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Soil chemical and biochemical properties under *Populus alba* growing: three years study in trace element contaminated soils.

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Abstract

Certain plant species have the ability to grow in trace element-polluted soils without showing any negative symptoms. These species could be considered for phytoremediation techniques and their presence might influence the abundance, activity and composition of soil microbial communities. In this work we investigated the root-induced changes in chemical (pH, soluble trace element concentrations, total organic C, water-soluble C, and nitrogen concentrations) and biochemical (microbial biomass C, β-glucosidase activity and protease activity) properties caused by *Populus alba* on two contaminated soils (one with neutral pH (AZ) and other with acid pH (DO)) for a period of over 36 months. The results were compared to those obtained with a non-contaminated soil. The experiment was carried out in containers. At the end of the experiment, samples of the soil directly adhered to the root and that located more than 5 cm from the root were also studied. The results showed that, in neutral soils, poplar did not influence soil pH; the greatest effect on pH due to plant growth was found in acid soil. Poplar presence increased C sources, through root exudates, in all soils. In AZ soil, poplar maintained chemical and biochemical properties, whereas an important decrease

in soil quality was observed in the same bare soils. The effect of poplar development on soil quality was even more appreciable in acid contaminated soil (DO), in which the tree also produced a strong increment of soil pH, a decrease in trace element concentrations and an improvement of chemical and biochemical properties. We concluded that *Populus alba* is a suitable plant for the phytoremediation of trace element contaminated soils. Moreover, root exudates of this species may be responsible for the improvement of soil quality in trace element contaminated soils.

Key words: Salicaceas, rhizosphere, microbial biomass carbon, enzymatic activities, soil quality.

INTRODUCTION

Trace elements can to be toxic to living organisms when present at excessive concentrations (Baath, 1989). Trace elements affect the growth, morphology and metabolism of microorganisms in soils, as well as protein denaturation or the destruction of the integrity of cell membranes (Leita et al., 1995). In case of plants, the general symptoms of trace element toxicity at the whole plant level are stunted growth, root growth inhibition, chlorosis, burning and necrosis, which are caused by the inhibition of cell division and cell elongation, reduced photosynthesis rates and water stress (Verkleij et al., 2009). However, metal tolerant plants can grow in trace element contaminated soils without any major adverse symptoms, and such plants can be used for soil phytoremediation (McGrath, 1998).

Plants influence the biomass, activity and composition of the soil microbial communities through their rhizodepositions containing a large variety of labile C compounds (Farrar et al., 2003), which are rapidly utilised by rhizosphere

microorganisms (Renella et al., 2007). It has been reported that microbial utilisation of root exudates by rhizosphere microorganisms may be reduced in trace element contaminated soils (Renella et al., 2006). However, plant roots may also change some major soil chemical properties in the rhizosphere (e.g. pH, nutrient availability, heavy metal complexation) (Neumann and Romheld, 1999; Wenzel, 2009), thus leading to a general amelioration, allowing microorganisms to increase the levels of their biochemical activity. It is well known that plant presence promotes soil development and nutrient cycling. These chemical, biological and physical improvements accelerate the development of a viable nutrient cycle and self-sustaining vegetative cover and restore the affected area to some acceptable steady-state condition for secondary land use (Norland and Veith, 1995).

For the restoration of heavy metal contaminated soils, different strategies can be adopted. Green plants have been employed in recent years as a means of stabilising and/or removing metals from contaminated soils. The re-vegetation activity aims to stabilise the site, to establish a cover crop that will prevent the dispersal of metal-contaminated particles by water or wind erosion and to reduce metal mobility by rhizosphere-induced adsorption and precipitation processes (Vangronsveld et al., 1991; Vangronsveld et al., 1993). Vegetative stabilisation also improves the chemical and biological characteristics of the contaminated soil by increasing the organic matter content, nutrient levels, cation exchange capacity and biological activity.

Fast growing trees with high transpiration rates, such as poplar, have been of particular interest for phytoremediation of trace element-polluted soils (Laureysens et al., 2005; Sebastiani et al., 2004). The long-term goal of this technique is the creation of a self-sustaining ecosystem that can support productive land use activities and is aesthetically pleasing.

We hypothesised that *Populus alba* growth under contaminated soils could induce positive changes on the chemical and biochemical properties of contaminated soil. Therefore, we carried out an experiment in semi-field conditions, and measured chemical (pH, soluble trace element, total organic C, water-soluble C and nitrogen concentrations) and biochemical parameters (microbial biomass C, β-glucosidase activity, protease activity) related to the C and N cycle during 36 months of poplar growth. At the end of the experiment, we also analysed rhizosphere and bulk soil separately.

2. MATERIAL AND METHODS

2.1 Experimental Design

The experiment was carried out in semi field conditions using three different soils: a non-polluted soils, (CO pH 7.5), and two trace-element contaminated soils, (AZ pH 7.4 and DO pH 3.0). Soil CO was collected in an experimental farm of the Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC) located in Coria del Rio, Seville (37º 17' 08'' N, 6º 04' 1.5''W).

The trace-element polluted soils, AZ and DO, were collected in the area affected by a mine spill (South West of Spain, Grimalt et al., 1999) in special spots where *Populus alba* trees were growing (37º18'4.7"N, 6º15'39.1''W, AZ and 37º23'42.5'' N, 6º13'36.4'' W, DO). Collected soils (January 2009) were affected by a mine spill containing high levels of As, Cd, Cu, Pb and Zn from a pyrite mine in 1998 (Cabrera et al., 1999). The toxic sludge covering the ground and a major portion of the contaminated soil surface were mechanically removed. In the more accessible areas (e.g., former croplands), a partial soil restoration was carried out by adding organic matter and calcium-rich amendments. However, despite these clean-ups and partial

restoration of the soils, the affected zone was still polluted consistently by trace metals with a fairly irregular distribution (Cabrera et al., 2008).

Containers filled with these soils were planted with white poplar saplings (6 months-old) in February 2009, treatments AZ-P, DO-P and CO-P. Containers without plants were also established as control (AZ, DO and CO). The 18 containers were distributed in a fully randomized scheme.

The main characteristics of the soils are shown in Table 1. Two of the studied soils presented sandy-loam texture (CO and DO) whereas AZ soil showed a loam texture.

Containers were irrigated daily during the growth phase (April to November), though a drip irrigation hose with two emitter of 4L/h per container. The mean water doses during this time were 4 mm irrigation per container and day, sufficient to meet the plant demand due to evapotranspiration, and to keep the soil moisture at the water holding capacity.

Soil samples were taken with an auger at 0-20 cm depth in spring and autumn of each year from April 2009 until October 2011 (6 samplings in total). In each sampling, three soil cores (2 cm diameter, 20 cm depth) regularly distributed were taken from each container to make a composite sample. Soils from the containers with plants were collected around the tree trying to collect the soil close to the root.

At the end of the experiment after the removal of the tree from the container the soil adhered to the root (RS) and soil located at more than 5 cm of the root (bulk soil) were collected and analysed.

2.2 Soil chemical properties determination

Soil pH was measured in a 1/2.5 sample/1M KCl extract after shaking for one hour (Hesse, 1971) using a pH meter (CRISON micro pH 2002). The 0.01M $CaCl_2$-

extractable trace element concentrations in soils were determined using a 1:10 w:v ratio, after shaking for three h (Houba et al., 2000). Pseudo-total trace element concentrations were determined by microwave (Microwave Laboratory Station Mileston ETHOS 900, Milestone s.r.l., Sorisole, Italy) assisted aqua regia digestion on the fraction < 60 μm fraction. Quantification of elements in the extracts was achieved using a Varian ICP 720-ES (simultaneous ICP-OES with axially viewed plasma). Total organic carbon (TOC) in soil was analysed by dichromate oxidation and titration with ferrous ammonium sulphate (Walkley and Black, 1934). Water-soluble carbon (WSC) content was determined on using a TOC-VE Shimadzu analyser after extraction with water using a sample-to-extractant ratio of 1:10.

Kjeldahl N (N-Kjel) of soil was determined after digesting the soil samples by the method described by Hesse (1971). Extractable NH_4-N^+ in soil was determined by shaking a fresh sub-sample (2.5 g on an oven-dry basis) in 25 mL of 1 M KCl for 1 h. Extractable NO_3-N^- in soil was determined by shaking a fresh sub-sample (5 g on an oven-dry basis) in 25 mL of distilled water for 1 h. Determination of NH_4-N^+ and NO_3-N^- in extracts were carried out in a Bran + Luebbe GmbH AA3 dual-channel, continuous-flow auto-analyser (Norderstedt, Germany).

2.3 Soil microbiological properties determination

Microbial biomass carbon (MBC) content was determined by the chloroform fumigation–extraction method modified by Gregorich et al. (1990). Concentration of C in the extract was measured by a TOC-VE Shimadzu analyser. An extraction efficiency coefficient of 0.38 was used to convert the difference in soluble C between the fumigated and the unfumigated soil to MBC (Vance et al., 1987). The detection limits

for the TOC-VE Shimadzu analyser were as follows: 0.5 mg kg^{-1} for TC (Total Carbon) and 0.04 mg kg^{-1} IC (Inorganic Carbon) contents.

The soil β-glucosidase activity was measured as reported by Tabatabai (1982) after soil incubation using p-nitrophenyl-β-D-glucopyranoside as substrate and by quantification of the p-nitrophenol (PNP) as enzymatic reaction product by UV/VIS spectrometry absorbance at 400 nm wavelenght.

Protease activity was measured using casein as substrate and colorimentric determination of the released tyrosine, according to Ladd and Butler (1972).

2.4 Statistical analysis

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 plant. A correlation matrix between all chemical and biochemical parameters was calculated. The significance levels reported (**$p<0.01$ and *$p<0.05$) are based on Spearman coefficients, because the variables required non-parametric analyses.

Sun ray plots were constructed to compare graphically the mean values of different studied parameters in each soil and treatment. The star shape and integrated area for each treatment allow a comparison of visual and statistical presentations of multivariate data. Soil chemical and biochemical properties for the three soils and soil contamination in the case of DO soil, were designed; the integrated area of the plot for each treatment was measured using the measuring tools of Adobe Acrobat 9® (Adobe Systems Incorporated, CA, USA).

3. RESULTS

3.1 Soil characteristics

The pH values of CO and AZ soils were close to neutral or slightly alkaline, whereas DO soil presented an acid pH (Table 2). Control soil (CO) presented trace element concentrations in the same order of magnitude of the background concentrations of the soils of the area, whereas the total concentrations of contaminated soils with regard to the contained trace elements were 5-fold higher for some elements (Table 1), and exceeded the current legislation limits for agricultural soils (Bowen, 1979). Values are higher than the background values of the area before the accident (Cabrera et al., 1999).

Trace element concentrations extracted with 0.01 M $CaCl_2$ were lower in neutral soils (CO and AZ) than in the acidic soil (DO), and significant negative correlations were found between pH and all of the measured trace elements, except for Pb (Table 2).

Values of pH in root soil (RS) and bulk soils in the last sampling (October 2011) are shown in Table 2. Generally, the pH in RS has always been higher than that obtained in bulk soil, close to one unit for AZ and DO (Table 2). The pH values in all RS samples were slightly alkaline. .

In this last sampling, available trace element concentrations extracted with 0.01 M $CaCl_2$ for RS were similar to the corresponding bulk soil (Table 2). Arsenic concentrations were below the detection limit (As 0.1 mg kg^{-1}).

3.2. Total soil carbon and nitrogen and biochemical properties

Total organic carbon (TOC) contents at different times of the experiment (0, 18 and 36 months) are shown in Table 3. The lowest values of TOC corresponded to non-contaminated soil. Values of TOC in soils with plants were higher than those detected in

bare soils (month 18 and 36), showing the largest differences at the end of the experimental period.

The presence of *P. alba* increased water soluble carbon (WSC) in all soils, although significant differences were only found in some samplings of AZ and DO soils (Figure 1). The time evolution of the WSC values was similar for all soils; constant values until October 2010 were followed by an increase in the next sampling (April 2011). In the last sampling, values of WSC decreased, especially in AZ soils. In general, the highest WSC values were observed for the AZ-P soil, reaching a maximum in April 2011 (221 mg kg^{-1}), and the lowest in DO soils, with acidic pH values and higher trace element contamination occurring in October 2011 (61.8 mg kg^{-1}); however, the lowest values were found for CO-P soil in the last sampling (74.3 mg kg^{-1}).

Microbial biomass carbon (MBC) was positively affected by plant growth and the highest concentrations were found in soils with poplars, except in DO soils where this effect was only observed at some sampling times (Figure 2). In the neutral soils (CO and AZ) higher mean values were presented compared with acid soil (DO). The highest values were found for CO-P soil in the second sampling (533 mg kg^{-1}) and the lowest for DO soil in October 2011 (39.4 mg kg^{-1}) (Figure 2). Clear seasonal trends were not identified. In general, WSC was significantly correlated with microbial biomass carbon (MBC) ($r= 0.443$; $p<0.01$).

Values of β-glucosidase activity are shown in Figure 3. For all cases, soils with poplars reached higher values than soils without plants. In the case of bare soils, a trend toward a decrease in time was observed. The highest β-glucosidase values were found in AZ soil and showed the greatest difference between treatments with and without poplars in all samplings. Moreover, in AZ-P soil, an increase was observed during April 2009 when compared with values of AZ soil at the same sampling time (Figure 3). All

of the parameters involved in the C cycle were positively correlated with pH values (r= 0.232 for WSC; $p<0.05$, r= 0.529 for MBC and r= 0.420 for β-glucosidase, respectively; $p<0.01$), whereas no correlations were found with soluble trace elements, except for negative correlations between MBC and Cd (r=-0.420; $p<0.01$) and between MBC and β-glucosidase activity and Zn (r= -0.518 for MBC and r= -0.282 for β-glucosidase; $p<0.01$).

For each soil, values of WSC and MBC were similar in RS to those found in the rest of the rhizosphere soil (bulk soil) (Table 4). In control soil (CO), values of β-glucosidase activity were higher in RS than in bulk soil, although significant differences were not found. For both contaminated soils (AZ and DO), similar contents of β-glucosidase activity were obtained in RS and bulk soil (Table 4).

Contents of Kjeldahl N fraction in the soils at the beginning of the experiment were higher in contaminated soils (AZ and DO) than in control soils. These values (with and without poplars) were similar throughout the studied period and, in general, were slightly lower in bare soils (Table 3). A slight increment was observed for acidic contaminated soil (DO) for both treatments in the last sampling (1.35 g kg^{-1} for DO and 0.99 g kg^{-1} for DO-P), especially in bare soil.

In neutral soils (CO and AZ), values of NH_4-N^+ ranged between 1.00 mg kg^{-1} and 8.00 mg kg^{-1}; however, significant differences between the soils with or without poplars were not found (Figure 4). These values tended to decrease at the end of the study. In DO soils, NH_4-N^+ concentrations were much higher, especially during the first sampling in which values reached a maximum of 14 mg kg^{-1} and then decreased to 5 mg kg^{-1} in the following samplings (Figure 4).

Contents of NO_3-N^- were lower in soils with poplar than those found in unplanted soils (Figure 4). Major differences were found between contaminated soils with and

without poplar in the sampling performed in October 2010 (Figure 4). However, in soils with poplars, NO_3-N^- values were similar to or below 20 mg kg^{-1}, except in DO soil, where values reached up to 60 mg kg^{-1}. For bare soils, higher values were obtained in contaminated soils, up to 60 mg kg^{-1} in AZ and up to 80 mg kg^{-1} in DO (Figure 4).

Poplar presence also stimulated the soil protease activity, although differences with unplanted soils were not significant due to the variability of the data (Figure 5). The maximum values of this activity were found at the end of the experiment in contaminated soils with poplar (AZ-P and DO-P) reaching values of 120 mg kg^{-1} and 100 mg kg^{-1} respectively (Figure 5). Higher protease activity was detected in soils planted with poplar than in bare soils. Positive correlations between this activity and some of the parameters related with the C cycle were found ($r= 0.292$ for β-gluc, $r= 0.327$ for MBC and $r= 0.355$ for TOC; $p<0.01$), whereas significant negative correlations with Cd and Zn were observed ($r= -0.229$ for Cd and $r= -0.284$ for Zn; $p<0.01$).

Protease values in the rhizosphere soils are shown in Table 4. In the non-contaminated soil, this activity was higher in RS compared to bulk soil, although the difference was not significant. However, in contaminated soils, similar values were found in RS and bulk soils (Table 4).

3.3 Evaluation of soil quality based on chemical and biochemical properties

For a general interpretation of all studied parameters referred in our samples, the data were represented in sunray plots and the values of each parameter were transformed to their corresponding standard scores in order to fit them into the graphs (Figures 6 and 7).

In general, in non-contaminated soil (CO) (Figure 6a), poplar presence did not produce any significant change in chemical and biochemical properties during the experiment. This was also proven by the calculation of the integrated area (IA) values of Figure 6a, which are reported in Table 5. Values of IA for CO without poplar soil were very similar during the experiment (around 600 mm^2) whereas a strong reduction was observed for the same soil with poplar (CO-P), from 1030 mm^2 to 463 mm^2. In neutral contaminated soils (AZ), poplar presence maintained chemical and biochemical properties, whereas an important decrease (a reduction of the IA value from 826 to 289 mm^2) in soil quality was observed in bare soils (Figure 6b and Table 5).

The effect of poplar development on the improvement of parameters related to soil quality was even larger in acid contaminated soil (DO), in which the tree also produced a strong increment of pH values (Figure 6c) and a decrease in trace element availability (Figure 7; Table 6). The increase in chemical and biochemical properties was also proven by their IA values (Table 5); poplars were capable of duplicating this value, passing from an initial area of 1051 mm^2 to a final area of 1812 mm^2.

4. DISCUSSION

In our study, at the end of the experiment, TOC increased in soils planted with poplar compared to bare soil (Table 4), likely due to the input of plant litter and root exudates; this exerted a positive influence on the measured soil biochemical properties.

Water soluble C originates mainly from release of organic substances from fresh material during decomposition and significantly contributes to soil nutrient cycling (Qualls et al., 1991). Our results (Figure 1) confirm the previous finding that WSC is significantly higher in vegetated than in bare soils (Zhang et al., 2011). The root system and litter presence might be important for readily soluble C sources. In addition, pH

values and soil and trace element availability seem to influence the WSC behaviour, as was observed in DO soils. Other authors have also reported the negative effect of both pH soil and trace element concentrations on WSC content.

Microbial biomass plays a crucial role in nutrient cycling (Nannipieri et al., 2002), and the measurement of MBC can be used as a parameter to assess the biotic impact of soil trace element contamination (Zhang et al., 2008). The lowest values of MBC were found in the DO soil, with the highest trace element concentration and more acidic pH values. Poplar growth sustained a higher microbial biomass (Figure 2), likely through the rhizodepositions, and utilisation of readily mineralisable low molecular weight organic compounds by rhizosphere microorganisms has been reported (Renella et al., 2007). It is also known that root exudates have an impact on trace element availability, either directly or indirectly through the stimulation of microbial activity (Puschenreiter et al., 2003). Indeed, several studies (Badalucco and Kuikman, 2001; Falchini et al., 2003) have confirmed that root exudation is the main factor controlling microbial activity and community structure in the rhizosphere. This is reflected in a raise in MBC values in soils with poplar, especially in contaminated soils (AZ-P and DO-P).

Values of β-glucosidase activity in soils with poplar were significantly higher than in bare soils, and the stimulation of various enzyme activities during the mineralisation of root exudates has been reported (Renella et al., 2007). In our study, the increase of β-glucosidase activity in trace element contaminated soils with plants might be related either to enzyme release by the plant roots and to the enhanced microbial enzyme synthesis in the rhizosphere (George et al., 2005). An increase of β-glucosidase activity in re-vegetated trace element contaminated soils has been also reported by Pérez de Mora et al. (2006). In contrast, no correlations between trace element availability and β-

glucosidase activity were found, thus confirming the results obtained by Renella et al. (2006).

Poplar growth also influenced the measured parameters related to N turnover in soil, thus confirming that N turnover is faster in planted than in bare soils (Clarholm, 1985), and the lower values of mineral N in planted than in bare soils were likely due to the N uptake by plants. Poplar rhizodeposition stimulated the N mineralisation processes, and led to N depletion in the rhizosphere mainly by NO_3-N^- uptake. Cloutier-Hurteau et al. (2011) reported higher NH_4-N^+ and lower NO_3-N^- in soils planted with *P. tremuloides* than in bare soils. In general, values of NO_3-N^- tended to be lower in spring samplings (April) in soils with plants because of the high demand for nutrients in this season, when maximum plant growth is registered. Moreover, in DO soils, higher amounts of ammonia and lower amounts of nitrate were observed compared to other soils, revealing a blockage in the nitrification reaction. Soil pH clearly affected the nitrification process in DO soil (Ste-Marie and Paré, 1999).

Protease activity is involved in N turnover in soil through the hydrolysis of proteins and peptides (Tate, 2002). In our study, protease activity was stimulated by the release of poplar root exudates (Figure 5). The stimulation of protease activity during decomposition of plant litter and root exudates has been previously reported (Plaza et al., 2004), even in trace element contaminated soils (Renella et al., 2006). Moreover, the increase of protease activity over time for DO-P soils might be related to the decrease in trace element availability. Other studies demonstrated that the reduction of exchangeable forms of several trace elements could have reduced the possible addictive or synergistic effects of different trace elements on soil microflora (Renella et al., 2003).

The general presentation of the data in the sun-ray plots (Figure 6a) confirmed the low fertility of control soil; although it was not contaminated with trace elements, soil

chemical and biochemical properties decreased over time. Poplar presence seems to improve chemical and biochemical fertility of only contaminated soils (Figures 6b, c), with the greatest effects seen for the acidic contaminated soils (DO). These results supported the hypothesis that soil microbial activity responds to a decrease of trace element availability and to soil pH neutralisation and an increase of WSC in re-vegetated trace element contaminated soils (Mench et al., 2006; Perez de Mora et al., 2006).

The fact that there were no relevant differences between the soil directly adhered to the roots and that located close to the roots could be due to the semi-field conditions of the experiment. Although several authors using *Populus* sp. under field conditions (Cloutier-Hurteau et al., 2011; Gamalero et al., 2012) have found differences between the soil directly adhered to the root and the rest of the soil surrounding the tree, under container conditions, the development of the root system encompassed the entire soil container by the end of the experiment. Therefore, the root system of the *Populus alba* influenced all of the soil in the container.

Poplar plantation increased MBC, β-glucosidase and protease activities of trace element contaminated soils likely through rhizodeposition, and aided in improving the quality of the contaminated soils. The presence of a root system might prove important for readily soluble C and N sources that influence the biochemical properties related to C and N cycles. Indeed, the fresh input of nutrients, by litter decomposition and root exudation, should stimulate microbial activity and accelerate the recovery process. Moreover, biochemical properties seemed to be the appropriate indicators of the available heavy metal fraction after *in situ* stabilisation of these metals with poplars.

Considering the complex role of the rhizosphere no relevant differences were observed between the soils directly adhered to the roots and bulk soil.

Our results suggest that *Populus alba* is a suitable plant for the remediation of trace element contaminated soils and that root exudates of this species may be responsible for the improvement in some chemical and biochemical properties of the soil. At the same time, these trees could produce an important biomass for energy purposes.

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Table 1. Some characteristic of the studied soils. Mean values ± standard errors (n=3).

Soil	pH	As	Cd	Cu	Pb	Zn
		Pseudototal content (mg kg^{-1})				
CO	7.74±0.059	8.40±1.07	0.59±0.03	19.4±1.17	15.7±0.24	52.0±1.92
AZ	7.41±0.050	112±3.05	3.82±0.09	166±3.61	236±7.80	506±8.84
DO	3.00±0.244	290±8.54	3.53±0.05	193±6.03	391±14.8	227±5.36
Background range*		0.1-40	0.01-2	2-250	2-30	1-900
		0.01 M $CaCl_2$-extractable contents (mg kg^{-1})				
CO			0.01±0.000	0.03 ±0.002	0.12±0.04	0.04±0.01
AZ			0.01±0.000	0.08 ±0.015	0.17±0.04	0.04±0.01
DO			0.33±0.004	15.2 ±6.16	0.47±0.33	69.5±2.18

*According to Bowen et al., 1979

Table 2. Values of 0.01M $CaCl_2$-extractable trace element concentrations (mg kg^{-1}) in root soil (RS) and bulk soil in October 2011. Mean values ± standard errors (n=3)

Soil	pH	Cd	Cu	Pb	Zn
CO-bulk soil	7.73±0.18	0.002±0.001	0.12±0.050	<0.02	0.17±0.03
CO-RS	7.81±0.64	0.001±0.001	0.08±0.009	<0.02	0.09±0.02
AZ-bulk soil	7.15±0.15	0.004±0.003	0.15±0.005	<0.02	0.12±0.03
AZ-RS	7.87±0.26	0.004±0.001	0.16±0.015	0.025±0.005	0.17±0.05
DO-bulk soil	6.79±0.53	0.005±0.001	0.239±0.027	<0.02	0.17±0.04
DO-RS	7.57±0.12	0.003±0.001	0.22±0.013	0.031±0.004	0.10±0.01

Table 3. Values of total organic carbon content (TOC) and Kjeldahl nitrogen (N-Kjel) in soils at different sampling times (initial, middle, and at the end of the experiment). Means values ± standard errors (n=3)

	Month=0		**Month=18**		**Month=36**	
Sample	**TOC ($g\ kg^{-1}$)**	**N-Kjel ($g\ kg^{-1}$)**	**TOC ($g\ kg^{-1}$)**	**N-Kjel ($g\ kg^{-1}$)**	**TOC ($g\ kg^{-1}$)**	**N-Kjel ($g\ kg^{-1}$)**
CO	5.92±0.13	0.53±0.02	4.85±0.41	0.43±0.16	4.59±0.31	0.46±0.17
CO-P	5.92±0.13	0.53±0.02	8.40±0.86	0.56±0.08	8.86±1.25	0.54±0.14
AZ	10.2±0.83	1.02±0.17	11.9±0.99	0.89±0.09	11.8±0.19	0.89±0.12
AZ-P	10.2±0.83	1.02±0.17	16.1±0.62	0.97±0.07	17.3±1.73	1.15±0.02
DO	10.0±0.50	0.81±0.01	11.1±0.66	1.00±0.20	10.4±0.17	1.35±0.21
DO-P	10.0±0.50	0.81±0.01	12.7±0.72	0.88±0.07	16.5±3.10	0.99±0.14

Table 4. Values of water soluble carbon (WSC), microbial biomass carbon (MBC) and β-Glucosidase and protease activities in soils adhered to the root (RS) and bulk soil in October 2011. Mean values ± standard errors (n=3).

Sample	WSC mg kg^{-1}	MBC mg kg^{-1}	β-Glucosidase mg PNF $kg^{-1}h^{-1}$	Protease mg Tyr kg^{-1}
CO-bulk soil	74.3±7.45	318±46.0	83.2±18.4	28.8±5.18
CO-RS	72.7±11.2	310±52.4	138±41.5	54.7±9.72
AZ-bulk soil	125±13.4	329±25.4	213±15.6	117±3.48
AZ-RS	121±24.4	313±21.6	243±45.5	109±11.2
DO-bulk soil	124±16.7	366±31.4	117±37.5	93.0±21.6
DO-RS	120±12.5	371±33.5	110±28.2	99.8±10.9

*$p<0.05$; significant differences for pairs between the same soils

Table 5. Index of the integrated area (IA, mm^2) of sun-ray diagrams plotting the different chemical (pH, Water Soluble Carbon, Total Organic Carbon, Kjeldahl Nitrogen) and biochemical (β-Glucosidase activity, Microbial Biomass Carbon and Protease activity) properties in each soil and treatment in the first (April 2009) and in the last (October 2009) sampling.

Soil	**Soil Quality (IA) (mm^2)**
CO (April-09)	633
CO-P (April-09)	1030
CO (Oct-11)	656
CO-P (Oct-11)	463
AZ (April-09)	826
AZ-P (April-09)	1466
AZ (Oct-09)	289
AZ-P (Oct-09)	1275
DO (April-09)	731
DO-P (April-09)	1052
DO (Oct-11)	1053
DO-P (Oct-11)	1812

Table 6. Index of the integrated area (IA, mm^2) of sun-ray diagrams plotting the 0.01M $CaCl_2$-extractable trace element concentrations in DO soil in the first (April 2009) and in the last (October 2009) sampling. According to the clockwise, starting at the 12 o'clock, the parameters are Cd, Cu, Pb and Zn

Soil	**Trace element contamination**
	IA (mm^2)
DO (April-09)	1656
DO-P (April-09)	879
DO (Oct-11)	57.7
DO-P (Oct-11)	56.8

Figure Captions

Figure 1. Evolution in time of water soluble carbon (WSC) in the three studied soils with and without poplar. Significant differences due to presence of plant in the same soil and time are marked with an asterisk.

Figure 2. Evolution in time of Microbial Biomass Carbon (MBC) in the three studied soils with and without poplar. Significant differences due to presence of plant in the same soil and time are marked with an asterisk.

Figure 3. Evolution in time of β-glucosidase activity in the three studied soils with and without poplar. Significant differences due to presence of plant in the same soil and time are marked with an asterisk.

Figure 4. Evolution in time of NO_3-N and NH_4-N in the three studied soils with and without poplar. NO_3-N concentration is represented by ●/○ and NH_4-N is represented by ■/□. Significant differences due to presence of plant in the same soil and time are marked with an asterisk.

Figure 5. Evolution in time of protease activity in the three studied soils with and without poplar. Significant differences due to presence of plant in the same soil and time are marked with an asterisk.

Figure 6. Sun ray plots of soil quality for different soil and treatments in soil in the first (April 2009) and in the last (October 2009) sampling. According to the clockwise, starting at the 12 o'clock, the parameters are pH, β-Glucosidase activity (β-Glucosidase), Microbial Biomass Carbon (MBC), Water Soluble Carbon (WSC), Total Organic Carbon (TOC), Kjeldahl Nitrogen (N Kjel) and Protease activity (Protease).

Figure 7. Sun ray plots of soil contamination for the two different treatments of DO soil at different sampling times (at the begging and at the end of the experiment). According to the clockwise, starting at the 12 o'clock, the parameters are $CaCl_2$-Cd (Cd), $CaCl_2$-Cu (Cu), $CaCl_2$-Pb (Pb), $CaCl_2$-Zn (Zn).

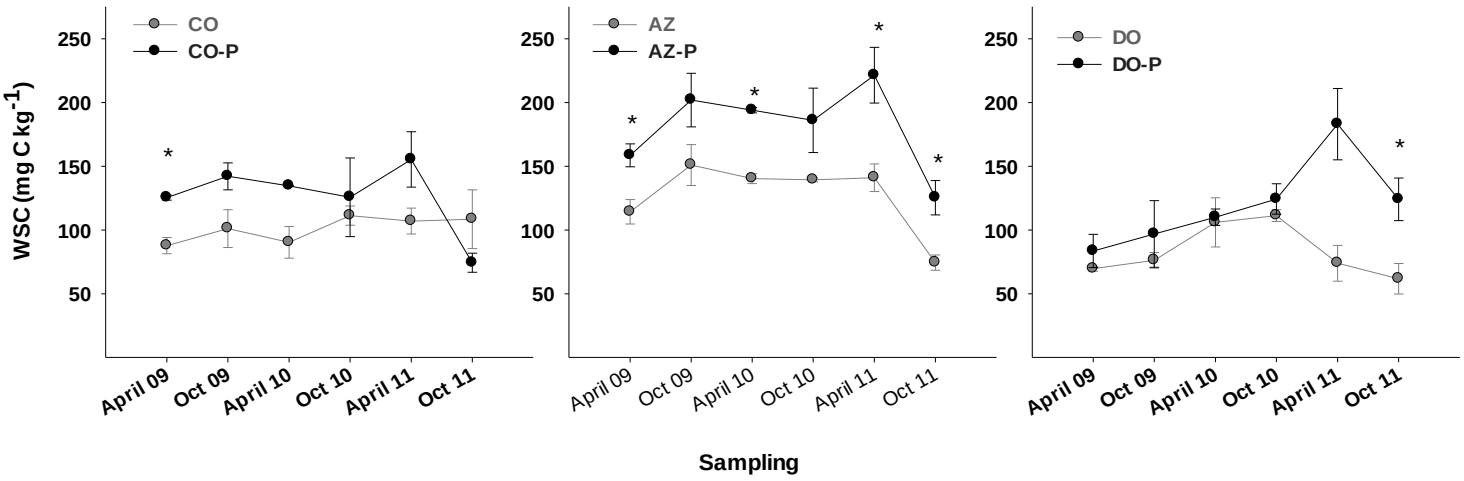
CO
CO-P
AZ
AZ-P
DO
DO-P
WSC (mg C kg-1)
250
200
150
100
50
*
April 09
Oct 09
April 10
Oct 10
April 11
Oct 11
Sampling

Figure 1.

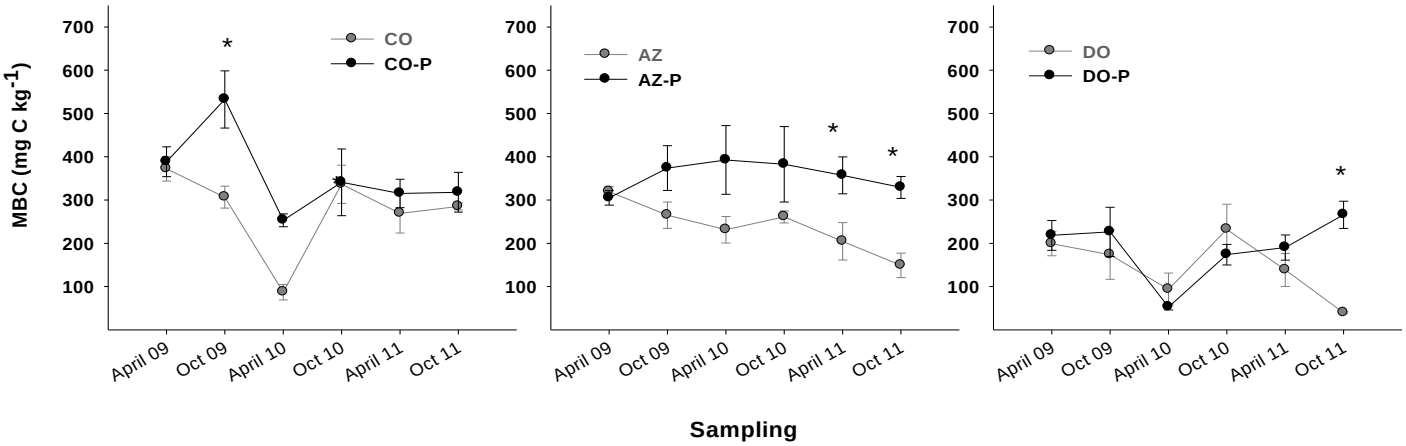
MBC (mg C kg-1)
700
600
500
400
300
200
100
CO
CO-P
AZ
AZ-P
DO
DO-P
*
April 09
Oct 09
April 10
Oct 10
April 11
Oct 11
Sampling

Figure 2.

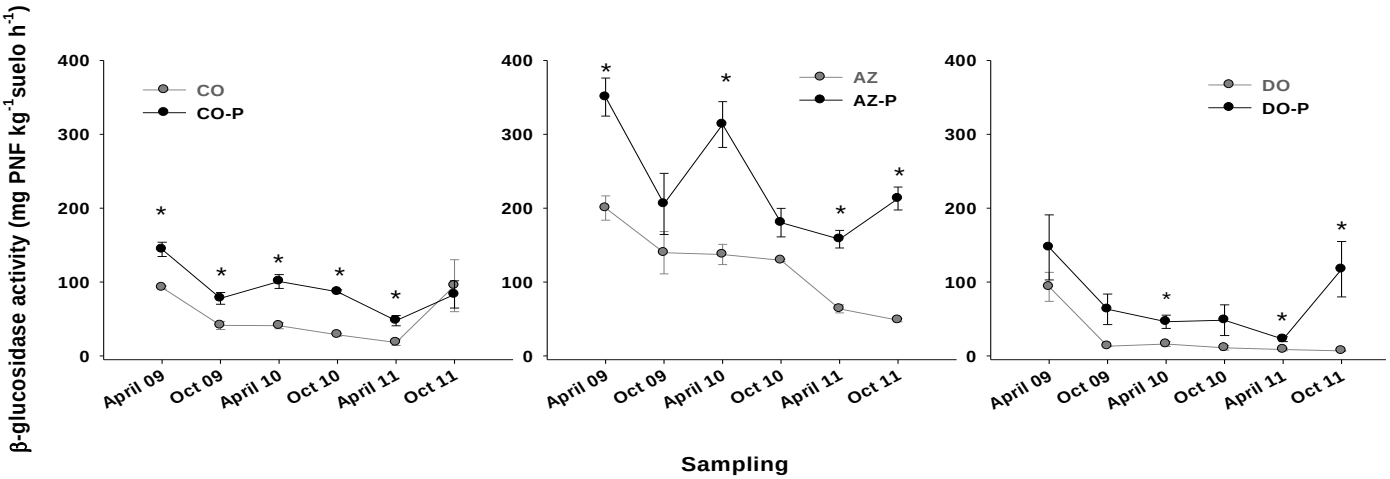
β-glucosidase activity (mg PNF kg-1suelo h-1)
400
300
200
100
0
CO
CO-P
AZ
AZ-P
DO
DO-P
*
April 09
Oct 09
April 10
Oct 10
April 11
Oct 11
Sampling

Figure 3.

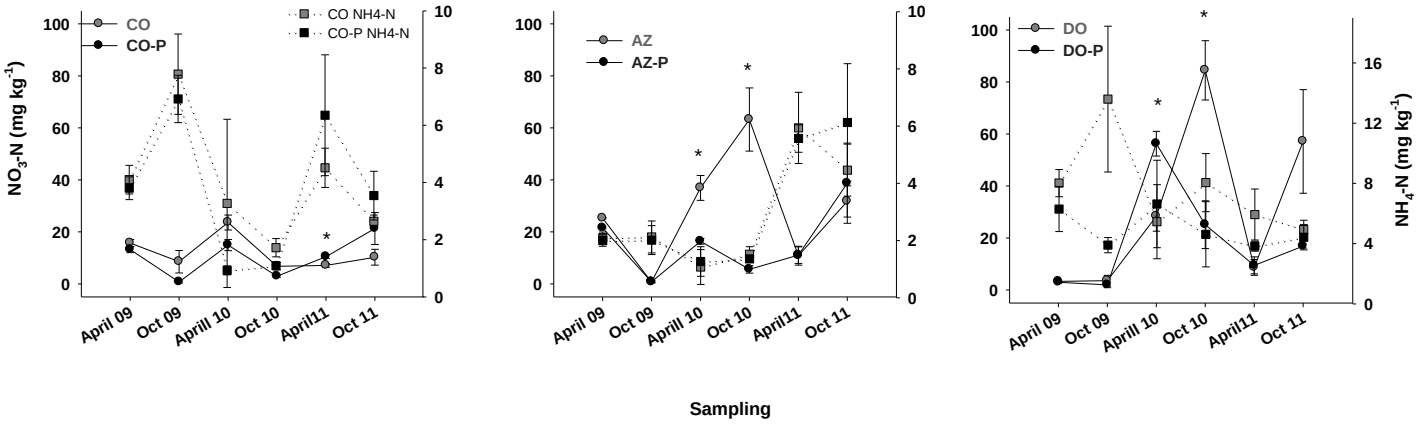
CO
CO-P
CO NH4-N
CO-P NH4-N
AZ
AZ-P
DO
DO-P
NO_3-N (mg kg^{-1})
NH_4-N (mg kg^{-1})
April 09
Oct 09
Aprill 10
Oct 10
April11
Oct 11
Sampling

Figure 4.

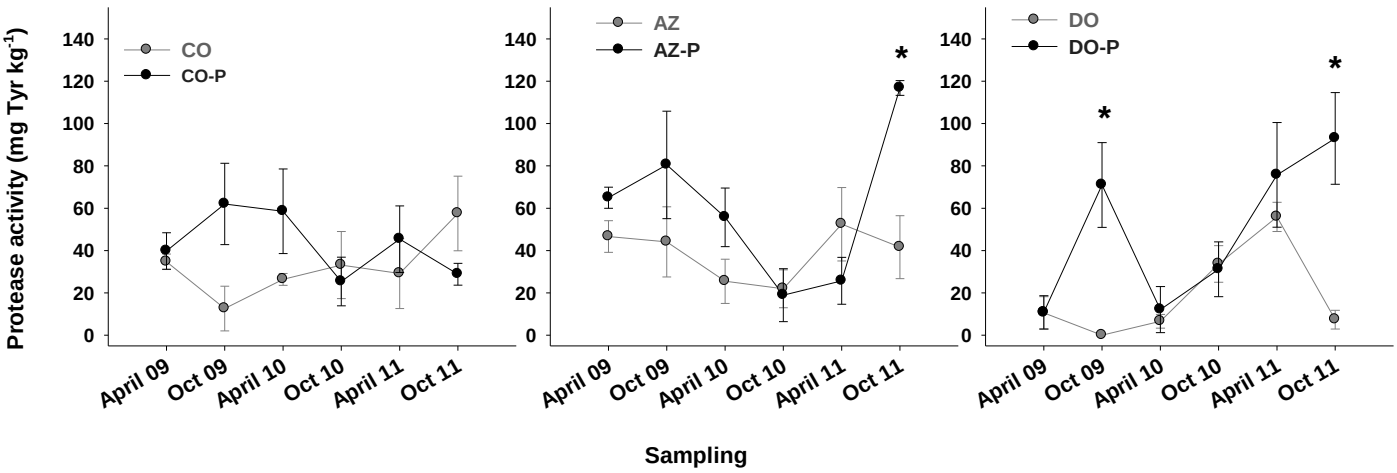
Protease activity (mg Tyr kg-1)
CO
CO-P
AZ
AZ-P
DO
DO-P
*
0
20
40
60
80
100
120
140
April 09
Oct 09
April 10
Oct 10
April 11
Oct 11
Sampling

Figure 5.

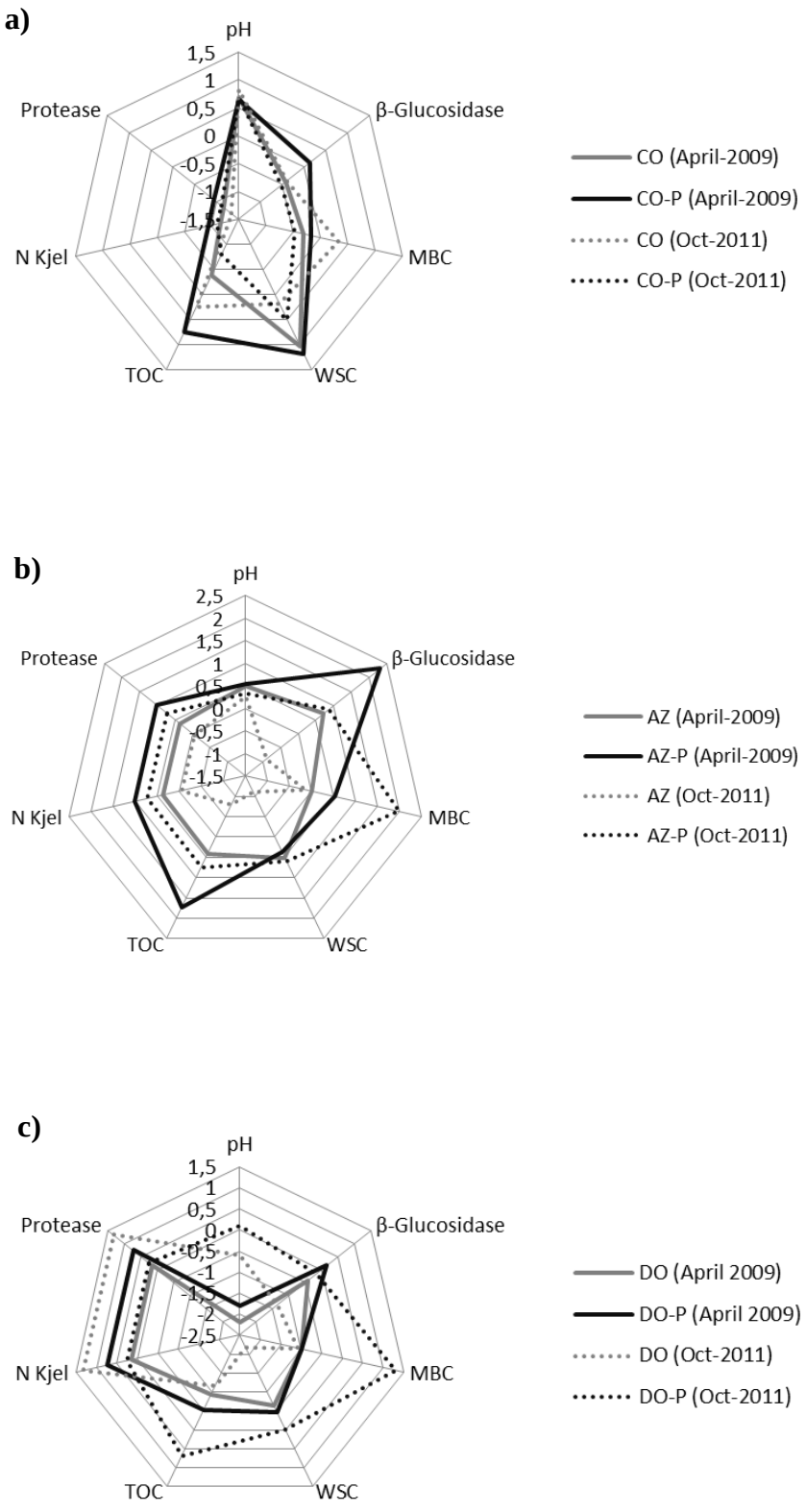
a)
pH
1,5
1
0,5
0
-0,5
-1
-1,5
β-Glucosidase
MBC
WSC
TOC
N Kjel
Protease
CO (April-2009)
CO-P (April-2009)
CO (Oct-2011)
CO-P (Oct-2011)
b)
pH
2,5
2
1,5
1
0,5
0
-0,5
-1
-1,5
β-Glucosidase
MBC
WSC
TOC
N Kjel
Protease
AZ (April-2009)
AZ-P (April-2009)
AZ (Oct-2011)
AZ-P (Oct-2011)
c)
pH
1,5
1
0,5
0
-0,5
-1
-1,5
-2
-2,5
β-Glucosidase
MBC
WSC
TOC
N Kjel
Protease
DO (April 2009)
DO-P (April 2009)
DO (Oct-2011)
DO-P (Oct-2011)

Figure 6.

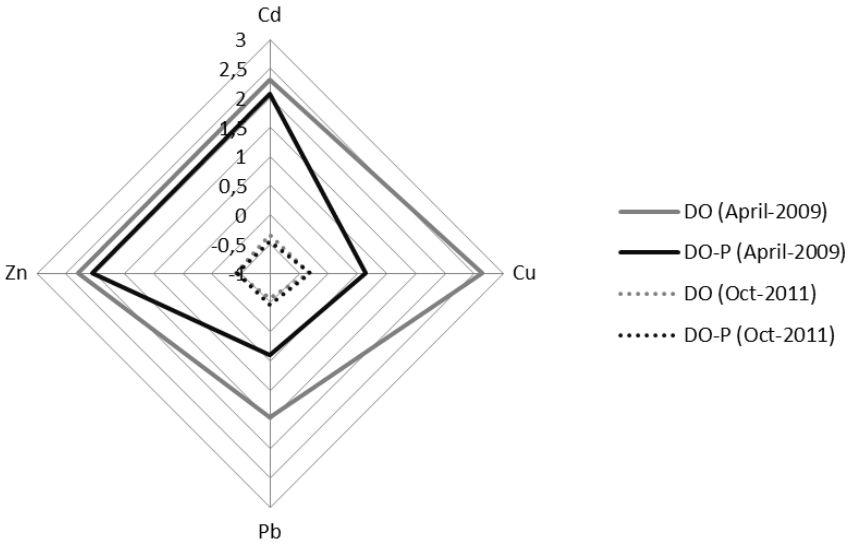
Cd
3
2,5
2
1,5
1
0,5
0
-0,5
-1
Zn
Cu
Pb
DO (April-2009)
DO-P (April-2009)
DO (Oct-2011)
DO-P (Oct-2011)

Figure 7.