce the heterogeneity between tubs the day after sampling. Three treatments were applied: an unheated control (unheated), heated at 200°C (T200), or heated at 450°C (T450). Heating was undertaken for 30 min to simulate different fire severities to the soil communities and was done by placing trays with 1–1.5 kg soil in a muffle furnace. Once all necessary soil was heated, all soil within each heating treatment was homogenised.

Soil was then placed into 30 square plastic tubs (34 × 34 cm^2, 14 cm height; 10 tubs per heating treatment) containing 3500 g soil each. Nine iButton® temperature loggers (model DS1402D-DR8+, Embedded Data Systems, Lawrenceburg, KY) were set to record temperature at 3 h intervals and buried at about 1 cm depth in nine randomly chosen samples. Soils were moistened to 35% of the water holding capacity of the unheated soil. Five tubs of each heating treatment were then sown with commercial *Lolium perenne* L. seeds, a native grass species that has been used for post-fire seeding. All tubs were then randomly placed outdoors under a polycarbonate roof to exclude the rain and allow for control of soil moisture and incubated for 142 days (28th March–17th August). The incubation site was at the outdoor facilities of the Forest Science and Technology Centre of Catalonia's main headquarters, in Solsona (42°00′38.0″ N, 1°31′9.8″ E, 700 m a.s.l.), NE Iberian Peninsula. During the incubation period, gravimetric water loss by evaporation and transpiration of each sample was measured every 1–3 days, and the equivalent amount of tap water was added to each pot. Mean soil daily temperature during the incubation period ranged between 7.7°C and 25.4°C (mean = 18.2°C).

2.3 | Above- and belowground biomass

At the end of the incubation period, aboveground biomass (AGB) in the planted samples was determined by clipping the grass to ground level. To determine belowground biomass (BGB), roots were first sorted by hand and then shaken in water to eliminate most of the remaining soil. They were then packed using a fine cloth and shaken under a continuous tap water flow to eliminate the finest soil particles. Both, AGB and BGB were oven-dried at 60°C to determine dry mass.

2.4 | Soil sampling

Two soil sampling were collected from each tub at days 72 and 142 of the incubation. The soil collected in the first sampling was only used to determine the functional profiles of soil communities. Soil collected at the end of the incubation (day 142) was used for both functional profiling and chemical analyses. Sampling was performed by inserting a soil corer (50 ml PVC tubes, 28 mm diameter) into the soil from the surface to ~5-cm depth. In the second sampling, three soil samples were collected from

TABLE 1 Substrates used for assessing the community-level physiological profiles

Substrate	Formula
Organic acids	
Citric acid	$C_6H_8O_7$
L-Malic acid	$C_4H_6O_5$
Oxalic acid	$C_2H_2O_4$
Carbohydrates	
D-Glucose	$C_6H_{12}O_6$
D-Fructose	$C_6H_{12}O_6$
D-Galactose	$C_6H_{12}O_6$
L-Arabinose	$C_5H_{10}O_5$
D-Trehalose dihydrate	$C_{12}H_{22}O_{11}{\cdot}2H_2O$
N-acetylglucosamine (NAG)	$C_8H_{15}NO_6$
Amino acids	
L-Arginine	$C_6H_{14}N_4O_2$
L-Lysine	$C_6H_{14}N_2O_2$
L-Cysteine hydrochloride	$C_3H_7NO_2S$-HCl
L-Alanine	$C_3H_7NO_2$
γ-aminobutyric acid (GABA)	$C_4H_9NO_2$
Phenols	
Orcinol	$C_7H_8O_2$

each pot: one was used to determine the functional profiles (stored at 4°C until use), while the soil of the other two corers were air-dried and sieved to 2 mm to be used for chemical analyses.

2.5 | Soil chemical analyses

Before their incubation in tubs, soil from each heating treatment (i.e., unheated, T200 and T450) was analysed for organic C and total N contents. Soil organic C was determined by dichromate oxidation following the method of Mebius (1960), as modified by Nelson and Sommers (1996). Total soil N was determined using an elemental analyser (Thermo Fisher Scientific).

All other soil chemical analyses were performed on the three (i.e., unheated, T200 and T450) non-incubated soils (initial soil characteristics) and also to the 30 incubated soils collected at the end of the incubation period. In these soils, 0.5 M K_2SO_4 extracts were obtained from 4 g soil (soil:extractant ratio 1:8). From these extracts, extractable organic C was quantified by dichromate oxidation following the method of Mebius (1960) and Nelson and Sommers (1996) but adapted for liquid samples. Total and mineral (NH_4^+ and NO_3^-) nitrogen were analysed colorimetrically from the same 0.5 M K_2SO_4 extracts. NH_4^+ concentration in the extracts were determined by the nitroprussiate method (Baethgen & Alley, 1989), and NO_3^- concentration by the Cataldo et al. (1975) method. Total extractable N was then determined by analysing NO_3^- after persulfate oxidation of an aliquot of the same 0.5 M K_2SO_4 extracts (Cabrera & Beare, 1993). Organic extractable N was calculated by subtracting mineral N from total N in the K_2SO_4 extracts. Finally, available phosphorus (P) was extracted from 2 g soil with 0.5 M $NaHCO_3$ (pH 8.5, solution ratio 1:20) and determined by the ascorbic acid blue method. Pseudo-total and retained organic and inorganic P were analysed by an ignition method (Saunders & Williams, 1955). Briefly, approximately 2 g of air-dried soil were extracted with 50 ml of 0.5 M H_2SO_4. This first extract included the retained inorganic P. Then another subsample of approximately 2 g of air-dried soil was calcined at 550°C in a muffle furnace for 1 h, and then extracted with 50 ml of 0.5 M H_2SO_4. The P in this latter extract contained all retained P, which made up the pseudo-total P pool. Finally, the difference between both extracts gave the retained organic P. Phosphorus in all extracts was quantified by the colorimetric method described by Murphy and Riley (1962).

2.6 | Community-level physiological profiling

Catabolic response profiles were determined by the Microresp™ technique (Campbell et al., 2003) to the unheated and heated soils after 72 and 142 days of the incubation. The method quantifies the substrate-induced respiration during a 6 h incubation in the laboratory after the addition of different C sources. It uses 96 deep-well plates that are homogeneously filled with fresh 2 mm sieved soil that is rewetted to 50% of water holding capacity. Every C substrate is added into three wells (replicates) at a rate of 30 mg substrate ml^{-1} of soil water, except for L-arginine, which was added at a rate of 7.5 mg ml^{-1} of soil water, following the recommendations of the Microresp™ Technical Manual. We used 15 substrates, which included six carbohydrates, five amino acids, three organic acids, and one phenol (Table 1). Carbohydrates and especially organic acids represent a source of labile C easily used by soil microbiota. Amino acids are also considered labile compounds (Jones, 1999), but they (and *N*-acetylglucosamine, a carbohydrate) also provide N to soil. By contrast, phenolic compounds are considered recalcitrant and a slow source of C, degraded by a narrower range of microorganisms that include fungi and bacteria (Waldrop

et al., 2000). Given that the studied soils contained a substantial amount of inorganic C (as $CaCO_3$), open-air plate pre-incubation period of 1 h was set to allow the abiotic release of CO_2 (García-Palacios et al., 2011). During the 6 h incubation, deep-well plates were assembled to detection plates containing agar with an indicator solution containing Cresol red. Then, the respiration rate was quantified colorimetrically in a microplate reader (Multiskan FC, Thermo Scientific) with a 570 nm filter. Respiration rate of soils with the only addition of deionised water was taken as a basal soil respiration rate.

Calibration curves were made by determining colour development of the agar in microplate strips (provided by the Microresp™ kit) with a microplate reader with a 570 nm filter during a 6 h incubation in sealed jars, after adding a H_3PO_4 solution to known amounts of $CaCO_3$.

2.7 | Calculations and statistics

Substrate-induced respiration (SIR) was calculated by the difference between CO_2 efflux from soils receiving every substrate and the mean CO_2 efflux from soils receiving only deionised water. A diversity index of catabolic functions of soil communities (H) was computed from the SIR values across all substrates using the Shannon diversity index as follows:

$$H = -\sum p_i \times \ln p_i,$$

where pi is the respiration response to the addition of each substrate i as a proportion of total respiration response of all substrates together (Derry et al., 1999; Zak et al., 1994). The response to a particular substrate is the CO_2 evolved (μgCO_2-C g^{-1} h^{-1}) during the 6 h incubation minus the mean CO_2 evolved from those samples with the only addition of deionised water.

Negative SIR values, which occurred with the addition of arginine, lysine, and occasionally orcinol, were changed to 10^{-6} to calculate the natural logarithm. In practice, this adjustment made the contribution of these substrates to the calculation of H negligible.

The effect of heating and grass on soil extractable N and C, on soil P, and on microbial catabolic functional diversity was analysed by one-way ANOVA with IBM SPSS Statistics v.19 for Windows. Tukey's multiple range test was used to determine significant differences between the three treatments (unheated, T200 and T450) when the ANOVA was significant ($p < 0.05$). Prior to the statistical analyses, normality was checked by the non-parametric Kolmogorov–Smirnov test. Those variables that did not follow a normal distribution were transformed by natural logarithm or square root. Pearson correlations were performed between substrate-induced responses and extractable C or nutrient levels across all treatments (with or without grass, $n = 15$).

The effect of heating and grass treatments was analysed on the mean SIR of each group of substrates (except phenol). Given the different behaviour of SIR to the addition of arginine and lysine compared to the other amino acids, they were analysed as separate groups. Both arginine and lysine contain more than one N per molecule. The treatment effects were analysed using a generalised linear mixed model (GLMM) assuming a Gaussian distribution of errors. Models for each substrate group shared the same full model structure: heating, grass, sampling times and their interactions were included in the fixed term as explanatory variables, while the two sampling times and the specific compounds of the substrate group were included as a random term nested to the tub (as we are not interested in the SIR of a specific compound). For phenol, as it was only one specific compound, the random term only includes the sampling time nested to the pot.

Backward selection of variables for inclusion in the final models was based on Akaike's criterion (AIC). Standard fitted versus residual plots and quantile-quantile plots for the final models were used to assess compliance with the modelling assumptions of normal independent residuals with constant variance. Visual inspection of plots did not reveal any obvious deviations from homoscedasticity or normality. We calculated the marginal R^2 and conditional R^2 of the model (Nakagawa & Schielzeth, 2013). The marginal R^2 indicates the model variance explained by fixed effects whereas conditional R^2 indicates the variance explained by the whole model. Mixed model analyses were performed using the "lme4" library (Bates et al., 2015) for R (version 4.0.4, R Core Team, 2021).

3 | RESULTS

3.1 | Immediate effect of heating on soil C and nutrients

A visual examination of heated soils just after the heating treatments were applied showed that aggregation was altered (i.e., disrupted macroaggregates) in T450 but not T200 treatments. Compared to the unheated soils and T200 treatment, T450 treatment reduced organic C content by 24.7% ($p = 0.002$), slightly increased the pseudo-total P by 7.8% ($p = 0.015$), and altered the retained P by increasing the inorganic fraction and reducing the organic fraction (Table 2). By contrast, the T200 treatment did not alter total carbon and nutrient contents (Table 2).

	n	Unheated	T200	T450
Organic C (mgC g^{-1})	3	44.2 ± 0.1 a	42.0 ± 0.6 a	33.3 ± 1.7 b
Total N (mgN g^{-1})	3	2.5 ± 0.06 a	2.5 ± 0.05 a	2.3 ± 0.04 a
Pseudo-total P (μgP g^{-1})	4	409.0 ± 7.48 b	405.9 ± 6.02 b	440.7 ± 5.16 a
Retained inorganic P (μgP g^{-1})	4	233.2 ± 9.05 b	257.5 ± 12.64 b	382.6 ± 9.78 a
Retained organic P (μgP g^{-1})	4	175.7 ± 2.37 a	148.3 ± 10.06 a	58.1 ± 7.41 b
pH_w	3	8.3 ± 0.03 b	8.2 ± 0.00 a	8.5 ± 0.00 c
pH_{KCl}	3	7.8 ± 0.02 b	7.7 ± 0.01 a	8.5 ± 0.01 c

TABLE 2 Initial (i.e., before incubation) properties of soils by heating treatments

Note: All values are means ± standard error. Different letters indicate significant differences between heating treatments (pairwise Tukey test).

TABLE 3 Extractable organic C, mineral, organic, and total N and inorganic available P contents of soils before and at the end of the incubation, by heating and grass treatments.

		n	Organic C_{extr} (μgC g^{-1})	Mineral N_{extr} (μgN g^{-1})	Organic N_{extr} (μgN g^{-1})	Total N_{extr} (μgN g^{-1})	Available P[a] (μgP g^{-1})
Unheated	Initial	3	632.8 ± 24.7	23.6 ± 4.62	39.9 ± 1.83	63.5 ± 3.81	6.4 ± 0.42
	No grass	5	626.5 ± 10.6	68.5 ± 3.85	52.5 ± 1.34	121.1 ± 2.91	5.6 ± 0.23
	Grass	5	628.0 ± 25.7	30.3 ± 4.86	46.0 ± 1.62	76.3 ± 5.45	5.4 ± 0.06
T200	Initial	3	1086.2 ± 12.6	25.1 ± 0.48	73.2 ± 0.33	98.2 ± 0.70	7.0 ± 0.22
	No grass	5	797.6 ± 14.7	113.4 ± 2.47	54.2 ± 3.29	167.6 ± 2.61	8.9 ± 0.11
	Grass	5	747.5 ± 8.7	15.2 ± 1.28	45.6 ± 1.76	60.9 ± 1.57	6.2 ± 0.26
T450	Initial	3	1911.8 ± 37.9	91.2 ± 2.51	190.9 ± 4.57	282.1 ± 2.18	52.6 ± 0.45
	No grass	5	912.3 ± 24.3	92.8 ± 2.23	92.2 ± 2.17	185.0 ± 3.19	28.2 ± 1.11
	Grass	5	918.2 ± 14.7	28.1 ± 1.92	79.8 ± 2.46	107.9 ± 2.59	23.8 ± 1.07
Source of variation:							
Heating (H)			*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Grass (G)			0.333	*<0.001*	*<0.001*	*<0.001*	*<0.001*
H × G			0.233	*<0.001*	0.422	*<0.001*	*<0.001*

[a]$n = 4$ for initial (i.e., before incubation) samples of available P.

Note: Values are means ± standard error. Results of ANOVA (p-values) indicate significant effects of heating and grass treatments only at the end of the incubation (i.e., not the initial). Significant ($p < 0.05$) p-values are in italics.

	AGB (g)	BGB (g)	Total biomass (g)	R/S
Unheated	4.7 ± 0.35 a	4.2 ± 0.86 a	9.0 ± 1.15 a	0.88 ± 0.14 b
T200	12.0 ± 0.79 b	10.8 ± 1.10 b	22.8 ± 1.85 b	0.90 ± 0.05 b
T450	15.2 ± 0.66 c	7.6 ± 0.57 b	22.8 ± 1.13 b	0.50 ± 0.03 a
p-value	*<0.001*	*0.001*	*<0.001*	*0.013*

TABLE 4 Above- (AGB), belowground (BGB) and total grass biomass, and root: Shoot ratios (R/S) in sowed soils at the end of the incubation period

Note: Values are means ± standard error ($n = 5$). Different letters indicate significant differences between treatments (Tukey test). Significant ($p < 0.05$) p-values are in italics.

Although total C and nutrients were not altered with the T200 treatment, extractable C and nutrient content in the soil changed significantly. Indeed, this treatment increased K_2SO_4-extractable organic C (by 72%) and total extractable N (by 55%) compared to unheated soils, while it did not alter the available P (Table 3). The increase in extractable N occurred only in organic forms, while the extractable inorganic N was similar to the unheated soils (Table 3). The T450 treatment caused stronger effects on extractable nutrients than T200 treatment. Indeed, extractable organic C increased by 202% and total extractable N by 344% compared to the unheated soils. The increase of extractable N occurred in both fractions, that is, organic and inorganic. Heating at 450°C also caused a

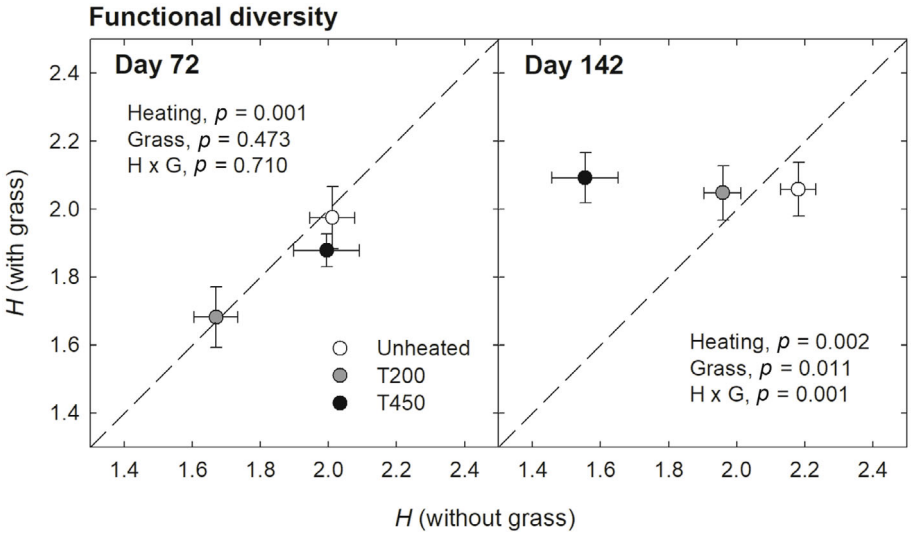

FIGURE 1 Catabolic functional diversity (mean ± standard error, $n = 5$) of soils with and without grass by heating treatments at the 72nd day and at the end of the incubation period (day 142). Catabolic functional diversity is expressed as the Shannon diversity index (H) of respiration responses to the addition of 15 organic substrates (Table 1) using a Microresp™ system

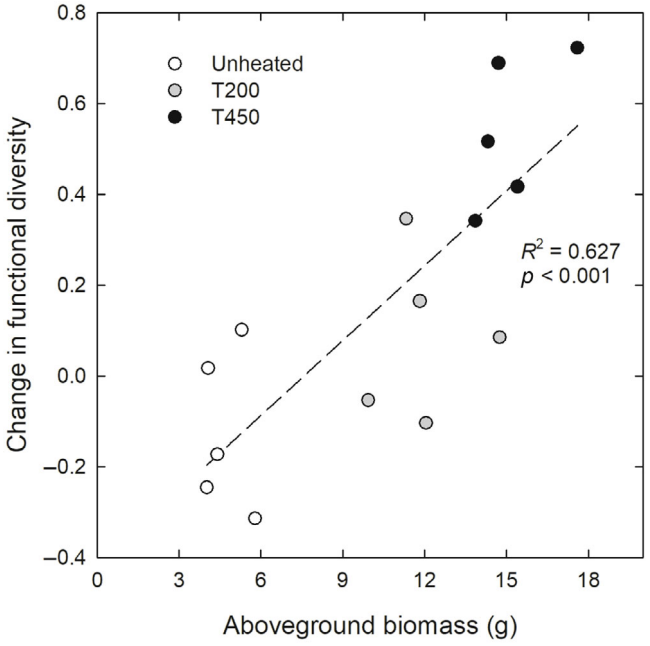

FIGURE 2 Correlation between the grass effect on functional diversity and aboveground biomass by heating treatments. The effect of grass on catabolic functional diversity was calculated as the difference in Shannon index of each soil with grass and the mean Shannon index of the corresponding (i.e., with the same heating treatment) soils without grass

huge increase in available P, resulting in values that were about 7–8 times higher than those in the unheated or T200 treated soils (Table 3).

3.2 | Above- and belowground biomass

At the end of the incubation period, both above- and belowground *Lolium perenne* biomass were significantly higher in the heated than in the unheated soils (Table 4). Total biomass (i.e., above- plus belowground biomass) did not differ between the two heating treatments (T200 and T450), but the root/shoot ratio (R/S) in T450 soils was lower than in T200 soils (R/S = 0.50 ± 0.03 vs. R/S = 0.90 ± 0.05, $p = 0.013$), which in turn was similar to those in unheated soils (R/S = 0.88 ± 0.14, $p > 0.05$) (Table 4).

TABLE 5 Basal respiration (μgC g^{-1} h^{-1}) at the two samplings by heating and grass treatments

		Day 72	Day 142
Unheated	No grass	0.027 ± 0.005	0.094 ± 0.028
	Grass	0.088 ± 0.048	0.067 ± 0.010
T200	No grass	0.045 ± 0.019	0.092 ± 0.015
	Grass	0.049 ± 0.016	0.126 ± 0.023
T450	No grass	0.045 ± 0.014	0.076 ± 0.015
	Grass	0.026 ± 0.003	0.117 ± 0.018
Source of variation:			
Heating (H)		0.855	0.350
Grass (G)		0.438	0.308
H × G		0.188	0.166

Note: All values are mean ± standard error of mean ($n = 5$). Results of ANOVA (p-values) are also indicated.

3.3 | Extractable carbon and nutrients in soils incubated with and without grass

After the 142 day incubation, K_2SO_4-extractable organic C content in grass-free unheated soils was similar to the pre-incubation values. In heated soils, in which heating treatment had caused a large increase of extractable C content,

it decreased during the incubation but still remained significantly higher than in unheated soils at the end of the incubation at both heating treatments (Table 3).

During the incubation period (142 days), total K_2SO_4-extractable N content in grass-free soils notably increased in both unheated and T200 soils but, in T450 soils, it decreased by 34% from the pre-incubation values. The increase in unheated and T200 was mainly due to large increases in the mineral fraction (mainly increases in NO_3^- which offset the little reductions in NH_4^+, data not shown), while the organic N fraction showed moderate increases (32%, unheated) or reductions (−26%, T200; −52%, T450) (Table 3). The large reduction of total extractable N in T450 soils during the incubation was mostly due to the large reduction in the organic fraction, while the mineral N content, which had already increased notably with heating, remained stable during the incubation.

Available P showed few changes during the incubation period in unheated and T200 samples, with small decreases in the former and mild increases in the later (Table 3). In T450 soils, whose initial available P values were much higher than in the other heating treatments, it decreased substantially. However, this decrease was not enough to reach the levels of unheated or T200 soils.

The presence of grass did not significantly alter the extractable organic C content in any of the heating treatments during the incubation (Table 3). In contrast, total extractable N content was reduced by grass in all treatments at the end of the incubation (Table 3). Most of this reduction can be attributed to the large decrease of the mineral fraction of the extractable N. The low extractable mineral N (i.e., mainly NO_3^--N) content in soils with grass at the end of the incubation resulted in values that were similar (unheated) or even lower (T200 and T450) than the mean pre-incubation values (Table 3).

Available P at the end of the incubation had not been altered by the presence of grass in unheated soils, but in both heating treatments, grass reduced it compared to the soils without grass (Table 3). In T450 soils, despite such reduction, the resulting available P values were still much higher than in unheated or T200 soils (Table 3).

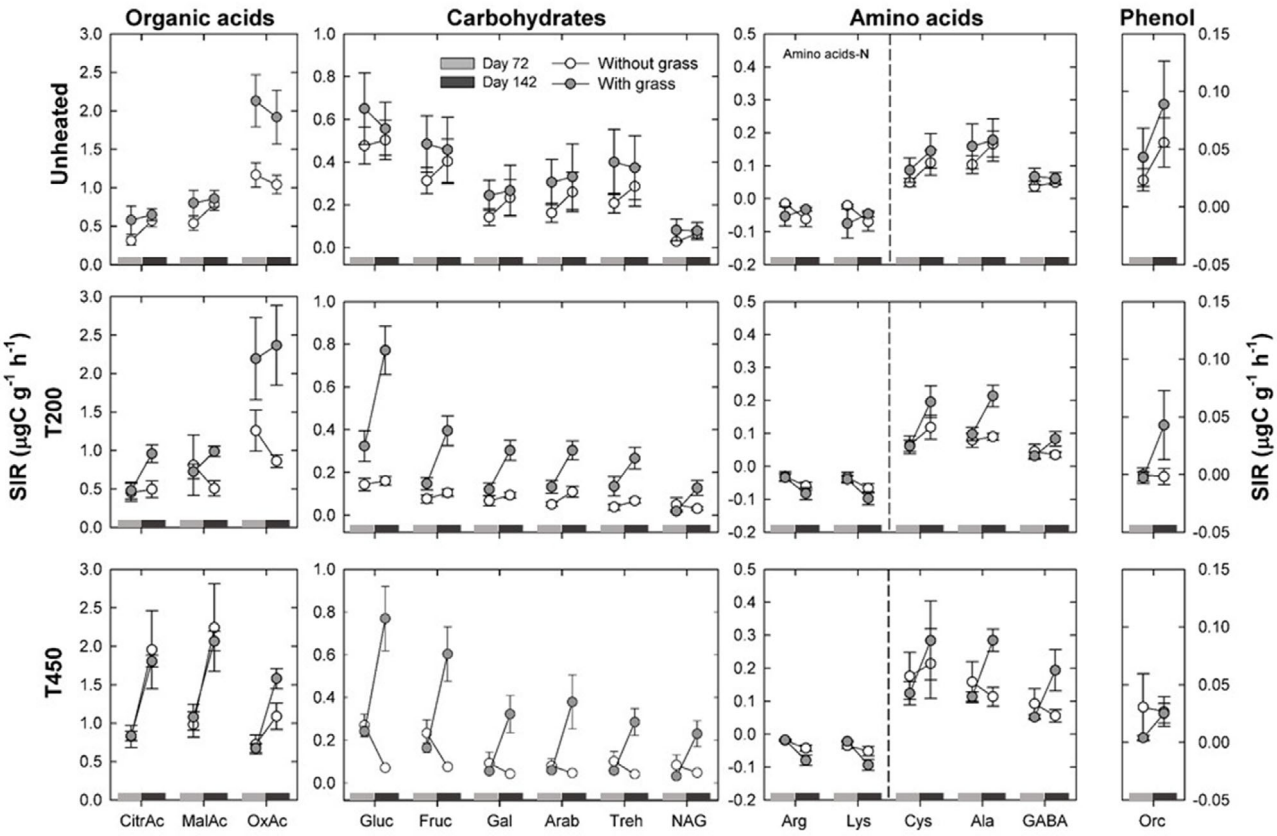

FIGURE 3 Substrate-induced respiration (SIR) (mean ± standard error, $n = 5$) of unheated and heated soils. For each heating and grass treatment, couples of SIR values for each substrate provide mean SIR ($n = 5$) at days 72 (left circles) and 142 (right circles) of the incubation. Given the observed different behaviour of arginine and lysine (i.e., those richest in N) from the other amino acids, they were analysed separately, as indicated by the vertical dashed line. Note the change in scale between types of substrate

TABLE 6 Final fixed effect model coefficient estimates of mean SIR (μgC g^{-1} h^{-1}) for each substrate group, and the marginal and conditional model variance (R^2m, R^2c) explained by the models

	Organic acids	Carbohydrates	Amino acids	Amino acids-N	Phenol
Model significance					
R^2m	0.211	0.282	0.142	0.290	0.201
R^2c	0.583	0.664	0.363	0.437	0.201
Fixed effects					
Intercept	0.7534*	0.2219**	0.0548	−0.0175	0.0412***
HeatingT200		−0.1507*		−0.0142	−0.0432**
HeatingT450	−0.0688	−0.0781	0.0542*	−0.0093	−0.0312*
Grass	0.3367**	0.1392^{+}	0.0376^{+}	−0.0465*	
Time2	0.0490	0.0703^{+}	0.0550^{+}	−0.0479**	0.0233*
HT200 × G		−0.0639		0.0417	
HT450 × G		-0.1815^{+}		0.0537*	
HT200 × T2		−0.0468		0.0166	
HT450 × T2	0.8877***	−0.1596**		0.0277	
G × T2		−0.0875		0.0728***	
HT200 × G × T2		0.2789***		−0.0948**	
HT450 × G × T2		0.5068***		−0.1191***	

Note: The intercept represents the coefficients for the unheated treatment, without grass, at time 1, and the other parameters indicate the additional contribution of each term to their coefficients. Arginine and lysine (amino acids-N) respiration responses were analysed as a separate group from other amino acids (cysteine, alanine and GABA) given their observed different behaviour from the other amino acids. ***$p < 0.001$, **$p < 0.010$, *$p < 0.050$, $^{+}p < 0.100$. Time2 or T2 refers to the second sampling (day 142).

3.4 | Catabolic functional diversity in soils with and without grass

After 72 days of incubation, soil catabolic functional diversity index was not affected by the presence of grass in any heating treatment (Figure 1). Irrespective of grass treatment, catabolic functional diversity in T200 soils was lower than in unheated or T450 soils, due to their lower respiration responses to most substrates (except to organic acids). But at the end of the incubation (day 142, Figure 1), T200 soils had recovered their catabolic functional diversity, resulting in values that were similar to that in unheated soils. By contrast, T450 soils without grass showed a low diversity of catabolic functions compared to T200 or unheated soils without grass at the end of the incubation, while catabolic functional diversity in heated soils with grass was similar to the unheated soils, irrespective of the heating temperature (Figure 1).

The effect of grass on catabolic functional diversity, as measured as the difference in the Shannon index values between each soil with grass and the mean Shannon index of soils with the same heating treatment without grass, increased with AGB ($r = 0.627$, $p < 0.001$, Figure 2). By contrast, the effect of grass on soil catabolic functional diversity did not correlate with BGB.

3.5 | Substrate-induced respiration in soils with and without grass

Basal soil respiration was not significantly affected by heating treatments or the presence of grass (Table 5). The addition of organic substrates caused a positive response at all treatments, except for the addition of arginine and lysine, which caused a small negative effect on CO_2 efflux (Figure 3). Organic acids were the substrates that caused the greatest response, followed by carbohydrates. Amino acids and phenol were the compounds that caused the smallest respiration response (Figure 3).

Overall, the response to all substrate types (i.e., organic acids, carbohydrates, amino acids and phenol) were altered, to a greater or lesser extent, by heat treatment (Figure 3, Table 6). Even though respiration responses to carbohydrate additions at the end of the incubation were depressed in soils heated at both temperatures, the magnitude of the effect was greater at T450 than at T200 soils. However, the presence of grass during the incubation caused a notable increase of the responses to these substrates at the last sampling in both T200 and T450 soils (Heating × Grass × Time2, $p < 0.001$; Table 6). Indeed, while at the end of the incubation, the respiration responses to carbohydrate additions in heated grass-free soils were similar or lower than those in the

first sampling, heated soils with grass had response values that were closer to the values in unheated soils (Figure 3). At the end of the incubation, across all heating treatments, mean responses to carbohydrate additions in grass-free soils were negatively correlated with the extractable C ($r = -0.670$, $p = 0.006$) and total extractable N ($r = -0.697$, $p = 0.004$). The significant correlation with available P ($r = -0.526$, $p = 0.044$) was mainly due to the very high values of available P in T450 soils compared to any other treatment (Table 3). None of these correlations were significant in soils with grass.

Among the amino acids, two different response patterns were found. While addition of cysteine, alanine, and GABA stimulated microbial activity (i.e., positive SIR), the addition of arginine and lysine inhibited the respiration (i.e., negative SIR) (Figure 3). These two groups also differed in the factors explaining the respiration responses to their addition (Table 6). The respiration response to the addition of cysteine, alanine, and GABA (Amino acids in Table 6) was greater ($p < 0.050$) in T450 soils than in T200 or unheated soils. The presence of grass, marginally significant (Amino acids in Table 6), also tended to increase the responses to these substrates. By contrast, the addition of arginine and lysine (i.e., Amino acids-N in Table 6) at the first sampling slightly stimulated the CO_2 efflux only in T450 soils with grass (HeatingT450 × Grass, $p < 0.05$). At the end of the incubation, in soils with grass, lower CO_2 effluxes were observed in both heated soils compared to unheated soils (Heating × Grass × T2, $p < 0.010$). In line with this, in the soils with grass ($n = 15$), the mean respiration responses to the addition of lysine and arginine across the three heating treatments correlated negatively with belowground biomass ($r = -0.770$, $p = 0.001$) and extractable C content ($r = -0.544$, $p = 0.036$). Hence, the additions of these two amino acids slightly inhibited soil respiration (i.e., negative SIR), but this inhibition was greater in soils containing higher root biomass and extractable organic C.

The respiration response to the organic acid additions were notably stimulated in T450 soils, at the end of incubation, in comparison to the unheated and T200 soils (HeatingT450 × Time2, $p < 0.001$). The presence of grass also stimulated the respiration responses irrespective of heating treatment ($p < 0.010$). Looking across all heating treatments, the mean response to the addition of organic acids increased with AGB ($r = 0.640$, $p = 0.010$). By contrast, the responses to the addition of a phenol compound were reduced by heating treatments at both temperatures (HeatingT200, $p < 0.010$; HeatingT450, $p < 0.050$), and they were not altered by the presence of grass (Table 6).

4 | DISCUSSION

Forest fires generally cause an immediate reduction of soil microbial biomass and a shift in microbial community structure (Bárcenas-Moreno et al., 2011; Pérez-Valera et al., 2019; Prendergast-Miller et al., 2017). Soil heating also causes a release of a substantial amount of soluble organic C and nutrients, which can be attributed to the labile organic matter released from dead microbes (Guerrero et al., 2005; Serrasolsas & Khanna, 1995). Additionally, in strongly heated soils, the disruption of aggregates further expose physically protected organic matter to leaching (Jian et al., 2018). These labile compounds are generally used rapidly by microorganisms, which may allow the recovery of microbial abundance and cause a rapid increase in post-heating soil respiration (Guerrero et al., 2005). Plants can also take advantage of released nutrients to regrow after fire (Kutiel & Naveh, 1987) which, in turn, will influence the post-fire recovery of microbial communities and their functions (Knelman et al., 2015).

4.1 | Fire heating and microbial functions

In the absence of grass, soil heating altered the microbial substrate utilisation patterns, a change that was still significant 5 months after treatment. Changes in the functional profiles have previously been described after experimental fires by D'Ascoli et al. (2005) in Mediterranean maquis soils, which were attributed to changes in the community structure as has often been described (e.g., Goberna et al., 2012; Prendergast-Miller et al., 2017). Generally, after an initial decline in microbial abundance in the surface mineral soil, opportunistic microorganisms take advantage of the high C and nutrient availability of recently burnt soils to quickly recolonise them. Fires tend to favour those microorganisms with heat-resistance capacities (e.g., spore formers) and those with potential fast-growth strategies (Bárcenas-Moreno et al., 2011), usually resulting in increases in bacterial predominance and declines in fungal abundance (Fultz et al., 2016; Rutigliano et al., 2007), which may have consequences at a functional level.

In our study, changes in the substrate utilisation patterns led to declines in the diversity of catabolic functions in T200 soils at the first sampling, and in T450 at the end of the incubation. Given the short-term (i.e., 72 days) effect of soil heating on catabolic functional diversity at T200 soils, the lack of significant effects at T450 was unexpected. In the short term, labile soluble organic matter release in heavily heated soils may briefly maintain relatively high

rates of some microbial functions compared to soils subjected to light or moderate heating. Higher respiration responses to carbohydrate additions in T450 than in T200 treatments at the first sampling may reflect this phenomenon. This observation is in line with previous studies that showed a short-term increase in soil β-glucosidase activity after fire, which is linked to the high levels of soluble carbohydrates in burnt soils (Goberna et al., 2012), an effect that, according to our results, may persist for at least some weeks. These high rates of some microbial functions in heavily heated soils led to relatively high catabolic functional diversity values observed in T450 soils compared to T200 soils in the first sampling. However, this effect may be transitory, as shown by the functional profiles and the low catabolic functional diversity of T450 soils at the end of the incubation.

At the end of the incubation, extractable organic C content was still notably higher in heated than in unheated soils. The persistence of soluble organic matter together with the low metabolic capacity of soil communities in T450 soils may indicate that a fraction of the released soluble organic matter was not as labile as is usually assumed. This result and the observed low phenol SIR in T450 soils indicate that recalcitrant aromatic compounds of pyrogenic origin are probably abundant in high-temperature heated soils (Hobley et al., 2019; Santos et al., 2016), which may have relevant implications in the post-fire microbial functions and C dynamics.

The high nutrient content of heated soils could also have a role in reducing microbial respiration responses to the addition of organic substrates. Indeed, as previous research demonstrated, nutrient enrichment has a negative impact on organic matter decomposition and CO_2 efflux in soils (Treseder, 2008; Zak et al., 2008). In line with this, it is indicative that our additions of amino acids with high N content (i.e., arginine and lysine) were the only substrates that caused a negative SIR. Hence, in strongly heated soils, the increase of nutrient availability, together with the aromatic nature of soluble organic compounds, and maybe an eventual alteration of the structure of the microbial communities may contribute to slow down the C cycling and to maintain the low catabolic functional diversity.

The high respiration responses to the addition of organic acids at the end of the incubation in T450 soils compared to T200 or unheated soils also contributed to reduce their catabolic functional diversity. Organic acids are generally rapidly degraded in soils, with an average half-life of about 2–3 h (Jones, 1998); these substrates always caused the highest SIR responses in our soils. This phenomenon may be partly due to the fact that citric and malic acids can be directly incorporated into the Krebs cycle, therefore avoiding the energy (and enzymes) needed for glycolysis. However, we do not know the mechanism by which the metabolisation of organic acids was enhanced in the T450 soils. One possible explanation is that microorganisms degrading the most labile substrates were the most favoured by heating. Indeed, opportunistic bacterial populations are rapidly recovered after fire, taking advantage of the release of labile organic matter (Bárcenas-Moreno et al., 2011; Bárcenas-Moreno & Bååth, 2009; Guerrero et al., 2005). Fire-induced increase of organic acids and carboxyl content in soils has been previously observed (Blank et al., 1994; Knicker et al., 2006), probably because of oxidation of pyrolysed residues (Knicker et al., 2006). This may explain why the increase in decarboxylase activity occurred in T450 soils, which is expected to contain more pyrolysed organic matter than T200 soils.

4.2 | Plant–soil interactions and microbial functions

Plants influence soil microbial communities through the regulation of the quantity and quality of organic substrates released to the soil, and through the competition for nutrients (Hart et al., 2005). After a fire, plants generally take advantage of the fire-driven increase of nutrient availability to regrow (Kutiel & Naveh, 1987; Pausas et al., 2003; Pereira-Silva et al., 2019). This also occurred in our heated soils, causing high above- and belowground biomass in heated soils compared to the unheated. The different plant biomasses, particularly photosynthetic biomass, in heated and unheated soils may have determined the plant influence on soil microbial functions, as it may affect the amount of rhizodeposits (Kuzyakov & Cheng, 2001). Plants release a substantial amount of labile organic substrates (Hütsch et al., 2002), providing resources to soil microbial communities. Rhizodeposition is key for microbial recolonisation and can shape the microbial community structure not only in the rhizosphere (Paterson et al., 2007) but also in the bulk soil (Brant et al., 2006). The wide range of chemical forms in rhizodeposits (i.e., from labile exudates to complex compounds coming from dead root tissues) may configure the community structure, as it is known that different compounds are used by distinct microbial taxa (Paterson et al., 2008).

In our heated soils, the presence of plants caused a notable influence on microbial communities at a functional level. Indeed, plants caused shifts in the patterns of microbial substrate use at the end of the incubation, particularly by stimulating the respiration response to carbohydrate additions. Previous research shows that plant colonisation after fire caused an increase in some soil

enzyme activities like β-glucosidase, acid phosphatase or *N*-acetylglucosaminidase in high severity fires (Knelman et al., 2015). This is consistent with our results, which show that the effect of grass on respiration responses to carbohydrate additions was stronger on T450 than on T200 soils in spite of having less root biomass (and more AGB). This stronger effect of grass on T450 than on T200 soils suggests that the effect of plants on microbial functions through their rhizodeposition may be more related to the photosynthetic activity than to the root biomass. Kuzyakov and Cheng (2001) showed that CO_2 efflux from the rhizosphere were tightly coupled with the photosynthetic activity of plants, suggesting that it is central in regulating plant rhizodeposition. In our planted T450 soils, this effect led to the recovery of microbial functions and catabolic functional diversity to levels similar to the unheated ones, while microbial communities in non-planted soils had substantially reduced their catabolic capacities (i.e., low catabolic activity and diversity). The plant-mediated recovery of microbial functions may be relevant for the post-fire recovery of soil fertility, which may subsequently prevent soil degradation. The effect of plants on post-fire recovery of soil microbial functions highlights the importance of plant–soil interactions as the mechanism shaping the activity of soil microbial communities and driving the post-fire functional recovery of soils, and the need to study such interactions under field conditions.

5 | CONCLUSIONS

Fire-induced soil heating notably alters the capacity of microbial communities to utilise organic substrates, which results in a reduction in the diversity of microbial catabolic functions. Moderate heating (T200) generally caused a reduction in the respiration responses, particularly after the addition of carbohydrates, some amino acids, and phenol. In spite of their low catabolic activity, the catabolic functional diversity in moderately heated soils recovered 5 months after the heating event. By contrast, in high-intensity fires with heavily heated soils (T450), the large release of soluble organic C allowed a relatively high catabolic activity (lower than unheated soils), which lead to transitory maintenance of catabolic functional diversity. However, both respiration responses to substrate additions and catabolic functional diversity were remarkably reduced 5 months post-heating.

Plant growth is enhanced by the fire-driven increase in soil nutrient availability, which is key for the post-fire recovery of microbial functions. The recovery of soil catabolic activity and functional diversity is coupled to post-fire plant development. Plants may supply organic substrates of diverse quality through the rhizodeposition, stimulating the catabolic activity in burned soils, particularly the utilisation of carbohydrates and organic acids. These results highlight the importance of plant–soil interactions for the post-fire recovery of soil functionality and, by extension, for the ecosystem functions.

AUTHOR CONTRIBUTIONS

Jordi Garcia-Pausas: Conceptualization (equal); data curation (lead); formal analysis (equal); investigation (lead); methodology (equal); writing – original draft (lead); writing – review and editing (lead). **Joan Romanyà:** Investigation (equal); supervision (equal); visualization (equal); writing – original draft (supporting); writing – review and editing (supporting). **Pere Casals:** Conceptualization (equal); formal analysis (equal); funding acquisition (lead); investigation (equal); methodology (equal); project administration (lead); supervision (lead); writing – original draft (supporting); writing – review and editing (supporting).

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Jordi Garcia-Pausas https://orcid.org/0000-0003-2727-3167
Joan Romanyà https://orcid.org/0000-0002-4519-9653
Pere Casals https://orcid.org/0000-0002-0372-988X

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