METHODS – or INTRODUCTION?

*Study area and sampling:*

Wide coverage, over multiple biomes, varying almost independently in temp and precip (Fig 1a-c)

**1.) Quantifying leaf proteins at the continental scale.** A total of 324 photosynthetically active Eucalypt leaf samples were collected from 32 species; four species were recorded at multiple location. For each species-location combination, three canopy leaves were collected from each of three individuals to make a total of nine samples.

a.) Sampling locations (triangles) were located along three latitudinal bands, spanning broad gradients of rainfall and temperature. The resulting coverage of climate space represents of much of the vegetated area of the Australian continent;

b.) Sampling locations lie within six of the eight biomes described by Whittaker (1967).

c.) Mean annual temperature (oC) and mean annual precipitation (mm, log scaled) of sampling sites (triangles) are distributed orthogonally with respect to one another (r = ).

RESULTS / DISCUSSION

*Protein composition of the average eucalypt leaf.*

In Fig 2a we show how protein resources were allocated to all major functions in an ‘average’ eucalypt leaf (based on 320 leaf samples). The majority (64%, SD X%) of protein was associated with photosynthesis: 36% was associated with the carbon fixing Calvin Cycle, 22% (SD X%) with the light reactions and 4% (SD X%) with photorespiration (Fig 2a). The most abundant individual protein complexes were Rubisco (30%, SD X%) of leaf protein, and photosystem II (X%, SD X%) (Fig 2b). Protein synthesis, folding and degradation was the second most abundant top-level category at X% (SD X%) (Fig 2a).

Our mass spectrometry approach allowed detection of X individual proteins per sample, on average. These proteins accounted for 99.9% of sample mass, among which the top 500 most abundant proteins represented 90% (Fig 2c). This is a higher degree of dominance by the top few proteins than observed in [comparison] (Fig 2d), reflecting the specialist nature of leaves as photosynthetic organs.

*Linking leaf protein abundances with environment and functional traits*

We were able to describe patterns of leaf protein abundance across environmental gradients, as well as in relation to key leaf functional traits ~~and physiological properties~~ (Fig 3a). Per leaf area abundances of all major protein functional categories were correlated with each other, as well as with leaf nitrogen per area (N\_area), leaf mass per area (LMA), and maximum photosynthetic rate (Amax). Patterns in proportional abundances of protein functional categories (indicating investment in a defined function relative to investment in all other functions) were considerably less general.

*b.) first scatterplot panel*

Given the level of detail in our protein abundance dataset, it would have been possible to test a plethora of specific environment-protein abundance relationships. We decided to focus on photosynthesis here due to the dominance in leaves of proteins catalysing this set of processes. We selected several specific relationships of interest to the vegetation modelling community for deeper analysis; to date these relationships have only been investigated via proxies.

Calvin cycle proteins per leaf area reduced notably as sites became warmer (stat, Fig. 3b-i), and to a lesser extent with increasing precipitation (Fig. 3b-iii). The per leaf area abundance of Calvin cycle proteins was highly correlated with the total abundance of protein per area (Pearson’s r = 0.97), and environmental trends in Calvin cycle protein abundance were essentially identical to trends in leaf protein abundance.

Photosystem proteins per leaf area showed a pronounced decline with increasing incident irradiance (Fig. 3b-v, X% per Y irradiance). Per leaf area photosystem protein abundance declined substantially with increasing MAT (Fig. 3b-i) and was also strongly correlated with total leaf protein abundance (Pearson’s r = 0.82). No per leaf area response to MAP was observed (Fig. 3b-iii), however. Since MAP and incident irradiance were negatively correlated (i.e. denser canopies at wetter sites, Pearson’s r = -0.59) the lack of protein response to MAP could be explained by changing light conditions.

Proportional allocation of protein resources to Calvin cycle protein did not adjust over gradients of MAP or MAT (Fig. 3b-ii,iv) but increased marginally (stat) with increasing incident radiation (Fig. 3b-vi). Proportional photosystem protein abundance increased with increasing MAP (Fig. 3b-iv) and decreased with increasing incident irradiation to a similar extent as the per leaf area measure (Fig . BLAH). This latter response may explain the observed decline in Calvin cycle proteins as incident irradiance increased. The range of interspecific variation in photosystem protein proportional abundance (0.09-0.23, 2.6-fold) was considerably higher than for Calvin cycle proteins (0.30-0.39, 1.3-fold). These observations provide robust evidence that eucalypt leaves specifically optimise protein allocation to light capture in response to environmental conditions (some stats and numbers), while adjustment of carboxylation capacity is largely achieved through bulk changes in per leaf area protein content.

*c.) second scatterplot panel*

One obvious way Calvin Cycle protein per leaf area can change is via changes in depth of mesophyll and of leaf, and indeed adjustments in per leaf area Calvin cycle protein abundance occurred to some extent via changes in leaf mass per area (LMA) (Fig. 3c-i). The substantial scatter around the Calvin cycle – LMA relationship indicates that LMA responded to other requirements in addition to carboxylation capacity. Photosystem abundance did not increase per leaf area with increasing LMA (Fig. 3c-ii) and declined as a proportion of total leaf protein. Light harvesting capacity thus appears to be optimised for a given leaf area independently from leaf thickness.

Leaf nitrogen per area was a strong predictor of both Calvin cycle and photosystem protein abundance per leaf area, and no relative changes in these protein categories occurred with increasing nitrogen per area.

*d.) protein abundance/concentration/LMA multiple regressions*

*Here we model the contributions of leaf protein fraction and LMA to per leaf area Calvin cycle protein abundance across gradients of temperature and precipitation.*

We hypothesised that Calvin cycle protein abundance would be driven by temperature dependence of enzyme kinetics, and that maximisation of CO2 drawdown at low stomatal conductance in water-limited environments. Fig 3d-i shows that these demands were in fact complementary: leaves sampled at cold dry sites required the most protein, while leaves from warm wet sites experienced neither constraint, having both low protein content per area and low LMA.

The role of LMA versus protein concentration (Calvin cycle protein as a fraction of leaf dry mass) in determining per leaf area protein abundance depended interactively on MAP and MAT (Fig 3d-ii,iii). Low per leaf area Calvin cycle protein abundance at warm, wet sites was more closely associated with low LMA than low protein concentration, while high per leaf area Calvin cycle protein abundance at cold, dry sites was strongly associated with high Calvin cycle protein concentration.

Plants are able to build cheaper leaves at warm wet sites, where photosynthetic reaction kinetics are increased and plants are not water limited.

This idea gives rise to several expectations about how abundances of the different pools of photosynthetic leaf proteins should respond across gradients of temperature, light and water availability. Abundance of both Calvin cycle enzymes and photosystems should increase towards colder environments, to compensate for lower enzyme activity at lower temperatures (Raven & Geider 1988). This effect has been observed for Rubisco in a number of studies (summarised by Hikosaka et al 2006). Rates of primary photochemistry performed by the light harvesting apparatus may be less temperature sensitive, however (Raven & Geider 1988).

Allocation to photosystem complex proteins should be greatest where photosynthesis is light-limited (Niinemets 2007), and investment in Calvin cycle enzymes should increase with light availability, since capacity for carboxylation of RuBP determines the rate of light-saturated photosynthesis (Farquhar et al. 1980).

Investment in Calvin cycle enzymes should increase towards drier sites. By effecting greater internal CO2 drawdown, rate of CO2 uptake can be maintained at lower stomatal conductance, reducing the water cost of photosynthesis for dryland plants (Wright et al. 2001a,b, Scalon & Wright 2017). No direct effect of precipitation on investment in photosystem proteins is expected, although cross-correlation between precipitation and vegetation canopy density could influence this relationship.

In line with these expectations, an increasing number of terrestrial biosphere models have incorporated a leaf nitrogen allocation component in an attempt to improve estimates of photosynthesis (Ghimire 2016 refs, Dong Ning refs).