

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

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1 <b>Title :</b>
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- 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 chemical applications. Exposure to xenobiotics and heavy metals causes biochemical, 31 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

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

## 52 Keywords :

- 53 Ecotoxicity, Field margin plants, Heavy metals, Persistent organic pollutants, Pesticides, Soil
- 54 pollution
- 55
- 56

### 57 **Highlights**:

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

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#### 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 result in yearly accumulations of 34-60 µg L<sup>-1</sup> by dispersion over a global Earth surface of 73  $510 \times 10^6$  km<sup>2</sup>. On a regional scale, average application rates of pesticides per hectare of arable 74 land can attain 6.5-60 kg ha<sup>-1</sup> in Asia and South America (Carvalho, 2017), which may result 75 76 in very high levels of expected environmental yearly accumulations of  $4,333-40,000 \ \mu g \ L^{-1}$ . 77 Actual measurements in pesticide-contaminated soils show that residual persistence of 78 pesticides (Alberto et al., 2017; Arias-Estévez et al., 2008; Jablonowsksi 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 et al., 2017) yields a glyphosate concentration of 2,600  $\mu$ g L<sup>-1</sup>. This theoretical value is lower 82 83 than the average measurement of 6,433  $\mu$ g.kg<sup>-1</sup> 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; Padgette 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).

However, the range of soil pollution effects on plants is difficult to apprehend and remains to be fully understood (Rodríguez-Eugenio et al., 2018). Agri-environmental plant communities in field margins, vegetative filter strips (VFS) or rotational fallows are necessarily confronted with agricultural pollutants through residual soil contamination and/or through drift, run-off 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 agricultural fields, the confrontation of plant communities with pesticides and other 116 117 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 landscapes (Billeter et al., 2008; Liira et al., 2008; Kuussaari et al., 2011; Stehle et al., 2011; 139

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 responses of growth stimulation, no adverse effect or growth inhibition, depending on plant 148 149 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* 

Seeds of *Arabidopsis thaliana* (Columbia, Col-0) were obtained from the Nottingham
Arabidopsis Stock Center, and propagated under laboratory-controlled conditions. Seeds of *Agrostis stolonifera* (cv. Penncross), *Anthemis tinctoria* (bulk seeds), *Centaurea cyanus* (bulk
seeds), *Fagopyrum esculentum* (bulk seeds), *Festuca rubra* (cv. Herald), *Lolium perenne* (cv.
Brio), *Lotus corniculatus* (cv. Leo), *Phleum pratense* (cv. Kaba), and *Trifolium pratense* (cv.
Violetta) were obtained from the Phytosem (Gap, Hautes-Alpes, France) seed company.
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 µmol m<sup>-2</sup> 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).

Seeds of Agrostis stolonifera, Anthemis tinctoria, Centaurea cyanus, Fagopyrum esculentum, 172 173 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 (glyphosate, AMPA, atrazine, hydroxyatrazine, tebuconazole) and heavy metals ( $Cu^{2+}$  added 182 as CuSO<sub>4</sub>, Zn<sup>2+</sup> added as ZnSO<sub>4</sub>) were axenically added to cooled-down melted agar-183 184 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

of *Centaurea cyanus* did not undergo any treatment of dormancy break. Sown Petri dishes or
Falcon tubes were then transferred to a control growth chamber at 22 °C/20 °C under a 16 h
light (100 µmol m<sup>-2</sup> s<sup>-1</sup>)/8 h dark regime. Analysis of physiological and growth parameters
was carried out after 7 (*Agrostis stolonifera*, *Anthemis tinctoria*, *Festuca rubra*, *Lotus corniculatus*, *Phleum pratense*, *Trifolium pratense*), 10 (*Fagopyrum esculentum*, *Lolium perenne*), 12 (*Centaurea cyanus*) or 14 (*Arabidopsis thaliana*) days of growth in the absence
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 [(Growth<sub>control</sub> – Growth<sub>treatment</sub>)/Growth<sub>control</sub>] 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

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

#### 214 2.5. Data analysis

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

**3. Results** 

# 3.1. Characterization of sublethal effects of root-level agricultural contaminants on the model 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 essential to reflect a realistic evaluation of comparative chemical stress sensitivity, as 231 232 advocated by Nuttens and Gross (2017).

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

inhibition curves. EC50 values (Table 1) were estimated graphically from these hyperbolic or
sigmoid curves of inhibition. The EC50 values for glyphosate, AMPA, atrazine, and
hydroxyatrazine agreed with previous analyses under similar modalities of exposure (Serra et
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 significant effect on primary root growth (Alkio et al., 2005), with an EC50 value (532  $\mu$ M) 255 256 that was within the range of inhibitory PAH concentrations (Alkio et al., 2005).

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

3.2. Effects of sublethal levels of root-level agricultural contaminants on root growth of agri 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, Table 1), except in the case of glyphosate and AMPA, which were applied at concentrations 272 273 of respectively 1  $\mu$ M (instead of EC50 = 0.75  $\mu$ M) and 50  $\mu$ M (instead of EC50 = 30  $\mu$ M).

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).

Tebuconazole and fluoranthene induced the highest level of responses, with highly positive effects on root growth of *Lolium perenne* and highly negative effects on root growth of *Anthemis tinctoria*, relatively to all or most of the other plant species (supplementary data 1). Zn gave the lowest level of responses, with NOAE interactions in *Trifolium pratense*, Agrostis stolonifera, Festuca rubra, Phleum pratense, Lolium perenne and Fagopyrum esculentum. On the other hand, species-specific behaviours were significantly contrasted. Root growth of yellow chamomile (*Anthemis tinctoria*) and cornflower (*Centaurea cyanus*) was negatively affected by all or most of xenobiotic or heavy metal treatments. Root growth of bird's-foot trefoil (*Lotus corniculatus*), creeping bentgrass (*Agrostis stolonifera*), English ryegrass (*Lolium perenne*) and red fescue (*Festuca rubra*) showed contrasted chemicalspecific 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 in the response to root-level exposure. 305

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 effets 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 compounds or root-shoot crosstalk of root-level exposure. 330

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

The comparison of root and shoot responses (Table 2) highlighted strikingly different behaviours of the nine agri-environmental plant species towards to the different xenobiotics and heavy metals, with cases of general tolerance (*Festuca rubra, Lolium perenne*) and cases of general sensitivity (*Lotus corniculatus, Centaurea cyanus, Anthemis tinctoria*). Analysis of the effects of xenobiotics and heavy metals on the levels of carotenoids and chlorophylls did 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 pollutantsensitive species, *Lotus corniculatus*, showed a 30% (± 5, SEM) decrease of carotenoid levels 344 345 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),

yellow chamomile (*Anthemis tinctoria*), white Dutch clover (*Trifolium pratense*) and common
 buckwheat (*Fagopyrum esculentum*), (iii) cornflower (*Centaurea cyanus*), (iv) bird's-foot
 trefoil (*Lotus corniculatus*). Cornflower (*Centaurea cyanus*) and bird's-foot trefoil (*Lotus 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 glyphosate, AMPA or hydroxyatrazine (Fig. 4C), thus highlighting the potentially important 372 373 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 (Padgette et al., 1991), as described in subsection 3.1. 380

#### **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  $\mu$ 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; Padgette et al., 1991; Sammons et al., 2018). 393 The present EC50 value for atrazine  $(0.5 \,\mu\text{M})$  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 (± 2.6) µM IC50 value reported for inhibition of purified 5-399 enolpyruvylshikimate-3-phosphate synthase (EPSPS) respectively from different plant species 400 (Padgette 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).

Moreover, the effects of tebuconazole, Zn, Cu and fluoranthene on root growth involved both inhibition and activation rather than mere inhibition as in the case of Arabidopsis, thus highlighting major species-specific differences of mechanisms and responses. However, all of these conditions affected root growth of at least two of the nine plant species under study, with wide-ranging impacts for tebuconazole and hydroxyatrazine and narrow impacts for atrazine, Zn and Cu. Moreover, glyphosate, atrazine, hydroxyatrazine, tebuconazole, Zn and 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 in415 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, hydroxyatrazine, tebuconazole), heavy metals (Cu, Zn) and polycyclic aromatic hydrocarbons 420 421 (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 section 1. The concentration of atrazine (100  $\mu$ g L<sup>-1</sup>) corresponded to environmental 424 concentrations commonly found in soils, waters and sediments (Alberto et al., 2017; 425 Jablonowski et al., 2010). The concentration of glyphosate (169  $\mu$ g L<sup>-1</sup>) was lower than 426 427 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  $[2,229 (\pm 476)]$ µg.kg<sup>-1</sup>] (Primost et al., 2017) and than the lowest experimental concentrations used by 432 Orcaray et al. (2010) (53 mg L<sup>-1</sup>) or Saunders et al. (2013) (10 mg kg<sup>-1</sup>). The concentration of 433 434 fluoranthene was also lower than observed PAH contaminating levels in the environment (Rodríguez-Eugenio et al., 2018). The concentration of AMPA (5,550 µg L<sup>-1</sup>) corresponded to 435 436 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 an average concentration of 4,204 ( $\pm$  2,258) µg.kg<sup>-1</sup>. The concentration of tebuconazole 438

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). In contrast, the concentration of hydroxyatrazine  $(5,000 \ \mu g \ L^{-1})$  was significantly higher than 442 currently-described (up to 640 µg.kg<sup>-1</sup>) environmental values (Alberto et al., 2017; 443 444 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.,

Such an impact of chemically-distinct compounds, with established bioactivity or supposed inactivation, and such a diversity of sensitivities emphasise the need to expand the range of pesticide tests in order to improve predictive power on the environmental impacts of pesticides (Milner and Boyd, 2017). Moreover, cumulative exposures can result in growth disruption that is not reducible to the sum of individual effects because of potential synergistic or antagonistic interactions between contaminants (Alberto et al., 2017). There are therefore
 environmental situations where the combined effects of mixtures of xenobiotics and/or heavy
 metals on plant growth and development may be more drastic than the predicted effects
 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

The present results under controlled conditions emphasised the direct action of chemical 470 471 stressors on plant tissues. The effects of chemical stressors have been experimentally characterized in a limited number of plant species, and their targets and mechanisms of action 472 473 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 chloroplast-localized canonical sites of action, such as glyphosate and atrazine, have 475 476 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 observed effects on the canonical targets of parent molecules, thus implying alternative 481 482 mechanisms of action (Alberto et al., 2017, 2018; Gomes et al., 2016; Serra et al., 2013, 483 2015).

The present comparative study confirmed the importance of noncanonical patterns of responses to glyphosate and atrazine. The significant effects on root growth in the absence of effects on shoot growth in several plant species (*Trifolium pratense*, *Anthemis tinctoria*, *Fagopyrum esculentum*) could be related to the root-specific effects that have been discovered 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 on Lotus corniculatus, Anthemis tinctoria and Centaurea cyanus emphasised the potentially 522 523 high toxicity of tebuconazole and Zn for natural plant communities.

In spite of the diversity of modes of action, all of the chemical stressors were found to induce, 524 525 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 interactions has revealed a significant impact of xenobiotics on carbon and nitrogen 530 531 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 CO<sub>2</sub> 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 studies have shown differences of responses to abiotic stresses within cultivars, for instance in 554 555 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.

The three most tolerant species (*Festuca rubra*, *Lolium perenne*, *Agrostis stolonifera*) were Poaceae that develop deep rooting systems (Roumet et al., 2008) and are used for phytostabilization and pollutant buffering of VFS and for phytoremediation of polluted soils (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, which were the most sensitive plant species, were likely to be impaired under a wide range of 587

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 pesticide treatment of crops grown on pesticide-contaminated soil (Alberto et al., 2016). 600 601 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.

605	<b>Conflict of interest</b>
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606 The authors do not have any commercial or financial conflict of interest regarding the present

article.

608

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912 Legends of Figures

Figure 1. Effects of root-level exposure to glyphosate on primary root growth of *Arabidopsis 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 [(Growth<sub>control</sub> – Growth<sub>treatment</sub>)/Growth<sub>control</sub>]. 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 [(Growth<sub>control</sub> – Growth<sub>treatment</sub>)/Growth<sub>control</sub>]. Negative values therefore reflect 927 growth increase under treatment conditions. Statistical significance of differences ( $P \leq 0.05$ ) between treatment and control is shown by asterisks above bars. The statistical significance of 928 929 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.

**Figure 4.** Principal component analysis and hierarchical clustering of species-specific plant responses to agricultural xenobiotics and heavy metals. PCA was carried out on the correlation matrix of growth response parameters (F: foliar growth; R: root growth) under the various conditions of treatment. Growth responses to agricultural xenobiotics and heavy metals were analysed in terms of percentage of inhibition (Fig. 2, Fig. 3). Plant species under 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.



Figure 1







Figure 4



Figure 4



Figure 4

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

4 5 6 7	Agricultural contaminant	EC50 (µM)	Experimental Concentration (µM)	Experimental Concentration (mg L <sup>-1</sup> )
8		0.75	1	0.160
9 10	Glyphosate	0.75	1	0.169
10 11 12	AMPA	30	50	5.550
12	Atrazine	0.5	0.5	0.1
14 15	Hydroxyatrazine	25	25	5
16 17	Tebuconazole	4	4	1.28
18 19 20	Zn	15	15	0.98
20 21 22	Cu	6	6	0.38
22 23 24	Fluoranthene	532	532	107.5
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Table 2. Differential tolerance and sensitivity of agri-environmental plant species towards agricultural xenobiotics and heavy metals. Agri-environmental plant species are ranked according to the numbers of tolerance (first number) and enhancement (second number) interactions relatively to the 8 xenobiotic (glyphosate, AMPA, atrazine, hydroxyatrazine, tebuconazole, fluoranthene) and heavy metal (Cu, Zn) treatments described in Fig. 2 and Fig.

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3.

Plant species	Root growth Tolerance + enhancement	Shoot growth Tolerance + enhancement
Festuca rubra	(8+0)/8	(6+1)/8
Lolium perenne	(6+2)/8	(6+0)/8
Agrostis stolonifera	(5+1)/8	(6+0)/8
Trifolium pratense	(5+0)/8	(6+1)/8
Fagopyrum esculentum	(5+0)/8	(7+0)/8
Phleum pratense	(5+0)/8	(6+0)/8
Lotus corniculatus	(4+1)/8	(2+0)/8 (4+0)/8
Centaurea cyanus	(2+0)/8	
Anthemis tinctoria	(0+0)/8	(6+0)/8

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