Changes in soil quality following poplar short-rotation forestry under different cutting cycles

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Abstract

In the last decade, the change of energy concept induced by global warming and fossil fuel depletion together with the advances in agriculture towards a multifunctional and a more sustainable use of rural areas promoted the development of biomass crops. In this regard, *Populus* is largely utilised in short-rotation forestry (SRF), as it is known to be a fast-growing tree, producing large yields and having a high energy potential. Most studies focused on economic-productive and energetic aspects of *Populus* plantations, whereas their impact on soil quality and health have been poorly investigated. In this study, the main soil chemical parameters, microbial biomass and activity were assessed aiming at evaluating the impact of *Populus* SRF under one, two and three-year cutting cycles (T1, T2 and T3) in comparison with an intensive food cropping system (wheat-soybean rotation, WS). In addition, arbuscular mycorrhizal (AM) fungal inoculum potential was measured using root colonisation (RC) and number of entry points (EP). In the 0-10 cm soil depth, pH, phosphorus (P), total nitrogen (N) and soil organic carbon (SOC) were significantly affected by the management. In comparison with WS, *Populus* SRF treatments produced significant pH decreases together with N and SOC increases, these last ones ranging from 11% to 34% and from 21% to 57%, respectively. Under T3 soil pH decreased of 0.25 units, while P, N and SOC increased of 10%, 34% and 57%, respectively, in comparison with WS. Microbial biomass and soil respiration under SRF showed also mean increases of 71% and 17%, respectively. Under SRF treatments, *Lolium perenne*, commonly observed in all field plots, was more than twofold colonised by AM fungi in comparison with WS, while the number of EP, observed on *Lactuca sativa* used as a test plant, showed values ranging from 8 to 21 times higher. The present study shows the potential of a *Populus* SRF to improve soil chemical, biochemical and biological quality parameters in comparison with an intensive food cropping system.

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Introduction

In the last years, the concept of multifunctional agriculture has obtained large attention from both scientists and policy makers due to its production of both agricultural commodities and ecological services. Such multifunctionality has been focused on an accurate revision of the management of rural areas and of conventional cropping systems both as process and as product (Renting *et al*., 2009). In this regard, bioenergy crops have recently gained great interest as a potential alternative to agri-food productions and as a clean and renewable energy source reducing greenhouse gas emissions (Wise *et al*., 2009; Popp *et al*., 2010). Within biomass crops, *Miscantus* and *Panicum*, which are perennial rhizomatous grasses, and fast-growing trees, as *Eucalyptus*, *Populus* and *Salix*, are grown worldwide for such bioenergy purposes (Bonari *et al*., 2004b; Tilman *et al*., 2006; Karp and Schield, 2008).

Traditionally, *Populus* (poplar) breeding has achieved large success for wood production in short-rotation forestry (SRF) due to the fact that the hybrids of poplar have a fast growth and produce a high yield, although they were not initially selected for growing as coppice (Bonari *et al*., 2004 a,b; Karp and Schield, 2008). Moreover, many poplar clones and species showed to have a high energy potential and to be able to grow in marginal lands and drought conditions (Hansen, 1991; Makeschin, 1994). Many studies have been performed on biomass productivity and quality, management intensity, economic balance and energy aspects of poplar SRF (Yue *et al*., 1999; Bonari *et al*., 2004 a,b; Vande Walle *et al*., 2007; Karp and Schield, 2008; Lemus *et al*., 2008; Guidi *et al*., 2008, 2009; Nassi o Di Nasso, 2010).

So far, less attention has been focused on the evaluation of soil quality changes under SRF management (Tolbert *et al*., 2002; Kahle *et al*., 2007; Zornoza *et al*., 2009; Mao and Zeng, 2010), while many studies were performed on the impact of alternative cropping systems, such as the organic and biodynamic farming, in comparison with high- and low-input conventional managements (Wood and Edwards, 1992; Schjønning *et al*., 2002; Hamer *et al*., 2008; Lagomarsino *et al*., 2009; Mazzoncini *et al*., 2010). As regard to soil quality evaluation, Doran and Parkin (1996) proposed a minimum data set of sensitive physical, chemical and biological indicators. Firstly, most studies on soil quality changes under alternative farming utilised chemical and biochemical indicators (Dick, 1983; Kingery *et al*., 1996; Omay *et al*., 1997; Carter, 2002), then the biological parameters became more and more important in evaluating such changes (Saviozzi *et al*., 2001; Bending *et al*., 2004; Parisi *et al*., 2005; Piotrowski and Rillig, 2008; Mazzoncini *et al*., 2010). Similarly, evaluating soil quality and health under SRF or following the conversion from food to biomass crops, physical and chemical indicators were largely used (Sartori *et al*., 2006; Berthrong *et al*., 2009; Laganière *et al*., 2010), while recently the attention has been also focused on the biochemical and biological parameters (Makeschin *et al*., 1994; Guo and Han, 2008; Zornoza *et al*., 2009; Kahle *et al*., 2007; 2010; Mao and Zeng, 2010).

The SRF management is associated with minimal mechanical disturbance of the soil and less agrochemical inputs in comparison with conventional cropping systems (Lemus and Lal, 2005; Dickmann, 2006). This, together with the leaf litterfall of deciduous trees, is likely to promote the increase of soil organic carbon (SOC), nitrogen (N) and phosphorus (P) content, as well as of soil microbial biomas (Lal, 2003; Liebig *et al*., 2005; Ritter, 2007; Iovieno *et al*., 2010). One of the fundamental components of microbial biomass, which might be affected by SRF, is represented by arbuscular mycorrhizal fungi (AMF) (Rooney *et al*., 2009). AMF are mutualistic associations between the roots of the majority of plant species and soil borne fungi belonging to Glomeromycota (Schüβler *et al*., 2001; Smith and Read, 2008). In response to such symbiosis, bioenergy crops could in turn benefit by an increased biomass yield and a greater cropping resistance (Rooney *et al*., 2009), since AMF are largely known to have a fundamental role in plant nutrition and protection against root and shoot pathogens (Smith and Read, 2008).

The aim of the present study was to evaluate the impact on soil quality of a bioenergy crop management, represented by a SRF poplar plantation under different coppicing frequencies, in comparison with an intensive food cropping system based on a wheat-soybean rotation.

Materials and Methods

Field site and experimental set-up

A long-term poplar (*Populus deltoides* Bartr.) SRF field experiment was started in 1996 at the "Enrico Avanzi" Interdepartmental Centre for Agro-Environmental Research of the University of Pisa (43°40' N lat; 10°19' E long), Italy. Before experimental set-up, the field site was conventionally cultivated with maize (*Zea mays* L.) - durum wheat (*Triticum durum* Desf.) rotation for more than 15 years. The soil showed the following physical and chemical characteristics: clay, 20.1%; silt, 40.5%; sand, 39.4%; available P, 8.8 mg Kg^{-1}; total N, 1.3 g Kg^{-1}; organic carbon, 10.4 g Kg^{-1}. Climatic conditions were typically Mediterranean. More details on climate conditions are given by Mazzoncini *et al*. (2008). The experiment was a completely randomised design, i.e. one, two and three - year cutting cycles (T1, T2 and T3), with three treatments and three replicates (n=3; plots of 500 m^2). Details on the poplar stands and their management are given by Nassi o Di Nasso *et al*. (2010). In addition, an adjacent intensively tilled (ploughing to 30 cm depth) wheat-soybean rotation (WS), showing similar physical and chemical characteristics in 1996, was selected and used as control. Details on the wheat-soybean experimental design and its management are given by Mazzoncini *et al*. (2008).

Soil and root sampling

In the spring of 2005, one combined soil sample, obtained by mixing three random soil cores, was collected (0-10 cm depth) from each plot and from three random areas within the WS rotation. The soil samples utilised for biochemical analyses were sieved through 2 mm sieve at the field moisture, whereas the samples used for chemical and AMF analyses were oven dried at 30°C before sieving. The root systems of perennial ryegrass (*Lolium perenne* L.), a common weed found in all the plots, known to be highly responsive to a wide range of AMF, were collected (one combined root sample per each SRF plot and WS area) at a depth of 15 cm and then rinsed and dried (70°C for 3 days).

Analytical procedures

Soil samples were analysed for pH, available P, total N, SOC, microbial biomass carbon (MBC) and soil respiration (SR). Soil pH was measured in deionised water (1:2.5 w/v) (McLean, 1982) and P and N were determined by colorimetry using the Olsen method (Olsen and Sommers, 1982) and by the macro Kjeldahl digestion procedure (Bremner and Mulvaney, 1982), respectively. SOC was evaluated using the modified Walkley-Black wet combustion method (Nelson and Sommers, 1982). MBC was determined by the Vance chloroform fumigation-extraction method, while SR was measured according to the Isermeyer method, described in Alef and Nannipieri (1995). MBC and SR were assessed on soil subsamples of 45 g and SR was determined after 10 days of incubation in closed jars maintained at 25°C. The percentage of AMF colonisation was determined by the gridline-intersect method (Giovannetti and Mosse, 1980) after clearing and staining the roots according to Phillips and Hayman (1970), using lactic acid instead of phenol. AMF infectivity was assessed using the mycorrhizal inoculum potential test (MIP) (Pellegrino *et al*., 2011) on lettuce (*Lactuca sativa* L.): three seedlings were grown for two weeks in 50 mL sterilised plastic tubes filled with 40 mL of soil obtained by each replicate plot (n=6). Lettuce root system was stained as described above, mounted on microscopic slides and examined under a Reichert-Jung (Vienna, Austria) Polyvar light microscope. The number of entry points (EP) was assessed at a magnification of 125-500x and of 1250x.

Statistical analyses

The soil quality parameters were expressed as percentage of variation in comparison with their values under the intensive wheat management (WS), used as control. For pH values, considering their logarithmic scale, we expressed the variation in units. Data were compared using a one-way (management as factor) analysis of variance (ANOVA). Data were ln- and arcsin-transformed when needed to fulfil the assumptions of ANOVA, which was carried out according to a completely randomised design. The Tukey-B procedure was used to means comparison. Soil chemical parameters showed neither a normal distribution of error terms nor constant error variance, therefore a non-parametric ANOVA was required. In this case, we used the Kruskal-Wallis test and the Mann-Whitney *U*-test as *post-hoc*. All statistics were performed with the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Ordination analysis (Redundancy Analysis, RDA) was carried out in Canoco for Windows v. 4.5 (ter Braak and Šmilauer, 2002) in order to investigate the influence of the management (used as explanatory variable) on the soil quality parameters (used as response variables). Additionally, Monte-Carlo permutation test was conducted using 499 random permutations in order to determine the statistical significance.

Results and Discussion

Chemical parameters

In the 0-10 cm soil depth, pH, P, N and SOC were significantly affected by the management (Figure 1). Soil pH, calculated as units of variation in comparison with the value under WS, ranged from -0.26 to -0.13 units in T3 and T1, respectively (Figure 1a). All the poplar SRF treatments produced significant pH decreases in comparison with WS and, within poplar, soil pH significantly decreased from

T1 to T3 (Figure 1a). In a recent meta-analysis, Berthrong *et al*. (2009) reported that *Eucalyptus* and *Pinus* plantations induced a strong and moderate acidification, respectively. A general decrease of pH was also observed in afforestations of a never-tilled soil or grasslands (Ross *et al*., 1999; Chen *et al*., 2000; Sartori *et al*., 2007). Consistently, Guo and Han (2008) reported significant decreases of pH at 0-10 cm and at 10-20 cm soil depth due to soil use conversion based on a 50-year-old *Populus davidiana* plantation. On the contrary, no changes in soil pH were revealed under *Salix* and *Populus* stands in comparison with arable land by Kahle *et al*. (2007, 2010). The reduction of soil pH has been suggested to be related to the higher organic or carbonic acid production, the latter due to an increased autotrophic respiration (Richter and Markewitz, 1995) and to the influence of tree root system on level of ground water and cation uptakes (Attiwell and Adams, 1993; Jobbágy and Jackson, 2003). Here, the differences in acidification among the different SRF treatments may be due to the larger production of tree root biomass, under less frequent cutting cycles, that may release a higher number of H^+ ions (Attiwell and Adams, 1993).

The variation of soil available P ranged from -17% to 10% in T1 and T3, respectively (Figure 1b). Significant soil P changes were observed between T1-T2 in comparison with WS and within poplar SRF, T3 showed a significant soil P increase, in comparison with T1 and T2. Some authors reported increases of soil P due to the afforestation with different tree species in comparison with grasslands or agricultural soils (Ritter, 2007; Zornoza *et al*., 2009), while some others observed higher P contents or no changes in arable lands or pastures than in adjacent forests (Koerner *et al*., 1997; Ross *et al*., 1999; Chen *et al*. 2000; Zhao *et al*., 2007). Such contrasting results might be due to variables influencing P dynamics, which may be associated with SOC changes as reported by Piccolo *et al*. (1996), and to previous land-use, time since land-use conversion, tree species planted and climatic conditions (Ross *et al*., 1999; Ritter, 2007; Zhao *et al*., 2007).

The total soil N variations ranged from 11% to 34% in T2 and T3, respectively (Figure 1c). All poplar SRF showed significant soil N increases in comparison with WS and, within such treatments, T3 produced a significantly higher increase than T1-T2 (Figure 1c). Consistently with our data, in other studies soil N concentration in the shallow layer was lower in agricultural lands than in *Betula* and *Larix* thinned closed canopy plantations and in *Populus* stands (Ritter *et al*., 2007; Sartori *et al*., 2007). Lower soil N was also detected in agricultural lands than in deciduous forests by Morris *et al*. (2007). By contrast, soil N decreases were reported under *Pinus* and *Eucalyptus* afforestations (Binkley and Resh, 1999; Berthrong *et al*., 2009).

The change of SOC due to the different treatments in comparison with WS ranged from 21% to 57% in T1 and T3, respectively (Figure 1d). A significant increase was observed under all poplar SRF treatments and within poplar stands, SOC under T3 was significantly higher than under T1 and T2 (Figure 1d). The increase of SOC under SRF may result in positive changes of soil structure, water retention, nutrient availability, biological diversity and C sequestration, since it is well known to affect directly or indirectly the overall soil quality parameters (Schjønning *et al*., 2004). Here, SOC showed a pattern similar to the soil N concentration as reported in other studies (Franzluebbers and Stuedemann, 2009; Yao *et al*., 2010). Such similarity between SOC and N patterns was previously explained by carbon inputs from plant production and outputs through microbial decomposition (Gill *et al*., 1999). Along with our results, several studies observed SOC increases due to the afforestation of agricultural soils (Park *et al*., 1994; Grigal and Berguson, 1998; Tolbert *et al*., 2002; Kahle *et al*., 2007; Laganière *et al*., 2010), while some others reported no changes or lower SOC concentration under forest than under adjacent grassland (Berthrong *et al*., 2009; Chen *et al*., 2010;

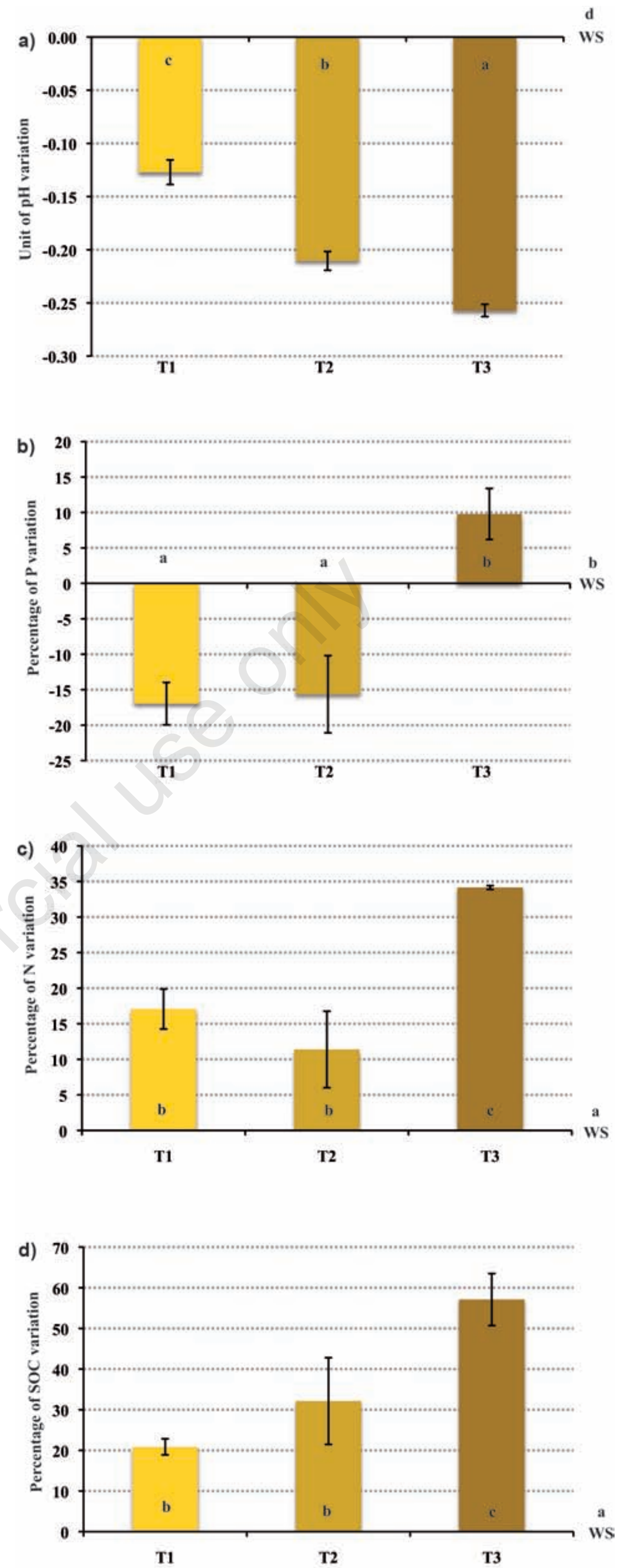

Figure 1. Soil pH a), available phosphorus (P); b), total nitrogen (N); c) and organic carbon (SOC); d) under poplar short-rotation forestry (one, two and three-year cutting cycles: T1, T2 and T3). The values are expressed as unit or percentage of variation in comparison with their values under a wheat-soybean rotation (WS). Different letters indicate significant differences as tested by the Kruskal-Wallis test (P≤0.05) and the Mann-Whitney *U*-test as *post-hoc* (P≤0.05).

Mao and Zeng, 2010). Climate, soil type, management, land use and time since land use conversion may explain these contrasting results (Paul *et al*., 2002; Laganière *et al*., 2010).

Biochemical parameters

Most studies have used biochemical indicators, such as the MBC and the SR, aiming to evaluate the impact of different managements on soil quality (Haynes, 1999; Dilly and Nannipieri, 2001; Lagomarsino *et al*., 2009; Iovieno *et al*., 2010), while under SRF such parameters have been less investigated (Guo and Han, 2008; Mao and Zeng, 2010). Here, MBC and SR showed to be significantly affected by the management (Figure 2). MBC was significantly increased by all poplar SRF treatments compared with WS (Figure 2a), showing variations from 43% to 93% (T1 and T3, respectively). Our results are in agreement with other studies evaluating the effects of agricultural land conversion to *Populus*, *Quercus* and *Salix* plantations on MBC (Makeschin, 1994; Zornoza *et al*., 2009; Kahle *et al*., 2010; Mao and Zeng, 2010). By contrast, other authors showed no changes or significant decreases of MBC under *Pinus* stands (Chen *et al*., 2000; Macdonald *et al*., 2009). The MBC increases observed here and in other studies may be explained by the increase in carbon available for microorganisms derived from rhizodeposition and from the high-quality litter of *Salicaceae* and *Fagaceae*, while the lower soil MBC under *Pinus* afforestation, as compared to the soil under the climax vegetation, was attributed to the low-quality litter of pine needle litter by Iovieno *et al*. (2010). In addition, the mean decrease of number of live bacteria in the soils amended with *Pinus* in comparison with *Quercus* observed by Grenni *et al*. (2009) may contribute to explain the differences of MBC changes commonly reported between *Pinus* and other trees.

SR percentages of variation ranged from 8% to 25% in T1 and T3, respectively (Figure 2b). SR values under T2 and T3 were significantly higher than that under WS and, within the different cutting cycles, T2-T3 and T1 produced significantly different effects on SR (T2-T3>T1) (Figure 2b). According to our data, Zornoza *et al*. (2009), studying the impact of different land use, observed higher values of SR under forest than under abandoned and agricultural systems. The SR pattern, similar to the MBC one, may be explained by the higher quantity and different quality of litter under the tree stands in comparisons with herbaceous-based systems (Singh and Singh, 1995; Chen *et al*., 2000).

Arbuscular mycorrhizal fungi measurements

L. perenne, the common plant species found in all the plots, showed root colonisation (RC) changes ranging from 141% to 170% in T1 and T2, respectively (Figure 3a). *L. perenne* grown under WS was significantly less colonised by AMF than that grown under poplar SRF and, within the different cutting cycles, the root colonisation under T1 and T3 was significantly lower than that under T2 (Figure 3a). The difference of AMF colonisation between SRF and WS may be attributed to the cultural operations carried out in order to prepare seedbed, to fertilise crops and to control weeds, pests and diseases as well as to the above- and belowground plant species diversity (Helgason *et al*., 1998; Vandenkoornhuyse *et al*., 2003; Leake *et al*., 2004). In addition, the highest root colonisation of the *L. perenne* grown under T2 might be explained by a large production of poplar fine roots observed in such management (Amato, 2000; Bonari and Masoni, 2000).

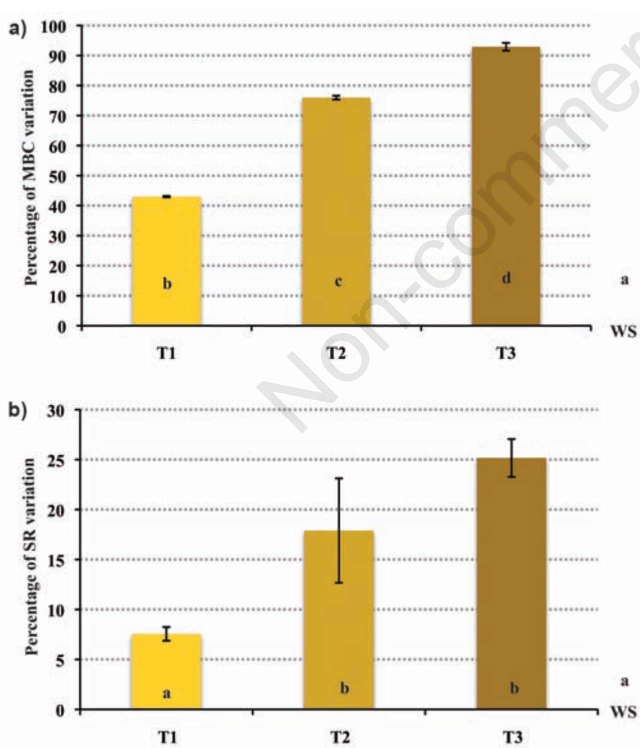

Figure 2. Soil microbial biomass (MBC) a) and soil respiration (SR) b) under poplar short-rotation forestry (one, two and three-year cutting cycles: T1, T2 and T3). The values are expressed as percentage of variation in comparison with their values under a wheat-soybean rotation (WS). Different letters indicate significant differences as tested by ANOVA (P≤0.001) and the Tukey-B test as *post-hoc*.

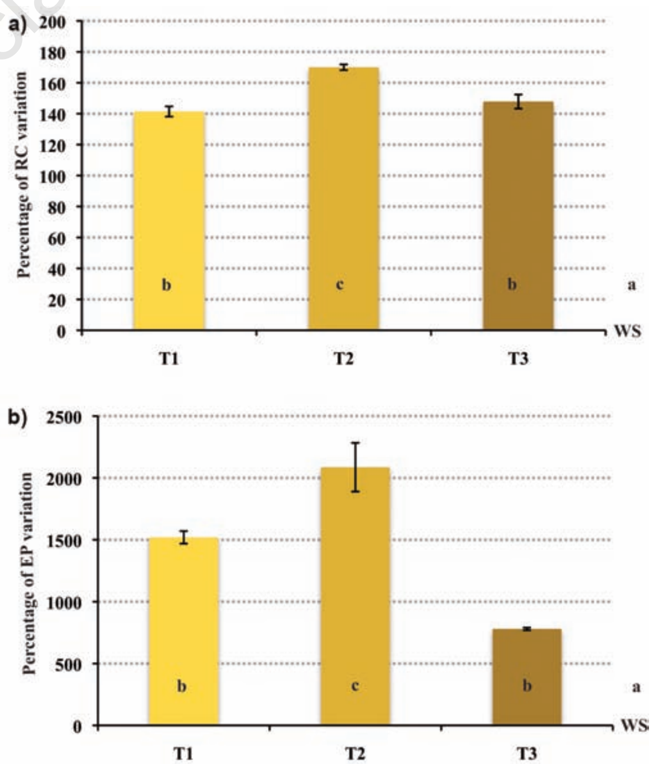

Figure 3. Arbuscular mycorrhizal fungal root colonisation (RC). a) and number of entry points (EP) b) under poplar short-rotation forestry (one, two and three-year cutting cycles: T1, T2 and T3). The values are expressed as percentage of variation in comparison with their values under a wheat-soybean rotation (WS). Different letters indicate significant differences as tested by ANOVA (P≤0.001) and the Tukey B test as *post-hoc*.

The number of EP under poplar SRF treatments showed values from 8 to 21 times higher than that reported under WS (Figure 3b). The number of EP under T1 and T3 was significantly lower than under T2 (T1-T3<T2) (Figure 3b). Our EP data under WS are consistent with the values reported by several authors, which measured such parameter assessing the AMF inoculum potential under shrubs, wild and cultivated plant species from semiarid ecosystem to boreal grasslands (Requena *et al*., 2001; Bharadwaj *et al*., 2007). Besides, EP values similar to those reported, here, under SRF were observed for different AMF inocula on several plant species (Liu and Luo, 1994). Such strong difference of AMF inoculum potential between a herbaceous-based system (WS) and the SRF may be explained by the different management, plant communities, patterns of root systems and hyphal networks, AMF communities in the soil and *in planta* (Bever *et al*., 1996; Helgason *et al*., 1998; van der Heijden *et al*., 1998; Daniell *et al*., 2001; Vandenkoornhuyse *et al*., 2003; Giovannetti *et al*., 2004).

Multivariate analysis of the soil chemical, biochemical and biological parameters

The RDA analysis, aiming at evaluating the impact of the different managements on the soil quality parameters, showed that management, used as explanatory variable, explained 69.9% (I and II axes) of the whole variance and that its effect on soil quality parameters (Figure 4), used as response variables, was significant (P=0.002). In detail, the Monte-Carlo permutation test pointed out significant differences on soil quality between WS and poplar SRF stands (P=0.01) and between T3 and the other cutting cycles (P=0.002), as showed by the distances of the centroids representing the managements. The biplot shows that the values of all soil quality parameters were higher under poplar SRF in comparison with WS, and that the differences among the T1, T2 and T3 were due to the fact that T1 and T2 increased the parameters linked to AMF, while T3 the soil chemical and biochemical variables. The short distance between the arrows representing RC and EP, as well as those representing SOC and N, shows the strong correlation between such parameters.

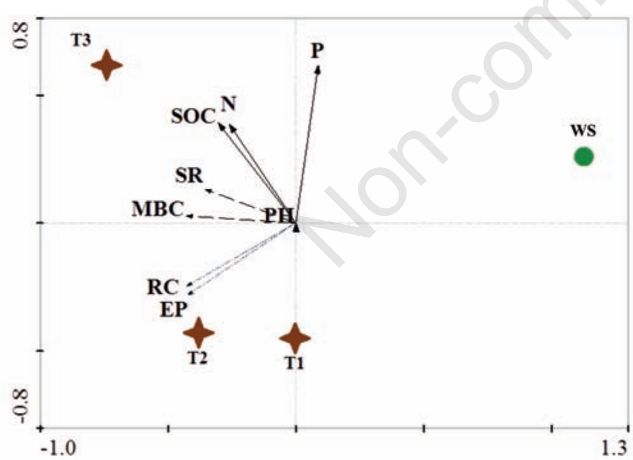

Figure 4. Redundancy Analysis (RDA) biplot based on: chemical, biochemical and AMF parameters (pH; P, available phosphorus; N, Kjeldahl nitrogen; SOC, soil organic carbon; SR, soil respiration; MBC, microbial biomass carbon; RC, mycorrhizal colonization of *Lolium perenne*; EP, number of entry points) and treatments (one, two and three-year cutting cycles poplar short-rotation forestry: T1, T2 and T3, respectively; wheat-soybean rotation, WS). Treatments are represented by stars (T1, T2 and T3) and circle (WS). The chemical, biochemical and AMF parameters are represented by arrows. The 1st and 2nd axis accounted for 49.0% and 69.9% of the variability explained by all canonical axes and were significant (P=0.002).

Conclusions

Since biomass is one of the most important sources of renewable energy, plant-microbial interactions under *Poplar* stands in comparison with conventional agricultural management are a cutting-edge issue. The present study shows the potential of a bioenergy crop management, represented by poplar SRF, to improve soil quality in comparison with an intensive food cropping system and a distinct behaviour of the different poplar cutting cycles in promoting soil organic carbon, microbial biomass and AMF inoculum potential. Such findings have important ecological and environmental implications, since the positive belowground effects observed here under poplar plantations could improve the viability of low-input SRF stands. The interactions between bioenergy crops and microorganisms need to be further investigated to explore their implications on plant-soil carbon sequestration, biomass production and nutrient uptake by mycorrhizas.

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