Supporting Information

Carbon-based nanomaterials alter the composition of the fungal endophyte community in rice (*Oryza sativa* L.)

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SI Text 1. Phytohormone measurement

After 20-day CNM exposure, the content of seven different phytohormones, namely indole-3-acetic acid (IAA), zeatin riboside (ZR), dihydrozeatin riboside (DHZR), brassinolide (BR), abscisic acid (ABA), gibberellic acid3 (GA3) and gibberellic acid4 (GA4) in rice shoots upon three typical CNMs exposure were measured. Briefly, 1.5 g of fresh rice shoot tissues was weighed and ground thoroughly in cold methyl alcohol. Plant homogenates were then incubated at 4 °C for 4 hours, and centrifuged at 4000 rpm for 15 minutes. The supernatant was subsequently passed through C18 Sep-Pak cartridges (Waters Corp., Millford, MA, USA), concentrated by nitrogen, and diluted 5000 folds with phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween-20 and 0.1% (w/v) gelatin. Phytohormones were determined by icELISA protocol simplified by Zhao et al.[1] Briefly, the microtiter plate was coated with coating antigen and coating buffer for 3 h at 37 ◦C, then the plate was washed with PBS for four times. After blocked with 3% non-fat dry milk in PBS for 30 min at 37 ◦C and washed again with PBST, the plate was then added with standard substance, tested plant sample and antibody, respectively. Plates were then washed with PBST after incubated for 0.5 h at 37 ◦C, and then the substrate solution was added into the plate. The reaction was stopped by adding 100 mL of 2 M H_2SO_4. Absorbance was read at 492 nm in the microplate reader. All relative reagents were provided by the Center of Crop Chemical Control, China Agricultural University. The concentrations of phytohormones were calculated using the formulas reported by Weiler et al.[2]

SI Text 2. Plant DNA extraction

The DNA of fresh rice shoots was extracted using Hi-DNAsecure Plant Kit as the procedures below.

1. 100 mg fresh plant sample were thoroughly ground in liquid nitrogen, homogenized with 400 μL of 400 Buffer GFA and 6 μL of RNase A (10 mg/mL); the mixture was vortexed for for 1 minute and incubated for 10 minutes.

2. 130 μL of Buffer LP2 was added into samples, shaken for 1 minute by vortex, then the mixture was centrifuged for 5 minutes at 12000 rpm (approximately 13400×g), and the supernatant was transferred to a clean tube.

4. 1.5 times volume of Buffer LP3 was added to the supernatant, and thoroughly mixed for 1 minute by vortex. All mixtures were filtered in the Spin Column CB3, and centrifuged for 30 second at 12000 rpm (approximately 13,400×g). After discarding the filtrate, the Spin Column CB3 was placed into the Collection Tube.

5. 600 μL of Buffer PW was added to the Spin Column CB3 to wash the membrane, and then the Spin Column CB3 was placed into the Collection Tube after removing filtrate.

6. Repeat the step 5, and then membrane was dried at ambient temperature.

7. 200 μL of TB buffer was added in The Spin Column CB3, which was placed into a 1.5 ml centrifuge tube. After 5-minute incubation at ambient temperature for 5 minutes, the sample was centrifuged at 12000 rpm (approximately 13400×g) for 2 minutes. All the filtrate was finally collected into 1.5 ml centrifuge tubes.

SI Text 3. Characterization of CNMs

The diameter of layered rGO was 0.5-3.0 μm and the thickness of the single layer varied from 0.5 to 3.7 nm (**Figure S1A and S1D**). For MWCNTs, the outer cross-sectional diameter of MWCNTs was approximately 25 nm (**Figure S1B and S1E)**. The diameter of C_{60} ranged from 30 to 60 nm with a spherical shape (**Figure S1C and S1F).** Both C_{60} and rGO dispersed well in DI water and 1/2 Kimura strength nutrient solution, while MWCNTs tended to agglomerate and

tangle. Nutrient solution did not significantly alter the CNM dispersion as compared to DI water. The zeta potential of the C_{60}, MWCNTs and rGO in DI water was -13.17±0.55, -15.87±0.67 and -9.65±1.73 mV, respectively, and the conductivity was 4.85±0.11, 5.22±2.42 and 12.66±6.18 μS/cm, respectively (**Table S1**). In 1/2 Kimura nutrient solution, the zeta potential of the C_{60}, MWCNTs and rGO was -22.33±2.06, -15.87±1.21 and -12.67±1.73 mV, respectively. In comparison with the conductivity of three CNMs in DI water, 1/2 Kimura nutrient solution significantly increased the conductivity of rGO, MWCNTs and C_{60} to 24.23±1.97, 61.63±0.15 and 59.6±0.40 μS/cm, respectively. Additionally, the oxygen content of rGO, MWCNTs and C_{60} was 20.56%, 5.83% and 2.93%, respectively (**Table S2**).

SI Text 4. Alpha diversity indices calculation in endophytic fungal communities

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The Shannon index was calculated as the formula:

$H=-\sum_{i=1}^{R} p_i ln p_i$

Where Pi represents the proportion of species i relative to the total number of species.

And the Simpson index was computed as followed:

$D=\frac{1}{\sum_{i=1}^{S} p_i^2}$

Where P_i is the proportion of species i relative to the total number of species, and s represents the total number of individuals of all species.

The Chao1 index was calculated as the formula:

$$S_{Chao1}=S_{obs}+\frac{F_1(F_1-1)}{2(F_2+1)}$$

Where F_1 represents the count of singletons, F_2 are doubleton counts, and Sobs represents the observed species number.

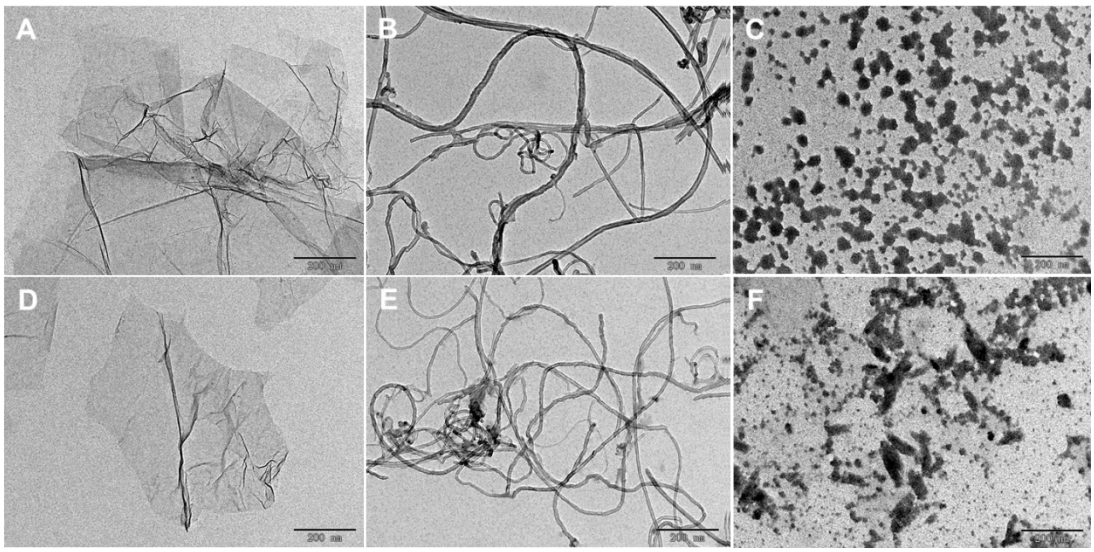

Figure S1. TEM images of MWCNTs (A), rGO (B) and C_{60} (C) in DI water and MWCNTs (A), rGO (B) and C_{60} (C) in 1/2 strength Kimura nutrient solution at 50 mg/L. The scale in each panel represents 200 nm.

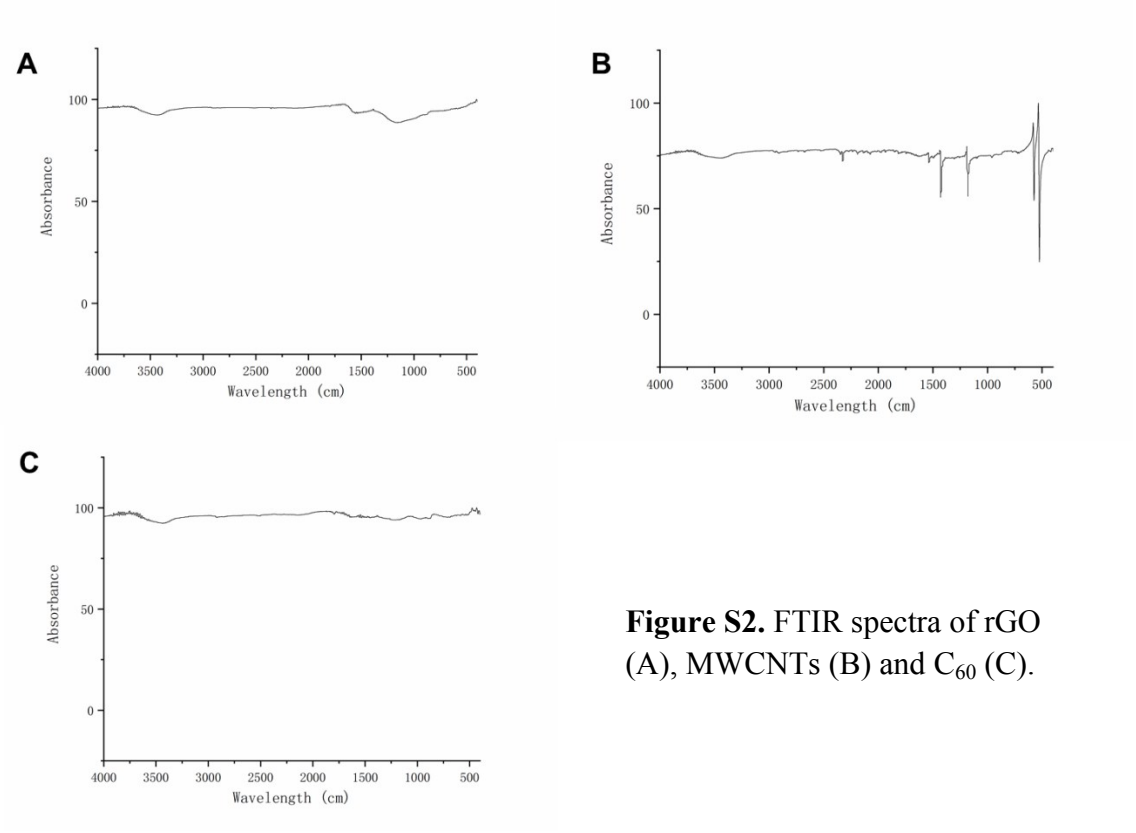

Figure S2. FTIR spectra of rGO (A), MWCNTs (B) and C_{60} (C).

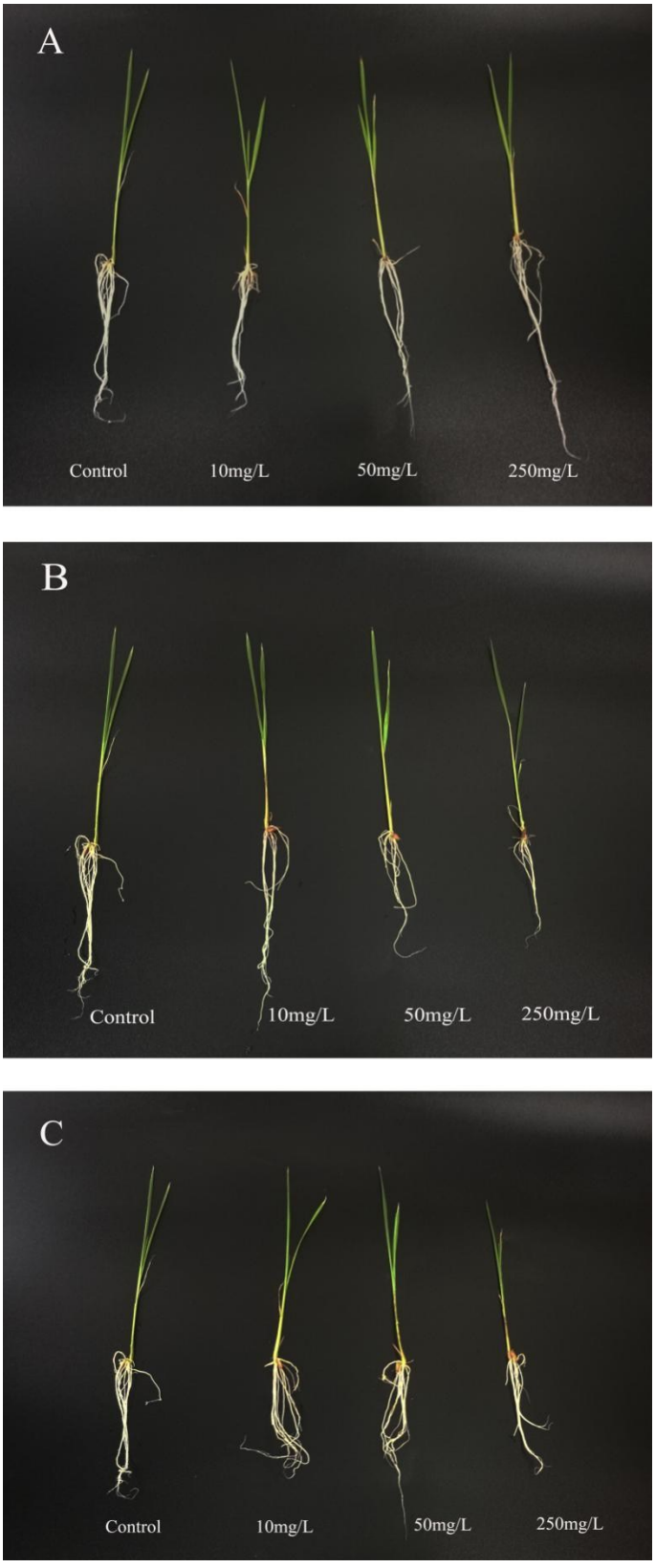

Figure S3. Phenotypic images of rice seedlings exposed to rGO (A), MWCNTs (B), and C_{60} (C) at 10, 50 and 250 mg/L.

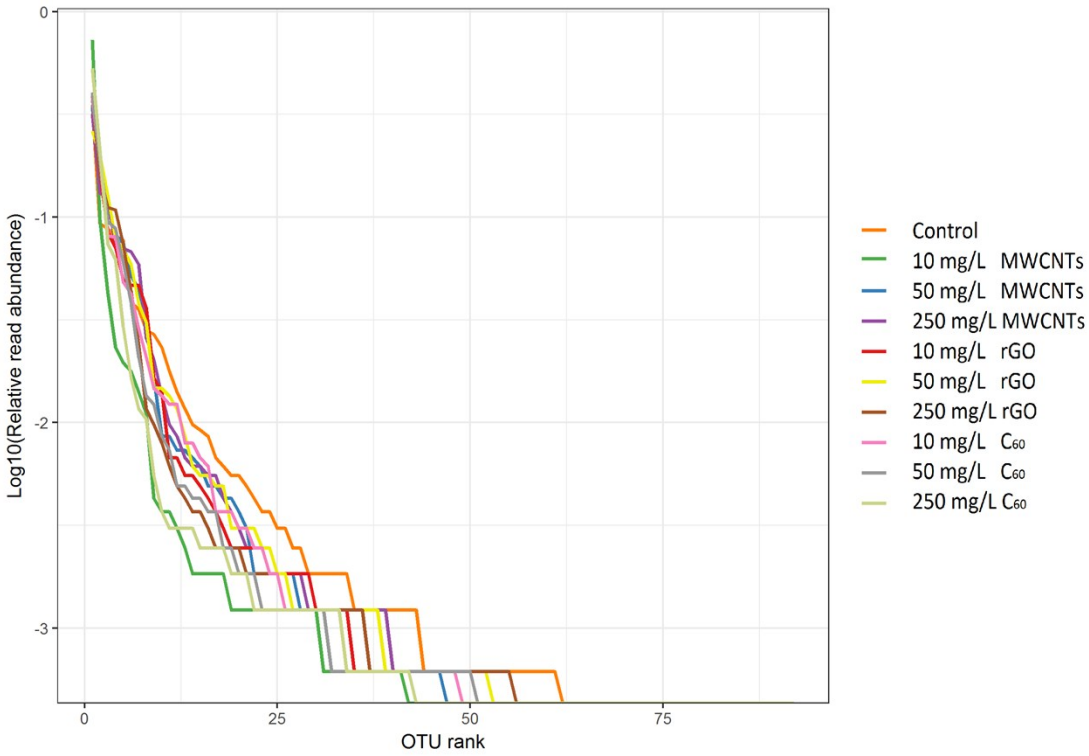

Figure S4. Rank abundance distribution curves of endophytic fungi across all the CNM treatments.

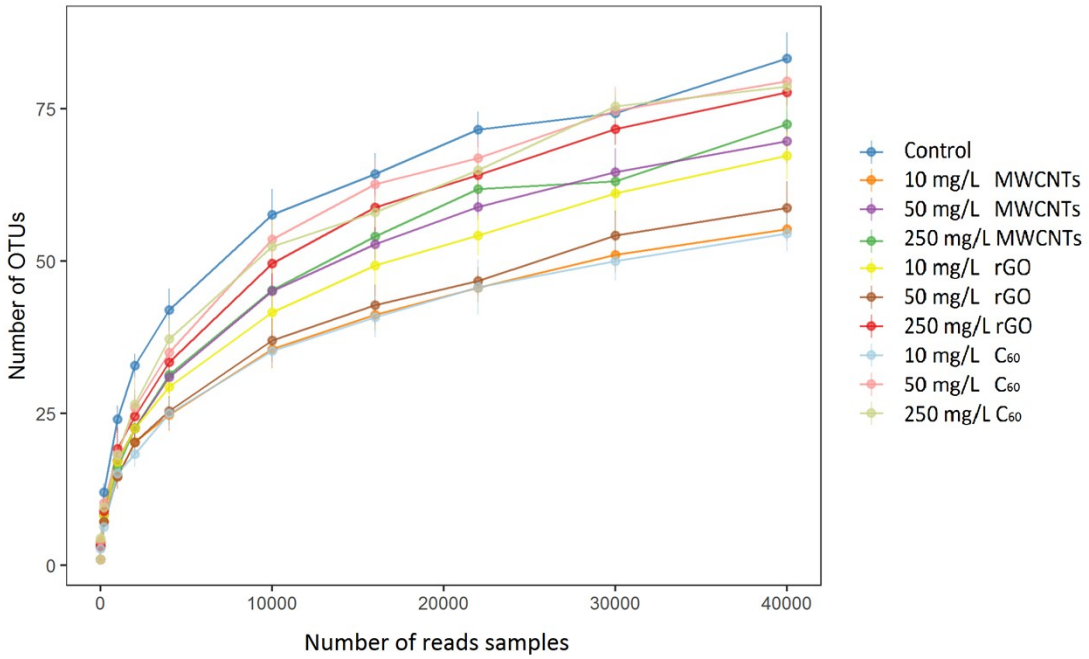

Figure S5. Rarefaction curves of OTUs clustered at 97% sequence identity among the endophytic fungi across all the CNM treatments.

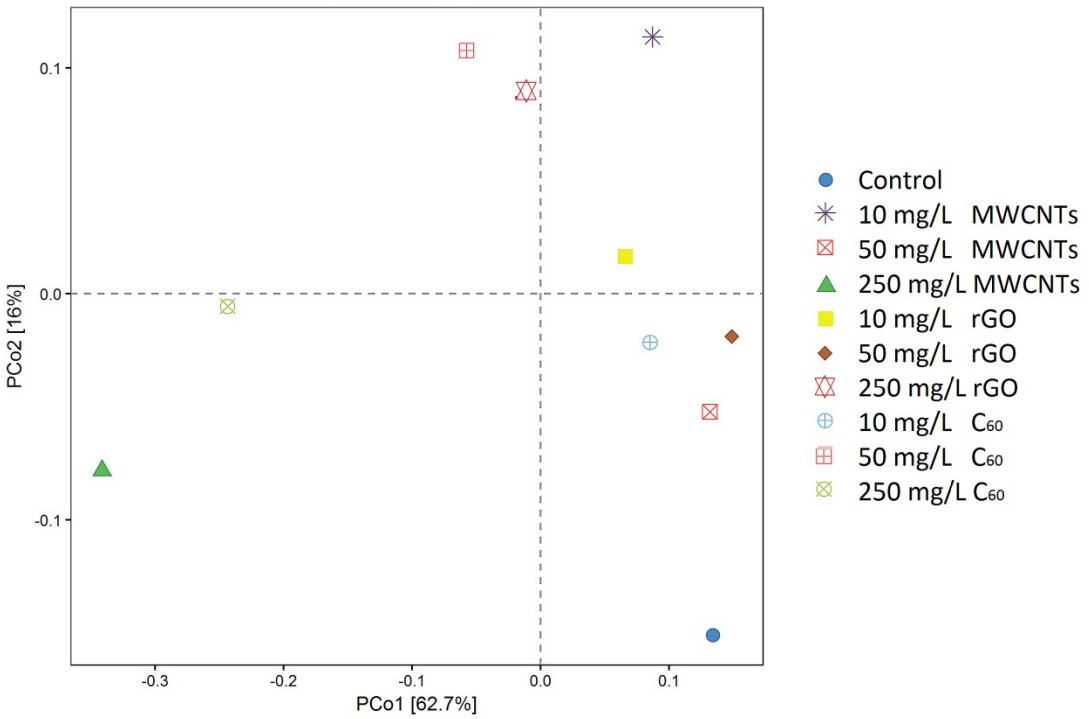

Figure S6. PCoA plot of endophytic fungi upon exposure to different types of CNMs at three doses based on weighted unifrac distance. Principal Co-ordinate (PCo) 1 and 2 represents 62.7 and 16 % of the variance, respectively.

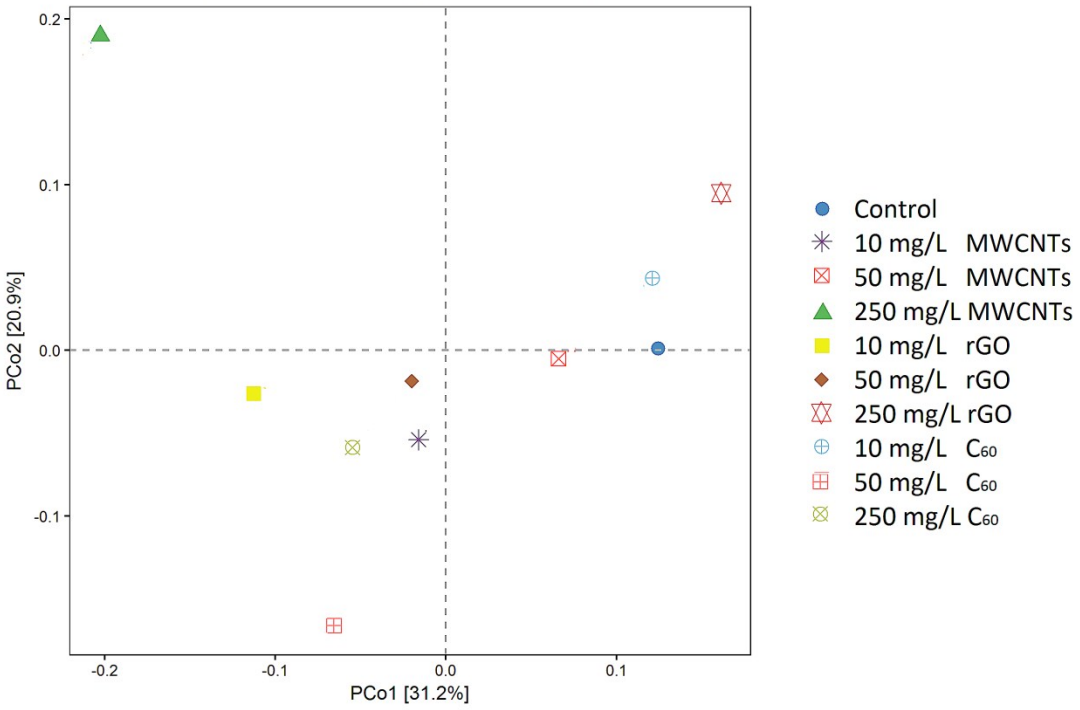

Figure S7. PCoA plot of rice samples exposed to three CNMs at different doses based on unweighted unifrac distance. Principal Co-ordinate (PCo) 1 and 2 represents 31.2 and 20.9 % of the variance, respectively.

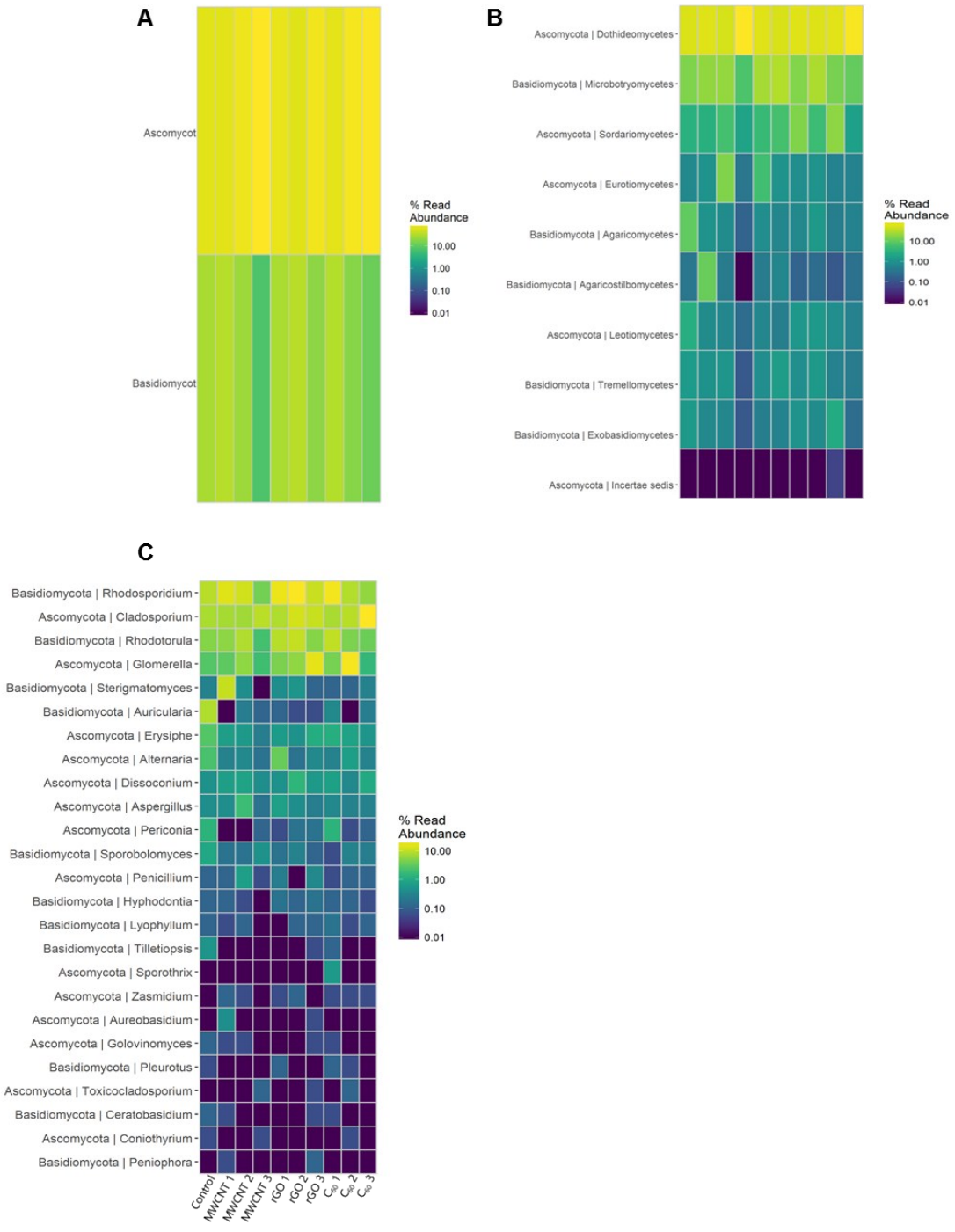

Figure S8. Heatmaps of rice shoots at the level of phyla (A), classes (B) and genera (C) upon exposure to different types of CNMs at three doses. The number 1, 2 and 3 represents the low (10 mg/L), medium (50 mg/L) and the high concentration (250 mg/L), respectively.

Table S1. Conductivity and zeta potential of 50 mg/L CNMs

	DI water		Nutrient solution (pH=6.5)	
Treatment	**Zeta potential (mV)**	**Conductivity (µS/cm)**	**Zeta potential (mV)**	**Conductivity (µS/cm)**
rGO	-9.65±1.73^{c}	12.66±6.18^{a}	-12.67±1.37^{b}	24.23±1.97^{b}
MWCNTs	-15.87±0.67^{a}	5.22±2.42^{a}	-15.47±1.21^{b}	61.63±0.15^{a}
C_{60}	-13.17±0.55^{b}	4.85±0.11^{a}	-22.33±2.06^{a}	59.6±0.40^{a}

Note: Different letters are statistically different at $p<0.05$.

Table S2. Element C and O contents of CNMs

	rGO		MWCNTs		C_{60}	
Element	**Weight percent (%)**	**Atomic percent (%)**	**Weight percent (%)**	**Atomic percent (%)**	**Weight percent (%)**	**Atomic percent (%)**
C	**74.36**	**74.36**	**92.39**	**94.17**	**96.13**	**97.07**
O	**25.64**	**25.64**	**7.61**	**5.83**	**3.87**	**2.93**
Total	**100**	**100**	**100**	**100**	**100**	**100**

Table S3. The Composition of 1/2 Kimura nutrient solution.

Composition	Concentration (mmol/L)
KNO_3	0.091
$Ca(NO)_2 \cdot 4H_2O$	0.183
$MnSO_4 \cdot H_2O$	0.274
KH_2PO_4	0.1
$(NH_4)_2SO_4$	0.183
$MnSO_4 \cdot H_2O$	1×10^{-3}
H_3BO_3	3×10^{-3}
$(NH_4)_6Mo_7O_{24} \cdot 5H_2O$	1×10^{-3}
$ZnSO_4 \cdot 7H_2O$	1×10^{-3}
$CuSO_4 \cdot 5H_2O$	2×10^{-4}
Fe-EDTA	6×10^{-2}

Table S4. The content of brassinolide (BR), dihydrozeatin riboside (DHZR), gibberellin acid4 (GA4) in rice shoots

Treatments	BR (ng/g. FW)	DHZR (ng/g. FW)	GA4+ (ng/g.FW)
Control	8.07±0.56^{a}	7.97±1.17^{ab}	7.43±0.94^{abc}
10 mg/L C_{60}	8.00±0.45^{a}	6.91±0.62^{ab}	7.78±1.18^{abc}
50 mg/L C_{60}	7.62±1.21^{a}	9.79±1.41^{ab}	7.21±0.29^{abc}
250 mg/L C_{60}	6.60±0.60^{a}	6.05±0.28^{b}	6.71±0.62^{c}
10 mg/L MWCNTs	7.66±1.24^{a}	6.81±0.59^{ab}	7.00±0.78^{ab}
50 mg/L MWCNTs	7.75±0.64^{a}	6.97±0.43^{ab}	7.33±1.55^{abc}
250 mg/L MWCNTs	7.04±0.75^{a}	6.15±0.76^{b}	5.71±0.58^{bc}
10 mg/L rGO	7.19±1.01^{a}	6.76±0.49^{ab}	5.94±0.98^{bc}
50 mg/L rGO	8.05±0.69^{a}	8.76±2.50^{a}	8.87±0.96^{a}
250 mg/L rGO	7.98±0.45^{a}	6.94±2.31^{ab}	7.30±1.19^{abc}

Note: The data are the averages of three replicates ± SE. Values followed by different letters are significantly different at $p < 0.05$.

Table S5. Alpha diversity indices of rice endophytic fungal communities exposed to three CNMs at different doses

Treatments	Reads	Observed phylotypes	Shannon	Simpson	Chao1	Coverage
Control	53226	62	2.55	0.84	79.1	0.99
10 mg/L MWCNTs	47064	46	2.30	0.83	54.25	0.99
50 mg/L MWCNTs	58290	56	2.41	0.84	67.33	0.99
250 mg/L MWCNTs	42992	42	1.25	0.46	47.08	0.99
10 mg/L rGO	55508	53	2.23	0.80	81.5	0.99
50 mg/L rGO	533716	53	2.42	0.85	61.08	0.99
250 mg/L rGO	52850	56	2.14	0.79	71.83	0.99
10 mg/L C_{60}	49740	48	2.20	0.80	59.67	0.99
50 mg/L C_{60}	50982	54	2.06	0.78	79.3	0.99
250 mg/L C_{60}	55292	42	1.65	0.67	44.77	0.99

References:

1. J. Zhao, G. Li, G. X. Yi, B. M. Wang, A. X. Deng, T. G. Nan, Z. H. Li and Q. X. Li, Comparison between conventional indirect competitive enzyme-linked immunosorbent assay (icELISA) and simplified icELISA for small molecules, *Anal Chim Acta*, 2006, **571**, 79-85.
2. E. W. Weiler, P. S. Jourdan and W. Conrad, Use of Immunoassay in Plant-Science .21. Levels of Indole-3-Acetic-Acid in Intact and Decapitated Coleoptiles as Determined by a Specific and Highly Sensitive Solid-Phase Enzyme-Immunoassay, *Planta*, 1981, **153**, 561-571.