Carbon nanotubes alter agrosystem multifunctionality

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Supporting information

2.1. CNT synthesis and characterization

In brief, a catalytic powder was prepared by impregnation of MgO powder (Honeywell, ref. 372493) by Co (cobalt nitrate hexahydrate) and Mo (ammonium heptamolybdate) salts to reach an elemental composition of $Mg_{0.99}(Co_{0.0075}Mo_{0.0025})O$. After incubation 1h at 50°C, the suspension was frozen in liquid nitrogen to limit sedimentation, before freeze drying. The obtained powder was calcined in air at 450°C for 1h in air flow. 1g of catalytic powder was introduced in an alumina boat, placed at the center of a quartz reactor. A N_2 flow (3 $L.h^{-1}$) was used to carry EtOH vapors by flowing in a flask containing 2 g of 100% ^{13}C ethanol (Sigma-Aldrich, CAS 14742-23-5, ref Sigma 427039). The flask was immerged in an oil bath thermostatically controlled at 25°C to avoid interferences from room temperature variations. A heating wire (60°C) was rolled around the hose connecting the EtOH reservoir to the reactor to avoid the condensation of EtOH vapors. The catalytic powder was heated at 850°C (5°C/min) in N_2 atmosphere (3 $L.h^{-1}$). During a 30 min dwell time at this temperature, a valve was

operated to force the flow of N_2 through the washing bottle containing the ^{13}C EtOH. Then, the temperature was decreased to room temperature (5°C/min) in N_2 atmosphere (3 $L.h^{-1}$). At the end of the synthesis, a black composite powder was obtained. CNTs were first extracted from the nanocomposite powder by processing in an aqueous solution of HCl to eliminate accessible metal catalyst, then washed to neutrality using deionised water. Then, the wet CNTs were oxidised in 3M HNO_3 by refluxing them at 130°C for 24h. This step allows to decrease the remaining catalyst amount of 90% leaving only metal catalyst tightly encapsulated in graphitic shell unlikely to leach from the CNTs under environmental conditions[1,2] which is supported by the fact that ICP-AES quantification of Mg and Co in soil failed to revealed any significant differences among groups (p=0.616, ANOVA 1-way for [Mg] = 60.79 $mg.kg^{-1}$ soil and p=0.99, Kruskal-wallis test, for [Co] = 0.18 $mg.kg^{-1}$ soil, Mo was under detection limit). Finally, the oxidized CNTs were washed to neutrality using deionised water and used wet.

2.5. Phytotoxicity parameters

The photochemical efficiency of photosystem II was determined by spectrofluorometry[3] on the basis of the effective quantum yield (EQY) $\phi_{PSII} = (F_m' - F_t)/F_m' = \Delta F/F_m'$ (F_m' is the steady state maximum fluorescence measured after a saturating pulse and F_t the steady-state fluorescence level after a period of constant actinic light) without dark adaptation using a portable field device (Diving-PAM, Walz, Germany). The DLC-8 leaf clips were used to keep a constant distance between the leaf and the optical fiber.

For other biomarkers, dry leaf material was ground twice in a ball mill for 1.5 minutes. 50 mg of this material was placed in a 2-ml microplate with 96 wells filled with 1.5 ml of 95% methanol. The plate was mixed for 2 min, incubated in the dark at 4°C for 24 and 48 h, respectively, for chlorophyll a and b and secondary metabolites (phenolic compounds, flavonoids and tannins). After incubation and

centrifugation (5 min at 4500 rpm), chlorophyll a and b were measured by transferring 100 µl of the supernatant to new microplates and measuring absorbance at 470, 652 and 666 nm.

For phenolic compounds, 200 µl reaction mixture contained 20 µl supernatant, 40 µl Folin reagent (10% v/v) and 0.10 mM sodium hydrogen carbonate ($NaHCO_3$). The mixture was allowed to stand for 2 hours at room temperature for color development. Then the absorbance was measured at 760 nm.

Flavonoids were determined with the reaction mixture (final volume 200 µL) containing 25 µL of methanolic extract, 7.25 µM sodium nitrite ($NaNO_2$), 0.11 µM aluminum chloride ($AlCl_3$) and 0.02 mM sodium hydroxide (NaOH). The mixture was homogenised for 1 minute and the absorbance was measured at 595 nm. For tannins, the reaction mixture (final volume of 100 µl) contained 50 µl of methanolic extract and 6.57 µmol of vanillin[4]. The mixture was left in the dark for 15 minutes and the absorbance was measured at 500 nm.

The concentrations of total phenolic compounds, flavonoid and tannin were calculated using standard gallic acid and catechin curves, respectively.

Figure S1: MWCNT characterization (A) observed by transmission electron microscope and (B) powder Raman scattering spectrum obtained using a 633 nm wavelength laser

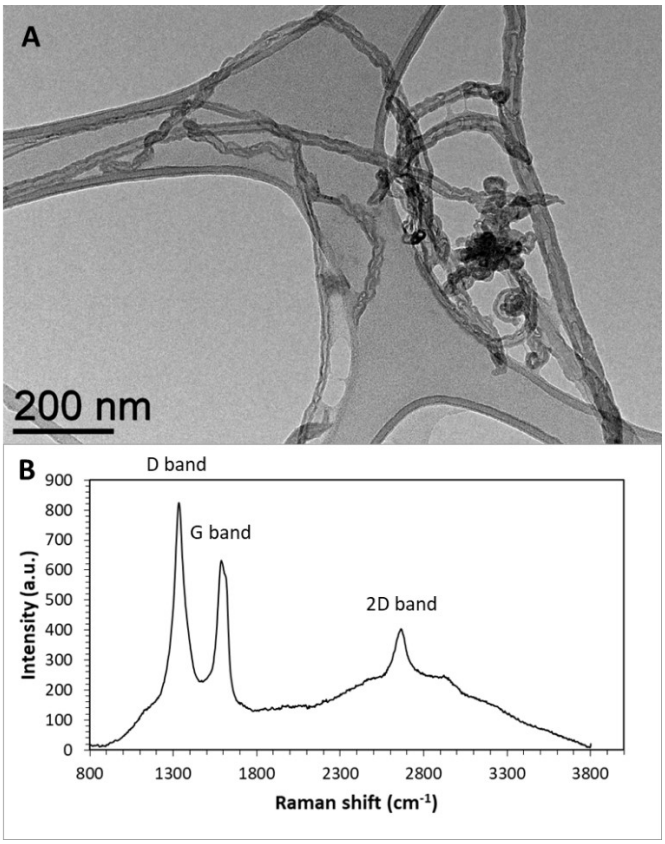


Figure S2: rRNA 16S, nosZ, nirK, AmoA-AOA and AmoA-AOB number of copies at the beginning of the exposure for the 3 CNT modalities (0 = 0 mg.kg^{-1}, 0.1 = 0.1 mg.kg^{-1}, 10 = 10 mg.kg^{-1}) as determined by qPCR. Same lowercase letters indicate treatments that do not differ significantly (p-value > 0.05) following an ANOVA.

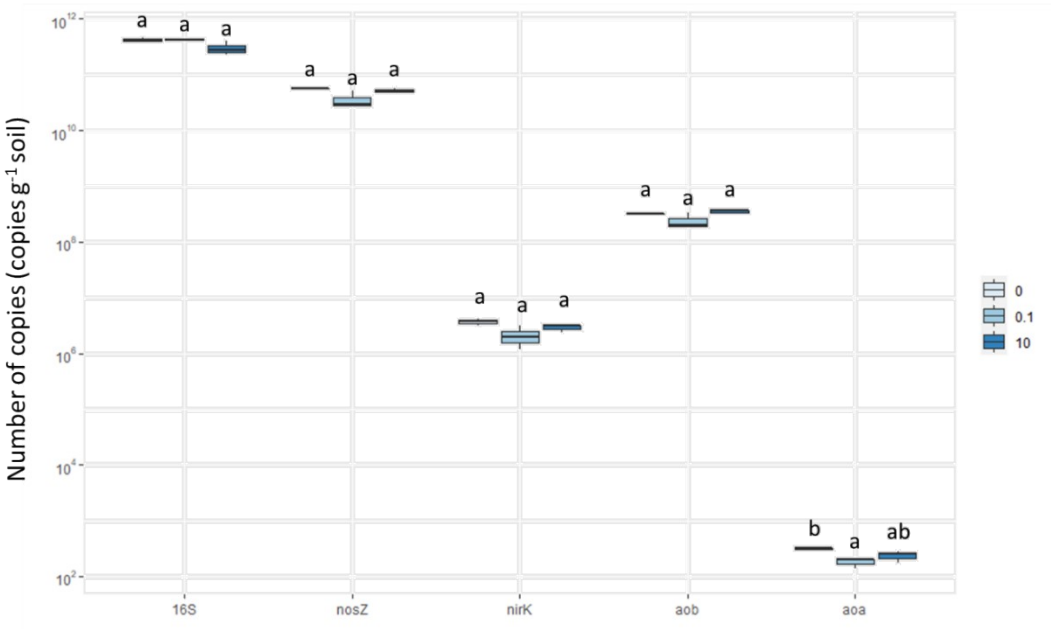


Figure S3: rRNA 16S, nosZ, nirK, amoA-AOA and amoA-AOB number of copies at the end of exposure for the 3 CNT modalities (0 = 0 mg.kg^{-1}, 0.1 = 0.1 mg.kg^{-1}, 10 = 10 mg.kg^{-1}) as determined by qPCR. Same lowercase letters indicate treatments that do not differ significantly (p-value > 0.05) following an ANOVA.

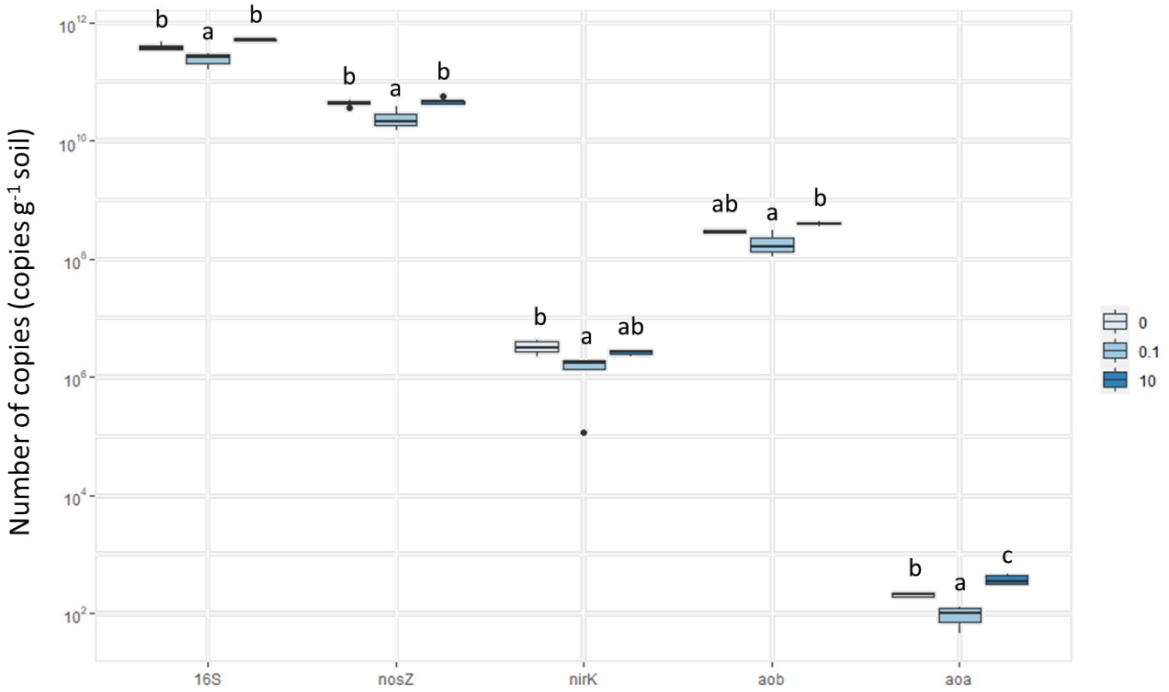


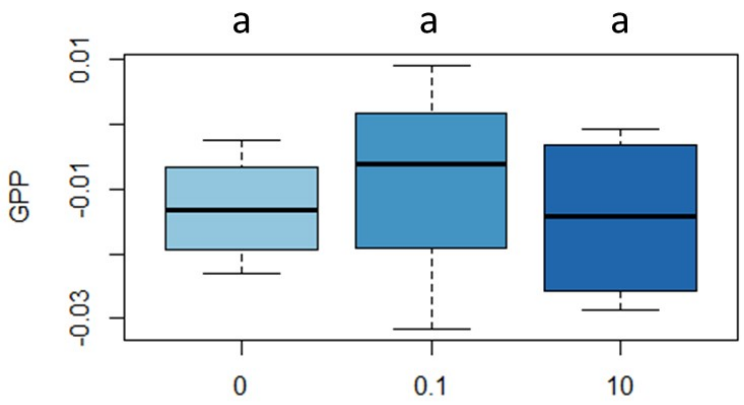
Table S1: Soil physico-chemical characterization after exposure. Average and standard deviation for clay, silt, sand, C/N, N, organic C (Corg) and pH for the 3 CNT modalities (0 = 0 mg kg^{-1}, 0.1 = 0.1 mg kg^{-1}, 10 = 10 mg kg^{-1}). Same lowercase letters indicate treatments that do not differ significantly (p-value > 0.05) following an ANOVA (pH, loam, C/N, N and C) or Kruskal-Wallis test (clay, sand and Corg) (n = 5)

CNT concentration	pH	Clay (%)	Silt (%)	Sand (%)	Corg (%)	N (%)	C (%)	C/N
0	5.1 ± 0.1 **a**	1.5 ± 0.1 **a**	12.7 ± 0.6 **a**	85.8 ± 0.6 **a**	1.3 ± 0.2 **a**	0.04 ± 0.005 **a**	0.5 ± 0.1 **a**	13.4 ± 0.9 **a**
0.1	5.0 ± 0.1 **a**	1.4 ± 0.1 **a**	11.6 ± 1.0 **a**	87.0 ± 1.0 **a**	1.3 ± 0.04 **a**	0.04 ± 0.006 **a**	0.5 ± 0.1 **a**	14.0 ± 0.7 **a**
10	5.0 ± 0.1 **a**	1.2 ± 0.1 **a**	13.5 ± 1.2 **a**	85.3 ± 1.2 **a**	1.5 ± 0.1 **a**	0.04 ± 0.004 **a**	0.58 ± 0.04 **a**	14.1 ± 0.4 **a**

Table S2: Elemental plant concentrations (measured by ICP-AES, in $mg.kg^{-1}$ DW) in maize leaves for the 3 CNT modalities (0 = 0 $mg.kg^{-1}$, 0.1 = 0.1 $mg.kg^{-1}$, 10 = 10 $mg.kg^{-1}$) after a six week-exposure in soil. Same lowercase letters indicate treatments that do not differ significantly (p-value > 0.05) following an ANOVA (B, Ca, Cd, Fe, Mg, Mn, Ni, P, Pb and Zn) or Kruskal-Wallis test (C, Na and S) (n = 5)

[CNT]	B	Ca	Cd	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	S	Zn
0	4.9 ± 0.7 a	1238.9 ± 91.6 a	0.01 ± 0.00 a	0.61 ± 0.02 a	5.83 ± 0.58 b	3642 ± 339 a	811.4 ± 8.0 a	16.0 ± 2.9 ab	16.0 ± 2.9 ab	0.12 ± 0.03 a	1321.1 ± 44.4 b	0.08 ± 0.01 a	189.7 ± 23.2 a	6.62 ± 1.54 a
0.1	5.5 ± 0.4 a	1370.6 ± 90.3 a	0.01 ± 0.00 a	0.61 ± 0.09 a	3.97 ± 0.07 a	4391 ± 801 b	962.2 ± 93.4 b	24.7 ± 4.4 a	12.4 ± 3.0 a	0.11 ± 0.02 a	1812.5 ± 160.3 a	0.09 ± 0.03 a	186.9 ± 19.0 a	11.18 ± 2.49 b
10	7.9 ± 0.5 b	1274.4 ± 63.5 a	0.01 ± 0.00 a	0.59 ± 0.05 a	5.27 ± 0.53 b	3646 ± 648 a	957.5 ± 42.6 b	19.7 ± 3.8 a	27.9 ± 5.8 b	0.09 ± 0.02 a	1399.5 ± 163.2 b	0.08 ± 0.02 a	203.5 ± 15.6 a	10.45 ± 1.66 b

Figure S4: Gross Primary Production (GPP in $mg_{CO2}.m^{-2}.h^{-1}$) of microcosms after 6 weeks of exposure to the 3 CNT modalities (0 = 0 $mg.kg^{-1}$, 0.1 = 0.1 $mg.kg^{-1}$, 10 = 10 $mg.kg^{-1}$). Same lowercase letters indicate treatments that do not differ significantly (p-value > 0.05) following an ANOVA (n=5).



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