Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information for:

Impacts of a Porous Hollow Silica Nanoparticle-Encapsulated Pesticide Applied to Soils on Plant Growth and Soil Microbial Community

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Table S1. List of the soil properties.

Analysis	**Result**
pH	7.0
C_{om} (%)	4.1
P (ppm)	152
K (ppm)	357
Mg (ppm)	291
Al (ppm)	759
Na (ppm)	14
CEC (meq/100 g)	16.9
Water Holding Capacity at 1/3 bar (%)[a]	22.9
Water Holding Capacity at 15 bars (%)[a]	13.26
Available Water Capacity (%)[a]	9.69
Sand (%)	31
Silt (%)	34
Clay (%)	35

Note: C_{om}: organic matter concentration, P: phosphate, K: potassium, Mg: magnesium, Al: aluminum, Na: sodium, CEC: cation exchange capacity, [a] USDA no. 42 method.

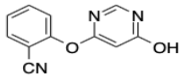
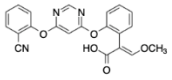
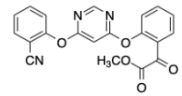
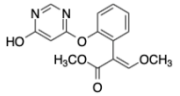
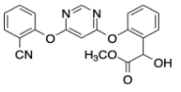
Matrix Effects on the Azoxystrobin Detection. To validate the azoxystrobin measurements from the biomass extract, recovery validation was determined by spiking control plant biomass after homogenization with azoxystrobin in different concentration ranges. The low-range and high-range concentration comprised in spiking azoxystrobin in homogenized biomass to a final concentration of 10 ppb and 500 ppb, respectively. The recoveries (N = 3) for the low range (RL) and high range (RH) can be found in Table S2.

Table S2. Azoxystrobin recoveries in homogenized biomass for low- and high-range concentrations.

Range	Sample ID	Recovery (%)	Average (%)	Standard Deviation (%)
Low	RL1	102	105	2.5
	RL2	108		
	RL3	106		
High	RH1	107	107	1.0
	RH2	106		
	RH3	108		

qPCR Thermal Cycling Parameters. The thermal cycling protocol for bacteria was as follows: denaturation at 95℃ for 3 minutes followed by 40 cycles of amplification (10 s at 95 ℃, 30 s at 50 ℃, 60 s at 72 ℃). For fungi, the denaturation step took place at 95℃ for 3 minutes followed by 40 cycles of amplification (20s at 95℃, 30s at 50℃, 90s at 72℃).

Table S3. List of suspected metabolites derived from azoxystrobin in tomato plants for different treatments and data points. The peaks were categorized by low, medium and high based on the comparison among them and do not have quantification purposes.

Suspected metabolites of azoxystrobin in *Solanum lycopersicum*							
Structure							
Formula		$C_{11}H_7N_3O_2$	$C_{21}H_{15}N_3O_5$	C_7H_5NO	$C_{20}H_{13}N_3O_5$	$C_{15}H_{14}N_2O_5$	$C_{20}H_{15}N_3O_5$
Ion m/z ratio		214.06166	390.10902	120.04494	376.09337	303.09813	378.10902
Treatment	**Replicate**	**Peak Intensity**					
Azo [Day 10]	1	Low	Low	Not detected	Not detected	Low	Low
	2	Low	Low	Not detected	Not detected	Low	Low
	3	Low	Low	Not detected	Not detected	Low	Low
Azo [Day 20]	1	Low	Low	Not detected	Low	Low	Low
	2	Low	Low	Not detected	Low	Low	Low
	3	Low	Low	Not detected	Low	Low	Low
Azo@PHSN [Day 10]	1	Medium	Medium	Not detected	Low	Low	Medium
	2	Medium	Medium	Not detected	Low	Low	Medium
	3	Medium	Medium	Not detected	Low	Low	Medium
Azo@PHSN [Day 20]	1	High	High	Low	High	High	High
	2	High	High	Low	High	High	High
	3	High	High	Low	High	High	High

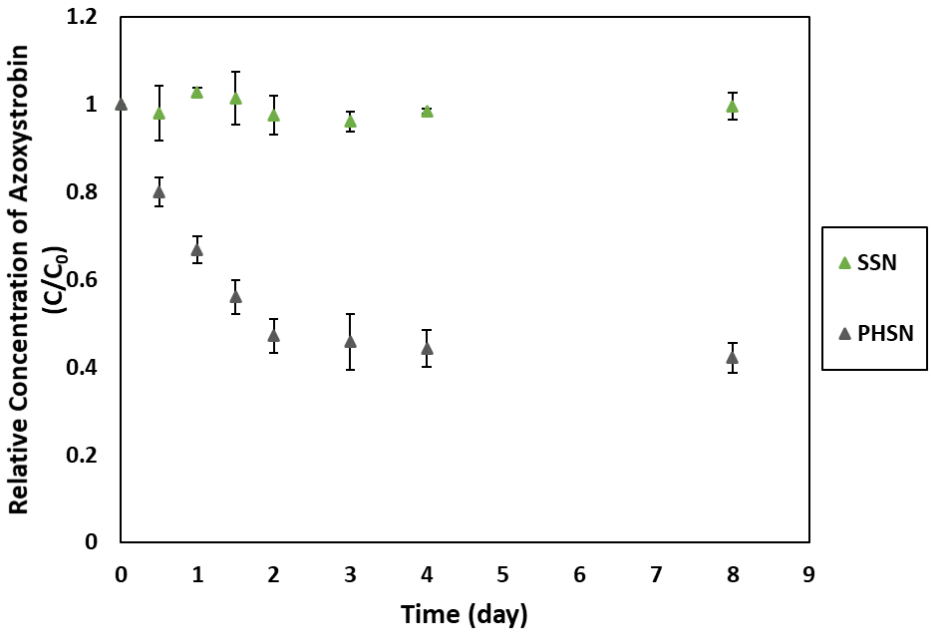


Figure S1. Relative concentration (C/C_0) of azoxystrobin in solution (20% v/v methanol) phase over time using solid SiO_2 NPs (SSN) and porous hollow SiO_2 NPs (PHSN) as nanocarriers.

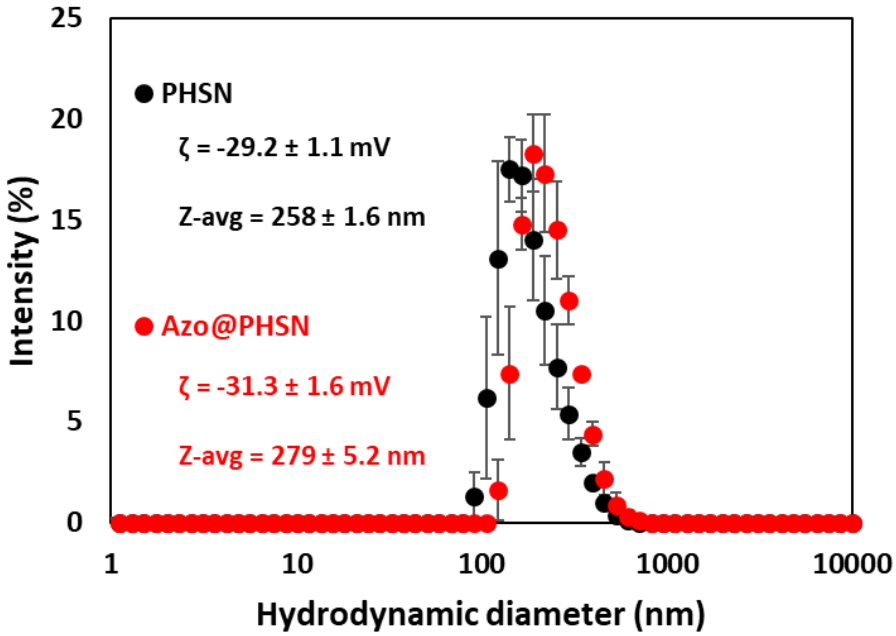


Figure S2. Hydrodynamic diameter distribution, zeta average and zeta potential of PHSN (●) and Azo@PHSN (●) measured at pH 6.5 and ionic strength 1 mM (NaCl).

Pielou's evenness index (J') and Faith's Phylogenetic Diversity (PD). The Pielou's evenness index (*J'*)[1] is the numerical representation that quantifies how equal the microbial communities are in the sample. In other words, it is the mathematical measurement of the biodiversity in the sample. At day 10, there was no significant statistical difference among the bacterial communities for all treatments, based on the *J'* (Figure S5a). The variance was analyzed through Kruskal-Wallis pairwise testing and no p-value was below the $p < 0.05$ threshold. Similarly, at day 20 there was no significant statistical difference among the bacterial communities (Figure S5b). The p-values of the pairwise comparison between control and Azo treatments, and control and Azo@PHSN treatments had the lowest p-values overall, 0.15 and 0.20, respectively. Faith's phylogenetic diversity (PD) is another way to measure biodiversity mathematically[2]. It, however, revealed the same conclusions as for the *J'*: no statistical different could be determined among the bacterial communities with different treatments at days 10 (Figure S5c) and 20 (Figure S5d).

For fungi communities, at 10 days, three pairwise Kruskal-Wallis combinations of the *J'* indicated significant statistical difference, (1) day zero and Azo treatments, (2) day zero and PHSN treatments, and (3) day zero and Azo@PHSN treatments (Figure S5e), indicating that except for the day zero and control, all other treatments showed significant differences in the biodiversity when compared to the initial stage. After 20 days, however, the variance analysis of the *J'* indicated that only two combinations were statistically different, day zero and Azo@PHSN treatments, and control and Azo@PHSN treatments (Figure S5f), suggesting that the soil microbial community biodiversity differences tended to diminish as the days progressed. Faith's PD showed a different trend from the *J'* at day 10, where there were statistical changes only between Azo treatment and other three treatments: control, PHSN and Azo@PHSN (Figure S5g). At day 20, Faith's PD variance analysis showed representative differences in biodiversity between Azo and Azo@PHSN treatments, control and Azo@PHSN treatments (Figure S5h).

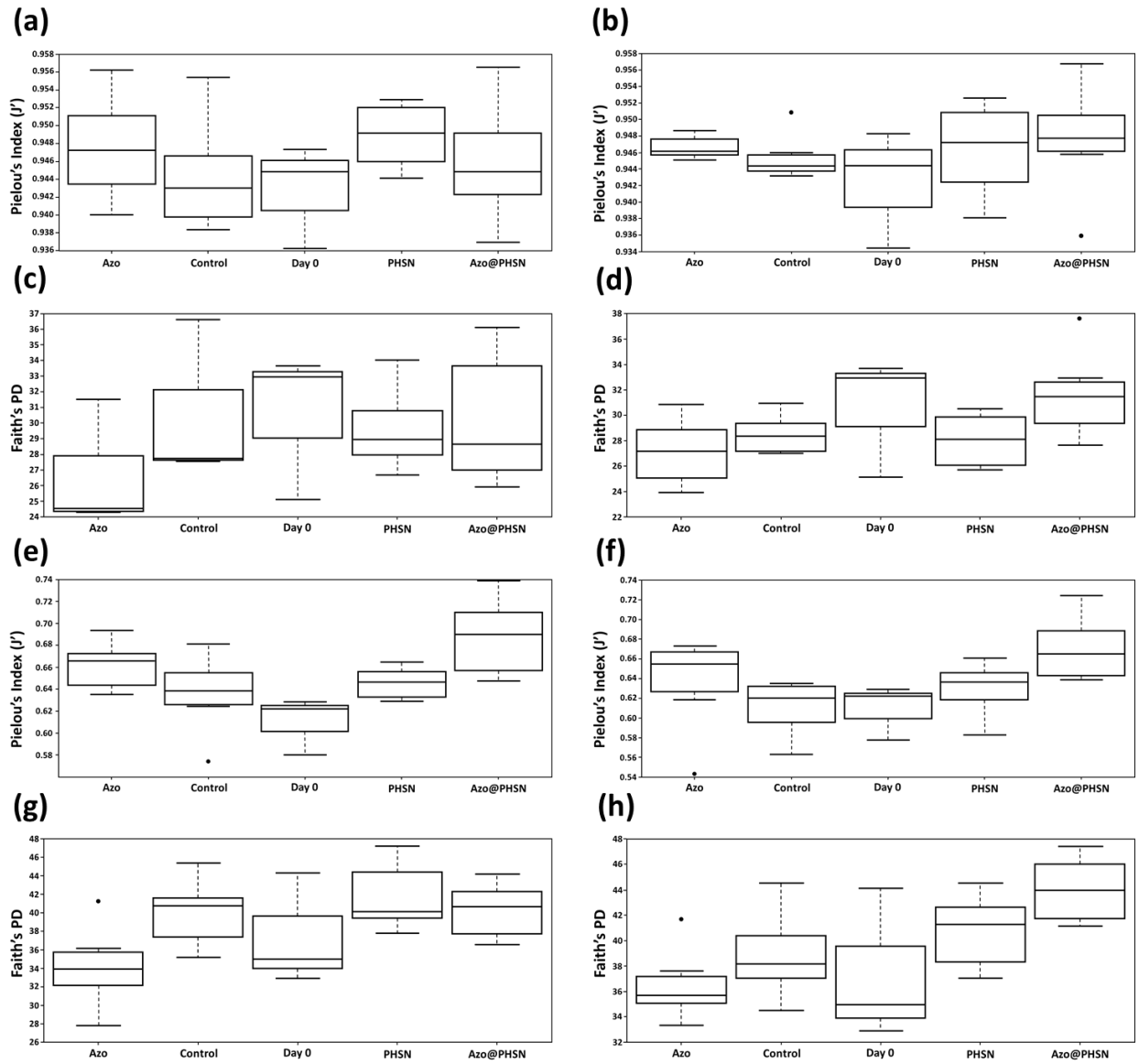


Figure S3. α-diversity boxplots for the bacterial and archaea communities represented by (a) Pielou's evenness index for samples at day 10, (b) Pielou's evenness index for samples at day 20, (c) Faith's PD for samples at day 10, and (d) Faith's PD for samples at day 20. α-diversity boxplots for the fungi communities represented by (e) Pielou's evenness index for samples at day 10, (f) Pielou's evenness index for samples at day 20, (g) Faith's PD for samples at day 10, and (h) Faith's PD for samples at day 20. The black dots above and below the error bars in some instances indicate outliers.

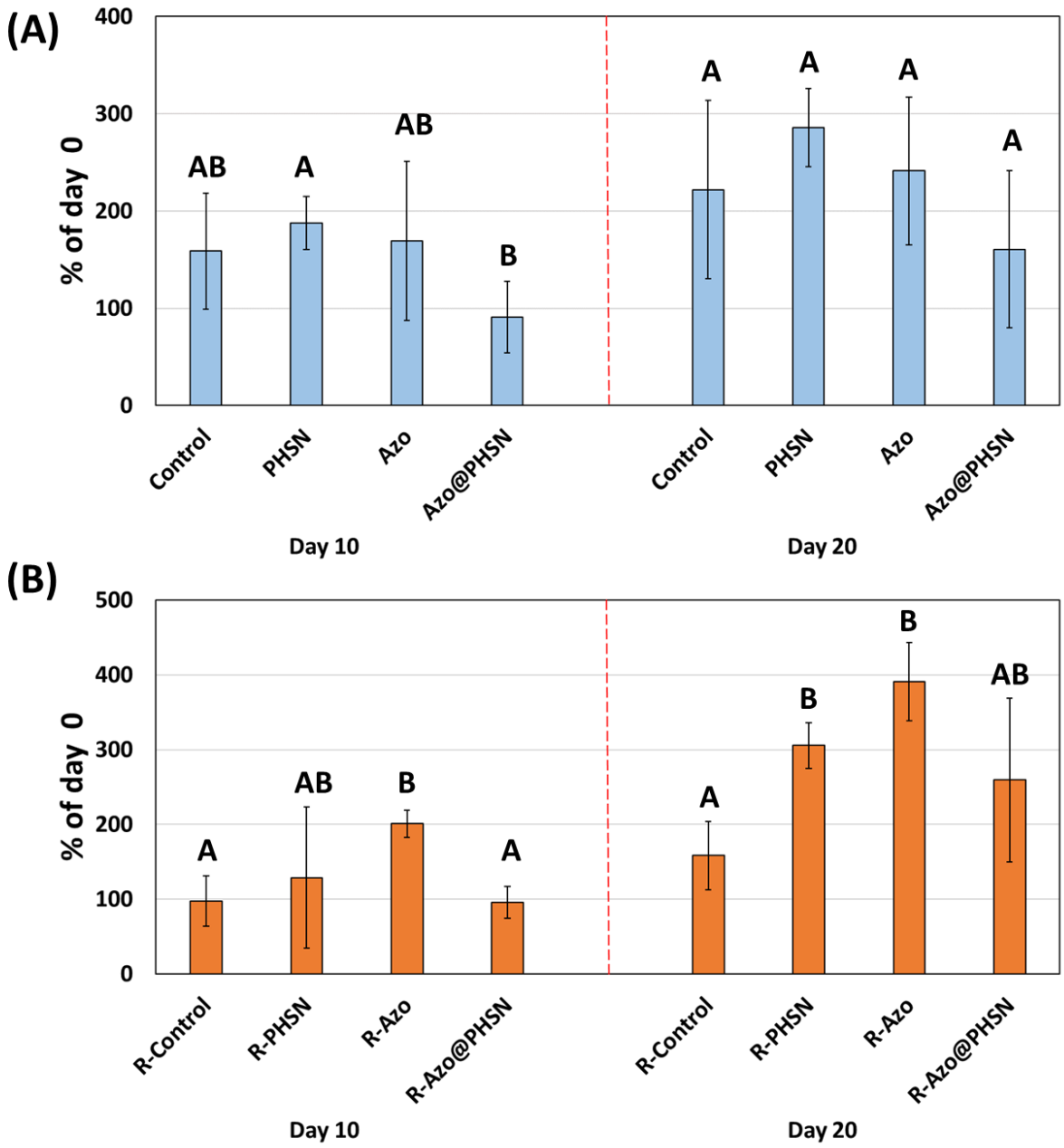


Figure S4. Percentage of day 0 soil microbial population in different treatments using qPCR targeting the gene 16S rRNA for total bacterial communities using (A) bulk soil and (B) soil loosely attached to the roots. Different letters (*A* and *B*) indicate significant statistical differences among samples (ANOVA one-way test, $p < 0.05$ threshold).

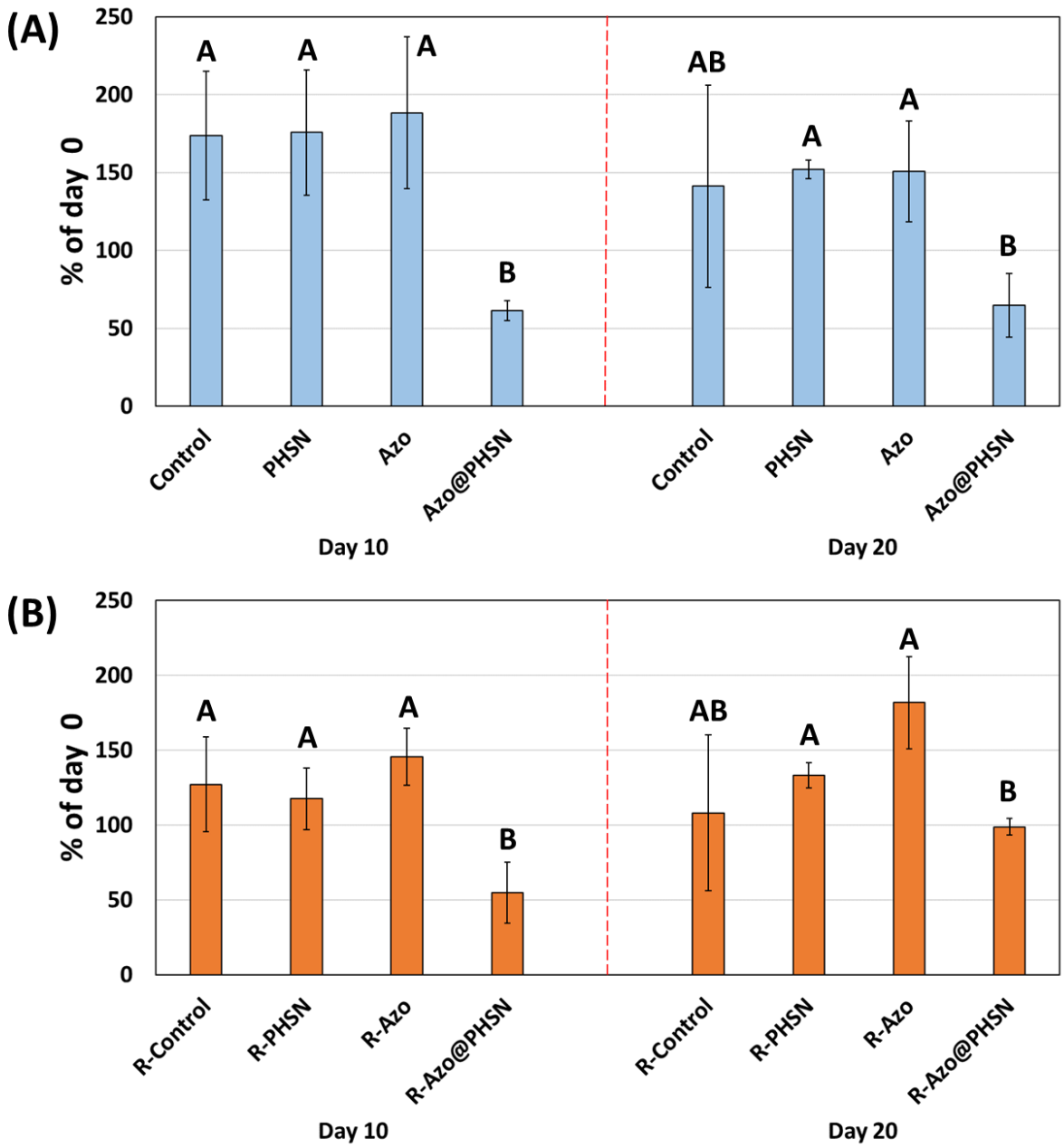


Figure S5. Percentage of day 0 soil microbial population in different treatments using qPCR targeting the gene 18S rRNA for total fungi communities using (A) bulk soil and (B) soil loosely attached to the roots. Different letters (*A* and *B*) indicate significant statistical differences among samples (ANOVA one-way test, $p < 0.05$ threshold).

REFERENCES

1. E. C. Pielou, The measurement of diversity in different types of biological collections, *Journal of theoretical biology*, 1966, **13**, 131-144.

2. D. P. Faith, Conservation evaluation and phylogenetic diversity, *Biological conservation*, 1992, **61**, 1-10.