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**Towards financially viable phytoextraction and production of plant-based palladium catalysts**

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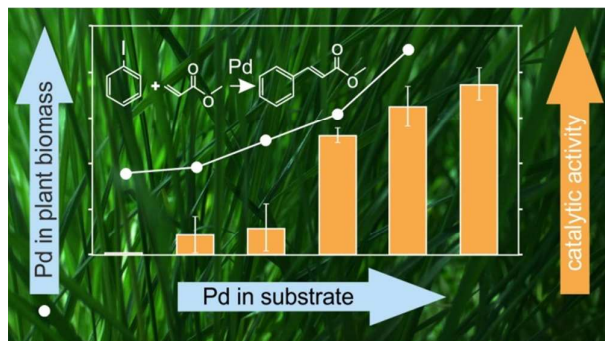
30 **ABSTRACT**

31 Although a promising technique, phytoextraction has yet to see significant  
32 commercialization. Major limitations include metal uptake rates and subsequent processing  
33 costs. However, it has been shown that liquid-culture-grown *Arabidopsis* can take up and  
34 store palladium as nanoparticles. The processed plant biomass has catalytic activity  
35 comparable to that of commercially available catalysts, creating a product of higher value  
36 than extracted bulk metal. We demonstrate that the minimum level of palladium in  
37 *Arabidopsis* dried tissues for catalytic activity comparable to commercially available 3%  
38 palladium-on-carbon catalysts was achieved from dried plant biomass containing between 12  
39 and 18 g·kg<sup>-1</sup> Pd. To advance this technology, species suitable for in-the-field application:  
40 mustard, miscanthus and sixteen willow species and cultivars, were tested. These species  
41 were able to grow, and take up, palladium from both synthetic and mine-sourced tailings.  
42 Although levels of palladium accumulation in field-suitable species are below that required  
43 for commercially available 3% palladium-on-carbon catalysts, this study both sets the target,  
44 and is a step towards, the development of field-suitable species that concentrate catalytically-  
45 active levels of palladium. Life cycle assessment on the phytomining approaches described  
46 here indicates that the use of plants to accumulate palladium for industrial applications has  
47 the potential to decrease the overall environmental impacts associated with extracting  
48 palladium using present-day mining processes.

49

50

51

52 **TOC/Abstract Art**

53

54

55 **INTRODUCTION**

56 A number of plant species have been found to take up gold and deposit it as gold  
57 nanoparticles (NPs) in their tissues<sup>1</sup>, a phenomenon that has also been demonstrated for  
58 platinum group metals (PGMs) in *Arabidopsis thaliana*<sup>2</sup>. Following a low-  
59 energy, pyrolysis treatment, the metal NP-containing plant biomass derived material can have  
60 catalytic activity comparable to that of commercially available catalysts and, critically,  
61 creates a product of higher value than the extracted bulk metal alone. These features present a  
62 potentially financially viable opportunity for the phytoextraction of these metals from PGM-  
63 rich sources and mine wastes<sup>3</sup>.

64 Phytoextraction was initially described in 1995 as ‘the use of metal-accumulating plants to  
65 remove toxic metals from soil’<sup>4</sup>, and expanded in 2001 as ‘the utilization of plants to  
66 transport and concentrate metals from the soil into the harvestable parts of roots and above-  
67 ground shoots’<sup>5</sup>. However, phytoextraction has now been around for several decades and,  
68 although a promising technique, it has yet to see significant commercialization<sup>6</sup>. A key factor  
69 for successful phytoextraction is that the value of the metal extracted needs to exceed the cost

70 of the recovery method. In the case of PGMs, the bulk value is relatively high. However, the  
71 costs of phytoextraction and subsequent harvesting, processing and smelting can still be  
72 inhibitory. By exploiting the natural ability of plants to accumulate PGMs as NPs,  
73 phytoextraction may be financially viable<sup>7</sup>.

74

75 The material potentially available for phytomining is the waste material (the ‘tailings’) that is  
76 separated from the valuable metals in mineral ores. Depending on the efficiencies of  
77 separation, tailings can contain variable amounts of a variety of metals, some valuable, and  
78 some toxic. Tailings, which typically consist of highly liquid slurries of silicates and other  
79 rock debris, are deposited in ponds during active ore processing and subsequently stabilized  
80 for long-term storage upon mine closure.

81 So far, the production of biomass which has catalytic activity has only been demonstrated in  
82 non-field conditions, using the non-crop species *Arabidopsis*<sup>2</sup>. There is a need to develop this  
83 technology in species suitable for field testing. While plants can grow in a diverse array of  
84 challenging environments, land for phytoextraction often contains deleterious traits, such as  
85 toxic levels of metals, sub-optimal pH, low organic matter content and low nutrient content.  
86 These properties result in suboptimal plant growth and low plant biomass. Even so, some  
87 species can be well-suited to the harsher conditions found in metal-rich land. Willows (*Salix*  
88 sp.), for example, are able to withstand concentrations of metals (copper, cadmium, nickel  
89 and zinc) considered toxic to many other plant species and can achieve high biomass in  
90 relatively poor soils<sup>8-12</sup>. Additionally, there is considerable genetic diversity within the  
91 willow genus that could be exploited to develop cultivars optimized for the environmental  
92 conditions present at a field site<sup>13</sup>. Other species with phytoextraction potential include

93 miscanthus (*Miscanthus x giganteus*)<sup>14</sup> and switchgrass (*Panicum virgatum*)<sup>15,16</sup>. These  
94 species are currently grown as bioenergy crops, and the necessary agricultural infrastructure  
95 for growing and harvesting is already in place<sup>17</sup>.

96 At the biological level, a major factor limiting the realization of phytoextraction is the low  
97 levels of PGMs taken up naturally by the plant. Significant progress towards the  
98 commercialization of nickel phytoextraction is being made with the use of hyperaccumulator  
99 species such as *Alyssum* sp.<sup>18,19</sup>; but, only a limited number of elements are known to be  
100 concentrated by hyperaccumulator species, and no suitable species have yet been found for  
101 PGMs.

102 At the physico-chemical level, poor metal uptake is linked to the chemical form of the metal.  
103 In the case of PGMs, these elements exist in field locations predominantly as chemically  
104 inert, zero-valent forms, or are bound to minerals. A highly effective recovery method for  
105 gold and PGMs is the use of cyanide for solubilization<sup>1</sup>.

106

107 However, irresponsible use of cyanide as a lixiviant in the mining industry has resulted in  
108 examples of serious, large-scale environmental pollution where toxic cyanide-containing  
109 complexes such as ferri- and ferro-cyanide, have accumulated in soils<sup>20, 21</sup>. Cyanide use is  
110 now tightly regulated in many countries, but is still the principal method used by the mining  
111 industry to recover gold, and silver, with this use representing approximately 15% of cyanide  
112 consumption globally<sup>22</sup>.

113 To achieve palladium NP formation in plants, and catalytic activity in the subsequently  
114 processed biomass, an as-yet-unknown threshold concentration of palladium in the tissues  
115 needs to be exceeded. In the present study, we have aimed to establish the minimum  
116 concentration of palladium needed in dry plant biomass to achieve catalytic activity  
117 comparable to that of commercially available 3% palladium-on-carbon catalysts. As an  
118 extension to previous studies in *Arabidopsis*<sup>2,23</sup>, we have extrapolated the experiments to  
119 plant species suitable for in-field application, including miscanthus and willow. The levels of  
120 palladium uptake by these species have been compared with target levels determined using  
121 our model *Arabidopsis* system.

122 Life cycle assessment (LCA) is a methodology that accounts for the environmental impacts  
123 associated with products and processes along the lifecycle of a material. In the mining  
124 industry, LCA has been utilized extensively<sup>24-28</sup>, and its application to understand the  
125 environmental implications of phytoaccumulation; and to highlight the related potential for  
126 opportunities of metal recovery from mine tailings, have been previously proposed<sup>3</sup>.  
127 Recently, LCA has been applied for nickel phytomining<sup>19</sup>. Here, we have used LCA to test if  
128 our phytomining approaches have the potential to decrease the overall environmental impacts  
129 associated with the current mining processes.

## 130 **MATERIALS AND METHODS**

### 131 *Synthetic and mine-collected tailings for plant growth*

132 Synthetic, palladium-rich tailings were created for the initial stages of this research, based on  
133 protocols for synthetic gold tailings, which have been extensively used for gold uptake  
134 experiments<sup>29</sup>. The elemental composition of the synthetic tailings, prior to dosing with

135 palladium, is shown in Table S1. Use of synthetic tailings enabled the concentration of  
136 palladium to be controlled in a background material that did not contain phytotoxic levels of  
137 metals, such as nickel, that are often present in mine samples and wastes. The use of synthetic  
138 tailings also meant that variables underpinning the process of palladium-uptake by plants  
139 (*i.e.*, lixiviant and metal concentration in soil) could be explored at a relatively low cost prior  
140 to using genuine mine wastes, which are more difficult to acquire in significant quantities.  
141 Mine-collected tailings were obtained from North American Palladium. Table S2 shows the  
142 elemental profiles, and Table S3 the gold and PGM profiles, of this material. Mustard plants  
143 were also grown on equivalent (v/v) amounts of vermiculite in palladium-free control  
144 experiments.

145 *Growing Arabidopsis, mustard, miscanthus, and willow*

146 *Arabidopsis* plants, ecotype Col0, were grown in liquid culture as described elsewhere<sup>2</sup>. For  
147 mustard, plastic P2 trays containing 1 kg of synthetic, or mine-collected, tailings were sown  
148 with 4.2g of mustard (*Brassica alba* L., cultivar Rivona) seed. The trays were placed in a  
149 glasshouse and watered as required. To measure the effect of a palladium solubilizing  
150 treatment on the uptake of palladium by the mustard plants, a potassium cyanide treatment  
151 was applied as follows: After seven days in the glasshouse, 100 ml of potassium cyanide (1  
152 mg ml<sup>-1</sup> cyanide) was applied to selected P2 trays, the plants were then harvested 24 h later  
153 and dried overnight at 60 °C.

154 *Miscanthus* (*Miscanthus x giganteus*) rhizomes and willow rods (*Salix* sp.; Yorkshire Willow  
155 Ltd, UK) were rooted in sand for four weeks. Rooted, in-leaf plants were transferred to 1.5l  
156 pots containing either 1 kg (for *miscanthus*), or 1.5 kg (for willow), of synthetic tailings or  
157 mine-collected tailings. A general purpose fertilizer was added weekly according to the

158 manufacturer's instructions and the plants grown for four weeks. To measure the effect of a  
159 palladium solubilizing treatment on the uptake of palladium by the plants, half of the plants  
160 were dosed with 100 ml (for miscanthus), or 150 ml (for willow) of potassium cyanide (1 mg  
161 ml<sup>-1</sup> cyanide). One week later, all the plant roots and shoots were harvested and dried  
162 overnight at 60 °C.

### 163 *ICP-MS analysis*

164 Plant tissues were ground to a fine powder using ball bearings in an end-over-end mixer. To  
165 0.5 g of tissue, 5 ml of aqua regia (3:1 hydrochloric acid (37%): nitric acid (70%)) was added.  
166 The samples were heated for 2 h at 70 °C then diluted to 50 ml with ultrapure water and  
167 filtered using a 0.45 µm filter. Metal content was determined using inductively coupled  
168 plasma mass spectrometry (ICP-MS) on an Agilent 7700x and calibrated against multi-  
169 element and precious calibration standards (Agilent Ltd).

170

### 171 *Testing catalytic activity*

172 To produce the catalyst, dried plant material was pyrolysed using the Barnstead Thermolyte  
173 6000 Furnace under N<sub>2</sub> (1 K m<sup>-1</sup>) at 573 K (300 °C) as described<sup>2</sup>. For the reaction of  
174 iodobenzene with methyl acrylate, 5.00 mmol iodobenzene, 6.25 mmol methyl acrylate, and  
175 6.25 mmol triethylamine in 1.75 mL of N-methyl-2-pyrrolidone, were added to a 25 ml round  
176 bottom flask. Once the flask had been heated to 393 K, 10 mg of palladium catalyst were  
177 added. For control experiments, no catalyst was added. Control reactions with 10 mg of  
178 pyrolysed, palladium-free plant material were also run. The reaction was allowed to proceed

179 for 2 hours at 393 K and the levels of substrate and product measured using a Gas  
180 Chromatography-Flame Ionization Detector (GC-FID) using diethyl succinate as a standard.

181 *Life cycle assessment comparisons*

182 Details of the two LCA models developed are shown in Supplementary Material.

183 Two scenarios were developed to conduct a comparative LCA. The first scenario compared  
184 the environmental impacts associated with the production of Arabidopsis catalyst material  
185 relative to the commercial route for activated carbon-palladium catalysts. A second scenario  
186 modeled the phytoaccumulation of palladium from mine tailings and the processing of the  
187 biomass for the production of valuable products such as bio-gas, bio-oil, and bio-char  
188 containing palladium.

189 The models were carried out in accordance with the ISO 14040 guidelines, and developed  
190 using the SimaPro 8.0 software. To enable a comparative LCA study with real systems, the  
191 investigated processes were scaled up to a hypothetical facility based on extrapolation from  
192 laboratory scale studies and literature data. Modules of inventory data for raw material and  
193 chemical production, heat generation and grid electricity production mix, and transportation  
194 were derived from the ecoinvent 3.0 database. Direct and indirect mass and energy flows  
195 were accounted for according to the system boundaries set for the two systems investigated.  
196 A selection of standardized indicators was used for the assessment of environmental impacts  
197 to midpoint and endpoint categories, and is shown in Table S4. A detailed description of the  
198 two LCA scenarios is reported in the Supplementary Material.

199 **RESULTS**

200 In previous studies, catalytic activity was recorded for pyrolysed *Arabidopsis* biomass  
201 containing 5 g·kg<sup>-1</sup> palladium. The biomass was derived from plants grown in liquid culture  
202 and dosed with 10 mM potassium tetrachloropalladate<sup>2</sup>. This experimental system was  
203 replicated in the current work as a model in which metal doses could be accurately controlled  
204 in a small-scale system. Using this model, the relationship between the *in planta* palladium  
205 concentration and catalytic activity of liquid-culture grown *Arabidopsis* plants dosed with a  
206 range of palladium concentrations (from 0.5 mM to 1 mM of potassium tetrachloropalladate)  
207 was investigated.

#### 208 *Testing catalytic activity in Arabidopsis*

209 The concentration of palladium in the dried *Arabidopsis* material was found to increase from  
210 0.18 to 18 g kg<sup>-1</sup> palladium with increasing concentrations (0.5 mM to 1 mM respectively) of  
211 potassium tetrachloropalladate (Figure 1). For pyrolysis of the plant biomass, the temperature  
212 chosen was 300 °C. This was based on an earlier study which demonstrated that at this  
213 temperature the mean nanoparticle diameter and frequency distributions were unaltered, with  
214 the remaining biomass comprising predominantly carbon and oxygen<sup>2</sup>. Previous studies using  
215 thermal gravimetric-infrared (TGIR) analysis of the post-pyrolysis material showed a 45%  
216 mass loss between 100 - 300°C, attributed to the loss of water and carbon dioxide<sup>2</sup>. Catalytic  
217 activity was tested using the Heck reaction between iodobenzene and methyl acrylate to form  
218 *trans*-methyl cinnamate. Figure 1 shows that there was no catalytic activity from plants dosed  
219 with 0.5 mM potassium tetrachloropalladate. However, product was observed from plants  
220 dosed with concentrations of 0.6 mM and above, with product yields increasing with higher  
221 concentrations of potassium tetrachloropalladate. Palladium on carbon 3% (Pd/C), a  
222 commercially available palladium catalyst, was used to compare with the performance of the

223 pyrolysed biomass. The yields obtained from plants dosed with 1 mM palladium were  
224 comparable to those obtained by Pd/C (64.9 % and 74.9 % respectively).

225 The results presented in Figure 1 indicate a palladium concentration of between 12 and 18  
226  $\text{g}\cdot\text{kg}^{-1}$  in dried *Arabidopsis* biomass is the target level above which catalytic activity would be  
227 comparable to that of commercially available catalysts. In order to assess if this target could  
228 be reached in species more suited to in-field application, mustard, willow, and miscanthus  
229 were tested in studies using synthetic tailings. To increase uptake by solubilizing the  
230 palladium, potassium cyanide was used. While there is currently no data available for  
231 palladium, a review comparing gold uptake by a number of plant species treated with a range  
232 of cyanide-based compounds indicated that potassium and ammonium thiocyanate yield the  
233 highest levels of gold uptake<sup>1</sup>. For *Brassica juncea*, the highest levels of uptake were  
234 observed using potassium cyanide<sup>1</sup>. As mustard and *Arabidopsis* are also in the Brassicaceae,  
235 and willow has been shown to effectively remediate potassium cyanide<sup>40</sup>, this compound was  
236 chosen as the lixiviant.

### 237 *Growth and palladium uptake by mustard*

238 The biomass of mustard plants grown on synthetic tailings containing up to  $50 \text{ mg}\cdot\text{kg}^{-1}$   
239 palladium was not significantly different to the biomass of plants grown on the no-Pd control  
240 material, but the biomass of plants grown on synthetic tailings containing  $100 \text{ mg}\cdot\text{kg}^{-1}$   
241 palladium was a third lower than in the absence of palladium ( $p < 0.05$ ; Figure 2A). The  
242 addition of cyanide significantly ( $p < 0.001$ ) increased the uptake of palladium by 26, 20, 30,  
243 and 23-fold in plants grown in synthetic tailings containing 5, 10, 50, and  $100 \text{ mg}\cdot\text{kg}^{-1}$   
244 palladium, respectively (Figure 2B and Table S5). The pie charts shown in Figure S1 and  
245 data in Table S5, illustrate both the effect of increasing levels of palladium on the uptake of

246 other metals present in the synthetic tailings and the effect of the exogenous application of  
247 cyanide. The mustard plants grown on the synthetic tailings in the absence of cyanide or  
248 palladium contained predominantly zinc and copper. With increasing palladium  
249 concentration, and in the absence of cyanide, levels of palladium in the plant increased  
250 predominantly at the expense of copper, whereas in the presence of cyanide, levels of copper,  
251 which is also solubilized by cyanide, were less affected.

252 However, mine sourced materials often contain other elements at concentrations inhibitory to  
253 plant growth. To test growth and palladium uptake in this inhibitory background, PGM-rich  
254 mine-collected tailings from North American Palladium were used. The mine-collected  
255 tailings contained approximately  $20 \text{ mg}\cdot\text{kg}^{-1}$  palladium; a concentration that the synthetic  
256 tailings study (Figure 2) indicated was below the phytotoxicity level. In agreement with this  
257 result, no toxicity symptoms were seen in the mustard plants grown on the mine material  
258 (Figure S2). The biomass of seven-day-old seedlings grown on the mine-collected tailings  
259 was higher than those grown on vermiculite alone. We speculate that this could be due to  
260 additional nutrients present in the mine-collected tailings that were lacking in the vermiculite.  
261 As seen with the palladium-dosed synthetic tailings experiment (Figure 2), the addition of  
262 cyanide significantly ( $p < 0.001$ ) increased the uptake of palladium by 89, 333, and 8.4-fold  
263 in plants grown in mine-collected tailings containing 5, 10, and  $20 \text{ mg}\cdot\text{kg}^{-1}$  palladium,  
264 respectively (Figure S2).

#### 265 *Growth and palladium uptake by miscanthus and willow*

266 Given the dramatic increase in the ability of mustard to take up palladium conferred by the  
267 application of cyanide, the potential of the more field-suitable species miscanthus and willow  
268 was next investigated. For miscanthus, the application of cyanide had no effect on the

269 biomass of root or shoot tissues (results not shown), whereas aerial tissues of the cyanide  
270 treated plants had almost 500-fold more palladium than those from untreated plants grown on  
271 synthetic tailings (undosed plants contained  $0.0013 \pm 0.0001 \text{ g}\cdot\text{kg}^{-1}$ ; cyanide-treated plants  
272 contained  $0.505 \pm 0.039 \text{ g}\cdot\text{kg}^{-1}$  palladium).

273 For the willow experiments, a fast-growing, bioenergy hybrid of *Salix viminalis*, 'Super  
274 Willow' was chosen. As seen with miscanthus, *S. viminalis* 'Super Willow' leaf biomass was  
275 unaltered by the cyanide treatment. At the lower concentrations of palladium-dosed synthetic  
276 tailings (5 and 10  $\text{mg}\cdot\text{kg}^{-1}$ ), leaf biomass was not significantly affected, however, at the  
277 higher concentrations of palladium (50 and 100  $\text{mg}\cdot\text{kg}^{-1}$ ) total leaf biomass significantly  
278 decreased ( $p < 0.05$ ; Figure 3A). The application of cyanide dramatically, and significantly ( $p$   
279  $< 0.001$ ), increased the uptake of palladium by 126, 127, 19 and 23-fold in plants grown at 5,  
280 10, 50 and 100  $\text{mg}\cdot\text{kg}^{-1}$  palladium respectively (Figure 3B). In agreement with the data for  
281 mustard, (Figure 2B and Table S5), the cyanide treatment was observed to be less effective at  
282 promoting palladium uptake at palladium concentrations above 20  $\text{mg}\cdot\text{kg}^{-1}$ .

283 *Salix viminalis* 'Super Willow' was grown on mine-collected tailings and growth and  
284 palladium uptake measured. Figure S3A demonstrates that the application of cyanide did not  
285 affect leaf or stem biomass, yet it dramatically, and significantly ( $p < 0.001$ ), increased the  
286 levels of palladium in the leaf and stem by 65 and 49-fold, respectively (Figure S3B and C).  
287 The effect of the cyanide treatment moderately increased uptake of other metals present in the  
288 mine-collected tailings (cobalt, nickel, copper, cadmium and lead), with the greatest effect  
289 observed for copper: the treatment of *S. viminalis* 'Super Willow' with cyanide increased  
290 copper levels by 9.5 and 12.5-fold, respectively, in the leaves and stems (Figure S3 and Table  
291 S6).

292 There is a wealth of genetic variability for metal uptake within the willow genus<sup>13</sup>. To  
293 investigate the variation in palladium uptake, 16 different species and cultivars of willow  
294 were selected. Figure 4A shows the variation in leaf and stem dry weights. Across the 16  
295 species and cultivars of willow, there was an 11.5 and 4.7-fold variation in leaf and stem  
296 biomass, respectively. *Salix alba*, *S. candida*, and *S. purpurea* (cv. Green Dicks) had  
297 consistently high leaf and stem biomass. In this experiment, the concentration of palladium in  
298 the leaves of *S. viminalis* was higher (Figure 4B, 0.313 g·kg<sup>-1</sup>) than in the previous  
299 experiment (Figure 3B, 0.094 g·kg<sup>-1</sup>), the discrepancy may have resulted from different  
300 environmental conditions in the glasshouse during the experiment for Figure 4: warmer,  
301 sunnier conditions would have increased transpiration rates and could lead to enhanced  
302 palladium uptake. Overall, across the 16 species and cultivars, there were 6.7 and 4.5-fold  
303 variations, respectively, in leaf and stem palladium content, with 6-fold more palladium  
304 present in the leaves than in the stems. Of the two species with consistently high palladium  
305 levels, *S. nigricans* and *S. purpurea* (cv. Green Dicks), the latter species also produced high  
306 leaf and stem biomass.

307 The palladium concentration in the non-Arabidopsis species tested in this work was below  
308 the 12 g·kg<sup>-1</sup> threshold established for catalytic activity. However, preliminary testing of the  
309 mustard, miscanthus, and willow (cv. Green Dicks) containing respectively 0.5, 1.5, and 0.8  
310 g·kg<sup>-1</sup> palladium post-pyrolysis resulted in catalytic yields of 5, 7, and 1.2 % respectively.

### 311 *Life Cycle Assessments*

312 To conduct the LCA, midpoint impact categories for the processes were defined (Table S4).  
313 This translated the total impact of a process into individual environmental themes. Figure 5A  
314 compares the commercial production route for Pd/C with that using liquid-culture grown

315 Arabidopsis for each selected midpoint impact category. The commercial production route  
316 for the Pd/C catalyst has higher impacts for climate change, metal depletion, fossil depletion,  
317 cumulative energy demand, and water scarcity single midpoints. The catalyst material  
318 obtained through the Arabidopsis process was found to have greater impacts for those  
319 categories associated with outputs to water and terrestrial compartments. Figure 5B shows  
320 aggregated scores for the two processes after weighting each (midpoint) impact category to  
321 damage impact categories (endpoint) for human health, ecosystems, and resources. Overall,  
322 the commercial Pd/C catalyst has a total environmental impact about three times higher than  
323 that produced through the Arabidopsis process.

324 The difference in environmental impacts between the two processes clearly favors the  
325 Arabidopsis process. However, phytoaccumulation efficiency is the most relevant parameter  
326 in influencing the LCA results. The Arabidopsis process was modeled by assuming that  
327 plants are dosed with the same amount of palladium required for the commercial production  
328 process. If the Arabidopsis process should require a greater input of palladium, or if part of  
329 the non-phytoaccumulated palladium is unrecovered, environmental impacts would increase  
330 significantly. Given that the environmental impacts favor the Arabidopsis process over the  
331 common production route for the Pd/C catalyst, the use of willow to make a Pd/C-equivalent  
332 catalyst is likely to be even more favorable. However, this would rely on the willow  
333 accumulating palladium to a level to be commercially viable as a catalyst.

334

335 Pyrolysis to 300 °C under N<sub>2</sub> is used for stabilization of the Arabidopsis Pd/C-equivalent  
336 catalyst. Whilst being very simple, this approach is not optimal to maximize value and  
337 process large volumes of biomass. Microwave assisted pyrolysis (MAP) is an alternative

338 approach that is being developed as a green technology for use as part of a holistic  
339 biorefinery<sup>30,31</sup>.

340 Using this technique the biomass is stabilized via microwave heating at lower temperatures  
341 and shorter times than needed for conventional pyrolysis. In addition to the solid bio-char  
342 catalyst, bio-oil and bio-gas are collected during MAP and this not only prevents the release  
343 of greenhouse gasses but also brings extra value to the process.

344 In the second scenario the impacts of bio-gas, bio-oil, and palladium-containing bio-char  
345 production from willow biomass (Pd-willow) were compared to the impacts that would  
346 derive from the production of the same amount of gas, oil, and palladium from common  
347 extraction routes. Figure 6A shows that production of these three products from Pd-willow  
348 decreases the overall impact for all midpoint categories, with the exception of particulate  
349 matter formation and agricultural land occupation. In most cases, the environmental impacts  
350 were reduced by up to 100 %. The environmental benefits associated with the avoided  
351 production of natural gas, oil, and palladium concentrate lead to a distinct improvement in  
352 total environmental performance, with the avoided damage to resources being the endpoint  
353 category for which the best score was derived (Figure 6B). The results of the sensitivity  
354 analysis support the confidence of the model.

## 355 **DISCUSSION**

356 Arabidopsis plants were grown hydroponically and dosed with solutions of potassium  
357 tetrachloropalladate, factors chosen to favour palladium uptake, and establish whether  
358 palladium concentrations in the biomass would be sufficient to obtain catalytically active  
359 material comparable to commercially available 3% carbon on palladium. The studies  
360 presented indicate that to obtain catalytically active material comparable to commercially

361 available 3% carbon on palladium, the dried plant biomass, prior to pyrolysis, needs to  
362 contain a minimum concentration of between 12 and 18  $\text{g}\cdot\text{kg}^{-1}$  palladium. At this  
363 concentration, we have shown previously that the Arabidopsis tissues contain palladium NPs  
364 which confer catalytic activity<sup>2</sup>. We infer that tissues from other species containing above 12  
365 to 18  $\text{g}\cdot\text{kg}^{-1}$  palladium would also contain palladium nanoparticles, but this should be tested  
366 in future studies. In combination with the application of cyanide, the highest concentrations  
367 of palladium achieved from plants grown on synthetic tailings in this study were 0.53  $\text{g}\cdot\text{kg}^{-1}$   
368 for mustard (with a KCN treatment 24 h pre-harvest), and 0.51 and 0.82  $\text{g}\cdot\text{kg}^{-1}$  palladium  
369 (with a KCN treatment 1 week pre-harvest) for miscanthus and willow (cv. Green Dicks),  
370 respectively. At these levels, catalytic activity, albeit low (5, 7, and 1.2 % yields,  
371 respectively), from subsequently pyrolysed material was observed. However, the levels of  
372 palladium accumulated in biomass from KCN-treated plants grown on mine-collected tailings  
373 were significantly lower: 0.0085  $\text{g}\cdot\text{kg}^{-1}$  for mustard and 0.0142  $\text{g}\cdot\text{kg}^{-1}$  for willow leaf.  
374 Although not tested, it was considered unlikely that pyrolysed material from the plants grown  
375 on mine-collected tailings would yield detectable catalytic activity. The technology  
376 developed in these studies is aimed at phytoextracting PGMs from wastes such as mine  
377 tailings which have levels of palladium typically between 0.7- 1  $\text{mg}\cdot\text{kg}^{-1}$ ; significantly lower  
378 than for the mine-collected tailings used here. Thus, achieving plant biomass with between 12  
379 and 18  $\text{g}\cdot\text{kg}^{-1}$  palladium is still a significant biotechnological challenge. However, our results  
380 demonstrate that in just one week, and following a single, non-optimized, cyanide treatment,  
381 the best performing willow cultivar had the biological capacity to take up approximately 10  
382 % of the palladium required to reach the target concentration. Given the extensive genetic  
383 resources available in this genus, screening or selective breeding programs would likely  
384 identify willow lines with further enhanced palladium uptake ability. Furthermore, this study

385 shows that willows and miscanthus are able to withstand the toxicities present in mine-  
386 sourced material; relative to ores and concentrates, tailings and other wastes will likely have  
387 correspondingly lower levels of phytotoxic metals. In field scenarios, species such as willow  
388 and miscanthus would be grown on significantly larger scale. Harvesting coppiced willow on  
389 a three to five year rotation in combination with repeated lixiviant treatments would be  
390 predicted to lead to further increases in palladium uptake.

391 On a global scale, implementing phytoaccumulation in field scenarios would maximize the  
392 extraction of valuable forms of palladium and other PGMs from natural deposits. Overall  
393 recovery rates of palladium from the processing of virgin ores are estimated at 80-90%<sup>32-35</sup>,  
394 resulting in considerable losses during comminution and concentration steps: the amount of  
395 cumulative palladium lost in mine tailings is estimated to represent about 5% of global  
396 reserve and reserve base values<sup>3</sup>. Thus, should palladium phytoaccumulation efficiency  
397 increase at levels to be financially viable, the exploitation of mine tailings would have a  
398 strong potential for supplementing primary palladium supply. From a perspective of resource  
399 conservation and environmental protection, the resulting potential benefits associated with  
400 recoverable palladium tailings would be greater still if phytoaccumulation led to lower  
401 environmental impacts of mining activity.

402 Results from the preliminary LCA reported in this paper are limited by the estimates used to  
403 enable the scaling of lab-based results up to a hypothetical full-scale facility. However, our  
404 analysis suggests that the use of plants to accumulate palladium, either for catalysts, or bio-  
405 gas, bio-oil, and palladium-containing bio-char production, as outlined here for willow, has  
406 the potential to decrease the overall environmental impacts (*i.e.*, computed as environmental  
407 impacts associated with the process, less the environmental burdens resulting from the

408 avoided production of equivalent products such as natural gas or oil) associated with  
409 palladium extraction by current mining processes.

410 Such a net-environmental performance improvement is of particular importance when  
411 considering the use of cyanide for palladium phytoaccumulation. In support of the use of  
412 cyanide in controlled phytoextraction conditions, it has been shown that plants can degrade  
413 cyanide, taking up both free cyanide and iron cyanides and metabolizing them to the amino  
414 acid asparagine<sup>36-40</sup>. These findings present the perhaps paradoxical situation whereby the  
415 application of cyanide might be the only mechanisms to enable the phytoextraction of PGMs  
416 to be financially viable.

417 However, in addition to environmental concerns, the use of cyanide treatments causes co-  
418 solubilization of significant levels of other metals such as gold, copper, and nickel. The  
419 presence of these metals in the pyrolysed biomass could affect subsequent catalytic activity<sup>41</sup>.  
420 Furthermore, the application of cyanide directly to the land results in the indiscriminate  
421 solubilization of the target metal throughout the material. This approach is inefficient for  
422 phytoextraction, because much of the solubilized metal is beyond the reach of the roots and is  
423 thus lost as leachate. Targeting solubilization to the rhizosphere, a microecological zone  
424 closely surrounding the roots, would enable more efficient metal uptake. This is an approach  
425 that could perhaps be combined with biomining, using precious metal accumulating bacteria  
426 such as *Cupriavidus metallidurans*<sup>42</sup> in the rhizosphere.

427 Whatever approaches to increase PGM accumulation are tested, there are large areas of land  
428 containing increasingly valuable PGM metals reserves that are currently un-vegetated and  
429 contain toxic levels of metals and wastes as by-products from the mining industry. The

430 combination of the phytoextraction process, in tandem with phytoremediation to re-vegetate,  
431 stabilize and restore ecological diversity, could be a win-win situation for the environment  
432 and for catalysis technologies. The approach described here increases the viability of green  
433 technologies and aids in promoting the use of biomass to replace fossil fuels.

434

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443 Meech.

444 **FIGURE LEGENDS**

445 **Figure 1.** Palladium uptake by *Arabidopsis* plants dosed with a range of concentrations of  
446 potassium tetrachloropalladate. Left axis (line), level of palladium in the dried plant biomass;  
447 right axis (column), catalytic activity of the pyrolysed plant biomass in the Heck reaction of  
448 iodobenzene and methyl acrylate to yield *trans*-methyl cinnamate (for Pd in dried plant  
449 material,  $n = 3$  biological replicates  $\pm$  s.e.m.; for % yield,  $n = 3$  technical replicates  $\pm$  r.s.d).

450 **Figure 2.** Growth and palladium uptake by mustard (*Brassica alba* L.) germinated and grown  
451 on synthetic tailings dosed with a range of palladium concentrations. After seven days,  
452 seedlings were dosed with  $100 \text{ mg}\cdot\text{kg}^{-1}$  cyanide (in the form of KCN), then harvested 24 h  
453 later. A) Biomass of aerial tissues, and B) concentration of palladium in the aerial tissues ( $n =$   
454 6 biological replicates  $\pm$  s.e.m.).

455 **Figure 3.** Growth and palladium uptake by *Salix viminalis*, 'Super Willow' grown on  
456 synthetic tailings dosed with a range of palladium concentrations. After four weeks, plants  
457 were dosed with  $100 \text{ mg}\cdot\text{kg}^{-1}$  cyanide (in the form of KCN), then harvested after seven days.  
458 A) Leaf dry weight, and B) concentration of palladium in the leaf tissues ( $n = 5$  biological  
459 replicates  $\pm$  s.e.m.).

460 **Figure 4.** Growth and palladium uptake by a range of willow species (*Salix* sp.). Rooted  
461 cuttings were grown for four weeks on synthetic tailings containing  $50 \text{ mg}\cdot\text{kg}^{-1}$  palladium,  
462 dosed with  $100 \text{ mg}\cdot\text{kg}^{-1}$  cyanide (in the form of KCN), then harvested after one week. A)  
463 Leaf and stem dry weight, and B) concentration of palladium, in the leaf and stem tissues ( $n =$   
464 5 biological replicates  $\pm$  s.e.m. except *Salix. alba*, *S. chermesina* and *S. alba vitellina* where  $n$   
465 = 2).

466 **Figure 5.** (A) Characterization results for midpoint categories. (B) Characterization results  
467 for endpoint categories; the y-axis reports absolute single points for total damage on  
468 resources, ecosystems, and human health according to the Europe ReCiPe H/A method.

469 **Figure 6.** (A) Characterization results for midpoint categories. (B) Characterization results  
470 for endpoint categories; the y-axis reports absolute single points (1 mPoint =  $10^{-3}$  Point) for  
471 total damage on resources, ecosystems, and human health according to the Europe ReCiPe  
472 H/A method. Negative values refer to avoided impacts.

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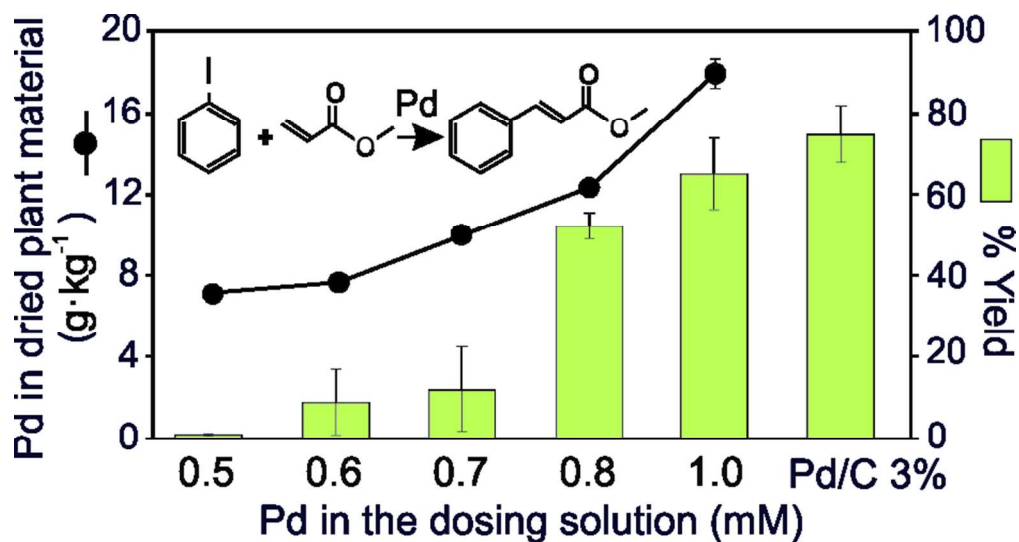


Figure 1. Palladium uptake by *Arabidopsis* plants dosed with a range of concentrations of potassium tetrachloropalladate. Left axis (line), level of palladium in the dried plant biomass; right axis (column), catalytic activity of the pyrolysed plant biomass in the Heck reaction of iodobenzene and methyl acrylate to yield trans-methyl cinnamate (for Pd in dried plant material,  $n = 3$  biological replicates  $\pm$  s.e.m.; for % yield,  $n = 3$  technical replicates  $\pm$  r.s.d).

Figure 1

79x41mm (300 x 300 DPI)

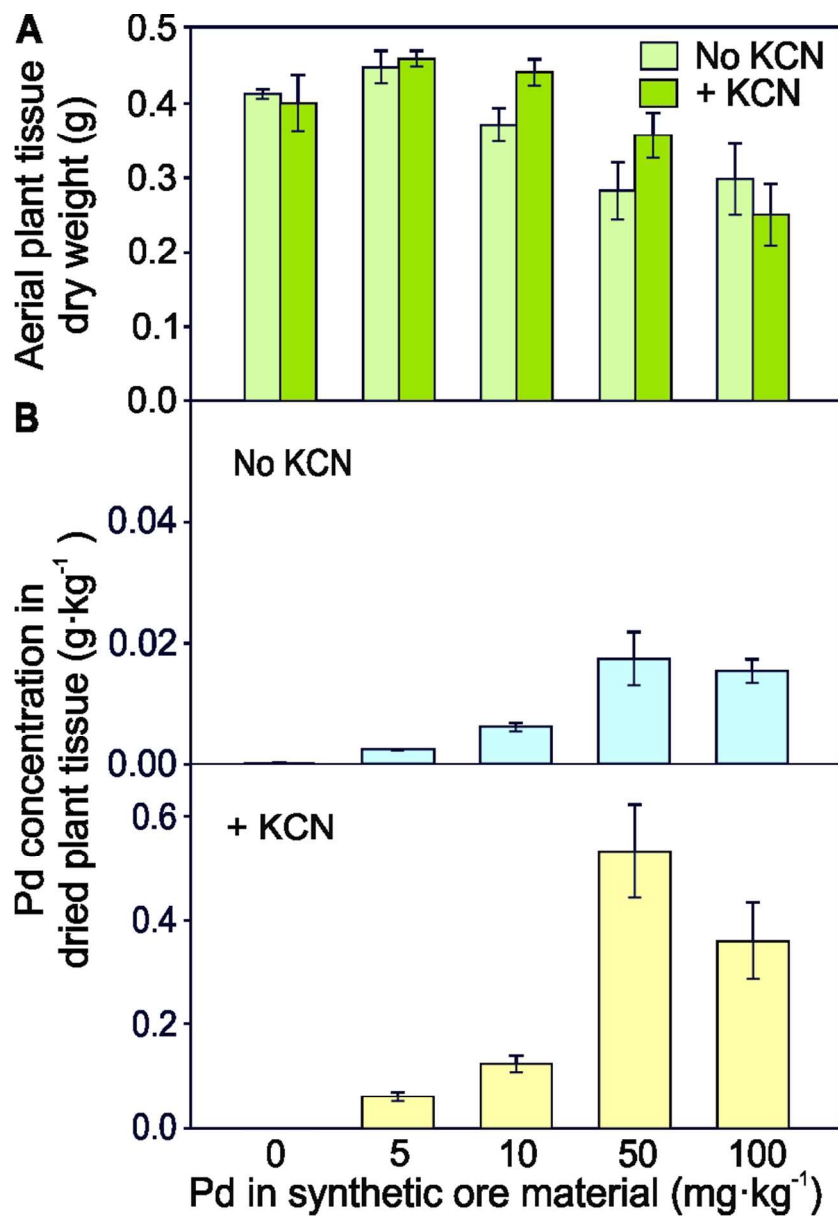


Figure 2. Growth and palladium uptake by mustard (*Brassica alba* L.) germinated and grown on synthetic tailings dosed with a range of palladium concentrations. After seven days, seedlings were dosed with 100 mg·kg<sup>-1</sup> cyanide (in the form of KCN), then harvested 24 h later. A) Biomass of aerial tissues, and B) concentration of palladium in the aerial tissues (n = 6 biological replicates ± s.e.m.).

Figure 2  
74x108mm (300 x 300 DPI)

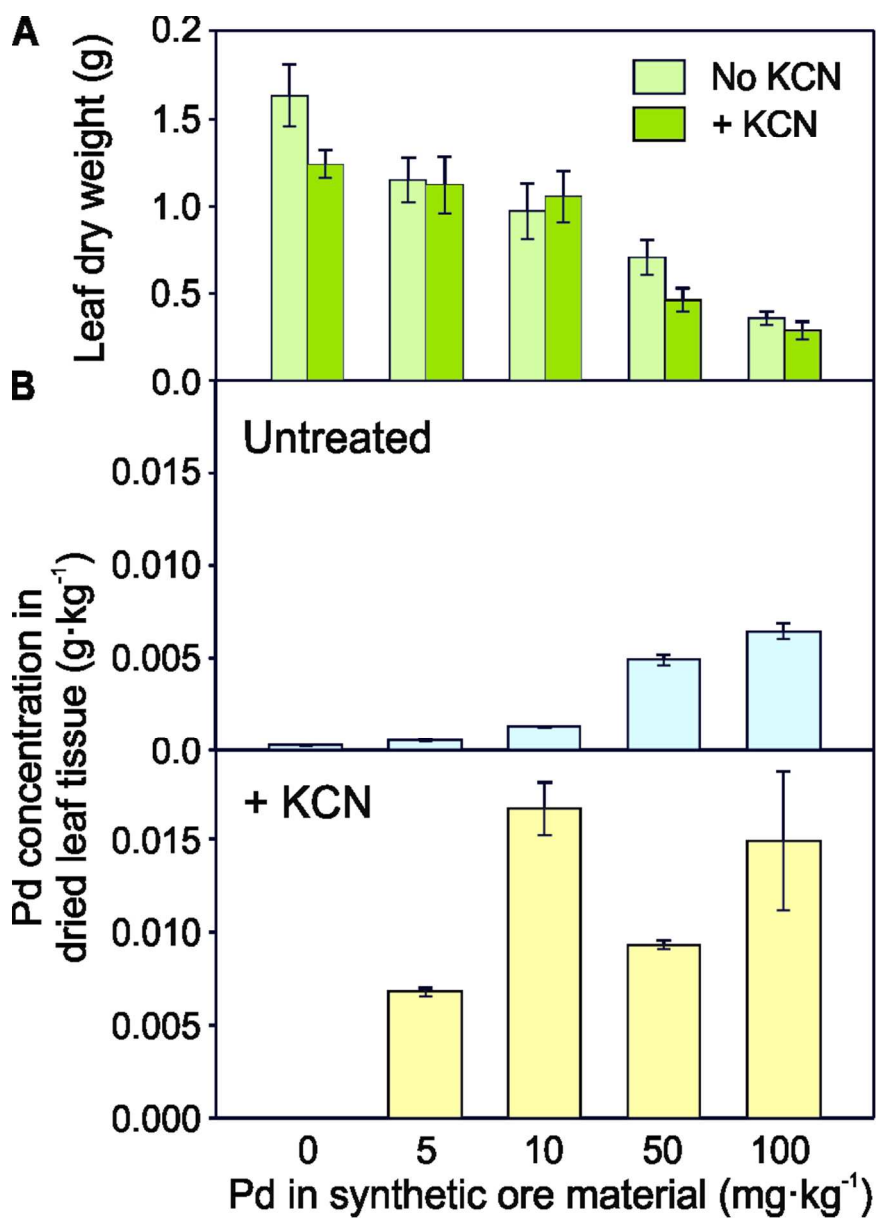


Figure 3. Growth and palladium uptake by *Salix viminalis*, 'Super Willow' grown on synthetic tailings dosed with a range of palladium concentrations. After four weeks, plants were dosed with 100 mg kg<sup>-1</sup> cyanide (in the form of KCN), then harvested after seven days. A) Leaf dry weight, and B) concentration of palladium in the leaf tissues (n = 5 biological replicates ± s.e.m.).

Figure 3  
76x106mm (300 x 300 DPI)

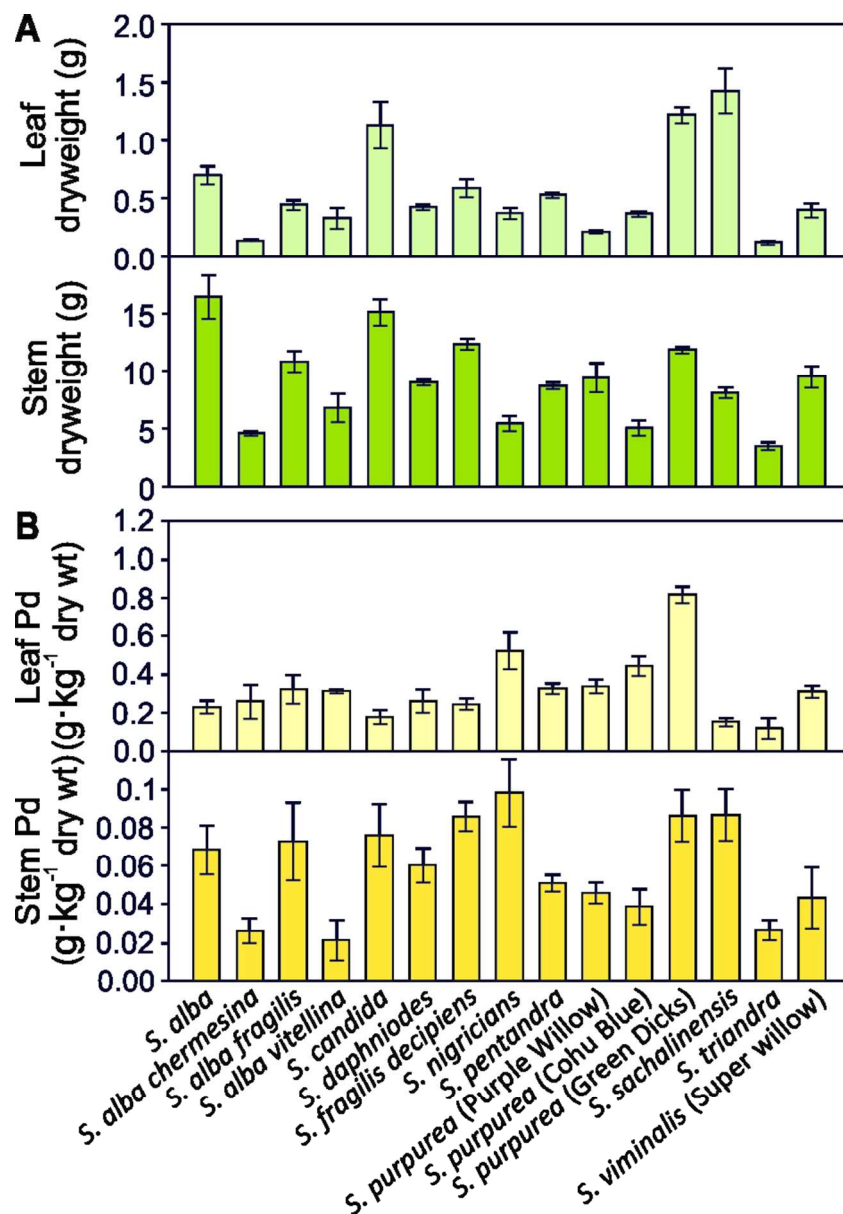


Figure 4. Growth and palladium uptake by a range of willow species (*Salix* sp.). Rooted cuttings were grown for four weeks on synthetic tailings containing 50 mg kg<sup>-1</sup> palladium, dosed with 100 mg kg<sup>-1</sup> cyanide (in the form of KCN), then harvested after one week. A) Leaf and stem dry weight, and B) concentration of palladium, in the leaf and stem tissues (n = 5 biological replicates ± s.e.m. except *Salix. alba*, *S. chermesina* and *S. alba vitellina* where n = 2).

Figure 4

81x113mm (300 x 300 DPI)

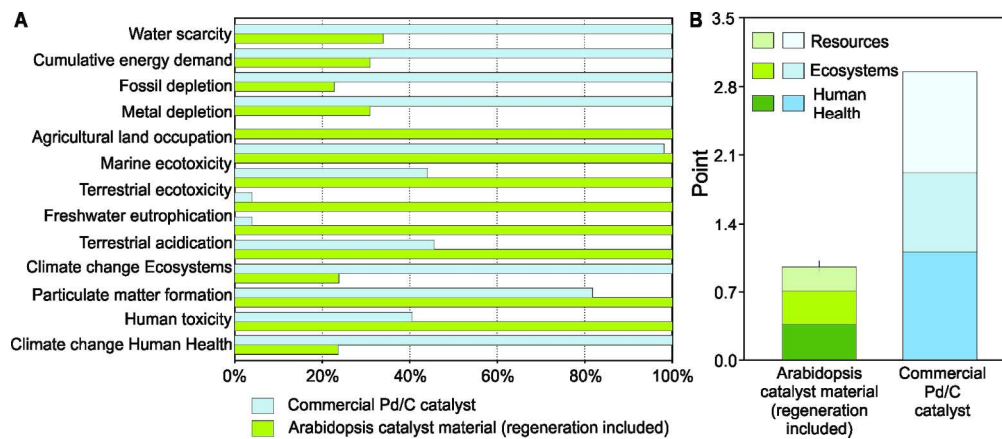


Figure 5. (A) Characterization results for midpoint categories. (B) Characterization results for endpoint categories; the y-axis reports absolute single points for total damage on resources, ecosystems, and human health according to the Europe ReCiPe H/A method.

Figure 5

173x73mm (300 x 300 DPI)

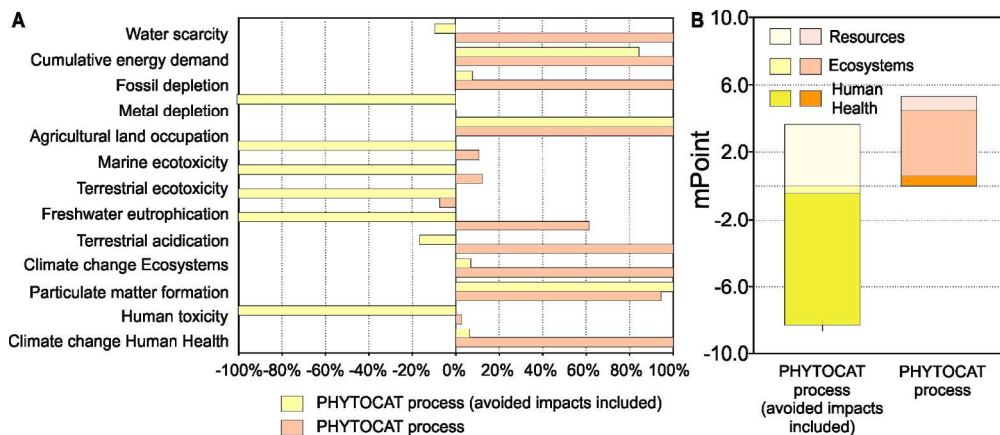


Figure 6. (A) Characterization results for midpoint categories. (B) Characterization results for endpoint categories; the y-axis reports absolute single points (1 mPoint = 10<sup>-3</sup> Point) for total damage on resources, ecosystems, and human health according to the Europe ReCiPe H/A method. Negative values refer to avoided impacts.

Figure 6  
174x74mm (300 x 300 DPI)