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Agro-forestry management of *Paulownia* plantations and their impact on soil biological quality: The effects of fertilization and irrigation treatments

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ABSTRACT

Short-rotation plantations for biomass production are wood systems in which fast growing tree species are grown under intensive agricultural practices to achieve high biomass yields. *Paulownia* sp. is an extremely fast-growing tree species which is commonly used to produce biomass for energetic purposes. This tree species has a great nutrient and water demand, and the establishment of its plantations may affect soil fertility and quality. For this fact, this study is focused on the evaluation of the responses of the soil microbial community – in terms of biomass, community structure, and activity – in a *Paulownia* plantation submitted to a semiarid climate. Particular attention was paid to the impact of the soil fertilization (mineral fertilizer or an organic-mineral amendment, BAN, obtained from the forestry industry) and level of irrigation on the soil microbial community. For both irrigation regimes, a significant increase in TOC was observed with the addition of the BAN to the soil. The content of water soluble C in the soil was affected by both factors (fertilization and irrigation) and their interaction. Both enzyme activities related with the C and P cycles in soil and dehydrogenase activity were significantly affected by the fertilization, irrigation, and their interaction. Regardless of the irrigation level, the microbial respiration rate was highest with the BAN treatment. The content of total PLFAs and PLFA biomarkers of microbial groups showed no significant differences between the fertilization treatments. However, the total PLFAs and the Gram-positive, fungal, and saturated PLFA concentrations differed significantly between the irrigation levels. In general, the use of residues generated in the forest biomass industry (pine bark and biomass ash) as a soil amendment had a positive effect on the soil microbial activity without altering the structure of the soil microbial community. Considering the water deficit of semiarid areas, such as SE Spain, the low level of irrigation tested in this study would be enough to maintain both the soil microbial activity and suitable production of biomass by *Paulownia* trees.

1. Introduction

Short-rotation plantations producing biomass for energetic purposes are one of the promising means to increase the amount of renewable energy and have been identified as the most energy-efficient C conversion technology with regard to reducing greenhouse gas emissions and sequestering CO_2. Short-rotation plantations for biomass production are wood systems in which fast-growing tree species – such as *Populus* spp., *Eucalyptus* sp., *Salix* sp., *Paulownia* sp., or *Robinia* sp. – are grown under intensive agricultural practices (weed control, fertilizers application, and irrigation) to achieve high biomass yields (Rodriguez-Pleguezuelo et al., 2015). The area of land dedicated to biomass crops is expected to increase considerably in the mid- to longer-term, due to energetic demands (Parmar et al., 2015; Rowe et al., 2011).

Paulownia sp. is an extremely fast-growing, deciduous tree species that is propagated vegetatively and is tolerant of different soil and climate conditions (Zhang et al., 2007). Due to their high capacity to assimilate nutrients from the soil, *Paulownia* sp. plantations, under intensive agricultural practices, may potentially affect the soil microbial community and its functionality (Lucas-Borja et al., 2011). One of the main concerns is the high water demand of this plant species, which

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could have a major impact on local water resources (Garcia-Morote et al., 2014; McKay, 2011). In consequence, the establishment of these plantations as a large-scale crop is limited in semiarid regions with significant water shortages, such as those predicted in climate change scenarios (IPCC, 2013).

Concerns about the effects of these intensive plantations on soil fertility and quality have arisen (Madejon et al., 2016; Vanguelova and Pitman, 2009), and extensive knowledge of the impacts of these plantations and their agro-forestry management on the soil microbial biomass and soil biogeochemical potential is necessary.

Given the fundamental role of soil microorganisms in global biogeochemical cycling and soil quality (Bastida et al., 2008; Nannipieri et al., 1990), a deep understanding of soil microbial processes is important for the suitable management of agroforestry systems (Szott et al., 1991). The effects of soil management − such as fertilization and irrigation − on the biomass, structure, and activity of the soil microbial community in semiarid *Paulownia* plantations have been scarcely studied (Lucas-Borja et al., 2012; Madejon et al., 2016). This study focuses on covering this gap in our knowledge of the agroforestry management of *Paulownia* plantations in semiarid lands. The reuse of by-products generated in the forestry industry as soil amendments in short-rotation forest plantations may be an alternative to the use of mineral fertilizers, decreasing both the impacts on the environment and the production costs as well as promoting the soil sustainability of *Paulownia* plantations. Furthermore, irrigation strategies must be considered also, in the face of water limitation in the coming decades in semiarid ecosystems (Bastida et al., 2017).

Here, we evaluate the soil microbial community – in terms of biomass, community structure, and activity − in a *Paulownia* plantation exposed to a semiarid climate and the impacts on it of the soil fertilization (mineral fertilizer or an organic-mineral amendment obtained from the forestry industry) and level of irrigation. In order to determine the activity of the soil microbial community, several enzyme activities (EAs), dehydrogenase activity, and soil microbial respiration were assessed, as indicators of soil microbial quality (Nannipieri et al., 1990). The biomass and structure of the soil microbial community were analyzed by the quantification of phospholipidic fatty acids (PLFAs) (Bastida et al., 2008; Frostegard et al., 1993; Zelles et al., 1992). We hypothesized that organic-mineral amendments from the forestry industry would fulfill the soil nutrient demands of the plantation and promote the development and activity of the soil microbial community at least as well as conventional fertilization. Moreover, we expected that the interaction between the type of fertilization and the level of irrigation would influence the soil microbial community. Particularly, given the lignocellulosic nature of the organic-the soil nutrient demands of the plantation and promote the development and activity of the soil microbial community at least as well as conventional fertilization. Moreover, we expected that the interaction between the type of fertilization and the level of irrigation would influence the soil microbial community. Particularly, given the lignocellulosic nature of the organic-the soil nutrient demands of the plantation and promote the development and activity of the soil microbial community at least as well as conventional fertilization. Moreover, we expected that the interaction between the type of fertilization and the level of irrigation would influence the soil microbial community. Particularly, given the lignocellulosic nature of the organic-mineral amendment from the forestry industry, we expect that fungal biomass would be influenced by this treatment.

2. Materials and methods

2.1. Experimental area characteristics

This experiment was carried out in Albacete (Castilla-La Mancha Region, SE Spain), on abandoned cropland (coordinates: 39° 12′ 38.28” N, 1° 56′ 37.86” W; 714 m.a.s.l.). The climate of this area is cold semi-arid, type BSk according to the Köppen Classification. The mean annual temperature and precipitation are 13.6 °C and 372.1 mm, respectively.

The soil was classified as a Calcic Vertisol according to the FAO-UNESCO Soil Map of the World (FAO, 1988). The soil is less than 30 cm deep, with a limestone crust. The soil analysis showed a very alkaline soil (pH = 8.5) with clay contents higher than 30% throughout the profile. Given the amount of clay (36.2%), this soil had a high cation exchange capacity (CEC), which ranged between 29.24 and 22.68 meq/100 g. Abundant organic matter (5.68%) also contributed to the CEC. The soils were rich in bases, being calcium (Ca)-dominated soils in which Ca and magnesium (Mg) clearly dominated sodium (Na) and potassium (K). Base saturation was 100% throughout all the profiles, due to the alkaline parent material and the climate. The abundant clay present in this soil can immobilize K as an exchangeable cation, and K could be limiting according to the K/Mg ratio. Phosphorus availability (18 mg kg^{-1}) was moderate. However, this soil is characterized by the high amounts of nitrogen (N) (0.26%) in the upper 10 centimeters of the soil profile. Additionally, the available soil mineral nitrogen (SMN), present as nitrate or ammonium, was estimated at 15 kg ha^{-1} (a high availability of N).

2.2. Experimental design

The trial was set up in a randomized, complete-block design, in which two crop factors (irrigation and fertilization), with two and three levels, respectively, were controlled. In this experimental area, one *Paulownia* plantation with a density of 1333 trees ha^{-1} (tree spacing: 3 × 2.5 m) was established. Irrigation with a low dose (LI) and a high dose (HI) of water was carried out during the growing season (1750 and 2400 m^3 ha^{-1} $year^{-1}$, respectively). The fertilization levels were: 1) unfertilized trees (UF); 2) conventional fertilization (CF) with 500 kg ha^{-1} of Nitroacid® (acid solution with 15% ureic N and 40% SO_3), 100 kg ha^{-1} of Fercristal® (water soluble fertilizer, NPK 15-5-30), and 35 kg ha^{-1} of Fercristal® (13-40-13) during the growing season; and 3) fertilization with crushed pine bark (4000 kg ha^{-1}), ash from forest prunings combustion (2000 kg ha^{-1}), and 500 kg ha^{-1} of Nitroacid, which was denominated BAN. The main chemical characteristics of the pine bark and ash are shown in Table 1. Every treatment was replicated in three plots. CF began in May 2011 and it was added yearly until 2015. Commercial fertilizers were applied at the start of the growing period (one half dose in April) and halfway through the period (another half dose in July). The bark and ash were applied in February 2010. The fertilizer granules and solid amendments were placed in each tree basin and were mixed with the soil, while liquid fertilizer was added by fertigation.

Table 1
Chemical characteristics of materials used in BAN treatment.

Element	Ash	Bark
	%	
C	nd	48.6
N	nd	0.1
Ca	16.2	1.12
	mg kg^{-1}	
Al	17551	980
Fe	8889	736.6
Mg	10108	950.1
P	2562	143.1
K	17143	1338
Na	597.0	73.63
Mn	25.78	25.58
Cd	1.88	0.49
Cu	26.78	2.81
Cr	24.11	20.23
Ni	n.d.	10.24
Pb	89.61	7.93
Zn	39.06	2.77

The clone used in the plantation was *in vitro* 112® (*Paulownia elongata* × *P. fortunei* hybrid), since it has been previously shown to display adaptability to semiarid climates. *Paulownia* seedlings were grown for 4 months in a nursery, in pots, before being planted in the study area, in spring (April 2010). They were cut in the second spring (March 2011) to encourage coppicing and better formation of stems, and to leave a single sprout per tree. This was done to achieve rapid growth during the following season (summer 2011). Pruning (30% live crown ratio, in the second spring) involved the removal of buds and lateral branches to promote upward growth and better quality stems. The water dose was controlled by an automatic drip irrigation system, thus avoiding waterloggingSoil sampling was carried out in October 2015, when it was supposed higher microbial activity than previous periods, and six subsamples of each plot were taken and then mixed to form a composite soil sample. Each sample was air dried, homogenized by sieving (2-mm mesh), and then divided into two parts. One part was stored at 4 °C for chemical and biochemical analyses, and the other was frozen at −20 °C until PLFA analysis.

2.3. Analysis of soil physical-chemical and chemical properties

The electrical conductivity and pH were measured for a 1/10 (w/v) aqueous extract in a conductivity meter (SensION EC71, HACH, Loveland, Col., USA) and pH-meter (GLP21 +, CRISON, Barcelona, Spain), respectively. The soil samples were submitted to nitric-perchloric acid digestion using a microwave oven, prior to determination of the total concentrations of macro and micronutrients in an ICP-OES analyzer (ICAP 6500 Duo, Thermo Scientific, Waltham, MA USA). The total contents of organic C (TOC) and N were determined by a Thermo Scientific CN Flash2000 analyzer, while the water soluble fraction of C was determined using a CN analyzer for liquid samples (Multi N/C 3100, Analytikjena, Germany).

2.4. Soil enzyme activity determination

Soil enzyme activities (EA) were determined by a microplate method and each enzyme activity was tested using specific substrates, by a technique modified from Allison and Jastrow, 2006. A 96-well microplate was used for each enzyme activity. For this, an extract of each soil sample was made previously, using Tris-HCl buffer (pH 7), and was homogenized using a mixer. Of this extract, aliquots were taken in triplicate and were placed in the wells of a microplate, adding the corresponding substrate. In the same plate, parallel controls (without substrate) were prepared in triplicate and each plate was incubated at 28 °C for the time corresponding to each enzyme activity. The substrates were 6 mM 4-nitrophenyl β-D-cellobioside for cellobiohydrolase (CBH), 6 mM 4-nitrophenyl N-acetyl-α-D-glucosaminide for N-acetyl-β-glucosaminidase (NAG), 15 mM *p*-nitrophenol β-D-glucopyranoside for β-glucosidase (BGA), 5 mM leucine p-nitroanilide predissolved in a small volume of acetone for leucine aminopeptidase (LEU), 20 mM *p*-nitrophenol phosphate for alkaline phosphatase (APA), and 50 mM pyrogallol for polyphenoloxidase (PPO) and peroxidase (POD). For the latter enzyme activity (POD), 10 µl of 30% H_20_2 were added together with the substrate. The incubation times for the enzyme activity determinations were: 1 h for BGA and APA, 2 h for PPO and POD, 4 h for LEU, and 6 h for NAG and CBH. After that, the microplates were centrifuged and 100 µl of the supernatant in each well were transferred to a new microplate. For BGA, APA, CBH, and NAG, 5 µl of 1 M NaOH were added to stop the enzymatic reaction. The absorbance of the solution in each well was read with a TECAN Infinite M200 spectrophotometer at the following wavelengths: 410 nm for BGA, APA, NAG, and CBH; 405 nm for LEU; 460 nm for PPO and POD.

2.5. Determination of microbial respiration in the soil

The microbial respiration (MR) was measured as reported by García et al. (2003). Soil samples (15 g) were placed in hermetically sealed flasks. They were moistened to 40–60% of their WHC and then incubated for 39 days at 28 °C. The CO_2 evolved was measured periodically (every day for the first week and then weekly) using an infrared gas analyzer (PBI Dansensor, Checkmate II). The cumulative amount of CO_2 evolved was calculated and the respiration rate was expressed as mg CO_2-C kg^{-1} soil day^{-1}.

2.6. PLFA analysis

Phospholipids were extracted from 6 g of soil using chloroform-methanol extraction, as described by Bligh and Dyer (1959), and were fractionated and quantified using the procedure described by Frostegard et al. (1993). They were transformed into fatty acid methyl esters (FAMEs) by alkaline methanolysis and were designated as described by Frostegard et al. (1993). The complete dried FAME fraction was dissolved in isooctane containing 0.23 mg ml^{-1} of 21:0 FAME as internal standard. The analysis was performed using a Trace Ultra Thermo Scientific gas chromatograph fitted with a 60-m capillary column (Thermo TR-FAME, 60 m x 0.25 mm ID x 0.25 µm film), using helium as the carrier gas. The following fatty acids are characteristic bacterial fatty acids and were chosen as bacterial biomarkers: i15:0, a15:0, 15:0, i16:0, i17:0, cy17:0, cy19:0, 16:1ω7c, 16:1ω7t, 18:1ω9c, and 18:1ω9 t (Dungait et al., 2011; Frostegard et al., 1993). The fatty acid 18:2ω6 was used as an indicator of fungal biomass (Brant et al., 2006; Rinnan and Baath, 2009). The Gram-positive representative fatty acids used were i15:0, a15:0, i16:0, and i17:0. The Gram-negative fatty acids used were cy17:0, cy19:0, 16:1ω7c, 16:1ω7t, 18:1ω9c, and 18:1ω9 t (Dungait et al., 2011; Frostegard et al., 1993).

2.7. Data analysis

The normality and variance homogeneity of the variables were tested by the Kolmogorov-Smirnov and Levene tests, respectively. Variables were transformed logarithmically when necessary. The data obtained from the chemical, biochemical, and PLFAs analyses were submitted to two-way ANOVA, to determine the significant effects of the irrigation and fertilization treatments and their interactions on the above mentioned soil parameters. Tukey's test was used to determine the Honestly Significant Differences (HSD) between treatment means ($P < 0.05$).

The procedure of bivariate correlation was carried out to obtain Pearson's correlation coefficients and their significance level, to analyze how the chemical, biochemical, and microbial biomass variables of the soil are correlated. Also, multivariate Factor Analysis (FA) was performed on the relative contents (molar percentages) of the different PLFA biomarkers, to determine the overall effects of crop fertilization and irrigation on the microbial community structure of the soil. All procedures for data analysis were computed using the IBM SPSS 23 statistical package.

3. Results

3.1. Soil chemical properties

The values of soil pH were in the alkaline range, from 8.39 to 8.68 (Table 2). There were no significant effects of the fertilization treatments and irrigation level on soil pH, but a significant interaction between these two factors was observed. The electrical conductivity (EC) of the soil was significantly affected by the fertilization and level of irrigation: an increase was found with the addition of pine bark, ash, and N (BAN treatment). The total N concentration in the soil was significantly affected by the BAN treatment, being increased in comparison to the non-fertilized soil (NF) and the soil receiving commercial fertilization (CF). The irrigation level had no effect on the total N concentration. The abundance of total organic C (TOC) was

Table 2
Values of pH and electrical conductivity (EC) and content of Total N, total organic C (TOC) and water soluble C (WSC) measured in soil of the different treatments.

Fertili-zation	Irriga-tion	p H		EC (μS/cm)		Total N (g/100 g)		TOC (g/100 g)		WSC (mg/kg)	
		Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
NF	low	8.68	*0.03*	149.3	*2.6*	0.11	*0.00*	0.98	*0.25*	42.3	*0.4*
CF	low	8.53	*0.04*	184.7	*8.7*	0.16	*0.02*	1.11	*0.10*	30.1	*0.1*
BAN	low	8.39	*0.03*	244.7	*6.6*	0.20	*0.02*	1.63	*0.15*	36.6	*3.3*
NF	high	8.45	*0.06*	174.3	*7.8*	0.13	*0.01*	1.24	*0.09*	30.5	*0.2*
CF	high	8.50	*0.05*	179.7	*16.2*	0.12	*0.01*	1.01	*0.01*	35.4	*3.5*
BAN	high	8.60	*0.01*	311.7	*16.9*	0.15	*0.03*	2.66	*0.35*	80.6	*5.1*

Two way ANOVA	F	p	F	p	F	p	F	p	F	p
Fertilizat.	1.6	NS	62.9	***	5.3	*	19.7	***	47.2	***
Irrigation	0.2	NS	10.3	**	4.5	NS	6.2	*	28.3	***
Fertili. x Irrigat.	15.9	***	5.3	*	2.9	NS	4.4	*	49.3	***

*p < 0.05; **p < 0.01; ***p < 0.001; NS: not significant.

Table 3
Nutrient and heavy metal contents in the soil of the different treatments assayed.

		Low irrigation			High irrigation			Two-way ANOVA			
		NF	CF	BAN	NF	CF	BAN		Fertili-zation	Irriga-tion	Inter-action
Cd (mg/kg)	Mean	0.03	0.04	0.09	0.09	0.07	0.26	F	17.79	20.92	4.86
	SE	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*0.05*	sig.	***	**	*
Cr (mg/kg)	Mean	13.2	15.9	13.5	14.7	13.5	19.2	F	3.56	4.47	10.00
	SE	*0.5*	*0.8*	*0.5*	*0.6*	*1.0*	*1.6*	sig.	NS	NS	**
Cu (mg/kg)	Mean	4.63	5.65	5.52	5.88	5.37	18.7	F	329.7	363.5	295.9
	SE	*0.19*	*0.24*	*0.22*	*0.26*	*0.31*	*0.5*	sig.	***	***	***
Fe (mg/kg)	Mean	5590	7042	6238	7743	7241	8057	F	0.89	16.82	3.17
	SE	*402*	*312*	*298*	*308*	*745*	*189*	sig.	NS	***	NS
K (g/100 g)	Mean	0.34	0.43	0.41	0.46	0.44	0.60	F	9.67	27.85	6.52
	SE	*0.01*	*0.01*	*0.02*	*0.02*	*0.04*	*0.04*	sig.	**	***	*
Mg (g/100 g)	Mean	0.18	0.17	0.16	0.13	0.13	0.18	F	2.59	8.59	7.58
	SE	*0.02*	*0.01*	*0.01*	*0.01*	*0.00*	*0.00*	sig.	NS	*	**
Mn (mg/kg)	Mean	220	272	234	278	260	341	F	2.35	12.32	0.02
	SE	*21*	*13*	*9*	*3*	*27*	*21*	sig.	NS	**	*
Na (mg/kg)	Mean	457	435	375	365	355	534	F	4.21	0.06	22.57
	SE	*21*	*18*	*16*	*10*	*9*	*38*	sig.	*	NS	***
Ni (mg/kg)	Mean	10.1	11.1	8.34	6.77	6.77	10.3	F	2.41	32.27	33.66
	SE	*0.2*	*0.6*	*0.49*	*0.36*	*0.07*	*0.4*	sig.	NS	***	***
Pb (mg/kg)	Mean	10.9	15.0	12.9	13.7	12.5	23.4	F	31.87	33.32	36.36
	SE	*0.30*	*1.27*	*0.51*	*0.33*	*1.1*	*0.4*	sig.	***	***	***
P (g/100 g)	Mean	0.03	0.03	0.03	0.03	0.03	0.05	F	6.88	3.20	4.36
	SE	*0.00*	*0.00*	*0.00*	*0.01*	*0.00*	*0.00*	sig.	**	NS	*
S (g/100 g)	Mean	0.11	0.13	0.14	0.10	0.10	0.13	F	11.02	14.64	3.38
	SE	*0.01*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	sig.	**	**	NS
Zn (mg/kg)	Mean	12.4	15.2	14.4	16.1	13.9	14.9	F	0.11	1.38	3.45
	SE	*1.2*	*1.1*	*0.5*	*0.7*	*1.2*	*1.0*	sig.	NS	NS	NS

Statistical significance level: *p < 0.05; **p < 0.01; ***p < 0.001; NS: not significant.

influenced significantly by both the fertilization treatment and the irrigation level, the effects of the former being different for LI and HI. For both irrigation regimes, a significant increase in TOC was produced by the addition of BAN to the soil. Significant effects of the fertilization treatment and irrigation level on the water soluble C (WSC) concentration were observed. Differing trends were also found in the WSC in relation to the effect of the fertilization treatment applied, depending on the irrigation level − as evidenced by the significant interaction between the two factors. With LI, the WSC was significantly higher in the NF and BAN soils than in the CF soil; for HI, it was significantly higher for BAN than for CF and NF. Regarding the concentrations of some nutrients and heavy metals in the soil (Table 3), significant increases in the Cd, Cr, Cu, K, Mg, Na, Ni, Pb, and P concentrations were found with BAN addition, with respect to the NF and CF soils, at HI.

3.2. Effects of the treatments on plant biomass

At both levels of irrigation, plant biomass productivity, leaf area index, and crown coverage were lower for the non-fertilized trees than for those fertilized with CF or BAN (Fig. 1). However, there were interactions between the level of irrigation and the fertilization treatment. Thus, with LI, the values of these plant biomass parameters were highest in the CF plots, being significantly higher than in the BAN plots, while the latter values were − in turn − significantly higher than those in the NF plots. With HI, the trend of the values of these parameters in the different treatments was similar, except that there were no significant differences between the NF and BAN plots. No significant effects of the irrigation dose on the plant biomass parameters were observed, except in the non-fertilized plots, where the higher level of irrigation produced a significantly higher (LSD method, $p < 0.05$) plant biomass.

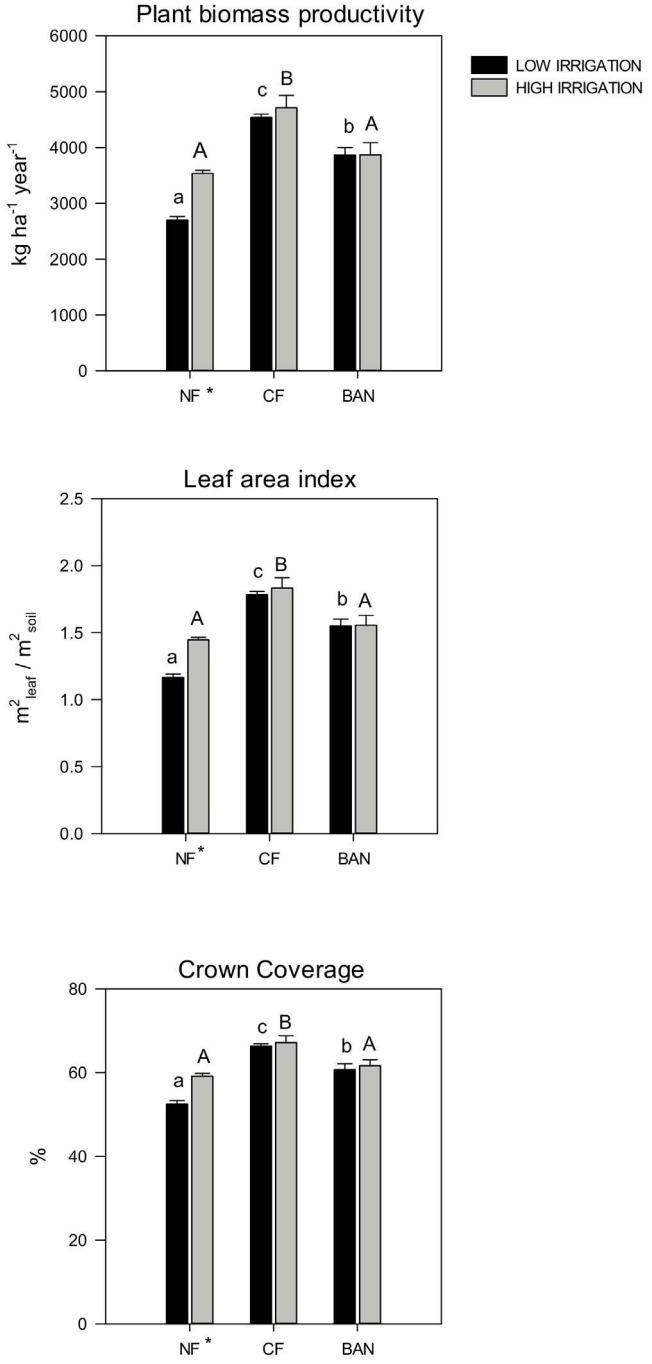

Fig. 1. Plant biomass parameters measured in the different soil treatments assayed in this study. Asterisks under bars denote significant differences between the value registered for the high dose irrigation and those in the low dose, for a given fertilizer treatment. Lower case and capital letters shared by treatments represent no significant differences of the values at P < 0.05, respectively for low and high irrigation. For each mean value, the error bar represents the standard error.

3.3. Soil enzyme activities and microbial respiration

Both the soil enzyme activities and the dehydrogenase activity (DHA) were significantly affected by both the fertilization and irrigation (Table 4). Moreover, a significant interaction between these two factors was observed. The single exception to this trend was the N-acetyl-β-D-glucosaminidase activity (NAG), which was significantly affected by the soil fertilization but not by the irrigation or the interaction of the two factors. For the low irrigation dose, increases

Table 4
Results of two-way ANOVA carried out on data of soil enzyme activities.

Factor	APA	BGA	CBH	NAG	POD	PPO	DHA
Fertilization	***	***	***	***	**	***	***
Irrigation	***	**	**	NS	***	***	*
Interaction	**	***	***	NS	*	***	***

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

in the alkaline phosphatase (APA), β-glucosidase (BGA), cellobiohydrolase (CBH), and NAG activities were observed in the soils with the CF and BAN additions (Fig. 2), these activities being significantly higher in the BAN treatment than for NF and CF. With HI the trends were different. Thus, APA activity was significantly lower with the CF treatment, relative to NF and BAN, and NAG activity was significantly higher in BAN soil than in the NF and CF soils. The CBH and BGA activities did not differ significantly among the fertilization treatments at the high level of irrigation. A significant effect of the irrigation level on BGA activity, independently of the fertilization treatment, was found, but only a positive effect (increase) was observed in the NF soil. Polyphenol oxidase (PPO) activity was significantly higher in soil receiving the CF or BAN treatment, relative to NF soil, for the low irrigation dose, but with high irrigation PPO activity was highest in BAN soil, being significantly higher than in NF. In the latter treatment, in turn, this enzyme activity was significantly higher than with CF. In the case of peroxidase activity (POD), only with HI was it significantly higher in BAN than in the other two treatments. It should be noted that for this high level of irrigation a significant decrease in POD activity was observed in comparison with the low irrigation dose, independently of the applied treatment. The response of dehydrogenase activity (DHA) to the fertilization treatment was different for the low and high irrigation doses (Fig. 3). Thus, for LI, DHA activity was significantly higher in the CF and BAN treatments than in the NF soil, being highest in the BAN treatment; but, at high irrigation, DHA activity was significantly decreased in CF soil, relative to NF and BAN. Regardless of the irrigation level, microbial respiration (MR) was highest for the BAN treatment (Fig. 3). With LI, the SRR in BAN soil was significantly higher than in the other treatments; with HI, the SRR in BAN soil was significantly higher than in CF soil, but did not differ significantly from that in NF soil.

Principal components analysis (PCA) of the EA data extracted two factors which explained 50.43 and 34.69%, respectively, of the data variability. There was a separation of the soil treatments scores in Factor 2 for the low and high irrigation (Fig. 4). However, with LI, the three treatments assayed were separated by their scores in Factor 1. The BGA and CBH activities were highly correlated with Factor 1, while PPO and APA were highly correlated with Factor 2.

3.4. Effects of the irrigation level and fertilization treatments on PLFA biomarkers

Two-way ANOVA of the data detected that, averaged across irrigation levels, there were no significant ($p \geq 0.05$) differences in the concentrations of total PLFAs or PLFA biomarkers of microbial groups between the two fertilization treatments and the control (Table 5). However, averaged across fertilization treatments, there was a significant difference ($p < 0.05$) in the total PLFAs and the Gram-positive, fungal, and saturated PLFAs concentrations between the irrigation levels. Moreover, significant interactions between the treatment and irrigation factors were observed. For each irrigation level, there was a significant effect of the fertilization treatment on the total PLFAs concentration (Fig. 5). Thus, the total PLFAs abundance was highest in NF soil with the higher irrigation level, being significantly greater than in the soil where CF was applied, but not significantly different from the BAN treatment. With LI the total PLFAs concentration in CF soil was significantly higher than in

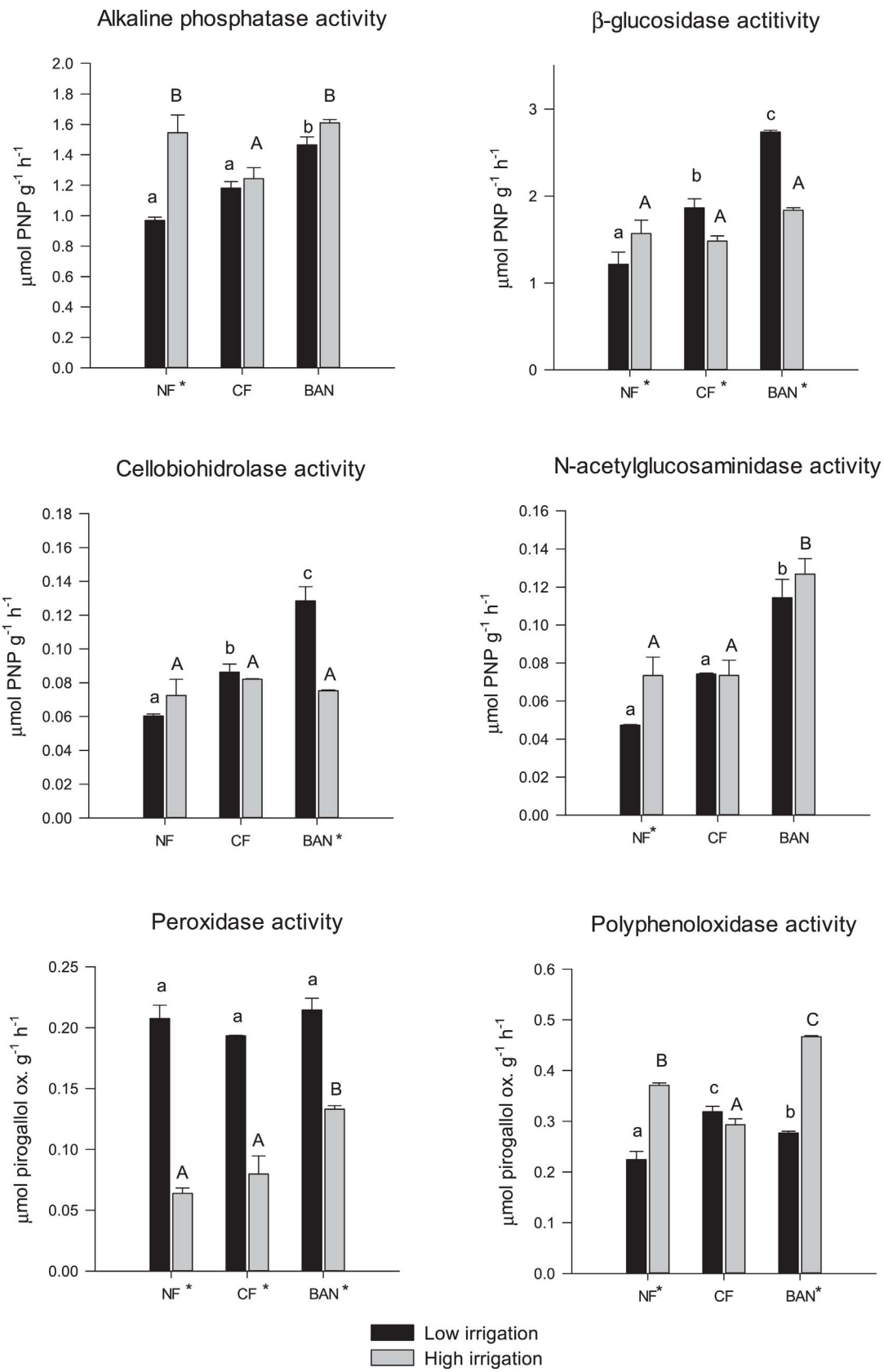

Fig. 2. Enzyme activities detected in the different soil treatments assayed in this study. Asterisks under bars denote significant differences between the value registered for the high dose irrigation and those in the low dose, for a given fertilizer treatment. Lower case and capital letters shared by treatments represent no significant differences of the values at $P < 0.05$, respectively for low and high irrigation. For each mean value, the error bar represents the standard error.

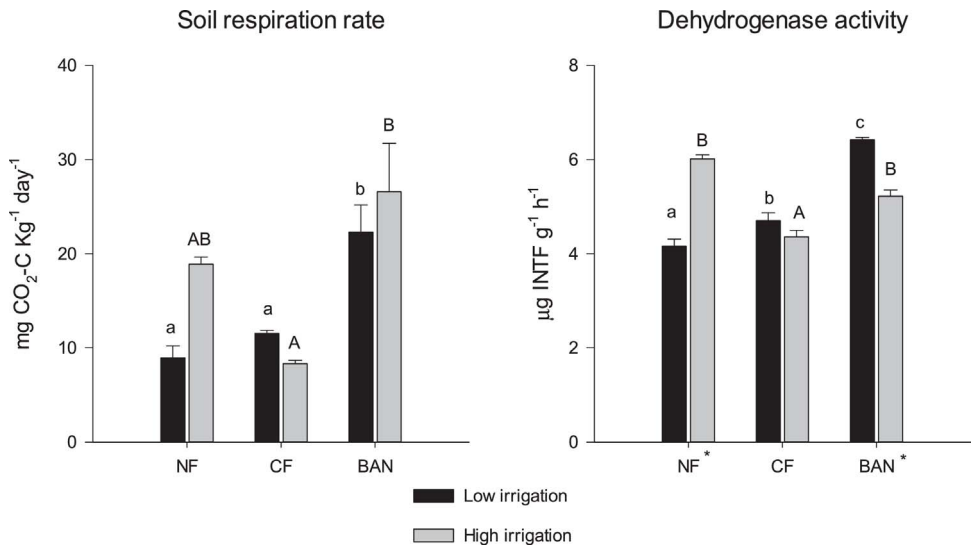

Fig. 3. Soil dehydrogenase activity and respiration rate registered in the different soil treatments assayed in this study. Asterisks under bars denote significant differences between the value registered for the high dose irrigation and those in the low dose, for a given fertilizer treatment. Lower case and capital letters shared by treatments represent no significant differences of the values at $P < 0.05$, respectively for low and high irrigation. For each mean value, the error bar represents the standard error.

NF soil, but did not differ significantly from that of the BAN soil. The irrigation dose significantly affected the total PLFAs concentration only in the NF soil; it increased with the amount of water applied. The absolute concentrations of bacterial PLFAs followed patterns similar to that of the total PLFAs, according to the treatment, for both irrigation levels. The fungal PLFAs followed a similar trend in relation to the irrigation factor, but there was not a significant effect of the fertilizer treatment. There was a significant increase in fungal PLFA abundance with the dose of water added to NF soil. The Gram-positive PLFAs exhibited a similar trend in relation to the treatments. Thus, their abundance was highest in NF soil receiving high irrigation, being significantly higher than in CF and BAN; but, for low irrigation, their abundance was significantly higher in CF than in NF and BAN. In the NF and CF soils there was a significant increase and decrease, respectively, in the abundance of these PLFA biomarkers due to the effect of the irrigation level. In contrast, the Gram-negative PLFAs concentration was not significantly affected by the treatment or irrigation. The abundance of actinobacterial PLFAs was highest in the NF soil with greater irrigation, being significantly different from that in the BAN soil, but there were no significant differences among treatments for the low irrigation level. Two-way ANOVA detected that, averaged across irrigation levels, the Gram-positive to Gram-negative (G+/G-), fungal to bacterial (F/B), saturated to monounsaturated (S/M), and *iso* to *anteiso* 15:0 (i/a 15:0) PLFA ratios were not significantly affected by the treatment (Table 5). Averaged across fertilization treatments, the G+/G-, S/M, and i/a 15:0 ratios were significantly affected by the irrigation level. Significant interactions between the two factors were observed for G+/G- and S/M.

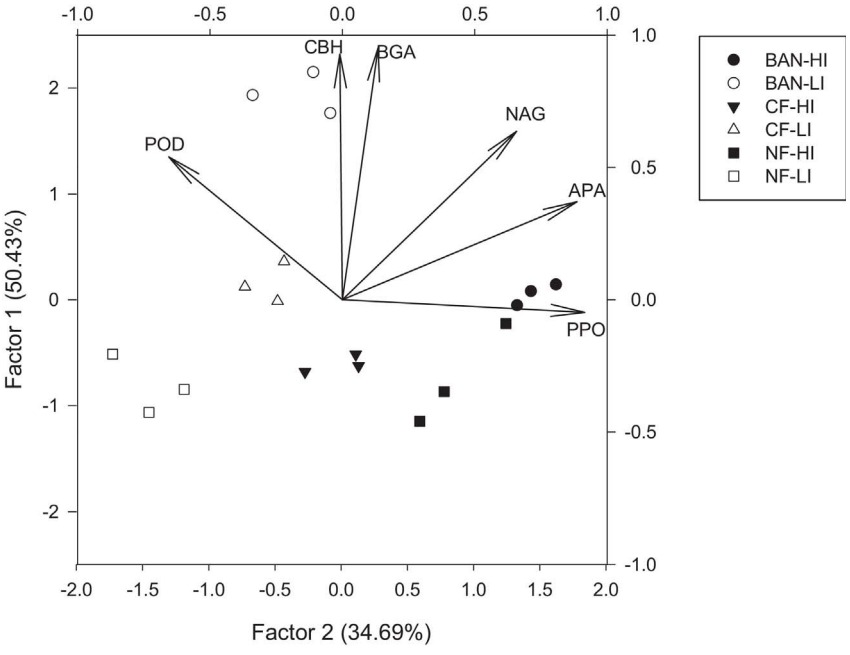

Fig. 4. Bi-plot showing similarities or differences between the assayed treatments according to the Factorial Analysis performed on the measured values of enzyme activities. Vectors represent the main correlations of these variables with factors 1 and 2. Values in brackets denote the percentage of the data variation explained for a given factor.

Table 5
Results of two-way ANOVA carried out on the concentrations of total PLFA and biomarker PLFAs.

PLFA biomarker	Fertilization		Irrigation		Fertiliz. x irrigation	
	F	Sig.	F	Sig.	F	Sig.
Gram_posit	1.47	NS	11.80	**	20.28	***
Gram_negat	0.53	NS	0.30	NS	3.18	NS
Fungi	0.46	NS	6.06	*	4.19	*
Bacteria	0.50	NS	4.61	NS	11.19	**
Saturated	0.89	NS	10.29	*	20.57	***
Monounsatur	0.72	NS	0.42	NS	2.52	NS
Actinobacterias	3.80	NS	0.39	NS	3.88	*
Total PLFAs	0.51	NS	6.07	*	13.20	**
G+/G-	3.03	NS	22.49	***	20.88	***
Fungi/Bact	0.08	NS	0.33	NS	0.08	NS
i/a 15:0	1.80	NS	6.12	*	0.43	NS
Sat/monosat	2.68	NS	14.34	**	18.15	***

***: $p < 0.001$; **: $p < 0.005$; *: $p < 0.05$.

3.5. Effects of the irrigation level and fertilization treatments on the microbial community structure

Shifts of the soil microbial community structure under the *Paulownia* plantation due to the effects of the fertilization treatment and irrigation level were observed in the two-dimensional ordination plot using FA of PLFAs, which contributed with a relative abundance higher than 1 mol% (Fig. 6). This PCA extracted two factors which, respectively, explained 29.1 and 23.5% of the data variation. Soil samples were separated along the Factor 1 axis; in particular, samples of BAN soil receiving high irrigation were separated from the rest on the basis of their scores in both factors. However, for the other samples, there was not a clear separation on the basis of fertilization treatment or irrigation level. Correlations of individual PLFAs with Factors 1 and 2 are plotted in Fig. 6. The relative abundances of 18:2ω6,9 and 10Me16:0 were positively and highly correlated with Factor 1, while a:15:0 (positively) and 15:0 (negatively) were highly correlated with Factor 2.

4. Discussion

4.1. Effects of fertilization and irrigation on soil chemical parameters and microbial activity

The amendment of the soil under a *Paulownia* plantation with BAN, which contains ashes from wood residues, produced an increase in the EC and in the concentrations of most macro and micronutrients and some heavy metals. Similar results were observed by Augusto et al. (2008), who quantified the effect of wood ash on the nutrient concentrations of a wide range of forest soils using a meta-analysis of data. The positive influence on soil chemical parameters of ash spreading under tree plantations was also reported by Marron (2015). However, it is necessary to note that with ash addition to soil there is a slight risk of increasing the total concentrations of some heavy metals, as was detected in our study. However, the alkaline pH of the tested soil prevents high bioavailability of these heavy metals (Kabata-Pendias and Mukherjee, 2007; Koptsik, 2014). Specifically for total N, there was a significant effect of the BAN treatment; its concentration was increased by the N-rich fertilizer (Nitroacid) added in this treatment. Also, the TOC concentration in the soil increased with the application of BAN. This can be explained by the high organic C content of the wood residues used in this soil amendment. Moreover, BAN induced higher MR in soil receiving greater irrigation. This might be related to the degradation of organic C compounds in the BAN residues and the incorporation of the products into the water soluble pool of soil organic matter, as reflected by the higher WSC concentration in this soil. Also, this finding may be a consequence of the higher β − glucosidase (BGA) activity in this soil. In fact, positive and significant ($p < 0.05$) correlations was observed between BGA and SR and also between SR and WSC (Supplementary data, Table S1). Hassan (2013) indicated that the level and status of SOM were affected by the quality and type of the organic inputs, with different C:N ratios and lignin contents. The fact that the TOC and WSC concentrations were greatest in soil receiving the BAN treatment and high irrigation dose was additionally due to the incorporation of organic C from the litter layer. A similar trend of this soil parameter was observed by Madejon et al. (2016), in an experiment where compost was added to the soil under a *Paulownia* plantation. The WSC represents the most active and dynamic C fraction of soil. Thus, the increase in soil organic C as a consequence of the BAN treatment is closely related to the overall improvement of the biological activity in this soil.

Enzyme activities have been proposed as indicators of the biological quality of the soil under forest plantations (Lucas-Borja et al., 2012). Application of BAN positively influenced all the hydrolytic enzyme activities under the low irrigation dose of the *Paulownia* trees. This stimulation of soil enzyme activities with compost amendment in a *Paulownia* plantation was also observed by Madejon et al. (2016). However, no clear effect of the irrigation dose on the activities of this kind of enzyme was observed in our experiment, and this effect depended on the treatment applied. Some authors have supposed that soil moisture determines the rates at which enzymes, substrates, and reaction products diffuse (Burns et al., 2013). Nevertheless, other factors (such as the enzyme substrate or inhibitors content) more important than the soil moisture should be kept in mind to explain enzyme activity trends. This fact can be confirmed by the significant correlations found between some hydrolases (APA, NAG and PPO) and TOC content (Supplementary data, Table S1). In addition, we detected a significant and positive correlation between soil enzymes activities (APA, BGA, NAG and PPO) and microbial activity measured by soil respiration and dehydrogenase activity (Supplementary data, Table S1). Hydrolytic enzyme activities have a key role in organic matter degradation and the production of bioavailable forms of nutrients such as C, N, and P (Allison and Vitousek, 2005; Burns et al., 2013). These available forms of nutrients are useful for both the growth of *Paulownia* and the soil microbial community under its plantation.

Polyphenol oxidase and peroxidase activities catalyze the oxidation of the most recalcitrant organic matter (lignocellulosic compounds) to produce precursors of humic substances, or other metabolites which then undergo mineralization to finally yield CO_2 (Sinsabaugh, 2010). The dehydrogenases are primarily intracellular oxidative enzymes that transfer hydrogen from organic substrates to electron acceptors such as NAD^+, and they are very suitable indicators of soil microbial activity and quality (Pascual et al., 2000). Pine bark, added in the BAN treatment, is fundamentally composed of lignocellulosic compounds; hence, the application of the BAN to the soil contributed to increasing the amount of specific substrates of these oxido-reductases. The net effect of these phenol oxidases is the partial degradation of lignocellulosic compounds and other phenolic compounds, and a small fraction of this OM input may accumulate as humic substances or be exported as WSC. The WSC is the most bioavailable C fraction and within the cells of microorganisms is oxidized by dehydrogenase activity, to provide energy.

The multivariate analysis confirmed that the fertilization treatment and irrigation affected the EA of the soil under the *Paulownia* plantation, BGA, CBH, and PPO being the most important activities that explain the variability of the data. Thus, the fertilization and irrigation treatments can potentially alter C-cyling in the soil of this *Paulownia* forestry plantation.

In the case of soil microbial respiration, only the fertilization factor had a significant effect, and the BAN had a positive influence on the microbial mineralization of SOC. In a similar experiment, Epron et al. (2004) observed greater respiration in the soil to which a higher

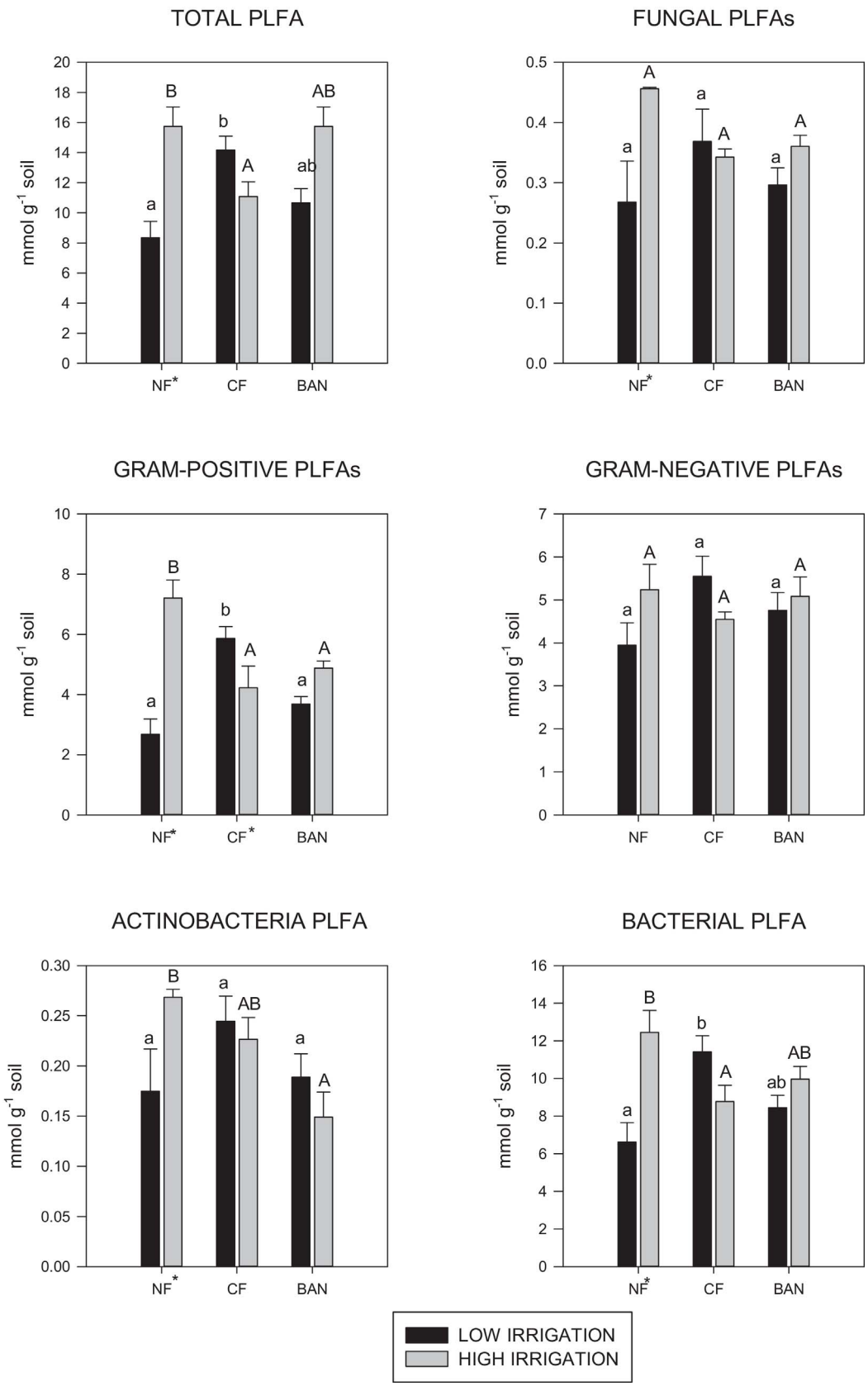

Fig. 5. Abundances of total PLFAs and PLFA biomarkers of the main microbial groups registered in the different treatments assayed in this study. Asterisks under bars denote significant differences between the value registered for the high dose irrigation and those in the low dose, for a given fertilizer treatment. Lower case and capital letters shared by treatments represent no significant differences of the values at $P < 0.05$, respectively for low and high irrigation. For each mean value, the error bar represents the standard error.

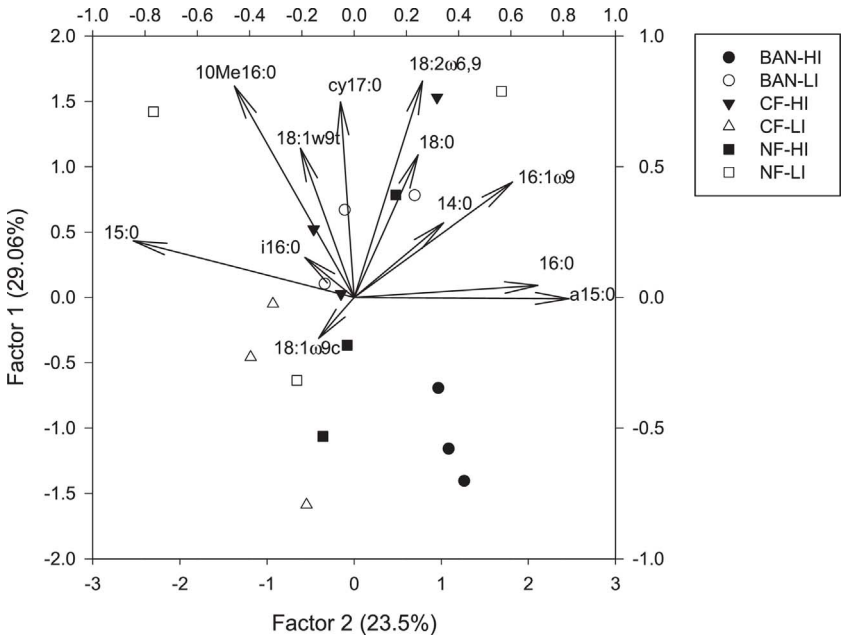

Fig. 6. Bi-plot showing the ordination of soil samples according to the factorial analysis of their relative concentrations of the different PLFA biomarkers. Vectors represent the correlations of every PLFA biomarker with factors 1 and 2. Values in brackets denote the percentage of the data variation explained for a given factor.

amount of plant detritus was added, and these researchers reported that the soil respiration was positively correlated with the total above-ground litter (leaf, bark, and woody debris).

4.2. Effects of the irrigation and fertilization treatments on the biomass and structure of the soil microbial community

Overall, the abundance of the PLFA biomarkers did not correlate with the hydrolase activity measured in the soil. However a negative and significant correlation was detected between APA and the total microbial biomass (Table S1). As well as negative and significant correlation was found between POD and fungi PLFA. In the case of PPO activity, we observed a negative and significant correlation with total microbial biomass; but a positive and significant correlation was observed with fungal and bacterial biomass. Sinsabaugh (2010) reported that the fungi and bacteria synthesize and release phenol oxidases in the environment in order to degrade phenol molecules.This suggests that biomass and functionality are not directly linked. Indeed, changes in community structure and composition, more than community biomass, have been associated with changes in microbial functionality (Nielsen et al., 2012). However, the F/B ratio was not influenced by the irrigation or the fertilization treatment. This indicates that agro-forestry management has a higher impact on soil hydrolases activities than on the composition and biomass of the microbial community. It can be argued that agro-forestry management modifies more the extracellular environment – that directly determines the activity of enzymes (i.e. humus-enzyme complexes) – without direct consequences for the microbial biomass (Nannipieri et al., 2012).

The fertilization treatments did not have a significant effect on the abundances of PLFA biomarkers, whereas the dose of water added did so for the total, Gram-positive, and fungal PLFAs. The microbial biomass was greater in the unfertilized soil receiving the high dose of irrigation than in the fertilized soils (CF or BAN). It is interesting to look at the relationships between the biomass and the activity of the community since these two concepts are not always fully connected. The microbial community was more active due to the BAN treatment, as the respiration and EA in this treatment reflected (higher C mineralization efficiency) – which resulted in a lack of correlation between the total PLFAs and the respiration rate (Table S1). Also, this indicates that the amount of C used by microbial cells to obtain energy was higher than for biomass production. The immobilization of enzymes within humic substances might be an important mechanism sustaining the higher activity of some enzymes (i.e. phosphatase, NAG, CBH, etc.) in BAN soil, despite the absence of an increase in biomass.

The ratio of the saturated and monounsaturated PLFAs (S/M) has been used as a bioindicator of C bioavailability (Zelles et al., 1992, 1995) and stress (Hinojosa et al., 2005) in the soil. High S/M ratios have been related to reductions in the microbial growth rate due to nutrient limitations (García-Sanchez et al., 2015). Bossio and Scow (1998) found that the S/M ratio increased with soil flooding. In our study the S/M ratio was highest (3.11) in the NF soil with a high dose of irrigation and lowest (1.75) in the NF soil with a low dose of irrigation. As Bossio and Scow (1998) reported, this increase in the S/M ratio with the dose of water added to soil indicates a change in the aerobic or anaerobic microbial abundance. The iso to anteiso 15:0 ratio was significantly decreased by the BAN treatment (values of 1.09 and 0.01, respectively, for LI and HI) relative to the control values (2.04 and 0.87). Garcia-Sanchez et al. (2015) and Chen et al. (2013) also reported that a high ratio of iso/anteiso PLFAs is indicative of nutrient deficiency in soil.

PLFA biomarkers such as 18:2ω6,9, 10Me16:0, i15:0, and a15:0 had greater importance with regard to differentiating soil samples according to the microbial community structure. However, the differences in the microbial community structure of the soil under the *Paulownia* plantation cannot be clearly explained by the level of irrigation or the fertilization treatment applied to this soil.

5. Conclusions

This study provides evidence that the fertilization treatment and the level of irrigation had a more significant influence on the soil microbial activity than on the biomass and structure of the soil microbial community under a *Paulownia* plantation. The EA – such as those of BGA, CBH, and PPO – were the most sensitive indicators of soil management (fertilization and irrigation level), thus showing that these two factors influenced SOC transformations (C mineralization and sequestration).

In general, the use of residues generated in the forest biomass industry (pine bark and biomass ash) as a soil amendment had a positive effect on the soil microbial activity, without altering the

structure of the soil microbial community. These residues may constitute an alternative to mineral fertilizers, to promote soil sustainability in agro-ecosystems of short-rotation tree species, such as *Paulownia* plantations.

However, the level of irrigation altered the biomass of the microbial community and the abundance of some PLFA biomarkers in soil that did not receive any fertilization. Considering the structural water deficit in SE Spain, it is worth concluding that the lower level of irrigation ($1750\ m^3\ ha^{-1}$) used here is suitable for maintaining both the soil microbial activity and suitable production of biomass by *Paulownia* trees.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2017.05.001

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