CURSO ACADÉMICO 2021-2022

UNIVERSIDAD DE CÓRDOBA

ETSIAM
Escuela Técnica Superior de Ingeniería Agronómica y de Montes

EFFECTS OF HIGH-INTENSITY CONTROLLED BURNING IN PHYSICAL-CHEMICAL AND BIOLOGICAL SOIL PROPERTIES IN "LOS BOQUERONES" AREA

TRABAJO FIN DE MÁSTER

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AKNOWLEDGEMENTS

First of all, I would like to thank the LABIF team, as well as CILIFO, for making this work possible.

for making this work possible, as well as for providing me with the necessary information and offering me the possibility to see first hand the burning process.

Many thanks also to my director of the Final Masters Project: Mr. Antonio Rafael Sánchez Rodríguez. Thank you very much, Antonio, for helping me at all times even from a distance with endless video calls, for solving my millions of doubts and encouraging me at all times.

Many thanks to Mrs. María del Campillo García for encouraging me to improve in all aspects of my life, for trusting me and for encouraging me to find solutions and face the situations I have faced throughout this year.

Many thanks to José María, for helping me in everything that was in your hands at any time and for making the mornings in the laboratory more pleasant. Also to the rest of the department for welcoming me as one more and making me participate in the life of this wonderful department.

I would like to thank my friends and colleagues, who have always been with me, and have supported me in the moments when I needed it most, as well as for celebrating the worthy moments with me. moments worth celebrating with me.

Last but not least, to my family, for teaching me every day to improve, for the advice and for being there at all times.

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El uso del fuego para la gestión de los ecosistemas forestales se ha hecho más frecuente en los últimos años en Europa. El fuego tiene un gran impacto en el suelo y, por tanto, es necesario entender cómo las quemas controladas afectan a este recurso no renovable, esencial para la vida en los ecosistemas (forestales). El propósito de este estudio fue evaluar las principales alteraciones en las propiedades fisicoquímicas y biológicas del suelo como consecuencia de una quema controlada de alta intensidad en la zona de "Los Boquerones" (municipio de Villaviciosa de Córdoba) en dos momentos diferentes, inmediatamente después de la quema y ocho meses después (recuperación). Los objetivos específicos fueron evaluar la heterogeneidad espacial de las alteraciones de las diferentes propiedades del suelo, así como abordar la relación entre la ocurrencia del fuego y las comunidades microbianas del suelo. Se estableció una malla de 12 puntos, representativos de 1,4 ha, en una ladera de Sierra Morena (Córdoba), con suelos desarrollados sobre areniscas, pizarras. En cada punto se colocaron sensores térmicos y se recogieron muestras de suelo a dos profundidades (0 - 2 y 2 - 5 cm) antes de la quema, inmediatamente después de la quema y ocho meses después (recuperación). Se analizaron la susceptibilidad magnética del suelo, el color, el pH, la conductividad eléctrica, la capacidad de intercambio catiónico, el contenido y/o la disponibilidad de nutrientes, entre otros, y sus variaciones espaciales y temporales. El pH del suelo, principal impulsor de los microorganismos del suelo aumentó sustancialmente en los primeros centímetros del suelo (0 - 2 cm) inmediatamente después de la quema hasta más de 2 unidades, y el aumento se mantuvo ocho meses después de la quema. Esto puede ser de interés para la gestión forestal (selección de especies vegetales) y el control y la prevención de enfermedades. Además, la quema de alta intensidad tuvo un efecto positivo a corto plazo sobre algunas de las propiedades del suelo, como la disponibilidad de nutrientes para las plantas, que aumentó considerablemente. En este sentido, el fósforo disponible se incrementó en más de 30 mg kg-1 después de la quema en los 2 cm superiores del suelo, y aunque hubo una disminución posterior, la cantidad de P disponible seguía siendo mayor que antes de la quema ocho meses después de ésta. Se observó una tendencia similar para la capacidad de intercambio catiónico. La magnitud de las alteraciones de los indicadores del suelo evaluados se explicaba espacialmente por el comportamiento del fuego durante la quema controlada. La quema también afectó a los microorganismos del suelo tanto directa como indirectamente. Así, tanto la intensidad del fuego como las alteraciones en las propiedades del suelo determinaron su comportamiento. En conclusión, los posibles efectos inmediatos y a corto y medio plazo de las quemas sobre el recurso no renovable suelo deben ser considerados para una gestión más holística del fuego en los ecosistemas forestales, ya que su funcionalidad y capacidad de proporcionar servicios ecosistémicos se ve ampliamente alterada por estos eventos en función de su intensidad.

Palabras clave: propiedades del suelo, quema controlada, fuego, servicios ecosistémicos, funcionalidad del suelo, manejo forestal.

ABSTRACT

In the last years, the use of fire to manage forest ecosystems has become more frequent in Europe. Fire has a great impact on the soil and therefore it is necessary to understand how controlled burns affect this non-renewable resource, essential for life in (forest) ecosystems. The purpose of this study was to evaluate the main alterations in the physical-chemical and biological properties of the soil as a result of a high intensity-controlled burn in the "Los Boquerones" area (municipality of Villaviciosa de Córdoba) at two different times, immediately after the burn and eight months later (recovery). The specific objectives were to assess the spatial heterogeneity of the alterations of the different soil properties as well as addressing the relationship between fire occurrence and soil microbial communities. A grid of 12 points, representative of 1.4 ha, was and stablished on a hillside in Sierra Morena (Córdoba), with soil developed on sandstones and slates. At each point, thermal sensors were placed, and soil samples were collected at two depths (0 – 2 and 2 – 5 cm) before burning, immediately after burning and eight months later (recovery). Soil magnetic susceptibility, colour, pH, electrical conductivity, cation exchange capacity, nutrient content and / or availability, between other, and their spatial and time variations were analysed. Soil pH, the main driver for soil microorganisms, was substantially increased in the first centimetres of the soil (0 – 2 cm) immediately after burning up to more than 2 units, and the increase was maintained eight months after the burn. This may be of interest for forest management (plant species selection) and disease control and prevention. In addition, the high-intensity burn had a positive short-term effect on some of the soil properties, such as nutrient availability for plants, which was considerably increased. In this sense, available phosphorus was increased more than 30 mg kg^{-1} after the burn in the uppermost 2 cm of soil, and although there was a decrease later, the amount of available P was still higher than before the burn eight months after the burn. A similar trend was observed for cation exchange capacity. The magnitude of the alterations in the soil indicators assessed was spatially explained by the behaviour of the fire during the controlled burning. The burn also affected soil microorganisms both directly and indirectly. Thus, the intensity of the fire as well as the alterations on soil properties determined their behaviour. In conclusion, the possible immediate and short to medium term effects of burning on the non-renewable resource soil should be considered for a more holistic management of fire in forest ecosystems, as its functionality and capacity to provide ecosystem services is largely altered by these events as a function of their intensity.

Key words: soil properties, prescribed fire, fire, ecosystem services, soil functionality, forest management.

INTRODUCTION

THE PROBLEM OF FOREST FIRES ON A GLOBAL SCALE

The planet is in a process of continuous change accelerated by human influence, which implies a variation in climatic and ecosystem conditions. In general, climate change leads to a rise in average temperature and a decrease in humidity globally, as well as an increase in the occurrence of extreme events, including heat waves, drought, storms and floods (Prichard et al., 2017). These changing conditions favour the occurrence of increasingly severe wildfires and the occurrence of wildfires in areas where they did not occur before, such as North Europe and Asia (Prichard et al. 2017; Pechony & Shindell, 2010). Along with climate change, another factor affecting the frequency and severity of wildfires is land-use change. In recent decades, many rural areas traditionally used for agricultural activities have been abandoned due to migration to cities (Alcañiz et al., 2018).

Most wildfires have an anthropogenic origin. In 2000, 350 million hectares were affected by forest fires, of which about 80% were caused by humans both intentionally and through negligence (Alcañiz et al., 2018). This fact implies that among the most important prevention measures is to raise awareness of the impacts that forest fires can have in our society and in the environment. The importance of forest resources and the impacts of fires are overshadowed and diluted in the society. The main negative effects of forest fires are forest degradation, soil erosion, increase in pest and disease attacks, greenhouse gas emissions, among others (Caon et al., 2014; de Faria et al., 2017). However, it should not be forgotten that some ecosystems are adapted to fires and need it for their dynamics to take place (Alcañiz et al., 2018; FAO, 2006; García-Chevesich, 2012; Pausas et al., 2008). Therefore, it is necessary to consider a holistic management of fire, beyond the simple suppression or minimisation of fires.

One of the major stumbling blocks of the global forest fire problem is the lack of reliable and up-to-date information, due to the difficulty of monitoring and recording fires (FAO 2006). However, new technologies allow us to have first-hand access to fire-related information through remote sensing and satellite imagery in relation with the fire severity effects in the vegetation, while further work is necessary to understand the effects of fire in the soil. Another factor influencing the unreliability of existing fire information is the absence of common and robust terminology. This in turn is very challenging to economically account for the effects of fire, making it difficult to prioritise the restoration of ecosystems and landscapes after a fire.

In terms of prevention in different parts of the world, the main objective is to raise awareness and sensitise people to the impacts of fire and its proper use (FAO 2006). Fire is a key tool in agricultural and forestry management, so a total ban on its use is misplaced (Pérez et al., 2018; Rodrigues et al., 2020). Despite this, there are still many places in the world where the use and management of controlled fire is prohibited due to the damage caused by uncontrolled forest fires (Quigley et al., 2020). This is due to a lack of research and experience in the use of fire as a tool in prevention and fire and wildland firefighting (Harper et al., 2018). Prescribed and controlled burns are useful tools to decrease the area affected by future wildfires as well as to create barriers to stop them (Prichard et al. 2017).

Commonly used suppression methods are based on ground fighting, using hand tools and organisation of firefighting patrols. Aerial mechanisms (light aircraft, helicopters, drones, etc.) are available to support ground fighting, but without good organisation on the ground, suppression may not be successful (FAO 2006). The way in which governments and administrations deal with forest fires varies from one country to another. In some countries, such as those in the Mediterranean area, the responsibility for forest fires lies with the forest authorities. Other countries have opted to fight forest fires with non-specialised firefighters, which makes interventions difficult in many cases (FAO 2006). In general, there is a considerable number of institutions involved in firefighting, which implies that coordination between them is essential, or firefighting will be deficient. Additionally, there are multiple international cooperation agreements concerning the problem of forest fires at a global level, coordinated by organisations such as the Food and Agriculture Organization (FAO), the World Health Organization (WHO) or the United Nations (UN). Some examples of actions that have taken place at the international level in the fight against forest fires are the creation of the World Summit on Sustainable Development, the Global Wildland Fire Network or the Global Fire Monitoring Centre (Goldammer, 2003).

Although prevention and suppression methods are necessary to prevent wildfires, there is a counterpart to their application, the so called “Fire paradox”(Rego & Rigolot, 2011). This paradox explains that with the current policies of suppression, fire events do not occur at all, meaning that areas that need fire for their natural dynamics accumulate high quantities of fuel, which leads to potential more severe wildfires when they happen. A possible way to address this paradox would be the use of fire as a tool of forest and fuel management (Arévalo & Naranjo-Cigala, 2018).

THE PROBLEM OF FOREST FIRES IN SPAIN

Spain is the country in the European Union where most forest fires occur (Greenpeace, 2020), and the situation may worsen given the vulnerability of Mediterranean ecosystems to climate change (Alcañiz et al., 2018; Rodrigues et al., 2020). The main causes that lead to changes in the ignition patterns throughout Spain include anthropic-related reasons as well as policies that prioritize fire suppression (Gonzalez et al., 2007). Until 1955, the situation was similar to that of other countries, i.e. there was insufficient information on fires (number of fires, severity, burnt area, etc.), but in that year the Forest Fire Service was created with the aim of systematising and standardising the collection of data through the so-called Fire Reports (Área de Defensa contra & Incendios Forestales (ADCIF), 2017). With the information obtained through the Fire Reports, statistics were compiled at national, regional, and provincial level. So, it is possible to assess how the incidence of forest fires evolves depending on the conditions. Thanks to this availability of information, the cause of practically all forest fires that have occurred in the country since the 1950s is known. As most of the fires for which the cause is known are human caused, an effective measure is to try to reduce as far as possible the social conflicts that can lead to fires.

In recent years, the number of forest fires in Spain has decreased (Greenpeace, 2021). However, the proportion of large forest fires, which are those affecting more than 500 hectares, is higher (WWF España, 2018; Greenpeace, 2021). The size of these fires is so large that it difficult their extinction, resulting in a major impact. The most effective strategy is to act to prevent them from occurring or spreading out of control, by raising public awareness, urban planning, or fuel treatments to avoid fuel continuity (Greenpeace, 2021). Moreover, given the scale of these fires, they not only affect the forest and rural environments, but they also have serious repercussions on urbanised areas, with all that this entails.

In Spain, the total area affected by forest fires has been reduced in recent years thanks to the experience of the extinguishing teams and services, however, the figures are still alarming (WWF Spain 2018). One measure to improve this situation is the use of prescribed and controlled burning, as well as other preventive forestry treatments, in which, in addition to seeking the natural regeneration of the forest under controlled conditions, extinguishing brigades are trained to know how to act in the event of an uncontrolled forest fire.

PRESCRIBED FIRE AND CONTROLLED BURNS

Given the current situation of the environment and forest fires, it is necessary to identify and evaluate the tools that can be applied at different scales to mitigate this problem. These tools include forest management at a certain level of specificity, i.e., it is vital to know the characteristics and vulnerability of each specific forest system to make the right decisions. Within this forest management is preventive silviculture, which seeks to reduce the continuity of fuel, both horizontally and vertically, and that includes prescribed and controlled burning.

A differentiation between the concepts of forest fire and burns should be done. The term wildfire is referred to the uncontrolled burning of an area that was not intended to burn (National Geographic Society, 2019b). On the other hand, burns imply the controlled use of fire on areas to reduce the amount of fuel or the flame length to be reached (Junta de Andalucía, 2020; National Geographic Society, 2019a). Prescribed burns mean the use of fire in a planned manner under specific conditions of time, fuel and topographic parameters (Alcañiz et al., 2018) and controlled burns are those in which an area is delimited and subjected to the effects of fire freely, without establishing specific parameters of temperatures, intensities, or speeds (Corporación Nacional Forestal, 2013). Among the purposes of this last type of burning (controlled burns) are the reduction of vegetation fuel, mitigation of the fire spread, the increase of biodiversity in the area, regeneration of grasslands and training of firefighting brigades (Santín and Doerr 2016; Harper et al. 2018).

Wildfire and prescribed burning have effects on the functionality of forest ecosystems, including the water cycle (changing runoff dynamics, water solute composition), the carbon cycle (affecting sequestered carbon pools and gaseous exchanges) and other nutrients, habitats and biodiversity (changes in the distribution of plant species and the dominance of some over others, as well as affecting fauna and their distribution), containing soil as a support and development of life in the ecosystems (Alcañiz et al., 2018; Caon et al., 2014).

EFFECTS OF FIRE ON SOIL

Soil is the support of most of the life on Earth; so understanding how it reacts to disturbances such as forest fires is necessary to determine their impacts on the natural environment (Alcañiz et al., 2018). Fire has direct impacts on biological, physical and chemical soil properties due to the processes that take place during temperature rise and soil organic matter combustion (Alcañiz et al., 2018; Certini, 2005).

A series of alterations occur in the soil depending on the temperature reached in the soil under the effects of fire, summarised in Fig. 1. In general, fire has a greater impact on the surface layers of the soil (Abakumov et al., 2020; Alcañiz et al., 2018; Francos et al., 2018) due to the low thermal conductivity of soils. It prevents the effects of the high temperatures from having an influence beyond the first few centimetres of soil (Enninful & Torvi, 2008), where the highest temperatures occur. However, it also depends on other variables such as soil moisture, root presence. Additionally, deeper depths may be indirectly affected.

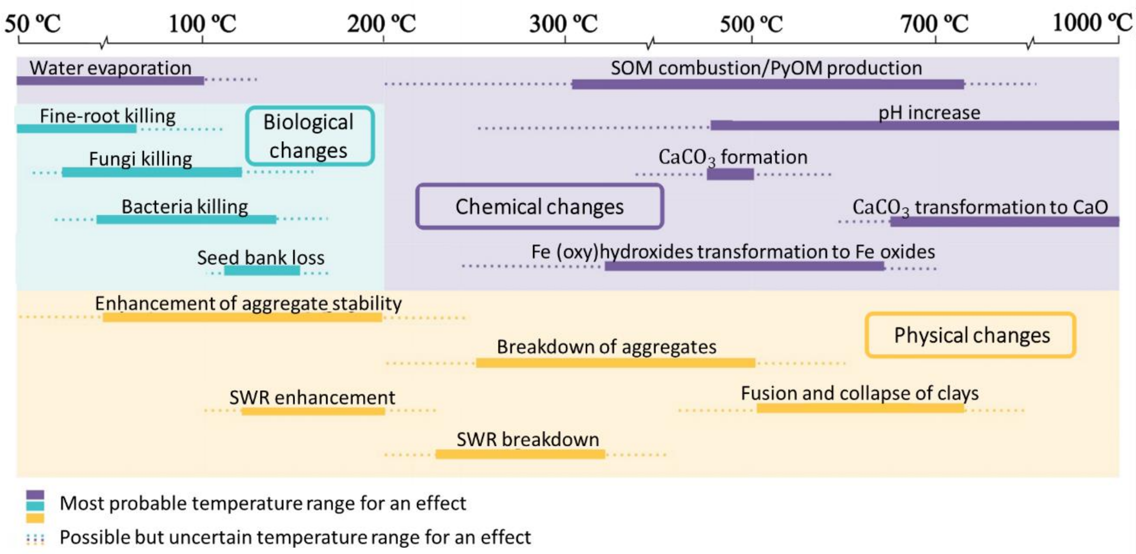

Figure 1 Effects of soil temperature on the variation of biological, physical and chemical soil properties (Adapted by me from: Santín & Doerr, 2016)

BIOLOGICAL PROPERTIES

At the biological level, plant roots, fungi, bacteria, seed banks and any form of life present in the soil are drastically affected when soil temperature exceeds 50 °C and especially when it reaches 150 °C (Adkins et al., 2020; Santín & Doerr, 2016), as shown in Fig. 1. Previous studies shown that the effects of high temperatures alter the biomass and composition of the microbial community, decreasing its activity in the soil (Múgica et al., 2018). The effects that fire can have on the biological properties of the soil mainly depend on the characteristics of the burn or fire, the amount of fuel, the microtopography and soil moisture (Alcañiz et al., 2018).

One popular approach nowadays to study the microbial community structure of a soil is by analysing the phospholipid fatty acids (PLFAs) or the neutral lipid fatty acids (NLFA), which are used to assess the conditions and alterations in the soil microbial structure

(Bååth, 2003; Veum et al., 2019). Several studies (Adkins et al., 2020; Fritze et al., 1993; Köster et al., 2021) concluded that microbial communities varied depending on the fire occurrence and burn severity.

PHYSICAL PROPERTIES

High temperatures during wildfires can lead to fragmentation or cracking of parent material, which increases weathering and, consequently, nutrient availability in a similar way to when rock gelifraction occurs (Santín & Doerr, 2016). This also leads to an increase of soil bulk density (Giorgis et al., 2021), although this effect is not clear as different authors have obtained different results (Alcañiz et al., 2018).

Soil structure is affected both by the combustion of the soil organic matter, and soil aggregates, as well as the content in clays (elements that favour soil structure). Usually, organic matter accumulates to a greater extent in the soil surface, so it is directly affected by high fire temperatures, being the major consumption of it in the temperature range from 200 °C to 460 °C (Alcañiz et al., 2018). In the short term, this could have a positive effect on vegetation, as it allows nutrients to be deposited in plant-available forms, although it can lead to a loss of fertility if the fire or burn is preceded by rainfall (Alcañiz et al., 2018). On the other hand, when the clay content of the first soil horizon is low, it will only be affected if this first horizon (O, A) does not exist, and the second horizon, which normally has a greater accumulation, is exposed. Even in this situation, clays are able to withstand high temperatures, up to 460 °C, without being altered, so they will only be affected by high intensity fires and with the B horizon exposed (Alcañiz et al., 2018; Caon et al., 2014; García-Chevesich, 2012).

Another physical property that is altered by high temperatures is soil porosity, which is directly related to the water infiltration capacity of the soil. Fire affects porosity by decreasing the proportion of macropores, so infiltration is also reduced, increasing runoff in the event of a rainfall event (García-Chevesich, 2012), and, finally, facilitating the loss of nutrients and fertility of the first horizon of the soil.

Another alteration produced by the passage of fire is the potential increase in the hydrophobicity of the soil, decreasing its permeability by the creation of a continuous water-repellent layer (Certini, 2005). The severity depends on the interactions between different soil properties and the temperature regimes reached during the fire (Huffman et al., 2001).

Fire not only affects soil properties while it is occurring but also has (indirect) effects after the fire has ceased (Santín and Doerr, 2016). The most palpable effect that takes place post-fire is an increase in soil erosion (Caon et al., 2014). This is due to the large impact that the removal of plant material has on hydrological dynamics, as well as the alteration of soil structure, which results in reduced transpiration and evaporation surfaces, reduced water retention capacity and obstacles to water flow (García-Chevesich, 2012; Santín & Doerr, 2016; Shakesby, 2011). These effects are compounded by the increased runoff discussed above. However, ash left on the ground after a fire can temporarily increase the infiltration capacity and prevent runoff to some extent, depending on the amount of precipitation received. In addition, the erosion promoted by runoff associated with ash accumulation could generate more fertile soils in the valley bottom (or in the first third of the slope) (Santín & Doerr, 2016).

CHEMICAL PROPERTIES

The first process that occurs with increasing soil temperature in the range of 50 - 100 °C is the evaporation of water. From 150 °C onwards, processes such as combustion of organic matter (300 - 700 °C), increase of soil pH (500 - 1000 °C), formation of calcium carbonate ($CaCO_3$) followed by transformation to calcium oxide (500 - 1000 °C) and transformation of iron hydroxides to iron oxides (300 - 600 °C), among others (Santín & Doerr, 2016).

The accumulation of ash, resulting from the combustion of vegetation and organic matter located on the soil surface, results in nutrient enrichment of the soil, increase in electrical conductivity and pH, as well as in the concentration of available phosphorus (P) and certain exchange cations (Ca, Mg, K and Na; Caon et al., 2014; Coates et al., 2018; Francos et al., 2018; Majder-łopatka et al., 2019). In addition, the increase in soil pH together with the high temperatures reached at the soil surface have effects on nitrogen (N) availability, increasing the amount of dissolved organic N and inorganic nitrogen forms (NH_4^+, NO_3^-) (Caon et al., 2014; Múgica et al., 2018).

The formation of ash together with gaseous emissions (CO_2) that take place during the combustion of vegetation result in the alteration of iron oxides, for example goethite and formation of hematite and maghemite, the second one with magnetic properties. These alterations could produce an increase in the magnetic susceptibility of the soil, especially in the first centimetres of the soil where the accumulation of particles and ash is much higher (Certini, 2005; Jordanova et al., 2019a). This increase in the magnetic properties of the soil is favoured by fires of higher intensity (Jordanova et al., 2019b).

SOIL FUNCTIONALITY AND ECOSYSTEM SERVICES

The concept of soil quality refers to productivity or fertility, which encompasses the interactions between the soil resource and humans, as well as the sustainability of ecosystems and the unique and irreplaceable value of soil (Bünemann et al., 2018). Soil is the life support for plants, animals and humans, and its conservation is therefore of vital importance. In this context, it is necessary to define the terms soil ecosystem services and soil functionality. Firstly, ecosystem services refer to the benefits that natural resources provide to living beings (Bünemann et al., 2018). Soil functionality encompasses the processes that take place in the soil and make it possible for ecosystem services to be provided (Bünemann et al., 2018). Soil must be considered a multifunctional resource, given its complexity and the wide variety of processes that take place in it. Actions taken on soil have, in most cases, a direct consequence on its functionality, as well as on the ecosystem services provided by the soil (Bünemann et al., 2018). Fig. 2 shows the relationships between soil threats (fire should be included in this list), soil functions and the ecosystem services provided by soil.

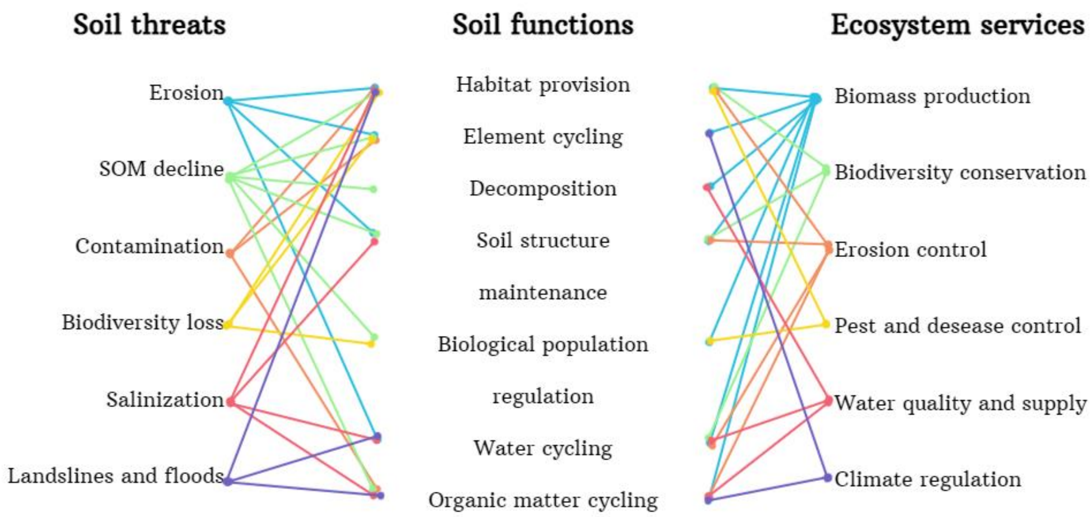

Figure 2 Relationships between soil hazards and soil functions and ecosystem services (Adapted by me from: Brussaard, 2012).

Objectives

The purpose of this study was to evaluate the main alterations in the physical-chemical and biological properties of the soil as a result of a high intensity-controlled burn in the "Los Boquerones" area (municipality of Villaviciosa de Córdoba) at two different times: immediately after the burn and eight months later (recovery), to compare with initial conditions (before the burn). In addition, specific objectives were to assess the spatial heterogeneity of the alterations of the different soil properties as well as addressing the relationship between fire occurrence and soil microbial communities.

MATERIALS AND METHODS

STUDY AREA

The experiment was conducted in an experimental area, where a controlled burn was developed (Fig. 3A). The study area is in a sunny 49.7% slope (Fig. 3B) in "Los Boquerones" (ETRS89 30N, 325212.60 m E 4202607.67 m N: geographic coordinates 37° 57' 12.3477'' N, 4° 59' 21.9754'' W, mean altitude: 402.44 m), within the "El Olivarejo" public land (Villaviciosa de Córdoba, Córdoba) (Fig. 3A).

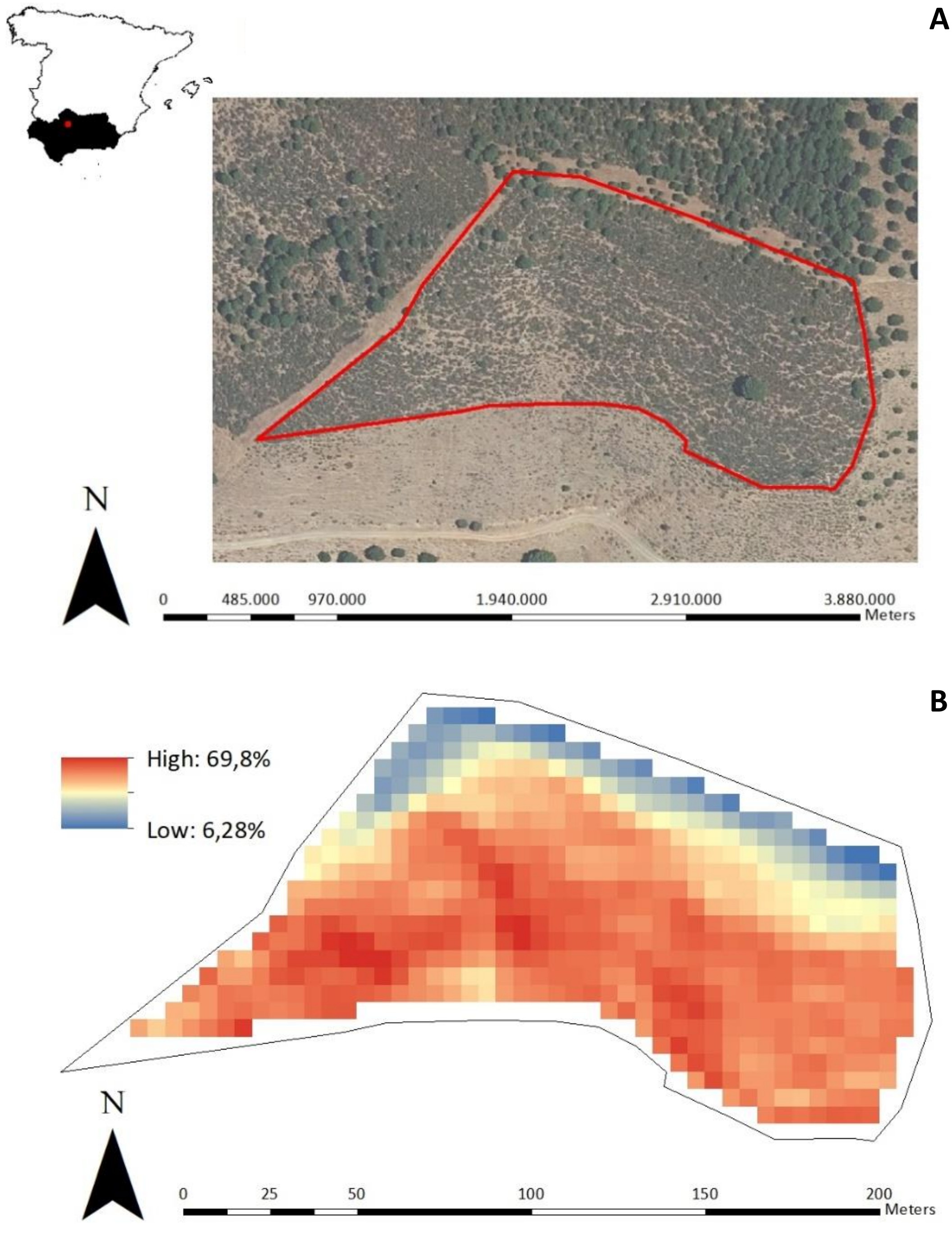

Figure 3 A) Location of the area of study. B) Slope of the study area

VEGETATION

The vegetation in the area is mainly dominated by pine trees (*Pinus pinea* L.) from reforestation and kermes oaks (*Quercus coccifera* L.), which grow naturally. There are also eucalyptus trees (*Eucalyptus camaldulensis* Dehnh) in watercourses or in the edges of firebreaks and woodlands. The plant composition of the experimental plot is mainly shrub, the most abundant species being *Genista hirsuta* Vahl*, Cistus ladanifer* L. and *Salvia rosmarinus* Spenn.

PARENT MATERIAL AND CLIMATOLOGY OF THE STUDY AREA

The study area, like the rest of the mountains of Sierra Morena Range, was formed due to the Hercynian orogeny. The substrate is predominantly siliceous from the Lower Cambrian, with the presence of slates. As a result, the soils are generally acidic and shallow, and are very easily eroded (Junta de Andalucía, 2011). The presence of stable vegetation in the area produces a layer of leaf litter in the first centimetres of the soil. The area is dominated by Eutric Regosols, Lithosols and Eutric Cambisols, greyish-brown or reddish-brown in colour, with a medium to coarse texture and stoniness (REDIAM, 2010).

The area is characterised by a Mediterranean climate (Csa according to Köppen classification), with temperatures that reach up to 33.5 °C and precipitations below 10 mm during the months of summer (Fig. 4). According to Allué's phytoclimatic classification, the area corresponds to type IV_4 Mediterranean genuine, warm, less dry and with warm winters (García & Allué, 2005). Table 1 shows the main climatic variables calculated from the period 2003-2021. The maximum temperature reached was 34.1 °C, with the average temperature during the summer months being 25.6 °C (Table 1). In terms of relative humidity (RH), the average value in summer was 37.8%, while the maximum values are reached in autumn and winter, with December being the wettest month with 79.6% (Table 1). In relation to reference evapotranspiration (ET0), the maximum values are reached in summer (Table 1), with an average of 215.83 mm month^{-1}.

Table 1 Monthly average data calculated from the values recorded at the Espiel Agroclimatic Station for the period 2003-2021. (Source:http://www.uco.es/grupos/meteo/)

	Tmed	Tmax	Tmin	Prec	ET0	Rn	Rs	RH	DPV	WIN
January	5.6	13.8	-2.4	49.8	35.8	3.0	8.5	77.5	0.4	1.2
February	7.5	16.4	0.5	60.8	42.0	4.9	11.5	73.5	0.3	1.4
March	10.2	19.3	1.4	71.9	71.8	7.8	15.8	68.4	0.5	1.6
April	13.2	21.5	4.7	76.5	93.7	10.8	19.4	67.1	0.6	1.5
May	17.7	26.5	8.0	47.4	137	13.4	23.9	56.4	1.1	1.4
June	23.3	32.1	12.4	7.2	173.3	14.8	27.0	43.4	2.0	1.5
July	27.0	33.5	18.5	1.3	203.1	14.6	28.1	33.4	2.7	1.5
August	26.6	34.1	16.7	8.2	271.1	15.1	24.9	36.7	5.7	1.5
September	21.7	31.8	12.6	43.0	175.3	11.3	19.3	68.0	3.4	1.3
October	16.1	23.9	3.9	92.7	75.8	6.2	13.6	64.4	0.8	1.3
November	9.8	19.2	1.9	78.0	72.3	4.6	9.4	75.3	1.5	1.3
December	6.4	14.8	-0.3	84.7	27.6	2.3	7.7	79.6	0.2	1.2

Tmed = Mean daily temperature (°C); Tmax = average daily maximum air temperature (°C); Tmin = average daily minimum air temperature (°C); Prec = Precipitation (mm month-1); ET0 = Reference evapotranspiration (Penman-Monteith) (mm month-1); Rn = Net radiation over meadow (MJ m-2 day-1); Rs = Solar radiation (MJ m-2 day-1); HR = Relative Humidity (%); DPV = Daily mean Vapour Pressure Deficit (kPa); WIN = Wind speed at 2 m (m s-1).

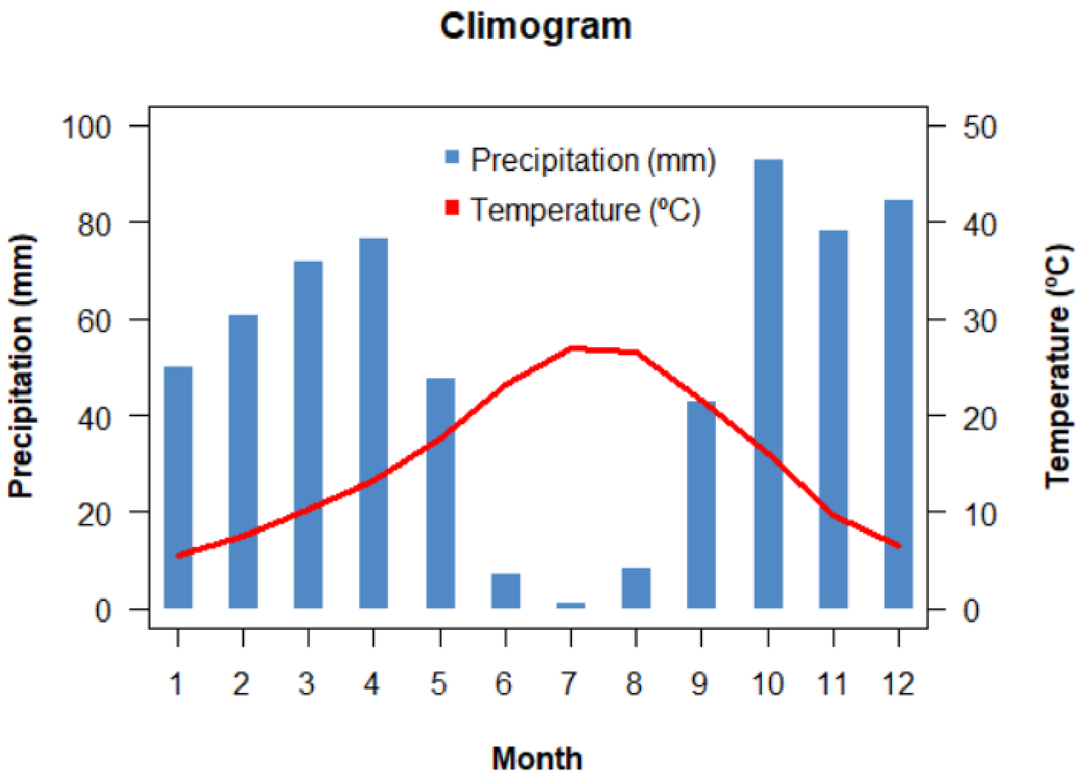

Figure 4 Average monthly temperatures and precipitation (the number corresponds to the month of the year, e.g., 1-January, 2-February, …, 12-December) of the Agroclimatic Station of Espiel for the period 2003-2021 ((Source: http://www.uco.es/grupos/meteo/), the closest station to the study area.

Meteorology during the following eight months after the controlled burn

Additionally, the same variables shown in Table 1 are shown in Table 2 for the eight months after the burn. The total precipitation was 402.5 mm in this period, being the two months that followed the burn the ones in which it rained the most, especially in December.

Table 2 Data for the eight months after the burning calculated from the values recorded at the Espiel Agroclimatic Station. (Source: http://www.uco.es/grupos/meteo/)

Year	Month	Tmed	Tmax	Tmin	Prec	ET0	Rn	Rs	HR	DPV	WIN
2019	November	10.7	19.2	4.6	82.6	33.0	2.9	7.0	82.2	0.3	2.0
	December	9.8	14.8	5.7	135.5	24.8	2.2	6.6	84.7	0.2	1.4
2020	January	7.5	12.4	4.7	31.5	24.8	2.5	7.6	84.4	0.2	1.1
	February	11.3	12.9	9.8	1.5	54.9	5.6	12.4	79.5	0.4	0.9
	March	11.8	17.9	6.0	59.8	66.9	7.4	14.4	74.7	0.4	1.7
	April	13.7	16.2	8.9	64.3	72.4	8.9	15.0	80.7	0.4	1.2
	May	19.5	25.2	11.8	15.0	127.2	12.7	22.0	62.4	1.2	1.0
	June	22.7	29.4	16.7	2.9	164.4	14.4	26.2	47.3	1.8	1.4
	July	29.2	32.0	25.0	9.4	194.4	13.7	26.1	35.4	3.0	1.3

Tmed = Mean daily temperature (°C); Tmax = average daily maximum air temperature (°C); Tmin = average daily minimum air temperature (°C); Prec = Precipitation (mm month-1); ET0 = Reference evapotranspiration (Penman-Monteith) (mm month-1); Rn = Net radiation over meadow (MJ m-2 day-1); Rs = Solar radiation (MJ m-2 day-1); HR = Relative Humidity (%); DPV = Daily mean Vapour Pressure Deficit (kPa); WIN = Wind speed at 2 m (m s-1).

CHARACTERISTICS OF THE CONTROLLED BURN

FIRE BEHAVIOUR

The burning was developed on 25th October 2019, starting at 13:00 hours when the optimal wind and temperature conditions were reached and lasted around 30 minutes. Based on the spread rate, fire-line intensity, heat per unit area, flame length and flame residence time, three different fire behaviours were established along the plot, represented in Fig. 5 and Table 3. The behaviour in grey had lower values for all fire characteristics and corresponded to the area where the burn was started. The main difference between the grey and green behaviours was that the second one presented bigger flame lengths and residence times. Lastly, the purple behaviour was the most severe, presenting the higher values for all characteristics (Table 3).

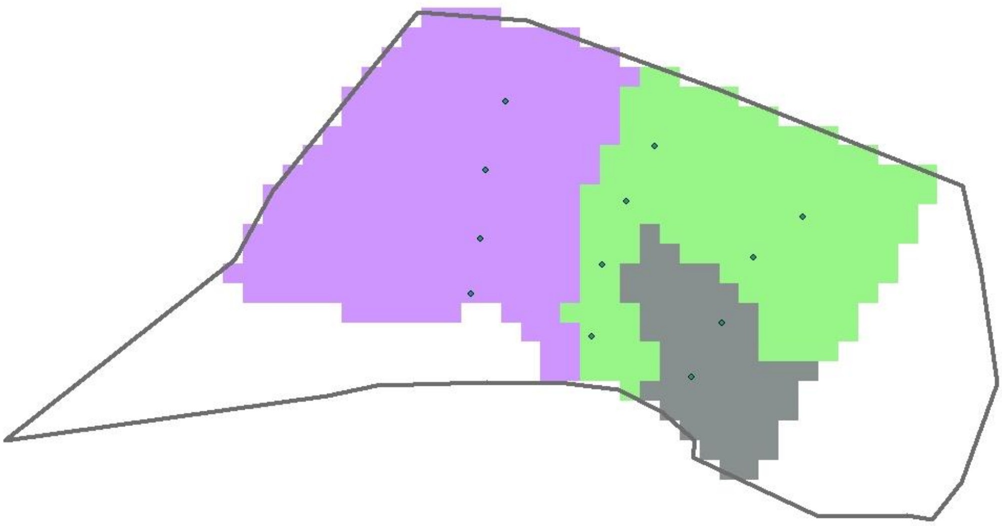

Figure 5 Fire behaviour along the study plot.

Table 3 Characterization of fire parameters across the plot (Source: Informe preliminar elaborado en relación con el fuego experimental realizado en la finca Los Boquerones (Córdoba) el 25 de octubre de 2019, Laboratorio de Gestión del Paisaje Forestal y Defensa contra incendios).

Behaviour	Spread rate (m min-1)	Fire-line intensity (kW m-1)	Heat per unit area (kcal m2)	Flame length (m)	Flame residence time (s)
	10.46	5116.97	6930.00	3.83	65.00
	11.31	5495.00	7035.00	5.76	160.91
	29.77	18185.21	8877.27	8.52	200.50

SOIL TEMPERATURES MEASURED DURING THE CONTROLLED BURN

The grid for soil sampling within the experimental plot was based on three transects (A, C and E), spaced 40 m apart from each other, and four points within each transect (1, up the slope, 2 and 3, mid-slope, and 4 with the lowest height of the transect points), spaced 20 m apart between them and 60 m between point 1 and point 4, as shown in Fig. 6. At these points, two temperature sensors (thermocouples) were placed on the surface (first one) and at a depth of 2 cm (second one), to record information during the prescribed fire (maximum surface temperature, maximum soil temperature and flame residence time).

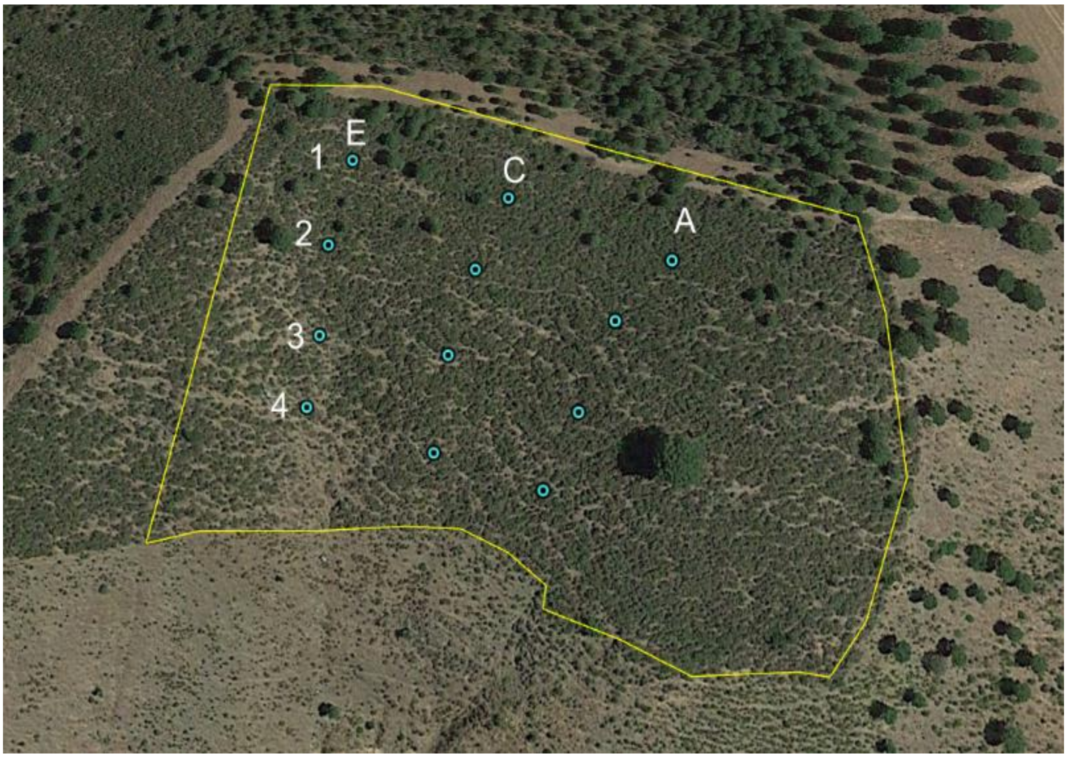

Figure 6 Sampling points matrix

Table 4 shows the values of (1) the maximum surface temperature, which is the maximum temperature reached by the sensors at the soil surface, (2) maximum soil temperature, which is the maximum temperature collected by the sensors located two centimetres deep in the soil, (3) the flame residence time, which is the time the flame stayed at each sampling point; (4) time at soil temperature higher than 60 °C, which is the time the flame stayed at those sampling points where the first two centimetres of soil reach 60 °C. The maximum soil temperature at 2 cm depth ranged between 36 °C (points A1 and A4) and 187 °C (point E4). At the surface (0 – 2 cm), it reached higher values, between 521 °C (point C2) and 1032 °C (point E4). As for the residence time, the maximum value recorded was 530 s (point A2), and the minimum 47 s (point A4). At 2 cm depth, only two points reached a temperature above 60 °C, and the time required for this exceeded 150 s, the maximum value being 223 s (Point C4).

Table 4 Temperatures reached in the soil surface (0 cm Depth) and at 2 cm depth.

Point	Maximum soil temperature (0 cm depth, °C)	Maximum soil temperature (2 cm depth, °C)	Flame residence time (s)	Soil temperature > 60°C (s)
A1	942.5	36.0	66	
A2	921.5	50.0	530	
A3	899.5	40.5	114	
A4	940.0	36.0	47	
C1	912.0	33.5	63	
C2	521.0	31.5	77	
C3	1001.0	37.5	83	
C4	966.5	93.0	115	223
E1	785.0	34.8 *	170	170
E2	928.3	54.5	116	
E3	493.0	43.0	132	
E4	1032.0	187.0	384	192

*There was an error in the maximum soil temperature at the soil depth of 2 cm for point E1, as the thermocouple recorded the same temperature at the surface (0 – 2 cm) of the soil. To carry out the statistical analysis and the elaboration of the maps, the average temperature of points A1 and C1 was considered, being the temperature recorded 735 °C for that point (E1).

EXPERIMENTAL DESIGN AND SAMPLE COLLECTION

Soil samples were collected at two different depths, the first one from 0 to 2 cm soil depth, and the second one at a depth of 2 to 5 cm, in the 12 points mentioned in the previous section (3 transects and 4 heights within each transect). The soil surface sampling was carried out using a shovel to collect 0.5-1.0 kg of soil (by scratching or scraping the soil), after removing the mulch, as shown in Fig. 7. On the same points where the surface samples were collected, the soil was sampled by introducing the shovel slightly, without exceeding 5 cm in depth with the help of a ruler (Fig. 7B), obtaining the deepest soil samples (between 2 and 5 cm). The sample collection area had a radius of 2.5 metres around each point (A1-A4, C1-C4 and E1-E4).

In addition, soil samples were also taken at three different times at the same points, (i) before and (ii) just after the burn (approximately three hours later, when the soil temperature allowed it, following the same criteria as before the burn), and (iii) 8 months after the burn. These three times are called "treatments" in the following sections.

In the laboratory, each soil sample was passed through a 2 mm-sieve. A subsample of each soil sample was frozen at -20 °C for microbiological analysis (phospholipid fatty acid analysis or PLFAs) and another subsample was used to determine soil moisture. Once sieved, extractions were performed with 0.5 M K_2SO_4 at a 1:5 (soil:extractant; w:v) ratio to determine nitrate (NO_3^-), ammonium (NH_4^+) and dissolved organic carbon (DOC), and the rest of the soil was dried at room temperature in the laboratory (approximately 25 °C) for one week. After drying, the samples were stored in 0.5 kg containers, duly labelled, for the analyses described in the following section.

Figure 7 On the left, image A shows the shallow soil sampling (0 – 2 cm). On the right, image B shows the deepest oil sampling (2–5 cm).

Soil Physical-chemical analysis

Soil moisture

A soil sample was placed in an aluminium container (previously weighed, P1, empty container) and weighed (P2, empty container plus fresh soil). Subsequently, the soil sample was dried in an oven at 105 °C for 24 h (P3, empty container plus dried soil). After this time, the container was removed from the oven and reweighed at room temperature. The percentage of soil moisture was calculated using the following equation:

Equation 1: $$\%\mathbf{Moisture} = \frac{\mathbf{P2-P3}}{\mathbf{P3-P1}} * \mathbf{100}$$

Magnetic susceptibility

When analysing the magnetism of a sample, two values must be considered, the specific mass magnetic susceptibility (χ_{lf}) related to the mineralogy and geochemistry of the sample, and the frequency-dependent susceptibility (χ_{fd} %), related to the presence of pedogenic ferromagnetic minerals (Bautista et al., 2014).

The χ_{lf} was determined using the MS2 Magnetic Susceptibility Meter (Bartington Instruments, Oxon, UK), in which a Falcon™ tube with approximately 10 ml of soil was introduced, and the low frequency susceptibility (χ_{lf}) is measured, and then the calculation is performed, considering the weight of the sample (Equation 2). On the other hand, the χ_{fd} % was determined by making a second measurement of the samples, but at a high frequency, and then performing the calculation shown in Equation 3:

Equation 2: $$\chi_{lf}\,\frac{\mathbf{m^3}}{\mathbf{kg}} = \frac{\mathbf{Reading}}{\mathbf{Soil\ weight}} * \mathbf{10^{-7}}$$

Equation 3: $$\chi_{\mathbf{fd}}\% = \frac{\chi_{\mathbf{lf}} - \chi_{\mathbf{hf}}}{\chi_{\mathbf{lf}}} * \mathbf{100}$$

Soil color

A subsample of each soil sample was grounded to obtain fine powder. Then, the samples were placed in a sample holder and the colour was determined using the Cary 5000 UV-Vis-NIR spectrophotometer (Varian), in which a wavelength scan from the ultraviolet (380 nm) to the near infrared (750 nm) is performed. After that, the Munsell Conversion software 2018 v12.18.5f (2010 WallkillColor) was used to obtain the corresponding hue, value and chroma values for each soil sample.

Soil pH

The analysis of soil pH was carried out using the pH-meter PH-Meter GLP 21 (Crison Instruments, S.A., Barcelona, Spain), preparing each soil sample in a 1:2.5 (w:w) soil:water suspension after shaking the mix once every 5 minutes during 25 minutes.

Soil electrical conductivity

Electrical conductivity in the soil solution (EC) was analysed by using a EC-Meter BASIC 30 (Crison Instruments, S.A., Barcelona, Spain). Five g of soil and 25 ml deionised water were shaken for 30 min in a container before the analysis (1:5; w:v, soil:water suspension).

Cation Exchange Capacity

To determine the exchangeable cations of the soil, 3 g of soil were weighed and 40 ml of 1N ammonium citrate ($AcNH_4$ pH=7 was added to Falcon™ tubes and shaken at 180 rpm for 30 minutes in the isothermal room at 25 °C, then centrifuged at 3000 rpm for 10 minutes and the supernatant was transferred to single-use tubes. A flame photometer (PFP7, Jenway) was used to measure Ca and Mg and an atomic absorption spectrometer (AANALYST 200, PerkinElmer) was used to measure K and Na. Cation exchange capacity in soil was calculated as the sum of these bases.

Oxalate-Extractable Iron in soil

For the determination of oxalate-extractable Fe (Fe_{ox}), 0.8 g of soil was weighed, to which 40 ml of 0.2 M ammonium oxalic acid at pH 3 was added in Falcon™ tubes and shaken at 180 rpm for 2 h. A blank was shaken together with the soil samples. After 2 h, the samples were centrifuged at 3000 rpm for 10 min and the supernatant was transferred to disposable tubes to prepare the samples for colorimetry. The extracted Fe was determined by measuring the absorbance with a spectrophotometer (Lambda 35, PerkinElmer).

Dithionite-extractable iron in soil

For the determination of iron to dithionite (Fe_{di}), 1.6 g of soil was weighed, to which 40 ml of citrate-bicarbonate 0.3 M and 1 g of sodium dithionite were added in Falcon™ tubes and shaken at 180 rpm for 16 hours at 25 °C. A blank was shaken together with the soil samples. After 16 h, the samples were centrifuged at 3000 rpm for 10 min and the supernatant was transferred to disposable tubes to prepare the samples for colorimetry. The extracted Fe was determined by measuring the absorbance with a microplate spectrophotometer at 508 nm (PoweWave HT Microplate Spectrophotometer, BioTek).

DETERMINATION OF DISSOLVED ORGANIC CARBON

The determination of dissolved organic carbon (DOC) was carried by weighting 5 g of soil and making extractions 25 ml of 0.5 M KCl 1:5 (w:v) (Total Organic Carbon Analyzer, Shimadzu). Dilutions were done (at least 1:5).

AMMONIUM IN SOIL

Five g of soil were weighed and extractions performed with 0.5 M K_2SO_4 1:5 (w:v). After colorimetry was developed by adding Na-nitroprusside, salicylic acid, NaOH, K_2HPO_4, Na-Hypochlorite and Na_2EDTA (Mulvaney, 1996), the extracted NH_4^+ was determined by measuring the absorbance with a microplate spectrophotometer at 667 nm (PoweWave HT Microplate Spectrophotometer, BioTek).

NITRATE IN SOIL

Five g of soil were weighed and extractions were performed with 0.5 M K_2SO_4 1:5 (w:v). After colorimetry was developed by adding vanadium chloride, NEDD and sulfanilamide (Mulvaney, 1996), the extracted NO_3^- was determined by measuring the absorbance with a microplate spectrophotometer at 540 nm (PoweWave HT Microplate Spectrophotometer, BioTek).

SOIL AVAILABLE PHOSPHORUS BY THE OLSEN METHOD

Available P in soil was extracted with 0.5 M sodium bicarbonate ($NaHCO_3$, buffered at pH 8.5) using the Olsen method (Olsen et al., 1954). The procedure consisted of extracting the P contained in 1.5 g of soil, previously weighed in 50 ml tubes, to which 30 ml of 0.5 M $NaHCO_3$ (adjusted at pH 8.5) were added. Subsequently, the tubes were shaken for 30 min at 180 rpm and 25 °C, immediately centrifuged and the supernatant transferred to 10 ml tubes. Later, 1 ml of the supernatant was mixed with 0.125 ml 5 N H_2SO_4, 4.125 ml deionised H_2O and 1 ml mixed reagent (consisting of a solution of 5.28 g of ammonium molybdate per litre of ascorbic acid). Six standards were prepared from a 40 ppm P solution (standard curve). The extracted P (P_{Olsen}) was determined by measuring the absorbance with a spectrophotometer (Lambda 35, Perkin Elmer).

AVAILABLE FE, CU_{DTPA}, MN_{DTPA} AND ZN_{DTPA} IN SOIL

Micronutrients were determined by weighting 10 g of soil and adding 20 ml of diethylenetriaminepentaacetic acid (DTPA) 0.005 M (pH= 7.3; Lindsay and Norvell, 1978) in Falcon™ tubes. They were shaken at 180 rpm for 2 hours in the isothermal room at 25 °C, then centrifuged at 3000 rpm for 10 minutes and the supernatant was transferred to single-use tubes. The measure was made by atomic absorption spectrophotometry at 240.3 nm (Fe), 324.8 nm (Cu), 279.5 nm (Mn) and 213.9 (Zn) (AANALYST 200, PerkinElmer).

PHOSPHOLIPID FATTY ACIDS (PLFAS) AND NEUTRAL LIPID FATTY ACIDS (NLFAS) IN SOIL

Phospholipid fatty acids (PLFAs) and neutral lipid fatty acids (NLFAs) were determined on 25 g soil samples according to Bartelt-Ryser et al. (2005). In the case of the NLFAs, the determination was made only in 6 samples, corresponding to points A1, C1 and E1 at both soil depths. Briefly, each soil sample was sieved to pass 2 mm and dried by freeze-drying. Fifty-six different fatty acids were detected in the soil samples used for PLFAs. These fifty-six fatty acids, classified per taxonomic group were: (gram+ bacteria) 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 16:0 anteiso, 17:0 iso, 17:0 iso 3OH, 18:0 iso, 19:0 anteiso, 17:0 anteiso, 15:1 anteisoω9c, 15:1 isow6c and 17:1 isoω9c (Veum et al., 2019); (gram− bacteria) 15:1 w6c, 16:1ω7c, 16:1ω9c, 17:1ω8c, 18:1ω5c, 18:1ω7c, 18:1ω9c, 19:1 w8c,20:1 w6c, 20:1 w8c, 20:1 w9c, 21:1 w3c, 21:1 w6c, 17:0 cycloω7c and 19:0 cycloω9c (Veum et al., 2019); (actinomycetes) 17:0 10 methyl, 17:1ω7c 10 methyl, 18:0 10 methyl, 18:1ω7c 10 methyl, 19:1ω7c 10 methyl and 20:0 10 methyl (Veum et al., 2019); (saprotrophic fungi) 18:2ω6c (Paul and Clark, 1996); (biomarker for putative arbuscular mycorrhizal fungi) 16:1ω5c (Olsonet al., 1999); (bacteria) 12:0, 14:0, 15:0, 16:0, 17:0, 20:0, 22:0 and 24:0 (Kujur & Patel, 2014); (eukaryote - protozoa) 15:4 w3c, 20:4 w6c, 18:3 w6c, 19:4 w6c, 20:5 w3c (Veum et al., 2019); (not assigned to a specific taxonomic group) 21:0 and 23:0 (Ratledgeand Wilkinson, 1988; Niklaus et al., 2003). Some PLFAs ratios were calculated to assess alterations in soil microbial communities (protozoa/bacteria or predator/prey, fungi/bacteria, gram+/ gram−, saturated/un-saturated fatty acids, mono/polyunsaturated fatty acids, and GNeg Stress, which is an indicator of hight stress based on 16ω/17 cyclo and 18ω/19 cyclo precursor/cyclopropane fatty acids (Knivett & Cullen, 1965)).

MAPS ELABORATION

The main soil properties were selected to study their spatial alteration immediately and 8 months after the prescribed fire (regarding the former values, before the burning). ArcGIS Desktop 10.8© was used to produce maps for the two soil depths sampled (0–2 cm and 2–5 cm). To study how properties varied across the experimental plot, we used interpolation according to the Cokriging method (Han et al., 2003), a geostatistical method that allows to use information on several layers. This method was chosen over other deterministic methods (based only on distances), because it allows to consider other variables such as the fire behaviour, the slope and the slope aspect in the plot. The prediction maps were elaborated for the soil properties pH, P_{Olsen}, Fe_{DTPA}, Fe_{ox}, CEC, DOC, NO_3^- and NH_4^+, and for the uppermost 2 cm of soil, considering the behavior of fire along the study area, the slope, and the slope aspect.

STATISTICAL ANALYSIS

The statistical analyses carried out to study the variation in soil properties with the different treatments (control / burned / after 8 months) and soil depths (0–2 cm / 2–5 cm) were as follows. Those variables that met the requirements for a parametric test (normality was assessed by Shapiro's test and homogeneity of variance by Levene's test) were subjected to a factorial analysis of variance (ANOVA) to determine the effect of time (called treatment; before burning, immediately and 8 months after the prescribed fire) and soil depth (0–2 cm and 2–5 cm), as well as to study possible interactions between both factors. Pairwise comparisons among treatments were made by applying the paired test to all properties. In the cases where the results of the ANOVA showed a non-significant interaction between treatment and soil depth, the Tukey’s test (with 5% significance) was applied to address the differences between the different samples, whose results are shown in Table AI in the Annexes. In addition, a Principal Component Analysis (PCA) was performed to study how the samples were grouped according to treatment and depth, as well as their relationship with the measured variables. Then, regression trees were performed to obtain prediction models of the different taxonomic groups based on sampling and fire characteristics. To prove the goodness of the models the root-mean-square error (RMSE) and mean absolute error (MAE) were used.

To evaluate the relationship between treatments, depths and PLFAs, permutational analysis of variance (PERMANOVA) on Bray-Curtis dissimilatory matrices, as well as redundancy analysis (RDA) were carried out. All statistical analyses were performed using R' software (R Core Team 2013) and the packages: "tidyverse", "stringr", "devtools", "dplyr", "car", "ggpubr", "rstatix", "vegan", “rpart” and "ggplot2".

RESULTS

GENERAL SOIL PROPERTIES

The PCA (Fig. 8) showed that the samples were well grouped according to both treatment and soil depth. The first component (PC1), which explained the 46.3% of the total variance, was able to differentiate the 0 – 2 cm samples from the 2 – 5 cm ones in the samples immediately after the burn and eight months later. The control samples (before the burn) were not differentiated regarding the soil depth. The second component (PC2) that explained the 17.9% of the total variance was able to group the samples according to their treatment (time), being the control and immediately after the fire closer than the samples taken eight months after the fire. The variables used for the PCA are shown on Fig. 8B. In the case of the PC1, the variables that contributed the most were DOC, P_{Olsen}, CEC, Zn_{DTPA} and soil pH in one sense and chroma and value in the opposite sense. For the PC2, the variables that contributed the most to PC2 were Fe_{di}, Fe_{ox}, χ_{fd} % (SM), χ_{lf} and, in the opposite sense, Fe_{DTPA}. Additionally, the PCA showed that the control and burned (2 – 5 cm) samples behaved in a similar way and were more related to higher soil contents of Fe_{di}, Fe_{ox}, χ_{fd} %, χ_{lf}, chroma, value and NO_3^-. Surface (0 – 2 cm) samples after the burn were related to higher DOC, P_{Olsen}, CEC, hue, soil pH, Zn_{DTPA}, Mn_{DTPA}, NH_4^+ and total PLFAs. The samples collected eight months after the burn partially returned to similar values to the ones observed on the first sampling regarding the PC1 but were more related to high contents in Fe_{DTPA} and lower contents in NO_3^-, Fedi, Feox and SM (PC2). Besides that, there was still a difference between the two depths (Fig. 8A).

A selection of the physical-chemical and biological properties was independently analysed (factorial ANOVA) and is shown in Tables 5 and 6. The χ_{fd} % and χ_{lf} were significantly reduced with the burn and it was kept eight months later. Initial soil colour (not included in tables or figures) was uniform both on the soil surface (0 – 2 cm) and at a greater depth (2 – 5 cm), 7.91YR 5.90/3.72. These values varied significantly in the first cm of the soil (0 – 2 cm) immediately after burning (hue, value and chroma, $p < 0.05$), going from 7.97 YR 5.84/3.62 to 8.27 YR 5.37/2.90. Eight months after the controlled burn, the difference was still significant compared to the former values (before the fire), with values of 8.21 YR 5.48/3.10. The variations of colour in the deeper layer (2 – 5 cm) were not significant.

Soil pH (Fig. 9) was significantly increased ($p < 0.05$) in the top 2 cm of soil immediately after burning, reaching its higher mean value (7.97 units) at this point. The surface (0 – 2 cm) samples collected eight months after burning and the ones collected immediately after the fire took place at the deeper layer (2 – 5 cm) showed similar values and lower than the mentioned above (0 – 2 cm). Samples collected from the 2 – 5 cm soil depth corresponding to the control and after eight months samplings had similar values significantly ($p < 0.05$) higher than the surface control samples (0 – 2 cm).

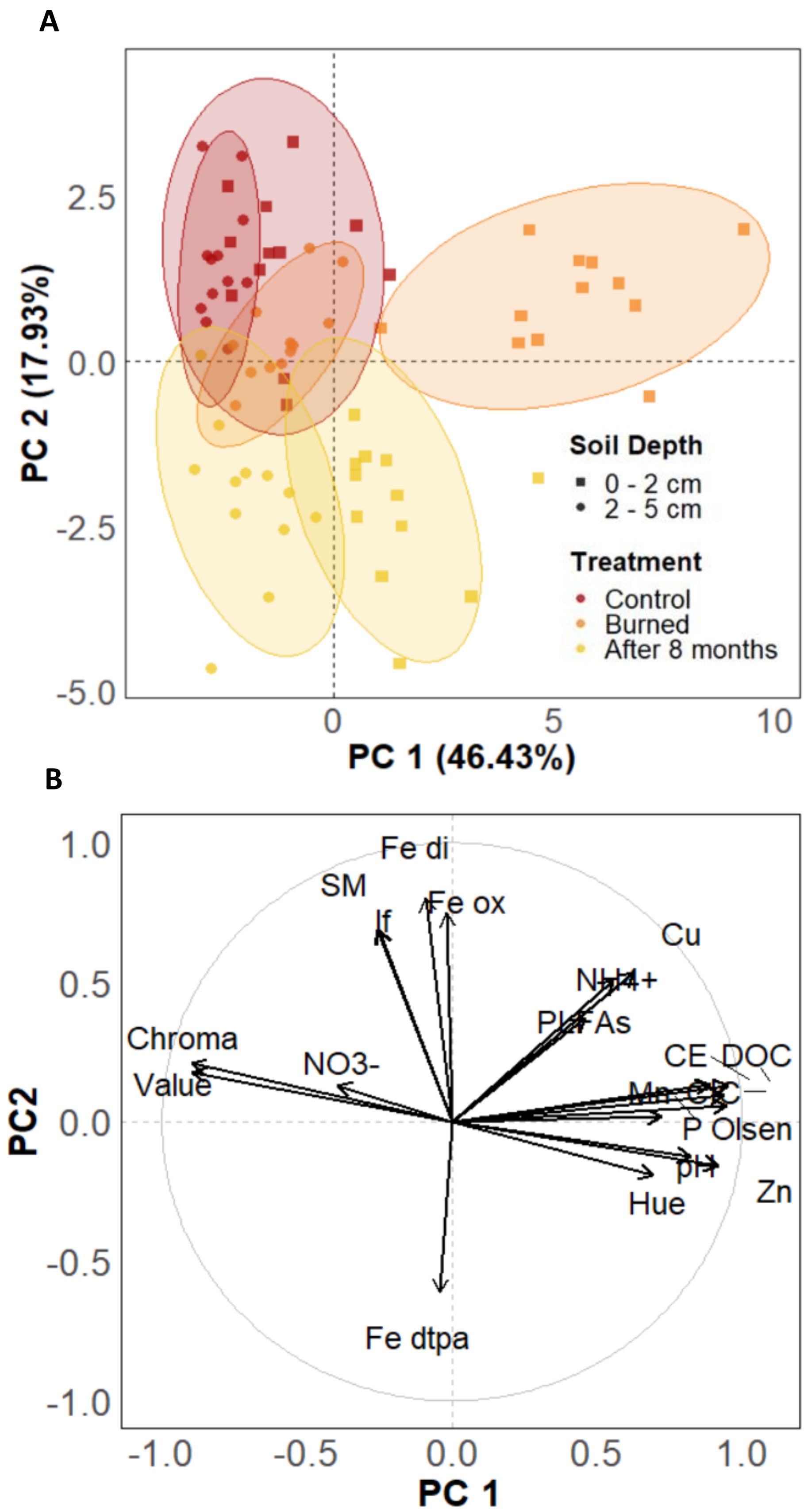

Figure 8 Principal Component Analysis performed on the samples from Los Boquerones as a function of treatment and soil depth (A) and the *variables* used for that (B)

Table 5 Factorial ANOVA for a selection of soil physical-chemical and biological properties as a function of the treatment [control (before burning), burned (immediately after burning) and 8 months after burning; *n* = 24] and soil depth (0 – 2 cm and 2 – 5 cm; *n* = 12). Different letters indicate significant differences according to the Tukey's test (0.05).

	χlf (m^3 kg^{-1})	χfd (%)	$pH_{1:2.5}$	$EC_{1:5}$ (µS cm^{-1})
Treatment				
Control	$1.09 \cdot 10^{-7} \pm 3.05 \cdot 10^{-10}$ a	8.53 ± 0.3 a	6.39 ± 0.05	118 ± 8.95
Burned	$1.07 \cdot 10^{-7} \pm 2.09 \cdot 10^{-10}$ b	6.54 ± 0.18 b	7.42 ± 0.14	513 ± 82.8
After 8 months	$1.07 \cdot 10^{-7} \pm 6.04 \cdot 10^{-10}$ b	6.18 ± 0.53 b	7.05 ± 0.06	185 ± 13.0
p (treatment)	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**
Soil depth (cm)				
0 – 2	$1.08 \cdot 10^{-7} \pm 4.09 \cdot 10^{-10}$ a	6.99 ± 0.36 a	7.19 ± 0.12	401 ± 61.18
2 – 5	$1.08 \cdot 10^{-7} \pm 3.85 \cdot 10^{-10}$ a	7.18 ± 0.33 a	6.73 ± 0.06	143 ± 11.14
p (soil depth)	0.779	0.663	**< 0.05**	**< 0.05**
p (treatment × soil depth)	0.709	0.635	**< 0.05**	**< 0.05**

	CEC (meq 100 g^{-1})	Fe_{ox} (g kg^{-1})	Fe_{di} (g kg^{-1})	PLFA (nmol g^{-1})
Treatment				
Control	4.96 ± 0.25	1.52 ± 0.06 a	2.03 ± 0.06 a	167 ± 9.74 a
Burned	7.96 ± 0.69	1.29 ± 0.10 b	1.86 ± 0.07 a	165 ± 7.62 ab
After 8 months	6.07 ± 0.20	0.92 ± 0.03 c	0.86 ± 0.01 b	141 ± 7.54 b
p (treatment)	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**
Soil depth				
0 – 2 cm	7.80 ± 0.45	1.22 ± 0.07	1.56 ± 0.09	181 ± 6.75
2 – 5 cm	4.86 ± 0.15	1.26 ± 0.07	1.61 ± 0.10	135 ± 4.93
p (soil depth)	**< 0.05**	0.628	0.463	**< 0.05**
p (treatment × soil depth)	**< 0.05**	0.883	0.917	0.776

χ_{lf}: specific mass magnetic susceptibility; χ_{fd} %: frequency dependent magnetic susceptibility; $EC_{1:5}$: electrical conductivity in the relationship 1:5 (soil:water) extract; CEC: cation exchange capacity; Fe_{ox}: iron extractable with oxalate; Fe_{di}: iron extractable with dithionite; PLFA: total phospholipid fatty acids as biomarker for soil microorganisms.

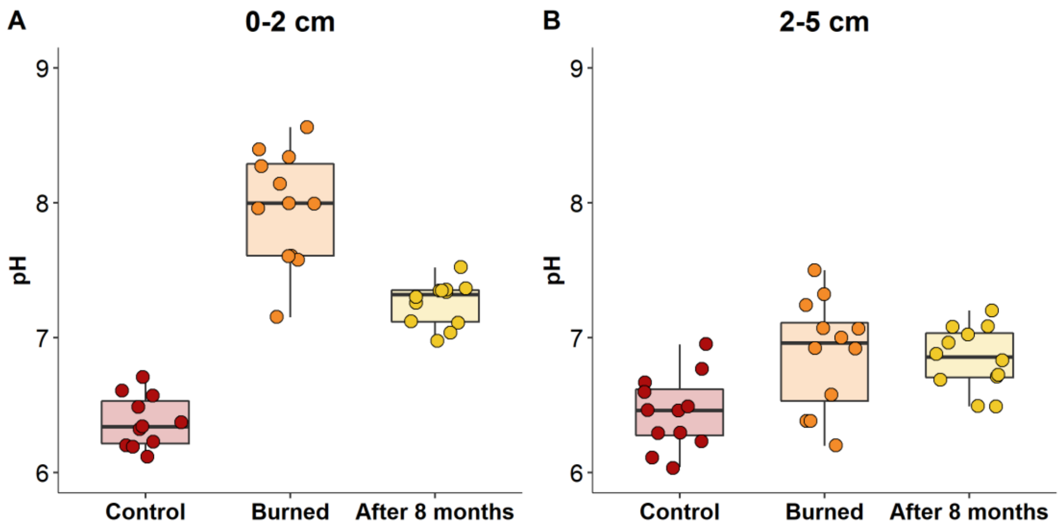

Figure 9 Soil pH (soil:water, 1:2.5) as a function of treatment [control (before burning), burned (immediately after burning) and 8 months after burning; *n* = 12] for the different soil depths.

Electrical conductivity (EC) showed a significantly ($p < 0.05$) higher value for the surface burned samples (immediately after the fire only) compared to the rest of the samples, which had similar values (Fig. 10).

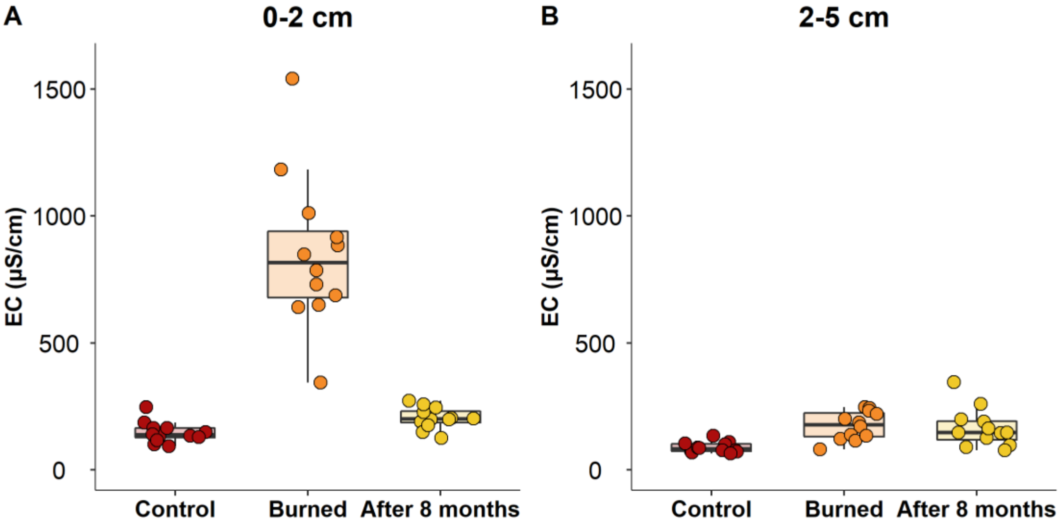

Figure 10 Electrical conductivity (soil:water, 1:5) as a function of treatment [control (before burning), burned (immediately after burning) and 8 months after burning; $n = 12$] for the different soil depths.

Likewise, an increase in CEC was observed after the controlled burn at both soil depths (Fig. 11) and the highest values were measured in the soil's samples collected from the first two cm of soil. After eight months, the values in the uppermost two centimetres decreased related to the ones obtained immediately after the burn but were still significantly higher ($p < 0.05$) than the control samples, while the CEC in the 2 – 5 cm depth was even higher than in the previous soil sampling (Fig. 11).

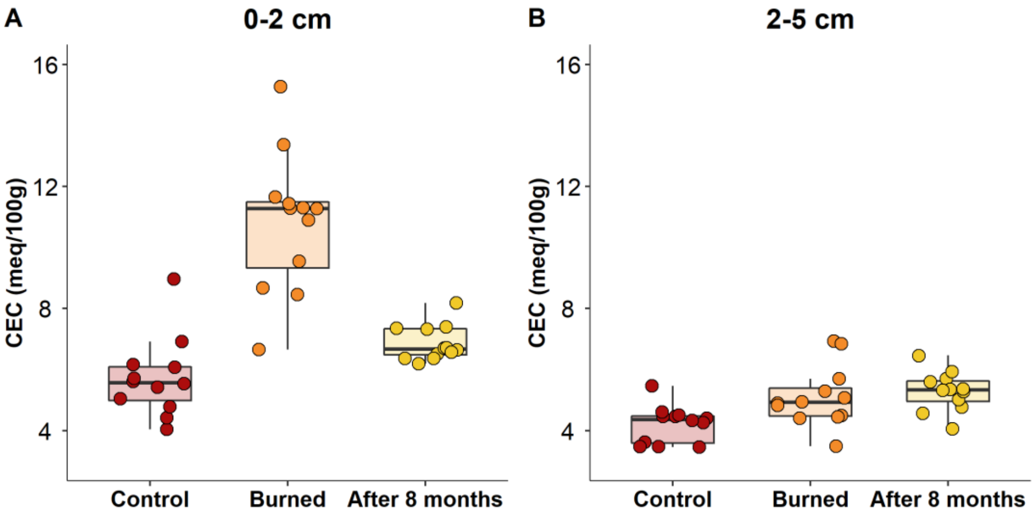

Figure 11 Cation exchange capacity as a function of treatment [control (before burning), burned (immediately after burning) and 8 months after burning; $n = 12$] for the different soil depths.

Fe_{ox} and Fe_{di} were significantly reduced with the treatment ($p < 0.05$) (Table 5).

Table 6 shows soil variables related to soil fertility and nutrient availability. The interaction between treatment and soil depth was significant for all these variables except for Fe_{DTPA}. The highest DOC contents in soil were reached in the soil surface (0 – 2 cm) for the burned samples and the lowest values were observed for the samples collected in the 2 – 5 cm depth, not being significantly different among them (with time; Fig. 12).

Table 6 Factorial ANOVA for soil variables related to fertility and nutrient availability as a function of the treatment [control (before burning), burned (immediately after burning) and 8 months after burning; *n* = 24] and soil depth (0 – 2 cm and 2 – 5 cm; *n* = 12). Different letters indicate significant differences according to the Tukey's test (0.05).

	DOC ($\mu g\ g^{-1}$)	NH_4^+ ($\mu g\ g^{-1}$)	NO_3^- ($\mu g\ g^{-1}$)	P_{Olsen} ($mg\ kg^{-1}$)
Treatment				
Control	270 ± 28.0	18.1 ± 1.19	33.6 ± 2.80	5.22 ± 0.35
Burned	796 ± 121	35.7 ± 2.66	19.9 ± 1.43	23.0 ± 3.60
After 8 months	402 ± 48.9	6.26 ± 0.98	26.9 ± 2.69	11.5 ± 1.44
p (treatment)	< 0.05	< 0.05	< 0.05	< 0.05
Soil depth				
0 – 2 cm	741 ± 82.7	22.4 ± 2.59	25.9 ± 2.10 a	20.4 ± 2.59
2 – 5 cm	237 ± 14.8	17.7 ± 2.35	27.6 ± 2.20 a	6.09 ± 0.34
p (soil depth)	< 0.05	< 0.05	0.421	< 0.05
p (treatment x soil depth)	< 0.05	< 0.05	< 0.05	< 0.05
	FeDTPA ($mg\ kg^{-1}$)	MnDTPA ($mg\ kg^{-1}$)	ZnDTPA ($mg\ kg^{-1}$)	CuDTPA ($mg\ kg^{-1}$)
Treatment				
Control	21.3 ± 2.66 b	63.0 ± 4.34	2.28 ± 0.36	0.92 ± 0.04
Burned	18.3 ± 1.12 b	91.2 ± 10.03	5.12 ± 0.62	1.04 ± 0.05
After 8 months	35.0 ± 1.88 a	74.8 ± 13.55	4.28 ± 0.48	0.65 ± 0.04
p (treatment)	< 0.05	< 0.05	< 0.05	< 0.05
Soil depth (cm)				
0 – 2	26.0 ± 2.13	105 ± 9.36	5.84 ± 0.41	1.02 ± 0.04
2 – 5	23.7 ± 1.90	48.0 ± 2.63	1.95 ± 0.16	0.72 ± 0.03
p (soil depth)	0.295	< 0.05	< 0.05	< 0.05
p (treatment x soil depth)	0.052	< 0.05	< 0.05	< 0.05

DOC: dissolved organic carbon; P_{Olse}: phosphorus soluble in sodium bicarbonate; Fe_{DTPA}, Mn_{DTPA}, Zn_{DTPA}, Cu_{DTPA}: iron, manganesum, zinc and copper extractables with DTPA.

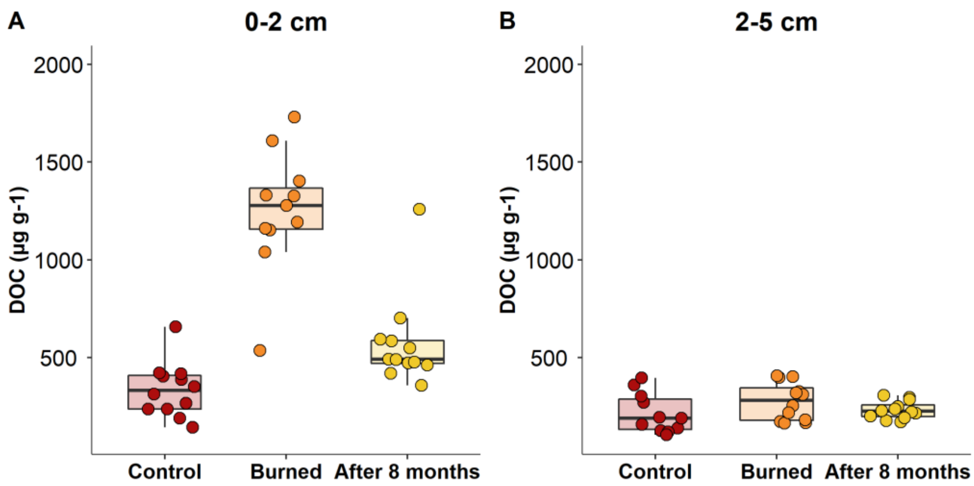

Figure 12 Dissolved organic carbon as a function of treatment [control (before burning), burned (immediately after burning) and 8 months after burning; *n* = 12] for the different soil depths.

Inorganic N forms in soil were also altered. Firstly, NH_4^+ was significantly increased (p < 0.05) immediately after the burn in the uppermost 2 cm of soil, and then (eight months since the burn) it was decreased below the initial value (Fig. 13) at both soil depths. The lowest NH_4^+ values were found for the deepest samples (2 – 5 cm) collected eight months after burning. Secondly, NO_3^- (Fig. 13) showed similar values for all samples at the 2 – 5 cm depth, with no significant differences between them. However, the control and after 8 months samples showed significantly (p < 0.05) higher NO_3^- values than the samples collected immediately after the burn in the uppermost 2 cm of soil.

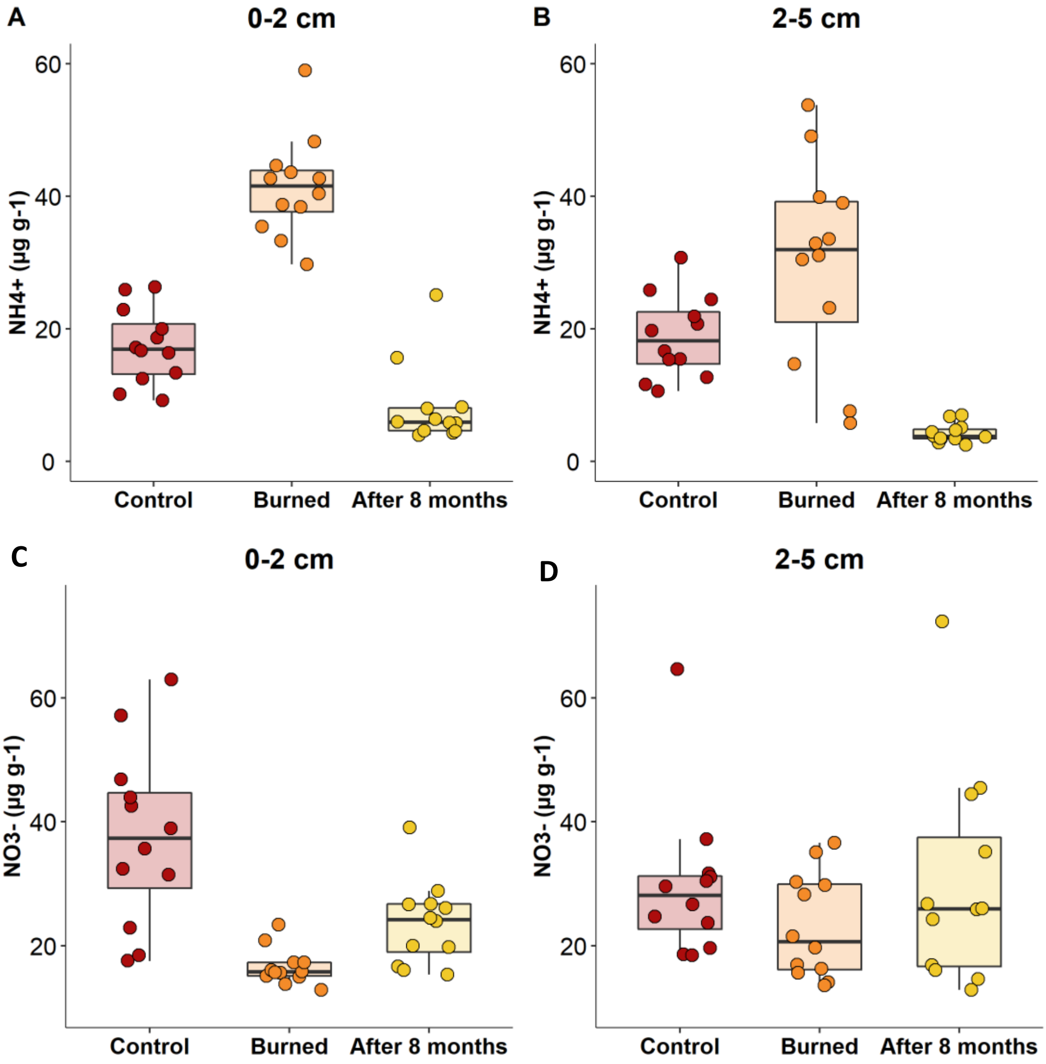

Figure 13 NH_4^+ (A, B) and NO_3^- (C, D) as a function of treatment [control (before burning), burned (immediately after burning) and 8 months after burning; n = 12] for the different soil depths.

The P_{Olsen} (Fig. 14) was significantly increased (p < 0.05) immediately after the fire in the top 2 cm of soil, while the rest of the samples did not show any significant differences among them. It should be highlighted that the lowest values were measured in the control samples before the controlled burn and that even after 8 months later these values were higher at both soil depths.

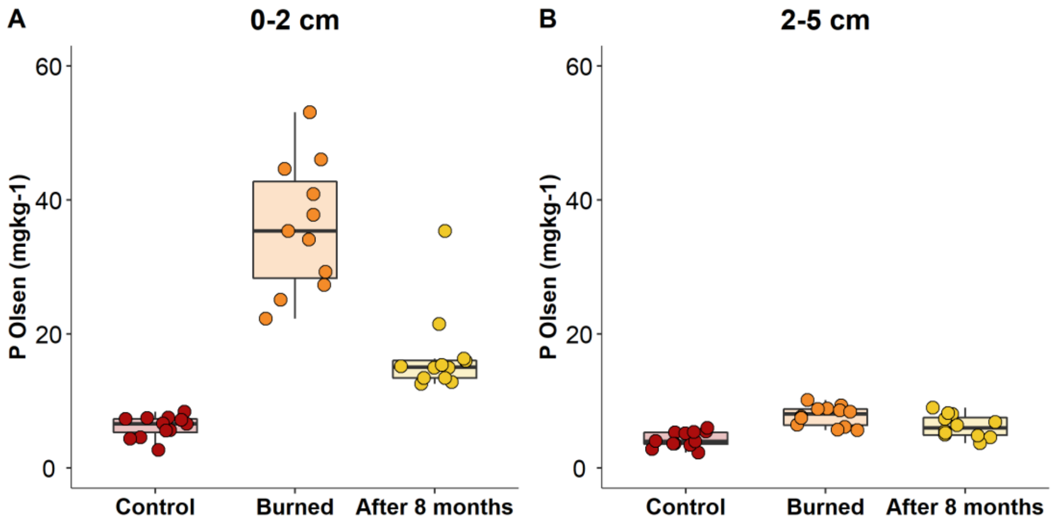

Figure 14 P_{Olsen} as a function of treatment [control (before burning), burned (immediately after burning) and 8 months after burning; *n* = 12] for the different soil depths.

Fe_{DTPA} was significantly reduced eight months after the soil was burnt only in relation with the treatment ($p < 0.05$, Table 6), varying its mean value from 21.3 mg kg^{-1} (control samples) to 18.3 mg kg^{-1} (immediately after burning) and finally to 35.0 mg kg^{-1} (eight months after the burn; Table 6). Fig. 15 shows the pattern followed by the different micronutrients. Mn_{DTPA} was significantly increased ($p < 0.05$) after the burn in the first 2 cm of soil, and drecreased eight months after the burn. All 2 – 5 cm samples had a similar value, lower than the amount of Mn_{DTPA} measured in the soil surface (0 – 2 cm). In the case of Zn_{DTPA}, the content in soil was increased with the fire and kept higher than the initial content eight months after the fire at both soil depths. Finally, Cu_{DTPA} was significantly increased ($p < 0.05$) after burning in the uppermost 2 cm of soil, reaching the highest values at this point. The control and burned samples collected from the soil surface (0 – 2 cm) after eight months and from the soil depth of2 – 5 cm showed significantly lower values than the contents, and the lowest Cu_{DTPA} were observed in the deepest soil samples (2 – 5 cm) collected eight months after the burn.

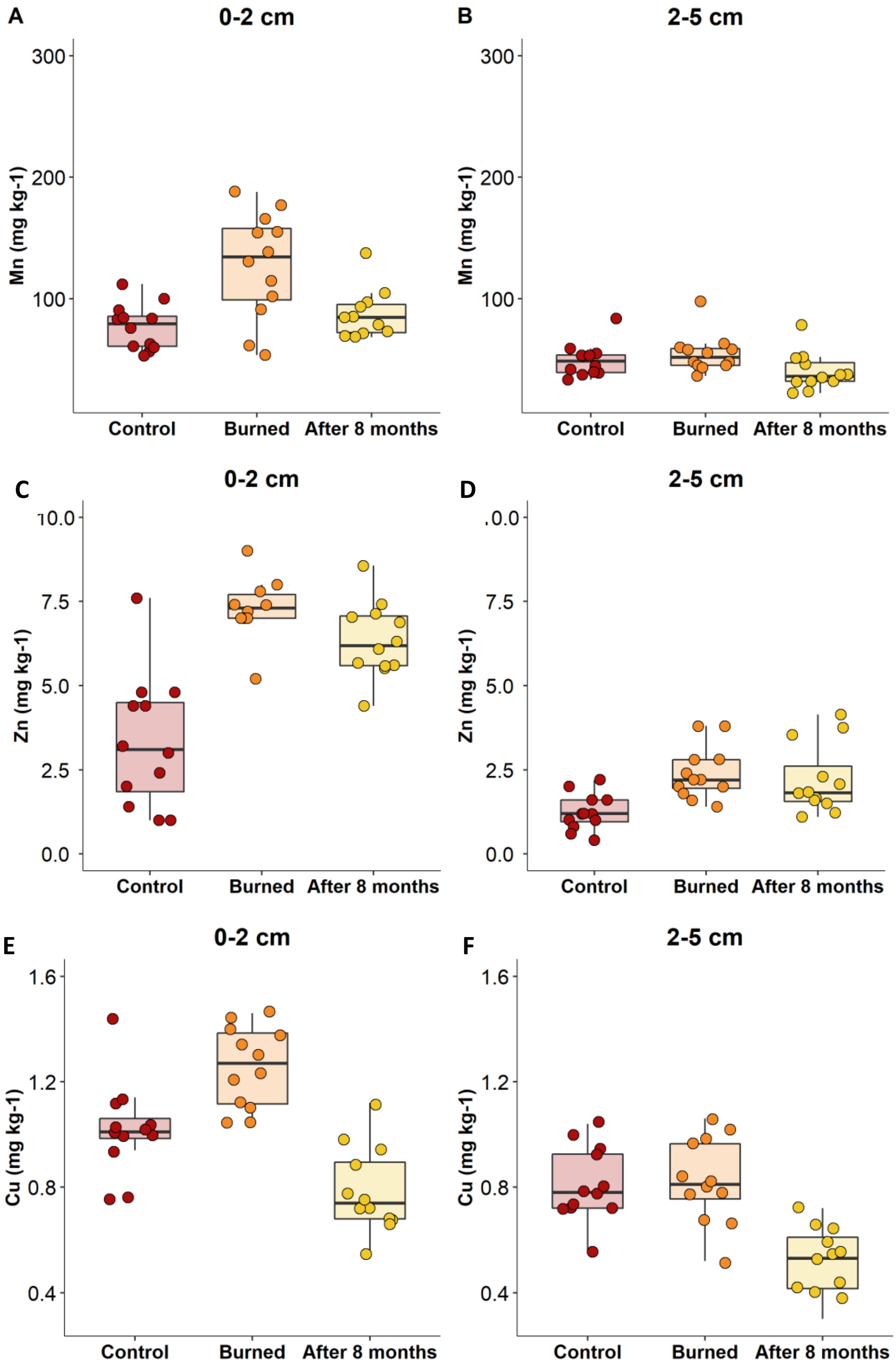

Figure 15 Mn_{DTPA} (A, B), Zn_{DTPA} (C, D) and Cu_{DTPA} (E, F) as a function of treatment [control (before burning), burned (immediately after burning) and 8 months after burning; *n* = 12] for the different soil depths.

PLFAs AND NLFAs

In general, the total amount of PLFAs in soil were significantly decreased ($p < 0.05$) due to the burn in comparison with the control samples. The lowest values were measured eight months after the burn at both soil depths (Table 6). The PLFA content in samples collected immediately after the burn did not significantly differ from the other samples (treatments). In addition, a higher content in PLFA was measured in the 0 – 2 cm than in the 2 – 5 cm soil depth (Table 6 and Fig. 16).

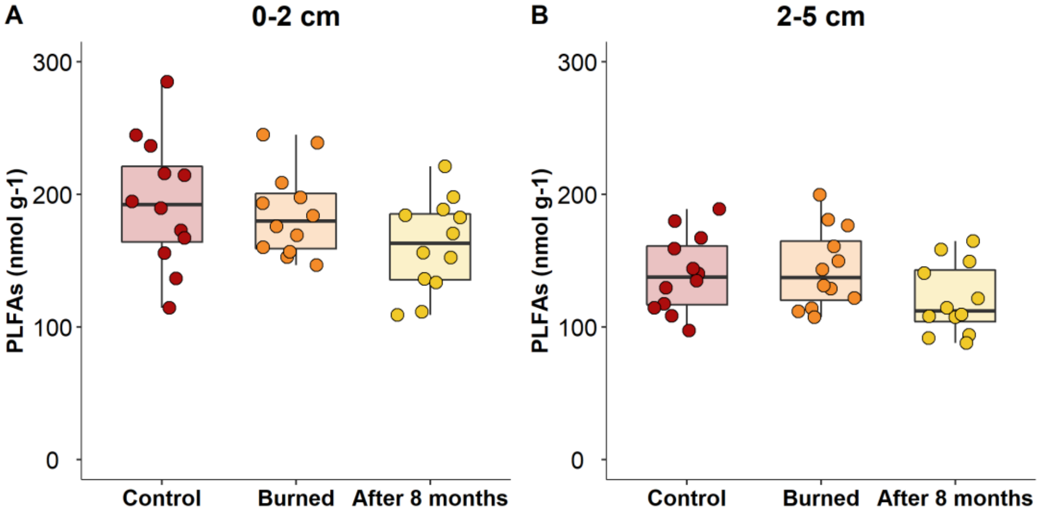

Figure 16 PLFAs as a function of treatment [control (before burning), burned (immediately after burning) and 8 months after burning; n = 12] for the different soil depths.

The results of the PERMANOVA are shown in Table 7. The different taxonomic groups were significantly affected by both, treatment, and soil depth in all cases. Although there were significant ($p < 0.05$) differences observed in relation to treatment and soil depth (as well as for their interaction), the model explains a reduced percent of the variability (small R^2 values). Other statistical analysis was thus needed to comprehend the differences observed and consider all the variability.

Table 7 PERMANOVA performed on a Bray-Curtis dissimilatory matrix for the different taxonomic groups, fatty acid types, ratios, total PLFA and total NLFA.

	Taxonomic group (%)	FA Type (%)	Ratios	PLFA (nmol/g)	NLFA (nmol/g)
R2 (treatment)	0.071	0.119	0.066	0.086	0.27
p (treatment)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
R2 (soil depth)	0.454	0.411	0.419	0.301	0.34
p (soil depth)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
R2 (interaction)	0.033	0.029	0.019	0.01	0.06
p (interaction)	< 0.05	0.052	0.272	0.656	< 0.05

Some patterns were seen in the RDA for the different taxonomic groups (Fig. 17): (i) most of the 0 – 2 cm soil samples were grouped in the left of the ordination plot while the 2 – 5 cm soil samples are on the right (RDA 1 explained 45.36% of the total variance); (ii) the burned soil samples at 0 – 2 cm were more related to a high content in fungi and these samples after eight months to AM fungi and gram negative bacteria. AM fungi were the only taxonomic group that showed no significant differences (p = 0.09) according to soil depth. Regarding the relationships between the different taxonomic groups and the environmental factors, fungi were related to higher soil contents in NH_4^+, and inversely related to NO_3^- content, while eukaryote, gram negative and AM fungi were mostly affected by the maximum temperature and NO_3^- content in soil. On the other hand, gram positive and actinomycetes behaved in the opposite direction to AM fungi, gram negative and eukaryote.

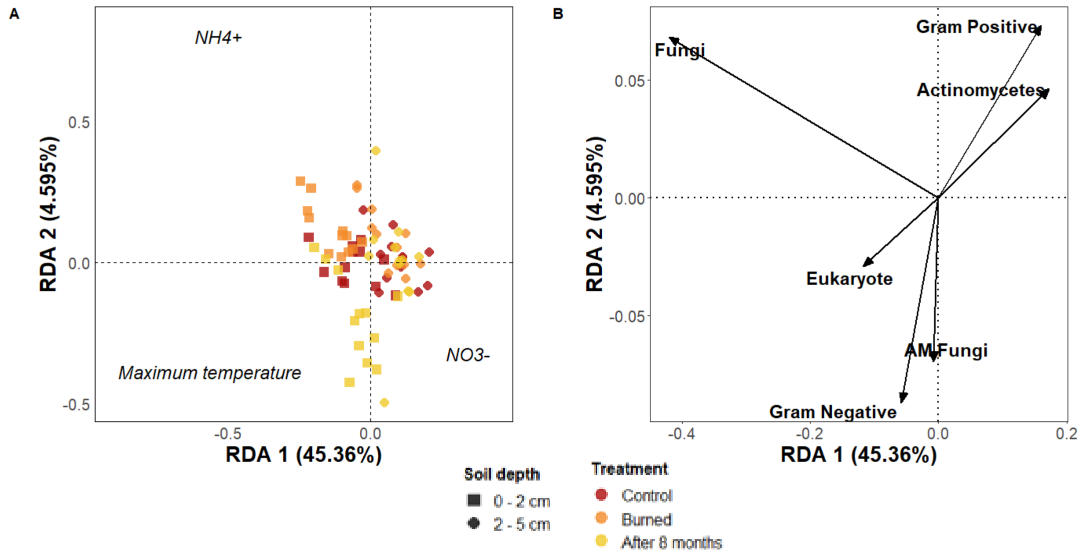

Figure 17 Redundant Analysis of the different taxonomic groups. (A) Distribution of samples along the two principal components and main correlated soil properties; (B) Distribution of taxonomic groups according to the two principal components.

In the case of the fatty acid ratios and indexes, the result of the RDA (Fig. 18) showed that they had different behaviours regarding soil depths, being the uppermost 2 cm of soil more related to Straight, MUFA and 18:2 w6c, 9c fatty acids, while the samples from the depth 2 – 5 cm were more related to 10 methyl, branched and cyclo fatty acid types. The samples collected eight months after the burn were clearly differentiated from the other samples in the RDA, being more related to Fe_{DTPA} and NO_3^-, while the rest of the samples were more related to NH_4^+, Fe_{ox}, Cu_{DTPA} and χ_{fd} %.

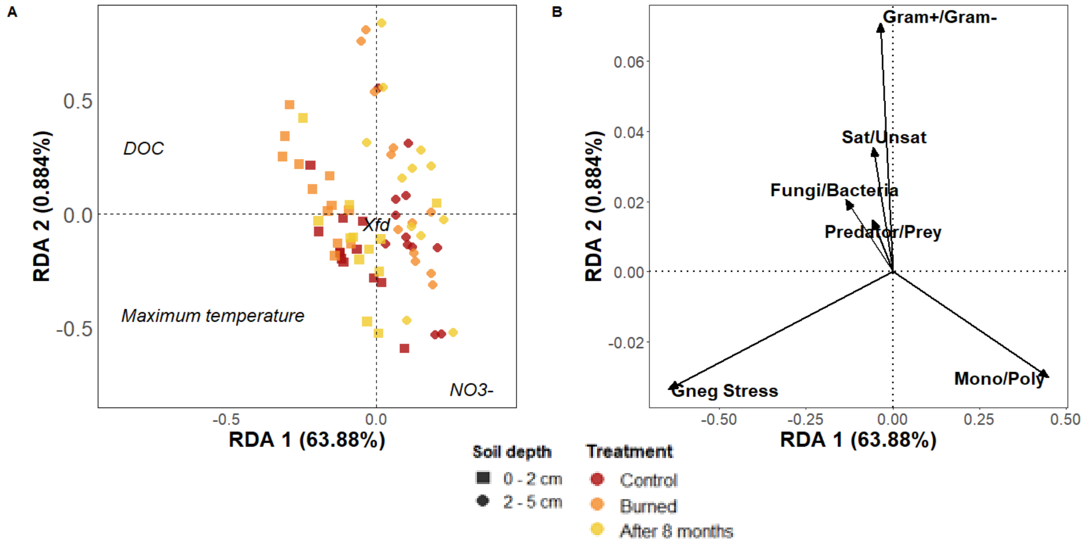

Figure 18 Redundant Analysis of the different ratios and indexes with PLFA. (A) Distribution of samples along the two principal components and main correlated soil properties; (B) Distribution of fatty acid types according to the two principal components.

Once again, as shown in Fig. 19 the samples behaved different in both soil depths regarding the different fatty acids type, being the samples from the uppermost 2 cm of soil more related to GNeg Stress, and the fungi/bacteria and Predator/Prey ratios. The deeper samples (2 – 5 cm) were more related to the gram+/gram- and mono/poly ratios. GNeg stress was the most related to maximum temperature, while mono/poly ratio was the most related to NO_3^-. The rest behaved similarly, being the predator/prey ratio more related to Xfd, and fungi/bacteria more affected by soil DOC content.

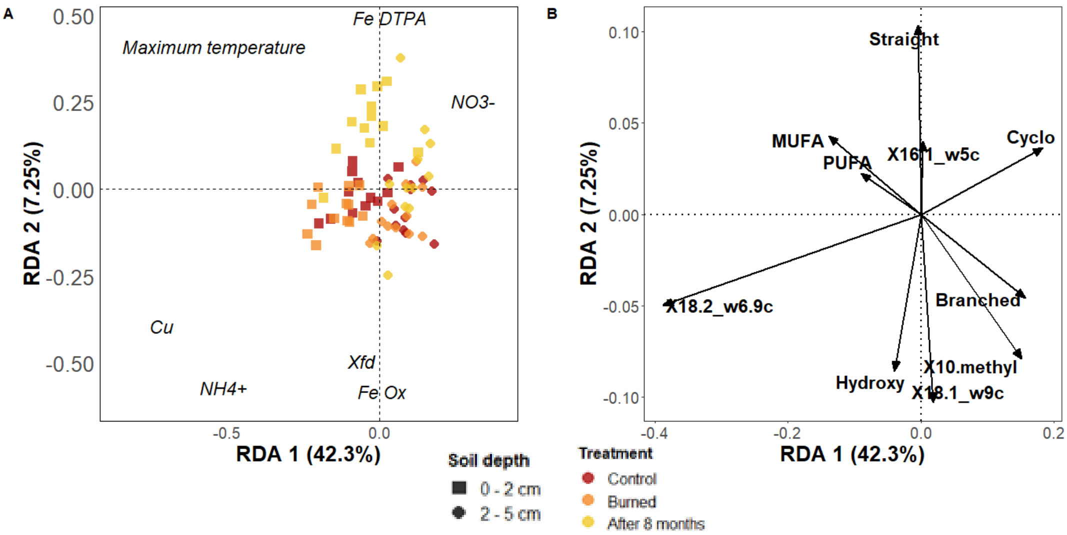

Figure 19 Redundant Analysis of the different fatty acids type. (A) Distribution of samples along the two principal components and main correlated soil properties; (B) Distribution of ratios according to the two principal components.

To address the status of the arbuscular mycorrhizal fungi (AM fungi) in the soil, the NLFA 16:1 w5c and the NLFA/PLFA 16:1 w5c ratio were used (Table 8). In both cases the trend followed was similar at both soil depths, increasing the value immediately after burning and decreasing below the initial value eight months after the burn. Although this general trend could be observed, the only significant alterations ($p < 0.05$) occurred for the NLFA/PLFA 16:1 w5c ratio in the 2 – 5 cm soil depth, where this value was significantly increased after the burn and decreased below the values of the control samples after eight months of recovery. In the case of the NLFA 16:1 w5c the only significant change occurred in the 2 – 5 cm soil depth during the eight months after the burn, where there was observed a significant decrease.

Table 8 Mean ± SE values of the NLFA 16:1 w5c and NLFA to PLFA 16:1 w5c ratio.

	Control (*n*=6)		Burned (*n*=6)		After 8 months (*n*=6)	
	0 – 2 cm	2 – 5 cm	0 – 2 cm	2 – 5 cm	0 – 2 cm	2 – 5 cm
NLFA 16:1 w5c (nmol/g)	12.4 ± 3.16	11.5 ± 2.19	13.9 ± 1.83	15.1 ± 1.92	11.5 ± 1.31	5.78 ± 1.19
NLFA/PLFA 16:1 w5c	1.52 ± 0.08	1.62 ± 0.08	2.01 ± 0.15	2.18 ± 0.07	1.44 ± 0.12	0.81 ± 0.03

REGRESSION TREES

The goodness of each model was tested by using the root-mean-square error (RMSE) and the mean absolute error (MAE), shown in Table 9. In general, all models showed acceptable values of both errors. A general trend that all models followed is that soil depth did not play a decisive role (not being significant) in any of them.

Table 9 Root-mean-square error and mean absolute error for each regression tree.

	RMSE	MAE
PLFA (nmol g^{-1})	35.3	29.6
16:1 w5c (nmol g^{-1})	1.43	1.22
AM fungi (%)	0.51	0.40
18:2 w6c (nmol g^{-1})	3.73	2.95
Fungi (%)	1.87	1.54
Gram - (%)	1.66	1.24
Gram + (%)	1.84	1.49
Eukaryote (%)	0.39	0.30
Actinomycetes (%)	0.97	0.70

The regression tree obtained for the total amount of PLFA is shown in Fig. 20. According to this prediction model, PLFAs mainly depended on the maximum temperature reached on the soil's surface. The other two most significant factors that affect the total amount of PLFAs were flame residence time and the treatment, being separated the control and burned samples from those collected eight months after the burn. The amount of total PLFAs increases as the temperature increases and residence time decreases. In this case, the soil depth does not affect significantly to the amount of PLFAs.

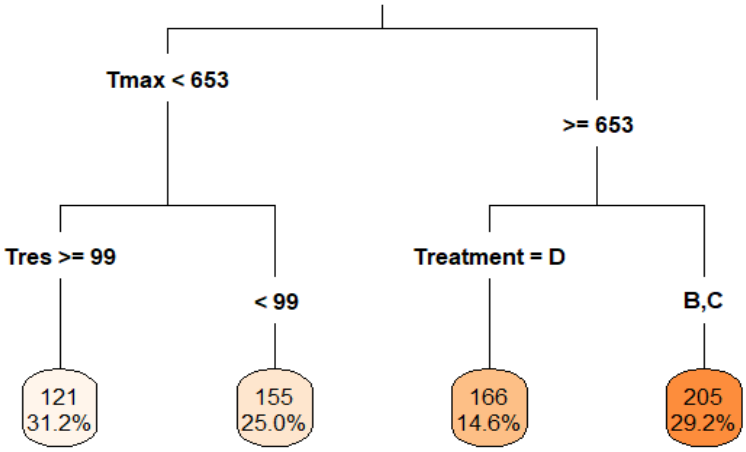

Figure 20 Regression tree for total PLFA (nmol/g). Tmax: maximum surface temperature (^{o}C); Tres: flame residence time (s), Treatment: (C) control, (B) immediately after burning, (D) after eight months.

The regression trees obtained for the fatty acid 16:1 w5c, which was used as a biomarker of arbuscular mycorrhizal fungi, and for the arbuscular mycorrhizal fungi (AM Fungi) communities (%) are shown in Fig. 21. According to this prediction model, arbuscular mycorrhizal fungi depended mainly on the maximum temperature reached on the soil's surface. The model for 16:1 w5c fatty acid was very similar to the one obtained for the total PLFAs, meaning that they behaved in a similar way. In the case of 16:1 w5c fatty acid, the main split occurred at a temperature of 653 °C, as in the case of the total PLFA, while for the AM fungi communities the main split regarding temperatures occurred at 931 °C, although they were already affected at a temperature of 40.3 °C. Despite this difference, it can be said that AM fungi was significantly affected once the temperatures exceed 600 °C. In the case of AM fungi communities, treatment seemed to have an important effect, separating the control, and burned samples from the samples collected eight months after the controlled burn. Other variable that affected significantly the 16:1 w5c fatty acid was the flame residence time, being the split at 99 seconds. In both cases soil depth didn't play an important role in the models.

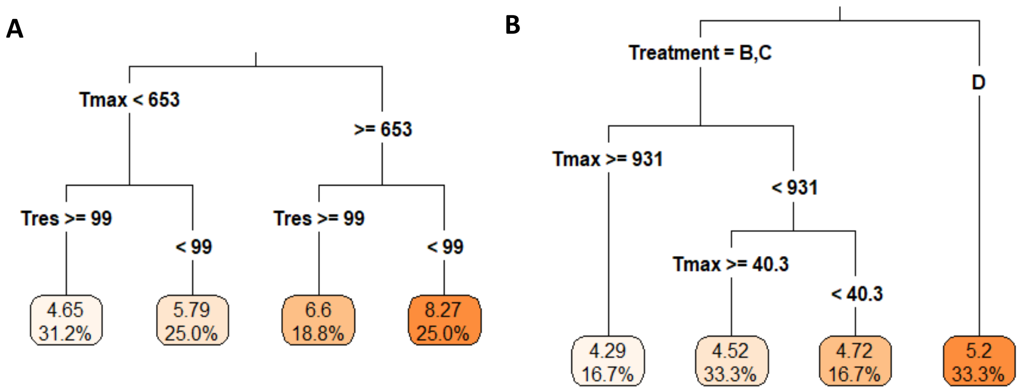

Figure 21 Regression tree for (A) 16:1 w5c fatty acid (nmol/g) and (B) AM fungi (%). Tmax: maximum surface temperature (^{o}C); Tres: flame residence time (s), Treatment: (C) control, (B) immediately after burning, (D) after eight months.

Like the total amount of PLFAs and the 16:1 w5c fatty acid, the 18:2 w6c fatty acid, a biomarker of saprotrophic fungi, depended mainly on the maximum temperature, being the main split as in both previous cases at 653 °C, as shown in Fig. 22. In this case, the treatment also played a significant role, but in a different way as it has been shown in the previous models. In this case, the samples collected immediately after the burn were differentiated from the other two samplings, meaning that in that moment the amount of 18:2 w6c fatty acid increased. Regarding the Fungal communities, the two factors that affected them were maximum temperature and treatment, like what happened with the 18:2 w6c fatty acid, unless in the case of fungi, the main split was at a temperature lower than before, 354 °C. Soil depth did not affect significantly either of these communities.

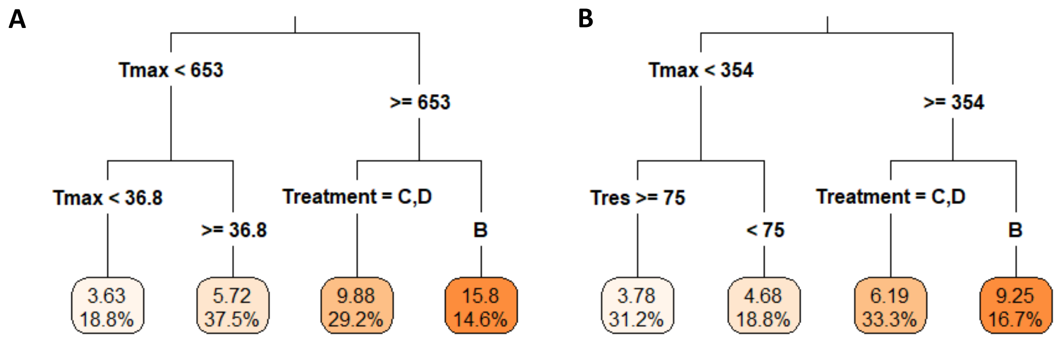

Figure 22 Regression tree for (A) 18:2 w6c fatty acid (nmol/g) and (B) fungi (%). Tmax: maximum surface temperature (^{o}C); Tres: flame residence time (s), Treatment: (C) control, (B) immediately after burning, (D) after eight months.

Gram-negative bacteria were mainly affected by maximum temperature as well, but in the case of this taxonomic group, at lower temperatures there were already alterations. As shown in Fig. 23A, the main split was for maximum temperature at 71.5 °C, meaning that as the burn took place, the gram-negative were the first taxonomic group to be altered. Like what happened with fungal communities, the treatment was significant, showing lower values the samples taken immediately after the fire. For the other two samplings, the control and eight months after the fire, the differentiating factor was the flame residence time, being the split at 116 seconds.

The behaviour of the gram-positive bacteria was like the fungi, being the most significant variable the maximum temperature at a split value of 354 °C (Fig. 23B). The main difference between these two taxonomic groups was the flame residence time, which has higher significant values in the case of gram positive, meaning that at the same temperature, fungal communities were affected sooner than gram-positive bacteria.

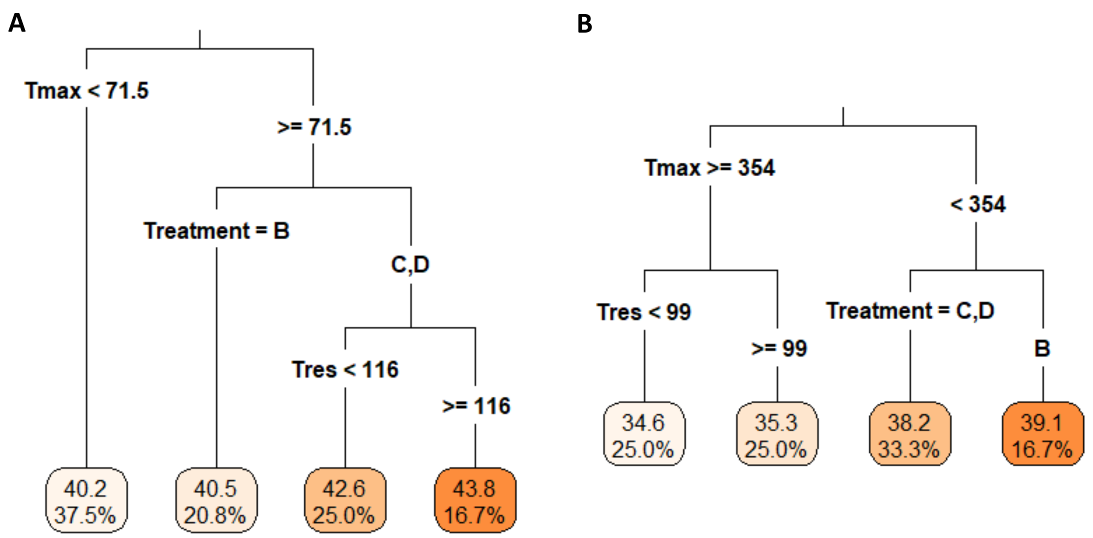

Figure 23 Regression tree for (A) gram negative (%) and (B) gram positive (%). Tmax: maximum surface temperature (^{o}C); Tres: flame residence time (s), Treatment: (C) control, (B) immediately after burning, (D) after eight months.

The model for eukaryote (Fig. 24A) showed that the main split occurred at a maximum temperature of 917 °C. However, although the main split was at a high temperature, another split was made according to temperature at a value of 140 °C. For actinomycetes (Fig. 24B), the regression tree was similar to the ones for gram positive and fungi, where the main split was at a temperature of 354 °C. The difference with actinomycetes was that regarding the treatment, the control samples were differentiated from the other two samplings, having the first one higher amount of actinomycetes. Flame residence time was also significant in this model, being the split at 116 seconds, showing that the shorter time the flame was in a point, the higher amount of actinomycetes was to be found at temperatures less than 354 °C.

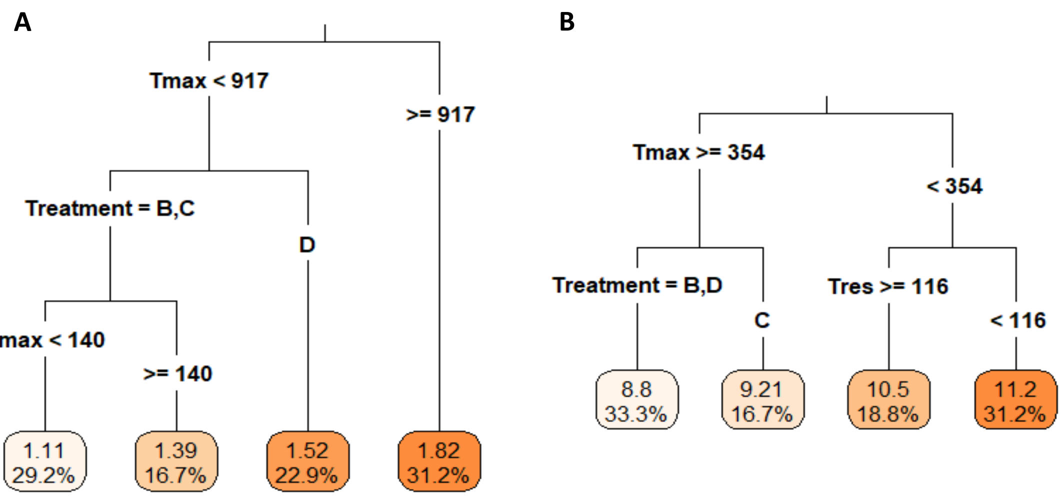

Figure 24 Regression tree for (A) eukaryote (%) and (B) actinomycetes (%). Tmax: maximum surface temperature (^{o}C); Tres: flame residence time (s), Treatment: (C) control, (B) immediately after burning, (D) after eight months.

SPATIAL VARIATION OF SOIL PROPERTIES – PREDICTION MAPS

For the different soil properties, each model is represented in the figures shown below. In all cases, the A figure shows the differences in the values / contents between control and immediately after the burn samples, and the B figure shows the differences in the values / contents between control samples and the ones collected eight months after the burn, for the 0 – 2 cm soil depth. The goodness of each model has been tested by using RMSE and MAE, whose values are shown in Table 10.

Table 10 Root-mean-square error and mean absolute error for each prediction map (only the values from the 0 – 2 cm soil samples were included here).

Soil Property	Treatments compared	RMSE	MAE
pH	C-B	0.12	0.32
pH	C-D	0.47	038
P_{Olsen}	C-B	4.14	7.55
P_{Olsen}	C-D	8.36	4.49
Fe_{Ox}	C-B	250	465
Fe_{Ox}	C-D	345	333
CEC	C-B	1.33	1.00
CEC	C-D	0.16	0.64
Fe_{DTPA}	C-B	5.55	16.3
Fe_{DTPA}	C-D	3.07	17.1
NO_3^-	C-B	2.07	8.44
NO_3^-	C-D	3.94	12.64
NH_4^+	C-B	4.17	6.82
NH_4^+	C-D	6.04	3.21
DOC	C-B	165	244
DOC	C-D	159	160

Treatments: C = Control; B = Immediately after burning; D = Eight months after the controlled burn

The spatial variation of pH and P_{Olsen} followed a similar trend when comparing both, control and burned samples, and control and after-eight-months samples, although the differences were smaller in the second case (Figs. 25 and 26). The magnitude of the differences in pH had an inverse relationship with fire behavior (Fig. 5), being greater the variation in the south-eastern corner of the study area (Fig. 25), matching with the area where the fire had lower values of spread rate, fire-line intensity, heat per unit area, flame length and flame residence time. The western part of the area showed smaller differences in both comparisons, corresponding with the areas where the fire was more intense.

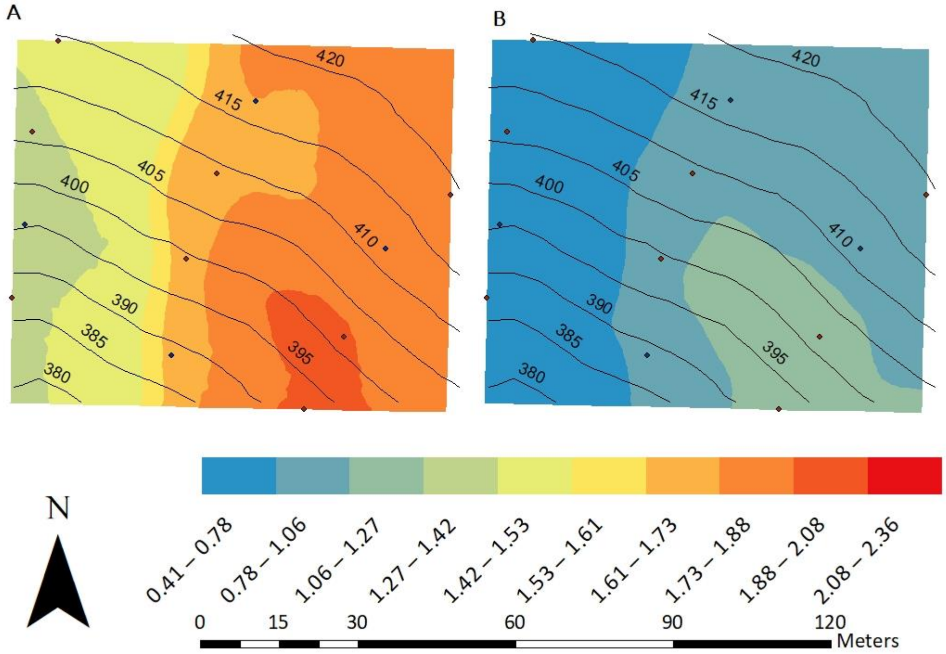

Figure 25 Prediction map for the difference of pH in the uppermost two cm of soil. (A) Differences between control and burned samples; (B) Differences between control and after eight months samples.

P_{Olsen} varied in a greater way in the eastern part of the study area, being homogeneous the variation in the Fig. 26A, and heterogeneous in the Fig. 26B. This shows that after a big increase of P_{Olsen}, the decrease that happened during the eight months that followed, happened according to the spatial characteristics of the study area, showing bigger differences in the areas were the slope was higher.

In the case of the cation exchange capacity, the pattern was the opposite to what happened with P_{Olsen}, being the spatial variation heterogeneous in Fig. 26C and becoming more homogeneous in Fig. 26D. It seems like the slope has a big influence in this variation, as the differences are less pronounced in the central western part of the area, where the burn started, the fire was less severe and there was less vegetation.

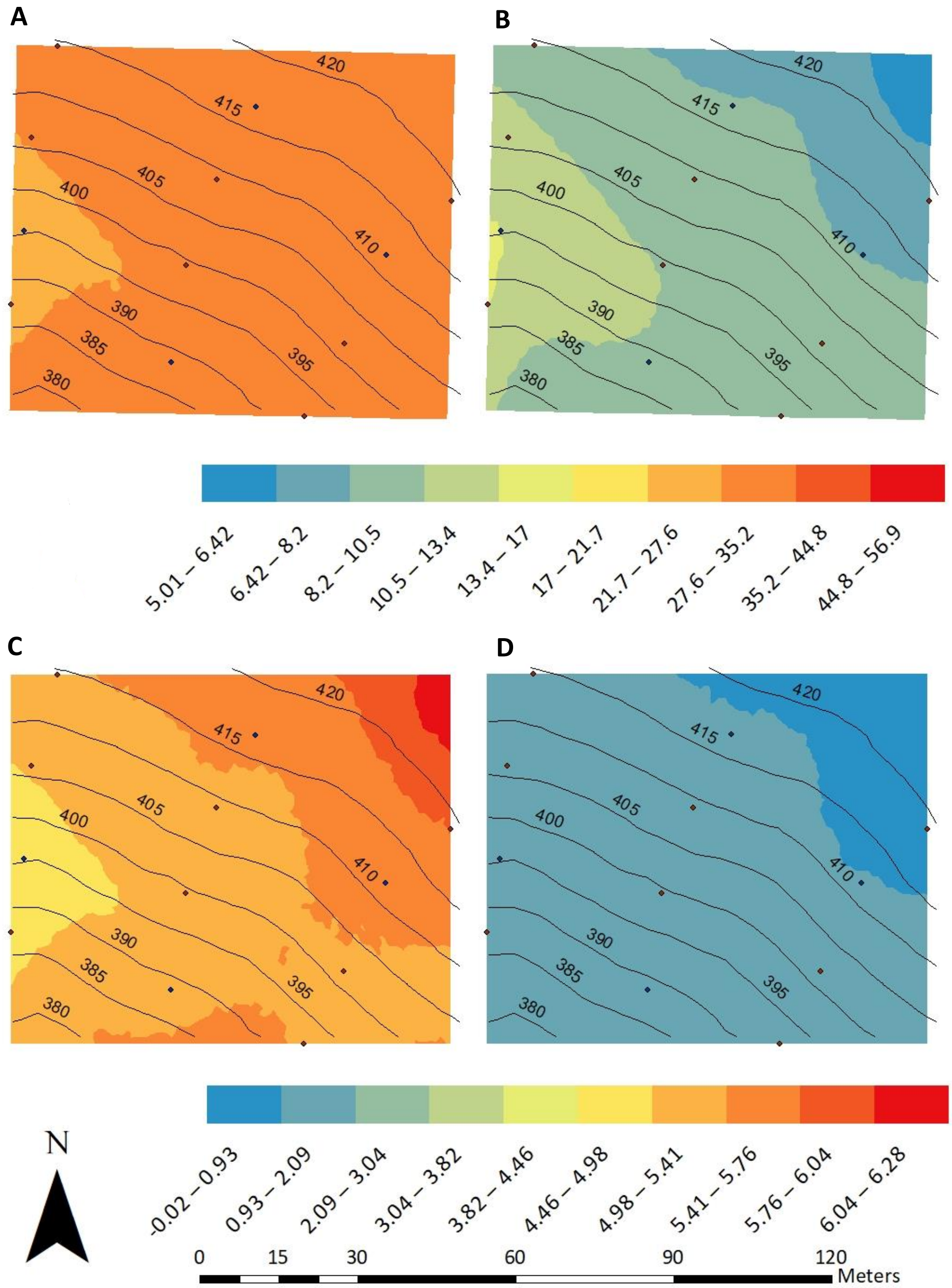

Figure 26 Prediction map for the difference of P_{Olsen} (A, B) and cation exchange capacity (C, D) in the uppermost two cm of soil. (A and C) Differences between control and burned samples; (B and D) Differences between control and after eight months samples.

Fe_{ox} variations showed bigger values in Fig. 27A than in Fig. 27B, being the areas with bigger differences those in which the area was affected by the most severe fire behaviour and being the alterations in the other two scenarios more homogeneous. In Fig. 27B it can be observed that the alterations follow a similar pattern as in the case of CEC mentioned above, which was related to the slope of the area, being smaller the changes in the mid-western part of the plot, where the fire reached bigger flame lengths.

The spatial behaviour of Fe_{DTPA} in Fig. 27C, was more homogeneous than in Fig. 27D. In Fig. 27C, there was a big increase in the areas corresponding to those in which the fire behaved less severely, while in the areas where the fire was more intense the decrease was of a lower magnitude. On the other hand, the areas where the fire was more severe were those in which the increase was bigger with respect to the control sampling (Fig. 27D).

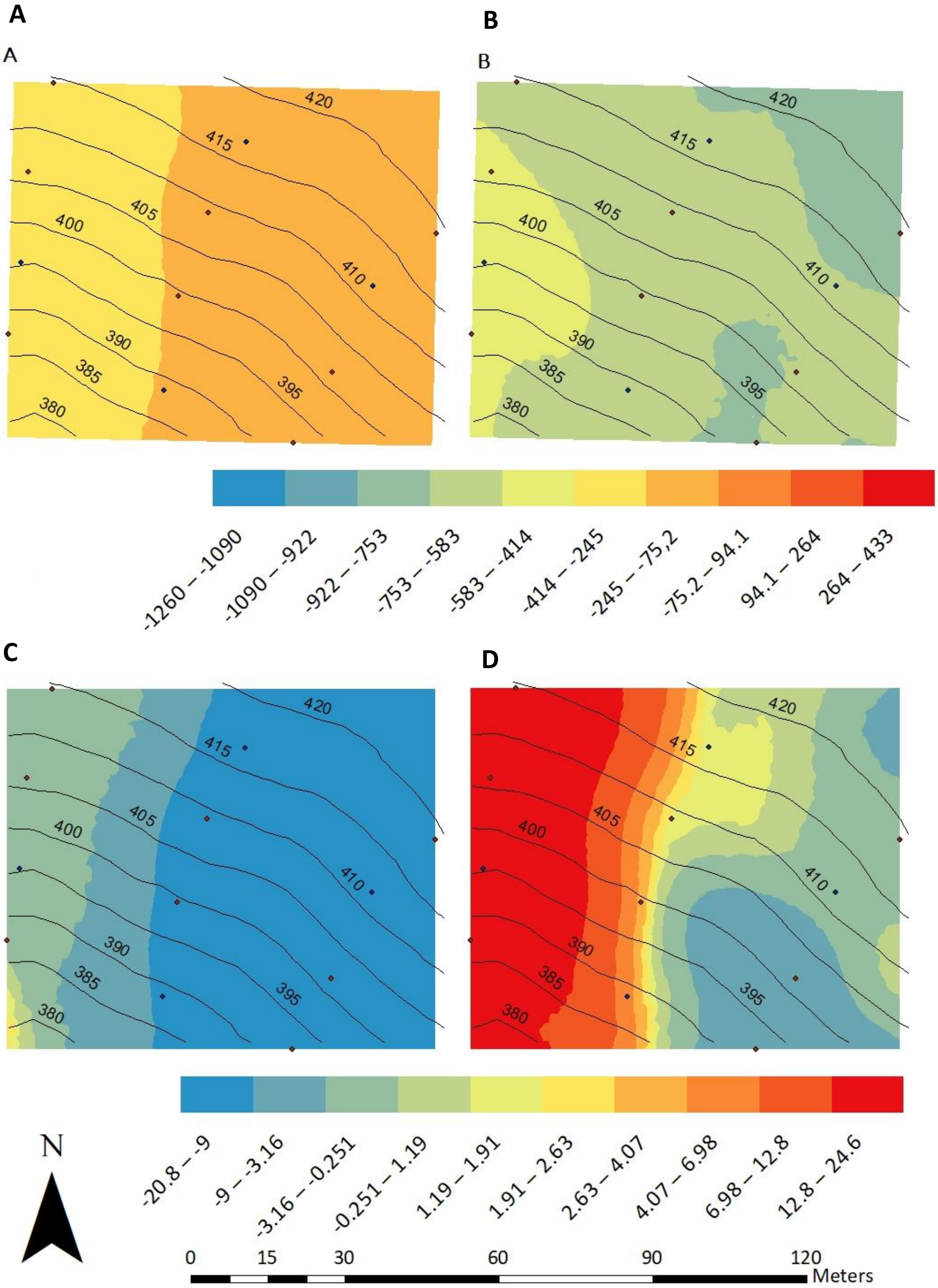

Figure 27 Prediction map for the difference of Fe_{ox} (A, B) and Fe_{DTPA} (C, D) in the uppermost two cm of soil. (A) Differences between control and burned samples; (B) Differences between control and after eight months samples.

As mentioned in previous sections, the soil content in DOC was greatly increased immediately after the controlled burn and then decreased during the following eight months but these values remained still significantly higher than before the burn. This pattern was also observed in Fig. 28A, where the differences in the amount of DOC are considerable, especially in the north-eastern corner of the plot. The areas in which the changes were smaller were the same as the areas where the fire was more severe, and the slope was more pronounced (Figs. 3, 5 and 28A). Additionally, more heterogeneity was observed in Fig. 27B but the differences were bigger in the areas where the fire was more intense.

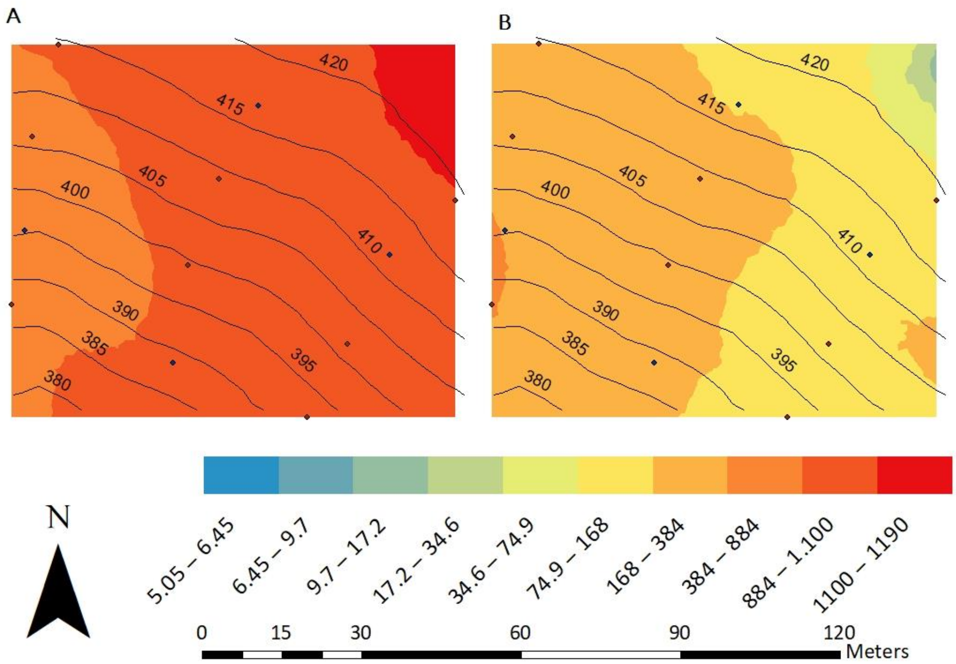

Figure 28 Prediction map for the difference of DOC in the uppermost two cm of soil. (A) Differences between control and burned samples; (B) Differences between control and after eight months samples.

Ammonium was homogeneously increased along the plot immediately after the fire, being the north-eastern corner the area where changes were smaller (Fig. 29A). On the other hand, the behaviour was the opposite, being the decrease more heterogeneous for the difference between the former values and the contents after 8 months of soil recovery (Fig. 29B). In this case, the biggest differences occurred in the north-eastern corner of the plot and the smallest in the areas where the fire was more severe.

The behaviour of soil NO_3^- content was similar when comparing control samples with both, immediately after (Fig. 29C) and eight months after the fire (Fig. 29D); the bigger decreases were observed in the southern part of the study area. The alterations follow a pattern similar to the level curves, showing a relationship between altitude and the alterations.

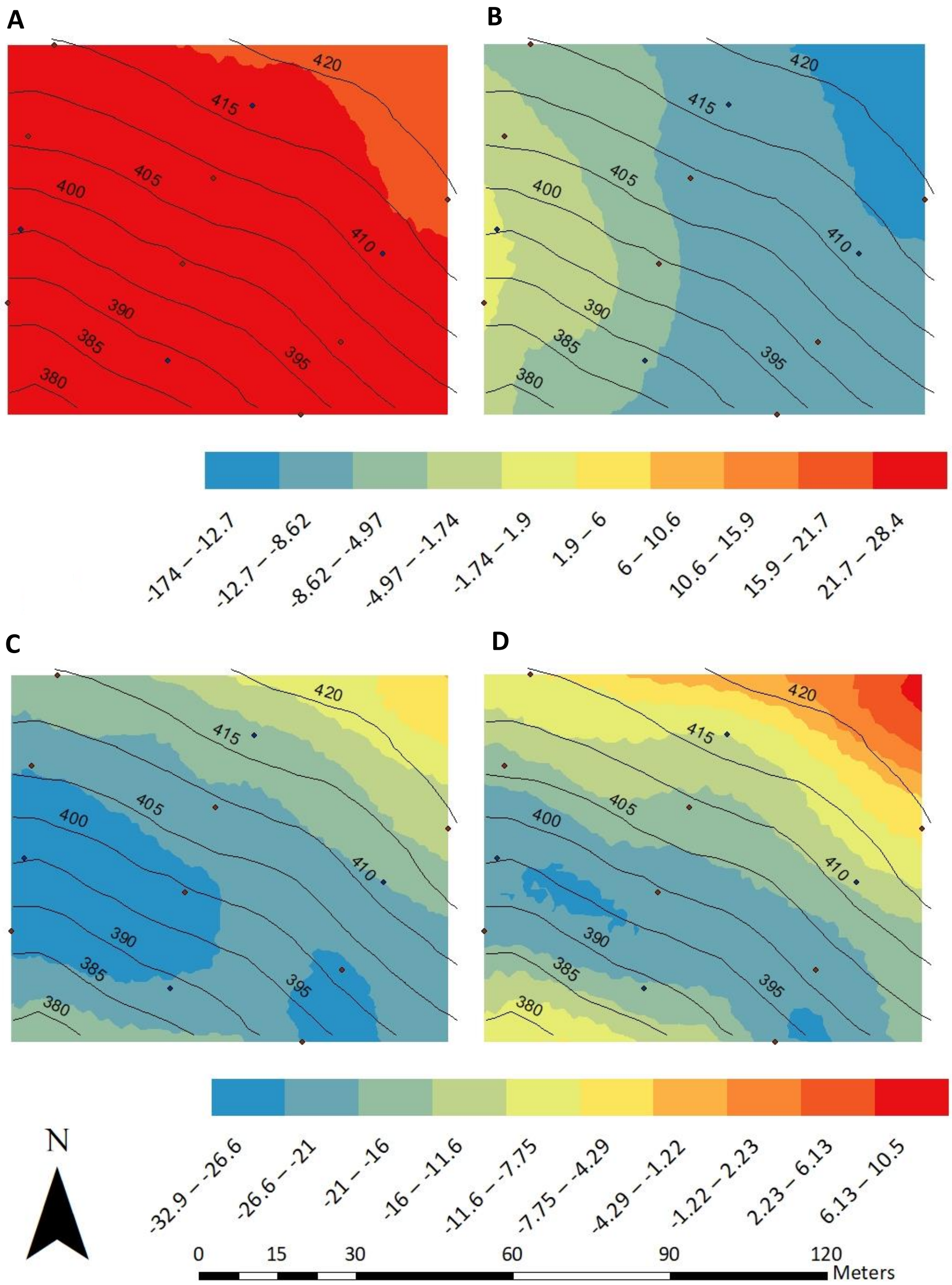

Figure 29 Prediction map for the difference of NH_4^+ (A, B) NO_3^- (C, D) in the uppermost two cm of soil. (A, C) Differences between control and burned samples; (B, D) Differences between control and after eight months samples.

DISCUSSION

Fire had different behaviours along the study area, and added to the characteristics of vegetation and topography, it resulted in a great variability in the alterations observed in soil properties among the different sampling points. Regarding the effects of the burn at the different soil depths, the temperatures reached in the soil surface are not related to the temperature in deeper depths beyond the first two or three centimetres of soil according to Fonseca et al. (2017). This is because surface temperature peaks do not occur where residence times are longer but are a temporary event. In addition, the soil is characterised by a low thermal conductivity (Abakumov et al., 2020; Enninful & Torvi, 2008).

ALTERATIONS IN SOIL PROPERTIES

The combustion of the vegetation and organic matter during the burn led to the accumulation of ash on soil's surface, which plays a very important role in the alteration of some soil properties such as soil pH (Arocena & Opio, 2003; Giorgis et al., 2021), electrical conductivity (Alcañiz et al., 2016; Certini, 2005), dissolved organic carbon (Revchuk & Suffet, 2014), cation exchange capacity (Úbeda et al., 2005), available P (Badía et al., 2014; Caon et al., 2014; Romanyà et al., 1994), and micronutrients availability (Parra et al., 1996). In general, the pattern observed for these soil properties was similar, increasing immediately after the fire, linked to the deposition of ashes, and decreasing after some time due to events of precipitation, which cause a surface overflow that trawls the ash away (or to deeper soil layers with infiltration), among other reasons (Hung et al., 2005; Notario del Pino et al., 2008; Úbeda et al., 2005; Vila-Escalé et al., 2007). In the case of this study, the burn took place in October, and during the month of December the precipitation was higher than in other months This could be one of the main influencing factors for the decrease observed in some of these properties eight months after the burn.

The different behaviours of the fire during the burn led to the partial combustion of vegetation in the areas where the fire was less intense, causing a greater accumulation of ash in comparison with the areas where the fire was more intense, in which the combustion of the vegetation was almost total. This fact affected the spatial variability of certain soil properties, which had an inverse relation with the fire severity, such as soil pH or available P (P_{Olsen}), that were more altered in the areas were the fire was less severe.

Regarding the magnetic properties, the variation in the magnetic susceptibility measured at low frequency (χ_{lf}) does not correspond to that observed in previous studies. Jordanova et al. (2019b) found an increase in the magnetic susceptibility measured at low frequency (χ_{lf}) was observed in the first centimetres of the soil after natural forest fires in Bulgary. This difference may be due to several factors, such as: (i) the parent material and the Fe content of the soil (Bautista et al., 2014; Jordanova et al., 2019b); (ii) the presence of vegetation cover that influences the amount of organic matter content in soil (Jordanova et al., 2019a),; or (iii) the severity of the fire, which determines the temperature reached in the soil, and therefore, the conditions the transformation of iron minerals to forms with different properties, including magnetism (Bautista et al., 2014; Jordanova et al., 2019a). There is a relationship between magnetic susceptibility and soil organic carbon, so that the magnetic susceptibility of a soil can be considered as an estimator of soil organic carbon Jordanova et al., 2019a). Therefore, the higher the magnetic susceptibility, the higher the organic carbon content. This relationship can be explained based on the behaviour of organic matter when combustion occurs, since when organic carbon undergoes this process, it is converted into its more pyrogenic form, which has a reducing character with great importance in the process of transformation of soil Fe oxides to more magnetic forms (magnetite, maghemite, pyrrhotite or magnetic pyrite) (Bautista et al., 2014; Jordanova et al., 2019b).

Additionally, the magnetic susceptibility (χ_{fd} %) is related to supraparamagnetic grains in the soil. According to Bautista et al. (2014), if the percentage is between 2 and 10%, the sample presents a mixture between these supraparamagnetic particles and coarse grains, which are found in similar proportions. In the case of this study, this percentage was slightly decreased, which implies that the proportion of supraparamagnetic particles was reduced due to the fire.

The darkening of soil colour observed in the results, a consequence of fire and the accumulation of charred plant debris and ash (Ulery & Graham, 1993), will affect the albedo, being reduced after burning (Dadi et al., 2013). However, the change in soil colour observed in the 0 – 2 cm soil depth was not as evident as in the first two centimetres due to the lower temperatures reached in deeper layers of soil (Ketterings & Bigham, 2000). This effect occurs in the short term, as once the post-fire recovery of the area begins, in which vegetation develops again, albedo increases, progressively returning to pre-fire values, if the severity of the fire was not extremely high (Dadi et al., 2013). A variation in soil colour (and albedo) leads to a variation in soil temperature, which conditions the processes that take place in the soil, such as vegetation recovery, microbial communities, evapotranspiration, nutrient cycling, among others (Sánchez et al., 2011). In the case of this study, there was a variation in colour, slightly decreasing the value and chroma, producing a slight darkening of the soil, so alterations in albedo are expected in the first months after the fire.

The content of amorphous iron oxides (Fe_{ox}) is related to the solubility and availability of Fe in the soil (Campillo & Torrent, 1992). In a study conducted by Norouzi & Ramezanpour, (2013), Fe_{ox} was increased after the occurrence of natural forest fires that took place in pine forests in Iran, while this soil index was slightly reduced in the case of this study. This may be due to differences in vegetation cover (ecological differences between pine stands and scrub stands, as is the case in this work) and different soil texture, as well as the different type of fire affecting the soil (Norouzi & Ramezanpour, 2013), in a similar way to this study. The evolution of the decrease in total iron oxides (Fe_{di}) in soil after eight months since the burn was sharper than in the case of Fe_{ox}. The increase in soil pH after fire is also related to the dispersion and promotion of small particles (Nørnberg et al., 2004), which explains the results obtained by Norouzi & Ramezanpour, (2013), in which both forms of Fe oxides were increased slightly after fire, contrary to what it was observed in this study.

The alteration in soil carbon and organic matter in the top layer depends on the fire severity, volatilisation of organic matter and oxidation processes. Incomplete consumption of vegetation can bring organic matter to the soil in the form of semipyrolyzed ash (Doerr et al., 2018), which can protect organic matter from bio-decomposition processes (Johnson & Curtis, 2001). As the soil temperature increases, organic matter decomposes. This includes nutrient mineralization and the death of soil microorganisms that have immobilised nutrients, for example, N inside (Yevdokimov & Blagodatsky, 1994) and inorganic forms of N, i.e. NH_4^+ and NO_3^- are released, increasing nutrient availability in the short term. However, part of the total N and other nutrients will be lost by volatilisation (of NH_4^+), being this process proportional to the temperature (the higher the temperature, the more N will be lost). After the fire, mineralisation could increase, as solar radiation is easily absorbed due to the increase in the albedo previously mentioned. Soil microorganisms 'work better' at higher temperatures and the NH_4^+ in the soil will increase (mineralisation) (DeLuca et al., 2002). Additionally, part of this NH_4^+ is transformed by these microorganisms into NO_3^- (through nitrification) (Ball et al., 2010), which would explain the drop in NH_4^+ in the uppermost two centimetres of soil and the associated increase in NO_3^- after 8 months at both depths (more noticeable at 0 – 2 cm). These transformations can explain the spatial behaviour of both NH_4^+ and NO_3^-, as they had opposite behaviour in soil. However, NO_3^- is susceptible to leaching (Meisinger & Delgado, 2002), which can also help explain the spatial behaviour observed in the prediction maps, in which greater differences were observed in the areas more susceptible to leaching at the lower part of the slope.

The content in soil available nutrients after a fire usually increases due to the accumulation of ash enriched and to the release of basic cations from the soil organic matter (Badía et al., 2014; Caon et al., 2014; Romanyà et al., 1994). However, this effect normally persists only in the short or medium term, since with the washing of ash (water, wind and even the effect of gravity) the available P (and other nutrients) will be drastically reduced (Ferreira et al., 2016; Pereira et al., 2012), and may reach lower values than the initial ones. It would have a negative impact on soil fertility (in the short or medium term depending on precipitation events, location of the area that has suffered the burn or fire, etc.). Furthermore, the conditions reached during the burn affected soil P, favouring its mineralisation from soil organic matter and even the release from the vegetation, releasing inorganic phosphorus into the soil solution. Then, this P can be absorbed by plants, microorganisms and be adsorbed on the surface of Al, Fe and Mn oxides and hydroxides (in acid soils), or the formation of calcium phosphates (in alkaline or neutral soils) (Badía et al., 2014; Ferreira et al., 2016), decreasing its availability in the soil and depleting reserves. Phosphorus plays a very important role in soil fertility, being one of the most limiting macronutrients for the regeneration of vegetation, and fire can cause the transformation of organic P into inorganic P, which is labile and bioavailable, meaning that the controlled burn has a positive effect on the fertility of soil regarding P availability (Elser et al., 2007; Schaller et al., 2015) and will contribute to the regeneration of the burned area.

ALTERATIONS IN SOIL MICROBIAL COMMUNITIES

Biological properties of soils are related directly and indirectly to the effect of controlled burns and/or fires by means of the different processes that occur after them, such as: combustion of the soil organic matter, the accumulation of ash and the changes of physic-chemical properties of soils, as well as climatic events such as precipitation that increase soil’s moisture (Longo et al., 2014).

According to the results of this study, soil microbial communities were also affected by different soil properties, such as soil pH and nutrient availability (Hart et al., 2005). The main taxonomic groups, as well as the different fatty acid types were related to soil inorganic N forms such as NO_3^- and NH_4^+ and to the maximum temperature reached on soil's surface, among others. Similar results were found by Cobo-Díaz et al. (2015), where a significant relationship was found between the alteration of bacterial communities and the N cycle. This indicates the vital role they played for soil microbial communities.

The relationship among the taxonomic groups and maximum temperature was also addressed by the regression trees, in which this variable had an important role on the alteration of the different taxonomic groups. Although microorganisms are adapted to a wide range of temperatures (Neary et al., 2005), the drastic changes of temperatures that result from a controlled burn have a direct effect on the soil microbiota in as much as it can exceed the vital temperature of the microorganisms (Certini et al., 2021), thus altering the presence of the different taxonomic groups. The microorganisms most affected by lower temperatures during the fire were mycorrhizal fungi and gram negative bacteria (more sensitive to the effect of the temperature) (Busse et al., 2005; Neary et al., 2005). The different taxonomic groups conforming the microbial communities of soil have different resilience and resistance capacities, being the fungal and mycorrhizal fungi communities the most sensitive to the effect of fire due to heating and the change in organic matter (Chanda, 2020; Köster et al., 2021). Bacteria and actinomycetes have a high resistance to the effects of controlled burns due to their adapting capacity to the burned environment, being the actinomycetes the most resistant group (Chanda, 2020). Regarding eukaryotes, their advantage resides in their recovery time after a fire event, which is shorter than in the case of the other taxonomic groups (Certini et al., 2021).

In our study, it was clear that the soil microbial community was affected by the fire and that this community was different to the former one eight months after the fire and even it was differently affected with soil depth. The development of this soil microbial community, and, consequently, the ecosystem services that will be provided by the forest system will depend on the postfire management and on the vegetation that will cover the soil.

FOREST ECOSYSTEMS MANAGEMENT

This study has shown that the use of controlled burns has effects on soil properties, which can be useful from the management of forest ecosystems point of view. The persistent rise in pH is helpful for the management of species such as *Cistus ladanifer*, that need to be controlled due to their invasive and allelopathic tendencies (Du Plessis et al., 2018), and that do not tolerate basic soil conditions (Núñez-Olivera et al., 1995). In addition, it has been also shown that the accumulation of ash after the burn leads to a rise in fertility in the short-term, which, in combination with the control of certain undesirable species, can benefit the rapid recuperation of the vegetation cover (Úbeda et al., 2005). In addition, the controlled burn benefits the growth of pasturelands and tender buds, increasing its livestock and hunting potential.

The use of controlled burning has both positive and negative aspects in terms of soil condition. Some of the negative effects include increased susceptibility to erosion, runoff, and consequent soil loss (Fonseca et al., 2017), not addressed in this study. However, considering the results of this research, the low costs and the possible effects of controlled burns, their use and application for the management of forest ecosystems can be of great interest.

CONCLUSIONS

The use of controlled burning for different purposes has implications on soil conditions in both the short and medium term. In this study, physical, chemical and biological properties of the 0 – 2 cm and 2 – 5 cm were directly influenced by the burn not only immediately after the fire but also in the mid-term. The burn increased the differences in soil properties and microbial community structure between the two assessed soil depths. All these changes condition soil quality and health and have implications on soil's functionality and the capacity to provide ecosystem services, not only immediately after burning but also in the medium term. For this reason, the effects in the soil described in these types of work related to high intensity controlled and prescribed burns should be considered when planning the use of fire to manage forest ecosystems. The postfire management and regeneration of the affected area will be a function of the positive and negative impacts of the burn. Further research in the long-term is needed to completely understand the effects of these burns in soil physical, chemical and biological properties and to include this information for a holistic management of fire and forest ecosystems.

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ANNEXES

Table AI Interactions between treatment [control (before burning), burned (immediately after burning) and 8 months after burning; *n* = 24] and soil depth (0 – 2 cm and 2 – 5 cm; *n* = 12) for the different soil physical-chemical properties.

	pH	**EC (µS cm -1)**	**CEC (meq100g-1)**	**DOC (µg g-1)**	**POlsen (mg kg-1)**
Control (0 – 2 cm)	6.34 ± 0.07 b	147 ± 12.0 b	5.72 ± 0.37 b	336 ± 39.7 b	6.18 ± 0.47 b
Burned (0 – 2 cm)	7.97 ± 0.12 a	852 ± 87.1 a	10.8 ± 0.66 a	1315 ± 108 a	38.2 ± 3.45 a
After 8 months (0 – 2 cm)	7.26 ± 0.05 c	204 ± 12.3 b	6.86 ± 0.17 bc	571 ± 67.6 bc	16.8 ± 1.82 c
Control (2 – 5 cm)	6.45 ± 0.08 d	88.5 ± 5.94 b	4.21 ± 0.17 cd	204 ± 30.2 c	4.26 ± 0.33 c
Burned (2 – 5 cm)	6.88 ± 0.12 c	174 ± 16.1 b	5.11 ± 0.29 cd	277 ± 27.7 c	7.77 ± 0.43 c
After 8 months (2 – 5 cm)	6.85 ± 0.07 d	166 ± 22.0 b	5.28 ± 0.18 d	232 ± 13.1 c	6.23 ± 0.48 c
ANOVA					
p (treatment)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
p (soil depth)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
p (interaction)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
	NH4+ (µg g-1)	**NO3- (µg g-1)**	**MnDTPA (mg kg-1)**	**ZnDTPA (mg kg-1)**	**CuDTPA (mg kg-1)**
Control (0 – 2 cm)	17.5 ± 1.63 b	37.6 ± 4.12 ab	76.8 ± 5.38 ab	3.33 ± 0.57 a	1.02 ± 0.05 b
Burned (0 – 2 cm)	41.4 ± 2.18 a	16.6 ± 0.85 a	128 ± 12.7 a	7.83 ± 0.44 a	1.26 ± 0.04 a
After 8 months (0 – 2 cm)	8.22 ± 1.78 c	23.6 ± 1.94 ab	110 ± 22.9 bc	6.35 ± 0.32 b	0.79 ± 0.05 c
Control (2 – 5 cm)	18.8 ± 1.78 de	29.7 ± 3.6 b	49.3 ± 3.92 c	1.23 ± 0.16 bc	0.81 ± 0.04 c
Burned (2 – 5 cm)	30.1 ± 4.35 cd	23.2 ± 2.43 b	54.9 ± 4.52 c	2.4 ± 0.22 bc	0.83 ± 0.05 c
After 8 months (2 – 5 cm)	4.29 ± 0.41 e	30.1 ± 4.96 b	39.9 ± 4.43 c	2.21 ± 0.3 c	0.52 ± 0.04 d
ANOVA					
p (treatment)	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*
p (soil depth)	*< 0.05*	*0.421*	*< 0.05*	*< 0.05*	*< 0.05*
p (interaction)	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*

EC: electrical conductivity in the relationship 1:5 (soil:water) extract; CEC: cation exchange capacity; DOC: dissolved organic carbon; P_{Olsen}: phosphorus soluble in sodium bicarbonate; Fe_{DTPA}: iron to DTPA.