Applied Soil Ecology 62 (2012) 42–49

Contents lists available at SciVerse ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Assessment of *Miscanthus* × *giganteus* for rhizoremediation of long term PAH contaminated soils

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ARTICLE INFO

Article history:
Received 11 October 2011
Received in revised form 8 June 2012
Accepted 24 July 2012

Keywords:
PAH
Rhizoremediation
Polluted soils
Miscanthus × *giganteus*
Rehabilitation
Industrial crop

ABSTRACT

The purpose of this study was to investigate the potential of *Miscanthus* × *giganteus* (M × G) for rhizoremediation of long term PAH-polluted soils. To evaluate its growth ability on contaminated substrates, a pot experiment was a pot experiment was conducted. Plant development was compared to that obtained in a reference soil. Pollutant dissipation with several other physico-chemical and microbiological parameters (including bacterial community diversity molecular analysis) were investigated in order to better characterize the rhizosphere effects of M × G. Field trials were also conducted to confirm the feasibility of crop installation in situ. Plants demonstrated a physiological adaptation to soils from various PAH contamination levels (from 26 to 364 mg PAH kg^{-1} dry soil) both in laboratory and in field scale conditions. Changes in rhizosphere bacterial community were observed via specific bacterial phylotype selection in the root vicinity. Despite the lack of conclusive trends for phytoremediation, slight decreases in total 4-ring PAH concentration would suggest a positive influence of growing plants in the long term. Furthermore, significant organic carbon inputs and nitrate losses were measured after 17 weeks of laboratory cultivation, indicating a global improvement of soil agronomic quality.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are composed of two or more fused benzene-rings in linear, angular or cluster arrangements (Blumer, 1976; Cerniglia, 1992). These pollutants are commonly found in air, water, sediment and soil subsurfaces of industrialized countries, since the most prominent source of emission is related to anthropogenic processing of fossil fuels (Blumer, 1976; Wilcke, 2007). Once introduced into soils, PAH show a strong affinity for organic phases due to their high hydrophobicity (Blumer, 1976; Bogan and Sullivan, 2003). They may dissolve in non-aqueous liquid phase (NAPL), partition onto coal tar particles or in organic matter and become progressively sequestered in soil micropores (Bogan and Sullivan, 2003). Such "aging" or "weathering" processes may conduce to PAH accumulation for many years (Bogan and Sullivan, 2003). Therefore, soils can act as "sinks" for these contaminants (Blumer, 1976; Bogan and Sullivan, 2003; Wilcke, 2007). Because of their hazard risk to human health and to living organisms, with potential carcinogenic and mutagenic properties, PAH removal from the environment is an important concern (Bouchez et al., 1995).

Among the several technologies that have been tested for PAH dissipation in soils during the last decades, bioremediation through bacterial degradation has been shown to be one of the most environmentally friendly and promising technique (Haritash and Kaushik, 2009). Indeed, the chemically stable PAH molecules become more reactive following the enzymatic action of microbial dioxygenases that catalyze successive ring oxidations and fissions, allowing for progressive mineralization of the mother compound (Cerniglia, 1992).

Biodegradation of organic contaminants in soils has been demonstrated to be stimulated in the vicinity of plant roots, mainly due to the enhancement of microbial growth and activity following root exudation (Aprill and Sims, 1990; Gerhardt et al., 2009). The interrelationships between plants and soil microorganisms involved in the breakdown of pollutants lead to the formulation of the "rhizoremediation technology" (Aprill and Sims, 1990; Nichols et al., 1997). Finally, it appears that such a plant-assisted remediation technology has the advantages of being: (i) less expensive than

Abbreviations: M × G, *Miscanthus* × *giganteus*; PAH, polycyclic aromatic hydrocarbon; PCR–TGGE, polymerase chain reaction–temperature gradient gel electrophoresis; ch., chlorophyll.

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http://dx.doi.org/10.1016/j.apsoil.2012.07.009

engineered physicochemical techniques due to its solar-energy driven approach (root exudation is dependent on initial CO_2 fixation by plants), (ii) particularly effect-efficient as a "polishing" method with generally no additional soil contamination, and (iii) substantial facilities to be implemented in situ when treating large surface areas of contaminated soils (Gerhardt et al., 2009; Haritash and Kaushik, 2009; Schwitzguébel et al., 2002).

In spite of its mentioned advantages, the efficiency of PAH rhizodegradation may be mitigated due to the presence of other inorganic and organic pollutants. Indeed, polluted soils located in former industrial sites involved in petroleum and coke transformation are often characterized by the presence of heavy metals, cyanides and other co-contaminants (Roy et al., 2005). These latter may induce harmful effects toward plant growth as well as root development and impede subsequent plant-assisted bioremediation in rhizosphere soils (Haritash and Kaushik, 2009; Roy et al., 2005). Therefore, special attention must be devoted to the choice of plants before implementing phytoremediation programs in situ.

Miscanthus × *giganteus* (M × G) is a sterile perennial grass producing a high-yield biomass under temperate climates. Furthermore, this Gramineae is capable of growing on a large variety of soils (Lewandowski et al., 2003). Previous works showed the ability of this crop to develop on domestic sludge amended soils (up to 200 t of material/ha), without any significant heavy metal bioconcentration in aerial part (Fernando et al., 2004). Arduini and his collaborators also reported its tolerance to chromium and cadmium (two metals often found in hydrocarbon industrially polluted soils) following hydroponic studies (Arduini et al., 2006a,b). Moreover, the potential of M × G root exudates to selectively enhance microbial growth and degradation activity of PAH-degrading bacterial consortia was recently demonstrated through in vitro assays (Técher et al., 2011).

The aim of this study was to investigate the growth performance of *Miscanthus* × *giganteus* (biomass production and chlorophyll contents) associated with subsequent PAH dissipation and changes in bacterial communities in two industrially polluted soils different in their hydrocarbon and heavy metal contents. For this purpose, pot experiments were conducted under controlled conditions in the laboratory. Moreover, to address the paucity of information available in the literature concerning simultaneous rhizoremediation with crops and soil quality improvements, several physicochemical parameters (pH, soluble organic carbon, nitrite, nitrate, phosphate and potassium contents) were investigated. Field trials were also conducted in order to test the feasibility of *Miscanthus* × *giganteus* crop cultivation at a regional scale.

2. Materials and methods

2.1. Soil collection and preparation

Two industrially polluted soils, respectively named M and H, were collected at a former coke plant in Northern France. According to the FAO (World Reference Base for Soil Resources, 2006), M and H were classified as Technosol soils. Soil type M came from a site with the following characteristics (BASOL, 2008): a total PAH concentration of 26 mg kg^{-1} dry soil, a heavy metal concentrations (mg kg^{-1} dry soil) in the range of 48–140 for Cr, 530–10,000 for Pb, 880–7700 for Zn and the presence of cyanides varying from 11 to 163 mg kg^{-1} dry soil. Soil type H came from a site with the following characteristics (BASOL, 2008): a total PAH concentration of 364 mg kg^{-1} dry soil, but a lower heavy metal contamination (mg kg^{-1} dry soil) in the range of 58–67 for Cr, 220–290 for Pb, 740–830 for Zn and no detectable cyanide contamination.

Soil preparation before initial (t_0) physicochemical analyses and pot experiments included air drying, sieving (<2 mm) and mixing. Moreover, an artificial soil was used as a referential for *Miscanthus* × *giganteus* growth comparison. This reference soil was reconstituted according to ISO standard protocols (International Standardization Organisation, ISO 11269-2, 1995) and was composed (w/w) of 55% of a commercial potting A3 soil (LUFA Speyer) (He et al., 1995), 42.5% acid-washed sand and 2.5% sphagnum peat. Physicochemical characteristics of the three soils were determined according to standard techniques (Cunningham et al., 1996). The reference soil was classified as a sandy loam soil with a texture of sand (62%), silt (18.5%), clay (19.5%) and a $pH_{water\ of}$ (7.16). The soil type M was a sandy loam soil and had the following composition: sand (66%), silt (20%) and clay (14%), total organic C (11.5%) and C/N (21). The soil type H was a loamy sand soil and presented the following composition: sand (79%), silt (15%), clay (6%), total organic C (8.6%) and C/N (16).

2.2. Experimental design and plant analyses

The greenhouse experiment had a completely randomized block design with five replications that had the following treatments: reference soil planted with M × G, soil types M and H planted with M × G and, soil types M and H unplanted. M × G growth in the two polluted soils M and H was monitored during 17 weeks and compared to growth measured in the reference soil. The unplanted control pots of the two polluted soils were set up in parallel for a better assessment of the effects of planting on soil quality (PAH dissipation, soil physicochemical characteristics and bacterial community changes).

Before planting, rhizomes of M × G from 7 to 10 cm were placed in quartz sand for shoot emergence during two weeks. Then plastic pots were filled with 700 g dry soil, receiving one germinated rhizome (for those planted) and brought to 70% of water-holding capacity using deionised water. Growth conditions included the emission of photosynthetically active radiation at 450 and 650 nm (OSRAM Fluora L36W/77) and a day/night cycle of 16/8 h at 20–23 °C. Stem length at the first internodes was measured weekly.

Plants were harvested after 4.5 months of growth. The last emerged leaf of each plant was weighed and immediately stored into liquid nitrogen for further determination of chlorophyll pigment contents. For this purpose, 80 mg of frozen plant material was mixed with 500 mg glass beads (0.1 mm diameter) and 750 μL N,N-dimethylformamide (DMF) during 5 min. Additional steps of washing (with 1 mL of DMF), centrifugation (12,000 × *g*; 90 s) and mixing were repeated five times to recover all photosynthetic pigments. Absorbances of chlorophyll a and b were read at 664 and 647 nm into a glass microtiter plate and chlorophyll contents calculated according to Inskeep and Bloom (1985) formula. The remaining harvested plant, i.e. rhizomes, roots, stems and leaves were separated to estimate their fresh weight and dry weight to the nearest 0.1 g after 48 h in an oven at 105 °C.

2.3. Soil chemical analyses

The content of the 16 PAH congeners listed as priority pollutants by the US Environmental Protection Agency (US-EPA) was analyzed according to the French standard methods using Soxhlet extraction and HPLC quantification (AFNOR XP X33-012, 2000).

In order to perform the analyses of water extractable soil nutrients, a soil water extraction procedure was set up, comprising 2 h mixing on an orbital shaker with ultrapure water (soil:water ratio (v:v) of 1:5) at ambient temperature. A settling period was maintained for 10 min prior to filtration (0.45 μm). Then, pH and electrical conductivity were directly measured, followed by dissolved organic carbon, nitrite, nitrate, phosphate and potassium contents.