

1 Tracing Copper Derived from Pig Manure in Calcareous Soils and Soil

2 Leachates by ^{65}Cu Labeling

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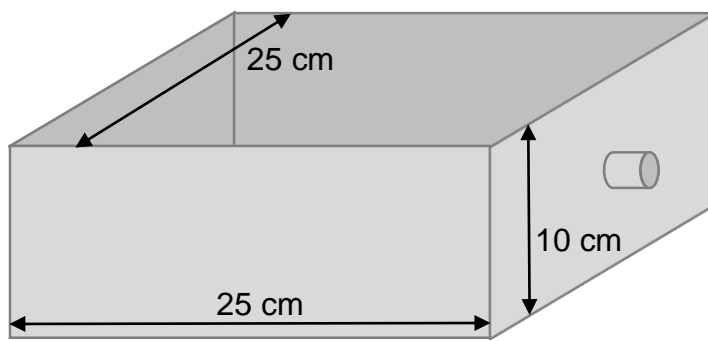
4 Munker, Qiaoyun Xue, Xianyong Lin, Wulf Amelung

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6 The following Supporting Information has 12 pages including two figures and and four

7 tables.

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SI Fig. 1: Schematic stainless steel lysimeter.

Lysimeter installation

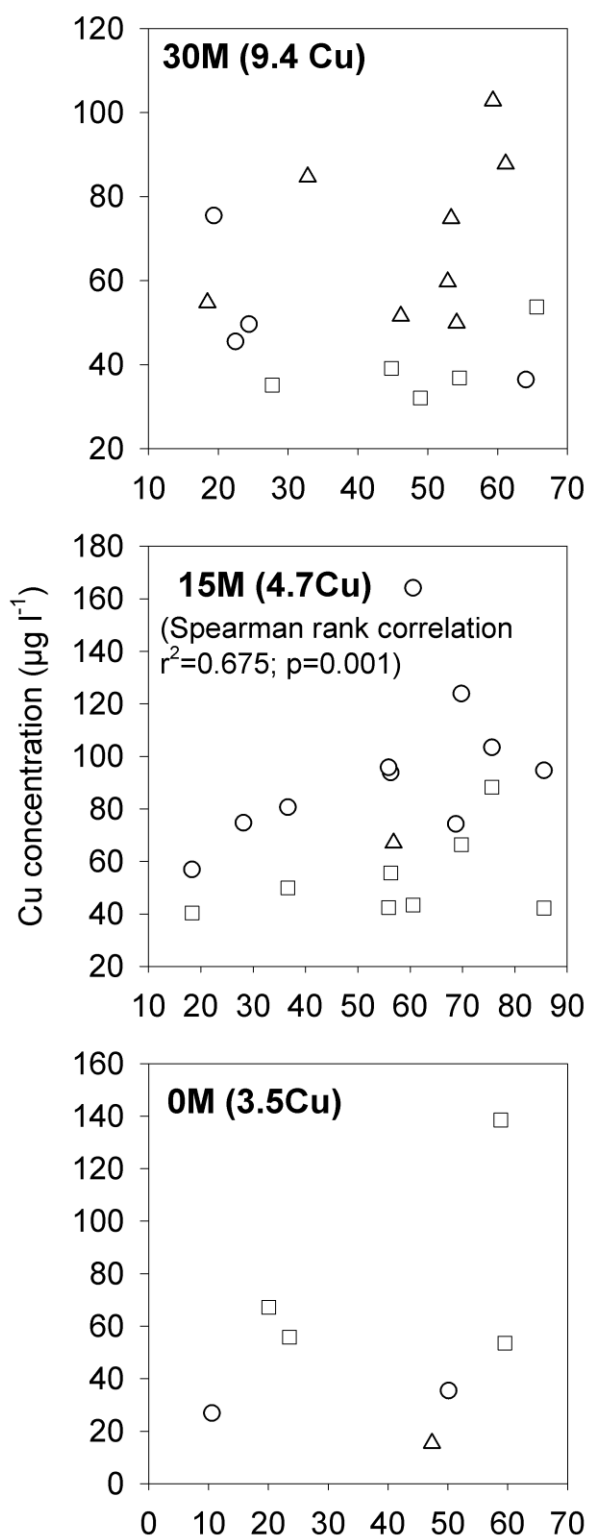
The lysimeters were filled with acid-washed pebbles (size decreasing from 20 mm in the bottom to 5 mm on top) and covered with acid-washed quartz sand (< 2 mm) up to 2 cm below the top edge. Before installation of each lysimeter four stainless steel plates (45 x 25 x 0.1 cm each) were inserted into the soil from the surface to separate a block of undisturbed soil from the surrounding soil. The lysimeters were then installed underneath this soil block by pressing two cm of the lysimeter frame into the overlying soil with a hydraulic jack. The strong force applied by the hydraulic jack ensured the contact between the quartz sand in the lysimeter and the overlying soil. The reason to shield off the soil above the lysimeter with stainless steel plates was to avoid lateral loss of leachate. To minimize the risk of preferential flow along these plates we protected the soil around the plates from direct rainfall and irrigation by placing strips of silicone cloth on the soil surface (covering 1 cm of soil to both sides of the plates). The horizontal distance between lysimeter and trench was 20 cm, and the distance in-between neighbouring lysimeters was 40 cm. The walls of the trench were covered with plastic foil to minimize evaporation.

Sample preparation for Cu isotope measurements

The laboratory condition and quality of reagents during sample preparation for isotopic analysis were similar to that described by Bigalke et al.¹ The volume of aqua regia digest for isotope analysis was based on the total Cu concentration (ICP-OES) in the sample and chosen to contain at least 400 ng Cu. In comparison to this the Cu amount in the blanks is insignificant as it never exceeded 4 ng. Aqua regia digests of manure and soil samples were transferred to Savillex® beakers and evaporated at 80°C to dryness. The dry sample was dissolved in 200 µl 6 M hydrochloric acid and evaporated at 100°C again. This procedure was repeated once. The dried sample was then re-dissolved in 1.1 ml 10 M hydrochloric acid and transferred to polyethylene vials and centrifuged for 15 min at 3000 x *g* to remove remaining solids. Leachate samples were treated in the same way, but due to large amounts of residues after the first evaporation, this residue was additionally boiled at 120°C in ten drops of concentrated hydrogen peroxide and 2 ml aqua regia for four hours. After a further evaporation step the procedure was identical to that used for the soil sample extracts. Copper was separated from the matrix to avoid matrix effects and interferences. Likewise, the sample Ni was removed, because Ni of a defined isotope composition was added later for mass bias drift correction (see below). The Cu separation procedure was based on a protocol modified from Borrok et al.², using 1 ml anion exchange resin (AG MP-1 resin, 100-200 mesh, Bio-Rad, München, Germany) in columns (PE) with 6 mm inner diameter (Bio-Rad, München, Germany). Before usage, the resin was cleaned using 2 ml of millipore water followed by 5 ml 0.56 M HNO₃ and another 2 ml of millipore water. The sample solution was loaded onto the resin in 1 ml 10 M HCl.

The metals in the sample matrix were subsequently eluted with 5 ml 10 M HCl, and Cu was eluted by the addition of another 5 ml of 10 M HCl. After the column separation, the Cu eluate was evaporated at 100°C and the residue was treated with 100 µl concentrated hydrogen peroxide and dried at 80°C, re-dissolved in 100 µl concentrated HNO₃, and dried again. These oxidation steps ensured to destroy organic matter deriving from the resin and to drive out any remaining hydrochloric acid.^{1,3} Finally, the evaporated samples were dissolved in 1 ml 0.14 M

62 HNO₃ and a Ni standard solution (1000 mg l⁻¹ in HNO₃ (5%), Johnson Matthey, United
63 Kingdom) was added. This solution was diluted to a final concentration of 150 µg l⁻¹ Cu and 1
64 µg l⁻¹ Ni.



Dissolved organic carbon concentration (mg l^{-1})

SI Fig. 2: Relation between the concentration of total Cu and dissolved organic carbon in the leachate. Different symbols represent the replications.

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69 *SI Table 1: Daily precipitation and percolation data (ml leachate per lysimeter) as well as*
 70 *concentrations of dissolved organic carbon (DOC) in the leachate.*

Time after application Days	Rainfall and irrigation mm	30 M (9.4Cu)					
		Replication 1		Replication 2		Replication 3	
		Leachate ml	DOC mg l ⁻¹	Leachate ml	DOC mg l ⁻¹	Leachate ml	DOC mg l ⁻¹
1	15						
3	14.4						
4	5.6						
6	14.4						
8	41.0	365	45	80	n.d.	1100	52.8
10	31.0	255	55			340	46.1
13	20.0						
14	10.0						
17	20.0						
18	14.4						
19	20.4						
20	14.4						
21	3.6						
22	17.8						
23	25.2						
24	14.4			70	22.4	195	61.1
25	3.6						
26	10.8						
28	23.4						
29	32.4						
30	3.6			100	24.4	540	59.3
31	3.6						
32	18.0						
33	18.0						
34	7.2						
37	31.0	120	66				
38	14.4						
39	14.4						
41	27	355	49	185	64.1	380	53.3
42	20						
43	20					80	18.4
44	20						
45	32					230	32.8
47	14.4	30	28	100	19.4	170	54.1
48	14.4						

71 *n.d. not determined*

Time after application days	Rainfall and irrigation mm	15 M (4.7Cu)					
		Replication 1		Replication 2		Replication 3	
		Leachate ml	DOC mg l ⁻¹	Leachate ml	DOC mg l ⁻¹	Leachate ml	DOC mg l ⁻¹
1	15						
3	14.4						
4	5.6						
6	14.4						
8	41.0	1100	56.3	405	56.8	895	39.4
10	31.0	500	85.5			350	49.8
13	20.0						
14	10.0						
17	20.0						
18	14.4						
19	20.4						
20	14.4						
21	3.6						
22	17.8						
23	25.2						
24	14.4	155	69.8			180	32.5
25	3.6	40	36.7				
26	10.8						
28	23.4						
29	32.4						
30	3.6	430	60.6			290	58.6
31	3.6						
32	18.0						
33	18.0						
34	7.2						
37	31.0	175	55.9			80	23.7
38	14.4						
39	14.4						
41	27	690	75.6			430	23.5
42	20						
43	20	100	18.3				
44	20						
45	32	180	28.2			150	47.1
47	14.4	220	68.7			130	15.6
48	14.4						

Time after application days	Rainfall and irrigation mm	0 M (3.5Cu)					
		Replication 1		Replication 2		Replication 3	
		Leachate ml	DOC mg l ⁻¹	Leachate ml	DOC mg l ⁻¹	Leachate ml	DOC mg l ⁻¹
1	15						
3	14.4						
4	5.6			40	n.d.		
6	14.4						
8	41.0	60	n.d.			170	n.d.
10	31.0						
13	20.0			220	10.6		
14	10.0						
17	20.0						
18	14.4						
19	20.4						
20	14.4						
21	3.6						
22	17.8						
23	25.2						
24	14.4					22	47.3
25	3.6						
26	10.8						
28	23.4						
29	32.4						
30	3.6	100	n.d.				
31	3.6						
32	18.0						
33	18.0						
34	7.2						
37	31.0	15	58.8				
38	14.4						
39	14.4						
41	27	270	59.5	130	50.1		
42	20						
43	20						
44	20						
45	32	20	23.5				
47	14.4	130	20.1				
48	14.4						

75 *n.d. not determined*

76 *SI Table 2: Instrumental settings for Cu isotope measurements.*

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Instrumental parameters

Cool gas flow (l min ⁻¹):	15.00
Aux gas flow (l min ⁻¹):	~0.70
Sample gas flow (l min ⁻¹):	1.05 - 1.10
Forward power (W):	1200
Baseline:	90 baselines á 1.0 s

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79 SI Table 3: Measured $\delta^{65}\text{Cu}$ values relative to NIST 976 ($^{65}\text{Cu}/^{63}\text{Cu} = 0.44498$, Equation 1), $^{65}\text{Cu}/^{63}\text{Cu}$ ratios relative to NIST 976, total Cu
80 concentration in soil, and percentage of Cu derived from experimentally added manure for all replications and sampled depths.

30 M (9.4Cu)						15 M (4.7Cu)				0 M (3.5Cu)			
	Depth	$\delta^{65}\text{Cu}$	$^{65}\text{Cu}/^{63}\text{Cu}$	Total Cu	Added Cu	$\delta^{65}\text{Cu}$	$^{65}\text{Cu}/^{63}\text{Cu}$	Total Cu	Added Cu	$\delta^{65}\text{Cu}$	$^{65}\text{Cu}/^{63}\text{Cu}$	Total Cu	Added Cu
	cm	‰		mg kg ⁻¹	%	‰		mg kg ⁻¹	%	‰		mg kg ⁻¹	%
Replication 1	0-2	534.3	0.6827	160.4	30.3	185.4	0.5275	179.2	12.7	151.6	0.5124	169.1	4.9
	2-4	31.2	0.4588	66.6	2.0	9.00	0.4490	594.5	0.6	2.62	0.4461	121.6	0.1
	4-6	4.9	0.4472	78.8	0.3	1.78	0.4458	100.2	0.1	1.39	0.4456	72.3	0.0
	6-8	3.03	0.4463	81.2	0.1	1.38	0.4456	97.7	0.0	0.68	0.4453	89.3	0.0
	8-10	1.55	0.4457	101.5	0.0	0.58	0.4452	80.4	0.0	0.28	0.4451	87.2	0.0
	10-15	1.43	0.4456	69.4	0.0	0.38	0.4451	58.5	0.0	0.25	0.4451	47.1	0.0
	15-20	0.25	0.4451	34.2	0.0	0.52	0.4452	29.3	0.0	0.07	0.445	41.7	0.0
	20-30	-0.10	0.4449	35.4	0.0	0.06	0.4450	30.2	0.0	0.18	0.4451	32.1	0.0
	30-40	-0.20	0.4449	15.5	0.0	-0.29	0.4448	12.0	0.0	-0.50	0.4448	8.1	0.0
Replication 2	0-2	502.2	0.6685	216.7	28.1	264	0.5625	179.9	16.4	234.2	0.5492	112.0	7.3
	2-4	75.5	0.4786	54.8	4.7	27.9	0.4574	591.2	1.8	8.07	0.4486	377.2	0.2
	4-6	10.6	0.4497	90.7	0.6	2.66	0.4462	54.9	0.1	2.27	0.4460	38.6	0.0
	6-8	3.61	0.4466	75.4	0.2	1.04	0.4454	89.1	0.0	1.13	0.4455	58.7	0.0
	8-10	2.98	0.4463	70.6	0.1	1.09	0.4455	75.8	0.0	0.73	0.4453	51.3	0.0
	10-15	1.47	0.4456	51.2	0.0	0.30	0.4451	90.5	0.0	0.62	0.4453	37.9	0.0
	15-20	0.39	0.4452	22.3	0.0	-0.08	0.4449	52.5	0.0	0.07	0.445	24.6	0.0
	20-30	0.38	0.4452	26.4	0.0	-0.59	0.4447	19.2	0.0	-0.39	0.4448	16.5	0.0
	30-40	-0.77	0.4446	9.7	0.0	-0.58	0.4447	7.4	0.0	-0.80	0.4446	6.9	0.0
Replication 3	0-2	581.4	0.7037	173.9	32.9	226.7	0.5458	100.7	14.3	188.5	0.5288	130.2	5.6
	2-4	53.6	0.4688	92.4	3.5	7.10	0.4481	65.1	0.4	8.07	0.4486	499.5	0.2
	4-6	94.8	0.4871	99.9	6.2	3.58	0.4466	46.5	0.2	1.94	0.4458	67.9	0.0
	6-8	2.81	0.4462	92.8	0.1	2.50	0.4461	67.6	0.1	1.34	0.4456	86.7	0.0
	8-10	1.05	0.4454	82.0	0.0	2.01	0.4459	64.7	0.1	0.92	0.4454	59.6	0.0
	10-15	0.80	0.4453	81.2	0.0	1.57	0.4457	46.0	0.0	0.88	0.4454	46.5	0.0
	15-20	0.22	0.4451	47.0	0.0	0.33	0.4451	45.7	0.0	0.62	0.4453	45.0	0.0
	20-30	0.03	0.4450	48.4	0.0	-0.22	0.4449	28.1	0.0	-0.13	0.4449	18.1	0.0
	30-40	-1.09	0.4445	7.8	0.0	-0.23	0.4449	11.5	0.0	-1.16	0.4445	7.2	0.0

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82 SI Table 4: Mean values of the distribution of the applied tracer KBr (105 mg Br per lysimeter
83 plot) from three replicates in the soil profile after 53 days of irrigation and the sum of Br⁻ found
84 in the leachate (40 cm depth), and recovery of the applied Br⁻ in each soil depth, leachate, and
85 the sum of recovery. Standard error is given as \pm .

	Soil depth (cm)	Br ⁻ (mg)	Recovery (%)	Sum recovery (%)
30 M (9.4Cu)	0-2	0.9 \pm 0.1	0.9 \pm 0.1	22.0 \pm 3.9
	2-4	0.7 \pm 0.1	0.7 \pm 0.1	
	4-6	0.8 \pm 0.1	0.8 \pm 0.1	
	6-8	0.7 \pm 0.2	0.7 \pm 0.2	
	8-10	0.6 \pm 0.1	0.6 \pm 0.1	
	10-15	1.3 \pm 0.2	1.3 \pm 0.2	
	15-20	2.2 \pm 0.8	2.1 \pm 0.7	
	20-30	4.2 \pm 1.1	4.0 \pm 1.0	
	30-40	5.5 \pm 0.6	5.2 \pm 0.5	
	Sum leachate	6.2 \pm 3.7	5.9 \pm 3.5	
15 M (4.7Cu)	0-2	1.8 \pm 0.2	1.7 \pm 0.2	33.8 \pm 5.2
	2-4	1.0 \pm 0.1	1.0 \pm 0.1	
	4-6	1.0 \pm 0.1	0.9 \pm 0.1	
	6-8	0.9 \pm 0.1	0.8 \pm 0.1	
	8-10	1.0 \pm 0.3	1.0 \pm 0.3	
	10-15	3.0 \pm 0.6	2.9 \pm 0.5	
	15-20	4.0 \pm 0.8	3.8 \pm 0.7	
	20-30	5.0 \pm 0.9	4.8 \pm 0.9	
	30-40	8.3 \pm 0.5	7.9 \pm 0.5	
	Sum leachate	9.5 \pm 4.1	9.0 \pm 3.9	
0 M (3.5Cu)	0-2	1.6 \pm 0.3	1.5 \pm 0.3	28.8 \pm 3.6
	2-4	1.1 \pm 0.1	1.1 \pm 0.1	
	4-6	1.1 \pm 0.2	1.0 \pm 0.2	
	6-8	0.9 \pm 0.1	0.9 \pm 0.1	
	8-10	0.8 \pm 0.1	0.8 \pm 0.1	
	10-15	2.3 \pm 0.3	2.2 \pm 0.3	
	15-20	2.8 \pm 0.4	2.7 \pm 0.4	
	20-30	7.2 \pm 1.0	6.8 \pm 1.0	
	30-40	11.9 \pm 2.2	11.3 \pm 2.1	
	Sum leachate	0.6 \pm 0.1	0.6 \pm 0.1	

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87 References - Supporting Information

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