Supporting Information

Antibiotic toxicity screening on seedling emergence: beyond traditional species

R. Silvana Cortés-Lagunes[a], Andrea-Lorena Garduño-Jiménez [b], Juan-Carlos Durán-Álvarez[a], Felicity C. T. Elder[a], Wenshi Bao [c], Joshua Greenwood[b], John H. Nightingale[b], Fan Zhang[c], Laura J. Carter [b,d]

[a.] Instituto de Ciencias Aplicadas y Tecnología, Universidad Nacional Autónoma de México, Circuito Exterior S/N, Ciudad Universitaria, 04510 Mexico City, Mexico.
[b.] School of Geography and Water@Leeds, Faculty of Environment, University of Leeds, LS2 9JT, UK.
[c.] College of Science, Nanjing Agricultural University, Nanjing 210085, China.

Table S1. Antibiotic abbreviations, purity and supplier details

No.	*Pharmaceutical*	*Abbreviation*	*Supplier and purity (UK)*	*Supplier and Purity (China)*	*Supplier and purity (Mexico)*
1	Azithromycin	AZI	Apollo Scientific (98%)	Aladdin (≥98%)	Sigma-Aldrich (94.7 %)
2	Ciprofloxacin	CPX	Sigma-Aldrich (98 %)	Aladdin (≥98%)	Sigma-Aldrich (94 %)
3	Chloramphenicol	CHL	Sigma-Aldrich (98 %)	Aladdin (≥98%)	-
4	Enrofloxacin	ERX	LGC Standards (98%)	Aladdin (≥98%)	-
5	Florfenicol	FLOR	Cayman chemical (98 %)	Aladdin (≥98%)	-
6	Lincomycin	LIN	Sigma-Aldrich (96 %)	Aladdin (≥98%)	-
7	Ofloxacin	OFX	LGC Standards (98%)	Aladdin (≥98%)	-
8	Oxytetracycline hydrochloride	OTC	Sigma-Aldrich (96.5 %)	Aladdin (≥98%)	Sigma-Aldrich (>98 %)
9	Sulfadimidine	SFD	-	Aladdin (≥99%)	-
10	Sulfamethazine	SFZ	LGC Standards (98%)	-	-
11	Trimethoprim	TRM	Sigma-Aldrich (98 %)	Aladdin (≥99%)	Sigma-Aldrich (98 %)
0	Reagent blank	Control	-	-	-

S1. Cleaning and sterilisation of petri dishes and seeds

Petri dishes

In the UK, petri dishes were washed with Decon 90 (Decon Laboratories, UK), rinsed with sterile and dried in a drying chamber. In Mexico regular soap was used to wash the Petri dishes (70.9 cm^2 surface area). They were thoroughly rinsed using sterile water In China disposable sterile Petri dishes were rinsed using sterile water. Following the sterile water rinse, all petri dishes were rinsed using methanol and air dried.

Seeds

In the UK the seeds were sterilised by soaking them in autoclaved water. In Mexico by placing them 10 mm away from a UVC Pen-Ray mercury lamp inside a dark box for 30 minutes. In China sterilisation was achieved by using hydrogen peroxide and the washed thoroughly with autoclaved deionised water (further details in Table S1).

Seed Type	*Hydrogen Peroxide Concentration*	*Soaking Time*	*Key Precautions*
Mung bean, Soybean	3%	≤20 minutes	Hard seed coat, strictly control the time
Rice, Wheat	3%	15 minutes	Rinse thoroughly to avoid liquid residue
Shasta daisy	1.5% - 2%	10 - 12 minutes	Air - dry to prevent mildew
Lettuce, Chinese Cabbage, Chicory	1.5% - 2%	10 - 15 minutes	Over - soaking may damage the embryo

Table S2. Sterilisation conditions for each seed in the China experiment

Table S3. Environmental concentrations of the target antibiotics reported in irrigation water and soil pore-water, compared with the nominal concentration used in this Tier I screening study (1 mg L^{-1}). Reported values represent dissolved-phase concentrations relevant to plant exposure.

Antibiotic	*Environmental concentration (mg L^{-1})*	*Exposure context*	*Concentration used in this study (mg L^{-1})*	*Reference*
Azithromycin	0.002 (maximum)	Reclaimed water used in agricultural irrigation	1	(1)
Ciprofloxacin	0.016	Pig slurry-impacted wastewater used in agricultural irrigation		(2)
Chloramphenicol	0.002 (average)	Reclaimed water used for greenhouse irrigation		(3)
	0.001	Reclaimed water used in agricultural irrigation		(4)
Enrofloxacin	Not detected	Soil pore-water from manure amended agricultural soil		(5)
	0.031	Pig slurry-impacted wastewater used in agricultural irrigation		(2)
Florfenicol	0.011-0.173	Soil pore-water from flooded paddy soils		(6)
Lincomycin	0.006-0.02	Soil-pore water in a soil-plant system		(7)
Ofloxacin	0.0004 (maximum)	Reclaimed water used in agricultural irrigation		(8)
Oxytetracycline	0.001-0.01	Soil-pore water in a soil-plant system		(7)
Sulfamethazine	0.04-0.06 (median)	Soil pore-water from grassland soil after manure application (top soil)		(9)
	0.003-0.105	Soil pore-water from flooded paddy soils		(6)
	0.0032 (maximum)	Soil pore-water from manure amended agricultural soil		(5)
Trimethoprim	0.001-0.01	Soil-pore water in a soil-plant system		(7)

Table S4. Selected reaction monitoring (MRM) transitions and optimized source/collision parameters for azithromycin, oxytetracycline, ciprofloxacin and trimethoprim for HPLC-MS/MS quantification.

Analyte	*Precursor ion (m/z)*	*Product ion (m/z)*	*Dwell*	*Fragmentor voltage (V)*	*Collison energy (eV)*	*Cell acceleration voltage (V)*
Azithromycin	749.6	591.4	200	160	30	1
		158.2	200	135	40	3
Oxytetracycline	461.1	443.2	200	100	7	1
		426.2	200	100	20	1
Ciprofloxacin	332.1	314.0	200	100	15	5
		288.2	200	135	15	7
Trimethoprim	291.3	261.3	200	100	20	7
		230.1	200	125	16	7

Table S5. Analytical validation results for the azithromycin, ciprofloxacin, oxytetracycline, and trimethoprim HPLC-MS/MS method quantification

	Linear range (µg/L)	*R^2*	*R*	*LOD (µg/L)*	*LOQ (µg/L)*	*Recovery (%)*					*Intra-day precision RSD (%)*
						1.0 *µg/L*	*5.0 µg/L*	*7.5 µg/L*	500.0 *µg/L*	*2 000.0 µg/L*	
Azithromycin	0.95 – 1 947.45	0.9995	0.9997	2.37	4.74	-	90.9 - 104.2	-	99.9 - 106.0	97.7 -101	13.69
Ciprofloxacin	7.05 -1 939.10	0.9995	0.9998	2.35	23.51	-	-	95.5 - 104.7	95.3 - 101.3	99.2 - 102.0	7.9
Oxytetracycline	0.90 – 1 900.0	0.9997	0.9998	0.90	0.05	95.0 - 105.3	-	-	99.2 -104.8	97.6 - 101.2	11.6
Trimethoprim	2.45 -1 735.76	0.9987	0.9993	0.98	4.91	-	94.7 - 100.3	-	95.3 - 100.1	99.8 - 101.8	12.1

Table S6. Germination results (percentage seed germination depending on antibiotic exposure)

	AZI	*CPX*	*CHL*	*ERX*	*FLO*	*LIN*	*OFX*	*OTC*	*SFD*	*SFZ*	*TRM*	*Control*
China												
Soybean	40.0	53.3	56.7	56.7	80.0	73.3	50.0	90.0	73.3	NT	36.7	66.7
Wheat	96.7	96.7	86.7	90.0	96.7	96.7	93.3	93.3	93.3	NT	93.3	100.0
Mung Bean	86.7	83.3	83.3	80.0	90.0	86.7	86.7	86.7	93.3	NT	86.7	100.0
Lettuce	90.0	93.3	96.7	100.0	90.0	96.7	96.7	100.0	100.0	NT	90.0	86.7
Rice	83.3	96.7	86.7	86.7	80.0	90.0	86.7	93.3	86.7	NT	90.0	90.0
Chinese Cabbage	83.3	76.7	83.3	93.3	16.7	73.3	90.0	86.7	93.3	NT	86.7	86.7
Chicory	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NT	0.0	0.0
Shasta daisy	16.7	16.7	26.7	33.3	13.3	23.3	26.7	13.3	13.3	NT	33.3	16.7
UK												
French bean	0	36.67	0	56.67	3.3	0	0	0	NT	6.7	0.0	3.3
Pea	40.0	76.7	40.0	63.3	50.0	33.3	40.0	40.0	NT	53.3	23.3	26.7
White clover	73.3	43.3	86.7	90.0	66.7	83.3	93.3	93.3	NT	60.0	73.3	76.7
Red clover	86.7	83.3	60.0	40.0	63.3	56.7	70.0	73.3	NT	86.7	86.7	66.7
Oats	50.0	20.0	43.3	13.3	66.7	30.0	50.0	13.3	NT	10.0	43.3	26.7
Wheat (Claire)	96.7	96.7	100.0	96.7	96.7	96.7	100.0	100.0	NT	100.0	93.3	100.0
Barley	100.0	93.3	96.7	96.7	93.3	96.7	96.7	100.0	NT	90.0	93.3	96.7
Sheeps Fescue	0.0	6.7	3.3	3.3	3.3	0.0	0.0	0.0	NT	0.0	0.0	0.0
Red Fescue	96.7	83.3	66.7	83.3	83.3	70.0	76.7	70.0	NT	93.3	70.0	86.7
Quaking grass	0.0	0.0	6.7	0.0	3.3	3.3	10.0	0.0	NT	0.0	0.0	6.7
Cos lettuce	93.3	90.0	100.0	83.3	90.0	96.7	83.3	93.3	NT	86.7	96.7	86.7
Oxeye Daisy	13.3	20.0	20.0	13.3	6.7	10.0	10.0	13.3	NT	13.3	3.3	3.3
Black Knapweed	23.3	3.3	10.0	6.7	6.7	30.0	0.0	6.7	NT	3.3	6.7	53.3
Cornflower	90.0	96.7	90.0	96.7	93.3	80.0	93.3	80.0	NT	56.7	73.3	86.7
Mexico												
Tomate	40.0 ± 10.0	40.0 ± 10.0	NT	NT	NT	NT	NT	53.3 ± 15.3	NT	NT	46.7 ± 20.8	40.0 ± 8.9
Cempasuchil	90.0 ± 10.1	86.7 ± 15.3	NT	NT	NT	NT	NT	86.7 ± 5.8	NT	NT	66.7 ± 5.8	78.3 ± 11.7
Carnation	46.7 ± 11.5	80.0 ± 10.0	NT	NT	NT	NT	NT	80.0 ± 10.0	NT	NT	83.3 ± 5.8	68.3 ± 14.7
Alfalfa	70.0 ± 10.0	73.3 ± 5.8	NT	NT	NT	NT	NT	100.0 ± 0.0	NT	NT	93.3 ± 5.8	93.3 ± 8.2

*Not tested

**Mexico results have the standard deviation with n = 3

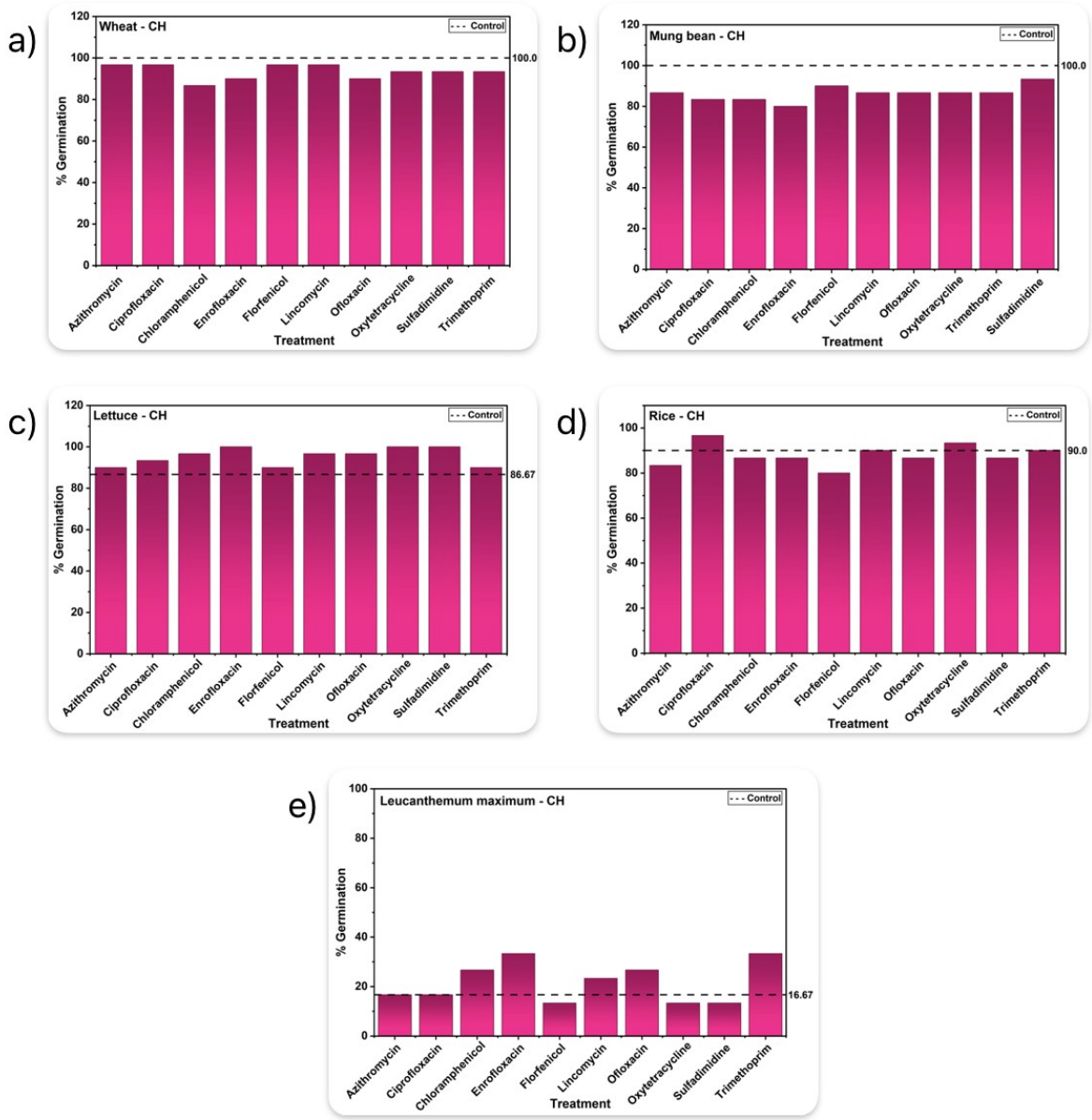

Figure S1. Effects on germination in five seeds treated with 10 antibiotics in China: a) wheat (*T. aestivum*), b) Mung bean (*V. radiata*), c) Lettuce (*L. sativa)*, d) Rice (*O. sativa*), and e) Shasta daisy (*L. maximum),* the dotted line represents the germination results in the control

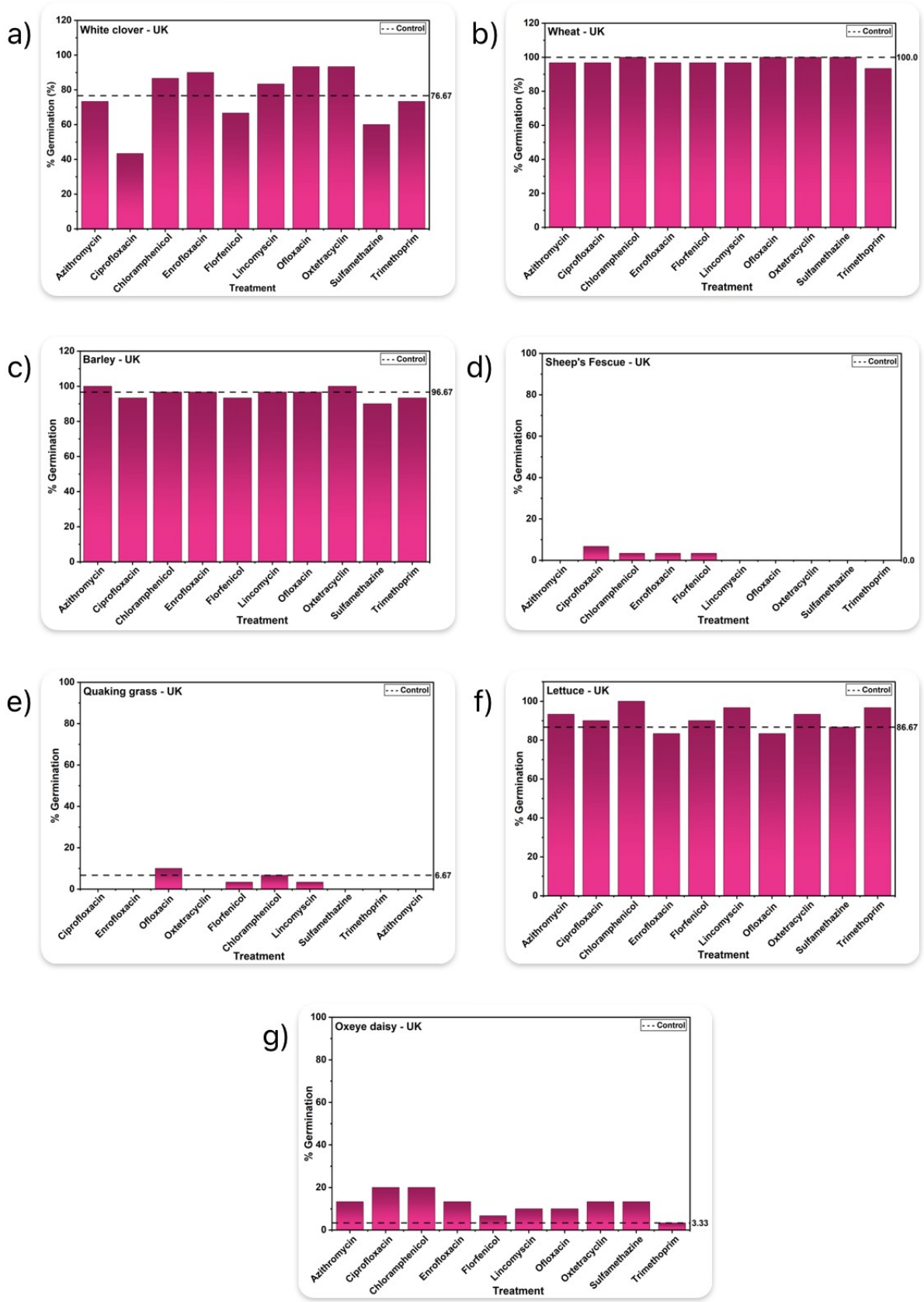

Figure S2. Effects on germination in seven seeds treated with 10 antibiotics in UK: a) wild clover (*T. repens*), b) wheat (*T. aestivum*), c) barley (*H.* vulgare), d) sheep's fescue (*F. ovina*), e) quaking grass (*B. rubra*), f) Lettuce (*L. sativa)*, and g) Oxeye daisy (*L. vulgare*), the dotted line represents the germination results in the control

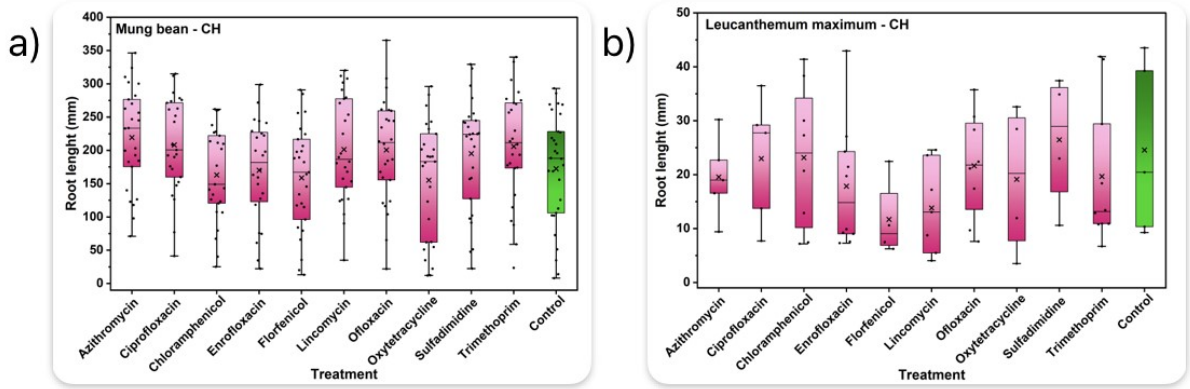

Figure S3. Effect of antibiotic treatments on root length of two seed species from China: a) mung bean (*V. radiata),* and b) Shasta daisy (*L. maximum)*

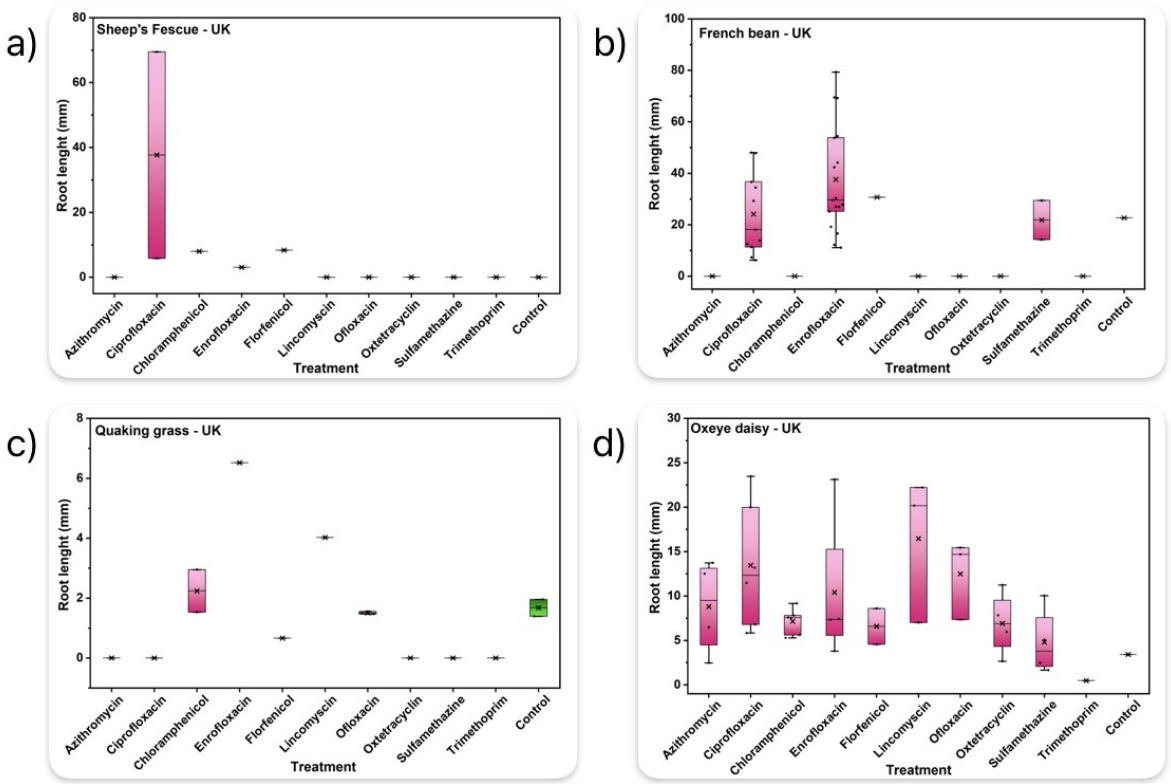

Figure S4. Effect of antibiotic treatments on root length of four seed species from England: a) French bean (*P. vulgaris)*, b) sheep's fescue (*F. Ovina)*, c) quaking grass (*B. media)*, and d) oxeye daisy (*L. vulgare)*

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