

Poplar rotation coppice at a trace element-contaminated phytomanagement site: A 10-year study revealing biomass production, element export and impact on extractable elements

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Abstract

 Growing lignocellulosic crops on marginal lands could compose a substantial proportion of future energy resources. The potential of poplar was explored, by devising a field trial of two hectares in 2007 in a metal-contaminated site to quantify the genotypic variation in the growth traits of 14 poplar genotypes grown in short-rotation coppice and to assess element transfer and export by individual genotypes. Our data led us to conclusions about the genotypic variations in poplar growth on a moderately contaminated site, with the Vesten genotype being the most productive. This genotype also accumulated the least amounts of trace 27 elements, whereas the Trichobel genotype accumulated up to 170 mg Zn kg⁻¹ DW in the branches, with large variation being exhibited among the genotypes for trace element (TE) accumulation. Soil element depletion occurred for a range of TEs, whereas the soil content of major nutrients and the pH remained unchanged or slightly increased after 10 years of poplar growth. The higher TE content of bark tissues compared with the wood and the higher proportion of bark in branches compared with the wood led us to recommend that only stem wood be harvested, instead of the whole tree, which will enable a reduction in the risks encountered with TE-enriched biomass in the valorization process.

Keywords: export of trace elements, biomass yield, phytomanagement, poplar genotypes

1. Introduction

 Renewable resources are the focal point of European Union (EU) initiatives to establish energy self-sufficiency in the EU. Woody biomass is a renewable resource and is almost carbon-neutral (Zanchi et al., 2012). Using wood as an energy source is certainly not new; however, second-generation biofuels utilize woody biomass as raw materials to develop liquid fuels, *i.e*., ethanol and diesel within biorefinery projects. Lignocellulosic perennial crops are indeed recognized as feedstock for platform chemicals and lignin-based building blocks (Rohde et al., 2018). The EU has been, and remains, the major global source of demand as a result of its predicted targets for renewable energy. This demand is largely met by the forest resources of the UE and supplemented by imports from the United States, Canada and Russia. Growing lignocellulosic crops on contaminated or marginal lands could make up a substantial proportion of future energy resources (Gelfand et al., 2013). Efforts have been made in the past ten years to link phytotechnologies to the production of valuable biomass products and to the fate of contaminants during industrial processes (Ancona et al., 2019; Asad et al., 2017; Bert et al., 2017; Chalot et al., 2012; Dastyar et al., 2019; Delplanque et al., 2013; Šyc et al., 2012). Despite these efforts, phytotechnologies have not been commercially applied to date (Conesa et al., 2012; Gerhardt et al., 2017) and the use of gentle remediation options (GRO) as practical onsite remedial opportunities is still limited in Europe, particularly at trace- element-contaminated sites (Cundy et al., 2016). This lack of application is mostly due to the lack of long-term field assessments under realistic conditions. A recent article discussed how phytotechnologies could be upgraded from a proven technology to a widely accepted practice (Gerhardt et al., 2017), including the implementation of large-scale field trials in long-term experiments.

 Among useful crops, poplar species and hybrids are considered low-input woody crops, with recognized adaptation capacities to a wide range of site and climatic conditions (Navarro et al., 2014; Verlinden et al., 2013; Yáñez et al., 2019). A useful list of publications on the use of poplar as a renewable resource is provided by the Food and Agriculture Organization of the United Nations (FAO, 2016). This document, to which recent reviews on the use of poplar biomass for platform chemicals (Rohde et al., 2018) and thermal energy (Pandey et al., 2016) can be added, listed the numerous industrial processes that may consider poplar biomass.

 Poplars also readily grow on marginal lands where biomass yields can fall within the range 8-12 tons per hectare per year (Ciadamidaro et al., 2017; Verlinden et al., 2013). The use of *Populus* species has been proposed for phytomanagement purposes because these woody species are characterized by fast-growth, deep-rooting systems and can be managed in short-rotation coppicing plantations (Ciadamidaro et al., 2017; Laureysens et al., 2005, 2004; Marmiroli et al., 2011; Migeon et al., 2009; Rohde et al., 2018; Vangronsveld et al., 2009; Yáñez et al., 2019). The genus *Populus* has traditionally been divided into six sections on the basis of the flower and leaf characters, with approximately 30–40 species occurring worldwide, mainly in the Northern Hemisphere (Polle and Douglas, 2010). Different genotypes of the same poplar species may exhibit variable tolerance to trace elements (TE) because of intraspecific genetic dissimilarity (Castiglione et al., 2009). For instance, a large variation in poplar genotypic responses to leachate irrigation has been found (Zalesny et al., 2007), and high variability occurs in foliar trace element concentrations within a selection of 14 poplar genotypes (Pottier et al., 2015).

 A number of works have been dedicated to the study of poplar responses to soil contamination in controlled pot or hydroponic (Cicatelli et al., 2010; De Oliveira and Tibbett, 2018; Langer et al., 2012; Lingua et al., 2008; Migeon et al., 2012), tank (Zalesny and Bauer, 2007), or lysimeter (Dimitriou and Aronsson, 2011, 2010) set ups. Such experiments are

 needed to study fundamental mechanisms (contaminant transfer between soil-aboveground and below ground plant tissues, interactions between plant and microbes) but may not reflect how trees will behave in natural situations in the long term. Field trials have been implemented, and short-term data are available for a few field experiments (Castiglione et al., 2009; French et al., 2006; Gamalero et al., 2012; Lettens et al., 2011; Sabatti et al., 2014). However, long-term studies are scarce (Evangelou et al., 2012; Foulon et al., 2016a, 2016b; Mertens et al., 2007) but are crucial to the study of long-term impacts of tree plantation on pedological and biological soil characteristics.

 The PHYTOPOP project aimed to explore the potential of poplar for phytomanagement purposes, by setting up a field trial of two hectares in 2007 to produce enough biomass for further valorization and estimate productivity at a realistic scale. The study was based on the utilization of a large panel of poplar cultivars, planted in a short rotation coppice (SRC) scheme. During the project, the harvesting process was also investigated from an economic point of view, and analyzed poplar behavior in a combustion unit (Chalot et al., 2012). The PHYTOPOP biomasses were further used to assess the fate of TE during pyrolysis (Bert et al., 2017) and bioethanol production (Asad et al., 2017). The mechanisms controlling metal content in the leaves and the expression of genes that may be involved in metal transport were also studied (Migeon et al., 2010; Pottier et al., 2015)*.* However, in none of these papers we evaluated biomass production at this specific site. We indeed wanted to obtain long-term data, which are lacking in the literature, to provide some recommendations to end-users.

 The present paper aimed to determine the impacts of a PHYTOPOP plantation on soil characteristics, TE accumulation and poplar yields after one rotation. The specific objectives were 1) to quantify the genotypic variation in the growth traits of 14 poplar genotypes grown in SRC systems at a TE contaminated site and 2) to assess element transfer and export by the different genotypes.

2. Materials and methods

2.1. Site description and experimental design

 The experimental site is located in the Northwest region of Paris at Pierrelaye (49°01'47.6"N, 2°10'34.2"E). The whole area has been irrigated with raw wastewaters from 119 the end of the $19th$ century until the beginning of the $21st$ century to increase the soil fertilization potential and has been dedicated to vegetable gardening for more than one century (Baize et al., 2002, Dère et al., 2007, Lamy et al., 2006). However, this resulted in the accumulation of TE such as Pb, Cu, Zn and Cd in the first 30-50 cm of soil layer. This leads to poly-metallic pollution characterized by TE concentrations that could reach levels more than 10-fold greater than those of a non irrigated local reference soil.

 Poplars were planted in April 2007 as a short-rotation coppice (SRC) at densities of 1000 stems per ha for bioenergy production as part of the PHYTOPOP project, as fully described in previous papers (Foulon et al., 2016a, 2016b; Pottier et al., 2015). Based on the soil characteristics at the experimental site, 14 poplar genotypes were chosen that belong to the two Aigeros and Tacamahaca sections and to 4 species *P. deltoides, P. deltoides* x *P. nigra, P. trichocarpa and P. trichocarpa* x *P. maximowiczii*. Each of the 14 genotypes was 131 grown on two independent plots of 389 m^2 , with 49 poplars per plot (7 rows of 7 trees), with spaces of 3.50 m per line and 2.85 m per row. Details on the genotypes are provided in Table S1.

2.2. Soil sampling and chemical analyses

 In April 2007 and January 2011, 25 topsoil samples were taken from all poplar genotypes with an auger (4 cm in diameter), to a depth of 20-25 cm, each being taken at 50

 cm from the planted poplar. The 25 samples were mixed in a large container; then, 2 to 3 kg of the mixture (approximately 50%) was taken to the laboratory as a composite sample and analyzed. Soil samples were further collected using the same procedure in October 2013 and October 2016 for the Skado genotype only. This genotype, together with the I214 genotype, has been selected for a subsequent field trial in 2011 within the BIOFILTREE project (Ciadamidaro et al., 2017), given preliminary growth assessments in 2010.

 Initial (2007) and later 2011 soil analyses were performed by the INRA Soil Analysis Laboratory (LAS-INRA, Arras, France), which is accredited by COFRAC (French 146 accreditation board). The soil pH (soil: water ratio $= 1:5$) was measured according to NF ISO 10390. For initial soils, the total Zn, Pb, Cu, and Cd were determined according to NF X31- 148 147 (tri-acid HF–HCl–HNO₃ digestion). In addition, TE availability in soils was determined using two extracting reagents: an organic chelating agent, diethylenetriamine-pentaacetic acid 150 (DTPA), and a neutral salt, ammonium nitrate (NH_4NO_3) . The NH_4NO_3 extraction was selected to estimate the easily exchangeable pool of elements (Pruess, 1998) and has been widely used (McLaughlin et al., 2000; Pérez-de-Mora et al., 2006; Madejón et al., 2006). Soil 153 was homogenized in a solution of $NH₄NO₃ 1 M (1/2.5 m/v) (DIN 19730, 1997)$. The aim of the DTPA extraction was to quantify exchangeable metals and also metals associated with organic matter and with Mn, Fe, and Al oxy-hydroxides (Baize, 2003). Extraction was done in 156 triethanolamine-CaCl₂-DTPA (0.005 M) solution at $pH = 7.3$ (NF X 31-121). After 157 extraction, the total, DTPA-, and $NH₄NO₃$ -extractable element concentrations in solution were obtained with inductively coupled plasma mass spectrometry analyses.

 The third and fourth samplings were carried out in October 2013 and October 2016 (as described above) but only under the Skado genotype, thus allowing 7 and 10 years, respectively, of poplar growth. To determine the TE extractable fractions of these soil samples, 5 g of 2-mm sieved soil was dried at 60°C for 48 h and incubated with 50 ml of 10 mM calcium chloride under agitation (40 rpm) for 2 h at room temperature. The mixture was first filtered with ash-free filters and subsequently passed through a 0.45-µm mesh and 165 acidified at 2% (v/v) with HNO₃ prior to inductively coupled plasma atomic emission spectrometry (ICP-AES, Thermo Fischer Scientific, Inc., Pittsburg, USA) analysis. The 2007 and 2011 samples were also reanalyzed by the same analytical procedure. The methods for measuring other physicochemical characteristics are those described by Zappelini et al. (2015).

2.3. Biomass harvest and measurements

171 In the experimental field, diameter at breast height (dbh) and tree total height (H_{tot}) were measured in October 2013, allowing 7 years of growth after establishment of the plantation (one rotation), on all individual trees from the 14 plots (*i.e*., 25 trees per plot, excluding the border trees). Allometric equations were calculated per genotype to identify genotypic variability, using trunk and branches dry weight (DW) of a tree as a function of stem dbh. The plot estimates were further extrapolated to determine DW per ha and per year. The proportion of bark dry mass of total biomass (wood and bark) was calculated for samples of various diameters. Allometric power equations between diameter and bark proportion were used to estimate whole tree bark dry biomass. The plot estimates were extrapolated to produce DW per ha and per year.

 To determine the best fitting allometric equation, a subset of trees was selected using an optimal design approach based on the best subset of trees, allowing the statistical model parameters to be estimated, as described in the "statistical analysis" section below. Damaged trees were removed at this selection step. Per genotype, between 5 and 7 trees were required 185 to meet the statistical criteria on the 2013 observed dbh and H_{tot} , considering the allometric assumption that these two variables are related to DW. In February 2014, selected trees were harvested, and fresh biomasses were determined for the two compartments, trunks (primary

 axis up to the top of tree) and branches (all secondary axes). Three trunk sections were 189 collected on each tree at their basis (DM_h) , at half of total height (DM_m) and at the tree 190 summit, at a diameter of approximately 4 cm (DM_s); branches were also collected (Fig. S1). Fresh samples were weighed immediately after harvest and dried in a forced air oven at 50°C until reaching constant weights. The dry biomass was then measured. The total trunk (trunkDW in kilograms) and branch (branchDW in kilograms) dry weights were calculated from fresh weight (FW in kilograms) data and desiccation rates (DR), considering the samples mentioned above, using the following equations (1-2):

196 (1) trunkFW $(kg)^*(DR_h+DR_m+DR_s)/3=trunkDW$ (kg)

197 (2) branchFW (kg)*
$$
DR_{br}
$$
 = branchDW (kg)

For allometric data, the following best fitting equation was used (3):

$$
199 \qquad (3) \text{ DW}=a^*dbh^b
$$

200 where DW is the aboveground biomass (excluding leaves) production (kg DW tree⁻¹), 201 dbh is the diameter at breast height (in cm), and a and b are the regression coefficients that were shown to fit the material properly for poplar (Johansson, 1999; Picard et al., 2012; Morhart et al., 2013).

2.4. Element concentrations in biomasses

 Poplar samples (branches, trunks, wood, and bark) were used for element analysis using previously published procedures (Ciadamidaro et al., 2017). Poplar samples were washed with distilled water, dried at 70°C for at least 48 h, and ground using a stainless-steel mill. The studied elements (Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, P, Pb and Zn) were determined 209 by ICP-AES following the acid digestion with concentrated $HNO₃$ (DigiPREP system). All samples were run together with certified reference materials, including oriental basma tobacco leaves (INCT-OBTL-5, LGC Promochem, Molsheim, France) and a loamy clay soil (CRM052, LGC Promochem, Molsheim, France). The percentage of recovery of the studied

 elements ranged between 77 and 110%. The element export from the soil was further evaluated by each poplar genotype by calculating the quantity of elements transferred to the woody biomass per hectare and per year.

2.5. Statistical analyses

 For allometric data, all statistical analyses were performed using R 3.4.1 software (R Development Core Team, 2015). Optimal subsets of trees were selected per genotype using R package AlgDesign (Wheeler 2014), which allowed estimation of statistical model parameters 221 without bias and with minimum variance of the relationship between dbh and H_{tot} , thereby maximizing the criteria established earlier (Fedorov, 1972). After providing the DW data, graphical exploration allowed for checking the sampling and drying procedure bias and showed the presence of heteroscedasticity in the nonlinear relationship between dbh and branchDW or trunkDW, as is the case for many taxa (Picard et al., 2012). For the model 226 calibration, goodness of fit was evaluated by the coefficient of determination (R^2) , and heteroscedasticity was assessed by plotting residuals against observed values and testing their normality (Shapiro-Wilk test). Logarithm transformation of data and power form 229 (DW=a*dbh^^b) with a and b as the regression coefficients were the most relevant models. When regressions were performed, slopes and intercepts were tested for differences among genotypes by analysis of covariance. Interactions between the dbh and genotypes have been considered but were never statistically significant in modeling. The two biomass components, branchDW and trunkDW, were modeled separately as two dependent variables without consideration of possible additivity (Vega-Nieva et al., 2015). However, when applying the models, the sum of the two components resulted only in an average of 2.3% higher than the observed total tree dried biomass.

 The effects of genotype and position of the sample in the branch or trunk wood on element concentrations and quantity of TE exported were tested with a two-way analysis of variance. All tests were considered significant when p< 0.05. Prior to analysis, outliers were discarded, and the normality of the variable or of the residual values was tested with the Shapiro-Wilk test, and homoscedasiticity, with the Bartlett test. The design was a randomized block design with genotype, branches and trunk as the fixed factors and plot as a random factor. For the selected genotype Skado, the effect of sample position on element concentration was tested for the branch, trunk wood and trunk bark.

3. Results and discussion

3.1. Soil characteristics and short-term impact of the poplar plantation

 The pedogeochemical characteristics of the entire Pierrelaye area has been thoroughly studied (Lamy et al., 2006; van Oort et al., 2008), and the authors concluded that irrigation with wastewaters for more than 100 years has deeply modified the macromorphology and the chemistry of the soil. The surface horizons of the Pierrelaye Luvisol have indeed accumulated large amounts of organic matter and have been markedly enriched with TE. The total Zn, Pb, Cu and Cd at the Pierrelaye site for the initial PHYTOPOP plots sampled in 2007 values were 20- to 317-fold higher than the natural pedogeochemical background values, as shown in Table 1, and are in the range of the TE content and surface content spatial heterogeneity recorded in that contaminated area (Lamy et al., 2006).

 The impact of the poplar plantation on the TE pools and pH was first evaluated after 4 growing seasons for all genotype plots, by comparing the ammonium nitrate -TE extractable fractions in 2007 and 2011, as well as the pH values (Table 2). Only the 3 major TE—Cd, Cu 260 and Zn—are provided (Pb values fell under the detection limits). The $NH₄NO₃-TE$ extractable fraction decreased in all plots between the two sampling periods (Table 2), whereas the DTPA-TE extractable fraction slightly increased for most poplar genotypes (Table S2). As the data provided are from composite samples, statistical analysis could not be performed on this dataset. The pH, which is an essential parameter in phytomanagement practices either evolved slightly upward or remained unchanged.

3.2. Longer-term impact of the poplar plantation

 Our work was further pursued on the poplar Skado plot only. This genotype was chosen in 2011, together with the I214 genotype, for a new phytomanagement plantation at the same site within the BIOFILTREE project (Ciadamidaro et al., 2017). Soil samples were collected in 2013 and 2016, from which the pH and the CaCl₂-extractable element fractions were measured. The 2007 and 2011 soil samples from under the Skado genotype were also reanalyzed; these samples had been stored while dry, with the same extraction and analytical methods used for the 2013 and 2016 soil samples as on earlier samples. The pH analysis after 7 and 10 years of growth under the Skado genotype confirmed the lack of impact of the 275 poplar trees on this key soil parameter (Fig. 1). A depletion of the $CaCl₂$ -extractable fraction for Cd, Cu, Mn, Ni and Zn was also evident between 2007 and 2016 (Fig. 1), which confirms 277 the first series of analyses (2007-2011) performed with the $NH₄NO₃$ -extractant (Table 2) for Cd, Cu and Zn. The Mn and Ni were not chosen as target elements in the analyses of the 2007 and 2011 soil samples. Other elements, such as K Mg, S and Si, increased significantly with time, which confirmed some of our previous data (Foulon et al., 2016b). Other elements (Na, Al, and Fe) followed the same trends; however, the changes were not statistically significant.

282 The depletion of the $NH₄NO₃$ (2007-2011, first series of analysis)- or CaCl₂ (2007- 2011-2013-2016, second series of analysis)-extractable TE fractions was not observed for the major elements and was low in absolute terms but high in relative terms, especially for Mn. Two main hypotheses could explain this decrease in the long term to confirm a depletion of the soil exchangeable compartment, with both hypotheses involving processes attributed to changes in TE speciation under the land use: i) an increase in leaching in the soil profile under trees without replenishment of the exchangeable pool of TE or ii) an increase in retention in the soil due to the so-called process of "natural attenuation." In the first case, a leaching of TE had been already observed in a soil profile of this area. But due to the constitution of the Luvisols, mobile TE were found trapped in the Bt horizon mainly constituted of clay before reaching the water table. Indeed, Dère et al (2007) observed that exogenous Zn and Cu but not Pb were found down to the base of the studied solum (i.e. around 1 m), mostly readsorbed on deep horizon's constituents. In the second case, the TEs that are easily phytoavailable would have been taken up by poplar roots. As no obvious differences were observed between planted and unplanted poplar (naturally revegetated) plots (data not shown), it can be hypothesized that the herbaceous layer under the poplar trees played a major role in changing 298 levels in the CaCl₂-extractable fraction. However, these depletions for Cd, Cu and Mn under the adjacent maize plots was noticed in one of our previous studies (Foulon et al., 2016a), which may indicate an overall phenomenon of land use with plant species efficient in the use of atmospheric carbon, which then resulted in high amounts of the soil organics able to change TE speciation rather than from the poplar having a specific impact on its underlying vegetation. Indeed, the indirect effect of the newly developed vegetation (poplar trees or the adjacent herbaceous layer), by regularly enriching the Pierrelaye soil with fresh organic material other than from the wastewaters, could result in the alteration of the distribution of TE in the various soil extractable fractions. Previous works have demonstrated the importance of leaf litter on the nutrient cycles and bioavailability of TE (Ciadamidaro et al., 2013, 2014), and hence on the element export capacity of trees. Litter or litter components represent the main route of entry of nutrients in the soil and is one of the key points of the recycling of organic matter (OM) and nutrients, and, in forest ecosystems, the main source of OM and nutrients for the topsoil (Kuzyakov and Domanski, 2000). In particular, it can cover between 60 and 80% of the nutritional requirements of the tree per year. We did not attempt to determine the TOC content of the soils under the various poplar clones, but future projects at this experimental site would need to address this key feature in the management of TE contaminated soils.

 Some changes between 2007 and 2011 indeed occurred in the DTPA fraction in poplar or unplanted (naturally revegetated) soils (Table S2). The small increase in the DTPA fraction, which tends to mimic an increase in the available TE pool, is unlikely to be solely responsible for the displacement of the extractable fraction, as these fractions were of much greater magnitude, but it may have partially contributed to it.

 The lack of change in the pH is an important indicator and could be associated with soil stabilization. It has to consider, however, that such soils rich in organic carbon have a high buffering effect against changes in pH, and data from a longer term could be useful for drawing conclusions. Due to these buffering effects, changes in soil pH are less important than those of TE availability seen either through DTPA, CaCl₂ or ammonium nitrate extractions. But the decrease in salt-exchangeable PTE compared to the increase in DPTA- extractable PTE is consistent with a change in PTE speciation in favor with PTE complexation with freshly organic matter brought by the aboveground vegetation.

 Nevertheless, such an indication is important to take into consideration when looking for future management of the site, as a reforestation of the Pierrelaye area is now being planned for the next few years. In a previous study, the soil pH under poplar stands of various genotypes, ages and tree density did not differ significantly from adjacent arable fields (Weih et al., 2003).

3.3. Genetic variability of poplar productivity at Pierrelaye

335 H_{tot} and dbh are frequently used as predictor variables, relating an easily measurable parameter to that of a less obtainable dependent factor such as tree dry weight. Both parameters are known to be strongly indicative of aboveground woody biomass (Morhart et al., 2013; Zianis, 2008) and are part of the equations detailed in the material and methods section. Individual genotypic allometric equations allowed establishing relationships between the diameter measured at breast height (dbh) in 2013 and total dry biomasses of harvested trunk and branches respectively, for the 14 poplar genotypes grown at the Pierrelaye site (Fig. 2). Although the equations gave similar curves, the differences between the poplar genotypes was evident and showed that among the poplar genotypes retained in the present study, some variability occurred in the dry weight distribution between trunk and branches and/or wood density.

 The significance of our datasets was further tested (Table 3). This analysis allowed us to conclude that the genotype effect was significant for the trunkDW parameter, whereas for the branchDW parameter, the regression coefficients were not statistically significant. As a consequence, specific equations for each genotype were used to calculate the trunkDW, whereas a single equation for all genotypes was used to calculate the branchDW. The 351 proportion of branch DW in the total biomass was significantly correlated $(r = 0.9)$ with the "b" regression coefficients of the trunkDW equations, indicating that these different equations reflected the architectural variability within a genotype. These data further allowed us to 354 estimate the productivity (expressed as oven dry tons or odt ha^{-1} year⁻¹) of each genotype, which was estimated in 2013 (7-year-old plantation).

 Over the entire 7-year rotation, huge genotypic variability was observed, with the 357 Vesten genotype producing the largest biomass $(8.5 \text{ odd ha}^{-1} \text{ year}^{-1})$ in the SRC production scheme, whereas the Dorskamp genotype produced 2.8 times less biomass (Fig. 3). In Europe, 359 the mean modeled yields for poplars ranged between 1.5 and 11.7 odt ha⁻¹ year⁻¹ for Aspen (Aylott et al., 2008; Johansson, 1999; Morhart et al., 2013; Verlinden et al., 2013). There was no clear relationship between the genetic origin and production potential. The Bakan and

362 Skado (TxM) genotypes showed a similar yield of approximately 7 odt ha⁻¹ year⁻¹ and were among the most productive genotypes, whereas the DxN genotypes exhibited large variations 364 ranging from 3.1 (Dorskamp) to 8.5 (Vesten) odt ha⁻¹ year⁻¹. Our data contrast with those from a previous study (Verlinden et al., 2013), where clustering of the poplar genotypes was clearly determined by genetic origin, with the poplar plantation being established on a normal, uncontaminated soil. Interestingly, in that study, the Bakan and Skado genotypes showed very 368 similar biomass production to that of our study, approximately 5.75 odt ha^{-1} year⁻¹. The Vesten genotype in our study was more productive than that in the Verlinden study (Verlinden et al., 2013). The trees were, however, harvested after only 2 growing seasons in this previous study. The Dorskamp genotype showed notably lower biomass productivity in our study than the other 13 genotypes. However, this genotype experienced more rabbit feeding than the other genotypes, and the mortality was much higher for that genotype (Table S1). A high variability in growth and in cutting quality (especially cutting diameter) during the first years is likely due to weed pressure and management practices (Broeckx et al., 2012). However, at our site, all plots received the same appropriate treatment for weed management, and cutting diameters were homogenous between all genotypes that were planted.

 The dry branch proportions ranged from 26 to 40% of the total tree biomass (Fig. 3). The highest proportions were found for the D (Dvina and Lena) and DxN (Triplo and Vesten) poplar genotypes, whereas the four T (Trichobel and Fritzi Pauley) or TxM (Bakan and Skado) genotypes exhibited the lowest branch proportions. The branch proportion in the TxM genotypes is close to that reported (22.2%) for another genotype (hybrid 275) of the same hybrid family (Morhart et al., 2013) grown in standard conditions. The quantification of branch dry biomass is an important factor, as it may greatly influence the overall quality of the final product. This is even more important in the context of phytomanagement, as the proportion of bark increased with decreasing diameter (see results below and Fig. 4).

3.4. Macronutrient concentrations in wood and branches of poplar genotypes

388 Macronutrient concentrations (expressed as mg kg^{-1} DW) were determined in poplar branches and bark-removed trunks (wood) after seven growing seasons (Table 4). Macronutrients were measured at three heights (summit, middle, and base), as described in the material and methods section, and the data provided are the averages of samples from the three sections.

 Overall, branches accumulated more Ca, K, Mg, and P than woody tissues (Table 4). The highest Ca concentrations in branches were measured for Bakan, which was 1.8 times significantly higher than for Vesten. This ratio was 1.6 for the wood of Dorskamp compared to that of Triplo (Table 4). The highest ratio for K in the wood was 1.4 between the Trichobel and Koster genotypes, when there was no effect of the genotype on the K concentration (ANOVA) in the branches (Table 4). The Mg concentration was about 1.7 times higher in the branches of Dvina or Triplo compared to Trichobel or Vesten. The Dvina, Dorskamp, and Soligo genotypes also had the highest Mg levels in their wood, and significantly higher than in Skado or Bakan, for example (Table 4). For most poplar genotypes, the K and P concentrations were the highest in the upper sections of the trunk (not shown). For instance, K and P doubled in the summit sections of the Bakan and Fritzi Pauley trunks compared to the basal sections.

 According to a study of Obernberger et al. (2006), typical woody biomasses from 406 deciduous species (from uncontaminated soils) contained, on average, 1200 mg kg⁻¹ Ca, 800 407 mg kg⁻¹ K, 200 mg kg⁻¹ Mg, and 100 mg kg⁻¹ P for the major elements. The K, Mg, and P concentrations were, on average, 1.5 to 5-fold higher than those given by Obernberger et al. (2006), whereas Ca concentrations were in the same range.

3.5. Trace element concentrations in trunk and branches of poplar genotypes

 The Bakan and Dorskamp genotypes accumulated significantly more Cd and/or Zn than the Fritzi Pauley, Triplo and Lena genotypes, both in the wood (Table 5) and in the branches (Table 6). The largest variations measured for these two TE were of the order of two times. For Cu, the highest values were measured for Dorskamp, they were significantly different from the Cu values measured for Fritzi Pauley. There were no significant variations in Cr and Pb concentrations in wood between genotypes (Table 5). Cr and Pb values were the lowest of all elements mentioned. For Fe, the highest levels were measured for the Lena genotype, 2.6 and 2.3 times higher in the wood (Table 5) and branches (Table 6), respectively, when compared to the I214 genotype. Mn accumulated the most in the branches of Dvina, Flevo and Dorskamp and in the wood of Dvina, Koster, Bakan, while the Fritzi Pauley, I214 or Trichobel genotypes accumulated significantly 1.8 (wood) to 2.7 (branches) times less.

 Even if it is difficult to obtain clear trends for all elements, it should be noted that the Dorskamp, Bakan and Dvina genotypes often appeared as the most accumulating genotypes (Mn, Cu, Fe, Zn, and Cd) for wood or branches. On the contrary genotypes such as Fritzi Pauley, Trichobel or Triplo were more often quoted as the less accumulating genotypes. Although the Fritzi Pauley and Trichobel genotypes belong to the same species, the Dorskamp, Bakan and Dvina genotypes belong to three different species (Table S1). It is therefore difficult to establish a link between the accumulation of elements and the taxonomic relationships of the genotypes.

 Branches accumulated more Cd, Cu, Fe, Mn and Zn than woody tissues (Tables 5 and 6). Pb and Cr concentrations were within narrow ranges, and no clear significant tendency was observed between the wood and branches. The largest variations between branches and woods were observed for Mn, with an order of magnitude difference observed for most genotypes. The major contaminants Cu, Zn and Cd were 3- to 4-fold higher in branches

 compared with wood. For most poplar genotypes, the TE concentrations were the highest in the upper sections of the trunk (not shown). For instance, Fe doubled in the summit sections of the Bakan and Fritzi Pauley trunks compared to the basal sections. The Pb concentration was always the highest in the basal section of the trunk, probably reflecting a simple contamination by aerosols, rather than an active transport, as already detailed in Migeon et al. (2009). No such clear tendency was observed for the other elements.

 Metal content in woody biomass can greatly affect the industrial process, as it may interfere with the thermal degradation by acting as catalysts. A previous paper reported for instance that Cl ions may change metal behavior by increasing metal volatilization (Bert et al., 444 2017). For TE, 0.1 mg kg⁻¹ Cd, 1 mg kg⁻¹ Cr, 2 mg kg⁻¹ Cu, 25 mg kg⁻¹ Fe, 83 mg kg⁻¹ Mn, 2 445 mg kg⁻¹ Pb, and 10 mg kg⁻¹ Zn are the concentrations usually reported (Obernberger et al., 2006). In our study, Cu, Cr, Fe, Mn and Pb concentrations in poplar wood, which range 447 between 0.1 and 3 mg kg^{-1} , are so low that they will not be discussed further herein. Cd 448 concentrations were also very low in wood $(< 0.5$ mg kg⁻¹); however, the volatile nature of Cd and its potential dissemination into the atmosphere when the biomass is burned should be considered (Chalot et al., 2012; Evangelou et al., 2012). When compared to the present dataset, Zn was accumulated at a much higher rate in a previous study, although large variations were observed among the poplar genotypes (Castiglione et al., 2009). The total Zn 453 concentration (879-950 mg kg^{-1}) was also higher compared to our soil data (Table 1). The authors also did not mention whether bark was included or not in the stem analysis.

 In a previous paper (Pottier et al., 2015), data on the leaf concentrations were provided from the same trees and on changes during seasons. These previous analyses demonstrated large variations in the TE concentrations of leaf tissues among the various genotypes. Phytomanagement of TE-contaminated sites strongly relies on the capacity of the plant cover to tolerate high concentrations of TE. Salicaceaous species respond to that constraint by

 storing TE in their aboveground biomass (Migeon et al., 2009), with potential sequestration of TEs within cells in the organelles. In poplar, this is illustrated by the sequestration of Mn in a Golgi-associated compartment through the transport protein PtMPT11 (Peiter et al., 2007) and by the vacuolar sequestration of Zn through the transport protein PtMTP1 (Blaudez et al., 2003). The potential function of other target transport genes were further underscore in TE management (Pottier et al., 2015) and their potential use in phytomanagement using *Populus* species. *PtNRAMP1* may, for instance, represent an interesting target for genetic engineering of poplar and modulation of leaf TE content.

3.6. Element accumulation in bark tissues

 Given the overall contribution of the bark tissues in element distribution within the whole tree, our study was further focused on this aspect on the Skado genotype. Bark was separated from the wood at different heights of the trunk and compared to branches. The bark content averaged 10-12% of dry trunk biomass, whereas branches < 2 cm in diameter are more than 30% bark (Fig. 4).

 Element concentrations were further determined in bark collected on the trunk from Skado (Table 7). Bark accumulated 2 to 14 times more Ca, Fe, Mn, and Zn than debarked- wood. Differences were also observed for Cd, K and Mg, but to a lesser extent. Other elements such as Cr, Cu and Pb did not accumulate differently in bark and wood, with ratios close to one. Very similar ratios between wood and bark were also measured in a field experiment where poplar were grown on TE-contaminated soils (Evangelou et al., 2012).

 In a previous study, the authors concluded that the quality of the final product is strongly impacted by the amount of bark in the biomass feedstock (Morhart et al., 2013). As wood chips from SRC are being used as recycling materials to enhance soil fertility, concern is growing over the fate of TE from bark tissues (Vandecasteele et al., 2013). This is even more meaningful for biomass harvested at phytomanagement sites, where the presence of TE in the bark tissues may have an impact on the industrial processes. Evangelou and collaborators (Evangelou et al., 2012) also concluded that harvesting biomass with smaller bark proportion in relation to trunk is desirable.

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3.7. Element export by poplar genotypes

 Given the biomass production data and the element contents, the amount of exported 491 element (expressed as kg^{-1} ha⁻¹ year⁻¹ or g^{-1} ha⁻¹ year⁻¹) was calculated after 7 growing seasons. The most productive genotypes facilitated the highest export of elements (Table 8). As a consequence of the variation in the element accumulations and biomass produced, the export capacities were highly variable among the genotypes. The Bakan, Triplo and Vesten genotypes showed higher export capacities, with Ca and Cd export greater than 20 kg^{-1} ha⁻¹ 496 year⁻¹ and 3 g^{-1} ha⁻¹ year⁻¹, respectively. Conversely, the Flevo and Dorskamp genotypes exported much less of these elements. These findings were not related to the *Populus* species, as the Bakan and Triplo, for instance, do not belong to the same species (Table S1).

 The ability to export these elements is an important consideration in phytomanagement, as the concentration of contaminants in the harvested part will greatly determine their potential valorization. Additionally, the export of nutrients is also a key consideration. The export potential was clearly greater for branches for Ca, Mn and Fe and to a lesser extent for K, P, Mg, Zn, Cd compared with the trunk. This difference was directly linked to the higher bark content of branches and higher content in these elements (Table 7). Conversely, wood tissues showed greater Cu, Cr and Pb export capacities, related to their equal amounts in wood and bark samples (Table 8). Previous studies have suggested that harvesting poplar as the whole tree from short-term rotations should cause soil nutrient depletion and induce degradation of soil and decreased biomass production (Ge et al., 2015). However, such a decrease in soil nutrient contents was not observed at our site when comparing total (not shown) or ammonium nitrate-extractable (Fig. 1) fractions of major elements in the 2007 and 2011, 2013 and 2016 samplings, perhaps because their levels were already high enough. The enrichment of the Pierrelaye soils with many cations, such as K and Mg, in poplar plots may indicate a thorough mobilization that occurs through microbial activities that are stimulated by the presence of poplar trees (Foulon et al., 2016b, 2016a). Conversely, whether these microbial communities are indeed expressing the capacity to mobilize these elements from the bound fraction is unknown.

4. Conclusions

 Our set of data clearly emphasizes genotypic variations in the growth of poplar at a moderately contaminated site, as well as in element accumulation, and hence in nutrient export capacities. The present dataset highlighted that soil element depletion occurred for a range of TE, whereas the soil content of macronutrients remained unchanged or increased after 10 years of poplar growth. Given i) the greater TE content of bark tissues compared with wood and ii) the higher proportion of bark in branches compared with trunk, it can be recommended choosing stem wood-only harvesting instead of whole-tree harvesting, which will allow the risks encountered with TE-enriched biomass in the valorization process to be reduced. In the long term, this approach may also allow only limited nutrient losses. However, the impact of element-enriched remnants on physicochemical and biological soil properties requires further investigation. Future research programs should also expand this type of investigation based on the use of various genotypes on a broader range of site conditions to further enhance the uptake of phytotechnologies by stakeholders.

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Figure captions

Fig. 1. CaCl₂-TE extractable fraction in Pierrelaye soils collected under the Skado genotypes. Values are means and were measured in samples collected in 2007, 2011, 2013 and 2016. The 799 different letters above the element $(n = 3)$ and pH $(n=3)$ values represent significant differences (p<0.05, Kruskal-Wallis). ns : not significant.

 Fig. 2. Comparison of measured data (2013) and modeled relationships for trunkDW (kg) and dbh (cm), considering genotypic variability for the 14 poplar genotypes grown at the Pierrelaye site. Data shown in color are for the Skado (red) and Trichobel (green) genotypes.

Fig. 3. Productivity (odt ha⁻¹ year⁻¹) for the 14 poplar genotypes grown at the Pierrelaye site after one rotation (2013). The proportion of branches to trunk are provided (right axis). D: *P. deltoides*; DxN: *P. deltoides* x *P. nigra*; T: *P. trichocarpa*; TxM: *P. trichocarpa* x *P. maximowiczii.*

Fig. 4. Relationship between bark content (%) and branch diameter for the Skado genotype.

matter and element concentration (in blue)

- **Table S1**
- Poplar genotypes grown at the experimental field of Pierrelaye D: *P. deltoides*; DxN: *P.*
- *deltoides* x *P. nigr*a; T: *P. trichocarpa*; TxM: *P. trichocarpa* x *P. maximowiczii*. B: Belgium;
- I: Italy, NL: the Netherlands.

Table S2

 DTPA-TE extractable fraction in Pierrelaye soils collected under the various poplar genotypes. Values presented were measured in 2007 and 2011 and the changes occurring between the two samplings are provided.