

Supplementary material for

Biochar for circular horticulture: feedstock related effects in soilless cultivation

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More details on Materials and Methods of the greenhouse strawberry trial

2.3.2. Experimental set-up

Strawberry plants were grown in 1.5 L pots containing Prelvex white peat 100% (AVEVE Lammens, Wetteren, Belgium), mixed with different PGMix fertilizer doses (0.70, 1.05, 1.40 and 1.75 g PGMix per L) and 1.43 g per L lime (RHP, MG's-Gravenzande, The Netherlands). For the biochar treatments, the peat substrate with 1.05 g PGMix per L was mixed with 2 g dry matter (DM) biochar per L peat substrate. Shortly after production and prior to use in the peat blends, the For650, Speat650 and Scoir650 biochars were moistened and allowed to equilibrate for several weeks to avoid any hydrophobic effects. All blends were wetted to obtain 40% water-filled pore space (WFPS), put in a closed bag and pre-incubated at 15 °C for 1 week. Cold-stored bare-root strawberry transplants (cultivar Elsanta) were planted in all pots with pre-incubated blends. The pots were arranged in a semi-randomized design in the greenhouse and plants were grown for 11 weeks at 20 °C. In total, 6 biological replicates per treatment were sampled at the end of the experiment.

Every week, the moisture content of the substrate was adjusted to 40% WFPS based on mass loss recorded for each pot separately. Nutrient or salt losses by leaching from pots was avoided. The water volume added per pot was recorded during the whole experiment. The total water use during the experiment was expressed per pot, thus expressing the net effect of both transpiration by the strawberry plant and evaporation from the growing medium. No additional fertilizer was applied during plant growth.

Chlorophyll content was estimated, as described in detail by Debode *et al.* {Debode, 2018 #1340; Vandecasteele, 2021 #1361} for each of the three separate leaflets of two fully grown compound leaves of these six plants per treatment using a CCM-200 chlorophyll content meter (Opti-Sciences Inc., Hudson, NH, USA). The output was expressed in Chlorophyll Concentration Index (CCI), defined as the ratio of transmission at 931 to 653 nm through a leaf (Opti-Sciences Inc., USA). Total plant CCI was calculated as a weighted average based on individual leaf area.

Plant leaves of 4 plants per treatment were inoculated after ten weeks of plant growth with *B. cinerea* isolate PCF895 [42] according to the method of Harel *et al.* [26] and described in detail by De Tender *et al.* {De Tender, 2016 #1215; Vandecasteele, 2021 #1361}. Two plants per treatment were inoculated with sterile potato dextrose agar plugs. The resulting lesions on the leaflets were recorded one week after inoculation using a 0-4 disease scale. This scoring was used to calculate the disease severity index (DSI) per plant (i), used as input for statistical analysis.

$$DSI_i = 100 \times \frac{1 \times n_{i1} + 2 \times n_{i2} + 3 \times n_{i3} + 4 \times n_{i4}}{4 \times n_i}$$

Where n_{i1}, \dots, n_{i4} represent the number of leaves of each infection score and $n_i = \sum_{l=0}^4 n_{il} = 9$ is the number of leaves measured for each plant. This index has values in the interval (0,100), with a minimum index if all leaves score 0 and a maximum when all leaves score 4. Inoculated leaves remained on the plant until the end of the experiment. For plant leaf infection, the DSI was used as response variable for disease score.

2.3.3. Chemical characterization of the growing media, plant leaves and strawberry fruits

In total, six biological replicates were studied per treatment. "Leaf" is defined as the aboveground vegetative biomass, including stalks and the three separate leaflets of the compound leaves. Leaves were dried at 70 °C and ground, and the material of one plant (one pot) was considered as one biological replicate. Fruits were collected during the trial, and one sample per plant for the whole trial was freeze-dried before grinding. Total N was determined by Thermo scientific – flash 4000 N analyzer (ISO 16634-1), total concentrations of P, K, Mg and Ca were determined by 5110 VDV Agilent ICP-OES in the extract following digestion of 0.5 g dried and ground material with 4 mL HNO₃ (p.a. 65 %) and 12 mL HCl (p.a. 37%) using a SCP Digiprep MS 200 heating block. Assessment of optimal range and nutrient deficiency for foliar composition is based on Pritts *et al.* [46]. Total uptake in the leaves and fruits was calculated by multiplying the measured concentration with the dry mass of leaves/fruits.

Table S1. Biochars tested in different experiments (codes of the biochars: see Table 1. Ref biochar: reference biochar based on stone oak wood)

Process	Chemical characterisation	Leaching experiment (10 volume% biochar/ 90% limed peat)	Greenhouse trial (2 g DM L ⁻¹ limed peat)
Reference biochar	x	x	x
Flax650	x	x	
Flax400	x		
Misc650	x	x	
Misc400	x		
For650	x	x	x
For400	x	x	
Speat650	x	x	x
Speat400	x		
Speat3-650	x		
Scoir650	x	x	x
Scoir4-650	x		

Table S2. Available elements extracted in water (H₂O) or ammonium acetate (aa) in mg (L biochar)⁻¹, of spent growing media (SGM) feedstocks (below the line) and biochars,(above the line) including the ref biochar (reference biochar based on stone oak wood). See m&m for full details.

Code	Matrix	Feedstock	NO ₃ -N	NH ₄ -N	SO ₄	Cl	C	Na	P	K	Ca	Mg	Mn
			H ₂ O					aa					
Ref biochar	biochar	Reference biochar	< 5	< 5	60	30	528	54.9	150	3139	11478	487	44.2
Flax650	biochar, 650 °C	Flax shives	< 5	< 5	45	51	2410	35.0	91	603	380	< 76	2.2
Flax400	biochar, 400 °C	Flax shives	< 5	< 5	25	< 10	195	<25	105	384	447	< 76	2.6
Misc650	biochar, 650 °C	Miscanthus straw	< 5	< 5	17	17	1612	<25	< 14	245	< 105,0	< 76	3.3
Misc400	biochar, 400 °C	Miscanthus straw	< 5	< 5	< 12	< 10	80	<25	< 14	83	< 105	< 76	5.3
For650	biochar, 650 °C	wood from forestry management	< 5	< 5	17	27	2109	32.8	< 14	455	221	< 76	<1.5
For400	biochar, 400 °C	wood from forestry management	< 5	< 5	30	26	1391	<25	19	220	196	< 76	1.6
Speat650	biochar, 650 °C	Spent peat, batch1	< 5	< 5	1220	136	665	155	57	667	1677	117	12.9
Speat400	biochar, 400 °C	Spent peat, batch1	< 5	< 5	1427	163	807	204	181	812	5598	552	35
Scoir650	biochar, 650 °C	Spent coir, batch2	< 5	< 5	378	151	1716	114	68	660	500	76	< 1.5
Speat3-650	biochar, 650 °C	Spent peat, batch3	< 5	< 5	735	229	347	141	57	1043	4036	344	37
Scoir4-650	biochar, 650 °C	Spent coir, batch4	< 5	< 5	170	270	351	221	60	465	1539	100	25
	SGM	Spent peat, batch1	127	< 5	734	33	313	12	32	320	2067	251	2
	SGM	Spent coir, batch2	120	26	147	56	115	8	41	247	639	76	< 1.5
	SGM	Spent peat, batch3	200	22	769	88	633	89	42	480	2594	337	8
	SGM	Spent coir, batch4	216	43	252	168	653	148	50	285	2042	240	21

Table S3. Characteristics of the white peat (not limed, except for the CEC and pH: these were measured on the limed peat) used in the leaching experiment. Available elements were extracted in water (H₂O) or ammonium acetate (aa). DM: dry matter, EC: electrical conductivity, CEC: cation exchange capacity

Parameter		
pH-H ₂ O	-	5.0
EC	μS cm ⁻¹	12
Bulk density	g (L fresh weight) ⁻¹	150
Total OM		949
Total N		10.5
Total P		0.25
Total K		0.25
Total Ca	g (kg DM) ⁻¹	5.40
Total Mg		0.65
Total Na		0.07
Total Fe		1.05
Total Al		0.79
NO ₃ -N (H ₂ O)		<5.0
NH ₄ -N (H ₂ O)		<5.0
SO ₄ (H ₂ O)	mg L ⁻¹	<11.7
Cl (H ₂ O)		<10
Na (aa)		<25
K (aa)		<36
P (aa)		<14
Ca (aa)	mg L ⁻¹	442
Mg (aa)		<76
CEC	cmol _c kg ⁻¹	127

Table S4. Mean water use (standard error in brackets) of the different treatments in the strawberry field trial

Treatment: limed peat +	Mean Water Use (g)
1.05 g PGMix L ⁻¹	6098 (147)
0.70 g PGMix L ⁻¹	6459 (110)
1.40g g PGMix L ⁻¹	6755 (85)
1.75 g PGMix L ⁻¹	6904 (193)
1.05 g PGMix L ⁻¹ + Ref Biochar (2 g L ⁻¹)	6641 (165)
1.05 g PGMix L ⁻¹ + For650 (2 g L ⁻¹)	6258 (263)
1.05 g PGMix L ⁻¹ + Speat650 (2 g L ⁻¹)	6201 (244)
1.05 g PGMix L ⁻¹ + Scoir650 (2 g L ⁻¹)	6621 (69)

Table S5. Initial and final pH, electrical conductivity (EC) and water-extractable nutrients at the start and the end of the strawberry trial, analyzed on a composite sample of the peat blend per treatment. Codes of the biochars: see Table 1. Ref biochar: reference biochar based on stone oak wood. PGMix: Multi-mix Potting Soil 14+16+18 mineral fertilizer.

Treatment: limed peat +	pH-H ₂ O	EC	NO ₃ -N	NH ₄ -N	SO ₄	Cl	P
	-	μS cm ⁻¹	mg (L blend) ⁻¹				
Initial							
0.70 g PGMix L ⁻¹	4.88	277	51	55	170	< 10	47
1.05 g PGMix L ⁻¹	4.83	446	93	108	367	< 10	97
1.40 g PGMix L ⁻¹	4.79	565	122	121	393	< 10	120
1.75 g PGMix L ⁻¹	4.77	570	122	130	405	< 10	127
1.05 g PGMix L ⁻¹ + Ref biochar (2 g L ⁻¹)	4.88	404	81	89	285	< 10	73
1.05 g PGMix L ⁻¹ + For650 (2 g L ⁻¹)	4.84	378	56	91	315	< 10	74
1.05 g PGMix L ⁻¹ + Speat650 (2 g L ⁻¹)	4.88	349	48	80	302	< 10	66
1.05 g PGMix L ⁻¹ + Scoir650 (2 g L ⁻¹)	4.85	339	52	71	254	< 10	68
Final							
0.70 g PGMix L ⁻¹	4.59	72	< 5.0	< 5.0	70	< 10	< 4.7
1.05 g PGMix L ⁻¹	4.18	119	< 5.0	< 5.0	162	< 10	19
1.40 g PGMix L ⁻¹	4.19	149	< 5.0	6	208	< 10	28
1.75 g PGMix L ⁻¹	4.16	236	19	14	294	< 10	58
1.05 g PGMix L ⁻¹ + Ref biochar (2 g L ⁻¹)	4.47	89	< 5.0	< 5.0	117	< 10	7
1.05 g PGMix L ⁻¹ + For650 (2 g L ⁻¹)	4.28	96	< 5.0	< 5.0	135	< 10	8
1.05 g PGMix L ⁻¹ + Speat650 (2 g L ⁻¹)	4.33	113	< 5.0	< 5.0	167	< 10	10
1.05 g PGMix L ⁻¹ + Scoir650 (2 g L ⁻¹)	4.29	112	< 5.0	< 5.0	165	< 10	16

Table S6. Ammonium acetate extractable nutrients at the start of the strawberry trial, analysed on a composite sample of the peat blend per treatment. Codes of the biochars: see Table 1. Ref biochar: reference biochar based on stone oak wood. PGMix: Multi-mix Potting Soil 14+16+18 mineral fertilizer.

Treatment: limed peat +	P	K	Ca	Mg	Na
	mg (L blend) ⁻¹				
0.70 g PGMix L ⁻¹	51	155	1043	184	< 25
1.05 g PGMix L ⁻¹	102	265	1083	193	< 25
1.40 g PGMix L ⁻¹	109	321	993	178	< 25
1.75 g PGMix L ⁻¹	143	386	933	171	< 25
1.05 g PGMix L ⁻¹ + Ref biochar (2 g L ⁻¹)	82	255	1021	162	< 25
1.05 g PGMix L ⁻¹ + For650 (2 g L ⁻¹)	75	188	903	155	< 25
1.05 g PGMix L ⁻¹ + Speat650 (2 g L ⁻¹)	77	226	961	160	< 25
1.05 g PGMix L ⁻¹ + Scoir650 (2 g L ⁻¹)	64	202	805	137	< 25

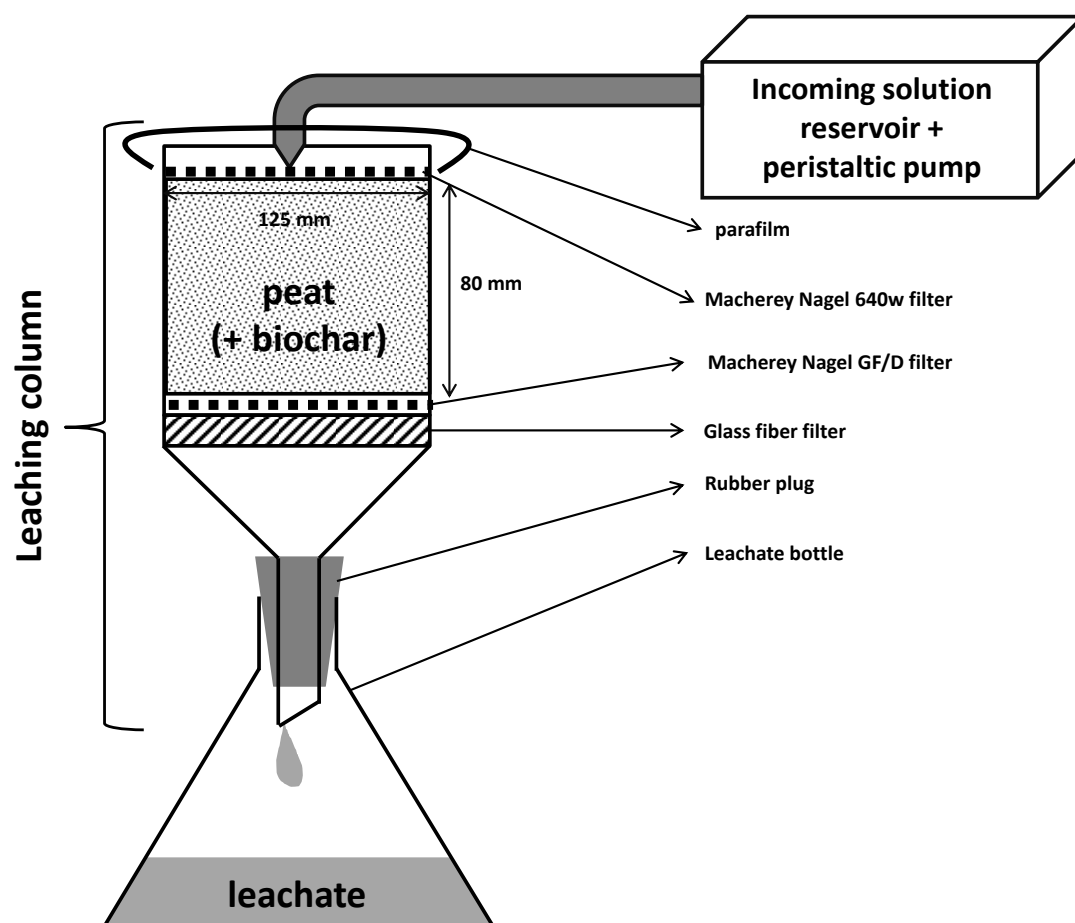
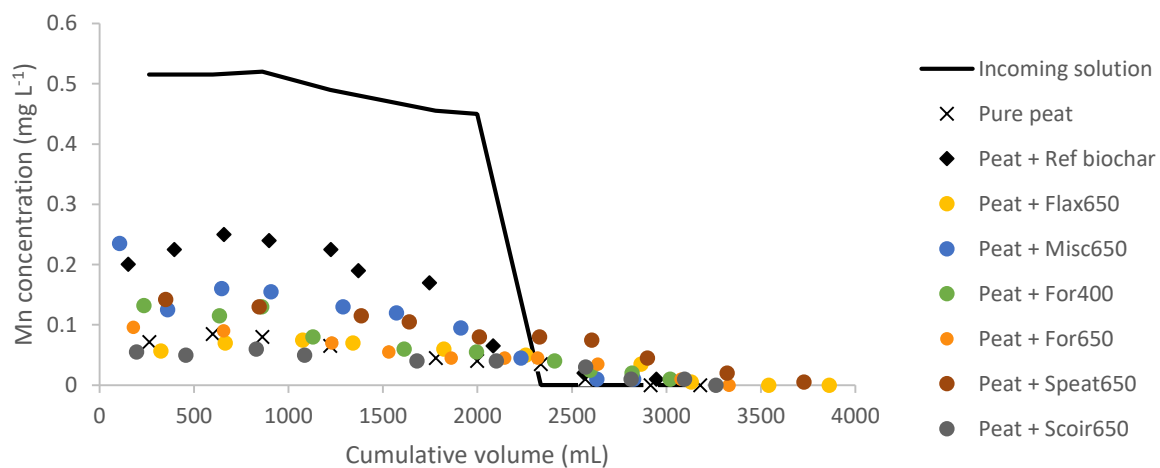
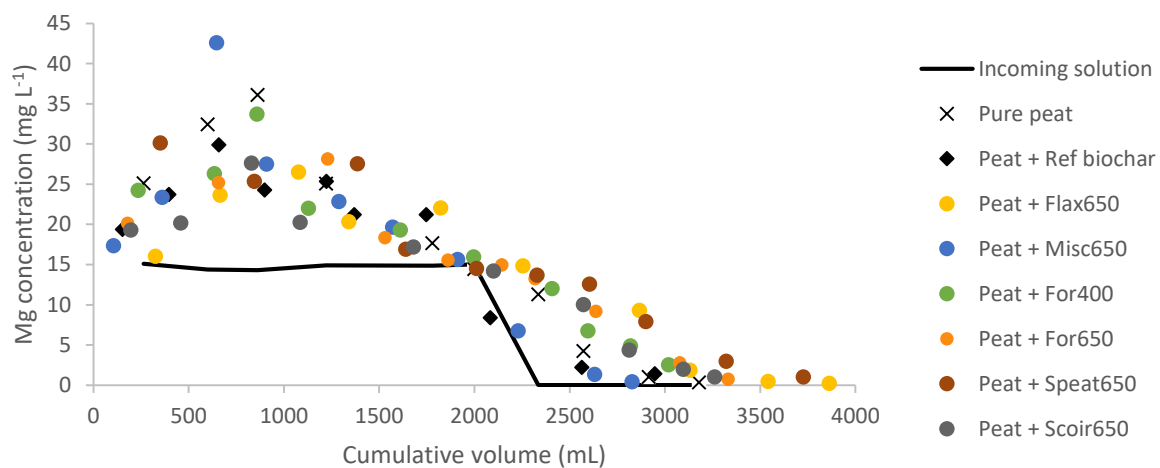
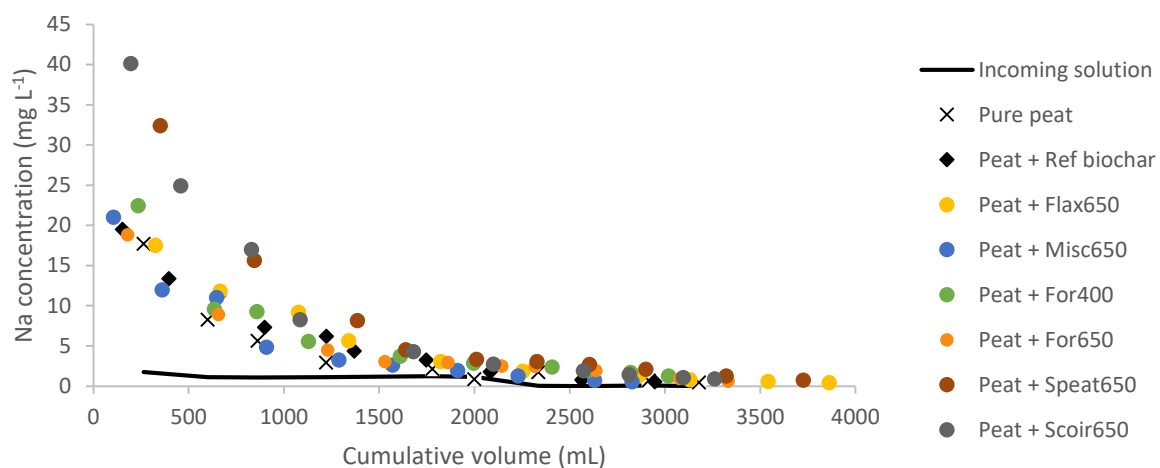
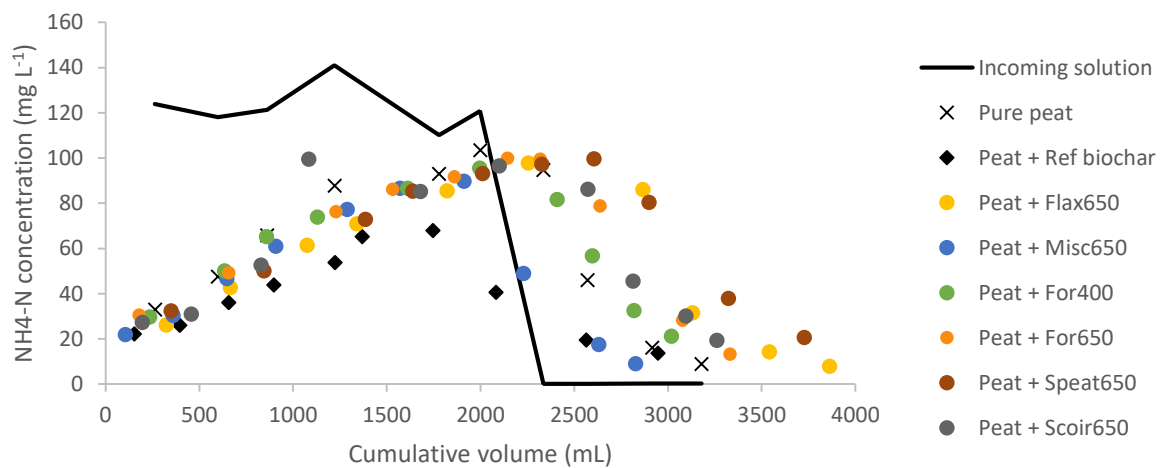
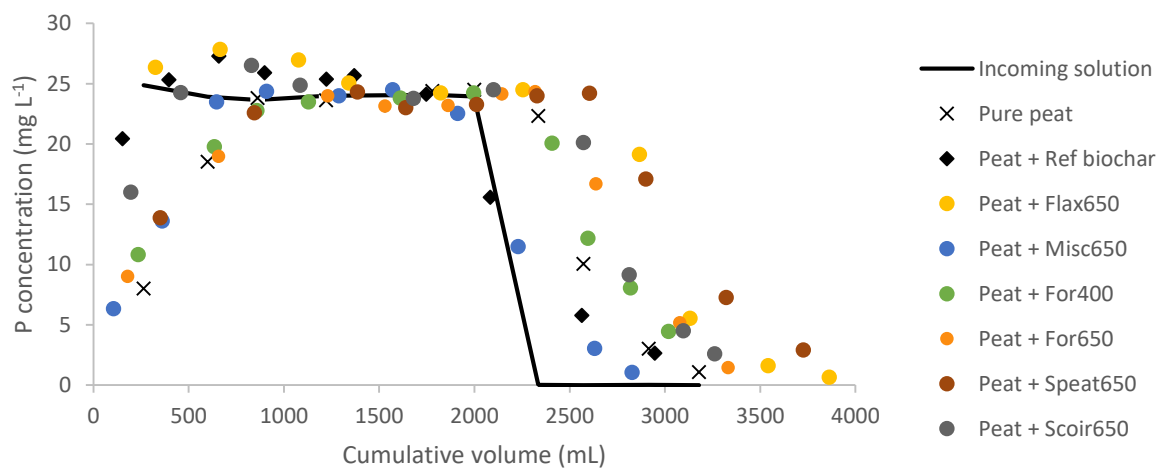
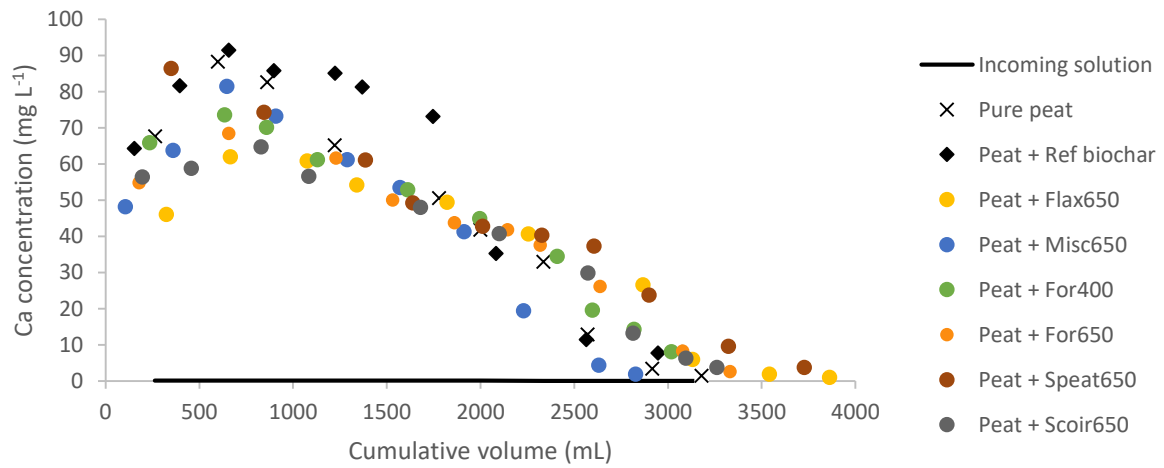


Figure S1. Overview of the setup of the leaching experiment (adapted from [1]).





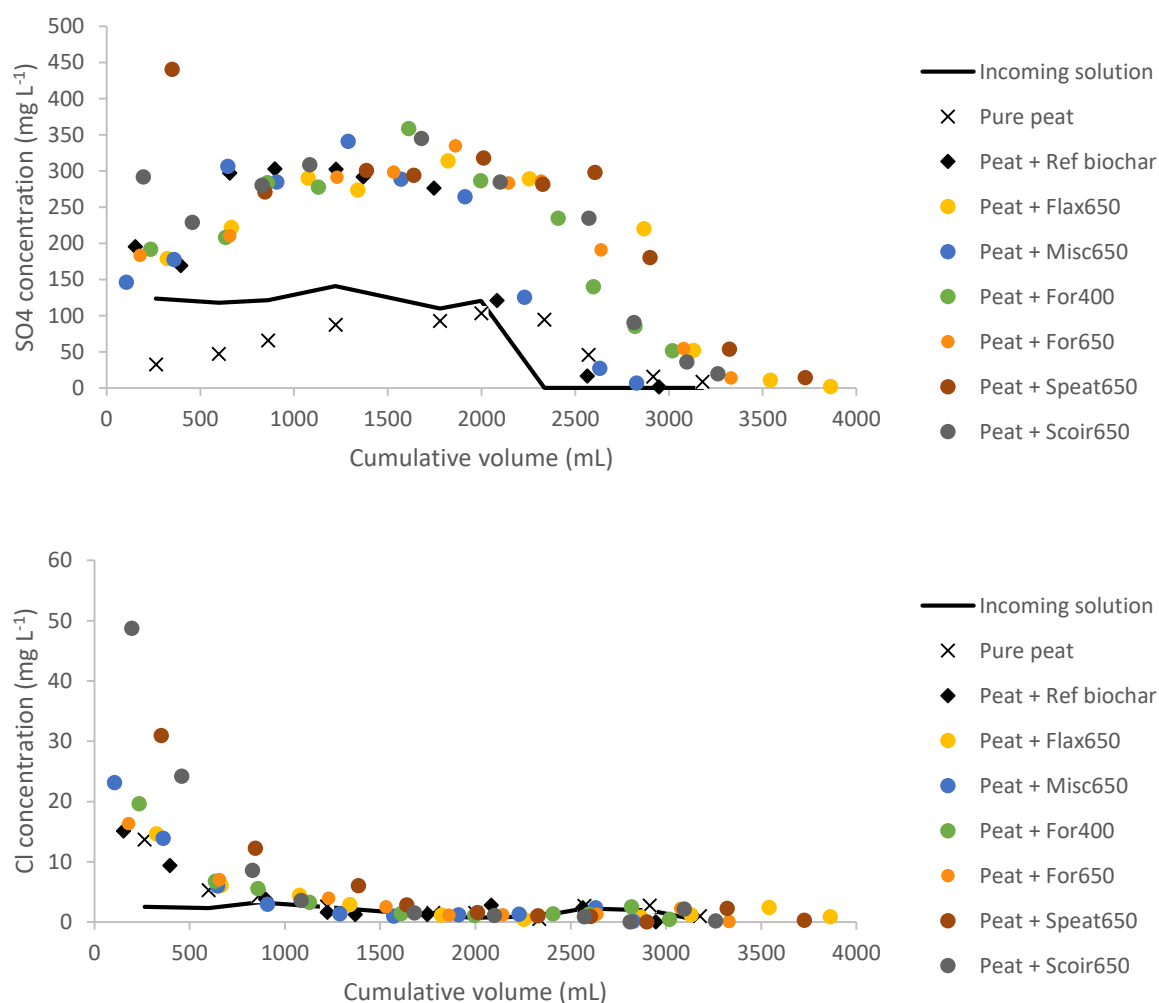


Figure S2. Average concentration (of 2 replicates) of Na, Mg, Mn, Ca, P, NH₄-N, SO₄ and Cl in the leachate of the peat and peat blend columns. The solid black line represents the incoming solution concentration (first phase: fertigation solution, second phase: water). Codes of biochars: see Table 1. Ref biochar: reference biochar based on stone oak wood.

Reference

1. Vanden Nest, T.; Vandecasteele, B.; Ruysschaert, G.; Cougnon, M.; Merckx, R. Effect of organic and mineral fertilizers on soil P and C levels, crop yield and P leaching in a long term trial on a silt loam soil. *Agriculture, Ecosystems and Environment* **2014**, *197*, 309-317.