Availability of ^{15}N from pioneer herbaceous plants to pine seedlings in reclaimed burnt soils

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A pot experiment was used to assess the N uptake by pine seedlings during two years on a burnt soil added with ^{15}N -labelled ryegrass, obtained from a ^{15}N -enriched sample of this soil after the fire. Nitrogen concentration in needles, stems and roots of seedlings decreased significantly from the first to the second growing period (from 2.55, 1.30 and 2.19% to 1.19, 0.47 and 1.00%, respectively), needles accounting for 53-58% of pine-N. At the end of the experiment, $98.87 \pm 1.12\%$ of the added ryegrass- ^{15}N was recovered: two thirds in the soil organic N pool and one third in the pine seedlings. Therefore, the post fire pulse of inorganic N, which was successfully kept in the burnt soil-plant system through its uptake by the pioneer species, is available for trees at the medium term. Pine seedlings assimilated 16.4% and 16.9% of the added ryegrass- ^{15}N in the first and the second year, respectively. This result contrasts with the usual yearly decrease of added N uptake by plants; a possible explanation is the transient increase of available N in burnt soils that would have modified the mineralization pattern of the ^{15}N -labelled phytomass. The pine N derived from the ryegrass-N decreased from 4.05% in the first year to 2.53% in the second one, being 3.10% the two-years weighed average. Besides the direct contribution of ryegrass to pine N nutrition

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reflected by these figures, the rapid post fire establishment of a herbaceous cover on the burnt soil also provides important indirect benefits for tree nutrition by reducing organic- and inorganic-N losses.

The ecological conditions produced by the Mediterranean climate are favourable to frequent fires of a natural origin, which influences the biota in all Mediterranean areas, and when fire is suppressed the habitat remains under non natural conditions.¹ However, nowadays fires are even more frequent due to human influence and they are normally considered as negative and damaging events in vegetation¹: in the Mediterranean basin about 50000 fires sweep through 7000-10000 km² of forest each year, causing huge economic and ecological damages.² Wildfires frequently promote or accelerate land degradation by enhancing soil and nutrient losses by erosion and leaching.³⁻⁷ Consequently, the development and improvement of burnt soil reclamation techniques, particularly to avoid post fire soil and nutrient losses, are a global concern.

In burnt soils, the increased risk of soil erosion is strongly related with the percentage of bare soil⁸ and lasts until vegetation has recovered⁹; therefore, soil re-vegetation must be promoted as soon as possible after the wildfire to reduce soil damage^{7,10}. Herbaceous plant sowing, combined with the addition of organic manures, is widely considered as a promising technique for the first step of burnt soil reclamation¹⁰⁻¹⁵ because these plants stabilize the ash layer, improve the soil structure and protect the soil against erosion^{5,10}. Moreover, these herbaceous plants retain, within the soil-plant system, the nutrients that otherwise would have been leached^{5,10}; for instance, Villar et al.¹¹ reported that, after a 3-month growing period, *Lolium perenne* up took 76% of the initial inorganic N content of a burnt soil (220 mg kg⁻¹), this figure being increased up to 92-95% in the treatments that received organic manures. Success of

reafforestation, i.e. the second step for burnt soils reclamation, will depend on the efficacy of the first step and on the availability for the trees of the nutrients derived from the uncropped dead herbaceous phytomass, after its recycling through the soil organic matter. This question is especially important in the early years of stand development, when the green crown is being constructed.¹⁶ Among tree nutrients, N is very important because: a) it is the major growth-limiting mineral element for higher plants;¹⁷ and b) its cycle is strongly altered by wildfires, which reduce dramatically the labile soil N reserves that are the major source of N for plants.^{18,19} Therefore, from the N cycle point of view, evaluating accurately the medium-term efficacy of the burnt soil restoration techniques is necessary; the usefulness of ¹⁵N tracers for this task has been recently recognized.^{11,12,19-21}

Taking into account these considerations, the aim of the present paper was to evaluate the availability of N from a ¹⁵N-labelled herbaceous phytomass for pine seedlings on a soil affected by a high intensity wildfire.

EXPERIMENTAL

Experimental design

The burnt soil was collected from the 0-5 cm depth layer of a Cambisol over granite under *Pinus sylvestris* L. (Cabeza de Manzaneda, Galicia, NW Spain), after a high intensity wildfire. The burnt soil was thoroughly homogenised after sieving at 4 mm; its main characteristics were: 14.11% of C, 1.07% of total N, 97 mg of NH₄⁺-N kg⁻¹, C/N 13 and pH(H₂O) 7.10.

Maritime pines (*Pinus pinaster* Aiton) were grown, in two consecutive years, from early February to late November in a set of 5 vitrified metallic pots (20 cm in diameter, 19 cm high) filled with 2700 g of dry soil; the experiment was run in a greenhouse under natural

illumination. Pots were brought to 75% of soil water-holding capacity (determined in a Richards membrane-plate extractor at a 10 kPa pressure) and watered gravimetrically with deionised H₂O to this moisture level each 1-2 days, as necessary.

Twenty-five one-year-old *P. pinaster* seedlings of similar high (28.9 ± 0.9 cm) were selected: a) 15 were planted in pots with an unburnt soil and kept for the second year of the experiment; b) 5 were destructively sampled and analysed; and c) 5 were planted in the burnt soil, each pot receiving 6.30 g of ¹⁵N-labelled *Lolium perenne* phytomass (a roots + shoots mixture, which contained 114.86 mg of ¹⁴N and 50.77 mg of ¹⁵N). As greenhouse conditions largely prevent the soil recolonization by fauna, which takes place after wildfires under field conditions, a reduction in the plant material breakdown and mixing with the soil would be expected. Taking into account that this effect will reduce the mineralization rate of the ¹⁵N-labelled *Lolium perenne*, its phytomass was milled (< 0.5 mm) and mixed with the upper 0.5 cm of the soil. After harvesting the pines grown during the first year, the set of pots with burnt soil + ¹⁵N-labelled phytomass was kept for the second phase of the experiment.

From the 15 seedlings previously kept for the second year, those 10 most similar were selected for the second growing period: a) 5 were destructively sampled and analysed; and b) 5 were transplanted to the set of pots with burnt soil + ¹⁵N-labelled phytomass and, after a 10-month growing period, they were harvested.

Elemental and isotopic N analysis

In all pine seedlings destructively sampled, needles, stems (including branches) and roots were separately harvested, dried at 60 °C, weighed, frozen with liquid-N₂ and finely ground (50-125 mm) in a stainless steel ball mill.

Dry matter content of soils and plants was assessed by oven-drying fresh material at 110 °C for 5 h. Total N content and ¹⁵N abundance of plant (*L. perenne* phytomass and pines) and soil samples were determined with a Finnigan MAT delta C isotopic ratio mass spectrometer (Finnigan, Bremen, Germany) operating in continuous flow on line with a CN elemental analyser (CE instruments, Milano, Italy).

The percentage of pine N derived from the ¹⁵N-labelled *Lolium* phytomass (%PNDFL) was calculated as follows:

$$mg\ N_p \times E_p = (mg\ N_s \times E_s) + (mg\ N_L \times E_L)$$

where N_p = pine N; N_s = pine N derived from the soil; N_L = pine N derived from the ¹⁵N-labelled *Lolium* phytomass; E_p = atom % ¹⁵N excess of pine N; E_s = atom % ¹⁵N excess of soil N assimilated by the pines; and E_L = atom % ¹⁵N excess of *Lolium* derived N assimilated by the pines. As $E_s = 0$, then:

$$mg\ N_p \times E_p = mg\ N_L \times E_L$$

$$\% \text{ PNDFL} = \frac{mgN_L}{mgN_p} \times 100 = \frac{E_p}{E_L} \times 100$$

The percentage of *Lolium*-N assimilated by the pines (%LNAP) was calculated as follows:

$$\% \text{ LNAP} = \frac{mgN_L}{mg\ \text{Lolium N applied}} \times 100$$

Statistical analysis

Data were statistically analysed by one-way ANOVA and the Levene's test was used for verifying the equality of variances among groups. Honestly significant differences were established at $P < 0.05$ using the Tukey's test when homocedasticity was observed. In the case of unequal variances, original data were subjected to Cox-Box transformations to obtain equality

of variances, and significant differences among the mean groups were then established at $P < 0.05$ using the Tukey's test.

RESULTS AND DISCUSSION

Foliar N concentration was significantly higher in the two-year-old seedlings ($2.55 \pm 0.18\%$) than in the three-year-old ones ($1.19 \pm 0.02\%$). These values were high and normal, respectively, compared with the 0.9-1.6% reported for other pine species.²²⁻²⁴ The decrease of N concentration with foliage age was also reported by Zhang and Allen.²⁵ Stems from the two-year-old pines had $1.30 \pm 0.09\%$ of N, a concentration higher than that of stems from the three-year-old pines (0.47 ± 0.07) and than that reported by Beets and Pollock²² for *Pinus radiata* (0.4-0.6%). As in needles and stems, N concentration in roots also decreased significantly from two- ($2.19 \pm 0.11\%$) to three-year-old seedlings ($1.00 \pm 0.19\%$). Similarly, during tree development, Miller¹⁶ reported a progressive shift of dry matter allocation from nutrient rich (leaves, twigs and fine roots) to nutrient poor (stem wood and structural roots) organs and tissues, eventually the only net increase being of very nutrient-poor heart wood (0.08-0.20% of N).²⁴

Pine seedlings stored more ryegrass-derived N in their needles than in stems and roots, although the differences were not significant for the first year; the stocks of stem- and root-N were not significantly different (Fig. 1). The distribution of the ryegrass-N taken up by the plants among needles, stems and roots was similar for years 1 and 2, needles accounting for a slightly higher proportion of the pine-N than stems and roots together. Melin and Nõnmik²⁶ also reported that half the supplied inorganic N recovered in trees was found in needles biomass. On average, 79-80% of the aboveground annual uptake of N in the two successive growing periods was accounted for by needles and 20-21% by stems + branches; these figures were, respectively, higher and lower than those reported by Beets and Pollock²² for *P. radiata* (65% and 35%).

At the end of the experiment, on average $98.87 \pm 1.12\%$ of the added ryegrass-N was recovered in the burnt soil-pine system, the soil organic N pool accounting for 65.5% of the ryegrass-N (see Fig. 1), a figure similar to that reported for inorganic fertilizers in forest stands.²⁷

During the two-year period considered, the pines assimilated one third of the ryegrass-N, this amount being equally distributed among the first (16.4%) and the second year (16.9%) (Fig. 2). This result contrasts with the yearly decrease of added-N plant uptake normally found in tracer experiments.²⁸ Two characteristics of the recently burnt soils could explain this result: a) the high amount of inorganic N at the beginning of the experiment (262 mg of NH_4^+ -N pot^{-1} ; i.e., 58% more than the total N added with the labelled phytomass) was large enough to supply around 40% of the pine N uptake during the first year; and b) wildfires increase the mineralization of the native soil N and reduce the potentially mineralizable soil organic N pool, contributing to a transient increase of available N in burnt soils.¹⁸ The transient high availability of mineral N in the burnt soil could have modified the mineralization rate of N from the labelled phytomass during the experiment.

The ^{15}N isotopic data showed a good availability of the ryegrass-N for the trees because it was: a) in the range of 22.5-42.4% found by Chang et al.²⁹ for ammonium sulfate- ^{15}N applied to four-year-old conifer stands (*Thuja plicata*, *Tsuga heterophylla* and *Picea sitchensis*); and b) higher than the 12-28% of the applied ^{15}N -ammonium nitrate assimilated by 120-140 year-old Scots pines after two growing seasons.²⁶ This remarkable result proves that the post fire pulse of inorganic N, which was successfully kept in the burnt soil-plant system through its uptake by pioneer herbaceous species, is available for trees at the medium term. On the other hand, the comparison of Figs. 1 and 2 showed that the ryegrass-N and the soil-N assimilated by the pines were stored in needles, stems and roots in similar proportion.

The percentage of pine N derived from the ryegrass-N decreased from 4.05% in the first year to 2.53% in the second one, the highest figures (around 4.5%) being found in the needles and the stem at the end of the first year (Fig. 3). For the two-years period, the average of pine N derived from the ryegrass-N was 3.10%, although this figure only reflects a direct ryegrass contribution to N nutrition of pines. A rapid post fire establishment of an herbaceous cover on the burnt soils also provides very important indirect benefits for tree nutrition. These indirect benefits are due to the highly-developed herbaceous rhizosphere that reduces soil erosion, avoiding soil organic N losses, and promotes the soil microbiota recovery that: a) can immobilize temporarily some fire-derived soil available N, and thus protect it against lixiviation; and b) is indispensable for the restoration of the N cycling in burnt ecosystems.

CONCLUSIONS

The transient pulse of fire-derived soil inorganic N, successfully retained by the pioneer herbaceous plants within the burnt soil-plant system, is widely assimilated by the trees (>33% in two years) after its recycling through the soil organic matter. Only up 4% of pine N derives directly from the herbaceous N pool; nevertheless, the rapid post fire establishment of a herbaceous cover on the bare burnt soil also provides very important indirect benefits for the N nutrition of trees by reducing organic- and inorganic-N losses through erosion and leaching.

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Fig. 1. Budget of the added Lolium-N after 1 and 2 pine growing periods: N taken up by pines (N, needles; S, stems+branches; R, roots); N incorporated into the soil; and N unaccounted for. All N data are in g m⁻² (mean±standard deviation; n=5).

Fig. 2. Percentage of ryegrass-N assimilated by seedlings and allocated to needles, stems and roots during 2 growing periods.

Fig. 3. Percentage of needles-, stem- and roots-N of the pine seedlings derived from ryegrass-N.