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SUPPLEMENTARY INFORMATION

Bioponics–An Organic Closed-Loop Soilless Cultivation System: Yields and Characteristics Compared to Hydroponics and Soil Cultivation

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Figure S5. Transmission of the nutrient solution in bioponics (BP) and hydroponics (HP).Supplementary methods

Nutrient solution analysis

Total organic carbon (TOC) was measured as non-purgeable organic carbon (NPOC), according to ASTM D7573 and total nitrogen (TN) according to DIN EN 12260 with a TOC-LCSH analyser equipped with a TNM-L unit (Shimadzu, Kyoto, Japan). The samples for TOC measurements were automatically acidified with HCl to pH < 3 and sparged with purified air to remove inorganic carbon. Purgeable organic carbon (POC) may also be lost during this sample treatment.

The NO_2^-, NO_3^-, SO_4^{2-}, Cl^-, PO_4^{3-}, NH_4^+, Na^+, K^+, Ca^{2+}, Mg^{2+} were determined by ion chromatography (IC) with specific settings (supplementary file: Table S2). The preparation of the eluents, the dilution of the calibration standards (SINGLE ELEMENT IC-Standard-Solutions 1000 mg l^{-1}, Carl Roth GmbH, Karlsruhe, Germany) and individual samples was done with ultrapure water. Additionally, a biogas digestate concentrate sample was analyzed by IC to measure the nutrient input to BP systems.

Iron and manganese in nutrient solution were determined by Inductively coupled plasma - optical emission spectrometry (ICP-OES). The dilution of the calibration standards (standards for ICP 1000 mg l^{-1}, Merck, Buchs, CH) and of individual samples was done with ultrapure water.

Consumer test

A consumer test was carried out to get an idea of how the lettuces from the different cultivation systems are rated by consumers. The 47 volunteer participants were 68% female and 32% male, 28% were 20-29 years old, 32% 30-59 years old and 30% 60-69 years old. 30% reported consuming lettuce daily, 62% several times a week and 9% once a week. The test group was thus not representative for the demographic variation in the population. When buying lettuce, 55% pay attention to the method of cultivation and for 34% of the participants lettuce from sustainable production is important and for 45% even very important. The consumer test showed no significant differences, and due to the insufficient sample size, only trends can be derived. The acceptance of the BP lettuces seemed to be like the ones grown in HP or SO (supplementary file: Table S9). Participants rated all three lettuces as equally popular overall. The popularity in terms of appearance, taste, texture was about the same for the HP and SO lettuces, while the BP lettuces scored slightly lower. In terms of bitterness, the lettuces were also the same, although some testers stated 'just right' for the BP variant. Lettuces from SO tended to be a little too weak in lettuce flavour. In terms of crispness, HP and SO tended to be too crunchy, while BP's lettuces were too delicate. While BP and SO tend to be too less juicy, lettuces from HP were 'just right'.

Supplementary tables

Table S1. Start and end date of each trial.

	Start date	End date
Trial A	June 15th 2020	July 10th 2020
Trial B	July 13th 2020	August 7th 2020
Trial C	August 10th 2020	September 4th 2020

Table S2. Commercially available mineral nutrients used for HP nutrient solution.

	Composition	Supplier
Potassium sulfate	51% K_2O, 18% S	Yara International ASA, Oslo, N
Calcium nitrate	15.5% N, 19% Ca,	Yara International ASA, Oslo, N
Potassium monobasic phosphate	52% P_2O_5, 34%K_2O	Yara International ASA, Oslo, N
Micromix	4.2% Mn*, 0.35% Cu*, 2.45% Zn*, 0.175% Mo, 0.385% B	Ökohum GmbH, Herrendorf, CH
Plantspeed Fe EDTA	6.7% Fe*	Ökohum GmbH, Herrendorf, CH

* from chelate of EDTA

Table S3. Instrument parameters used for ion chromatography (IC) on the 930 Compact IC Flex (Metrohm Schweiz AG, Zofingen, CH).

	Anions	Cations
Eluent (isocratic)	3.2 mM sodium carbonate / 2 mM sodium hydrogencarbonate	7 mM nitric acid
Column	Metrosep A Supp 5 – 250/4.0	Metrosep C6 – 250/4.0
Flow	0.6 ml min^{-1}	0.9 ml min^{-1}
Detector	Conductvity detector @ 30°C	Conductvity detector @ 30°C
Suppressor	on (regeneration with 0.1 M sulfuric acid)	off

Table S4. Iron concentration in nutrient solution reported as MEAN ± SD (n = 66). Significant differences were obtained between cultivation systems regarding UV-C application (Kruskal-Wallis), and located using a Tukey's range test (α = 5%).

	BP	HP
With UV-C [mg l^{-1}]	1.4 ± 1.5 [a]	1.7 ± 1.6 [a]
Without UV-C [mg l^{-1}]	4.3 ± 3.4 [b]	3.3 ± 2.1 [b]
With *Trichoderma* [mg l-1]	2.8 ± 2.7	2.7 ± 2.0
Without *Trichoderma* [mg l-1]	2.8 ± 3.3	2.2 ±2.0

Table S5. Chlorophyll- and flavonoid content and nitrogen balance index (NBI) of lettuce in bioponics (BP) and hydroponics (HP) reported as MEAN ± SD. Significant differences for NBI, chlorophyll, and flavonoid (Kruskal-Wallis) were obtained, and located using a Tukey's range test (α = 5%). Anthocyanin contents were below detection limit.

	BP	HP	SO
n =	97	106	54
NBI [-]	248.94 ± 169.49 [c]	145.37 ± 133.10 [a]	87.99 ± 50.03 [b]
Chlorophyll [μg cm^{-2}]	23.21 ± 3.76 [a]	23.13 ± 3.61 [a]	20.76 ± 3.75 [b]
Flavonoid [μg cm^{-2}]	0.17 ± 0.09 [b]	0.27 ± 0.09 [a]	0.31 ± 0.14 [a]

Table S6. Nitrate content in lettuce leaves fresh weight (ZHAW) in bioponics (BP), hydroponics (HP), and soil (SO) cultivation reported as MEAN ± SD. Significant differences were obtained between cultivation systems (one-way ANOVA), and located using a Tukey's range test (α = 5%).

	BP	**HP**	**SO**
n =	24	24	12
Nitrate [mg kg^{-1}]	396.99 ± 274.02 ^{b}	598.65 ± 181.63 ^{a}	690.29 ± 132.44 ^{a}

Table S7. Comparison of two nitrate (in plant sap; NovaCropControl, and extract; ZHAW), chlorophyll, and NBI measurement methods (Trial C). Significant strong positive correlations (*) between obtained for sap of young and old leaves, sap young leaves and nitrate in extract, and sap of old leaves and nitrate in extract (*n* = 3).

	Correlation [r-value]	**Significance** [p-value]
NBI (Dualex) – Nitrate in sap young leaves	-0.56	0.001
NBI (Dualex) – Nitrate in extract of mixed leaves	-0.46	0.011
NBI (Dualex) – Nitrate in sap old leaves	-0.50	0.005
NBI (Dualex) – Chlorophyll (Dualex)	0.18	0.316
Nitrate in sap young leaves – Nitrate in extract of mixed leaves	0.94	2.514E-14 *
Nitrate in sap young leaves – Nitrate in sap old leaves	0.96	1.195E-16 *
Nitrate in sap young leaves – Chlorophyll (Dualex)	-0.59	0.001
Nitrate in sap old leaves – Nitrate in extract of mixed leaves	0.91	5.606E-12 *
Nitrate in sap old leaves – Chlorophyll (Dualex)	-0.49	0.006
Nitrate in extract of mixed leaves – Chlorophyll (Dualex)	-0.50	0.005

Table S8. UV-C dose applied to the nutrient solution in bioponics (BP) and hydroponics (HP) reported as MEAN ± SD (*n* = 8).

	UV-C dose [mW s cm^{-2}]
BP2 – UV-C	56 ± 27
BP4 - *Trichoderma* & UV-C	88 ± 83
HP1 - *Trichoderma* & UV-C	140 ± 109
HP3 – UV-C	188 ± 147

Table S9. Presence (+) or absence (-) of *T. atrobrunneum* strain T720 in seedling production substrate and bioponics (BP), hydroponics (HP), and soil (SO) cultivation. Systems, in which *Trichoderma* was applied, are highlighted dark **bold**.

	Trial A			**Trial B**			**Trial C**		
Date	**25.06.**	**01.07.**	**09.07.**	**18.07.**	**29.07.**	**06.08.**	**13.08.**	**24.08.**	**03.09.**
Seedlings Trial A - *Trichoderma*; Substrate	**N.A.**	**N.A.**	**N.A.**	**-**	**N.A.**	**N.A.**	**-**	**N.A.**	**N.A.**
Seedlings Trial A Substrate	N.A.	N.A.	N.A.	-	N.A.	N.A.	-	N.A.	N.A.
Seedlings Trial A - *Trichoderma*; Rockwool	**+**	**N.A.**	**N.A.**	**+**	**N.A.**	**N.A.**	**+**	**N.A.**	**N.A.**
Seedlings Trial A Rockwool	-	N.A.	N.A.	-	N.A.	N.A.	-	N.A.	N.A.
Seedling substrate before-use	-	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Cultivation substrate before-use	-	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	+
BP1	-	-	-	-	-	-	-	-	-
BP2 – UV-C	-	-	-	-	-	-	-	-	-
BP3 - *Trichoderma*	**+**	**+**	**+**	**-**	**-**	**-**	**-**	**+**	**+**
BP4 - *Trichoderma* & UV-C	**-**	**+**	**+**	**-**	**+**	**-**	**+**	**+**	**+**
HP1 - *Trichoderma* & UV-C	**+**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**
HP2 - *Trichoderma*	**+**	**-**	**+**	**+**	**-**	**-**	**+**	**-**	**+**
HP3 – UV-C	-	-	-	-	-	-	-	-	-
HP4	-	-	-	-	-	-	-	-	-
SO1 - *Trichoderma*; Irrigation water	**+**	**+**	**+**	**-**	**-**	**+**	**+**	**-**	**+**
SO2; Irrigation water	-	-	-	-	-	-	-	-	-
SO1 - *Trichoderma*; Substrate	**N.A.**	**-**	**+**	**N.A.**	**-**	**-**	**N.A.**	**-**	**-**
SO2 Substrate	N.A.	-	-	N.A.	-	-	N.A.	-	-

Presence (+), absence (-) or no data available (N.A.)

Table S10. Overview consumer test (acceptance test) of lettuce grown in bioponics (BP), hydroponics (HP), and soil (SO) cultivation (n = 47).

Parameter	BP	HP	SO	p-value
Overall popularity	6.34	6.98	6.89	0.37
Popularity appearance	7.34	7.23	7.53	0.43
Popularity taste	6.30	7.04	6.62	0.22
Popularity texture	6.72	7.11	7.11	0.20
Bitterness	All three are perceived similarly in terms of bitterness; for BP, few consumers indicated 'just right'.			
Flavour	SO tended to be a bit too weak in lettuce flavour.			
Crispness	HP and SO tended to be too crisp.			
Delicateness	BP polarized (too delicate), the other two 'just right'.			
Juiciness	BP and SO tend to be too less juicy, HP 'just right'.			

Supplementary figures

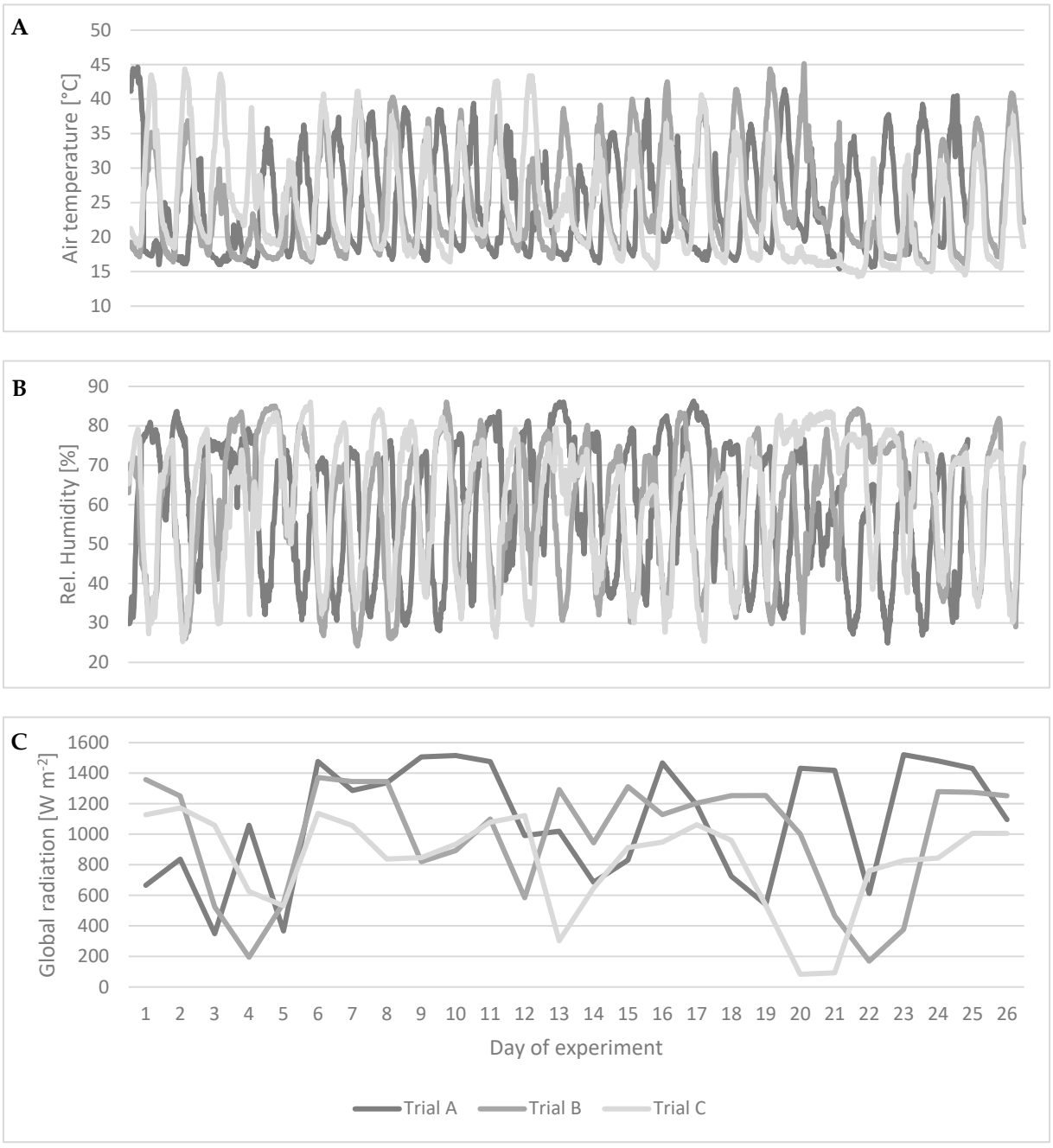


Figure S1. A) Air temperature, B) rel. humidity and C) global radiation in the foliar greenhouse.

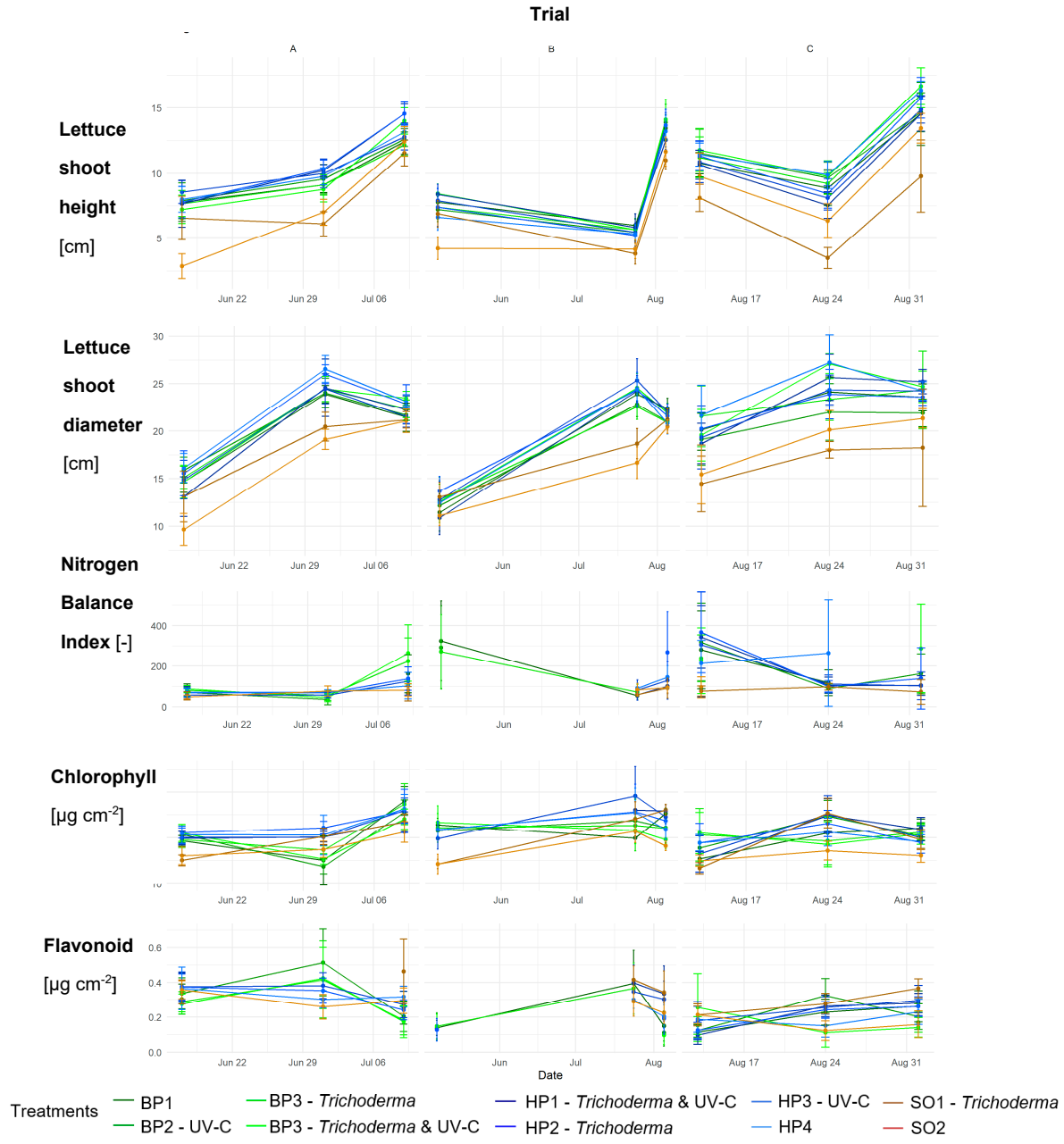


Figure S2. Development of plant growth (lettuce shoot height and diameter, nitrogen balance index, chlorophyll, and flavonoid content) over time in bioponics (BP), hydroponics (HP), and soil (SO) cultivation.

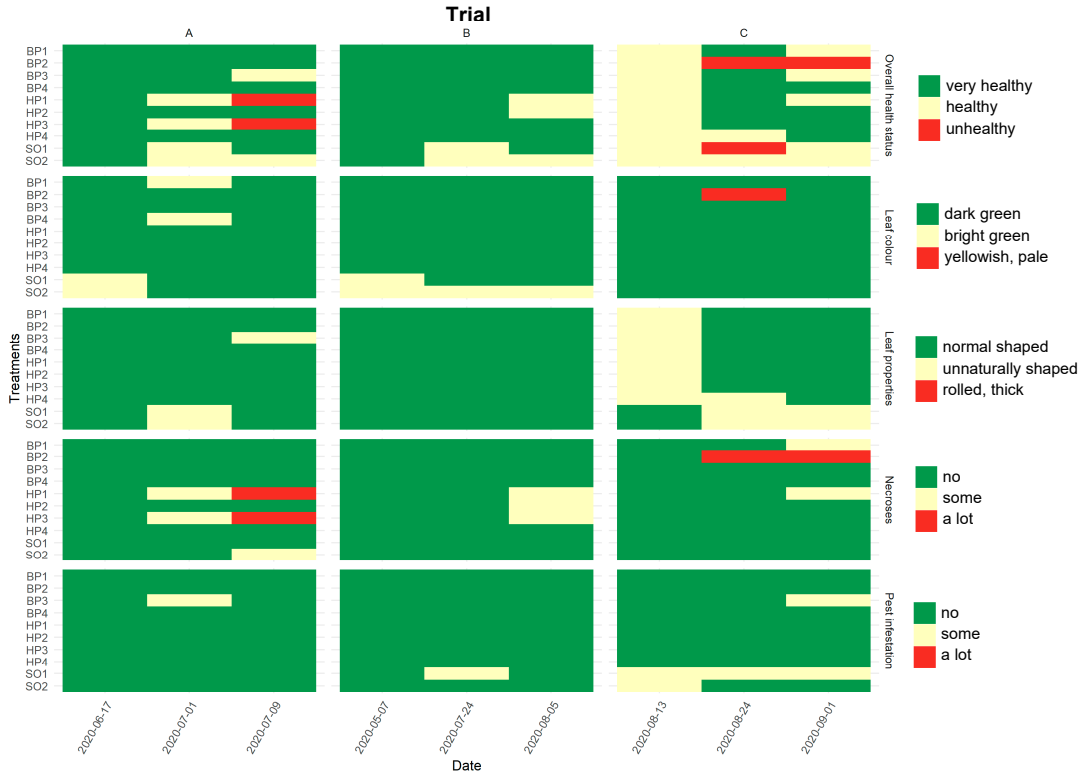
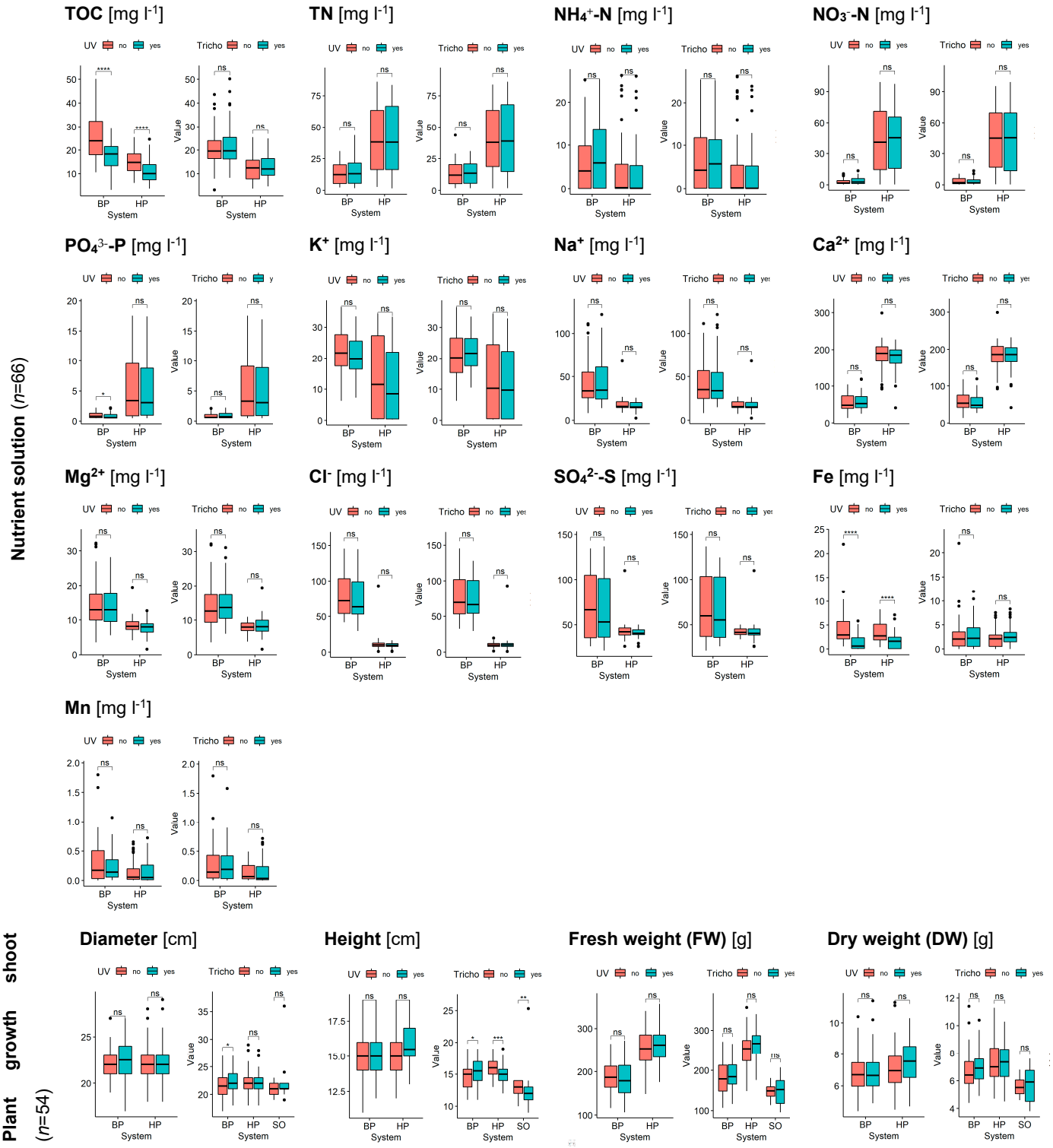
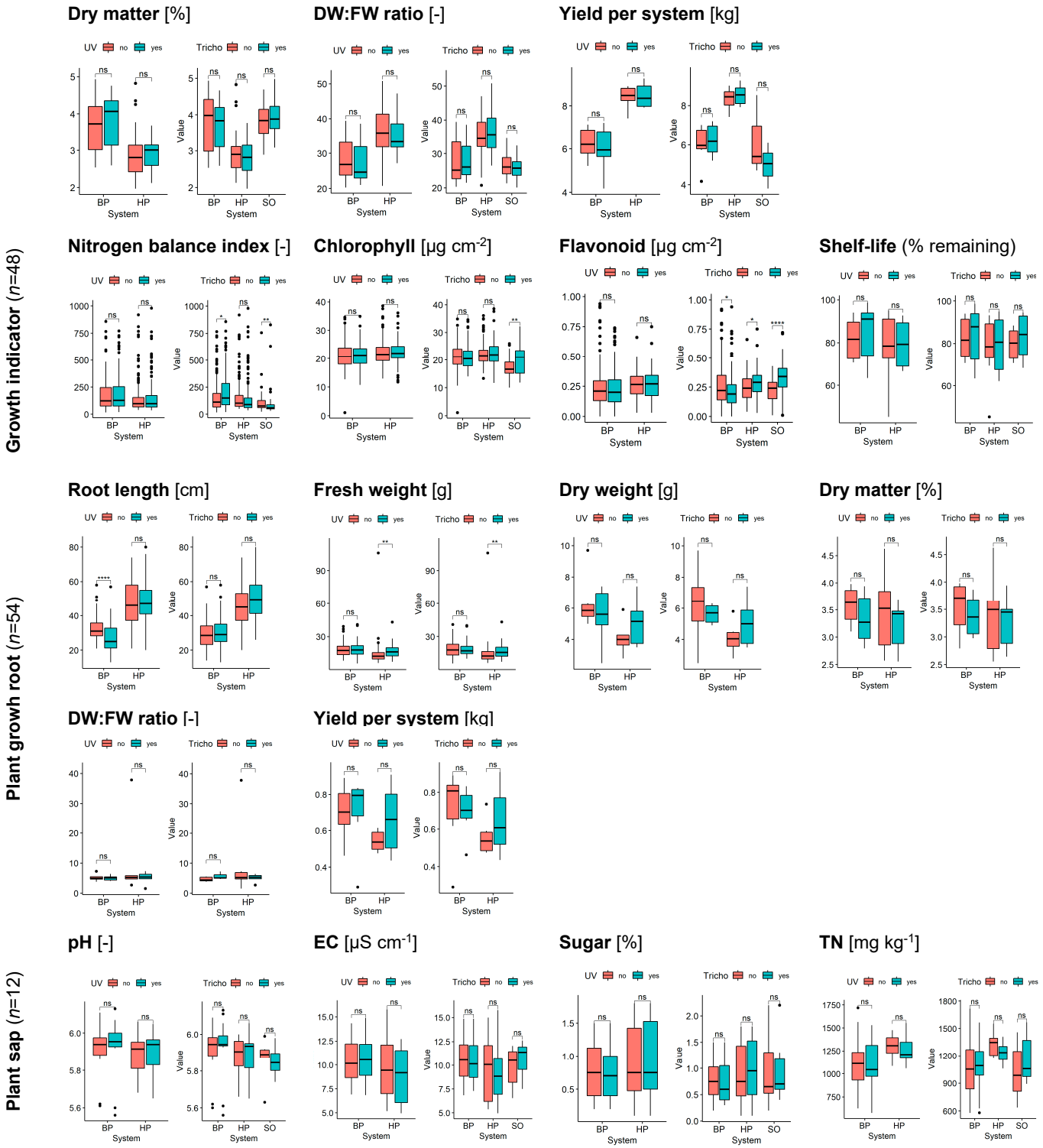


Figure S3. Development of plant health (overall health status, leaf color, leaf properties, necroses, pest infestation) over time in bioponics (BP), hydroponics (HP), and soil (SO) cultivation.


Nutrient solution (n=66)
TOC [mg l-1]
TN [mg l-1]
NH4+-N [mg l-1]
NO3--N [mg l-1]
PO43--P [mg l-1]
K+ [mg l-1]
Na+ [mg l-1]
Ca2+ [mg l-1]
Mg2+ [mg l-1]
Cl- [mg l-1]
SO42--S [mg l-1]
Fe [mg l-1]
Mn [mg l-1]
Plant growth shoot (n=54)
Diameter [cm]
Height [cm]
Fresh weight (FW) [g]
Dry weight (DW) [g]
UV
Tricho
no
yes
BP
HP
SO
System
Value
ns


Growth indicator (n=48)
Plant growth root (n=54)
Plant sap (n=12)
Dry matter [%]
DW:FW ratio [-]
Yield per system [kg]
Nitrogen balance index [-]
Chlorophyll [µg cm-2]
Flavonoid [µg cm-2]
Shelf-life (% remaining)
Root length [cm]
Fresh weight [g]
Dry weight [g]
Dry matter [%]
DW:FW ratio [-]
Yield per system [kg]
pH [-]
EC [µS cm-1]
Sugar [%]
TN [mg kg-1]
UV no yes
Tricho no yes
ns
Value
BP HP SO
System

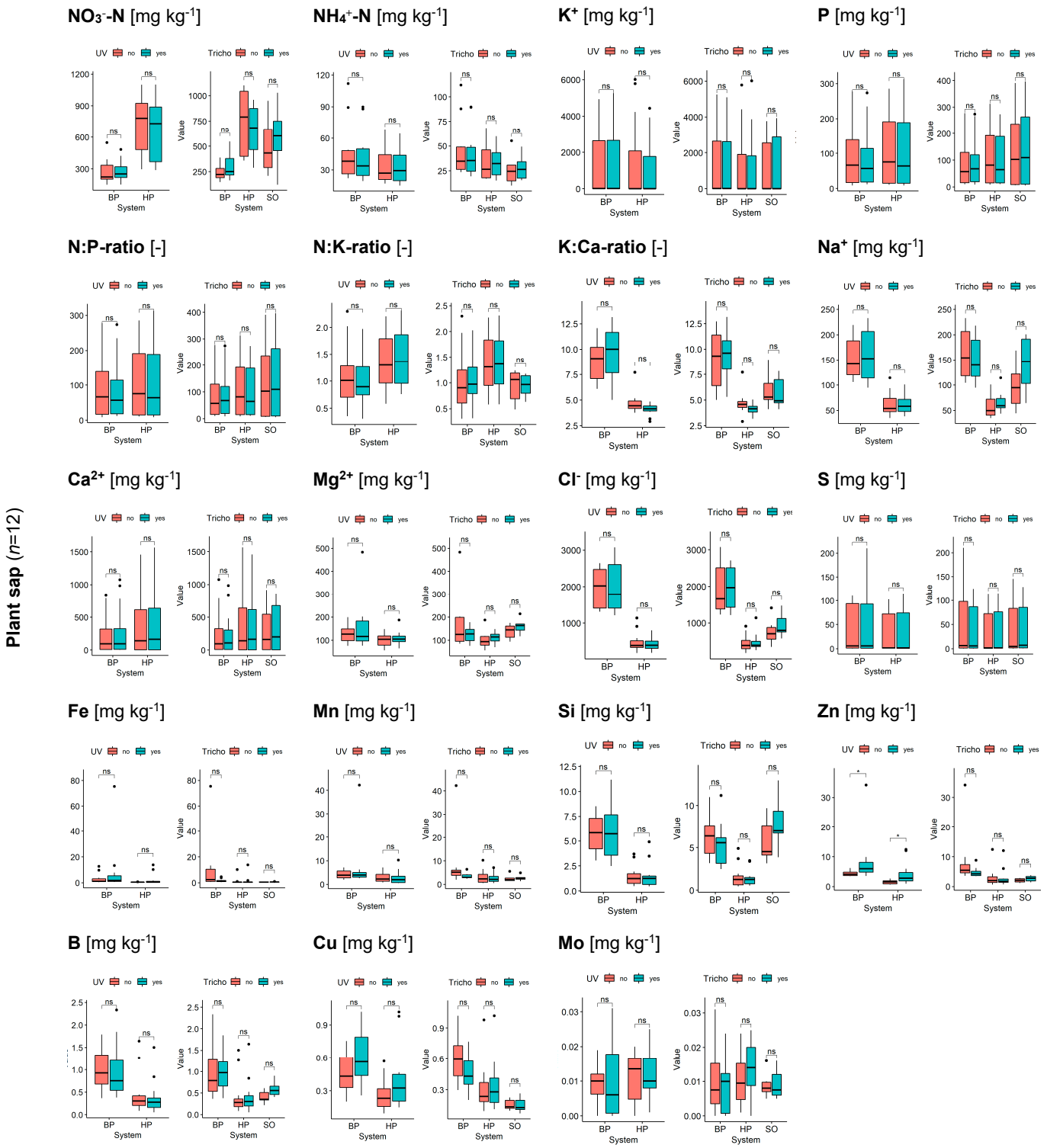


Figure S4. Comparison of water chemistry, plant growth and plant leaf sap regarding UV-C treatment and *T. atrobrunneum* application in bioponics (BP) and hydroponics (HP) based on Wilcoxon Signed Ranks Tests using a Bonferroni correction.

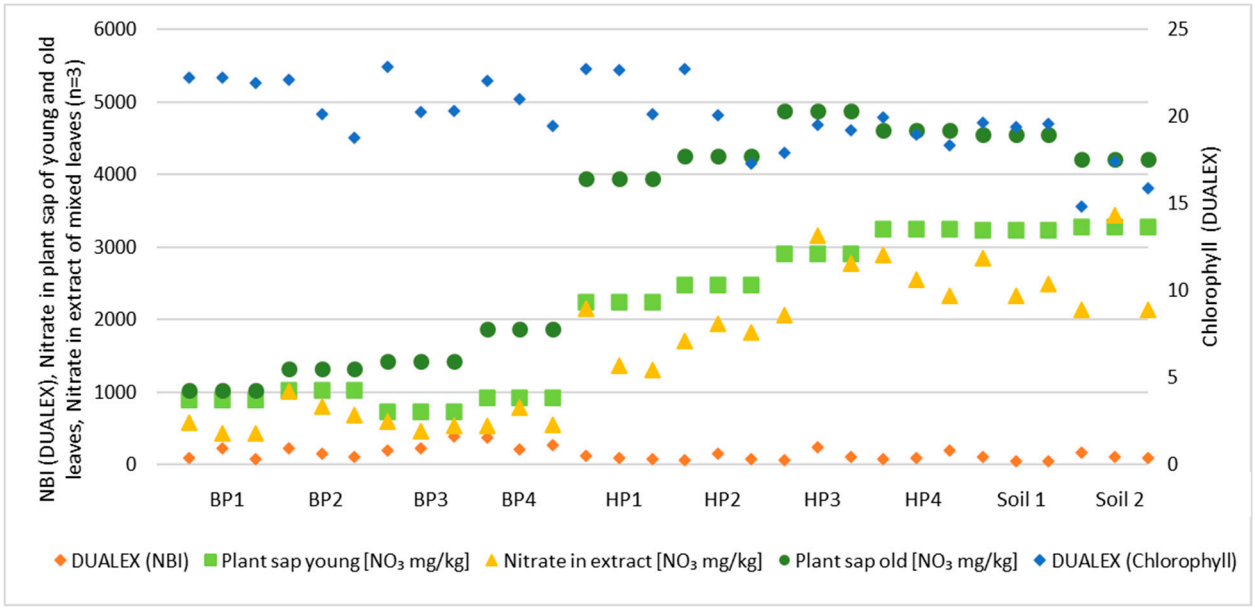


Figure S5. Comparison of two nitrate (in plant sap of young and old leaves; NovaCropControl, and in extract of mixed leaves; ZHAW), chlorophyll, and NBI measurement (Dualex) methods (Trial C).

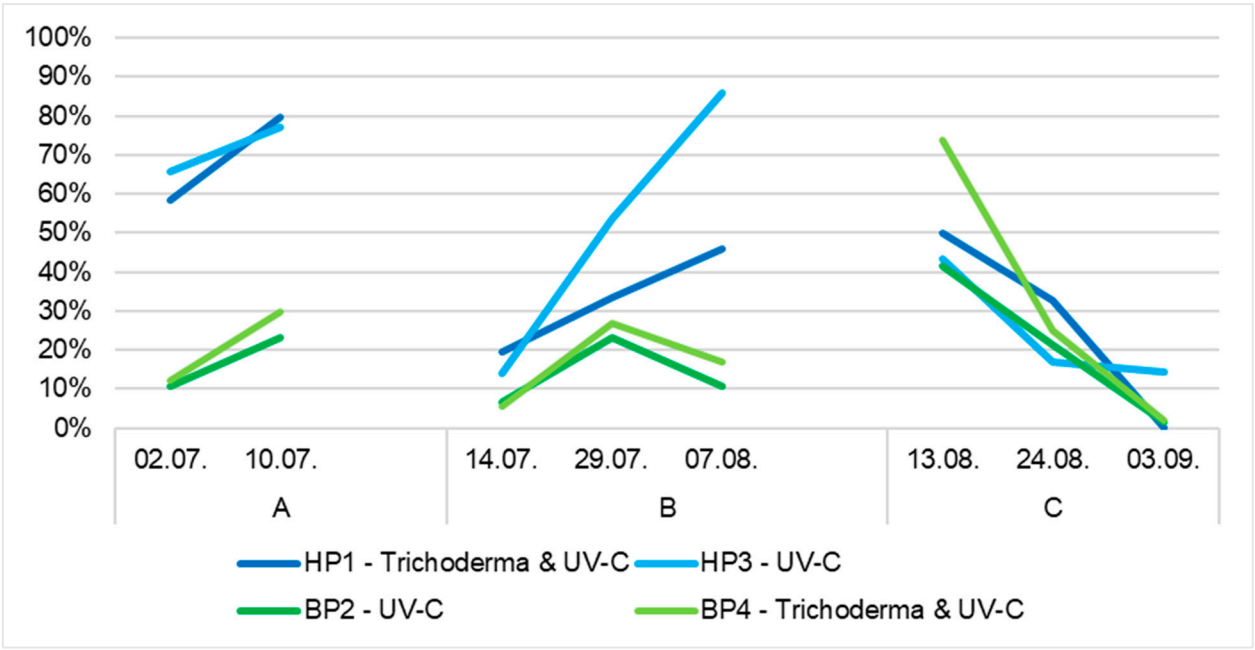


Figure S6. Transmission of the nutrient solution in bioponics (BP) and hydroponics (HP). Bioponic (green) usually had higher turbidity (lower transmission, higher extinction), with exception of Trial C.