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**FERNÁNDEZ-FERNÁNDEZ, M.; GOMEZ-REY, M.X.*; GONZÁLEZ-PRIETO, S.J. (2015). Effects of fire and three fire-fighting chemicals on main soil properties, plant nutrient content and vegetation growth and cover after 10 years.** ***Science of the Total Environment*** **515-516, 92-100.**

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**Abstract**

The study addresses a knowledge-gap in the long-term ecological consequences of fire and fire-fighting chemicals. Ten years after a prescribed fire and the application of three fire-fighting chemicals, their effects on the soil-plant system were evaluated. Five treatments were established: unburnt soils (US) and burnt soils treated with water alone (BS), foaming agent (BS+Fo), Firesorb (BS+Fi) and ammonium polyphosphate (BS+Ap). Soils (0-2 cm depth) and foliar material of shrubs (*Erica umbellata, Pterospartum tridentatum* and *Ulex micranthus*) and trees (*Pinus pinaster*) were analysed for total N, $\delta^{15}N$, and soil-available and plant total macronutrients and trace elements. Soil pH, $NH_4^+$-N and $NO_3^-$-N; pine basal diameter and height; and shrub cover and height were also measured. Compared with US plots, burnt soils had less nitrates and more Mo. Although differences were not always significant, BS+Ap had the highest levels of soil available P, Na and Al. Plants from BS+Ap plots had higher values of $\delta^{15}N$ (*P. pinaster* and *E. umbellata*), P (all species), Na (*P. tridentatum* and *U. micranthus*) and Mg (*E. umbellata* and *P. tridentatum*) than other treatments; while K in plants from BS+Ap plots was the highest among treatments for *P. pinaster* and the lowest for the shrubs. Pines in US plots were higher and wider than in burnt treatments, except for BS+Ap, where the tallest and widest trees were found, although half of them were either death (the second highest mortality after BS+Fi) or had a distorted trunk. BS+Ap was the treatment with strongest effects on plants, showing *E. umbellata* the lowest coverage and height, *P. tridentatum* the highest coverage, *U. micranthus* one of the lowest coverages and being the only treatment where *Genista triacanthos* was absent. Consequently, it is concluded that both fire and ammonium polyphosphate application had significant effects on the soil-plant system after 10 years.

**Keywords:** flame retardants; $\delta^{15}N$; nutrients; shrubs; trace elements; trees.

## 1. Introduction

Wildfires are an important issue due to their impacts not only on the burnt ecosystems but also on the adjacent systems (agricultural, urban, transports, watersheds...) (Birot 2009). Moreover, the time span of their effects ranges from the combustion period up to a few decades afterwards (Birot 2009). Climate change and the shift in land use will very likely contribute to an increase in the extent and number of wildfires (Birot 2009; Pereira et al. 2011).

The application of water added with fire-fighting chemicals (FFCs) helps to extinguish wildfires; therefore, this strategy has been widely used since the 1930s (Giménez et al. 2004). Research on FFCs has been mainly focused on their effectiveness as fire extinguishers (see Giménez et al. (2004)). As most FFCs are used in natural areas, sometimes with high wilderness or landscape values, more attention should be paid to their potential effects on the ecosystems (Adams and Simmons 1999; Basanta et al. 2002). Most of the times, FFCs are applied by aircraft and it is virtually impossible to target the chemicals exclusively to the burning area. Consequently, there are three possible scenarios under realistic wildfire-fighting conditions: (1) the retardant reaches soils in the fire line and both soil and retardant are affected by the heating and oxidation caused by the fire; (2) the retardant reaches soils in the burnt area which are still hot or warm; and (3) the retardant reaches soils close to the fire line but not affected by the fire. Most studies are focused on the last two scenarios, probably due to the problems derived from studying the narrow and very heterogeneous area of the first scenario (Couto-Vázquez et al. 2011).

In the 1980s it was already suggested that the high amounts of N and P present in many FFCs could have a fertilizing effect on plants in burnt areas (Neary and Currier 1982). Although longer-term studies would be better to assess the consequences of FFC application on vegetation (Giménez et al. 2004; Couto-Vázquez et al. 2011), some studies already available show that some plant communities are affected in different ways by FFCs in the short-term. Larson and Duncan (1982) studied the effects of application of diammonium phosphate (DAP) as a fire retardant on burnt and unburnt grassland areas in California. They found that native legumes failed to establish on the plots treated with DAP, possibly due to the high amounts of N it released, and that treated areas doubled their biomass and were more intensively grazed by cattle than the untreated ones. Bradstock et al. (1987) found that an ammonium sulphate retardant applied on a forest of *Eucalyptus* and *Angophora* in New South Wales produced leaf (but not tree) death and varied levels of foliage damage to understorey shrubs, depending on the species and the extent of retardant coverage. Compared to untreated areas, they also found a decrease in vegetation cover for 19 of 45 species in the plots treated with the FFC. Larson and Newton (1996) conducted some experiments in North Dakota prairies with Phos-Check G75-F (an N- and P-based FFC) and the foam suppressant Silv-Ex (an N-based FFC) alone and in combination with fire. Results showed that the former increased biomass, decreased species richness, inhibited leaf production in some species and triggered dominance of the weed *Poa pratensis*; and the latter decreased species richness and increased insect herbivory. In a subsequent study with the same treatments in a North Nevada shrub steppe, Larson et al. (1999) found that although species richness was affected in the short-term by Phos-Check, after a year the larger impacts on plant communities were due to the burning rather than to any chemical treatment. Bell et al. (2005) studied the effect of Phos-Check on an Australian heathland and found that a single application did not change species composition substantially, although it caused plant death of some species and weed invasion at high concentrations of retardant. Laboratory experiments with unheated soils treated with Fire-Trol 934 (an N- and P-based FFC) showed that seed germination and seed viability of several Mediterranean species decreased when exposed to the chemical (Cruz et al. 2005; Luna et al. 2007). Recently, Song et al. (2014) found that three fire suppressant foams (Forexpan S, Phos-Chek-WD881, and Silv-ex) reduced seed germination rates in the laboratory, but did not affect seedling emergence rate of the tested species in the field.

Despite the important role soils play in terrestrial ecosystems, the effects of FFCs on soils have been scarcely studied and only in recent years (Giménez et al. 2004). In a 3-month laboratory experiment with heated and unheated soils treated with the fire retardant Firesorb (a terpolymer of acrylic acid and acrylamide), there were no major adverse effects of the retardant on the soil microbial community, the heating itself being a greater factor affecting the microorganisms (Basanta et al. 2002; Díaz-Raviña et al. 2006). García-Villaraco et al. (2009) assessed the effects of Fire-Trol 934 during one year on burnt and unburnt Mediterranean pastures and also concluded that the application of the fire retardant caused minimal or negligible negative effects on functional diversity and total activity of soil microorganisms. Hopmans et al. (2007) applied Phos-Check to an Australian heathland and found that the addition of the retardant caused a transient change of pH (lower), and salinity and available N (both higher), although pre-treatment levels were reached again one year later. Phosphorus also increased considerably and P levels one year after Phos-Check application were still high despite evidence of P leaching into the subsoil, suggesting possible long-term impacts on growth and composition of the heathland vegetation which is very sensitive to high concentrations of P. The influence of fire, Fire-Trol 931 (an N- and P-based FFC) and pine seedlings on leachates from pots was evaluated by Pappa et al. (2006), Pappa et al. (2008) and Koufopoulou et al. (2014) in a laboratory experiment. These authors found that the FFC increased the leaching of N (substantially) and P, as well as that of Na, Fe and Si which were mobilized by the FFC-derived $NH_4^+$; the fertilizer effect of the FFC also favoured the growth of pines planted in the pots, where N and P leaching was consequently reduced. Therefore, the effects of N- or P-based FFCs on soil and water are cause of increasing concern and have led to the search for more environmentally friendly FFCs, as magnesium carbonates (Liodakis and Tsoukala 2010).

Up to now, the most comprehensive field experiment on the long-term influence of FFCs on terrestrial ecosystems assessed throughout five years the effects of fire and FFCs on soils (chemical, biochemical and microbial properties) and plants (size, coverage and nutrient content of dominant species) (Couto-Vázquez and González-Prieto 2006; García-Marco and González-Prieto 2008; Barreiro et al. 2010; Couto-Vázquez et al. 2011). These authors set up the so-called 'Tomiño experiment' in a medium size experimental area (1000 $m^2$) on a NW Spain shrubland, where they applied three FFCs (foaming agent RFC-88, acrylic acid-acrylamide terpolymer Firesorb and ammonium polyphosphate FR Cros 134 P) immediately after a prescribed fire. Their results showed an increase in available N and P, which was especially strong and long-lasting in the ammonium polyphosphate treatment; a factor that could delay vegetation recovery in these plots (Couto-Vázquez and González-Prieto 2006; Couto-Vázquez et al. 2011). Five years after the fire, shrub cover and height, as well as pine size and mortality, were affected by the ammonium polyphosphate and to a lesser extent by the terpolymer (Couto-Vázquez et al. 2011). Changes in the microbial community structure were also detected five years after the prescribed fire, being more noticeable in the terpolymer and the ammonium polyphosphate plots (Barreiro et al. 2010).

These results suggest that the effects of FFCs on soils and plants can persist even after five years, making it necessary to broad the time span of the studies on this topic. Therefore, the aim of this paper is to extend from 5 to 10 years the 'Tomiño experiment' and to analyse total N, $\delta^{15}N$ and soil-available and plant total macronutrient and trace element concentrations on soils and foliar material of the dominant shrubs and tree species. Moreover, soil pH and inorganic forms of soil N were measured, as well as plant size and coverage.

## 2. Materials and methods

The experimental area, located at Alto da Pedrada (Tomiño, Galicia, NW Spain; UTM coordinates 29T 05182 – 46509; altitude of 455 m asl), has been previously used by the authors' research team (Couto-Vázquez and González-Prieto 2006; García-Marco and González-Prieto 2008; Barreiro et al. 2010; Couto-Vázquez et al. 2011). The soil, developed over a parent material of paragneisses and with a slope of 18–19%, had in 2003 a vegetation cover of approximately 50-60 cm height dominated by *Pterospartum tridentatum, Erica umbellata* and *Ulex europaeus*, and there were also some individuals of *Ulex micranthus, Ulex minor* and *Erica cinerea*. In the summer of 2003, within a total surface of 40 x 25 m, five in-situ treatments were established: plots in the unburnt area (US) as a control; and plots in the burnt area that would receive 2 L $m^{-2}$ of water alone (BS) or water with foaming agent at 1% (BS+Fo), with Firesorb at 1.5% (BS+Fi) or with ammonium polyphosphate at 20% (BS+Ap). After a prescribed fire (i.e. with the fire extinguished but with the soil still warm), burnt soil treatments were arranged in a fully randomized design with four replications per treatment and 1 m separation around each plot (4 × 4 m), whereas the four unburnt soil replicates were established along the slope and adjacent to the burnt ones (Couto-Vázquez and González-Prieto 2006). As the area is under a high grazing pressure, the plots were fenced after the prescribed fire.

The firefighting chemicals were selected among the most widely used in countries of the Mediterranean basin: RFC-88 is a foaming agent produced by Auxquimia SA; Firesorb is a light cross-linked terpolymer of acrylic acid, acrylamide and acrylamidopropanesulfonic acid sodium salt, manufactured by Evonik Stockhausen Gmbh; and FR Cros 134 P is an ammonium polyphosphate produced by Chemische Fabrik Budenheim KG. Data on density, total nutrient concentrations and $\delta^{15}N$ of the three firefighting chemicals are available in previous papers (Couto-Vázquez and González-Prieto 2006; García-Marco and González-Prieto 2008).

Seven months after the prescribed fire, four 1-year old pine seedlings (supplied by a forest nursery from a genetically similar stock) were planted in each plot to follow their development and to assess how the fire and flame retardants affect post-fire reforestation. Height and basal diameter of pines were measured quarterly during the first 3 years and then 4, 5 and 10 years after the prescribed fire.

The soil–plant system was characterised 10 years after the fire as it was done 5 years before (i.e. 5 years postfire) (Couto-Vázquez et al. 2011). After removing the plant litter layer, soil samples were taken from the A horizon (0–2-cm depth). Five 15 x 15 cm squares, uniformly distributed within each plot, were sampled, sieved at 4 mm, mixed and thoroughly homogenised. The soil was divided into fresh subsamples, which were kept at 4 ºC for inorganic-N measurements, and air-dried sub-samples for the other analyses. For total N and $\delta^{15}N$ determination, aliquots of air-dried subsamples were finely ground (< 100 µm) in a planetary ball mill (Retsch PM100, Retsch GmbH, Haan, Germany, with cups and balls of zirconium oxide). The presence or absence of the dominant shrub species (*Erica umbellata, Genista triacanthos, Pterospartum tridentatum, Ulex europaeus* and *Ulex micranthus*), as well as their maximum height, was recorded within each plot along three down-slope transects with sampling points every 25 cm (avoiding 50 cm on each plot-side to prevent possible edge effects). Plant material from the upper half of *Pinus pinaster* (50 pairs of needles from the youngest branches), *Ulex micranthus, Erica umbellata* and *Pterospartum tridentatum* (~10 g of spines, leaves and twigs, and whorls respectively) was also collected (one composite sample per species per plot). *Genista triacanthos* and *Ulex europaeus* were not sampled because they were not analysed in the previous studies and they were lacking in some plots. The plant material was washed successively with tap and then deionised water, oven-dried at 60 ºC for 48 h and finely ground in the same way as soils.

The dry matter content of soils and plant material was assessed by oven-drying sub-samples at 110 ºC for 5 h. Soil pH was measured with a pH meter (Metröhm, Switzerland) in 0.1 M KCl employing a soil:solution ratio of 1:2.5. Total N and $\delta^{15}N$ of soils and plants were measured in ground samples with an elemental analyser (Carlo Erba, Milano, Italy) 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 isotopic standards (IAEA-N1 and IAEA-N2, 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. For soil $NH_4^+$-N and $NO_3^-$-N analysis, an extraction-diffusion method described previously (Couto-Vázquez and González-Prieto 2006) was used, but with 48-h diffusion periods at 50 ºC; three blanks and three standards ($NH_4NO_3$) were included in each batch to subtract N from reagents and to check for N recovery. Instead of separate extraction with acetic acid for macronutrients and DTPA-$CaCl_2$-TEA for trace elements, as done in previous samplings of the study (Couto-Vázquez and González-Prieto 2006; García-Marco and González-Prieto 2008), soil Na, K, Mg, Ca, P, Al, Fe, Mn, Zn, Cu and Mo were jointly extracted by shaking soils for 2 hours with a solution of $NH_4$Ac 1 M and DTPA 0.005 M (soil:solution ratio 1:5). Compared with the respective traditional soil-tests, $NH_4$Ac-DTPA extracts similar quantities of Ca and higher amounts of K, Mg, Al, Cu, Fe, Mn and Zn (1.3x, 2.5x, 13x, 3x, 2.4x, 1.8x and 2.5x, respectively; S. García-Marco, pers. comm.). The soil extracts and the corresponding blanks were then filtered through cellulose paper (Filter-Laboratory 1242, 90-mm diameter) and analysed by simultaneous inductively coupled plasma optical emission spectrometry (ICP-OES, Varian Vista Pro, Mulgrave, Australia). A calibration curve prepared with certified standards of all elements was measured beforehand and one of the calibration solutions was routinely included in each set of 30 samples as a quality control and, when necessary, the calibration curve was measured again. In order to measure the total nutrient content of plant material, aliquots of 500 mg of plant samples were digested in a high performance digestion unit (Milestone 1200 Mega, Sorisole, Italy) for 55 min with 8 mL of 65% $HNO_3$ and 25 mL of 30% $H_2O_2$. Blanks and reference materials (hay powder No. 129, Community Bureau of References, EU; apple leaves No. 1515, National Institute of Standards and Technology, USA) were also included in each digestion batch to subtract elements from reagents and to check for element recovery. Once cooled, the solutions were filtered through quantitative filter paper (Filter-laboratory 1242, 90-mm diameter), transferred to 25 mL volumetric flasks, made to volume with water and analysed by ICP-OES as previously described for soils. Due to analytical interferences for determining total P in pine needles by this method, they were digested with $HNO_3$, HCl and $H_2O_2$ on a laboratory hot plate. The first day, 2 mL of $HNO_3$ 70% were added to 150 mg aliquots of ground pine needles and the mixture was kept at room temperature for two days. Afterwards, 1 mL of HCl 37% was added, the sample tubes were slowly heated up to 130 ºC and then the hot plate was turned off. The heating process was repeated twice (without the addition of HCl) the following two days. Then, the samples were treated with 4 mL of $H_2O_2$ 30% (2 mL per day during two days) and slowly heated to 90 ºC. As for the other elements, blanks and reference material (hay powder No. 129, Community Bureau of References, EU) were also included in each digestion batch. The digested samples were transferred to 200 mL volumetric flasks, the pH was adjusted within the range 3 to 5 by adding NaOH pellets, made to volume with water and their total P content was measured by the method of Murphy and Riley (1962). Analytical-grade chemicals and type I water (ASTM 2008) were used for analyses.

All analyses were carried out in duplicate and means were used in the statistical procedures, after doing a third analysis if the coefficient of variation was higher than 5 %. The effects of treatments on soil and plant variables 10 years after the prescribed fire were statistically analysed by one-way ANOVA. After checking the normal distribution of variables (Shapiro-Wilk's W test) and the equality of variances among treatment groups (Levene's test), significant differences among the group means were established at $p<0.05$ using: (1) Tukey's test in the case of homoscedasticity of the original data (or data after Tukey's ladder of power or Box–Cox transformations); or (2) Games– Howell's test if variances remained heterogeneous after data transformations. All statistical analyses were performed with SPSS 15.0. software (SPSS Inc., Chicago, IL).

## 3. Results

### *3.1. Effects of fire and FFC on soil properties*

Table 1 shows data on the main soil properties 10 years after the fire. No effect of fire or FFC on soil $pH_{KCl}$ was found. Nitrate concentration was significantly higher in US plots (12 mg $kg^{-1}$ dw) than in the burnt treatments (about 3 mg $kg^{-1}$ dw) except for BS+Fi (6 mg $kg^{-1}$ dw), while no differences among treatments in soil total N content, $\delta^{15}N$ and $NH_4^+$-N were found. BS+Ap plots had significantly more available P (14 mg $kg^{-1}$ dw) than the other treatments (5 mg $kg^{-1}$ dw). The concentration of Na was significantly higher in BS+Ap than in BS+Fi, with the other treatments having intermediate values, while no differences among treatments were found for Ca, K and Mg.

**Table 1.** Effects of fire and firefighting chemicals on soil properties (mean ± S.D.) 10 years after the prescribed fire. Key: US, unburnt soil; BS, burnt soil; BS+Fo, burnt soil + foaming agent; BS+Fi, burnt soil + Firesorb; BS+Ap, burnt soil + ammonium polyphosphate; dw, dry weight. Within each row, treatments with different letters are significantly different (p<0.05).

| | US | BS | BS+Fo | BS+Fi | BS+Ap |
|---|---|---|---|---|---|
| $pH_{KCl}$ | 3.16 ± 0.08[a] | 3.19 ± 0.02[a] | 3.24 ± 0.09[a] | 3.25 ± 0.06[a] | 3.12 ± 0.05[a] |
| $NH_{4+}$-N (mg $kg^{-1}$ dw) | 44.5 ± 6.2[a] | 41.9 ± 6.8[a] | 43.5 ±6.1[a] | 42.9 ± 10.8[a] | 34.9 ±3.1[a] |
| $NO_{3-}$-N (mg $kg^{-1}$ dw) | 12.1 ± 3.8[a] | 2.8 ±1.5[b] | 3.5 ± 2.3[b] | 6.3 ± 2.9[ab] | 2.8 ± 0.4[b] |
| Total N (g $kg^{-1}$ dw) | 8.95 ± 1.48[a] | 7.92 ± 1.04[a] | 8.20 ± 0.51[a] | 7.71 ± 0.96[a] | 8.54 ± 0.68[a] |
| $\delta^{15}N$ (‰) | 1.87 ± 0.25[a] | 1.73 ± 0.27[a] | 1.51 ± 0.21[a] | 1.59 ± 0.21[a] | 1.86 ± 0.33[a] |
| Available Al (mg $kg^{-1}$ dw) | 194 ± 19[b] | 243 ± 15[ab] | 245 ± 32[ab] | 262 ± 23[a] | 269 ± 26[a] |
| Available Ca (mg $kg^{-1}$ dw) | 118 ± 45[a] | 118 ± 48[a] | 119 ± 38[a] | 105 ± 47[a] | 69 ± 20[a] |
| Available Cu (mg $kg^{-1}$ dw) | 0.55 ± 0.09[a] | 0.55 ± 0.12[a] | 0.60 ± 0.07[a] | 0.47 ± 0.06[a] | 0.58 ± 0.18[a] |
| Available Fe (mg $kg^{-1}$ dw) | 900 ± 31[a] | 791 ± 14[b] | 796 ± 43[b] | 750 ± 48[b] | 723 ± 53[b] |
| Available K (mg $kg^{-1}$ dw) | 95.3 ± 11.9[a] | 96.5 ± 15.3[a] | 103.3 ± 15.8[a] | 101.0 ± 21.8[a] | 92.0 ± 6.2[a] |
| Available Mg (mg $kg^{-1}$ dw) | 76.7 ± 28.1[a] | 82.8 ± 30.2[a] | 86.0 ± 32.3[a] | 74.3 ± 32.1[a] | 56.5 ± 9.3[a] |
| Available Mn (mg $kg^{-1}$ dw) | 3.73 ± 1.33[a] | 4.11 ± 1.68[a] | 3.91 ± 0.21[a] | 4.41 ± 0.50[a] | 4.18 ± 1.27[a] |
| Available Mo (mg $kg^{-1}$ dw) | 0.44 ± 0.03[b] | 0.53 ± 0.03[a] | 0.53 ± 0.07[ab] | 0.56 ± 0.03[a] | 0.57 ±0.04[a] |
| Available Na (mg $kg^{-1}$ dw) | 38.0 ± 8.9[ab] | 31.0 ± 3.9[ab] | 36.0 ± 6.6[ab] | 28.3 ± 2.5[b] | 43.3 ± 6.7[a] |
| Available P (mg $kg^{-1}$ dw) | 4.77 ± 0.21[b] | 4.68 ± 0.71[b] | 4.60 ± 0.43[b] | 5.15 ± 0.68[b] | 13.80 ± 1.24[a] |
| Available Zn (mg $kg^{-1}$ dw) | 2.53 ± 0.64[a] | 2.46 ± 0.72[a] | 1.90 ± 0.18[a] | 3.34 ± 1.66[a] | 1.45 ± 0.51[a] |

In unburnt soils, Fe concentration was significantly higher than in burnt soils. The available Mo differs slightly but significantly among treatments, being higher in BS+Ap, BS+Fi and BS than in US. For the available Al the only significant differences found were those among BS+Ap and BS+Fi plots (highest) and US (lowest). Neither the fire nor the addition of FFC affected the concentration of Cu, Mn and Zn.

*3.2. Effects of fire and FFC on plant elemental composition*

*Erica umbellata* and *P. pinaster* had $\delta^{15}N$ values ranging from -3 to 0 ‰, whereas *P. tridentatum* and *U. micranthus* values were between -0.5 and 0.5 ‰ (Fig. 1). Significant differences among treatments for $\delta^{15}N$ were found in *U. micranthus* (BS and BS+Fo > US) and *P. pinaster* (BS+Ap > BS+Fi), while in *P. tridentatum* neither fire nor FFC effects were observed. For *E. umbellata* an additional test was done to compare BS+Ap with the other burnt treatments considered together, having the former significantly higher $\delta^{15}N$ values than the latter. Total N content of all the studied plant species was between 10 and 25 g $kg^{-1}$ dw and was neither affected by fire nor by FFCs 10 years after the fire (see Supplementary data).

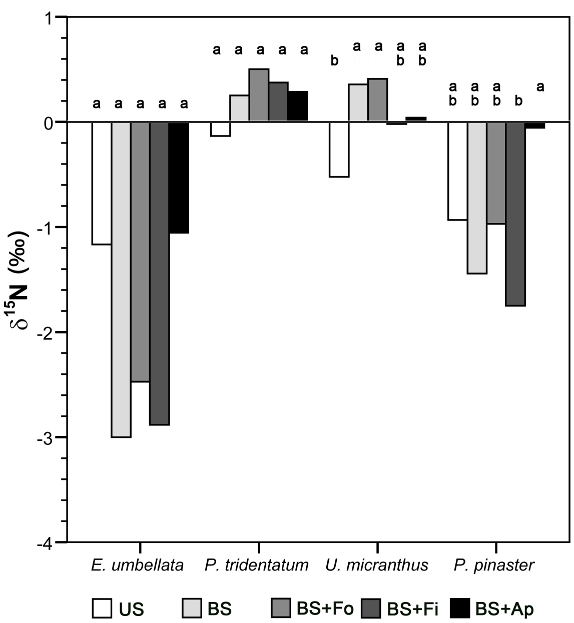


**Fig. 1.** $\delta^{15}N$ in foliar material 10 years after the prescribed fire. For each plant species different letters indicate significant differences among treatments ($p<0.05$). Key: US, unburnt soil; BS, burnt soil; BS+Fo, burnt soil + foaming agent; BS+Fi, burnt soil + Firesorb; BS+Ap, burnt soil + ammonium polyphosphate; dw, dry weight.

Regardless of the species, plant total P in BS+Ap plots was approximately twice as high as in the rest of the treatments ($p< 0.05$; Fig. 2a). For plant total K (Fig. 2b) significant differences were found between BS plots (higher) and BS+Ap plots (lower) in all shrub species and, moreover, in *P. tridentatum* all treatments were significantly higher than BS+Ap. In *P. pinaster* the highest K levels were found in BS+Ap and BS+Fi and the lowest in US and BS ($p< 0.05$). The highest Na levels were found in BS+Ap plots for *P. tridentatum* and *U. micranthus* and in US plots for *E. umbellata*, whereas in *P. pinaster* no significant differences due to either fire or FFCs were found (Fig. 2c). Whereas *U. micranthus* and *P. pinaster* showed no significant differences among treatments in plant total Mg, *E. umbellata* from BS+Ap plots had higher levels of Mg than plants from the other plots ($p< 0.05$), and *P. tridentatum* showed a similar tendency to that of *E. umbellata*, although differences were not always significant (Fig. 2d). Plant total Ca was neither affected by fire nor FFCs in any of the studied species (Supplementary data).

Significant differences among treatments for Cu and Mn were only found in *E. umbellata* (Cu: BS > US ≈ BS+Ap; Mn: BS > BS+Ap) and for Fe and Zn in *P. tridentatum* (US > burnt treatments) (Fig. 2e, f and Supplementary data). Plant total Al (Supplementary data) was neither affected by fire nor FFCs in *E. umbellata* and *P. pinaster*, whereas small but significant differences were found in *P. tridentatum* (US > BS ≈ BS+Fo) and *U. micranthus* (US ≈ BS+Ap > BS). Molybdenum concentration was close to the

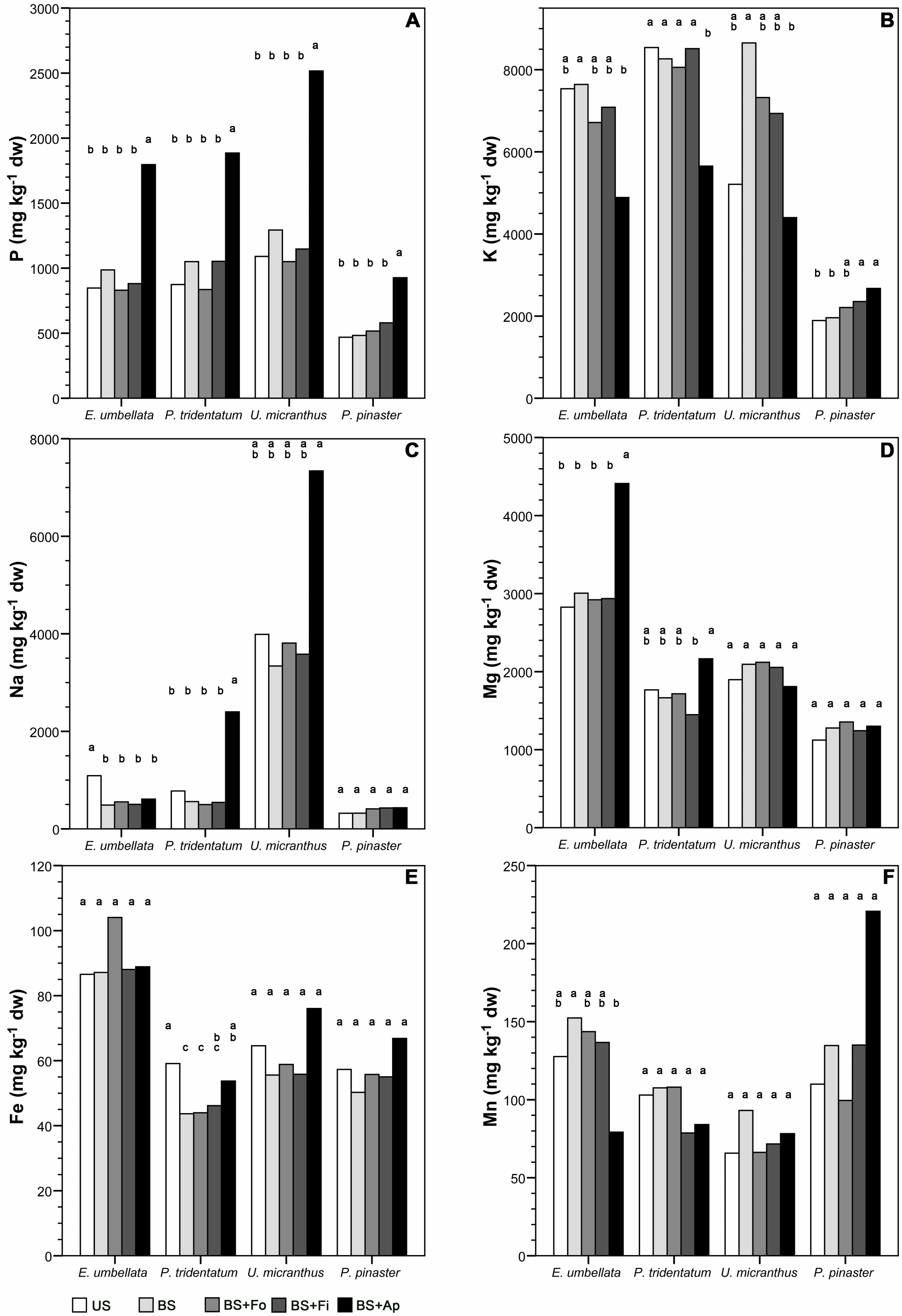


**Fig. 2.** Plant total nutrient concentration in foliar material 10 years after the prescribed fire: a) phosphorus; b) potassium; c) sodium; d) magnesium; e) iron; and f) manganese. For each plant species different letters indicate significant differences among treatments ($p<0.05$). Key: US, unburnt soil; BS, burnt soil; BS+Fo, burnt soil + foaming agent; BS+Fi, burnt soil + Firesorb; BS+Ap, burnt soil + ammonium polyphosphate; dw, dry weight.

detection limits in shrubs and in pines and its variance was very high and heterogeneous (data not shown).

*3.3. Effects of fire and FFC on vegetation cover*

Pines growing in BS+Ap plots were the tallest and had the largest basal diameter, followed by pines from US, BS+Fi, BS+Fo and BS for both variables (Fig. 3). The highest pine mortality was in BS+Fi plots (only half of the 16 planted pines were alive at t=10 years). In BS+Ap plots less pines had died (3 out of 16), but 5 had distorted trunks. In US, BS and BS+Fo the pine mortality was lower (3, 3 and 2 dead pines respectively). Although almost 25% of the pines had died by t=10 years, there were only one BS+Fi and one BS+Ap plot without living pines. Between years 5 and 10 after the fire, two pines from a US plot died and the trunks of 5 pines from BS+Ap plots became distorted.

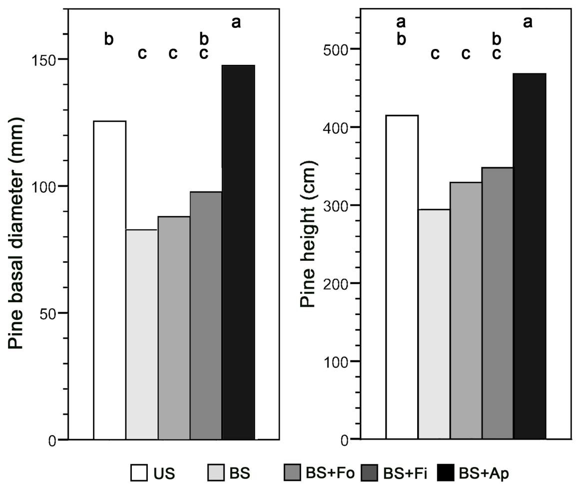


**Fig. 3.** Basal diameter (left) and height (right) of *P. pinaster* 10 years after the prescribed fire. Different letters indicate significant differences among treatments ($p<0.05$). Key: US, unburnt soil; BS, burnt soil; BS+Fo, burnt soil + foaming agent; BS+Fi, burnt soil + Firesorb; BS+Ap, burnt soil + ammonium polyphosphate.

Figures 4 and 5 show cover and height of the studied shrubs. Ten years after the fire, both plant cover and height of *E. umbellata* were significantly lower in BS+Ap plots (plant cover around 30%; mean height 60 cm) than in the other treatments (plant cover > 85%; mean height 70-80 cm). *Genista triacanthos*, not previously recorded in the experimental plots or the surrounding area, appeared in plots of all treatments except BS+Ap and covered 7-19% of soil surface with a mean plant height between 70 and 90 cm, without significant differences in any of the two variables among treatments. For *P. tridentatum* the plant cover ranged between 40% and 95%, being significantly higher in BS+Ap plots, and mean plant height was around 70-90 cm without significant differences among treatments. *Ulex europaeus* covered a small proportion of the soil (< 10%) but these plants were the tallest among the shrubs (90 to 150 cm). There were no significant differences in *U. europaeus* cover among treatments and no statistical analysis could be done for its height (this species was only present in 8 plots). The cover of *U. micranthus* was between 20% and 50%, decreasing in the order BS+Fo, BS, BS+Fi, BS+Ap and US, while its mean height ranged from 70 to 90 cm and did not differ significantly among treatments.

## 4. Discussion

*4.1. Effects of fire and FFCs on soil properties and plant elemental composition*

During the first post-fire months Couto-Vázquez and González-Prieto (2006) reported significantly higher soil $pH_{KCl}$ in burnt plots, which resumed to pre-fire values after one year. Five years after the fire all plots became significantly acidified compared to t=0 (Couto-Vázquez et al. 2011) and no subsequent changes were recorded after 10 years. As the area of the field experiment is under a high grazing pressure and the plots were fenced after the prescribed fire, Couto-Vázquez et al. (2011) attributed the soil acidification to grazing exclusion as also reported in other studies (Basher and Lynn 1996; Dormaar and Willms 1998).

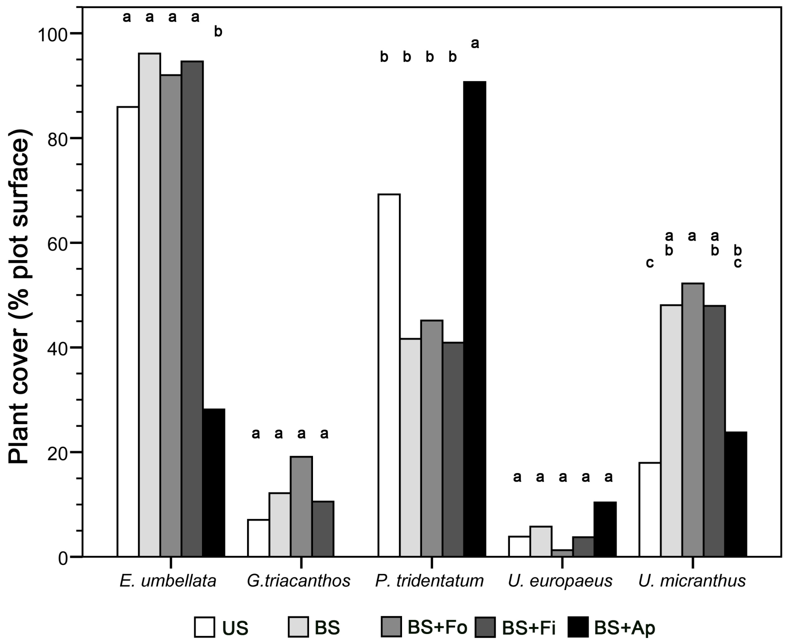


**Fig. 4.** Plant cover of the shrub species 10 years after the prescribed fire. For each shrub species different letters indicate significant differences among treatments ($p<0.05$). Key: US, unburnt soil; BS, burnt soil; BS+Fo, burnt soil + foaming agent; BS+Fi, burnt soil + Firesorb; BS+Ap, burnt soil + ammonium polyphosphate.

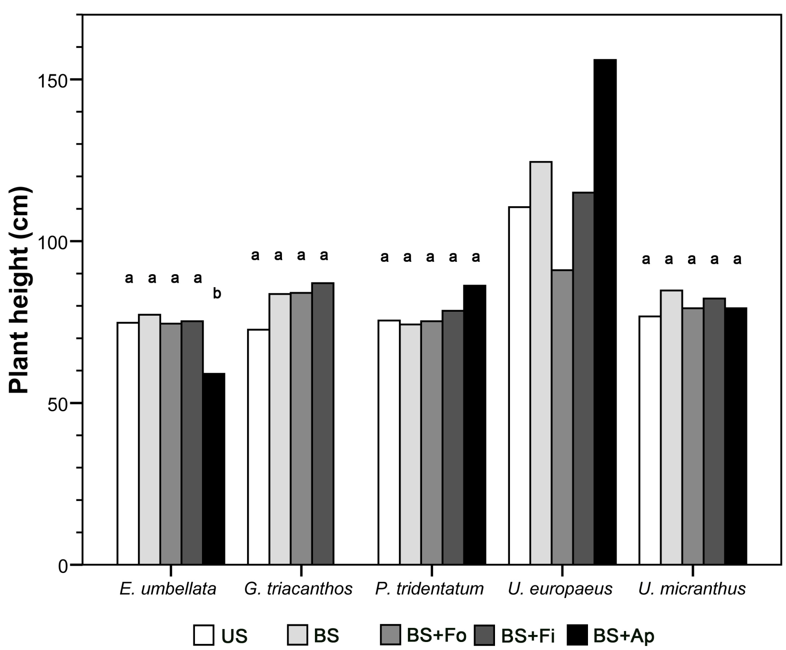


**Fig. 5.** Plant height of the shrub species 10 years after the prescribed fire. For each shrub species different letters indicate significant differences among treatments ($p<0.05$). For U. europaeus no statistical analysis could be done because it was only present in 8 plots. Key: US, unburnt soil; BS, burnt soil; BS+Fo, burnt soil + foaming agent; BS+Fi, burnt soil + Firesorb; BS+Ap, burnt soil + ammonium polyphosphate.

Although the meta-analysis done by Johnson and Curtis (2001) showed a significant positive effect of fire on soil N 10 years after the fire, in our case it was fairly constant throughout the whole field experiment. The higher total N concentration in plants from BS+Ap plots recorded at t=5 years (differences not significant for all species; Couto-Vázquez et al. 2011) contrasted with the lack of significant differences 10 years after the fire; indeed, *E. umbellata* from the BS+Ap treatment had the lowest total N content. Therefore, it can be concluded that the N-fertilizing effect that ammonium polyphosphate had on the studied species during the first 5 years is not noticeable 10 years after the fire, likely due to the substantial N leaching as Pappa et al. (2006) and Pappa et al. (2008) reported in pots added with an N- and P-based FFC.

After an increase of soil $\delta^{15}N$ in burnt soils during the first post-fire year, attributed to N losses triggered by the fire (Couto-Vázquez and González-Prieto 2006), soil $^{15}N$ abundance increased in all treatments at t=5 years (Couto-Vázquez et al. 2011) and decreased at t=10 years but remained higher than pre-fire levels. The lack of differences among treatments both five and 10 years after the fire suggests that factor(s) other than fire or FFCs are influencing the soil $\delta^{15}N$, possibly changes in the N cycle of the soil-plant system due to grazing exclusion. For most species, plant $\delta^{15}N$ values increased slightly from five (Couto-Vázquez et al. 2011) to 10 years after the fire, although differences among treatments more or less prevailed for all species. In shrubs there were no differences in *P. tridentatum*, small differences in *U. micranthus* and significant differences among burnt treatments in *E. umbellata* due to ammonium polyphosphate addition. In *P. pinaster* the BS+Ap plots showed the highest $\delta^{15}N$ values at t=10 years, as at 5 years earlier, but differences with the other treatments were no longer significant except for BS+Fi.

Both *E. umbellata* and *P. pinaster* can establish mycorrhizal symbioses that lead to $^{15}N$-depleted plants and $^{15}N$-enriched fungi due to a strong isotopic fractionation (West et al. 2006). The high $\delta^{15}N$ values recorded for BS+Ap plots and the positive correlations between $\delta^{15}N$ and total plant P in these species ($r= 0.535$ to $0.655$; $p< 0.02$) suggest that, even 10 years after the application of ammonium polyphosphate, the N transfer from the fungi to the plants was affected by either: a) a reduction in soil mycorrhizal abundance; or b) a rise in the direct uptake of soil N by pines, probably because ammonium polyphosphate addition increased the labile soil N pool even in the long-term.

The soil $NH_4^+$-N concentrations varied greatly with time (especially in BS+Ap plots) but at t=10 years they were similar among treatments and to prefire levels. Contrastingly, from one to 10 years after the fire the $NO_3^-$-N levels were higher in unburnt than in burnt plots (except BS+Ap at t=5 years); therefore, data from the present study suggests a decrease of net nitrification in the burnt soil even in the long-term. As highlighted by Gómez-Rey and Gonzalez-Prieto (2013), published results about the effects of fire on gross and net nitrification are contradictory, with some studies reporting a decrease in post-fire nitrification (see Bastias et al. (2006) and references therein) and others the opposite (Kaye and Hart 1998; DeLuca et al. 2006; Koyama et al. 2010, 2012).

Despite a marked decrease with respect to the 24-fold higher values recorded at t=5 years (Couto-Vázquez et al. 2011), the available P in BS+Ap soils was still three times higher than in the other treatments 10 years after the fire. Total P in plants from BS+Ap plots was approximately twice as high as in the other treatments both 5 and 10 years after the fire (Couto-Vázquez et al. 2011). Several authors have suggested that the application of P-based fire retardants could have long-lasting effects on soil and plant P and therefore long-term studies of at least 10 years are needed (Larson et al. 1999; Giménez et al. 2004). Our results filled this gap of knowledge showing a significant effect of the ammonium polyphosphate treatment on soil available and plant total P concentrations even 10 years after the fire. Therefore, despite the substantial P leaching reported after adding an ammonium polyphosphate based FFC (Pappa et al. 2006; Pappa et al. 2008), our results showed that the remaining amount of P was enough to substantially modify the availability and plant uptake of these nutrients for a long time.

The high concentration of soil P in BS+Ap plots was the main and most long-lasting factor affecting vegetation development during the experiment, although other factors such as pH, available N and certain nutrient ratios (Fe/Mn, P/Fe, P/Zn) have also played an important role in earlier stages

(Couto-Vázquez and González-Prieto 2006; García-Marco and González-Prieto 2008; Couto-Vázquez et al. 2011). For the leguminous species (*P. tridentatum* and *U. micranthus*) there was a positive correlation between plant total N and P ($r= 0.621$ to 0.691; $p< 0.005$). Although contradictory results have been reported about the effects of P fertilization on $N_2$ fixation rates in legumes (see Cavard et al. (2007) and references therein), the N-P correlation we found could be due to an enhanced $N^2$ fixation due to P fertilization because biological $N_2$-fixation is a P-demanding process (Rodríguez-Echeverría et al. 2009).

For soil Na, we found a significant and unexplained difference, not reported 5 years before, between BS+Ap (highest) and BS+Fi (lowest), and no significant differences among treatments for soil K. Nevertheless, the Na/K ratio in *P. tridentatum* and *U. micranthus* was significantly higher in BS+Ap plots than in the other treatments both at t=5 years (based on data from Couto-Vázquez et al. 2011) and t=10 years. Therefore, the addition of ammonium polyphosphate probably had an influence on the Na/K ratio of the leguminous plants, although no information regarding how P availability affects the mechanisms of Na and K regulation in legumes has been found. Moreover, *E. umbellata* in BS+Ap plots showed the lowest concentration of K (without significant differences in the Na/K ratio) and Mn, as well as the highest Mg concentration. Again, the ammonium polyphosphate seems to have an impact on plant nutrition of this species, although more data and information are needed to ascertain the mechanisms involved.

A short-term decrease in available Fe has usually been reported after wild and prescribed fires (González-Parra et al. 1996; Certini 2005; García-Marco and González-Prieto 2008; Close et al. 2011; Gómez-Rey et al. 2013). Results from our study site showed that this fire-derived Fe depletion is a long-lasting effect, as both five years (Couto-Vázquez et al. 2011) and 10 years after the fire the unburnt soil had 10-18% more available Fe than the burnt treatments. Plant total Fe 10 years after the fire showed no significant differences among treatments for all species except *P. tridentatum*, as also reported 5 years earlier (Couto-Vázquez et al. 2011). However, if all the species are considered together, Fe concentration in plants from BS+Ap plots was significantly higher than in BS ($p< 0.05$), being intermediate in the other treatments. Although burnt soils tend to be depleted in Fe compared to unburnt soils (García-Marco and González-Prieto 2008), soil Fe availability in the study area seems to be high enough to preclude a fire-triggered Fe deficiency in plants. However, if the long-term decrease we found in available Fe concentration is also a rule in burnt soils, forest fires would pose a fertility problem in ecosystems with low levels of this nutrient, as previously suggested by García-Marco and González-Prieto (2008).

For the other elements (Ca, Cu, Al and Mo) no clear effect of fire or FFCs was observed. In the case of soils, the available Al and Mo were strongly correlated ($r= 0.964$; $p< 0.001$), suggesting that the available Mo, which is an essential constituent of enzymes involved in the N cycle (Williams and Fraústo da Silva 2000; Fageria et al. 2002), could be associated to Al (oxy) hydroxides.

### *4.2. Effects of fire and FFC on vegetation cover*

The pines with the best trade-off between size and survival were those in the unburnt plots (second highest and widest pines, second lowest mortality). The significant differences in pine height and basal diameter among US and BS treatments indicate that the negative effect of fire on pine growth already reported during the first 5 years (Couto-Vázquez et al. 2011) persisted even 10 years after the fire. The BS+Fo and BS+Fi treatments did not show any effects on pine growth, but the highest pine mortality was recorded in the BS+Fi plots, as for 5 years before (Couto-Vázquez et al. 2011). At t=10 years, pines in BS+Ap plots were the biggest, both in height and diameter (as for 5 years after the fire, see Couto-Vázquez et al. 2011), although they had the second highest mortality and one third of them were clearly distorted (useless for timber production) and probably no longer viable; a problem that was recorded neither 5 years earlier in BS+Ap plots nor at t=10 years in the other plots. Ammonium polyphosphate based FFCs are known to have a fertilizing effect on pines (Pappa et al. 2008; Couto-Vázquez et al. 2011); however, lower relative amounts of root tissue in response to high resource conditions have previously been reported (see Coleman 2007, and references therein). These results suggest that the problem we found in BS+Ap plots could originated from an unfavourable shoot-to-root ratio due to the high amount of N and P supplied by the ammonium polyphosphate. If this hypothesis is correct, the root system of BS+Ap pines could be insufficient to sustain the trunks beyond a certain size, when the wind would bend the pines over at the root neck leading to a distorted growth form.

Compared to the shrub data obtained 5 years after the fire (Couto-Vázquez et al. 2011), the cover of: a) *E. umbellata* decreased in BS+Ap plots and increased slightly in the others; b) *P. tridentatum* increased in all treatments; c) *U. micranthus* decreased in BS+Ap and US, whereas it increased in BS, BS+Fo and BS+Fi; and d) *U. europaeus* decreased in BS+Ap, increased in BS and was detected for the first time in the other treatments. While at t=5 years plants in BS+Ap plots were significantly higher than those growing in the other treatments, 5 years later there was either no difference among treatments *(P. tridentatum* and *U. micranthus*) or plants growing in BS+Ap were smaller than the others (*E. umbellata*). At t=10 years *G. triacanthos* was recorded for the first time in all treatments except BS+Ap.

Ten years after the fire, no fire effect on shrub cover or height was found except for a higher cover of *U. micranthus* in BS compared to US plots. Regarding FFCs, the data indicates that BS+Fi and BS+Fo have no long-term effects on plant communities, consistent with observations for another foaming agent (Larson et al. 1999), whereas the BS+Ap treatment still affected the shrub community, apparently preventing the development of *E. umbellata* and the establishment of *G. triacanthos*. Negative effects of ammonium polyphosphate on seed germination and viability have already been reported (Cruz et al. 2005; Luna et al. 2007; Song et al. 2014), although it has been suggested that its effect on plants is negligible under field conditions (Song et al. 2014) or that it might disappear once the retardant is gone (Angeler et al. 2004). However, our data shows that the BS+Ap treatment still had an effect on some soil and plant properties 10 years after its application, as P concentration in the BS+Ap plots was still three times higher in soils and two times higher in plants than in the other treatments. The shrubs *G. triacanthos* and *E. umbellata* are the only obligate seeders studied in the experiment (Reyes et al. 2009) and therefore the most sensitive to hampered germination due to the application of ammonium polyphosphate. In contrast, *U. europaeus, U. micranthus* and *P. tridentatum* are strong resprouters (Reyes et al. 2009) and they had benefited from the fertilizing effect of the fire retardant up to 5 years after the prescribed fire (Couto-Vázquez et al. 2011). Nevertheless, data from t=10 years (shorter *P. tridentatum* plants; lower cover of *U. europaeus* and *U. micranthus*; high soil and plant P levels) suggests that the fertilizing effect was counterbalanced by other side effects of ammonium polyphosphate.

Although plant cover data from this study should be cautiously compared with that in the pre-fire plant survey (Pesqueira et al. 2005) as the latter is not given in a quantitative form, two differences must be highlighted: a) *G. triacanthos* appeared in our plots (except BS+Ap) between 5 and 10 years after the fire; and b) the coverage of *U. micranthus* was larger than that of *U. europaeus* in our experimental plots, while the reverse was true in the surrounding area. These differences can not be attributed to the fire or the fire-fighting chemicals, because they were also observed in the control unburnt plots. As our experimental plots were fenced but the surroundings were under a high grazing pressure, we hypothesize that grazing exclusion is an important factor influencing the plant communities growing in the area. On the one hand, grazing is a selective perturbation for plant communities as it often exerts a higher pressure on legumes due to their higher nutrient content (Pesqueira et al. 2005) and, thus, grazing exclusion may favour the establishment of *Genista triacanthos*. On the other hand, *U. micranthus* with weaker spines seems to be more palatable than *U. europaeus*.

Competition between different shrub species could also have shaped the plant community in the experimental plots. Irrespectively of the treatment, the resprouter species need less time to recover after the fire than the obligate seeders (Calvo et al. 2002; Pesqueira et al. 2005) and, therefore, the former *(U. europaeus, U. micranthus* and *P. tridentatum*) can displace the latter (*E. umbellata* and *G. triacanthos*). In the BS+Ap treatment, the obligate seeders were at a disadvantage due to the negative effect of ammonium polyphosphate on seed germination and viability (Luna et al. 2007), whereas the resprouters did not suffer this deleterious impact and were favoured by the fertilizing effect in the medium- to long-term (Couto-Vázquez et al. 2011).

## 5. Conclusions

After 10 years, the main effects of the experimental fire in the soils were a reduction in nitrate levels and Fe availability, although no symptoms of plant Fe deficiency were detected, likely due to the iron-rich soils. Concerning the vegetation, it is noteworthy that the 10-year-old pines still showed the negative effects of the prescribed fire on their growth. No other remarkable effects of the fire on the soil-plant system were found.

Among the studied fire-fighting chemicals, the only one with long lasting effects was ammonium polyphosphate, which even 10 years after its application had profound effects on the ecosystem: a) increased the concentrations of soil available and plant total P; b) depressed K uptake in shrubs and increased Na uptake in legumes; c) reduced pine's viability; d) modified the species composition of the shrub community (obligate seeders hindered by resprouters); and e) played an important role in N nutrition in plants, probably by affecting their mycorrhizal (*E. umbellata* and *P. pinaster*) and rhizobial (*U. micranthus* and *P. tridentatum*) symbioses.

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**SUPPLEMENTARY DATA.** Elemental concentration in foliar material 10 years after the prescribed fire of: a) nitrogen; b) calcium; c) aluminium; d) copper and e) zinc. For each plant species different letters indicate significant differences among treatments (p<0.05). Key: US, unburnt soil; BS, burnt soil; BS+Fo, burnt soil + foaming agent; BS+Fi, burnt soil + Firesorb; BS+Ap, burnt soil + ammonium polyphosphate; dw, dry weight.