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# Poplar biomass production at phytomanagement sites is significantly enhanced by mycorrhizal inoculation

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## ABSTRACT

Afforestation of trace element (TE)-contaminated soil, notably with fast growing trees producing large biomass has been demonstrated to be an attractive option for their bioremediation. Mycorrhizal fungi can form symbiotic associations with plants, contributing to TE tolerance and participating actively in bioremediation processes. We studied the effects of mycorrhizal inoculation on the growth of two poplar clones (Skado and I214), to large-scale. Two TE-contaminated sites of *ca.* 1 ha (Pierrelaye and Fresnes-sur-Escaut, France) were planted with 2200 trees, and were either inoculated with a mixed commercial inoculum or not-inoculated and allometric parameters were determined after 2 and 4 years of growth. The height diameter relationships remained linear overtime, although the second period of the experiment has been more favorable to growth of the Skado clone and its survival rates were higher compared than those of the I214 clone, at both sites. The inoculation with mycorrhizal significantly impacted the biomass production of the Skado clone at both sites, despite striking differences in soil structure and contamination. In overall, this bioaugmentation option with mycorrhizal fungi influenced more poplar growth than soil contamination and highly improved its biomass production.

## 1. Introduction

Phytomanagement options are attractive alternatives to other remediation technologies due to their relatively low cost, potential effectiveness and the inherently aesthetic nature of using plants to remediate contaminated sites when there is no short-term pressure (Pilon-Smits, 2005). Integrated bioremediation is rapidly gaining acceptance throughout developed countries about pollutant linkage deriving from industrial, agricultural and urban activities (Adriano, 2001). In the United States and Canada specialized firms are patenting and selling plants particularly suited for different phytoremediation and bioremediation purposes (Raskin, 2002). Hyperaccumulator plants are recognised to tolerate and accumulate high concentrations of trace element (TE) in their shoots, which are now regarded as valuable biomasses (Conesa et al., 2012). However, some of these hyperaccumulators are sometimes plant species with reduced growth capability, poor biomass production, highly specific environmental adaptations, such as *Noccaea caerulescens* (Pawlowska et al., 2000; Pilon-Smits, 2005; Borghi et al., 2007). Alternatively, many trees and shrubs show strong environmental adaptations, produce large biomass and can have a significant practical or economical value (Calfapietra et al., 2010). Afforestation is a potentially sustainable reclamation strategy for contaminated land (Dickinson et al., 2009), post-mining sites (Pietrzykowski and Krzaklewski, 2007), and mine spoil (Ciadamidaro et al., 2014), in addition to having several environmental benefits, such as soil stabilization. As a phytostabilization system, woody species can minimize TE uptake by plants and its cycling in the woodland ecosystem on polluted soils. Phytostabilization is not just stabilization of the soil material, but can result in a lower TE mobility and bioavailability for entry into the food web (Vandecasteele et al., 2008). In addition to phytostabilisation, Salicaceous species can also promote phytoextraction of Cd and Zn in their leaves (Konlechner et al.,

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2013), and rhizodegradation of xenobiotics (Nzegung et al., 2004). In particular, poplar is currently one of the most studied species, not only for its phytoremediation activity, but also for its capacity to be a feedstock for the energy sector, through biofuel and green energy (Chalot et al., 2012; Fernandez et al., 2016; Bert et al., 2017). Many authors (*e.g.* Searle and Malins, 2014; Fang et al., 2013) highlighted the advantages of poplar used in short rotation coppice (SRC), such as high biomass and energy yield, ecological interest and comparatively low biomass production costs.

Several studies have been focused on the benefits of microorganisms symbioses with plants growing on TE-contaminated sites, with the aim of possible biotechnological applications such as phytoremediation (Kozdrój et al., 2007; Krupa and Kozdrój, 2007; Gadd, 2010). It is now widely accepted that rhizospheric microorganisms may actively participate to phytoremediation processes, and might be useful in extending the application of phytoremediation to additional TE-contaminated sites (Kuffner et al., 2008). Hosting different mycorrhizal types might be of functional importance for plant nutrition (Smith and Read, 2008) and has been shown to contribute to metal tolerance of host plants as fungi can reduce the metal uptake of plant by sequestration, extracellular precipitation and biosorption to the cell walls (Hildebrandt et al., 2007; Ciccatelli et al., 2014). Numerous data suggest that mycorrhizal fungi play a filtering or sequestering role on plant roots (Leung et al., 2007; Lingua et al., 2008; Arriagada et al., 2010; Li et al., 2014), enhancing root to shoot metal ratio and increasing survival rate in harsh conditions (Laureysens et al., 2005).

Most importantly, selection of adequate trees and microbes is a key feature in a phytoremediation strategy. Significant clonal differences in accumulation were found for most metals within poplar clones (Zalesny et al., 2007; Borghi et al., 2008; Ciccatelli et al., 2014). Extensive variation in clonal responses to leachate irrigation was demonstrated for poplar (Ciccatelli et al., 2014). A high variability occurred for foliar metal concentrations within a selection of 14 poplar clones (Pottier et al., 2015). Black alder is more adapted to the harsh conditions of the post-mining substrate than silver birch and Scots pine (Kuznetsova et al., 2010). Experimental data underline the importance of endogenous microbes, which are presumably more adapted to toxic compounds than exogenous microbes (Colpaert et al., 2004; Sprocati et al., 2006; Hildebrandt et al., 2007). For instance, the isolate *Rhizophagus intraradices* Br1 obtained from a contaminated soil was more effective in transferring TE tolerance to tomato, maize or *Medicago truncatula* than the isolate *R. intraradices* Sy167, which was routinely used in laboratory experiment (Hildebrandt et al., 2007).

Within the present paper, we propose the implementation of an integrated bioremediation option that combines poplar together with arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi, exploiting the complex interactions evolved for the mutual benefit of both organisms, in which plant roots provide habitat, nutrients and exudates to microbial populations, whereas mycorrhizae, increasing mineral and water nutritions, as well as tolerance to TE contamination, improve biomass production of poplars.

## 2. Materials and methods

### 2.1. Site description

Experimental sites are located in Pierrelaye (Ile de France, France, 49° 1′ 47″ N, 2° 10′ 29″ E) and Fresnes-sur-Escaut (Hauts-de-France, France, 50° 25′ 47″ N, 3° 35′ 7″ E) (Fig. 1). The Pierrelaye (P) site was irrigated with raw wastewaters of Paris conurbation from 1899 to 2002, therefore becoming highly enriched in nutrients and OM, thus offering an opportunity for the production of market vegetables, until the late 90's. However, TE accumulated into the soil leading to multi-pollution characterized by Pb, Cu, Zn and Cd concentrations 10 times higher than in a non-irrigated reference soil (Lamy et al., 2006). Fresnes-sur-Escaut (F) is a deposit site of sediments from the nearby Escaut canal. Due to past transport of metal ores and metal smelting industries, sediments were contaminated with TE such as Cd, Zn, As and Pb. Soils at both sites differ in their composition (Table 1). The P soil is sandy whereas the F anthroposol derived from the sediments has a silt loam texture. Climatic conditions during the experimental period are reported in Fig. 2.

### 2.2. Experimental design

Four replicate field blocks were established in February (P) and March (F) 2011 using unrooted poplar cuttings Skado (*P. trichocarpa x P. maximowiczii* section Tacahamaca) and I214 (*P. deltoides x P. nigra* section Aigeiros) of 1.5 m length, planted at an initial spacing of 1.8 m × 2.75 m, with a final plant density of 2.200 trees $ha^{-1}$; each block therefore consisted in 48 trees (6 × 8). Both clones were selected for their growth yield and low TE accumulation capacities in leaves, from a previous clonal assay (PHYTOPOP, Pottier et al., 2015). In each block, half of the stems were inoculated (4 inoculated plots per clone per site) with a mix of commercial inocula as described below, or left not-inoculated (4 control plots per clone per site).

### 2.3. Inoculation protocol

The fungal inoculum consisted of a mixture of commercial inocula provided by two companies. The Agronutrition (Toulouse, France) provided the AM *Rhizophagus irregulare* DAOM 197198 strain. The Symbiom Company (Czech Republic) provided the Symbivit® commercial preparation containing 6 AM fungi (*Rhizophagus intraradices* BEG140, *Funneliformis mosseae* BEG95, *F. geosporum* BEG199, *Claroideoglomus claroideum* BEG96, *C. etunicatum* BEG92, *Glomus microaggregatum* BEG56), as well as the Ectovit® commercial preparation containing 6 ECM fungi (*Hebeloma mesophaeum*, *Amanita rubescens*, *Laccaria proxima*, *Paxillus involutus, Pisolithus arrhizus* and *Scleroderma citrinum*). The average number of propagules was 250,000 per liter and 1800 per liter in Symbivit® and Ectovit®, respectively. The Symbivit® product was first mixed with water (1L of product in 5L of water). The Ectovit® liquid product was added to that preparation (200 mL of product in 5L of water), as well as the Agronutrition inoculum. 100 mL of that mixture was applied to each rootless poplar (for the inoculated treatment), just before recovering the hole with soil. Each tree therefore received approximately 26,000 (25,000 Symbivit® + 180 Ectovit® + 900 Agronutrition) propagules, as recommended by the manufacturers.

### 2.4. Soil chemical analysis

In 2011, in each plot, 10 top-soil (0–20 cm) samples were collected with a hand auger, each being taken at 20 cm from the unrooted planted poplar, and mixed together to form a composite sample which was analyzed. From the 32 soil composites, 8 samples per site were selected based on the total TE concentrations to perform the agronomic characterization. The 8 samples were sent to an external laboratory (Galys, Blois, France) which performed the following analyses: particle size distribution, exchangeable Ca (CaO), Mg (MgO), K ($K_2O$) and Na ($Na_2O$), organic matter (OM) (NF ISO 14235), total nitrogen ($N_t$) (NF ISO 13878), phosphorus ($P_t$) (NF ISO 11263), total $CaCO_3$ (NF ISO 10693), cation exchange capacity (CEC) (NF X 31-130) and soluble boron (B) (NF X 31-122).

Total TE concentrations in soil and sediment samples were determined in 2011, before plantation. The soil samples were dried at 40 °C in a forced air oven to a constant weight, ground with a grinder (agate mortar, Retsch RM100) and sieved to < 200 µm (Retsch AS 200 digit). Total TE concentrations (As, Cd, Cr, Pb, Zn, Cu, Ni) were measured using ICP-OES (Ultima 2, Jobin Yvon Horiba) after acid digestion of 500 mg of sample in a microwave digestion system (Mars Xpress, CEM) using a mix of 2 mL of 67% nitric acid, 6 mL of 34% hydrochloric acid and 2 mL of 48% hydrofluoric acid. To assess the analytical quality, a standard reference sediment material (Buffalo River Sediment,