

Electronic Supplementary Material

Titel: “Assessment of a Cu applications in two contrasting soil – effects on soil microbial activity and the fungal community structure”

Journal: Ecotoxicology

Authors: Katharina M. Keiblinger^{1*}, Martin Schneider^{1,2,#}, Markus Gorfer^{3,#}, Melanie Paumann¹, Evi Deltedesco^{1,3}, Harald Berger⁴, Lisa Jöchlinger¹, Axel Mentler¹, Sophie Zechmeister-Boltenstern¹, Gerhard Soja², Franz Zehetner¹

**Corresponding:* Katharina Keiblinger, Institute for Soil Research, University of Natural Resources and Life Sciences (BOKU), Peter Jordanstrasse 82, 1190 Vienna, Austria; email: katharina.keiblinger@boku.ac.at; Tel. +43-1-47654-91141, Fax: +43 1 47654 91109

Supplementary Tables

Supplementary Table S1 Trace metal contents of the fungicide.

	mean [mg kg ⁻¹]	SEM	n
Cu	334620.64		1
Mn	23.25 ±	2.07	2
Fe	67.30 ±	55.75	2
Ni	31.75 ±	12.86	5
Zn	136.50 ±	44.16	2
Sr	12.61 ±	3.77	4
Pb	7.87 ±	1.91	4

^a SEM - standard error of mean

Supplementary Table S2 Basic characterization of the fungicide.

Parameter	mean
C _{org}	6.38%
N	0.3%
C/N	21.5
pH	6.3
EC	26.3 mS
Ash content	22.25%

Supplementary Table S3 Primer sequences for amplification of fungal DNA.

ITS3-Mix

ITS31_NeXTf TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CATCGATGAAGAACGCAG**
 ITS32_NeXTf TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CAACGATGAAGAACGCAG**
 ITS33_NeXTf TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CACCGATGAAGAACGCAG**
 ITS34_NeXTf TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CATCGATGAAGAACGTAG**
 ITS35_NeXTf TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CATCGATGAAGAACGTGG**

ITS4-Mix

ITS4_NeXTr GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCCTCCGCTTATTGATATGC**
 ITS43S_NeXTr GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCCTSSCTTATTGATATGC**

Primers contain fungal specific sequences at the 3' end (highlighted in bold) and a 5'-tail for sequencing at the Illumina MiSeq platform. Primers target the ITS2 region and are composed of five different forward primers – ITS31-ITS35 as specified by (Tedersoo et al., 2014) – and two different reverse primers – ITS4 (White et al., 1990) and ITS43S, a degenerate version of ITS4. Forward and reverse primers were mixed in equimolar ratios to obtain ITS3-Mix and ITS4-Mix.

Supplementary Table S4 Dose Response Relationship of microbial biomass (SMBC and Ergosterol) fitted with Sigma Plot using logistic curve fit with four parameters. EC₅₀ values are given in mg Cu kg⁻¹ dw, min and max of SMBC and Ergosterol are also given in mg kg⁻¹ dw.

		SMBC		Ergosterol	
		soil D	soil L	soil D	soil L
1st sampling	EC ₅₀	76	142	49	9.2
	min	80	80	0.30	1.08
	max	161	132	10.4	15.4
	R ²	0.99	0.92	0.98	0.99
2nd sampling	EC ₅₀	84	80	94	57
	min	85	88	4.32	1.60.
	max	137	153	4.78	2.00
	R ²	0.92	0.85	0.99	0.76

Supplementary Table S5 Dose Response Relationship of microbial activity (Respiration rate and Peroxidase as well as Phenoloxidase activity) for soil L at the second sampling, fitted with Sigma Plot using logistic curve fit with four parameters. EC₅₀ values are given in mg Cu kg⁻¹ dw, min and max of Respiration are given in µg g⁻¹ h⁻¹, while min and max of Peroxidase and Phenoloxidase activity are given in nmol g⁻¹ h⁻¹.

	Respiration	Peroxidase	Phenoloxidase
EC ₅₀	188	265	37
min	0.39	3268	6151
max	1.15	9753	9651
R ²	0.86	0.99	0.88

Supplementary Table S6 Pearson's product-moment correlation coefficients (r) for the measured parameters at the **second sampling** in both soils, within soil L and within soil D. Bold numbers indicate significant correlations with p-values below 0.001. Bold italic numbers indicate significant correlations with p-values below 0.05.

Both	<i>EDTA-Cu</i>	<i>pH</i>	<i>SMBC</i>	<i>Respiration</i>	<i>qCO₂</i>	<i>Peroxidase</i>	<i>Phenoloxidase</i>
pH	0.255	1.000					
SMBC	-0.587	-0.380	1.000				
Respiration	-0.255	0.774	0.053	1.000			
qCO ₂	0.058	0.748	-0.451	0.800	1.000		
Peroxidase	-0.491	-0.846	0.640	-0.426	-0.600	1.000	
Phenoloxidase	0.049	-0.663	0.244	-0.356	-0.251	0.706	1.000
shoot DW	-0.556	0.473	0.205	0.745	0.473	-0.178	-0.416
root DW	-0.574	0.464	0.328	0.731	0.351	-0.180	-0.496

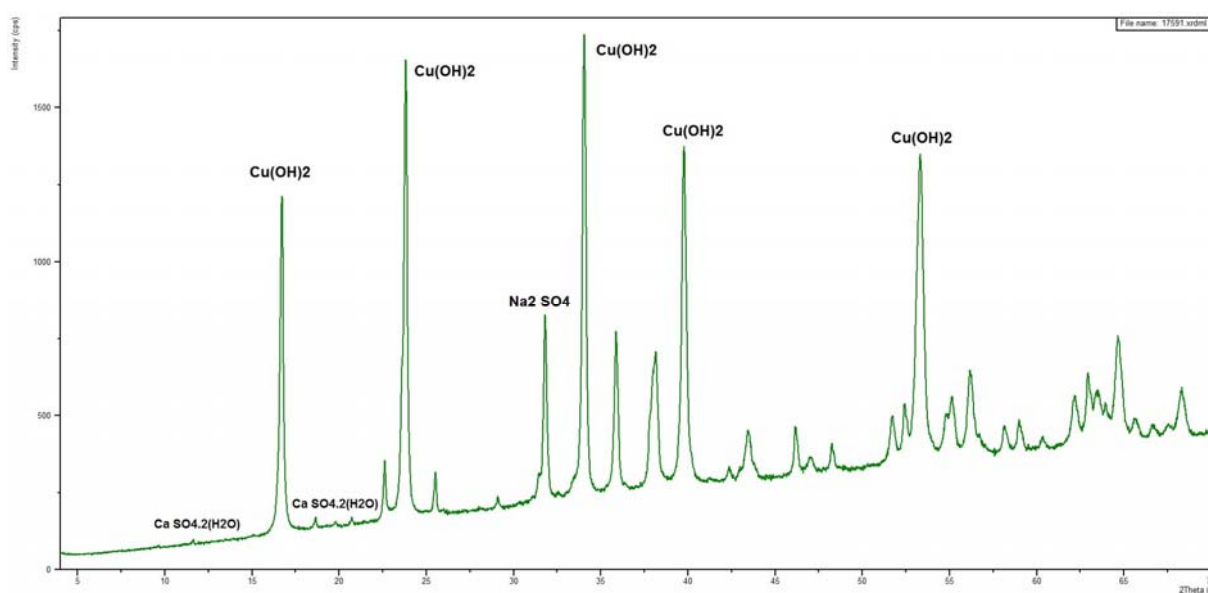
L	<i>EDTA-Cu</i>	<i>pH</i>	<i>SMBC</i>	<i>Respiration</i>	<i>qCO₂</i>	<i>Peroxidase</i>	<i>Phenol-oxidase</i>
pH	0.818	1.000					
SMBC	-0.696	-0.736	1.000				
Respiration	-0.826	-0.900	0.846	1.000			
qCO ₂	-0.615	-0.455	0.118	0.516	1.000		
Peroxidase	-0.759	-0.754	0.814	0.925	0.380	1.000	
Phenoloxidase	-0.448	-0.727	0.755	0.826	0.038	0.833	1.000
shoot DW	-0.606	-0.706	0.944	0.893	0.243	0.857	0.826
root DW	-0.593	-0.754	0.930	0.908	0.236	0.837	0.866

D	<i>EDTA-Cu</i>	<i>pH</i>	<i>SMBC</i>	<i>Respiration</i>	<i>qCO₂</i>	<i>Peroxidase</i>	<i>Phenoloxidase</i>
pH	0.863	1.000					
SMBC	-0.464	-0.616	1.000				
Respiration	-0.245	0.196	-0.354	1.000			
qCO ₂	0.209	0.570	-0.822	0.809	1.000		
Peroxidase	-0.684	-0.836	0.517	-0.385	-0.578	1.000	
Phenoloxidase	0.740	0.770	-0.875	0.228	0.709	-0.606	1.000
shoot DW	-0.893	-0.805	0.176	0.322	0.007	0.571	-0.466
root DW	-0.945	-0.906	0.515	0.203	-0.271	0.608	-0.727

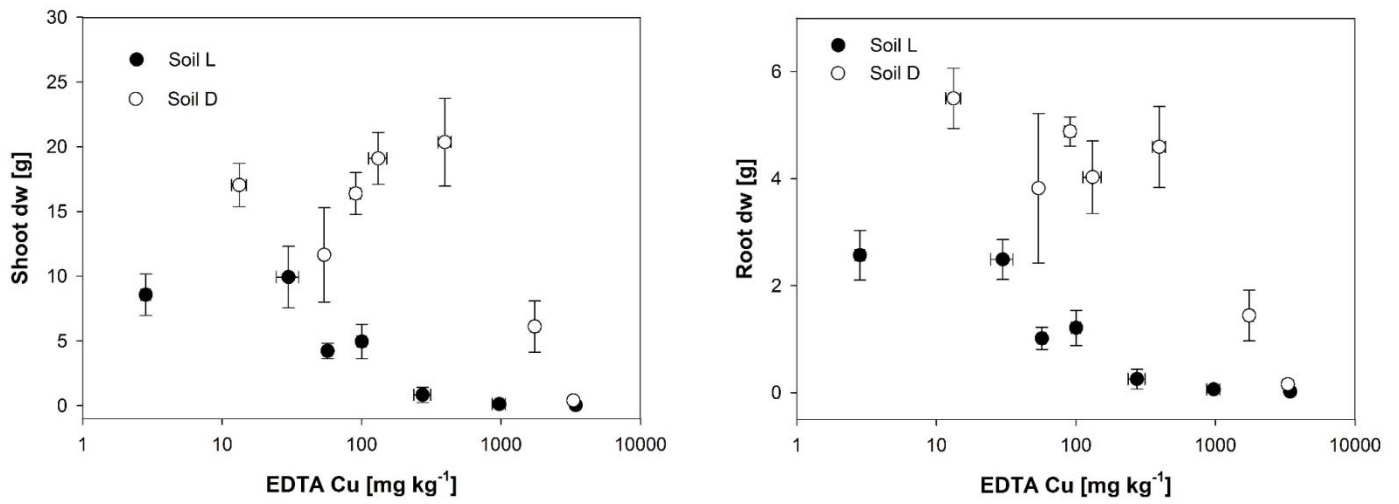
Supplementary Table S7 Dose Response Relationship of the relative Abundance of fungal genera (Acremonium, Dactylonectria and Fusarium) for soil L at the second sampling, fitted with Sigma Plot using logistic curve fit with four parameters. EC₅₀ values are given in mg Cu kg⁻¹ dw, min and max are relative Abundance in %.

	Acremonium	Dactylonectria	Fusarium
EC ₅₀	3.5	6.6	55
min	0.23	0.265	2.61
max	4.08	9.94	9.76
R ²	0.98	0.85	0.69

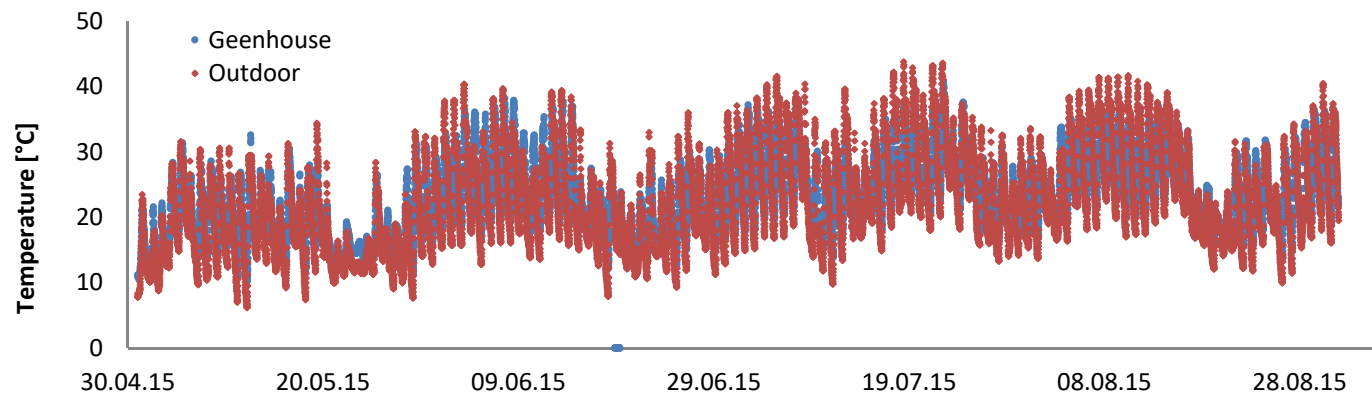
Supplementary Figures



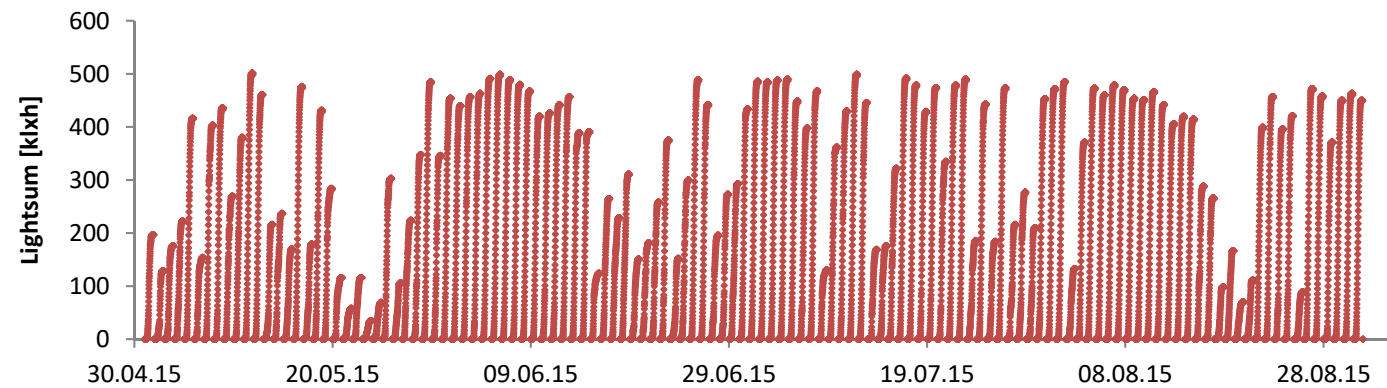
Supplementary Fig. S1 XRD results of the fungicide powder.



Supplementary Fig. S2 Shoot biomass [g] (left) and root biomass [g] (right) in the two different soils at the 2nd sampling in dependence of EDTA-extractable Cu. Error bars indicate standard error; n=5.



Supplementary Fig. S1 Temperature profile in the greenhouse and outdoors, over the course of incubation



Supplementary Fig. S2 Lightsum profile in the greenhouse over the course of incubation