



Perennial energy cropping systems affect soil enzyme activities and bacterial community structure in a South European agricultural area



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ABSTRACT

In recent decades, perennial rhizomatous grasses have been introduced in the Po Valley (Northern Italy), not only to produce bioenergy, but also to face the loss of soil organic carbon due to intensive crop management. Given the dual purpose of perennial energy crops, this work was intended to evaluate changes induced by the introduction of these crops on soil microbial community structure and on soil functionality. We compared a 9 year-old land conversion to two perennial energy crops, giant *Miscanthus* (*Miscanthus sinensis* × *giganteus*) and giant reed (*Arundo donax* L.), with two 40-year old annual arable systems, continuous wheat and maize/wheat rotation. The structure of the bacterial community was studied by the fingerprinting method of denaturing gradient gel electrophoresis (PCR-DGGE) amplifying 16S rRNA fragments, while the functional aspects of soil were investigated through the determination of three soil enzyme activities involved in soil carbon, nitrogen, and phosphorous cycles (β -glucosidase, urease, and alkaline phosphatase, respectively). Introduction of perennial energy crops positively stimulated the three soil enzymes, especially in the shallow soil layer (0–0.15 m), where accumulation of carbon and nitrogen was stronger. Enzyme activities were also positively correlated with organic carbon, apart from β -glucosidase. A significant but weaker correlation was also observed between enzyme activities and total nitrogen. The DGGE profiles revealed the relationship between crop types and soil microbial communities. Community richness was higher in perennial than in annual crops, but no effect of soil depth was observed. In opposition, Shannon index of diversity was not influenced by crop type, but only by soil depth with a 32% increase in the shallow layer. We conclude that the introduction of perennial energy crops in a South European soil increases both soil biochemical activity and microbial diversity, related to the ability of these crops to stabilize organic matter in soil. It is thereby evidenced that perennial rhizomatous grasses for energy uses could represent a sustainable choice for the recovery of soils depleted by intensive agricultural management.

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1. Introduction

Perennial energy crops, such as giant *Miscanthus* (*Miscanthus sinensis* × *giganteus* Greef and Deuter) and giant reed (*Arundo donax* L.) have been proposed as potential biofuel feedstocks in alternative to fossil fuels. The interest in these crops as a

component in a portfolio of climate mitigation measures is promoting their diffusion in many agricultural areas of the world (Sims et al., 2006; Rechberger and Lötjönen, 2009; International Energy Agency, 2013).

The introduction of perennial species is expected to take place at the expense of traditional annual crops with consequent changes in applied agricultural techniques. Main differences in management of perennial crops with respect to traditional arable ones are represented by reduced soil disturbance by tillage and lower needs of fertilizers and irrigation (Fazio and Barbanti, 2014). While the effects of the land conversion to perennial energy crops on soil organic carbon (SOC) accumulation and on

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soil microbial activity is well documented (Hakey et al., 2010; Mao et al., 2011; Piotrowska and Wilczewski, 2012), little information concerning the impact of perennial energy crops on soil microbial functionality is available.

This knowledge is crucial for the development of a sustainable agriculture, as soil microorganisms represent an integrative component of soil quality because of their involvement in many ecosystem processes (e.g., organic matter decomposition and nutrient cycling, N₂ fixation, aggregate formation and stabilization) (Schutter et al., 2001; Mao et al., 2011; Werling et al., 2014). Due to this reason, soil microbiota represents an early and sensitive indicator of soil quality changes (Schlotter et al., 2003; Bending et al., 2004; Winding et al., 2005).

It has been demonstrated that perennial crop residues and plant root exudates may either stimulate or inhibit the growth and activity of different fractions of the soil microbial community; thus, the cultivation of different crops may result in distinct microbial communities (Mao et al., 2011). Previous studies have shown that, when perennial grasslands sustain more microbial biomass than annual cropping systems (Culman et al., 2010; Werling et al., 2014), these differences are mainly due to two mechanisms: perenniality and tillage (Frey et al., 1999; Drijber et al., 2000). Annual crops, like maize or wheat, have a shallower root apparatus with a lower density than perennial species (Jackson et al., 1996). On the contrary, through permanent root turnover, perennial crops can allocate more resources belowground and maintain more complex food webs, accommodating a larger number of beneficial microorganisms. Secondly, under perennial energy crops, soil disturbance by tillage is limited to the year of their establishment, while it is annually required by annual crops. As demonstrated by many authors, soil tillage is assumed to be deleterious to microbial biomass (Frey et al., 1999; Drijber et al., 2000; Van Groenigen et al., 2010) and diversity (Acosta-Martinez et al., 2010; Cañizares et al., 2012).

Based on the literature, most of the studies on the introduction of perennial species to improve soil quality refer to giant *Miscanthus* and they are largely carried out in Atlantic and Continental areas of Northern Europe. In contrast, there is a lack of information on the effects of these crops in warm-temperate environments of Southern Europe. This aspect is fundamental, considering that the wide range of climatic conditions from Northern to Southern Europe influences biomass production of perennial energy crops, and therefore, soil organic matter input (Lewandowski et al., 2003). As the potential of giant *Miscanthus* and giant reed to increase organic C storage also in agricultural soils of Southern Europe has already been demonstrated (Cattaneo et al., 2014), what we focused in this study were the effects of these crops on soil biological functionality and microbial communities. This study was performed in the South-Eastern Po Valley (Northern Italy), a warm-temperate site, included in the Mediterranean-North environmental zone (Metzger et al., 2005). Compared to more continental areas, this agro-ecosystem is characterized by a rapid C turnover in soil and by low SOC levels (<10 g C kg⁻¹ in the upper 0.30 m; ARPA, 2009), due to intensive anthropic practices and to warmer climatic conditions. We compared a 9 year-old land conversion to giant *Miscanthus* and giant reed with two 40-year old annual systems. We investigated similarities and differences in composition and diversity of soil microbial communities between soils under annual and perennial species, by the fingerprinting method of PCR-DGGE.

Because of their key role in the biochemical functioning of soils, we measured the activity of three soil enzymes involved in soil carbon, nitrogen and phosphorous cycles (β -glucosidase, urease, and alkaline phosphatase, respectively), to evaluate the functional aspects of soil under annual and perennial crops.

2. Materials and methods

2.1. Experimental site and crop management

The field experiment was located at the experimental farm of the University of Bologna (Italy), in Cadriano (44°33'N, 11°21'E, 32 m a.s.l.). The soil is classified as a fine silty, mixed, mesic udic Ustochrepts (USDA Soil Taxonomy). The climate is warm-temperate with mean annual precipitation of 700 mm, mainly concentrated in the cold semester, and mean temperature of 13.3 °C. In this study, we compared a 9-year old land conversion to the perennial energy crops, giant *Miscanthus* (*Miscanthus sinensis* × *giganteus* Greef and Deuter) and giant reed (*Arundo donax* L.) (names according to IAPT, 2012), with two respective 40-year old annual systems, continuous wheat (*Triticum aestivum* L.) and maize/wheat (*Zea mays* L./*Triticum aestivum* L.) crop rotation. Giant *Miscanthus* is a perennial rhizomatous C₄ grass, native to East Asian tropical and subtropical regions, with a considerable biomass potential in a variety of environmental conditions (Lewandowski et al., 2000). Giant reed is a perennial rhizomatous C₃ grass that tolerates severe and prolonged drought conditions and can grow in different soil conditions (Lewandowski et al., 2003). The two annual crop systems were established in 1966, in 32 m² experimental plots with four replicates. The two perennial energy crops, giant *Miscanthus* and giant reed, were established in 2002, in 90 m² experimental plots with four replicates, in soils previously cultivated with annual C₃ and C₄/C₃ species, respectively. In both annual and perennial crops, 120 kg of N ha⁻¹ was annually applied as urea, during the spring time. Moreover, 100 kg P₂O₅ ha⁻¹, as triple superphosphate, was annually applied in maize/wheat and 50 kg P₂O₅ ha⁻¹ in continuous wheat. In perennial crops, 100 kg P₂O₅ ha⁻¹ was added prior to planting in 2002. No K fertilizer was applied, given the sufficient level of this nutrient. Soil tillage at 0.30 m was carried out every year in annual crops; only prior to planting (autumn 2001) in perennial ones. In both annual and perennial species, the rest of crop husbandry reflected the normal practices followed in the experimental area. Perennial crops were annually harvested in September/October, while annual crops were harvested at maturity (wheat, early summer; maize, late summer), and the recoverable fraction of their residues was always removed from the field.

2.2. Soil sampling

Soil sampling took place in March 2011 at the beginning of the 10th growing season of perennial energy crops, using a 85 mm diameter soil corer up to a depth of 0.60 m, divided in three layers (0–0.15, 0.15–0.30, 0.30–0.60 m). For both annual and perennial crops, two replicates were taken according to a completely randomized design. Each replicate was composed by three subsamples, which were put together and transported to the laboratory the same day. Soil samples for microbiological analysis were subsequently wet sieved (4 mm mesh sieve) and stored at –20 °C. Soil samples for chemical analysis (C_{ORG}, N_{TOT} and P_{AVAIL} contents) were wet sieved (2 mm mesh sieve), removing visible debris, and then air-dried.

A chemical and textural characterization of the studied soils was carried out, according to the current Italian methods of soil analysis (D.M. 13/09, 1999). Results were grouped for crop type because very similar and averaged:

- Annual crops: pH_{H₂O} 6.1; sand 34.5%; silt 42%; clay 23.5%; active/total limestone <0.1/<0.5, respectively; organic carbon 6.9 g kg⁻¹; total nitrogen 0.9 g kg⁻¹; C:N ratio 8; available phosphorous 26.4 mg kg⁻¹, exchangeable K 152 mg kg⁻¹.

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