**Quantifying protein amounts**

While substantial progress has been made in characterising allocation of leaf protein to different functional pools, there some important limitations of approaches taken so far. Most crucially, protein quantification depends on complete extraction from leaves. The difficulty of extracting protein varies according to protein solubility (e.g. soluble, membrane-associated or cell wall-associated fractions) and between species (REF), and different extraction methods may also preferentially extract different protein fractions (Makino & Osmond 1990). Extraction of protein from leaves with high phenol content (e.g. eucalypts) is especially challenging due to the chemistry involved (see Warren 2000 for ref). Furthermore, most studies do not quantify what fraction of total protein was actually extracted from leaves (Warren refs?). Thus meaningful comparisons of protein abundances can be difficult to make even within a sample, still more difficult between species, and may not be reliable across studies.

Secondly, protein amounts are often estimated by measuring a proxy (e.g. chlorophyll for ‘pigment protein complexes’), and then calculating the protein amount using a ‘stock’ ratio of proxy amount to protein amount (Niinemets & Tenheunen 1997, Ghimire 2016). For example, Evans & Poorter (2001) estimated amounts of ‘pigment protein complex’ by measuring chlorophyll and using values of 38·5 and 41 moles of pigment protein nitrogen per mole of chlorophyll, for low- and high-light-grown plants, respectively. These values were sourced from measurements made on model organisms in the 1970’s and 80’s. Amounts of rubisco and electron transport proteins can similarly be estimated using equations that relate gas exchange parameters to protein amounts (Niinemets & Tenheunen 1997, Ghimire 2016, Evans & Poorter 2001). This approach is problematic for rubisco, since it only quantifies rubisco in its active conformation (REF). In a study of Australian species, the concentration of rubisco measured by radioimmune assay varied between 40% and 600% of that estimated from enzyme kinetics and gas exchange measurements (Warren et al 2000).

Finally, work to date has either made accurate measurements of a small number of species grown in controlled conditions, used plant traits, gas exchange measurements or environmental variables to estimate nitrogen allocation to the different functions of photosynthesis (Dong 2016, Ghimire 2016, Dechant 2017). These limitations prevent us from accurately parameterising regional scale models of vegetation function.

Because we are able to comprehensively extract leaf protein and rapidly quantify over 2000 individual leaf proteins, our approach represents a substantial increase in accuracy and specificity with which we can investigate allocation of protein to functions of interest, and greatly expands the scope of what is possible ~~in plant proteomics and related disciplines~~.