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Supplementary Materials

The Sustainability of Basil Production in Hydroponics Can Be Increased Using Low-Nutrient Regimes Incorporating Biofertiliser from Microbial Fuel Cells

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FOR GROWING BASIL HYDROPONICALLY
This is 'Modified Sonneveld's Solution'
Using this solution because:
1. A good ratio of atoms in it (see sheet 'Key Solns') - we can then minimise 'excess' use.
2. Essentially a nitrate + some NH4+ solution for growth of 'leafy' herbaceous plant
3. pH of solution will rise, adjusted with HCl which provides necessary chlorine

10 mM = 140 mg
9.84mM = 137.76 mg

1. MAKING STOCK SOLUTIONS

STOCK SOLUTION A (in 5 litres)		
Ca(NO3)2.3H2O	244.81 g	31.43
NH4NO3	19.16 g	6.70
KNO3	222.59 g	30.82
10%Fe-DTPA	1.00 g	
total N (g)		69.0
STOCK SOLUTION B (in 5 litres)		
KH2PO4	68.52 g	
MgSO4.7H2O	123.87 g	
STOCK SOLUTION C (In 1 litre)		
MnSO4.7H2O	0.772 g	
H3BO3	0.937 g	
Na2MoO4.2H2O	0.061 g	
ZnSO4.7H2O	0.577 g	
CuSO4.5H2O	0.093 g	
CATHOLYTE SOLUTION D (1 Liltre)		
Total N	0.001 g	

2. ADDING NUTRIENT STOCKS TO RESEVOIR TANK

	Tank Volume (L)	Number of Plants	Excess of N
adjust	*40*	*10*	*1.5*

mL per hydroponic set-up

% N Catholyte = 80
mg per plant

	STOCK SOLUTION (mL) INTO TANK							
days	A	B	C	D	N demand	Sonne N supply	+ Cath N supply	N Supply
0-12	0.00	0.00	0	0				
7 to 13	9.93	9.93	0.10	547514	45.63	13.69	54.75	68.44
14-20	20.33	20.33	0.20	1121305	93.44	28.03	112.13	140.16
21 to 27	19.04	19.04	0.19	1050382	87.53	26.26	105.04	131.30
28 to 34	8.45	8.45	0.08	466049	38.84	11.65	46.60	58.26
35 to 41	2.51	2.51	0.03	138497	11.54	3.46	13.85	17.31
42 to end	0.60	0.60	0.01	33272	2.77	0.83	3.33	4.16
totals	61	61	1	3357018	279.75	83.93	335.70	419.63
							From model	437.24 mg

3. CONTROLLING NUTRIENT SOLUTION PROPERTIES WHEN + CATHOLYTE

pH = control with HCl addition
Eh = control with Venturi pump or aquarium aeration pump.
Solution top-up/change - top solution up to 40 Litres as required, do not refresh water in one generation of basil.

(**A**): Nutrient dozing template

Based on Cornell University (US) Controlled Environment Agriculture Group solution for Lettuce (link) — Controlled Environment Agriculture (cornell.edu)
It is for one US gallon = 3.758 litres
For stock at 100x final — Nutrient Solution Calculator - WUR
(to two decimal places)

	g/litre	RMM	stock conc (M)	mL/L of stock	final conc		NO3 (mM)		General ratio	Solution conc	solution ratio
STOCK 1								Nitrogen	1000000	9.85	38869
								Potassium	250000	5.41	21347
Ca(NO3)2.3H2O	48.96	218.1	0.224	10	2.24		9.36	Calcium	125000	2.24	8858
NH4NO3	3.83	80.02	0.048	10	0.48			Magnesium	80000	1.01	3968
KNO3	44.52	101.11	0.440	10	4.40		NH4 (mM)	Phosphorus	60000	1.01	3973
Fe-DTPA							0.48	Sulphur	30000	1.01	3968
								Silicon	30000		
STOCK 2							*NO3/NH4*	Chlorine	3000		
KH2PO4	13.70	136.1	0.101	10	1.01		*19.5*	Iron	2000		
MgSO4.7H2O	24.77	246.38	0.101	10	1.01			Boron	2000	0.01515	60
MnSO4.7H2O	0.08	276.95	0.000	10	2.79	uM		Manganese	1000	0.00279	11
H3BO3	0.09	61.81	0.002	10	15.15	uM		Sodium	400	0.00025	1
Na2MoO4.2H2O	0.01	241.5	0.000	10	0.25	uM		Zinc	300	0.00201	8
ZnSO4.7H2O	0.06	287.45	0.000	10	2.01	uM		Copper	100	0.00044	2
CuSO4.5H2O	0.01	209.62	0.000	10	0.44	uM		Nickel	2		
								Molybdenum	1	0.00025	1

(**B**): Nutrient dozing template

Figure S1. Modified Sonneveld solution dozing template.

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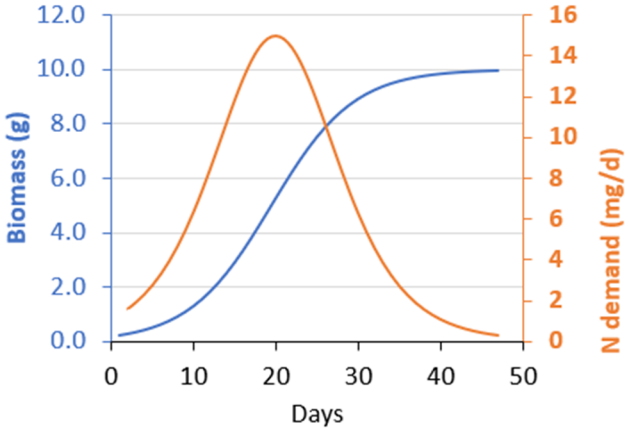

Figure S2. standard sigmoidal growth curve that assumes 3% N in final biomass used for nutrient dozing above (Table S1).

Experiment 1 Chlorophyll Florescence Statistical Analysis

Treatment: 100% NS, 75% NS, 50% NS, 25% NS

Nutrient solution (NS)

Table S1. (A,B,C,D,E): Experiment 1 chlorophyll fluorescence statistical analysis.

Two Way Anova Table						
(A)						
Trait	**Term**	**df**	**SS**	**MS**	**F**	**P**
Alpha (Fv/Fo)	Treatment	3	0.003090	0.001030	5.23	0.002730
Alpha (Fv/Fo)	DAP	7	0.002560	0.000366	1.86	0.091200
Alpha (Fv/Fo)	Treatment:DAP	21	0.002910	0.000139	0.704	0.813000
Alpha (Fv/Fo)	Residual	64	0.012600	0.000197	NA	NA
(B)						
95% TUKEY HSD, Alpha (Fv/Fo)						
100%NS	**75%NS**		**50%NS**		**25%NS**	
0.85 ± 0.01 a	0.84 ± 0.01 ab		0.84 ± 0.02 a		0.83 ± 0.01 b	
(C)						
Trait	**Term**	**df**	**SS**	**MS**	**F**	**P**
Y (Fm)	Treatment	3	0.002770	0.000924	2.62	0.0581
Y (Fm)	DAP	7	0.027400	0.003920	11.1	4.10×10^{-9}
Y (Fm)	Treatment:DAP	21	0.004480	0.000213	0.606	0.899
Y (Fm)	Residual	64	0.022500	0.000352	NA	NA
(D)						
Trait	**Term**	**df**	**SS**	**MS**	**F**	**P**
ETR	Treatment	3	747.000000	249.000000	26.0	4.06×10^{-11}
ETR	DAP	7	3476.000000	497.000000	51.8	5.74×10^{-24}
ETR	Treatment:DAP	21	779.000000	37.100000	3.87	1.49×10^{-5}
ETR	Residual	64	613.000000	9.580000	NA	NA
(E)						
95% TUKEY HSD, ETR						
DAP	**100%NS**	**75%NS**	**50%NS**		**25%NS**	
24	19.87 ± 1.26 ab	17.97 ± 1.80 b	22.27 ± 1.36 a		17.87 ± 1.02 b	
26	18.50 ± 1.31 a	13.87 ± 1.27 b	17.43 ± 1.10 a		11.87 ± 0.51 b	
28	26.30 ± 2.36 a	22.37 ± 0.85 a	26.33 ± 2.12 a		20.77 ± 3.07 a	
31	12.77 ± 2.11 a	12.10 ± 2.04 a	13.27 ± 1.62 a		13.07 ± 1.90 a	
33	41.40 ± 5.14 a	22.53 ± 1.79 b	25.33 ± 2.48 b		23.63 ± 8.80 b	
35	16.73 ± 1.88 a	10.70 ± 3.82 ab	6.87 ± 1.34 b		11.90 ± 1.40 ab	
38	33.37 ± 3.79 a	17.47 ± 6.65 b	20.67 ± 3.43 b		21.67 ± 4.18 ab	
39	14.30 ± 1.78 a	9.63 ± 1.31 a	11.03 ± 5.37 a		10.27 ± 1.50 a	

Different letters (a and b) in each row represent significant differences ($p < 0.05$). Chlorophyll fluorescence parameters of basil (*Ocimum basilicum* cv Genovese) measured under different % Sonneveld's solution (n = 3): effective PSII quantum yield (Y(Fm)), a proxy for PSII efficiency (Alpha (Fv/Fo), and electron transport rate (ETR, μmol e^- m^{-2} s^{-1}).

Experiment 2 Chlorophyll Florescence Statistical Analysis

Treatment: 80% NS, 60% NS, 40% NS, 20% NS

Nutrient solution (NS)

Table S2. (A,B,C,D,E,F): Experiment 2 chlorophyll fluorescence statistical analysis.

TWO WAY ANOVA TABLE						
(A)						
Trait	**Term**	**df**	**SS**	**MS**	**F**	**P**
Alpha (Fv/Fo)	Treatment	3	0.0217	0.00723	15.0	1.11×10^{-6}
Alpha (Fv/Fo)	DAP	4	0.00166	0.000414	0.857	0.498
Alpha (Fv/Fo)	Treatment:DAP	12	0.00969	0.000807	1.67	0.111
Alpha (Fv/Fo)	Residual	40	0.0193	0.000483	NA	NA
(B)						
95% TUKEY HSD, Alpha (Fv/Fo)						
80%NS	**60%NS**		**40%NS**		**20%NS**	
0.84 ± 0.01 a	0.79 ± 0.04 b		0.83 ± 0.01 a		0.83 ± 0.01 a	
(C)						
Trait	**Term**	**df**	**SS**	**MS**	**F**	**P**
Y (Fm)	Treatment	3	0.0465	0.0155	22.2	1.29×10^{-8}
Y (Fm)	DAP	4	0.0772	0.0193	27.6	4.91×10^{-11}
Y (Fm)	Treatment:DAP	12	0.0196	0.00163	2.33	0.0226
Y (Fm)	Residual	40	0.028	0.000699	NA	NA
(D)						
95% TUKEY HSD, Y (Fm)						
DAP	**80%NS**	**60%NS**	**40%NS**		**20%NS**	
31	0.65 ± 0.03 a	0.56 ± 0.02 b	0.64 ± 0.03 ab		0.61 ± 0.05 ab	
33	0.70 ± 0.02 a	0.63 ± 0.02 b	0.69 ± 0.01 a		0.69 ± 0.01 a	
35	0.72 ± 0.01 a	0.58 ± 0.07 b	0.67 ± 0.02 ab		0.71 ± 0.00 a	
38	0.74 ± 0.01 a	0.70 ± 0.04 a	0.74 ± 0.01 a		0.74 ± 0.01 a	
40	0.69 ± 0.03 a	0.67 ± 0.02 a	0.68 ± 0.01 a		0.69 ± 0.01 a	
(E)						
Trait	**Term**	**df**	**SS**	**MS**	**F**	**P**
ETR	Treatment	3	1649	550	3.24	0.032
ETR	DAP	4	14,559	3640	21.5	1.63×10^{-9}
ETR	Treatment:DAP	12	1472	123	0.723	0.721
ETR	Residual	40	6787	170	NA	NA
(F)						
95% TUKEY HSD, ETR						
80%NS	**60%NS**		**40%NS**		**20%NS**	
25.49 ± 17.13 b	39.10 ± 26.48 a		28.03 ± 17.00 ab		33.49 ± 18.61 ab	

Different letters (a and b) in each row represent significant differences ($p < 0.05$). Chlorophyll fluorescence parameters of basil (*Ocimum basilicum* cv Genovese) measured under different % Sonneveld's solution (n = 3): effective PSII quantum yield (Y(Fm)), a proxy for PSII efficiency (Alpha (Fv/Fo), and electron transport rate (ETR, μmol e^{-} m^{-2} s^{-1}).

Experiment 3: Chlorophyll Florescence Statistical Analysis

Treatment**:** 100% NS, 50% NS + 50% WW, 50% NS + 50% WW + 0.46 mM Gypsum

Nutrient solution (NS)

Table S3. (A,B,C,D,E,F): Experiment 3 chlorophyll fluorescence statistical analysis.

TWO WAY ANOVA TABLE						
(A)						
95% TUKEY HSD, Alpha (Fv/Fo)						
Trait	**Term**	**df**	**SS**	**MS**	**F**	**P**
Alpha (Fv/Fo)	Treatment	2	0.00923	0.00462	9.41	0.00042
Alpha (Fv/Fo)	DAP	6	0.00493	0.000822	1.68	0.151
Alpha (Fv/Fo)	Treatment:DAP	12	0.00641	0.000534	1.09	0.393
Alpha (Fv/Fo)	Residual	42	0.0206	0.00049	NA	NA

Table S3. *Cont.*

TWO WAY ANOVA TABLE						
(B)						
95% TUKEY HSD, Alpha (Fv/Fo)						
100% NS	**50% NS + 50% WW**			**50% NS + 50% WW + Gypsum**		
0.86 ± 0.02 a	0.83 ± 0.03 b			0.84 ± 0.02 b		
(C)						
Trait	**Term**	**df**	**SS**	**MS**	**F**	**P**
Y (Fm)	Treatment	2	0.00137	0.000687	0.545	0.584
Y (Fm)	DAP	6	0.0292	0.00486	3.86	0.00375
Y (Fm)	Treatment:DAP	12	0.0392	0.00327	2.59	0.0113
Y (Fm)	Residual	42	0.0529	0.00126	NA	NA
(D)						
95% TUKEY HSD, Y (Fm)						
DAP	**100% NS**	**50% NS + 50% WW**		**50% NS + 50% WW + Gypsum**		
21	0.73 ± 0.01 a	0.70 ± 0.01 c		0.72 ± 0.01 b		
25	0.70 ± 0.02 a	0.67 ± 0.03 a		0.66 ± 0.02 a		
27	0.65 ± 0.04 a	0.66 ± 0.04 a		0.69 ± 0.00 a		
29	0.72 ± 0.06 a	0.73 ± 0.01 a		0.73 ± 0.02 a		
32	0.66 ± 0.08 a	0.71 ± 0.01 a		0.65 ± 0.05 a		
34	0.68 ± 0.07 a	0.73 ± 0.02 a		0.70 ± 0.04 a		
36	0.73 ± 0.02 a	0.61 ± 0.05 b		0.69 ± 0.02 ab		
(E)						
Trait	**Term**	**df**	**SS**	**MS**	**F**	**P**
ETR	Treatment	2	35.2	17.6	3.42	0.0421
ETR	DAP	6	1309	218	42.3	2.96×10^{-16}
ETR	Treatment:DAP	12	283	23.6	4.57	1.09×10^{-4}
ETR	Residual	42	216	5.15	NA	NA
(F)						
95% TUKEY HSD, ETR						
DAP	**100% NS**	**50% NS + 50% WW**		**50% NS + 50% WW + Gypsum**		
21	6.93 ± 0.42 ab	8.00 ± 1.04 a		6.00 ± 0.26 b		
25	14.20 ± 5.54 a	17.23 ± 1.65 a		19.87 ± 4.95 a		
27	17.17 ± 1.36 a	15.03 ± 2.42 a		18.25 ± 1.95 a		
29	8.17 ± 0.59 ab	6.07 ± 1.36 b		8.70 ± 0.17 a		
32	16.50 ± 2.26 a	17.13 ± 3.26 a		13.60 ± 0.35 a		
34	17.60 ± 2.36 a	13.67 ± 1.85 ab		12.60 ± 1.15 b		
36	19.57 ± 0.12 b	14.70 ± 1.22 c		25.43 ± 2.76 a		

Different letters (a, b and c) in each row represent significant differences ($p < 0.05$). Chlorophyll fluorescence parameters of basil (*Ocimum basilicum* cv Genovese) measured under different % Sonneveld's solution, with/without catholyte substitution (n = 3): effective PSII quantum yield (Y(Fm)), a proxy for PSII efficiency (Alpha (Fv/Fo), and electron transport rate (ETR, μmol e^{-} m^{-2} s^{-1}).