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Instituto de Investigaciones Agrobiológicas de Galicia, CSIC. Apartado 122, E-15780 Santiago de Compostela, Spain.

ABSTRACT

The evolution of the soil organic-N forms and their bio-availability was studied in a ^{15}N labelled and burnt soil (BLS) after two successive reclamation steps under greenhouse conditions: a 3-month growing period of *Lolium*, without (BLS-L) or with poultry manure addition (4 and 8 Mg ha^{-1} : BLS+PM4-L and BLS+PM8-L), followed by a 12-month growing phase of pine seedlings (BLS-P, BLS+PM4-P and BLS+PM8-P). The results were compared with those obtained for the homologous labelled unburnt soil (LS, LS-L and LS-P) to evaluate the efficacy of these reclamation techniques in the mitigation of the drastic post-fire changes exhibited by the major biologically available N pool in terrestrial ecosystems: the soil organic N. The significant and steady decrease of the ^{15}N enrichment observed in the unburnt soil during the successive plant growth cycles ($\text{LS} > \text{LS-L} > \text{LS-P}$) contrasts with the lack of significant changes, in both the content of total organic ^{15}N and the atom % ^{15}N in excess, among the treatments with the burnt soil ($\text{BLS} \geq \text{BLS-L} \geq \text{BLS-P}$). These results showed that: a) in LS, N mineralization proceeds faster for the recently incorporated N (^{15}N enriched) than for the native N, supplying the growing vegetation with inorganic N more ^{15}N enriched than the bulk soil N; and b) in BLS, soil combustion has reduced the usually higher biological availability of the recently added N to levels similar to those of the endogenous N.

The re-vegetation with *Lolium* and *Pinus* and the addition of poultry manure mitigated the high differences observed in the size of the amino acid and the organic derived NH_4^+ N pools due to the combustion process, which are usual between burnt and unburnt soils. Conversely, these burnt soil reclamation techniques (re-vegetation and poultry manure addition), even jointly used, were unable to reduce the huge differences observed between the burnt and the unburnt soils for the other N fractions considered (amides, amino sugars, hydrolysable unidentified-N, hydrolysable organic N and un-hydrolysable N) that accounted for more than 80 % of the soil organic N. Consequently, it seems that without the introduction of N_2 -fixing microorganisms or plants in the burnt soils the recovery of the natural soil organic N composition will take place slowly.

Key words: *Lolium perenne*, *Pinus pinaster*, poultry manure, step-wise hydrolysis, wildfires.

1. Introduction

As a result of its high concentration factor in the living organisms compared with their environment (Haynes, 1986; Nilsson et al., 1995), nitrogen (N) has been considered as the major growth-limiting mineral element since the evolution of higher plants (Ericsson, 1995). Only around 0.02 % of the earth's nitrogen reserves are within the biosphere, but this small fraction, which is mainly concentrated (> 94 %) into the

soil organic matter in the case of the terrestrial ecosystems, is the most biologically available N pool (Haynes, 1986). Consequently, in the terrestrial biosphere, nitrogen fertility is very closely associated with the dynamics of the soil organic N.

Wildfires trigger losses of soil N that are important, both quantitative and qualitatively, from a point of view of N biological availability (DeBano et al., 1979; Giovannini et al., 1990;

Villar et al., 1998; Fynn et al., 2003; Prieto-Fernández et al., 2004; Castro et al., 2005). Moreover, wildfires also strongly modify the distribution of the soil organic N forms, reducing the most labile fractions and increasing the most recalcitrant pools (Carballas et al., 1993; Knicker et al., 1996; Sánchez and Lazzari, 1999; Prieto-Fernández et al., 2004; Castro et al., 2005). Consequently, wildfires modify the short- and long-term soil N fertility after the fire (Chandler et al., 1983; Carballas et al., 1993; Prieto-Fernández et al., 1993; Fynn et al., 2003), the restoration of the N cycle being a basic step for the post fire reclamation of soils in the burnt soil-plant system (Castro et al., 2005).

During the last decade, the addition of organic amendments combined with plant sowing has emerged as a promising technique for reclaiming burnt soils, particularly to avoid post-fire soil erosion, due to its beneficial effects on soil microbial populations, soil aggregate stability and the growth of pioneer plants (Vázquez et al., 1996; Villar et al., 1998; Castro et al., 2000; Guerrero et al., 2001; Meyer et al., 2004; Villar et al., 2005), but relevant studies on the transformation of the soil organic-N forms and their bio-availability during the post-fire reclamation steps are still lacking. For these studies, the use of isotopic tracers (i.e., to work with a ^{15}N labelled burnt soil: Villar et al., 1998; González-Prieto et al., 1999; Castro et al., 2005) is very useful, specially when combined with the step-wise hydrolytic fractionation of the organic N, a method that accurately discriminates among different N pools that have contrasting lability in burnt and unburnt soils (González-Prieto et al., 1992, 1997; Prieto-Fernández et al., 2004) and in other organic materials (Takahashi et al., 2004).

In this paper we report, for the first time, the changes produced in the ^{15}N and ^{14}N distribution among the step-wise hydrolytic fractions of a ^{15}N labelled and burnt soil during two successive reclamation steps: sowing of an herbaceous pioneer species, with or without poultry manure addition, followed by the implantation of pine seedlings; the results were compared with those obtained for the corresponding unburnt labelled soil. By monitoring the evolution of the soil organic-N

forms and their bio-availability, the objective of this study was to evaluate the efficacy of the proposed reclamation techniques in the mitigation of the drastic post-fire changes exhibited by the major biologically available N pool in terrestrial ecosystems: the soil organic N.

2. Materials and methods

2.1. Soil samples

Unburnt soil samples were taken from the A horizon (0-15 cm depth) of a sandy, base desaturated Cambisol over granite under *Pinus pinaster* Aiton located at Salgueiras Hill (Galicia, NW Spain). Six samples, which represented a total of 240 kg of soil, were taken at random from a surface area of 1000 m², mixed to obtain a composite sample, sieved at 4 mm and thoroughly homogenized. The unburnt soil was acid (pH_{KCl} 4.0), contained 66.6 g C kg⁻¹ soil, 4.31 g N kg⁻¹ soil, 2.5 mg inorganic N kg⁻¹ soil in NH_4^+ -N form and had a C/N ratio of 15.5 (González-Prieto et al., 1999).

The unburnt soil was firstly labelled with ^{15}N (LS) and then burnt (BLS) by the procedures described in a preceding paper (Castro et al., 2005). The ^{15}N tracer was introduced in the soil as $(^{15}\text{NH}_4)_2\text{SO}_4$ (66 atom % ^{15}N), at a dose equivalent to 325 kg N ha⁻¹, and then *Lolium perenne* was sowed six times and harvested four weeks after each sowing. Prior to the 5th sowing, the labelled phytomass produced during the first four crops was ground (< 50 μm) and thoroughly mixed with the soil; the same was made with the phytomass of the 5th crop, previously to the 6th sowing. After the 6th cropping, the ^{15}N labelled soil (LS) was obtained. The LS was heated at the laboratory in a furnace under programmed conditions simulating those of high severity wildfires (385 °C for 10 min) to obtain the BLS.

2.2. Organic wastes

Poultry manure (PM) was obtained from an industrial farm, collected at random in plastic bags 3 days before the beginning of the experiment and kept in a refrigerator at 4°C. Before use, poultry manure was thoroughly homogenized; subsamples were slightly triturated and, after removing big feathers, they

were sieved (≤ 4 mm) and homogenized again. Poultry manure contained 3.447 % of total N and 0.444 % of inorganic N (Castro et al., 2000).

2.3. Vegetative pot experiments

The experiments were run in a greenhouse, under natural illumination. Pottery pots (17 cm diameter, 14 cm high, 227 cm² surface) were filled with 2 kg of dry soil. Six treatments were set-up (Castro et al., 2000): ¹⁵N-labelled soil (LS), ¹⁵N-labelled burnt soil without poultry manure (BLS) and ¹⁵N-labelled burnt soil with poultry manure (BLS+PM) at doses equivalent to 1, 2, 4 and 8 Mg of dry PM ha⁻¹ (PM1, PM2, PM4 and PM8 treatments, respectively); the evolution of the organic N forms was not followed in the BLS+PM1 and BLS+PM2 treatments, because the amount of PM derived N was too low to evaluate its contribution to each organic N pool. The poultry manure doses PM4 and PM8 supplied 45.5 and 91.0 mg N kg⁻¹ soil, respectively (87 % in organic forms and 13 % as inorganic N). All pots were brought to 75 % of soil water-holding capacity and watered gravimetrically to this moisture level every 1-2 days, as necessary.

Lolium perenne seeds (n= 200 pot⁻¹) were sown without pregermination and covered with a 0.5 cm layer of soil, which was previous and thoroughly mixed with the PM in the manured treatments. Five replicates of each treatment were prepared separately and inoculated with fresh soil. Inocula was extracted by shaking 100 g of fresh soil with 1 L of distilled water for 1 h; 10 ml of inocula were added to each pot (Castro et al., 2000). After 3 months of growing, the *Lolium perenne* phytomass (shoots and roots) was harvested, a representative aliquot of soil from each pot was kept separately for N analyses and the rest of soil from the 5 replicates of the same treatment was mixed and thoroughly homogenized to prepare the 4 replicates per treatment used in the following vegetative experiment in which one *Pinus pinaster* seedling was planted in each pot. After 12 months of growing, the pine seedlings were harvested and representative aliquots of soil from each pot were taken for N analyses. To differentiate them from the initial soils (without suffix), the post-*Lolium* and the post-*Pinus* soils were identified with the suffixes «-L» and «-P»,

respectively.

2.4. Physical, chemical and ¹⁵N isotopic analyses

The dry matter content of soils, poultry manure and plants was assessed by oven-drying fresh material at 110°C for 5 h. Soil water-holding capacity was determined in a Richards membrane-plate extractor at a pressure corresponding to a matrix potential of 10 kPa.

Soil total C and total N were measured on finely ground samples with an elemental analyser (EA) coupled on-line with an isotopic ratio mass spectrometer (Finnigan Mat, delta C, Bremen, Germany).

For organic-N distribution analyses, three samples of each soil treatment containing 50 mg of N were fractionated by a simple stepwise acid hydrolysis (González-Prieto and Carballas, 1992), involving refluxing samples with 1M HCl for 3 h (hydrolysate H1) and then with 3M HCl for 3 h (hydrolysate H2). Both the neutralization of the hydrolysates and the determination of the various forms of organic N were made by the methods described in González-Prieto and Carballas (1988). Under the specific conditions for each form of N, the resulting ammonia was steam distilled, collected into 10 ml of 0.02M H₂SO₄ and measured by back titration of the excess of H₂SO₄ with 0.01M NaOH (see Castro et al., 2005, for a more detailed description). Two replicates were distilled and titrated for each form of N. The resulting (NH₄)₂SO₄ solutions were then acidified with 2 ml of 0.02M H₂SO₄ and oven dried at 80 °C. Aliquots of 40-60 µg N as (NH₄)₂SO₄ were taken for ¹⁵N analyses with an elemental analyser (EA) on-line with an isotopic ratio mass spectrometer (Finnigan Mat, delta C, Bremen, Germany). Total N and ¹⁵N analyses of soils and hydrolysis residues were also made with the elemental analyser on-line with the isotopic ratio mass spectrometer. To prevent cross-contamination between samples: (1) glassware was cleaned in a dishwasher and then immersed in 36M H₂SO₄ for 1 h; (2) two aliquots of each sample were distilled consecutively, and the data obtained from the first distillate was discarded if cross-contamination with the preceding sample was detected (Mulvaney, 1986); (3) 25 ml of 95% ethanol were distilled for 3 min between

samples (Mulvaney, 1986); (4) NaOH and H₂SO₄ titrated solutions were prepared from extra pure concentrated solutions to prevent contamination from reagents; (5) standards with a known isotopic ratio were included to detect, and to correct if necessary, for the isotopic dilution of the sample N by the natural abundance N derived from the reagents or from ambient NH₃ (Kelley et al., 1991). Thereafter, endogenous N refers to the native soil N and exogenous N to the ¹⁵N incorporated into the soil organic N pool after the labelling process.

Data were statistically analysed by one-way ANOVA and least significant ranges (L.S.R.) were determined at $P < 0.05$ using Tukey's test.

3. Results and discussion

3.1. Total organic-N

The endogenous organic N content of the labelled soil (LS) did not show any clear trend during the growing cycles of *L. perenne* (LS-L) and *P. pinaster* (LS-P), whereas that of the burnt labelled soil showed a non significant decreasing tendency from BLS to BLS-P (Fig. 1A). Therefore, the soil total organic N content of all treatments remained within the normal range for forest soils from the same (González-Prieto and Carballas, 1991; Prieto Fernández and Carballas, 2000; González-Prieto and Villar, 2003) and other temperate-humid regions (Pritchett and Fisher, 1987).

Fig. 1. Changes in the endogenous (A) and exogenous (B) soil organic N content during the successive growing cycles of *Lolium perenne* and *Pinus pinaster* in the ¹⁵N labelled soil (LS), the burnt ¹⁵N labelled soil (BLS), and in BLS with 4 (BLS+PM4) and 8 Mg ha⁻¹ of poultry manure (BLS+PM8). Different small letters (a, b, c) indicate significant differences ($P \leq 0.05$) among soil treatments.

The significant and steady decrease of the exogenous organic N (¹⁵N enriched) observed in the labelled unburnt soil during the successive plant growth cycles (LS > LS-L > LS-P; Fig. 1B) contrasts with the lack of significant changes among the treatments in the labelled and burnt soil (BLS ≥ BLS-L ≥ BLS-P). Consequently, the differences in ¹⁵N content between the homologous unburnt and burnt soils (LS vs BLS, LS-L vs BLS-L and LS-P vs BLS-P) decreased

after each plant growth period. These results showed that N mineralization proceeds faster for the recently incorporated N (¹⁵N enriched) than for the native N, supplying the growing vegetation, either herbaceous or arboreous, with inorganic N more ¹⁵N enriched than the bulk soil N in the case of the unburnt soil but not in the burnt soil; this different behaviour was due to soil combustion, which reduced the usually higher biological availability of the recently

added N (Kelley and Stevenson, 1987; Clay et al., 1990; González-Prieto et al., 1997) to levels similar to those of the endogenous N, likely due to the increase of the proportion of heterocyclic N compounds (Knicker et al., 2005) which are more stable (Vairavamurthy and Wang, 2002)“.

The addition of 4 or 8 Mg ha⁻¹ of poultry manure (PM) at the beginning of the first re-vegetation phase did not have significant effects on the total organic N content of the burnt soil both after the first (BLS-L $\approx$ BLS-PM4-L $\approx$ BLS-PM8-L) and the second (BLS-P $\approx$ BLS-PM4-P $\approx$ BLS-PM8-P) plant growth cycles (Fig. 1A). However, the PM-derived N diluted the soil ¹⁵N enrichment (1.0688, 1.0551 and 1.0430 atom % ¹⁵N in excess in BLS-L, BLS-PM4-L and BLS-PM8-L, respectively), although the effect was only significant for the higher PM dose.

3.2. Amide-N

During the development of the *L. perenne* vegetation cover, the amount of amide-N increased slightly in the labelled soil and

significantly in the burnt labelled soil (Fig. 2A). Compared with BLS, this fraction decreased in the treatments with poultry manure (Fig. 2A); the reduction was more pronounced for the amide-¹⁵N (Fig. 2B) probably due to the “added N interaction” (trough soil N pool displacement, isotopic substitution between N fertilizer and soil native N pools and the stimulation of organic matter mineralization, usually reported on fertilization experiments; see Léon et al., 1995, and Kuzyakov et al., 2000), that could affect more strongly the recently incorporated N which has a higher biological availability. After the growing period of *P. pinaster*, the importance of the amide-N decreased in LS till values that, compared to those before the *L. perenne* growing phase, were similar for the endogenous N (Fig. 2A) and significantly lower for the exogenous N (Fig. 2B). A parallel, but stronger, decreasing trend was reported in all BLS treatments (Fig. 2A,B), all post-*Pinus* values being significantly lower than the corresponding initial and post-*Lolium* values.

Fig. 2. Changes in the endogenous (A) and exogenous (B) soil amide N pool during the successive growing cycles of *Lolium perenne* and *Pinus pinaster* in the ¹⁵N labelled soil (LS), the burnt ¹⁵N labelled soil (BLS), and in BLS with 4 (BLS+PM4) and 8 Mg ha⁻¹ of poultry manure (BLS+PM8). Different small letters (a, b, c...) indicate significant differences ($P \leq 0.05$) among soil treatments.

3.3. Organic derived NH_4^+ -N

Irrespectively of the soil treatment, during the 3 month growing phase of *Lolium* the endogenous organic N recovered as NH_4^+ -N hardly varied (Fig. 3A) but this fraction lost recently incorporated N (^{15}N enriched; Fig. 3B). Also in all treatments, the endogenous and exogenous organic N recovered as NH_4^+ -N hardly varied during the 12 mo growing period of *Pinus* seedlings, although it showed a slight tendency to decrease except for the endogenous N in LS (Fig. 3A,B). The slight increase in the amount of organic derived NH_4^+ -N from LS-L to

LS-P, jointly with its slight decrease from BLS-L to BLS-P, reduce to one third the difference between LS and BLS showing that soil re-vegetation substantially mitigated the combustion effects on this N fraction, usually reported in burnt soils (DeBano et al., 1979; Sánchez and Lázzari, 1999; Prieto-Fernández et al., 2004; Castro et al., 2005). The decrease of NH_4^+ - ^{15}N in LS-P reflects the steady decrease of ^{15}N enrichment from LS to LS-P, which was likely due to the higher plant availability of the recently incorporated N (^{15}N enriched).

Fig. 3. Changes in the endogenous (A) and exogenous (B) soil organic N derived NH_4^+ -N pool during the successive growing cycles of *Lolium perenne* and *Pinus pinaster* in the ^{15}N labelled soil (LS), the burnt ^{15}N labelled soil (BLS), and in BLS with 4 (BLS+PM4) and 8 Mg ha^{-1} of poultry manure (BLS+PM8). Different small letters (a, b, c...) indicate significant differences ($P \leq 0.05$) among soil treatments.

3.4. Amino sugar-N

In all treatments, the total amount of amino sugar-N in H1 and H2 decreased during the 3 month growing period of *Lolium* (except in H2 of LS) and the subsequent 12 month growing period of *Pinus* seedlings (Fig. 4A-D); the reduction, not always statistically significant, was generally higher for the amino sugar N recently incorporated to the soil (enriched in ^{15}N ; Fig. 4C,D). These results showed the

susceptibility of the amino sugar fraction to the reorganization processes in other N bearing compounds and/or to the mineralization-assimilation processes. Neither the re-vegetation nor the addition of poultry manure reduced the differences in this N fraction between LS and BLS (Figs. 4A,B), derived from the combustion process (Castro et al., 2005) and usual between burnt and unburnt soils (DeBano et al., 1979; Prieto-Fernández et al., 2004).

Fig. 4. Changes in the soil hexosamine N pool during the successive growing cycles of *Lolium perenne* and *Pinus pinaster* in the ¹⁵N labelled soil (LS), the burnt ¹⁵N labelled soil (BLS), and in BLS with 4 (BLS+PM4) and 8 Mg ha⁻¹ of poultry manure (BLS+PM8). A, B: amount of endogenous α-amino acid N recovered in hydrolysates 1 and 2, respectively; C, D: amount of exogenous organic N recovered as α-amino acids in hydrolysates 1 and 2, respectively. Different small letters (a, b, c...) indicate significant differences ($P \leq 0.05$) among soil treatments.

3.5. α-amino acid-N

The amount of α-amino acid-N, both endogenous and exogenous, hydrolysed in H1 decreased slightly, but significantly, from LS to LS-L and LS-P (Fig. 5A,C), whereas the fraction extracted in H2 remained without significant changes (Fig. 5B,D). Conversely, the α-amino

acid-N fraction (specially the endogenous pool) increased, slowly but steadily, during the growing phases of *Lolium* and *Pinus* in all BLS treatments, with or without poultry manure addition (Fig. 5A-D). Jointly, these contrasting tendencies in LS and BLS treatments slightly mitigated the huge differences that the soil combustion established between both

treatments in this fraction, as was reported for this (Castro et al., 2005) and other soils (DeBano et al., 1979; Carballas et al., 1993; Sánchez and Lázari, 1999; González-Vila and Almendros, 2003; Prieto-Fernández et al., 2004). The atom % ^{15}N in excess for the α -amino acid-N of both H1

and H2 steadily decreased during the *Lolium* and *Pinus* growing periods in the unburnt soil ($\text{LS} > \text{LS-L} > \text{LS-P}$) whereas in the burnt soil there was no a clear trend.

Fig. 5. Changes in the soil a-amino acid N pool during the successive growing cycles of *Lolium perenne* and *Pinus pinaster* in the ^{15}N labelled soil (LS), the burnt ^{15}N labelled soil (BLS), and in BLS with 4 (BLS+PM4) and 8 Mg ha⁻¹ of poultry manure (BLS+PM8). A, B: amount of endogenous a-amino acid N recovered in hydrolysates 1 and 2, respectively; C, D: amount of exogenous organic N recovered as a-amino acids in hydrolysates 1 and 2, respectively. Different small letters (a, b, c...) indicate significant differences ($P \leq 0.05$) among soil treatments.

3.6. Hydrolysable unidentified-N

The importance of this N pool increased in the order $LS < LS-L < LS-P$ for the endogenous N (Fig. 6A,B) and decreased in the same order for the exogenous N (Fig. 6C,D), with the relative exception of the H2 fraction. In the burnt soil, the endogenous hydrolysable unidentified N also increased in the same order ($BLS < BLS-L < BLS-P$), the increase being usually higher in the treatments with poultry manure addition (Fig. 6A,B), whereas the changes in the exogenous N of this pool were small and, in its H2 fraction, rather erratic (Fig. 6C,D). Consequently, although the joint use of both burnt soil reclamation techniques (re-vegetation and poultry manure addition) had a synergistic effect on the recovery of the hydrolysable unidentified-N pool, these techniques were unable to reduce the huge differences in this fraction with the unburnt soil (Castro et al., 2005).

In the unburnt soil, the atom % ^{15}N in excess in both H1 and H2 steadily decreased during the *Lolium* and *Pinus* growing periods ($LS > LS-L > LS-P$) whereas in the burnt soil there was a transient increase after the first re-vegetation phase and a decrease after the second one, except in the BLS+PM4 treatment.

3.7. Total hydrolysable organic-N

During the growing phases of *Lolium* and *Pinus*, there were no noticeable changes in the total amount of organic N hydrolysed in H1 in the labelled soil (Fig. 7A), but that extracted in H2 increased in the order $LS \leq LS-L < LS-P$ (Fig. 7B). Conversely, the amount of hydrolysable organic ^{15}N and the atom % ^{15}N in excess decreased significantly from LS to LS-L (H1 and H2), decreasing again (H1) or remaining quite stable (H2) from LS-L to LS-P (Fig. 7C,D); these results, together with the minimal N transfers to other more recalcitrant fractions, showed a steady export of N from the most labile soil N pool (that recently incorporated) to the growing vegetation. Therefore, the biological availability of the exogenous, recently incorporated, N was higher than that of the endogenous N, in

agreement with the results of several authors (Kelley and Stevenson, 1987; Clay et al., 1990; González-Prieto et al., 1997).

The organic N hydrolysed in H1 did not follow any clear trend from BLS to BLS-P (Fig. 7A) whereas that extracted in H2 increased, slowly but steadily, over the two plant growing cycles (Fig. 7B). These tendencies were similar to those observed in the unburnt soil and, consequently, the huge differences established by the soil combustion between LS and BLS for the hydrolysable organic N fractions (Castro et al., 2005) were not mitigated during the 15 months re-vegetation phases ($LS-L$ vs $BLS-L$; $LS-P$ vs $BLS-P$). This result agrees with that of Prieto-Fernández et al. (2004), who found that the strong changes observed in the lability of the organic N remained for at least 2 years after the wildfires, and also with the altered soil organic matter found 1-2 y after soil burnings by other authors (Almendros et al., 1990, 1992; Fernández et al., 1997). The fire-derived decrease of total hydrolysable organic N was not mitigated even in the treatments with poultry manure (Fig. 7A,B), likely due to the greater plant growth and N uptake found in these treatments (Castro et al., 2000), although under natural conditions this will lead to higher litter (and hydrolysable N) inputs to the added poultry manure treatments. The amount of exogenous hydrolysable organic ^{15}N recovered in H1 (Fig. 7C) and H2 (Fig. 7D) increased, although not significantly, from BLS to BLS-L whereas during the growing phase of *Pinus* seedlings it tended to decrease, the reduction being only statistically significant for that extracted in H1; the same tendencies were found in the treatments without and with poultry manure addition. The differences in the atom % ^{15}N in excess for the hydrolysable organic N (both H1 and H2) between the unburnt and the burnt soil decreased after the 3 month *Lolium* growing phase and converged to similar values in all treatments after the 12 month *Pinus* growing period, showing that, according to this variable, the burnt soil reclamation techniques employed were useful.

Fig. 6. Changes in the soil hydrolysable unidentified N pool during the successive growing cycles of *Lolium perenne* and *Pinus pinaster* in the ¹⁵N labelled soil (LS), the burnt ¹⁵N labelled soil (BLS), and in BLS with 4 (BLS+PM4) and 8 Mg ha⁻¹ of poultry manure (BLS+PM8). A, B: amount of endogenous a-amino acid N recovered in hydrolysates 1 and 2, respectively; C, D: amount of exogenous organic N recovered as a-amino acids in hydrolysates 1 and 2, respectively. Different small letters (a, b, c...) indicate significant differences ($P \leq 0.05$) among soil treatments.

3.8. Non hydrolysable-N

During the plant growing phases, the amount of organic N in non hydrolysable forms showed a slight decreasing tendency in both the LS and the BLS treatments (Fig. 8A), suggesting that a small proportion of this recalcitrant N pool was mobilized to hydrolysable forms and/or assimilated by the plants, as also

reported Prieto-Fernández et al. (2004). Although the un-hydrolysable N is considered to be much less biologically available than the hydrolysable N (González-Prieto et al., 1992, 1997; Prieto-Fernández and Carballas, 2000; Takahashi et al., 2004), it can be biologically transformed (Schulten and Schnitzer, 1998). In the case of the exogenous un-hydrolysable N, there were no significant changes when

expressed in absolute values (mg kg^{-1} ; Fig. 8B), but after the *Pinus* growing period the percentage of the whole soil ^{15}N in un-hydrolysable forms increased significantly in all treatments (data not shown); these two results showed that the relative importance of the non hydrolysable ^{15}N increased due to a preferential plant uptake of ^{15}N from the hydrolysable fractions. The addition of poultry manure to the BLS did not modify substantially this trend.

Jointly considered, these results showed that neither plant re-vegetation nor poultry manure addition mitigated the important differences in the size of the un-hydrolysable N pools between the unburnt and the burnt soils reported for both the endogenous and the exogenous N in these soils by Castro et al. (2005) and for total soil N in other soils by Sánchez and Lázzari (1999) and Prieto-Fernández et al. (2004).

Fig. 7. Changes in the soil hydrolysable N pool during the successive growing cycles of *Lolium perenne* and *Pinus pinaster* in the ^{15}N labelled soil (LS), the burnt ^{15}N labelled soil (BLS), and in BLS with 4 (BLS+PM4) and 8 Mg ha^{-1} of poultry manure (BLS+PM8). A, B: amount of endogenous a-amino acid N recovered in hydrolysates 1 and 2, respectively; C, D: amount of exogenous organic N recovered as a-amino acids in hydrolysates 1 and 2, respectively. Different small letters (a, b, c...) indicate significant differences ($P \leq 0.05$) among soil treatments.

Fig. 8. Changes in the endogenous (A) and exogenous (B) soil un-hydrolysable N pool during the successive growing cycles of *Lolium perenne* and *Pinus pinaster* in the ¹⁵N labelled soil (LS), the burnt ¹⁵N labelled soil (BLS), and in BLS with 4 (BLS+PM4) and 8 Mg ha⁻¹ of poultry manure (BLS+PM8). Different small letters (a, b, c...) indicate significant differences ($P \leq 0.05$) among soil treatments.

4. Conclusions

The increasing differences in ¹⁵N enrichment between the homologous unburnt and burnt soils after each plant growth period (LS vs BLS < LS-L vs BLS-L < LS-P vs BLS-P) showed that N mineralization proceeds faster for the recently incorporated N (¹⁵N enriched) than for the native N, supplying the growing vegetation with inorganic N more ¹⁵N enriched than the bulk soil N in the case of the unburnt soil but not in the burnt soil. This different behaviour was due to soil combustion, which reduced the usually higher biological availability of the recently added N to levels similar to those of the endogenous N.

The re-vegetation with *Lolium* and *Pinus* and the addition of poultry manure mitigated the important differences, due to the combustion process, observed in the size of the amino acid and the organic derived NH₄⁺ N pools and that are usual between burnt and unburnt soils. However, although these burnt soil reclamation techniques (re-vegetation and poultry manure addition) are very useful to avoid post-fire soil erosion and to fix the nutrients from the ash

layer, they were unable, even jointly used, to reduce the huge differences with the unburnt soil in the other N fractions considered (amides, amino sugars, hydrolysable unidentified-N, hydrolysable organic N and un-hydrolysable N) that accounted for more than 80 % of the soil organic N. Consequently, it seemed that without the introduction of N₂-fixing microorganisms or plants in the burnt soils the recovery of the natural soil organic N composition will take place slowly.

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