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## Abstract

Wildfires often modify soil properties, including the N status and net N mineralization rates, but their impacts on gross N fluxes have been scarcely evaluated. We aimed to ascertain the immediate effects of a medium-high severity wildfire on soil N transformations. Net and gross N rates were analytically and numerically (FLUAZ) quantified in burned (BS) and unburned (US) topsoils from the temperate-humid region (NW Spain). Analytical and numerical solutions were significantly correlated for both gross N mineralization ( $m$ ) ( $r^2=0.815$ ;  $p<0.001$ ) and gross nitrification ( $n$ ) ( $r^2=0.950$ ;  $p<0.001$ ). In BS, all  $\text{NH}_4^+$ -N fluxes (net  $m$ , gross  $m$  and gross  $\text{NH}_4^+$ -N immobilization,  $ia$ ) increased, while those of  $\text{NO}_3^-$ -N decreased (gross  $n$  and gross  $\text{NO}_3^-$ -N immobilization,  $in$ ) or did not vary (net  $n$ ). In US and BS, gross  $m$  (0.26-3.60 and 4.70-15.42  $\text{mg N kg}^{-1} \text{d}^{-1}$ , respectively) predominated over gross  $n$  (0.026-2.45 and 0.001-0.002  $\text{mg N kg}^{-1} \text{d}^{-1}$ , respectively), and the same was true for the net fluxes. Compared with the few available data on recently burned soils ( $m=8-55 \text{ mg N kg}^{-1} \text{d}^{-1}$ ;  $n=0.50-1.83 \text{ mg N kg}^{-1} \text{d}^{-1}$ ), our gross  $m$  and  $n$  rates were similar and very low, respectively; gross  $n$  showed that nitrifiers were active in US and also in BS, despite the 98 % reduction observed immediately after the fire. For gross fluxes,  $m$  increased more than  $ia$  suggesting an  $\text{NH}_4^+$ -N accumulation, but there is no risk of  $\text{NO}_3^-$ -N leaching because  $n$  decreased more than  $in$ .

**Keywords:** N mineralization; gross N fluxes; net N fluxes;  $\text{NH}_4^+$ -N immobilization;  $\text{NO}_3^-$ -N immobilization; extractable nutrients

## Introduction

Fire is a strong widespread driving force of renewal in some forests, but also a factor of forest destruction and soil degradation by accelerating the processes of soil and nutrient losses through volatilization, leaching and erosion (Certini 2005). Nitrogen, the major growth-limiting nutrient, is also the element most prone to direct losses to the atmosphere by combustion,  $\text{NH}_3$  volatilization or denitrification (Fisher and Binkley 2000); consequently, important particulated and gaseous ( $\text{NH}_3$ ,  $\text{NO}_x$ ) N losses due to wildfires have been frequently reported (Prieto-Fernandez et al. 2004; Certini 2005). Wildfires also modify the soil organic N distribution, increasing the most recalcitrant forms and decreasing the most labile ones, which are the major source of N for soil microorganisms and plants (Prieto-Fernández et al. 1993).

Soil inorganic N usually increase after fires and, among the factors involved, the organic- to inorganic-N conversion by the heating/combustion processes and an enhancement of N mineralization rate have

been reported (Prieto-Fernández et al. 1993; Certini 2005). However, the underlying mechanisms are not well understood due to the dynamic nature of soil inorganic N cycling and to assess these mechanisms gross N transformation rates must be measured (Koyama et al. 2010). Quite surprisingly, the impact of fires on gross N fluxes has been scarcely studied. The revision of published works on this topic shows that in all cases analytical methods instead of numerical procedures were used to calculate the gross N fluxes, despite the advantages of the latter (Murphy et al. 2003). Only the two first published articles quantified the immediate effects of fire on gross N fluxes, while three studies measured the gross N rates 6-12 months after the fire and the other six papers focussed on fire effects at the long-term (see Table 1). Most studies reported an increase of gross N mineralization during the first 1-2 years after the fire, followed by a decrease in subsequent years (Table 1). No short-term information is available about fire effects on  $\text{NH}_4^+$ -N immobilization, while from 1 year after the fire the published results showed either no changes or a decrease (Table 1).

Irrespectively of the time after fire, a lack of changes has been usually reported for the gross nitrification rate, but decreases and increases have also been found (Table 1). Like for  $\text{NH}_4^+$ -N, no short-term data

are available about fire effects on gross  $\text{NO}_3^-$ -N immobilization, whereas three out four published paper reported a significant decrease one year after the fire and the other indicated no changes (Table 1).

Table 1. Summary of cited research about fire effects on gross N fluxes.

	Time from fire	Ecosystem	Soil depth (cm)	Incubation	Soil C ( $\text{g kg}^{-1}$ )	Soil N ( $\text{g kg}^{-1}$ )	Fire effect			
							<i>m</i>	<i>ia</i>	<i>n</i>	<i>in</i>
Neill et al. (1999) <sup>a</sup>	5 d	Tropical forest and pasture	0-5	lab	13.5	1.3	ns		ns	
Anderson and Poth (1998) <sup>a</sup>	17-45 d	Dry tropical shrubland	0-5	<i>in situ</i>			↗		↘	
Aranibar et al. (2003)	6 m	Woodland, savanna	0-5	<i>in situ</i>	13.5	1.2	↗		ns	
Dannemann et al. (2011)	6 m	Mediterranean shrubland	0-4	lab			↗		↗	
Kaye and Hart (1998) <sup>b</sup>	1 y	Montane pine forest	0-15	<i>in situ</i>	39	1	↗	ns	ns	↘
Koyama et al. (2010) <sup>c</sup>	2 y	Coniferous montane forest	0-10	<i>in situ</i>			↘	↘	ns	↘
Koyama et al. (2012)	2 y	Coniferous montane forest	0-10	lab		1.3-2.7	↘	↘	ns	↘
Bastias et al. (2006)	2-4 y	Wet sclerophyll forest	0-10	lab	37-66	1-1.2	↘		↘	
LeDuc and Rothstein (2007)	3-6 y	Pine forest	0-10	lab	23	1	ns	ns	ns	ns
Ball et al. (2010)	12 y	Dry montane forest	0-5	<i>in situ</i>					↗	
Smithwick et al. (2005) <sup>d</sup>	50-350 y	Coniferous montane forest	0-15	lab	24-26	0.7-0.8				

Note: <sup>a</sup>Unburned and burned soils sampled in April and September, respectively. <sup>b</sup> Prescribed fire. <sup>c</sup> Only wildfire data were considered. <sup>d</sup> No available data on unburned soils.

The scarcity of studies about the immediate or short-term effects of fire on soil gross N fluxes contrasts with the almost balanced proportion between short- and long-term studies devoted to burned soils. Besides the difficulties for quick action (site selection and soil sampling) after burning events, the widely unbalanced proportion may be due to the difficulties of meeting the prerequisites of  $^{15}\text{N}$  pool dilution in recently burned soils. In contrast to net measurements, the  $^{15}\text{N}$  pool dilution technique provides information on N actually mineralized, nitrified and immobilized as separated processes (Murphy et al., 2003). One of the three key assumptions of this technique is the uniform distribution of the applied  $^{15}\text{N}$ -label throughout the soil, to achieve uniform  $^{15}\text{N}$ -enrichment of the inorganic N pool in question (Murphy et al. 2003). To meet this assumption can be a serious challenge in

recently burned soils due to water repellency, which reduces the affinity of soils to water such that they resist wetting for periods up to hours, days or weeks. Soil water repellency is a world wide phenomena caused partly or entirely by hydrophobic, long-chained organic molecules, released from decomposing or burning plant litter (Doerr et al. 2000). Fire can induce, enhance or destroy soil water repellency depending on the temperature reached and its duration, but surface water repellency is usually higher in burned than unburned land (Doerr et al. 2000). Moreover, in contrast to what was generally assumed, Bodí et al. (2011) recently reported that one third of ash samples from wildfire sites exhibited water repellency ranging from low to extreme.

Accordingly, once solved the problem of soil water

repellency, the main objectives of present paper were: a) to quantify gross N (ammonification, nitrification,  $\text{NH}_4^+$ -N immobilization,  $\text{NO}_3^-$ -N immobilization) transformation rates immediately after a wildfire, calculated by employing both the classical analytical method of Kirkham and Bartholomew (1954) and the numerical procedure of FLUAZ model (Mary et al. 1998); and b) to compare the patterns of gross and net N. Equations of Kirkham and Bartholomew (1954) were the first proposed to calculate two simultaneous N fluxes (production and consumption), while FLUAZ combines a numerical integration method with a nonlinear fitting method to calculate the fluxes between four N pools ( $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, organic N and biomass N).

## Material and methods

### Site description

The experimental field was located close to the Montes do Invernadeiro Natural Park (UTM 29 T PG34168-71422, 1566 m asl) at Laza (Galicia, NW Spain). The area has a temperate (mean annual temperature: 6-8 °C) and rainy (1600-1800 mm  $\text{y}^{-1}$ ) climate. In the first four days of September 2010, the area was affected by a wildfire (1700 ha) that consumed the continuous litter layer (4-8 cm thick), leading to a burned surface with 42 % of bare soil and 58 % of soil covered by a 0-1 cm layer of white and black ashes and charred plant debris.

A hillslope area was selected for study taking advantage of its unusual characteristics in the case of wildfires: within a radius of 50 m, an unburned control soil and a soil affected by a fire of medium-high severity were available. The whole studied area was very homogeneous in terms of: a) orientation (245-250° WSW); b) slope (26-30 %); c) soil type, an Entisol (IUSS Working Group 2006) developed over metamorphic rocks (phyllites) and with silt loam texture; and d) vegetation cover, dominated by a 7-year-old *Pinus sylvestris* L. plantation (1.0-1.6 m height) with shrubland mainly of *Erica* spp., *Vaccinium myrtillus* L., *Pterospartum tridentatum* Willk. and *Cistus* spp. Two treatments with three replications were arranged in six experimental plots (4 x 20 m with 3 m separation), established between planting rows with the longest dimension parallel to the maximum slope. Three of them were installed in the burned area (BS) and the other three in that unburned (US).

### Soil sampling

A week after the wildfire and before any rain event, top soil was sampled from the A horizon (0-2 cm depth). In each plot, ten squares (15 x 15 cm) uniformly distributed were taken after removing the plant litter of unburned (US) or the ash layer of burned plots (BS), and they were combined to obtain a composite sample. The field-moist soil was sieved (< 2 mm), homogenised and stored at 4 °C for soil incubation purposes. Sub-samples were air-dried for chemical analysis.

### Chemical analysis

Soil pH was measured with a pH-meter (MetröhM, Switzerland) in  $\text{H}_2\text{O}$  and KCl employing a soil:solution ratio of 1:2.5. Electrical conductivity was measured in 1:5 soil:water filtered extracts with a conductometer (MetröhM, Switzerland) using 0.01 M KCl as reference solution and type I water (electrical conductivity max: 0.056  $\mu\text{S cm}^{-1}$  at 25 °C) for all aqueous solutions. Total C, total N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were measured on finely ground soil samples (< 100  $\mu\text{m}$ ) with an elemental analyser (Carlo Erba CNS 1508) coupled on-line with an isotopic ratio mass spectrometer (Finnigan Mat, delta C, Bremen, Germany). For analyses of extractable Na, K, Mg, Ca, P, Al, Fe, Mn, Zn, Cu, Co, B and Mo, soils (10 g) were shaken for 2 h with a mixture of 1 M  $\text{NH}_4\text{Ac}$  and 0.005 M DTPA as extracting solution (1:5 soil:solution ratio); the extracts were filtered through cellulose paper (Filter-Laboratory 1242, 90-mm diameter) and then analysed by simultaneous ICP-OES (Varian Vista Pro, Mulgrave, Australia). All analyses were carried out in duplicate and the mean of both analyses for each replicate was used in the statistical procedure.

### Soil incubation and gross N transformation rates

The Water Drop Penetration Time (WDPT) test (Wessel 1988) was used to measure the water repellency of the soils (with initial moisture content of 22% and 10% for US and BS, respectively): three drops of distilled water (~0.05 mL) were placed on the surface of each fresh burned or unburned soil sample and the time required for their complete penetration was recorded; sample WDPT being the average value of the three drops. With a WDPT between 11000 and 14000 s, both the unburned and the burned soil exceeded three- to four-fold the threshold of extreme water repellency (WDPT > 3600 s; Bodí et al. 2011); under these circumstances, soil

rewetting to an adequate soil moisture level for the incubation experiment will take several days, leading to an undesired soil preincubation.

After discarding the use of any wetting agent or the employ of a hot vapour source, which will inevitably alter the soil, we carried out a preliminary test with an ultrasonic humidifier (35 W), observing that both the unburned and the burned soil were wettable (WDPT < 5 s; Bodí et al. 2011) by the tiny drops of water vapour (1-10 mm vs 0.3-1 mm for drops of nebulized water). Consequently, a wetting system was setup as follows: the water vapour exit of a humidifier, equipped with an *ad hoc* joint, was connected by flexible tubes (1.1 cm int. Ø) to three plastic bells (35 cm Ø), that were placed over three plastic trays, each one containing 350 g of soil. By supplying an excess of water vapour (that was easily evacuated through the space between bells and trays), the unburned and the burned soils absorbed the amount of water (65-70 g) needed for adjusting soil humidity prior <sup>15</sup>N labelling; the wetting process, monitored by weighting periodically the soil+tray, took around 5-6 h.

Gross N transformation rates were determined by the <sup>15</sup>N isotope dilution technique, using a paired labelling experiment with NH<sub>4</sub>NO<sub>3</sub> in which half the plots receive it as <sup>15</sup>NH<sub>4</sub>NO<sub>3</sub> and the other half as NH<sub>4</sub><sup>15</sup>NO<sub>3</sub>; the size and <sup>15</sup>N abundance of the soil NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N pools were determined at different times of incubation (Barraclough 1991), allowing to calculate not only the gross rates of mineralization and nitrification, but also to quantify the immobilization of ammonium and nitrate separately. After wetting up to 60% of WHC (equivalent to a soil moisture level of 51-57% and 35-37% for US and BS, respectively), thirty subsamples of each composite soil sample (fresh soil equivalent to 20 g dw) were placed in 250-mL flasks, totalling 180 flasks: 2 treatments (burned and unburned) × 3 experimental plots × 2 labels × 3 replicates × 5 extraction times. Half of the flasks were labelled with 1 mL of <sup>15</sup>NH<sub>4</sub>NO<sub>3</sub> by flask and the other set with 1 mL of NH<sub>4</sub><sup>15</sup>NO<sub>3</sub>, equivalent to a N addition of 312 µg kg<sup>-1</sup> dw with 98 atom % excess of <sup>15</sup>N. This N addition increased by 17-18% the soil NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N pools in US and by 0.2% (NH<sub>4</sub><sup>+</sup>-N) and 20% (NO<sub>3</sub><sup>-</sup>-N) in BS. The <sup>15</sup>N-labelled solution was added uniformly over the soil surface with an automatic pipette; the solution readily disappeared (< 5 s) from soil surface. Then, to facilitate the distribution of label in the soil and for adjusting soil moisture to 70% of water

holding capacity, 2 mL of dionized water were added with an automatic pipette. The flasks were covered with polyethylene film and incubated in the dark at 24 °C. Five extraction times were selected within a 14 days period: 0.5 h and 1, 3, 7 and 14 days. Each incubated soil sample was shaken for 1 h with 100 mL of 2 M KCl and filtered through glass microfibre filters (Whatman GF/A, 125 diameter). After repeated washes with deionized water to remove the KCl and inorganic N, the soil retained in the filter was oven dried (105 °C), finely ground (< 100 µm) and analysed for total N and <sup>15</sup>N as previously described. The inorganic N content was analyzed by a modified diffusion method (Khan et al. 1997). In order to sequentially liberate NH<sub>4</sub><sup>+</sup>-N and NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup>-N in the form of NH<sub>3</sub>, 50 ml aliquots of soil extracts, placed in a 500 ml wide-mouth glass jar, were treated successively (24 h periods at 55 °C) with MgO (0.1 g) to release and trap NH<sub>3</sub> into H<sub>2</sub>SO<sub>4</sub> and followed by a second dose of MgO (0.1 g) plus Devarda's alloy (0.4 g) to the same extract for conversion of NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>-N to NH<sub>3</sub>. Gaseous NH<sub>3</sub> was recovered into 10 ml of 0.004 M H<sub>2</sub>SO<sub>4</sub> in a Teflon bottle attached to the glass jar and measured by back titration of the excess of H<sub>2</sub>SO<sub>4</sub> with 0.004 M NaOH. After titration, the resulting (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solutions were acidified with 1 mL of 0.004 M H<sub>2</sub>SO<sub>4</sub> and evaporated to dryness at 60 °C in a vacuum oven (Mettler VO400, PM400) for obtaining (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> crystals. To accelerate the drying process, the oven was alternatively under vacuum (15 kPa) and atmospheric pressure; in order to trap possible traces of atmospheric NH<sub>3</sub>, the incoming air was passed through a column of activated charcoal. The (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> crystals were finally packed into tin capsules and analyzed for <sup>15</sup>N as previously explained for soils.

#### *Calculation and statistical analysis*

Mean values (N amount and <sup>15</sup>N enrichment) of the three subsamples destructively sampled at t= 0, 1, 3, 7 and 14 days were used to calculate the gross N rates [mineralization (*m*), nitrification (*n*), immobilization of ammonium (*ia*) and immobilization of nitrate (*in*)] using the classical analytical method of Kirkham and Bartholomew (1954) and the numerical model FLUAZ, version 8 (Mary et al. 1998). For the FLUAZ model, the *m* and *i* rates were considered constant during each interval period (zero order kinetics), while *n* was assumed to follow a first-order kinetic (Mary et al. 1998). A fixed value of 0.05 was used for the parameter *b*, which denotes the relative proportion of NH<sub>4</sub><sup>+</sup>-N

immobilization compared with total immobilization (Mary et al. 1998). The fit of the experimental data was calculated by minimizing the MWE (mean weighted error) criterion and, following Mary et al. (1998) indications, we verified that all partial derivatives  $\partial\text{MWE}/\partial P$  were positive and that the correlation matrix between the fitted rate parameters did not have values close to 1. Initially, FLUAZ derived gross remineralization rates were also simulated to provide an estimate of remineralization. The simulated rates obtained showed a small remineralization only in the intervals 2, 3 and 4 of unburned soils, but all estimated rates were significantly correlated and the MWE was high. Thus, we used a fixed remineralization rate of  $0.01 \text{ mg kg}^{-1} \text{ d}^{-1}$  (the minimum input data accepted by FLUAZ) for these intervals.

Net N mineralization (net  $m$ ) for the whole incubation period was calculated as the difference between gross N immobilization and gross N mineralization derived from FLUAZ with changes in N pools between  $t=0$  and  $t=14$  d. Similarly, net nitrification (net  $n$ ) rates were calculated as the difference between gross  $\text{NO}_3^-$ -N immobilization and gross nitrification. Additionally, net rates were calculated by difference between  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N at each incubation time and their respective initial levels.

Data on extractable elements were statistically analysed by one-way ANOVA and those of gross N fluxes by two-way ANOVA (with treatment and time interval as factors). The Levene's test was used for verifying the equality of variances among groups. In the case of homocedasticity, significant differences among the mean groups were established at  $p < 0.05$  using the Bonferroni's test. In the case of unequal variances, the original data were subjected to the Tukey's ladder of power, or to Cox-Box transformations to obtain equality of variances and then significant differences among the mean groups were established at  $p < 0.05$  using the Bonferroni's test.

## Results

### *Chemical properties*

Compared to the unburned soil, significant changes in most soil characteristics were observed immediately after the wildfire (Table 2). The fire provoked a significant increase (about 0.5 units) in soil pH and electrical conductivity, while a significant

decrease was observed for organic C, total N contents and  $\delta^{15}\text{N}$  values and  $\delta^{13}\text{C}$  became slightly less negative. Among available macro-nutrients, the wildfire enhanced significantly the soil content of  $\text{NH}_4^+$ -N (70 times), Na, Ca and P, while no significant differences were found for  $\text{NO}_3^-$ -N and extractable K and Mg contents. Wildfire effects on available Al and micro-nutrients ranged from a significant reduction for Al, Fe and Co, to no effects for Zn, Cu, B and Mo, and to a significant increase for Mn.

Taking into account the percentage of variance explained ( $\eta^2$ , Table 2), the wildfire was responsible for 85-99 % of the variation in soil pH (in  $\text{H}_2\text{O}$  and KCl), electrical conductivity and contents of organic C, total N,  $\delta^{15}\text{N}$ ,  $\text{NH}_4^+$ -N, Ca, P and Mn ( $p < 0.05$  to  $p < 0.001$ ). Wildfire influence was lower on  $\delta^{13}\text{C}$  (76 % of variance explained), moderate on contents of Na, Al, Fe and Co (40-50% of variance explained;  $p < 0.10$ ) and not significant for the other studied nutrients.

### *N pools and atom % $^{15}\text{N}$ excess*

For each incubation period, Fig. 1 shows the  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N contents and their isotopic excess measured and FLUAZ-modelled. The measured values of pools and atom %  $^{15}\text{N}$  excess were generally well fitted with the FLUAZ model, with the exception of  $\text{NO}_3^-$ -N contents in US at  $t=1$  day (Fig. 1c) and  $^{15}\text{N}$ org excess in BS at  $t=3$  days (Fig. 1e). The observed recovery of  $^{15}\text{N}$  was approximately 100 % (Fig. 1f). Contents of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N gradually increased with time in burned soils and remained relatively stable in unburned soils.

The %  $^{15}\text{N}$  excess in the  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N pools decreased continuously during the incubation of unburned and burned soils (Fig. 1b), showing a continuous input of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N pools, respectively, at natural abundance that diluted the added tracer. During the first incubation interval, the faster decrease of the %  $^{15}\text{N}$  excess in the  $\text{NH}_4^+$ -N pool of unburned than burned soil was not due to a faster mineralization in the former, but to differences in the initial  $\text{NH}_4^+$ -N content. As the initial  $\text{NO}_3^-$ -N content was similar in unburned and burned soil (Fig. 1c), the faster decrease of the atom %  $^{15}\text{N}$  excess in the  $\text{NO}_3^-$ -N pools observed in the former (Fig. 1d) indicated a more active nitrification process.

The %  $^{15}\text{N}$  excess in the organic-N rose rapidly during the first incubation period (Fig. 1e), suggesting a fast immobilization of the added  $^{15}\text{NH}_4$ .

Table 2. Main initial soil characteristics (WHC, pH, EC,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and nutrient concentrations) in the unburned soil (US) and burned soil (BS) and results of the one way ANOVA. Values are mean  $\pm$ s.d (n=3). WHC: water holding capacity; EC: electrical conductivity.

	US	BS	<i>p</i>	$\eta^2$
WHC (g H <sub>2</sub> O kg <sup>-1</sup> )	899 $\pm$ 44	603 $\pm$ 12	0.001	0.974
pH H <sub>2</sub> O	3.68 $\pm$ 0.15	4.14 $\pm$ 0.06	0.001	0.857
pH KCl	2.81 $\pm$ 0.03	3.19 $\pm$ 0.08	0.001	0.912
EC ( $\mu\text{S cm}^{-1}$ )	15.8 $\pm$ 0.6	102.2 $\pm$ 7.3	0.001	0.982
Total C (g kg <sup>-1</sup> )	221 $\pm$ 7.2	169 $\pm$ 3.2	0.001	0.970
$\delta^{13}\text{C}$ (‰)	-25.8 $\pm$ 0.06	-25.7 $\pm$ 0.06	0.05	0.758
Total N (g kg <sup>-1</sup> )	12.1 $\pm$ 0.61	9.04 $\pm$ 0.30	0.001	0.939
$\delta^{15}\text{N}$ (‰)	1.69 $\pm$ 0.10	1.31 $\pm$ 0.03	0.05	0.907
NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> )	0.92 $\pm$ 0.18	65.82 $\pm$ 35.60	0.001	0.997
NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> )	0.85 $\pm$ 0.23	0.77 $\pm$ 0.03	n.s.	0.079
Extractable Na (mg kg <sup>-1</sup> )	28.5 $\pm$ 1.9	23.1 $\pm$ 3.1	0.10	0.439
Extractable K (mg kg <sup>-1</sup> )	182 $\pm$ 14	191 $\pm$ 12	n.s.	0.326
Extractable Mg (mg kg <sup>-1</sup> )	161 $\pm$ 13	167 $\pm$ 8	n.s.	0.208
Extractable Ca (mg kg <sup>-1</sup> )	428 $\pm$ 29	583 $\pm$ 43	0.001	0.873
Extractable P (mg kg <sup>-1</sup> )	8.3 $\pm$ 0.9	14.4 $\pm$ 1.0	0.001	0.955
Extractable Al (mg kg <sup>-1</sup> )	271 $\pm$ 20	220 $\pm$ 27	0.10	0.474
Extractable Fe (mg kg <sup>-1</sup> )	764 $\pm$ 81	644 $\pm$ 47	0.10	0.495
Extractable Mn (mg kg <sup>-1</sup> )	9.1 $\pm$ 1.7	29.9 $\pm$ 4.9	0.001	0.883
Extractable Zn (mg kg <sup>-1</sup> )	3.5 $\pm$ 0.4	3.2 $\pm$ 0.3	n.s.	0.061
Extractable Cu (mg kg <sup>-1</sup> )	0.63 $\pm$ 0.15	0.44 $\pm$ 0.04	n.s.	0.471
Extractable Co (mg kg <sup>-1</sup> )	0.31 $\pm$ 0.05	0.23 $\pm$ 0.05	0.10	0.416
Extractable B (mg kg <sup>-1</sup> )	0.31 $\pm$ 0.05	0.37 $\pm$ 0.07	n.s.	0.061
Extractable Mo (mg kg <sup>-1</sup> )	0.09 $\pm$ 0.01	0.09 $\pm$ 0.01	n.s.	0.363

#### *N transformation rates*

For all plots and time intervals (n=24), a significant correlation ( $r^2=0.815$ ) was found between the gross N mineralization rates obtained with the equation of Kirkham and Bartholomew (1954) (Fig. 2a) and the FLUAZ model (Fig. 3a), the relationship being almost perfect ( $r^2=0.996$ ) when one value (burned soil plot 3, interval 2) was removed. For the gross nitrification rate ( $n$ ), the correlation between the values obtained with the analytical (Fig. 2b) and the numerical (Fig. 3b) method was also significant (n=24;  $r^2=0.950$ ). Therefore, hereafter only data on FLUAZ modelled  $m$  and  $n$  were used. In all unburned plots, but not in the burned ones, best fittings with the FLUAZ model were achieved when a gross N remineralization rate,

fixed at 0.01 mg kg<sup>-1</sup> d<sup>-1</sup>, was included in time intervals 2, 3 and 4. The inclusion of such fixed rate leads to higher mineralization and immobilization rates in these intervals than without it.

Gross N mineralization rates decreased sustainedly from the beginning to the end of the incubation (Fig. 2a). The two-way ANOVA showed a strong effect of treatment (T), incubation interval (I) and T  $\times$  I interaction (partial  $\eta^2=0.978, 0.941$  and  $0.710$ , respectively;  $p < 0.001$ ) on the gross N mineralization rate ( $m$ ), which was significantly higher ( $p < 0.001$ ) in the burned than unburned soils for the four time intervals considered (Fig. 2a), although the differences decreased progressively from the first to the last one.

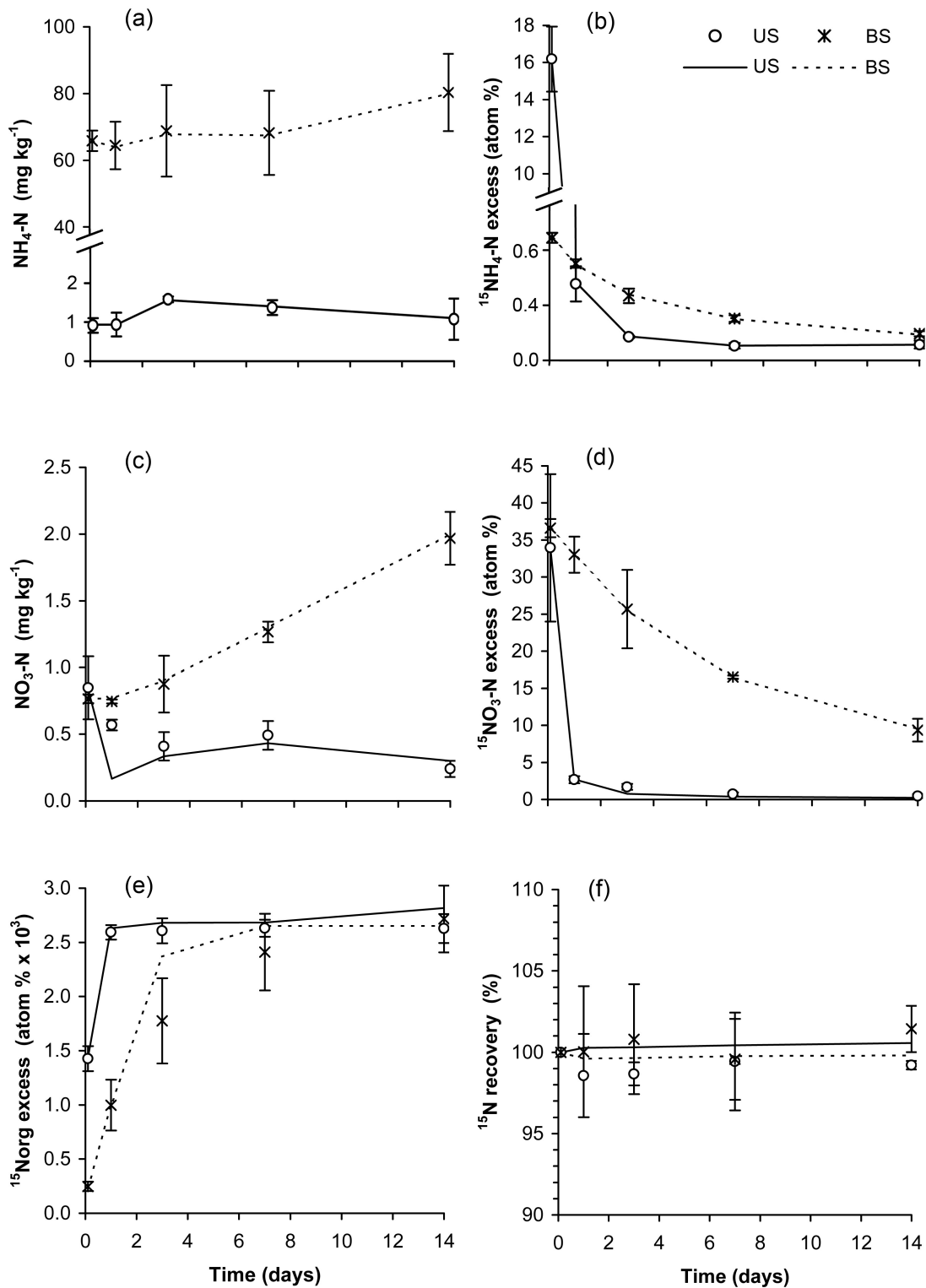


Figure 1. Soil content ( $\text{mg kg}^{-1}$ ) in  $\text{NH}_4^+\text{-N}$  (a) and  $\text{NO}_3^-\text{-N}$  (c), atom %  $^{15}\text{N}$  excess of  $\text{NH}_4^+\text{-N}$  (b),  $\text{NO}_3^-\text{-N}$  (d) and organic-N pools, and percentage of added  $^{15}\text{N}$  recovery (f), measured (symbols) and FLUAZ modelled (lines) during the incubation of unburned (US) and burned (BS) soils. Figures were made with mean values ( $n=3$ ) from  $^{15}\text{NH}_4\text{NO}_3$  experiment [(a), (b)],  $\text{NH}_4^{15}\text{NO}_3$  experiment [(c), (d)] and both experiments [(e), (f)].

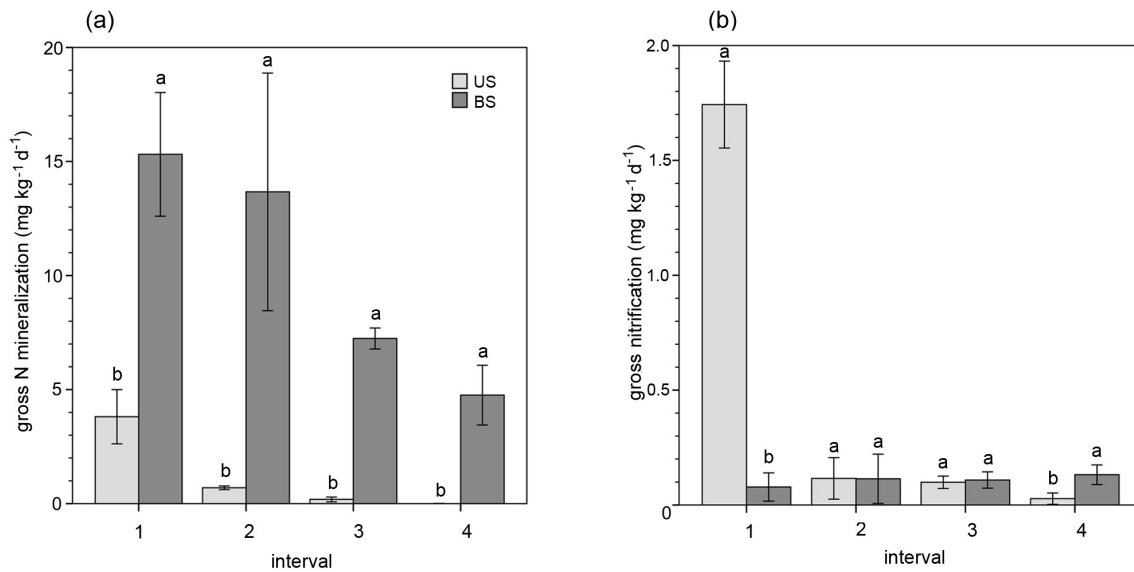


Figure 2. Gross rates ( $\text{mg N kg}^{-1} \text{ soil d}^{-1}$ ) of N mineralization (a) and nitrification (b) for each incubation period (interval 1: 0.5h-1d; interval 2: 1-3 d; interval 3: 3-7 d; interval 4: 7-14 d) in unburned (US) and burned (BS) soil estimated using the equations of Kirkham and Bartolomew (1954). Bars show  $\pm 1$  standard deviation. Within each time interval, different letters indicate significant differences among means ( $n=3$ ) at the  $p = 0.05$  level.

The paired  $^{15}\text{NH}_4\text{NO}_3$  and  $\text{NH}_4^{15}\text{NO}_3$  treatments allowed to calculate the individual  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N immobilization rates with the FLUAZ model. The  $\text{NH}_4^+$ -N immobilization accounted for 64-99% of total N immobilization in all intervals, except in the first one in unburned soils where  $\text{NO}_3^-$ -N immobilization represented 59% of the total immobilization. For both unburned and burned soil, the highest rates of gross  $\text{NH}_4^+$  immobilization were observed in the first interval. Patterns of gross  $\text{NH}_4^+$  immobilization rate ( $ia$ ) obtained with FLUAZ model were similar to that observed for  $m$ : the two-way ANOVA showed a strong effect of treatment, incubation interval and  $T \times I$  interaction (partial  $\eta^2 = 0.970, 0.892$  and  $0.724$ , respectively;  $p < 0.001$ ),  $ia$  being ever significantly higher ( $p < 0.001$ ) in the burned than unburned soils, although the differences decreased progressively from the first to the last time interval considered (Fig. 3c).

The same was true for the gross nitrification rate ( $n$ ), with partial  $\eta^2 = 0.970, 0.818$  and  $0.830$  for treatment, incubation and  $T \times I$  interaction, respectively (ANOVA-2). Nevertheless, contrarily to  $m$ ,  $n$  was ever significantly higher ( $p < 0.001$ ) in the unburned than burned soil and during the incubation it decreased continuously in the unburned soil while it remained quite stable at very low values in the

burned soil (Fig. 3b).

Like for the other gross N transformation rates, the two-way ANOVA also showed strong effects of treatment, incubation interval and  $T \times I$  interaction (partial  $\eta^2 = 0.934, 0.975$  and  $0.970$ , respectively;  $p < 0.001$ ) on the gross  $\text{NO}_3^-$ -N immobilization rate ( $in$ ) calculated with FLUAZ. Nonetheless, only one significant difference between treatments was found for  $in$ , higher values being observed in unburned than burned soil in the first incubation interval (Fig. 3d).

In unburned soils, gross N immobilization rates were lower than gross N mineralization rates, resulting in net mineralization (Table 3). In contrast, the relatively higher gross N immobilization of burned soils lead to net immobilization during the first half of the incubation, although a strong variability among plots was observed. Except for the first interval in unburned soils,  $\text{NO}_3^-$ -N immobilization was higher than nitrification, leading to negative net nitrification. Similar trends were obtained when rates were calculated by differences in the mineral N content (Table 3 and Online Resource 1). Data for the whole incubation period, obtained by running FLUAZ with change in pools between  $t=0$  and  $t=14$  d, showed that both  $m$  ( $\eta^2 = 0.992$ ;  $p < 0.001$ ) and  $n$  ( $\eta^2 = 0.875$ ;  $p < 0.01$ ) were strongly influenced by



the wildfire, with the highest values in the burned and the unburned soil, respectively (Table 3). A similar result was found for the net N mineralization rate calculated with changes in N pools between  $t=0$  and  $t=14$  d ( $\eta^2= 0.928$ ;  $p< 0.01$ ), but not for the other

intervals and for the net nitrification that was not significantly affected by the fire in all intervals period (Table 3).

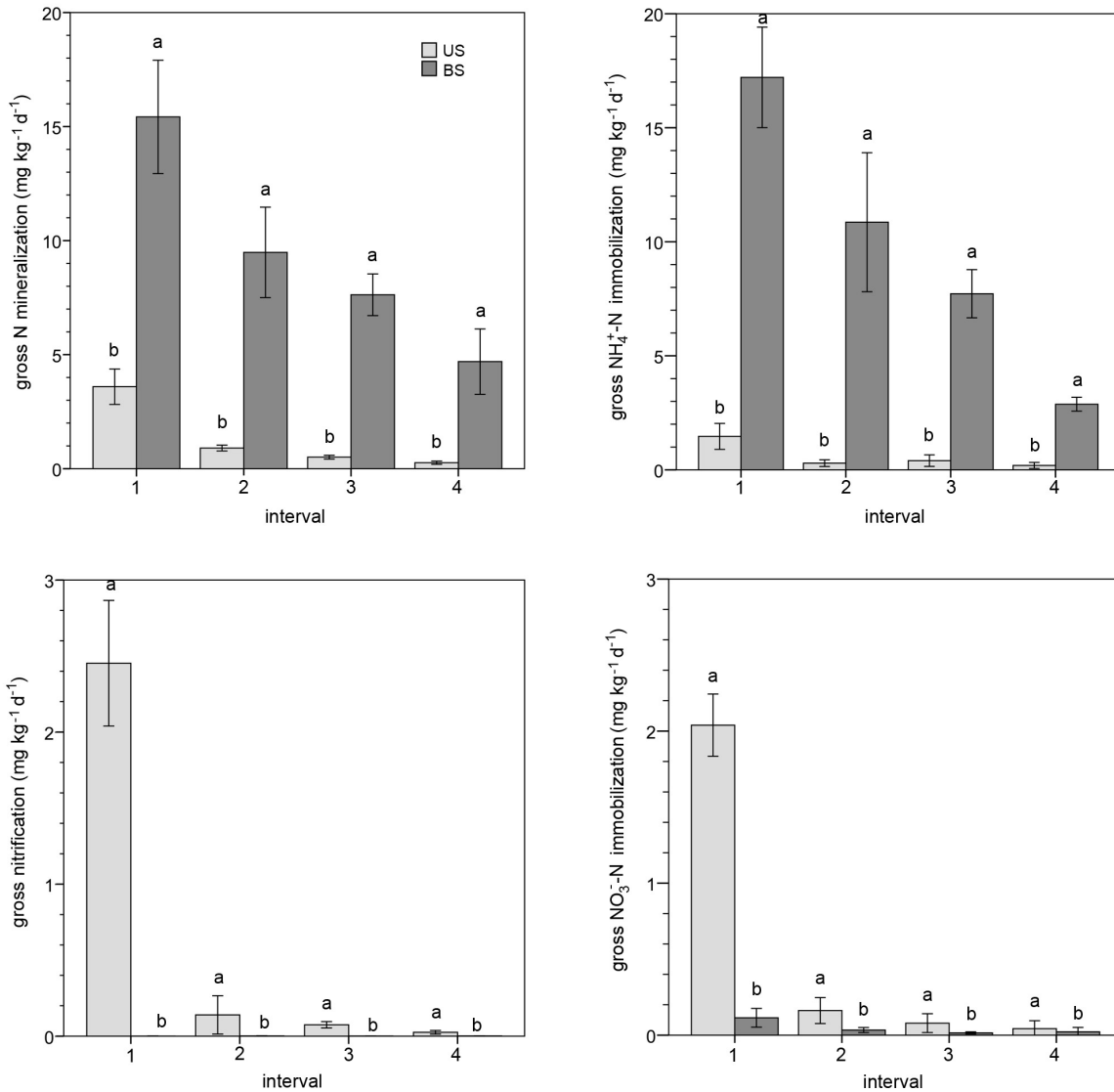


Figure 3. FLUAZ estimated gross rates (mg N kg<sup>-1</sup> soil d<sup>-1</sup>) of N mineralization (a), NH<sub>4</sub><sup>+</sup>-N and immobilization (b), nitrification (c), and NO<sub>3</sub><sup>-</sup>-N immobilization (d) for each incubation period (interval 1: 0.5h-1d; interval 2: 1-3 d; interval 3: 3-7 d; interval 4: 7-14 d) in unburned (US) and burned (BS) soil. Bars show  $\pm 1$  standard deviation. Within each time interval, different letters indicate significant differences among means ( $n=3$ ) at the  $p = 0.05$  level.

Table 3. Rates ( $\text{mg N kg}^{-1} \text{ d}^{-1}$ ;  $n=3$ , mean  $\pm$  s.d.) of gross (FLUAZ modelled) and net N mineralization and nitrification for each incubation interval calculated from the gross N rates (FLUAZ) and results of the one way ANOVA, in the unburned soil (US) and burned soil (BS).

Process	Interval (days)	US	BS	<i>p</i>	$\eta^2$
Gross N mineralization	0-14	0.496 $\pm$ 0.125	7.949 $\pm$ 0.581	0.001	0.992
Gross nitrification	0-14	0.099 $\pm$ 0.033	0.002 $\pm$ 0.001	0.01	0.865
Gross $\text{NH}_4^+$ -N immobilization	0-14	0.368 $\pm$ 0.102	6.884 $\pm$ 0.492	0.001	0.992
Gross $\text{NO}_3^-$ -N immobilization	0-14	0.142 $\pm$ 0.015	0.033 $\pm$ 0.012	0.001	0.959
Net N mineralization	0-1	2.128 $\pm$ 0.254	-1.784 $\pm$ 4.689	n.s.	0.342
Net N mineralization	1-3	0.613 $\pm$ 0.189	-1.373 $\pm$ 2.567	n.s.	0.309
Net N mineralization	3-7	0.102 $\pm$ 0.024	-0.094 $\pm$ 0.294	n.s.	0.168
Net N mineralization	7-14	0.070 $\pm$ 0.117	1.811 $\pm$ 1.716	n.s.	0.014
Net N mineralization	0-14	0.128 $\pm$ 0.025	1.065 $\pm$ 0.671	0.01	0.928
Net nitrification	0-1	0.413 $\pm$ 0.617	-0.113 $\pm$ 0.061	n.s.	0.351
Net nitrification	1-3	-0.022 $\pm$ 0.064	-0.032 $\pm$ 0.016	n.s.	0.018
Net nitrification	3-7	-0.004 $\pm$ 0.042	-0.014 $\pm$ 0.007	n.s.	0.036
Net nitrification	7-14	-0.017 $\pm$ 0.041	-0.020 $\pm$ 0.028	n.s.	0.003
Net nitrification	0-14	-0.043 $\pm$ 0.019	-0.031 $\pm$ 0.012	n.s.	0.185

## Discussion

Taking into account the almost complete consumption of the thick litter layer, the high percentage of bare soil and the 25 % reduction of the organic matter content (C and N) in the 0-2 cm soil layer, the wildfire affected the soil with a moderate to high severity. In agreement with the findings of other authors, results showed that our burned soil had: a) higher soil pH and electrical conductivity, due to the oxides and carbonates of basic ions supplied by ashes and the combustion of organic acids, that lead to increased extractable Na, Ca and P (see, for example, the review by Certini, 2005); b) lower contents of soil C and N, due to volatilization (Certini 2005), and also lower values of soil  $\delta^{15}\text{N}$ , related with the deposition of  $^{15}\text{N}$ -depleted charred materials from  $\text{N}_2$ -fixing legumes and/or ectomycorrhized ericaceous (Gómez-Rey et al. 2013b); and c) lower levels of Fe and higher of Mn (Certini 2005; García-Marco and González-Prieto 2008; Close et al. 2011; Gómez-Rey et al. 2013a). Contrastingly, although several authors reported that fire increases the availability of K, Mg, Zn and Cu in soils (Certini 2005; García-Marco and González-Prieto 2008; Close et al. 2011; Gómez-Rey et al. 2013a), we did not find a significant effect on these nutrients.

Values of daily net N mineralization and nitrification rates observed in the unburned soils

(Table 2) were comparable to the mean values of 14 bush soils from the same region (0.119 and 0.005  $\text{mg kg}^{-1} \text{ d}^{-1}$ , respectively; González-Prieto et al. 1996). In agreement with previous studies from the same area (González-Prieto et al. 1996; González-Prieto and Villar 2003), net N fluxes we found showed that  $\text{NH}_4^+$ -N dominated the inorganic N pools in forest soils and that net ammonification largely predominated over net nitrification. The rates of gross N mineralization and nitrification also supported this pattern. Highlighting, once again, the need of the isotope dilution techniques for true evaluations of the mineralization-immobilization turnover, our results show that, despite being significantly correlated, net N mineralization underestimated about four-fold the gross rates of  $\text{NH}_4^+$ -N production. The absence of net nitrification did not coincide with the gross rates of nitrification and microbial immobilization of  $\text{NO}_3^-$ -N we observed; consequently, gross  $\text{NO}_3^-$ -N fluxes found in the present study showed that  $\text{NO}_3^-$ -N production in these soils is a more important process than would be expected from net nitrification measurements. Taking into account that the initial  $\text{NH}_4^+$ -N pool in BS was increased only 0.2% by the added  $\text{NH}_4^+$ -N, their effects on gross  $\text{NH}_4^+$ -N fluxes must be negligible compared with the effects of fire. In the same way, considering that both gross nitrification and gross  $\text{NO}_3^-$ -N immobilization were

strongly reduced by fire, without noticeable differences among the four incubation periods, we can conclude that tracer addition had little or no effect on gross  $\text{NO}_3^-$ -N fluxes.

As previously observed Prieto-Fernández et al. (1993), a short-term stimulation of net N mineralization after fire (8-fold) was found, the increase being even higher (16-fold) for the gross N mineralization (Table 2). Our results agree with those of Anderson and Poth (1998) and Neill et al. (1999) on post-fire gross N mineralization rates in Brazilian soils, although in our case the effect was stronger than the two- or three-fold increase reported by these authors. Although differences in fire severity and ecosystem characteristics (climate, vegetation) should be taken into account, the high increase in gross rates we found must be related with the lower sampling depth (0-2 vs 0-5 cm) and higher SOM content (10 to 20-fold) of our soils. As Prieto-Fernández et al. (1993) reported for soil net N mineralization after a wildfire, the deposition of partially burned vegetation and the transient increase of labile organic matter and nutrients could explain the stimulation of gross mineralization due to the enhanced activity of surviving soil microorganisms during the incubation under optimal conditions of temperature and humidity. Six months after a natural fire in a Mediterranean shrubland ecosystem (Dannenmann et al. 2011) and a South African woodland (Aranibar et al. 2003), gross ammonification in the 0-5 cm soil layer was still two- or three-times higher in burned than in unburned plots. However, this transient post-fire increase of gross N mineralization disappears 2-3 years after the fire, according with studies of several authors in the 0-10 cm soil layer (Bastias et al. 2006; Leduc and Rothstein 2007; Koyama et al. 2010, 2012). Leduc and Rothstein (2007) reported similar rates in burned and unburned soils from North American pine forests, while Koyama et al. (2010, 2012) in the same ecosystem and Bastias et al. (2006) in Australian wet sclerophyll forests found lower values in burned soils, likely due to reduced C availability to soil microbes (Koyama et al. 2010, 2012).

No published information about the immediate effects of fire on gross  $\text{NH}_4^+$  immobilization was found, but the strong (10-fold) increase we observed on the burned plots one week after the fire agrees with the three-fold higher rates in the 0-4 cm soil layer of a Mediterranean shrubland studied by Dannenmann et al. (2011) six months after the fire. Like gross N mineralization, the post-fire increase of

gross  $\text{NH}_4^+$  immobilization might disappeared with time, considering that similar (Leduc and Rothstein 2007) or lower values (Koyama et al. 2010, 2012) than in unburned soils have been reported in soils burned 2-3 years ago; such reduction being most likely caused by reduced C availability to soil microbes.

Although net measurements were unable to detect the effects of fire on nitrifiers, the gross nitrification was almost suppressed (98 % reduction) in our study area immediately after the fire. The higher decrease of gross nitrification in our soils than in Brazilian cerrado soils (-40%, Anderson and Poth 1998) and Amazonian forests soils (no significant changes, Neill et al. 1999) was likely due to differences in sampling depth and/or fire severity because: a) the nitrifying microorganisms are disfavoured by burning (Acea and Carballas 1996); b) the nitrification process was suppressed by exposure to temperature higher than 40 °C, probably due to thermal denaturation of enzymes (Luxhoi et al. 2008); and c) fire effects disappeared with depth. Published results about the medium- and long-term effects of fire on gross nitrification were also contradictory. No differences were reported in a South African woodland half a year after the fire (Aranibar et al. 2003) and in North American pine forests after 2-6 years (Leduc and Rothstein 2007; Koyama et al. 2010, 2012). Conversely, around two-fold higher rates in soil of burned plots were found in Mediterranean shrubland ecosystem 6 months after a natural fire (Dannenmann et al. 2011) and in North American pine forests exposed to fire 12-years ago (Ball et al. 2010). The latter authors also observed that the number of ammonia-oxidizing bacteria was 4-12 times higher in burned than in control soils; this result agrees with those of DeLuca et al. (2006) who found that the charcoal induced increase in net nitrification is rather a result of greater activity of the nitrifying community (i.e. increased gross nitrification) than of reduced microbial immobilization.

As for  $\text{NH}_4^+$ -N, just post-fire data on gross  $\text{NO}_3^-$ -N immobilization were not previously available. Gross  $\text{NO}_3^-$ -N immobilization rates similar to that in control soils were found by Dannenmann et al. (2011) and Leduc and Rothstein (2007) six months and 3-6 years after the fire, respectively. Contrasting with these results, we observed a strong decrease (76 %; Table 2) of the gross  $\text{NO}_3^-$ -N immobilization rate immediately after the fire, that was of similar magnitude than the reported by Koyama et al. (2010, 2012) two years after wildfires. As fire reduced gross  $\text{NO}_3^-$ -N

immobilization to a lesser extent than gross nitrification, the consequence is an increase of  $\text{NO}_3^-$ -N concentrations under BS.

## Conclusions

Globally considered, our results pointed to a faster  $\text{NH}_4^+$ -N and a slower  $\text{NO}_3^-$ -N turnover in burned soils immediately after fire, whose effects on N fluxes are greater than the estimated with net N rates. As gross mineralization increased more than  $\text{NH}_4^+$ -N immobilization, an accumulation of  $\text{NH}_4^+$ -N with time is expected, but without a concurrent increased risk of  $\text{NO}_3^-$ -N leaching because gross nitrification decreased more than  $\text{NO}_3^-$ -N immobilization. The high  $\text{NH}_4^+$ -N availability must be beneficial for the recolonization of burned soils by plants, although those species that prefer or rely mostly on  $\text{NO}_3^-$ -N nutrition could be disfavoured. More research is needed on fire effects at longer term and in different soil layers to accurately know the post-fire changes in soil N biogeochemistry.

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