Responses in soil carbon and nitrogen fractionation after prescribed burning in Pla de la Llacuna, Montseny

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Abstract

Prescribed fire is one of the most widely-used management tools to achieve precise and clearly defined objectives related to recover encroached rangelands. To my knowledge, there is no study that examines the legacy effect of the plant species on soil carbon (C) and nitrogen (N) particle size fractions after prescribed burning. Thus, this study aimed to assess the impact of prescribed burning on the C and N contents in the different fractions in topsoil (0-5cm and 5-10cm) in Pla de la Llacuna, Montseny, particularly to examine the legacy effect of the former extant plant species on soil carbon and nitrogen fraction distribution. Five vegetation patch types dominated by different plant functional types were identified. Multivariate analysis showed that patches dominated by different plant functional types show variability in the soil C and N contents, with legumes containing higher C and N proportions compared with other patch types, and most differentiated from biocrust soils. Soils under the legume *Cytisus scoparius* and the Atlantic shrub *Calluna vulgaris* were the ones showing a higher response to burning compared to the soil under the other species. According to univariate response analysis, C and N in total soil and in the sand fraction in the upper layer (0-5cm) decreased after prescribed burning, but increased in silt and clay fractions in the deeper layer (5-10 cm) likely due to downwards translocation and accumulation of C and N. The C/N ratio of total soil and sand fraction in the upper layer (0-5 cm), as well as silt fraction in both layers decreased after fire indicating soil C in the study site is labile. No significant interactions were found between plant species and burning in the total soil, neither in the three soil C and N fractions. But there were some tendencies for vegetation patch types to respond differently to burning, *C. scoparius* and *C. vulgaris* always being the most differentiated. This study will be helpful in terms of ecological, as well as management aspects, in terms of understanding ecological legacy effects and their possible consequences when planning prescribed burning.

Keywords: Prescribed burning; Soil particle size fractions; Plant species-fire interactions; *Cytisus scoparius*; *Calluna vulgaris*; biocrusts

1. Introduction

Fire is an important driver of environmental changes in an ecosystem, responsible for altering nutrient pools by changing the physical, chemical and biological properties of soils and nutrient cycling (González-Pérez et al. 2008; Certini 2005). Prescribed burning is considered as the deliberate application of fire under selected conditions to achieve precise and clearly defined management objectives (Fernandes et al. 2013). It is a widely-used practice to reduce the risk of wildfire (Reverchon et al. 2012). Combustion of litter and soil organic matter due to prescribed burning increase plant available nutrients that results in rapid growth of herbaceous plants and a significant increase in plant storage of nutrients (Rau et al. 2008). Prescribed burning is characterized by lower temperature, intensity and severity compared to wildfires and considered to affect only the upper centimetres of the soil (San Emeterio et al. 2014).

Soil includes different carbon (C) and nitrogen (N) pools where soil labile C and N pools are characterized by their small quantities and fast turnover rates (Hu et al. 1997). With longer turnover rates, recalcitrant organic C and N pools are physically or chemically protected (Poirier et al. 2000). The influence of prescribed fires on soil C and N depends on fire frequency and intensity. It may also affect microbial community composition and function that lead to altered C and N cycling in an ecosystem (Artz et al. 2009). Soils are considered as a major reservoir of C in terrestrial ecosystems (Nair et al. 2010). Soil organic carbon (SOC) is responsible for soil fertility and it is used as an indicator of soil health. Fire can influence N availability by its effect on N mineralisation. Low intensity fire can stimulate mineralisation of N, whereas high intensity fire often reduces N mineralisation (Serrasolsas and Khanna 1995; Guerrero et al. 2005). According to the findings of several studies, it is normal to find increased C and N content in low intensity and severity fires because of the incorporation of unburned or partially unburned slash fragments into the soil (Soto and Díaz-Fierros 1993; Úbeda et al. 2005; Roaldson et al. 2014), while other studies report no change (Boyer and Miller 1994; Switzer et al. 2012). The C/N ratios of soil after burning are typically lower than those in the original soils (Vega 1986).

Generally, around 50–75% of total Organic Carbon is associated with clay-sized particles (<2 μm), about 20–40% with silt sized particles (2-63 μm) and 10% with sand-sized particles (>63 μm) in temperate top soils (Christensen 2001). Soil particle size fractions are helpful in maintaining stability of ecosystems (Six et al. 2004). These fractions are better indicators of soil C sequestration potential and thus a tool for environmental monitoring (Scott Howlett et. al. 2011). SOM within the sand fraction is allocated to the active (labile) pool and in silt and clay fractions to the passive (recalcitrant) pool. The labile pool is the most sensitive pool because it is easily affected by fluctuation in environmental conditions. The labile pool is decomposed rapidly and get oxidized with any changes in land use practice easily (Haynes 2005). While the passive/non-labile pool is more stable and recalcitrant, and therefore this fraction is decomposed slowly by microbial activity (Wiesenberg et al. 2010).

Plants determine the quantity and the quality of residues, soil organic matter, as well as soil structure (Wardle et al. 2004). Thus, soil functions are also affected by plant functional diversity (Castro et al. 2010; Debouk et al. 2020). Plant functional types (PFTs) have proved to be a useful tool for predicting soil processes like C and N cycles (Wang et al. 2014). Phenology and vegetation structure may be determinant for the Net Ecosystem Exchange (NEE) as the aboveground living biomass directly takes-up (Nakano and Shinoda 2014) and releases CO_2 (Thakur and Eisenhauer 2015). Legume is one plant functional group that have potential to modify soil nutrient availability as legumes have the capacity to fix symbiotic nitrogen (Craine et al. 2001). Through increasing available soil N by higher N mineralisation and nitrification, legumes can affect community N budgets (Johnson et al. 2004). In addition, biological soil crusts (BSCs), which are assemblages of lichens, fungi, cyanobacteria, and mosses that colonize the soil surface, exert a great influence on ecosystem functioning by playing a key role in the N cycle, as N-fixing lichens and free-living, heterotrophic bacteria forming part of BSCs, are able to fix substantial amounts of atmospheric N (Evans and Ehleringer 1993).

Soil C and N deposits in heathlands may be vulnerable to fire (Davies et al. 2016). Many studies have focused on the dynamics of topsoil C and N stocks after prescribed burning. A few studies have focused on the effects of fire on soil C and N contents in clay, sand, and silt fractions (Granged et al. 2011; Girona-García et al. 2018). There are several studies conducted on the fire effect in seed germination, natural regeneration, changes in vegetative composition. To my knowledge, there is no study that examines the legacy effect of the plant species on soil carbon and nitrogen particle size fractions after prescribed burning. Ecological legacy is a concept focused mainly on community or ecosystem-level phenomena, on changes due to past events (Vogt et al. 1997). Therefore, the aim of this study was to assess the impact of prescribed burning on the C and N contents in the different fractions in topsoil (0-5cm and 5-10cm) in Pla de la Llacuna, Montseny, particularly to examine the legacy effect of the former extant plant species on soil carbon and nitrogen fractions after prescribed burning. I hypothesise that due to low to moderate prescribed fire, (1) the C and N contents in different soil fractions will be increased because of accumulation of burnt material; (2) patches dominated by different plant functional types will show variability in the soil C and N contents, with legumes containing higher C and N proportions compared with other species; and (3) burning effects on soil C and N distribution will be modified by plant species, that is, there will be ecological legacy effects of former extant species.

2. Materials and methods

2.1. Study area

The study was conducted at the Pla de la Llacuna (longitude 2º 18′ to 2º 22′ east, latitude 41º 44′ to 41º 47′ north), located in the Pla de la Calma, an elevated plateau that ranges between 1250 and 1300 m a. s. l. This occupies an irregular area of about 974 hectares in the Montseny Natural Park, in the Northern Catalan Pre-Littoral Range, north-eastern Iberian Peninsula (Fig. 1). This park comprises Mediterranean and Central Europe landscapes with different biomes having local influence of metropolitan conurbations nearby.

The plateau is characterized by humid Mediterranean climate; mean annual precipitation is approximately 700 mm where snow accounts for around 10% of the annual precipitation. The mean annual temperature is 10 ºC. Bedrock is a metamorphic schist where the major minerals are quartz albite, muscovite and chlorite. Soils are acidic, with a pH of 4.5 to 5.5, and characterised by a sandy-loam texture (Belillas and Roda 1991). The topographic location and humidity circulation lead a general situation that favors the presence of Atlantic vegetation, where the shrubs *Erica scorparia* L., *Erica arborea* L. and *Calluna vulgaris* L. are widespread. The area is covered principally by shrubs and grass (Bolòs et al. 1986), including biological crusts dominated by various species of the lichen *Cladonia*. For centuries, the hills of the Montseny mountains have been used as pastures. Montseny was declared as a Natural Park in 1977; since then many traditional practices have declined, and some, such as shepherds’ burning, have completely ceased (Bartolomé et al. 2005). Many small flocks composed mainly of sheep and goats grazed in Montseny in former times. There are now fewer but larger flocks, having a similar total number of cattle (Bartolomé et al. 1998).

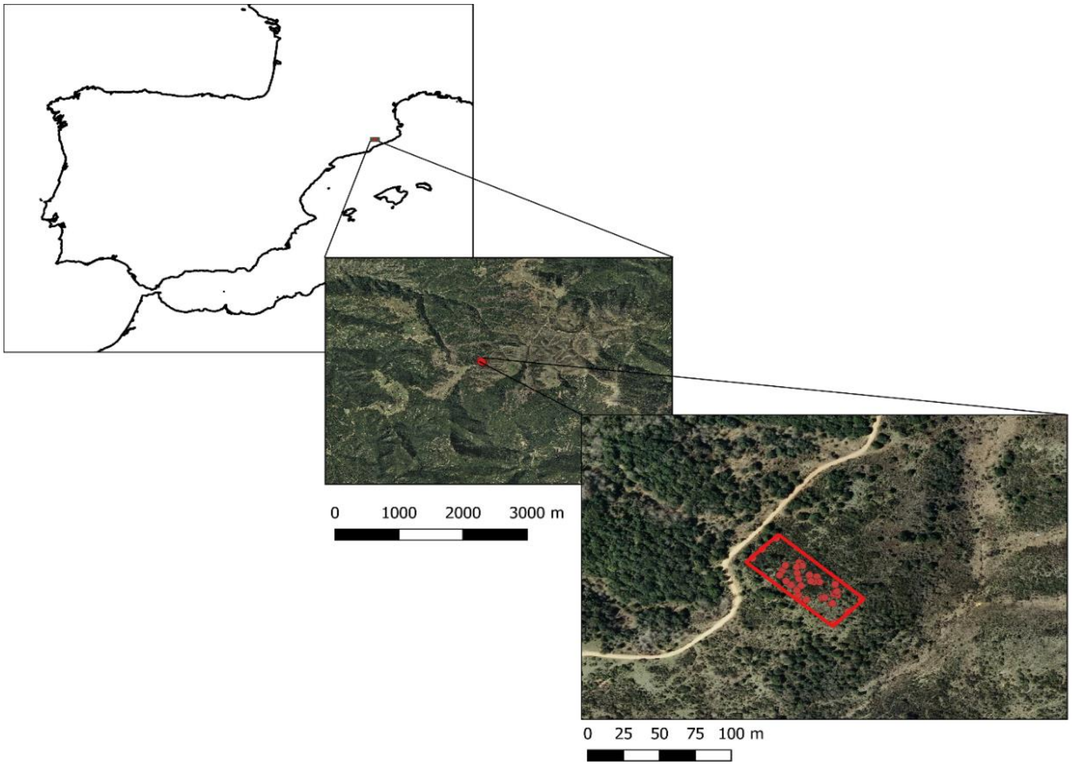

Fig. 1 Map of the Study Area (Courtesy of José Manjón-Cabeza Córdoba)

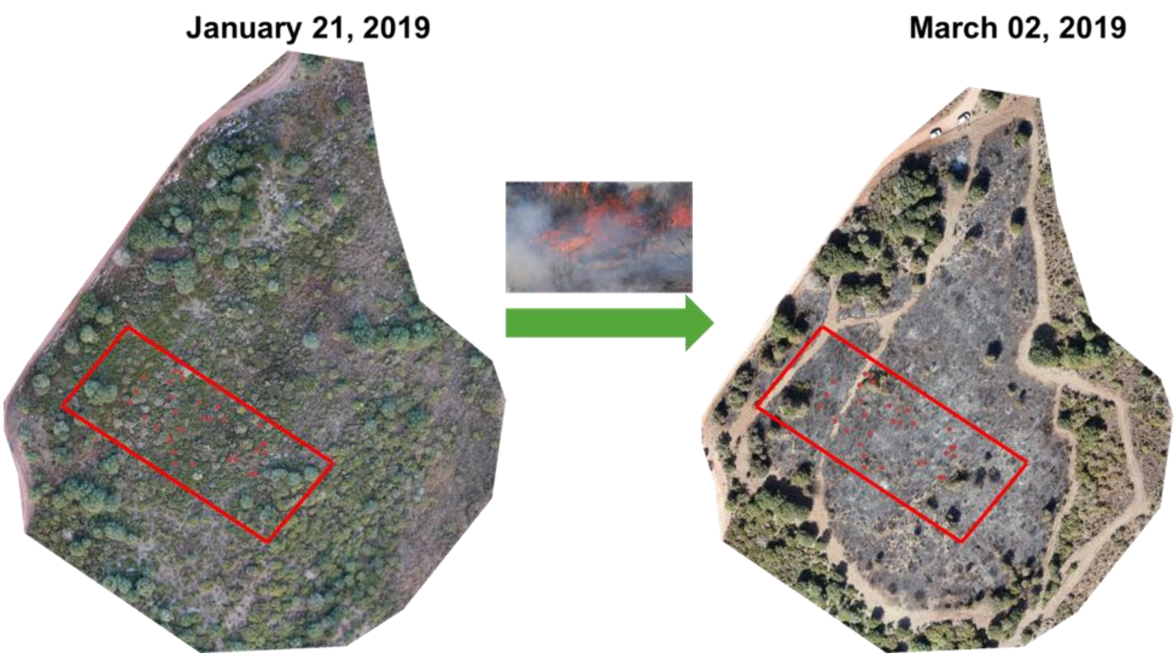

Fig. 2. The study area before and after the prescribed burning (Courtesy of Josefina Plaixats)

2.2 Field sampling and laboratory determinations

The area selected for the prescribed burning experiment had a surface of 3200 m^2 (Figs. 1 and 2). A smaller area of 30 x 90 m within this surface was selected for the soil sampling (Fig. 2). Thirty georeferenced sampling points were distributed throughout this smaller area using a stratified directed sampling. Slope was used as stratifying variable, with three areas separated according to the different general slope; and soils sampled within each slope area according to the required criteria: different vegetation dominated patches. Two samplings were performed, one before the burning event (pre-burning) and a re-sampling in the same 30 sampling points afterwards (post-burning). The pre-burning sampling was carried out in January 30th, 2019, and the post-burning sampling was carried out in March 5th, 2019. The burning event was conducted in February 13th, 2019. The fire intensity was low to moderate in our study area. There was rain after burning in February 21 (0.1 mm). Fig. 2 shows the study area before and after the prescribed burning.

A plot of 30 x 90 m was selected and three additional subareas with different slope were determined as stratification levels (strata) of the variable slope: the top area with gentle slope; the intermediate area with medium slope; and the bottom area with the steepest slope. Slope was obtained using the 5m DEM (Digital Elevation Model) from the IGN (www.ign.es), and a general slope value provided for each of the three sampled subareas.

Five vegetation patch types were initially identified as the most abundant vegetation patches in the shrubland. Each type was dominated by different species: the vascular plants *Calluna vulgaris*, *Erica arborea*, *Cytisus scoparius* and *Pteridium aquilinum* L.; and the biocrust dominated by lichens mostly of the genus *Cladonia*, and including some grasses. They belong to different plant functional types, where the shrubs *C. vulgaris* and *E. arborea* are Ericaceae and *C. scoparius* is a legume; *Pteridium* is a fern; and the *Cladonia* patch is a lichen-dominated biocrust. Henceforth, I will use the terminology: Calluna (CV) for *Calluna vulgaris* patches; Erica (EA) for *Erica arborea* patches; Cytisus (CS) for *Cytisus scoparius* patches; Pteridium (PA) for *Pteridium aquilinum*; and Cladonia (CSP) for the biocrusts with *Cladonia*.

One replicate of each vegetation patch type (5 types) was selected at each plot (2 plots) and slope stratum (3 strata), yielding a total of 30 replicates. Each replicate was georeferenced during the pre-burning sampling with a highly precise GNSS Leica Zeno 20 (Leica Geosystems AG, Heerbrugg, Switzerland) with a differential correction Real Time Kinematic (RTK) broadcast system that was connected to the RTKAT service provided by the Institut Cartogràfic i Geològic de Catalunya (ICGC). And also re-sampled after burning, in the post-burning sampling, using the same GNSS instrument.

Soil samples were extracted in two different layers corresponding to two different soil depths (0-5 cm and 5-10 cm) in each georeferenced sampling point using a 4 x 4 cm^2 coring probe; afterwards soils were transported to the laboratory and oven dried at 60ºC until constant weight. Soil samples were physically fractionated using the method developed by Six et al. (2002). The three soil fraction samples of known moisture content were analysed by a LECO C.N.H.S. Elemental Analyzer for the percentage of C and N. Total percentage of soil C and N was also measured in the two different soil depth layers. This results in eight variables including: C in total, C in clay, C in silt, C in sand in the upper layer (0-5 cm) and C in total, C in clay, C in silt, C in sand in the deeper layer (5-10 cm), as well as N in total, N in clay, N in silt, N in sand in the upper layer (0-5 cm) and N in total, N in clay, N in silt, N in sand in the deeper layer (5-10 cm). The C/N ratio was also calculated for each of the fractions and layers described above.

2.3. Statistical Analysis

Multivariate indirect ordination analysis was applied to the totality of the soil variables analysed in this study. In particular, I applied Principal Component Analysis (PCA). In addition, I tested multivariate direct ordination methods including Redundancy Analysis (RDA). Nonetheless, PCA provided a clearer picture of the patterns, probably due to the high topographical variability in the study, and I describe those PCA results. Results from RDA are shown in Annex. Multivariate analysis was conducted with CANOCO 5.1 (Braak and Šmilauer 2018).

In addition to multivariate mathematical analysis, I performed multivariate statistical regression on each of the study variables of interest, or response variables. In particular, linear mixed effect models were performed with the identity of the sampling point as random factor, and with burning, slope, and vegetation patch type as fixed factors. Then the best model was selected according to AIC (Akaike Information Criterion). I included slope as a quantitative covariate (block) factor. All statistical analyses were conducted in the R version 4.0.2 (R Core Team 2020).

3. Results

In the study area, silt was the fraction including the highest soil C and N contents in both layers (Table 1, Table 2).

3.1 Overall soil C and N distribution

Considering together all the C and N variables, including C and N content in the total soil and in the three soil fractions, the Principal Component Analysis (PCA) explained 31% of the total variability. PCA axis 1 explained 60 % of the explained variability and mainly separated samples according to the plant species under which the soil was originally extracted (Fig. 3A). Soils under *Cytisus* were distributed along the most negative part on PCA axis 1, followed by those under *Pteridium* (Fig. 3A); while soils under *Cladonia* distributed mostly on the positive side (Fig. 3A). Soils under the two Ericaceae had low responses to this axis, with *Erica* being particularly unresponsive, placed close to 0 on the PCA Axis 1 (Fig. 3A). On the other hand, Axis 2 added 20% to the explained variability. This axis mostly explained the variability due to burning (Fig. 3A). Soils under *Cytisus* and *Calluna* were the ones showing a higher response to burning compared to the soil under the other species (Fig. 3A). In particular, soils under *Calluna* strongly varied in response to burning, in terms of overall soil fractional C and N properties (Fig. 3A). Finally, PCA axis 3 explained 8 % of the total explained variability. Axes PCA2 and PCA3 suggest differences among vegetation patches in overall soil C and N responses to burning, soils under *Calluna*, *Cytisus*, and *Cladonia* being the ones most differentiated between unburned and burned conditions (Fig. 3A; Fig. 3B). The high variability shown in the overall responses (high values of SE around the mean for the three axes shown in Fig. 3) seems to be related to the high topographical heterogeneity in the sampled terrain, as suggested by the results of the direct RDA (Annexes; Fig. 7), where slope, in addition to species identity and burning treatment, show a high correlation to the three axes (Annexes; Fig. 7).

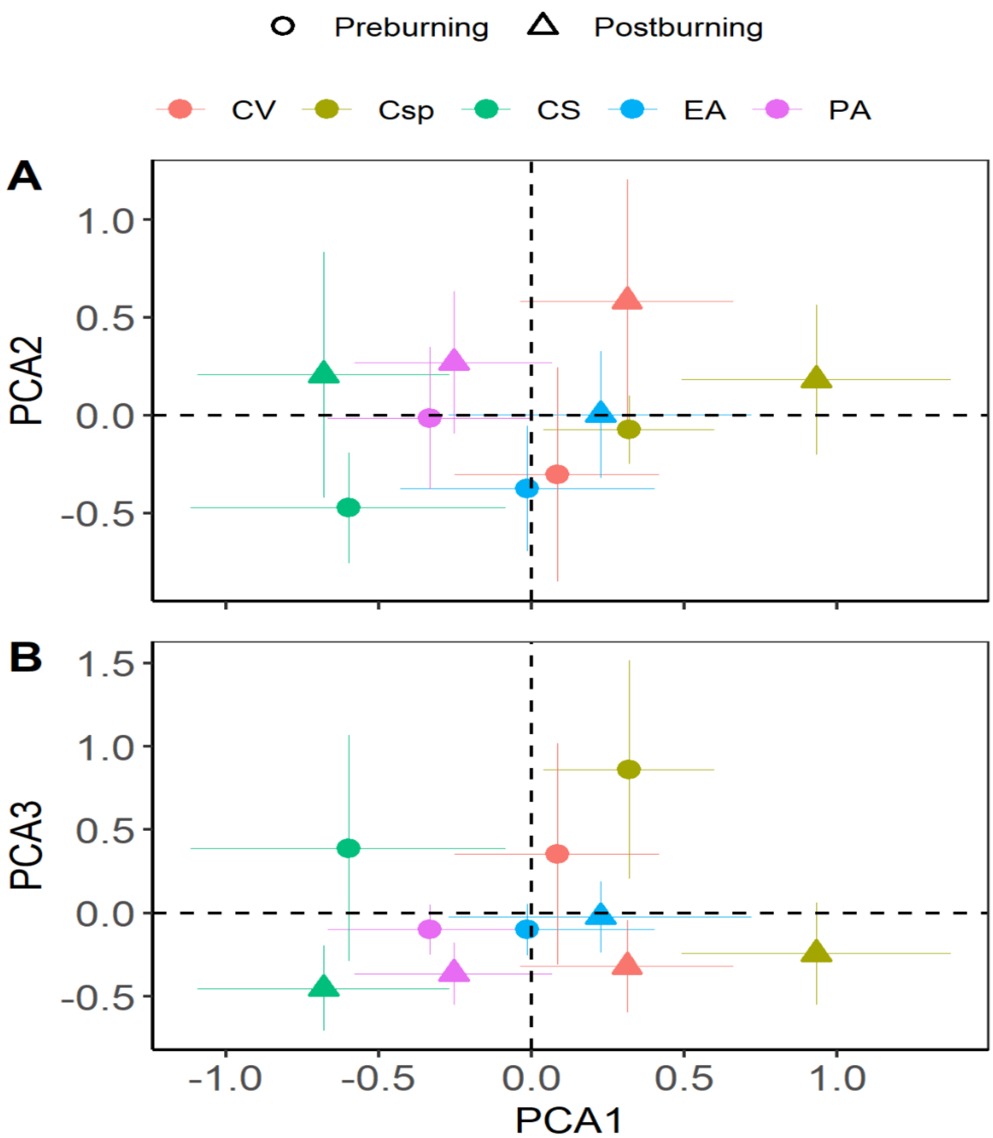

Fig. 3. Sample distribution along the three first axes (PCA1 and PCA2 (A), above; PCA1 and PCA3 (B), below) of Principal Component Analysis (PCA) performed on the overall soil C and N variables (total and fractional). Samples are clumped into groups according to vegetation patch type and prescribed burning treatment. The mean value ± 1 SE, standard error, per each species and treatment is represented by different symbols and whiskers. The five vegetation types are: *Calluna vulgaris* (CV); *Cladonia* (CSP); *Cytisus scoparius* (CS); *Erica arborea* (EA); *Pteridium aquilinum* (PA). Circles (o), pre-burning; triangles (Δ), post-burning.

3.2 Total and fractional soil C

Both total (Fig. 4A) and sand fraction C (Fig. 4D) decreased after burning in the upper layer (0-5 cm) (Table 1); conversely C in clay fraction showed an increasing trend (Table 1, Fig. 4F), and C in silt fraction increased with burning in the deeper layer (5-10 cm; Table 1, Fig. 4G). Soil C generally increased with slope in almost all soil fractions in both soil layers (Table 1; Annexes; Fig. 8, Fig. 9).

Cytisus patches showed significantly higher C in sand in the upper than in the deeper layer (Table 1, Fig. 4D), but no significant interactions were found between plant species and burning in the total carbon, neither in the three soil C fractions (Table 1). However, we could detect tendencies in the particular response of some plant species to the burning (Fig. 4). Thus, after burning, total soil C in the upper layer (0-5 cm) showed a decreasing trend in all species, which was more pronounced in *Cladonia* and *Erica* patches compared to the other patch types (Table 1, Fig. 4A). C in sand also decreased in all the species, but especially in the *Calluna* and *Cytisus* patches in the upper soil layer (0-5 cm) (Table 1, Fig. 4D). In contrast, C in the silt fraction increased after burning more remarkably in *Calluna* and *Cytisus* than in the other patch types in the deeper layer (5-10 cm; Table 1, Fig. 4G).

3.3 Total and fractional soil N

N in the total soil (Fig. 5A) and in the sand fraction (Fig. 5D) at the upper layer (0-5 cm) decreased after fire (Table 2). Conversely, soil N significantly increased with prescribed burning in the clay (Fig. 5F) and silt fractions (Fig. 5G) in the deeper soil layer (5-10 cm) (Table 2). Similarly to C, N also mainly increased with slope in total and in fractions (Table 2; Annexes, Fig. 10, Fig. 11).

Significantly higher N content was found in sand fraction under *Cytisus* than under the other vegetation patches in the 0-5 cm soil depth layer before and after burning (Table 2, Fig. 5D). There were no significant interactions between plant species and burning in the total soil, neither in the three soil N fractions (Table 2). But there were some tendencies for vegetation patch types to respond differently to burning (Fig. 5). Total soil N decreased in almost all vegetation patch types, more remarkably in *Cladonia* in the upper layer (0-5 cm) after burning (Table 2, Fig. 5A). Also, N in sand decreased in all species, but mainly in *Calluna*, followed by *Cytisus* and *Erica* in the upper soil layer (0-5 cm depth) after burning (Table 2, Fig. 5D). On the other hand, N in the clay fraction increased especially in *Cytisus*, but also slightly increased in *Calluna* and *Cladonia* after burning in the deeper layer (5-10 cm, Table 2, Fig. 5F). This increasing trend was also found in the silt fraction, in which N increased more markedly in *Calluna* and *Cytisus* in the deeper layer (5-10 cm depth) compared to the other vegetation patch types (Table 2, Fig. 4G).

3.4 Total and fractional soil C/N ratio

Prescribed fire significantly affected the C/N ratio of total soil (Fig. 6A) and sand fraction (Fig. 6D) in the upper layer (0-5 cm), as well as silt fraction (Fig. 6C; Fig. 6G) in both layers (0-5 cm and 5-10 cm) (Table 3). A significant burning x species interaction on C/N was observed in total soil in the deeper soil layer (5-10 cm), with *Cladonia* decreasing markedly its C/N ratio after fire compared to the other patches (Table 3, Fig. 6E).

Table 1:

Mean ± 1 standard error (SE) of all C variables in the study, by soil depth layer. P-values from the mixed model regressions on C variables, for the following tested explanatory variables: slope, burning and species.

			Explanatory variables (P-value)		
Carbon variables	**Soil depth (cm)**	**Mean ± 1 SE**	**Slope**	**Burning**	**Species**
Carbon in total	0-5	10.7 ± 0.4	**0.001**	**0.001**	0.158
	10	4.3 ± 0.2	**0.030**	0.548	0.394
Carbon in clay	0-5	8.8 ± 0.2	**0.003**	0.609	0.201
	10	6.5 ± 0.2	**0.008**	*0.081*	0.372
Carbon in silt	0-5	11.4 ± 0.3	**0.006**	0.521	0.142
	10	6.7 ± 0.2	0.092	**0.005**	0.502
Carbon in sand	0-5	8.8 ± 0.4	**0.001**	**0.016**	**0.038**
	10	4.1 ± 0.2	**< 0.001**	0.696	0.281

P-values in bold indicate < 0.05; P-values < 0.1 are in italics

Table 2:

Mean ± 1 standard error (SE) of all N variables in the study, by soil depth layer. P-values from the mixed model regressions on N variables, for the following tested explanatory variables: slope, burning and species.

			Explanatory variables (P-value)		
Nitrogen variables	**Soil depth (cm)**	**Mean ± 1 SE**	**Slope**	**Burning**	**Species**
Nitrogen in total	0-5	0.6 ± 0.0	**0.003**	**0.009**	0.193
	10	0.3 ± 0.0	**0.015**	0.445	0.308
Nitrogen in clay	0-5	0.7 ± 0.0	**0.019**	0.280	0.275
	10	0.5 ± 0.0	**0.028**	**0.031**	0.230
Nitrogen in silt	0-5	0.8 ± 0.0	**0.013**	0.481	0.203
	10	0.5 ± 0.0	**0.043**	**0.000**	0.409
Nitrogen in sand	0-5	0.5 ± 0.0	**0.002**	**0.025**	**0.028**
	10	0.3 ± 0.0	< **0.001**	0.911	0.193

P-values in bold indicate < 0.05

Table 3:

Mean ± 1 standard error (SE) of all C/N variables in the study, by soil depth layer. P-values from the mixed model regressions on C/N variables, for the following tested explanatory variables: slope, burning, species and burning x species.

			Explanatory variables (P-value)			
C/N ratio	**Soil depth (cm)**	**Mean ± SE**	**Slope**	**Burning**	**Species**	**Burning × Species**
Total	0-5	16.8 ± 0.2	0.212	**< 0.001**	0.872	
	10	14.4 ± 0.2	0.893	0.370	0.927	**0.015**
Clay	0-5	12.5 ± 0.1	0.239	*0.073*	0.841	
	10	12.2 ± 0.1	**0.019**	0.163	0.953	
Silt	0-5	14.8 ± 0.1	*0.098*	**< 0.001**	0.590	
	10	13.6 ± 0.1	0.549	**< 0.001**	0.820	
Sand	0-5	16.4 ± 0.2	**0.031**	**0.016**	0.272	
	10	14.2 ± 0.1	0.581	0.253	0.601	

P-values in bold indicate < 0.05; P-values < 0.1 are in italics

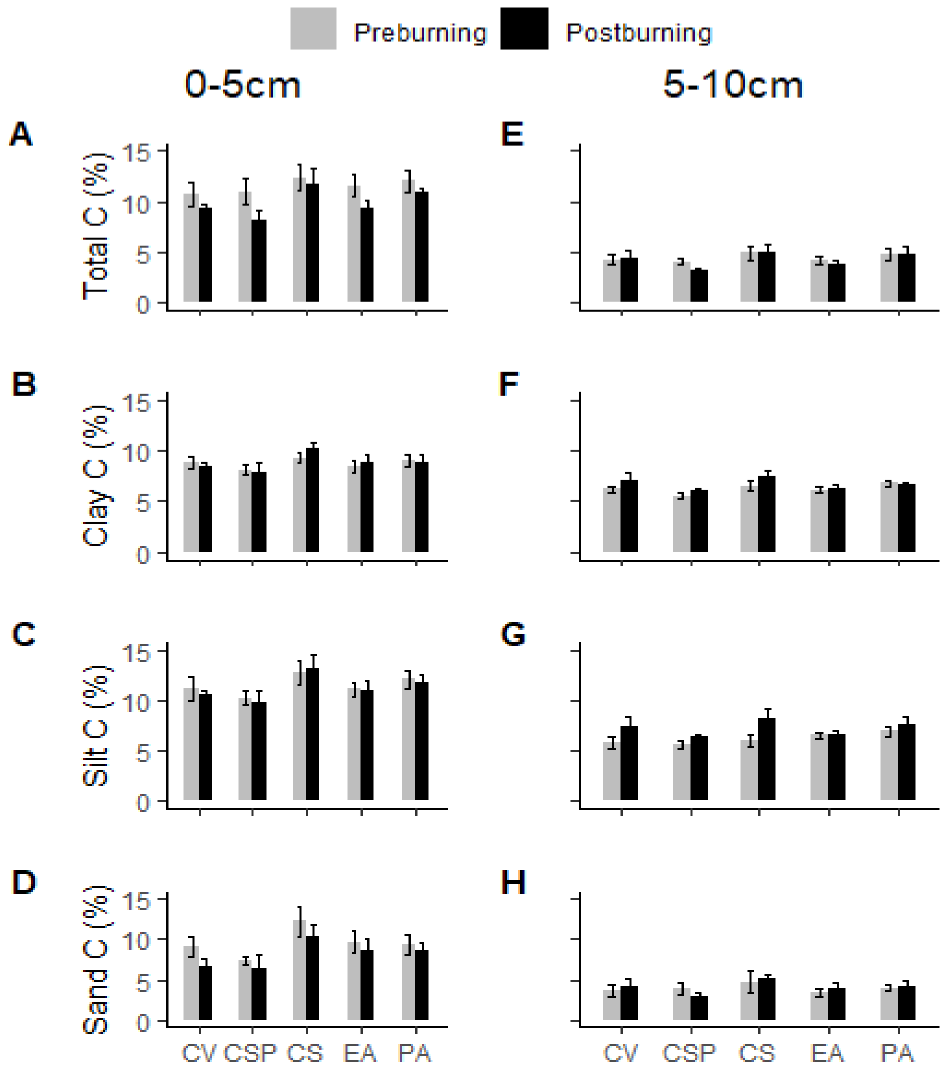

Fig. 4. Mean ± 1 SE total and fractional (top to bottom) soil C distribution in the upper (0-5 cm; left) and deeper (5-10 cm; right) soil layers per vegetation patch type (*Calluna vulgaris* (CV); *Cladonia* (CSP); *Cytisus scoparius* (CS); *Erica arborea* (EA); *Pteridium aquilinum* (PA)) and burning treatment.

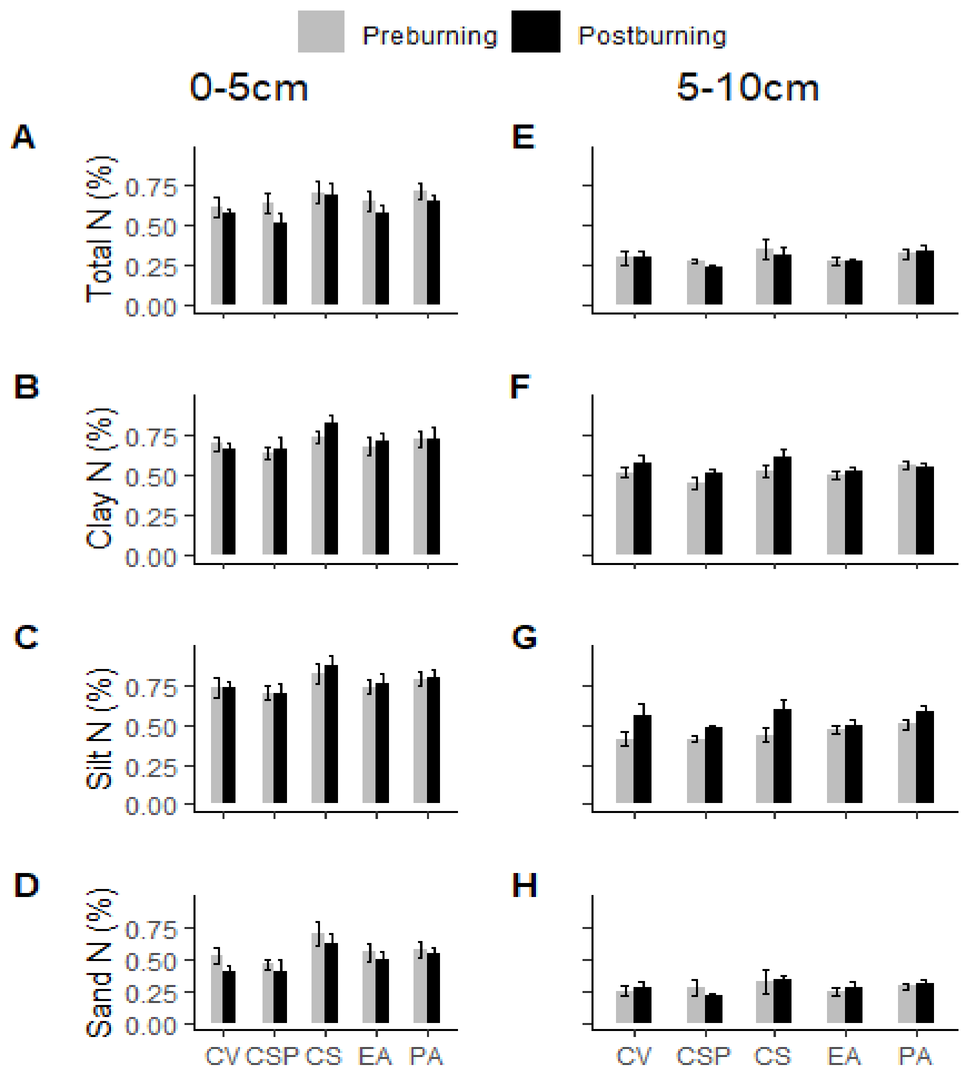

Fig. 5. Mean ± 1 SE total and fractional (top to bottom) soil N distribution in the upper (0-5 cm; left) and deeper (5-10 cm; right) soil layers per vegetation patch type (*Calluna vulgaris* (CV); *Cladonia* (CSP); *Cytisus scoparius* (CS); *Erica arborea* (EA); *Pteridium aquilinum* (PA)) and burning treatment.

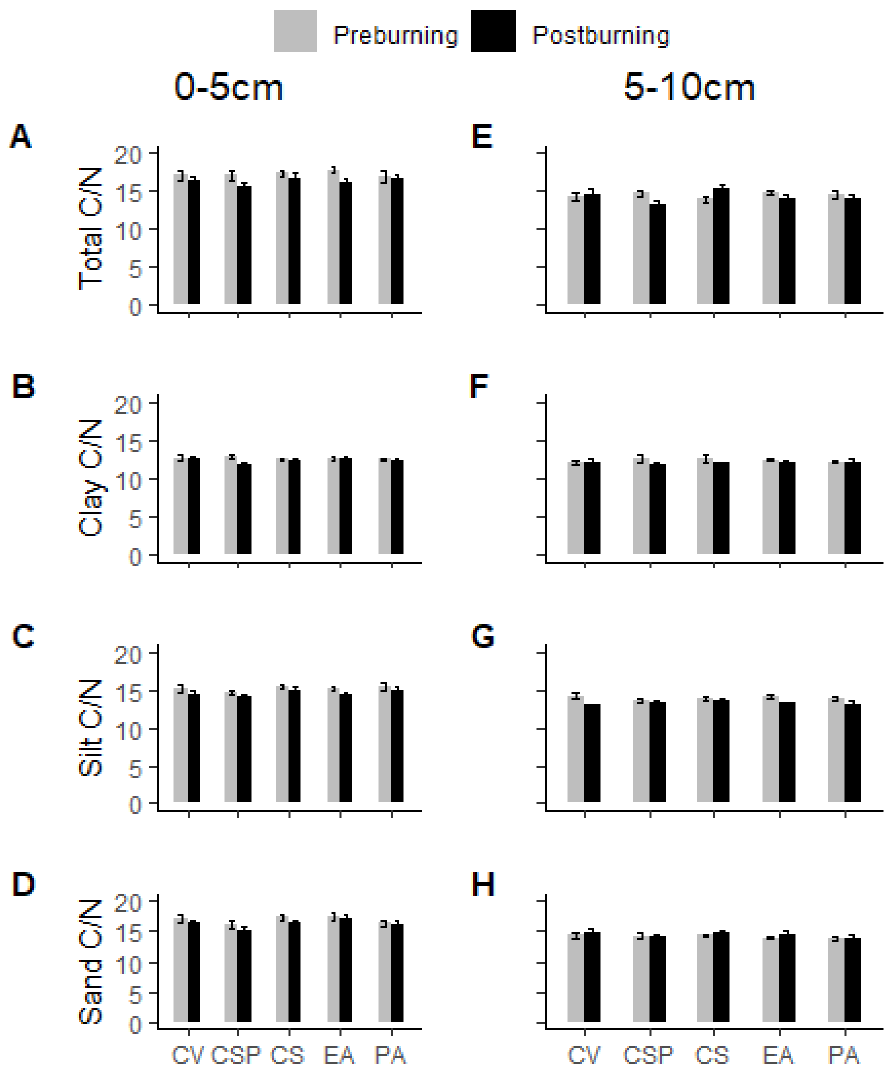

Fig. 6. Mean ± 1 SE total and fractional (top to bottom) soil C/N ratio distribution in the upper (0-5 cm; left) and deeper (5-10 cm; right) soil layers per vegetation patch type (*Calluna vulgaris* (CV); *Cladonia* (CSP); *Cytisus scoparius* (CS); *Erica arborea* (EA); *Pteridium aquilinum* (PA)) and burning treatment.

4. DISCUSSION

The results show significant decrease in soil C and N content of coarser fractions from upper layer (0-5 cm), but increase in finer fractions in deeper layer (5-10 cm). Patches dominated by different plant functional types show variability in the soil C and N contents, with legumes containing higher C and N proportions compared with other species. The results show an important effect of burning and species composition on total and fractional soil C and N dynamics (Table 1, Table 2; Figs. 3

to 5). The species legacy effects in the soil C and N responses to burning were weak and best revealed when analysing jointly all the C and N variables in the study by multivariate analysis (Fig. 3). The topographical variability of the study area (Fig. 2), reflected by the significance of slope in most regression models, probably masked the interacting effects between plant species and burning (Table 1 and 2). Indeed, the representation of most soil response variables suggested some tendencies in legacy effects (Figs. 3 to 5), but few significant interactions, except for C/N (Tables 1 and 2). The results suggest, thus, that topographical variability in addition to the species and burning is prone to mask biodiversity legacy effects (species x burning) on soil C and N dynamics after fire. The consideration of more than one response variable (C and N join variation, C/N; or the ensemble of study variables in multivariate spaces) seems to rend the legacy trends strong enough to overcome statistical shortcomings of univariate response analysis.

4.1 Total and fractional soil C and N distribution before and after burning

C and N in the total soil (Fig. 4A; Fig. 5A) and in the sand fraction (Fig. 4D; Fig. 5D) at the upper layer (0-5 cm) decreased after fire (Table 1, Table 2). The soil C and N can be substantially decreased when temperature increases to a range of 200–250 °C (Certini 2005), which may explain the remarkable decrease of soil C and N observed at 0–5 cm depth in Pla de la Llacuna. The decrease in total C in low severity prescribed burning might be attributed to C loss as CO_2 into the atmosphere, while the decrease of total N might be attributed to N loss as volatilization of N (Weast 1988), as well as direct convective transfer of ash (Smith 1970). The reduction of soil C and N during the fire affected mainly the sand fraction, maybe because combustion can be more intense in this size range due to the oxygen present in macropores (Jordán et al. 2011).

In contrast, soil C and N significantly increased with prescribed burning in the clay (Fig. 4F; Fig. 5F) and silt fractions (Fig. 4G, Fig. 5G) in the deeper soil layer (5-10 cm) (Table 1, Table 2). This suggests the redistribution of C and N in soil fractions after prescribed burning by promoting C and N enrichment in finer fractions in the study area. The increase of C and N in the clay and silt fractions may be due to the downwards translocation and accumulation of C and N at deeper layers compared to the upper layer (Leal et al. 2019), including leaching of C and N from the sand fraction from the upper layer to fine fractions to the deeper layer (Fowells and Stephenson 1934). There were no significant changes in other variables. Martí-Roura et al. 2014 suggest this could indicate the compensation of losses via volatilization by the incorporation of new C and N into ashes and partially charred plant residues. It could also suggest reduced mobility of C and N in these other fractions in front of burning disturbances.

So, the results didn't support the first hypothesis, because not every C and N fractional variable increased with burning.

4.2 Plant species and species legacy effects on total and fractional soil C and N distribution after burning

PCA Axis 1 differentiated vegetation patches according to initial soil C and N conditions, while PCA Axis 2, and particularly, Axis 3 showed that the differences on overall soil C and N properties before burning were bigger than post-burning, and that patches separated differently on those axes according to burning (Fig. 3B). This suggests that species had very strong effects on soils before burning, but left legacy effects on soil fractional C and N after burning in dissimilar intensity. However, when analysing the soil C and N total and fractional response variables separately in the regression models, there were no significant interactions between plant species and burning in the total soil, neither in the three soil C and N fractions (Table 1, Table 2). But there were some tendencies for vegetation patch types to respond differently to burning (Fig. 4; Fig. 5).

The legume shrub *Cytisus* showed the highest differentiation when considering overall composition of soil C and N parameters compared to other species, both before and after burning (PCA axis 1; Fig. 3A), In addition, *Cytisus* patches showed significantly higher C and N in sand in the upper than in the deeper layer before and after burning (Table 1, Table 2; Fig. 4D, Fig. 5D). This could be related, among other facts, to the root system of *Cytisus*, characterised by root splitters (Chmelíková and Hejcman 2012). The sand-size fraction is basically consisted of the particulate organic matter, which is coherent with the fact that this fraction is the entrance pool of organic material in soil (Diekow et al. 2005). Thus, it is in the agreement with my second hypothesis.

Very importantly, legumes are capable to fix atmospheric nitrogen (Reich et al. 2003). Thus, to avoid nutrient limitation, those species can access atmospheric nitrogen and produce high-nitrogen content biomass (Craine et al. 2002). Due to their effectiveness in transferring aminoacids between nodules and roots, legumes favour organic N sources compared to other plant functional types (Moran-Zuloaga et al. 2015). Legumes possess higher leaf nitrogen content (Reich et al. 1998) and higher specific leaf area compared with other plant functional types (PFT), traits related with increased photosynthetic rates (Reich et al. 1998). This will enhance net CO_2 uptake compared to other PFT, and increase the photosynthetic rates of the whole community (Ibañez et al. 2020), resulting in enhanced soil organic matter accumulation. Soils under legumes have higher litter quality and litter decomposition rates, larger effects on N availability, and N supply rates than non-legumes due to symbiotic relationships (Tilman and Wedin 1991; Fornara and Tilman 2008). Zhao et al. (2014) found that there were significant correlations between legumes and soil properties, and the presence of legumes increased soil C and N content. Also according to Debouk et al. (2020) soil under legume possess more nitrogen than other plant functional type combinations. Furthermore, complex interactions between plant herbage quality and soil organic C dynamics have been reported (Rodriguez et al. 2000).

Cladonia patches also showed important differences in soil C and N distribution compared to the other patch types (PCA axis 1; Fig. 3A). A study conducted in Siberian forests by Webb et al. (2017) found that soil C storage was lower in lichen, likely due to lower rates of C fixation (Turetsky et al. 2010), or higher rates of decomposition of vascular plant litter lichen patches (Wardle et al. 2003). Castillo-Monroy et al. (2010) found that NO_3 was lower in the site dominated by lichens than those composed by other plant functional types.

Soils under *Cytisus* and *Calluna* were the ones showing a higher response to burning compared to the soil under the other species (PCA axis 2; Fig. 3A). The important response of *Cytisus* after burning is not surprising. It is well known that, after burning, legumes modify soil C and N (Hendricks and Boring 1999; Newland and DeLuca 2000). The presence of legumes influences the amount of extractable inorganic N after burning. N-rich plant litter and burned residues of legume can stimulate N mineralization and nitrification after low intensity fires (Madritch and Cardinale 2007). Legumes also respond positively to fire in other ecosystems, including the tallgrass prairie and pine forests of the southeastern US (Hendricks and Boring 1999; Newland and DeLuca 2000). On the other hand, a large proportion of the nutrients can be mobilized as ash and smoke from the soil under *Calluna* after burning (Evans and Allen 1971).

After burning, total soil C decreased in all the species, but this decrease was more pronounced in *Cladonia* and *Erica* patches (Table 1, Fig. 4A). Total soil N also decreased in almost all vegetation patch types, more remarkably in *Cladonia* patches in the upper layer (0-5 cm depth) compared to the other patch types (Table 2, Fig. 5A). Sedia and Ehrenfeld (2005) found that the accumulation of organic matter and the mineral N, predominantly in the form of ammonium, were lower on the soil beneath lichens compared with grasses and mosses after fire.

C in sand also showed a decreasing trend in all species after burning, but especially in the *Calluna* and *Cytisus* patches (Table 1, Fig. 4D); as well as decreased N in all patches, but mainly in *Calluna*, followed by *Cytisus* and *Erica* in the upper soil layer (0-5 cm depth) after burning (Table 2, Fig. 5D). A large proportion of the nutrients can be mobilized as ash and smoke from the soil under *Calluna* after burning (Evans and Allen 1971).

On the other hand, N in the clay fraction increased especially in *Cytisus*, but also slightly increased in *Calluna* and *Cladonia* after burning in the deeper layer (5-10 cm; Table. 2, Fig. 5F). This increasing trend was also found in the silt fraction, in which C and N increased after burning more remarkably in *Calluna* and *Cytisus* in the deeper layer (5-10 cm) (Table 1, Table 2; Fig. 4G, Fig. 5G). Nutrients under *Calluna* not exported in smoke might be translocated downward on the soil as ash (Evans and Allen 1971; Leal et al. 2019). And, this might be the result of an increase in belowground deposition under *Cytisus* (Birouste et al. 2012). This trend is also likely due to the incorporation of ashes under *Cladonia* in the deeper layer (Soto and Díaz-Fierros 1993). Therefore, it partially agrees with my 3rd hypothesis.

Slope significantly modified almost all soil carbon and nitrogen fractions in both soil layers (Table 1, Table 2). The accumulation of particulates transport downslope can lead to increased soil C and N in depositional areas (Nitzsche et al. 2017), responsible for higher rates of heterotrophic respiration and denitrification (Groffman and Tiedje 1989). In our case, higher C and N contents were found in steeper slopes by the bottom of the hill side, suggesting a more pronounced effect of microtopography than of slope itself. Topography is known to alter soil properties that control soil biogeochemical processes (Sebastià 2004).

Therefore, according to our second hypothesis, different species dominated patches had different soil C and N total and fractional contents, and legumes contained higher C and N proportions than species. In addition, there were some tendencies for some species to respond more strongly to burning than other species, trends that were revealed more clearly when considering multiple response variables ensemble.

4.3 Total and fractional soil C/N ratio

Prescribed fire significantly affected the C/N ratio of total soil (Fig. 5A) and sand fraction (Fig. 5D) in the upper layer (0-5), as well as in the silt fraction (Fig. 5C; Fig. 5G) in both layers (0-5 cm and 5-10 cm) (Table 3). A significant burning x species interaction on C/N was observed in total soil in the deeper soil layer (5-10 cm), with *Cladonia* decreasing markedly its C/N ratio after fire compared to the other patches (Table 3, Fig. 5 E). The C/N ratio of slope in the clay fraction in the deeper layer and in the sand fraction in the upper layer was also significant (Table 3). This decreasing trend of the C/N ratio indicating high N input over C could be an indicator of a high content of microbial debris (González-Pérez et al. 2004) which is related with microbial decomposition. Decomposition leads to net mineralization of nitrogen (FOG 1988). Increased N inputs leads to the primary production and soil C storage (Nave et al. 2009). Though soil C and N significantly increased with prescribed burning in the silt fractions in the deeper soil layer (5-10 cm), the C/N ratio decreased. This can be causing a change in the recalcitrancy of the C in the soil.

5. Conclusion

C and N in total soil and in the sand fraction in the upper layer (0-5cm) decreased after prescribed burning, but increased in silt and clay fractions in the deeper layer (5-10 cm), which is likely due to downwards translocation and accumulation of C and N from coarse fractions in superficial soil to fine fractions in deeper soil layers. Hence, it is a recommendation for further study to assess the translocation process of C and N in the different fractions in this ecosystem. The C/N ratio of total soil and sand fraction in the upper layer (0-5 cm), as well as silt fraction in both layers decreased after fire indicating soil C in the study site is labile. There were differences both in the way different species distributed C and N among fractions and total C and N, and in how different patches responded to burning. Differences in the response to burning were stronger when considering more than one soil variable together, including multivariate ordination analysis and the C/N ratio, and remaining elusive in the univariate response analysis. The legume shrub *Cytisus* showed the highest differential overall composition of soil C and N parameters compared to other species as legume possess more C and N than other species. So, legume can help in maintaining soil fertility in the site (Barthes et al. 2004), as well as provide support in the harsh conditions and compensate nutrient loss (Chaer et al. 2011). Soils under *Cytisus* and *Calluna* were the most responsive to burning compared to other species. Therefore, the effect of fire is dependent on the species composition of the ecosystem, and it is important in ecological as well as in management aspects.

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Annexes

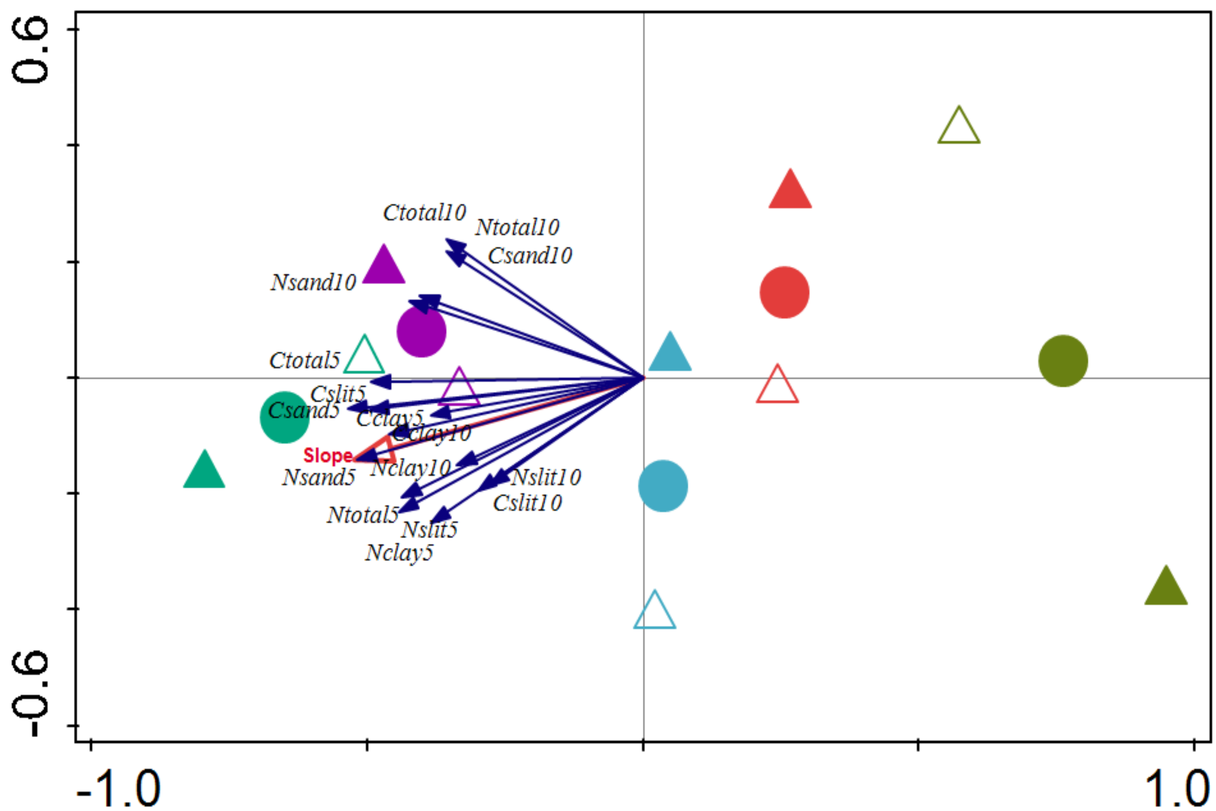

Fig. 7 Representation of the first two axes from Redundancy Analysis (RDA) on soil total and fractional C and N variables as descriptor variables (labels and arrows in blue). Explanatory variables are slope, burning, species, and species x burning. Symbols represent: circles, species; solid triangles, post-burnig treatment per species; open triangles, pre-burning treatment per species. Colors represent: Red, *Calluna vulgaris*; light green, *Cytisus scoparius*; deep green, *Cladonia* biocrust; blue, *Erica arborea*; and purple, *Pteridium aquilinum*.

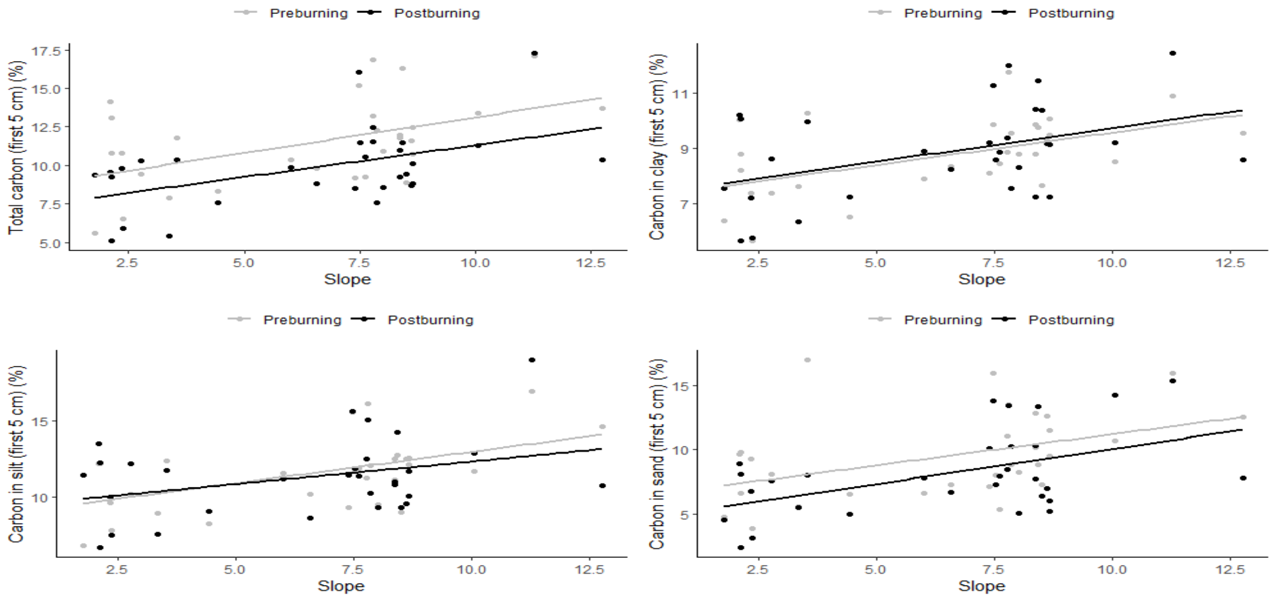

Fig. 8 Relationship between slope and C in total and fractions in the upper layer (0-5 cm)

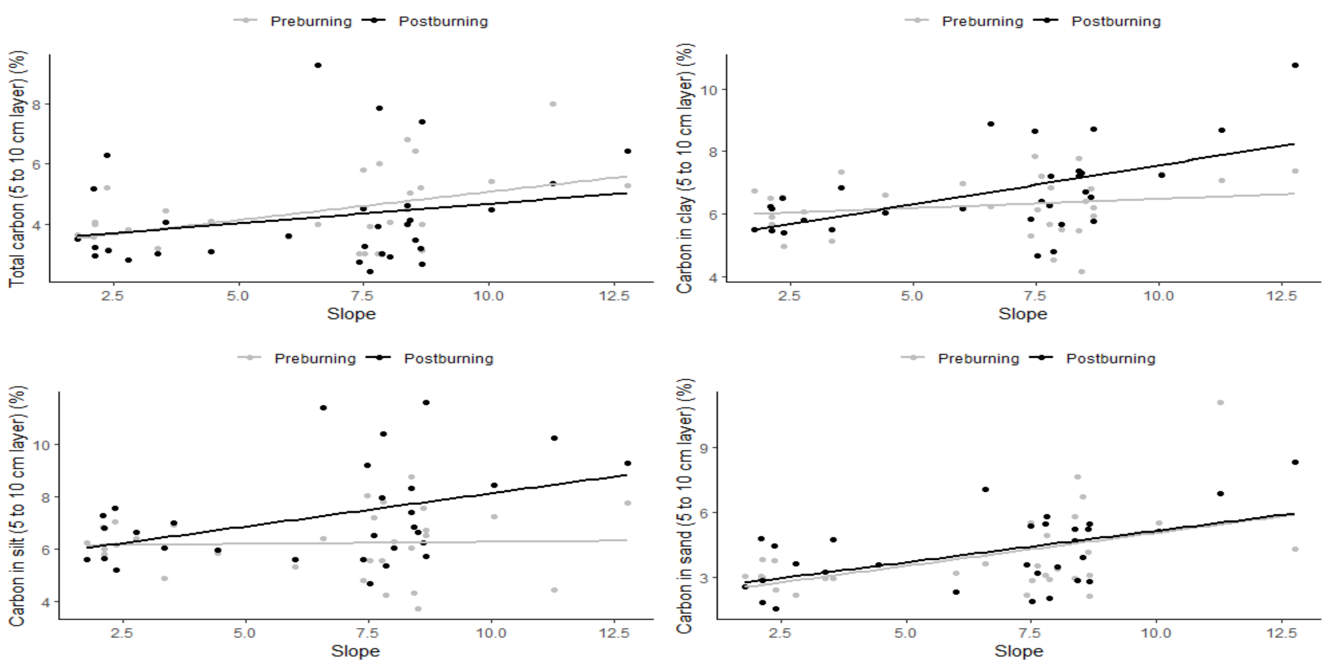

Fig. 9 Relationship between slope and C in total and fractions in the deeper layer (5-10 cm)

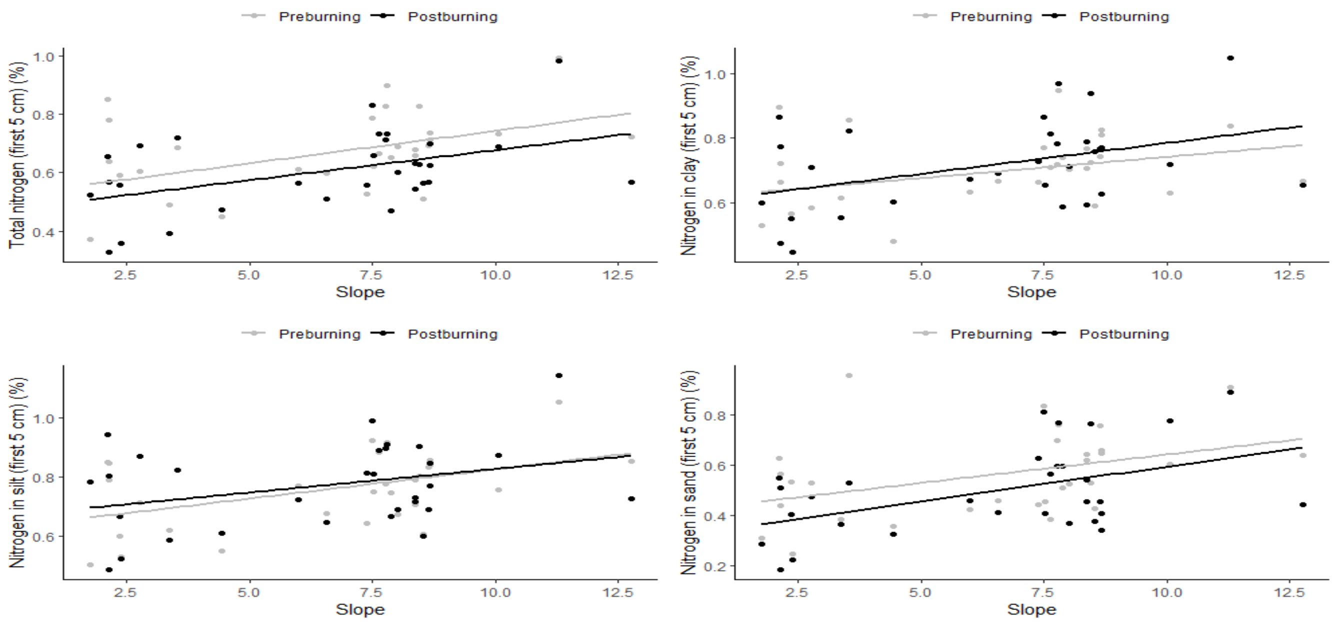

Fig. 10 Relationship between slope and N in total and fractions in the upper layer (0-5 cm)

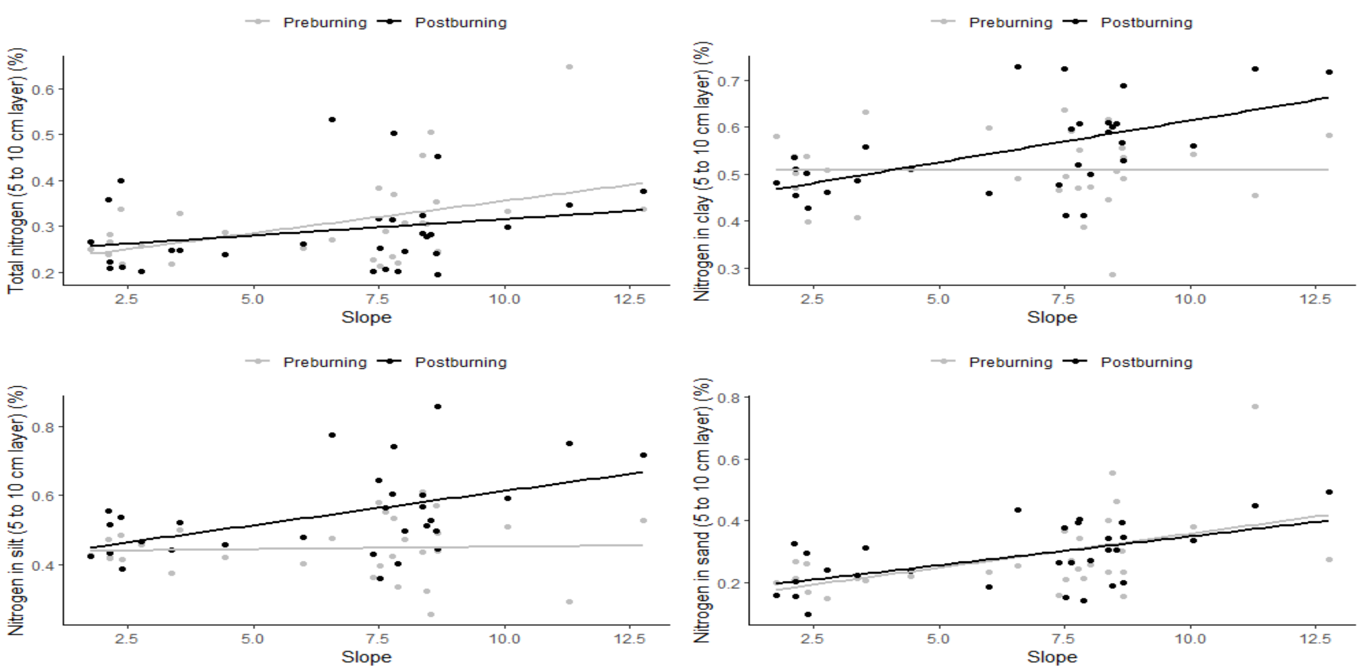

Fig. 11 Relationship between slope and C in total and fractions in the deeper layer (5-10 cm)