Post-fire mineral N allocation and stabilisation in soil particle size fractions in Mediterranean grassland and shrubland

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ABSTRACT

In the Mediterranean region, long-term post-fire soil N dynamics may be relevant in the stabilisation of soil organic matter and N cycling in the plant-soil system. Post-fire recycling of N may be retained by the retention of N in physically and/or chemically protected fractions of soil organic matter. We studied the allocation of post-fire ^{15}N-tracer among different soil organic matter fractions (coarse sand, fine sand, coarse silt and fine silt + clay) and ^{15}N-tracer dynamics for 12 years after prescribed fires in three different Mediterranean plant communities (grassland, mixed shrub-grassland and shrubland). We selected 6 plots for each community and we set experimental fires. Directly after the fires, we applied ^{15}NH$_4^+$-N and we monitored the fate of ^{15}N-tracer over a period of 12 years. For this purpose, we carried out a physical size fractionation and we analysed the biochemical recalcitrance of N and ^{15}N by acid hydrolysis in the size fractions obtained. In both burned and unburned plots the finest soil particles (<20 μm) accounted for most soil N. Fire promoted N increases in the medium size fractions while the N pool in the finest and coarsest fractions did not change after the fires. Interestingly, ^{15}N-tracer was quickly incorporated into fine fractions from which, in the case of plant communities free of legumes, it was remobilised in the following years. Fire did not promote changes in recalcitrant N, but shrubland showed marked decreases in N recalcitrance 6 years after the fires. Despite the fact that the primary effects on soil fractions were detected just after the fires, these persisted for 12 years post-fire. Newly incorporated ^{15}N-tracer was less recalcitrant than total N and, surprisingly, fine fractions had very low recalcitrant ^{15}N values, similar to the coarse fractions. Apparently, the N transformations in the finest fraction (<20 μm) were mainly regulated by the quality of the ^{15}N compounds retained in the fraction.

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1. Introduction

Fire can cause complex alterations to the physical and chemical properties of soil. Fires immediately decrease the amount of labile organic matter (either by direct combustion or thermal breakdown) and enhance both the availability of plant N and the recalcitrance of organic matter pools (Almendros et al., 1992; Fernández et al., 1999). Although N volatilization is produced at relatively low temperatures (200 °C) and large amounts of N can be lost during fires, mineral N pools, mainly ammonium, increase in the uppermost soil layers as a consequence of temperature induced mineralization and ash deposition (Rapp, 1990; Martí-Roura et al., 2013). On the other hand, soil heating during fires can promote changes in soil N-forms to more chemically recalcitrant forms (Sánchez and Lázzari, 1999) by increasing heterocyclic and aromatic N (Knicker et al., 2005). The high biochemical stability of these fire-induced recalcitrant N-forms can render them difficult to mobilize by microbial processes, and therefore are able to remain in soils for years (Rovira et al., 2012). This effect may hold back primary production in the long-term (Knicker and Skjemstad, 2000).

Post-fire soil mineral N, if not lost by leaching or volatilization, can be assimilated by regrowing plants or incorporated into soil organic matter (SOM) and stabilized. SOM stabilisation can occur through physical protection or by chemical recalcitrance (Dungait et al., 2012). Physical protection can take place through SOM incorporation within aggregates through nanopores too small for organisms or enzymes to enter and function (Skjemstad et al., 1993; Mayer, 1994) by encapsulation into hydrophobic macromolecules (Knicker and Hatcher, 1997), or by the formation of organo-mineral-associates (Baldock and Skjemstad, 2000). This latter mechanism has been considered the most important for the physical protection of organic matter (Kögel-Knabner et al., 2008).

The chemical recalcitrance of decomposition residues can also delay SOM decomposition. Aromatic and/or O-alkyl compounds can be difficult to be metabolized by soil microbia (Almendros et al., 1984; Kalbitz et al., 2003). The prevailing view is that chemical recalcitrance plays a secondary role in SOM stabilisation (Mikutta et al., 2006; Marschner et al., 2008; Rovira et al., 2010; Dungait et al., 2012). However, in a recent field study it has been shown that N-rich labile organic matter from an incubation of labelled roots within the soil was recovered primarily in the protected fractions whereas the more recalcitrant fraction remained mostly unprotected (Garcia-Pausas et al., 2012). Therefore, physical protection and chemical recalcitrance may work simultaneously and sometimes in a complex manner since labile and recalcitrant organic pools are not evenly distributed among the physical fractions (Rovira and Vallejo, 2007; Rovira et al., 2010).

Quick plant regeneration and N use post-fire can largely reduce N losses from the system. After this, N will return to the soil through plant litter deposition and, depending on SOM quality, will be retained in the soil over the short and long-term. The interactions between plant litter and SOM pools of different nutrient richness or chemical recalcitrance can shape the microbial processes thereby transforming the pools of soil organic matter and nutrients (Madritch and Cardinale, 2007). These interactions are likely to occur in diverse plant communities with contrasting litter quality such as in those with a significant presence of legumes or a combination of woody and green plant material. The quality of the soil organic matter pools and of the new plant litter produced post-fire in the regrowing plant communities can regulate the post-fire allocation and stabilisation of mineral N in SOM (Zhu and Wang, 2011).

We hypothesise that after fire the stabilisation of the mineral N pool allocated in soil particle size fractions will depend both on the physical protection and the chemical recalcitrance of the SOM pools in each fraction. While N allocated to coarse fractions will be stabilized mainly by its biochemical recalcitrance, N hold in fine fractions will mostly be physically protected with little influence on its biochemical quality. Different plant communities and especially those with the presence of legumes can also influence the quality of the organic sources and SOM pools and, consequently, N stabilisation processes.

Our aim was to study the allocation and physical and chemical stabilisation of the N pool which appears shortly after fire among different particle size fractions over a 12-year period in three different Mediterranean plant communities (grassland, mixed shrub-grassland, shrubland). With this purpose in mind, we applied ^{15}N isotopic labelling on ashes just after a fire and we assessed changes in the total N and ^{15}N-tracer in different physical size fractions and in the recalcitrant pool of each fraction in the following 12 years post-fire.

2. Material and methods

2.1. Study site

The study was carried out at three different plant communities located in abandoned fields in the North East of Iberian Peninsula (41° 56′ N, 0° 37′ E, 460 m a.s.l.). These terraced fields were abandoned in early 1960s. The three different plant communities which now occupy the old fields are representative of Mediterranean set-aside land: grassland dominated by the perennial grass *Brachypodium retusum* (Pers.) Beauv; mixed shrub-grassland dominated by the legume shrub *Genista scorpius* L. in Lam et DC and the grass *B. retusum* and shrubland dominated by *Rosmarinus officinalis* L. The climate is dry Mediterranean continental, with a mean annual temperature of 13.5 °C and mean annual precipitation of 516.9 mm

(observation period: 1996–2008; Monestir de les Avellanes, 41° 52′ N, 0° 45′ E, 580 m a.s.l.). Rainfall distribution is markedly seasonal, with maximum values in spring and autumn and a dry season that usually lasts from June to September. The soils are Calcaric Cambisol (FAO, 2006), developed from a fine textured Eocenic limestone and marl colluvium, with a pH (H_2O) of 8.2, 60% calcium carbonate content and the soil is loam. The study areas had been free of fire and grazing by domestic animals for at least 10 years before the study began.

2.2. Experimental design, soil $^{15}NH_4^+$ labelling and soil sampling

Six pairs of plots (from 20 to 60 m^2) of each vegetation type were selected in different terraces (18 pairs of plots in total). In 1996 a plot of each pair was experimentally burned while the other remained as a control plot, representing the undisturbed state. Fire intensity was low to medium, with maximum temperatures at ground level from 300 to 526 °C. See Romanyà et al. (2001) for more details.

Three days after the fires, one subplot of 2 × 2 m was bordered in each burned plot and a highly ^{15}N-enriched solution ($^{15}NH_4Cl$, 99 atom%, 1 kg N ha^{-1}) was applied on the ashes of these subplots in order to label the post-fire mineral N pool. Subplots were divided in 64 squares (25 × 25 cm); each square was sprinkled with 18.75 ml of $^{15}NH_4Cl$ solution (1 l m^{-2} of 0.333 mg 15 N l^{-1}) in order to homogenize ^{15}N labelling on the subplots surface. See Casals et al. (2005) for more details.

From each plot, three soil cores (5 × 5 cm) from 0 to 5 cm depth were taken and pooled into one sample. Sampling was carried out 3 days (just 10 min after labelling), 6 years and 12 years post-fire in ^{15}N-labelled and burned plots and 3 days post-fire in unburned plots. Then soil samples were air-dried and sieved (2 mm) before fractionation.

2.3. Particle size fractionation of soil organic matter

The size fractionation method follows essentially that given in Rovira et al. (2010), with the main difference that, as in this experiment we did not attempt to separate fine silt from clay, the strength of the ultrasonic treatment was lower than in the original procedure (600 J ml^{-1} vs 1200 J ml^{-1}). Size fractionation methods by wet sieving have been successfully used as alternative methods to other fractionation methods such as density fractionation (Rovira and Vallejo, 2007; Garcia-Pausas et al., 2012; Lopez-Sangil and Rovira, 2013). This simple, reliable and inexpensive method uses water as the only solvent that may affect the obtained fractions and, for this reason, fractions are chemically unaltered and can be used in further analyses.

Briefly, 10 g of air dried 2 mm sieved soil were softly shaken for 1 h with 50 ml water and two glass beads. Then the beads were removed, and the soil-water mixture was placed in an ice bath and dispersed with a sonifier Branson model 250 (5 min, 600 J ml^{-1}). The dispersed mixture was then passed through a column of meshes (200, 50 and 20 μm) under magnetic agitation and an extensive water flush, thus obtaining the 2000–200 (coarse sand; CSa), 200–50 (fine sand; FSa), and 50–20 μm (coarse silt; CSi) size fractions. All of these fractions were collected in 20 ml flasks. The <20 μm fraction (fine silt plus clay; FSi + C) was collected in 1-l jar, flocculated with a minimum amount of a saturated solution of AlK(SO$_4$)$_2$ and recovered by centrifugation. After the fractionation method 4 fractions were obtained: CSa, FSa, CSi and Fsi + C. All fractions were dried at 60 °C, until constant weight. A subsample of each obtained size was finely ground to analyse the total organic C (TOC), and the total N and ^{15}N enrichment. TOC was analysed by dichromate oxidation (Mebius, 1960) adapted to a colorimetric

method (Soon and Abboud, 1991). Total N and enriched ^{15}N were analysed by an elemental analyzer (PDZ Europa ANCA-GSL) interfaced to a continuous flow isotope ratio mass spectrometer (IRMS) (20–20 isotope ratio mass spectrometer; PDZ Europa, Sercon Ltd., Cheshire, UK).

The combined study of the physical protection of SOM and the biochemical recalcitrance of this organic matter can suggest a more natural approach to understand the organic matter stabilization in soil. For this reason a subsample of each fraction obtained after the fractionation method (CSa, FSa, CSi and FSi + C) was further hydrolysed to obtain the recalcitrant N pool of each fraction (see Section 2.5 for more details).

2.4. Recovery of ^{15}N calculations

To calculate total recovery of ^{15}N in each soil fraction, we considered the first sampling date (10 min after the labelling) as the initial level. To calculate the atom % [15]N excess in bulk soil and soil fractions we used the following equation:

$$\text{atom }\%^{15}\text{N excess} = \text{atom }\%^{15}\text{N}_L - \text{atom }\%^{15}\text{N}_C \qquad (1)$$

where atom % [15]N$_L$ is the concentration of ^{15}N-tracer in the labelled burned plots and atom % [15]N$_C$ is the concentration of ^{15}N-tracer in the unlabelled burned (control) plots. Atom% [15]N excess of fractions was referred to the [15]N$_C$ from each respective unlabelled fraction. To calculate the percentage of ^{15}N-recovery in soils at each fraction (i) we used the following mass-balance equation that relates the initial ^{15}N-tracer concentration ($t = 0$) in the bulk soil to the ^{15}N-tracer concentration in each i soil fraction at each sampling date (t):

$$\% \text{ Recovery of }^{15}\text{N at the } i \text{ fraction}$$
$$= \frac{\text{atom }\%^{15}\text{N excess}_{t_i} * \text{N}_{t_i}}{\text{atom }\%^{15}\text{N excess}_0 * \text{N}_0} \times 100 \qquad (2)$$

N$_{ti}$ is the total amount of N (g N kg^{-1}) in the i soil fraction at time t and N$_0$ is the total amount of N in the bulk soil (g N kg^{-1}) at time 0.

2.5. Biochemical recalcitrance

For the analysis of the biochemical recalcitrance of SOM in each soil fraction (CSa, FSa, CSi and FSi + C) we applied the two-step acid hydrolysis with sulphuric acid of Rovira and Vallejo (2002). Briefly, 200 mg of dry ground soil material from each fraction was refluxed with 10 ml of 2.5 M H$_2$SO$_4$ for 30 min at 105 °C in an aluminium heating block. The liquid was decanted after centrifugation; the unhydrolysed residue was washed with 10 ml of water, recovered by centrifugation and dried at 60 °C. This residue was gently soaked overnight with 1 ml of H$_2$SO$_4$ 70–72%, at room temperature. Then, 9 ml of water were added and the residue was refluxed again, for 3 h at 105 °C. After centrifugation, the liquid phase was decanted again. The unhydrolysed residue was recovered and washed with 10 ml of water in order to eliminate the residual acid, after which the centrifugation plus decantation process was repeated. The unhydrolysed residue was finally dried at 60 °C, weighed and was taken as the recalcitrant fraction. This recalcitrant fraction was analysed for total C, N and ^{15}N with an elemental analyzer (PDZ Europa ANCA-GSL) interfaced with a continuous flow isotope ratio mass spectrometer (IRMS) (20–20 isotope ratio mass spectrometer; PDZ Europa, Sercon Ltd., Cheshire, UK). From the obtained values, the recalcitrance indices for N (RIN) and ^{15}N-tracer (RI[15]N) were calculated for each fraction:

$$\text{RIN}(\%) = (\text{Unhydrolysed N/Total N}) \times 100$$

$$\text{RI}^{15}\text{N}(\%) = (\text{Recovery of unhydrolysed }^{15}\text{N}/$$
$$\text{Recovery of total }^{15}\text{N}) \times 100$$

2.6. Statistical analysis

All statistical analyses were run using PASW statistics 17. All percentages were arc-sin (sqrt) transformed prior to statistical analysis following Town (2002). Data were checked for normal distribution (Kolmogorov–Smirnov test) and homogeneity of variances (Levene's test) before statistical analysis. Analysis of variance (ANOVA) was performed to test the differences between the fractions, time (day 3, year 6 and year 12 post- fires) and also between burned (day 3 post-fire) and unburned plots (day 3 post-fire) in the distribution of N in fractions, N content, ^{15}N recovery, RIN, RI[15]N, C:N ratio and recalcitrant C:N ratio. Duncan multiple range comparison test was applied when the significance level reached $p \leq 0.05$.

3. Results

3.1. Total N and recalcitrant N in fractions

After the fractionation, the mass recovery rate was between 94 and 98% and the mass distribution for the different fractions was: coarse sand (CSa), 23.8 ± 0.6%; fine sand (FSa), 16.2 ± 0.3%; coarse silt (CSi), 18.5 ± 0.3% and fine silt plus clay (Fsi + C), 38.1 ± 0.6%. Soil N was mostly located in the finest fraction —FSi + C— with values of between 50 and 60% of the total N in all plant communities, without differences throughout the experiment (Fig. 1). In contrast, the distribution of soil N after the fires in the other fractions was different depending on the plant community. In grassland no effects in total N after fire were observed in any fraction. In contrast, in shrubland and mixed shrub-grassland total N was higher in FSa and CSi fractions 3 days post-fire and remained constant up until year 12 post-fire, except FSa in shrubland which decreased over time. The coarse fraction (CSa) behaved differently. No changes in total N were observed in the CSa fraction just after fires, whereas it increased from 6 years to 12 years post-fire in the shrubland and mixed shrub-grassland.

The recalcitrance of N (RIN) was higher in the fine fractions (CSi and FSi + C) than in the coarse fractions (CSa and FSa) (Table 1). In general, no differences existed just after fires between burned and unburned plots, except for the decrease observed in the CSi fraction of mixed shrub-grassland ($p < 0.05$) and grassland ($p < 0.1$). Over the course of the next 12 years, we did not detect significant changes in the RIN in any fraction in grassland and mixed shrub-grassland plots. In contrast, in shrubland we observed a decrease in the RIN of all fractions from day 3 to year 6 post-fire; after 12 years however, only the coarse sand (CSa) fraction recovered its initial values.

The C:N ratio in soil fractions ranged from very low values in FSi + C to quite high values in coarse fractions (Fig. 2). In general the C:N ratio in fine fractions (CSi and FSi + C) did not show any change after the fires and only grassland showed a higher C:N ratio in unburned FSi + C when compared to the burned plots. The C:N ratio of coarse fractions was lower in burned plots immediately after the fires compared with the unburned plots in all three communities. After fires, the C:N ratio of coarse fractions did not change over time in shrubland whereas it increased in grassland and mixed shrub-grassland communities.

Recalcitrant C:N ratios were much higher than the C:N ratio of the respective fraction (Fig. 2). This difference was particularly large in coarse fractions. Just after fires, the recalcitrant C:N ratio in the CSa fraction was lower in mixed shrub-grassland and in shrubland
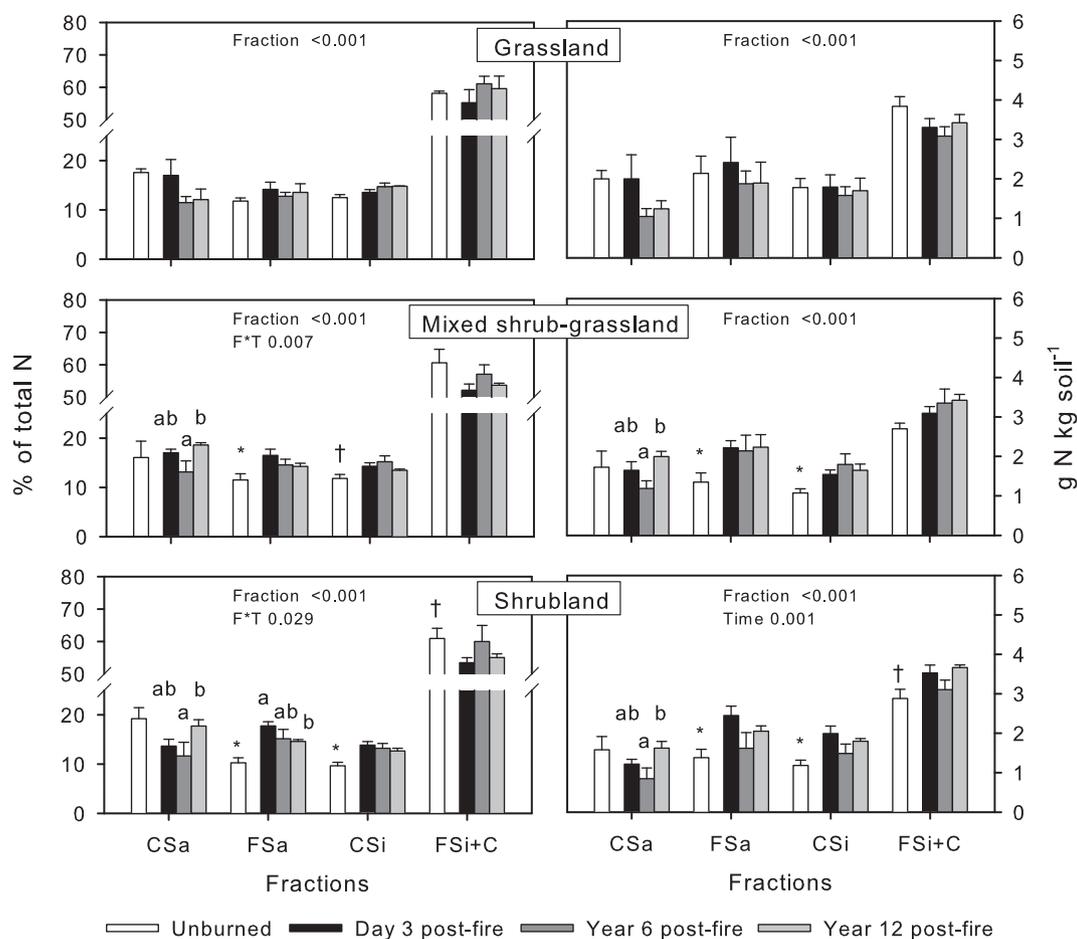
Fig. 1. Total N distribution (% of total N and kg N soil^{-1}) in fractions (coarse sand (CSa), fine sand (FSa), coarse silt (CSi) and fine silt + clay (FSi + C)) from unburned and burned soils (day 3, year 6 and year 12 post-fire) in three different plant communities (grassland, mixed shrub-grassland and shrubland). Differences between burned and unburned plots 3 days post-fire are labelled with †$p < 0.1$ and *$p < 0.05$. Significant factors and interactions are indicated. Within a given graph, time differences in each fraction are labelled with a different lowercase letter, at $p < 0.05$. Values are means ± SE ($n = 5$–6).

Table 1
Values of RIN (Recalcitrant Index for N) in fractions from unburned and burned soils (day 3, year 6 and year 12 post fire) and RI^{15}N (Recalcitrant Index for ^{15}N) from burned soils in three different plant communities (grassland, mixed shrub-grassland and shrubland). Differences between burned and unburned plots 3 days post-fire are labelled with†$p < 0.1$ and *$p < 0.05$. Time differences are labelled with a different lowercase letter, at $p < 0.05$. Values are means ± SE ($n = 5$–6).

Plant community	RIN (%)				RI^{15}N (%)		
	Unburned	Day 3	Year 6	Year 12	Day 3	Year 6	Year 12
Grassland							
CSa	37.97 (2.22)	32.16 (2.31)	28.49 (0.92)	28.54 (1.39)	26.97 (2.46)	20.67 (2.30)	25.53 (1.08)
FSa	38.83 (2.04)	34.33 (1.93)	35.35 (1.33)	32.97 (1.52)	**ab** 30.88 (3.06)	**a** 25.69 (2.48)	**b** 29.14 (1.51)
CSi	† 42.50 (1.49)	38.50 (1.28)	40.85 (1.12)	39.27 (0.95)	35.57 (3.24)	27.17 (2.62)	32.62 (0.78)
FSi + C	41.89 (2.00)	40.87 (1.46)	42.39 (2.73)	40.53 (2.69)	26.02 (1.20)	26.91 (1.20)	29.39 (1.59)
Mixed shrub-grassland							
CSa	† 39.53 (2.06)	33.25 (1.70)	30.77 (1.66)	30.72 (1.02)	32.93 (3.32)	27.59 (1.86)	32.32 (0.37)
FSa	36.30 (1.60)	35.54 (0.65)	36.90 (1.84)	33.62 (0.91)	37.24 (3.17)	33.38 (2.72)	34.46 (1.40)
CSi	* 48.85 (1.85)	41.75 (2.15)	45.37 (1.60)	43.63 (2.28)	45.49 (6.18)	40.16 (3.45)	42.64 (1.54)
FSi + C	45.26 (2.96)	44.10 (2.44)	43.48 (3.68)	40.65 (1.64)	32.78 (4.30)	31.00 (2.57)	34.90 (1.47)
Shrubland							
CSa	36.01 (2.59)	**a** 31.53 (0.67)	**b** 27.18 (1.80)	**a** 31.40 (0.63)	29.03 (1.59)	22.99 (1.16)	29.16 (0.92)
FSa	35.11 (2.56)	**a** 39.34 (1.97)	**b** 32.56 (0.70)	**b** 33.26 (0.83)	**a** 36.00 (1.91)	**b** 26.63 (0.96)	**b** 29.72 (1.35)
CSi	44.56 (4.71)	**a** 48.06 (2.94)	**b** 39.89 (1.10)	**b** 38.94 (1.26)	**a** 42.62 (2.29)	**b** 30.59 (1.42)	**b** 33.61 (1.08)
Fsi + C	47.54 (3.33)	**a** 45.01 (1.13)	**b** 39.89 (1.27)	**b** 37.54 (1.01)	**a** 29.07 (1.67)	**b** 25.28 (1.52)	**a** 27.83 (0.66)

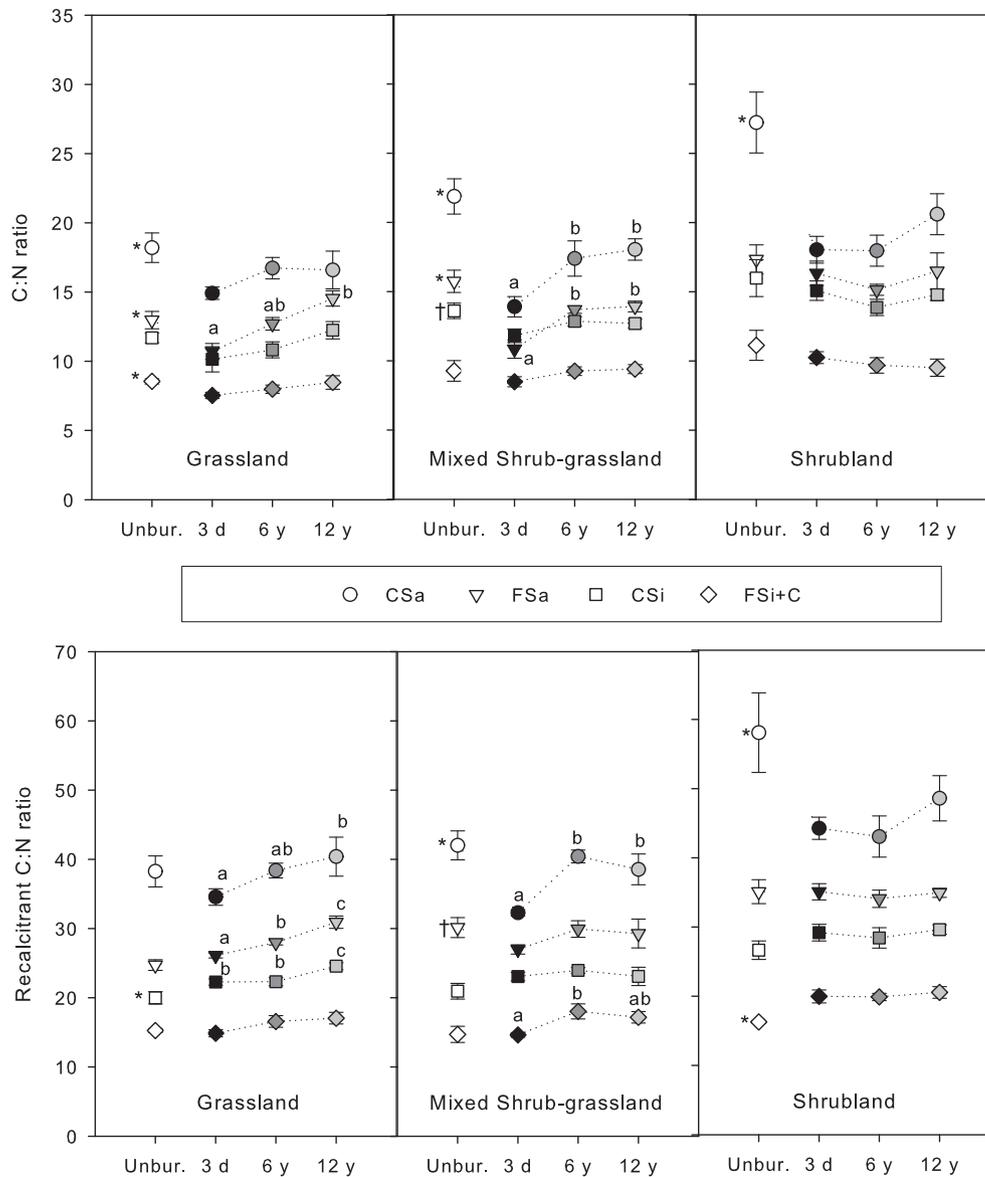|	RIN			RI^{15}N		
	Fraction	Time	F * T	Fraction	Time	F * T
Grassland	<0.001	n.s.	n.s.	0.001	0.001	n.s.
Mixed shrub-grassland	<0.001	n.s.	n.s.	<0.001	0.009	n.s.
Shrubland	<0.001	<0.001	n.s.	<0.001	<0.001	n.s.

Fig. 2. C:N ratio and recalcitrant C:N ratio in fractions (coarse sand (CSa), fine sand (FSa), coarse silt (CSi) and fine silt + clay (FSi + C)) from unburned and burned soils (day 3, year 6 and year 12 post-fire) in three different plant communities (grassland, mixed shrub-grassland and shrubland). Differences between burned and unburned plots 3 days post-fire are labelled with †$p < 0.1$ and * $p < 0.05$. Within a given graph, time differences are labelled with a different lowercase letter, at $p < 0.05$. Values are means ± SE ($n = 5–6$).

in burned plots when compared to control plots while no differences were detected in grassland. In the time after a fire, the recalcitrant C:N ratio increased in coarse fractions in grassland and mixed-shrubland (only in the CSa) while there was no change in shrubland. Only in mixed shrub-grassland did these changes in recalcitrant C:N ratio in the finest fraction (FSi + C) also occur over time.

3.2. Recovery of ^{15}N and recalcitrant ^{15}N in fractions

[15]N-tracer recovered in soil fractions just after the ^{15}N-labelling ranged from 22 to 53%. A significant part of the ^{15}N-NH$_4^+$ in soil was lost during the fractionation process most likely as part of the soluble fraction that was discarded. Just after the fires, a substantial fraction of the added ^{15}N-tracer was found to be located in the finest fraction (FSi + C) in grassland and shrubland (25 and 33% of ^{15}N recovery at day 3 post-fire, respectively). In the mixed shrub-grassland the figures were lower (14% at day 3 post-fire) (Fig. 3).

Statistical differences ($p < 0.05$) and marginally significant differences ($p < 0.1$) in ^{15}N-recovery were found over the 12 years in shrubland and grassland after fires. The behaviour of both legume-free plant communities (grassland and shrubland) was remarkably similar. Thus, we repeated the analysis of the evolution over time of the ^{15}N-tracer in both plant communities together (Table 2): we observed a decrease in significant quantities of ^{15}N-tracer from day 3 to year 6 post-fire in CSa and in FSi + C fractions whereas ^{15}N-recovery increased in the CSa and CSi fractions in year 12 post-fire (Table 2).

A significant proportion of ^{15}N-tracer was retained in the recalcitrant fraction of all fractions immediately after fire (Fig. 3, Table 1), and was higher in the grassland and shrubland than in the mixed shrub-grassland (Fig. 3). In contrast, the highest RI[15]N was found in mixed shrub-grassland (RI[15]N between 33% and 45% on day 3 post-fire; Table 1). On day 3 for all plant communities the highest RI[15]N occurred in medium size fractions (FSa and CSi) while the lowest occurred in the coarsest (CSa) and finest (FSi + C)
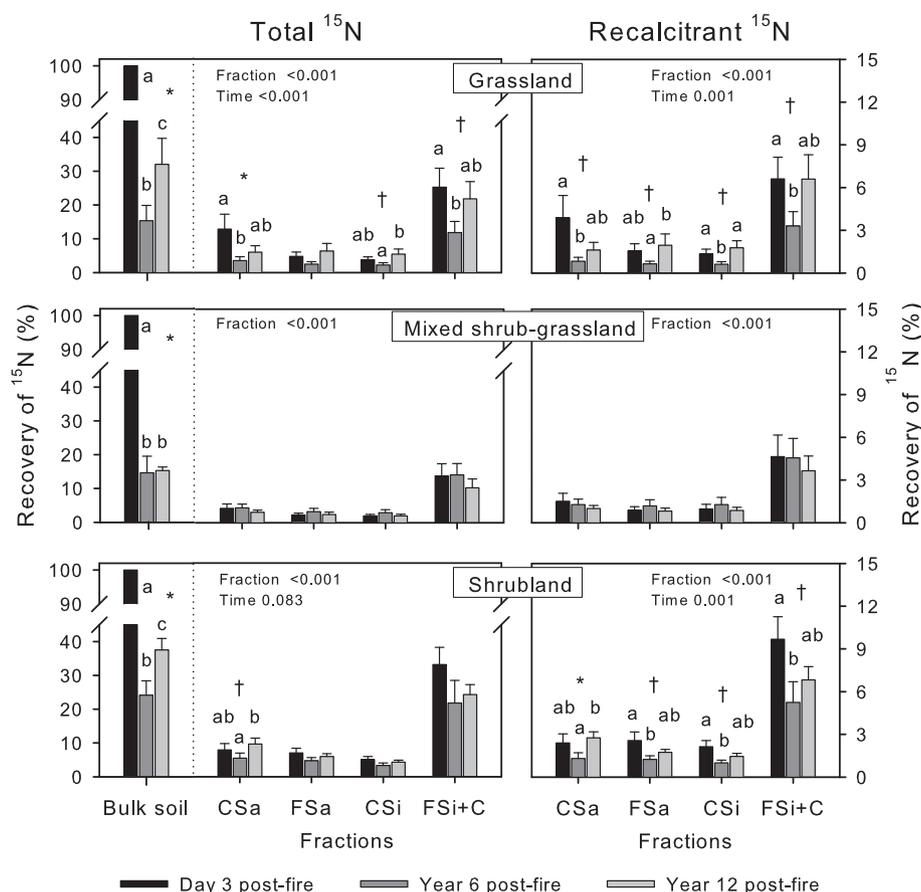
Fig. 3. Recovery of ^{15}N in soil (%) in fractions (coarse sand (CSa), fine sand (FSa), coarse silt (CSi) and fine silt + clay (FSi + C)) from burned soils (day 3, year 6 and year 12 post-fire) in three different plant communities (grassland, mixed shrub-grassland and shrubland). Significant factors and interactions are indicated. Time differences within a fraction are labelled with †*p* < 0.1 or * *p* < 0.05 and different letters indicate the significant differences. Values are means ± SE (*n* = 5–6).

fractions. This retention pattern contrasted with the total N recalcitrance of this fraction (see RIN; Table 1).

The changes over time of the recovery of recalcitrant ^{15}N in each fraction showed different patterns depending on the plant community (Fig. 3). While no differences over time were found in mixed shrub-grassland, grassland and shrubland showed a highly significant effect of time with a similar pattern among these two vegetation types and fractions (Fig. 3). To gain resolution in interpreting the effects of time in each particle size fraction these two non

legume plant communities were analysed together (Table 2). Then we observed that, for each particle size fraction, the recovery of ^{15}N in the recalcitrant fraction decreased from day 3 to year 6 post-fire in all fractions but, after that, the values increased again. From day 3 to year 6 post-fire, all plant communities showed general decreases in recalcitrance (RI[15]N) (Table 1). Then, from years 6–12 the RI[15]N changes were not significant except for an increase in the FSi + C fraction of shrubland.

4. Discussion

4.1. Changes in total N

Fire promoted a redistribution of N in soil fractions and mainly in woody plant communities (mixed shrub-grassland and shrubland). Fire has a shredding effect on plant litter (Scharenbroch et al., 2012). Thus, after fires N increased in medium size fractions (FSa, CSi) and remained constant over the whole period of study. This effect was only detectable in woody plant communities with higher biomass and with higher fire intensity (Romanyà et al., 2001). In contrast, N in the coarsest fraction (CSa) did not change shortly after fire compared with unburned plots in shrubland, which may be related with the highest temperatures reached at the soil surface in this community. The C:N ratio in coarse fractions was much lower in the burned plots, indicating large losses of organic C and therefore changes in the quality of organic matter after fire. The lack of net changes in total N observed directly after the fire was probably a result of the compensation between losses through

Table 2
Changes over time (day 3, year 6 and year 12 post-fire) on the total and recalcitrant ^{15}N recovery (%) in grassland and shrubland analysed together showing the differences in time for each soil fraction (coarse sand (CSa), fine sand (FSa), coarse silt (CSi), fine silt + clay (FSi + C)). Time differences are labelled with a different lowercase letter, at *p* < 0.05 (*P*-values are shown in the table).

	Time post-fire			*P*-value
	3 days	6 years	12 years	
Recovery of total [15]N (%)				
CSa	10.40 (2.39) b	4.43 (0.92) a	8.03 (1.35) b	0.05
FSa	5.91 (0.96)	3.50 (0.66)	6.15 (1.05)	0.08
CSi	4.42 (0.63) ab	2.75 (0.49) a	4.83 (0.73) b	0.05
FSi + C	29.21 (3.81) b	16.36 (3.67) a	23.17 (2.68) ab	0.03
Recovery of recalcitrant [15]N (%)				
CSa	3.14 (0.84) b	1.05 (0.24) a	2.24 (0.37) b	0.03
FSa	2.06 (0.41) b	0.92 (0.17) a	1.84 (0.36) b	0.03
CSi	1.74 (0.29) b	0.79 (0.14) a	1.60 (0.24) b	0.01
FSi + C	8.14 (1.15) b	4.19 (0.87) a	6.72 (0.88) b	0.02

volatilization and the incorporation of new N into ashes and partially charred plant residues. In the burned woody communities, the total N in the CSa increased towards the end of sampling period, in year 12. This pattern may be related to an increase of organic matter deposition due to a period of drought between years 6 and 12, as described previously (Martí-Roura et al., 2011). Thus, the N that was generated during or shortly post-fire was located in medium particles (FSa and Csi) while N resulting from a severe drought, mostly particulated, was mainly held in the coarsest fraction (CSa) and was likely to have come from dead coarse root deposition.

In our experiment N recalcitrance did not change after fire in any of the soil fractions. In coarse fractions of burned woody plant communities the recalcitrant C:N ratio was lower than in unburned plots, indicating that values of recalcitrant C were lower after fire. These findings are in contrast to those of Rovira et al. (2012) that showed increases in N and C recalcitrance post-fire in the bulk soil. In the first years following fire, N recalcitrance decreased only in shrubland, the only plant community dominated by seeders. Seedling litter deposition was enriched in non-woody plant material in contrast to that of mature shrubs. This would have contributed to the increase of the labile N fraction in this plant community.

As expected, most N was found in the finest fraction (FSi + C) and, in agreement with the findings of Rovira et al. (2010), this fraction and the fine silt fraction showed a higher recalcitrance than in coarse fractions (Table 1). Unlike in coarse fractions, where the stabilisation of organic matter is only controlled by the inherent molecular-level resistance to microbial breakdown, the stabilisation in fine fractions is also controlled, by the association of organic matter with aggregates or minerals (Christensen, 2001; Swanston et al., 2002).

4.2. Recovery of ^{15}N in soil fractions

The greatest proportion of ^{15}N-tracer applied after fire was quickly retained in fine fractions <20 µm (FSi + C). Hilscher and Knicker (2011) also observed that most of the ^{15}N-tracer applied after a burning process was found in the finest fraction (<2 µm fraction). This could partly explained by the quick sorption of ^{15}N-NH_4^+ into the cation exchange complex. However, an important part of the ^{15}N-tracer was also retained in the coarsest fraction. As coarse organic debris are believed to be the main source of C for soil microflora (Hagedorn et al., 2003; Rovira et al., 2010) we suggest that ^{15}N-NH_4^+ could be quickly retained in this fraction through microbial processes incorporating part of the ^{15}N-tracer into this particulate organic matter fraction.

As expected, this newly incorporated ^{15}N-tracer was less recalcitrant ($RI^{15}N$) than the total recalcitrant N (RIN) suggesting that in the case of microbial accessibility this N pool would be more prone to biological transformations than the older N in the fraction. Knicker and Skjemstad (2000) found the presence of peptide-like structures, N-rich particles traditionally considered chemically and physically labile fractions of soil organic matter in residues of fine soil fractions (<53 µm). The presence of these N-rich compounds in fine fractions could therefore lower the recalcitrance of these fractions. This was indeed the case in our study where values for the finest fraction were very low and even lower than those found in FSa and CSi. Garcia-Pausas et al. (2012) found a rapid incorporation of less recalcitrant compounds into the finest fractions (FSi + C) that were particularly rich in N, likely coming from microbial resynthesis (von Lützow et al., 2006; Rumpel et al., 2010). In our experiment, low recalcitrance of newly incorporated ^{15}N-tracer was found in both the finest (FSi + C) and the coarsest fractions (CSa) but not in intermediate fractions.

Thus, as we can observe in Fig. 4, during the first 6 years post-fire, changes in the recalcitrant ^{15}N-tracer in most particle size fractions negatively related to its initial recalcitrance ($RI^{15}N$) (day 3 post-fire). The steepest slopes of these relationships were seen for the finest and coarsest fractions (FSi + C and CSa) while the medium size fractions with higher recalcitrance showed much more gradual slopes or non-significant relationships. This suggested high sensitivity of changes in the recalcitrant ^{15}N pool to the substrate recalcitrance which occurred in these two fractions. As previously mentioned, the finest and coarsest fractions showed the most prominent changes over time of total ^{15}N recovery indicating that recalcitrant ^{15}N in these fractions was more readily transformed than in the medium size particle fractions (FSa and CSi).

Plant communities free of legume shrubs over time showed decreases of the ^{15}N-tracer pool that affected both the coarsest (CSa) and the finest (FSi + C) fractions. It appears therefore that the physical protection provided by the fine particles did not prevent the ^{15}N-tracer from being exported from this fraction. Interestingly, these changes which occurred over time affected all soil fractions, when taking into account recalcitrant ^{15}N. This suggests that the ^{15}N-tracer transformations in the medium size fraction (FSa) mainly involved the recalcitrant fraction whereas in CSi, FSi + C and CSa fractions this involved the entire pool (labile and recalcitrant) of ^{15}N and coincided with a particularly low ^{15}N recalcitrance at day 3 post-fire (Table 1).

In contrast, the retention of ^{15}N-tracer did not show any changes over time for mixed shrub-grassland. It is interesting to note that this plant community showed the highest $RI^{15}N$ in all soil fractions as compared to other plant communities. This was already so three days after fire suggesting that in this plant community ^{15}N tracer readily incorporated into more recalcitrant forms. Overall N recalcitrance was quite similar among all vegetation types except for mixed shrub-grasslands which seemed to readily incorporate ^{15}N-tracer into more N recalcitrant pools. These processes might be influenced by the specificity of the soil microbial communities which occur in plant communities with mixed legumes (Ladygina and Hedlund, 2010; Zhou et al., 2012). From our data set we can infer that the retention of the ^{15}N-tracer even in the fine fraction (FSi + C), during the years following fire, was mainly regulated by the recalcitrance of the substrate rather than by physical protection. Consequently, differences in soil organic matter quality i.e. changes
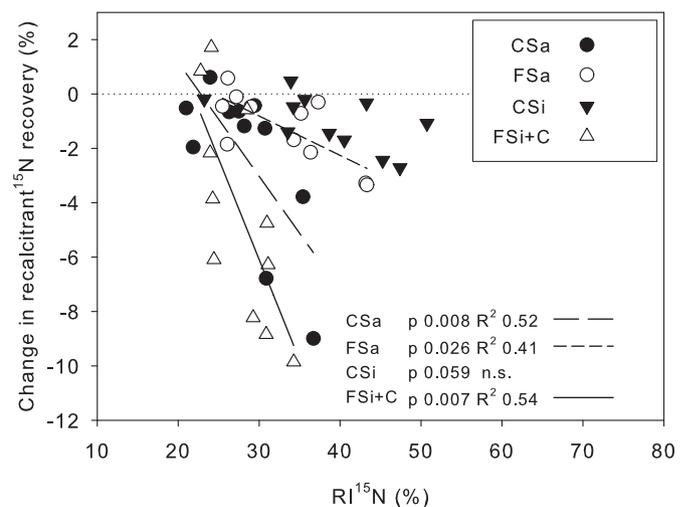
Fig. 4. Relationship between $RI^{15}N$ (%) at day 3 post-fire and the changes in recalcitrant ^{15}N recovery (%) between year 6 and 3 days post-fire in each soil fraction (coarse sand (CSa), fine sand (FSa), coarse silt (CSi) and fine silt + clay (FSi + C)).

in vegetation type seem to decide the fate of the ^{15}N-tracer in soil after fires.

In agreement with the drought-induced total N increases that were observed (between 6 and 12 years post-fire) (Martí-Roura et al., 2013), increases in ^{15}N-tracer were also observed during the period of drought between 6 and 12 years post-fire. These mainly occurred in the coarsest fraction (CSa) and only in legume-free plant communities. Increases in the finer fractions were not significant. This indicates that N in root deposition during drought periods is partly retained in coarser debris.

5. Conclusions

Fire promotes a fast incorporation of soluble mineral N into both the fine fraction and the coarsest fraction likely as a consequence of an increased N mineralization. It was found that the ^{15}N-tracer incorporated into these fractions (finest and coarsest) was a more labile N than N retained in medium size fractions. As a result, N transformations in the coarsest and finest fractions (CSa and FSi + C) were greater than in medium size fractions (FSa and CSi) and affected both labile and recalcitrant ^{15}N forms.

Our data suggest that significant part of N transformations occur in physically protected soil environments. These transformations can affect both labile and recalcitrant N forms. Differences in recalcitrance associated with the chemical quality of plant debris may control the mineralisation of N in both physically protected and unprotected environments.

Acknowledgements

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