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Responses in Soil Carbon and Nitrogen Fractionation after Prescribed Burning in the Montseny Biosphere Reserve (NE Iberian Peninsula)

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Abstract: Prescribed fire is one of the most widely-used management tools to recover encroached rangelands. Fire has been reported to cause changes in the soil physical and chemical properties. However, the legacy effects of former plant species on soil responses to fire remains unknown. The legacy effect of the former extant plant species on soil carbon (C) and nitrogen (N) fractionation distribution after prescribed burning in topsoil (0–5 cm and 5–10 cm) was investigated in Mediterranean shrublands in Montseny. We sampled soils under five vegetation patch types: *Cytisus scoparius* L., *Calluna vulgaris* L., *Erica arborea* L., *Pteridium aquilinum* L., and *Cladonia* biocrusts, pre- and post-burning. Multivariate analysis on soil C and N fractions showed that soils under the legume *Cytisus* and the biocrust were the most differentiated. Vegetation patch types tended to respond differently to burning, soils under *Cytisus*, *Cladonia* and *Calluna* showing the strongest response. Total C and N, and C and N in sand decreased after burning in the 0–5 cm soil layer. Conversely, C in silt, as well as N in clay and silt, increased with soil depth after burning. This study will be helpful for 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

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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 [1,2]. Prescribed burning is considered as the deliberate application of fire characterized by lower temperature, intensity and severity compared to wildfires [3,4]. This practice is a widely-used to reduce the risk of wildfire and recover shrublands [5].

Soils are considered as a major reservoir of C in terrestrial ecosystems [6]. Soil includes different carbon (C) and nitrogen (N) pools where soil labile C and N pools are characterized by their small size and fast turnover rates, with large size and slow turnover rates, recalcitrant organic C and N pools are physically or chemically protected [7]. Soil organic carbon (SOC) is responsible for soil fertility, and it is used as an indicator of soil health. Decreased soil C/N ratio led to a decline in SOC and an increase in soil N [8,9]. The influence of prescribed fires on soil C and N depends on fire frequency and intensity, in addition to climatic factors [3,10]. Furthermore, prescribed burning is responsible to affect only the upper centimetres of the soil [4]. Prescribed fires may also affect microbial community composition and function that lead to altered C and N cycling in an ecosystem [10,11]. Some studies have described increased upper soil C and N content after low intensity fires because of the incorporation of unburned or partially unburned slash fragments into the soil [12–14], while other studies report no change [15]. Soil organic matter decreased with short-term low intensity fire in topsoil in Mediterranean Shrubland [16,17]. [18] observed decreases in soil nutrient concentrations with greater fire intensity on topsoil in a mixed Chaparral in USA. Soil carbon and nitrogen were lost from the first 5 cm with high intensity and long-term burning in a shrubland of Eastern Spain [19].

Soil particle size fractions are helpful in maintaining the stability of ecosystems [20].

Soil organic matter within the sand fraction is allocated to the active (labile) pool, and that in the silt and clay fractions, to the passive (recalcitrant) pool. The labile pool is easily affected by fluctuation in environmental conditions as well as decomposed rapidly and becomes oxidized easily with changes in land use [21]. The passive/non-labile pool is more stable and recalcitrant, and therefore this fraction is decomposed slowly by microbial activity [22].

Furthermore, plants determine the quantity and the quality of residues, soil organic matter, as well as soil structure [23]. Thus, soil functions are also affected by plant functional diversity [24]. Plant functional types (PFTs) have proved to be a useful tool for predicting soil processes including C and N cycles [25]. Legumes have the potential to modify soil nutrient availability as legumes have the capacity to fix symbiotic N [26]. In addition, biological soil crusts (BSCs), which are assemblages of lichens, fungi, cyanobacteria, and mosses that colonize the soil surface, play a key role in the N cycle, because N-fixing lichens and free-living heterotrophic bacteria forming part of BSCs are able to fix substantial amounts of atmospheric N [27].

Many studies have focused on the dynamics of topsoil C and N stocks after prescribed burning. There are several studies conducted on fire effects on seed germination, natural regeneration, and changes in vegetation composition. However, very few studies have focused on the effects of fire on soil C and N fractionation, that is, C and N contents in clay, sand, and silt fractions [16,28]. Furthermore, to our knowledge, this is the first study that examines the legacy effects of the prior 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, related to memories of the ecosystem to past events [29]. To understand present biodiversity patterns and predict the future ecological impacts of ongoing human practices on ecosystem services and functions, it is important to understand the carry-overs, or legacies, of the past events [30]. 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 (0−5 cm and 5−10 cm) 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. We 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 soil under legume containing higher C and N contents compared with other species; and (3) burning effects on soil C and N distribution will be modified by prior 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 2°18′ to 2°22′ east, latitude 41°44′ to 41°47′ north), located in the Pla de la Calma, an elevated plateau that ranges between 1000 and 1350 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 (Figure 1). This park comprises Mediterranean and Central European landscapes including different biomes, having local influence of metropolitan conurbations nearby.

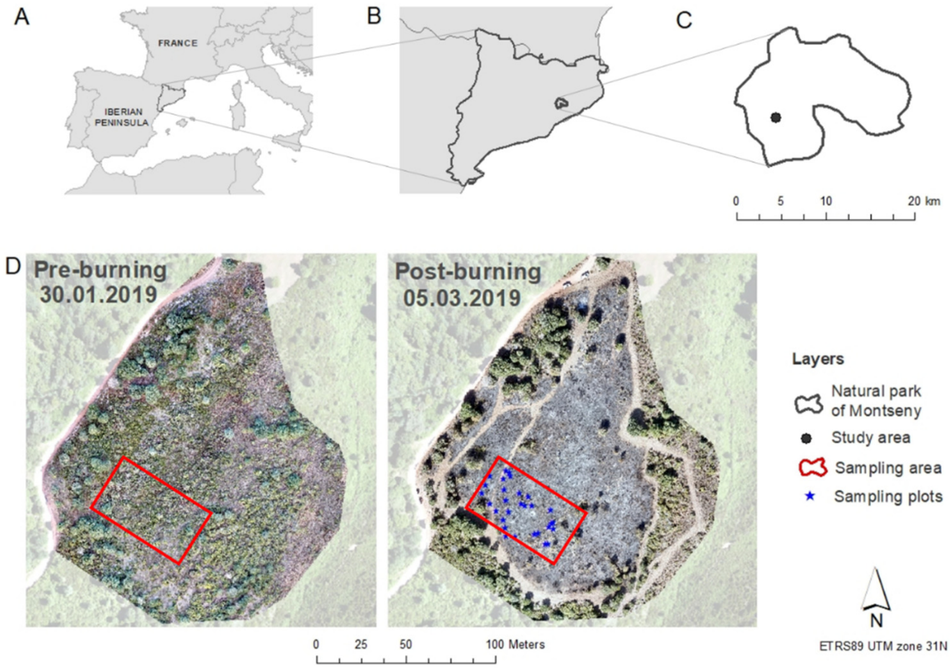

Figure 1. (**A**) Study area located in the NE of the Iberian Peninsula, (**B**,**C**) in the Natural Park of Montseny (Catalonia). (**D**) RGB orthomosaics representing the pre-burned and burned area of the Pla de la Llacuna experimental area (courtesy of the OPEN2PRESERVE project, https://open2preserve.eu/ accessed on 17 November 2021).

The plateau is characterized by humid Mediterranean climate; mean annual precipitation approximately ranges between 700 and 1000 mm, where snow accounts for around 10% of the annual precipitation. The mean annual temperature is 11 °C according to the Tagamanent meteorological station (https://www.meteo.cat accessed on 17 November 2021). 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 [31]. The topographic location and humidity circulation lead to 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 [32], including biological crusts dominated by various species of the lichen *Cladonia* sp pl., usually accompanied by some grasses. 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. Many small flocks composed mainly of sheep and goats grazed in Montseny in former times. The lack of direct grazing has caused a change in the vegetation structure and open grasslands are now covered by shrubs [33].

2.2. Field Sampling and Laboratory Determinations

The area selected for the prescribed burning experiment had a surface of 1.7 ha (Figure 1). A smaller area of 30 × 90 m within this surface was selected for the soil sampling (Figure 1). The burning event was conducted in 28 February 2019. Two samplings were performed in the study area, one before the burning event (pre-burning) which was carried out in 30 January 2019; and a re-sampling afterwards (post-burning) in 5 March 2019. The fire intensity was low to moderate in our study area. There was a small rain after burning in February 21 (0.1 mm).

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* L., *Erica arborea* L., *Cytisus scoparius* L. and *Pteridium aquilinum* L.; and the biocrust dominated by lichens mostly of the genus *Cladonia*. These vegetation patches belong to different plant functional types, where the shrubs *C. vulgaris* and *E. arborea* are Ericaceae and *C. scoparius* is a legume; *P. aquilinum* is a fern; and the *Cladonia* patch is a lichen-dominated biocrust, often mixed with grasses. Henceforth, we 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.*

The sampling was conducted using a stratified directed sampling. Soil samples were extracted in two different soil layers (0−5 cm and 5−10 cm) using a 4×4 cm^2 coring probe. The treatments used for stratification (burning, vegetation patch and soil layer) were replicated six times across the sampling area, resulting in 120 samples. Sampling points were georeferenced throughout the sampling area 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.

In addition, we measured the slope at each soil sampling point using the 5 m DEM (Digital Elevation Model) from the IGN (www.ign.es accessed on 17 November 2021). Slope was calculated in QGIS based on the DEM provided by the Institut Cartogràfic Geogràfic Català (ICGC, 2021), and a slope value provided for each sampling point. Slope was included in the modelling as a way of controlling the possible effects of spatial microtopographical heterogeneity.

Afterwards soils were transported to the laboratory and oven dried at 60 °C until constant weight. Soil samples were physically fractionated using the method developed by Six et al. [34]. 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: total C, C in clay, C in silt, C in sand, as well as total N, N in clay, N in silt, N in sand. The C/N ratio was also calculated for each of the fractions described above.

2.3. Data Analysis

Multivariate indirect ordination analysis was applied to the ensemble of the soil variables analysed in this study. In particular, we applied Principal Component Analysis (PCA). Multivariate analysis was conducted with CANOCO 5.1 [35].

In addition to multivariate analysis, we performed univariate statistical regression on each study variable, using linear mixed effect models, with the identity of the sampling point as random factor, and with burning, vegetation patch type, soil layers and slope as fixed factors. We included the interactions among those factors, except for slope, which was used as a covariate. Then the best model was selected according to AIC (Akaike Information Criterion) and stepwise method (both forward and backward). We did the normality test of all variables by Shapiro-Wilk Normality Test. All the variables followed the normal distribution except C/N ratio in the clay fraction. In this case, we used a generalized linear mixed model with inverse gaussian distribution. Then we performed Tukey's post hoc analysis at the $p < 0.05$ significance level. All statistical analyses were conducted in the R version 4.0.2 [36].

3. Results

In the study area, silt was the fraction including the highest soil C and N contents (Tables 1 and 2). We found that most of the study variables increased with slope as well as soil depth (Tables 1–3).

Table 1. Final models for all C variables in the study. *p*-values from the mixed model regressions on C variables, for the following tested explanatory variables: slope, burning, species, and soil depth.

Explanatory Variables	Carbon Variables			
	Total Soil Carbon	Carbon in Clay	Carbon in Silt	Carbon in Sand
Slope	0.001 **	0.001 **	0.002 **	<0.001 ***
Burning	0.004 **	0.175	0.191	0.085
Species	0.095	0.061	0.076	0.024 *
Soil depth	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
Burning * Soil depth	0.021 *	-	0.039 *	0.034 *
Species * Soil depth	-	-	-	0.076

$p < 0.1$; $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***.

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 65% of the total variability. PCA axis 1 explained 88% of the explained variability and mainly separated samples according to the plant species under which the soil was originally extracted (Figure 2A). Soils under the legume shrub *Cytisus* were distributed along the most positive part on PCA1, followed by those under *Pteridium* (Figure 2A); while soils under *Cladonia* distributed mostly on the negative side (Figure 2A). Soils under the two Ericaceae had low responses to this axis, suggesting intermediate soil C and N trends (Figure 2A). In addition, PCA axis 2 added 6% to the explained variability. This axis mostly explained the variability due to burning (Figure 2A). Soils under *Cytisus* and *Cladonia* followed by *Calluna* were the ones showing a higher response to burning compared to the soil under the other species (Figure 2A). Finally, PCA axis 3 explained 3% 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 *Cytisus*, *Calluna* and *Cladonia* being the ones most differentiated between unburned and burned conditions (Figure 2A,B).

3.2. Total and Fractional Soil C

Both total C (Figure 3A; Table 1) and C in the sand fraction in the 0–5 cm soil layer (Figure 3D; Table 1) decreased after burning; conversely, C in the silt fraction (Figure 3C; Table 1) increased with prescribed burning in the 5–10 cm layer, according to the regression model. However, this was not captured by the Tukey test, suggesting that the increase of C in the silt fraction with burning is relatively weak.

The highest C content was found in the soils under *Cytisus* patches, while the lowest C content was found under *Cladonia* patches in the sand fraction (Figure 4D; Table 1). 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 burning (Figure S1 in Supplementary Materials). After burning, total soil C in the 0–5 cm soil layer showed a decreasing trend in all species, which was more pronounced in *Cladonia* and *Erica* patches compared to the other patch types (Figure S1A in Supplementary Materials). C in sand also decreased in all the species, but especially in the *Cytisus* and *Calluna* patches in the 0–5 cm soil layer (Figure S1D in Supplementary Materials). In contrast, soil C increased after burning more remarkably in *Cytisus* and *Calluna* in the silt fraction in the 5–10 cm layer (Figure S1G in Supplementary Materials).

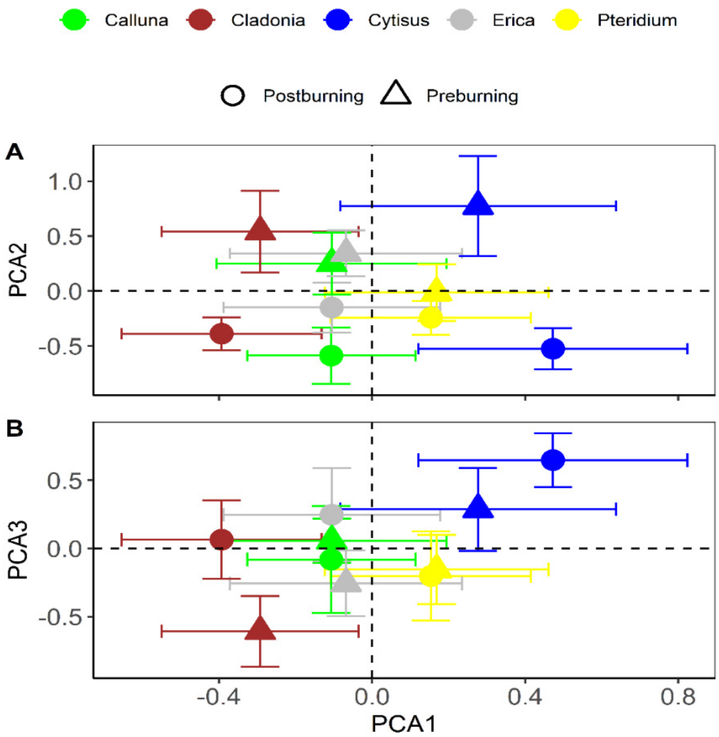

Figure 2. Samples distribution along the two first axes (PCA1 and PCA2 (**A**); and the first and third axes, PCA1 and PCA3 (**B**) of Principal Component Analysis (PCA) performed on the overall soil C and N variables (total and fractional) in 0–5 and 5–10 soil layers. Samples are clumped into groups according to vegetation patch type and prescribed burning treatment. The mean value ± 1 standard error of each axis variable is represented for each species and treatment by different symbols and whiskers.

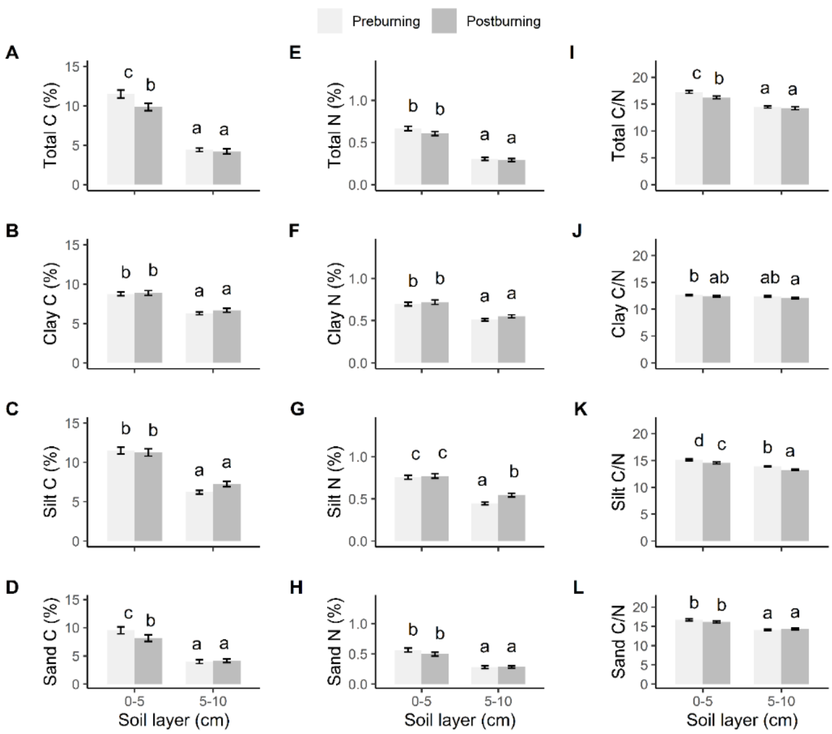

Figure 3. Mean ± 1 SE total and fractional (top to bottom) soil C (**A**–**D**), N (**E**–**H**) and C/N ratio (**I**–**L**) distribution in the 0–5 cm and 5–10 cm soil layers. Different letters indicate significant differences ($p < 0.05$) among treatments according to multiple Tukey mean comparison tests (soil layers and burning). Sample size $n = 120$.

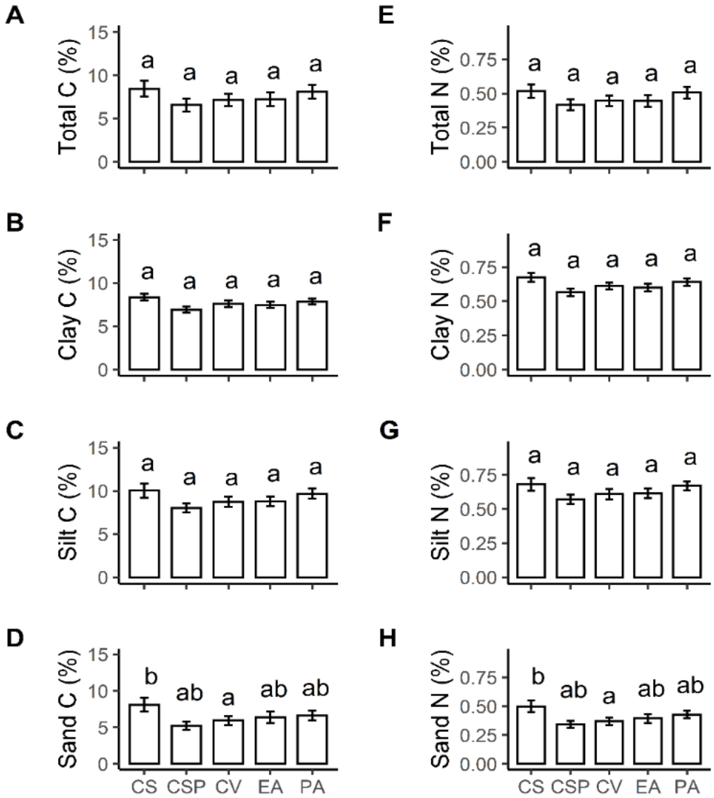

Figure 4. Mean ± 1 SE total and fractional (top to bottom) soil C (**A**–**D**) and N (**E**–**H**) distribution per vegetation patch type; *Cytisus scoparius* (CS); *Cladonia* biocrust (CSP); *Calluna vulgaris* (CV); *Erica arborea* (EA); *Pteridium aquilinum* (PA). Different letters indicate significant differences ($p < 0.05$) among treatments according to Tukey mean comparison tests. Sample size $n = 120$.

Soil C content in the 5–10 cm layer was less than half than that in the 0–5 cm layer under *Cytisus*, *Erica* and *Pteridium*. While soil C content in the 5–10 cm layer was half than that in the 0–5 cm layer under *Cladonia* and *Calluna* (Figure 5; Table 1).

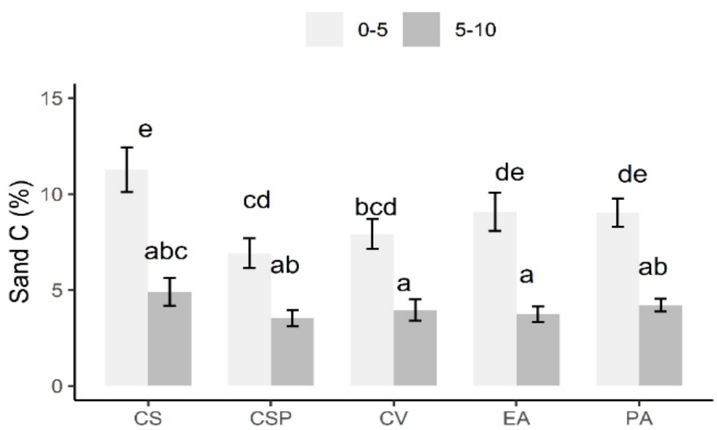

Figure 5. Mean ± 1 SE soil C distribution in sand fraction in 0−5 cm and 5−10 cm soil layers per vegetation patch type; *Cytisus scoparius* (CS); *Cladonia* biocrust (CSP); *Calluna vulgaris* (CV); *Erica arborea* (EA); *Pteridium aquilinum* (PA). Different letters indicate significant differences ($p < 0.05$) among treatments according to multiple Tukey mean comparison tests (soil layers and species). Sample size $n = 120$.

3.3. Total and Fractional soil N

Total soil N (Figure 3E; Table 2), and N in the sand fraction in the 0–5 cm soil layer (Figure 3H; Table 2) decreased after burning. Conversely, soil N in both the clay (Figure 3F; Table 2) and the silt fractions (Figure 3G; Table 2) significantly increased with prescribed burning in the 5–10 cm layer.

Significantly highest and lowest N content were found in sand fraction under *Cytisus* and *Cladonia* patches, respectively, compared to the other vegetation patches (Figure 4H; Table 2). There were no significant interactions between plant species and burning in the total soil N, neither in the three soil N fractions (Table 2). However, there were some tendencies for vegetation patch types to respond differently to burning (Figure S2 in Supplementary Materials). Total soil N decreased in almost all vegetation patch types, but more remarkably in *Cladonia* in the 0–5 cm layer after burning (Figure S2A in Supplementary Materials). N in sand decreased in all species, but mainly in *Calluna*, followed by *Cytisus* and *Erica* in the 0–5 cm soil layer after burning (Figure S2D in Supplementary Materials). On the other hand, N in the clay fraction increased especially in *Cytisus*, but also slightly in *Cladonia* and *Calluna* after burning in the 5–10 cm layer (Figure S2F in Supplementary Materials). This increasing trend was also found for the silt fraction, where N increased after burning more remarkably in *Cytisus* and *Calluna* in the 5–10 cm layer (Figure S2G in Supplementary Materials).

Table 2. Final models for all N variables in the study. *p*-values from the mixed model regressions on N variables, for the following tested explanatory variables: slope, burning, species, and soil depth.

Explanatory Variables	Nitrogen Variables			
	Total Soil Nitrogen	**Nitrogen in Clay**	**Nitrogen in Silt**	**Nitrogen in Sand**
Slope	0.001 **	0.003 **	0.003 **	<0.001 ***
Burning	0.028 *	0.047 *	0.002 **	0.108
Species	0.075	0.088	0.102	0.013 *
Soil depth	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
Burning * Soil depth	-	-	0.022 *	0.082

$p < 0.1$; $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***.

3.4. Total and Fractional Soil C/N Ratio

The soil C/N ratio decreased with soil depth (Figure 3I). In addition, there was a tendency for total soil C/N to decrease after burning, particularly from the soil under *Cladonia* biocrusts, followed by *Erica.* patches (significant burning x species interaction; Figure 6; Table 3).

Table 3. Final models for all C/N variables in the study. *p*-values from the mixed model regressions on C/N variables, for the following tested explanatory variables: slope, burning, species, and soil depth.

Explanatory Variables	C/N Ratio			
	Total C/N Ratio	**C/N Ratio in Clay**	**C/N Ratio in Silt**	**C/N Ratio in Sand**
Slope	-	0.031 *	-	0.104
Burning	0.002 **	0.024 *	<0.001 ***	0.415
Species	0.821	-	-	-
Soil depth	<0.001 ***	0.014*	<0.001 ***	<0.001 ***
Burning * Soil depth	0.051	-	-	0.033 *
Burning * Species	0.023 *	-	-	-

$p < 0.1$; $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***.

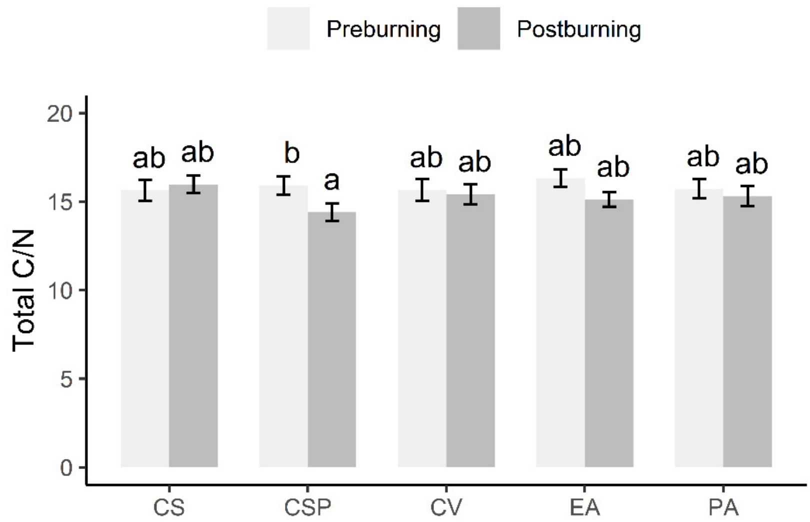

Figure 6. Mean ± 1 SE soil C/N ratio distribution in total soil per vegetation patch type; *Cytisus scoparius* (CS); *Cladonia* biocrust (CSP); *Calluna vulgaris* (CV); *Erica arborea* (EA); *Pteridium aquilinum* (PA) and burning treatment. Different letters indicate significant differences ($p < 0.05$) among treatments according to multiple Tukey mean comparison tests (burning and species). Sample size $n = 120$.

Prescribed fire significantly decreased the C/N ratio of total soil (Figure 3I) and sand fraction (Figure 3L) in the 0–5 cm layer, as well as in clay (Figure 3J) and silt fractions (Figure 3K) (Table 3).

4. Discussion

Our results show an important effect of burning and species composition on total and fractional soil C and N dynamics (Figures 2 and 3) (Figures S1 and S2 in Supplementary Materials) which was unknown in previous literature. Part of the variability in the soil C and N contents can be attributed to the specific vegetation patch, with soils under the legume species performing higher C and N contents compared with other species. Furthermore, after burning, we found a significant decrease in soil C and N content in the coarser soil fractions in the 0−5 cm soil layer but an increase in the finer fractions in the 5–10 cm layer.

We found that most of the study variables increased with slope (Tables 1–3) maybe due to the spatial microtopographical heterogeneity in the distribution of soil parameters [37].

4.1. Total and Fractional Soil C and N Distribution before and after Burning

Total soil C (Figure 3A; Table 1) and N (Figure 3E; Table 2), and C (Figure 3D; Table 1) and N (Figure 3H; Table 2) in the sand fraction in the 0–5 cm soil layer decreased after burning. The soil C and N can be substantially decreased with low to moderate fire intensity [2], 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 [38], as well as direct convective transfer of ash [39]. Ref. [17] also found a significant decrease in total soil organic C (SOC) and in total N content in the uppermost soil layer immediately after prescribed burning. In our study, 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 [40]. Our findings contrast the results obtained in a study by [13], where SOC at 0–5 cm depth increased immediately after low-intensity prescribed burning in Mediterranean grassland in the northeastern Iberian Peninsula.

In contrast, soil N in the clay fraction (Figure 3F; Table 2) as well as C (Figure 3C; Table 1) and N (Figure 3G; Table 2) in the silt fraction significantly increased with prescribed burning in the 5–10 cm layer. 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 N in the clay as well as C and N in the silt fraction may be due to the downwards translocation and accumulation of C and N at 5–10 cm layer compared to the 0–5 cm layer [41].

These results partly support the first hypothesis that the C and N contents in different soil fractions will be increased due to the accumulation of burnt material. However, we have found that coarser fractions generally losing C and N after fire; while finer fractions tend to increase their C and N content (Figure 3; Tables 1 and 2).

4.2. Plant Species and Species Legacy Effects on Total and Fractional Soil C and N Distribution after Burning

PCA1 differentiated vegetation patches according to initial soil C and N conditions (Figure 2A), while PCA2 (Figure 2A), and PCA3 (Figure 2B) showed that patches separated differently on those axes according to burning. This suggests that species had very strong effects on soils before burning but left imprint on soil fractional C and N after burning in dissimilar intensity, suggesting that species legacy effects were uneven after burning. This is shown by the observed tendency of vegetation patch types to respond differently to burning (Figures S1 and S2 in Supplementary Materials). Furthermore, the effects of species were not as strong as the effects of other treatment factors (Tables 1–3).

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 (PCA1; Figure 2A). Legumes are capable to fix atmospheric N and allocate it to the plant in exchange for carbohydrates. Therefore, legumes can strongly enhance the input of N into the soil ecosystem [42]. Due to their effectiveness in transferring aminoacids between nodules and roots, legumes favour organic N sources compared to other plant functional types (PFT) [43]. In addition, legumes possess higher leaf nitrogen content and higher specific leaf area compared with other plant functional types, traits related with increased photosynthetic rates, which increase net CO_2 uptake [44,45]. Thus, legumes enhance plant productivity, which in turn lead to increased C sequestration in soil [42]. Those legumes' traits result in higher litter quality and litter decomposition rates than non-legumes due to symbiotic relationships [46]; which in turn can result in higher soil C and N. The study by [47] agreed with our results, showing how soil C and N pools were enhanced by the presence of two legume species in an experiment carried out over 2 years. However, the enhancement effect on soil C has been reported to disappear at high legume proportions [48].

Cladonia patches also showed important differences in soil C and N distribution compared to the other patch types (PCA1; Figure 2A). A study conducted in Siberian forests by [49] found that soil C storage was lower in lichen patches, likely due to lower rates of C fixation [50], or higher rates of decomposition of lichen patches [23]. Ref. [51] found that NO_3 was lower in the site dominated by lichens than those composed by other plant functional types.

Soils under *Cytisus* and *Cladonia*, followed by *Calluna*, were the soils showing a higher response to burning compared to the soil under the other species (PCA2 and PCA3; Figure 2A,B). Burned residues of N-rich legume can stimulate N mineralization and nitrification after low intensity fires [52]. Legumes have also been reported to respond positively to fire in other ecosystems, as for instance tallgrass prairies and pine forests of the southeastern US [53,54]. On the other hand, maybe because lichens have lower accumulation rates of organic matter content and mineral N, predominantly ammonium, compared with grasses and mosses, [55] found that these soil characteristics were also maintained after fire. According to [56], a large proportion of the nutrients can be mobilized with such fire intensity from the soil under *Calluna* after burning as smoke, where smoke is defined as including the gases and volatile products of combustion together with suspended solid particles.

These results are in the agreement with our second hypothesis, which states that different species-dominated patches will have different soil C and N total and fractional contents, and legumes will contain higher C and N proportions than other species. Furthermore, they also agree with our third hypothesis that burning effects on soil C and N distribution will be modified by former plant species. However, the legacy effects of prior vegetation patches were not equally intense in all patches, neither they were necessarily linked to prior soil differences among plants (Figure 2)

4.3. Total and Fractional Soil C/N Ratio

The decreasing trend of the C/N ratio after burning found in this study agrees with what has been traditionally reported in the literature, generally attributed to preferential immobilization of N over C after prescribed burning [1,57]. However, to our knowledge, no study has previously reported the dependency that this parameter showed on prior vegetation patch, according to our results (Figure 6). The total soil C/N ratio was the soil parameter showing the strongest legacy effect compared to other studied soil C and N variables, strongly supporting our third hypothesis.

5. Conclusions

We have found that soil C and N contents are dependent on soil fractions, with the sand fraction being more vulnerable to lose C and N than clay and silt. In particular, C and N in total soil and in the sand fraction in the 0–5 cm layer decreased after short-term prescribed burning but increased in silt and clay fractions in the 5–10 cm layer, which is likely due to downwards translocation and accumulation of C and N from coarse fraction in 0–5 cm soil to fine fractions in 5–10 cm soil layer. 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 decreasing trend of the C/N ratio in this study suggests that soil C in the study site could be more labile than N. 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. That means the composition of species matters. The species legacy effects in the soil C and N responses to burning were best revealed when analysing jointly all the C and N variables in the study by multivariate analysis, with total soil C/N being the single variable most influenced by ecological legacies. The legume shrub *Cytisus* showed the highest differential overall composition of soil C and N parameters compared to other species as legumes possess more C and N than other species. So, legumes can help in maintaining soil fertility in the site [58], as well as provide support in the harsh conditions and compensate nutrient loss [59]. Soils under *Cytisus* and *Calluna* were the most responsive to burning compared to other species. Therefore, the effect of short-term prescribed burning is dependent on the species composition of the ecosystem, and it is important in ecological as well as in management aspects.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su14074232/s1, Figure S1: Mean ± 1 SE total and fractional (top to bottom) soil C distribution in the 0–5 cm and 5–10 cm soil layers per vegetation patch type (Cytisus scoparius (CS); Cladonia (CSP); Calluna vulgaris (CV); Erica arborea (EA); Pteridium aquilinum (PA) and burning treatment, Figure S2: Mean ± 1 SE total and fractional (top to bottom) soil N distribution in the 0–5 cm and 5–10 cm soil layers per vegetation patch type (Cytisus scoparius (CS); Cladonia (CSP); Calluna vulgaris (CV); Erica arborea (EA); Pteridium aquilinum (PA) and burning treatment, Figure S3: Mean ± 1 SE total and fractional (top to bottom) soil C/N ratio distribution in the 0–5 cm and 5–10 cm soil layers per vegetation patch type (Cytisus scoparius (CS); Cladonia (CSP); Calluna vulgaris (CV); Erica arborea (EA); Pteridium aquilinum (PA) and burning treatment.

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supervision, M.-T.S., M.I. and J.M.-C.; project administration, M.-T.S. and J.P.; funding acquisition, M.-T.S. and J.P. All authors have read and agreed to the published version of the manuscript.

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