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Species- and organ-specific responses of agri-environmental plants to residual agricultural pollutants

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1 **Title :**

2 Species- and organ-specific responses of agri-environmental plants to residual agricultural
3 pollutants

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25 **Abstract**

26 Soil pollution by anthropogenic chemicals is a major concern for sustainability of crop
27 production and of ecosystem functions mediated by natural plant biodiversity. The complex
28 effects on plants are however difficult to apprehend. Plant communities of field margins,
29 vegetative filter strips or rotational fallows are confronted with agricultural pollutants through
30 residual soil contamination and/or through drift, run-off and leaching events that result from
31 chemical applications. Exposure to xenobiotics and heavy metals causes biochemical,
32 physiological and developmental effects. However, the range of doses, modalities of
33 exposure, metabolization of contaminants into derived xenobiotics, and combinations of
34 contaminants result in variable and multi-level effects. Understanding these complex plant-
35 pollutant interactions cannot directly rely on toxicological or agronomical approaches that
36 focus on the effects of field-rate pesticide applications. It must take into account exposure at
37 root level, sublethal concentrations of bioactive compounds and functional biodiversity of the
38 plant species that are affected. The present study deals with agri-environmental plant species
39 of field margins, vegetative filter strips or rotational fallows in European agricultural
40 landscapes. Root and shoot physiological and growth responses were compared under
41 controlled conditions that were optimally adjusted for each plant species. Contrasted
42 responses of growth inhibition, no adverse effect or growth enhancement depended on
43 species, organ and nature of contaminant. However, all of the agricultural contaminants under
44 study (pesticides, pesticide metabolites, heavy metals, polycyclic aromatic hydrocarbons) had
45 significant effects under conditions of sublethal exposure on at least some of the plant species.
46 The fungicide tebuconazole and polycyclic aromatic hydrocarbon fluoranthene, which gave
47 highest levels of responses, induced both activation or inhibition effects, in different plant
48 species or in different organs of the same plant species. These complex effects are discussed

49 in terms of dynamics of agri-environmental plants and of ecological consequences of
50 differential root-shoot growth under conditions of soil contamination.

51

52 **Keywords :**

53 Ecotoxicity, Field margin plants, Heavy metals, Persistent organic pollutants, Pesticides, Soil

54 pollution

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57 **Highlights :**

- 58 • Agri-environmental plants are directly confronted to soil agricultural pollutants.
- 59 • Diverse residual chemicals affect agri-environmental plants at sublethal levels.
- 60 • These effects are not only species-specific, but also root- and shoot-specific.
- 61 • Pollutant-specific effects lead to differential root-shoot responses.
- 62 • Root-shoot disequilibrium is one major consequence of agricultural soil pollution.

63

64

65 **1. Introduction**

66 Extensive use of chemicals in agriculture and industry has resulted in widespread
67 environmental contamination with potential impact on environmental quality, human well-
68 being and planetary sustainability (Arias-Estévez et al., 2008; Persson et al., 2013; MacLeod
69 et al., 2014; Rodríguez-Eugenio et al., 2018; Silva et al., 2019). On the basis of expected
70 environmental concentration calculations taking into account a 15-cm depth of affected soil or
71 waterbody (Peterson et al., 1994), worldwide consumption of 2.6-4.6 Tg of pesticides per year
72 (Wilson and Tisdell, 2001; Zhang et al., 2011; Bringel and Couée, 2018) could theoretically
73 result in yearly accumulations of 34-60 $\mu\text{g L}^{-1}$ by dispersion over a global Earth surface of
74 $510 \times 10^6 \text{ km}^2$. On a regional scale, average application rates of pesticides per hectare of arable
75 land can attain 6.5-60 kg ha^{-1} in Asia and South America (Carvalho, 2017), which may result
76 in very high levels of expected environmental yearly accumulations of 4,333-40,000 $\mu\text{g L}^{-1}$.
77 Actual measurements in pesticide-contaminated soils show that residual persistence of
78 pesticides (Alberto et al., 2017; Arias-Estévez et al., 2008; Jablonowski et al., 2010; Primost
79 et al., 2017; Serra et al., 2013; Silva et al., 2018) can fall within these ranges of theoretical
80 values of potential accumulation. In Argentinian agrosystems, expected environmental
81 concentration calculation (Peterson et al., 1994) on the basis of annual applications (Primost
82 et al., 2017) yields a glyphosate concentration of 2,600 $\mu\text{g L}^{-1}$. This theoretical value is lower
83 than the average measurement of 6,433 $\mu\text{g.kg}^{-1}$ for glyphosate and its metabolite
84 aminomethylphosphonate (AMPA) in soils of these Argentinian agrosystems (Primost et al.,
85 2017), thus emphasising the importance of persistence and pluri-annual accumulation.
86 Moreover, agricultural and livestock activities are not only sources of agrochemicals, but also
87 of heavy metals and polycyclic aromatic hydrocarbons (PAHs) that can accumulate in the
88 environment as agriculture-related contaminants in parallel with pesticides and pesticide
89 residues (Rodríguez-Eugenio et al., 2018).

90 Root-level exposure to such residual levels of xenobiotics and heavy metals causes a variety
91 of biochemical, physiological and developmental effects on agricultural or natural plant
92 species (Alberto et al., 2017; Serra et al., 2013, 2015a, 2015b; Zhang et al., 2014). Herbicides
93 such as atrazine, glyphosate or imazethapyr are known to act through a major biochemical
94 target (Holländer and Amrhein, 1980; Padgett et al., 1991; Rutherford & Krieger-Liszkay,
95 2001; Qian et al., 2015; Sammons et al., 2018), that can be considered to be the canonical
96 target mediating their mechanism of action (Piya et al., 2019). However, root-level effects of
97 herbicides have often been shown to be disconnected from usual effects on canonical targets,
98 thus suggesting alternative and noncanonical mechanisms of action under such conditions of
99 exposure (Alberto et al., 2017, 2018; Qian et al., 2015; Serra et al., 2013, 2015a, 2015b).
100 Moreover, exposure to sublethal doses, the diverse modalities of contaminant exposures,
101 environmental and plant metabolization of contaminants into novel metabolites that can also
102 be harmful and the presence of contaminant mixtures in the environment result in variable and
103 multi-level effects (Armendáriz et al., 2016; Busi and Powles, 2009; Reeves et al., 2001;
104 Serra et al., 2013). Soil pollution by anthropogenic chemicals is therefore a major concern for
105 the sustainability of crop production (Rodríguez-Eugenio et al., 2018; Silva et al., 2019). Soil
106 pollution is also a major concern for the efficiency of ecosystem services mediated by natural
107 plant biodiversity (Rodríguez-Eugenio et al., 2018; Silva et al., 2019), such as the
108 maintenance of pollinator diversity (Kuussaari et al., 2011), soil stabilization of field margins
109 or pollution buffering (Mench et al., 2010; Serra et al., 2016).
110 However, the range of soil pollution effects on plants is difficult to apprehend and remains to
111 be fully understood (Rodríguez-Eugenio et al., 2018). Agri-environmental plant communities
112 in field margins, vegetative filter strips (VFS) or rotational fallows are necessarily confronted
113 with agricultural pollutants through residual soil contamination and/or through drift, run-off
114 and leaching events that result from chemical applications (Gove et al., 2007; Helander et al.,

115 2012; Rodríguez-Eugenio et al., 2018). On the one hand, in the context of VFS at the edge of
116 agricultural fields, the confrontation of plant communities with pesticides and other
117 agricultural contaminants is designed to mitigate the dispersion and the impact of pollutants in
118 terrestrial and aquatic environments (Collins et al., 2014; Krutz et al., 2005; Serra et al., 2016;
119 Stehle et al., 2011). On the other hand, plant biodiversity and functional group composition in
120 agriculturally-intensive landscapes are affected by land-use practices and application rates of
121 fertilizers and pesticides (Billeter et al., 2008; Liira et al., 2008). Understanding these
122 complex plant-pollutant interactions cannot directly rely on toxicological or agronomical
123 studies that focus on the effects of field-rate applications of pesticides on selected plant
124 species. It must take into account the specificities of environmental exposure, such as
125 exposure at root level, sublethal concentrations of bioactive compounds, exposure to derived
126 metabolites and the functional biodiversity of the plant species that are affected.

127 Application of such conditions has revealed that root growth was particularly sensitive to
128 supposedly non-phytotoxic levels of xenobiotics or of their metabolized derivatives (Serra et
129 al., 2013, 2015a, 2015b; Alberto et al., 2017). In parallel, major agricultural pollutants such as
130 atrazine and glyphosate (Rodríguez-Eugenio et al., 2018; Silva et al., 2018, 2019) act on shoot
131 chloroplastic targets. The present study therefore aims to characterize the potential
132 perturbations of root and shoot growth under conditions of exposure to agricultural
133 contaminants, taking into account the chemical diversity of contaminants and the diverse
134 responses of plants [bird's-foot trefoil (*Lotus corniculatus*), common buckwheat (*Fagopyrum*
135 *esculentum*), cornflower (*Centaurea cyanus*), creeping bentgrass (*Agrostis stolonifera*),
136 English ryegrass (*Lolium perenne*), red fescue (*Festuca rubra*), timothy grass (*Phleum*
137 *pratense*), white Dutch clover (*Trifolium pratense*), yellow chamomile (*Anthemis tinctoria*)]
138 that are commonly found in field margins, VFS or rotational fallows in European agricultural
139 landscapes (Billeter et al., 2008; Liira et al., 2008; Kuussaari et al., 2011; Stehle et al., 2011;

140 Toivonen et al., 2013; Serra et al., 2016). Root and shoot physiological and growth responses
141 were compared under controlled conditions that were optimally adjusted for each plant
142 species, with the parallel analysis of the unrelated model plant species *Arabidopsis thaliana* as
143 a reference species. The herbicide glyphosate, the glyphosate metabolite AMPA, the herbicide
144 atrazine, the atrazine metabolite hydroxyatrazine, the fungicide tebuconazole, copper, zinc
145 and the PAH fluoranthene all had significant effects under conditions of sublethal exposure
146 on at least two of the plant species under study. However, this diversity of agricultural
147 contaminants (pesticides, pesticide metabolites, heavy metals, PAHs) revealed contrasted
148 responses of growth stimulation, no adverse effect or growth inhibition, depending on plant
149 species, plant organ and nature of contaminant. Tebuconazole and fluoranthene, which gave
150 the highest level of responses, could induce both activation or inhibition effects, in different
151 plant species or in different organs of the same plant species. These complex effects are
152 discussed in terms of plant dynamics in agri-environmental contexts and of ecological
153 consequences of differential root-shoot growth under conditions of soil contamination.

154 **2. Materials and methods**

155 *2.1. Plant material*

156 Seeds of *Arabidopsis thaliana* (Columbia, Col-0) were obtained from the Nottingham
157 Arabidopsis Stock Center, and propagated under laboratory-controlled conditions. Seeds of
158 *Agrostis stolonifera* (cv. Penncross), *Anthemis tinctoria* (bulk seeds), *Centaurea cyanus* (bulk
159 seeds), *Fagopyrum esculentum* (bulk seeds), *Festuca rubra* (cv. Herald), *Lolium perenne* (cv.
160 Brio), *Lotus corniculatus* (cv. Leo), *Phleum pratense* (cv. Kaba), and *Trifolium pratense* (cv.
161 Violetta) were obtained from the Phytosem (Gap, Hautes-Alpes, France) seed company.
162 These seeds did not have any pesticide treatment coating.

163 *2.2. Growth conditions*

164 Seeds of *Arabidopsis thaliana* were surfaced sterilized in 3% bayrochlore:ethanol (1:1, v/v),
165 rinsed in absolute ethanol, and dried overnight. Germination and growth were carried out
166 under axenic conditions in square Petri dishes. After seed sowing, Petri dishes were placed in
167 the dark at 4 °C for 72 h in order to break dormancy and homogenize germination, and were
168 then transferred to a control growth chamber at 22 °C/20 °C under a 16 h light (100 $\mu\text{mol m}^{-2}$
169 s^{-1})/8 h dark regime. Growth medium consisted of 0.8% (w/v) agar in Hoagland basal salt mix
170 (H2395, Sigma-Aldrich) adjusted to pH 6, in the absence of any additional sucrose or soluble
171 carbohydrate (Ramel et al., 2009b; Serra et al., 2013, 2015a; Nuttens and Gross, 2017).

172 Seeds of *Agrostis stolonifera*, *Anthemis tinctoria*, *Centaurea cyanus*, *Fagopyrum esculentum*,
173 *Festuca rubra*, *Lolium perenne*, *Lotus corniculatus*, *Phleum pratense*, and *Trifolium pratense*
174 were surface-sterilized in ethanol (1 min) and bayrochlore:tween at various concentrations of
175 bayrochlore (20 min), and rinsed in sterile distilled water. Germination and growth were
176 carried out under axenic conditions in square Petri dishes (*Agrostis stolonifera*, *Anthemis*
177 *tinctoria*, *Festuca rubra*, *Lotus corniculatus*, *Phleum pratense*, *Trifolium pratense*) or in 30-
178 mL Falcon tubes (*Centaurea cyanus*, *Fagopyrum esculentum*, *Lolium perenne*). Growth
179 medium in Petri dishes consisted of 0.8% (w/v) agar in Hoagland basal salt mix (H2395,
180 Sigma-Aldrich) adjusted to pH 6. Growth medium in 30-mL Falcon tubes consisted of 0.3%
181 (w/v) agar in Hoagland basal salt mix (H2395, Sigma-Aldrich) adjusted to pH 6. Xenobiotics
182 (glyphosate, AMPA, atrazine, hydroxyatrazine, tebuconazole) and heavy metals (Cu^{2+} added
183 as CuSO_4 , Zn^{2+} added as ZnSO_4) were axenically added to cooled-down melted agar-
184 Hoagland medium prior to pouring into Petri dishes or Falcon tubes. Xenobiotic and heavy
185 metal treatments were carried out by direct exposure starting at early development by seed
186 sowing on chemical-containing growth medium.

187 After seed sowing, Petri dishes or Falcon tubes were placed in the dark at 4 °C for 3 to 8 days
188 (according to plant species) in order to break dormancy and homogenize germination. Seeds

189 of *Centaurea cyanus* did not undergo any treatment of dormancy break. Sown Petri dishes or
190 Falcon tubes were then transferred to a control growth chamber at 22 °C/20 °C under a 16 h
191 light (100 $\mu\text{mol m}^{-2} \text{s}^{-1}$)/8 h dark regime. Analysis of physiological and growth parameters
192 was carried out after 7 (*Agrostis stolonifera*, *Anthemis tinctoria*, *Festuca rubra*, *Lotus*
193 *corniculatus*, *Phleum pratense*, *Trifolium pratense*), 10 (*Fagopyrum esculentum*, *Lolium*
194 *perenne*), 12 (*Centaurea cyanus*) or 14 (*Arabidopsis thaliana*) days of growth in the absence
195 or presence of xenobiotics or heavy metals.

196 2.3. Analysis of growth responses

197 Plantlets in Petri dishes were directly photographed. Seedlings in Falcon tubes were aligned
198 on glass plates and photographed. Lengths of main root and elongating leaf were measured
199 using ImageJ software. Results were expressed as root or leaf length or as percentage of
200 inhibition of growth $[(\text{Growth}_{\text{control}} - \text{Growth}_{\text{treatment}})/\text{Growth}_{\text{control}}]$ relatively to the control in
201 the absence of xenobiotic or heavy metal. The action of xenobiotic and heavy metal
202 treatments on *Arabidopsis thaliana* root growth was characterized by median effective
203 concentrations (EC50) resulting in a 50% inhibitory response. The nine agri-environmental
204 plant species (*Agrostis stolonifera*, *Anthemis tinctoria*, *Centaurea cyanus*, *Fagopyrum*
205 *esculentum*, *Festuca rubra*, *Lolium perenne*, *Lotus corniculatus*, *Phleum pratense*, *Trifolium*
206 *pratense*) were exposed to the eight xenobiotics and heavy metals at the EC50 inhibitory
207 concentrations determined for *Arabidopsis thaliana* root growth.

208 2.4. Analysis of physiological parameters

209 Pigments were extracted by grinding shoots of seedlings in 80% (v/v) acetone, and the
210 absorbance of the resulting extracts was measured at three wavelengths: 663, 646, and 470
211 nm. Levels of chlorophylls and total carotenoids (xanthophylls and carotenes) in these
212 extracts were determined from the equations given by Lichtenthaler and Wellburn (1983), as
213 previously described (Serra et al., 2013).

214 2.5. *Data analysis*

215 Physiological and growth parameters were measured on five to thirty independent biological
216 replicates. Results were given as the mean (\pm SEM) of these determinations. Statistical
217 analysis was carried out with the R version 3.1.3 software. Pairwise comparisons of means
218 used the non-parametric Mann–Whitney–Wilcoxon test. In order to test and visualize
219 relationships between treatments and response parameters, principal component analysis
220 (PCA) based on the correlation matrix (Ramel et al., 2009a) was carried out using the
221 FactoMineR package of R.

222 **3. Results**

223 *3.1. Characterization of sublethal effects of root-level agricultural contaminants on the model*
224 *plant species Arabidopsis thaliana*

225 In line with the molecular effects of soluble sugars on xenobiotic stress responses (Ramel et
226 al., 2007, 2009a, 2009b), the toxicological impact of xenobiotics and heavy metals on
227 Arabidopsis plantlets was significantly modified in the presence of additional sucrose (data
228 not shown). Whereas the inhibitory effects of atrazine were lifted by exogenous sucrose,
229 glyphosate showed the same level of toxicity in the absence or presence of exogenous sucrose
230 (data not shown). Cultivation in the absence of exogenous soluble sugars was therefore
231 essential to reflect a realistic evaluation of comparative chemical stress sensitivity, as
232 advocated by Nuttens and Gross (2017).

233 Since exposure was carried out at root level, and given the sensitivity of roots to xenobiotic
234 and heavy metal stress (Alberto et al., 2017; Serra et al. 2013, 2015a), dose-response
235 relationships were derived from measurements of primary root growth (Fig. 1). All of the
236 xenobiotics and heavy metals under study, glyphosate (Fig. 1), AMPA, atrazine,
237 hydroxyatrazine, tebuconazole, copper, zinc and fluoranthene (data not shown), had inhibitory
238 effects on the root growth of Arabidopsis plantlets, with typical hyperbolic or sigmoid

239 inhibition curves. EC50 values (Table 1) were estimated graphically from these hyperbolic or
240 sigmoid curves of inhibition. The EC50 values for glyphosate, AMPA, atrazine, and
241 hydroxyatrazine agreed with previous analyses under similar modalities of exposure (Serra et
242 al., 2013; Sulmon et al., 2004).

243 In contrast, in the case of tebuconazole, the EC50 value was much higher than the inhibitory
244 concentrations described by Serra et al. (2013). In the case of Zn, Cu and fluoranthene, direct
245 comparisons with previous studies could not be carried out because of major discrepancies in
246 the modalities of exposure. The much lower EC50 value for Zn relatively to that described by
247 Richard et al. (2011) reflected a higher sensitivity to Zn toxicity that could be ascribed to the
248 absence of exogenous sucrose in the present study. In contrast, the EC50 for Cu was very
249 similar to that resulting from exposure of 3-week-old Arabidopsis plants under hydroponics
250 conditions in the absence of exogenous sucrose (Lequeux et al., 2010). As previously
251 described for the analysis of phenanthrene effects on Arabidopsis (Alkio et al., 2005), the
252 relatively high range of fluoranthene concentrations (up to 550 μM) exceeded water
253 solubility. However, the phytotoxicity of PAHs is also induced by contact (Paškova et al.,
254 2009). Thus, in line with the effects of phenanthrene (Alkio et al., 2005), fluoranthene had a
255 significant effect on primary root growth (Alkio et al., 2005), with an EC50 value (532 μM)
256 that was within the range of inhibitory PAH concentrations (Alkio et al., 2005).

257 Finally, the fungicide tebuconazole and Cu were the most highly effective non-herbicide
258 chemical stressors, in agreement with previously-described side effects of tebuconazole
259 treatment (Serra et al., 2013) and the high toxicity of copper (Lequeux et al., 2010) in plants.

260 *3.2. Effects of sublethal levels of root-level agricultural contaminants on root growth of agri- 261 environmental plant species*

262 Xenobiotic and heavy metal exposure of the agri-environmental plant species was carried out
263 in the absence of exogenous soluble sugars, under growth conditions that were similar to

264 those of the *Arabidopsis* study. Certain conditions of seed sterilisation (percentage of
265 bayrochlore; duration of treatment) and stratification (duration of low temperature treatment)
266 were adjusted for each plant species in order to optimise germination efficiency and axenic
267 growth (data not shown). In order to take into account the growth characteristics of each
268 species, experiments were carried out either in Petri dishes or in 30-mL Falcon tubes as
269 described in Materials and Methods. The nine agri-environmental plant species were
270 subjected during germination and early growth to xenobiotic and heavy metal concentrations
271 corresponding to the EC50 values of impact on root growth of *Arabidopsis* plantlets (Fig. 1,
272 Table 1), except in the case of glyphosate and AMPA, which were applied at concentrations
273 of respectively 1 μM (instead of EC50 = 0.75 μM) and 50 μM (instead of EC50 = 30 μM).
274 All of the nine agri-environmental species showed significant root responses to at least some
275 of the xenobiotics or heavy metals (Fig. 2), except red fescue, which showed responses that
276 were not significantly different from control. Treatment with herbicide compounds and
277 metabolites, glyphosate, AMPA, atrazine and hydroxyatrazine, resulted in either a no
278 observable adverse effect (NOAE) situation or inhibition of root growth. Tebuconazole, Zn,
279 Cu or fluoranthene showed significant inhibitory effects on root growth, for instance in the
280 case of yellow chamomile (*Anthemis tinctoria*) and cornflower (*Centaurea cyanus*) (Fig. 2).
281 However, in parallel, tebuconazole, Zn, Cu or fluoranthene enhanced root growth of bird's-
282 foot trefoil (*Lotus corniculatus*), creeping bentgrass (*Agrostis stolonifera*), and English
283 ryegrass (*Lolium perenne*) (Fig. 2; supplementary data 1), thus suggesting the induction of
284 hormetic effects (Belz et al., 2014; Dyer, 2018; Velini et al., 2008).
285 Tebuconazole and fluoranthene induced the highest level of responses, with highly positive
286 effects on root growth of *Lolium perenne* and highly negative effects on root growth of
287 *Anthemis tinctoria*, relatively to all or most of the other plant species (supplementary data 1).
288 Zn gave the lowest level of responses, with NOAE interactions in *Trifolium pratense*,

289 *Agrostis stolonifera*, *Festuca rubra*, *Phleum pratense*, *Lolium perenne* and *Fagopyrum*
290 *esculentum*. On the other hand, species-specific behaviours were significantly contrasted.
291 Root growth of yellow chamomile (*Anthemis tinctoria*) and cornflower (*Centaurea cyanus*)
292 was negatively affected by all or most of xenobiotic or heavy metal treatments. Root growth
293 of bird's-foot trefoil (*Lotus corniculatus*), creeping bentgrass (*Agrostis stolonifera*), English
294 ryegrass (*Lolium perenne*) and red fescue (*Festuca rubra*) showed contrasted chemical-
295 specific responses, with a mixture of negative and positive responses.

296 3.3. Effects of sublethal levels of root-level agricultural contaminants on shoot growth of 297 agri-environmental plant species

298 The impact of xenobiotics and heavy metals on shoot growth was characterized under the
299 same conditions of cultivation and treatment as those described in subsection 3.2. Root-level
300 exposure can result in xenobiotic or heavy metal translocation throughout the plant and
301 targeting of shoot and leaf mechanisms and/or root-level interactions or perturbations can lead
302 to modifications of shoot growth and development (Serra et al., 2013., 2015a; Sulmon et al.,
303 2007). In all of the nine species, shoot growth significantly responded to at least some of the
304 xenobiotics or heavy metals (Fig. 3), thus showing the involvement of root-shoot interactions
305 in the response to root-level exposure.

306 Treatment with herbicide compounds and metabolites, glyphosate, AMPA, atrazine and
307 hydroxyatrazine, resulted in either a NOAE situation or inhibition of shoot growth. AMPA
308 gave the lowest level of responses, with NOAE shoot growth interactions in all of the nine
309 agri-environmental plant species. In contrast with their effects on root growth (Fig. 2),
310 tebuconazole and fluoranthene did not show any enhancement effects on shoot growth (Fig. 3),
311 but induced either a NOAE situation or inhibition of shoot growth (Fig. 3). In contrast, Zn and
312 Cu had significant enhancement effects on shoot growth of respectively white Dutch clover
313 (*Trifolium pratense*) and red fescue (*Festuca rubra*) (Fig. 3; supplementary data 1), thus

314 suggesting the induction of hormetic effects (Belz et al., 2014; Dyer, 2018; Velini et al.,
315 2008). Tebuconazole induced the highest level of shoot growth inhibition, especially in
316 bird's-foot trefoil (*Lotus corniculatus*) and cornflower (*Centaurea cyanus*) (Fig. 3;
317 supplementary data 1). Moreover, tebuconazole was the only contaminant that affected shoot
318 growth of nearly all of the nine agri-environmental plant species (Fig. 3). Zn treatment gave
319 the most contrasted responses with highly positive effects on shoot growth of *Trifolium*
320 *pratense* and highly negative effects on shoot growth of *Lotus corniculatus* and *Centaurea*
321 *cyanus* (Fig. 3; supplementary data 1).

322 On the other hand, species-specific behaviours were significantly contrasted. Shoot growth of
323 bird's-foot trefoil (*Lotus corniculatus*) and cornflower (*Centaurea cyanus*) was negatively
324 affected by a range of xenobiotic or heavy metal treatments (Fig. 3). Shoot growth of white
325 Dutch clover (*Trifolium pratense*) and red fescue (*Festuca rubra*) showed contrasted
326 chemical-specific responses, with a mixture of negative and positive responses (Fig. 3).
327 Moreover, in bird's-foot trefoil (*Lotus corniculatus*) and English ryegrass (*Lolium perenne*),
328 significant inhibition of shoot growth by tebuconazole, Zn or fluoranthene did not correlate
329 with inhibition of root growth (Fig. 3), thus demonstrating root-shoot translocation of toxic
330 compounds or root-shoot crosstalk of root-level exposure.

331 *3.4. Species-specific analysis of the effects of sublethal levels of root-level agricultural* 332 *contaminants on growth and development of agri-environmental plant species*

333 The comparison of root and shoot responses (Table 2) highlighted strikingly different
334 behaviours of the nine agri-environmental plant species towards to the different xenobiotics
335 and heavy metals, with cases of general tolerance (*Festuca rubra*, *Lolium perenne*) and cases
336 of general sensitivity (*Lotus corniculatus*, *Centaurea cyanus*, *Anthemis tinctoria*). Analysis of
337 the effects of xenobiotics and heavy metals on the levels of carotenoids and chlorophylls did
338 not reveal any clear relationship between the decrease or increase of carotenoids and

339 chlorophylls and growth responses (data not shown). For example, both pollutant-sensitive
340 species, such as *Centaurea cyanus* and *Anthemis tinctoria*, and the pollutant-tolerant species
341 *Festuca rubra* showed stable levels of chlorophylls and carotenoids across the different
342 xenobiotic and heavy metal treatments. Moreover, negative or positive variations of
343 chlorophylls and carotenoids were found to be xenobiotic-specific. Another pollutant-
344 sensitive species, *Lotus corniculatus*, showed a 30% (± 5 , SEM) decrease of carotenoid levels
345 in the presence of atrazine. In the case of pollutant-tolerant species, chlorophyll levels showed
346 a 55% (± 6 , SEM) increase in *Lolium perenne* under conditions of tebuconazole treatment and
347 a 67% (± 15 , SEM) increase in *Agrostis stolonifera* under conditions of glyphosate treatment.
348 This differential behaviour was further analysed by PCA of root and shoot growth responses
349 (Fig. 4). Distribution along the two axes on the first plane (Dim1 and Dim2) identified five
350 types of plant-contaminant interactions (Fig. 4A): (i) highly-tolerant red fescue (*Festuca*
351 *rubra*), which was negatively affected exclusively by the effects of tebuconazole on shoot
352 growth (Fig. 3), (ii) a central cluster comprising creeping bentgrass (*Agrostis stolonifera*),
353 timothy grass (*Phleum pratense*) and English ryegrass (*Lolium perenne*), which maintained
354 significant growth in the presence of the different agricultural contaminants (Fig. 3), (iii) a
355 cluster comprising white Dutch clover (*Trifolium pratense*), yellow chamomile (*Anthemis*
356 *tinctoria*) and common buckwheat (*Fagopyrum esculentum*), which showed significant
357 sensitivity of root growth to glyphosate and tebuconazole (Fig. 3), (iv) cornflower (*Centaurea*
358 *cyanus*), which showed high sensitivity of root growth to AMPA and copper (Fig. 3), (v)
359 bird's-foot trefoil (*Lotus corniculatus*), which showed high sensitivity of shoot growth to
360 glyphosate, hydroxyatrazine, tebuconazole and zinc (Fig. 3). Hierarchical classification (Fig.
361 4B) revealed a slightly different clustering of four types of responses: (i) highly-tolerant red
362 fescue (*Festuca rubra*), (ii) a central cluster comprising moderately tolerant English ryegrass
363 (*Lolium perenne*), creeping bentgrass (*Agrostis stolonifera*), timothy grass (*Phleum pratense*),

364 yellow chamomile (*Anthemis tinctoria*), white Dutch clover (*Trifolium pratense*) and common
365 buckwheat (*Fagopyrum esculentum*), (iii) cornflower (*Centaurea cyanus*), (iv) bird's-foot
366 trefoil (*Lotus corniculatus*). Cornflower (*Centaurea cyanus*) and bird's-foot trefoil (*Lotus*
367 *corniculatus*) showed the highest level of shoot growth sensitivity.

368 *Festuca rubra* and the *Agrostis stolonifera-Phleum pratense-Lolium perenne* cluster showed a
369 common response pattern towards the 8 different agricultural contaminants (Fig. 4C). In
370 contrast, the *Trifolium pratense-Anthemis tinctoria-Fagopyrum esculentum* cluster, *Centaurea*
371 *cyanus* and *Lotus corniculatus* showed patterns of responses that were strongly driven by
372 glyphosate, AMPA or hydroxyatrazine (Fig. 4C), thus highlighting the potentially important
373 action of herbicide metabolites on plant community dynamics in agricultural landscapes.
374 Whereas most root-related and shoot-related vectors showed parallel effects, Zn and
375 glyphosate showed strikingly different effects on root and shoot growth (Fig. 4C), thus
376 suggesting differential modes of action at root and shoot level. This would agree with the
377 multiple roles and multiple sites of action of Zn (Richard et al., 2011; Rouached, 2013) and
378 with the involvement of glyphosate in other processes (Serra et al., 2013; Orcaray et al. 2010;
379 Vivancos et al., 2011) than its canonical inhibition of EPSPS (Padgett et al., 1991), as
380 described in subsection 3.1.

381 **4. Discussion**

382 *4.1. Agri-environmental plant species can be affected by a large array of root-level* 383 *agricultural contaminants at residual concentrations*

384 The nine agri-environmental plant species of the present study were generally less sensitive to
385 xenobiotics and heavy metals than the reference species *Arabidopsis thaliana*, except for
386 *Anthemis tinctoria* and *Centaurea cyanus* which were oversensitive to respectively
387 tebuconazole and AMPA. In *Arabidopsis thaliana*, atrazine and glyphosate were the most
388 effective chemical stressors with EC50 values lower than 1 μ M, which could be ascribed to

389 the impact of their herbicide activity. The canonical targets of their mode of action are well
390 characterized, with atrazine targeting the D1 protein of photosystem II (PSII) (Rutherford &
391 Krieger-Liszkay, 2001), and glyphosate targeting 5-enolpyruvylshikimate-3-phosphate
392 synthase (EPSP) (Holländer and Amrhein, 1980; Padgett et al., 1991; Sammons et al., 2018).
393 The present EC50 value for atrazine (0.5 μM) was within the range of half maximal inhibitory
394 concentrations (IC50=0.4-0.8 μM) for inhibition of PSII fluorescence in isolated pea
395 thylakoids (Jursinic & Stemler, 1983), and in agreement with previously-described
396 correlations between PSII inhibition and root growth inhibition (Alberto et al., 2017). In
397 contrast, the present EC50 value for glyphosate (0.75 μM) was lower than the 6-37 μM IC50
398 values or the 16.1 (\pm 2.6) μM IC50 value reported for inhibition of purified 5-
399 enolpyruvylshikimate-3-phosphate synthase (EPSPS) respectively from different plant species
400 (Padgett et al., 1991) or from *Arabidopsis thaliana* (Sammons et al., 2018). This discrepancy
401 suggested that glyphosate affected additional noncanonical targets in parallel with its action
402 on EPSPS. Indeed, glyphosate toxicity in plants has been hypothesised to involve multiple
403 mechanisms in addition to EPSPS inhibition (Serra et al., 2013; Orcaray et al. 2010; Vivancos
404 et al., 2011). In contrast with *Arabidopsis thaliana*, many of the agri-environmental species
405 under study showed no decrease of root growth in response to these EC50 levels of atrazine
406 and glyphosate (Fig. 2).

407 Moreover, the effects of tebuconazole, Zn, Cu and fluoranthene on root growth involved both
408 inhibition and activation rather than mere inhibition as in the case of *Arabidopsis*, thus
409 highlighting major species-specific differences of mechanisms and responses. However, all of
410 these conditions affected root growth of at least two of the nine plant species under study,
411 with wide-ranging impacts for tebuconazole and hydroxyatrazine and narrow impacts for
412 atrazine, Zn and Cu. Moreover, glyphosate, atrazine, hydroxyatrazine, tebuconazole, Zn and
413 fluoranthene affected both root growth and shoot growth. All of the conditions of exposure

414 resulted in sublethal effects, in accordance with the general effects of residual contaminants in
415 the environment.

416 The eight xenobiotic and heavy metal compounds of the present study are found in
417 agricultural landscapes and field margins (Alberto et al., 2017; He et al., 2005; Hildebrandt et
418 al., 2009; Jiang et al., 2018; Primost et al., 2017; Serra et al. 2013, 2016; Silva et al., 2018).

419 The parallel analysis of pesticide-related compounds (glyphosate, AMPA, atrazine,
420 hydroxyatrazine, tebuconazole), heavy metals (Cu, Zn) and polycyclic aromatic hydrocarbons
421 (fluoranthene) thus gave a more realistic view of potential agriculture-related contaminations
422 than the exclusive study of pesticide contaminants (Serra et al., 2016). The levels of exposure
423 were lower than or similar to the theoretical values of potential accumulation described in
424 section 1. The concentration of atrazine ($100 \mu\text{g L}^{-1}$) corresponded to environmental
425 concentrations commonly found in soils, waters and sediments (Alberto et al., 2017;
426 Jablonowski et al., 2010). The concentration of glyphosate ($169 \mu\text{g L}^{-1}$) was lower than
427 observed environmental values in soils and suspended particulate matter (Ghanem et al.,
428 2007; Primost et al., 2017; Silva et al., 2018) and than experimental concentrations used in
429 mechanistic (Orcaray et al., 2010) or ecotoxicological (Saunders et al., 2013; Soares et al.,
430 2019) studies of root-zone impact of residual concentrations. It was for instance much lower
431 than the average concentration of glyphosate in Argentinian agrosystems [$2,229 (\pm 476)$
432 $\mu\text{g.kg}^{-1}$] (Primost et al., 2017) and than the lowest experimental concentrations used by
433 Orcaray et al. (2010) (53 mg L^{-1}) or Saunders et al. (2013) (10 mg kg^{-1}). The concentration of
434 fluoranthene was also lower than observed PAH contaminating levels in the environment
435 (Rodríguez-Eugenio et al., 2018). The concentration of AMPA ($5,550 \mu\text{g L}^{-1}$) corresponded to
436 environmental values that have been measured in agricultural landscapes (Ghanem et al.,
437 2007; Primost et al., 2017; Silva et al., 2018), such as Argentinian agrosystems, which show
438 an average concentration of $4,204 (\pm 2,258) \mu\text{g.kg}^{-1}$. The concentration of tebuconazole

439 corresponded to high environmental levels that have been described for individual fungicides
440 (Rial-Otero et al., 2004). The concentrations of Cu or Zn also corresponded to high
441 environmental levels that have been described in industrial polluted soils (Dazy et al., 2008).
442 In contrast, the concentration of hydroxyatrazine (5,000 $\mu\text{g L}^{-1}$) was significantly higher than
443 currently-described (up to 640 $\mu\text{g.kg}^{-1}$) environmental values (Alberto et al., 2017;
444 Jablonowski et al., 2010; Lerch et al., 1999). Moreover, none of the nine agri-environmental
445 plant species under study showed any oversensitivity to hydroxyatrazine (Fig. 2) relatively to
446 that of *Arabidopsis thaliana*.

447 Thus, the present results indicated that glyphosate, AMPA, atrazine, tebuconazole, Zn, Cu and
448 fluoranthene could not be considered as innocuous in terms of plant-contaminant interactions
449 under environmental conditions. Residual environmental levels of both glyphosate and its
450 metabolite AMPA were likely to affect *Trifolium pratense*, *Anthemis tinctoria*, *Centaurea*
451 *cyanus* and *Fagopyrum esculentum*, and residual environmental levels of atrazine were likely
452 to affect *Lotus corniculatus* and *Anthemis tinctoria*. The significant sensitivity of *Trifolium*
453 *pratense*, *Anthemis tinctoria*, *Centaurea cyanus* and *Fagopyrum esculentum* to low levels of
454 glyphosate suggested the possibility that effects under environmental conditions are more
455 drastic than observed in the present study. The significant sensitivity of *Centaurea cyanus* to
456 AMPA (Fig. 2) and the influence of AMPA and hydroxyatrazine on the patterns of responses
457 of *Trifolium pratense*, *Anthemis tinctoria*, *Fagopyrum esculentum* and *Lotus corniculatus*
458 (Fig. 4) underlined the potential environmental impact of herbicide metabolites.,

459 Such an impact of chemically-distinct compounds, with established bioactivity or supposed
460 inactivation, and such a diversity of sensitivities emphasise the need to expand the range of
461 pesticide tests in order to improve predictive power on the environmental impacts of
462 pesticides (Milner and Boyd, 2017). Moreover, cumulative exposures can result in growth
463 disruption that is not reducible to the sum of individual effects because of potential synergistic

464 or antagonistic interactions between contaminants (Alberto et al., 2017). There are therefore
465 environmental situations where the combined effects of mixtures of xenobiotics and/or heavy
466 metals on plant growth and development may be more drastic than the predicted effects
467 construed from single contaminant exposure experiments.

468 *4.2.. Modes of action of root-level agricultural contaminants and risk assessment of* 469 *differential root-shoot growth under conditions of soil contamination*

470 The present results under controlled conditions emphasised the direct action of chemical
471 stressors on plant tissues. The effects of chemical stressors have been experimentally
472 characterized in a limited number of plant species, and their targets and mechanisms of action
473 remain to be fully investigated (Alberto et al., 2017, 2018; Orcaray et al., 2010; Serra et al.,
474 2013, 2015; Vivancos et al., 2011). Even widely-used herbicides with well-known
475 chloroplast-localized canonical sites of action, such as glyphosate and atrazine, have
476 additional noncanonical effects that particularly affect signaling mechanisms, hormone
477 dynamics, developmental processes and root growth (Alberto et al., 2017, 2018; Couée et al.,
478 2013; Dogramaci et al., 2015; Orcaray et al., 2010; Ramel et al., 2012; Serra et al., 2013,
479 2015; Vivancos et al., 2011). Moreover, the corresponding metabolites, AMPA and
480 hydroxyatrazine, can significantly affect plant metabolism and growth in the absence of
481 observed effects on the canonical targets of parent molecules, thus implying alternative
482 mechanisms of action (Alberto et al., 2017, 2018; Gomes et al., 2016; Serra et al., 2013,
483 2015).

484 The present comparative study confirmed the importance of noncanonical patterns of
485 responses to glyphosate and atrazine. The significant effects on root growth in the absence of
486 effects on shoot growth in several plant species (*Trifolium pratense*, *Anthemis tinctoria*,
487 *Fagopyrum esculentum*) could be related to the root-specific effects that have been discovered
488 in *Arabidopsis thaliana* and *Lolium perenne* (Alberto et al., 2017, 2018; Serra et al., 2013,

489 2015), and showed that root-specific effects of glyphosate and atrazine through root-level
490 exposure can affect natural plant communities. The exclusive effects of AMPA on root
491 growth, rather than on shoot growth, were coherent with a similar differential impact
492 previously described in *Lolium perenne* (Serra et al., 2015). The effects of AMPA were
493 usually similar to or weaker than the effects of glyphosate (Fig. 2, Fig. 3). However, AMPA
494 decreased root growth in the same plant species (*Trifolium pratense*, *Anthemis tinctoria*,
495 *Centaurea cyanus*, *Fagopyrum esculentum*) that were affected by glyphosate at root level
496 (Fig. 2), thus suggesting potential additive effects on natural plant communities given that the
497 co-occurrence of both compounds appears to be ubiquitous in soil (Primost et al., 2017; Silva
498 et al., 2018).

499 In contrast, the effects of atrazine and hydroxyatrazine did not exactly follow the same
500 pattern, with parallel effects of both compounds on root and shoot growth of *Lotus*
501 *corniculatus* and exclusive effects of hydroxyatrazine on root and shoot growth of *Agrostis*
502 *stolonifera* and *Phleum pratense* (Fig. 2, Fig. 3). Moreover, in several cases (Fig. 2, Fig. 3),
503 hydroxyatrazine showed greater toxicity than atrazine. These important and specific effects of
504 hydroxyatrazine on root growth, especially in *Agrostis stolonifera*, *Phleum pratense*, and
505 *Centaurea cyanus*, were coherent with root-level effects (Alberto et al., 2017) and regulation
506 effects on root development genes (Alberto et al., 2018) that have been previously described
507 in *Arabidopsis thaliana*. The case of *Trifolium pratense*, where hydroxyatrazine decreased
508 shoot growth in the absence of root growth inhibition, suggested that hydroxyatrazine may
509 also have a shoot-related target. The mechanisms involved in the high toxicity of
510 hydroxyatrazine remain to be characterized. They may be related to interferences of the
511 triazine structure with cytokinin signaling (Couée et al., 2013; Alberto et al., 2017). The
512 effects of tebuconazole, fluoranthene, Cu and Zn on plant growth are not related to actions on
513 specific biochemical targets, and must involve a mixture of nutritional, biochemical, oxidative

514 stress and regulatory modes of action (Alkio et al., 2005; Lequeux et al., 2010; Paškova et al.,
515 2006; Rouached, 2013; Serra et al., 2015; Shishatskaya et al., 2018; Sverdrup et al., 2003).
516 The diverse pattern of responses (Fig. 2, Fig. 3) could not therefore be ascribed to a specific
517 mechanism. Moreover, in species-specific cases, all of these compounds could induce
518 enhancement of root or shoot growth (Fig. 2, Fig. 3), thus emphasising a complex action on
519 plant growth mechanisms. The root growth enhancement effect of fluoranthene in *Lolium*
520 *perenne* has been shown to occur in parallel with accumulation of fructose, glucose and the
521 cell-wall metabolite arabinose (Serra et al., 2015). On the other hand, strong negative effects
522 on *Lotus corniculatus*, *Anthemis tinctoria* and *Centaurea cyanus* emphasised the potentially
523 high toxicity of tebuconazole and Zn for natural plant communities.

524 In spite of the diversity of modes of action, all of the chemical stressors were found to induce,
525 in at least one plant species, a disconnection of shoot and root growth. Thus, tebuconazole and
526 fluoranthene significantly decreased shoot growth and increased root growth in *Lolium*
527 *perenne*, and glyphosate significantly decreased shoot growth without affecting root growth in
528 *Lotus corniculatus*. Such whole-plant modifications are likely to influence carbon and
529 nitrogen status of the plant. Metabolomics and transcriptomics analysis of plant-xenobiotic
530 interactions has revealed a significant impact of xenobiotics on carbon and nitrogen
531 metabolisms (Armendáriz et al., 2016; Orcaray et al., 2010; Serra et al., 2013, 2015a, 2015b;
532 Vivancos et al., 2011; Zulet et al., 2013). In *Lolium perenne*, besides affecting root-shoot
533 balance (Fig. 2, Fig. 3), fluoranthene treatment induces carbon metabolism modifications and
534 nitrogen metabolism disturbances in both leaves and roots (Serra et al., 2015), thus suggesting
535 that xenobiotic-induced enhancement of root growth may not be necessarily adaptive. Further
536 work will determine whether carbon and nitrogen metabolites could be robust biomarkers of
537 exposure to subtoxic chemical stress in the field.

538 Moreover, given the effects of carbon status on xenobiotic stress responses (Ramel et al.,
539 2007, 2009a, 2009b), modifications of carbon/nitrogen balance may affect exposure to
540 additional xenobiotics, as occurs during sequential or concomitant contaminations. The
541 responses to constraints associated with climate change, such as temperature, elevated CO₂
542 and salinity depend on adjustments of the levels of soluble sugars (Bigot et al., 2018).
543 Simultaneous or sequential exposure to xenobiotic and climate-change-related abiotic stresses
544 might elicit complex modifications of soluble sugar dynamics. Besides immediate impacts on
545 the plant, xenobiotic-induced changes of carbon and nitrogen status could therefore have
546 cascading effects on abiotic stress and climate change responses, and thus more generally on
547 plant ecosystem functioning under climate change.

548 *4.3. Differential responses of agri-environmental plant species and plant community* 549 *dynamics under conditions of agriculture-related chemical contaminations*

550 The comparative analysis of the nine plant species established that the dynamics of plant-
551 contaminant interactions was highly contrasted. The plant cultivars studied in the present
552 work are commonly used in the design and establishment of VFS and rotational fallows, and
553 as such, could be considered to be reference cultivars in this context. However, comparative
554 studies have shown differences of responses to abiotic stresses within cultivars, for instance in
555 *Agrostis stolonifera* (Xu et al., 2010) and *Festuca rubra* (Davies et al., 1995). Beyond
556 interspecific differences, further work on cultivars or populations of the plant species under
557 study will be necessary to reveal the determinants of root-shoot responses to agricultural
558 contaminants.

559 The three most tolerant species (*Festuca rubra*, *Lolium perenne*, *Agrostis stolonifera*) were
560 Poaceae that develop deep rooting systems (Roumet et al., 2008) and are used for
561 phytostabilization and pollutant buffering of VFS and for phytoremediation of polluted soils
562 (Bidar et al., 2007; Mench et al., 2010; Serra et al., 2016). *Festuca rubra* and *Lolium perenne*

563 have thus been found to colonize highly-contaminated soils containing Cu, Zn and
564 fluoranthene (Dazy et al., 2008). Moreover, *Festuca rubra*, *Lolium perenne* and *Agrostis*
565 *stolonifera* are able to adjust carotenoid and chlorophyll levels under conditions of xenobiotic
566 and heavy metal exposure (subsection 3.4; Serra et al., 2015a). Given that vegetation
567 characteristics are important for the efficiency of VFS (Hénault-Ethier et al., 2017) or
568 rotational fallows (Kuussaari et al., 2011; Toivonen et al., 2013), these plant species are likely
569 to be useful candidates for designing agri-environmental schemes (Ma and Herzon, 2014) and
570 sustaining related ecosystem functions (Serra et al., 2016) in agriculturally-intensive
571 landscapes under a wide range of agricultural pollution conditions. However, the effects of
572 agricultural contaminants on plant growth and development through indirect effects on plant-
573 plant competition or plant-microbe associations should also be taken into account (Damgaard
574 et al., 2014; Helander et al., 2018; Van Bruggen et al., 2018).

575 *Fagopyrum esculentum*, *Lotus corniculatus*, *Phleum pratense*, and *Trifolium pratense* have
576 often been used for designing VFS (Krutz et al., 2015; Serra et al., 2016; Stehle et al., 2011).
577 However, their intermediary sensitivity and tolerance to xenobiotics and heavy metals
578 indicated that their establishment and growth in agricultural landscapes, especially as part of
579 pesticide-exposed VFS, were likely to be strongly influenced by pollution and environment
580 conditions. Shoot growth of *Lotus corniculatus* was highly sensitive to glyphosate,
581 hydroxyatrazine, tebuconazole and Zn, although these compounds have very distinct
582 mechanisms of action on plants (Richard et al., 2011; Rouached, 2013; Serra et al., 2013).
583 Root growth of *Fagopyrum esculentum* was significantly affected by glyphosate, which is
584 intensively applied (Silva et al., 2018, 2019), thus suggesting that the phytostabilization role
585 of *Fagopyrum esculentum* in VFS may be limited under conditions of agricultural
586 pollution. Establishment and growth of Asteraceae *Anthemis tinctoria* and *Centaurea cyanus*,
587 which were the most sensitive plant species, were likely to be impaired under a wide range of

588 agricultural pollution conditions. *Anthemis tinctoria* and *Centaurea cyanus*, which show a
589 high degree of plant-insect interactions, are important components of wild flower seed
590 mixtures (Ditner et al., 2013; Stehlik et al., 2007; Toivonen et al., 2013; Warzecha et al.,
591 2018) for field margin sowing, intersowing or VFS in order to increase pollinator biodiversity
592 and regulate predator dynamics. Their sensitivity to all or most xenobiotics and heavy metals
593 may hamper such ecological engineering in agriculturally-intensive landscapes. Conversely,
594 plant species with greater sensitivity to xenobiotics and heavy metals may be useful
595 bioindicators of soil pollution in agroecosystems. Impairment of *Lotus corniculatus*, *Anthemis*
596 *tinctoria* and *Centaurea cyanus* establishment and growth is thus likely to reflect
597 contaminating levels of glyphosate and tebuconazole. Given the complex interactions
598 between the multiple effects of diverse pesticides, bioindication of residual contaminating
599 levels in soils may be useful to prevent crop injury or weed control failure due to excessive
600 pesticide treatment of crops grown on pesticide-contaminated soil (Alberto et al., 2016).
601 Moreover, the general sensitivity of *Anthemis tinctoria* and *Centaurea cyanus* to xenobiotics
602 and heavy metals entails that their cultivation as specialty crops should be carried out in a
603 clean environment requiring at least a restricted use of pesticide treatments.

604

605 **Conflict of interest**

606 The authors do not have any commercial or financial conflict of interest regarding the present
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608

609

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912 **Legends of Figures**

913 **Figure 1.** Effects of root-level exposure to glyphosate on primary root growth of *Arabidopsis*
914 *thaliana* plantlets.

915 **Figure 2.** Effects of root-level exposure to agricultural xenobiotics and heavy metals on main
916 root growth of agri-environmental plant species. Responses of each plant species to the range
917 of xenobiotics and heavy metals are expressed in values (mean \pm SEM) of percentage of
918 inhibition $[(\text{Growth}_{\text{control}} - \text{Growth}_{\text{treatment}})/\text{Growth}_{\text{control}}]$. Negative values therefore reflect
919 growth increase under treatment conditions. Statistical significance of differences ($P \leq 0.05$)
920 between treatment and control is shown by asterisks above bars. The statistical significance of
921 plant-plant differences of responses to a given xenobiotic or heavy metal treatment is given in
922 supplementary data 1. The Figure in colour is available online.

923 **Figure 3.** Effects of root-level exposure to agricultural xenobiotics and heavy metals on shoot
924 growth of agri-environmental plant species. Responses of each plant species to the range of
925 xenobiotics and heavy metals are expressed in values (mean \pm SEM) of percentage of
926 inhibition $[(\text{Growth}_{\text{control}} - \text{Growth}_{\text{treatment}})/\text{Growth}_{\text{control}}]$. Negative values therefore reflect
927 growth increase under treatment conditions. Statistical significance of differences ($P \leq 0.05$)
928 between treatment and control is shown by asterisks above bars. The statistical significance of
929 plant-plant differences of responses to a given xenobiotic or heavy metal treatment is given in
930 supplementary data 1. The Figure in colour is available online.

931 **Figure 4.** Principal component analysis and hierarchical clustering of species-specific plant
932 responses to agricultural xenobiotics and heavy metals. PCA was carried out on the
933 correlation matrix of growth response parameters (F: foliar growth; R: root growth) under the
934 various conditions of treatment. Growth responses to agricultural xenobiotics and heavy
935 metals were analysed in terms of percentage of inhibition (Fig. 2, Fig. 3). Plant species under
936 study are described by their generic name. (A) Distribution of plant species, (B) Hierarchical

937 classification of plant species, (C) Position of the different growth response parameters in the
938 presence of xenobiotics and heavy metals on the first plane (Dim1 and Dim2). The Figure in
939 colour is available online.

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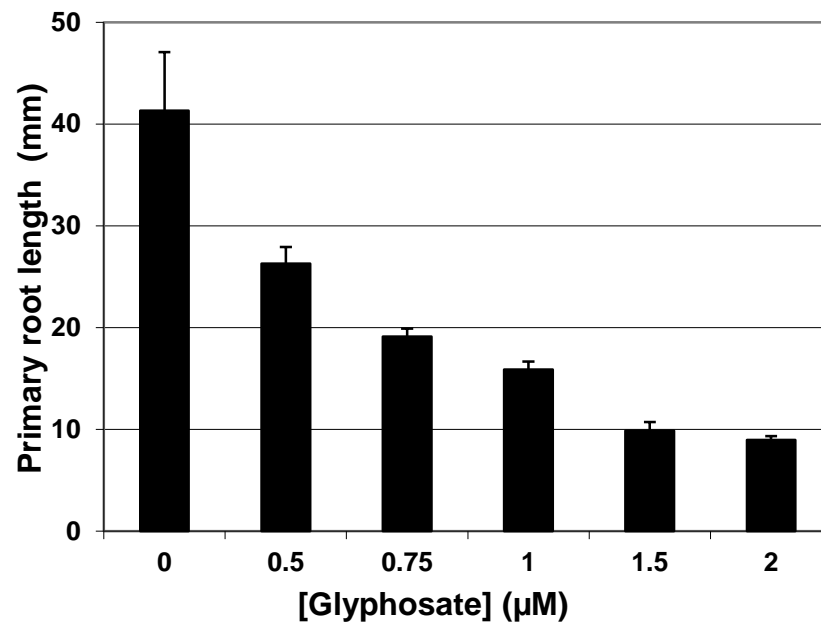


Figure 1

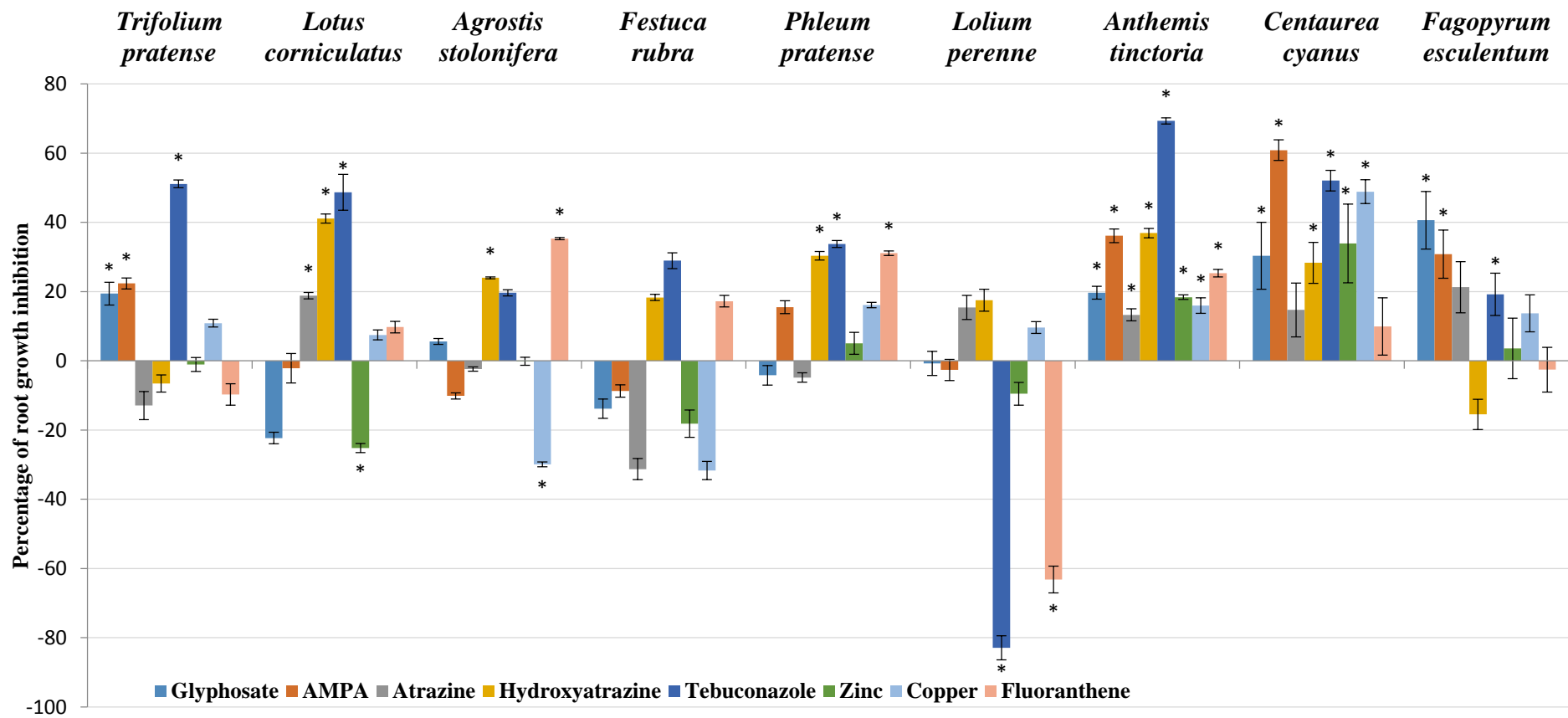


Figure 2

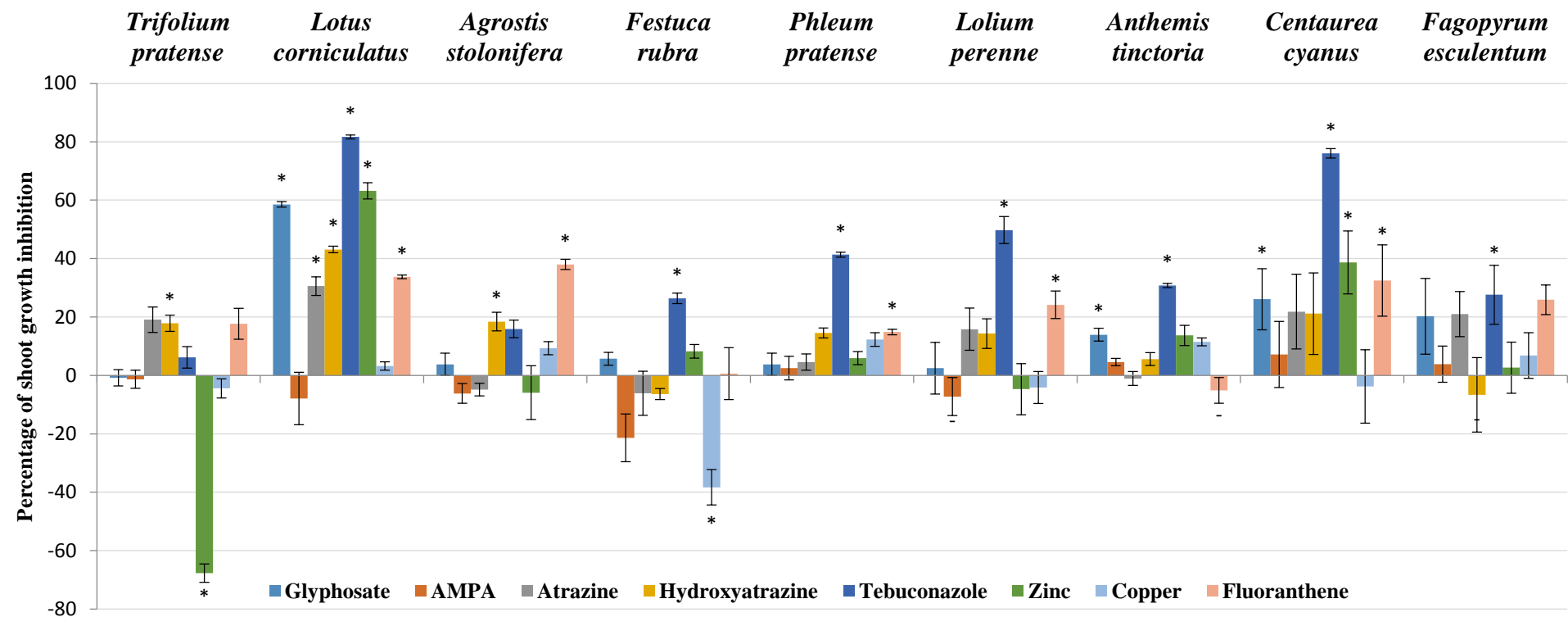


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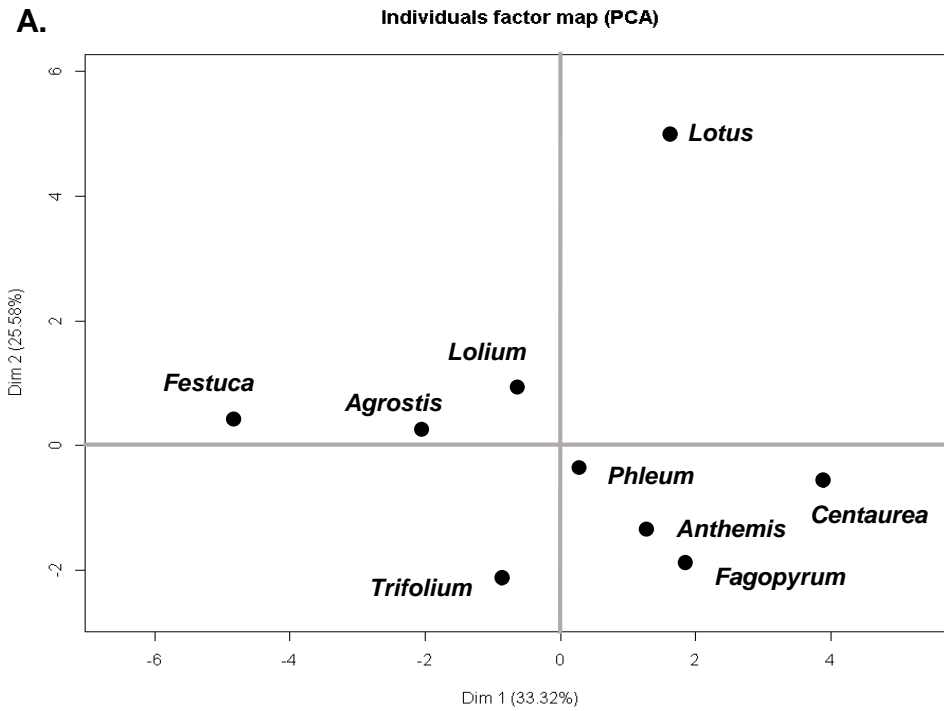


Figure 4

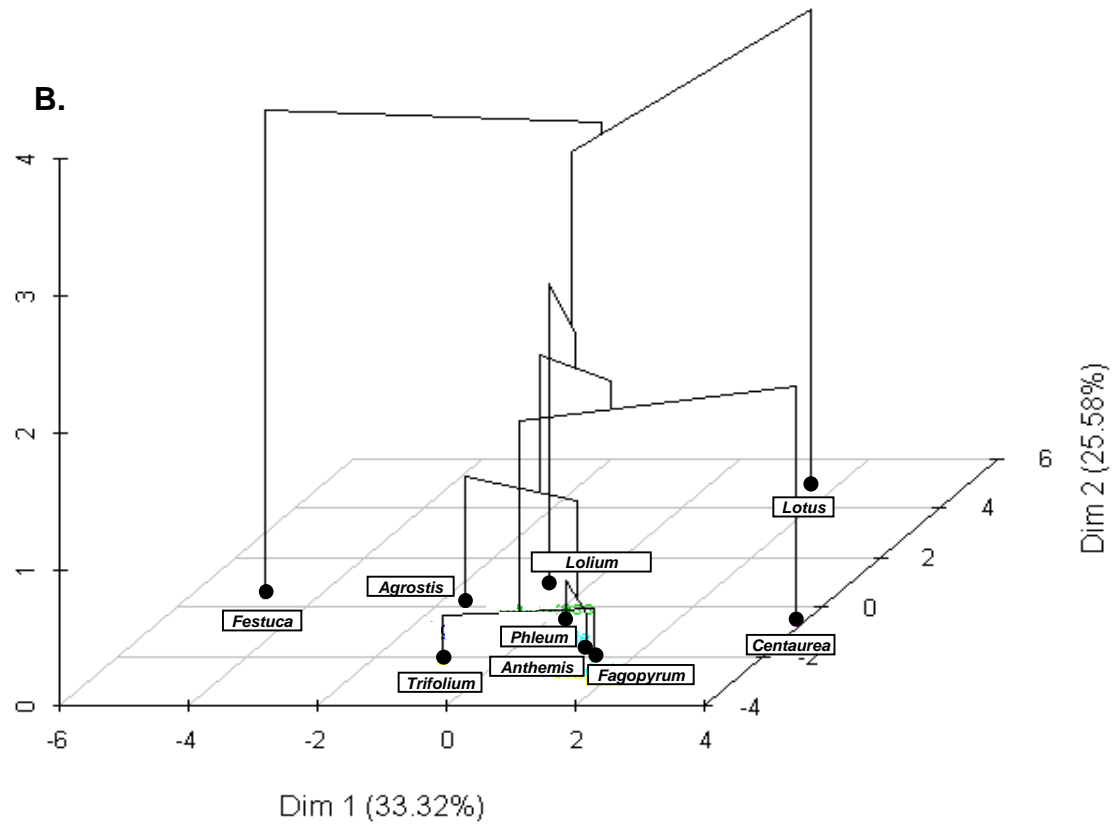


Figure 4

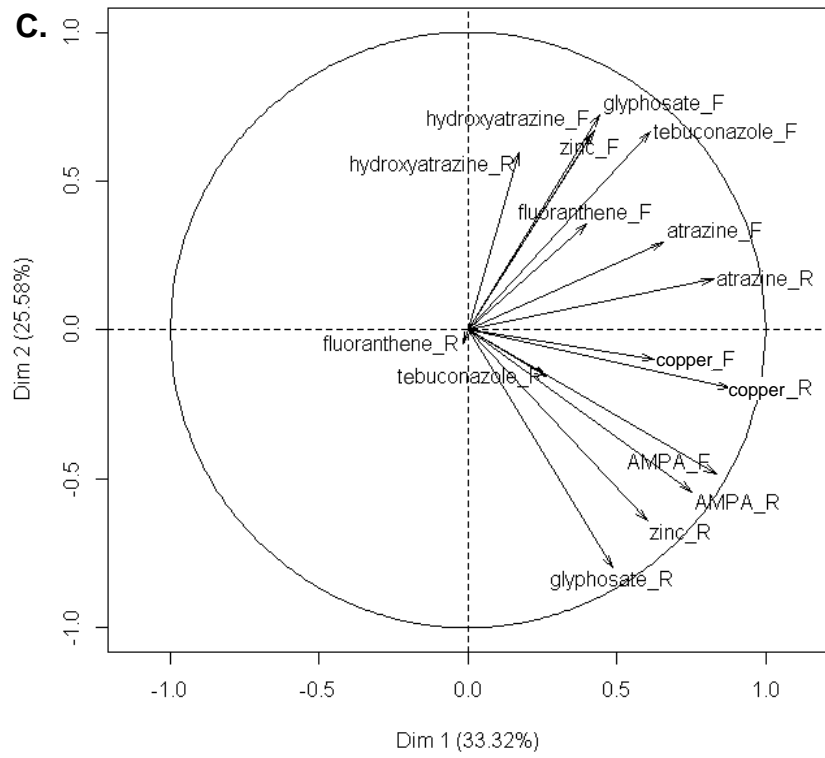


Figure 4

1 **Table 1.** Median effective concentrations (EC50) of the impact of agricultural xenobiotics
2 and heavy metals on primary root growth in *Arabidopsis thaliana* seedlings. Concentrations
3 used in subsequent experiments are given.

4	5	6	7	
Agricultural contaminant	EC50 (μM)	Experimental Concentration (μM)	Experimental Concentration (mg L^{-1})	
8				
9	Glyphosate	0.75	1	0.169
10				
11	AMPA	30	50	5.550
12				
13	Atrazine	0.5	0.5	0.1
14				
15	Hydroxyatrazine	25	25	5
16				
17	Tebuconazole	4	4	1.28
18				
19	Zn	15	15	0.98
20				
21	Cu	6	6	0.38
22				
23	Fluoranthene	532	532	107.5

24
25
26
27

28 **Table 2.** Differential tolerance and sensitivity of agri-environmental plant species towards
 29 agricultural xenobiotics and heavy metals. Agri-environmental plant species are ranked
 30 according to the numbers of tolerance (first number) and enhancement (second number)
 31 interactions relatively to the 8 xenobiotic (glyphosate, AMPA, atrazine, hydroxyatrazine,
 32 tebuconazole, fluoranthene) and heavy metal (Cu, Zn) treatments described in Fig. 2 and Fig.
 33 3.

34	35	36	37
Plant species	Root growth Tolerance + enhancement	Shoot growth Tolerance + enhancement	
38			
39	<i>Festuca rubra</i>	(8+0)/8	(6+1)/8
40			
41	<i>Lolium perenne</i>	(6+2)/8	(6+0)/8
42			
43	<i>Agrostis stolonifera</i>	(5+1)/8	(6+0)/8
44			
45	<i>Trifolium pratense</i>	(5+0)/8	(6+1)/8
46			
47	<i>Fagopyrum esculentum</i>	(5+0)/8	(7+0)/8
48			
49	<i>Phleum pratense</i>	(5+0)/8	(6+0)/8
50			
51	<i>Lotus corniculatus</i>	(4+1)/8	(2+0)/8
52			
53	<i>Centaurea cyanus</i>	(2+0)/8	(4+0)/8
54			
55	<i>Anthemis tinctoria</i>	(0+0)/8	(6+0)/8
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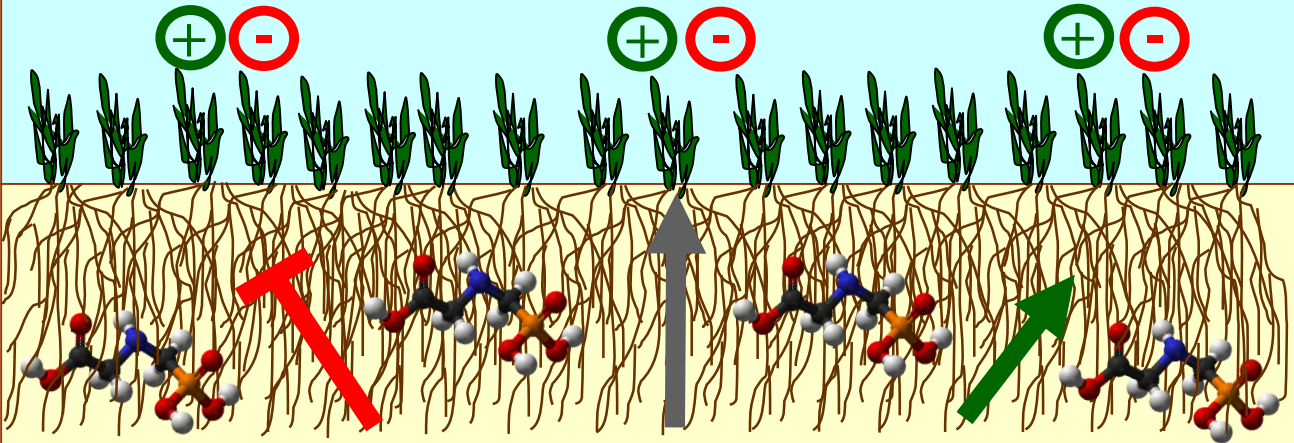
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BIOLOGICAL AND CHEMICAL DIVERSITY OF ROOT-SHOOT IMPACTS



ROOT EXPOSURES TO AGRICULTURAL POLLUTIONS