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## Contrasting response of nutrient acquisition traits in wheat grown on bisphenol A 1 contaminated soils

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### ► To cite this version:

A. Yacoumas, Nicolas Honvault, David Houben, H. Meglouli, Benoît Tisserant, et al.. Contrasting response of nutrient acquisition traits in wheat grown on bisphenol A 1 contaminated soils. *Water, Air, and Soil Pollution*, 2020, 231 (1), pp.23. 10.1007/s11270-019-4383-7. hal-02898111

**HAL Id: hal-02898111**

**<https://hal.science/hal-02898111>**

Submitted on 13 Jul 2020

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24 soil contamination using wheat (*Triticum aestivum*) as plant model. After 7 weeks, root and  
25 leaf traits were not markedly affected by the exposure to BPA at low and high concentrations  
26 (0.1 and 1000 mg. kg<sup>-1</sup> soil). Significant change on average root diameter and aerial biomass  
27 were only observed at the highest dose. BPA contamination had no influence on nutrients  
28 acquisition traits and root associated microbiota. Specific root length, carboxylates exudation  
29 and P, Ca, K concentrations in leafs were similar irrespective of the treatments. In addition,  
30 total microbial biomass, bacteria and fungi abundance measured by phospholipid fatty acid  
31 analysis did not differ among controls and contaminated soils. In summary, this experiment  
32 suggests a limited influence of BPA contaminated soils on traits involved in nutrient  
33 acquisition in wheat.

34

35 Keywords : bisphenol A, functional traits, nutrient acquisition, root exudation, soil  
36 microbiota, wheat

37

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40

## 41 INTRODUCTION

42

43 In recent years, the cumulative use of numerous chemical substances in agriculture, industry,  
44 household and cosmetic products has led to the build-up of emerging contaminants in soils,  
45 surface water , and groundwater with unpredictable consequences for the ecosystems. In  
46 Europe, contaminants of emerging concern refer to natural or synthetic chemicals recently  
47 identified or detected with increased concentration in soils and water which are not framed by

48 legislative regulation for environmental monitoring programs (Farré et al. 2008). Emerging  
49 contaminants include pharmaceuticals, cosmetics, personal care products, nutrients, food  
50 additives, pesticides and manufacturing materials for which limited knowledge is available on  
51 their environmental fate and impacts on ecosystems (Naidu et al. 2016).

52 Bisphenol A [2,2-bis(4-hydroxyphenyl)propane ; BPA] is a typical emerging contaminant  
53 with biological activities posing irreversible adverse effects in wildlife and humans. BPA has  
54 been extensively used since the 1940s for the formulation of plastics, thermal sensitive  
55 papers or raw materials intended to sport, electronic, automotive and medical equipment  
56 (Michałowicz 2014). BPA is one of the world's highest production volume chemicals with a  
57 worldwide consumption and production exceeding 6 million tons per year (Owczarek et al.  
58 2018). This chemical does not belong to the class of persistent organic pollutant, possesses  
59 short elimination half-lives under aerobic conditions and is easily removed from the  
60 contaminated matrices (Kang et al. 2006). However, BPA is recognized as a major  
61 environmental pollutant and a serious concern for ecosystems protection and human health.

62 BPA is widespread in oceans, rivers, sediments and soils because of its large-scale  
63 employment, the continuous emissions with effluents and the decomposition of plastic wastes  
64 (Im and Löffler 2016; Teuten et al. 2009). Occurrence of BPA residues have been evidenced  
65 in soils at low concentrations and reported at highest levels up to 150 µg/kg on cultivated soils  
66 (Careghini et al. 2015). In addition, agricultural soils, frequently irrigated and fertilized with  
67 treated sewage water and sludge respectively, can accumulate and transfer BPA to vegetation  
68 and food chains and ultimately expose vulnerable populations to this reprotoxic agent with  
69 endocrine disrupting properties (Firmin et al. 2016; Mercogliano and Santonicola 2018;  
70 Staples et al. 2010). Associations between BPA exposure and foetal development, immunity,  
71 metabolism, reproductive functions or its relation with chronic diseases have been mostly

72 explored last decades (Bókony et al. 2018; Flint et al. 2012; Jörg et al. 2009; Shafei et al.  
73 2018).

74 Toxic effects of BPA have been more recently observed on terrestrial plants such as broad  
75 bean, carrot, wheat, lettuce, tomato, soybean and *Arabidopsis Thaliana* (Ferrara et al. 2006;  
76 Jiao et al. 2017; Li et al. 2018; Pan et al. 2013; Sun et al. 2013). In these studies generally  
77 mainly carried out in hydroponic conditions, BPA inhibited seed germination, growth and  
78 reproductive development by interfering with mechanisms regulating nitrogen assimilation,  
79 photosynthesis, anti-oxidative defenses or hormone secretion. In some such experiment, plant  
80 roots exhibited characteristics of abnormal development with reduced growth and lower  
81 energy metabolism under exposure to BPA suggesting that the global pollution of agricultural  
82 soils may dramatically alter the mineral nutrition of food crop culture and threaten human  
83 food safety (Xia et al. 2017).

84 The effects of BPA on plant nutrition cannot be exclusively approached by hydroponic  
85 experiments (Neumann and George 2009). Indeed, soil properties can mediate BPA mobility  
86 and its effect on plant growth (Fent et al. 2003). Moreover, plants have evolved sophisticated  
87 strategies that manifest as functional traits ameliorating nutrient acquisition to cope with  
88 constraining spatial variability and poorly available elements in soil (Faucon et al. 2017).  
89 Traits involved in nutrient acquisition include morphological, physiological and biotic root  
90 traits, i.e. specific root length, exudation of protons and various metabolites as carboxylates  
91 (Li et al. 2014) and beneficial associations with soil microbial communities which promotes  
92 nutrient availability (Campos et al. 2018; Chen and Liao 2017). However, few data are  
93 available about BPA pollution effects on the fate of nutrients in the soil-plant system.

94 Given the importance of the mineral nutrition in the productivity and sustainability of  
95 agroecosystems, challenge is to understand the influence of BPA on plant functional traits  
96 involved in the nutrient acquisition. For this purpose, the stress response of wheat, an

97 economic global cultivated crop model was tested in soils artificially contaminated with BPA.  
98 To determine whether BPA concentration gradients could drive changes of specific above-  
99 and belowground plant traits related to the uptake of nutrients, we examined plant biomass,  
100 morphological root traits, root exudation of major carboxylates involved both in stress  
101 response and in nutrient mobilization, essential nutrient contents in leaves, and soil microbial  
102 biomass using PhosphoLipid Fatty Acids (PLFA) analysis techniques.

### 103 MATERIALS AND METHODS

#### 104 Soil properties and contamination

105 The soil used in the present study was collected in the 10-20 cm depth horizon of cultivated  
106 soil (wheat crop) in the farm of UniLaSalle Institute in Beauvais, France (N 49°28'13.88", E  
107 2°4'0.45") and was classified as a Haplic luvisol (IUSS Working Group WRB, 2015). Soil  
108 properties are presented in Table 1. The soil was sieved through a 5mm stainless mesh and  
109 mixed with river sand (22% ; v:v). Then a sub-sample of soil was spiked with 50 ml solution  
110 of bisphenol A dissolved in acetone. After evaporating acetone overnight at room  
111 temperature, the spiked soil was homogenized and mixed with unspiked soil using a roller  
112 mixer to reach concentrations of 0.1 and 1000 mg BPA . kg<sup>-1</sup> dry soil. A solvent control soil  
113 was prepared with BPA free acetone solution using similar procedure. The highest  
114 concentration was selected to improve knowledge of the BPA toxic effects on wheat model at  
115 a comparable scale level known to provoke marked phytotoxicity for other plants (Kim et al.  
116 2018; Nie et al. 2015). However, toxicological data obtained at high BPA doses which are  
117 valuable in regulatory hazard and risk assessment purpose, cannot be considered as  
118 representative of the actual environmental situation. A lower dose was also tested, which was  
119 based on the average values found in surveys, allowing us to elucidate relevance of BPA  
120 contamination in agricultural field (Tran et al. 2015).

121 Greenhouse experiment and plant cultivation

122 A greenhouse experiment was conducted from April to May 2018. The plant growth was  
123 conducted in a pot experiment using polypropylene pots filled with 4L of uncontaminated soil  
124 (control), solvent control soil (acetone) or soil contaminated with BPA at 0.1 and 1000 mg.kg<sup>-1</sup>  
125 dry soil. All treatments were performed in four replicates. Pots were sown with 10 wheat  
126 seeds (*Triticum aestivum* cultivar pakito) and randomly positioned in a greenhouse with  
127 controlled conditions (photoperiod 16h light/8 h dark, light intensity of 10 Watts.m<sup>-2</sup>,  
128 day/night temperature of 20 °C). Plants were watered three times a week with tap water. Half  
129 of seedlings were removed after one week of exposure. Other seedlings (5 per pot) were  
130 grown and harvested after 6 additional weeks. At the harvest, roots were gently rinsed with  
131 tap water to remove adhering soil and cleaned with adsorbing paper. After collecting root  
132 exudates, root systems were scanned. Roots and shoots were then dried at 65°C for 48h for  
133 the determination of biomass and nutrient concentration. Rhizospheric soil (i.e. only the  
134 adhering soil to 2 mm on roots that is removed by subsequent brushing after bulk soil falling  
135 off when shaking the root system) was sampled for PLFA analysis. Bulk soil sampling was  
136 performed at the beginning and the end of the experiment for BPA analysis.

137 Plant functional traits

138 Measurement of plant functional traits representative of plant nutrient acquisition were made  
139 after 7 weeks of cultivation. Three types of functional traits (morphological, physiological,  
140 and biotic characteristics) were assessed on roots, leaves and rhizospheric soil.

141 Root exudates were collected by incubating 15mL CaCl<sub>2</sub> solution (0.2mM) for 1h.  
142 Then, 2mL exudate solution was centrifuged (2 min, 10000 g) and 1.5mL supernatant solution  
143 was filtered through a sterile millipore 0.22 µm filter. Fifty microliters of filtrate was injected  
144 into an HPLC system (Thermo Fisher, San Jose, CA, USA) equipped with an auto-injector, a

145 degasser, a diode array detector. Carboxylates were separated using the method described by  
146 Nobile et al. (2019) on a reverse phase Luna 5 $\mu$ m C18 column 250mm x 4 mm (Phenomenex,  
147 Le Pecq, France) at 25°C by using an isocratic mobile phase : KH<sub>2</sub>PO<sub>4</sub> 25mM/methanol  
148 (99 :1 ; v:v) buffered at pH 2.40. Flow rate was set at 1 mL.min<sup>-1</sup>. The UV signal was  
149 recorded at 210 nm.

150 Root morphological trait measurements were performed according to Garnier et al.  
151 (2013). Shoots and roots of the plant were separated and weighted. Length, average volume  
152 and diameter of root were analysed using a root scanner and WinRHIZO® software (Regent  
153 Instruments Inc., Québec, Qc, Canada).

154 The concentrations of calcium, potassium and phosphorus in shoot were determined  
155 using elemental mass spectrometry after an acidic digestion in microwave as detailed by  
156 Lange et al. (2016). Briefly, 0.2 g of dry leaf was ground and mixed with 8 mL concentrated  
157 HNO<sub>3</sub> and 2 mL concentrated HCl. The mixture was introduced in a microwave Teflon vessel  
158 and heated to 185 °C for 1h. After cooling, the digested solution was filtered through  
159 Wathman paper, then diluted to 30 g with ultrapure water and injected into the ICP–MS  
160 system (Thermo Scientific XSERIES2). Quality control was performed using the SRM1573a  
161 CRM (tomato leaves).

162 After removing plant debris from soil samples, 3 g of freeze-dried soil (from each soil  
163 sample) were analyzed to measure the PLFA content. Lipid extraction was performed  
164 according to Frostegård et al. (1991) . The extracted lipids were fractionated into neutral  
165 lipids, glycolipids and polar lipids on a silica acid column by successively eluting with  
166 chloroform, acetone and methanol neutral lipids and phospholipids were subjected to a trans-  
167 esterification using a base solution (KOH (0.2 M) prepared in methanol) to transform the  
168 PLFA and the Neutral Lipid Fatty Acids (NLFA) into free fatty acid methyl esters. Final  
169 extracts were analyzed with the use of a PerkinElmer Autosystem gas chromatograph (GC)



170 equipped with a flame-ionization detector (Norwalk, CT) and an EC-1000 (Alltech) capillary  
171 column (30 m  $\times$  0.53 mm i.d.) with hydrogen as carrier gas (3.6 mL min<sup>-1</sup>). Fatty acid  
172 quantification was made by using nonadecanoic acid methyl ester (C19:0) as an internal  
173 standard. Their identification relied on the retention times of a wide range of standards (fatty  
174 acid methyl ester mixtures C4-C24:1, Sigma– Aldrich) and mass analysis. The  
175 characterization of FA was performed by Fast GC–MS on a Shimadzu 2010 Plus system  
176 equipped with a Shimadzu QP 2010 Ultra mass spectrometer detector. The separation was  
177 performed on a Zebron ZB-WAX capillary column (10 m  $\times$  0.1 mm i.d., 0.1 mm film  
178 thickness, Phenomenex). The system was operated in the constant linear velocity (70 cm s<sup>-1</sup>)  
179 using helium as a carrier gas. Fatty acids were identified by comparing their mass spectra with  
180 the standard mass spectra in the NIST MS library. The amounts of the PLFA 16:1v5 and the  
181 NLFA 16:1v5 in the soil of the different experimental plots were determined and used as  
182 indicators of the AMF biomass. The PLFA 18:2v6,9 was used as an indicator of saprophytic  
183 fungi biomass (Frostegård et al. 1991). Gram-positive (GP) bacteria biomass was estimated  
184 by the quantification of the PLFA: i15:0, a15:0, i16:0, i17:0, a17:0 amounts and Gram-  
185 negative (GN) bacteria biomass by the quantification of the PLFA: cy17:0, C18:1v7 and  
186 cy19:0 amounts in the soil.

#### 187 BPA dissipation analysis

188 BPA dissipation in soil was studied according to the HPLC method adapted from Matsumura  
189 et al. (2015). Briefly, each frozen soil sample was dried 4 h at 50°C then 500 mg of the dried  
190 soil was mixed with 600 $\mu$ l of ethylacetate in a 2mL polypropylene centrifuge tube. After 10  
191 min shaking, the mixture was centrifuged (30s, 9000g) and 300 $\mu$ l of the supernatant was  
192 recovered. The extraction step was repeated by three successive washing with 300 $\mu$ l of  
193 ethylacetate. The combined supernatants were dried overnight in the dark at room  
194 temperature. The extracts were reconstituted in 500 $\mu$ l acetonitrile–water (60:40) and 50 $\mu$ l

195 were injected into the HPLC system described above. Separation and quantification of BPA in  
196 the standards and sample extracts was achieved using a mobile phase consisting of  
197 acetonitrile/water/acetic acid (40:59 :1, v/v) with a flow rate of 1 mL min<sup>-1</sup>. The column  
198 temperature and UV signal were set at 25 °C and 230 nm, respectively. The standard  
199 calibration curve exhibited linearity in the range 0.025–100 mg. L<sup>-1</sup> BPA ( $r > 0.998$ ).

200

## 201 5. Statistical analysis

202 Data were analysed by Kruskal-Wallis test and pairwise comparison using IBM SPSS  
203 statistics 25.0 software. Differences between the treatments were declared significant at the *p*-  
204 value of 0.05 ( $p < 0.05$ ).

205

## 206 RESULTS

### 207 Leaf biomass and mineral content

208 Leaves did not share visible symptoms of BPA induced stress over the experimental period.  
209 However, the dry plant weight differed among treatment groups after 7 weeks of cultivation  
210 (figure 1). The dry shoot weight significantly decreased by 40 % at 1000 mg.kg<sup>-1</sup> BPA  
211 compared to the controls while shoot biomass production was not affected at 0.1mg .kg<sup>-1</sup>  
212 BPA. In contrast, soil contamination had no effect on the leaf mineral composition. For all  
213 treatments, leaf P, Ca, K concentrations exhibited similar values (table 3).

214

### 215 Root morphology, biomass and carboxylate exudates

216 Root diameter increased by 38% at 1000 mg.kg<sup>-1</sup> BPA compared to the control (table 2),  
217 while no change was observed in the group that received low BPA dose. For all the  
218 conditions, roots displayed similar values of length, volume, specific root length (SRL) (table  
219 2) and dry weight biomass (figure 1). BPA addition in the soil had no significant effect on  
220 carboxylates exudation pattern (figure 2). The main carboxylates released were a  
221 combination of malate (up to 2.3 nmol.g<sup>-1</sup>), malonate (up to 2.7 nmol.g<sup>-1</sup>) and citrate (up to  
222 1.5 nmol.g<sup>-1</sup>) while fumarate was less abundant (up to 0.4 nmol.g<sup>-1</sup>). Malate, citrate, malonate,  
223 fumarate production were comparable among treatment groups and the sum of carboxylates  
224 accounted for up to 6.6 nmol.g<sup>-1</sup>.

225

#### 226 Soil PLFA analysis

227 The composition and contents of microbial PLFA were similar in rhizosphere of plants grown  
228 in contaminated soil and uncontaminated soil (table 4). Total amount of microbial PLFA  
229 ranged in average from 2.78 to 4.82 µg.g<sup>-1</sup> dry roots. The predominant compounds were GN  
230 bacteria and AM biomasses indicators. GP bacteria, saprotrophic and ectomycorrhizal fungi  
231 PLFA were also detected in soil and represented less than 12 % of the total amount. There  
232 was a tendency for PLFA contents specific to all microbial functional groups investigated and  
233 their cumulative sum to reduce at 1000 mg.kg<sup>-1</sup> BPA ( $P < 0.1$ ). In soil sample treated with 0,1  
234 mg.kg<sup>-1</sup> BPA, the amount of PLFA markers of the bacterial and fungal community were not  
235 significantly different from controls. The ratio of GN:GP bacteria and the ratio of bacterial to  
236 fungi varied from 1.2 to 2.5 and were unaffected by soil treatments.

237

#### 238 BPA soil analysis

239 BPA contamination was not detectable in control and soils spiked at  $0.1 \text{ mg.kg}^{-1}$  with our  
240 analytical method (data not shown). BPA was nearly totally removed from soil spiked at  
241  $1000 \text{ mg.kg}^{-1}$  after 7 weeks of culture as its final concentration decreased by 90% compared  
242 with its initial concentration, indicating an estimated half-life of 15 days (figure 3).

243

## 244 DISCUSSION

245

246 Effects of BPA on plant nutrition were mainly studied using hydroponic approaches so far.  
247 However, in soils, plants behave differently than in hydroponic condition. Optimisation of  
248 resource acquisition via anatomical root changes, exudation and contribution of telluric  
249 microorganisms may reverse the negative effects of BPA stress on nutrient uptake and  
250 vegetative growth and enhance plant tolerance for survival in contaminated soil. Therefore  
251 this study was conducted to explore how these key functional traits involved in nutrient  
252 acquisition respond to BPA contaminated soil and affect plant functioning.

253

254 Effects of BPA addition on root morphological and leaf traits

255 Root functional traits which are highly variable, provide a protective response to contaminants  
256 in soils allowing to highlight their ecotoxicity. This response can be manifested by  
257 morphological and architectural remodelling of the root systems dependent to plant species,  
258 the nature of the contaminant and the exposure conditions (Potters et al. 2009). For wheat,  
259 reduction of the principal roots has been documented in plants exposed to toxic elements or  
260 organic chemicals (Lamhamdi et al. 2011; Qu et al. 2010). In our study, no evidence of BPA  
261 effects was observed on roots functional traits response at environmentally relevant  
262 concentration (figure 1, table 2). In the same way, BPA did not exert influence on root  
263 biomass and length at high dose. Root traits response to soil contamination was only

264 observed by changes in root diameter at 1000 mg.kg<sup>-1</sup>. This observation could be explained  
265 by changes in cortical cell number and size/width of stele diameter due to BPA induced stress  
266 hormones production, reactive oxygen species generation, cell wall and organelles damages as  
267 revealed by others studies (Wang et al. 2015; Xiao et al. 2019). This plasticity of cortical and  
268 stelar tissue was mainly evidenced in plants limiting the surface contact with metallic  
269 pollutant of the soil (Sofa et al. 2017). In our experiment, morphological root traits did no  
270 play a role of protective barrier against BPA penetration as roots length and volume were not  
271 reduced significantly with diameter increase. We postulated that changes in root morphology  
272 may have been beneficial to the transfer of BPA contaminant in above ground biomass of  
273 wheat. Indeed, several studies have reported the presence of high amounts of BPA in shoot of  
274 wheat, tomato and lettuce seedlings following root or foliar exposure (Lu et al. 2015; Wang et  
275 al. 2018). Once absorbed by root systems, this organic compound does not accumulate in root  
276 cells but rather distributes to stem and leaves of the plants and exerts toxic effects on  
277 chlorophyll synthesis, photosynthetic activity of chloroplast and stomatal guard cells (Ali et  
278 al. 2017; Jiao et al. 2017; Qiu et al. 2013). In the current study, no effects were shown in leaf  
279 traits of plants exposed to the environmental dose (figure 1, table 3). Unlike root, shoot  
280 growth was severely inhibited by the soil contamination at 1000 mg.kg<sup>-1</sup> BPA. Our findings  
281 are in agreement with several studies which reported that BPA disturbs the shoot development  
282 of various species including wheat with reduction of leaf biomass, area or anatomy  
283 abnormalities (Ali et al. 2016; Frejd et al. 2016; Staples et al. 2010). Exposure to BPA was  
284 also reported to reduce shoot growth more than root growth for *Vigna radiata* in another  
285 experiment using artificially contaminated soil (Kim et al. 2018). Root systems were not  
286 altered at BPA exposure below 1000 mg.kg<sup>-1</sup> while the threshold level of toxicity for shoot  
287 was evidenced for 500 mg.kg<sup>-1</sup> BPA. Based on the current data, it is difficult to solely ascribe  
288 the reduction of shoot biomass to deleterious events on chloroplastic cells in shoot of plant

289 grown in the presence of  $1000 \text{ mg.kg}^{-1}$  BPA. Perturbation of nutrients acquisition of the roots  
290 is another determining factor that could be influenced by BPA to promote inhibition of leave  
291 growth. Indeed, previous studies have already pointed the better maintenance of root growth  
292 than shoot in wheat experiencing mineral starvation (Shen et al. 2018). Nutrient exchanges in  
293 developing plant result from a complex coordination between leaves and roots metabolisms.  
294 Shoots produce metabolites, mainly carbohydrates, which are transferred and oxidized by  
295 respiratory enzymes of the roots to sustain energy demand of the cell growth, active transport  
296 of soluble elements or release of exudates at the soil/root interface (Jones 1998). Changes in  
297 roots activity, by controlling the absorption and translocation of nutrients, including elements  
298 necessary for the chlorophyll synthesis and photosynthesis, can have negative impact on the  
299 vegetative growth.

300 To understand whether defects of nutrient supply could explain shoot biomass reduction in the  
301  $1000 \text{ mg.kg}^{-1}$  BPA treatment, the concentrations of P and others major nutrients necessary for  
302 cell growth (Ca, K) were analysed. The treatment effects on leaf P, Ca, and K contents were  
303 not significant (table 3), suggesting that absorption function was not impaired in plant grown  
304 on environmentally contaminated soil. Moreover, nutrient uptake by roots in our  
305 experimental conditions seems less sensitive to BPA exposure than in artificial hydroponic  
306 culture showing reduced roots activity, respiration enzymes, absorption of mineral elements  
307 (P, K, Mg, Mn, and Zn) in soybean seedlings treated above  $3 \text{ mg L}^{-1}$  BPA (Xia et al. 2017).  
308 These discrepancies might result from morphological and physiological strategies developed  
309 by plants in solid matrices in interaction with soil microbiome to efficiently take up nutrients.  
310 Previous observations of root systems under abiotic stress have revealed that the effects of  
311 roots architecture on nutrients absorption are significant in soil but not in hydroponic  
312 solutions (Aibara and Miwa 2014) while other findings pointed the importance of root

313 diameter changes in adaptive response of plants to fluctuations of nutrients availability in  
314 soils (Strock et al. 2018).

315 Effects of BPA addition on plant-microbiome interactions

316 Plant–microbiome interactions are mainly explained by a range of plant-derived exudates,  
317 microbe–microbe interactions that could be enhanced in BPA contaminated soil to promote  
318 nutrient acquisition by the root. Plant-microbiome interactions are determined at distance by  
319 carbon assimilation capacity of the developing leaves. Keiluweit et al. (2015) have estimated  
320 that up to 40-60% of photosynthetically fixed carbon in shoots is allocated to the roots and  
321 microorganisms of the rhizosphere via border cells, tissue, mucilage, and the release of  
322 sugars, amino acids, phenolic compounds and carboxylates. Secretion of organic acids in the  
323 rhizosphere soil can have direct and strong involvement on chelation, dissolution, desorption  
324 of ionized elements and communication with beneficial microorganisms contributing to plant  
325 nutrition (Zhang et al. 2013). Because plants which increased root/shoot ratio, direct higher  
326 carbohydrates amounts from shoots to roots (Hermans et al. 2006), BPA-induced shoot  
327 biomass reduction was expected to influence soil microbial communities and carboxylate  
328 exudates that can optimize nutrient access to the roots. However, no effect on organic acids  
329 composition and release was observed at both high and low doses in our study. Malate,  
330 malonate, citrate and fumarate were detected in all wheat root exudates (figure 2) as generally  
331 reported in previous environmental studies carried out with wheat species (Lyu et al. 2016;  
332 Pearse et al. 2007; Vančura 1964). Low production rates of exudate were consistent with  
333 other findings indicating that wheat release slow and largely constitutive carboxylates  
334 amounts which do not always play a determinant role in improving nutrient mobility (Pearse  
335 et al. 2005; Wang 2019). It has also been reported that the proportion of malate and citrate  
336 exuded in wheat were dependent on nutrient shoot status and were not systematically affected  
337 by fluctuating nutrients availability in the soil (Pang et al. 2018; Shen et al. 2018; Wang

338 2019). Nevertheless, the unresponsiveness of carboxylates to BPA exposure in our  
339 experimental conditions cannot be interpreted as a lack of effect on plant exudation and  
340 nutrients acquisition efficiency. Syranidou et al (2017) detected a variation of carboxylates  
341 exudates of *Junctus Acutus* soil leachates 2 hours after incubation with BPA. Formic acid was  
342 the only carboxylate affected by BPA addition. In our situation, exudates were not collected  
343 within similar short term period. Moreover, we did not investigated all substances exuded  
344 with selected carboxylates that could be influenced by BPA exposure to drive changes in  
345 structure and diversity of microbial communities. In this line, previous researches have  
346 proved that BPA used as carbon source can cause direct effects on abundance, structural  
347 composition and enzymatic activities of bacteria communities of waste water treatment plant  
348 sludge and sediments (Huang et al. 2017; Li et al. 2019). BPA in contaminated soil could  
349 exert a selective pressure on microbial population at the interface of the root system to  
350 optimize acquisition of nutrients but this effect has not received much attention in the  
351 literature. Among, microorganisms living in the plant rhizosphere, plant growth promoting  
352 rhizobacteria, saprotrophic, ectomycorrhizal and AM fungi play an important role in nutrients  
353 capture from the soil and plant development (Bardgett et al. 2014; Wang et al. 2017).

354 In our study, the quantification of microbial biomass of the control and contaminated  
355 rhizosphere using PLFA analyses revealed the domination of GN bacteria and AM biomass  
356 in agreement with the literature (Field et al. 2019; Zhang et al. 2019) (table 4). The  
357 introduction of BPA in soil did not cause a pronounced disruption of microbial biomass at the  
358 end of the experimental period. Environmental exposure to BPA did not caused any damage  
359 to soil PLFA. A global trend of reducing bacteria and fungal PLFA concentrations was only  
360 observed when high dose was applied. Our findings were in line with earlier results showing  
361 that microorganisms were tolerant to low BPA concentration in sediment but could not  
362 survive and proliferate when exposed to high loading (Matsumura et al. 2015). The soil



363 contamination at both environmental and toxic concentrations failed to change bacterial to  
364 fungi ratio and GN:GP bacteria ratio. Comparable general observation was made in  
365 bioreactors by Pilna et al (2019), reporting no differences of PLFA ratios of GN:GP, fungi  
366 and bacteria in BPA contaminated sewage sludge. Some previous studies have also shown  
367 that microbial inhibition or promotion were dependent to the initial concentration of BPA  
368 added and the duration of the experiment (Li et al. 2019; Wei et al. 2019). In other reports,  
369 none persistent effects on microorganisms growth were evidenced following introduction of  
370 antibiotics, organic chemicals or inoculation of living bacteria (Liu et al. 2016; Magdalena et  
371 al. 2019). In these disturbed soils where strong effects on microbial biomass accompanied  
372 shift from bacterial to fungi, difference in PLFA markers of the first days reduced gradually to  
373 be not significant after several weeks of incubation. A similar situation, which implies that  
374 microbial population was temporary affected at the beginning of the experiment, could have  
375 occurred despite that no long-term effects were reported by our PLFA analyses in soil after 7  
376 weeks of cultivation. The catabolic activity of microorganisms degrading BPA is another  
377 important factor responsible to the changes of microbial equilibrium in BPA contaminated  
378 soil (Zhao et al. 2015). BPA is generally prone to a rapid decomposition and utilization in soil  
379 from many bacterial and fungal strains producing ligninolytic enzymes able to catalyse  
380 oxidation of aromatic compounds (Prieto 2016; Zhang et al. 2013). The presented PLFA  
381 results do not rule out an active participation of such microorganisms to the removal of BPA  
382 in soil within the experimental period. In our study, an important dissipation of BPA in soil  
383 was confirmed with HPLC analysis by comparing soil collected prior and at the end of the  
384 experiment (figure 3). This result, consistent with published values (Tran et al. 2015; Yu et al.  
385 2013), suggests that BPA weakly bound to soil particle, was bioavailable to microorganisms  
386 and plant. While further experiments are required to identify biodegradation processes of BPA

387 in soil, their mobilization did not influence plant-microbiome interactions mediating nutrient  
388 availability.

389

## 390 CONCLUSION

391 Our work demonstrates that plant trait responses to BPA exposure dose-dependent. While no  
392 influence on plant functional traits was found with BPA at environmental concentration,  
393 remodelling of morphological traits was the main strategy used by wheat plants to ensure  
394 nutrient acquisition in response to increasing BPA dose. Plant maintain optimum P, Ca, and K  
395 contents in shoot by favouring root growth rather than shoot growth and by producing more  
396 large roots under high BPA concentration. Root carboxylate exudation and microbial structure  
397 of the rhizosphere did not take part to BPA-induced stress adaptation.

398 This first attempt to identify long-term effects of environmentally relevant BPA soil  
399 contamination did not focus on early events that may transiently occur during developmental  
400 stages of the plant. Therefore, the traits response of plant under BPA stress need to be  
401 confirmed by extending experiments to more impoverished soil, i.e. more nutrient deficient as  
402 for the P in which carboxylate exudation and root-microbial interactions will probably be  
403 more important to cover nutritional requirements of the plant. A better understanding of  
404 processes that control nutrient uptake through an accurate determination of functional traits  
405 provide a benefit approach to successfully assess environmental risk of BPA and others  
406 emerging contaminants under field conditions.

407

408

## 409 TABLES & FIGURES

410

411 Table 1 : Characterization of the soil

412

	Unit	Luvisol
clay	%	22
silt	%	63
sand	%	15
pH-water <sup>a</sup>		8.1
CEC <sup>b</sup>	cmol <sub>c</sub> kg <sup>-1</sup>	12.1
C org <sup>c</sup>	g kg <sup>-1</sup>	7
Pi-water <sup>d</sup>	mg kg <sup>-1</sup>	12
P total <sup>e</sup>	mg kg <sup>-1</sup>	450
K total <sup>e</sup>	g kg <sup>-1</sup>	16.3
Ca total <sup>e</sup>	g kg <sup>-1</sup>	5.4

413

414 <sup>a</sup>NF ISO 10390

415 <sup>b</sup>NF ISO 23470

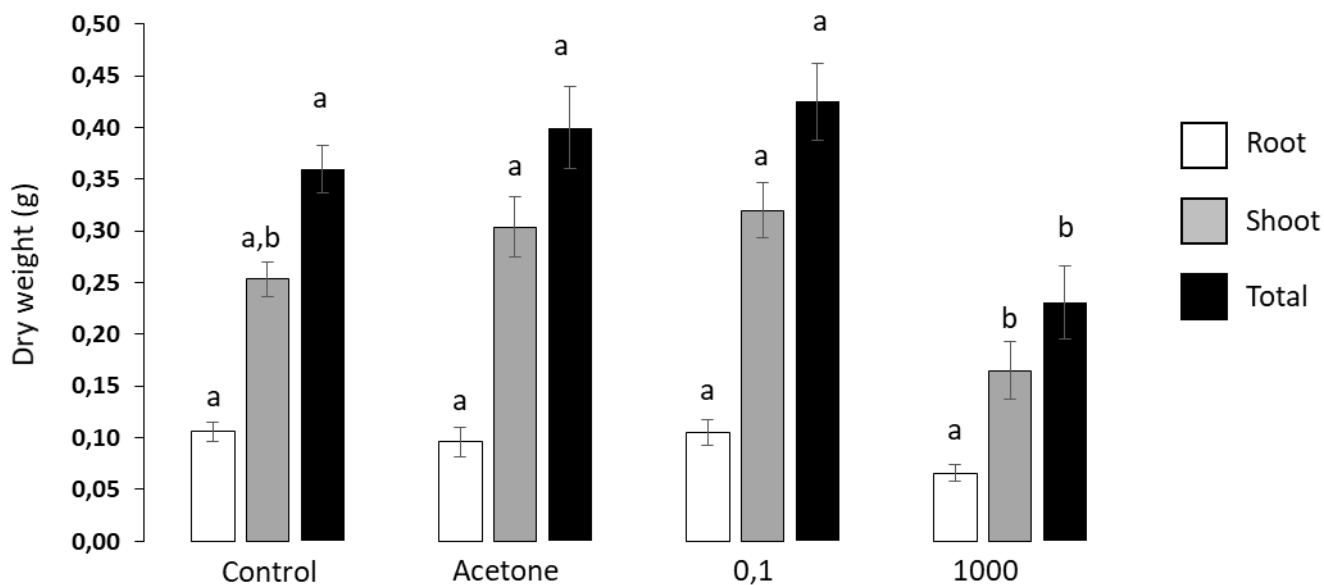
416 <sup>c</sup>NF ISO 10694

417 <sup>d</sup>Inorganic P extracted with water (Brabant wallon, Belgique)

418 <sup>e</sup>Acid digestion and ICP-ES analysis (Veritas Mineral Laboratories, Canada)

419

420



421

422 Figure 1 : Root, shoot and total plant dry weights (g) of wheat grown on the soil, in the  
 423 absence and in the presence of 0,1 or 1000 mg BPA /kg of dry soil. The values are expressed  
 424 as Mean  $\pm$  SEM; N=20.

425

426 Table 2 : Root morphological traits of wheat grown on the soil in the absence and in the  
 427 presence of 0,1 or 1000 mg BPA /kg of dry soil. The values are expressed as Mean  $\pm$  SEM;  
 428 N=3.

	Control without acetone	Acetone	0,1mg BPA / kg	1000 mg BPA / kg
<i>Length (cm)</i>	507,6 $\pm$ 110,5 a	672,2 $\pm$ 182,6 a	806,8 $\pm$ 89,1 a	606,6 $\pm$ 158,0 a
<i>Diameter (mm)</i>	0,28 $\pm$ 0,03 a	0,25 $\pm$ 0,02 a	0,26 $\pm$ 0,01 a	0,36 $\pm$ 0,02 b
<i>Volume (cm<sup>3</sup>)</i>	0,25 $\pm$ 0,02 a	0,28 $\pm$ 0,04 a	0,44 $\pm$ 0,03 a	0,66 $\pm$ 0,22 a
<i>Specific root lenght (cm.g<sup>-1</sup>)</i>	4291 $\pm$ 1792 a	7737 $\pm$ 1838 a	7108 $\pm$ 1038 a	7041 $\pm$ 1326 a

429

430

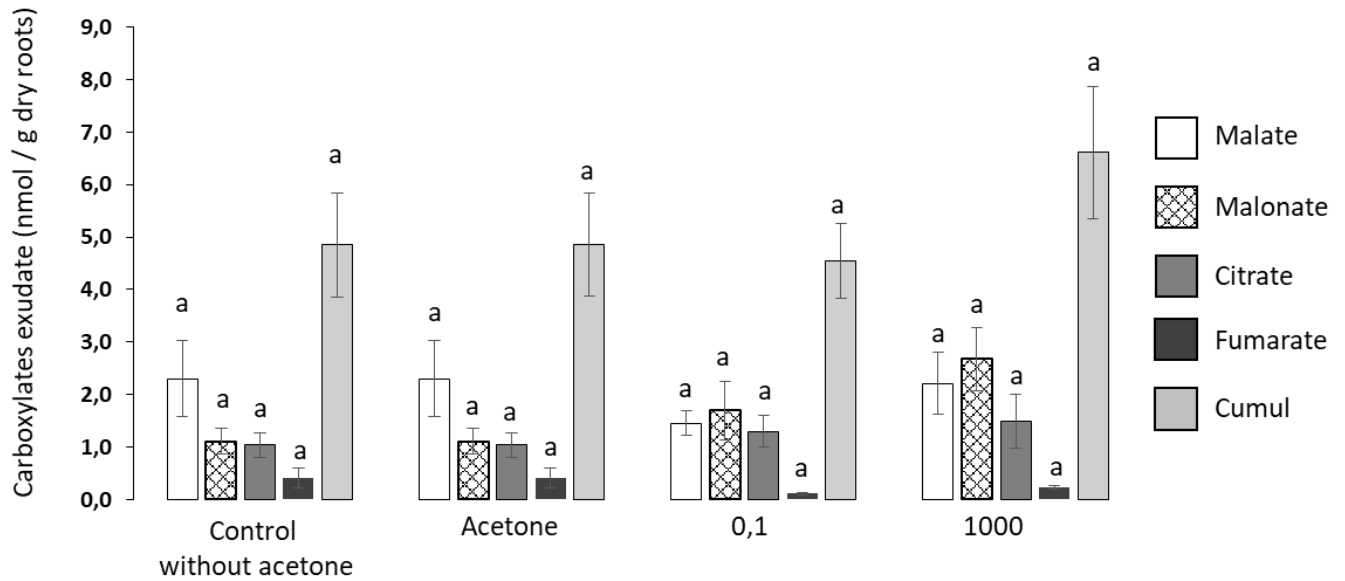
431 Table 3 : Mineral composition (mg / g of dry matter) of wheat shoots grown on the soil in the  
 432 absence and in the presence of 0,1 or 1000 mg BPA /kg of dry soil. The values are expressed  
 433 as Mean  $\pm$  SEM; N=3.

434

	Control without acetone	Acetone	0,1mg BPA / kg	1000 mg BPA / kg
<i>Phosphorus</i>	2,3 $\pm$ 0,3 a	3,0 $\pm$ 0,4 a	2,1 $\pm$ 0,3 a	2,9 $\pm$ 0,5 a
<i>Potassium</i>	42,7 $\pm$ 4,0 a	51,2 $\pm$ 5,2 a	39,4 $\pm$ 4,0 a	44,5 $\pm$ 6,2 a
<i>Calcium</i>	11,3 $\pm$ 0,8 a	12,7 $\pm$ 1,1 a	10,1 $\pm$ 1,1 a	12,4 $\pm$ 0,7 a

435

436



437

438 Figure 2 : Root exudate composition in carboxylates of plants wheat grown on the soil in  
 439 the absence and in the presence of 0,1 or 1000 mg BPA /kg of dry soil. The values are  
 440 expressed as Mean  $\pm$  SEM; N=12.

441

442

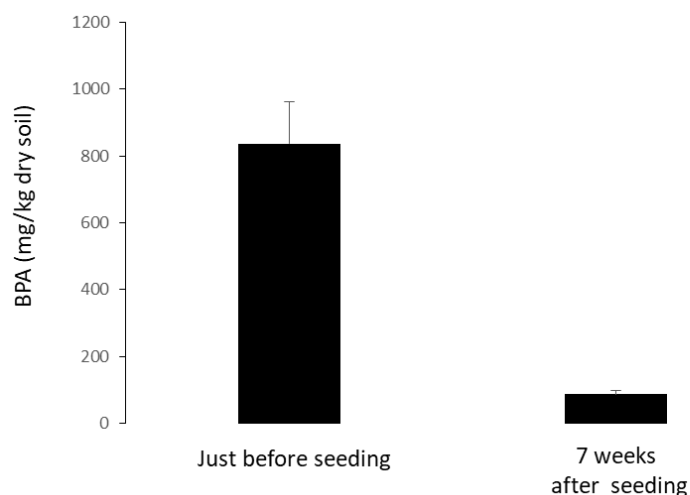
443

444 Table 4 : Bacterial and fungal biomass estimated by PLFA ( $\mu\text{g}$  /g dry roots) analysis in the  
 445 soil in the absence and in the presence of 0,1 or 1000 mg BPA /kg of dry soil. The values are  
 446 expressed as Mean  $\pm$  SEM; N=3.

Treatments	Bacterial			Fungi			Bacterial:Fungi	Total
	Gram Negative (GN)	Gram Positive (GP)	GN:GP	Arbuscular Mycorrhiza (AM)	Saprotrophic+EctoMycorrhiza (S+EM)	AM+ S+ EM		
Control without acetone	0,43 $\pm$ 0,10 a	0,24 $\pm$ 0,13 a	2,35 $\pm$ 0,06 a,b	1,31 $\pm$ 0,08 a,b	0,63 $\pm$ 0,05 a	1,94 $\pm$ 0,04 a	1,20 $\pm$ 0,05 a	4,27 $\pm$ 0,19 a,b
acetone	0,38 $\pm$ 0,06 a	0,23 $\pm$ 0,10 a	2,50 $\pm$ 0,10 a	1,46 $\pm$ 0,07 a,b	0,48 $\pm$ 0,03 a,b	1,94 $\pm$ 0,10 a	1,27 $\pm$ 0,07 a	4,40 $\pm$ 0,17 a,b
0,1 mg BPA / kg	0,42 $\pm$ 0,07 a	0,22 $\pm$ 0,11 a	2,50 $\pm$ 0,30 a,b	1,53 $\pm$ 0,02 a	0,45 $\pm$ 0,04 a,b	1,98 $\pm$ 0,04 a	1,52 $\pm$ 0,07 a	4,82 $\pm$ 0,35 a
1000 mg BPA / kg	0,24 $\pm$ 0,03 a	0,14 $\pm$ 0,05 a	1,57 $\pm$ 0,03 b	0,94 $\pm$ 0,04 b	0,21 $\pm$ 0,02 b	1,16 $\pm$ 0,07 a	1,36 $\pm$ 0,07 a	2,72 $\pm$ 0,01 b

447

448



449

450 Figure 3 : Concentration of BPA just before and 7 weeks after wheat seeding in spiked soil  
 451 at 1000 mg BPA /kg. The values are expressed as Mean  $\pm$  ET ; N=3.

452

453

#### 454 ACKNOWLEDGEMENTS

455 This research was supported by the SFR Condorcet and was carried out in the framework of  
 456 the Alibiotech project, which is financed by European Union, French State and the French  
 457 Region of Hauts-de-France.

458

459

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