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Impact on soil quality of a 10-year-old short-rotation coppice poplar stand compared with intensive agricultural and uncultivated systems in a Mediterranean area

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ABSTRACT

Bioenergy crops play an ecologically and economically fundamental role as an alternative to agri-food productions and as renewable energy sources. Little attention has been focused on soil quality following conversion of agricultural lands to biomass crops. Here, we assessed the impact of a 10-year-old short-rotation coppice (SRC) poplar stand on the main soil chemical parameters, microbial biomass carbon, soil respiration, and arbuscular mycorrhizal fungi (AMF), compared with intensive agricultural and uncultivated systems. Three different harvest frequencies of poplar SRC (annual T1, biannual T2 and triennial T3 cutting cycles) were evaluated. Multivariate analysis showed that poplar SRC improved soil quality compared with intensive agricultural and uncultivated systems. T1 and T2 positively affected AMF inoculum potential and root colonisation of a co-occurring plant species, while T3 improved the majority of soil chemical and biochemical parameters. Moreover, three different AMF morphospecies belonging to the genera *Glomus* and *Scutellospora* were found in poplar SRC, while morphospecies belonging exclusively to genera *Glomus* were recorded in intensive agricultural and uncultivated systems. Such aspects have agro-ecological implications, since the positive changes of soil nutrient availability and carbon content together with a high abundance and diversity of soil biota show clear soil sustainability of poplar SRC.
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1. Introduction

During the last 10 years the concept of multifunctional agriculture has become established (Van Huylenbroeck and Durand, 2003). Such multifunctionality includes the conversion into low-input cultivation models and non-food productions, and into practices aiming to protect the rural landscape. In this context, bioenergy crops play an ecologically and economically fundamental role as a potential alternative to agri-food production and as renewable energy sources, when they are integrated in an optimised and sustainable territorial management of resources (Jordan et al., 2007). Large emission of greenhouse gases, and in particular of CO_2, induced by human activities, promoted a change in energy concept and the request for alternative sources, such as biomass crops (Lemus and Lal, 2005). These crops are mainly perennial rhizomatous grasses or fast-growing trees, such as *Eucalyptus*, *Populus* and *Salix* (Karp and Shield, 2008; Hinchee et al., 2009).

Many *Populus* (poplar) species have high energy potential, produce high yield, reduce soil erosion and are able to grow in marginal lands and drought conditions, as in Mediterranean areas (Makeschin, 1994; Guidi et al., 2008; Guo et al., 2010). Most species used in Europe belong to *P. nigra* and *P. deltoides*, although several hybrids are also successfully cultivated (Hinchee et al., 2009). Many studies have been performed on poplar short-rotation coppice (SRC) biomass production and quality, energy balance and management intensity, mainly in relation to fertilisation, irrigation, tillage, crop age and cutting cycles (Kauter et al., 2003; Deckmyn et al., 2004; Nassi o Di Nasso et al., 2010). Some of these studies have compared the benefits of poplar SRC with other bioenergy crops (Bonari et al., 2004; Karp and Shield, 2008), and others have evaluated the conversion of agricultural lands from food production to biomass crops, in particular to poplar SRC (Boehmel et al., 2008; Gasol et al., 2010). So far, less attention has been focused on the impact of poplar SRC on soil health and quality (Makeschin, 1994; Coleman et al., 2004; Zornoza et al., 2009; Kahle et al., 2010; Mao and Zeng, 2010), whereas the impact of forestry and of conventional and alternative cropping system managements have been largely studied (Carter and Rennie, 1982; Pietikaïnen and Fritze, 1995; Fließbach et al., 2007; Mazzoncini et al., 2010).

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Table 1
Mean soil physical and chemical characteristics at the experimental site in 1996 at 0–30 cm of depth.

Physical and chemical characteristics[a]	1996
Sand [%]	39.4
Silt [%]	40.5
Clay [%]	20.1
P [mg kg^{-1}]	8.8
Total N [g kg^{-1}]	1.3
SOC [g kg^{-1}]	10.4
C/N	8.4

[a] P: available Olsen phosphorus; Total N: Kjeldahl nitrogen; SOC: soil organic carbon; C/N: carbon/nitrogen ratio.

Soil quality has been defined as 'the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation' (Karlen et al., 1997). Soil quality evaluation criteria should meet physical, chemical and biological parameters that are sensitive to changes in soil conditions (Doran, 2002). The concept of a minimum data set of indicators was first proposed by Larson and Pierce (1991) and seven key physical and chemical parameters were chosen as the most adequate. Within such parameters, soil organic carbon (SOC) represents the main chemical indicator, directly influencing aggregation, water retention, nutrient availability, C store and biological diversity (Baldock and Nelson, 2000). Later, Doran and Parkin (1996) proposed an array of soil physical, chemical and biological characteristics. As regards biological parameters, biochemical indicators such as microbial biomass, soil respiration, Cmic/Corg ratio and metabolic quotient (qCO_2) were suggested for their rapidity of reaction to environmental changes and human activities, ease of measurement and reproducibility (Bloem et al., 2006; Kutsch et al., 2009). Recently, other studies proposed as biological indicators the community diversity and activity of certain taxonomic groups of soil biota (Brussaard et al., 1997). Within soil microbes, arbuscular mycorrhizal fungi (AMF) have been shown to be responsive soil quality indicators both in crop- and woodlands (Helgason et al., 1998; Daniell et al., 2001; van der Heijden and Sanders, 2002; Oehl et al., 2003), although several tree species, including poplar, are exclusively or also colonised by ectomycorrhizal fungi (ECMF) (Lopez-Aguillon and Garbaye, 1990; Rooney et al., 2009). AMF colonise the roots of most plant species and play a key role on soil fertility and on plant health and fitness (Smith and Read, 2008).

The aim of this study was to evaluate the impact of a 10-year-old short-rotation coppice poplar stand, under different harvest frequencies, on soil quality in comparison with an intensive agricultural system and an uncultivated soil. For this purpose, we used some of the most reliable and sensitive soil chemical and biochemical parameters, and with regard to the biological ones, AMF were chosen as indicators.

2. Materials and methods

2.1. Field experimental site

The field experimental site was located at the "Enrico Avanzi" Interdepartmental Centre for Agro-Environmental Research of the University of Pisa (43°40′N lat; 10°19′E long, with 1 m above sea level and 0% slope), Italy. The soil was a poorly drained alluvial loam, classified as *Typic Xerofluvent* by USDA system (Soil Survey Staff, 1975) and as *Fluvisol* by FAO (IUSS, 2006). Soil physical and chemical characteristics are shown in Table 1. Climatic conditions were typically Mediterranean: rainfall mainly concentrated from autumn to spring (mean 948 mm $year^{-1}$) and mean monthly air temperature ranging from 11 °C in February to 30 °C in August (mean of 14.5 °C $year^{-1}$). More details on climate conditions are given by Mazzoncini et al. (2008). Before experimental set-up, the field site was conventionally cultivated with maize (*Zea mays* L.)–durum wheat (*Triticum durum* Desf.) rotation (one crop per year). The cropping system was annually tilled according to local standard practices: 30–35 cm deep ploughing followed by secondary tillage for seedbed preparation. Fertilisation was applied and incorporated into the soil during seedbed preparation for both maize (335 kg ha^{-1} N, 150 kg ha^{-1} P and 150 kg ha^{-1} K) and wheat (175 kg ha^{-1} N, 90 kg ha^{-1} P and 135 kg ha^{-1} K). Chemical pre-emergence and mechanical post-emergence weed controls were applied during the maize growth, while pre- and post-emergence chemical controls were performed for the wheat.

2.2. Field experimental set-up

A long-term experiment was initiated in winter 1996. The experimental field was a completely randomised design with five management treatments and three replicates ($n = 3$; plots of 500 m^2): (1) intensive agricultural system (maize–wheat cropping system, MW): plots were still conventionally cultivated with maize–durum wheat rotation (one crop per year); (2) uncultivated system (US): plots were left to develop under natural succession vegetation, where perennial ryegrass (*Lolium perenne* L.), rough meadowgrass (*Poa trivialis* L.), bermuda grass (*Cynodon dactylon* L.), orchard grass (*Dactylis glomerata* L.), and red fescue (*Festuca rubra* L.) mainly grew. No fertiliser or other agricultural practices were applied. Forage was annually removed; (3) 1-year cutting cycle poplar SRC (T1): plots were established using 20 cm long unrooted *Populus deltoides* Bartr. (Lux clone) cuttings with a density of 10,000 plants ha^{-1}. The soil was tilled according to the standard practices: deep ploughing as the main tillage in the autumn, and disk and rotary harrowing before planting. Chemical weed control was performed before planting by applying 2.5 kg ha^{-1} of glyphosate and NPK fertilisation was incorporated into the soil (48 kg ha^{-1} N, 144 kg ha^{-1} P and 144 kg ha^{-1} K). T1 plots were harvested every year at the end of February, before vegetative re-growth; (4) 2-year cutting cycle poplar SRC (T2): plots were cultivated and managed as T1, except for the harvesting frequency which was set every 2 years according to the rotation; (5) 3-year cutting cycle poplar SRC (T3): plots were cultivated and managed as T1 and T2, except for the harvesting cycle of 3 years.

2.3. Sampling

In April 2005, during wheat growth in MW plots, one combined soil sample, resulting from three random soil cores pooled together, was collected from each replicate plot (0–10 cm depth) in order to control physical and chemical soil spatial variability which has been shown also to affect at different scales several AMF proprieties (Hart and Klironomos, 2002; Wolfe et al., 2007; Mummey and Rillig, 2008). With regard to temporal variability of the soil quality parameters, in several climatic conditions, including our areas, chemical characteristics slightly change during the year, while biochemical and biological parameters, including AMF, have been shown to consistently maintain the same patterns of variability among systems differently managed although they changed with the season (Helgason et al., 1999; Vandenkoornhuyse et al., 2002; Di Bene, 2003; Oehl et al., 2003; Stukenbrock and Rosendahl, 2004; Bastida et al., 2006; Pellegrino, 2007; Pellegrino et al., 2010). In addition, after long-term changes, in the evaluation of stable systems it is important to avoid sampling close to soil treatments such as tillage, fertilisation, weed control and harvest (Picci and Nannipieri, 2002). Therefore, we sampled only once in April since early spring sampling was the best choice with respect to the above

advice. Actually, all the management operations were concentrated in autumn–winter (tillage, fertilisation and weed control) and late spring–summer (cutting and harvest). Moreover, such a sampling period is also considered the best time to assess microbiological parameters since soil characteristics are relatively stable, the land is dry enough to access, all the plants are actively growing and the identification of AMF spores is easier than in autumn due to the fewer amounts of young and immature spores (Oehl et al., 2003; Bloem et al., 2006). We take all these facts as a strong justification for choosing early spring as single sampling date.

Soil samples used for the chemical analyses and for the assessment of the mycorrhizal infection potential were oven dried at 30 °C and passed through a 2 mm sieve, whereas samples used for biochemical analyses were sieved at field moisture. *L. perenne* was identified as a common and co-occurring plant species. Soil and roots of such plant were sampled by extracting turfs ($n = 3$), approximately 7–10 cm across and 10 cm deep, from all the plots. In addition, turfs across the main crops, durum wheat and poplar, were sampled from the plots under the MW and SRC treatments. After transport to the laboratory, each turf was carefully washed in water taking care to avoid breaking the roots. Only the fine roots attached to the main roots of the target plants (*L. perenne*, *T. durum* and *P. deltoides*) were collected and stored in a cool dry place prior to assessment of the AMF root colonisation.

2.4. Soil chemical and biochemical analyses

Soil samples were analysed for: pH; cation exchange capacity, CEC; electrical conductivity, EC; available phosphorus, P; total nitrogen, total N; organic carbon, SOC; microbial biomass carbon, MBC and soil respiration, SR. All such analyses were carried out in three replicates in order to control the variability. Soil pH and EC were measured in deionised water (1:2.5 and 1:2, w/v, respectively) (McLean, 1982) and CEC was evaluated by the Rhoades method (1982). P was determined by colorimetry using the Olsen method (Olsen and Sommers, 1982). Total N was evaluated by macro Kjeldahl digestion procedure (Bremner and Mulvaney, 1982) and SOC using the modified Walkley–Black wet combustion method (Nelson and Sommers, 1982). MBC and SR were assessed on soil samples adjusted to 55% of the field capacity on the basis of the ideal water content for an evaluation of microbial activity. MBC was determined by the Vance chloroform fumigation-extraction method, while SR was estimated according to the Isermeyer method, described in Alef and Nannipieri (1995). Such biochemical parameters were assessed using titration on soil subsamples of 45 g after 10 days of incubation in closed jars maintained at 25 °C.

Soil C/N ratio was calculated dividing SOC by organic N. Cmic/Corg and Cmic/SR (metabolic quotient, qCO_2) ratios were calculated dividing MBC by SOC and SR, respectively, and utilised as indices of microbial biomass contribution to soil organic carbon and respiration (Anderson and Domsch, 1989).

2.5. AMF measurements

The percentage of AMF colonisation was assessed after clearing and staining with lactic acid instead of phenol (Phillips and Hayman, 1970), using the gridline intersect method (Giovannetti and Mosse, 1980).

Mycorrhizal infection potential of soil was evaluated by a rapid test to assess AMF infectivity (mycorrhizal infection potential, MIP). Lettuce (*Lactuca sativa* L.) seeds were sown in 50 mL sterilised plastic tubes filled with 40 mL of soil obtained by each plot and six replicate tubes were used. After emergence, the lettuce plants were thinned to three. The plants were removed from the tubes after 2 weeks' growth and the root systems were stained as described above, mounted on microscope slides and examined under a Reichert-Jung (Vienna, Austria) Polyvar light microscope. Root length and colonised root length were measured using a grid eyepiece. Number of infection units, measured as hyphae with entry points, and number of entry points were assessed at a magnification of 125–500× and verified at a magnification of 1250×.

AMF spores were assessed in 50 g of soil per each plot by wet sieving and decanting, followed by sucrose centrifugation (Sieverding, 1991). After centrifugation, the supernatant was sieved by 50 μm mesh and quickly rinsed with tap water. Spore number was counted using a Petri dish under a dissecting microscope and presented as spore density (number of spores per gram of soil). AMF spores, spore clusters and sporocarps were separated on the basis of size and colour, and then mounted on microscope slides using polyvinyl–lactic acid–glycerol (PVLG) or PVLG mixed 1:1 (v/v) with Melzer's reagent. The slides were examined under a Reichert-Jung (Vienna, Austria) Polyvar light microscope and morphospecies were identified following current species descriptions and identification manuals (Schenck and Pérez, 1990; International Culture Collection of Vesicular and Vesicular-Arbuscular Endomycorrhizal Fungi [http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm]). At least 50 spores of each morphospecies were observed and measured using a grid eyepiece. Relative abundance of the identified AMF morphospecies was calculated by dividing the number of spores belonging to each species by the total number of spores (at least 100 randomly selected spores per each plot). Shannon's index (H_0), as an additional measure of AMF diversity, was calculated by the formula: $H_0 = -\sum p_i \ln p_i$ (p_i is the relative abundance of the ith species compared with all species identified in a sample).

2.6. Statistical analyses

Data were analysed by one-way analysis of variance (ANOVA), using management as the factor. Data were ln- or arcsine transformed when needed to fulfil the assumptions of ANOVA, which was carried out according to the completely randomised design. Tukey-B procedure was used for comparing means. H_0 data showed neither a normal distribution of error terms nor constant error variance, therefore a non-parametric ANOVA was required. We used the Kruskal–Wallis test and then Mann–Whitney *U*-test as post-hoc. Means and standard errors given in the tables are for untransformed data. Linear regression analysis was used to test whether there was a correlation between number of entry points and AMF colonised root length. All the analyses were performed on SPSS 17.0 software (SPSS Inc., Chicago, IL, USA).

All parameters utilised to evaluate soil quality and AMF morphospecies data were separately evaluated in constrained ordination analyses (redundancy analysis, RDA), in order to investigate the influence of different managements (used as explanatory variables) either on soil quality parameters or the AMF morphospecies structure (used as response variables). A detrended canonical correspondence analysis (DCCA) for AMF morphospecies structure suggested the use of linear method, as the lengths of gradients were < 3. RDAs were conducted in Canoco for Windows version 4.5 (ter Braak and Šmilauer, 2002). Additionally, Monte–Carlo permutation tests were conducted using 499 random permutations in order to determine the statistical significance of the relation between the different managements and the two data matrixes.

3. Results

3.1. Chemical parameters

After 10 years of different land use, in the 0–10 cm soil depth, CEC, EC and C/N did not show any significant impact of manage-

Table 2
Soil chemical parameters measured in poplar SRC, intensive agricultural and uncultivated systems 10 years after experimental set-up (0–10 cm depth).

Chemical parameters[a]	Poplar SRC[b]			MW	US
	T1[c]	T2	T3		
pH (H_2O, 1:2.5)*	8.19 ± 0.01[d] bc	8.11 ± 0.01 ab	8.06 ± 0.01 a	8.19 ± 0.05 bc	8.23 ± 0.03 c
CEC [cmol kg^{-1}]	10.23 ± 0.18	9.63 ± 0.84	11.58 ± 0.47	7.64 ± 0.42	12.98 ± 4.01
EC [μS cm^{-1}]	92.00 ± 3.46	113.33 ± 7.22	100.00 ± 2.31	101.00 ± 5.20	103.67 ± 6.06
P [mg kg^{-1}]**	12.05 ± 0.40 a	12.24 ± 0.79 a	15.93 ± 0.52 b	9.33 ± 0.10 a	12.25 ± 1.61 a
Total N [g kg^{-1}]***	1.44 ± 0.03 b	1.37 ± 0.07 b	1.65 ± 0.00 c	1.16 ± 0.03 a	1.34 ± 0.07 ab
SOC [g kg^{-1}]**	12.37 ± 0.20 ab	13.50 ± 1.10 bc	16.07 ± 0.66 c	10.03 ± 0.54 a	12.07 ± 0.92 ab
C/N	8.95 ± 0.06	10.23 ± 0.33	10.13 ± 0.43	8.98 ± 0.39	9.33 ± 0.27

[a] CEC: cation exchange capacity; EC: electrical conductivity; P: available phosphorus; Total N: Kjeldahl nitrogen; SOC: soil organic carbon; C/N: carbon/nitrogen ratio.
[b] Poplar SRC: poplar short-rotation coppice; MW: intensive agricultural system, based on maize–wheat crop rotation; US: uncultivated system.
[c] T1: 1-year cutting cycle; T2: 2-year cutting cycle; T3: 3-year cutting cycle.
[d] Values are means ± SE of three plots for each treatment. Values in each row not followed by the same letters are significantly different as tested by one-way ANOVA (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

ment, whereas pH, P, total N and SOC were significantly affected by the treatments (Table 2). Soil pH value was significantly higher in US than in T3, while the amount of P, ranging from 9.33 to 15.93 mg kg^{-1} in MW and T3, respectively, produced two different groups with high and low P concentration: T3 and other treatments (Table 2). Total N was significantly higher in poplar SRC than in MW, while T2 and T3 significantly increased SOC compared with MW (Table 2).

3.2. Biochemical parameters

Microbial biomass carbon (MBC) and soil respiration (SR) were significantly affected by the management (Table 3). In contrast, Cmic/Corg ratio did not differ among the treatments (Table 3). MW and T3 showed the lowest and the highest values of both MBC and SR (Table 3). In detail, MBC ranged from 91.20 and 148.35 mg C kg^{-1} soil, while SR ranged from 60.72 to 163.27 mg CO_2-C kg^{-1} soil (Table 3). In MW and in US, MBC was significantly lower than in poplar SRC (Table 3). Among poplar SRC, three different groups were observed: T1 (low MBC), T2 (medium MBC), and T3 (high MBC) (Table 3). Similarly to MBC, SR in MW was significantly lower than in poplar SRC and US (Table 3). In detail, two different groups can be distinguished: T1 (low SR) and T3 (high SR) (Table 3).

The Cmic/Corg ratio did not significantly differ among the treatments, ranging from 0.89% to 1.79% in T1 and the US, respectively (Table 3). On the other hand, the mineralised C per unit of microbial biomass C (qCO_2) was significantly influenced by the management, showing values ranging from 0.66 to 1.35 mg CO_2-C mg Cmic^{-1} d^{-1} in MW and US, respectively (Table 3). As regard to poplar SRC, qCO_2 increased from T3 to T1, which showed values significantly different between each other and similar to T2 (Table 3).

3.3. AMF measurements

3.3.1. Mycorrhizal colonisation

The percentage of colonised root length of *P. deltoides* was not significantly affected by the cutting cycle ($P = 0.09$), ranging from 15.3% to 18.7% (in T2 and T3, respectively). *L. perenne* in US and *T. durum* in MW showed mycorrhizal colonisation values of 3.8% and 8.8%, respectively. All poplar samples, carefully analysed along the fine root tips, did not show any outer sheath-like structures characteristic of the ECMF.

In all replicate plots of poplar SRC, of US and of MW, *L. perenne* was consistently detected. Mycorrhizal colonisation of such common and co-occurring plant species weed was significantly affected by management (Table 4). The highest mycorrhizal colonisation of *L. perenne* was observed in T2, and such value significantly differed from those recorded in T1 and T3 (Table 4). In addition, *L. perenne* grown in MW and US was significantly less colonised by AMF, showing values ranging from 5.9% to 9.4% (Table 4).

3.3.2. Mycorrhizal infection potential

Mycorrhizal infection potential (MIP) was assessed using three different parameters: colonised root length, number of infection units and number of entry points of *L. sativa* roots. Colonised root length showed values ranging from 0.41% to 3.68% in US and T2, respectively (Table 5). The differently managed poplar SRC showed colonised root lengths significantly different from each other, and higher than those reported in US and MW (Table 5). Infection units values ranged from 0.13 to 3.18 units cm^{-1} root length in MW and T2, respectively, while the number of entry points from 0.11 to 3.28 cm^{-1} root length in US and T2, respectively (Table 5). Among the managements, three different groups with low, medium and high infectivity can be discriminated using the infection unit parameters: MW and US (low infectivity), T3 (medium infectivity),

Table 3
Soil biochemical parameters measured in poplar SRC, uncultivated soil and maize–wheat cropping system 10 years after experimental set-up (0–10 cm depth).

	MBC mg C kg^{-1} soil	SR[a] mg CO_2-C kg^{-1} soil	Cmic/Corg (%)	qCO_2 mg CO_2-C mg Cmic^{-1} d^{-1}
Poplar SRC[b]				
T1[c]	109.97 ± 0.17[d] b	140.30 ± 0.90 bc	0.89 ± 0.02	1.28 ± 0.01 cd
T2	135.32 ± 0.49 c	153.79 ± 6.82 cd	1.02 ± 0.08	1.14 ± 0.05 bc
T3	148.35 ± 1.01 d	163.27 ± 2.47 d	0.93 ± 0.03	1.10 ± 0.01 b
Uncultivated soil	94.46 ± 1.95 a	127.75 ± 4.32 b	1.79 ± 0.07	1.35 ± 0.06 d
MW cropping system	91.20 ± 2.90 a	60.72 ± 4.40 a	0.91 ± 0.03	0.66 ± 0.04 a

[a] MBC: microbial biomass carbon; SR: soil respiration; Cmic/Corg: microbial carbon/soil organic carbon ratio; qCO_2: metabolic quotient, SR/MBC.
[b] Poplar SRC: poplar short-rotation coppice; MW cropping system: maize–wheat cropping system.
[c] T1: 1-year cutting cycle; T2: 2-year cutting cycle; T3: 3-year cutting cycle.
[d] Values are means ± SE of three plots for each treatment. Values in each column not followed by the same letters are significantly different as tested by one-way ANOVA ($P < 0.001$).

Table 4
Mycorrhizal colonisation of *Lolium perenne*, spore density and arbuscular mycorrhizal fungal morphotype diversity estimated using Shannon's measure (H_0) revealed in poplar SRC, uncultivated soil and maize–wheat cropping system 10 years after experimental set-up (0–10 cm depth).

	Mycorrhizal colonisation (%)	Number of spores g^{-1} soil	H_0
Poplar SRC[a]			
T1[b]	16.90 ± 0.23[c] c	11.70 ± 0.46 b	0.97 ± 0.00 d
T2	18.90 ± 0.13 d	12.40 ± 0.17 b	1.01 ± 0.03 d
T3	17.35 ± 0.32 c	16.90 ± 1.93 c	0.68 ± 0.00 b
Uncultivated soil	5.93 ± 0.12 a	4.63 ± 0.19 a	0.79 ± 0.00 c
MW cropping system	9.37 ± 0.55 b	5.68 ± 0.32 a	0.42 ± 0.01 a

[a] Poplar SRC: poplar short-rotation coppice.
[b] T1: 1-year cutting cycle; T2: 2-year cutting cycle; T3: 3-year cutting cycle; MW cropping system: maize–wheat cropping system.
[c] Values are means ± SE of three plots for each treatment. Values in each column not followed by the same letters are significantly different as tested by one-way ANOVA ($P < 0.001$) and by the Kruskall–Wallis non-parametric test ($P = 0.012$).

and T1 and T2 (high infectivity) (Table 5). Using the number of entry points a similar trend was observed: MW and US (low infectivity), T3 (medium infectivity), T1 (medium–high infectivity), and T2 (high infectivity) (Table 5). The number of entry points and colonised root length were positively correlated with each other ($R^2 = 0.62$, $P = 0.001$).

3.3.3. AMF spore population

The spore density significantly differed among managements, ranging from 4.6 to 16.9 spore g^{-1} of soil in US and T3, respectively (Table 4). US and MW showed a similar spore density, which was significantly lower than that reported in poplar SRC (Table 4). Although no differences were observed between T1 and T2, such managements significantly reduced the spore density compared with T3 (Table 4).

Overall, on the basis of spore morphology, we detected six different AMF species belonging to the genera *Glomus* and *Scutellospora*: *Glomus etunicatum* (Beck. & Gerd.), *Glomus geosporum* (Nicol. & Gerd.), *Glomus intraradices* (Schen. & Smith), *Glomus mosseae* (Nicol. & Gerd.), *Scutellospora calospora* (Nicol. & Gerd.) and an unidentified *Glomus* species (Fig. 1). In T1 and T2 three AMF species were recorded (*G. mosseae*, *Glomus* sp. and *S. calospora*), while in T3 only two species were observed (*G. mosseae* and *S. calospora*) (Fig. 1). In US and in MW four (*G. etunicatum*, *G. geosporum*, *G. intraradices* and *G. mosseae*) and three AMF species (*G. geosporum*, *G. intraradices* and *G. mosseae*) were detected, respectively (Fig. 1). The AMF community of soil under different managements were

Table 5
Colonised root length, number of infection units and number of entry points of *Lactuca sativa* grown in poplar SRC, uncultivated soil and maize–wheat cropping system 10 years after experimental set-up (0–10 cm depth).

	Colonised root length (%)	Infection units cm^{-1} root length	Entry points cm^{-1} root length
Poplar SRC[a]			
T1[b]	1.63 ± 0.07[c] b	2.46 ± 0.09 c	2.43 ± 0.09 c
T2	3.68 ± 0.31 d	3.18 ± 0.52 c	3.28 ± 0.42 d
T3	2.30 ± 0.04 c	1.35 ± 1.12 b	1.32 ± 0.08 b
Uncultivated soil	0.41 ± 0.12 a	0.13 ± 0.02 a	0.11 ± 0.03 a
MW cropping system	0.63 ± 0.17 a	0.13 ± 0.03 a	0.13 ± 0.02 a

[a] Poplar SRC: poplar short-rotation coppice; MW cropping system: maize–wheat cropping system.
[b] T1: 1-year cutting cycle; T2: 2-year cutting cycle; T3: 3-year cutting cycle.
[c] Values are means ± SE of three plots for each treatment. Values in each column not followed by the same letters are significantly different as tested by one-way ANOVA ($P < 0.001$).

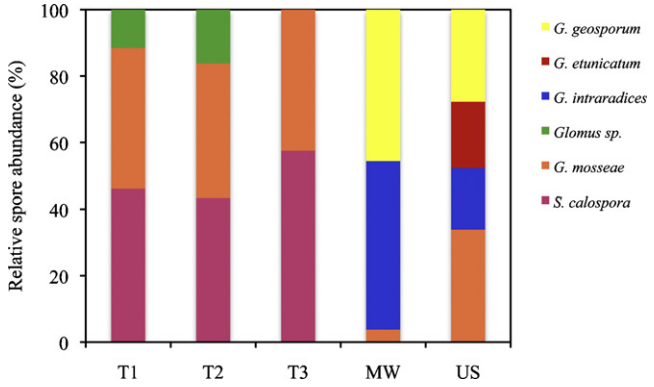

Fig. 1. Relative spore abundance in 1-, 2- and 3-year cutting cycle (T1, T2 and T3) poplar short-rotation coppice; intensive agricultural system: maize–wheat cropping system, MW; uncultivated system: US. The values are means of three replicates per treatment. Each colour corresponds to an AMF species: *Glomus geosporum*, *Glomus etunicatum*, *Glomus intraradices*, *Glomus* sp., *Glomus mosseae* and *Scutellospora calospora*.

determined on the basis of the relative spore abundances of the six AMF species (Fig. 1). The most common species in poplar SRC were *S. calospora* and *G. mosseae*. *S. calospora* ranged from 43% to 58% in T2 and T3, respectively, while *G. mosseae* from 40% to 42% in T2 and T1–T3, respectively (Fig. 1). In US, the most abundant species were *G. geosporum* and *G. mosseae* (28% and 34%, respectively), while in MW were *G. geosporum* and *G. intraradices* (46% and 51%, respectively) (Fig. 1). H_0, ranging from 0.42 to 1.01 in MW and T2, was significantly affected by the management ($P = 0.012$), as shown by the Kruskall–Wallis test (Table 4). According to our initial hypothesis, the AMF diversity was lowest in MW, but unexpectedly T1 and T2 showed higher diversity ($H_0 \geq 0.97$) than US ($H_0 = 0.79$) (Table 4).

RDA showed that management explained 88.1% (I and II axes) of the whole variance and that its effect on AMF communities was significant ($P = 0.004$) (Fig. 2a). In detail, the Monte–Carlo permutation test showed that all treatments were significantly different among each other ($P \leq 0.004$). In the biplot of the RDA (Fig. 2a) the centroids of MW and US are distanced from each other and from poplar SRC, while the centroids representing the differently managed poplar SRC cluster, share *S. calospora* and *G. mosseae*. The biplot also shows that T1 and T2 centroides are closer to each other than to T3, sharing *Glomus* sp. (Fig. 2a). In addition, the arrow representing *G. etunicatum* points to US and the arrow representing *G. intraradices* and *G. geosporum* point to MW, showing their preferential presence in such managements (Fig. 2a).

3.4. Main patterns of chemical, biochemical and AMF as affected by agroecosystem management

RDA showed that management explained 59.6% (I and II axes) of the whole variance and that its effect on the whole soil quality parameters was significant ($P = 0.002$) (Fig. 2b). In detail, the Monte–Carlo permutation test showed that all treatments were significantly different to each other ($P \leq 0.004$). In the biplot of the RDA (Fig. 2b), the centroids of MW and US are distant from each other and from poplar SRC. The centroids representing the differently managed poplar SRC are also distant from each other (Fig. 2b). Soil quality parameter arrows point to poplar SRC, clearly showing their higher values compared with US and MW (Fig. 2b). In addition, the arrows show that values of mycorrhizal infection potential are higher in T1 and T2 than in T3, while the values of MBC, SR, SOC, P, total N and CEC are highest in T3 (Fig. 2b). The diagram points out correlations between mycorrhizal infection potential parameters, between SOC and SR, and between P and total N (Fig. 2b). The biplot also shows that AMF spore density and MBC represent the most

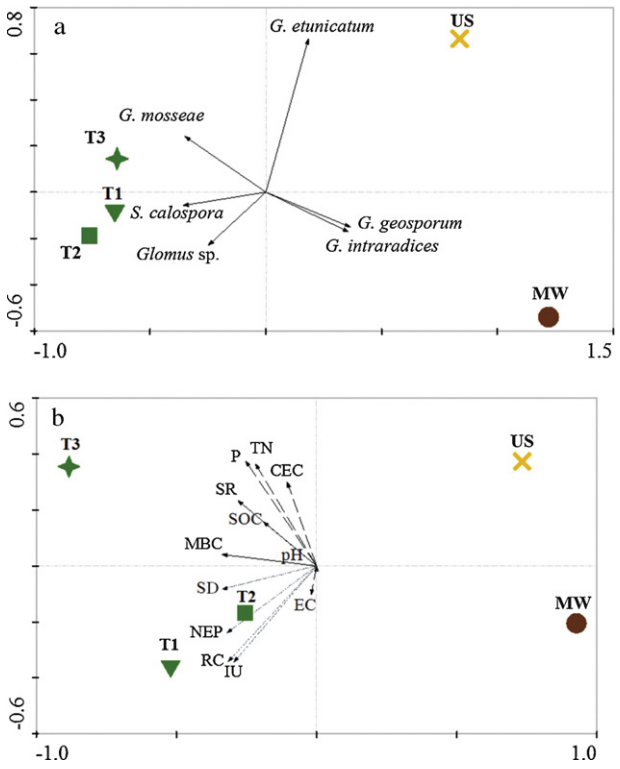

Fig. 2. Redundancy analysis (RDA) biplot based on: (a) arbuscular mycorrhizal fungal (AMF) spore number per gram of soil (*Scutellospora calospora*, *Glomus mosseae*, *Glomus* sp., *Glomus intraradices*, *Glomus etunicatum* and *Glomus geosporum*); (b) chemical, biochemical and AMF parameters (pH; CEC: cation exchange capacity; EC: electrical conductivity; P: available Olsen phosphorus; total N: Kjeldahl nitrogen; SOC: soil organic carbon; SR: soil respiration; MBC: microbial biomass carbon; mycorrhizal colonisation of *Lolium perenne*: RC; spore density: SD; number of entry points: NEP; number of infection units: IU) and treatments (1-, 2- and 3-year cutting cycle poplar short-rotation coppice: T1, T2 and T3, respectively; intensive agricultural system: maize–wheat cropping system, MW; uncultivated system: US). Treatments are represented by down-triangle (T1), square (T2), star (T3), cross (US) and circle (MW). The AMF morphospecies in (a) and the chemical, biochemical and AMF parameters in (b) are represented by arrows. (a) The 1st and 2nd axis accounted for 72.2 and 88.1 of the variability explained by all canonical axes and were significant ($P = 0.002$); (b) the 1st and 2nd axis accounted for 50.3 and 59.6 of the variability explained by all canonical axes and were significant ($P = 0.002$).

discriminating variables between US and MW vs. SRC treatments, while CEC, total N and P between T1 and T2 vs. T3 (Fig. 2b).

4. Discussion

In this work, for the first time, we assessed the impact of a 10-year-old bioenergy crop management based on a SRC poplar plantation on soil quality, using the main chemical parameters, microbial biomass, soil respiration and AMF root colonisation, infectivity, spore density, community composition and structure, compared with intensive agricultural (MW) and uncultivated (US) systems. Multivariate analyses showed that: (i) poplar SRC improved soil quality compared with MW and US; (ii) poplar SRC under the three harvest frequencies differentially increased soil quality: T1 and T2 affected AMF positively, except for spore production, while T3 improved chemical and biochemical parameters.

4.1. Chemical parameters

With the conversion from maize–wheat cropping system (MW) to poplar SRC and uncultivated system (US), only 2- (T2) and 3-year (T3) cutting cycle poplar SRC topsoil layer revealed higher SOC concentration compared with the plots maintained under MW, which had SOC level similar to the initial value. A recent meta-analysis, evaluating several tree species in different stand age afforestation programmes of agricultural soils, reported SOC increases ranging between 2% and 25% due to coniferous and broadleaf plantations (Laganière et al., 2010). With regard to poplar, Hansen (1993) and Coleman et al. (2004) found a mean annual carbon (C) increase of about 1.60 and 3 Mg ha^{-1}. Our data are consistent with the findings of several authors, who in general reported long-term positive SOC changes due to poplar afforestation of former cultivated lands (Hansen, 1993; Makeschin, 1994; Coleman et al., 2004). On the contrary, a meta-analysis on soil property changes due to afforestation by different tree genera showed contrasting results, since *Pinus* reduced SOC content by 15%, whereas some other conifers and *Eucalyptus* did not significantly increase SOC levels (Berthrong et al., 2009a). Moreover, recent studies on *Populus* reported no significant changes in 0–15 cm layer in the first 15 years following afforestation of agricultural soils (Mao and Zeng, 2010). Such contradictory reports may be due to variables controlling SOC dynamics, such as previous land use, time since land-use conversion, tree species planted, soil clay content, pre-planting disturbance and climatic conditions (Laganière et al., 2010).

Here, as observed in most studies, soil nutrient concentrations and pH were strictly related to SOC patterns (Paustian et al., 1997; Lemus and Lal, 2005). All SRC poplar treatments had higher soil total N compared with MW, while soil P concentration significantly increased only under T3, which showed the lowest pH value. T3 increased total N by 42% compared with MW, while T1 and T2 increased such value by a mean of 22%. Accordingly, in several afforestations, including poplar plantations, stand age was reported to increase soil N content (Ritter, 2007; Sartori et al., 2007; Mao and Zeng, 2010). In particular, 10-, 12- and 20-year poplar afforestations increased soil total N by 40%, 20% and 23% compared with agricultural lands (Kahle et al., 2007; Sartori et al., 2007; Mao and Zeng, 2010). By contrast, Berthrong et al. (2009a) showed a negative change of soil N, similarly to SOC trend, in *Pinus* afforestation and no changes in *Eucalyptus* and other tree genera. Moreover, soil N decreases were revealed in *Eucalyptus* and *Pinus* by other authors (Binkley and Resh, 1999; Smal and Olszewska, 2008). In our study, N increase in SRC poplar soils, compared with other treatments, may be due to less soil disturbance, high plant litter quantity and intensive and deep rooting, decreasing percolation water with nutrients and especially soil N losses. Inconsistent results concerning soil N changes may be a consequence of tree species planted, soil type and management (Sartori et al., 2007; Berthrong et al., 2009a).

Soil P content is known to be the one of the major growth-limiting elements, because P is often bound in insoluble forms or is physically retained by aggregates (Ritter, 2007). Here, T3 increased soil available P, by 30%, 31% and 71% in comparison with US, other poplar SRC and MW, respectively. Interestingly, Zornoza et al. (2009) comparing soil available P in *Pinus* forest, agricultural and uncultivated soils in a Mediterranean area, observed increases of 41% and 91% under forest management compared with the other land uses. Moreover, as regards total P, which is correlated with the available P, Ritter (2007) reported soil increases of 3% and 15% following afforestation with *Larix* and *Betula* compared with grazed, treeless areas.

In T3 plots we observed a decrease of 0.13 pH units compared with MW. Berthrong et al. (2009a) reported that plantations with *Eucalyptus*, *Pinus* and other conifers decreased soil pH of 0.30 units. Although such changes are similar to our data, they observed variation of pH from 5.6 to 5.3, due to the fact that the control soil was already acidic. In *Populus* and *Salix*, a minor decrease in soil pH was found even 3 years after tree plantation and in *Populus* stands also along with length of rotation (Makeschin, 1994; Sartori et al., 2007). On the contrary, Kahle et al. (2007, 2010) did not observe any soil pH change due to poplar plantation.

4.2. Biochemical parameters

In the present study, all poplar SRC treatments increased MBC and SR by a mean of 44% and 151%, respectively, compared with the maize–wheat cropping system, while the uncultivated system showed MBC and SR increases of 4% and 110%, respectively, in comparison with MW. Our results are consistent with previous works studying MBC or SR changes under forestry management compared with agricultural lands, supporting the hypothesis that soil MBC increases following afforestation of agricultural lands (Makeschin, 1994; Zornoza et al., 2009; Kahle et al., 2010; Mao and Zeng, 2010). Only a few studies have evaluated SRC poplar impact on microbial biomass and activity assessed on the basis of SR. Makeschin (1994), comparing the effect of energy forestry and of an intensive wheat cropping system on biochemical parameters, reported increases of MBC 9 years after land use change. Consistently, Kahle et al. (2010), studying the impact of afforestation of agricultural lands on microbial proprieties, observed positive MBC changes. As regards MBC shifts, Mao and Zeng (2010) reported an initial decline and then a significant increase with stand age. In contrast with our findings, several authors have shown that forest management determines a decline in soil MBC ranging from −27% to −43% (Pietikaïnen and Fritze, 1995; Chen et al., 2003; Berthrong et al., 2009b).

Increases in soil MBC due to poplar afforestation was not unexpected because of higher energy and nutrient sources for decomposer microorganisms, supplied by a larger quantity of leaf and root litter in comparison with crop residues. Moreover, soil MBC increases may depend also on no-tillage practices during the plantation period and on high plant coverage (Gupta et al., 1994). Concerning such factors, it is well established that ploughing negatively affects MBC by reducing SOC content in the shallow layer (Carter and Rennie, 1982).

The similarity of Cmic/Corg ratio among the different managements might show that soil microorganisms have a similar metabolic behaviour in all treatments and a similar efficiency in the conversion of organic C into microbial biomass C, in agreement with the concentrations of the soil organic carbon, which were similar in all the plots, except for the T3 (Bastida et al., 2006; Zornoza et al., 2009; Frazão et al., 2010). The Cmic/Corg values in the SRC poplar under different cutting cycles were 53% and 62% lower than that reported under *Pinus* and *Populus* plantations, respectively (Bastida et al., 2006; Zornoza et al., 2009; Mao and Zeng, 2010). In the MW and US systems, the values of this index were comparable to that observed by Frazão et al. (2010) in similar systems, although lower than those reported by Zornoza et al. (2009) and by Fließbach et al. (2007). Moreover, in conventional and organic systems adjacent to our field sites, Cmic/Corg values lower than that observed here were found (Mazzoncini et al., 2010).

The metabolic quotient (qCO_2) is an index that estimates the activity and efficiency of decomposition by soil microorganisms by evaluating the CO_2 loss through respiration: low respiration per unit of microbes represents high efficiency (Anderson and Domsch, 1990; Kutsch et al., 2009). Here, the MW system showed a lower qCO_2 than poplar SRC treatments. Litter inputs in *Populus* plantations, which represent the substrate for respiration, could determine the increase of this index as suggested also by Bastida et al. (2006). Moreover, the differences between MW and US might be due to changes in species composition within microbial communities which is largely reported to be highly related to changes in plant diversity (Smalla et al., 2001; Johnson et al., 2003; Öpik et al., 2006). Actually, the AMF species richness in US was twofold than that observed in MW.

The limitation of a single sampling date is that biochemical parameters can vary through the season due to the effects of factors such as temperature, moisture, photosynthate production and/or their interactions (Luo and Zhou, 2006; Kutsch et al., 2009). The consequence is that we cannot assume that the values observed in the different treatments will remain constant through the year. In fact, in adjacent conventionally tilled and no-tilled plots, values of MBC and SR higher and similar than that obtained here in the intensive agricultural and uncultivated systems were observed in autumn and spring, respectively (Di Bene, 2003). Nevertheless, this does not invalidate our data on the impacts of management on biochemical parameters since several authors observed variable values of MBC, SR, Cmic/Corg and qCO_2 through the season but consistent differences among treatments (Di Bene, 2003; Bastida et al., 2006; Frazão et al., 2010).

4.3. AMF measurements

L. perenne has a fibrous root system, with thick main roots and thinner lateral branches, which are usually colonised by AMF. Such a common and co-occurring plant species was chosen as a suitable test plant, due to its well known responsiveness to a wide range of AMF and soil conditions (Oehl et al., 2003; Gollotte et al., 2004). Here, we observed that *L. perenne*, growing in SRC poplar plots, showed an AMF colonisation 1–2 times higher than the colonisation of the same plant species occurring in the maize–wheat (MW) rotation and in the uncultivated system (US). The differences between SRC and MW *L. perenne* colonisation rate can be well explained mainly by ploughing, fertilisation or fungicide application, while those between SRC and US by root system architecture and development of the plant species growing in the different treatments. As to AMF root colonisation, several authors have shown that ploughing and disturbance reduce the extent and interconnectedness of AMF extraradical mycelium spreading from mycorrhizal roots into the surrounding soil (McGonigle and Miller, 1996; Helgason et al., 1998) and, in some cases, disturbance was also shown to negatively affect root colonisation rates (Koske and Gemma, 1997). Moreover, the production and mortality dynamics of fine roots, which are an essential component of the poplar root system (Block et al., 2006), might determine a greater AMF colonisation of *L. perenne* in poplar SRC compared with US, where only herbaceous species were growing.

Two main methods have been developed in order to evaluate AMF inoculum potential by means of infective propagule or spore number (Porter, 1979). In our study, infective propagule number, measured using lettuce AMF colonisation, infection units, number of entry points and AMF spore richness were consistently higher in poplar SRC than in MW and US. Changes of AMF abundance and infectivity due to disturbance, fertilisation or pesticide applications, plant community composition, have been previously shown in several studies (Bever et al., 1996; Oehl et al., 2003, 2004). In particular, Baum and Makeschin (2000) and Chifflot et al. (2009) studying AMF formation under poplar plantation, observed an AMF spore density consistent with our data.

Ten years after starting the long-term field experiment, the AMF spore density revealed under MW and US showed values lower than under SRC. The values observed under MW are consistent with the data reported in conventionally or organically agricultural fields belonging to the same area and with those found in microcosms using soil originating from arable lands under a similar crop rotation (Oehl et al., 2004; Pellegrino, 2007; Mazzoncini et al., 2010). Nevertheless, MW and US spore density were lower than in arable lands and grassland in UK and Central Europe (Eason et al., 1999; Oehl et al., 2003).

Intensive harvesting and herbivory were shown to reduce C accumulation rates below-ground, as a result of above-ground plant re-growth (Barto and Rillig, 2010). Such C decreases may explain the reduction of AMF spore occurrence in T1 and T2 compared with T3 due to the fact that AMF are dependent on plants for their C nutrition. So far, we have no information about C allocation

to fungal component following coppicing. Concerning C allocation, Rooney et al. (2009) suggested that coppicing initially determines a plant retain aiming at tree regeneration. According to their suggestions, Nassi o Di Nasso et al. (2010) observed dry yield and stem diameter increases of 38% and 73%, respectively, under the 3-year cutting cycle poplar SRC in comparison with T1 and T2.

Our data showed that significant AMF spore population changes, due to poplar SRC management, occurred 10 years after poplar plantation. Three different AMF morphospecies belonging to the genera *Glomus* and *Scutellospora* were found in SRC poplar plots, while three and four morphospecies belonging to the genera *Glomus* were recorded in MW and US, respectively.

This finding may reflect the edaphic and climatic homogeneity of the studied area. Similar spore richness were observed in arable soils and tree plantations (Del Val et al., 1999; Calvente et al., 2004), while higher values were reported in adjacent fields subjected to conventional and organic managements (Mazzoncini et al., 2010) and in several systems from monocropping to grasslands in Europe and USA (Bever et al., 1996; Oehl et al., 2003; Calvente et al., 2004). Such differences may be due to a low sampling effort in comparison with the other studies, which is known to affect the observed AMF community richness (Renker et al., 2006).

The presence of the genera *Glomus* in MW may depend on the high responsiveness of the *Scutellospora* genera to tillage, monocropping, fertilisation and pesticide applications (Helgason et al., 1998; Daniell et al., 2001; Oehl et al., 2004). By contrast, other studies reported the occurrence of *Scutellospora calospora* in organic and low-input agroecosystems based on crop rotation and in conventional maize monoculture (Oehl et al., 2003, 2004; Mazzoncini et al., 2010). The lower AMF diversity in MW and US than in T1 and T2, evaluated here also by H_0, is mainly caused by the low number of spores and would result in low functional contribution to hosts due to AMF functional complementarity (Koide, 2000; Avio et al., 2006).

Here, homogenization of the field soil in the sampling enabled us to reduce spatial variation that may influence AMF root colonisation, infectivity, spore density, community composition and structure, changing the available pool of AMF (Lekberg et al., 2007; Wolfe et al., 2007; Mummey and Rillig, 2008). AMF seasonality has been studied by several authors in different ecosystems and dates. Most research has revealed temporal dynamics in resting spores in the soil, which may reflect variation in sporulation rather than in fungal presence (Gemma et al., 1989; Schultz et al., 1999). In addition, Helgason et al. (1999), evaluating the AMF diversity within the roots of bluebell by morphological and molecular techniques, revealed clear seasonal patterns dependent on fungal species and, then, Vandenkoornhuyse et al. (2002) found that the AMF communities within *Trifolium repens* and *Agrostis capillaris* changed over time, although the authors suggested that a change of management may be responsible for this, observing also differences dependent on plant and fungal species. By contrast, the sampling season had no influence on AMF composition and patterns of diversity both in the field as spores and within the roots as active colonisation (Oehl et al., 2003; Stukenbrock and Rosendahl, 2004).

5. Conclusions

This study shows that 10 years after land management change, soil quality under poplar SRC is improved compared with an intensive agricultural cropping system and, unexpectedly, to an uncultivated system. These aspects have agro-ecological implications, since the positive changes of soil nutrient availability and C content together with a high abundance and diversity of soil biota reflect low risk of nutrient losses, contribution to climate protection and biodiversity, showing a clear soil sustainability of poplar SRC. Differences in soil chemical, biochemical and AMF parameters also observed among the three SRC poplar cutting cycles indicate that the choice of a suitable harvest frequency is important to preserve soil fertility and health. Overall, multivariate analysis showed that T1 and T2 determine changes of parameters related to AMF, except for spore production, while T3 improved chemical and biochemical traits. As mycorrhizal symbiosis establishment and extraradical mycelium development is dependent on plant fixed C, the potential reduction of available C following clipping would result in a high mycelium growth from AMF spores and from already colonised poplar roots aiming to establish symbiosis with other plant species seeking C sources. Our findings of plant–soil biota interactions under different SRC harvest intensity may contribute to sustainable bioenergy crop management. Further study on biomass crops is required to investigate subsurface layer SOC dynamics and shifts of soil microbial community composition, activity and function using also molecular techniques.

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References

Alef, K., Nannipieri, P., 1995. Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London.
Anderson, T.H., Domsch, K.H., 1989. Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil Biol. Biochem. 21, 471–479.
Anderson, T.H., Domsch, K.H., 1990. Application of eco-physiological quotients (qCO_2 and qD) on microbial biomass from soils of different cropping histories. Soil Biol. Biochem. 22, 251–255.
Avio, L., Pellegrino, E., Bonari, E., Giovannetti, M., 2006. Functional diversity of arbuscular mycorrhizal fungal isolates in relation to extraradical mycelial networks. New Phytol. 172, 347–357.
Baldock, J.A., Nelson, P.N., 2000. Soil organic matter. In: Sumner, M.E. (Ed.), Handbook of Soil Science. CRC Press, Boca Raton, USA, pp. 25–84.
Barto, E.K., Rillig, M.C., 2010. Does herbivory really suppress mycorrhiza? A meta-analysis. J. Ecol. 98, 745–753.
Bastida, F., Moreno, J.L., Hernández, T., García, C., 2006. Microbiological activity in a soil 15 years alter its devegetation. Soil Biol. Biochem. 38, 2503–2507.
Baum, C., Makeschin, F., 2000. Effects of nitrogen and phosphorus fertilization on mycorrhizal formation of two poplar clones (*Populus trichocarpa* and *P. tremula* × *tremuloides*). J. Plant Nutr. Soil Sci. 163, 491–497.
Berthrong, S.T., Jobbágy, E.G., Jackson, R.B., 2009a. A global meta-analysis of soil exchangeable cations, pH, carbon, and nitrogen with afforestation. Ecol. Appl. 19, 2228–2241.
Berthrong, S.T., Schadt, C.W., Piñeiro, G., Jackson, R.B., 2009b. Afforestation Alters the composition of functional genes in soil and biogeochemical processes in South American Grasslands. Appl. Environ. Microb. 75, 640–6248.
Bever, J.D., Morton, J.B., Antonovics, J., Schultz, P.A., 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. J. Ecol. 84, 71–82.
Binkley, D., Resh, S., 1999. Rapid changes in soils following *Eucalyptus* afforestation in Hawaii. Soil Sci. Soc. Am. J. 63, 222–225.
Block, R.M.A., Van Rees, K.C.J., Knight, J.D., 2006. A review of fine root dynamics in *Populus* plantations. Agroforest. Syst. 76, 73–84.
Bloem, J., Hopkins, D.W., Benedetti, A., 2006. Microbiological Methods for Assessing Soil Quality. CABI, Wallingford, UK.
Boehmel, C., Lewandowski, I., Claupein, W., 2008. Comparing annual and perennial energy cropping systems with different management intensities. Agric. Syst. 96, 224–236.
Bonari, E., Picchi, G., Ginanni, M., Fraga, A., Guidi, W., 2004. Poplar short rotation coppice behaviour under different harvesting treatments. In: Van Swaaij, W.P.M., Fjällström, T., Helm, P., Grassi, A. (Eds.), Second World Biomass Conference. ETA, Florence, pp. 237–239.
Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen – total. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, 2nd ed. Agronomy Monograph 9. American Society of Agronomy, Madison, WI, pp. 595–624.
Brussaard, L., Behan-Pelletier, V.M., Bignell, D.E., Brown, V.K., Didden, W., Folgarait, P., Fragoso, C., Freckman, D.W., Gupta, V.V.S.R., Hattori, T., Hawksworth, D.L.,

Klopatek, C., Lavelle, P., Malloch, D.W., Rusek, J., Soderstrom, B., Tiedje, J.M., Virginia, R.A., 1997. Biodiversity and ecosystem functioning in soil. Ambio 26, 563–570.
Calvente, R., Cano, C., Ferrol, N., Azcon-Aguilar, C., Barea, J.M., 2004. Analysing natural diversity of arbuscular mycorrhizal fungi in olive tree (*Olea europaea* L.) plantations and assessment of the effectiveness of native fungal isolates as inoculants for commercial cultivars of olive plantlets. Appl. Soil Ecol. 26, 11–19.
Carter, M.R., Rennie, D.A., 1982. Changes in soil quality under zero tillage farming systems: distribution of microbial biomass and mineralisation C and N potentials. Can. J. Soil Sci. 62, 587–597.
Chen, C.R., Condron, L.M., Davis, M.R., Sherlock, R.R., 2003. Seasonal changes in soil phosphorus and associated microbial properties under adjacent grassland and forest in Zew Zealand. Forest Ecol. Manage. 177, 539–557.
Chifflot, V., Rivest, D., Olivier, A., Cogliastro, A., Khasa, D., 2009. Molecular analysis of arbuscular mycorrhizal community structure and spores distribution in tree-based intercropping and forest systems. Agric. Ecosyst. Environ. 131, 32–39.
Coleman, M.D., Isebrands, J.G., Tolsted, D.N., Tolbert, V.R., 2004. Comparing soil carbon of short rotation poplar plantations with agricultural crops and woodlots in North Central United States. Ecol. Manage. 33, S299–S308.
Daniell, T.J., Husband, R., Fitter, A.H., Young, J.P.W., 2001. Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. Fems Microbiol. Ecol. 36, 203–209.
Deckmyn, G., Laureysens, I., Garcia, J., Muys, B., Ceulemans, R., 2004. Poplar growth and yield in short rotation coppice: model simulations using the process model SECRETS. Biomass Bioenerg. 26, 221–227.
Del Val, C., Barea, J.M., Azcón-Aguilar, C., 1999. Diversity of arbuscular mycorrhizal fungus populations in heavy-metal-contaminated soils. Appl. Environ. Microbiol. 65, 718–723.
Di Bene, C., 2003. No-Tillage Management as Instrument to Mitigate CO_2 Emissions by Increasing Organic Carbon Pool in Agricultural Soils. MSc thesis. University of Pisa, Pisa, Italy.
Doran, J.W., 2002. Soils health and global sustainability: translating science into practice. Agric. Ecosyst. Environ. 88, 119–127.
Doran, J.W., Parkin, T.B., 1996. Quantitative indicators of soil quality: a minimum data set. In: Doran, J.W., Jones, A.J. (Eds.), Methods for Assessing Soil Quality. SSSA, Inc., Madison, WI, USA.
Eason, W.R., Scullion, J., Scott, E.P., 1999. Soil parameters and plant responses associated with arbuscular mycorrhizas from contrasting grassland management regimes. Agric. Ecosyst. Environ. 73, 245–255.
Fließbach, A., Oberholzer, H.R., Gunst, L., Mäder, P., 2007. Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agric. Ecosyst. Environ. 118, 273–284.
Frazão, L.A., Piccolo, M.C., Feigl, B.J., Cerri, C.C., Cerri, C.E.P., 2010. Inorganic nitrogen, microbial biomass and microbial activity of a sandy Brazilian Cerrado soil under different land uses. Agric. Ecosyst. Environ. 135, 161–167.
Gasol, C.M., Brun, F., Mosso, A., Rieradevall, J., Gabarrell, X., 2010. Economic assessment and comparison of acacia energy crop with annual traditional crops in Southern Europe. Energ. Policy 38, 592–597.
Gemma, J.N., Koske, R.E., Carreiro, M., 1989. Seasonal dynamics of selected species of VA mycorrhizal fungi in a sand dune. Mycol. Res. 92, 317–321.
Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84, 489–500.
Gollotte, A., van Tuinen, D., Atkinson, D., 2004. Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. Mycorrhiza 14, 111–117.
Guidi, W., Piccioni, E., Bonari, E., 2008. Evapotranspiration and crop coefficient of poplar and willow short rotation coppice used as vegetation filter. Bioresour. Technol. 99, 4832–4840.
Guo, X.Y., Zhang, X.S., Huang, Z.Y., 2010. Drought tolerance in three hybrid poplar clones submitted to different watering regimes. J. Plant Ecol. 3, 79–87.
Gupta, V.V.S.R., Roper, M.M., Kirkegaard, J.A., Angus, J.F., 1994. Changes in microbial biomass and organic matter levels during the first year of modified tillage and stubble management practices on a red earth. Aust. J. Soil Res. 32, 1339–1354.
Hansen, E.A., 1993. Soil carbon sequestration beneath hybrid poplar plantations in the north central United States. Biomass Bioenerg. 5, 431–436.
Hart, M., Klironomos, J.N., 2002. Diversity of arbuscular mycorrhizal fungi and ecosystem functioning. In: Van Der Heijden, M.G.A., Sanders, I.R. (Eds.), Mycorrhizal Ecology. Springer-Verlag, Heidelberg, Germany, pp. 225–242.
Helgason, T., Daniell, T.J., Husband, R., Fitter, A.H., Young, J.P.W., 1998. Ploughing up the wood-wide web? Nature 394, 431.
Helgason, T., Fitter, A.H., Young, J.P.W., 1999. Molecular diversity of arbuscular mycorrhizal fungi colonising *Hyacinthoides non-scripta* (bluebell) in a seminatural woodland. Mol. Ecol. 8, 659–666.
Hinchee, M., Rottmann, W., Mullinax, L., Zhang, C., Chang, S., Cunningham, M., Pearson, L., Nehra, N., 2009. Short-rotation woody crops for bioenergy and biofuels applications. In Vitro Cell. Dev. Biol.-Plant 45, 619–629.
IUSS Working Group WRB, 2006. World reference base for soil resources 2006. World Soil Resources Reports No. 103. FAO, Rome.
Johnson, D., Vandenkoornhuyse, P.J., Leake, J.R., Gilbert, L., Booth, R.E., Grime, J.P., Young, J.P.W., Read, D.J., 2003. Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. New Phytol. 161, 503–515.
Jordan, N., Boody, G., Broussard, W., Glover, J.D., Keeney, D., McCown, B.H., McIsaac, G., Muller, M., Murray, H., Neal, J., Pansing, C., Turner, R.E., Warner, K., Wyse, D., 2007. Sustainable development of the agricultural bio-economy. Science 316, 1570–1571.
Kahle, P., Baum, C., Boelcke, B., Kohl, J., Ulrich, R., 2010. Vertical distribution of soil properties under short-rotation forestry in Northern Germany. J. Plant Nutr. Soil Sci. 173, 737–746.
Kahle, P., Hildebrand, E., Baum, C., Boelcke, B., 2007. Long-term effects of short rotation forestry with willows and poplar on soil properties. Arch. Acker. Pfl. Boden. 53, 673–682.
Karlen, D.L., Mausbach, M.J., Doran, J.W., Cline, R.G., Harris, R.F., Schuman, G.E., 1997. Soil quality: a concept, definition, and framework for evaluation. Soil Sci. Soc. Am. J. 61, 4–10.
Karp, A., Shield, I., 2008. Bioenergy from plants and the sustainable yield challenge. New Phytol. 179, 16–32.
Kauter, D., Lewandowski, I., Claupein, W., 2003. Quantity and quality of harvestable biomass from *Populus* short rotation coppice for solid fuel use – a review of the physiological basis and management influences. Biomass Bioenerg. 24, 411–427.
Koide, R., 2000. Functional complementarity in the arbuscular mycorrhizal symbiosis. New Phytol. 147, 233–235.
Koske, R.E., Gemma, J.N., 1997. Mycorrhizae and succession in plantings of beachgrass in sand dunes. Am. J. Bot. 84, 118–130.
Kutsch, W.L., Schimel, J., Denef, K., 2009. Measuring soil microbial parameters relevant for soil carbon fluxes. In: Kutsch, W., Bahn, M., Heinemeyer, A. (Eds.), Soil Carbon Dynamics. An Integrated Methodology. Cambridge University Press, Cambridge, UK, pp. 169–186.
Laganière, J., Angers, D.A., Paré, D., 2010. Carbon accumulation in agricultural soils after afforestation: a meta-analysis. Global Change Biol. 16, 439–453.
Larson, W.E., Pierce, F.J., 1991. Conservation and enhancement of soil quality. In: Dumanski, J., Pushparajah, E., Latham, M., Myers, R. (Eds.), Evaluation for Sustainable Land Management in the Developing World. Vol. 2. Technical Papers. Proceedings of International Workshop, Chiang Mai, Thailand, 15–21 September 1991. International Board for Soil Research and Management, Bangkok, Thailand, pp. 175–203.
Lekberg, Y., Koide, R.T., Rohr, J.R., Aldrich-Wolfe, L., Morton, J.B., 2007. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. J. Ecol. 95, 95–105.
Lemus, R., Lal, R., 2005. Bioenergy crops and carbon sequestration. Crit. Rev. Plant Sci. 24, 1–21.
Lopez-Aguillon, R., Garbaye, J., 1990. Some aspects of a double symbiosis with ectomycorrhizal and VAM fungi. Agric. Ecosyst. Environ. 29, 263–266.
Luo, Y., Zhou, X., 2006. Soil Respiration and the Environment. Academic Press/Elsevier, San Diego, CA, USA.
Makeschin, F., 1994. Effects of energy forestry on soils. Biomass Bioenerg. 6, 63–79.
Mao, R., Zeng, D.H., 2010. Changes in soil particulate organic matter, microbial biomass, and activity following afforestation of marginal agricultural lands in a semi-arid area of Northeast China. Environ. Manage. 46, 110–116.
Mazzoncini, M., Canali, S., Giovannetti, M., Castagnoli, M., Tittarelli, F., Antichi, D., Nannelli, R., Cristani, C., Bàrberi, P., 2010. Comparison of organic and conventional stockless arable systems: A multidisciplinary approach to soil quality evaluation. Appl. Soil Ecol. 44, 124–132.
Mazzoncini, M., Di Bene, C., Coli, A., Petri, M., Antichi, D., Bonari, E., 2008. Rainfed wheat and soybean performance in a long-term tillage experiment in central Italy. Agron. J. 100, 1418–1429.
McGonigle, T.P., Miller, M.H., 1996. Development of fungi belowground in association with plants growing in disturbed and undisturbed soils. Soil Biol. Biochem. 28, 263–269.
McLean, E.O., 1982. Soil pH and lime requirement. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, 2nd ed. Agronomy Monograph 9. American Society of Agronomy, Madison, WI, pp. 199–224.
Mummey, D.L., Rillig, M.C., 2008. Spatial characterization of arbuscular mycorrhizal fungal molecular diversity at the sub-metre scale in a temperate grassland. FEMS Microbiol. Ecol. 64, 260–270.
Nassi o Di Nasso, N., Guidi, W., Ragaglini, G., Tozzini, C., Bonari, E., 2010. Biomass production and energy balance of a 12-year old short-rotation coppice poplar stand under different cutting cycles. Global Change Biol. Bioenerg. 2, 89–97.
Nelson, D.W., Sommers, L.E., 1982. Total carbon, organic carbon and organic matter. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, 2nd ed. Agronomy Monograph 9. American Society of Agronomy, Madison, WI, pp. 539–579.
Oehl, F., Sieverding, E., Ineichen, K., Mader, P., Boller, T., Wiemken, A., 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. Appl. Environ. Microbiol. 69, 2816–2824.
Oehl, F., Sieverding, E., Mader, P., Dubois, D., Ineichen, K., Boller, T., Wiemken, A., 2004. Impact of long-term conventional an organic farming on the diversity of arbuscular mycorrhizal fungi. Oecologia 138, 574–583.
Olsen, S.R., Sommers, L.E., 1982. Phosphorus. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, 2nd ed. Agronomy Monograph 9. American Society of Agronomy, Madison, WI, pp. 403–430.
Öpik, M., Moora, M., Liira, J., Zobel, M., 2006. Composition of root-colonising arbuscular mycorrhizal fungal communities in different ecosystems around the globe. J. Ecol. 94, 778–790.
Paustian, K., Collins, H.P., Paul, E.A., 1997. Management controls on soil carbon. In: Paul, E.A., Elliott, E.T., Paustian, K., Cole, C.V. (Eds.), Soil Organic Matter in Temperate Agroecosystems. CRC Press, Boca Raton, FL, pp. 15–49.
Pellegrino, E., 2007. Mycorrhiza for the Sustainable Management of Agroecosystems. Field Studies on Molecular and Functional Biodiversity of

Indigenous and Introduced Arbuscular Mycorrhizal Fungi. PhD Thesis. University of Pisa, Pisa, Italy.
Pellegrino, E., Bedini, S., Avio, L., Bonari, E., Giovannetti, M., 2010. Field inoculation effectiveness of native and exotic arbuscular mycorrhizal fungi in a Mediterranean agricultural soil. Soil Biol. Biochem., doi:10.1016/j.soilbio.2010.11.002.
Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55, 158–161.
Picci, G., Nannipieri, P., 2002. Metodi di analisi microbiologica del suolo. Franco Angeli, Roma, Italy.
Pietikaïnen, J., Fritze, H., 1995. Clear-cutting and prescribed burning in coniferous forest, comparison of effects on soil fungal and total microbial biomass, respiration and nitrification. Soil Biol. Biochem. 27, 101–109.
Porter, W.M., 1979. The most probable number method for enumerating infective propagules of vesicular arbuscular fungi in soil. Aust. J. Soil Res. 17, 515–519.
Renker, C., Weißhuhn, K., Kellner, H., Buscot, F., 2006. Rationalizing molecular analysis of field-collected roots for assessing diversity of arbuscular mycorrhizal fungi: to pool, or not to pool, that is the question. Mycorrhiza 16, 525–531.
Rhoades, J.D., 1982. Soluble salts. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, 2nd ed. Agronomy Monograph 9. American Society of Agronomy, Madison, WI, pp. 167–179.
Ritter, E., 2007. Carbon, nitrogen and phosphorus in volcanic soils following afforestation with native birch (*Betula pubescens*) and introduced larch (*Larix sibirica*) in Iceland. Plant Soil 295, 239–251.
Rooney, D.C., Killham, K., Bending, G.D., Baggs, E., Weih, M., Hodge, A., 2009. Mycorrhizas and biomass crops: opportunities for future sustainable development. Trends Plant Sci. 14, 542–549.
Sartori, F., Lal, R., Ebinger, M.H., Eaton, J.A., 2007. Changes in soil carbon and nutrient pools along a chronosequence of poplar plantations in the Columbia Plateau, Oregon, USA. Agric. Ecosyst. Environ. 122, 325–339.
Schenck, N.C., Pérez, Y., 1990. Manual for the Identification of VA Mycorrhizal Fungi, 3rd ed. Synergistic Publications, Gainesville, FL, USA.
Schultz, P.A., Bever, J.D., Morton, J., 1999. *Acaulospora colossica* sp. nov. from an old field in North Carolina and morphological comparisons with similar species, A. laevis and A. koskei. Mycologia 91, 676–683.
Sieverding, E., 1991. Vesicular-Arbuscular Mycorrhiza Management in Tropical Agrosystems. GTZ, Eschborn, Germany, p. 371.
Smal, H., Olszewska, M., 2008. The effect of afforestation with Scots pine (*Pinus silvestris* L.) of sandy post-arable soils on their selected properties. II. Reaction, carbon, nitrogen and phosphorus. Plant Soil 305, 171–187.
Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., Roskot, N., Heuer, H., Berg, G., 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing Gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Appl. Environ. Microb. 67, 4742–4751.
Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis, 3rd ed. Academic Press, Amsterdam.
Soil Survey Staff, 1975. Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys. USDA-SCS Agric. Handb. 436. U.S. Gov. Print. Office, Washington, DC.
Stukenbrock, E.H., Rosendahl, S., 2004. Clonal diversity and population genetic structure of arbuscular mycorrhizal fungi (*Glomus* spp.) studied by multilocus genotyping of single spores. Mol. Ecol. 14, 743–752.
ter Braak, C.J.F., Šmilauer, P., 2002. CANOCO Reference Manual and CanoDraw for Windows User's guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power, Ithaca.
van der Heijden, M.G.A., Sanders, I.R., 2002. Mycorrhizal Ecology. Springer-Verlag, Berlin.
Van Huylenbroeck, G., Durand, G., 2003. Multifunctional Agriculture: a New Paradigm for European Agriculture and Rural Development. Ashgate Publishing Limited, Aldershot.
Vandenkoornhuyse, P., Husband, R., Daniell, T.J., Watson, I.J., Duck, J.M., Fitter, A.H., Young, J.P.W., 2002. arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. Mol. Ecol. 11, 1555–1564.
Wolfe, B.E., Mummey, D.L., Rillig, M.C., Klironomos, J.N., 2007. Small-scale spatial heterogeneity of arbuscular mycorrhizal fungal abundance and community composition in a wetland plant community. Mycorrhiza 17, 175–783.
Zornoza, R., Mataix-Solera, J., Guerrero, C., Arcenegui, V., Mataix-Beneyto, J., 2009. Comparison of soil physical, chemical, and biochemical properties among native forest, maintained and abandoned almond orchards in mountainous areas of Eastern Spain. Arid Land Res. Manage. 23, 267–282.