

White poplar (*Populus alba* L.) – Litter impact on chemical and biochemical parameters related to nitrogen cycle in contaminated soils

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Abstract

Aim of study: The aim of this study was to determine the effect of litter from *Populus alba* on chemical and biochemical properties related to the N cycle in soils with different pH values and trace element contents. We hypothesized that this litter would influence several parameters related to the N cycle and consequently to soil health.

Area of study: we collected two reforested contaminated soils of different pH values (AZ pH 7.23 and DO pH = 2.66) and a non-contaminated soil (RHU pH 7.19).

Materials and methods: Soil samples were placed in 2,000 cm³ microcosms and were incubated for 40 weeks in controlled conditions. Each soil was mixed with its corresponding litter, and soils without litter were also tested for comparison. Ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N) content, potential nitrification rate (PNR), microbial biomass nitrogen (MBN), protease activity, and several chemical properties such as pH, available trace element concentrations (extracted with 0.01 M CaCl₂) were determined at different times of incubation.

Main results: Values of available trace elements did not vary during the incubation and were always higher in acid soil. In neutral soils litter presence increased values of Kjeldahl-N, NO₃⁻-N content, potential nitrification rate (PNR), microbial biomass nitrogen (MBN) and protease activity. Presence of trace elements in neutral soils did not alter the parameters studied. However, acidic pH and high content of available trace elements strongly affected NH₄⁺-N and NO₃⁻-N, microbial biomass N and protease activity.

Research highlights: Our results showed the negative effect of the acidity and trace element availability in parameters related with the N-cycle.

Key words: microbial biomass N; protease activity; soil pH; N mineralization; nitrification; phytoremediation.

Introduction

Nitrogen occupies a unique position among the soil-derived elements essential for plant growth because of the large amounts required by plants in comparison to other elements. In most terrestrial ecosystems N is the limiting nutrient for plant growth (Robertson and Groffman, 2007). Humans are altering the global cycle of N via combustion of fossil fuels, production of N fertilizers, cultivation of N-fixing legumes and other actions (Vitousek *et al.*, 1997). Among anthropogenic activities, industrial countries have been increasingly contaminating soil with inorganic pollutants. Some authors have shown the adverse effects of these pollutants on microbial processes in soils (Babich and

Stotzky, 1980; Tyler, 1981) that can affect some steps of the N cycle. In this regard it has been evidenced that high levels of trace elements in soils can affect the number, diversity and activity of soil organisms, affecting enzyme activities and nitrogen mineralization processes (Gil-Sotres *et al.*, 2005). Several researchers have investigated the influence of inorganic pollutants, mainly heavy metals, on N mineralization and nitrification and have reported somewhat mixed results (Wilke, 1989).

Litter decomposition is a key process that facilitates the cycling of nitrogen in terrestrial ecosystems. Litter decomposition is largely controlled by litter quality, dominant weather features and composition and activity of microorganisms in the soil and forest floor (Berg and Matzner, 1997; Heal *et al.*, 1997; Fierer and Schimel, 2002; Borken *et al.*, 2003). Therefore, biodegradation of organic residues rich in metals might be influenced

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by the presence of metals, producing indirectly negative effects on N cycle.

Soil pH is another important factor regulating N transformation in soil. According to Zhang *et al.* (2011), in many ecosystems soil pH is the major factor controlling nitrification. Some authors suggest that nitrification activity in soil declines markedly below pH 6.0 (Aciego Pietri and Brookes, 2008). Watson and Watson (1989) reported that nitrification does not occur at pH < 6.5. However, some studies have shown the adaptation of the autotrophic bacteria to low pH in soils (Islam *et al.*, 2006).

Contaminated soils are in most of the cases unsuitable for the production of conventional crops or grasslands but they have the potential to be used for energy crops such as willow or poplar (Mertens *et al.*, 2004; Robinson *et al.*, 2000). These are deciduous trees and their development in trace elements contaminated areas would cause, after the autumnal fall, the presence of an extensive “carpet” of litter loaded with trace elements, whose entire removal is not always viable. There is little information regarding the influence of this heavy metal rich litter on the properties of the reforested soils (Sheid *et al.*, 2009), especially on the chemical and biochemical properties related to the N-cycle. Short-rotation forest plantations have been introduced with the aim of producing biomass for energy and industry over the past two decades (Lucas-Borja *et al.*, 2011). In this respect, poplars are largely used, as they are known to be fast-growing trees, producing large yields and having a high energy potential (Calfapietra *et al.*, 2010).

The aim of this study was to determine the effect of *Populus alba* L. litter on chemical and biochemical properties related to the N cycle in soils with different pH values and trace element (As, Cd, Cu and Zn) contents. We hypothesized that this litter would influence several parameters related to the N cycle. To test this hypothesis microcosm studies were carried out. We studied the evolution of various parameters: pH, available trace element concentrations, nitrogen mineraliza-

tion by the analysis of NH_4^+ -N and NO_3^- -N contents and microbiological properties (PNR, potential nitrification rate, MBN, microbial biomass N and protease activity) as a tool to evaluate the effects of the litter on trace element-polluted soils with different pH.

Materials and methods

Soil and litter collection

The experiment was carried out using three different soils collected in January 2009. The soil RHU (pH 7.19) was collected in a non-contaminated riparian forest in La Algaba, Seville (37° 29' 4.8" N, 6° 01' 34" W) where *Populus alba* was growing. Two trace element polluted soils, AZ (pH 7.23) and DO (pH 2.66) were collected in the area affected by a mine spill in 1998 (South West of Spain, Grimalt *et al.*, 1999) in spots where *Populus alba* trees were growing (37° 18' 4.7" N, 6° 15' 39.1" W, AZ and 37° 23' 42.5" N, 6° 13' 36.4" W, DO) (see Table 1). Soils were collected from the first 0-25 cm soil. Values of pH in DO soils were extremely acidic due both to the acidic origin of the soil and to the effect of the mine spill (very acidic) (Cabrera *et al.*, 1987, 1999). Moreover, soil from DO is located in a riparian area where the removal of sludge from soil was very difficult, and remains of sludge were left on the soil surface. Similar pH values of the soils in the area have been reported by Domínguez *et al.* (2008). The most relevant characteristics of the soils are shown in Table 1.

Afforestation in the study area started in 1999, after the purchase of affected lands by the regional administration. Depending on the local habitat conditions, the target tree and shrub species to afforest were those typical of riparian forests, such as *Populus alba*, *Fraxinus angustifolia* and *Salix atrocinerea* or those typical of drier upland forests, such as *Quercus ilex* subsp. *ballota*, *Olea europaea* var. *sylvestris*, *Ceratonia siliqua*, *Phillyrea angustifolia*, *Pistacia lentiscus*, *Rosma-*

Table 1. Some characteristics of the studied soils. Standard deviations are in brackets (n = 3)

Soil	OM	Sand	Silt	Clay	Texture	As	Cd	Cu	Pb	Zn
	%	g kg ⁻¹				Total, mg kg ⁻¹				
RHU	1.57	612	214	174	Sandy loam	9.18 (0.81)	<0.01	25.8 (2.7)	17.9 (0.9)	69.1 (6.3)
AZ	2.42	393	346	263	Loam	112 (5)	3.82 (0.15)	166 (6)	236 (14)	506 (15)
DO	1.51	790	60	150	Sandy loam	290 (15)	3.53 (0.10)	193 (10)	391 (25)	227 (9)

Table 2. Some characteristics of litter samples. Standard deviations are in brackets (n = 6). Kjeldahl-N (Kjel-N), lignin (Lig), available (0.01 M CaCl₂ soluble)

Litter	pH	Kjel-N	Lig	As	Cd	Cu	Pb	Zn	Cd	Cu	Zn
		g 100 g ⁻¹	g kg ⁻¹	Total, mg kg ⁻¹						Available, mg kg ⁻¹	
RHU	5.20 (0.21)	2.73 (0.02)	32.4 (0.09)	2.61 (0.31)	0.74 (0.10)	13.9 (1.59)	2.74 (0.23)	163 (8.74)	0.46 (0.09)	4.61 (0.25)	32.5 (6.52)
AZ	5.25 (0.12)	1.10 (0.02)	33.9 (1.66)	2.68 (0.21)	3.20 (0.91)	17.9 (3.64)	4.05 (2.00)	289 (69.2)	1.31 (0.22)	7.11 (0.35)	69.3 (9.36)
DO	5.53 (0.25)	1.37 (0.02)	35.3 (0.35)	22.4 (9.31)	5.26 (0.97)	82.0 (25.0)	49.4 (11.2)	647 (117)	1.92 (0.45)	51.2 (1.58)	364 (51.2)

rinus officinalis and *Retama sphaerocarpa* (Dominguez et al., 2010).

Litter samples of *Populus alba* were collected at each sampling site (RHU, AZ and DO) in January 2009. This material was brought to the laboratory for its characterization and for its use in the microcosm studies. Table 2 shows the most relevant characteristics of the litter samples.

Experimental design

Each soil sample (1,500 g; crushed and sieved <2 mm) was placed in a container of 2000 cm³ (30 × 30 cm). Each soil was mixed with its corresponding litter (RHU-L, AZ-L, DO-L). The doses of litter were calculated according to the litter wet weight at field conditions in RHU, AZ and DO in an area of 25 × 25 cm. This weight was extrapolated to the microcosm area, resulting in a mean weight of 85 g of wet litter per container. Soils without litter addition were also tested. A randomised complete block design with three replicates per treatment and soil was used. Deionized water was added to bring the soil moisture to 70% of its water holding capacity. The incubation was performed in a growth chamber at 28°C. Water losses were compensated by adding deionized water during the experiment. A subsample of soil (100 g per sampling time) from each container was taken after 0, 2, 4, 8, 16, 24, 32 and 40 weeks of incubation.

Soil and litter analysis

For the initial characterization, soil samples were oven-dried (40°C) after removing plant material and stones, crushed and sieved through a 2-mm sieve, then ground to < 60 μm. For initial analysis litter material was oven-dried (70°C) to constant weight, and sieved and ground to pieces of around 2-5 cm. Soil and litter pH were measured in a 1/2.5 (w:v) sample/1 M KCl extract after shaking for one hour (Hesse, 1971) using

a pH meter (CRISON micro pH 2002). Soil organic carbon content was determined by the method of Walkley and Black (1934). Kjeldahl Nitrogen of soil and litter was determined after digesting the soil and litter samples by the method described by Hesse (1971). Klason lignin content was determined according to Tappi T222 om-88 regulation, with some modifications (Tappi Standard, 2004): final concentration of H₂SO₄, 4%, and final extraction in the autoclave (121°C) for 1 h. Pseudo-total trace element concentrations in soil samples (< 60 μm) were obtained by digestion with *aqua regia* (1:3 v:v conc. HNO₃:HCl) in a microwave oven (Micro-wave Laboratory Station Mileston ETHOS 900, Mile-stone s.r.l., Sorisole, Italy). The term pseudo-total accounts for the *aqua-regia* digestion, because it does not completely destroy silicates. Recovery of pseudo-total elements was assessed against total elements in a BCR reference sample (CRN, 277). Recovery rates were: As 83%, Cd 66%, Cu 103%, Pb 72% and Zn 93%. Total trace elements were calculated on a dry weight basis using the above recovery factors. The total of trace elements in litter was determined after digesting by wet oxidation with concentrated HNO₃ under pressure in a microwave digester. Recovery rates for plant material (litter) were assessed against the poplar leaves reference material (CS DC73350) and were between 90 and 110%.

Availability of trace elements in soils and litter were determined in 1/10 (w:v) sample/0.01 M CaCl₂ extracts after shaking for three hours (Houba et al., 1996). Following Kabata-Pendias (2004) trace elements extracted with 0.01 M CaCl₂ is the most labile fraction subject to leaching and uptake of plants and microorganisms. Trace elements (As, Cd, Cu, Pb and Zn) in all the extracts were determined by ICP-OES (inductively coupled plasma-optical emission spectrometry) using an IRIS Advantage spectrometer (Thermo Jarrel Ash Corporation, MA USA). The detection limits for the method was 0.1 mg kg⁻¹ for As, 0.03 mg kg⁻¹ for Cd, 0.03 mg kg⁻¹ for Cu, 0.3 mg kg⁻¹ for Pb and 0.03 mg kg⁻¹ for Zn. Ammonium in soil was extracted by

shaking a fresh sub-sample (2.5 g on an oven-dry basis) in 25 ml of 1 M KCl for 1 hour. Nitrate in soil was extracted by shaking a fresh sub-sample (5 g on an oven-dry basis) in 25 ml of distilled water for 1 hour. Determination of NH_4^+ -N and NO_3^- -N in the extracts were carried out in a Bran + Luebbe GmbH AA3 dual-channel, continuous-flow auto-analyser (Norderstedt, Germany). Potential nitrification rate (PNR) was measured by method of Kandeler (1996) and modified by Hoffmann *et al.* (2007). Moist soil samples (5 g) were incubated for 5 h at 25°C, using ammonium sulfate as substrate, and adding sodium chlorate to repress the oxidation to nitrate. Subsequently accumulated nitrite during incubation period was extracted with 2M KCl, determined colorimetrically by the method Griess-Ilosvay (Keeney and Nelson, 1982), and expressed as NO_2^- -N $\mu\text{g g}^{-1} (\text{dm}) \text{d}^{-1}$.

Microbial Biomass Nitrogen (MBN) content was determined in moist subsamples by the chloroform fumigation-extraction method modified by Gregorich *et al.* (1990). Concentration of N in the extract was measured by a TOC-VE Shimadzu analyser. A K_{EN} (measure of the efficiency of the extraction procedure) of 0.54 was used to calculate MBN. Protease activity was measured after incubation of soil with casein and measurement of the absorbance of the extracted tyrosine at 700 nm (Ladd and Butler 1972). Protease activity is expressed as mg of tyrosine $\text{kg}^{-1} 2\text{h}^{-1}$.

Nitrogen mineralization simulation model

The N mineralization process was adjusted to a one-pool model proposed by Stanford and Smith (1972), in which the process follows the first order kinetic (Equation 1):

$$N_m = N_0 \times (1 - e^{-kt}) + N_i \quad [1]$$

where N_m is the cumulative amount of N mineralized at a specific time t , N_0 is the potentially mineralizable N (representing the pool of the organic N that might be mineralized), k is the mineralization rate coefficient and N_i is the mineral N at time 0. This equation has been widely used to describe N mineralization (Cabrera *et al.*, 2005; Madrid *et al.*, 2011). The equation was fitted to data using the Marquardt iteration method in order to obtain the N_0 and k .

The rate of mineralization which is the first derivative of Eq. 1 is:

$$\frac{dN_m}{dt} = N_0 \times k \times e^{-kt} \quad [2]$$

The rate of mineralization decreases with time and at $t=0$ is equal to $N_0 \times k$, that is called “the initial potential rate of N-mineralization”.

Statistical analyses

All statistical analyses were carried out with the program SPSS 15.0 for Windows. A Student's t -test ($p \leq 0.05$) was used to assess differences between the same soil with and without litter addition.

Results

Evolution of pH, Kjeldahl-N and available trace element concentration

Soil pH values, Kjeldahl-N and available trace element concentrations in soils at three representative times of incubation (0, 24 and 40 weeks) are shown in Table 3. Generally, in RHU and AZ litter presence did not affect pH values, however values of DO-L were always significantly higher than those of DO, although values were still very acidic. As a rule, comparing the initial and final pH values during the incubation period, a little acidification was observed in all soils.

Kjeldahl-N concentrations were significantly higher (around 0.50-1.00 g kg^{-1}) due to litter addition, especially in RHU soil, and tended to decrease throughout the incubation (Table 3).

In non-contaminated soil (RHU) available Cd and Cu elements were very low (below the detection limits of the used method) (Table 3). The presence of litter did not significantly alter the concentration of available Cd, Cu and Zn in this soil. Neutral contaminated soil (AZ) also presented low concentrations of available Cd, Cu and Zn during the whole incubation period. Litter addition to AZ had little effect on available trace elements, although a significant increase of the concentration of available Cu in AZ-L compared to AZ was observed at the initial time ($T=0$) and after 24 weeks of incubation ($T=24$) (Table 3). The behavior of the acid contaminated soil (DO) differed from that of the neutral soils. Concentrations of available Cd, Cu, and Zn were much higher in this soil than in the other two soils. In this acid soil, litter addition increased available Cd and Zn from the beginning of the experiment ($T=0$), but it did not increase over time (from $T=0$ to $T=40$) (Table 3). In DO available Cu

Table 3. Changes in pH values, Kjeldahl-N (Kjel-N) and available (0.01M CaCl₂-soluble) trace element concentrations at three different incubations times

Soil	Week	pH	Kjel-N (g 100 g ⁻¹)	Cd (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Zn (mg kg ⁻¹)
RHU	0	7.19	0.090	<0.001	0.010	0.068
	24	6.54	0.090	<0.001	0.006	0.421
	40	6.99	0.087	<0.001	0.033	0.179
RHU-L	0	7.18	0.231*	<0.001	0.010	0.028
	24	6.72	0.171*	<0.001	0.005	0.774
	40	6.97	0.163*	0.002	0.048	0.084
AZ	0	7.23	0.126	0.025	0.22	0.16
	24	7.22	0.118	0.001	0.14	0.01*
	40	7.14	0.102	0.004	0.08	0.05
AZ-L	0	7.21	0.174*	0.003	0.51*	0.16
	24	7.43*	0.168*	0.008*	0.27*	0.002
	40	7.16	0.158*	0.002	0.13	0.08
DO	0	2.66	0.097	0.28	31.5	78.4
	24	2.24	0.089	0.22	32.3	60.5
	40	2.40	0.081	0.18	23.6	46.4
DO-L	0	3.07*	0.154*	0.49*	16.1 *	103*
	24	2.56*	0.151*	0.48*	28.3	109*
	40	2.73*	0.151*	0.50*	21.0	104*

* $p < 0.05$. Significant differences between same soils and time with and without litter addition.

decreased after the addition of litter, but only at the beginning of the experiment ($T = 0$) the differences between DO and DO-L were significant. In general, concentrations of available trace elements in soils (with and without litter) tended to decrease slightly or remain constant over time (Table 3). Available As and Pb in the three soils were below the detection limits for these elements of the used method (As 0.01 mg l⁻¹; 0.1 mg kg⁻¹; Pb 0.03 mg l⁻¹; 0.3 mg kg⁻¹).

Evolution of NH₄⁺-N, NO₃⁻-N and potential nitrification rates (PNR)

The evolution of NH₄⁺-N, NO₃⁻-N concentrations and the potential nitrification rates (PNR) values are shown in Figs. 1, 2 and 3, respectively. In the neutral soils (RHU and AZ), NH₄⁺-N concentration did not change by the litter addition. In these soils, concentrations of NH₄⁺-N were generally lower than 4 mg kg⁻¹ (Fig. 1). In the acid soil (DO) NH₄⁺-N concentrations were higher than those found in neutral soils. Concentrations of NH₄⁺-N increased significantly with the addition of litter during the period between 2 and 32 weeks of incubation. Both DO and DO-L showed a maximum in that period.

In neutral soils (RHU and AZ), especially in those with litter application, after an initial immobilization period an increase of NO₃⁻-N was observed (Fig. 2). In AZ-L, NO₃⁻-N concentrations continued increasing up to 46 weeks of incubation. In the acid soil (DO) no significant differences in NO₃⁻-N concentrations were found with addition of litter, whose values were similar over time (Fig. 2).

In general, litter addition increased the potential nitrification rate (PNR) during the whole incubation period in the neutral soils (RHU and AZ) (Fig. 3). Values of PNR went on increasing up to 24 weeks of incubation for RHU (15 µg N g⁻¹ dry matter day⁻¹) and 40 weeks for AZ (25 µg N g⁻¹ dry matter day⁻¹), remaining approximately constant and at very low values for DO.

N-Mineralization model

N mineralization for RHU without litter addition fit to the first kinetic equation model throughout the entire experimental time, however for AZ the model was only fitted during 24 weeks of incubation (Fig. 4 and Table 4). No significant differences were found for the values of potentially mineralizable N (N_0) for these soils (mean value ± SE; 38.0 ± 10.5 and 16.6 ± 6.4 mg N kg⁻¹, for RHU and AZ respectively).

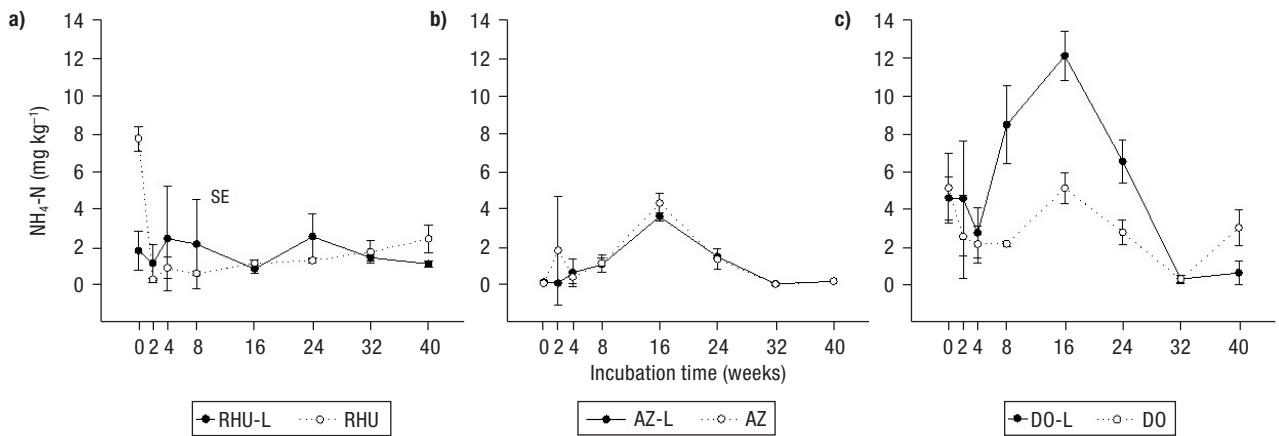


Figure 1. Evolution of $\text{NH}_4\text{-N}$ concentration in the different soils with and without litter addition (RHU pH = 7.19; AZ pH = 7.23 y DO pH = 2.66).

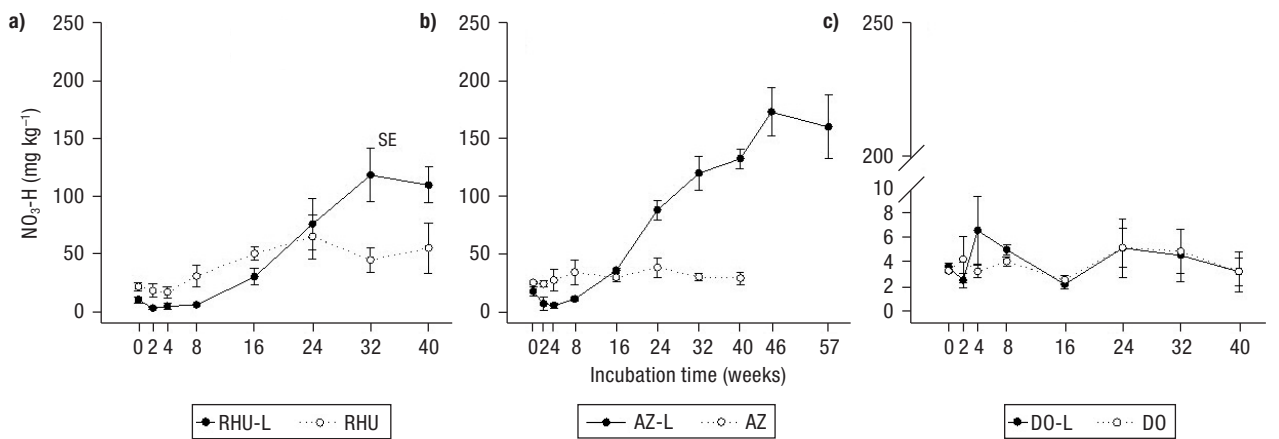


Figure 2. Evolution of $\text{NO}_3\text{-N}$ concentration in the different soils with and without litter addition.

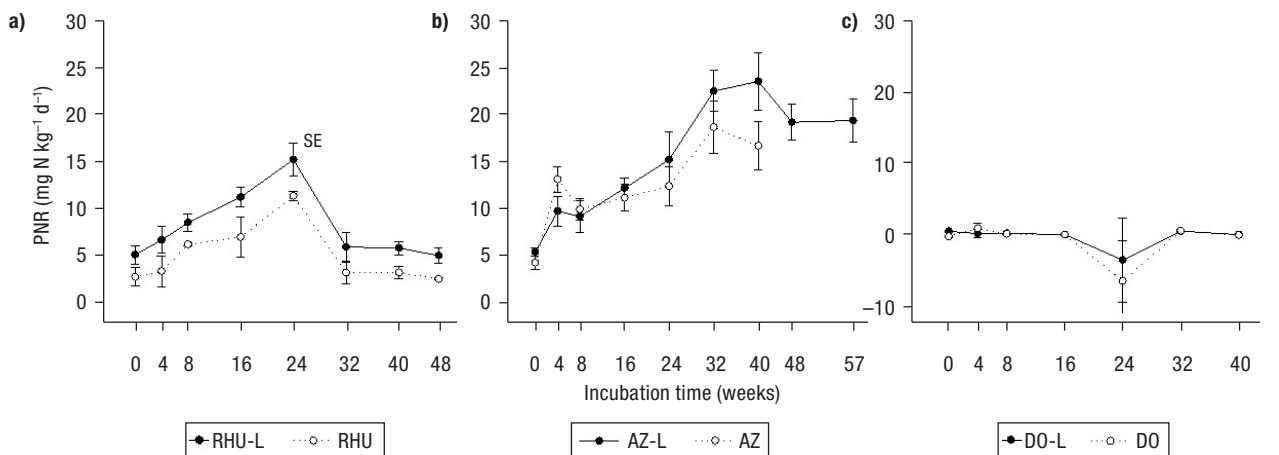


Figure 3. Potential Nitrification Rate (PNR) evolution in the different soils with and without litter addition.

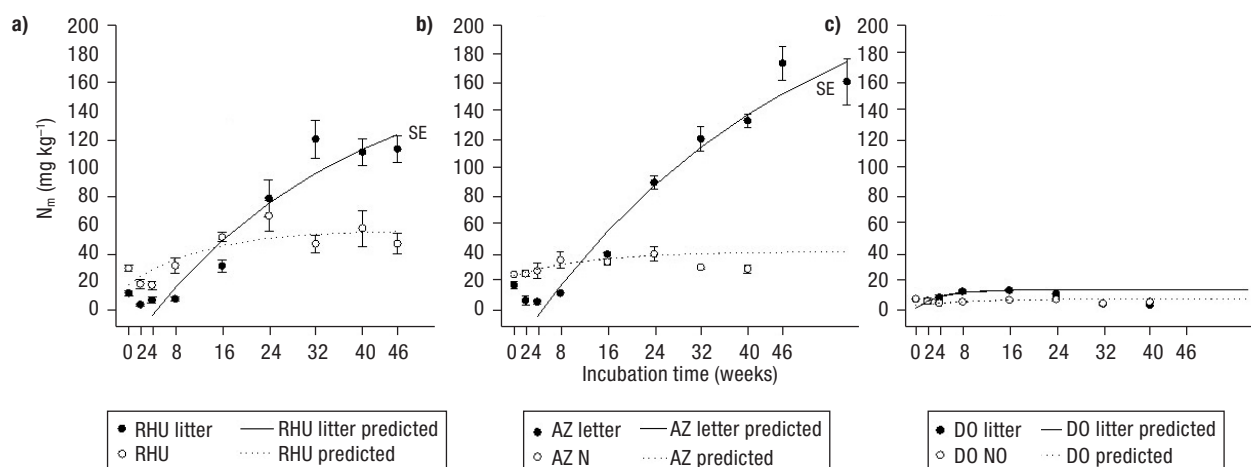


Figure 4. Experimental and predicted values of net mineralized N in the different soils.

Table 4. Net mineral nitrogen at time 0 (N_i), potentially mineralizable nitrogen (N_0), rate constant of mineralization (k), potential rate of mineralization ($N_0 \times k$), half-time for mineralization ($t_{1/2}$) and the coefficient of determination (r^2)

	N_i	N_i SE	N_0	N_0 SE	k	k SE	$N_0 \times k$	$N_0 \times k$ SE	$t_{1/2}$	r^2
	mg kg ⁻¹		mg kg ⁻¹		Week ⁻¹		mg kg ⁻¹ week ⁻¹		Week	
RHU	18.3	8.5	38.0	10.5	0.0796	0.0704	3.02	2.80	8.71	0.7025
RHU-L	-25.3	24	203	85	0.0284	0.0269	5.68	2.38	24.8	0.9238
AZ	24.7	2.2	16.6	6.4	0.0788	0.0742	1.31	0.50	8.79	0.8839
AZ-L	-27.6	17.7	285	82	0.0215	0.0126	6.13	1.76	32.2	0.9637

SE: standard error.

For both soils with litter addition, Eq. 1 could be also applied from the first 4 weeks of incubation. Differences between N_0 (203 ± 85 and 285 ± 82 mg N kg⁻¹ for RHU-L and AZ-L respectively) were not significant.

Values of k were similar for the both soils without litter addition (RHU and AZ; ca. 0.08 week⁻¹), while for soils with litter addition (RHU-L and AZ-L) values of k were smaller (0.02-0.03 week⁻¹). The potential rate of N mineralization ($N_0 \times k$) increased with the litter: from 3.02 ± 2.80 to 5.68 ± 2.38 mg kg⁻¹ week⁻¹ for RHU and RH-L, and from 1.31 ± 0.50 to 6.13 ± 1.76 mg kg⁻¹ week⁻¹ for AZ and AZ-L. Differences between $N_0 \times k$ values of RHU and AZ, and between values of RH-L and AZ-L were not significant. For DO-L, net N mineralization values were much smaller than for the other soils and did not fit any equation model (Fig. 4).

Evolution of the biochemical properties: Microbial Biomass Nitrogen (MBN) and protease activity

Evolution of the values of MBN was similar in all the soils, tending to decrease throughout the incubation

period (Fig. 5). In general, litter presence increased MBN of neutral soils, especially during the first 24 weeks of incubation. Lower values of MBN were found for the acid soil (DO), the application of litter only caused a rise of MBN in the first very early stage of the incubation.

The evolution of the soil protease activity values during the incubation period in AZ and DO shows a rapid and significant increase during the first two weeks of incubation (Fig. 6), decreasing to an approximately constant value afterwards. Evolution of protease values in RHU shows instead a decrease up to the week 8 and a maximum at 24 weeks. Protease values in the acid soil (DO) were lower compared with those for the other soils. Litter addition only caused increases of the protease activity in neutral soils: during all incubation period in AZ-L soil and only during the 16 first weeks in RHU-L. Protease activity values in RHU-L and AZ-L tend to decrease to values close to those of the soils without the addition of litter.

Discussion

The addition of litter did not cause alkalisation in neutral soils (RHU and AZ) due to the moderately

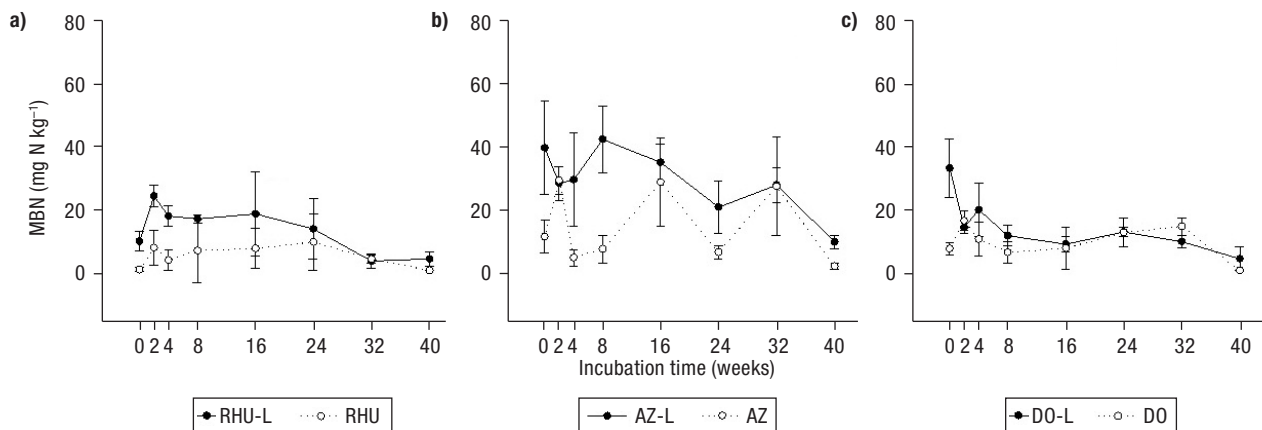


Figure 5. Evolution of Microbial Biomass Nitrogen (MBN) contents in the different soils with and without litter addition.

acidic nature of the litter (Table 2), but produce a slight increase of pH values in the acid soil DO (Table 3). Similar results were observed by Pérez de Mora *et al.* (2006) in an acid trace element contaminated soil amended with litter from a deciduous forest.

The available fraction of Cd, Cu and Zn measured using 0.01 M CaCl_2 , following Kabata-Pendias (2004) was higher in DO soil than in the other two soils due to its low pH. This hypothesis was supported by previous studies by Brallier *et al.* (1996) and Ciadamidaro *et al.* (2013) among others, who reported that the availability of cationic trace elements increases as acidity increases. The increase of available Cd and Zn found in DO-L compared to DO, and in some instances the increase of available Cd, Cu and Zn in the case of AZ-L compared to AZ, can be explained by the relative high concentrations of available trace elements added through the litter (Table 2 and 3). On the other hand, time-related decomposition of litter in the soil could lead to the solubilisation of trace elements, increasing

their bioavailability (Table 2). Generally, in this experiment we did not find significant increases of the concentrations of available trace elements between T = 0 to T = 40 in the litter treated soils (Table 3), therefore it seems that the changes on the soil trace elements due to the addition of litter happened in the first stage of the incubation. Afterwards trace elements in the litter are stabilized.

The rate-limiting step in nitrification is the oxidation of ammonia to nitrite, with nitrite typically being rapidly oxidized to nitrate by nitrite-oxidizing organisms (Mertens *et al.*, 2009). Soil pH is the major factor regulating the nitrification process in soil. Nitrification takes place in soil at pH ranging 5.5 to about 10.0 with optimum around 8.5. However, some authors have reported the occurrence of nitrification at pH as low as 3.8 (Sahrawat, 2008). The low values of $\text{NH}_4^+\text{-N}$ in neutral soils (RHU and AZ) demonstrated its rapid transformation into $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ during the whole incubation period (Fig. 1). In neutral soils, after

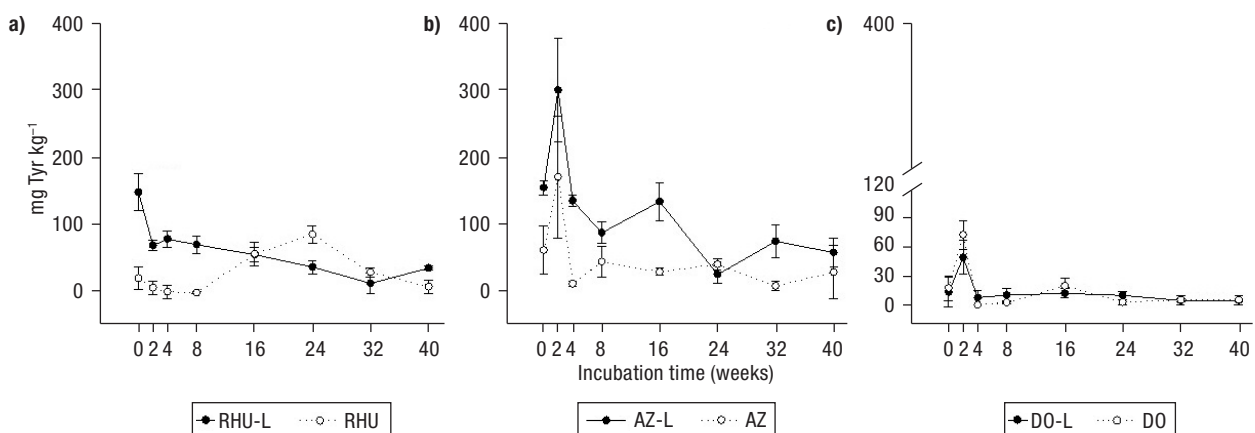


Figure 6. Evolution of protease activity in the different studied soils with and without litter.

an initial phase of N immobilization, NO_3^- -N concentrations increased exponentially (Fig. 2), indicating that NH_4^+ -N was oxidized to NO_3^- -N (He *et al.*, 2000). Other authors found the same results in aerobic incubation experiments (Cabrera *et al.*, 2005; Madrid *et al.*, 2011). According to Nannipieri and Eldor (2009) most mineral soils are affected by immobilization during early incubation, thus longer term incubations describe more realistically the release of soil N. Nitrate immobilization period of N coincides with the observed increase of MBN at initial times of incubation (Figs. 2 and 5). The N cycle in soil involves immobilization of inorganic N with the synthesis of protein by micro-organisms (Brady, 1990).

Nitrification in neutral soils with litter (RHU-L and AZ-L) was higher than that observed for soils without litter because the nitrogen provided by litter (Fig. 2). Despite the trace element contents in AZ soil, the nitrification process of AZ-L soil not only was not affected, but it was observed that the concentrations of NO_3^- -N during incubation were higher than in RHU-L. Differences can be explained in base on their different texture and organic matter (OM) contents, because nitrification proceeds most rapidly where there is abundance of exchangeable bases, and exchangeable bases generally increases with OM and clay contents (Brady, 1990).

In the acid soil (DO) higher concentrations of ammonia and lower concentrations of NO_3^- -N were observed during the incubation compared to the neutral soils (Fig. 1), revealing a blockage in the nitrification reaction. Application of litter had no effect on nitrification in this soil. There are two main differences between DO and neutral soil (RHU and AZ) soil pH and concentrations of available trace elements. Both soil pH and concentrations of available trace elements are known to affect the nitrification process in soils (Smolders *et al.*, 2004), but it is not possible to determine which of these two factors is most important in this case. On the other hand, denitrification and ammonia volatilization losses could also have resulted in an underestimation in net mineralization in this soil (Premi and Cornfield 1969; Stevens *et al.* 1998). At low pH the ability of nitrifiers to absorb N is very limited and this could also explain the absence of response of net nitrification in acidic soils (Ste-Marie and Paré, 1999).

Nitrogen mineralization was analysed by the model of Stanford and Smith (1972) that provide interesting conclusions about the behaviour of the native soil N and litter N. First of all, the value of N_o of RHU (poten-

tially mineralization N) representing the pool of organic N that might be mineralized, was higher though not significantly than that of AZ despite the OM and Kjeldhal-N content were higher in AZ (Table 4; Fig. 4). Probably the greater content of clay of AZ account for that difference, so that clays confer some degree of “protection” through their association with native soil organic matter (Hassink, 1994). The addition of litter increases the values of N_o in both soils (Table 4). Despite the lower N content of the litter of AZ compared to that of RHU, values of N_o for AZ-L was some higher than for RHU-L although differences were not significant. In this case MO of the litter is not protected by the clay and the mineralization of its nitrogen is supposed to be higher in AZ a loam soil more fertile than RHU a sandy loam soil. Values of MBN higher in AZ-L than in RHU-L confirm this hypothesis (Fig. 5). Mineralization rates (k) were similar for both soils (RHU and AZ) and higher for soils with allochthonous MO (RHU-L and AZ-L) (Table 2).

Campbell *et al.* (1991) defined $N_o \times k$ as the initial potential rate of the N mineralization process and an excellent index of the N-availability of the soil. As for N_o values of $N_o \times k$ were higher in RHU than in AZ due to the different nature of the soils. Litter addition increased the values of this parameter. Values of $N_o \times k$ for the original neutral soils were similar to that reported by Cabrera *et al.* (2005) for a sandy and a sandy-clay-loam soil; litter application increased $N_o \times k$ to similar values to those reported by the same authors for soil amended with olive mill wastewater compost. Nevertheless, values of $N_o \times k$ were lower than the range of values obtained for several soils by Simard and N'Dayegamiye (1993) (7.4-37.3 $\text{mg kg}^{-1} \text{week}^{-1}$), Hernández *et al.* (2002) (70-198 $\text{mg kg}^{-1} \text{week}^{-1}$).

The potential nitrification rate (PNR) was clearly affected by soil pH—revealing that nitrifying organisms are acid-sensitive in these soils (Dancer *et al.* 1973)—and by the litter addition. However effect of trace element on the soil cannot be inferred from this figure. Smolders *et al.* (2001) concluded that the effect of metals on PNR rate is smaller than the effect of soil properties, mainly pH. Moreover, they concluded that the PNR assay should be considered as a test that indicates the existence of a stress factor in soil. However, these results could also related with the fact that soluble trace elements in soils were also inhibitory factors on some steps involved in the N cycle, probably because toxic metals cause protein denaturation or

damage the integrity of microbial cell membranes, which consequently influences their growth, morphology and metabolism (Leita *et al.*, 1995).

Different aspects of abiotic toxicity towards microorganisms and microbial process in forest soils have been reviewed by Baath (1989). The adverse effects of acidity and heavy metal on soil biological properties are well known (Yang *et al.*, 2006); such studies have focused mainly in establishing the influence of the total concentrations of trace metals (Papa *et al.*, 2010). Soil functional quality, however, is more dependent on bioavailable trace metal concentration since this is the only fraction that affects soil-quality-related parameters such as enzyme activities or microbial related processes (Speir *et al.*, 1995). In fact, in this study, the two contaminated soils showed very different behaviours: in the case of AZ soil with neutral pH and low trace element availability, MBN and protease activity values were similar or even higher than those observed for non-contaminated soil, whereas DO soil with acid pH and high metal availability presented a poor microbiological status (Figs. 5 and 6).

Even though the pH was the most determinant soil property, the role of the litter input in microbiological properties was also noticeable. Neutral soils with litter addition presented higher values of MBN at the initial times of incubation. Protease activity was in general higher in soil with litter addition than soil without this addition during the entire incubation period. This extracellular enzymatic activity is closely related with protein N content and catalyses the release of amino acids (Nannipieri and Eldor, 2009). The factors regulating protease activity have been studied extensively with single bacterial and fungal species (Kumar and Takagi, 1999), and the results have shown that N source is one of the most important factors. Litter was an important source of N to soils (Tables 2 and 3) and its decomposition was accompanied by stimulation of a succession of soil enzymes (Moorhead *et al.*, 1999).

Conclusions

Despite its trace element content, white poplar-litter positively modified soil properties related with the N cycle, even in trace element contaminated soils, showing the importance of the litter in forest ecosystems. White poplar-litter increased the availability of Cd and Zn in acidic soil, but had little effect in neutral soils. Our

results showed the negative effect of the acidity and trace element availability in parameters related with the N-cycle.

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