



HAL
open science

Carbon nanotubes alter agrosystem multifunctionality

Mathieu Leroy, Vincent Jassey, Jérôme Silvestre, Maiaalen Barret, Emmanuel Flahaut, Camille Larue

► **To cite this version:**

Mathieu Leroy, Vincent Jassey, Jérôme Silvestre, Maiaalen Barret, Emmanuel Flahaut, et al.. Carbon nanotubes alter agrosystem multifunctionality. *Environmental science.Nano*, 2024, Celebrating the 10th anniversary of Environmental Science: Nano, 11, pp.4126-4137. 10.1039/d4en00195h . hal-04684639

HAL Id: hal-04684639

<https://hal.science/hal-04684639v1>

Submitted on 3 Sep 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Carbon nanotubes alter agrosystem multifunctionality

Leroy Mathieu^{1,2}, Jassez Vincent E.J.¹, Silvestre Jérôme¹, Barret Maialen¹, Flahaut Emmanuel², Larue
Camille^{1*}

¹Centre de Recherche sur la Biodiversité et l'Environnement (CRBE), Université de Toulouse, CNRS, IRD,
Toulouse INP, Université Toulouse 3 – Paul Sabatier (UT3), Toulouse, France, ²CIRIMAT, Université de Toulouse,
CNRS, INPT, UPS, UMR CNRS-UPS-INP N°5085, Université Toulouse 3 Paul Sabatier, Bât. CIRIMAT, 118, route de
Narbonne, 31062 Toulouse cedex 9, France

* corresponding author: Avenue de l'Agrobiopole, F-31326 Castanet-Tolosan. camille.larue@cnr.fr

Abstract

Since the discovery of the potential of carbon nanotubes (CNTs) in agriculture to improve crop yield, many studies have been conducted to understand which effects CNTs could have on agrosystem components. However, to date, very little is known about their impacts on ecosystem functions such as biogeochemical cycles or primary production and consequently on the multifunctionality of agrosystems. In this study, we aimed at understanding the impact of CNTs in microcosms including soil bacteria and a crop plant (maize) with a special focus on H₂O, C and N cycles as well as crop nutrition and resistance. After a 6-week exposure, bacterial concentration was increased by 82% in soils exposed to 10 mg.kg⁻¹; in parallel, the organic matter decomposition rate was also significantly enhanced. An increase of nitrifier abundance was quantified with archaeal *amoA* gene copy numbers reaching +144% in soils exposed to 10 mg.kg⁻¹ of CNTs. Maize exposed to 0.1 mg.kg⁻¹ of CNTs had in average 34%, 18% and 12% lower chlorophyll, tannin and phenolic compounds, respectively but no impact was detected on the biomass production. Maize plants were water-stressed after exposure to 10 mg.kg⁻¹ of CNTs with a significant 17% increase of the dry matter compared to unexposed maize.

25 CNT exposure also led to a significant decrease of H₂O flux in the system. As a result, the
26 multifunctionality of the agrosystem was significantly decreased at 0.1 mg.kg⁻¹. Structural equation
27 modeling suggested that CNT impact on bacteria population in general, and on bacteria implied in
28 denitrification and CO₂ emission were the main factors influencing the multifunctionality index.

29 **1. Introduction**

30 Nanotechnology feeds the hopes of several research domains due to its many potential applications.
31 Indeed, nanotechnology could be an important lever to the convergence of knowledge and
32 technology for the benefit of the society and could represent an opportunity for progress in the
33 twenty-first century¹. To develop nanotechnology, the production and use of nanomaterials have
34 increased over the years ². Among nanomaterials, particular attention from industries is focused on
35 carbon nanotubes (CNTs) because of their remarkable physical, electronic and chemical properties ³,
36 and mainly because they can combine several of them. Consequently, CNTs are now found in
37 products such as electronics, tires or sport equipment ⁴, and subsequently end up into the
38 environment during their journey from production to recycling ⁵. In addition, they tend to
39 accumulate in sewage sludge used as fertilizer in agriculture ⁵ and modelling predicts that
40 concentration of carbonaceous nanomaterials in biosolids could reach up to 10 mg.kg⁻¹ while in
41 natural soil it could be up to 0.01 mg.kg⁻¹ ⁶. Furthermore, CNTs might be used as nano-fertilizer as
42 their application on plants (for biofuel applications) and crops leads to increased yield according to
43 several studies ^{7,8}. Thus, their concentration in ecosystems, in particular in agrosystems, will possibly
44 increase in the future and their effects on this type of ecosystems need to be further examined in
45 order to ensure a sustainable use of CNTs.

46 According to the literature, CNTs can be taken up by plants^{7,9,10} and have contrasted impacts on
47 plants ⁷. They can act on plants from the germination stage with an activation of this process ¹¹⁻¹⁴.
48 Furthermore, upon exposure, plant growth was reported to either increase, decrease, or even
49 unmodified (based on root systems, biomass production, and number of flowers and fruits)^{8,15}. When
50 toxicity was detected, it was mostly associated with an overproduction of reactive oxygen species
51 (ROS), antioxidant enzymatic activities, necrotic lesions of cells and leaf tissues as well as root and
52 leaf morphological changes, decreased chlorophyll, and programmed cell death ¹⁶⁻¹⁹. These divergent
53 results might be related with the influence of confounding factors which have been understudied so

54 far such as plant species ²⁰ or soil type ²¹. Additionally, CNTs can also influence gas exchanges of
55 plants with their environment. For example, CNT exposure at 250 mg.L⁻¹ increased carbon (C)
56 assimilation rate of *Zea mays* and also decreased stomatal conductance and transpiration rate
57 possibly as a result of H₂O use modulation ²². The same results were observed on *Orthosiphon*
58 *stamineus* with a decrease in transpiration rate, stomatal conductance and net photosynthesis
59 following a dose-dependent relationship with the increase of CNT concentration (0, 700, 1400 and
60 2100 mg.L⁻¹)²³. Plants were also water-stressed as demonstrated by the positive correlation between
61 water use efficiency and CNT concentration ²³. At lower concentrations (25 mg.L⁻¹ and 54 mg.L⁻¹), no
62 effects of CNT exposure were observed on *Arabidopsis thaliana* on ambient photosynthetic rate,
63 stomatal conductance, transpiration, intrinsic water use efficiency and instantaneous water use
64 efficiency ²⁴. It is also worth mentioning that these reported studies were performed in hydroponics
65 which is not a realistic exposure scenario for agrosystems; usually not taking into account
66 interactions with other biotic and abiotic components of the environment.

67 In parallel, interactions of CNTs with micro-organisms have also been investigated. Microscopy
68 observations have evidenced that CNTs can interact with bacterial cell membranes; they can disrupt
69 integrity of the membrane structure by oxidative stress and/or physical damage ²⁵⁻²⁸. In addition,
70 most studies have reported a decrease in soil microbial activity following exposure to CNTs and a loss
71 of viability ²⁸⁻³². Furthermore, CNT and other carbon nanomaterial application can also cause changes
72 in microbial community structure ^{29,32-34}. Finally, microbial functions such as organic matter
73 decomposition, nitrogen (N) cycle and CO₂ fixation could be modified ³⁵⁻³⁸.

74 From this brief overview of the literature, we can see that if the impact of CNTs in single-organism
75 exposure condition has been studied so far, very little has been done at the ecosystem scale ^{39,40}.
76 However, having a functional overview of contaminant impacts at this scale is of tremendous
77 importance as plant/soil micro-organism interactions drive multiple processes and functions
78 supporting ecosystem services such as food production ⁴¹. In particular, aboveground-belowground

79 interactions drive biogeochemical cycles (C, N, P) through several processes such as photosynthesis,
80 mineralisation, nitrogen fixation, nitrification, denitrification, among others. One way to tackle this
81 gap of knowledge is the use of multifunctionality index as developed in ecology⁴²⁻⁴⁴. This score
82 provides an easily interpretable measure of the ability of different entities composing an ecosystem
83 (bacteria and plants, for example) to sustain multiple functions simultaneously by gathering the
84 selected functions in an index⁴⁵.

85 The objective of this study was thus to investigate the impacts of CNTs at both the organism (crop
86 plant) or community (micro-organisms) scales and at the microcosm scale by implementing a
87 multifunctionality score. More specifically, after exposure to CNTs their toxicity was assessed on (1)
88 the abundance of microbial communities and in particular those related to the N cycle by quantifying
89 marker genes by qPCR, (2) the organic matter decomposition process using tea bags, (3) the plant
90 growth (height, biomass, foliar surface area, dry matter content), (4) the plant physiology
91 (photosynthetic pigment and secondary metabolite concentrations by spectrophotometry and plant
92 mineral nutrition) and (5) the greenhouse gas exchanges of the system (CO₂, H₂O, CH₄). From these
93 different variables, the impact of CNTs on ecosystem functioning was determined by an integrative
94 approach based on multifunctionality scores and, structural equation modeling was used to better
95 understand the relationships among the different drivers and multifunctionality.

96

97 **2. Material and methods**

98 **2.1. CNT synthesis and characterization**

99 CNTs were synthesized at the Interuniversity Center of Materials Research and Engineering (CIRIMAT,
100 Toulouse University). More information is available in Supporting Information.

101 CNTs were characterized using transmission electron microscopy (JEOL 1400F, operated at 120 kV),
102 Raman spectroscopy (Labram HR 800 Yvon Jobin equipped with a He laser at 633 nm) and BET

103 method (on freeze-dried samples). They were oxidized multiwalled CNTs (MWCNTs) with length
104 varying from 1 to 20 μm , an average diameter of 22.5 ± 5.0 nm (Figure S1) and a specific surface area
105 of $60 \text{ m}^2.\text{g}^{-1}$. CNTs were suspended in deionised water at $100 \text{ mg}.\text{L}^{-1}$ and homogenized for 15 min in
106 an ultrasonic bath before use.

107

108 **2.2. Soil characterization and contamination**

109 The experiment was performed with a sandy soil (LUFA-Speyer 2.1) sieved to 2 mm with 86.0% of
110 sand, 12.6% of loam and 1.4% of clay. It contained 0.58 wt. % of organic carbon, 0.04 wt. % of
111 nitrogen, with a pH of 5.0 ± 0.1 (H_2O extraction according to NF ISO 10390:2004), a water retention
112 capacity of 30g/100g and a cation exchange capacity of $2.9 \pm 0.2 \text{ meq}.\text{100g}^{-1}$. More details on soil
113 characteristics are available in supporting information (Table S1). Soils were contaminated by the
114 addition of the required volume of CNT suspension to reach a final concentration of 0.1 or $10 \text{ mg}.\text{kg}^{-1}$
115 dry soil while maintaining the water holding capacity at 25%; the first concentration being close to
116 environmentally predicted concentrations ⁶ and the second one representing a worst-case scenario
117 (use of nano-fertilizer, for instance). They were then mixed manually for 10 min every day for a week
118 to ensure that CNTs were homogeneously distributed in the soil.

119

120 **2.3. Microcosm set-up**

121 Three hundred grams of soil were distributed in each microcosm (L = 45 cm, D = 10 cm). A tea-bag
122 (©Lipton green tea sencha, 1.13 ± 0.32 g) was introduced after the addition of 50 g of soil in each
123 microcosm to study organic matter decomposition. Prior to introduction, tea bags were infused in
124 water at 90°C to remove tea molecules which could have induced bias in microbial community
125 behaviour, dried at 70°C during 48 h and weighted. One seed of *Zea mays* L. subsp. *mays* was added,
126 plants were watered *ad libitum*. For each condition (CNT concentration of 0, 0.1 and $10 \text{ mg}.\text{kg}^{-1}$),

127 microcosms were replicated five times with a total of 15 microcosms. The experiment lasted for 6
128 weeks in an environmental chamber with controlled parameters (10 h/14 h day/night cycle,
129 24°/18 °C and a hygrometry rate of 85%).

130

131 **2.4. Bacterial abundance, functional genes and litter decomposition**

132 The bacterial abundance (16S rRNA) and the abundance of selected genes indicators for N cycling
133 (nitrification and denitrification) were analysed by qPCR ⁴⁶. To summarize, after DNA isolation from
134 contaminated soil using the extraction DNeasy PowerSoil kit ©QIAGEN, the total abundance of soil
135 bacteria was measured by targeting the universal 16S rRNA gene. The following genes related to N
136 cycle were quantified: ammonia monooxygenase A gene (*amoA*) of ammonia oxidizing archaea (AOA)
137 and ammonia oxidizing bacteria (AOB), nitrite reductase (*nirK*) and nitrous oxide reductase (*nosZ*)
138 genes. The same experimental procedure as described in Vijayaraj *et al.* ⁴⁷ was used. Results were
139 expressed relative to the initial gene abundances at the beginning of the experiment to account for
140 initial variability among microcosms (Figure S2; absolute results after exposure are also available in
141 Figure S3).

142 To study litter decomposition (C cycle), tea bags were removed and weighted after the 6 weeks of
143 exposure which permitted to calculate a decomposition average rate as follows:

$$\text{Decomposition rate} = \frac{M_i - M_f}{\text{Duration of exposure}}$$

144 where M_i and M_f represent the initial and final mass (mg dry weight) of the tea bag and the duration
145 of exposure is expressed in days.

146

147 **2.5. Plant morphology and metabolism**

148 Upon exposure, indicators of the C cycle and biomass production were gathered through the
149 determination of the effective quantum yield of maize plants (Diving-Pam underwater fluorometer,
150 Walz, United Kingdom), the height, the fresh and dry biomasses and the leaf surface area (using
151 Image J software). Part of the leaf biomass was oven-dried (60°C until constant weight) to determine
152 elemental concentrations by ICP-AES (see section 2.7). The remaining biomass was used fresh for
153 methanol-based extraction to quantify photosynthetic pigments (chlorophyll a and b) and secondary
154 metabolites *i.e.* total phenolic compounds, flavonoids and tannins as described in Leroy et al.⁴⁸ More
155 details on the phytotoxicity markers are available in SI.

156

157 **2.6. Greenhouse gas exchange measurements and analysis**

158 When plants were sufficiently high (*i.e.* sixth week), H₂O, CO₂ and CH₄ exchanges were recorded
159 every second for 3 minutes. To do so, an airtight flux chamber ($V = 0.06 \text{ m}^3$) was connected to a trace
160 gas analyser (LI-7810, ©LI-COR inc, United States).

161 Net ecosystem exchange of CO₂ (NEE) was measured using a transparent chamber and a LED light
162 while ecosystem CO₂ respiration (RECO) was measured using a darkened chamber. Gross primary
163 productivity (GPP: CO₂ uptake as a result of photosynthesis), was calculated as the difference
164 between NEE and RECO.

165 The slope of gas concentration was calculated and used to determine CO₂ flux rates ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$).
166 using R software and the “flux”⁴⁹ and “gasfluxes”⁵⁰ packages. For NEE, calculations used an
167 exponential nonlinear function to calculate the change in the concentration in the chamber over time
168 ⁵¹. RECO was calculated using linear regression of gas concentrations in the chamber over time. The
169 same was done with H₂O and CH₄ to obtain fluxes in presence of light and in the dark. Positive values
170 for the CO₂ and CH₄ fluxes indicate C release from the system while negative values indicate C uptake
171 by the mesocosm.

172

173 **2.7. Sample preparation and chemical analysis by ICP-AES**

174 Plant samples were digested using 1 ml of a mix of HNO₃ and HCl (3:1 v/v) and 1 ml of H₂O₂ in a close
175 vessel in a microwave system (MARS 2, ©CEM corporation, USA: ramp time: 20-25 min; holding
176 time: 10 min; temperature: 190 °C). Digested samples were then diluted in 5% HNO₃ before analysis
177 on an ICP-AES (ARCOS FHX22, ©AMETEK Spectral, United States) along with control samples (blanks
178 with only chemicals and standard reference material NIST SRM 1570a: Trace Elements in Spinach
179 Leaves).

180

181 **2.8. Statistical analyses**

182 For statistical analyses, 1-way ANOVAs were performed on the toxicity endpoints to test the effects
183 of the addition of CNTs. The normality, independence and homoscedasticity of the residues were
184 checked using Shapiro, Durbin-Watson and Breusch-Pagan tests, respectively. Data were
185 transformed to log or square root if one of the three conditions of validity was not met. A non-
186 parametric Kruskal-Wallis test was performed in the case of failed transformations. All statistical
187 analyses were performed on RStudio software (version 3.4.1). Principal component analyses (PCA)
188 were performed on the elemental concentrations in plants to assess the impact of CNTs on plant
189 nutrition.

190 Different functions with corresponding indicators were selected to be representative of the two main
191 types of functions provided by agroecosystems: ecological functions and nutritional functions⁵².

192 Ecological functions of agroecosystems include fluxes of energy and nutrients such as primary
193 production, decomposition, and biological fixation by plants. These functions are basic ecosystem
194 processes but are key to defining the global functioning of the system. As an extension of these basic
195 functions, nutritional functions have to be measured alongside their ecological counterparts as

196 agroecosystems' primary goal is food production. Nutritional functions encompass a broad suite of
197 functions such as crop production, nutrient quality or resistance to environmental stress. In our case,
198 we gathered the functions and variables as follows: N cycle including both nitrification (with *amoA*-
199 AOA and *amoA*-AOB gene abundances as indicators) and denitrification (*nirK*, *nosZ*), C cycle including
200 litter decomposition, ecosystem respiration, methane emission, and gross primary production
201 (related to bacterial abundance, quantum yield, chlorophyll, crop biomass), H₂O cycle (plant dry
202 matter content), plant nutrition (Z-score based on all elements quantified by ICP-AES), and plant
203 resistance (total phenolic compounds and tannins). Using the packages "dplyr"⁵³ and "tidyverse"⁵⁴
204 as well as the code library "multidiv"⁵⁵, the data were standardized and implemented in a unique Z-
205 score, an index representing the multifunctionality of the ecosystem (Allan et al., 2015, Delgado-
206 Baquerizo et al., 2016). A negative Z-score indicates a low multifunctionality and a positive Z-score a
207 high multifunctionality of the considered agrosystem.

208 We then combined CNTs, the multifunctionality of the agrosystem and its drivers to determine which
209 function was influenced most by CNTs and then drove a change in the multifunctionality of the
210 agrosystem. Specifically, we constructed a structural equation model (SEM) using the package
211 "lavaan"⁵⁶, which draws from a hypothesis-driven *a priori* model based on agrosystem theory and
212 accounting for our expectation that individual agrosystem functions may respond either positively or
213 negatively to CNTs, and therefore differently influence agrosystem multifunctionality. We considered
214 all available variables to investigate how individual indicators of each function related to agrosystem
215 multifunctionality (that is the full model). Then, the full model was simplified by step-wise exclusion
216 of variables with non-significant weights and/or significant collinearity, as estimated by AIC and chi-
217 squared statistics, until a minimal adequate model showing specific linkages remained⁵⁷. We
218 diagnosed model fits using chi-squared statistics ($P > 0.05$), root-mean-square error of approximation
219 index (RMSEA ≤ 0.1), standardized root-mean-square residual index (SRMR ≤ 0.1) and comparative fit
220 indices (CFI ≥ 0.95), and included variables and paths in the final model based on chi-squared
221 statistics ($P < 0.05$) and AIC values of the model.

222

223

224 **3. Results**

225

226 **3.1. Microbial responses to CNT exposure**

227 In soils exposed to 10 mg.kg⁻¹ of CNT, 16S rRNA and *amoA*-AOA gene abundances exhibited
228 significant differences ($p_{ARN\ 16S} < 0.01$ and $p_{amoA-AOA} < 0.01$, Figure 1A), with a 82% and 144% increase
229 in comparison to the control at the end of the experiment ranging from $3.87.10^{11}$ to $5.02.10^{11}$ cells.g⁻¹
230 for 16S rRNA and from $2.04.10^2$ to $3.68.10^2$ cells.g⁻¹ for *amoA*-AOA gene while no difference was
231 detected at 0.1 mg.kg⁻¹. Also, no significant difference was found for *nosZ*, *nirK* and *amoA*-AOB gene
232 copies with on average $4.05.10^{10}$, $2.37.10^6$ and $1.5.10^6$ cells.g⁻¹ across conditions, respectively ($p_{nosZ} =$
233 0.76 , $p_{nirK} = 0.39$, $p_{amoA-AOB} = 0.13$).

234 Decomposition rate of the litter was faster in soils exposed to 10 mg.kg⁻¹ of CNT compared to the
235 other conditions ($p_{litter\ decomposition} < 0.01$) with on average 9.1 mg.j⁻¹ vs. 7.8 mg.j⁻¹ (Figure 1B).

236

237

238 **3.2. Crop plant response to CNT exposure**

239 At the end of exposure, the proportion of dry weight was higher for plants exposed to 10 mg.kg⁻¹ of
240 CNT with on average +17.4% compared to control plants ($p_{DW/FW} < 0.01$, Figure 2A). No significant
241 difference was found on the other growth parameters tested: height, fresh weight (FW) and leaf area
242 with on average 54.23 cm, 3.54 g and 126.34 cm², respectively whatever the condition ($p_{height} = 0.7$,
243 $p_{FW} = 0.6$, $p_{leaf\ area} = 0.5$; Figure 2 B-C-D).

244 At the metabolic level, no difference on the quantum yield was detected between maize grown on
245 control soils and those exposed to CNTs ($p_{quantum\ yield} = 0.3$, Figure 3A). However, the concentration of
246 total chlorophyll was on average 34% and 9% lower in maize exposed to 0.1 and 10 of mg.kg⁻¹ CNT
247 than in maize in control condition ($p_{Chlorophyll} < 0.01$, Figure 3B). Furthermore, phenolic compounds

248 decreased by 15% when exposed to 0.1 mg.kg^{-1} of CNT compared to control condition ($p_{\text{phenolic compounds}}$
249 < 0.01 , Figure 3C). Similarly, maize exposed to the two CNT concentrations (0.1 and 10 mg.kg^{-1})
250 decreased by 18% their tannin content in leaves compared to control soils ($p_{\text{tannin}} = 0.01$, Figure 3D).
251 CNT exposure impacted significantly plant mineral nutrition. Indeed, the PCA on maize leaf elemental
252 composition (Ca, Cu, Fe, Mg, Mn, Na, P, S, Zn) after exposure suggested that plants exposed to
253 0.1 mg.kg^{-1} CNT were depleted in Fe but enriched in P, K while plants exposed to 10 mg.kg^{-1} were
254 enriched mainly in S; both exposure concentrations led to plant enrichment in Mg and Zn (Figure 3E,
255 Table S2).

256

257 **3.3. Greenhouse gas exchange**

258 At the 6th week of exposure, the NEE, RECO and CH₄ (in light and dark conditions) were similar
259 whatever the CNT concentration with on average 0.04 and $0.07 \text{ mg}_{\text{CO}_2} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ and -0.004 and -0.002
260 $\text{mg}_{\text{CH}_4} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ respectively ($p_{\text{NEE}} = 0.6$, $p_{\text{RECO}} = 0.2$, $p_{\text{CH}_4 \text{ Light}} = 0.4$, $p_{\text{CH}_4 \text{ darkness}} = 0.9$ Figure 4A-B-E-F).
261 Additionally, the GPP was also similar whatever the CNT concentration with on average -0.16
262 $\text{mg}_{\text{CO}_2} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ ($p_{\text{GPP}} = 0.7$, Figure S4). In contrast, the emission of H₂O in microcosms measured with
263 light (*ie* the evapotranspiration gathering both leaf transpiration and soil evaporation) was around
264 twice lower in the presence of CNTs at the two concentrations studied than in controls with on
265 average 16.4 and $37.2 \text{ mg}_{\text{H}_2\text{O}} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, respectively ($p_{\text{H}_2\text{O light}} < 0.01$, Figure 4C). This was not the case
266 without light: the emission of H₂O was on average $10.5 \text{ mg}_{\text{H}_2\text{O}} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ whatever the CNT
267 concentration ($p_{\text{H}_2\text{O dark}} = 0.16$, Figure 4D).

268

269 **3.4. Multifunctionality and structural equation modeling**

270 The agrosystem multifunctionality significantly decreased from 0.18 ± 0.10 in control condition and
271 0.17 ± 0.22 at 10 mg.kg^{-1} CNT to -0.35 ± 0.14 with the addition of 0.1 mg.kg^{-1} ($p < 0.001$, Figure 5A).

272 The SEM model revealed that CNT addition impacted the multifunctionality of the agrosystem mostly
273 indirectly (path = 0.193, $p > 0.05$; Figure 5B). In particular, multifunctionality was altered through the
274 impact of CNTs on microbial properties which impaired the C and N cycles. Indeed, CNTs directly
275 increased bacterial abundance (path = 0.420), which in turn positively influenced *amoA*-AOA (path =
276 0.773) and *nirK* (path = 0.705) gene abundances, as well as CO₂ respiration (path = 0.519) and organic
277 matter decomposition (path = 0.349) rates. Increasing nitrification (*amoA*-AOA, path = 0.888),
278 denitrification to a lower extent (*nirK* gene abundance; path = 0.285) and, CO₂ respiration (path =
279 0.552) then positively and significantly influenced agrosystem multifunctionality. CNTs also directly
280 increased the ratio between dry and fresh plant weight (path = 1.155), which in turn negatively
281 influenced methane emissions (path = -0.493) but positively influenced H₂O fluxes (path = 0.682) and
282 decomposition (path = 0.622). Additionally, CNT also directly impacted other plant parameters such
283 as plant nutrition with a positive influence on mineral content (path = 0.516) or on plant defense
284 with a negative influence on tannins (path = -0.621) or on water cycle overall (path = -1.327). Plant
285 related parameters contributed to a lesser extent to the multifunctionality with either non-significant
286 paths or paths <0.4.

287

288

289 **4. Discussion**

290 **4.1. Impacts of CNTs on C cycle**

291 Organic matter decomposition was 17% faster with the addition of 10 mg.kg⁻¹ of CNT compared to
292 control microcosms. This increase was related to the increase in the relative number of universal 16S
293 rRNA gene copies (+82%) showing an increase in bacterial density at this same condition (Figure 5B).

294 In parallel, plant photosynthesis did not seem to be impacted (slight trend of a decrease in the
295 quantum yield of exposed maize, although not significant) even though there was a significant

296 decrease in total chlorophyll pigments for plants exposed to CNTs. Plant growth (fresh biomass
297 production and height) was also not impacted after 6 weeks of exposure.

298 In the literature, it has been reported in different articles that nanomaterials can increase organic
299 matter decomposition process^{58,59}. A study with similar exposure duration (56 days) highlighted the
300 same increase in 16S rRNA copies in the presence of CNTs (both single-walled CNTs and multi-walled
301 CNTs, between 0.7 and 2.6% O) at 10 and 50 g.kg⁻¹⁶⁰. Although, there was no significant differences
302 in 16S RNA gene copy between the control condition and the soil exposed to 0.1 mg.kg⁻¹ CNT after
303 the 6 weeks of exposure; in this last condition, there was a significant decrease of gene copy
304 between the beginning and the end of exposure (T-test; p=0.041) suggesting toxicity at this lower
305 concentration. This higher toxicity of CNTs at lower concentrations has already been evidenced with
306 similar reduction of microbial biomass after exposure to carbon nanomaterials such as graphene^{32,61},
307 graphene oxide^{32,61,62} and CNTs^{29,32}. The homo and hetero-agglomeration of CNTs in soil might
308 explain this effect⁴⁸. Indeed, when the ionic strength of the medium and/or the CNT concentration
309 increase, CNT tend to agglomerate and become less mobile and less prone to interactions with other
310 environmental components⁶³. Despite the decrease in bacteria concentration over time at 0.1 mg.kg⁻¹
311 of CNTs, no effect on decomposition potential was noticeable suggesting that decomposition was
312 maybe compensated by others decomposers.

313 Interestingly, in our exposure conditions the difference detected at the organisms and community
314 levels (bacteria population and chlorophyll pigments) did not lead to any significant difference in gas
315 exchange (CO₂ and CH₄) at the microcosm level, suggesting some compensation mechanisms at this
316 scale.

317

318 **4.2. Impacts of CNTs on N cycle**

319 The quantification of genes involved in the nitrogen cycle showed an increase in *amoA*-AOA gene
320 copies up to 144% in soils exposed to 10 mg.kg⁻¹ of CNT compared to the control. A similar trend was

321 noticeable on *amoA*-AOB gene copies, suggesting a dose-dependent induction of the nitrifier
322 bacterial communities. In the literature, a study demonstrated a similar increase in *amoA*-AOA and
323 *amoA*-AOB gene abundances after 120 days of 0.5 mg.L⁻¹ and 5 mg.L⁻¹ exposure to GO in constructed
324 wetlands, while the increase was only significant for *amoA*-AOA gene abundance after exposure to
325 0.5 mg.L⁻¹ after 30 days³⁶. In contrast, in another study after 180 days of wastewater exposure, the
326 addition of carbon-based nanomaterials (single-walled CNTs, multi-walled CNTs and fullerene, 10 and
327 1000 µg.L⁻¹) led to the decline of the relative abundance of *Nitrosomonas* which was the dominant
328 AOB and is mainly responsible for the oxidation of ammonia to nitrate³⁸. Different parameters such
329 as exposure media, the physico-chemical characteristics of nanomaterials or different initial
330 microbial communities could explain the discrepancies among studies⁶⁴. These contrasted results
331 highlight the need for more mechanistic studies considering the influence of these confounding
332 factors for a better environmental risk assessment. However, there was no impacts of CNT exposure
333 on denitrifier communities (*nosZ* and *nirK*).

334 Additionally, this increase in nitrifier abundance could also be an indirect effect related to the
335 increase of organic matter decomposition leading to an enhanced release of NH₄⁺ which is the
336 nitrifier substrate.

337 These different results suggest that the population of nitrifiers could be favored under exposure to
338 carbon nanomaterials compared to denitrifiers (*nosZ* and *nirK* genes unchanged abundance). This
339 nitrification potential increase could lead to enhanced nitrate release and could thus result in higher
340 plant uptake and therefore higher growth along the life cycle, in the eutrophication of water bodies
341 as well as in biodiversity loss. If over the time course of this experiment, no significant impact was
342 detected on plant fresh biomass, it would be interesting to assess this parameter after a full plant
343 life-cycle exposure. This highlights also the interest of working with microcosm to be able to
344 investigate cascading effects of a contaminant on an organism assemblage. In the literature, Mondal

345 *et al.*¹⁸ evidenced similar results on *Brassica juncea* with an increase in DW/FW ratio after exposure
346 to CNTs and oxidized CNTs at 2.3 and 23 $\mu\text{g}\cdot\text{L}^{-1}$, respectively.

347

348 **4.3. Impacts of CNTs on H₂O cycle**

349 Concerning maize, the dry matter was on average 17.4% higher for plants exposed to 10 $\text{mg}\cdot\text{kg}^{-1}$ of
350 CNT compared to control plants while the fresh weights were similar, suggesting a decrease in water
351 content. This dry matter increase has already been demonstrated to be a common response to other
352 types of stress^{65,66}. It can be also correlated with a decrease in some secondary metabolites in maize
353 exposed to CNTs confirming the fact that plants are suffering stress conditions. Decrease in
354 secondary metabolites such as phenolic compounds and tannins could impair plant defense
355 capabilities as they are implied in many stress defense mechanisms such as high light, low
356 temperatures, pathogen infection, herbivores, nutrient deficiency and exposure to metals^{67,68}.

357 Alternatively, this decreased water content in maize may also be associated with CNT water
358 adsorption capabilities, decreasing water bioavailability for maize^{69,70}. However, the dry matter
359 content results obtained in this study do not follow the general trend observed in the literature
360 which described an increase in water content with CNT exposure^{71,72}. However, as demonstrated by
361 Line *et al.* crop species have very contrasted response to the exposure to CNTs, which can partly be
362 explained by different plant characteristics (morphology and/or physiology)⁷³.

363 The results related to water use obtained at the plant organism scale were confirmed at the
364 ecosystem scale with a decrease in H₂O flux in light condition in the microcosms exposed to CNTs. It
365 might be related with a decreased stomatal opening in water-stressed CNT-exposed maize. Indeed,
366 several studies demonstrated a dose-dependent decrease in stomatal conductance with the
367 concentration of CNTs in *Orthosiphon stamineus*²³ and *Zea mays*²².

368

369 **4.4. Impacts of CNTs on agrosystem multifunctionality**

370 As stated by Holden et al in 2012 while writing about ecological nanotoxicology, “standard toxicity
371 testing anchored in single-organism, dose-response characterizations does not adequately represent
372 real-world exposure and receptor scenarios and their complexities”⁷⁴. A proper risk assessment
373 should thus derive from ecology: *ie.* the study of organisms' interactions with each other and their
374 environments and account for ecological interactions across scales from within organisms to whole
375 ecosystems. Indeed, impact at the organism scale could propagate to the population with
376 consequences on community and in turn on ecosystem functions. Even though this paradigm has
377 been stated more than 10 years ago, there are only few nano-ecotoxicological studies that use this
378 approach; probably because the upscaling is not so straightforward as it has been demonstrated also
379 for other types of contaminants⁷⁵. In our study, to account for different scale processes, we used
380 two types of markers: the biomarkers directed towards organism functioning and the so-called
381 ecomarkers (in particular gas exchange) who reflect the ecosystem functioning.

382 Also in the 2010's in the field of ecology, an interesting way to approach this question has emerged
383 with the development of multifunctionality indices that permits to gather in a same index different
384 metrics related to the different components of a considered ecosystem⁴³. Here, it allows to conclude
385 that the addition of CNT could lead to contrasted consequences on agrosystem multifunctionality
386 according to CNT concentration with a decreased multifunctionality at low concentration (0.1 mg.kg⁻¹).
387 At the highest concentration though, the index remained similar to the control conditions but this
388 might be related to the fact that opposite effects were averaged, thus hiding individual effect
389 contribution. Additionally, it is also interesting to mention that an increased multifunctionality would
390 not necessarily indicate a benefit for the agrosystem. For instance, an increase in total phenolic
391 compounds suggests that the plant is undergoing stress⁶⁷. Therefore, such an increase could not be
392 considered as an “improvement” for agrosystem functioning. More particularly, the SEM model
393 showed that increasing multifunctionality in response to CNT was mostly driven by increasing nitrate

394 reductase and CO₂ respiration. suggesting that CNT addition could promote the release of
395 greenhouse gases (CO₂, CH₄ and N₂O) from the system through direct effects on the microbiome.

396

397 **5. Conclusion**

398 In conclusion, despite some articles of the literature suggesting that CNTs could be used as fertilizer
399 to increase crop production, our results obtained using an integrative approach in microcosms
400 demonstrated an impairment of some of the major biogeochemical cycles with consequences both at
401 the organism and at the ecosystem level. It is also worth mentioning that unlike more “traditional”
402 contaminants, CNT toxicity was not dose-dependent: the highest impact on the ecosystem
403 multifunctionality was observed at the lowest exposure concentration (0.1 mg.kg⁻¹, which is
404 environmentally relevant in agrosystems according to modeling studies available so far) rather than
405 at the highest one (10 mg.kg⁻¹, simulating a worst-case scenario).

406

407 **Conflicts of interest**

408 There are no conflicts of interest to declare.

409

410 **Acknowledgement**

411 This work was supported by the Région Occitanie, Toulouse Federal University (NANOMETAGRO),
412 and EC2CO program CARBOSTRESS (CNRS-INSU). Authors are thankful to the FBil and PAPC platforms
413 of the laboratory for functional ecology and environment for ICP-AES analysis and soil physico-
414 chemical characterization, respectively. We are also grateful to Morgan Légnani for CNT synthesis,
415 Mélissa Sontag for bacterial gene quantification and Laure Gandois for providing the LICOR
416 instrument.

417 **Bibliography**

- 418 1. Malik, S., Muhammad, K. & Waheed, Y. Nanotechnology: a revolution in modern industry.
419 *Molecules* **28**, (2023).
- 420 2. Piccinno, F., Gottschalk, F., Seeger, S. & Nowack, B. Industrial production quantities and uses
421 of ten engineered nanomaterials in Europe and the world. *J. Nanoparticle Res.* **14**, 1109
422 (2012).
- 423 3. O’Connell, M. J. *Carbon nanotubes: properties and applications*. (2006).
424 doi:10.1201/9781315222127.
- 425 4. Danish Consumer Council, The Ecological council, D. E. Welcome to The Nanodatabase.
426 <https://nanodb.dk/en/search-database/> (2020).
- 427 5. Gottschalk, F., Sun, T. & Nowack, B. Environmental concentrations of engineered
428 nanomaterials: review of modeling and analytical studies. *Env. Pollut* **181**, 287–300 (2013).
- 429 6. Holden, P. A. *et al.* Evaluation of exposure concentrations used in assessing manufactured
430 nanomaterial environmental hazards: are they relevant? *Environ. Sci. Technol.* **48**, 10541–
431 10551 (2014).
- 432 7. Liné, C., Larue, C. & Flahaut, E. Carbon nanotubes: impacts and behaviour in the terrestrial
433 ecosystem - A review. *Carbon N. Y.* **123**, 767–785 (2017).
- 434 8. Rudakiya, D., Patel, Y., Chhaya, U. & Gupte, A. Carbon nanotubes in agriculture: production,
435 potential, and prospects. in *Nanotechnology for Agriculture: Advances for Sustainable*
436 *Agriculture* (eds. Panpatte, D. G. & Jhala, Y. K.) 121–130 (Springer, 2019).
- 437 9. Larue, C. *et al.* Quantitative evaluation of multi-walled carbon nanotube uptake in wheat and
438 rapeseed. *J. Hazard. Mater.* **227–228**, (2012).
- 439 10. Petersen, E. *et al.* Evaluation of bioaccumulation of nanoplastics, carbon nanotubes,
440 fullerenes, and graphene family materials. *Environ. Int.* **173**, 107650 (2023).
- 441 11. Oleszczuk, P., Joško, I. & Xing, B. The toxicity to plants of the sewage sludges containing
442 multiwalled carbon nanotubes. *J. Hazard. Mater.* **186**, 436–442 (2011).
- 443 12. Ratnikova, T. A., Podila, R., Rao, A. M. & Taylor, A. G. Tomato seed coat permeability to
444 selected carbon nanomaterials and enhancement of germination and seedling growth. *Sci.*
445 *World J.* **2015**, 419215 (2015).
- 446 13. Lahiani, M. H., Dervishi, E., Ivanov, I., Chen, J. & Khodakovskaya, M. Comparative study of
447 plant responses to carbon-based nanomaterials with different morphologies. *Nanotechnology*
448 **27**, (2016).
- 449 14. Lahiani, M. H. *et al.* Interaction of carbon nanohorns with plants: Uptake and biological
450 effects. *Carbon N. Y.* **81**, 607–619 (2015).
- 451 15. Wang, Q., Li, C., Wang, Y. & Que, X. Phytotoxicity of graphene family nanomaterials and its
452 mechanisms: a review. *Front. Chem.* **7**, (2019).
- 453 16. Lin, C., Fugetsu, B., Su, Y. B. & Watari, F. Studies on toxicity of multi-walled carbon nanotubes
454 on Arabidopsis T87 suspension cells. *J. Hazard. Mater.* **170**, 578–583 (2009).
- 455 17. Shen, C.-X., Zhang, Q.-F., Li, J., Bi, F.-C. & Yao, N. Induction of programmed cell death in
456 Arabidopsis and rice by single-wall carbon nanotubes. *Am. J. Bot.* **97**, 1602–1609 (2010).

- 457 18. Mondal, A., Basu, R., Das, S. & Nandy, P. Beneficial role of carbon nanotubes on mustard plant
458 growth: An agricultural prospect. *J. Nanoparticle Res.* **13**, 4519–4528 (2011).
- 459 19. Begum, P. & Fugetsu, B. Phytotoxicity of multi-walled carbon nanotubes on red spinach
460 (Amaranthus tricolor L) and the role of ascorbic acid as an antioxidant. *J. Hazard. Mater.* **243**,
461 212–222 (2012).
- 462 20. Liné, C., Manent, F., Wolinski, A., Flahaut, E. & Larue, C. Comparative study of response of
463 four crop species exposed to carbon nanotube contamination in soil. *Chemosphere* **274**,
464 129854 (2021).
- 465 21. Larue, C. *et al.* Influence of soil type on TiO₂ nanoparticle fate in an agroecosystem. *Sci. Total*
466 *Environ.* **630**, 609–617 (2018).
- 467 22. Alp, F. N. *et al.* Multi-walled carbon nanotubes influence on gas exchange, redox reaction and
468 antioxidant system in Zea mays exposed to excessive copper. *J Plant Growth Regul* **41**, 3169–
469 3184 (2022).
- 470 23. Izad, A. I., Ibrahim, M. H., Abdullah, C. A. C. & Zain, N. A. M. Growth, leaf gas exchange and
471 secondary metabolites of Orthosiphon stamineus as affected by multiwall carbon nanotubes
472 application. *Annu. Res. Rev. Biol.* 1–13 (2018) doi:10.9734/ARRB/2018/38113.
- 473 24. Voleti, R. *Effects of low concentrations of carbon nanotubes on growth and gas exchange in*
474 *Arabidopsis thaliana.* (2015).
- 475 25. Jackson, P. *et al.* Bioaccumulation and ecotoxicity of carbon nanotubes. *Chem. Cent. J.* **7**, 1–21
476 (2013).
- 477 26. Zheng, X. *et al.* Carboxyl-modified single-walled carbon nanotubes negatively affect bacterial
478 growth and denitrification activity. *Sci. Rep.* **4**, (2014).
- 479 27. Hu, W. *et al.* Graphene-based antibacterial paper. *ACS Nano* **4**, 4317–4323 (2010).
- 480 28. Giraud, L., Tourrette, A. & Flahaut, E. Carbon nanomaterials-based polymer-matrix
481 nanocomposites for antimicrobial applications: a review. *Carbon N. Y.* **182**, 463–483 (2021).
- 482 29. Jin, L. *et al.* Single-walled carbon nanotubes alter soil microbial community composition. *Sci.*
483 *Total Environ.* **466–467**, 533–538 (2014).
- 484 30. Chung, H., Son, Y., Yoon, T. K., Kim, S. & Kim, W. The effect of multi-walled carbon nanotubes
485 on soil microbial activity. *Ecotoxicol. Environ. Saf.* **74**, 569–575 (2011).
- 486 31. Jin, L. *et al.* High concentrations of single-walled carbon nanotubes lower soil enzyme activity
487 and microbial biomass. *Ecotoxicol. Environ. Saf.* **88**, 9–15 (2013).
- 488 32. Ge, Y. *et al.* Long-term effects of multiwalled carbon nanotubes and graphene on microbial
489 communities in dry soil. *Environ. Sci. Technol.* **50**, 3965–3974 (2016).
- 490 33. Kerfahi, D. *et al.* Effects of functionalized and raw multi-walled carbon nanotubes on soil
491 bacterial community composition. *PLoS One* **10**, e0123042 (2015).
- 492 34. Evariste, L. *et al.* Graphene-based nanomaterials modulate internal biofilm interactions and
493 microbial diversity. *Front. Microbiol.* **12**, (2021).
- 494 35. Wu, F. *et al.* Carbon nanomaterials affect carbon cycle-related functions of the soil microbial
495 community and the coupling of nutrient cycles. *J. Hazard. Mater.* **390**, 122144 (2020).
- 496 36. Yan, C. *et al.* Assessment on the treatment of nitrogen contaminant by constructed wetland
497 exposed to different concentrations of graphene oxide. *J. Clean. Prod.* **338**, 130567 (2022).

- 498 37. Das, P., Davis, K., Penton, C. R., Westerhoff, P. & Bi, Y. Impacts of graphitic nanofertilizers on
499 nitrogen cycling in a sandy, agricultural soil. *J. Nanoparticle Res.* **24**, 120 (2022).
- 500 38. Yang, X. *et al.* Impacts of carbon-based nanomaterials on nutrient removal in constructed
501 wetlands: microbial community structure, enzyme activities, and metabolism process. *J.*
502 *Hazard. Mater.* **401**, 123270 (2021).
- 503 39. Caixeta Oliveira, H., Barozzi Seabra, A., Kondak, S., Adedokun, O. P. & Kolbert, Z. Multilevel
504 approach to plant–nanomaterial relationships: from cells to living ecosystems. *J. Exp. Bot.* **74**,
505 3406–3424 (2023).
- 506 40. Carboni, A. *et al.* Aquatic mesocosm strategies for the environmental fate and risk assessment
507 of engineered nanomaterials. *Environ. Sci. Technol.* **55**, 16270–16282 (2021).
- 508 41. Morrissey, J. P., Dow, J. M., Mark, G. L. & O’Gara, F. Are microbes at the root of a solution to
509 world food production? *EMBO Rep.* **5**, 922–926 (2004).
- 510 42. Delgado-Baquerizo, M. *et al.* Microbial diversity drives multifunctionality in terrestrial
511 ecosystems. *Nat. Commun.* **7**, 10541 (2016).
- 512 43. Maestre, F. T. *et al.* Plant species richness and ecosystem multifunctionality in global drylands.
513 *Science (80-.).* **335**, 214–218 (2012).
- 514 44. Liu, Y.-R. *et al.* Identity of biocrust species and microbial communities drive the response of
515 soil multifunctionality to simulated global change. *Soil Biol. Biochem.* **107**, 208–217 (2017).
- 516 45. Lefcheck, J. S. *et al.* Biodiversity enhances ecosystem multifunctionality across trophic levels
517 and habitats. *Nat. Commun.* **6**, 6936 (2015).
- 518 46. Simonin, M. *et al.* Titanium dioxide nanoparticles strongly impact soil microbial function by
519 affecting archaeal nitrifiers. *Sci. Rep.* **6**, 33643 (2016).
- 520 47. Vijayaraj, V. *et al.* Transfer and ecotoxicity of titanium dioxide nanoparticles in the terrestrial
521 and aquatic ecosystems : a microcosm study. *Environ. Sci. Technol.* **52**, 12757–12764 (2018).
- 522 48. Leroy, M. *et al.* Interactive effects of metals and carbon nanotubes in a microcosm
523 agrosystem. *J. Hazard. Mater.* **431**, 128613 (2022).
- 524 49. Jurasinski, G., Koebisch, F., Guenther, A. & Beetz, S. *Flux rate calculation from dynamic closed*
525 *chamber measurements.* (2014).
- 526 50. Fuss, R. & Hueppi, R. *Gasfluxes: greenhouse gas flux calculation from chamber measurements.*
527 (2023).
- 528 51. Kutzbach, L. *et al.* CO₂ flux determination by closed-chamber methods can be seriously biased
529 by inappropriate application of linear regression. *Biogeosciences* **4**, 1005–1025 (2007).
- 530 52. Stratton, A. E., Kuhl, L. & Blesh, J. Ecological and nutritional functions of agroecosystems as
531 indicators of smallholder resilience. *Front. Sustain. Food Syst.* **4**, (2020).
- 532 53. Wickham, H., François, R., Henry, L., Müller, K. & Vaughan, D. *dplyr: a grammar of data*
533 *manipulation.* (2023).
- 534 54. Wickham, H. *et al.* Welcome to the tidyverse. *J. Open Source Softw.* **4**, 1686 (2019).
- 535 55. Allan, E. *et al.* Land use intensification alters ecosystem multifunctionality via loss of
536 biodiversity and changes to functional composition. *Ecol. Lett.* **18**, 834–843 (2015).
- 537 56. Rosseel, Y. lavaan: an R package for structural equation modeling. *J. Stat. Softw.* **48**, 1–36

- 538 (2012).
- 539 57. Jassey, V. E. J. *et al.* Plant functional diversity drives niche-size-structure of dominant
540 microbial consumers along a poor to extremely rich fen gradient. *J. Ecol.* **102**, 1150–1162
541 (2014).
- 542 58. Du, J. *et al.* Co-exposures of acid rain and ZnO nanoparticles accelerate decomposition of
543 aquatic leaf litter. *J. Hazard. Mater.* **426**, 128141 (2022).
- 544 59. Evariste, L. *et al.* Assessment of graphene oxide ecotoxicity at several trophic levels using
545 aquatic microcosms. *Carbon N. Y.* **156**, 261–271 (2020).
- 546 60. Wu, F. *et al.* Effects of various carbon nanotubes on soil bacterial community composition and
547 structure. *Environ. Sci. Technol.* **53**, 5707–5716 (2019).
- 548 61. Liu, S. *et al.* Antibacterial activity of graphite, graphite oxide, graphene oxide, and reduced
549 graphene oxide: membrane and oxidative stress. *ACS Nano* **5**, 6971–6980 (2011).
- 550 62. Chung, H. *et al.* Effects of graphene oxides on soil enzyme activity and microbial biomass. *Sci.*
551 *Total Environ.* **514**, 307–313 (2015).
- 552 63. Ghosh, D. *et al.* The effects of pH, ionic strength, and natural organics on the transport
553 properties of carbon nanotubes in saturated porous. *Colloids Surfaces A Physicochem. Eng.*
554 *Asp.* **647**, 129025 (2022).
- 555 64. Leroy, M., Flahaut, E. & Larue, C. Carbon based nanomaterial interactions with metals and
556 metalloids in terrestrial environment: a review. *Carbon N. Y.* **206**, 325–339 (2023).
- 557 65. Tollenaar, M., Deen, W., Echarte, L. & Liu, W. Effect of crowding stress on dry matter
558 accumulation and harvest index in maize. *Agron. J.* **98**, 930–937 (2006).
- 559 66. Turhan, E. & Eris, A. Changes of micronutrients, dry weight, and chlorophyll contents in
560 strawberry plants under salt stress conditions. *Commun. Soil Sci. Plant Anal.* **36**, 1021–1028
561 (2005).
- 562 67. Lattanzio, V. *Natural products: phytochemistry, botany and metabolism of alkaloids, phenolics*
563 *and terpenes.* (2013). doi:10.1007/978-3-642-22144-6_57.
- 564 68. War, A. R. *et al.* Mechanisms of plant defense against insect herbivores. *Plant Signal. Behav.* **7**,
565 1306–1320 (2012).
- 566 69. Chen, Z. *et al.* Opportunities for graphene, single-walled and multi-walled carbon nanotube
567 applications in agriculture: a review. *Crop Des.* **1**, 100006 (2022).
- 568 70. Striolo, A., Chialvo, A. A., Gubbins, K. E. & Cummings, P. T. Water in carbon nanotubes:
569 adsorption isotherms and thermodynamic properties from molecular simulation. *J. Chem.*
570 *Phys.* **122**, 234712 (2005).
- 571 71. Martínez-Ballesta, M. C., Zapata, L., Chalbi, N. & Carvajal, M. Multiwalled carbon nanotubes
572 enter broccoli cells enhancing growth and water uptake of plants exposed to salinity. *J.*
573 *Nanobiotechnology* **14**, 1–14 (2016).
- 574 72. Tiwari, D. K. *et al.* Interfacing carbon nanotubes (CNT) with plants: enhancement of growth,
575 water and ionic nutrient uptake in maize (*Zea mays*) and implications for nanoagriculture.
576 *Appl. Nanosci.* **4**, 577–591 (2014).
- 577 73. Liné, C., Manent, F., Wolinski, A., Flahaut, E. & Larue, C. Comparative study of response of
578 four crop species exposed to carbon nanotube contamination in soil. *Chemosphere* **274**,
579 (2021).

- 580 74. Holden, P. A. *et al.* Ecological nanotoxicology: Integrating nanomaterial hazard considerations
581 across the subcellular, population, community, and ecosystems levels. *Acc. Chem. Res.* **46**,
582 813–822 (2013).
- 583 75. Vijayaraj, V. *et al.* Evaluating multiple stressor effects on benthic–pelagic freshwater
584 communities in systems of different complexities: challenges in upscaling. *Water* **14**, (2022).
- 585

586

Figure captions

587

588 **Figure 1:** A. Gene copy quantification for the universal gene 16S and for selected genes involved in N
589 cycle: *nosZ*, *nirK*, *amoA-AOB* and *amoA-AOA* by qPCR (normalized by the initial number of gene
590 copies) and litter decomposition (B), for the 3 CNT modalities (0 = 0 mg.kg⁻¹, 0.1 = 0.1 mg.kg⁻¹, 10 = 10
591 mg.kg⁻¹) after a 6-week exposure in soil. Same lowercase letters indicate treatments that do not
592 differ significantly (p-value > 0.05) following an ANOVA or a Kruskal-Wallis test (n = 5).

593

594 **Figure 2:** Plant morphological parameter assessment: dry weight/ fresh weight proportion (% of dry
595 matter) (A), height (B), fresh weight (FW, C), and leaf area (D) of maize exposed for 6 weeks in soil
596 contaminated with 3 CNT modalities (0 = 0 mg.kg⁻¹, 0.1 = 0.1 mg.kg⁻¹, 10 = 10 mg.kg⁻¹). Same
597 lowercase letters indicate treatments that do not differ significantly (p-value > 0.05) following an
598 ANOVA (n = 5).

599

600 **Figure 3:** Quantum yield (A), total chlorophyll (B), total phenolic compounds (C), tannin
601 concentration (D) and principal component analysis (PCA, E) on the micro- and macro-nutrient
602 content (Ca, Cu, Fe, Cu, Mg, Mn, Na, P, S, Zn) represented by individuals and correlation circle graphs
603 for maize leaves exposed to the 3 CNT modalities (0 = 0 mg.kg⁻¹, 0.1 = 0.1 mg.kg⁻¹, 10 = 10 mg.kg⁻¹)
604 after a 6-week exposure in soil. Cos2 corresponds to the level of variable contributions (elements) to
605 dimensions 1 and 2. Same lowercase letters indicate treatments that do not differ significantly (p-
606 value > 0.05) following an ANOVA (n = 5).

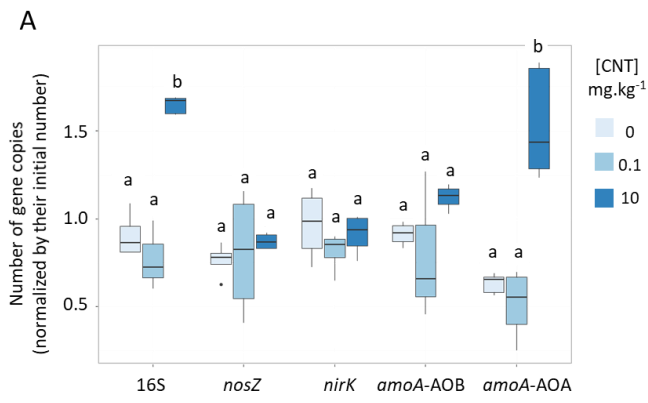
607

608 **Figure 4:** GreenHouse Gas exchange. Net Ecosystem Exchange (NEE, A), Ecosystem respiration (RECO,
609 B), H₂O emission measured in the light (C) or in the dark (D) and CH₄ emission measured in the light
610 (E) or in the dark (F) for microcosms exposed to the 3 CNT modalities (0 = 0 mg.kg⁻¹, 0.1 = 0.1 mg.kg⁻¹,

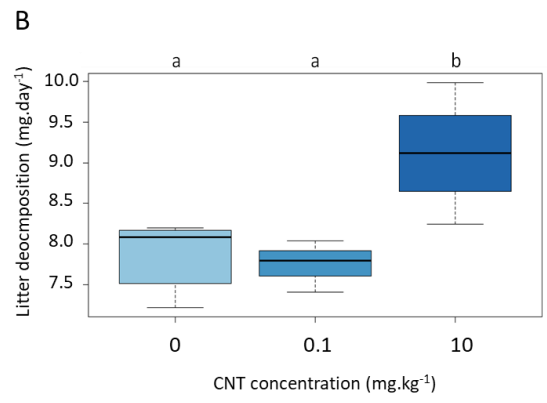
611 10 = 10 mg.kg⁻¹) after a 6-week exposure. Same lowercase letters indicate treatments that do not
612 differ significantly (p-value > 0.05) following an ANOVA or a Kruskal-Wallis (CH₄ in darkness) (n = 5).

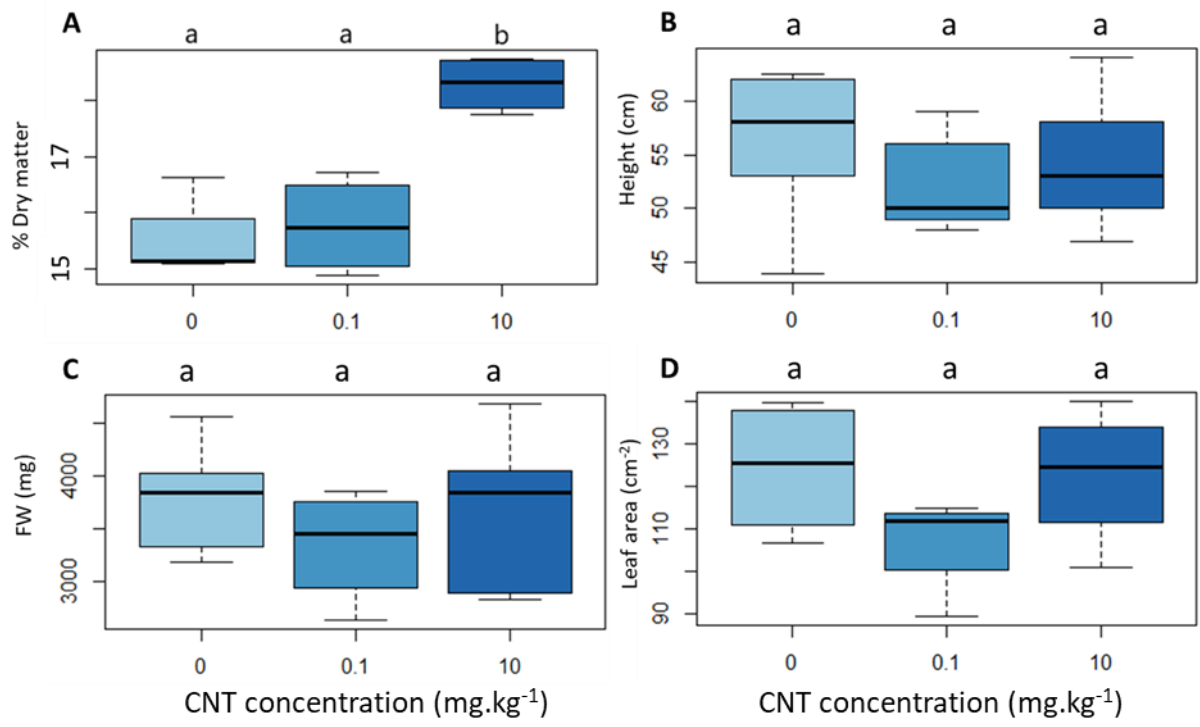
613 **Figure 5:** A. Multifunctionality index of microcosms exposed to the 3 CNT modalities (0 = 0 mg.kg⁻¹,
614 0.1 = 0.1 mg.kg⁻¹, 10 = 10 mg.kg⁻¹) after a 6-week exposure. Same lowercase letters indicate
615 treatments that do not differ significantly (p-value > 0.05) following an ANOVA (n=5). B. Structural
616 Equation Modeling investigating the impact of carbon nanotube (CNT) addition on agrosystem
617 multifunctionality through different functions: N cycle (nitrification represented by *amoA*-AOA gene
618 expression and denitrification represented by *nirK* gene expression, in yellow), H₂O cycle (with H₂O
619 flux in the system, in blue), C cycle (including organic matter decomposition, ecosystem respiration –
620 RECO – and, CH₄ emission, in green) and crop resistance and nutrition (represented by tannin and
621 mineral concentrations in plants, in red). Dashed arrows indicate a non-significant path (p values >
622 0.05), plain arrows indicate a significant positive correlation (p values < 0.05). The numbers adjacent
623 to arrows are standardized path coefficients. Model fit was overall good with P = 0.436, AIC=184.0,
624 RMSEA = 0.090, CFI = 0.988, SRMR=0.104.

625 **Figure 1**

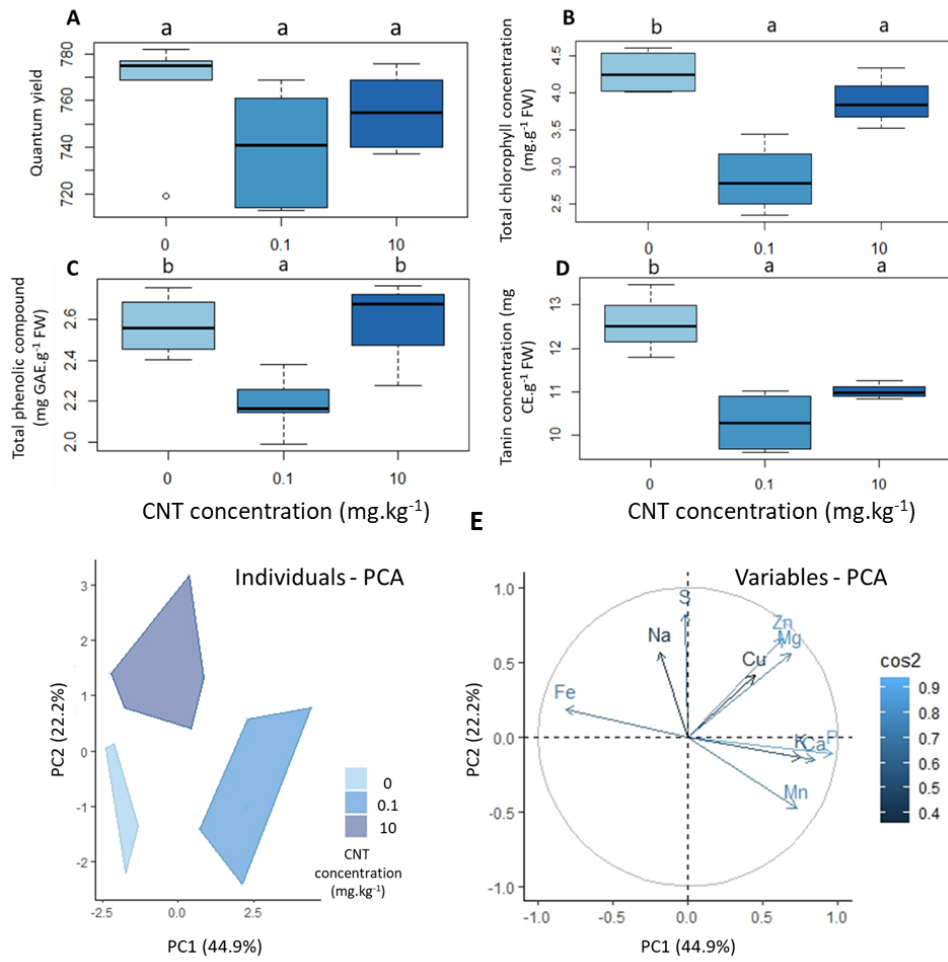


626
627



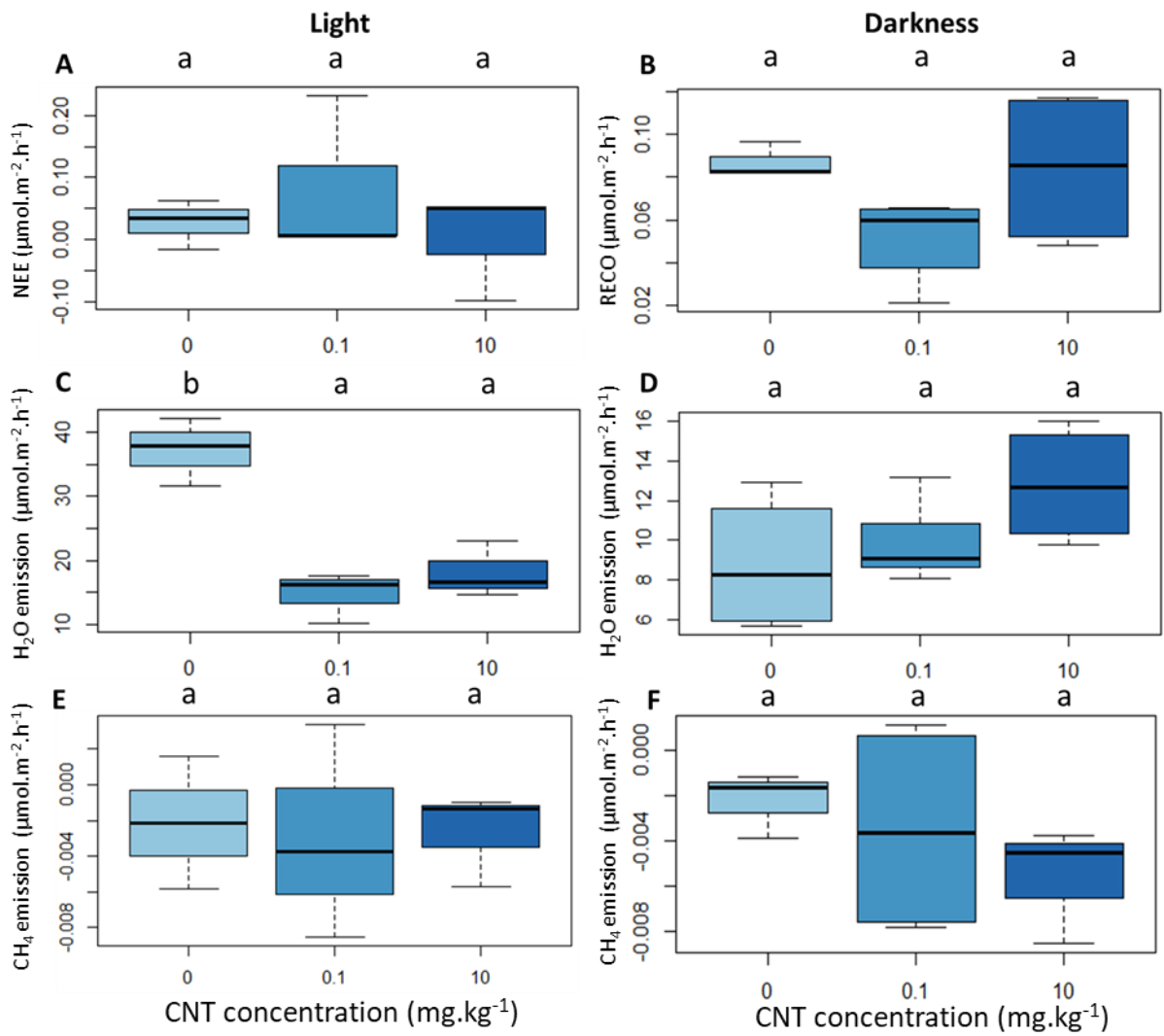


630 **Figure 3**

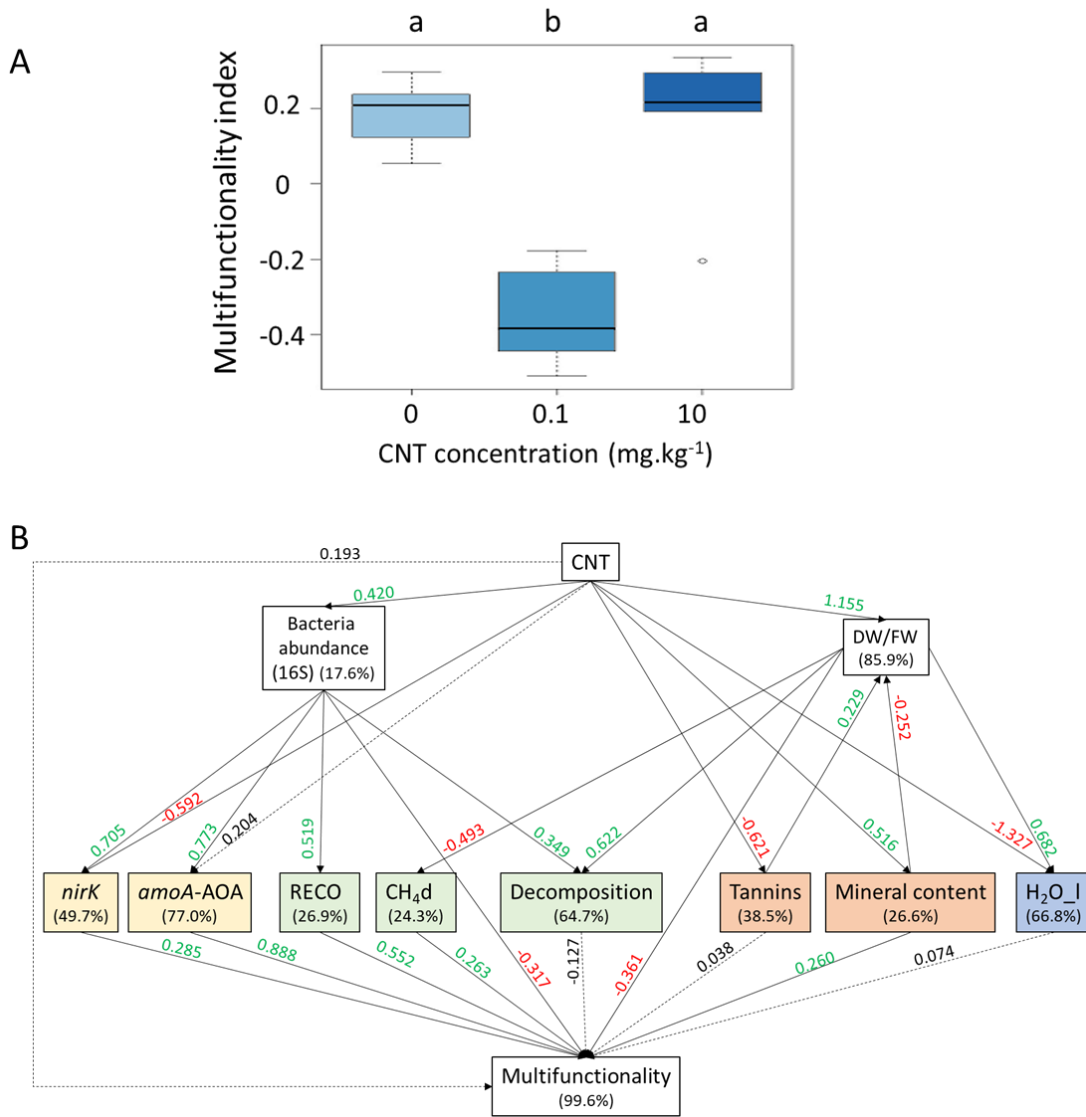


631

632



634
635



637

638

639