How can I frame the intro/discussion?

- N is a key plant nutrient and is essential for building protein. We know photosynthesis scales with Narea but there is substantial variation associated with this relationship.

- Lots of work been done to understand how N is allocated within the leaf, and what subpools of leaf N best predict photosynthetic capacity.

- we have had estimates for the sizes of different functional pools of protein in leaves since the 80's - achieved using classical biochemical wizardry (see Evans & Seeman 1989). These estimates were made using domesticated plants like spinach, plantago, phaseolus, curcumis, from which it was relatively straight forward to extract protein.

- allocation varies by species

- roughly 17% light harvesting, 31% carbon assimilation, 20% biosynthesis, 7% bioenergetics,

- talk about allocation equations and where the numbers have typically come from (Evans 1989 a/b, see Quebbeman 2016 review paper for an overview

- to date, studies have quantified protein functional pools accurately in the lab, for a limited number of species, or combined functional trait, gas exchange and environmental data to parameterise equations that estimate allocation

- Now using cutting edge mass spectrometric methods we can accurately and rapidly measure amounts of protein at any level of functional organisation, from high level functional categories down to individual protein subunits. We've done this for 32 Eucalypt species spanning broad ranges of environmental conditions across half the Australian continent.

- We provide an updated, completed quantification of protein allocation to all major leaf protein functional categories.

- we know there are some fundamental relationships between N allocation and major environmental variables - temperature, water, light

- Responses of major protein groups to these env vars have been investigated extensively as a means to understanding the fundamentals of how the photosynthetic apparatus is optimised to its environment – ‘coordination’ and ‘optimality theories’

  - light response (Evans & Poorter 2001 would do, Niinemets too)

  - temperature response (see below, Berry and Bjorkman 1980)

  - precip response (Wright paper, forget which one)

  - Evans & Poorter 2001, ref Niinemets and a few others, have directly determined Rubisco content and electron transport proteins in wild plants, but the species coverage remains limited and generally to glasshouse grown plants.

  - 'Pigment associated proteins' aren't usually quantified directly - measurements are made by measuring chlorophyll and then multiplying by a stock 'N per chlorophyll' number derived from Evans work (1989, paper with pie chart and tables?)

- It is possible to test a vast range of environment-function relationships using this dataset. In this initial analysis, we have opted to address variation in the abundance of photosynthesis proteins across fundamental environmental gradients: MAT, MAP and canopy irradiance. These relationships are of longstanding interest across multiple disciplines in the plant sciences.

- We present protein abundances as both fractional allocations and absolute amounts per leaf area, and explore the relationships between carboxylation enzymes and leaf anatomy across gradients of temperature and precip

-By combining of comparative field ecology and a novel quantitative proteomics approach, we describe drivers of variation in photosynthesis protein abundances with unprecedented accuracy, at the continental scale.

**What are the literature numbers on what proportion of protein each category comprises?**

**Evans & Seeman 1989 - The allocation of protein nitrogen in the photosynthetic apparatus: costs, consequences, and control**

Light reactions (thylakoid membrane bound proteins associated with light harvesting, electron transport and photophosphorylation), represent ~25% of leaf protein

Photosynthetic carbon reduction cycle (Calvin cycle), includes soluble proteins involved in CO2 assimilation, photorespiration, RuBP regeneration and starch & sucrose synthesis. Also represents ~1/4 of leaf nitrogen (what sort of plants are we talking about here?)





Warren 2000 – photosynthesis / rubisco / N relationships in Australian natives

“**Differences between species in the proportion of total N found in Rubisco, other soluble proteins and thylakoid proteins are large (Evans 1989 [above figure], and Table 1).** In Oryza sativa, 22–33% of total N is found in Rubisco (Makino et al. 1997) compared with about 20% for Triticum aestivum (Evans and Seemann 1984), 22–28% for Eucalyptus cladocalyx seed- lings (Gleadow et al. 1998), 6–20% in seedlings of four conifer species (Gezelius 1986; Brown et al. 1996), and 4–9% for Alocasia macrorrhiza (Seemann et al. 1987). Our data encompass this range of values and we observed no clear pattern between species or functional groups in the pro- portion of total N found in Rubisco or soluble proteins. Moreover, partitioning of N among different compounds exhibits significant phenotypic plasticity and was strongly affected by altering the rate and form of N applied. In four related tree species, for example, the proportion of foliar N found in Rubisco varied as follows: 24.0% (E. camaldu- lensis), 21.2% (E. decipiens), 9.3% (C. calophylla) and 8.5% (E. torquata, Table 1).”

**What are the ‘proxies’ for protein amounts?**

In Evans & Poorter 2001: ‘Pigment associated protein’ is calculated from chlorophyll measurements, using a stock average ratio of chlorophyll-‘soluble N’.

Rubisco content was assessed using a 14CABP binding method (Mate et al 1993).

Steve said this is probably pretty good. But only for 10 species grown in the glasshouse.

In Hikosaka 1998, Rubisco is calculated by:

“For determination of RuBPCase content, the frozen leaf was homogenized in a 100 mM Na-phosphate buffer (pH 7·5) containing 0·4 M sorbitol, 10 mM NaCl, 2 mM MgCl2, 5 mM iodine acetate, 1 mM phenylmethyl sulfonyl fluoride, 5 mM dithiothreitol and 2% (w/v) polyvinylpyrrolidon. After filtration through 20 μm mesh, concentration of chl in the filtrate was determined with 80% acetone (Porra et al. 1989). Then, the filtrate was applied to SDS-PAGE. The gel was stained with Coomassie Brilliant Blue R-250 (CBB). The band of the large subunit of RuBPCase was extracted with formamide for spectrophotometric determination of the RuBPCase content (Makino, Mae & Ohira 1986). Calibration curves were made with the RuBPCase purified from Spinacia oleracea L. and the protein concentration was determined using the method of Lowry et al. (1951). The RuBPCase content per unit leaf area was calculated as a product of the RuBPCase/chl ratio and chl content per unit leaf area.”

Evans & Seemann 1984 (‘Differences between Wheat Genotypes in Specific Activity of Ribulose-1,5-bisphosphate Carboxylase and the Relationship to Photosynthesis’) cite Collatz1978 – this approach appears to measure rubisco activity rather than absolute amount?

**Allocation of leaf N**

**From: “Optimal allocation of leaf-level nitrogen: implications for covariation of Vcmax and Jmax and photosynthetic downregulation”**

*There is no parameter in Evans 1989 equations (or later updates) to account for for Photosystems – we show that Photosystems are a really substantial component of leaf N and should be directly accounted for. (need to do some further reading on this to confirm)*

2.2. Nitrogen and Photosynthetic Capacity Nitrogenis critical fordevelopmentandmaintenanceof all componentsof thephotosynthetic system[Poorter et al., 1995] and is allocated to its various components as follows [Evans, 1989]

Norg = NP + NE + NR + NS + NO, (4)

where NP is the nitrogen invested in pigment proteins such as chlorophyll a and b, NE is nitrogen allocated to the electron transport system including cytochrome f and coupling factor, NR is the nitrogen allocated to RuBisCO, NS is nitrogen in soluble proteins other than RuBisCO, and NO is additional organic leaf nitrogen not invested in photosynthetic functions such as in cell walls (all terms in equation (4) are in units ofmmolNm−2).

Evans 1989: “Generally, a greater proportion of nitrogen is partitioned into the thylakoids when plants are grown at lower irradiance, increasing from 20 to 40% of total leaf nitrogen at low irradiance.” – this fold-change is similar to what we observed (we saw ~2.5 fold change, although it was more from 0.1-0.25 of total protein)

**Evans & Poorter 2001 \*\* this paper is essential to understanding photosynthetic capacity in terms of allocation light harvesting**

 “plants grown in low light partitioned a larger fraction of leaf nitrogen into light harvesting.”

By combining Pf and SLA into the same equation, we have been able to assess the relative importance of both param- eters. Whereas previous studies have shown that changes to f due to acclimation to growth irradiance improve photo-Psynthesis per unit leaf nitrogen, we have shown that changes in SLA have a greater impact under both low- and high-light conditions. Niinemets & Tenhunen (1997) took a different approach, combining data from different experi- ments and linking all of the parameters to leaf dry mass per unit area via empirical regressions through data collected on leaves at different canopy positions. They concluded that in low light, nitrogen re-allocation within the leaf was at least as important as changes to SLA. By contrast, we sug- gest that under low light, changes to P increased relative daily photosynthesis per unit leaf dry mass by only 2% in comparison with a 29% increase by changing SLA (Fig. 8). *It was under high light that re-allocation of nitrogen had a greater impact, enabling a 10% increase compared to a 22% increase by changing SLA (Fig. 8,9).*

The reason for this is that light capture by a leaf is very efficient at low chlorophyll contents and to increase absorp- tance requires a lot of additional pigment-protein com- plexes (Fig. 3a). More light can be captured by spreading a given amount of pigment-protein complexes over a greater area than by concentrating it in a given area. Under high light, increasing daily photosynthesis requires greater amounts amounts of nitrogen allocated to electron transport capac- ity and the soluble proteins. Nitrogen per unit leaf area is increased by decreasing SLA. As none of the additional nitrogen needs to be allocated to pigment-protein to main- tain absorptance, all of the additional nitrogen can be allo- cated towards increasing Jmax. Thus under high light, the two parameters interact and daily photosynthesis increases by more if the increase in 1/SLA is accompanied by a decrease in f.

“In previous papers, it has been shown that nitrogen partitioning within leaves changes with growth irradiance in such a way that it almost maximizes photosynthesis (Evans 1989b, 1989c, 1993a, 1993b; Hikosaka & Terashima 1996; Hikosaka et al . 1998; Niinemets, Kull & Tenhunen 1998).”

“These data were evaluated in a model that revealed a far greater sensitivity of daily photosynthesis per unit leaf dry mass to changes in SLA than to nitrogen par- titioning.”

“the data of Niinemets et al. (1998) that was collected on four deciduous tree species, in which allocation of nitro- gen to Rubisco and bioenergetics was relatively indepen- dent of the light environment of the leaf, whereas allocation to pigment-protein complexes was dramatically higher in leaves collected from low irradiance sites.”

**Is allocation to Calvin cycle responsive to irradiance?**

Evans 1989 photosynth nitrogen relationships in C3 plants:

*The response to different irradiance during growth, in terms of partitioning of nitrogen into RuBP carboxylase, depends on the species.* Changing the irradiance during growth did not alter the relationship between RuBP carbox- ylase and nitrogen for Phaseolus or Alocasia (Seemann et al. 1987). Growth of spinach under lower irradiance decreased the amount of soluble protein per unit of nitrogen, while RuBP carboxylase: soluble protein remained constant (Ter- ashima and Evans 1988). When Atriplex patula was grown at 3% sunlight, RuBP carboxylase as a proportion of solu- ble protein dropped by 20% (Medina 1971). A drop of 20% was also seen in Solidago virgaurea when grown under low light (Bj6rkman 1968).

LeRoux et al 1999 – “Photosynthetic light acclimation in peach leaves: importance of changes in mass:area ratio, nitrogen concentration, and leaf nitrogen partitioning.”

“The major conclusion from these studies is that changes in both [LMA] and leaf nitrogen partitioning play a role in photosynthetic light acclimation in peach trees, and our study also shows that the relative role of variation in Pc is particu- larly important at low irradiance. This”

**Temperature response:**

* **Hikosaka et al 2006 – “Temperature acclimation of photosynthesis: mechanisms involved in the changes in temperature dependence of photosynthetic rate” J Exp Botany**
  + Based on the biochemical model of photosynthesis, change in the photosynthesis–temperature curve is attributable to four factors: intercellular CO2 concentration, activation energy of the maximum rate of RuBP (ribulose-1,5-bisphosphate) carboxylation (Vc max), activation energy of the rate of RuBP regeneration (Jmax), and the ratio of Jmax to Vc max. In the survey, every species increased the activation energy of Vc max with increasing growth temperature. Other factors changed with growth temperature, but their responses were different among species. *Among these factors, activation energy of Vc max may be the most important for the shift of optimal temperature of photosynthesis at ambient CO2 concentrations.*
  + *“*In particular, photosynthesis at the optimal temperature is limited by Pc, irrespective of growth temperature (Figs 6, 7a; Hikosaka et al., 1999). Therefore the changes in temperature dependence of photosynthesis may be explained mainly by Vc max.”
* **“As nitrogen is a limiting resource of plant growth in many ecosystems, efficient use of nitrogen is believed to contribute to plant fitness. Since about half of leaf nitrogen is allocated to the photosynthetic apparatus, photosynthetic acclimation has been analysed in terms of nitrogen partitioning among photosynthetic components (Evans, 1989; Hikosaka and Terashima, 1995; Hikosaka, 2004). *For example, shade leaves allocate more nitrogen to chlorophyll–protein complexes for light harvesting, while sun leaves have more nitrogen in Calvin cycle enzymes and electron carriers to achieve high photosynthetic capacity at high light (Boardman, 1977; Chow and Anderson, 1987; Evans, 1987; Terashima and Evans, 1988; Hikosaka, 1996; Hikosaka and Terashima, 1996; Makino*et al*., 1997; Muller*et al*., 2005*a*).* Nitrogen reallocation from a non-limiting to a limiting process contributes to the efficient use of nitrogen in the photosynthetic apparatus (Evans, 1989; Hikosaka and Terashima, 1995; Hikosaka, 1997).”**
* ***Higher amounts of photosynthetic proteins in low-temperature-grown leaves have also been reported in many studies (Holaday*et al*., 1992; Huner*et al*., 1993, 1998; Steffen*et al*., 1995; Strand*et al*., 1999; Hikosaka, 2005). It may be a compensatory response to low temperature, which decreases enzyme activity.***
* Recently Muller et al. (2005b) discussed temperature response in absolute photosynthetic rates in relation to nitrogen use. The ecological and evolutionary significance of the environmental response in leaf nitrogen content per unit area have been analysed from the viewpoint of the optimization theory. Daily carbon gain as a function of leaf nitrogen content shows a saturating curve and there is a leaf nitrogen content that maximizes daily carbon gain per unit nitrogen (nitrogen use efficiency: Hirose, 1984; Hirose and Werger, 1987). The optimal leaf nitrogen content is higher at higher growth irradiance and there is a strong correlation among the optimal and actual nitrogen content (Hirose and Werger, 1987). Muller et al. (2005b)studied seasonal change in the photosynthesis–nitrogen relationship in Aucuba japonica, an understorey shrub. The optimal nitrogen content was higher in winter than in summer and was strongly correlated with the actual nitrogen content. It should be noted that the photosynthetic rate at the growth temperature was not constant in this study. **Therefore, *absolute photosynthetic rates may be regulated* not to keep a certain value, but to *maximize nitrogen use efficiency at the growth condition*.**

Hunera et al 1998 – ‘Energy balance and acclimation to light and cold’ “Changes in environmental conditions such as light intensity or temperature result in an imbalance between the light energy absorbed through photochemistry versus the energy utilized through metabolism. Such an energy imbalance is sensed through alterations in photosystem II excitation pressure, which reflects the relative reduction state of the photosystem.”

Photosystem temperature sensitivity:

* PSII is temperature sensitive to deviations from a temperature optimum which is set during leaf development (Yamasaki 2002 ‘Temperature Acclimation of Photosynthesis and Related Changes in Photosystem II Electron Transport in Winter Wheat’)
* Ensminger 2006 ‘Photostasis and cold acclimation: sensing low temperature through photosynthesis’ - “Optimum plant performance requires a balance in the rates of source versus sink processes. Low temperatures, however, can inhibit electron transport by increasing membrane viscosity through alterations in the biophysical properties of thylakoid lipids and decrease the rates of the enzymatic reactions involved in C, N and S reduction more strongly than they inhibit photophysical and photochemical processes involved in light absorption, energy transfer and transformation (Huner et al. 1998).”
* Huner et al 1993 ‘Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants’ “Exposure of fully expanded leaves of winter cereals to short-term, low temperature shifts inhibits whereas low temperature growth stimulates electron transport capacity and carbon assimilation.”
* This all said, I cant find any references which specifically describe changes in photosystem abundance over temperature gradients. May need to look harder.

Raven & Geider 1988 (‘temperature and algal growth’) the light-harvesting and reaction centre apparatus which catalyses the temperature-insensitive processes of light absorption, excitation energy transfer and primary photochemistry, and which is present (as assayed by photosynthetic pigment per unit biomass) in smaller relative amounts during resource-saturated growth at lower temperatures

**Ghimire et al 2016 ‘A global trait-based approach to estimate leaf N functional allocation from observations’**

Interesting work, calculates/estimates allocation from measurements of Vcmax, Jmax, leaf N and various environmental variables. Huge ‘residual N pool’ of various unquantified sources of N.

“This study integrated observa- tions from global databases with photosynthesis and respiration models to determine plant- functional-type-specific allocation patterns of leaf nitrogen for photosynthesis (Rubisco, elec- tron transport, light absorption) and respiration (growth and maintenance), and by difference from observed total leaf nitrogen, an unexplained “residual” nitrogen pool.”

“The relative allocation of leaf nitrogen to these component processes and structures has been examined in detail for a few model species (Chapin et al. 1986, Evans 1989, Takashima et al. 2004, Xu et al. 2012) but *little is known about how plants in natural ecosystems partition nitrogen resources, especially at regional and global scales.”*

“However these studies relied on a few measurements (i.e., data at only three sites) for evaluating the behavior of their optimal nitrogen allocation model (e.g., Xu et al. 2012), considered limited PFTs (mostly short-lived non-woody plants or woody juveniles) that lacked explicit representation of inter- species difference in nitrogen allocation (e.g., Chapin et al. 1986, Evans 1989, Makino and Osmond 1991, Onoda et al. 2004, Takashima et al. 2004, Guan and Wen 2011), or did not consider nitrogen allocation for a complete range of processes including carboxylation, light harvesting, bioenergetics, maintenance respiration, and growth respiration (e.g., Coste et al. 2005, Dela- grange 2011).”

**What is explained by leaf N?**

**“Estimation of photosynthesis traits from leaf reflectance spectra: Correlation to nitrogen content as the dominant mechanism”**

Good paper to reference ‘measuring leaf N from space’…

Both Vcmax and Jmax are strongly correlated to leaf N (as measured remotely in this study – could do with some more refs), we can infer that Vcmax and Jmax are a function of the amounts of protein involved in these processes – that is, carboxylation and ‘electron transport’. Well yeah sure… we get nowhere if we don’t have good gas exchange data though…

“It is well known that the Rubisco content in the leaf is closely related to Vcmax,25 (Jacob et al., 1995; Makino et al., 1994; Onoda, 2005) and that cytochrome f content has a strong linear relationship to Jmax,25 (Onoda, 2005; Sudo et al., 2003; Terashima and Evans, 1988).”

So the correlations between leaf N, Rubisco and cytochrome b6f might be useful?

Calvin cycle vs leaf N, R2 = 0.7652

Cytochrome b6f vs leaf N, R2 = 0.5916

Photosystems vs leaf N, R2 = 0.5445

Adding in leafrad\_mean and tavg (respectively) helps the models for (adjR2 = 0.64) photosystems and cytochrome b6f (adjR2 = 0.70), but straight leaf N is the best predictor of calvin cycle protein abundance.

This could be cool to add to the end of the results? Why is it better than just adding other variables to multiple regressions (which try to estimate Vcmax and Jmax from reflectances / leaf N) anyway?

Discussion fodder: we’ve put boundaries on how closely protein amounts can be linked to leaf N. The correlations are pretty strong for Calvin cycle proteins (give rubisco specifically as well) but

*Above is all for aggregated data. If we use unaggregated data to try and predict protein amounts from leaf N, we really don’t get much above 0.5 (calv = 0.5148, cyt = 0.4817, phot = 0.412) and adding envvars/LMA doesn’t get us much further.*

*This would be good for supplementary.*

**Evans 1989**

Across a large number of species, Bjorkman (1981) found a correlation coefficient of 0.96 between the light saturated rate of CO2 assimilation in air and the fully activated RuBP carboxylase activity.