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Experiments for *in silico* evaluation of Optimality of Photosynthetic Nitrogen Distribution and Partitioning in the Canopy: an Example Using Greenhouse Cucumber Plants

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[Abstract] Acclimation of leaf traits to fluctuating environments is a key mechanism to maximize fitness. One of the most important strategies in acclimation to changing light is to maintain efficient utilization of nitrogen in the photosynthetic apparatus by continuous modifications of between-leaf distribution along the canopy depth and within-leaf partitioning between photosynthetic functions according to local light availability. Between-leaf nitrogen distribution has been intensively studied over the last three decades, where proportional coordination between nitrogen concentration and light gradient was considered optimal in terms of maximizing canopy photosynthesis, without taking other canopy structural and physiological factors into account. We proposed a mechanistic model of protein turnover dynamics in different photosynthetic functions, which can be parameterized using leaves grown under different levels of constant light. By integrating this dynamic model into a multi-layer canopy model, constructed using data collected from a greenhouse experiment, it allowed us to test *in silico* the degree of optimality in photosynthetic nitrogen use for maximizing canopy carbon assimilation under given light environments.

Keywords: Functional partitioning, Light fluctuation, Mechanistic model, Nitrogen reallocation, Optimality, Photosynthetic acclimation

[Background] Intra-canopy nitrogen distribution in response to light has been intensively studied (Hirose and Werger, 1987; Werger and Hirose, 1991; Anten *et al*., 1995; Dreccer *et al*., 2000; Moreau *et al*., 2012; Hikosaka, 2016) and many studies demonstrated that, although the actual nitrogen distribution resulted in higher canopy photosynthesis than uniform nitrogen distribution, it was still suboptimal (Field, 1983; Evans, 1993; Hollinger, 1996; Hirose *et al*., 1997; Meir *et al*., 2002; Wright *et al*., 2006; Hikosaka, 2016). This discrepancy between optimum and reality could, on one hand, be explained by physiological limitations (Niinemets, 2012; Hikosaka, 2016). On the other hand, it might result from incorrect predictions by over-simplified models, where the effects of variations in the structural characteristics on light interception, age-dependent modifications of leaf biochemistry and photoacclimation in functional nitrogen partitioning were neglected. To incorporate these factors into the acclimation processes, a mechanistic model based on the concept of protein turnover (synthesis and degradation) was proposed to simulate the dynamics of photosynthetic nitrogen in carboxylation, electron transport and light harvesting functions along the development and ageing of the leaf (Pao *et al*., 2019a and 2019b). Leaf elevation angle and leaf area distribution in the canopy was measured to construct a multi-layer canopy model for simulating more realistic intra-canopy light distribution, which

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is used as input for the protein turnover model. By manipulating the parameters controlling nitrogen distribution and partitioning, it is possible to quantify the degree of optimality in photosynthetic nitrogen use for maximizing canopy carbon assimilation *in silico*.

Materials and Reagents

1. 25-L plastic boxes, as container for hydroponic system
2. Polystyrene foam boards for fixing plants onto plastic boxes
3. Rockwool cubes (10 cm × 10 cm × 6.5 cm), as growth medium in the hydroponic system (Grodan Delta; Grodan, Roermond, The Netherlands)
4. Rockwool cubes (3.6 cm × 3.6 cm × 4 cm), as seed sowing medium (Grodan A-OK Starter Plugs; Grodan, Roermond, The Netherlands)
5. Plastic plant support clips
6. N-P-K fertilizer (Ferty 2 MEGA; Planta, Regenstauf, Germany)
7. P-K fertilizer (Ferty Basisdünger 1; Planta, Regenstauf, Germany)
8. N fertilizer (YaraLiva Calcinit; Yara, Oslo, Norway)
9. Paper bags (size should be enough to contain a single cucumber lamina, which is about 15 cm × 20 cm or larger)
10. Rockwool slabs (100 cm × 20 cm × 7.5 cm), as growth medium in the greenhouse (Grodan GT Expert; Grodan, Roermond, The Netherlands)
11. Seed (*Cucumis sativus* 'Aramon', Rijk Zwaan, De Lier, The Netherlands)
12. 1% H_2SO_4 (96% H_2SO_4, Carl Roth, catalog number: 4623; preparation: 6 ml 96% H_2SO_4 mix with 1 L H_2O)

Equipment

1. Walk-in growth chambers with aeration system and controlled air temperature and humidity (Vötsch Industrietechnik, Balingen, Germany) and light source using metal halide lamps (HQI-BT 400 W/D PRO; Osram, Munich, Germany)
2. Quantum sensor LI-190R coupled with light meter LI-250A (LI-COR, Lincoln, NE, USA)
3. Light sensor logger (LI-COR, model: LI-1000, LI-1400 or LI-1500)
4. Temperature data logger (Tinytag; Gemini Data Loggers, Chichester, UK)
5. Portable photosynthesis system LI-6400XT (coupled with 6400-40 leaf chamber fluorometer) or LI-6800 (LI-COR, Lincoln, NE, USA)
6. Chlorophyll meter (Konica Minolta Sensing, model: SPAD-502)
7. Leaf area meter (LI-COR, model: LI-3100C)
8. Laboratory balance with resolution of 0.01 g (Sartorius, model: ED4202S) or with resolution of 0.1 mg for mass below 1 g (Sartorius, model: ED224S)

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9. Vacuum freeze dryer (Alpha 1-4 LSC; Martin Christ Gefriertrocknungsanlagen, Osterode am Harz, Germany)
10. 3D digitizer (Fastrak; Polhemus, Colchester, USA)

Software

1. *R* (ver. 3.3.0 or later; R Foundation for Statistical Computing, https://www.r-project.org/); *R* scripts and simulated example data sets are provided to facilitate data analysis (https://github.com/yichenpao/bio-protocol/)
   a. R script 1 [data processing] (see section Data analysis A, B)
   b. R script 2 [model parameterization] (see section Data analysis C, D); required packages are '*DEoptim*', '*deSolve*', '*ggplot2*', '*reshape2*', '*xlsx*'
   c. R script 3 [simulation and in silico test] (see section Data analysis E-H); required packages are '*DEoptim*', '*deSolve*', '*dplyr*', '*ggplot2*', '*magrittr*', '*xlsx*'
2. Digitool (customized software for 3D-digitizer, availability upon request)

Procedure

A. Raising seedlings for experiments
   1. Sow one cucumber seed (*Cucumis sativus* 'Aramon', Rijk Zwaan, De Lier, The Netherlands) in each rock-wool cube (3.6 cm × 3.6 cm × 4 cm, Figures 1A and 1B) and water sufficiently until the cubes are completely wet.
   2. Sow 20-40% more seeds (lower the germination rate and quality, more the additional amount) than the number of plants required for the experimental design in order to select for uniform seedlings in 7-10 days.
   3. Set environmental conditions to 10-15 mol m^{-2} d^{-1} photosynthetically active radiation (PAR) at the seedling level with 12 h light period, 24 °C day/20 °C night air temperature and 70% relative humidity.
   4. Eight days after sowing, transfer each rockwool cube into a larger rockwool cube (10 cm × 10 cm × 6.5 cm, Figure1C) and irrigate with nutrient solution of N-P-K fertilizer (0.5 g L^{-1} Ferty 2 MEGA; Planta, Regenstauf, Germany; 5.7 mM N, 2.7 mM K, 0.35 mM P, 0.45 mM Mg in working solution) once every day.

B. Growth chamber experiment to parameterize the protein turnover model
   1. Transplanting and starting experiment
      a. Prepare chambers with at least three constant light intensities (one < 5, one between 10-15 and one > 25 mol photon m^{-2} d^{-1} PAR for cucumber) at the plant level, which should cover most variation found in the light environment during crop production.

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b. Prepare nutrient solution with at least three levels of nitrogen (one < 2.8, one 3.5-5 and one > 8.5 mM NO_3^- for cucumber Aramon) using N fertilizer (YaraLiva Calcinit; Yara, Oslo, Norway) and P-K fertilizer (Ferty Basisdünger 1; Planta, Regenstauf, Germany; 5.2 mM K, 1.3 mM P, 0.82 mM Mg in working solution) if the effect of nitrogen is of interest.
c. Transplant the seedlings when their second true leaves reach a length of 3 cm (*ca.* eight days grown in the larger rockwool cubes) into hydroponic system (Figure 1D), consisting of a 25-L plastic box and a piece of polystyrene foam board that fixes a rockwool cube containing a plant.
d. Fill 25-L boxes with nutrient solution and supply the solution with air from aeration system (Figure 1E).
e. Prepare polystyrene foam boards for supporting the plants in the hydroponic system.
    i. Cut polystyrene foam boards to make them fit onto 25-L boxes.
    ii. Cut a squared opening (9.5 cm × 9.5 cm) in the middle of the boards.
    iii. Fix the plants into the openings in polystyrene foam boards and position them into the 25-L boxes.
f. Select the healthy and unshaded leaves within leaf ranks four to eight (counted acropetally) as sampled leaves in each plant and record their dates of appearance.
g. Record light condition at the level of the sampled leaves using quantum sensor LI-190R and light meter LI-250A (LI-COR, Lincoln, NE, USA).

2. Plant care and monitoring environmental conditions
    a. Prepare custom-made leaf holders.
        i. Make leaf holders using plastic coated metal wires to form a loop structure, consisting of a circular part which supports the leaf and a stick part which can be fixed to the stem and petiole (Figure 1F).
        ii. Combine each holder with two plastic plant support clips at the stick part.
        iii. Prepare leaf holders in different sizes and lengths in order to support leaves at various developmental stages.
    b. Allow plants to establish vegetative growth by removing flowers below the seventh node.
    c. Keep plants to single stem by cutting all side shoots and train the rest of the shoot above the sampled leaves downward to avoid mutual shading (Figure 1G).
    d. Renew the nutrient solution completely and record the nitrogen level in the nutrient solution once a week, fill the solution once between two times of solution renewal, and adjust pH value to 6.0-6.5 by 1% H_2SO_4 twice a week.
    e. Place data loggers Tinytag (Gemini Data Loggers, Chichester, UK) around the sampled leaves and record daily mean air temperature (T_{mean}, °C).
    f. Measure and record the PAR at the center of the sampled leaves (Figure 1a in Wiechers *et al.*, 2011) weekly and adjust the angle of the leaves using leaf holders (Figure 1F) to make sure they are horizontally and fully exposed to the light, not shaded by other leaves, in order to achieve target PAR level at the leaves.

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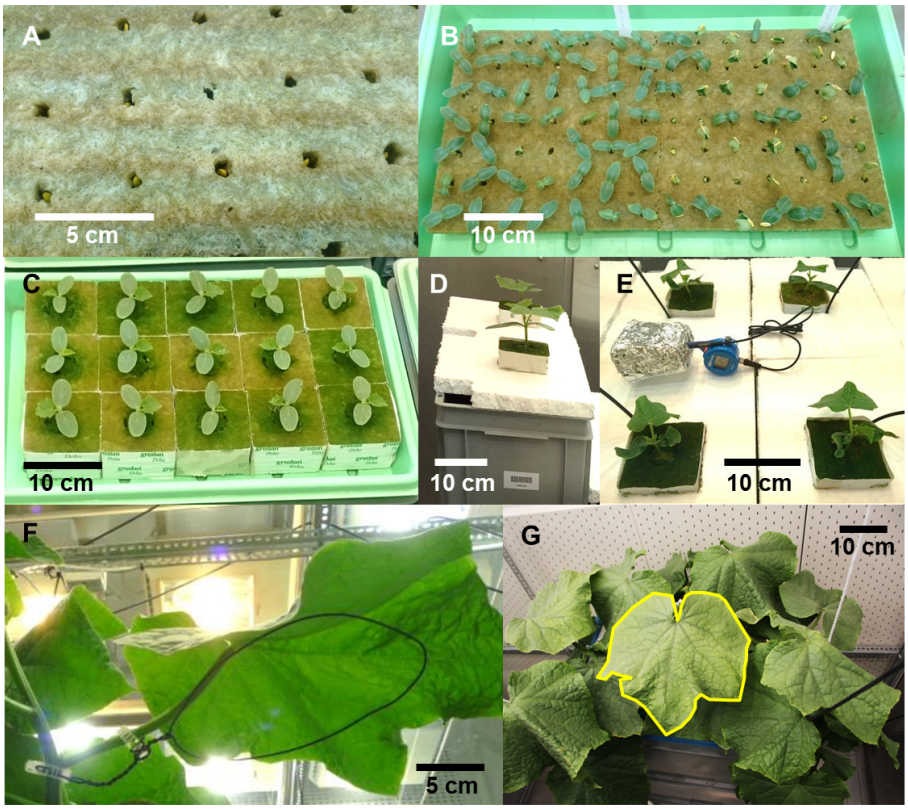

Figure 1. Raising seedlings and setup of growth chamber experiment. A-C. Raising seedlings in rockwool cubes. D-E. Transplanting seedlings into hydroponic system. F. Custom-made leaf holders fixed to a plant on its stem and petiole with support clips. G. Avoiding shading of the sampled leaf (marked yellow) by training rest of the shoot downward.

3. Collecting data of the sampled leaves
   a. Gas exchange
      i. Conduct measurements for each environmental condition at an interval of three to four days, starting with the youngest leaves (*ca.* three days after leaf appearance) and then the older ones to obtain data from leaves with a wide range of ages (*ca.* 40-550 °Cd).
      ii. Estimate age (t, °Cd) of individual leaves days from the day of its appearance to the day of measurement using daily mean temperature and a base temperature (T_{base}, 10 °C for cucumber):

$$t = \sum_{\text{day of appearance}}^{\text{day of measurement}} (T_{\text{mean}} - T_{\text{base}}) \quad \text{(G1)}$$

      iii. Measure net photosynthesis rate (A_n, µmol CO_2 m^{-2} s^{-1}, Table 1), intercellular CO_2 concentration (C_i, µmol mol^{-1}), photosynthetic photon flux density (*PPFD*, µmol m^{-2} s^{-1}) and quantum efficiency of photosystem II electron transport (ϕ_{PSII}) using a portable photosynthesis system LI-6400XT or LI-6800 (LI-COR, Lincoln, NE, USA).

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Table 1. Labeling of variables in the output file from portable photosynthesis systems LI-6400XT and LI-6800 (LI-COR, Lincoln, NE, USA). Necessary variables for data processing are net photosynthesis rate (A_n, µmol CO_2 m^{-2} s^{-1}), intercellular CO_2 concentration (C_i, µmol mol^{-1}), photosynthetic photon flux density (*PPFD*, µmol m^{-2} s^{-1}) and quantum efficiency of photosystem II electron transport (ϕ_{PSII}).

System	A_n	C_i	*PPFD*	ϕ_{PSII}
LI-6400XT	Photo	Ci	PARi	PhiPS2
LI-6800	Pn	Ci	Qin	PhiPS2

iv. Collect data to a *.csv* file (Figure 2 and Table 2), which will be used in the data analysis sections A and B for data processing (in this example *'example_chamber_gas_exchange_data.csv'*).

	A	B	C	D	E	F	G
1	ExpID	MeasureDate	LeafID	An	Ci	PPFD	PhiPS2
2	GC1	29.11.2016	N2-I2-V1-L4	14.538	326.5	1299.89	0.2156
3	GC1	29.11.2016	N2-I2-V1-L4	12.883	287.9	999.18	0.2841
4	GC1	29.11.2016	N2-I2-V1-L4	11.596	298.4	499.81	0.4675
5	GC1	29.11.2016	N2-I2-V1-L4	6.346	344.4	200.08	0.6463
6	GC1	29.11.2016	N2-I2-V1-L4	4.561	352.3	124.04	0.6881
7	GC1	29.11.2016	N2-I2-V1-L4	3.098	361.9	98.37	0.7066
8	GC1	29.11.2016	N2-I2-V1-L4	2.110	368.9	80.36	0.7189
9	GC1	29.11.2016	N2-I2-V1-L4	1.041	379.7	58.58	0.7294
10	GC1	29.11.2016	N2-I2-V1-L4	0.598	384.8	40.59	0.7360
11	GC1	29.11.2016	N2-I2-V1-L4	-0.145	393.1	27.99	0.7410
12	GC1	29.11.2016	N2-I2-V1-L4	-1.437	410.2	0.54	0.7740

Figure 2. Format of gas exchange data file. See Table 2 for explanation for column name, description and unit used.

Table 2. Column name, description and unit used in the gas exchange data file

Column name	Description	Unit
ExpID	ID of the experiment	unitless
MeasureDate	Date of measurement	unitless
LeafID	ID of the measured leaf	unitless
An	Net photosynthesis rate	µmol CO_2 m^{-2} s^{-1}
Ci	intercellular CO_2 concentration	µmol CO_2 mol^{-1}
PPFD	photosynthetic photon flux density	µmol photon m^{-2} s^{-1}
PhiPS2	quantum efficiency of photosystem II electron transport	unitless

v. Cut the lamina directly after the measurement for further analyses.

b. Harvest data

i. Measure relative chlorophyll content (SPAD value) using chlorophyll meter SPAD-502 (Minolta Camera, Japan) and leaf area by area meter LI-3100C (LI-COR, Lincoln, NE, USA) of the harvested lamina.

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ii. Keep each lamina in individual paper bag and freeze them under -20 °C for storage.

iii. Precool sample shelves in the vacuum freeze dryer (Alpha 1-4 LSC; Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) to 10 °C and the ice condenser to -50 °C. Freeze dry lamina samples for 48 h under pressure of 1.030 mbar and then measure the mass of freeze-dried lamina. Please note that most samples can be dried to 1-5% residual moisture; therefore, the measured dry mass should be corrected to exclude the weight of residual moisture.

iv. Grind the lamina into fine powder and analyze total nitrogen (*e.g.*, Nelson and Sommers, 1980) and chlorophyll (*e.g.*, Lichtenthaler, 1987) content.

v. Collect data to a *.csv* file (Figure 3 and Table 3; in this example *'example_chamber_harvest_data.csv'*), which will be used in data analysis sections A and B for data processing.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	ExpID	LeafID	NitrogenID	LightID	VarietyID	AppearanceDate	HarvestDate	LightLevel_mol_m2_d	NitrogenLevel_Mm	MeanTemp_oC	SPAD	LeafArea_cm2	DryMass_g	TotalN_mg_g	Chl_a_mg_g	Chl_b_mg_g
2	GC1	N3-I1-V1-L6	3	1	1	30.10.2016	25.11.2016	4.2	10.0	21.4	45.8	1029.8	2.79	53.53	15.19	5.15
3	GC1	N1-I1-V1-L8	1	1	1	02.11.2016	15.11.2016	4.3	2.5	21.4	42.4	493.8	0.96	54.43	16.94	5.67
4	GC1	N2-I1-V1-L5	2	1	1	28.10.2016	25.11.2016	4.0	5.0	21.4	53.9	788.4	2.42	47.39	13.3	4.77
5	GC1	N1-I2-V1-L8	1	2	1	31.10.2016	22.11.2016	12.0	2.5	22.3	56.4	1050.2	6.37	23.85	7.97	2.99
6	GC1	N1-I2-V1-L4	1	2	1	25.10.2016	18.11.2016	14.3	2.5	22.3	50.3	870.6	5.13	22.43	6.44	2.29
7	GC1	N3-I1-V1-L7	3	1	1	31.10.2016	22.11.2016	4.2	10.0	21.4	48.8	1146.2	2.53	52.38	16.71	6.09
8	GC1	N1-I2-V1-L7	1	2	1	30.10.2016	25.11.2016	12.0	2.5	22.3	49.5	956.9	4.47	26.85	7.64	2.74
9	GC1	N3-I1-V1-L6	3	1	1	31.10.2016	11.11.2016	4.3	10.0	21.4	40.3	800.3	1.57	63.83	16.26	5.26
10	GC1	N2-I3-V1-L6	2	3	1	27.10.2016	29.11.2016	27.7	5.0	22.4	66.7	875.7	9.21	32.89	4.26	1.45
11	GC1	N3-I3-V1-L7	3	3	1	29.10.2016	11.11.2016	26.6	10.0	22.4	60.4	1064.6	6.48	46.73	8.78	2.94
12	GC1	N1-L3-V1-L8	1	3	1	30.10.2016	29.11.2016	28.6	2.5	22.4	42.9	911.8	13.00	23.20	3.20	1.02

Figure 3. Format of harvest data file. See Table 3 for explanation for column name, description and unit used.

Table 3. Column name, description and unit used in the harvest data file

Column name	Description	Unit
ExpID	ID of the experiment	unitless
LeafID	ID of the harvested leaf	unitless
VarietyID	ID of the variety	unitless
LightID	ID of the light treatment	unitless
NitrogenID	ID of the nitrogen treatment	unitless
AppearanceDate	Date of appearance of the harvested leaf	unitless
HarvestDate	Date of harvest	unitless
LightLevel_mol_m2_d	Level of the light treatment	mol photon m^{-2} d^{-1}
NitrogenLevel_Mm	Level of the nitrogen treatment	mM N
MeanTemp_oC	Mean air temperature during growth of the harvested leaf	°C
SPAD	Relative chlorophyll content (SPAD value)	unitless
LeafArea_cm2	Leaf area of the harvested leaf	cm^2
DryMass_g	Leaf dry mass of the harvested leaf	g
TotalN_mg_g	Total nitrogen content of the harvested leaf	mg N g^{-1} dry mass
Chl_a_mg_g	Chlorophyll a content of the harvested leaf	mg Chl a g^{-1} dry mass
Chl_b_mg_g	Chlorophyll b content of the harvested leaf	mg Chl b g^{-1} dry mass

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c. Quantify the empirical relationship between SPAD value and leaf chlorophyll concentration per area to facilitate non-destructive estimation in the greenhouse experiment, using a linear (Chl = a + b × SPAD) or power (Chl = a × $SPAD^b$) function.

C. Gas exchange measurement using portable photosynthesis system LI-6400XT or LI-6800 (LI-COR, Lincoln, NE, USA)

1. Allow leaves to adapt for 10-20 min under measurement conditions of:
   a. Photosynthetic photon flux density (*PPFD*) 1,300 μmol m^{-2} s^{-1},
   b. Sample CO_2 400 μmol mol^{-1},
   c. Leaf temperature 25 °C,
   d. Relative humidity 55-65%,

   until Rubisco is fully activated and photosynthesis rate, stomatal conductance and fluorescence (*F'*) equilibrate to steady states, then read light-saturated net photosynthesis rate (A_{sat}, μmol CO_2 m^{-2} s^{-1}).
2. Measure maximum chlorophyll fluorescence (F_m') using the multiphase flash (MPF) approach (Loriaux *et al*., 2013; Moualeu-Ngangue *et al*., 2017):
   a. Phase 1 with constant maximum irradiance for 320 ms,
   b. Phase 2 with irradiance attenuation (30% ramp depth) over 350 ms,
   c. Phase 3 with constant maximum irradiance as in phase 1 for 200 ms.
3. Measure light response curves of net photosynthesis rate (A_n, μmol CO_2 m^{-2} s^{-1}) under *PPFD* 900, 500, 250, 150, 100, 85, 70, 60, 50, 40, 0 μmol m^{-2} s^{-1}.
4. Total duration of this measurement is 30-40 min per leaf; notice that for old leaves or leaves grown under low light, the time of adaptation is generally longer than for young and high light-grown leaves.
5. Quantum efficiency of photosystem II electron transport (ϕ_{PSII}) is computed using fluorescence data (Murchie and Lawson, 2013):

$$\phi_{PSII} = (F_m' - F')/F_m' \quad \text{(P1)}$$

D. Greenhouse experiment to obtain canopy structural information and data to evaluate the protein turnover model

1. Transplanting and starting experiment
   a. Record daily mean air temperature (T_{mean}, °C) near the seedlings using data logger Tinytag and transplant the seedlings when their third true leaves reach a length of 3 cm (*ca.* two weeks grown in the larger rockwool cubes).
   b. Transfer two plants onto one rockwool slab (100 cm × 20 cm × 7.5 cm) with a distance of 50 cm between them and 150 cm between rows (density of 1.33 plants m^{-2} in a greenhouse with 96 m^2 of cultivation area).
   c. Supply plants with nutrient solution by drip irrigation system with nitrogen levels of interest.

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2. Plant care and monitoring environmental conditions
   a. Train the plants vertically onto wires and remove all side shoots as well as flowers below the seventh node.
   b. Record daily mean air temperature using data logger Tinytag in the greenhouse and daily integral of PAR above the canopies using quantum sensor LI-190R and light meter LI-250A.
   c. Analyze nitrate (Navone, 1964) and ammonium (following German standard methods for the examination of water, waste water and sludge, DIN 38406-5) in the nutrient supply and nitrogen concentration remained in the rockwool slabs weekly.
   d. Collect data to a *.csv* file (Figure 4 and Table 4; in this example '*example_greenhouse_environment_data.csv*'), which will be used in data analysis sections E-H for simulation and *in silico* test.

	A	B	C	D	E	F	G	H	I	J
1	ExpID	Date	DPI_L_L	DPI_L_H	Supply_N_L	Substrate_N_L	Supply_N_H	Substrate_N_H	Tmean_L_L	Tmean_L_H
2	GH1	04.04.2017	10.1	23.6	2.9	2.9	9.7	7.9	22.9	23.6
3	GH1	05.04.2017	5.5	12.7	2.9	2.9	9.7	7.9	23.9	22.6
4	GH1	06.04.2017	9.9	23.1	2.9	2.9	9.7	7.9	22.2	22.1
5	GH1	07.04.2017	5.3	12.4	2.9	2.9	9.7	7.9	22.4	22.5
6	GH1	08.04.2017	6.7	15.6	2.9	2.0	11.9	9.7	22.7	23.2
7	GH1	09.04.2017	6.5	15.0	2.9	2.0	11.9	9.7	23.8	24.6
8	GH1	10.04.2017	6.5	15.0	2.9	2.0	11.9	9.7	22.7	23.1
9	GH1	11.04.2017	5.8	13.5	2.9	2.0	11.9	9.7	22.2	22.3
10	GH1	12.04.2017	4.3	10.0	2.9	2.0	11.9	9.7	21.8	21.5
11	GH1	13.04.2017	7.1	16.5	2.9	2.0	11.9	9.7	22.3	22.3
12	GH1	14.04.2017	8.5	19.9	3.5	0.1	10.9	7.0	23.0	22.9

Figure 4. Format of greenhouse environmental data file. See Table 4 for explanation for column name, description and unit used.

Table 4. Column name, description and unit used in the greenhouse environmental data file

Column name	Description	Unit
ExpID	ID of the experiment	unitless
Date	Date	unitless
DPI_L_***L***	Daily photon integral under light teatment ID ***L****	mol photon m^{-2} d^{-1}
DPI_L_***H***	Daily photon integral under light teatment ID ***H****	mol photon m^{-2} d^{-1}
Supply_N_***L***	Nitrogen level in the nutrient supply under nitrogen treatment ID ***L****	mM N
Substrate_N_***L***	Nitrogen level in the substrate under nitrogen treatment ID ***L****	mM N
Supply_N_***H***	Nitrogen level in the nutrient supply under nitrogen treatment ID ***H****	mM N
Substrate _N_***H***	Nitrogen level in the substrate under nitrogen treatment ID ***H****	mM N
Tmean_L_***L***	Daily mean air temperature under light treatment ID ***L****	°C
Tmean_L_***H***	Daily mean air temperature under light treatment ID ***H****	°C

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*ID of light and nitrogen treatments are named by users and should be identical as the treatment ID in greenhouse structural data.

3. Collecting plant data
   a. Measure leaf number, leaf elevation angle, leaf area and leaf area index non-destructively using a 3D digitizer (Chen *et al*., 2014) at a weekly interval (equates to roughly 100 °Cd difference between two measurements under the greenhouse condition described above) to obtain static canopy structures at various developmental stages.
   b. Estimate age (*t*, °Cd) of individual leaves in the canopy.
      i. Calculate total growing degree days (GDD_{canopy}) from the day of transplanting into greenhouse (when leaf *x* appeared; in this example *x* = 3) to the day of measurement using Eq. G1.
      ii. Divide GDD_{canopy} by the number of leaves appeared after transplanting (excluding the first *x-1* leaves) to estimate phyllochron (°Cd per leaf, interval between appearance of successive leaves), assuming constant phyllochron during the experimental period:

$$phyllochron = GDD_{\text{canopy}}/[total\ leaf\ number - (x-1)] \quad \text{(G2)}$$

      iii. Estimate the age of leaf *n* using phyllochron in relation to leaf *x*:

$$t_{\text{leaf}\,n} = GDD_{\text{canopy}} - (n-x) \times phyllochron \quad \text{(G3)}$$

   c. Measure gas exchange and relative chlorophyll content (SPAD value, used to estimate *Chl* non-destructively) to evaluate the performance of the functional model of photosynthetic protein turnover in the leaf.
   d. Conduct digitization and gas exchange measurement for the same plants within 2-3 days.
   e. Collect data to a *.csv* file (Figure 5 and Table 5; in this example '*example_greenhouse_structure_data.csv*'), which will be used in data analysis sections E-H for simulation and *in silico* test.

	A	B	C	D	E	F	G	H	I
1	ExpID	MeasureDate	PlantID	VarietyID	LightID	NitrogenID	LeafNo	LA_cm2	EA_degree
2	GH1	03.05.2017	219	1	H	H	1	381.3	128.9
3	GH1	03.05.2017	219	1	H	H	2	618.9	77.2
4	GH1	03.05.2017	219	1	H	H	3	873.4	59.5
5	GH1	03.05.2017	219	1	H	H	4	948.8	24.7
6	GH1	03.05.2017	219	1	H	H	5	1100.9	10.5
7	GH1	03.05.2017	223	1	H	H	1	269.2	25.9
8	GH1	03.05.2017	223	1	H	H	2	633.3	70.6
9	GH1	03.05.2017	223	1	H	H	3	1344.1	108.5
10	GH1	03.05.2017	223	1	H	H	4	862.2	13.8
11	GH1	03.05.2017	223	1	H	H	5	1018.8	7.6
12	GH1	03.05.2017	223	1	H	H	6	1307.1	28.3

Figure 5. Format of greenhouse plant structural data file. See Table 5 for explanation for column name, description and unit used.

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Table 5. Column name, description and unit used in the greenhouse plant structural data file

Column name	Description	Unit
ExpID	ID of the experiment	unitless
MeasureDate	Date of measurement	unitless
PlantID	ID of the plant digitized	unitless
VarietyID	ID of the variety digitized	unitless
LightID	ID of the light treatment	unitless
NitrogenID	ID of the nitrogen treatment	unitless
LeafNo	Rank number of the leaf digitized	unitless
EA_degree	Elevation angle of the leaf digitized	
LA_cm2	Area of the leaf digitized	cm^2

E. Digitizing plant structure and converting coordinates into structural data
   1. Digitize the structures of at least two representative plants for each treatment using a 3D digitizer (Fastrak; Polhemus, Colchester, USA).
   2. Obtain the structural information as Cartesian coordinates in a standardized sequence of points on the individual plant organs from bottom to top of a plant (modified from Kahlen and Stützel, 2007; Wiechers *et al*., 2011):
      a. Digitize ‘node 0’ at the base of the stem at its insertion point to the rockwool cube.
      b. Digitize ‘node 1’ opposite to the base of petiole of the first true leaf (‘Node’ in Figure 6A).
      c. Digitize ‘axil 1’ at the insertion point of the first true leaf to the stem (‘Axil’ in Figure 6A).
      d. Digitize ‘leaf 1’ with a predefined sequence and spatial arrangement of 13 points on the lamina surface (Figure 6).
      e. Continue digitizing in the sequence of ‘node *n* - axil *n* - leaf *n*’ until all leaves are digitized.
      f. Neglect flowers and fruits.
   3. Convert Cartesian coordinates into structural data.
      a. Leaf area: area sum of a predefined structure of triangles (Figure 6A).
      b. Leaf elevation angle (*EA*, °): the angle between the orientation of the leaf tip with respect to the base of the leaf (points 1 and 2 in Figure 6B) and the horizontal plane.

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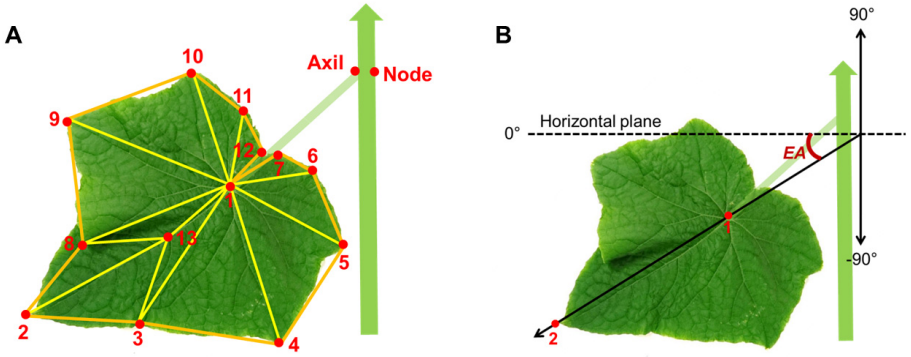

Figure 6. Configurations for extracting leaf area and elevation angle from digitized data. A. Predefined positions of digitized points on the cucumber stem for a node, leaf axil, lamina and structure of triangles defined on the lamina. B. Leaf elevation angle (EA).

4. Quantify the empirical relationships between leaf area index (*LAI*), *EA* and leaf age (*t*, °Cd) to simulate the dynamics of canopy structure in the *in silico* experiment, for example:

$$LAI = lai_{\max}/\{1 + exp[(lai_{t0} - t)/lai_{scal}]\} \quad \text{(G4)}$$

$$EA = 90 - es_{\min} \times exp\{-0.5 \times [ln(t/es_{t0})/ea_{scal}]^2\} \quad \text{(G5)}$$

Data analysis

R script 1 [data processing] (Figure 7)

```
  1› # 1.0.0. Set working directory to current path
  6› # 1.1.0. General constants
 33› # 1.2.0. Load data
 38› # 1.2.1. Merge harvest and gas exchange data
 44› # 1.3.0. Data processing
 95› # 1.3.1. Finalizing data processing and estimate photosynthetic nitrogen
116› # 1.4.0. Output processed data to file
```

Figure 7. Overview of *R* script 1 for data processing. Input data files for this script are *'example_chamber_harvest_data.csv'* and *'example_chamber_gas_exchange_data.csv'* from growth chamber experiment.

A. Estimate photosynthetic parameters using gas exchange data (Figure 7, # 1.3.0)

1. Estimate leaf absorptance (*abs*, unitless) using leaf chlorophyll concentration (*Chl*, mmol m^{-2}) given by Evans (1993):

$$abs = Chl/(Chl + 0.076) \quad \text{(P2)}$$

2. Estimate electron transport rate (*J*, μmol e^- m^{-2} s^{-1}) under various photosynthetic photon flux density (*PPFD*, μmol m^{-2} s^{-1}):

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$$J = abs \times \beta \times PPFD \times \phi_{\mathrm{PSII}} \quad \text{(P3)}$$

where β (0.5, unitless) is the partitioning fraction of photons between photosystem II and I.

3. Estimate maximum electron transport (J_{max}) by least squares fitting to a nonrectangular hyperbola:

$$J = \{J_{\max} + \phi \times PPFD - [(J_{\max} + \phi \times PPFD)^2 - 4\theta \times J_{\max} \times \phi \times PPFD]^{0.5}\}/(2\theta) \quad \text{(P4)}$$

where ϕ (0.425 μmol e^- μmol^{-1} photon; Chen *et al*., 2014) is the conversion efficiency of photons to *J*, and θ (0.7, unitless; Chen *et al*., 2014) is a constant convexity factor describing the response of *J* to *PPFD*.

4. Estimate daytime respiration rate (R_d, μmol CO_2 m^{-2} s^{-1}) using the linear portion ($40 \leq PPFD \leq 100$ μmol m^{-2} s^{-1}) of the light response curve (Kok, 1948) since the light compensation point in cucumber leaf is observed at *ca.* 40 μmol photon m^{-2} s^{-1}.
5. Estimate mesophyll conductance to CO_2 (g_m, mol m^{-2} s^{-1}) using the variable *J* method (Harley *et al*., 1992):

$$g_m = A_n/\{C_i - [\Gamma^* \times (J + 8A_n + 8R_d)/(J - 4A_n - 4R_d)]\} \quad \text{(P5)}$$

where Γ^* is CO_2 compensation point in the absence of mitochondrial respiration (43.02 μmol mol^{-1} for cucumber; Singsaas *et al*., 2003) and C_i is intercellular CO_2 concentration (μmol mol^{-1}).

6. Estimate chloroplastic CO_2 concentration (C_c, μmol mol^{-1}):

$$C_{\mathrm{c}} = C_{\mathrm{i}} - A_{\mathrm{n}}/g_{\mathrm{m}} \quad \text{(P6)}$$

7. Estimate maximum carboxylation rate (V_{cmax}, μmol CO_2 m^{-2} s^{-1}) using the one-point method (De Kauwe *et al*., 2016):

$$V_{\mathrm{cmax}} = (A_{\mathrm{sat}} + R_{\mathrm{d}}) \times (C_{\mathrm{c}} + K_{\mathrm{m}})/(C_{\mathrm{c}} - \Gamma^*) \quad \text{(P7)}$$

where K_m (mmol mol^{-1}) is given by K_c (404 μmol mol^{-1}) and K_o (278 mmol mol^{-1}), Michaelis-Menten constants of Rubisco for CO_2 and O_2, and O_c (210 mmol mol^{-1}) is the mole fraction of O_2 at the site of carboxylation:

$$K_m = K_c \times (1 + O_c/K_o) \quad \text{(P8)}$$

8. Parameterize empirical relationships between R_d, g_m and leaf age (*t*, °Cd, estimated using Eq. G1), mean daily photon integral over the last four days of leaf growth (DPI_{4d}, mol m^{-2} d^{-1}) and

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leaf photosynthetic nitrogen (N_{ph}, mmol m^{-2}) using, for example, Eq. 10 and Eqs.16 and 17 in Pao *et al*. (2019a):

$$R_d = r_{max} \times DPI_{4d} \times exp(-r_g \times DPI_{4d} \times t) + r_m \times DPI_{4d} \times t \quad \text{(P9)}$$

$$g_m = (g_{mm} \times N_{ph} + g_{mm0}) \times exp\{-0.5 \times ln[(t/g_{mt0})/g_{mscal}]^2\} \quad \text{(P10)}$$

B. Estimate photosynthetic nitrogen pools using photosynthetic parameters (Figure 7, # 1.3.1)

1. Estimate nitrogen involved in carboxylation (N_V, mmol N m^{-2}), electron transport (N_J, mmol N m^{-2}) and light harvesting (N_C, mmol N m^{-2}) following Buckley *et al*. (2013):
   a. N_V includes Rubisco and represents the nitrogen investment in carboxylation capacity:

$$N_V = V_{cmax}/\chi_V \quad \text{(M1a)}$$

   b. N_J includes electron transport chain, photosystem II core and Calvin cycle enzymes other than Rubisco:

$$N_J = J_{max}/\chi_J \quad \text{(M1b)}$$

   c. N_C includes photosystem I core and light harvesting complexes I and II:

$$N_C = (Chl - N_J \times \chi_{CJ})/\chi_C \quad \text{(M1c)}$$

   where χ_V (μmol CO_2 mmol^{-1} N s^{-1}) is the carboxylation capacity per unit Rubisco nitrogen, and χ_J (μmol e$^-$ mmol^{-1} N s^{-1}) is the electron transport capacity per unit electron transport nitrogen. χ_{CJ} (mmol Chl mmol^{-1} N) and χ_C (mmol Chl mmol^{-1} N) are the conversion coefficients for chlorophyll per electron transport nitrogen and per light harvesting component nitrogen, respectively.
2. Photosynthetic nitrogen (N_{ph}, mmol N m^{-2}) is defined as biologically active nitrogen in the proteins involved in photosynthetic functions, including nitrogen involved in carboxylation, electron transport and light harvesting:

$$N_{ph} = N_V + N_J + N_C \quad \text{(M2)}$$

3. Photosynthetic nitrogen partitioning fraction of a pool *X* (p_X) is determined as the ratio of nitrogen in the pool *X* (N_X, mmol N m^{-2}) to N_{ph}:

$$p_X = N_X/N_{ph} \quad \text{(M3)}$$

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4. Output processed data to a *.csv* file (Figure 8; in this example '*chamber_processed_data.csv'*) (Figure 7, # 1.4.0), which will be used in data analysis sections C and D for model parameterization.

	A	B	C	D	E	F	G	X	Y	Z	AA	AB	AC	AD
1	LeafID	ExpID.x	VarietyID	LightID	NitrogenID	AppearanceDate	HarvestDate	LMA_g_m2	Chl_a_b_mmol_m2	abs	J	Jmax	Jmax_se	Jmax_pv
2	N1-I3-V1-L5	GC1	1	3	1	27.10.2016	08.11.2016	66.5	0.639	0.894	201.2	223.9	8.62	1.64E-10
3	N1-I3-V1-L7	GC1	1	3	1	29.10.2016	01.11.2016	54.2	0.448	0.855	162.0	167.2	9.91	1.12E-08
4	N1-I3-V1-L5	GC1	1	3	1	27.10.2016	18.11.2016	76.5	0.616	0.890	158.7	174.7	6.64	1.45E-10
5	N1-I3-V1-L7	GC1	1	3	1	29.10.2016	11.11.2016	63.6	0.486	0.865	191.8	218.3	7.29	4.01E-11
6	N1-I3-V1-L8	GC1	1	3	1	30.10.2016	29.11.2016	142.6	0.671	0.898	152.1	164.5	5.57	4.63E-11
7	N1-I1-V1-I5	GC1	1	1	1	29.10.2016	01.11.2016	26.0	0.396	0.839	76.1	76.3	3.99	3.32E-09
8	N1-I2-V1-L5	GC1	1	2	1	27.10.2016	04.11.2016	41.8	0.543	0.877	214.1	247.3	9.73	2.05E-10
9	N1-I2-V1-L6	GC1	1	2	1	29.10.2016	01.11.2016	37.2	0.433	0.851	142.4	153.2	7.24	1.23E-09
10	N3-I2-V1-L8	GC1	1	2	3	31.10.2016	25.11.2016	61.0	0.922	0.924	162.6	184.2	5.71	1.94E-11
11	N2-I2-V1-L6	GC1	1	2	2	28.10.2016	01.11.2016	34.4	0.378	0.833	151.9	158.6	8.49	4.16E-09
12	N3-I3-V1-L8	GC1	1	3	3	31.10.2016	09.12.2016	111.0	0.574	0.883	191.7	208.6	9.13	5.79E-10

AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AQ
Rd	gm_mol_m2_s	Cc_umol_mol	Vc	Vcmax	NV_mmol_m2	NJ_mmol_m2	NC_mmol_m2	Nph_mmol_m2	pV	pJ	pC	LeafAge_oCd
1.47	0.343	181.2	167.6	167.6	37.3	23.6	18.6	79.5	0.470	0.297	0.233	148.8
6.18	0.075	155.7	144.9	144.9	32.3	17.6	13.0	62.9	0.513	0.280	0.207	37.2
0.71	0.162	181.2	132.2	132.2	29.4	18.4	17.9	65.8	0.447	0.280	0.273	272.8
2.38	0.473	218.6	146.0	146.0	32.5	23.0	14.1	69.6	0.467	0.331	0.202	161.2
1.60	0.087	117.5	154.4	154.4	34.4	17.4	19.6	71.3	0.482	0.243	0.275	372.0
2.59	0.069	198.9	60.6	60.6	13.5	8.0	11.6	33.2	0.407	0.243	0.350	34.2
2.59	0.316	238.9	156.1	156.1	34.8	26.1	15.7	76.6	0.454	0.341	0.205	98.4
2.96	0.094	167.5	123.1	123.1	27.4	16.2	12.6	56.1	0.488	0.288	0.224	36.9
0.98	0.148	180.8	135.5	135.5	30.2	19.4	27.0	76.6	0.394	0.254	0.352	307.5
4.36	0.112	190.9	123.4	123.4	27.5	16.7	10.9	55.2	0.498	0.303	0.198	49.2
2.21	0.128	141.2	179.4	179.4	39.9	22.0	16.7	78.6	0.508	0.280	0.212	483.6

Figure 8. Format of processed data file output from *R* script 1. This file will be used for model parameterization.

R script 2 [model parameterization] (Figure 9)

```
  1 # 2.0.0. Set working directory to current path
  6 # 2.1.0. Library
 13 # 2.2.0. Load data
 17 # 2.3.0. Protein turnover model
 34 # 2.4.0. Function for fitting
150 ####------------------------------------------------------####
151 ####----- Arguments CHANGABLE for parameterization ------####
152 ####------------------------------------------------------####
153 # 2.4.1. Parameterization step 1 fitting
177 # 2.4.2. Parameterization step 2 fitting
215 # 2.4.3. Parameterization step 3 fitting
250 # 2.5.0. Output parameterization result to file
```

Figure 9. Overview of *R* script 2 for model parameterization. Input data file for this script is *'chamber_processed_data.csv'* from script 1.

C. Description of protein turnover model (Figure 9, # 2.3.0)

The rate of change of a functional nitrogen pool N_X is determined by the instantaneous protein synthesis rate ($S_X(t)$, mmol N m^{-2} $°Cd^{-1}$) and degradation rate ($D_X(t)$, mmol N m^{-2} $°Cd^{-1}$) of the corresponding enzymes and protein complexes at a given leaf age (t, °Cd):

$$dN_X/dt = S_X(t) - D_X(t) \quad \text{(M4)}$$

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Protein synthesis as an age-dependent and zero-order process (Li *et al*., 2017), is described by a logistic function and independent of the current N_X state:

$$S_X(t) = 2\,S_{\max,X}/\left[1 + \exp\left(t \times t_{d,X}\right)\right] \quad \text{(M5)}$$

where $S_{max,X}$ (mmol N m^{-2} $°Cd^{-1}$) is the maximum protein synthesis rate of N_X which occurs at the early stage of leaf development. The constant $t_{d,X}$ ($°Cd^{-1}$) describes the relative decreasing rate of the protein synthesis over time. At age of $1/t_{d,X}$, S_X reduces to 53.8% of $S_{max,X}$.

The degradation rate D_x is governed by first-order kinetics (Li *et al*., 2017) with a degradation constant $D_{r,X}$ ($°Cd^{-1}$):

$$D_X(t) = D_{r,X} \times N_X(t) \quad \text{(M6)}$$

The variable $S_{max,X}$ is a function of daily leaf PAR interception ($DPI_{interceptLeaf}$, mol photon m^{-2} d^{-1}):

$$S_{\max,X} = S_{mm,X} \times k_{I,X} \times DPI_{\text{interceptLeaf}}/\left(S_{mm,X} + k_{I,X} \times DPI_{\text{interceptLeaf}}\right) \cdot r_{N,X} \quad \text{(M7)}$$

where $S_{mm,X}$ (mmol N m^{-2} $°Cd^{-1}$) is potential maximum protein synthesis rate and $k_{I,X}$ is rate constant describing the increase of $S_{max,X}$ with light. The factor $r_{N,X}$ increases with nitrogen level in the nutrient solution (N_S, mM) by a Michaelis-Menten constant, $k_{N,X}$ (mM):

$$r_{N,X} = N_S/\left(k_{N,X} + N_S\right) \quad \text{(M8)}$$

D. Parameterizing protein turnover model using data from growth chamber experiment (Figure 9)

Solve differential Eqs. M4-M6 to obtain $S_{max,X}$, $t_{d,X}$ and $D_{r,X}$ in *R* using an algorithm programed with *lsoda*() function from '*deSolve*' package and *DEoptim*() function from '*DEoptim*' package, which minimizes the sums of squares of the residuals between observations and simulations (Figure 9, # 2.4.0). There are three steps to quantify the parameters in Eqs. M5-M8:

1. Quantify $t_{d,X}$ (Eq. M5) and $D_{r,X}$ (Eq. M6) for each photosynthetic nitrogen pool using data of all environmental conditions, assuming $D_{r,X}$ and $t_{d,X}$ being species- and function-specific and not influenced by the light and nitrogen availabilities (Figure 9, # 2.4.1).
2. Quantify $S_{max,X}$ (Eq. M5) with the determined values of $t_{d,X}$, and $D_{r,X}$ for each environmental condition (Figure 9, # 2.4.2).
3. Determine $S_{mm,X}$, $k_{I,X}$ (Eq. M7) and $k_{N,X}$ (Eq. M8) from $S_{max,X}$ by nonlinear least squares fitting using *nls*() function from '*stats*' package, and the standard errors (se) and *P* values (pv) for the estimates are calculated as well (Figure 9, # 2.4.3).
4. Output results (Figure 9, # 2.5.0) to a *.csv* file (Figure 10; in this example '*parameterize_result_output.csv*'), which will be used in data analysis sections E-H for simulation and *in silico* test.

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	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	ExpID.x	VarietyID	NX	td	Dr	Smm	kI	kN	Smm_se	kI_se	kN_se	Smm_pv	kI_pv	kN_pv
2	GC1	1	NV	0.00153	0.02565	0.8776	0.1847	0.5791	0.06283	0.02895	0.27114	0	0	0.0335
3	GC1	1	NJ	0.00197	0.01376	0.8776	0.1847	0.5791	0.06283	0.02895	0.27114	0	0	0.0335
4	GC1	1	NC	0.00158	0.02029	0.8776	0.1847	0.5791	0.06283	0.02895	0.27114	0	0	0.0335

Figure 10. Format of parameterization result file output from *R* script 2. This file will be used for simulation and *in silico* test.

R script 3 [simulation and *in silico* test] (Figure 11)

```
# 3.0.0. Set working directory to current path
# 3.1.0. Library
####------------------------------------------------------####
####---- CHANGABLE Constants and empirical functions ----####
####------------------------------------------------------####
# 3.2.0. Constants for simulation process
# 3.2.1. General constants
# 3.3.0. Empirical functions of LAI and EA, Equations G4 and G5
# 3.3.1. Empirical functions of Rd and gm, Equations P9 and P10
####------------------------------------------------------####
####------------------ Data preparation ------------------####
####------------------------------------------------------####
# 3.4.0. Load data
# 3.4.1. Data preparation
####------------------------------------------------------####
####----------------- Defined functions ------------------####
####------------------------------------------------------####
# 3.5.0. Functions for simulation process
# 3.5.1. Functions for phyllochron, leaf age and light extinction, Equations G1, G2, G3 and P11
# 3.6.0. Function for simulating daily canopy carbon assimilation (DCA)
####------------------------------------------------------####
####-- Defined function to simulate Nph with various fd -####
####------------------------------------------------------####
# 3.7.0. Function for simulating photosynthetic nitrogen (Nph) with various fd
####------------------------------------------------------####
####----- IN SILICO TEST simulation with various fd -----####
####------------------------------------------------------####
# 3.7.1. Simulate Nph with various fd
# 3.7.2. Simulate DCA with various fd
# 3.7.3. Output DCA comparison with various fd to file
# 3.7.4. Plot DCA comparison between various fd
# 3.7.5. Output detailed results with various fd to file
# 3.7.6. Output control nitrogen partitioning in the canopy
####------------------------------------------------------####
####- Defined functions to simulate Nph with various fp -####
####------------------------------------------------------####
# 3.8.0. Function for simulating photosynthetic nitrogen (Nph) with various fp
# 3.8.1. Function for maximizing DCA with given fp
# 3.8.2. Function for optimizing Nph partitioning with various fp
####------------------------------------------------------####
####----- IN SILICO TEST simulation with various fp -----####
####------------------------------------------------------####
# 3.8.3. Optimize Nph partitioning with various fp
# 3.8.4. Output DCA comparison with various fp to file
# 3.8.5. Plot DCA comparison with optimal fp
# 3.8.6. Output detailed results with optimal fp to file
```

Figure 11. Overview of R script 3 for simulation and *in silico* test. Input data files for this script are '*example_greenhouse_structure_data.csv*' and '*example_greenhouse_environment_data.csv*' from greenhouse experiment and '*parameterize_result_output.csv*' from script 2.

E. Simulating leaf photosynthesis (Figure 11, # 3.6.0)

In order to evaluate daily canopy carbon assimilation, net photosynthesis rate (A_n, μmol CO_2 m^{-2} s^{-1}) of individual leaves in the canopy should be simulated. A_n is defined as the minimum of RuBP carboxylation-limited (A_c, mmol CO_2 m^{-2} s^{-1}) and RuBP regeneration-limited (A_j, mmol CO_2 m^{-2} s^{-1}) net photosynthesis rate (Farquhar *et al*., 1980). The steady-state A_c can be solved analytically with Eqs. 9b, 14 and 15, and A_j with Eqs. 9c, 14 and 15 in Pao *et al*. (2019a) with given values of

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leaf-to-air vapor pressure deficit (*D*, kPa), atmospheric CO_2 concentration (C_a, μmol mol^{-1}), photosynthetic photon flux density (*PPFD*, μmol m^{-2} s^{-1}) at leaf level and photosynthetic parameters.

1. Leaf level *PPFD* is simulated (Figure 11, # 3.4.1) following Beer-Lambert's law (Monsi and Saeki, 2005) with canopy light extinction coefficient (*k*) and leaf area index (*LAI*) and adjusted by the cosine of leaf elevation angle (*EA*, °), which are estimated with leaf age using Eqs. G4 and G5 (Figure 11, # 3.3.0):

$$PPFD = PPFD_{\text{aboveCanopy}} \times exp(-k \times LAI) \times \cos(EA) \quad \text{(P11)}$$

where diurnal *PPFD* above the canopy ($PPFD_{\text{aboveCanopy}}$, μmol m^{-2} s^{-1}) at a given time (t_{hour}, h) during the day is calculated by a simple cosine bell function (Kimball and Bellamy, 1986) with daily PAR integral above the canopy ($DPI_{\text{aboveCanopy}}$, mol m^{-2} d^{-1}) and day length (*DL*, h):

$$PPFD_{\text{aboveCanopy}} = DPI_{\text{aboveCanopy}} \times \frac{\pi}{2DL} \times \frac{10^6}{3600} \times \cos\left[\frac{\pi \times (t_{\text{hour}} - 12)}{DL}\right] \quad \text{(P12)}$$

2. Photosynthetic parameters J_{max}, V_{cmax}, *abs*, R_d and g_m
   a. Electron transport rate J_{max} under a given *PPFD* is calculated using Eq. P4.
   b. Carboxylation rate (V_c, μmol CO_2 m^{-2} s^{-1}) is calculated based on the amount of activated Rubisco under a given *PPFD* (Qian *et al*., 2012):

$$V_c = V_{cmax} \cdot \left\{0.31 + \frac{0.69}{1+\exp[-0.009 \cdot (PPFD - 500)]}\right\} \quad \text{(P13)}$$

   c. *abs* is calculated using Eq. P2.
   d. R_d and g_m are simulated using empirical relationships Eqs. P9 and P10 (Figure 11, # 3.3.1).

F. Simulating daily canopy carbon assimilation (Figure 11, # 3.6.0)

Daily canopy carbon assimilation during daytime (DCA, mol d^{-1}) on day *d* is simulated with input data of:

1. environmental information (from the appearance the leaf three until day *d*): T_{mean} (Eq. G1), $DPI_{\text{aboveCanopy}}$ (Eq. P10), and nitrogen concentration in the supply solution and rockwool slabs;
2. greenhouse canopy characteristics (on day *d*): leaf area (digitized data, Figure 7A) and leaf age (Eqs. G1-G3; Figure 11, # 3.5.1).

Each leaf in a canopy is first simulated for its photosynthetic nitrogen pools until day *d* using Eqs. M4-M8 (Figure 11, # 3.7.0 and # 3.8.0) and photosynthetic parameters using Eqs. M1a-M1c. $DPI_{\text{interceptLeaf}}$ during the growth is simulated using Eq. P11 (Figure 11, # 3.5.1). The mean value of $DPI_{\text{aboveCanopy}}$ during the plant growth (from transplanting to measurement day) is used as $DPI_{\text{aboveCanopy}}$ on day *d* to simulate DCA. Nitrogen level in the nutrient solution (N_S) is assumed to be

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the mean value of nitrogen concentration in the supply solution and rockwool slabs (Figure 11, # 3.4.1). In order to test the effect of incoming light condition on day *d* on the optimality of N_{ph} distribution and partitioning, $DPI_{aboveCanopy}$ is multiplied by a factor '*DPI multiplier*' assigned by users (Figure 11, # 3.7.2 and # 3.8.3).

Leaf net photosynthesis is simulated for a time step of 0.1 h on day *d*, and summed up for every 0.1 h over the daytime to obtain daily leaf carbon assimilation (DLA, mol d^{-1}). DCA is calculated as the sum of DLA of all leaves in the canopy.

G. *In silico* experiment to test the optimality of nitrogen distribution in the canopy (Figure 11)

To evaluate the effects of between-leaf distribution of N_{ph} on DCA, a distribution factor f_d is introduced into Eq. M5 to create variations in the rate of protein synthesis (Figure 11, # 3.7.0):

$$S_X(t) = 2\,S_{\max,X}/\left[1+\exp\left(t\times t_{d,X}\times f_d\right)\right] \quad \text{(S1)}$$

A control condition is defined with f_d = 1. Increasing f_d accelerates the decrease in the rate of protein synthesis and enhances acropetal N_{ph} reallocation, but it also reduces total N_{ph} in a canopy (N_{canopy}). To obtain the leaf photosynthetic nitrogen content ($N_{leaf,i}$, mmol N in leaf *i*) with comparable N_{canopy}, simulated $N_{leaf,i}$ with $f_d = n$ (denoted as $N'_{leaf,i}$) is adjusted proportionally to the ratio between N_{canopy} obtained with f_d = 1 and N_{canopy} obtained with $f_d = n$:

$$N_{\mathrm{leaf},i}(f_d=n) = N'_{\mathrm{leaf},i}(f_d=n)\times\left[N_{\mathrm{canopy}}(f_d=1)/N_{\mathrm{canopy}}(f_d=n)\right] \quad \text{(S2)}$$

Photosynthetic nitrogen partitioning fraction of a pool *X* in leaf *i* ($p_{X,i}$) is set equal to the control value:

$$p_{X,i} = N_{X,i}(f_d=1)/N_{\mathrm{ph},i}(f_d=1) \quad \text{(S3)}$$

These adjustments assure the same amount of N_{canopy} while changing the distribution pattern. The factor f_d is varied from 0.5 to 5.0 (Figure 11, # 3.7.1), which gives values of N_{ph} comparable to those observed in cucumber leaves (< 150 mmol N m^{-2}). Values of DCA produced by various f_d (Figure 11, # 3.7.2) are then compared with the control DCA (f_d = 1) under a given environmental condition and output (Figure 11, # 3.7.3) to a *.xlsx* file (in this example '*Test_fd_result.xlsx*') and plotted (Figure 11, # 3.7.4 and Figure 12). Detailed results with N_{ph} and p_X at leaf level can be output (Figure 11, # 3.7.5) to a *.xlsx* file (in this example '*Test_fd_result_detailed.xlsx*').

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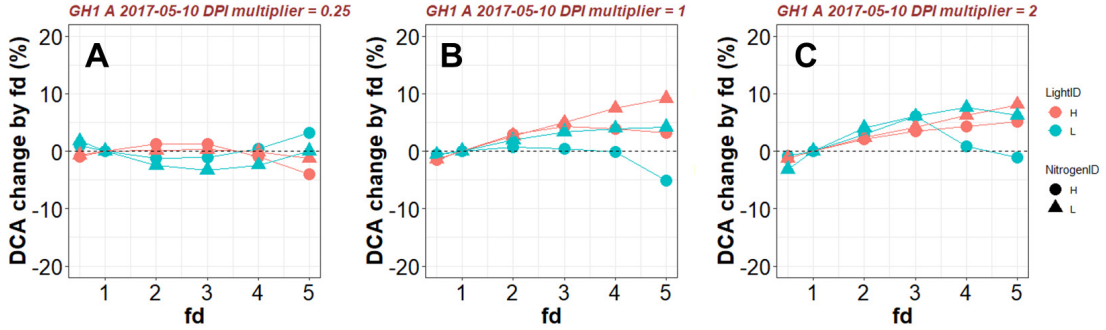

Figure 12. Example results of percentage change in daily canopy carbon assimilation during daytime (DCA, mol d^{-1}) with various values of photosynthetic nitrogen (N_{ph}) distribution factor f_d under a given daily photosynthetically active radiation integral above the canopy (DPI, mol m^{-2} d^{-1}). A. DPI = mean DPI during plant growth multiplied by 0.25. B. DPI = mean DPI during plant growth. C. DPI = mean DPI during plant growth multiplied by 2. Positive change in DCA resulted from varying f_d indicates that the control N_{ph} distribution (f_d = 1) is sub-optimal.

H. *In silico* experiment to test the optimality of nitrogen partitioning in the leaf (Figure 11)

To evaluate the effects of within-leaf partitioning of N_{ph} on DCA, a partitioning factor $f_{p,X}$ is introduced into Eq. M7 to modify maximum protein synthesis $S_{max,X}$, in order to create variations in partitioning pattern between the three photosynthetic nitrogen pools (Figure 11, # 3.8.0):

$$S_{\mathrm{max},X} = \left[S_{\mathrm{mm},X} \times f_{\mathrm{p},X} \times k_{\mathrm{I},X} \times I_{\mathrm{Ld}} / \left(S_{\mathrm{mm},X} \times f_{\mathrm{p,X}} + k_{\mathrm{I},X} \times I_{\mathrm{Ld}}\right)\right] \cdot r_{\mathrm{N},X} \quad \text{(S4)}$$

A control condition is defined by $f_{p,X}$ = 1. An increase in $f_{p,X}$ results in a higher rate of synthesis of N_X and increases the partitioning to pool X. The potential maximal protein synthesis rate for pool X ($S_{mm,X}$) is modified by a factor $f_{p,X}$, ranging from 0.2 to 2.0, to find the optimal within-leaf N_{ph} partitioning between functions which maximizes DCA (Figure 11, # 3.8.3). Partitioning pattern which maximizes DCA under a given environmental condition is identified as 'optimal' and then compared with the control DCA ($f_{p,X}$ = 1), and the results are output (Figure 11, # 3.8.4) a *.xlsx* file (in this example '*Test_fp_result.xlsx*') and plotted (Figure 11, # 3.8.5 and Figure 13). Detailed results with optimal N_{ph} partitioning at leaf level can be output (Figure 11, # 3.8.6) to a *.xlsx* file (in this example '*Test_fp_result_detailed.xlsx*').

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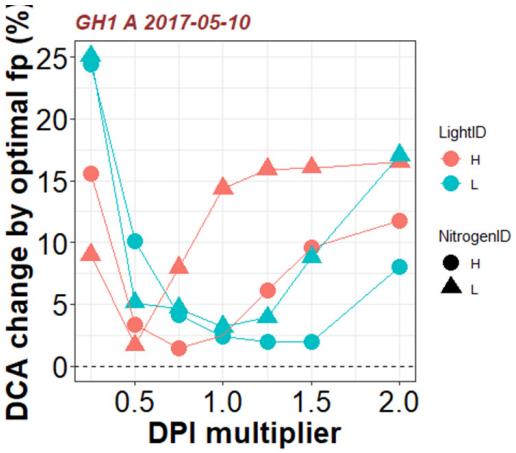

Figure 13. Example results of percentage change in daily canopy carbon assimilation during daytime (DCA, mol d^{-1}) with various values of photosynthetic nitrogen (N_{ph}) partitioning factor $f_{p,X}$ under mean incoming daily photosynthetically active radiation integral above the canopy (*DPI*, mol m^{-2} d^{-1}) during plant growth multiplied by DPI multiplier between 0.25 and 2.0. Positive change in DCA resulted from optimal $f_{p,X}$ indicates that the control N_{ph} partitioning ($f_{p,X}$ = 1) is sub-optimal. In this example, N_{ph} partitioning of the canopy grown under light treatment H and nitrogen treatment L is sub-optimal under its growing light environment, and DCA can be improved almost 15% if N_{ph} partitioning is optimized.

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Competing interests

The authors declare no conflict of interest.

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