FERNÁNDEZ-FERNÁNDEZ, M.¹; RÜTTING, T.²; GONZÁLEZ-PRIETO, S.J.^{1*} (2017). Effects of a high-severity wildfire and post-fire straw mulching on gross nitrogen dynamics in Mediterranean shrubland soil. *Geoderma* 305, 328-335.

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Abstract

Little is known about the combined impacts of fire and straw mulching, a widely used post-fire emergency measure, on the soil nitrogen (N) cycle. Unburnt (US) and severely-burnt soils without (BS) and with straw mulching (BSM) were preincubated (3 and 6 months) in the laboratory before fire and mulching effects on gross N transformations were investigated with a paired ¹⁵N-labelling experiment. The ammonium-to-nitrate (NH₄⁺/NO₃⁻) ratio of burnt soils decreased with preincubation time from 21 to 1.3, consistent with a shift of the N cycle towards net nitrification. After 3 months of preincubation, gross mineralisation (*M*_{SON}) and gross NH₄⁺ immobilisation (*I*_{NH4}) in BS more than doubled compared to US, in the latter being *M*_{SON} 4.82 mg N kg⁻¹ day⁻¹ and *I*_{NH4} 3.01 mg N kg⁻¹ day⁻¹. Mulching partly mitigated this stimulation in the mineralisation-immobilisation turnover (MIT). After 6 months, MIT differences among treatments disappeared and gross rates approached those in US after 3 months. After three months, autotrophic nitrification (NH₄⁺ oxidation) in all treatments was 0.41-0.52 N kg⁻¹ day⁻¹, while after 6 months it remained similar in US but increased 8-fold in burnt soils. Heterotrophic nitrification of organic N only occurred in burnt soils, and its importance was similar to autotrophic nitrification after 3 months, but around 4-fold lower after 6 months. To conclude, burning opened up the N cycle and NO₃⁻ accumulated, increasing the potential for ecosystem N losses. In the short term, straw mulching slightly mitigates the effects of fire on the N cycle.

Keywords: autotrophic nitrification; emergency stabilisation techniques; heterotrophic nitrification; mineralisation-immobilisation turnover; ¹⁵N tracing

1. Introduction

Most of the current large-scale and intense wildfires are caused by human activities and nowadays wildfires affect more land area than any other natural disturbance (Bento-Gonçalves *et al.*, 2012; Caldararo, 2002; Pausas and Keeley, 2009). As a consequence of climate change and land use change, the number, extent and severity of wildfires is very likely to increase considerably (Bento-Gonçalves *et al.*, 2012; Birot, 2009; Pereira *et al.*, 2011). Moreover, there is evidence of a substantial positive feedback of wildfires on the climate system (Bowman *et al.*, 2009). Elucidating the effects of wildfires on both the carbon (C) and nitrogen (N) cycles is crucial as these elements are fundamental to sustain primary production and some of their forms are among the main greenhouse gases. However, little is known about the impacts of fire and post-fire management activities on soil

gross N dynamics (Bowman *et al.*, 2009; Wang *et al.*, 2014).

The main repercussions of wildfires on the soil N cycle are the significant removal of soil organic matter (SOM); the redistribution of the remaining soil organic N (SON), with an increase of recalcitrant forms and decrease of the most labile ones; the conversion of organic to inorganic N due to heating and combustion; considerable losses of N by leaching, erosion and volatilisation; as well as a marked alteration of both quantity and specific composition of microbial communities (Castro *et al.*, 2006; Certini, 2005; Fisher and Binkley, 2000; Prieto-Fernandez *et al.*, 2004). Both increased soil inorganic N concentrations and altered net N transformation rates after fires have been widely reported [see Wang *et al.* (2014) and references therein]. Nevertheless, net N mineralisation is the outcome of the counteracting and simultaneous

gross mineralisation and gross immobilisation processes (the so-called mineralisation-immobilisation turnover, MIT) and, similarly, net nitrification is the result of gross nitrification and gross NO_3^- immobilisation. Consequently, in order to gain a better understanding of the underlying mechanisms, we need to investigate gross N transformation rates together with the environmental variables controlling these fluxes (Koyama *et al.*, 2010; Wang *et al.*, 2014).

One of the emergency stabilization techniques most widely used after wildfires is straw mulching, which has shown an immediate effectiveness in increasing ground cover and thus reducing soil erosion during the first critical months after the fire (Bento-Gonçalves *et al.*, 2012). Both the soil composition and soil processes might be altered by mulching, as it enhances vegetation recovery (and thus nutrient uptake by plants), decreases the runoff-infiltration ratio, reduces erosion and increases the topsoil C and N content. In Gómez-Rey *et al.* (2013, 2014) straw application after fire effectively reduced erosion but did not affect the soil nutrient concentrations. Mulching has been shown to affect gross N fluxes in unburnt soils (Cheng *et al.*, 2012; Huang *et al.*, 2008), but we are aware of only one experiment testing the effect of straw application on gross N rates in burnt soils (Gómez-Rey and González-Prieto, 2015). This study with a soil affected by a moderate to highly severe wildfire showed that mulching resulted in a short-lived stimulation of gross mineralisation and gross immobilisation of ammonium (NH_4^+). The aim of the present study was to investigate if this is also true for soils exposed to high severity fires, i.e., with even more extended consumption of surface organic matter, higher proportion of white/grey ashes and also with damage to soil structure [based on Parson *et al.* (2010) and Vega *et al.* (2013)].

In the reviews by Gómez-Rey & González-Prieto (2013) and Wang *et al.* (2014) no common response of gross N transformations to fires (wildfires and prescribed fires) was found, most probably due to the large number of influencing factors (e.g. forest and soil type, severity and recurrence of fire, climate, time between fire and sampling). In the present study, we investigated wildfire and post-fire mulching effects on gross N dynamics in a shrubland soil from NW Spain. We conducted a ^{15}N tracer experiment using the numerical ^{15}N tracing model *Ntrace* (Müller *et al.*, 2007; Rütting and

Müller, 2007) with a severely-burnt soil (with and without surface applied straw) and a control unburnt soil, previously preincubated for 3 and 6 months. Our hypotheses are: a) fire stimulates gross N transformations; b) mulching partially reverts the changes on N fluxes caused by a very high severity fire; and c) time after fire also affects N rates.

2. Materials and methods

2.1. Site description

Soil samples for the laboratory experiments were collected in the Tamega valley (42°4'45" N, 7°28'36" W, Galicia, NW Spain; 520 m asl). The area has a mesic and sub-humid climate with a mean annual temperature of 13-14 °C and a mean annual precipitation of 600-800 mm. The dominating vegetation is a shrubland of *Ulex europaeus* and *Erica arborea* with scattered *Pinus pinaster* trees. In this area fire recurrence is high and shrubland burns every 5-10 years. In August 2014 the area was affected by a high severity wildfire that burnt approximately 6 ha of shrubland. Within a distance of 25 m from the burnt place, an unburnt area with the same topography, orientation, vegetation cover and soil type was identified as control for the experiment. Burnt and unburnt soils were both characterized as Dystric Leptosol (IUSS Working Group, 2014).

2.2. Soil sampling

As the aim was to specifically study the effects of fire and post-fire mulching on the most severely affected area, the day after the fire and with the soil still warm, top soil (0-2 cm depth) was selectively sampled in 30 spots, of variable size (80-250 cm²), burnt with high or very high severity [based on Parson *et al.* (2010) and Vega *et al.* (2013)] considering the following characteristics: a) almost all the pre-fire ground cover and surface organic matter (litter, duff, and fine roots) was consumed; b) the whole burnt surface was covered either with white (or grey) ashes or with bare greyish soil (with reddish spots) exposed; and c) topsoil structure damaged or destroyed. With this sampling strategy we collected relatively homogeneous sub-samples which were combined into a composite sample, and thus pseudoreplication can restrict the generalization of our results. For the unburnt control, ten sampling squares (15 x 15 cm) were selected at random and the litter layer (O horizon)

and the mineral topsoil (0-2 cm) were separately sampled; soil subsamples were mixed into a composite sample and the same was done with the litter. In the laboratory the soils were sieved (< 2 mm), homogenised and stored at 4 °C for soil incubation purposes. For the mulching treatment, wheat straw (from an agricultural field nearby) was selected. Straw is widely used for this purpose in Galicia and elsewhere since a small dose (200-250 g m⁻²) ensures the 60-70% of ground cover needed to effectively protect burnt soils against erosion (Gómez-Rey *et al.*, 2013; Robichaud, 2009; Vega *et al.*, 2013).

2.3. Soil, litter and straw characterization

Subsamples of each material were air-dried and finely ground (< 100 µm) in a planetary ball mill (Retsch PM100, Retsch GmbH, Haan, Germany, with cups and balls of zirconium oxide). The dry matter content of all samples was assessed by oven-drying sub-samples at 105 °C for 5 h and the water holding capacity (WHC) of fresh soils was determined in a Richards' membrane-plate extractor at a pressure of 10 kPa.

Soil pH was measured on air-dried samples with a pH meter (MetröhM, Switzerland) in KCl employing a 1:2.5 soil/solution ratio. Organic N and C of soils, litter and straw were measured on ground samples with an elemental analyser (Carlo Erba CNS 1508). The method for quantifying soil content of NH₄⁺-N and nitrate NO₃⁻-N is described in the following section. All analyses were carried out in duplicate (if the coefficient of variation was higher than 5 % the analyses were repeated) and the mean of both analyses for each replicate was used.

2.4. Soil incubation and gross N transformation rates

To assess the effect of burning and straw addition on gross N transformation rates on a temporal basis, an experiment with three treatments (unburnt soil, US; burnt soil, BS; and burnt soil with straw mulching, BSM) and two preincubation times (3 and 6 months) was set up. These preincubation times were chosen to cover the most critical period after the fire, i.e. the first months, with the soil mostly bare and exposed to N losses due to the high inorganic N levels and very low plant uptake. After treatment application, soils were preincubated in the laboratory instead of in the field because, as usual, fire severity was extremely heterogeneous throughout the burnt site (in the scale of decimeters)

and, consequently, it would be impossible to find the areas burnt with high severity 3-6 months after applying the straw mulching without disturbing the whole burnt area. We are aware of the potential limitations of such an experimental design as it precludes some processes which play an important role in N cycling (physical mixing of soil and straw, plant growth, variable weather conditions). Nonetheless, short- or medium-term incubations are still considered an adequate method to get a picture of the potential capability of the different soils to transform N under optimal conditions [see Abadín *et al.* (2011) and references therein] and to test the effects of straw on soil N cycling. With the wetting system described in Gómez-Rey & González-Prieto (2013), which allows easily wetting highly hydrophobic soils, the soils were humidified to slightly below 70% of their WHC before starting the preincubation, so when soils received the labelled solution for the tracing experiment they were at 70% of their WHC. Approximately 2000 g of wet soil (corresponding to 1600 g of dry soil) were placed in six plastic trays (one per treatment and incubation time) of 20 x 20 x 2 cm. For the US and BSM treatments, a piece of nylon mesh (1 mm² mesh size) was placed on top of the soil to facilitate the separation of litter and straw from the soil. We added an amount of litter equivalent to 9.59 kg m⁻² (as in the field) to the US soils, while the BSM received 250 g m⁻² of straw, a frequently used dose that ensures a soil cover higher than 60% and, thus, effectively reduces erosion. The trays were covered (but not sealed) with filter paper and aluminium foil to regulate water evaporation and incubated in a chamber in darkness at 25 °C. Throughout the preincubation the humidity of the soils was adjusted to the initial value two times a week by adding around 20 ml water as simulated rain, i.e. allowing lixiviation through the litter layer (US) and the straw mulch (BSM).

After the 3 months preincubation period, the soil contained in one of the two plastic trays of each treatment was thoroughly homogenised and destructively sampled; then, aliquots equivalent to 30 g of dry soil were placed into 24 centrifuge bottles (250 ml) for a paired ¹⁵N labelling experiment with two tracers (¹⁵ NH₄NO₃ and NH₄¹⁵NO₃), four incubation times (0.5 h, 1, 3 and 7 days) and three replicates per tracer and incubation time. The same was done after 6 months of preincubation, totalling 144 incubated samples. The

bottles were filled with 4 successive layers of soil, each of which received 1 ml of ^{15}N -tracer solution added uniformly over the soil surface with an automatic pipette (i.e. 4 ml per bottle), equivalent to an N addition of 1 mg kg^{-1} dry soil with a ^{15}N excess fraction of 49 %. After labelling, soils were incubated at 25°C in darkness. Once incubated, soils were extracted with 150 ml KCl 2M (1:5 soil:solution ratio) shaken for 1 h and passed through glass microfibre filters (Whatman GF/A, 125 mm diameter). For $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ quantification, an extraction-diffusion method described in Fernández-Fernández *et al.* (2015) was used. After the two titrations (for NH_4^+ and NO_3^-), the resulting $(\text{NH}_4)_2\text{SO}_4$ solutions were evaporated to dryness at 60°C in a vacuum oven (Memmert VO400, PM400) for obtaining $(\text{NH}_4)_2\text{SO}_4$ 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_3 , the incoming air was passed through a column of activated charcoal. The $(\text{NH}_4)_2\text{SO}_4$ crystals were finally packed into tin capsules and analysed for ^{15}N . The soil remaining in the flasks and the filters during the extraction procedure was washed with deionized water until no chlorides were detected (silver nitrate test), oven-dried at 105°C , finely ground ($<100 \mu\text{m}$) and packed into tin capsules for organic N analyses. The ^{15}N enrichment of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, as well as the organic N content and its ^{15}N enrichment were measured with an elemental analyser (Carlo Erba CNS 1508) coupled on-line with an isotopic ratio mass spectrometer (Finnigan Mat, delta C, Bremen, Germany). An elemental reference material (Soil 3 from Eurovector, Milano, Italy) and an isotopic standard (IAEA-N1, IAEA-N2, IAEA 305-A, IAEA 305-B and IAEA 311, alternately, from the International Atomic Energy Agency, Vienna, Austria) were included in each set of 10 samples to check the accuracy of the results; if necessary, drift correction was made against internal standards during the run.

2.5. ^{15}N -tracing model

Gross N transformation rates have been calculated with either analytical or numerical methods by adding ^{15}N -tracers to the soil and then measuring the N pool sizes and their ^{15}N enrichment over a certain incubation period (Murphy *et al.*, 2003). With numerical solutions more N transformations can be estimated

simultaneously, different process kinetics can be considered and there is no amplification of errors. However, most of the studies on gross N rates in burnt soils have used analytical methods instead of numerical models (Gómez-Rey and González-Prieto, 2013). We used the ^{15}N tracing model *Ntrace* (Müller *et al.*, 2007; Rütting and Müller, 2007), which is set up in Simulink and summoned by the MCMC optimization routine programmed in MatLab (The Math Works Inc.); it is one of the latest developments and allows a more complex description of the soil N processes as a large number of parameters can simultaneously be estimated (Rütting and Müller, 2007). It basically consists of a set of differential equations solved by numerical methods and non-linear fitting routines which search for rate parameters that minimize the misfit between modelled data and a set of observed data and avoid trapping in local minima (Rütting and Müller, 2007). The main differences between the gross rates we present here and those reported in other studies on burnt soils are: 1) we estimated gross autotrophic and heterotrophic nitrification separately, the latter referring to NO_3^- production from SON without mixing into the free soil NH_4^+ pool (Fig. 1); 2) we quantified gross NH_4^+ immobilisation and immobilisation of NO_3^- to SON separately; and 3) rates are estimated for the whole incubation period instead of sequentially.

To quantify gross N transformation rates for each of the treatments and preincubation times, a *Ntrace* compartment model with different N pools and possible N transformations was suggested as a start point. To identify the best option describing the measured data, the model was run several times changing the number of considered processes and the Akaike information criterion was used to choose the final model (Staelens *et al.*, 2012). The parameters of the selected transformation rates were estimated using a Markov chain Monte Carlo (MCMC) method by fitting the model values to the measured contents and ^{15}N enrichments of NH_4^+ and NO_3^- (Müller *et al.*, 2007). The optimization procedure continues until the probability density functions (PDFs) are well characterised for all parameters (50000 iterations in our case). Thereafter, parameter averages and standard deviations were calculated from PDFs (Müller *et al.*, 2007). For transformations following first order kinetics, average rates were calculated by integrating gross N rates over the experimental period divided by the

total time (Rütting and Müller, 2007). Standard errors of means were calculated based on autocorrelation as described in Harmon & Challenor (1997). Because of the high number of iterations of the ^{15}N -tracing model, statistical tests are inappropriate for the comparison of results; therefore, statistical significance in differences between treatments was tested by an overlap of the 85% confidence intervals (CI) (Payton *et al.*, 2000; Rütting *et al.*, 2010).

The final model considered three N pools in all treatments: SON, NH_4^+ and NO_3^- (Fig. 1). Initial NH_4^+ and NO_3^- pool sizes and ^{15}N enrichments were estimated by back-extrapolation of the measured values from the first two time-points (0.5 hours and 1 day) (Müller *et al.*, 2004). Unburnt controls (US) retained four transformation rates: mineralisation of SON to NH_4^+ (M_{SON} ; zero order kinetics), immobilisation of NH_4^+ to SON (I_{NH_4} ; first order kinetics), immobilisation of NO_3^- to SON (I_{NO_3} ; first order kinetics) and oxidation of NH_4^+ to NO_3^- (autotrophic nitrification, O_{NH_4} ; first order kinetics), whereas burnt soils (BS and BSM treatments) also presented oxidation of SON to NO_3^- (heterotrophic nitrification, O_{SON} ; zero order kinetics).

3. Results

Compared with US, the burnt soil had less total C (136 vs 146 g kg^{-1} d.w; data from Barreiro *et al.*, 2016), and also a lower WHC (619 ± 7 vs 753 ± 9 g $\text{H}_2\text{O kg}^{-1}$ dw; mean $\pm$ s.d., $n=2$), but higher values of organic N (9.18 ± 0.12 vs 7.01 ± 0.05 g kg^{-1} dw) and pH_{KCl} (4.10 ± 0.01 vs 3.41 ± 0.01). The initial content of NH_4^+ -N was lower in US than in burnt soils (52.9 ± 0.3 and 130.4 ± 0.6 mg kg^{-1} dw, respectively), while the reverse was true for the NO_3^- -N (26.0 ± 0.5 and 9.2 ± 2.7 mg kg^{-1} d.w, respectively). Consequently, the $\text{NH}_4^+/\text{NO}_3^-$ ratio in BS (13:1) was much higher than in US (2:1). The C/N ratios of soils and added materials were: unburnt control, 21; burnt soils, 25; litter, 29; and straw, 72.

During the preincubation, the content of inorganic N (NH_4^+ -N plus NO_3^- -N) increased in all soils. At $t=3$ months burnt soils had 1.2 times more inorganic N than the unburnt control, but 3 months later the opposite pattern was recorded. The content of NH_4^+ -N in US increased with preincubation time (Fig. 1). Both BS and BSM showed similar contents

of NH_4^+ -N, which were 2-fold ($t=3$ months) and 1.3-fold ($t=6$ months) higher than at $t=0$ months (130.4 ± 0.6 mg kg^{-1} dw; mean $\pm$ s.d., $n=2$). Compared to initial values (26.0 ± 0.5 mg kg^{-1}), the unburnt soil had 3.7 and 5.2 times more NO_3^- -N after 3 and 6 months, respectively (Fig. 1), being the $\text{NH}_4^+/\text{NO}_3^-$ ratio relatively constant during the whole incubation. Contrastingly, BS and BSM had approximately 1.4 times more NO_3^- -N at $t=3$ months compared to initial values (9.2 ± 2.7 mg kg^{-1}), but up to 14 times more NO_3^- -N after 6 months of preincubation (Fig. 1) and, consequently, the $\text{NH}_4^+/\text{NO}_3^-$ ratio strongly decrease with the preincubation time. Therefore, for US the contribution of NH_4^+ -N to the inorganic N pool increased with preincubation time from 59% ($t=3$ months) to 65% ($t=6$ months), whereas for BS and BSM treatments NH_4^+ -N accounted for most of the total inorganic N at $t=3$ months (95%) but much less after 6 months of preincubation (57%).

In general, there was a good fit between the output of the ^{15}N tracing model and the measured data (Supplementary Information 1 to 6). All gross transformations are expressed on a soil dry weight (dw) basis. For each N transformation rate, the percentage of difference between treatments and preincubation times is calculated considering as reference value the following: a) $t=3$ months when comparing the rate of each treatment between the two preincubation times; b) US when assessing the effect of fire; and c) BS when assessing the effect of mulching. For each gross N transformation rate, all differences tested (by an overlap of the 85% CI) were significant both between treatments (same preincubation time) and between preincubation times (same treatment).

The mineralisation rate of SON to NH_4^+ (M_{SON}) at the 3 months preincubation in the unburnt control was 4.82 ± 0.08 mg N kg^{-1} dw day^{-1} , and burning had a positive effect on the rate, which was 133% higher in BS than US (Fig. 1). Mulching decreased M_{SON} by 37% (BSM compared to BS). At $t=6$ months, M_{SON} was reduced in the three treatments compared to $t=3$ months, but to a different extent: 16% in US, 70% in BS and 42% in BSM. As a result, the differences between treatments in M_{SON} after 6 months of preincubation were always below 20% (Fig. 1).

Fig. 1. Gross N transformation rates ($\text{mg N kg}^{-1} \text{ dw day}^{-1}$; mean $\pm$ 85% confidence interval, CI; if two CI do not overlap, the corresponding means are significantly different, Payton *et al.*, 2000; Rütting *et al.*, 2010) and size of N pools ($\text{mg N kg}^{-1} \text{ dw}$; mean $\pm$ s.d.; $n=3$) for US, BS and BSM after 3 and 6 months of preincubation. Box size is proportional to the N pool size (1:1) except for SON (1:10). Except for the SON pool (1:10), box size and arrows width are proportional (1:1) to the N pool size and to the estimated gross N transformation rates. For each gross N transformation rate, all differences were significant both between treatments (same preincubation time) and between preincubation times (same treatment). Significant differences in the size of the three N pools were only found between US and both burnt soils. Key: US, unburnt soil; BS, burnt soil; BSM, burnt soil with mulching; SON, soil organic-N; M_{SON} , mineralisation of SON to NH_4^+ ; $I_{NH_4^+}$, immobilisation of NH_4^+ to SON; $I_{NO_3^-}$, immobilisation of NO_3^- to SON; $O_{NH_4^+}$, oxidation of NH_4^+ to NO_3^- ; O_{SON} , oxidation of SON to NO_3^- .

Gross immobilisation of NH_4^+ to SON (I_{NH_4}) followed a similar pattern as M_{SON} (Fig. 1). At $t=3$ months BS had 216% higher I_{NH_4} than US ($3.01 \pm 0.12 \text{ mg N kg}^{-1} \text{ dw day}^{-1}$) and in BSM it was 128% higher than in US, but 28% lower than in BS. Three months later, I_{NH_4} in BS and BSM was around 60% lower while in US it remained similar. Therefore, by the end of the experiment there were relatively small differences ($<11\%$) in I_{NH_4} among treatments.

As all plausible N rates, oxidation of SON to NO_3^- (heterotrophic nitrification) was considered during the modelling of all treatments. In US, O_{SON} was discarded after several runs of the model because the values yielded by the model (usually 10000-50000 iterations) were close to 0 and did not follow a normal distribution; moreover, the misfit function was 11% higher when O_{SON} was included in the model. However, in BS and BSM, O_{SON} estimates were above zero and normally distributed; and the inclusion of this rate increased the global performance of the model (higher percentage of parameter sets accepted, around 15% reduction of the misfit function and better fit of the modelled and experimental data). At $t=3$ months O_{SON} was $0.55 \pm 0.06 \text{ mg N kg}^{-1} \text{ dw day}^{-1}$ for BS and $0.41 \pm 0.08 \text{ mg N kg}^{-1} \text{ dw day}^{-1}$ for BSM, whereas three months later it was 95% (BS) to 167% (BSM) higher (Fig. 1).

The differences between treatments in the oxidation rate of NH_4^+ to NO_3^- (autotrophic nitrification) were small at $t=3$ months and became larger in burnt soils after 6 months of preincubation (Fig. 1). Autotrophic nitrification at $t=3$ months was the same for US and BS ($0.53 \pm 0.02 \text{ mg N kg}^{-1} \text{ dw day}^{-1}$), whereas in BSM it was 20% lower. At $t=6$ months US showed a slightly lower autotrophic nitrification, but both BS and BSM showed higher rates (6- and 8-fold respectively) compared with the previous preincubation time. As a result, at $t=6$ months burnt soils had on average 7-fold higher nitrification rates than US and mulching had no effect on this rate. In BS and BSM, the $O_{\text{NH}_4}/O_{\text{SON}}$ ratio increased from 1 at $t=3$ months to 3.4 after 6 months of preincubation.

Compared to I_{NH_4} , I_{NO_3} behaved in a quite different way (Fig. 1). In the $t=3$ months experiment I_{NO_3} in US was $0.60 \pm 0.10 \text{ mg N kg}^{-1} \text{ dw day}^{-1}$, whereas in BS it was 15% higher. Straw mulching decreased I_{NO_3} by 55% when compared to BS and by 48% when compared to US. At $t=6$ months I_{NO_3} was

34% lower than at $t=3$ months in US, but 142-274% higher in BS and BSM, leading to larger differences in this rate between treatments at $t=6$ months (+321% for BS vs. US; +194% for BSM vs. US; and -30% for BSM vs. BS).

4. Discussion

Both high-severity fire and preincubation length affected the total inorganic N, $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ content in soil. The higher inorganic N content and six-time higher $\text{NH}_4^+/\text{NO}_3^-$ ratio in the burnt soil right after the fire is in agreement with the widely reported increased soil inorganic N concentrations after fire [see Wang *et al.* (2014) and references therein], especially that of NH_4^+ , a direct product of combustion, whereas NO_3^- requires nitrification to be formed (Karhu *et al.*, 2015). In incubation experiments without plants and under optimal conditions for N mineralisation, in which N losses are largely reduced, an increase in inorganic N concentration is usually reported (Abadín *et al.*, 2011). In US, the sustained parallel increase in both NH_4^+ and NO_3^- and the rather stable $\text{NH}_4^+/\text{NO}_3^-$ ratio during the whole experiment suggested that both net mineralization and net nitrification were fairly constant. On the contrary, in burnt soils the total amount of inorganic N increased up to 3 months and then remained stable, and the evolution of NH_4^+ (rapid increase followed by rapid decrease) and NO_3^- (initially stable and then fast increase) diverged during the whole preincubation. Both the reduction of the NH_4^+ pool and the larger NO_3^- pool in burnt soils might indicate that the source of labile organic matter is being depleted, slowing down heterotrophic mineralisation and/or benefiting autotrophic nitrification which do not depend on organic C [see González-Prieto *et al.* (1991) and Verhagen *et al.* (1995)].

These changes in inorganic N content in soils after fires are due to alterations (quantitative and qualitative) in gross N fluxes, most probably related to a changed microbial community and activity, as reported for the same soils by Barreiro *et al.* (2016). These authors found that burning had a large impact on the microbial community structure and negatively affected total microbial biomass, but increased soil respiration, as well as bacterial and fungal growth. Likewise, Goberna *et al.* (2012) recently demonstrated that archaea, bacteria and fungi respond differently to fire and that alterations

in the microbial community structure are the main driver of post-fire changes in microbial biomass and total activity, determining the alteration of soil biogeochemical processes. Both positive and negative effects of fire on soil microbial biomass and activity have been reported (Acea and Carballas, 1996; Díaz-Raviña *et al.*, 2012; Goberna *et al.*, 2012; Vazquez *et al.*, 1993; Yeager *et al.*, 2005). The meta-analysis by Dooley and Treseder (2012) showed that, across the 42 published papers, fire reduced both microbial and fungal abundance (by 33 and 48%, respectively), but with contrasting results depending on site-specific characteristics (e.g. wildfire or prescribed fire, fire severity, soil moisture, pH, vegetation, fuel loading). The positive effect of fire on the mineralisation-immobilisation turnover (MIT; M_{SON} and I_{NH_4}) after 3 months of preincubation agreed with most studies dealing with short-term fire impacts on gross N rates (Anderson and Poth, 1998; Neill *et al.*, 1999). However, we observed that enhanced MIT after fire was short-lived, as after 6 months of preincubation no difference between treatments was observed (Fig. 1). Indeed, a study in a Mediterranean ecosystem also showed a transient short-term increase of gross N mineralisation, which was no longer noticeable 6 months after burning (Karhu *et al.*, 2015). In the study by Bastias *et al.* (2006) in Australian forest soils, 3 months after fire gross N mineralisation was lower in burnt soils compared to the unburnt control, but 2 years later the difference was no longer significant. In a study conducted close to our sampling site (Gómez-Rey and González-Prieto, 2013, 2015), MIT was higher in burnt plots from the first week up to a year after the fire, and differences decreased with time as in our study. Similarly, in several experiments spanning from 6 to 12 months after burning gross N mineralisation was higher in burnt soils (Aranibar *et al.*, 2003; Dannenmann *et al.*, 2011; Kaye and Hart, 1998), although higher gross NH_4^+ immobilisation was only found in Dannenmann *et al.* (2011). However, all these studies calculated NH_4^+ immobilisation as NH_4^+ consumption minus nitrification and thus NH_4^+ immobilisation might be underestimated if heterotrophic nitrification of organic N was taking place (Dannenmann *et al.*, 2011).

Enhanced MIT is usually explained by higher post-fire availability of organic matter and inorganic N, lower plant competition and absence of plant

allelopathic compounds inhibiting microbial activity (Dannenmann *et al.*, 2011) or by the higher activity of the soil microorganisms that survived the fire (Díaz-Raviña *et al.*, 2012). Other studies assessing the short-term effects of burning on soil microbial communities show greatly increased microbial densities compared to the unburnt control, particularly of ammonifying (or N mineralizing) microorganisms (Acea and Carballas, 1996; Goberna *et al.*, 2012; Vazquez *et al.*, 1993). However, the positive effect on ammonifiers almost disappeared 1 year after the fire (Acea and Carballas, 1996), most probably due to the reduction of labile SON in burnt soils (Prieto-Fernandez *et al.*, 2004), which agrees with our observation of no stimulation of M_{SON} and I_{NH_4} after 6 month of preincubation.

Post fire mulching led to a decreased MIT compared to non-mulched burnt soils in the 3 months preincubation, but these differences disappeared three months later. Contrastingly, in Gómez-Rey and González-Prieto (2015) the MIT was higher in mulched plots compared to the controls (unburnt and burnt without mulching) 4 months after the fire, although there were no mulching effects on soil microbial biomass and activity (Díaz-Raviña *et al.*, 2012). Although in both studies ^{15}N incubations were in the laboratory (and used straw from the same field), it should be pointed out that Gómez-Rey and González-Prieto (2015) sampled soils from the field site 4, 8 and 12 months after fire and mulching, whereas we preincubated in the laboratory for 3 and 6 months soils sampled just after the fire. Other studies testing the effect of mulching in unburnt soils (Huang *et al.*, 2008; Shindo and Nishio, 2005; Watkins and Barraclough, 1996) also report higher gross mineralisation, gross N immobilisation and increased microbial functional diversity in mulched soils due to the extra supply of organic C and N from straw.

Gross oxidation of NH_4^+ to NO_3^- (O_{NH_4} ; autotrophic nitrification) was not affected by fire after short-term preincubation, whereas after long-term preincubation burnt treatments showed a much higher O_{NH_4} than US. These results suggest that, short after the fire, the enhanced activity of heterotrophs prevent an increase in autotrophic nitrification as heterotrophy outcompete autotrophic nitrifiers in NH_4^+ uptake (Verhagen *et al.*, 1995). However, as the increase of heterotrophic

activity was not sustained over time, the autotrophic nitrifiers take advantage of the abundant NH_4^+ supply and the increase of pH in burnt soils after 6 months of preincubation. Accordingly, Acea and Carballas (1996) found that the nitrifiers density in a forest soil one month after fire did not differ from the unburnt controls, but one year later ammonium oxidizers were positively affected. In another study on forest soils, ammonium-oxidizing bacteria were more abundant in burnt soils even 12 years after fire (Ball *et al.*, 2010). While Goberna *et al.* (2012) found a short-term increase of the nitrifying population, Yeager *et al.* (2005) have reported a decrease of soil biomass in general and of ammonia-oxidizers in particular in an experiment spanning from 1 to 14 months after the fire, especially in severely-burnt soils. The second nitrification pathway quantified in our experiment, oxidation of SON to NO_3^- (O_{SON}) [see Barraclough and Puri (1995) for assumptions and possible limitations], only took place in burnt soils. A possible explanation for this finding could be that burning favoured the community of heterotrophic nitrifiers due to the increased nutrient availability, changes in quality/quantity of SON or changes in the soil microbial community (no/less competitors in burnt soils). While at $t=3$ months O_{SON} was slightly lower in BSM than BS, three months later no differences between the two burnt soils were observed. Although O_{SON} doubled from 3 to 6 months, its contribution to NO_3^- production was similar to autotrophic nitrification at $t=3$ months, but around 4-fold lower at $t=6$ months, indicating an increased relative importance with time of autotrophic nitrification as source of NO_3^- .

As most studies quantify gross nitrification by the ^{15}N pool dilution technique, which cannot distinguish between autotrophic and heterotrophic nitrification (Barraclough and Puri, 1995), there is no available data with which to compare our results. Therefore, we calculated gross total nitrification ($O_{\text{NH}_4} + O_{\text{SON}}$), which was 2-fold ($t=3$ months) and 11-fold ($t=6$ months) higher in burnt soils compared to US. These results contrast with those from another experiment in Galicia, in which gross nitrification rates were very low in the unburnt control and not detectable in burnt plots (Gómez-Rey and González-Prieto, 2013, 2015). In Brazilian soils burning also reduced gross nitrification, although a fast recovery was observed after 45 days (Anderson and Poth, 1998). Other

studies report no significant differences in gross nitrification between burnt and unburnt soils (Aranibar *et al.*, 2003; Kaye and Hart, 1998; Neill *et al.*, 1999). However, our results agree with the higher gross nitrification rates reported 6 months after a fire in an Italian shrubland (Dannenmann *et al.*, 2011). Due to the low number of studies and their contrasting results, it is not possible to draw a generalization about fire effects on gross nitrification, likely due to big differences among the studied sites (climate, altitude, orientation, slope, vegetation, soil type, soil depth), fire characteristics (type, severity, recurrence), time after the fire and sampling strategy (soil or soil+ashes; soil depth). Neither gross autotrophic nitrification nor gross total nitrification was affected by mulching in our study. This finding is consistent with the results of a similar experiment by Gómez-Rey and González-Prieto (2015), and those of Shindo and Nishio (2005) and Huang *et al.* (2008) in unburnt soils. However, Cheng *et al.* (2012) found a suppression of gross nitrification by mulching in unburnt soils.

In unburnt soils, I_{NO_3} was nearly equal to total gross nitrification, while nitrification exceeded I_{NO_3} in burnt soils, even though I_{NO_3} after 6 months of preincubation was higher in burnt soils than US. Although our soils have been preincubated under optimal conditions in a closed system (i.e. without N leaching and plant uptake), the fast build-up of the NO_3^- pool in burnt soils between 3 and 6 months supports the evidence of long-term NO_3^- accumulation in burnt soils (Koyama *et al.*, 2012), which exacerbates the risk of ecosystem N losses via NO_3^- leaching (Bladon *et al.*, 2008; Stephan *et al.*, 2012) or denitrification (Karhu *et al.*, 2015). Contrastingly, Gómez-Rey and González-Prieto (2013, 2015) observed a sharp decrease in NO_3^- - immobilisation immediately after the fire that persisted at least for a year. Despite the wide differences in the experimental design and the model used, mulching decreased I_{NO_3} both in our study and in the one by Gómez-Rey and González-Prieto (2015), although in the latter experiment Díaz-Raviña *et al.* (2012) did not find an effect of mulching on soil microbial biomass and activity at $t=4$ months. These results with burnt soils contrast with those with unburnt soils in which gross NO_3^- immobilisation was higher in the mulched soils than in the control ones (Cheng *et al.*, 2012), likely because straw increases C availability

and enhances the activity of microbial communities responsible of NO_3^- immobilisation (Recous *et al.*, 1990).

The evolution of NH_4^+ and NO_3^- concentrations in US suggests that there is no substrate limitation in this soil and that N mineralization and nitrification could be sustained for a longer period. On the contrary, in burnt soils the shortage of available substrate for N mineralization and the active nitrification would probably lead to increased NO_3^- levels until the NH_4^+ pool is depleted. However, care must be taken when extrapolating these results and predictions to field conditions as soils preincubated in the laboratory might behave differently. In general, forest ecosystems and undisturbed grasslands have a conservative N cycle with low net mineralisation and low autotrophic nitrification (Davidson *et al.*, 1992). However, N additions and disturbance can alter this balance by stimulating autotrophic nitrification (Vitousek *et al.*, 1979) and, if plant uptake does not increase, NO_3^- will probably be lost through leaching and denitrification (Stockdale *et al.*, 2002). Similarly, burnt soils have large amounts of NH_4^+ due to combustion and high mineralisation rates, and plant uptake is negligible immediately after the fire. The relative dominance of the two main processes using NH_4^+ (O_{NH_4} and I_{NH_4}) has been suggested as a good indicator for potential N losses from soils (Stockdale *et al.*, 2002). In the present study, the $O_{\text{NH}_4}/I_{\text{NH}_4}$ ratio, was approximately 0.1 for all treatments at $t=3$ months and for US at $t=6$ months, while BS and BSM showed a much higher ratio (1.2) after 6 months of preincubation due to both the decrease in I_{NH_4} and the increase in O_{NH_4} . These results point to a significantly increased risk for N losses from the ecosystem after fire. As N losses in our laboratory experiment are low, the NO_3^- pool sharply increased in the long-term preincubation, but in field conditions, if that amount of NO_3^- is produced, the potential risk of N losses would be high. Whether N losses through leaching or denitrification dominate in burnt soils is unknown, but emissions of the greenhouse gas nitrous oxide (N_2O) have been studied in burnt soils (Karhu *et al.*, 2015). Available data shows either increased (Fierro and Castaldi, 2011; Karhu *et al.*, 2015), negative or unaltered N_2O emissions due to fire (Anderson and Poth, 1998; Dannenmann *et al.*, 2011; Inclan *et al.*, 2012). In a laboratory experiment, Karhu *et al.* (2015) identified denitrification as the main source of N_2O in burnt

soils, most probably due to the increased nitrification supplying NO_3^- (Castaldi and Aragosa, 2002; Karhu *et al.*, 2015). The high N_2O emissions from burnt soils might not be recorded in the field due to drier conditions, but they could be triggered by heavy rains (Karhu *et al.*, 2015).

4.1. Conclusions

To sum up, after 3 months of preincubation gross mineralisation and gross NH_4^+ immobilisation dominate in severely burnt soils; whereas after 6 months the soil N cycle opens up and NO_3^- accumulates in burnt soils, increasing the risk of N losses. Whether this is also the case in field conditions becomes paramount as it would have important consequences extending beyond the burnt ecosystem: N losses on-site, increased risk of eutrophication off-site and N_2O emissions due to denitrification. This study also shows that in laboratory conditions straw mulching application, very effective in reducing post-fire erosion, has only slight effects on gross N transformation rates but does not modify the increased risk of N losses in burnt soils, although this should be further investigated under field conditions.

Acknowledgements

We thank Cristina Vieites, María José Freire, Ana Quintáns and Jorge Benítez for their technical assistance in the laboratory. This work was supported by the Spanish Ministry of Economy and Competitiveness through the project AGL2012-39686-C02-01. M. Fernández-Fernández has a pre-doctoral fellowship (FPU-2012) by the Spanish Ministry of Education and her stay at Göteborgs Universitet was supported by a short-term fellowship by the same Ministry. Tobias Rütting is supported by the Swedish strategic research area "Biodiversity and Ecosystem services in a Changing Climate - BECC" (www.becc.lu.se/). The isotopic ratio mass spectrometer was partly financed by the European Regional Development Fund (EU).

Compliance with Ethical Standards

Conflicts of interest: The authors declare that they have no conflict of interest.

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Supplementary Information S1. Model fit for the unburnt soil (US) after 3 months of preincubation. Symbols represent measured concentrations of NH_4^+ -N (a) and NO_3^- -N (b) and their ^{15}N enrichment (c and d respectively) (mean $\pm$ standard deviation), and modelled data is shown with lines. Blue stands for incubations with $^{15}\text{NH}_4\text{NO}_3$ and red for incubations with $\text{NH}_4^{15}\text{NO}_3$.

Supplementary Information S2. Model fit for the burnt soil without mulching (BS) after 3 months of preincubation. Symbols represent measured concentrations of NH_4^+ -N (a) and NO_3^- -N (b) and their ^{15}N enrichment (c and d respectively) (mean $\pm$ standard deviation), and modelled data is shown with lines. Blue stands for incubations with $^{15}\text{NH}_4\text{NO}_3$ and red for incubations with $\text{NH}_4^{15}\text{NO}_3$.

Supplementary Information S3. Model fit for the burnt soil with mulching (BSM) after 3 months of preincubation. Symbols represent measured concentrations of NH_4^+ -N (a) and NO_3^- -N (b) and their ^{15}N enrichment (c and d respectively) (mean $\pm$ standard deviation), and modelled data is shown with lines. Blue stands for incubations with $^{15}\text{NH}_4\text{NO}_3$ and red for incubations with $\text{NH}_4^{15}\text{NO}_3$.

Supplementary Information S4. Model fit for the unburnt soil (US) after 6 months of preincubation. Symbols represent measured concentrations of NH_4^+ -N (a) and NO_3^- -N (b) and their ^{15}N enrichment (c and d respectively) (mean $\pm$ standard deviation), and modelled data is shown with lines. Blue stands for incubations with $^{15}\text{NH}_4\text{NO}_3$ and red for incubations with $\text{NH}_4^{15}\text{NO}_3$.

Supplementary Information S5. Model fit for the burnt soil without mulching (BS) after 6 months of preincubation. Symbols represent measured concentrations of NH_4^+ -N (a) and NO_3^- -N (b) and their ^{15}N enrichment (c and d respectively) (mean $\pm$ standard deviation), and modelled data is shown with lines. Blue stands for incubations with $^{15}\text{NH}_4\text{NO}_3$ and red for incubations with $\text{NH}_4^{15}\text{NO}_3$.

Supplementary Information S6. Model fit for the burnt soil with mulching (BSM) after 6 months of preincubation. Symbols represent measured concentrations of NH_4^+ -N (a) and NO_3^- -N (b) and their ^{15}N enrichment (c and d respectively) (mean $\pm$ standard deviation), and modelled data is shown with lines. Blue stands for incubations with $^{15}\text{NH}_4\text{NO}_3$ and red for incubations with $\text{NH}_4^{15}\text{NO}_3$.