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# 1 **Assessment for combined phytoremediation and biomass production on a** 2 **moderately contaminated soil**

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7 **Key words: phytomanagement, biomass production, pollution attenuation, ecosystem services,**  
8 **contaminated soil, organic pollution**

## 9 **Abstract**

10 Once previous industrial activity has ceased, brownfields are found in urban and suburban environments and  
11 managed in different ways ranging from being left untouched to total reconversion. These situations apply to large  
12 surface areas often impacted by residual diffuse pollution. Though significant and preventing any sensitive use,  
13 residual contamination does not necessarily require treatment. Moreover, conventional treatments show their  
14 technical and economic limits in these situations and gentle remediation options such as phytomanagement might  
15 appear more relevant to the management those sites. Thus, these sites face up two major issues: managing moderate  
16 contamination levels and providing an alternative use of economic interest. This work proposes to assess a  
17 management strategy associating the phytoremediation of organic pollution along with the production of biomass  
18 for energy generation production. A 16-week controlled growth experiment was conducted on a soil substrate  
19 moderately impacted by multiple pollution, by associating rhizodegradation with *Medicago sativa* or biomass  
20 production with *Robinia pseudoacacia* or *Alnus incana* in monocultures. The effect of a microbial inoculum  
21 amendment on the performances of these treatments was also evaluated. Results showed total hydrocarbons (TH),  
22 and to a lesser extent polycyclic aromatic hydrocarbons (PAH), concentrations decreased over time, whatever the  
23 plant cover. Good biomass production yields were achieved for both tree species in comparison with the control  
24 sample, even though *R. pseudoacacia* seemed to perform better. Furthermore, the quality of the biomass produced  
25 was in conformity with the thresholds set by the legislation concerning its use as a renewable energy source.

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## 37 Introduction

38 Brownfields found in urban and suburban environments present human-altered soils also called SUITMAs (Soils  
39 of Urban, Industrial, Traffic, Mining, and Military Areas) (Morel et al. 2015). After the end of previous anthropic  
40 activities, these soils have been left untouched or might have already faced various steps of reconversion including  
41 pollution treatments. In the European Union, it is estimated that there are almost one million potential brownfields  
42 (Oliver et al. 2005). In France, there are 9538 polluted or potentially polluted sites requiring government action  
43 (BASOL 2021). Authors generally agree that slightly and mildly impacted sites represent a large majority of those  
44 concerned by pollution issues (Bardos et al. 2011; Chiang and Gu 2015; Cundy et al. 2016). Despite these modest  
45 impacts, these sites require management to avoid any risk of pollutants being transferred to the various  
46 environmental compartments. Broadly speaking, classic soil pollution management operations involve strong  
47 actions such as excavation, backfilling, and/or physico-chemical treatments. These are costly operations,  
48 especially when applied to large land areas where contamination is a source of concern but is not present at highly  
49 elevated levels. For situations where residual pollution is diffuse and spread over large areas, conventional  
50 techniques show their technical and economic limits and gentle remediation options such as phytomanagement  
51 might appear relevant when managing such sites (Mench et al. 2010; Cundy et al. 2016).

52 Phytomanagement includes, i) the management of contaminants by plant species or phytoremediation, ii) the  
53 valorization of the land through a usage that is compatible with both the risk associated to the residual contaminants  
54 and general site environment (e.g. soil quality, topography of the site, landscape inclusion) (INERIS 2019).  
55 Phytoremediation aims at reducing pollutant concentration but also at controlling its impacts by limiting its  
56 transfer: reduction of dust emissions, limited leaching, reducing contact between the impacted soil and users.  
57 Phytomanagement thus uses phytoremediation strategies to secure brownfield sites through revegetation, but also  
58 has the ambition to develop economic activities generating value chains in areas that were previously deprived of  
59 them (French et al. 2006; Gerhardt et al. 2009; Gomes 2012; Bert et al. 2017; Dudai et al. 2018). This has been a  
60 well-documented technique since the 1990s (Anderson and Walter 1992) and many studies have been conducted  
61 to identify suitable plant species that can both meet the requirement of risk management and be valorized through  
62 biomass production (e.g. heat generation or bioproducts) (Jiang et al. 2015, 2022; Ciadamidaro et al. 2019; Padoan  
63 et al. 2020; Moreira et al. 2021; Guidi Nissim and Labrecque 2021). Tree species such as poplar, willow,  
64 eucalyptus, black locust and alder have shown to be capable of meeting the afore-mentioned criteria in a number  
65 of studies (Lorenc-Plucińska et al. 2013; ADEME and Bert 2016; McIntosh et al. 2016; Foulon et al. 2016;  
66 ADEME et al. 2017; Chalot et al. 2020; Deng et al. 2020).

67 In this context, a thorough knowledge of pollutants is not sufficient and it is necessary to consider the agronomic  
68 value of these soils, whose properties may have been altered by former anthropic activities. Indeed, many of the  
69 soils found on brownfields belong to the Technosols reference group “*whose properties and pedogenesis are*  
70 *dominated by their technical origin. They contain a significant amount of artefacts or are filled with a hard*  
71 *engineering material or contain a geomembrane*” (IUSS Working Group WRB 2015). The physical, chemical,  
72 and biological properties of these highly anthropized soils will reflect this diversity of human activities impacts.  
73 In turn, the degradation of soil properties will generate significant ecosystem dysfunctions: altered plant growth,  
74 loss of biodiversity, reduced water infiltration and increased runoff, lower quality of groundwater supply (Jeannée  
75 2001; Morel et al. 2005, 2015). The restoration of soil functions should generate ecosystem products and services

76 that are beneficial to human well-being and are referred to as ecosystem services (e.g., provision, regulation and  
77 cultural) (Millenium Ecosystem Assessment 2005).

78 Morel et al. (2015) proposed a categorization of SUITMAs based on the ecosystem services they provide. These  
79 soils were organized into four groups as follows, (1) pseudo-natural soils, (2) vegetated engineered soils, (3)  
80 dumping site soils, and (4) sealed soils. Vegetated engineered soils are soils deliberately built to fulfil specific  
81 functions such as the capacity to support plant growth and favor plant development in the long term. Dumping site  
82 soils are soils developed on a range of solid wastes originating from human activity and landfilled at specific areas  
83 such as brownfields, landfills and settling ponds. Groups of soils were given scores with 4 levels from "o" which  
84 refers to soils that poorly contribute to a given service and produce significant disservices to "+++" for soils that  
85 strongly contribute to the corresponding service based on qualitative analysis of their properties and the state of  
86 their functions. For the ecosystem services of pollution attenuation and biomass production, vegetated engineered  
87 soils were given a score of high value '+++' and '++(+)' respectively and dumping sites soils were given a score of  
88 medium value '++' and '++' respectively. Thus for brownfield sites, even though their initial potential for biomass  
89 production is rather low, ecological management and creation of vegetated engineered soils can strongly increase  
90 their fertility (Morel et al. 2015) and optimize the ecosystem services of the sites. Phytomanagement can be one  
91 of those interventions. Taken through the scope of modifying a specific land cover, phytomanagement can then be  
92 seen as a potential successful mean to optimize ecosystem services (Guidi Nissim and Labrecque 2021).

93 A laboratory experiment was set up to assess the performances of a phytomanagement strategy of a moderately  
94 impacted soil from a former metallurgical plant site. Two ecosystem services were evaluated: biomass production  
95 for energy-generation purposes and residual pollution mitigation. The growth experiment was conducted under  
96 controlled conditions in a phytotronic chamber for 16 weeks.

- 97 1. The biomass production service was evaluated by growing two ligneous species in monospecific  
98 treatments: black locust (*Robinia pseudoacacia*) and white alder (*Alnus incana*). The choice of these two  
99 species was based on (i) their ability to grow under short rotation coppicing and being suitable for heat-  
100 generation purposes (Uri et al. 2002; Grünewald et al. 2009; Mizaras et al. 2011; Alesso et al. 2021;  
101 Rebola-Lichtenberg et al. 2021), and (ii) their ability to grow in a degraded environment (e.g. pioneer  
102 species capable of atmospheric N<sub>2</sub> fixation via symbiotic association (Uri et al. 2002, 2011; Gentili 2003;  
103 Rice et al. 2004; Aosaar et al. 2012; Marron et al. 2018), tolerance of contamination (Krzaklewski et al.  
104 2012; Lorenc-Plucińska et al. 2013; Fan et al. 2018; Woś et al. 2020; Deng et al. 2020; Băbău et al.  
105 2021)).
- 106 2. For residual contamination mitigation, the performance of alfalfa (*Medicago sativa*) was assessed  
107 regarding the rhizodegradation of organic contaminants, i.e., total hydrocarbons (TH) and polycyclic  
108 aromatic hydrocarbons (PAH) since this species has shown its ability to promote such degradation (Teng  
109 et al. 2011; Dubrovskaya et al. 2017; Alves et al. 2018; Bourceret et al. 2018; Shahzad et al. 2020; Li et  
110 al. 2022).
- 111 3. In addition, the effect of a microbial inoculum amendment on the performances of these treatments was  
112 also evaluated.

113 The biomass yields, the quality of mycorrhization, the potential transfers of trace elements to plants as well  
114 as the evolution of soil contaminant concentrations were determined at the end of the experiment.

## 115 **Materials and Methods**

### 116 **Soils, plants, and microbial inoculum**

117 The main soil substrate used in this study comes from the brownfield of a former metallurgical site located in the  
118 suburbs of Caen, France. The geological context of the site is divided into an eastern half composed mainly of silt,  
119 with thick loamy, not very stony soils and a western half composed mainly of Ranville Limestone, associated with  
120 shallower, more calcareous stony soils. The soil material used in this study was collected from a heap of moderately  
121 contaminated soils (CS) excavated during multiple rehabilitation efforts on the site. An uncontaminated reference  
122 soil (unCS) was collected from an unpolluted agricultural field on a farm located in Chenevières, France. Both the  
123 CS and unCS substrates were air-dried and sieved (2 mm mesh) before use.

124 *Medicago sativa* seeds were sterilized as follow: the seeds were immersed in a 20% TFD9 detergent solution (v/v)  
125 for 15 min then rinsed twice with deionized water. The seeds were then immersed in a 10% H<sub>2</sub>O<sub>2</sub> solution (v/v)  
126 for 2 min, rinsed twice with deionized water and slightly dried with clean dry tissue paper before sowing.

127 *Alnus incana* and *Robinia pseudoacacia* seedlings of 1 year old and 60 cm in height were obtained from  
128 “Pépinières Levavasseur & fils” (Ussy, France). The roots of both species were thoroughly washed with tap water  
129 and then with deionized water to remove any adhering soil particles from their packaging before planting.

130 Microbial inoculum (MI) (TEAM HORTO<sup>®</sup> solution – Itapollina France) was purchased from Alsavit'  
131 (Kaysersberg, France). MI comes under the format of a dry rounded tablet of compressed powder containing two  
132 species of mycorrhizal fungi *Glomus intraradices* and *Glomus mossae*, a species of antagonistic fungus  
133 *Trichoderma atroviride* and rhizosphere bacteria.

### 134 **Experimental design**

135 The culture experiment was conducted in 4 L polypropylene cultivation pots (23 cm high x 17.3 cm diameter)  
136 lined with polypropylene plastic bags and filled with 4 kg of either CS or unCS dry substrate. One of the 2  
137 strategies was then applied in four replicates resulting in 11 treatments, as follows:

- 138 1. Rhizodegradation: CS substrate planted with *M. sativa*. Ten seeds were sown in each pot. After 4 weeks,  
139 6 seedlings were removed, leaving only the 4 most developed ones.
- 140 2. Biomass production: both unCS and CS substrates were planted with either *A. incana* or *R. pseudoacacia*,  
141 with one seedling per pot.

142 In addition, for all these conditions, an inoculation strategy was tested. For alfalfa treatments, one inoculum pellet  
143 (4.5 g) was crushed and mixed with the 3 cm topsoil. For tree treatments, one crushed pellet was spread over the  
144 moist roots of each seedling before planting.

145 In total, these conditions led to 11 treatments: 4 on the uncontaminated soil, all cultivated with one of the two tree  
146 species (*A. incana* or *R. pseudoacacia*) with or without microbial inoculation; 7 on the contaminated soil ; 8  
147 cultivated with one of the plant species (*M. sativa*, *A. incana* or *R. pseudoacacia*) with or without microbial  
148 inoculation ; one unvegetated control.

149 The substrate water content was maintained at 80% of the water holding capacity (WHC) by daily weighing of the  
150 pots and addition of deionized water. Regarding plant growth, the pots were placed in phytotronic chambers

151 (Experimental phytotronic platform of Lorraine, PEPLor, Université de Lorraine) for 16 weeks during which the  
152 hygrometry was maintained at 70%, the photoperiod was a 16h day and 8h night, and the temperature was set at  
153 23 °C during the day and 18 °C at night. The experimental set-up of the trial composed of 4 replicates of 11  
154 treatments was randomly distributed in 4 in-line blocks. Weekly rotation of the cultivation pots within each block  
155 was carried out and each block's position was switched out every 4 weeks to minimize position influence on the  
156 results. The duration of the experiment was defined by the optimal life cycle of *Medicago sativa*. After 16 weeks  
157 plants, were harvested and soil samples were collected for characterization.

## 158 **Soil characterization**

159 Soil samples were oven-dried to a constant weight at 45 °C for 72 h, homogenized, and then stored at 4 °C prior  
160 to analysis. Their physicochemical characteristics were determined following normalized methods. Soil texture  
161 was determined using the NF X 31-107 procedure. The pH was determined in a substrate-water mix with a ratio  
162 of 1:5 following the NF ISO 10390 procedure. Cation exchange capacity (CEC) was determined using  
163 cobaltihexamine trichloride extraction according to the NF ISO 23470 procedure. Total nitrogen ( $N_{\text{total}}$ ) and  
164 organic carbon ( $C_{\text{org}}$ ) contents were respectively obtained by elemental analysis following dry combustion after  
165 250  $\mu\text{m}$  additional grinding (Vario MICRO CUBE, Elementar; NF ISO13878 and NF ISO 10694). Available  
166 phosphorus ( $P_2O_5$ ) was estimated using the Olsen extraction method NF ISO 11263. Trace elements and major  
167 elements contents were determined by ICP-OES (iCAP 6300 Duo, Thermo Scientific) following the mineralization  
168 of 5 g 250  $\mu\text{m}$  ground material with aqua regia using SCP Science system (*DigiPREP MS*) (NF ISO 11446 and  
169 NF ISO 22036). Organic pollution characterization was performed by "EUROFINS Analyses pour  
170 l'environnement France SAS" which is COFRAC accredited. Total hydrocarbons and polycyclic aromatic  
171 hydrocarbon contents were determined following hexane/acetone extraction by GC/MS (NF ISO 16073) and  
172 GC/MS/MS (NF ISO 18287) respectively. Measurements for the reference uncontaminated soil were performed  
173 by the "Laboratoire d'analyse des sols d'Arras" which is COFRAC accredited for these analyses.

## 174 **Plant biomass**

175 Following the harvest, aerial and roots parts of the plants were separated. Root parts were washed gently with tap  
176 water and then with deionized water to remove adhering soil particles and the excess water blotted off roots with  
177 clean dry tissue paper. The fresh plant materials were weighed for fresh biomass calculation. Roots were observed  
178 and sampled for symbiotic association assessment (nodules, mycorrhiza). After weighing, the plant materials were  
179 oven-dried to a constant weight at 60 °C for 72 h and weighed for dry biomass calculation.

## 180 **Qualitative assessment of nodules**

181 Root nodule assessment was done qualitatively by observation of the full root system and using the following  
182 scoring system: 0, no infection; 1, less than 10%; 2, from 11 to 20%; 3, from 21 to 40%; 4, from 41 to 60%; and  
183 5, more than 60%. For each treatment, the nodulation frequency (NF) was determined by the following equation:  
184  $NF\% = 100 * (N - n_0)/N$ , where  $N$  is the number of root systems observed and  $n_0$  is the number of those root  
185 systems with no trace of nodulation. The nodulation intensity (NI) was determined by the following equation:  
186  $NI\% = (80n_5 + 50n_4 + 30n_3 + 15n_2 + 5n_1)/N$  where  $n_5, n_4...n_1$  denotes respectively the numbers of  
187 root systems scoring 5, 4...1.

## 188 **Mycorrhization quality assessment**

189 Mycorrhization assessment was done following the Trouvelot et al. (1986) procedure. After washing, roots were  
190 cut into pieces of about 1 cm with scissors, then the pieces were mixed, and a sample of these roots (about 100  
191 fragments) was placed in a pill box containing 50% ethanol. The pill boxes were then stored at 4 °C until staining.  
192 The mass of each sample was weighed to take into account in the overall biomass calculation. The roots were  
193 washed again with deionized water and then treated in a 10% KOH solution at 90 °C for 40 minutes, then stained  
194 with 0.05% trypan blue in lactoglycerol and water for 15 minutes at 90 °C. The stained roots were then rinsed with  
195 a 25% lactoglycerol-water solution. Thirty root fragments of about 1 cm were randomly selected and mounted  
196 between slide and lamina in a drop of lactoglycerol, at a rate of 10 pieces per lamina for observation with a photonic  
197 microscope (MZFL III, Leica). The quality of mycorrhization was assessed using a scoring system. This system  
198 is based on the overall assessment of each of the 30 fragments to which a class score of between 0 and 5 is given,  
199 corresponding to the estimated proportion of cortex colonized by the mycorrhizal symbiote. The scores are as  
200 follows: 0, no infection; 1, traces; 2, less than 10%; 3, from 11 to 50%; 4, from 51 to 90%; and 5, more than 90%.  
201 The mycorrhization frequency (F) was determined by the following equation:  $F\% = 100 * (N - n_0)/N$ , where  
202  $N$  is the number of fragments observed and  $n_0$  is the number of those fragments with no trace of mycorrhization.  
203  $F$  reflects the extent of contamination. The intensity of mycorrhization (M) is determined by the following  
204 equation:  $M\% = (95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1)/N$  where  $n_5, n_4...n_1$  denotes respectively the  
205 numbers of fragments scoring 5, 4...1.

## 206 **Element content in plants**

207 Plant trace and major elements contents in aerial parts were determined as follow: 0.5 g of ground plant material  
208 was digested in a solution of 2.5 ml nitric acid (14.65 M) and 5 ml hydrogen peroxide (12.7 M) under an extractor  
209 hood for 16 hours and then heated at 95 °C for 120 min regia using SCP Science system (*DigiPREP MS*). After  
210 heating, the volume was adjusted to 20 ml with ultrapure water, filtered to 0.45 µm and then adjusted again to  
211 25 ml with ultrapure water. A 10 ml aliquot was collected for the measurement of trace elements and 1 ml was  
212 diluted in 9 ml ultrapure water for the measurement of major elements using ICP/OES (iCAP 6300 Duo, Thermo  
213 Scientific). Total nitrogen and organic carbon contents of the plant material were determined as follows (NF  
214 ISO13878 and NF ISO 10694): 7 mg of ground plant material was weighed into a tin capsule, which was then  
215 sealed and placed in a 96-well microplate for storage prior to analysis using dry-burning elemental analysis (Vario  
216 MICRO CUBE, Elementar). Quality control was ensured using a certified plant sample which followed all the  
217 steps of the protocol. A control chart was used to verify and validate that the certified sample did not deviate by  
218 more than one standard deviation.

## 219 **Potential availability of elements in the soil**

220 Potential availability of elements in the soil samples were determined as follows using NF ISO 14870 and NF ISO  
221 23036 procedure: 5 g of sieved soil (2 mm mesh) were placed in a buffered solution (pH = 7.3) of 10 ml  
222 triethanolamine 0.1 M, calcium chloride 0.01 M and diethylenetriamine-pentaacetic acid (DTPA) 0.005 M and  
223 agitated for 2 hours at 20 °C. After agitation the solution was centrifugated at 3000 g for 10 minutes and filtered  
224 to 0.45 µm. A 1 ml aliquot was diluted in 9 ml ultrapure water for the measurement of available elements using  
225 ICP/OES (iCAP 6300 Duo, Thermo Scientific)



226 **Statistical analyses**

227 Data are presented as mean and standard error of replicate samples. Statistical analyses were carried out using  
228 either the Student's T-test or ANOVA followed by a Tukey Honest Significant Difference (HSD) post hoc test at  
229 a significance level of 0.05 when parametric assumptions were met. Wilcoxon's test or the Kruskal-Wallis test at  
230 a significance level of 0.05 were used when parametric assumptions were not met. Statistical treatment was  
231 conducted using the RStudio desktop software (RStudio Team 2020).

## 232 **Results**

### 233 **Soil characteristics**

234 The substrate textures were very different, CS being of clay loam type (clay: 27.6%; loam: 33.5%, sand: 38.9%)  
235 and unCS being of sandy loamy type (clay: 10%; loam: 31.4%; sand: 58.6%) according to the NRCS Soil Texture  
236 Calculator (USDA) (Table 1). CS was overall richer in  $N_{\text{total}}$ , CaO, MgO than unCS, the latter having higher  $K_2O$   
237 and  $P_2O_5$  (126 vs 95.0 mg  $kg^{-1}$ ) contents. pH was neutral for unCS (7.14), and alkaline in for CS (8.30).

238 Total trace elements and organic pollutant contents were significantly higher in CS than in unCS (Table1). The  
239 potential availability of these trace elements was however low, ranging from 0.3 mg  $kg^{-1}$  for Ni to 20.7 mg  $kg^{-1}$   
240 for Zn. This was expected given the relatively low total concentration and alkaline pH.

### 241 **Plant survival**

242 At the end of the experiment, no mortality was observed regarding *M. Sativa* seedlings, each pot containing 4 well-  
243 developed apparently healthy plants. For tree seedlings some mortality was observed. For the *A. incana*, only 75%  
244 of the seedlings grown on CS survived, whatever the inoculation condition. On the unCS treatments, 50% of non-  
245 inoculated *A. incana* seedlings survived, whereas all died in the inoculated treatment. For *R. pseudoacacia*, 75%  
246 and 100% of seedlings respectively survived on the non-inoculated and inoculated CS, while 75% survived on  
247 unCS, inoculated or not. Mortality did not seem to correlate with substrate or treatment types and was rather  
248 attributed to the initial quality of the plant material and possible stresses due to the transplantation procedure. *R.*  
249 *pseudoacacia* appeared more resistant to these experimental stresses.

### 250 **Nodulation**

251 Nodules were found on all root systems (NF = 100%). Nodulation intensities (NI) varied greatly from 11.7% for  
252 inoculated *A. incana* grown on unCS to 70% for non-inoculated *R. pseudoacacia* grown on unCS. Qualitatively,  
253 a trend of higher NI was observed with plants grown on unCS than on CS. Overall, inoculation with MI did not  
254 seem to have an effect on NI, regardless of the substrate nor the species.

### 255 **Inoculation**

256 Mycorrhization was observed in all the vegetated treatments regardless of the substrate and addition of the  
257 inoculum (Table 2). Both non-inoculated and inoculated *M. sativa* displayed high mycorrhization frequencies (F)  
258 at 96% and 91% respectively and moderate cortex mycorrhization intensities (M) at 29% and 58% respectively,  
259 with no significant differences between treatments ( $p$ -values of 0.14 and 0.052 respectively).

260 Regarding *A. incana*, the mycorrhization frequency was relatively high for both non-inoculated treatments unCS  
261 (82%), and CS (60%). For inoculated CS treatment, measured value was lower, though not statistically different  
262 (31%) (one-way ANOVA  $p$ -value = 0.18). No data were available for the unCS inoculated treatment due to  
263 mortality. Cortex mycorrhization intensities were rather moderate and not statistically different for either non-  
264 inoculated treatments unCS (33%), or CS (12%). For inoculated CS treatment, the measured value was statistically  
265 lower (3%) (one-way ANOVA  $p$ -value = 0.03).

266 Regarding *R. pseudoacacia*, both non-inoculated and inoculated CS treatments displayed the same high  
267 mycorrhization frequencies at 97% and moderate cortex mycorrhization intensities at 33% and 29% respectively.  
268 For unCS treatments, both non-inoculated and inoculated treatments displayed moderate mycorrhization  
269 frequencies at 33% and 39% respectively and very low cortex mycorrhization intensities at 0.4% and 0.5%  
270 respectively. Both mycorrhization frequencies and cortex mycorrhization intensities were statistically higher in  
271 CS treatments in comparison with unCS treatments.

272 In summary, mycorrhization frequencies (one-way ANOVA  $p$ -value = 0.00053) and cortex mycorrhization  
273 intensities (one-way ANOVA  $p$ -value = 0.002) were significantly higher in CS treatments. Moreover, inoculation  
274 with MI did not have any effect on mycorrhization frequencies or cortex mycorrhization intensities, regardless of  
275 plant species. Both substrates provided favorable conditions for infection and colonization of root systems by  
276 endogenous microbial consortia, but CS seemed to offer better overall conditions.

### 277 **Plant biomass yield**

278 Regarding biomass growth (Table 3), inoculation with MI did not have any effect on the plant biomass yields  
279 whatever the species or the substrates. This result was expected since as mentioned above both inoculated and  
280 non-inoculated treatments displayed similar mycorrhizal colonization. In the following sections, inoculated  
281 treatments will not be considered and results of non-inoculated treatments alone will be presented and discussed.

282 The contamination slightly affected *R. pseudoacacia* growth with a statistically non-significant reduction of 43%  
283 of aerial biomass yield production compared to the reference uncontaminated soil (Fig. 1). *A. incana* appeared  
284 more sensitive to the abiotic stresses of CS with a statistically significant reduction of aerial biomass production  
285 of 2.6 factor when compared to unCS. Since symbiotic associations, nodulations and mycorrhization were present  
286 at similar levels for both conditions alternative explanations shall be sought for these differences, such as trace  
287 elements' preferential uptake or kinetics effect. Whatever the cause, at this stage, *R. pseudoacacia* appeared to be  
288 more resistant and thus more fit for energy-generation purposes.

### 289 **Plant biomass quality**

290 Since all plant species tested can develop an atmospheric nitrogen fixation strategy and nodulation was found on  
291 all root systems no issue regarding nitrogen supply was expected. Indeed, for CS treatments, they all had significant  
292 total nitrogen ( $N_{\text{total}}$ ) in their aerial parts: 28.5 g kg<sup>-1</sup> for *M. sativa*, 22.4 g kg<sup>-1</sup> for *R. pseudoacacia*, 17.2 g kg<sup>-1</sup> for  
293 *A. incana*. For *M. sativa* the value was in the range of those found for agricultural purposes (COMIFER 2013).  
294 For *R. pseudoacacia* and *A. incana*, values for both species on the reference soil unCS were similar at 19 g kg<sup>-1</sup> in  
295 average. For *A. incana* only, the measured value was statistically higher in unCS treatment correlating with the  
296 qualitative observation of better nodulation intensities with this substrate. For both species measured values were  
297 lower than  $N_{\text{total}}$  average ranges reported in the literature (20 – 40 g kg<sup>-1</sup> for *A. incana* (Uri et al. 2002; Kuznetsova  
298 et al. 2011) and 27 – 45 g kg<sup>-1</sup> for *R. pseudoacacia* (Hacskeylo and Finn 1969; Athey and Connor 1989; Sambeek  
299 et al. 2008; Woś et al. 2020)). Here again, *R. pseudoacacia* seemed less affected by soil contamination than *A.*  
300 *incana*. For the latter, the nitrogen deficit might explain biomass reduction.

301 None of the plant species seemed to accumulate trace elements. For all plant species, only Cu, Pb, Ni and Zn were  
302 detected in low concentrations in the aerial parts of the seedlings (Table 4). Ratios between plant and soil trace

303 elements concentrations ranged from 0.11 (*R. pseudoacacia*) to 0.24 (*M. sativa*) for Cu and from 0.08 (*R.*  
304 *pseudoacacia*) to 0.24 (*M. sativa*) for Zn, which indicated no accumulation of any sort. Cu and Zn concentrations  
305 in aerial parts of the plants were not statistically different between unCS and CS either for *R. pseudoacacia* and *A.*  
306 *incana*. Pb was detected in *M. sativa* (0.79 mg kg<sup>-1</sup>) and in *A. incana* (0.74 mg kg<sup>-1</sup>). Ni was detected in *R.*  
307 *pseudoacacia* grown on both substrates (approx. 1 mg kg<sup>-1</sup>). Moreover, the alkaline pH of CS is known to be a  
308 limiting factor for trace element uptake.

309 For most major elements in *A. incana* and *R. pseudoacacia* aerial parts, concentrations did not differ from one  
310 substrate to another (Table 5). Only Mg and P behaved differently. For *R. pseudoacacia* treatment, Mg  
311 concentration was almost twice as high when grown on CS than on unCS, which correlated with higher  
312 exchangeable concentration in CS. Similarly, for *A. incana* treatment, P concentration was about twice as high  
313 when grown on unCS than on CS substrate, which correlated with the higher available P concentration in unCS.

### 314 **Soil organic pollution**

315 Total hydrocarbon concentration [C10 – C40] decrease was statistically significant in the rhizodegradation  
316 treatment (CS vegetated with *M. sativa*) in which it reached 33% on average (Table 6) in comparison with initial  
317 TH concentration (277 vs 414 mg kg<sup>-1</sup> respectively). More specifically, the TH content decrease was statistically  
318 significant in the lighter fractions [C10 – C16] (22%) and [C16 – C22] (25%), although a trend was also observed  
319 in the heavier fractions, as shown in Fig. 2. However, residual TH concentration in the unvegetated treatment (301  
320 mg kg<sup>-1</sup>) was not statistically different from either the initial value and the final concentration of the  
321 rhizodegradation treatment. While being only statistically significant in the alfalfa planted treatment, a trend of  
322 TH concentration decrease was observed in all planted treatments. Interestingly, this might indicate a potential  
323 positive effect of the two tree species for the stimulation of bio/rhizodegradation of TH. It is suggested that a  
324 longer experiment time could have allowed for a statistically significant effect on the TH concentration decrease.

325 For PAH, a generally decreasing trend was observed between initial and final concentrations. However, no  
326 statistical difference was found whatever the treatment nor the molecule (Table 6). This was somewhat expected  
327 given the recalcitrant nature of these contaminants and the expected low availability in old contaminated soils.

## 328 Discussion

### 329 A representative substrate

330 Due to their industrial past, brownfield soils often display multiple impacts in the immediate and distant  
331 compartments of the environment, such as soil, groundwater and biosphere. They are transformed by human  
332 activities and although they are subject to the same evolutionary factors as natural soils, anthropogenic  
333 disturbances impose very rapid cycles of transformation on these soils and affect their composition and therefore  
334 their properties (Norra et al. 2008; Morel et al. 2014; Huot et al. 2015; Greinert 2015). Generally speaking, strong  
335 changes in the physicochemical properties disrupt the development of vegetation and the activity of organisms in  
336 highly anthropized soils (Hiller 2000). In this experiment, the contaminated soil used was no exception. It was the  
337 result of an assemblage of multiple soil materials after multiple deconstruction work and stored as a heap fulfilling  
338 no function whatsoever. Literature reports that anthropic disturbances often lead to alkaline pH, trace elements  
339 and organic contaminations and high organic carbon content (Morel et al. 2005; Kuppusamy et al. 2017; Allory et  
340 al. 2021), which were all displayed by the contaminated soil used in this study. Originating from the introduction  
341 of various types of exogenous materials in anthropized soils these disturbances are very common in SUITMAs  
342 and brownfield soils especially (Jeannée 2001; Howard et al. 2013; Joimel et al. 2016; Greinert and Kostecki  
343 2019). In particular, artefacts often come from industrial by-products of former activities such as fossil fuel  
344 combustion residues (e.g., coal, hydrocarbons, etc.). These are the source of mineral (trace elements) and organic  
345 (e.g., hydrocarbons, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, etc.) pollution, which  
346 accumulate in the soil. This contaminant toxicity might then negatively impact the biological compartment (Baek  
347 et al. 1991; El Khalil et al. 2013; He et al. 2017). In this experiment, the CS substrate displayed alkaline pH (8.4),  
348 rather low trace elements concentrations, high organic carbon concentration  $C_{org}$  ( $63.1 \text{ g kg}^{-1}$ ), medium total  
349 hydrocarbon concentration ( $414 \text{ g kg}^{-1}$ ) and medium polycyclic aromatic hydrocarbon concentration ( $92 \text{ mg kg}^{-1}$ ).  
350 The organic content of this soil would qualify it to be eliminated to a non-hazardous waste storage facility,  
351 according to the French legislation on the matter:  $C_{org} = 30 - 50 \text{ g kg}^{-1}$ ,  $TH = 500 - 1000 \text{ g kg}^{-1}$ , and  $PAH = 50 -$   
352  $100 \text{ mg kg}^{-1}$ . Moderately impacted soils constitute a large majority of those concerned by pollution issues and the  
353 costs associated with their management can quickly rise to very high levels (Bardos et al. 2011; Chiang and Gu  
354 2015; Cundy et al. 2016), hence, low-cost remediation options such as phytomanagement might appear relevant  
355 for those sites (Mench et al. 2010).

### 356 Biomass production performances in terms of quantity and quality

357 Over the course of the experiment, the CS substrate proved to be a relatively suitable growing medium for the  
358 three plant species tested. *A. incana* and *R. pseudoacacia* biomass production was impaired on CS in comparison  
359 with unCS. The multiple abiotic stresses of CS substrate might explain the differences in biomass production  
360 observed. While *R. pseudoacacia* is a relatively undemanding, competition-intolerant, fast-growing and relatively  
361 tall tree pioneer species, it is also capable of adapting to difficult site conditions (e.g., dry climates, nutrient-poor  
362 and dry soils) (Nicolescu et al. 2020). *A. incana* is also pioneer species exhibiting rapid growth through vegetative  
363 asexual propagation (Francis et al. 2005; Uri et al. 2011), and is partially tolerant to drought, as well as having  
364 shown its ability to grow in low-nutrient environments, which makes it well suited for reclamation strategies for  
365 degraded sites (Lorenc-Plucińska et al. 2013). Both species are capable of atmospheric  $N_2$ -fixation via symbiotic

366 interaction (Uri et al. 2002; Gentili 2003; Nicolescu et al. 2020), so initial  $N_{\text{total}}$  concentration of the substrates was  
367 in theory not a major issue for these two species.  $N_{\text{total}}$  concentration in the aerial parts of both species was lower  
368 than what could be expected under classic growth conditions, according to the literature (Athey and Connor 1989;  
369 Uri et al. 2002). Nodulation and mycorrhization were detected in all treatments and were not significantly affected  
370 by the addition of the MI. However, nodulation intensities were qualitatively higher on plants grown on unCS  
371 treatment which might explain lower  $N_{\text{total}}$  in *A. incana* aerial parts grown on CS. Mycorrhization frequencies and  
372 intensities were statistically higher with the CS treatment than with unCS treatment. This difference between  
373 substrates might originate from their different  $P_2O_5$  concentrations. The literature reports that  $P_2O_5$  concentrations  
374 in a soil exceeding  $150 \text{ mg kg}^{-1}$  can limit symbiotic association by mycorrhizal fungi. Neither nodulation nor  
375 mycorrhization were significantly affected by the addition of the MI. MI failed to optimize biomass production as  
376 was initially intended. Differences in biomass production between substrates could find an explanation in the lower  
377  $P_2O_5$  concentration and alkaline pH, as *R. pseudoacacia* is known to grow best in slightly acidic to almost neutral  
378 soil (5.5-7.0) (Keresztesi 1988) and in the presence of both trace elements and organic contaminants in CS. Overall,  
379 *R. pseudoacacia* still performed better than *A. incana* on the contaminated soil.

380 Adverse effects on biomass production from organic contaminants such as PAHs and total hydrocarbons have  
381 already been described in the literature, though concentrations were higher than in our experiment (Reilley et al.  
382 1996; Shirdam et al. 2009; Afegbua and Batty 2018). Authors have suggested that these pollutants could have  
383 negatory effects through a decrease in water and nutrient uptake by the plant (Gudin and Syrratt 1975; Reilley et  
384 al. 1996; Adam and Duncan 2002; Cheema et al. 2010; Oguntimehin et al. 2010). In our situation, no such effects  
385 were observed, the slight decrease in P and N uptake for *A. incana* being attributed to soil supply differences and  
386 possible delay in symbiotic association kinetics. At the end of the experiment, both *R. pseudoacacia* and *A. incana*  
387 were still at very early stages of their development and thus, differences in biomass yields between plants grown  
388 on CS and unCS might be the expression of a potential delay in growth caused by the less favorable edaphic  
389 conditions provided by CS. Nevertheless, a longer cultivation period such as short rotation coppicing could  
390 potentially erase this delay in growth. For example, *R. pseudoacacia* being an economically important  
391 multipurpose species in many parts of Europe, it is used for biomass production/bioenergy generation and its  
392 biomass yield can reach up to  $14 \text{ dry matter t}$  when managed as short rotation coppice (Nicolescu et al. 2020). A  
393 similar use of *A. incana* is also reported given its suitability for this type of culture (Uri et al. 2002, 2011).

394 Initial trace element contents in CS were not an issue regarding French legislation on substrates for culture support,  
395 its concentrations being well below the thresholds ( $\text{Cd} = 2 \text{ mg kg}^{-1}$ ,  $\text{Cu} = 100 \text{ mg kg}^{-1}$ ,  $\text{Ni} = 50 \text{ mg kg}^{-1}$ ,  $\text{Pb} = 100$   
396  $\text{mg kg}^{-1}$ , and  $\text{Zn} = 300 \text{ mg kg}^{-1}$ ) (AFNOR 2002b). Both *A. incana* and *R. pseudoacacia* have been used in studies  
397 of phytoremediation of metal-contaminated sites and proved their usefulness in terms of biomass production, the  
398 two species being either phytoextractant or promoting phytostabilization of trace elements depending on the  
399 situation (French et al. 2006; Dadea et al. 2017; Padoan et al. 2020; Deng et al.). In this experiment, no  
400 accumulation of any sort was highlighted and bioconcentration factors were well below 1. The two most commonly  
401 found elements, Cu and Zn were in low concentrations in both species and in similar orders of magnitude whatever  
402 the substrate. This highlights the initial low availability of trace elements in CS which can be linked to its alkaline  
403 pH. This has a particular interesting consequence regarding the thresholds of trace element concentrations in  
404 biomass used as an energy source. In this experiment, biomass trace element concentrations did not exceed the  
405 relevant French legislation threshold, namely:  $\text{Cd} = 5 \text{ mg kg}^{-1}$ ,  $\text{Cu} = 30 \text{ mg kg}^{-1}$ ,  $\text{Pb} = 50 \text{ mg kg}^{-1}$ , and  $\text{Zn} = 200$

406 mg kg<sup>-1</sup> (Ministère de la Transition Ecologique et Solidaire 2018). However, even though no accumulation of trace  
407 element in aerial parts was highlighted, this experiment was not as long as short rotation coppice strategies, hence  
408 further monitoring and follow-up of trace elements accumulation in the plant aerial parts are recommended.

#### 409 **Assessment of pollution attenuation**

410 Although an overall decrease in total hydrocarbon concentration could be demonstrated after 16 weeks of  
411 experimentation, this was not attributable to any specific treatment. The lightest fractions [C10 - C16] and [C16 -  
412 C22] decreased by about 20% whatever the treatment, being easier to degrade than the heavier ones (Ouvrard et  
413 al. 2014). Regarding PAH concentration, no significant decrease was shown in any specific treatment. In fact, the  
414 presence of plants had no direct significant effect on the decrease in total hydrocarbon content, since residuals  
415 concentrations in the unvegetated treatment correlated to the rhizodegradation treatment. The same was seen with  
416 residual PAHs concentrations, which did not change significantly. These kinds of results have been demonstrated  
417 in a long term field experiment (Ouvrard et al. 2011) in which plant cover alone did not affect contaminant  
418 concentrations in soil. Overall, total hydrocarbon content decrease seemed to be due to biodegradation from  
419 endogenous microorganism consortia.

420 Therefore, regarding the objectives of PAH dissipation, the limiting factor here seems to be their bioavailability  
421 for the degrading bacteria (Harvey et al. 2002; Barnier 2009; Wenzel 2009). In a previous study (unpublished) the  
422  $\Sigma 16$  PAH bioavailability was measured via Tenax resin in CaCl<sub>2</sub> solution in a subsample originating from the  
423 same CS soil (Barnier et al. 2014). Results showed a very low PAH availability of 4.5% of the total soil  
424 concentration. It has been shown that the age of the organic pollution coupled with the strong presence of organic  
425 matter does not favor its bioavailability. PAHs are highly reactive to soil organic matter and aging processes in  
426 the soil (e.g., physical trapping, chemical bonding, and incorporation), which further reduces the bioavailable  
427 fraction of the pollution and mitigates its toxic effects. This leads to a long-term stabilization of the pollution that  
428 is considered an effective remediation by some authors, considering it rhizoattenuation rather than  
429 rhizodegradation (Ouvrard et al., 2014). However, numerous works have shown the effectiveness of strategies  
430 coupling phytoremediation and microbial inoculation in an intensity sometimes even superior to phytoremediation  
431 alone, allowing for better bioavailability and degradation of organic pollutants (Gao et al. 2010, 2017; Agnello et  
432 al. 2016; Dubrovskaya et al. 2017; García-Sánchez et al. 2018; Kong et al. 2018). In this experiment, the  
433 inoculation, which was directed towards the optimization of biomass production, had no significant effect on the  
434 decrease in organic pollutant content or on biomass production.

435 Overall, these results, considered through the scope of the ecosystem service of pollution attenuation, showed that  
436 it is impossible to conclude that the presence of a plant alone favored the decrease in organic pollutant. This would  
437 suggest that biodegradation by endogenous microorganism consortia was indeed responsible for this phenomenon.  
438 Considering that the soil substrate used in this study came from a heap fulfilling almost no function whatsoever it  
439 is safe to say that phytomanagement can be an efficient way of optimizing specific ecosystem services such as  
440 residual pollution dissipation or stabilization. More specifically, this heap was engendered to water run-off, eolian  
441 transport of soil particle and dust, that could lead to potential pollutant transfer to the immediate environment.  
442 Even though organic pollutant content was not significantly decreased by revegetation alone, the latter could act  
443 as a mitigator of the transfer processes that are taking place, as has previously been demonstrated (French et al.  
444 2006; Gerhardt et al. 2009; Mench et al. 2010; ADEME et al. 2017).

## 445 **Conclusion**

446 With regards to the results obtained, production of a satisfactory quantity and quality of biomass to meet energy  
447 production requirements, seems to be an achievable goal. In the long term, mitigation of organic pollution also  
448 appears possible, especially for total hydrocarbons. PAH contamination seeming particularly recalcitrant and very  
449 poorly bioavailable, it is not expected to be subjected to transfer processes to the different compartments of the  
450 environment. Overall, and considering the initial state of the soil substrate used in this study, it is suggested that  
451 the implementation of one, if not all, of the strategies tested, could only lead to an improvement of the soil functions  
452 of the CS substrate, thereby optimizing the provisioning of the ecosystem services of residual pollution attenuation  
453 and biomass production. As many authors and studies have suggested in previous works, revegetation of derelicts  
454 soils can be an efficient way of optimizing ecosystem services in highly anthropized areas allowing for new  
455 environmental and economic value creation. When implemented in a rational manner, phytomanagement strategies  
456 not only allows for the refunctionalization of a soil, but also permit the establishment of potential economic  
457 activities in a framework of circular economy and with a sustainable development mindset.

458



459 **Author's contribution**

460 Guillaume Hostyn, Christophe Schwartz, Jean-Marie Côme and Stéphanie Ouvrard all contributed to the study  
461 conception and design. Material preparation, data collection and analysis were performed by Guillaume Hostyn.  
462 The first draft of the manuscript was written by Guillaume Hostyn. Christophe Schwartz, Jean-Marie Côme and  
463 Stéphanie Ouvrard commented on further versions of the manuscript. Guillaume Hostyn, Christophe Schwartz,  
464 Jean-Marie Côme and Stéphanie Ouvrard all read and approved the final manuscript.

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470 **Data availability**

471 The datasets used and/or analyzed during the current study are available from the corresponding author on  
472 reasonable request.

473 **Declarations**

474 **Ethics approval**

475 Not applicable.

476 **Consent to participate**

477 Not applicable.

478 **Consent for publication**

479 Not applicable.

480 **Competing interests**

481 **The authors declare that they have no competing interests.**

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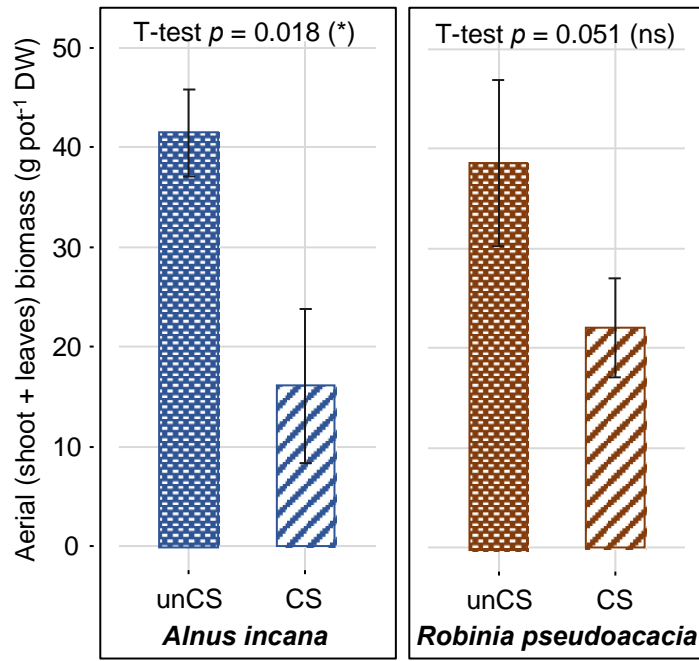
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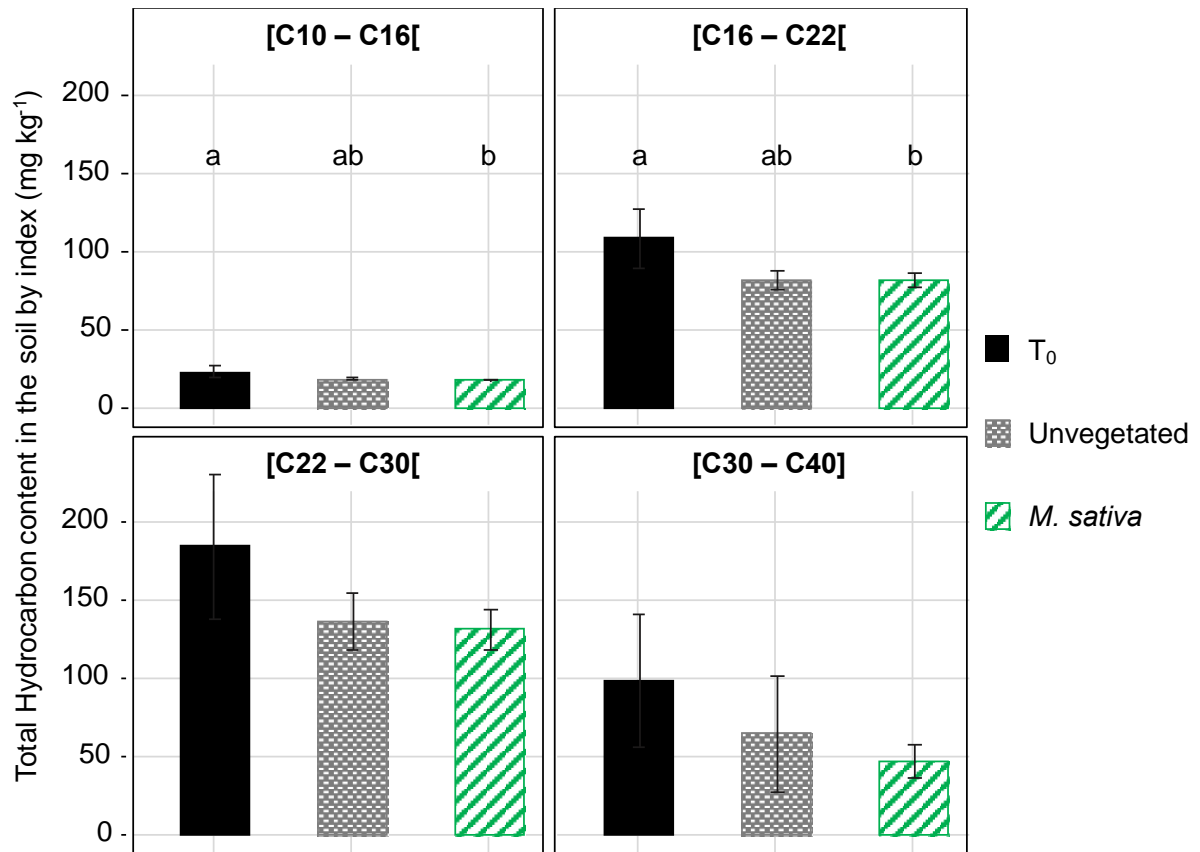
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742 **Fig. 1** *A. incana* and *R. pseudoacacia* aerial (shoot + leaves) biomass (g pot<sup>-1</sup> DW) at the end of the experiment  
 743 (T<sub>final</sub>)



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**Fig. 2** Soil concentration of total hydrocarbons by fraction ( $\text{mg kg}^{-1}$ ) in non-inoculated CS treatments at the start ( $T_0$ ) and at the end ( $T_{\text{final}}$ ) of the experiment. Dark bars indicate  $T_0$ ; dotted grey bars indicate unvegetated treatment; and striped green bars indicate *M. sativa* treatment. Different letters indicate a significant difference at  $p < 0.05$  according to Tukey's HSD test

749 **Table 1** Initial agronomical and pollution parameters of the soil substrates used in the study

Substrate	pH	C/N	N <sub>total</sub>	C <sub>org</sub>	CEC	clay	loam	sand	P <sub>2</sub> O <sub>5</sub> Olsen	TH	∑16 PAH	Cd	Cu	Ni	Pb	Zn
	g kg <sup>-1</sup>				cmol+ kg <sup>-1</sup>	%			mg kg <sup>-1</sup>							
CS	8.30	31.6	2.00	63.1	8.53	27.6	33.5	38.9	95.0	414	92.3	1.10	55.4	23.9	49.4	191
unCS	7.14	11.7	1.03	12.1	7.75	10.0	31.4	58.6	126	nd	nd	0.12	10.7	11.2	38.8	50.3

750 nd: not detected ; CEC : cation exchange capacity

751

752 **Table 2** Mycorrhization quality assessment at T<sub>final</sub> for each planted treatment. NF, Nodulation Frequency (%);  
753 NI, Nodulation Intensity (%); F, mycorrhization frequency (%); M, cortex mycorrhization intensity (%)

Plant species	Substrate	Inoculation	Nodulation Frequency	Nodulation Intensity	Mycorrhization quality assessment	
			NF	NI	F	M
<i>M. sativa</i>	CS	-	100	47.5	90.8 ± 4.19	28.7 ± 18.7
		+		16.3	95.8 ± 4.19	57.8 ± 14.3
<i>A. incana</i>	CS	-		16.7	60.0 ± 14.5 a	11.9 ± 12.0 ab
		+		11.7	31.1 ± 36.6 a	3.12 ± 2.77 b
	unCS	-		65.0	81.7 ± 11.8 a	32.7 ± 6.69 a
		+		No data	No data	No data
<i>R. pseudoacacia</i>	CS	-		31.3	96.7 ± 4.71 b	32.6 ± 12.1 b
		+		43.3	96.7 ± 3.33 b	29.1 ± 13.5 b
	unCS	-		70.0	33.3 ± 26.5 a	0.42 ± 0.35 a
		+		43.3	38.9 ± 18.4 a	0.48 ± 0.18 a

754 For each plant, different letters indicate a significant difference at  $p < 0.05$  according to Tukey's HSD test. '+'  
755 sign indicates inoculation with MI, '-' sign indicates no inoculation

756

757 **Table 3** Fresh and dry biomass (g pot<sup>-1</sup>) of root and aerial (shoot + leaves) parts at T<sub>final</sub> for each planted treatment

Plant species	Substrate	Inoculation	Fresh weight		Dry weight	
			Root	Aerial	Root	Aerial
<i>M. sativa</i>	CS	-	104 ± 46.3	26.2 ± 5.95	35.9 ± 13.4	15.0 ± 3.66
		+	110 ± 18.3	27.2 ± 5.39	40.4 ± 7.56	15.7 ± 3.42
<i>A. incana</i>	CS	-	78.5 ± 39.2 a	26.3 ± 11.2 a	36.8 ± 21.3 a	16.1 ± 7.72 a
		+	69 ± 18.3 a	26.1 ± 13.5 a	26.3 ± 7.19 a	15.2 ± 8.38 a
	unCS	-	159 ± 4.86 b	69.3 ± 3.37 b	58.3 ± 0.06 b	41.6 ± 4.38 b
		+	No data	No data	No data	No data
<i>R. pseudoacacia</i>	CS	-	65.1 ± 20.6 a	39.4 ± 8.47 a	18.8 ± 6.17 a	22.1 ± 5.02 a
		+	49.9 ± 11.0 a	33.5 ± 4.41 a	14.6 ± 4.07 a	18.7 ± 2.91 a
	unCS	-	68 ± 45.2 a	65.3 ± 12.9 b	24.0 ± 19.2 a	38.8 ± 8.28 a
		+	65.4 ± 17.4 a	65.3 ± 24.4 b	26.7 ± 6.05 a	40.2 ± 17.6 a

758 For each plant, different letters indicate a significant difference at  $p < 0.05$  according to Tukey's HSD test. '+'  
759 sign indicates inoculation with MI, '-' sign indicates no inoculation

760 **Table 4** Trace element concentration (mg kg<sup>-1</sup>) in aerial parts (shoot + leaves) at T<sub>final</sub> in non-inoculated treatments

Plant species	Substrate	Trace elements				
		Cd	Cu	Ni	Pb	Zn
<i>M. sativa</i>	CS	< QL	12.0 ± 2.72	< QL	0.79	46.6 ± 13.8
<i>A. incana</i>	CS	< QL	8.78 ± 6.13	< QL	0.74	28.3 ± 14.9
	unCS	< QL	9.83 ± 1.04	< QL	< QL	38.8 ± 1.67
<i>R. pseudoacacia</i>	CS	< QL	6.86 ± 1.05	0.74	< QL	18.0 ± 5.64
	unCS	< QL	6.80 ± 2.19	1.18	< QL	13.8 ± 1.54

761 For each plant, asterisks (\*) indicate a significant difference at  $p < 0.05$  according to Student's T-test

762

763 **Table 5** Major elements (g kg<sup>-1</sup>) in aerial parts (shoot + leaves) at T<sub>final</sub> in non-inoculated treatments

Plant species	Substrate	Major elements					
		Ca	Fe	K	Mg	P	S
<i>M. sativa</i>	CS	29.3 ± 6.88	0.28 ± 0.19	8.71 ± 0.78	3.14 ± 0.29	1.32 ± 0.14	1.92 ± 0.05
<i>A. incana</i>	CS	7.80 ± 5.01	0.09 ± 0.10	3.83 ± 1.81	1.30 ± 0.96	0.64 ± 0.31	0.91 ± 0.55
	unCS	9.12 ± 0.80	0.05 ± 0.00	3.90 ± 0.03	1.28 ± 0.15	1.24 ± 0.05*	0.95 ± 0.00
<i>R. pseudoacacia</i>	CS	22.8 ± 5.01	0.06 ± 0.01	5.66 ± 1.01	1.15 ± 0.16*	1.34 ± 0.30	1.30 ± 0.43
	unCS	15.1 ± 4.68	0.06 ± 0.03	6.02 ± 1.57	0.66 ± 0.19	1.53 ± 0.37	0.92 ± 0.31

764 For each plant, asterisks (\*) indicate a significant difference at  $p < 0.05$  according to Student's T-test

765

766 **Table 6**  $\Sigma$ 16 PAHs and total hydrocarbons fractions dissipation in unplanted and planted treatments

		Mean residual concentration (mg kg <sup>-1</sup> ) - Total loss %										
		[C10-C16]	[C16-C22]	[C22-C30]	[C30-C40]	[C10-C40]	$\Sigma$ 16 PAH					
Initial concentrations		22.6 ± 3.9 a	108 ± 18.7 a	184.7 ± 45.9 a	98.7 ± 42.1 a	414 ± 104a	92.3 ± 18.0 a					
Plant species	none	18.2 ± 0.6 ab	19	81.3 ± 6 ab	25	136.5 ± 18.7 a	26	64.9 ± 37.5 a	34	301 ± 53.2 ab	27	80.3 ± 9.00 a
	<i>M. sativa</i>	17.7 ± 0.7 b	22	81.2 ± 4.4 b	25	131.5 ± 13 a	29	46.7 ± 10.9 a	53	277 ± 20.9 b	33	90.3 ± 9.60 a
	<i>A. incana</i>	19.7 ± 2.4 a	13	86.7 ± 8.8 a	20	163.3 ± 21.9 a	12	93.7 ± 11.4 a	0	385 ± 56.5 a	7	75.8 ± 6.13 a
	<i>R. pseudoacacia</i>	18.4 ± 3.2 ab	19	84.4 ± 17.5 a	22	140.7 ± 44.3 a	24	54.6 ± 43.6 a	45	298 ± 107 a	28	81.5 ± 13.4 a

767 For each plant, different letters indicate a significant difference at  $p < 0.05$  according to Tukey's HSD test