**Results**

Summary

How much protein was there, of each time, what were the variances?

Total protein trends

*How do abundances of photosynthetic light harvesting and carbon assimilation proteins vary across environmental gradients?*

We found strong support for the hypothesis that the abundance of light harvesting proteins would be highest in light-limited environments. Photosystem protein abundance was best predicted by canopy openness (scaled by leaf age to account for self-shading) (Fig. Xa, R2 = 0.38, p < 0.001), with a modelled reduction of 54 % as the canopy opened (38 – 90 % canopy gap fraction) - approximately 1% protein per % gap fraction. Photosystem protein abundance similarly tracked mean annual radiation (again scaled by leaf age) (Fig. Xb, R2 = 0.27, p = 0.001), declining by 42 % over the observed range of 6.7 – 20.1 MJ m-2 yr-1. Photosystem abundance also increased with mean annual precipitation (Fig. Xc, R2 = 0.28, p < 0.001), which while correlated with canopy openness (Pearson’s r = 0.54) and radiation (Pearson’s r = 0.56), represents a synoptic environmental gradient of general interest.

We also predicted that carbon assimilation proteins would be more abundant in high light conditions, as they determine the rate of light-saturated photosynthesis. This hypothesis was somewhat supported by a weak, shallow relationship between Calvin cycle enzyme abundance and mean annual radiation (Fig. Xf, R2 = 0.15, p = 0.020, modelled increase of 8%). Canopy gap fraction did not predict Calvin cycle enzyme abundance, however (Fig. Xe).

Calvin cycle enzyme abundance was not significantly associated with mean annual rainfall, but was inversely related to rainfall during the driest month (Fig. Xg, R2 = 0.17, p = 0.013), with a modelled decrease of 11 % across a range of 0.7 to 90.8 mm of rainfall. This relationship supports our prediction that Calvin cycle proteins will be most abundant at low rainfall sites, so as to effect greater internal [CO2] (Ci) drawdown when stomates are closed.

To test hypotheses derived from the temperature-dependency of enzyme kinetics, we also looked at relationships between mean annual temperature and absolute protein amounts. Absolute photosystem (Fig. Xy) and Calvin cycle protein abundances (Fig. Xz) both declined with temperature, but no significant relationships with temperature were found in multiple regression models which account for declining total protein as temperatures increase.

Compared with photosystem proteins, Calvin cycle enzyme abundance was only weakly influenced by environmental conditions, in terms of both total variance explained and the magnitude of the effect. It is noteworthy that the absolute abundance of Calvin cycle enzymes is tightly bound by the total amount of leaf protein (Pearson’s r = 0.98, Fig. Za supp info), suggesting that Eucalypts tend to invest in carbon assimilation machinery to the maximum extent they are able. Absolute abundance of photosystem proteins also strongly tracks total protein amount, but with more room for variation (Pearson’s r = 0.82, Fig Zb supp info).

*How do protein abundances change as leaves age?*

Total protein differed significantly across leaf age classes: there was no significant difference between new and middle aged leaves (mean difference 3.6 %, p = 0.75), but old leaves contained considerably less protein on average than middle aged leaves (16.2 %, p = 0.002) and new leaves (13 %, p = 0.023).

We tested two competing hypotheses concerning the effect of leaf age on abundance of photosynthetic proteins. In the first, we predicted that the abundance of light harvesting proteins would increase with leaf age to counter the effect of shading associated with canopy development. The second hypothesis was that proportional abundance of photosynthetic proteins should decline with age, as nitrogen is progressively allocated to recalcitrant structural and defensive proteins over the lifespan of the leaf.

Light harvesting proteins were found to increase in abundance as leaves aged: middle and old age leaves contained significantly more protein on average than new leaves (17.1 %, p > 0.001; and 24.9 %, p > 0.001) but were not significantly different from each other (mean difference 6.7 %, p = 0.11). This trend remained significant in an ANCOVA model including canopy gap fraction as a covariate. Variance partitioning identified most variation explained in this model by leaf age as shared with gap fraction (0.09). Leaf age made only a minor independent contribution to explained variance (0.03), while gap fraction explained a larger portion independently (0.19). Thus the change in light harvesting proteins can be mostly attributed to the effect of shading.

* Total protein trends
* Photosystems
  + Yes, even when leafrad\_mean is taken into account
* Calvin cycle

Hypotheses:

* Abundance of light harvesting proteins increases with age to counter reduced light interception
  + Is there any effect of leaf age independent of increased shading? Can’t answer this directly but worth discussing
* Nitrogen is progressively allocated to recalcitrant structural and defensive protein throughout leaf lifespan, so older leaves contain proportionally less photosynthetic protein
  + Re: Onoda et al. 2003 “Allocation of nitrogen to cell walls decreases photosynthetic nitrogen-use efficiency”
  + But see Hikosaka & Shigeno 2009 “nitrogen allocation to cell walls does not explain the variation in PNUE”
  + Have not quantified structural / cell-wall associated proteins here