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REGULAR ARTICLE

Long-term retention of post-fire soil mineral nitrogen pools in Mediterranean shrubland and grassland

Mireia Martí-Roura · Pere Casals · Joan Romanyà

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Abstract
Background and Aims The post-fire mineral N pool is relevant for plant regrowth. Depending on the plant regeneration strategies, this pool can be readily used or lost from the plant–soil system. Here we studied the retention of the post-fire mineral N pool in the system over a period of 12 years in three contrasted Mediterranean plant communities.
Methods Three types of vegetation (grassland, mixed shrub-grassland and shrubland) were subjected to experimental fires. We then monitored the fate of ^{15}N-tracer applied to the mineral N pool in soils and in plants over 12 years.
Results The plant community with legumes (mixed shrub-grasslands) showed the lowest soil retention of ^{15}N-tracer during the first 9 months after fire. Between years 6 and 12 post-fire, a drought promoted plant and litter deposition. Coinciding with this period, ^{15}N-recovery in the first 15 cm of the soil increased in all cases, except in mixed shrub-grassland. This lack of increase may be attributable to the input of impoverished ^{15}N plant residues and enhanced leaching and denitrification, possibly by N_2-fixing shrubs. After the drought, the deepest soil layer showed large decreases in total N and ^{15}N-recovery, which were possibly caused by N mineralization.
Conclusions Twelve years after the fires, plant communities without N_2-fixing shrubs recycled a significant part of the N derived from the post-fire mineral N and this pool continued to interact in the plant–soil system.

Keywords ^{15}N-recovery · Drought · Soil organic C · Soil N · Legume · N_2-fixing plant

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M. Martí-Roura (✉) · J. Romanyà
Departament de Productes Naturals, Biologia Vegetal i Edafologia, Universitat de Barcelona,
Joan XXIII s/n, 08028 Barcelona Spain
e-mail: mmarti@ub.edu

P. Casals
Centre Tecnològic Forestal de Catalunya,
Ctra. St. Llorenç de Morunys, Km 2, 25280 Solsona Spain

Introduction

Fire is a natural or anthropogenic disturbance that causes short- and long-term changes in soil nutrient stocks and dynamics (MacKenzie and DeLuca 2006). The main changes in the N cycle in fire-affected ecosystems are detected either during or shortly after fires. Significant amounts of N are lost during fires, as N volatilization occurs at relatively low temperatures (200 °C) (Castro et al. 2006; Fisher and Binkley 2000; González-Pérez et al. 2004). Mineral nitrogen (N) increases in soil immediately after fire, mainly as ammonium (NH_4^+) released as a result of ash deposition and soil heating (Raison 1979; Rapp 1990). Vegetation mortality or damage after fire will reduce plant nutrient uptake and increase the potential for the loss of N by leaching. However, the N cycle is also vulnerable mid-term after fires since erosion,

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leaching, and N_2O emission may also contribute to the loss of this mineral N from the ecosystem (Levine et al. 1988; Neary et al. 1999).

Studies on the long-term effects of fires on soil have enhanced our understanding of N dynamics after these disturbances. Soil total N and organic C may decrease in the long-term (Duguy et al. 2007; Ojima et al. 1994), and N mineralization may also slow down in the long-term after fires, starting at 6–15 years after fires (Grady and Hart 2006) and continuing until at least 250 years after the fire (Polglase et al. 1992). While the low biochemical quality of the organic matter resulting from fire may favour long-term accrual of soil organic C and N (Rovira et al. 2012), post-fire changes in vegetation structure may slow down the long-term recovery of soil N stocks (Raison et al. 2009).

Changes in N forms occurring during or shortly after fires may have a strong influence on the long-term N cycle. Fire intensity and the onset of plant regeneration can be crucial processes for initial N dynamics. The high temperatures reached during high-intensity fires may chemically transform part of the N residues into highly recalcitrant organic N products, such as "Black Nitrogen" (Knicker 2007; Knicker 2010), which are stabilized into the most recalcitrant soil organic matter (SOM) pool (Knicker 2011). In contrast, low-intensity fires enhance the transformation of organic N forms into more available forms of this mineral N forms (Christensen 1973; Prieto-Fernández et al. 2004; Weston and Attiwill 1990), as well as increase labile and dissolved organic matter (Prieto-Fernández et al. 2004). Thus, N pools from low-intensity forest fires can be either leached to deep soil layers, recycled through microbial communities and incorporated into the soil organic N reserve, or used to build up new plant biomass.

The post-fire regeneration strategies of plants and their use of nutrients may have long-term effects on the resulting ecosystem. While seeders are nutrient-dependent and can readily use nutrients in freshly enriched ecosystems, resprouters can regrow faster after fires, as they can rely on their nutrient reserves and extensive rooting systems (Bell and Ojeda 1999; Verdaguer and Ojeda 2002). In contrast, a high abundance of legumes with a high rate of atmospheric N fixation has been reported shortly after fire in temperate ecosystems (Arianoutsou and Thanos 1996), at least in the short-term after fires (Casals et al. 2005). Thus, legumes improve soil fertility by increasing the availability of soil N (Vandermeer 1989; Vandermeer 1990). Post-fire N_2 fixation can fully replace lost N and prevent post-fire N limitation (Binkley et al. 1982; Johnson et al. 2004). However, other studies suggest that the regrowth of N_2-fixing vegetation after fires is insufficient to compensate the N loss caused by fires, although it may partially contribute to long-term N accretion in ecosystems with low fire frequency (Johnson and Curtis 2001; Perakis et al. 2011; Wells 1971).

In this context, here we studied the fate of the post-fire mineral N pool on the plant and soil system over 12 years in three contrasted plant communities growing in abandoned fields in the Mediterranean region. The first community was dominated by resprouting grassy sward (*Brachypodium retusum*), the second combined the grassy sward with a resprouting N_2-fixing shrub (*Genista scorpius*), and the third was dominated by an obligate seeder shrub (*Rosmarinus officinalis*). We hypothesized that the fate of the N-NH_4^+ released after fires differs depending on the plant community and the presence of N_2-fixing plants, which are able to incorporate atmospheric N into the soil N cycle. Other parameters, such as fire intensity or drought, may also affect the long-term fate of N in these plant–soil systems.

Material and methods

Study site

The study was carried out in a set of abandoned fields located in the NE Iberian Peninsula (41º 56′ N, 0º 37′ E, 460 m.a.s.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, 41º 52′ N, 0º 45′ E, 580 m.a.s.l.). The rainfall distribution is markedly seasonal, with maximum values occurring in spring and autumn, broken by a dry season that usually lasts from June to September. Monthly climatic data were obtained from the meteorological station mentioned previously above (10 km away from the experimental area), and the monthly water deficit was calculated using Thornthwaite and Mather's (1957) equations, assuming 100 mm of available water-holding capacity (Table 1).

The fields were on terraces that were abandoned in the early 1960s. At the beginning of the study, in 1996,

Table 1 Mean Temperature (°C), rainfall (mm) and water deficit (mm). Values are annual mean for the periods between sampling dates: first 9 months after fire, from month 9 to year 6 after fire, from year 6 to year 12 after fire; and for the 4-year drought (2004–2008). Meteorological station: Os de Balaguer-Monestir d'Avellanes (41º 52′ N, 0º 45º E, 580 m a.s.l.)

	Period between samplings			Drought
	Oct. 1996–Jun. 1997 (To 9th month)	Jul. 1997–Feb. 2002 (9th month to 6th year)	Mar. 2002–Feb. 2008 (6th to 12th year)	Jan. 2004–Feb. 2008
Mean Temp. (°C)	11.1	13.1	13.1	12.6
Rainfall (mm)	566	450	451	349
Water deficit (mm)	6.5	281.0	311.4	352.5

the fields were colonized by three plant communities that are widely distributed in the Mediterranean basin, namely grassland, mixed shrub-grassland, and shrubland. The grassland was dominated by the perennial resprouting grass *Brachypodium retusum* (Pers.) Beauv. In the mixed shrub-grassland, the N_2-fixing shrub *Genista scorpius* L. in Lam et DC. was scattered over a grassy sward of *B. retusum*. Finally, the shrubland was dominated by the evergreen obligate seeder *Rosmarinus officinalis* L. The study areas had been free of fire and grazing by domestic animals for at least 10 years before the study. The soils are Calcaric Cambisol (FAO-UNESCO 1988), developed from a fine-texture Eocenic limestone and marl colluviums, with a pH (H_2O) of 8.2 and containing 60 % of calcium carbonate.

Experimental design

For each community, six pairs of plots (from 20 to 60 m^2) were selected and distributed in various terraces. In October 1996, 12 experimental fires were set in the grassland and mixed shrub-grassland plots; 21 days later (November) six shrubland plots were burned after cutting all shrubs and letting them dry for a week. For each burned plot there was a paired unburned control plot, which represented vegetation and soil in an undisturbed state. During fires, temperature was measured at ground level (surface) every 30 s with four thermocouples per plot. The time-temperature curve for each type of vegetation was obtained as the mean of the four thermocouples per plot and the six plots per vegetation type (24 measurements per vegetation type). Fire intensity was low to medium, with temperatures at ground level from 300 to 526 °C. A detailed description on the initial soil N forms and fire intensity can be found in Romanyà et al. (2001). After the fires, a homogenously burned area of 2×2 m within each burned plot was selected for isotopic labelling. In all cases this area was covered by ashes.

Soil $^{15}N\text{-}NH_4^+$ labelling and plant and soil sampling

Given that NH_4^+ is a direct product of combustion (Covington and Sackett 1992), we applied $^{15}N\text{-}NH_4^+$ tracer on the soil surface of each 2×2 m subplot to label the post-fire pool of NH_4^+ in the surface soil layer, which holds the ashes. To prevent the volatilization of ammonia, labelling was applied 3 days after the fires by adding 1 kg N ha^{-1} of high ^{15}N-enrichment $^{15}NH_4Cl$, 99 atom % excess. The amount of N added to the soil surface was considered negligible compared to the N changes occurring in the soil during the days after the fires. To achieve the maximum homogeneity in the labelling, we divided the subplot into 64 squares of 25×25 cm and sprinkled each area with 18.75 ml of $^{15}NH_4Cl$ solution (1 l m^{-2} of 0.333 mg ^{15}N l^{-1}) (see Casals et al. (2005) for more details).

Soil samples were taken from burned plots in labelled and unlabelled subplots with a volumetric prismatic auger (5×5×30 cm) at the following times: 3 days (10 min after the labelling), 9 months, 6 years and 12 years after the fires. Soil cores were divided into four layers: 0–2.5; 2.5–5; 5–15 and 15–30 cm for the samples taken in 2002 and 2008. In 1996 and 1997, soils were sampled only to a depth of 5 cm and split into the two first layers. Bulk density was measured per each sampling date and layer by a volumetric auger with a 5×5 cm cross section.

Plant samples were collected 9 months and 12 years after the fires. We sampled only the aboveground biomass; belowground biomass was not considered. *B. retusum* aboveground biomass was sampled using four squares of 33×33 cm and all the biomass was collected. For *G. scorpius* and *R. officinalis*, we calculated allometries with 40 individuals of each species using the basal diameter and the dry weight, and then we measured the basal diameter of all the shrubs located in a 1×1 m square. See Martí-Roura et al. (2011) for more details.

Soil and plant measurements

Soil samples were air-dried and sieved (2 mm) before analysis. Soil NH_4^+ and NO_3^- were extracted with 2 M KCl (1:5 w:v) and determined colorimetrically using a Technicon Autoanalyzer (Technicon Instruments Corp. New York, USA). A subsample of the fine earth of each sample was finely ground in an agate mortar to analyse ^{15}N-enrichment and total N. Aboveground plant samples were dried (60 °C for 48 h) and also finely ground. Soil and plant samples were then encapsulated and analysed for total N and ^{15}N-enrichment. The content (as a percentage of dry mass) and the stable isotope ratios of N were measured by an elemental analyser (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).

^{15}N-recovery calculations

To estimate the proportion of ^{15}N-tracer recovered in soils and plants, we used a calculation based on N mass, amount of ^{15}N in soil at the initial level, and ^{15}N- enrichments in soils and plants. We considered the first sampling date (10 min after the labelling) as the initial level (t=0). At this moment, the recovery of the ^{15}N in the soil from the theoretically applied ^{15}N was between 74 and 87 %. The loss of ^{15}N during the labelling application was not further considered. To calculate the atom % ^{15}N excess in each sampling, we used the following formula:

$$\text{atom\% }^{15}\text{N excess} = \text{atom\% }^{15}\text{N}_\text{L} - \text{atom\% }^{15}\text{N}_\text{C} \quad (1)$$

where atom% $^{15}N_L$ is the concentration of ^{15}N in the labelled plots, and atom% $^{15}N_C$ is the concentration of ^{15}N in the unlabelled (control) plots.

To calculate the ^{15}N-recovery in soils, we used the following mass-balance equation, which relates the initial ^{15}N concentration (t=0) to the ^{15}N concentration at each sampling date (t):

$$\%\ ^{15}\text{N recovery in soils} = \frac{\text{atom\% }^{15}\text{N excess}_\text{t} * \text{N}_\text{t}}{\text{atom\% }^{15}\text{N excess}_0 * \text{N}_0} \times 100 \quad (2)$$

N_t and N_0 are the total amount of N in soils (kg N m^{-2}) at time t and time 0. Similarly, to calculate the ^{15}N-recovery in plants, we used the following equation:

$$\%\ ^{15}\text{N}-\text{recovery in plants} = \frac{\text{atom\% }^{15}\text{N excess plant} * \text{Nplant}}{\text{atom\% }^{15}\text{N excess}_0 * \text{N}_0} \times 100 \quad (3)$$

where atom% ^{15}N excess plant is the concentration of ^{15}N in plants, which grew in labelled burned plots, corrected by reference samples. As reference samples we used the same plant species but grown in non-labelled burned plots. N plant is the total amount of N (kg N m^{-2}) in plants and N_0 the total amount of N in soils (kg N m^{-2}) at time 0.

Statistical analysis

We used a General Linear Model (GLM)-Repeated Measures analysis to test the effects of time on N total, ^{15}N-enrichment and ^{15}N-recovery in soil and plant material for each community. Our data were normally distributed, and we used Levene's test to check homogeneity of variances before statistical analysis. When variances were unequal, original data were log-normal transformed. As the data did not fail the sphericity test, the assumed sphericity correction was used. We used the Bonferroni method to test the significance of multiple comparisons. The probability threshold used to determine significance was p<0.05.

We also used a One-Way Analysis of Variance (ANOVA) to test differences in ^{15}N-enrichment, biomass and %^{15} N-recovery between vegetation types. The same method was used to test the differences in organic C, total N and C:N ratio between burned and unburned plots. Duncan multiple range comparisons were applied for each significant factor.

Results

Changes in C and N stocks of soil

Bulk density was not altered by fire (data not shown). Three days after the fires, organic C and total N contents in the first 5 cm of soil in grassland and shrubland showed no changes compared with unburned plots (Table 2). In contrast, soil organic C increased about 42 % in burned mixed shrub-grassland. While no changes were observed in total N, mineral N increased after fire in the mixed shrub-grassland and shrubland. This increase was mainly to changes in $N\text{-}NH_4^+$, which largely increased (Table 3).

From day 3 to month 9 post-fire, organic C decreased in burned mixed shrub-grassland in the top 5 cm of soil, while no changes were found in the grassland (Fig. 1). Soil organic C in shrubland was also depleted during this period but only in the 0–2.5 cm layer (from 710 to 550 g C m^{-2} on day 3 and in month 9 post-fire, respectively). Similarly, during that time total N in mixed shrub-grassland also decreased 15 % in the top 5 cm of soil, while the other plant communities showed no changes (Fig. 1).

From month 9 to year 6 post-fire, organic C decreased slightly in the top 5 cm of soil in the grassland and shrubland, while no change was detected in total N (Fig. 1). In contrast, in the mixed shrub-grassland, organic C did not change while total N increased slightly.

From 6 to 12 years post-fire, the soil organic C content increased in the top 5 cm of soil in all three plant communities (28 % in grassland and 45 % in mixed shrub-grassland and shrubland) (Fig. 1). However, this increase occurred in both burned and unburned plots. Thus 12 years after the fires, no differences were found between burned and unburned plots (Table 2). For all plant communities, total N in top 5 cm of soils showed a mild increasing trend from month 9 to year 12 post-fire (Fig. 1). In the mixed shrub-grassland, this trend was already significant at year 6.

In the deeper layers (5–15 cm and 15–30 cm), soil organic C increased from year 6 to 12 post-fire in mixed shrub-grassland ecosystems (from 1100 to 1300 g C m^{-2} in year 6 and 12, respectively) and in shrubland (from 800 to 1100 g C m^{-2} in year 6 and 12, respectively) (Fig. 1). In contrast, the behaviour of total N during this period differed for each layer. For all the ecosystems, total N in the 5–15 cm layer was similar for years 6 and 12. In contrast, this parameter decreased significantly from year 6 to 12 in the 15–30 cm layer (Fig. 1).

Changes in soil ^{15}N-enrichment

Overall, ^{15}N-enrichment decreased in the 0–2.5 cm soil layer of the three communities, mostly in the first year after fire. In contrast, the 2.5–5 cm layer showed an increasing trend only in the mixed shrub-grassland and shrubland (Fig. 2).

The recovery of ^{15}N-tracer in soil largely decreased, mostly during the first year (Fig. 2). Mixed shrub-grassland showed the greatest decrease, with less than half the recovery of ^{15}N compared to the other plant communities, and this low value persisted until the end of the experiment. Thus, at 9 months

Table 2 Organic C, total N and C:N ratio in the top 5 cm of soil. Different letters indicate significant differences between burned and unburned plots per time point ($p<0.05$). Values are means (±SE) ($n=5–6$)

	Time after fires			
	3 days		12 years	
	Unburned	Burned	Unburned	Burned
Grassland				
Organic C (g m^{-2})	899.6 (61.0)	1010.9 (42.5)	887.6 (91.2)	1026.4 (70.2)
Total N (g m^{-2})	98.6 (5.3)	102.1 (8.9)	119.8 (11.4)	105.6 (7.9)
C:N	9.1 (0.4)	10.2 (0.7)	7.5 **a** (0.4)	9.8 **b** (0.4)
Mixed shrub-grassland				
Organic C (g m^{-2})	772.7 **a** (90.0)	1098.2 **b** (51.4)	1373.8 (151.9)	1127.4 (70.9)
Total N (g m^{-2})	74.2 (6.1)	88.3 (4.3)	123.3 (10.9)	104.3 (5.0)
C:N	9.8 (1.1)	12.4 (0.5)	10.7 (0.5)	10.8 (0.5)
Shrubland				
Organic C (g m^{-2})	919.7 (60.5)	1141.4 (99.6)	1248.0 (131.9)	1187.4 (60.0)
Total N (g m^{-2})	74.8 (7.3)	86.0 (6.4)	111.0 (12.1)	101.3 (5.0)
C:N	12.7 **a** (1.1)	13.1 **b** (0.7)	11.1 (0.5)	11.6 (0.6)

Table 3 N-NH_4^+, N-NO_3^- and N mineral in the top 5 cm of soil in unburned and burned unlabelled plots 3 days after fire. Different letters indicate significant differences between unburned and burned plots ($p<0.05$). Values are means (±SE) ($n=6$)

	N-NH_4^+ (μg g soil^{-1})		N-NO_3^- (μg g soil^{-1})		N mineral (μg g soil^{-1})	
	Unburned	Burned	Unburned	Burned	Unburned	Burned
Grassland	2.79 (0.37)	3.85 (0.62)	1.16 (0.46)	2.56 (0.53)	3.95 (0.75)	6.41 (1.07)
Mixed shrub-grassland	2.44 **a** (0.32)	8.30 **b** (1.24)	1.44 (0.43)	2.59 (0.57)	3.89 **a** (0.60)	10.89 **b** (1.17)
Shrubland	0.18 **a** (0.04)	3.28 **b** (0.90)	0.36 (0.16)	0.36 (0.10)	0.55 **a** (0.18)	3.64 **b** (0.98)

post-fire, ^{15}N-recovery in the top 2.5 cm of soil was 23 % in grassland, 9 % in mixed shrub-grassland and 17 % in shrubland. At 6 years post-fire, between 10 and 17 % of the ^{15}N-recovery (depending on the vegetation) remained in the uppermost layer while between 22 and 34 % was recovered from the deeper layers (2.5–5; 5–15 and 15–30 cm). In the deepest layers, we observed slight decreases in ^{15}N-recovery between years 6 and 12 post-fire in grassland (15–30 cm layer) and in mixed shrub-grassland (5–30 cm layer) (Fig. 2). For grassland and shrubland, pooling of the top three layers (0–15 cm soil) showed a significant increase in ^{15}N-recovery from year 6 to 12 post-fire ($p=0.039$ and $p=0.027$ respectively) while for mixed shrub-grassland this parameter remained unchanged ($p=0.205$).

^{15}N-enrichment in aboveground plant biomass

Twelve years after the fires, ^{15}N-enrichment was still high in plants in the labelled subplots. *R. officinalis* (the main shrub of the shrubland community) showed the highest values of ^{15}N-enrichment at 9 months and 12 years post-fire, followed closely by *B. retusum* (the main grass of grassland and mixed shrub-grassland communities) (Table 4). The N_2-fixing plant *G. scorpius* (the main shrub of mixed shrub-grassland) showed the lowest ^{15}N values, which, at the end of the experiment, were close to natural abundance. *B. retusum* growing in the same plots as *G. scorpius* (mixed shrub-grassland communities) showed slightly lower ^{15}N-enrichment and higher N content than *B. retusum* grown without the legume shrub.

Long-term ^{15}N-recovery in aboveground plant biomass and soils

Twelve years after fires, large amounts of ^{15}N-tracer, between 27 % and 63 %, were still retained in the first 30 cm of the soil profile (Table 5), whereas the recovery in aboveground plant was very low (<2 %; Table 5). Aboveground plant in shrubland showed the highest ^{15}N-recovery (1.7 %; Table 5), while the other plant species registered negligible recovery values (<0.1 %). The poor recovery values are attributed to either low ^{15}N-enrichment, as in *G. scorpius* in the mixed shrub-grassland, or low biomass values, as in *B. retusum* in the mixed shrub-grassland and also the pure grassland.

Discussion

Post-fire responses

As expected after fires of low to medium intensity (Almendros et al. 1988; Almendros et al. 1990; Knicker et al. 2005), the short-term changes in organic C and total N in the upper 5 cm of soil were negligible (Table 2). However, N-NH_4^+ in the top 5 cm of soil increased by 340 % in the mixed shrub-grassland and by 1784 % in the shrubland 3 days post-fire compared with unburned plots. These increases could be explained by the deposition of ashes and the increases in soil temperature (Raison 1979; Rapp 1990). No changes were detected in the grassland. This finding could be attributed to the lower fire intensity in this community and its higher initial NH_4^+ content. A considerable amount of this pool remained in the first 30 cm of the soil 12 years after fire, with differences related to plant community, while the amount remaining in aboveground biomass was considerably lower (Table 5).

Most of the changes in N occurred in the first months after fire, possibly because of the loss of this mineral N by volatilization or leaching (Chorover et al. 1994; Guillon and Rapp 1989; Mackensen et al. 1996; Murphy et al. 2006) or plant uptake. Between

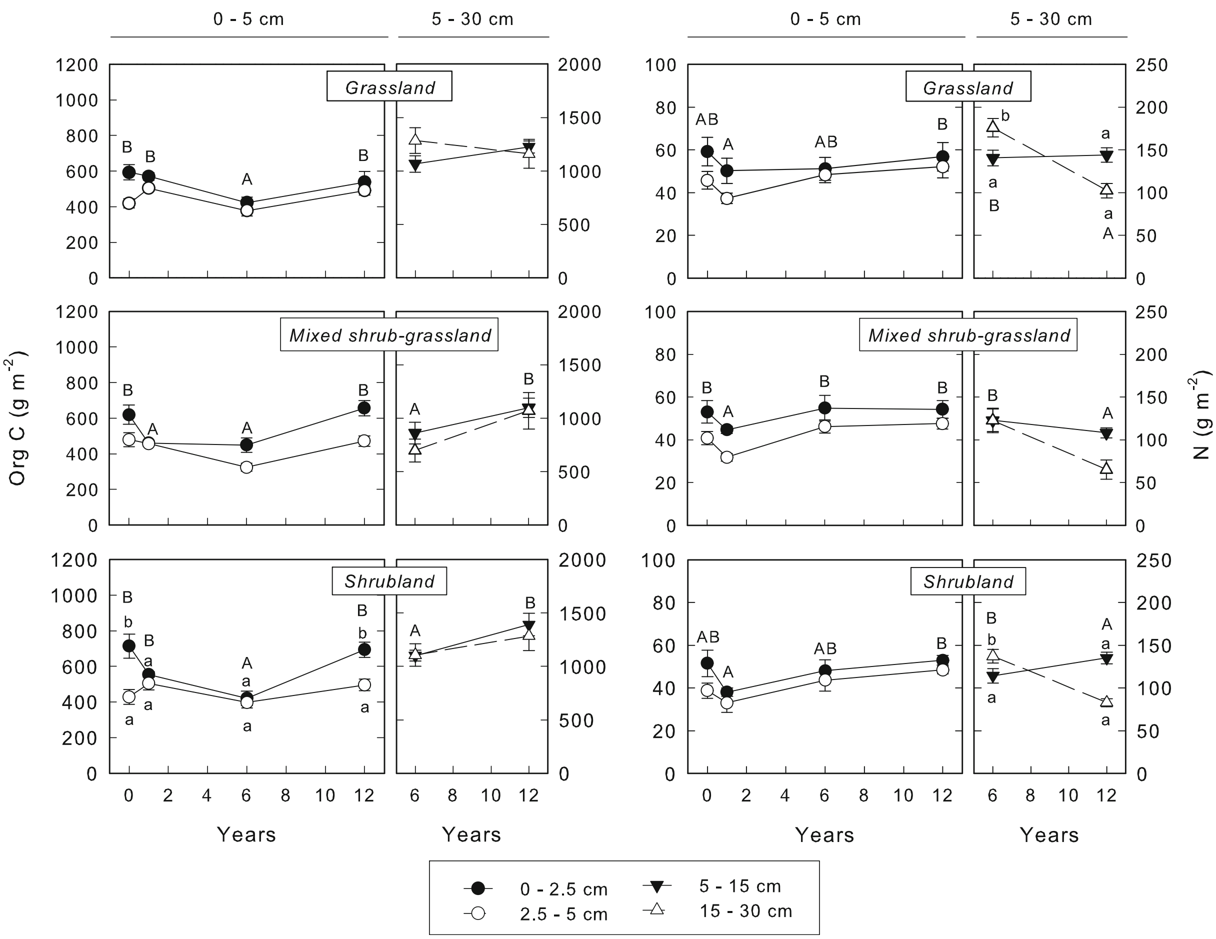

Fig. 1 Organic C and total N in soil at various depths and for each plant community (grassland, mixed shrub-grassland and shrubland) 3 days, 9 months, 6 years and 12 years after the fires. Capital letters indicate significant differences over time, including the two layers shown in each graph, and lower case letters indicate significant differences in each layer over time. Values are means (±SE) (n=5–6)

74 and 87 % of the labelled pool of N was lost from the top 5 cm soil layer during the first 9 months. Other studies on soil N retention after ^{15}N labelling have also reported large losses (up to 40 %) during the first days of the experiment (Seely and Lajtha 1997). In our study, nitrification processes readily occurred during the first weeks after fire, followed by N-NO_3^- leaching (Romanyà et al. 2001), as heavy rains (376 mm) fell during the 3 months after labelling.

Post-fire plant regrowth can also partly account for decreases in the soil pools of ^{15}N-tracer. However, given that at 9 months post-fire the grassland and mixed shrub-grassland showed similar plant cover (Casals et al. 2005), it would appear that the highest ^{15}N-tracer losses in soils of mixed shrub-grassland are not attributable to differences in plant regrowth and the consequent nutrient uptake. In contrast, these differences may be explained by soil processes. N-rich plant litter and burned residues of *G. scorpius* could increase N mineralization and nitrification. Increased N mineralization as a result of the addition of N-rich substrates has been widely reported (e.g. Madritch and Cardinale 2007; Kuzyakov et al. 2000). Furthermore, it has been proposed that N-enriched residues of N_2-fixing plants promote N loss in soil by denitrification (Baggs et al. 2000; Huang et al. 2004; Millar et al. 2004; Zhong et al. 2011). Romanyà et al. (2001) did not find a short-term increase in nitrification in a mixed shrub-grassland community after fire, but this plant community showed much higher values of mineral N (Table 3) after fires than the others. Thus leaching and denitrification processes could be

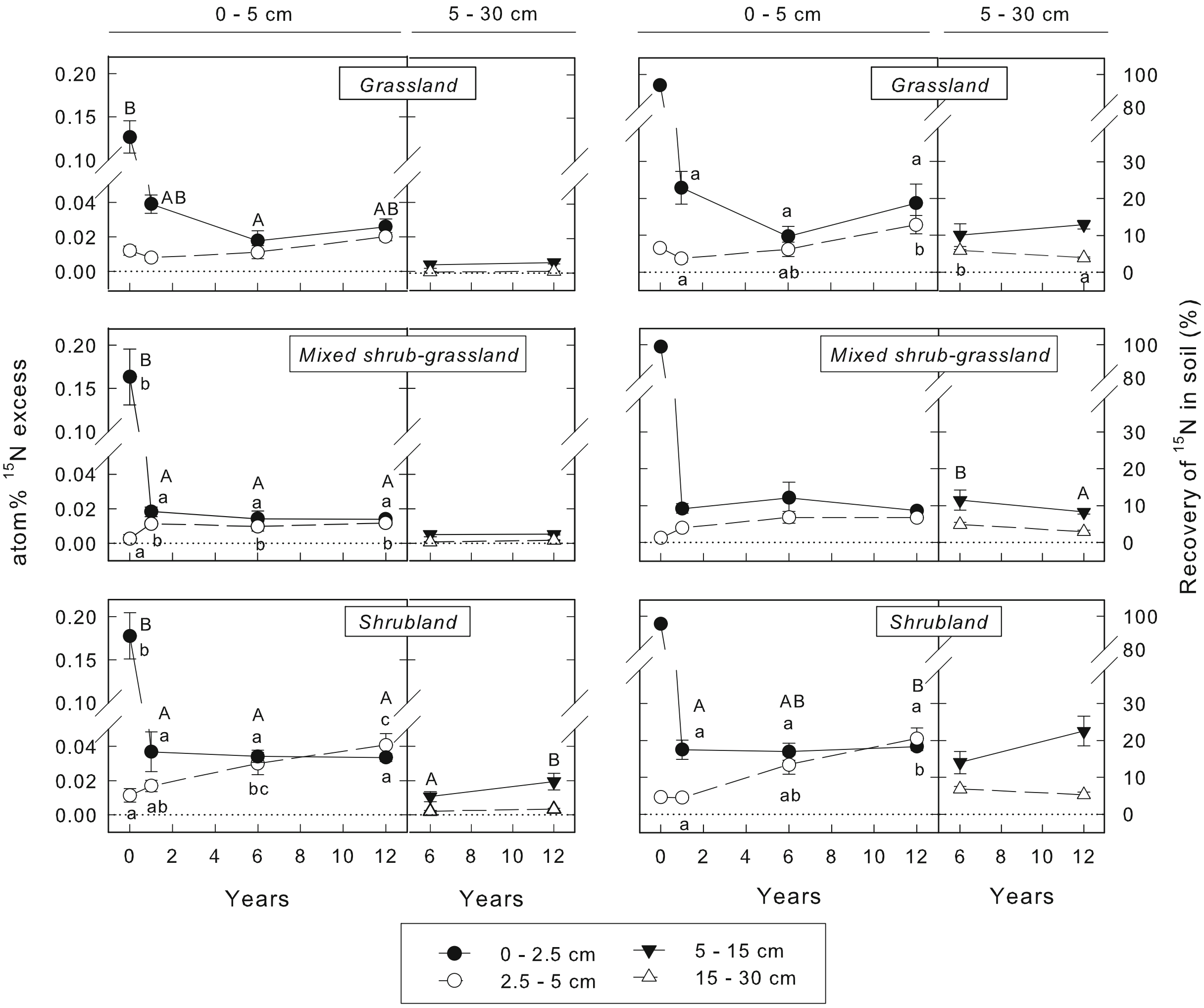

Fig. 2 ^{15}N-enrichment (Atom% ^{15}N excess) and recovery of ^{15}N (%) of the surface soil 3 days after fire, at various depths and for each plant community (grassland, mixed shrub-grassland and shrubland) 3 days, 9 months, 6 years and 12 years after fire. Capital letters indicate significant differences over time, including the two layers shown in each graph, and lower case letters indicate significant differences in each layer over time. Values are means (±SE) (n=5–6)

involved in ^{15}N-tracer (as $^{15}N\text{-}NH_4^+$ or $^{15}N\text{-}NO_3^-$) loss, as high amounts of available mineral N were in the soil during the first months after fire when no plant uptake was possible.

Long-term changes in soil C and N pools and recycling of ^{15}N-tracer: effects of drought periods

The organic C in the top soil increased in all plant communities between years 6 and 12 post-fire; however, similar increases for total N were not observed (Fig. 1). As suggested in Martí-Roura et al. (2011), large amounts of organic matter might have been deposited in this time as a result of a severe drought period (Table 1), which affected mainly plant communities with shrubs. These changes might be the result of an increase in belowground deposition, thus affecting the various soil layers. In the pure grassland, the increases in organic C affected only the top 5 cm of soil, where the rhizomes of this resprouting species occur. We therefore suggest that the drought-induced belowground deposition includes coarse roots in plant communities with woody plants and rhizomes of the *B. retusum* grassland. Coarse belowground plant material is generally rich in cellulose, hemicellulose or lignin and has a high C:N ratio (Birouste et al. 2012;

Table 4 Aboveground plant ^{15}N enrichment 9 months after the fires and biomass, total N and ^{15}N enrichment 12 years after the fires in plants growing in labelled plots. Values are from the dominant plants per each plant community. Within a column, values with different letter are significantly different ($p<0.05$). Values are means (±SE) (n=5–6)

	Time after fires			
	9 months	12 years		
	$\delta^{15}N$ (‰)	Biomass ($g\ m^{-2}$)	Total N ($mg\ N\ g\ plant^{-1}$)	$\delta^{15}N$ (‰)
Grassland				
B. retusum	381.3 **b** (49.0)	76 **a** (14)	7.8 **a** (0.1)	34.0 **b** (5.0)
Mixed shrub-grassland				
G. scorpius	24.7 **a** (7.3)	334 **b** (53)	8.2 **b** (0.2)	2.8 **a** (0.2)
B. retusum	326.5 **b** (22.2)	121 **a** (50)	8.5 **b** (0.2)	23.1 **b** (4.4)
Shrubland				
R. officinalis	916.7 **c** (142.0)	1361 **c** (284)	6.6 **a** (0.4)	47.6 **c** (4.1)

Goebel et al. 2011; Pregitzer et al. 2002). Thus, coarse roots and rhizome deposition contribute little to the soil N pool. Moreover, new inputs of labile organic matter may promote microbial activity and mineralization of the soil N reserve. This mineralized N can be immobilized in the soil organic matter, lost by leaching or denitrification or taken up by plants. Net N mineralization will be especially high in soils with the lowest C:N ratio, as is the case of the 15–30 cm layer. In this case, increased N mineralization could account for the large decreases in N pools detected in the 15–30 cm layer in all the vegetation types. These decreases barely affected the ^{15}N-tracer pool, which showed minor or no decreases in the deepest layer. In contrast, in the top 15 cm of soil, we did not observe any effect of drought on total N but ^{15}N-recovery increased during the drought in shrubland and grassland communities (Fig. 2). This increase in ^{15}N indicates the incorporation of ^{15}N pulses from plant necromass in soil organic matter and points to immobilization processes occurring in the first 15 cm of the soil. Furthermore, these observations support our hypothesis to explain soil organic C increases during this period (Martí-Roura et al. 2011). This increase in ^{15}N-recovery occurred only in plant communities without legume shrubs. Although the inputs of ^{15}N-impoverished plant litter of the N_2-fixing *G. scorpius* could partly explain these low values, it must be considered that the *B. retusum* sward growing in these mixed shrub-grassland community showed high $\delta^{15}N$ values, close to those of the pure grassland, and a similar biomass and ^{15}N-recovery in plants at 12 years post-fire (Table 4). Thus, we could expect that ^{15}N-recycling in the mixed shrub-grassland through *B. retusum* litter would behave similarly to that of the pure grassland. As this was not the case, we propose that the low retention of the ^{15}N-tracer in this N_2-fixing community is caused by the enhancement of ^{15}N loss during the decomposition of plant debris.

Legumes are an important component of post-fire successional communities (Crews 1999). These plants are able to use the increased mineral N pools caused by fire (Vitousek and Howarth 1991), but they can also provide a post-fire net N input by introducing fixed atmospheric N_2 (Hendricks and Boring 1999). The enhanced loss of N in the N-fixing plant community post-fire was negligible (less than 0.2 kg m^{-2}) in terms of plant–soil N-recycling rate. Net N input

Table 5 Recovery of ^{15}N (%) in soil and plants 12 years after fire. Values are means (±SE) (n=5–6)

	Soil	Aboveground plant		
	0–30 cm	*R. officinalis*	*G. scorpius*	*B. retusum*
Grassland	48.4 (7.8)	–	–	0.09 (0.01)
Mixed-Shrub-grassland	26.6 (1.5)	–	0.07 (0.01)	0.10 (0.03)
Shrubland	62.7 (7.8)	1.70 (0.32)	–	–

provided by legumes post-fire will far exceed this loss (Casals et al. 2005). Indeed, no loss of total N was observed in this plant community, and 12 years after fire the non-N-fixing plant *B. retusum* in this community had a higher N content than that found in pure grassland. This observation supports the notion that legumes enhance the regeneration of the whole community.

In conclusion, 12 years after the fires, more than half of the initial ^{15}N-tracer was still retained in the soils in communities with no legume shrubs. At that time, plants still contained part of the labelled ^{15}N-NH_4^+ released after fires, with the exception of N_2-fixing shrubs. The presence of legume shrubs favoured loss of the ^{15}N-tracer during the first year after fire and during the drought period, which took place several years post-fire. In Mediterranean grassland and shrubland without N_2-fixing shrubs, extreme climatic conditions, such as drought, favours the soil incorporation of plant N pools that were taken up from soils shortly after fire. The accumulation of belowground necromass under these conditions may favour the accrual of soil organic C to a greater extent than that of N.

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