The nitrogen cost of photosynthesis

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JXB expert view

Abstract

Global food security depends on three main cereal crops (wheat, rice and maize), achieving and maintaining their high yields as well as increasing future yields. Fundamental to the production of this biomass is photosynthesis. The process of photosynthesis involves a large number of proteins which together account for the majority of the nitrogen in leaves. As large amounts of nitrogen are removed in the harvested grain, this needs to be replaced from either synthetic fertilizer or biological nitrogen fixation. Knowledge about photosynthetic properties of leaves in natural ecosystems is also important, particularly when we consider the potential impacts of climate change. While the relationship between nitrogen and photosynthetic capacity of a leaf differs between species, leaf nitrogen content provides a useful way to incorporate photosynthesis into models of ecosystems and the terrestrial biosphere. This review provides a generalised nitrogen budget for a C3 leaf cell and discusses the potential for improving photosynthesis from a nitrogen perspective.

Keywords: fertilizer, leaf traits, light capture, bioenergetics, Rubisco, chlorophyll protein complexes, photosynthetic electron transport

Introduction

Just over a century has passed since the discovery of the Haber Bosch method to reduce atmospheric dinitrogen and produce ammonia which paved the way for large scale production of nitrogenous fertilizer. There is a close correlation between the production of nitrogenous fertilizer and the production of the three key cereals that dominate the human diet (wheat, rice and maize) (http://www.fao.org/faostat). Crop production reflects photosynthesis integrated over the life of the crop. The process of photosynthesis requires a system that is comprised of many proteins and which accounts for the majority of nitrogen in any plant. It is this large nitrogen requirement to construct a photosynthetic system that results in the need for nitrogenous fertilizer by highly productive crops.

The photosynthetic rate and other leaf attributes have been measured for an extensive number of species. By combining two attributes, nitrogen content and the leaf dry mass, both expressed per unit leaf area, it is possible to predict the photosynthetic capacity. This has proved a useful way of parameterising photosynthesis over large areas of natural ecosystem that is necessary for global models (Rogers *et al.*, 2017a). There are differences between species in the relationship between photosynthesis and leaf nitrogen content (Kattge *et al.*, 2011). These reflect underlying differences in the allocation of nitrogen between proteins, their properties, or a consequence of anatomical differences. Nitrogen and photosynthesis are central to each of these interrelated topics (Box 1) which are considered in this review.

Leaf nitrogen budget

It is timely to revisit the nitrogen budget of a leaf. Firstly, X-ray crystallography of protein complexes reveals atomic resolution, providing accurate pigment to protein stoichiometries. Secondly, a vast number of proteins and their relative abundance can now be determined using mass spectrometry.

Dividing nitrogen between different pools can take several directions. At a cellular level, one can separate soluble and membrane fractions from a cell wall pool. Alternatively, one can partition nitrogen between different organelles. These two approaches rely on different methodologies and generally no approach accounts for all of the nitrogen. Consequently, melding together these disparate pieces of information requires adjustments to reach an average total. This average may not apply to a particular leaf due to effects of age, environment and species, but it provides a useful common starting point for C3 species.

With mass spectrometry, thousands of proteins and their relative abundance in a range of organisms have been measured. The PaxDb resource (Wang *et al.*, 2015) provides estimates of protein abundance derived from spectral counts across many experiments and tissue types. The *Arabidopsis thaliana* database comprises 46 datasets, covering 76% of the expected proteome. More than 90% of protein is accounted for by the 1000 most abundant proteins. However, protein quantification by mass spectrometry has an inherent bias, over representing more abundant proteins when low abundance proteins fall below the instrument detection limits. Identification of proteins by mass spectrometry can also be biased due to a range of factors affecting peptide detection such as peptide solubility, enzymatic digestion efficacy and differing ion efficiencies (reviewed in Lundgren *et al.*, 2010).

Consequently, the PaxDb values cannot be taken at face value (Li *et al.*, 2017). For example, the abundance of Rubisco large subunits outnumbers that of the small subunit by more than eightfold. One would expect that the amounts of these two subunits should be similar as the mature Rubisco enzyme contains 8 large and 8 small subunits. Rubisco represents about 40% of soluble protein (Eckardt *et al.*, 1997), or 20% of leaf nitrogen (Evans and Seemann, 1984), which equates to about 119,000 ppm for each subunit (see supplementary information). Because Rubisco is such an abundant protein, this potentially introduces a significant bias unless it is corrected (Li *et al.*, 2017). Further, the stoichiometry in PaxDb of proteins within and between complexes does not necessarily match expectations, perhaps reflecting the fact that not all proteins are quantitatively captured during the tissue preparation and subsequent measurement. However, the data available from mass spectrometry allows a deeper understanding of N distribution between proteins than previous techniques have afforded. Moving forward, new data independent acquisition proteomic techniques, such as SWATH mass spectrometry (Law and Lim, 2013) will allow greater accuracy and a much finer resolution of leaf nitrogen allocation between proteins within leaves.

Thylakoid N costs

Within the chloroplast, protein complexes in the thylakoid membranes are involved with light capture, photosynthetic electron transport from water to NADP, and ATP synthesis. The relative abundance of these protein complexes varies in response to growth irradiance, which also changes the electron transport capacity per unit of chlorophyll. It is convenient to divide thylakoid nitrogen between two pools: light capture and bioenergetics. Both photosystem II and I reaction centres capture light and perform electron transport, but under unstressed conditions, neither determine the electron transport capacity. Consequently, it is appropriate to place them in the pool associated with light capture, together with the light harvesting chlorophyll a/b complexes (LHC). The distribution of chlorophyll between these complexes can be used to estimate the nitrogen associated with each, if one knows the chlorophyll to protein stoichiometry (Table 1). The majority of chlorophyll is associated with the LHC (56%), each of which binds 14 chlorophylls (Liu *et al.*, 2004). Photosystem I with its 4 associated LHC accounts for 30% of leaf chlorophyll in complexes that bind 156 chlorophylls (Caspy and Nelson, 2018; Scheller *et al.*, 2001). Photosystem II with CP26 and CP29 bind 63 chlorophyll (Wei *et al.*, 2016) and account for the remaining 14% of chlorophyll. Putting these three fractions together results in an average nitrogen cost for light capture of 37.3 mol N (mol Chl)$^{-1}$ (Table 1).

The second thylakoid nitrogen pool, bioenergetics, is associated with photosynthetic electron transport and ATP synthesis. The relative abundance of the cytochrome b6f and ATP synthase complexes covary, depending on the growth irradiance and are directly correlated with the electron transport capacity (Evans, 1987; Yamori *et al.*, 2011). Consequently, cytochrome f content provides a way to link photosynthetic performance to the nitrogen cost of these complexes. As quantitative measures of ATP synthase were lacking when the thylakoid nitrogen budget was first assembled, a ratio of 1 cyt f: 1 FNR: 1.2 ATP synthase was assumed which resulted in a nitrogen cost for bioenergetics of 8.85 mol N (mmol cyt f)$^{-1}$ (Evans and Seemann, 1989). Now with the PaxDb (Li *et al.*, 2017; Wang *et al.*, 2015), we have reassessed this assumption (see supplementary information) and obtained a ratio of cyt f: FNR: ATP synthase of 1: 0.85:1.35 which leads to a revised cost for bioenergetics of 10.86 mol N (mmol cyt f)$^{-1}$. The actual ratio assumed for ATP synthase makes a significant impact on the total nitrogen cost of bioenergetics as it represents about 80% of this pool.

The nitrogen cost of thylakoids with respect to their electron transport capacity can be represented graphically. In Box 2, cytochrome f content per unit chlorophyll, which is directly proportional to the electron transport capacity per unit chlorophyll, varies along the x axis. The total thylakoid nitrogen cost per unit chlorophyll is presented on the y axis. Symbols represent actual measurements taken from spinach and pea leaves that were grown under different irradiances, as well as several C4 species where mesophyll and bundle sheath cells were separately analysed (Evans, 1987; Evans and Seemann, 1989; Ghannoum *et al.*, 2005; Terashima and Evans, 1988). The green rectangle represents the average nitrogen cost of light capture associated with LHC and the two photosystem complexes (37.3 mol N (mol Chl)$^{-1}$). For simplicity, minor variation in chlorophyll distribution between pigment protein complexes has been ignored here (Leong and Anderson, 1984). The yellow triangle represents the increasing cost of nitrogen associated with bioenergetics as the electron transport capacity increases per unit chlorophyll. Two upper bounds are shown depending on the nitrogen cost assumed for bioenergetics (8.85 and 10.86 mol N (mmol cyt f)$^{-1}$ being the original and revised estimates, respectively). On average for a leaf growing in sunlight, there are about 55 mol N (mol Chl)$^{-1}$ in chloroplast thylakoid membranes.

Nitrogen distribution within the cell

To establish the relative distribution of nitrogen between the cellular organelles, it is necessary to juggle different sources of information as none provide the complete picture. An average distribution for mature leaves of C3 plants is: chloroplast 75%, mitochondria 5%, peroxisomes 2.5%, cytosol 7.5% and cell walls 10% (Li *et al.*, 2017; Makino and Osmond, 1991; Onoda *et al.*, 2017; Wang *et al.*, 2015). Alternatively, one can group the nitrogen distribution by function and superimpose this onto the organellar structure (Box 3). The relative size of each pool related to photosynthesis has been scaled to represent the fraction of leaf nitrogen associated with it, in total accounting for 54% of leaf nitrogen. In the case of the photorespiratory cycle, this occurs across three organelles. Within chloroplasts, about 16% of the nitrogen is associated with other proteins and molecules not directly associated with photosynthesis and protein synthesis. For the remainder of the cell, another 13% is left in the ‘other’ category that includes the nucleus, cytosol and non-photorespiratory mitochondrial processes.

Scaling to the ecosystem

Given the diversity of plant species and ecosystems, it is a challenge to represent them through generalisations. Leaf dry mass and nitrogen contents per unit area have been determined for samples collected in the field for many species. For those leaves which also had photosynthetic attributes measured in the field, relationships have emerged. Linear relationships between photosynthetic capacity and leaf nitrogen content per unit area exist for different plant types (Kattge *et al.*, 2009), although perhaps surprisingly, nitrogen fixing legumes overlap with non leguminous dicotyledonous crop species (Adams *et al.*, 2018). Since there are many more measurements of leaf nitrogen than photosynthesis on field grown material, these relationships between photosynthesis and leaf nitrogen are widely embedded into ecosystem and global models. However, given the variability in the slope relating photosynthetic capacity to leaf nitrogen content per unit area between plant types, ground truthing is still required, e.g. arctic biomes (Rogers *et al.*, 2017b). Field gas exchange can establish the relationship between Rubisco capacity and leaf nitrogen content, although this may not reflect the actual allocation of nitrogen in Rubisco (Bahar *et al.*, 2017). Improvements in remote sensing capability are increasing our ability to estimate plant characteristics from reflectance spectra (Martin *et al.*, 2018). Whether it is possible to use hyperspectral reflectance to derive estimates of Rubisco capacity directly (Serbin *et al.*, 2015; Silva-Perez *et al.*, 2018; Yendrek *et al.*, 2017) or indirectly by first predicting nitrogen content (Dechant *et al.*, 2017), is currently an active area of research.

Analysis of multiple publications revealed four features associated with increasing leaf mass per unit area between species (Onoda *et al.*, 2017). Firstly, there was an apparent decrease in nitrogen allocated to Rubisco. Secondly, there was a decrease in mesophyll conductance per unit of mesophyll cell surface exposed to intercellular airspace. Thirdly, the draw-down in CO_2 partial pressure between intercellular airspaces and the sites of carboxylation inside chloroplasts during photosynthesis increased with increasing LMA. Fourthly, there was an increase in the fraction of leaf nitrogen associated with the cell wall. The combination of these features reduces photosynthetic capacity per unit of leaf N in species with greater LMA. Given that LMA is associated with leaf lifespan, rather than achieving an instantaneous high photosynthetic rate per unit leaf nitrogen, species with high LMA may instead achieve greater lifetime photosynthetic return from a given investment of N into a leaf.

Fertilizer - photosynthesis - food

In the forty years 1962 - 2002, the combined global production of wheat, rice and maize increased from 682 to 1752 Mt a^{-1} and nitrogen fertilizer production increased from 13.6 to 88.2 Mt a^{-1} (http://www.fao.org/faostat/en/#data). There was a close linear relationship between these two, with 13.8 tonnes of grain produced per tonne of nitrogen fertilizer. Assuming an average grain nitrogen content for wheat, rice and maize of 1.9% (Jaksomsak *et al.*, 2017; Rapp *et al.*, 2018; Uribelarrea *et al.*, 2008), harvested grain accounts for one quarter of global N fertilizer. This is remarkable given that the fertilizer is not only applied to these three crops, that the harvested grain represents only part of the nitrogen in the crop at maturity, that there are losses of nitrogen from leaching, erosion and denitrification and there is some residual nitrogen left in the soil. However, the environmental costs associated with nitrogen escape are a growing cause for concern and there are pressing demands for improving the efficiency in the use of nitrogen applied in agriculture to reduce environmental damage, economic cost and atmospheric greenhouse gas consequences both during the production of fertilizer and NOx emissions from fields.

Plants need to balance carbon gain with the synthesis of organic nitrogen compounds. As a consequence of the oxygenation reaction catalysed by Rubisco, the photorespiratory pathway recycles 2 molecules of phosphoglycolate to produce one PGA. At the same time, one molecule of ammonia is released in mitochondria and is refixed by GS GOGAT. The widely used Farquhar, von Caemmerer and Berry biochemical model of C3 photosynthesis (Farquhar *et al.*, 1980) assumes complete recycling, although this may not always be the case (Abadie *et al.*, 2017; Bloom and Lancaster, 2018; Busch *et al.*, 2018). At 25 °C and current atmospheric CO_2 concentrations, approximately 6 carbon atoms are fixed per ammonia recycled (see supplementary information). By comparison, new biomass requires 33 carbon to be fixed for each new N, assuming the plant contains 2% N, 40% C and respires 30% of daily carbon fixed during the production of this new biomass. Incorporation of ammonia during photorespiration or de novo incorporation in leaves uses the same GS GOGAT enzymatic pathway. Therefore, for plants converting inorganic N into organic compounds in their leaves, 85% of the GS GOGAT flux is dealing with photorespiration on average. At any instant, this proportion would change as it is affected by temperature, irradiance and CO_2 concentration. One consequence of rising atmospheric CO_2 concentrations is that the C:N balance of plant tissue is changing. Elevated CO_2 reduces photorespiration and with the exception of legumes that can fix atmospheric nitrogen symbiotically, plants grown under elevated atmospheric CO_2 have lower nitrogen concentrations (Feng *et al.*, 2015). This translates into lower grain protein concentrations which may have dietary implications in future (Myers *et al.*, 2014; Zhu *et al.*, 2018).

Engineering photosynthesis to improve crop yield

The detailed knowledge of photosynthesis has led to the identification of many proteins that can be targeted to increase carbon gain. A selection of targets that have been identified are presented in Box 4. In some cases, initial proof of concept has been obtained with transformed model plants (Driever *et al.*, 2017; Kromdijk *et al.*, 2016; Lopez-Calcagno *et al.*, 2018; Salesse-Smith *et al.*, 2018). Field trials with crop plants are underway and their outcome is eagerly awaited. Given the central importance of Rubisco in determining the rates of CO_2 assimilation and photorespiration, and because it accounts for so much of leaf nitrogen, much attention is focussed on ways to improve its performance. Approaches fall into two categories. Firstly, those where the catalytic properties of Rubisco are altered (e.g. from C4 species or other organisms, (Orr *et al.*, 2016)). Secondly, those where the CO_2 partial pressure around Rubisco is increased (e.g. CO_2 concentrating mechanisms such as carboxysomes (Hanson *et al.*, 2016; Long *et al.*, 2018; Rae *et al.*, 2017), greater mesophyll conductance (Groszmann *et al.*, 2017) or photorespiratory bypass (Peterhansel and Maurino, 2011)). While some variation in kinetic properties of Rubisco between wheat relatives has been identified (Prins *et al.*, 2016), detailed crop modelling is needed to assess the impact and cost/benefit from engineering an alternative form into elite wheat. While there are several crop models available (Song *et al.*, 2017; Wu *et al.*, 2018; Yin and Struik, 2017), it is a complex task to deal with plant functions that are not necessarily well represented or fully parameterised. The perennial debate about whether plant growth and yield is determined by source photosynthesis or sink demand continues. In the case of rice, increasing sink capacity led to a dramatic increase in yield (Ashikari *et al.*, 2005). The current focus on improving photosynthesis is because the gains in harvest index (grain yield / above ground biomass) associated with the introduction of dwarfing genes have been largely maximised, but maintaining or increasing both sink strength and harvest index is also crucial.

If a plant could be engineered to fix more carbon per unit of nitrogen associated with photosynthesis, then unless de novo incorporation of nitrogen was also enhanced, there would be a lowering of the nitrogen concentration of the plant and most likely the protein content of the grain. An increase in carbon gain per unit photosynthetic N could free up N for investment in new tissues elsewhere and increase growth. This is observed when plants are grown under elevated atmospheric CO_2 (Ainsworth and Long, 2005). However, unless additional organic N is incorporated into other tissues, the conversion of that increased growth into greater yield would result in lower grain protein. If the additional organic N incorporated elsewhere in the plant could not provide any improvement above that gained from greater photosynthesis per unit of photosynthetic nitrogen, what is the benefit from raising photosynthetic rate per unit N?

A second concern is that for cereal crops, nitrogen is remobilised from leaves and stems during grain filling. At maturity, the grain can account for 80-90% of aboveground N (Barraclough *et al.*, 2010; Gaju *et al.*, 2014). For a crop yielding 10 tonnes per hectare with a 2.5% N concentration in the grain, this represents 250 kg N ha^{-1}. To contain this within a crop canopy with a leaf area index of 7 (Shearman *et al.*, 2005), the leaf nitrogen content would need to be 3.6 g m^{-2}. This is close to the maximum leaf nitrogen content that is observed (Silva-Perez *et al.*, 2018). If increasing photosynthesis per unit N resulted in lower N contents per unit leaf area, then a greater fraction of this remobilisable N would need to be present in the sheath and stem fractions. In the case of wheat, the ear can also make a substantial photosynthetic contribution to the grain (Maydup *et al.*, 2012; Zhou *et al.*, 2016). While these tissues can contribute to canopy photosynthesis, the relative efficiency of leaf and stem needs to be investigated in order to assess the consequences. The point is, that to increase yield while maintaining grain protein concentration requires increasing both photosynthetic carbon gain and de novo N incorporation. In addition, the crop canopy has to be capable of holding the vast majority of that N in its leaves to enable its relocation into developing grain. An alternative is to continue de novo N incorporation during grain filling which requires continued root growth, N uptake (perhaps associated with a late application of fertilizer) and incorporation into protein while leaves are senescing.

Future

Given that Rubisco constitutes the largest fraction of nitrogen in leaves of C3 plants, it justifiably attracts great attention. In the absence of complete kinetic information to describe the performance of Rubisco from different species, the default has frequently been to assume kinetic values of tobacco Rubisco (Bernacchi *et al.*, 2002). However, the kinetic properties of Rubisco from diverse species need to be determined. Some of the variation between species in the apparent Rubisco activity per unit leaf nitrogen might be associated with variation in kinetic properties, but other factors could also be involved, such as different allocation of nitrogen towards Rubisco and different activation state. With improved quantification of relative protein abundance, the extent to which variation in nitrogen allocation to pigment protein complexes is associated with Rubisco performance will be revealed. The limited number of species for which thylakoid N cost has been quantified should be expanded. In particular, the N allocated to ATP synthase needs attention, given its apparent significant cost.

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Box 1. Key developments relating photosynthesis and nitrogen

- Leaf nitrogen budget: A tradeoff is apparent between nitrogen allocated to Rubisco versus cell walls amongst plant functional types

In a meta analysis of C_3 species, Onoda et al. (2017) showed that with increasing leaf dry mass per unit area, the fraction of leaf nitrogen allocated to Rubisco declined while that allocated to cell wall material increased. Short lived leaves have greater photosynthetic rates per unit leaf nitrogen.

- Scaling to the ecosystem: Rubisco capacity per unit leaf nitrogen

Rubisco capacity (V_{cmax}) is commonly derived from gas exchange measurements, but this does not always equate to Rubisco protein. For tropical rainforest trees (Bahar *et al.*, 2017) and Arctic tundra (Rogers *et al.*, 2017b) new field data improves ecosystem models.

- Fertilizer, photosynthesis, food security: Rising atmospheric CO_2 reduces grain protein concentration

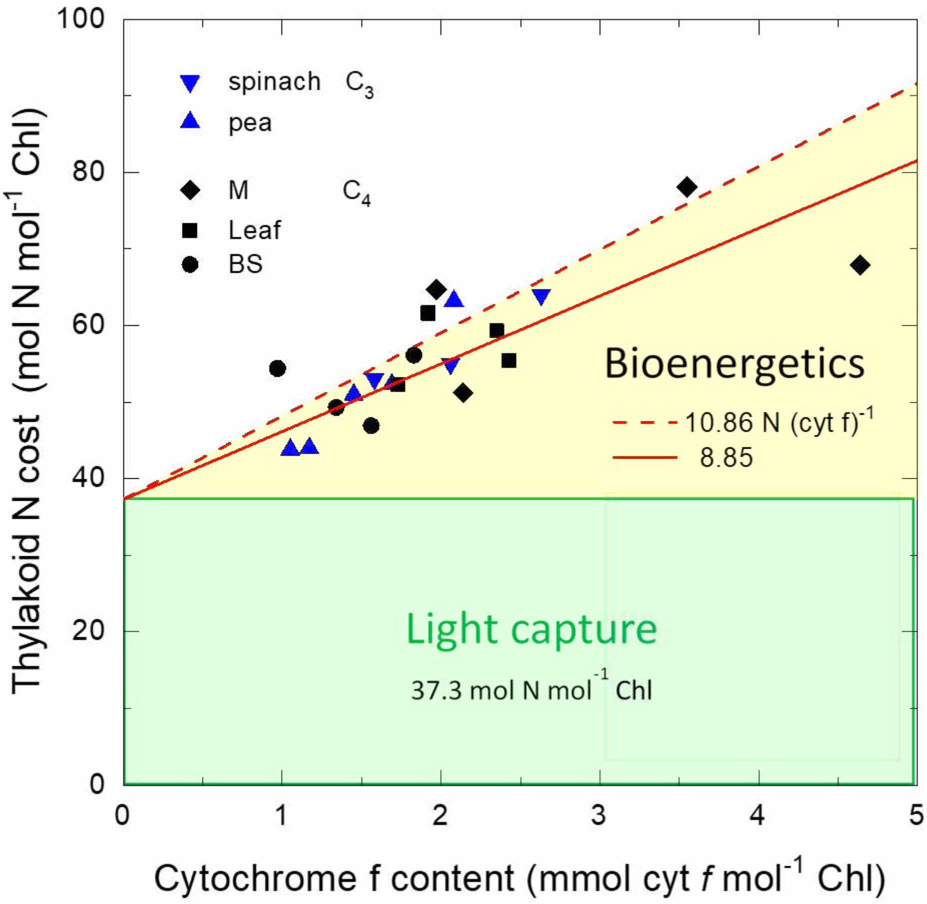
Achieving and maintaining high cereal yields requires the use of nitrogen fertilizers, yet rising atmospheric CO_2 is diminishing the grain quality (Zhu *et al.*, 2018). How can we diminish the negative impact of fertilizer use while maintaining protein?

- Engineering photosynthesis: Protein targets that increase photosynthesis and biomass

Increasing a photosystem II protein and two enzymes that interconvert carotenoids to regulate energy dissipation led to increased biomass production in field trials (Kromdijk *et al.*, 2016). There are a growing number of candidate genes being investigated to enhance photosynthesis.

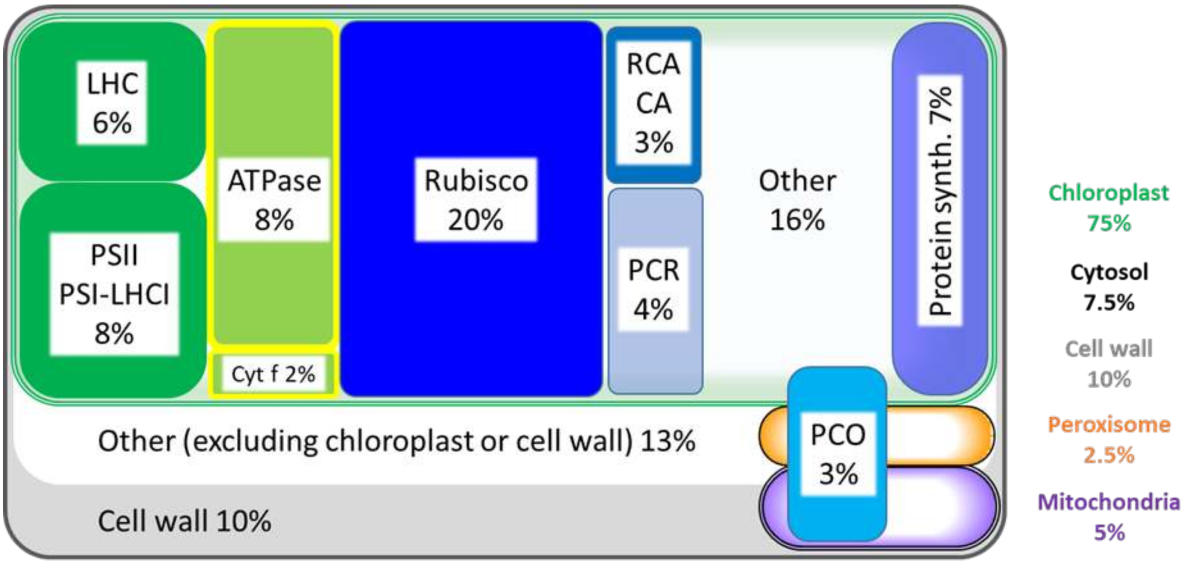
Box 2. The nitrogen cost of thylakoids in relation to their electron transport capacity.

Photosynthetic electron transport capacity is directly proportional to the cytochrome f content, 155 mol e^- (mol cyt f)$^{-1}$ s^{-1} (Evans, 1988; Niinemets and Tenhunen, 1997). A constant N cost associated with pigment protein complexes of 37.3 mol N (mol Chl)$^{-1}$ is assumed (green rectangle). Thylakoid nitrogen associated with photosynthetic electron transport (yellow triangle) is shown for two different assumed costs (red lines). Data from (Evans, 1987; Evans and Seemann, 1989; Ghannoum *et al.*, 2005; Terashima and Evans, 1988).



Box 3. Nitrogen budget for a C3 leaf cell.

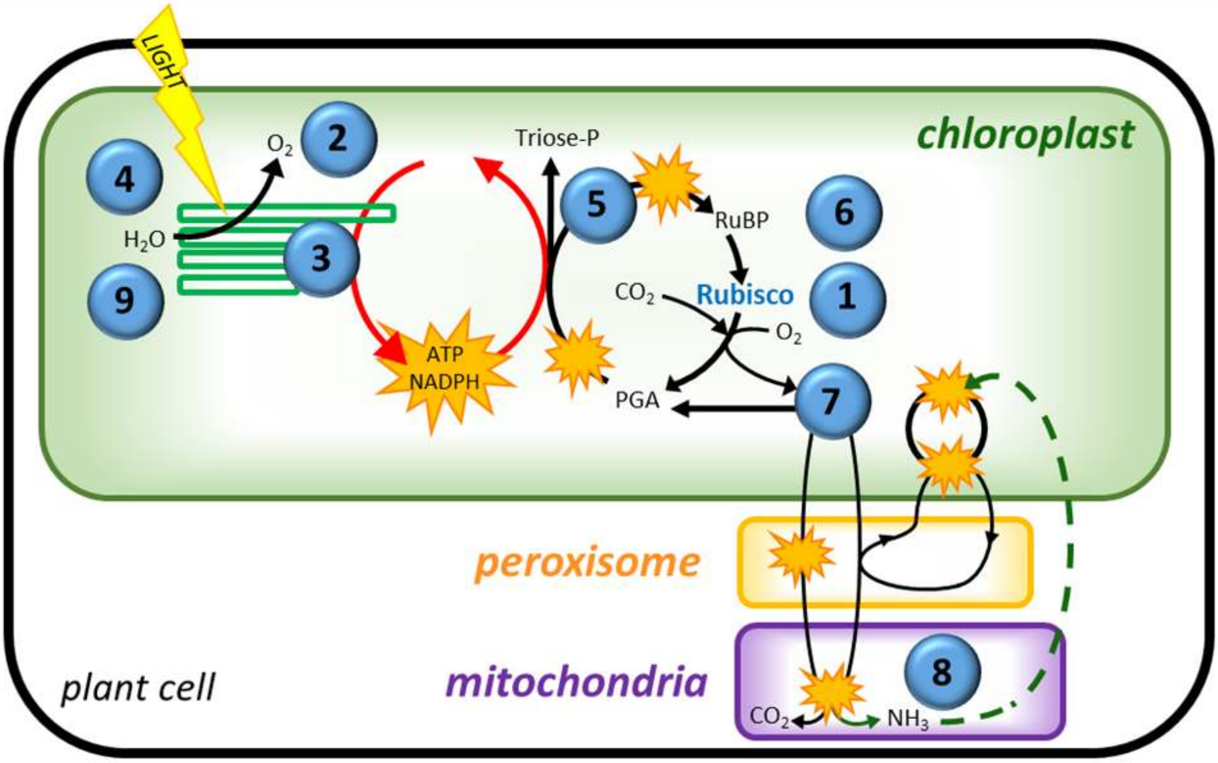
The coloured shapes are scaled relative to their proportion of leaf N. The distribution of nitrogen between different organelles is shown on the right hand side (see supplementary information). LHC light harvesting chlorophyll a/b complex, PSII photosystem II reaction centre, PSI-LHCI photosystem I reaction centre with its light harvesting chlorophyll a/b complex, ATPase ATP synthase, cyt f cytochrome b_6f Rieske iron sulphur complex, RCA Rubisco activase, CA carbonic anhydrase, PCR enzymes of the photosynthetic carbon reduction cycle excluding Rubisco, PCO enzymes in the photosynthetic carbon oxidation cycle, Protein synth. N associated with protein synthesis including amino acids.



Box 4. Targets for improving photosynthesis

Many proteins have been identified which could potentially increase carbon gain and a selection is shown. The numbering order reflects the nitrogen cost of adding additional proteins, beginning with the greatest N requirement for Rubisco or ATP synthase. The protein cost associated with increased expression of targets 3 to 6 is likely to be small. In the case of light harvesting complex, a reduction in chlorophyll content per unit area frees up nitrogen that could be invested in other more rate limiting photosynthetic proteins.

[refs – included for endnote referencing, not for printing
1 (Sharwood *et al.*, 2016a; Sharwood *et al.*, 2016b), 2 ATP synthase (Yamori *et al.*, 2011), 3 cytochrome b6f (Simkin *et al.*, 2017b), 4 PsbS VDE ZEP (Kromdijk *et al.*, 2016), 5 SBPase, FBP aldolase (Driever *et al.*, 2017; Simkin *et al.*, 2017a), 6 Rubisco activase , 7 photorespiratory bypass (Ahmad *et al.*, 2016; Dalal *et al.*, 2015; Kebeish *et al.*, 2007), 8 Glycine decarboxylase – H (Lopez-Calcagno *et al.*, 2018; Simkin *et al.*, 2017a), 9 light harvesting complex (Slattery *et al.*, 2017; Walker *et al.*, 2018)]



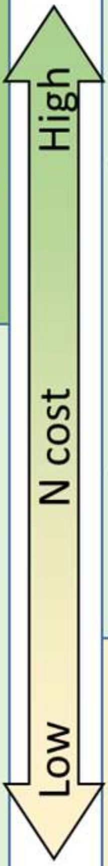
① **Rubisco** (Sharwood *et al.*, 2016a,b)
Transferring superior Rubiscos into C_3 crops could increase photosynthesis.

② **ATP synthase** (Yamori *et al.*, 2011)
Antisense reduction of ATP synthase suggests that while this complex may not limit electron transport rate, an increased content would be needed to obtain the maximum benefit from increasing cyt b_6f content.

③ **Cytochrome b_6f** (Simkin *et al.*, 2017b)
Overexpressing the Rieske FeS protein increased the content of cyt b6f complex, electron transport capacity and plant biomass in Arabidopsis.

④ **PsbS VDE ZEP** (Kromdijk *et al.*, 2016)
Overexpression of these three proteins in tobacco increased the capacity for NPQ and the speed with which photoprotection cycles under dynamic light conditions in the field, leading to increased plant growth.

⑤ **SBPase, FBP aldolase**
Two-fold overexpression of SBPase in wheat (Driever *et al.* 2017) and overexpression of SBPase / FBP aldolase in Arabidopsis (Simkin *et al.* 2017a) increased biomass.



⑥ **Rubisco activase**
Varying RCA by overexpression or antisense led to a decrease or increase, respectively, in Rubisco content in rice (Fukayama *et al.*, 2018).

⑦ **Photorespiratory bypass**
Three enzymes in the glycolate catabolic pathway from *E. coli* bypass the normal photorespiratory cycle, releasing CO_2 within the chloroplast (Kebeish *et al.*, 2007). Both *Camelina* (Dalal *et al.* 2015) and potato (Ahmad *et al.* 2016) plants transformed with these genes produce more biomass.

⑧ **Glycine decarboxylase – H**
Overexpression of GDC-H in Arabidopsis (Simkin *et al.*, 2017a) and tobacco (Lopez-Calcagno *et al.* 2018) led to an increase in biomass.

⑨ **Light harvesting complex**
Soybean mutants with reduced chlorophyll contents demonstrate that reallocation of N away from chlorophyll protein complexes towards other photosynthetic proteins could increase crop canopy photosynthesis (Slattery *et al.*, 2017; Walker *et al.*, 2018).

Complex	MW (kDa)	# Chl	N/Chl mol N (mol Chl)$^{-1}$	% total Chl	N/Chl mol N (mol Chl)$^{-1}$
LHC	28.8	14	23.5	56	13.2
PSI - LHCI	388	156	28.4	30	8.5
PSII	456	63	82.7	14	11.6
Chl					4
Light harvesting					37.3

Table 1. Molecular weight, number of chlorophyll molecules per complex, protein nitrogen cost per chlorophyll in the complex, percentage of the total chlorophyll associated with each complex and nitrogen cost of each component weighted by abundance giving a total nitrogen cost associated with light harvesting (updated from Evans and Seemann, 1989).

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Supplementary information

1. Rescaling PaxDb to account for Rubisco abundance

The fraction of leaf nitrogen accounted for by Rubisco varies between C3 species, ranging from 10 to 30% and decreasing with increasing leaf dry mass per unit leaf area e.g. (Onoda *et al.*, 2017). For Arabidopsis, Rubisco represents 40% of soluble protein (Eckardt *et al.*, 1997). In wheat, Rubisco represents about 20% of leaf nitrogen (Evans and Seemann, 1984). Assuming 7% of leaf nitrogen is not associated with protein (RNA and DNA, 3.6% of leaf nitrogen (rice, (Suzuki *et al.*, 2001)), chlorophyll 1.6- 2.4% of leaf nitrogen, 1-2% other (e.g. other lipids, amino acids, alkaloids), then Rubisco represents 20/0.93 = 21.5% of total protein. In the PaxDb, the abundance (ppm) is multiplied by the MW of each protein and summed to estimate total protein. By increasing the abundance of both the large and small subunits of Rubisco in PaxDb to 119,000, Rubisco represented 21.5% of total protein, or 20% of leaf nitrogen.

2. Nitrogen cost of bioenergetics.

Three protein complexes are combined: cytochrome b$_6$f complex, ATP synthase and Fd NADP reductase. The relative abundance of the protein subunits is taken from the PaxDb (Wang *et al.*, 2015) and normalised to PETC. For ATP synthase, the abundance is calculated by averaging four of the protein subunits, assuming each ATP synthase contains 3 alpha, 3 beta, 1 delta and 1 epsilon subunits. For FNR, there are two subunits and the average relative abundance is assumed.

Complex			PaxDb (ppm)	complex (ppm)	Complex ratio to PETC	MW (kDa)	N cost (mol N (mol cyt f)$^{-1}$)
cyt b6f	AT4G03280	PETC	3921	3921	1	101	1.15
ATP synthase	ATCG00120	3 ATPase alpha	15621	5207			
	ATCG00480	3 ATPase beta	16540	5513			
	AT4G09650	1 ATPase delta	5238	5238			
	ATCG00470	1 ATPase epsilon	5277	5277			
avg				**5309**	1.35	575	8.90
Fd NADP reductase	AT5G66190	FNR1	3244				
	AT1G20020	FNR2	3507				
avg				**3376**	0.86	82	0.81
Total							10.86

3. Nitrogen distribution within the cell

75%	chloroplast (75-80%, pea, (Makino and Osmond, 1991); PaxDb (Wang et al. 2015 with gene annotation by (Li *et al.*, 2017), Arabidopsis plastid 77% of total protein)
10%	cell wall (Onoda et al., 2017)
5%	mitochondria (5-10%, pea, Makino & Osmond, 1991; PaxDb (Wang et al. 2015 with gene annotation by Li et al. 2017) Arabidopsis mitochondria 3.7% of total protein)
2.5%	peroxisome (PaxDb (Wang et al. 2015 with gene annotation by Li et al. 2017) Arabidopsis)
7.5%	other (cytosol, nucleus)

4. Nitrogen fixed per carbon assimilated

The ratio of Rubisco carboxylations to ammonia recycled during photorespiration is derived from $2V_c/V_o = C/\Gamma^*$ (von Caemmerer, 2000) Eq 2.16, 2.18, where the CO_2 partial pressure in the chloroplast, C, is assumed to be 60% of ambient (400 x 0.6 μbar) and the CO_2 compensation point in the absence of mitochondrial CO_2 release, Γ^* = 40 μbar. One ammonia is recycled per two oxygenations, occurring every 6 carboxylations. By contrast, if new plant biomass contains 40% C and 2% N, i.e. C;N ratio of 23.3, and 30% of daily fixed carbon is respired during the construction of new biomass, 23.3/0.7 = 33.3 C need to be fixed per N gained in new biomass. These 33.3 C were associated with the recycling of 33.3/6 = 5.6 ammonia. For plants converting ammonia to organic compounds only in their leaves, the photorespiratory flux of ammonia thus represents 5.6/(5.6 + 1) = 0.85, or 85% of the GS GOGAT flux.

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