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

Michel Chalot, Olivier Girardclos, Lisa Ciadamidaro, Cyril Zappelini, Loïc Yung, Alexis Durand, Stéphane Pfendler, Isabelle Lamy, Vincent Driget, Damien Blaudez

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1 Poplar rotation coppice at a trace element-contaminated  
2 phytomanagement site: a 10-year study revealing biomass production,  
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4

5 **Michel Chalot<sup>a,b</sup>, Olivier Girardclos<sup>a</sup>, Lisa Ciadamidaro<sup>a</sup>, Cyril Zappellini<sup>a</sup>, Loic Yung<sup>a</sup>,**  
6 **Alexis Durand<sup>a</sup>, Stéphane Pfendler<sup>a</sup>, Isabelle Lamy<sup>c</sup>, Vincent Driget<sup>a</sup>, Damien Blaudez<sup>d</sup>**

7

8 <sup>a</sup>Université de Bourgogne Franche-Comté, CNRS, Laboratoire Chrono-environnement, F-  
9 25250, Montbéliard, France

10 <sup>b</sup>Université de Lorraine, F-54000, Nancy, France.

11 <sup>c</sup> INRA, AgroParisTech, UMR1402 ECOSYS, Ecotoxicology division, F-78026 Versailles  
12 cedex, France

13 <sup>d</sup>Université de Lorraine, CNRS, LIEC, F-54000, Nancy, France

14

15 \*Corresponding author: Laboratoire Chrono-Environnement, UMR 6249, Université de  
16 Bourgogne Franche-Comté, 4 place Tharradin, BP71427, F-25211 Montbéliard, France

17 Email: [michel.chalot@univ-fcomte.fr](mailto:michel.chalot@univ-fcomte.fr)

18

19 **Abstract**

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

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

39

## 40 **1. Introduction**

41 Renewable resources are the focal point of European Union (EU) initiatives to establish  
42 energy self-sufficiency in the EU. Woody biomass is a renewable resource and is almost  
43 carbon-neutral (Zanchi et al., 2012). Using wood as an energy source is certainly not new;  
44 however, second-generation biofuels utilize woody biomass as raw materials to develop liquid  
45 fuels, *i.e.*, ethanol and diesel within biorefinery projects. Lignocellulosic perennial crops are  
46 indeed recognized as feedstock for platform chemicals and lignin-based building blocks  
47 (Rohde et al., 2018). The EU has been, and remains, the major global source of demand as a  
48 result of its predicted targets for renewable energy. This demand is largely met by the forest  
49 resources of the UE and supplemented by imports from the United States, Canada and Russia.  
50 Growing lignocellulosic crops on contaminated or marginal lands could make up a substantial  
51 proportion of future energy resources (Gelfand et al., 2013). Efforts have been made in the  
52 past ten years to link phytotechnologies to the production of valuable biomass products and to  
53 the fate of contaminants during industrial processes (Ancona et al., 2019; Asad et al., 2017;  
54 Bert et al., 2017; Chalot et al., 2012; Dastyar et al., 2019; Delplanque et al., 2013; Šyc et al.,  
55 2012). Despite these efforts, phytotechnologies have not been commercially applied to date  
56 (Conesa et al., 2012; Gerhardt et al., 2017) and the use of gentle remediation options (GRO)  
57 as practical onsite remedial opportunities is still limited in Europe, particularly at trace-  
58 element-contaminated sites (Cundy et al., 2016). This lack of application is mostly due to the  
59 lack of long-term field assessments under realistic conditions. A recent article discussed how  
60 phytotechnologies could be upgraded from a proven technology to a widely accepted practice  
61 (Gerhardt et al., 2017), including the implementation of large-scale field trials in long-term  
62 experiments.

63

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

71           Poplars also readily grow on marginal lands where biomass yields can fall within the  
72 range 8-12 tons per hectare per year (Ciadamidaro et al., 2017; Verlinden et al., 2013). The  
73 use of *Populus* species has been proposed for phytomanagement purposes because these  
74 woody species are characterized by fast-growth, deep-rooting systems and can be managed in  
75 short-rotation coppicing plantations (Ciadamidaro et al., 2017; Laureysens et al., 2005, 2004;  
76 Marmioli et al., 2011; Migeon et al., 2009; Rohde et al., 2018; Vangronsveld et al., 2009;  
77 Yáñez et al., 2019). The genus *Populus* has traditionally been divided into six sections on the  
78 basis of the flower and leaf characters, with approximately 30–40 species occurring  
79 worldwide, mainly in the Northern Hemisphere (Polle and Douglas, 2010). Different  
80 genotypes of the same poplar species may exhibit variable tolerance to trace elements (TE)  
81 because of intraspecific genetic dissimilarity (Castiglione et al., 2009). For instance, a large  
82 variation in poplar genotypic responses to leachate irrigation has been found (Zalesny et al.,  
83 2007), and high variability occurs in foliar trace element concentrations within a selection of  
84 14 poplar genotypes (Pottier et al., 2015).

85           A number of works have been dedicated to the study of poplar responses to soil  
86 contamination in controlled pot or hydroponic (Cicatelli et al., 2010; De Oliveira and Tibbett,  
87 2018; Langer et al., 2012; Lingua et al., 2008; Migeon et al., 2012), tank (Zalesny and Bauer,  
88 2007), or lysimeter (Dimitriou and Aronsson, 2011, 2010) set ups. Such experiments are

89 needed to study fundamental mechanisms (contaminant transfer between soil-aboveground  
90 and below ground plant tissues, interactions between plant and microbes) but may not reflect  
91 how trees will behave in natural situations in the long term. Field trials have been  
92 implemented, and short-term data are available for a few field experiments (Castiglione et al.,  
93 2009; French et al., 2006; Gamalero et al., 2012; Lettens et al., 2011; Sabatti et al., 2014).  
94 However, long-term studies are scarce (Evangelou et al., 2012; Foulon et al., 2016a, 2016b;  
95 Mertens et al., 2007) but are crucial to the study of long-term impacts of tree plantation on  
96 pedological and biological soil characteristics.

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

110         The present paper aimed to determine the impacts of a PHYTOPOP plantation on soil  
111 characteristics, TE accumulation and poplar yields after one rotation. The specific objectives  
112 were 1) to quantify the genotypic variation in the growth traits of 14 poplar genotypes grown

113 in SRC systems at a TE contaminated site and 2) to assess element transfer and export by the  
114 different genotypes.

## 115 **2. Materials and methods**

### 116 **2.1. Site description and experimental design**

117 The experimental site is located in the Northwest region of Paris at Pierrelaye  
118 (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 19<sup>th</sup> century until the beginning of the 21<sup>st</sup> century to increase the soil  
120 fertilization potential and has been dedicated to vegetable gardening for more than one  
121 century (Baize et al., 2002, Dère et al., 2007, Lamy et al., 2006). However, this resulted in the  
122 accumulation of TE such as Pb, Cu, Zn and Cd in the first 30-50 cm of soil layer. This leads  
123 to poly-metallic pollution characterized by TE concentrations that could reach levels more  
124 than 10-fold greater than those of a non irrigated local reference soil.

125 Poplars were planted in April 2007 as a short-rotation coppice (SRC) at densities of  
126 1000 stems per ha for bioenergy production as part of the PHYTOPOP project, as fully  
127 described in previous papers (Foulon et al., 2016a, 2016b; Pottier et al., 2015). Based on the  
128 soil characteristics at the experimental site, 14 poplar genotypes were chosen that belong to  
129 the two Aigeros and Tacamahaca sections and to 4 species *P. deltoides*, *P. deltoides* x *P.*  
130 *nigra*, *P. trichocarpa* and *P. trichocarpa* x *P. maximowiczii*. Each of the 14 genotypes was  
131 grown on two independent plots of 389 m<sup>2</sup>, with 49 poplars per plot (7 rows of 7 trees), with  
132 spaces of 3.50 m per line and 2.85 m per row. Details on the genotypes are provided in Table  
133 S1.

134

### 135 **2.2. Soil sampling and chemical analyses**

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

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

144 Initial (2007) and later 2011 soil analyses were performed by the INRA Soil Analysis  
145 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  
147 10390. For initial soils, the total Zn, Pb, Cu, and Cd were determined according to NF X31-  
148 147 (tri-acid HF–HCl–HNO<sub>3</sub> digestion). In addition, TE availability in soils was determined  
149 using two extracting reagents: an organic chelating agent, diethylenetriamine-pentaacetic acid  
150 (DTPA), and a neutral salt, ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>). The NH<sub>4</sub>NO<sub>3</sub> extraction was  
151 selected to estimate the easily exchangeable pool of elements (Pruess, 1998) and has been  
152 widely used (McLaughlin et al., 2000; Pérez-de-Mora et al., 2006; Madejón et al., 2006). Soil  
153 was homogenized in a solution of NH<sub>4</sub>NO<sub>3</sub> 1 M (1/2.5 m/v) (DIN 19730, 1997). The aim of  
154 the DTPA extraction was to quantify exchangeable metals and also metals associated with  
155 organic matter and with Mn, Fe, and Al oxy-hydroxides (Baize, 2003). Extraction was done in  
156 triethanolamine-CaCl<sub>2</sub>-DTPA (0.005 M) solution at pH = 7.3 (NF X 31-121). After  
157 extraction, the total, DTPA-, and NH<sub>4</sub>NO<sub>3</sub>-extractable element concentrations in solution  
158 were obtained with inductively coupled plasma mass spectrometry analyses.

159 The third and fourth samplings were carried out in October 2013 and October 2016 (as  
160 described above) but only under the Skado genotype, thus allowing 7 and 10 years,  
161 respectively, of poplar growth. To determine the TE extractable fractions of these soil  
162 samples, 5 g of 2-mm sieved soil was dried at 60°C for 48 h and incubated with 50 ml of

163 10 mM calcium chloride under agitation (40 rpm) for 2 h at room temperature. The mixture  
164 was first filtered with ash-free filters and subsequently passed through a 0.45- $\mu\text{m}$  mesh and  
165 acidified at 2% (v/v) with  $\text{HNO}_3$  prior to inductively coupled plasma atomic emission  
166 spectrometry (ICP-AES, Thermo Fischer Scientific, Inc., Pittsburg, USA) analysis. The 2007  
167 and 2011 samples were also reanalyzed by the same analytical procedure. The methods for  
168 measuring other physicochemical characteristics are those described by Zappellini et al.  
169 (2015).

### 170 **2.3. Biomass harvest and measurements**

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

181 To determine the best fitting allometric equation, a subset of trees was selected using an  
182 optimal design approach based on the best subset of trees, allowing the statistical model  
183 parameters to be estimated, as described in the “statistical analysis” section below. Damaged  
184 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  $H_{\text{tot}}$ , considering the allometric  
186 assumption that these two variables are related to DW. In February 2014, selected trees were  
187 harvested, and fresh biomasses were determined for the two compartments, trunks (primary

188 axis up to the top of tree) and branches (all secondary axes). Three trunk sections were  
189 collected on each tree at their basis ( $DM_b$ ), 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).  
191 Fresh samples were weighed immediately after harvest and dried in a forced air oven at 50°C  
192 until reaching constant weights. The dry biomass was then measured. The total trunk  
193 (trunkDW in kilograms) and branch (branchDW in kilograms) dry weights were calculated  
194 from fresh weight (FW in kilograms) data and desiccation rates (DR), considering the samples  
195 mentioned above, using the following equations (1-2):

196 (1)  $\text{trunkFW (kg)} * (\text{DR}_b + \text{DR}_m + \text{DR}_s) / 3 = \text{trunkDW (kg)}$

197 (2)  $\text{branchFW (kg)} * \text{DR}_{br} = \text{branchDW (kg)}$

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

199 (3)  $DW = a * dbh^b$

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

#### 204 **2.4. Element concentrations in biomasses**

205 Poplar samples (branches, trunks, wood, and bark) were used for element analysis  
206 using previously published procedures (Ciadamidaro et al., 2017). Poplar samples were  
207 washed with distilled water, dried at 70°C for at least 48 h, and ground using a stainless-steel  
208 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  $\text{HNO}_3$  (DigiPREP system). All  
210 samples were run together with certified reference materials, including oriental basma tobacco  
211 leaves (INCT-OBTL-5, LGC Promochem, Molsheim, France) and a loamy clay soil  
212 (CRM052, LGC Promochem, Molsheim, France). The percentage of recovery of the studied

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

216

## 217 **2.5. Statistical analyses**

218 For allometric data, all statistical analyses were performed using R 3.4.1 software (R  
219 Development Core Team, 2015). Optimal subsets of trees were selected per genotype using R  
220 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  
222 maximizing the criteria established earlier (Fedorov, 1972). After providing the DW data,  
223 graphical exploration allowed for checking the sampling and drying procedure bias and  
224 showed the presence of heteroscedasticity in the nonlinear relationship between dbh and  
225 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  
227 heteroscedasticity was assessed by plotting residuals against observed values and testing their  
228 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.  
230 When regressions were performed, slopes and intercepts were tested for differences among  
231 genotypes by analysis of covariance. Interactions between the dbh and genotypes have been  
232 considered but were never statistically significant in modeling. The two biomass components,  
233 branchDW and trunkDW, were modeled separately as two dependent variables without  
234 consideration of possible additivity (Vega-Nieva et al., 2015). However, when applying the  
235 models, the sum of the two components resulted only in an average of 2.3% higher than the  
236 observed total tree dried biomass.

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

### 245 **3. Results and discussion**

#### 246 **3.1. Soil characteristics and short-term impact of the poplar plantation**

247 The pedogeochemical characteristics of the entire Pierrelaye area has been thoroughly  
248 studied (Lamy et al., 2006; van Oort et al., 2008), and the authors concluded that irrigation  
249 with wastewaters for more than 100 years has deeply modified the macromorphology and the  
250 chemistry of the soil. The surface horizons of the Pierrelaye Luvisol have indeed accumulated  
251 large amounts of organic matter and have been markedly enriched with TE. The total Zn, Pb,  
252 Cu and Cd at the Pierrelaye site for the initial PHYTOPOP plots sampled in 2007 values were  
253 20- to 317-fold higher than the natural pedogeochemical background values, as shown in  
254 Table 1, and are in the range of the TE content and surface content spatial heterogeneity  
255 recorded in that contaminated area (Lamy et al., 2006).

256  
257 The impact of the poplar plantation on the TE pools and pH was first evaluated after 4  
258 growing seasons for all genotype plots, by comparing the ammonium nitrate -TE extractable  
259 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  $\text{NH}_4\text{NO}_3$ -TE extractable  
261 fraction decreased in all plots between the two sampling periods (Table 2), whereas the

262 DTPA-TE extractable fraction slightly increased for most poplar genotypes (Table S2). As the  
263 data provided are from composite samples, statistical analysis could not be performed on this  
264 dataset. The pH, which is an essential parameter in phytomanagement practices either evolved  
265 slightly upward or remained unchanged.

### 266 **3.2. Longer-term impact of the poplar plantation**

267 Our work was further pursued on the poplar Skado plot only. This genotype was chosen  
268 in 2011, together with the I214 genotype, for a new phytomanagement plantation at the same  
269 site within the BIOFILTREE project (Ciadamidaro et al., 2017). Soil samples were collected  
270 in 2013 and 2016, from which the pH and the CaCl<sub>2</sub>-extractable element fractions were  
271 measured. The 2007 and 2011 soil samples from under the Skado genotype were also  
272 reanalyzed; these samples had been stored while dry, with the same extraction and analytical  
273 methods used for the 2013 and 2016 soil samples as on earlier samples. The pH analysis after  
274 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 CaCl<sub>2</sub>-extractable fraction  
276 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 NH<sub>4</sub>NO<sub>3</sub>-extractant (Table 2) for  
278 Cd, Cu and Zn. The Mn and Ni were not chosen as target elements in the analyses of the 2007  
279 and 2011 soil samples. Other elements, such as K Mg, S and Si, increased significantly with  
280 time, which confirmed some of our previous data (Foulon et al., 2016b). Other elements (Na,  
281 Al, and Fe) followed the same trends; however, the changes were not statistically significant.

282 The depletion of the NH<sub>4</sub>NO<sub>3</sub> (2007-2011, first series of analysis)- or CaCl<sub>2</sub> (2007-  
283 2011-2013-2016, second series of analysis)-extractable TE fractions was not observed for the  
284 major elements and was low in absolute terms but high in relative terms, especially for Mn.  
285 Two main hypotheses could explain this decrease in the long term to confirm a depletion of  
286 the soil exchangeable compartment, with both hypotheses involving processes attributed to

287 changes in TE speciation under the land use: i) an increase in leaching in the soil profile under  
288 trees without replenishment of the exchangeable pool of TE or ii) an increase in retention in  
289 the soil due to the so-called process of “natural attenuation.” In the first case, a leaching of TE  
290 had been already observed in a soil profile of this area. But due to the constitution of the  
291 Luvisols, mobile TE were found trapped in the Bt horizon mainly constituted of clay before  
292 reaching the water table. Indeed, Dère et al (2007) observed that exogenous Zn and Cu but not  
293 Pb were found down to the base of the studied solum (i.e. around 1 m), mostly readsorbed on  
294 deep horizon’s constituents. In the second case, the TEs that are easily phytoavailable would  
295 have been taken up by poplar roots. As no obvious differences were observed between  
296 planted and unplanted poplar (naturally revegetated) plots (data not shown), it can be  
297 hypothesized that the herbaceous layer under the poplar trees played a major role in changing  
298 levels in the CaCl<sub>2</sub>-extractable fraction. However, these depletions for Cd, Cu and Mn under  
299 the adjacent maize plots was noticed in one of our previous studies (Foulon et al., 2016a),  
300 which may indicate an overall phenomenon of land use with plant species efficient in the use  
301 of atmospheric carbon, which then resulted in high amounts of the soil organics able to  
302 change TE speciation rather than from the poplar having a specific impact on its underlying  
303 vegetation. Indeed, the indirect effect of the newly developed vegetation (poplar trees or the  
304 adjacent herbaceous layer), by regularly enriching the Pierrelaye soil with fresh organic  
305 material other than from the wastewaters, could result in the alteration of the distribution of  
306 TE in the various soil extractable fractions. Previous works have demonstrated the importance  
307 of leaf litter on the nutrient cycles and bioavailability of TE (Ciadamidaro et al., 2013, 2014),  
308 and hence on the element export capacity of trees. Litter or litter components represent the  
309 main route of entry of nutrients in the soil and is one of the key points of the recycling of  
310 organic matter (OM) and nutrients, and, in forest ecosystems, the main source of OM and  
311 nutrients for the topsoil (Kuzyakov and Domanski, 2000). In particular, it can cover between

312 60 and 80% of the nutritional requirements of the tree per year. We did not attempt to  
313 determine the TOC content of the soils under the various poplar clones, but future projects at  
314 this experimental site would need to address this key feature in the management of TE  
315 contaminated soils.

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

321 The lack of change in the pH is an important indicator and could be associated with soil  
322 stabilization. It has to consider, however, that such soils rich in organic carbon have a high  
323 buffering effect against changes in pH, and data from a longer term could be useful for  
324 drawing conclusions. Due to these buffering effects, changes in soil pH are less important  
325 than those of TE availability seen either through DTPA, CaCl<sub>2</sub> or ammonium nitrate  
326 extractions. But the decrease in salt-exchangeable PTE compared to the increase in DTPA-  
327 extractable PTE is consistent with a change in PTE speciation in favor with PTE  
328 complexation with freshly organic matter brought by the aboveground vegetation.

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

### 334 **3.3. Genetic variability of poplar productivity at Pierrelaye**

335 H<sub>tot</sub> and dbh are frequently used as predictor variables, relating an easily measurable  
336 parameter to that of a less obtainable dependent factor such as tree dry weight. Both

337 parameters are known to be strongly indicative of aboveground woody biomass (Morhart et  
338 al., 2013; Zianis, 2008) and are part of the equations detailed in the material and methods  
339 section. Individual genotypic allometric equations allowed establishing relationships between  
340 the diameter measured at breast height (dbh) in 2013 and total dry biomasses of harvested  
341 trunk and branches respectively, for the 14 poplar genotypes grown at the Pierrelaye site  
342 (Fig. 2). Although the equations gave similar curves, the differences between the poplar  
343 genotypes was evident and showed that among the poplar genotypes retained in the present  
344 study, some variability occurred in the dry weight distribution between trunk and branches  
345 and/or wood density.

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

356 Over the entire 7-year rotation, huge genotypic variability was observed, with the  
357 Vesten genotype producing the largest biomass ( $8.5 \text{ odt ha}^{-1} \text{year}^{-1}$ ) in the SRC production  
358 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  $11.7 \text{ odt ha}^{-1} \text{year}^{-1}$  for Aspen  
360 (Aylott et al., 2008; Johansson, 1999; Morhart et al., 2013; Verlinden et al., 2013). There was  
361 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<sup>-1</sup> year<sup>-1</sup> and were  
363 among the most productive genotypes, whereas the DxN genotypes exhibited large variations  
364 ranging from 3.1 (Dorskamp) to 8.5 (Vesten) odt ha<sup>-1</sup> year<sup>-1</sup>. Our data contrast with those  
365 from a previous study (Verlinden et al., 2013), where clustering of the poplar genotypes was  
366 clearly determined by genetic origin, with the poplar plantation being established on a normal,  
367 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<sup>-1</sup> year<sup>-1</sup>. The  
369 Vesten genotype in our study was more productive than that in the Verlinden study  
370 (Verlinden et al., 2013). The trees were, however, harvested after only 2 growing seasons in  
371 this previous study. The Dorskamp genotype showed notably lower biomass productivity in  
372 our study than the other 13 genotypes. However, this genotype experienced more rabbit  
373 feeding than the other genotypes, and the mortality was much higher for that genotype (Table  
374 S1). A high variability in growth and in cutting quality (especially cutting diameter) during  
375 the first years is likely due to weed pressure and management practices (Broeckx et al., 2012).  
376 However, at our site, all plots received the same appropriate treatment for weed management,  
377 and cutting diameters were homogenous between all genotypes that were planted.

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

### 387 **3.4. Macronutrient concentrations in wood and branches of poplar genotypes**

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

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

405       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<sup>-1</sup> Ca, 800  
407 mg kg<sup>-1</sup> K, 200 mg kg<sup>-1</sup> Mg, and 100 mg kg<sup>-1</sup> P for the major elements. The K, Mg, and P  
408 concentrations were, on average, 1.5 to 5-fold higher than those given by Obernberger et al.  
409 (2006), whereas Ca concentrations were in the same range.

### 410 **3.5. Trace element concentrations in trunk and branches of poplar genotypes**

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

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

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

435 compared with wood. For most poplar genotypes, the TE concentrations were the highest in  
436 the upper sections of the trunk (not shown). For instance, Fe doubled in the summit sections  
437 of the Bakan and Fritzi Pauley trunks compared to the basal sections. The Pb concentration  
438 was always the highest in the basal section of the trunk, probably reflecting a simple  
439 contamination by aerosols, rather than an active transport, as already detailed in Migeon et al.  
440 (2009). No such clear tendency was observed for the other elements.

441 Metal content in woody biomass can greatly affect the industrial process, as it may  
442 interfere with the thermal degradation by acting as catalysts. A previous paper reported for  
443 instance that Cl ions may change metal behavior by increasing metal volatilization (Bert et al.,  
444 2017). For TE, 0.1 mg kg<sup>-1</sup> Cd, 1 mg kg<sup>-1</sup> Cr, 2 mg kg<sup>-1</sup> Cu, 25 mg kg<sup>-1</sup> Fe, 83 mg kg<sup>-1</sup> Mn, 2  
445 mg kg<sup>-1</sup> Pb, and 10 mg kg<sup>-1</sup> Zn are the concentrations usually reported (Oberberger et al.,  
446 2006). In our study, Cu, Cr, Fe, Mn and Pb concentrations in poplar wood, which range  
447 between 0.1 and 3 mg kg<sup>-1</sup>, are so low that they will not be discussed further herein. Cd  
448 concentrations were also very low in wood (< 0.5 mg kg<sup>-1</sup>); however, the volatile nature of Cd  
449 and its potential dissemination into the atmosphere when the biomass is burned should be  
450 considered (Chalot et al., 2012; Evangelou et al., 2012). When compared to the present  
451 dataset, Zn was accumulated at a much higher rate in a previous study, although large  
452 variations were observed among the poplar genotypes (Castiglione et al., 2009). The total Zn  
453 concentration (879-950 mg kg<sup>-1</sup>) was also higher compared to our soil data (Table 1). The  
454 authors also did not mention whether bark was included or not in the stem analysis.

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

460 storing TE in their aboveground biomass (Migeon et al., 2009), with potential sequestration of  
461 TEs within cells in the organelles. In poplar, this is illustrated by the sequestration of Mn in a  
462 Golgi-associated compartment through the transport protein PtMPT11 (Peiter et al., 2007) and  
463 by the vacuolar sequestration of Zn through the transport protein PtMTP1 (Blaudez et al.,  
464 2003). The potential function of other target transport genes were further underscore in TE  
465 management (Pottier et al., 2015) and their potential use in phytomanagement using *Populus*  
466 species. *PtNRAMP1* may, for instance, represent an interesting target for genetic engineering  
467 of poplar and modulation of leaf TE content.

### 468 **3.6. Element accumulation in bark tissues**

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

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

480 In a previous study, the authors concluded that the quality of the final product is  
481 strongly impacted by the amount of bark in the biomass feedstock (Morhart et al., 2013). As  
482 wood chips from SRC are being used as recycling materials to enhance soil fertility, concern  
483 is growing over the fate of TE from bark tissues (Vandecasteele et al., 2013). This is even  
484 more meaningful for biomass harvested at phytomanagement sites, where the presence of TE

485 in the bark tissues may have an impact on the industrial processes. Evangelou and  
486 collaborators (Evangelou et al., 2012) also concluded that harvesting biomass with smaller  
487 bark proportion in relation to trunk is desirable.

488

### 489 **3.7. Element export by poplar genotypes**

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

499 The ability to export these elements is an important consideration in phytomanagement,  
500 as the concentration of contaminants in the harvested part will greatly determine their  
501 potential valorization. Additionally, the export of nutrients is also a key consideration. The  
502 export potential was clearly greater for branches for Ca, Mn and Fe and to a lesser extent for  
503 K, P, Mg, Zn, Cd compared with the trunk. This difference was directly linked to the higher  
504 bark content of branches and higher content in these elements (Table 7). Conversely, wood  
505 tissues showed greater Cu, Cr and Pb export capacities, related to their equal amounts in  
506 wood and bark samples (Table 8). Previous studies have suggested that harvesting poplar as  
507 the whole tree from short-term rotations should cause soil nutrient depletion and induce  
508 degradation of soil and decreased biomass production (Ge et al., 2015). However, such a  
509 decrease in soil nutrient contents was not observed at our site when comparing total (not  
510 shown) or ammonium nitrate-extractable (Fig. 1) fractions of major elements in the 2007 and

511 2011, 2013 and 2016 samplings, perhaps because their levels were already high enough. The  
512 enrichment of the Pierrelaye soils with many cations, such as K and Mg, in poplar plots may  
513 indicate a thorough mobilization that occurs through microbial activities that are stimulated  
514 by the presence of poplar trees (Foulon et al., 2016b, 2016a). Conversely, whether these  
515 microbial communities are indeed expressing the capacity to mobilize these elements from the  
516 bound fraction is unknown.

517

#### 518 **4. Conclusions**

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

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796 Figure captions

797 **Fig. 1.** CaCl<sub>2</sub>-TE extractable fraction in Pierrelaye soils collected under the Skado genotypes.

798 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  
800 differences (p<0.05, Kruskal-Wallis). ns : not significant.

801

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

805

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

810

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

812

813

814 Supplemental materials

815

816 **Fig. S1.** Variables measured in the field for individual trees and location of sampling for dry  
817 matter and element concentration (in blue)

818

819 **Table S1**

820 Poplar genotypes grown at the experimental field of Pierrelaye D: *P. deltoides*; DxN: *P.*

821 *deltoides* x *P. nigra*; T: *P. trichocarpa*; TxM: *P. trichocarpa* x *P. maximowiczii*. B: Belgium;

822 I: Italy, NL: the Netherlands.

823

824 **Table S2**

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

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