Building better climate change vegetation models: How do leaves allocate nitrogen among photosynthesis and stress proteins in future climate scenarios?

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The lack of understanding about how native plants respond to climate change is a major source of uncertainty in predictions of future climates. Photosynthesis is performed by enzymes in leaves that are made of nitrogen. Total leaf nitrogen is used in ecology as a predictor for photosynthetic capacity of vegetation. That relationship has long been known to be an oversimplification, assuming all leaves to have similar composition of photosynthesis enzymes, but it has not previously been possible to dissect the aggregate quantity of total leaf N on a wide enough scale to draw broad conclusions about variations in enzyme compositions.

The three achievements of this project are:

1) New methods that rapidly quantify individual leaf proteins and place them into functional groups
2) Determining leaf protein composition in natural vegetation across continent-scale environmental gradients
3) Determining response of leaf protein composition to short-term environmental stress

New methods. This project made a major technical advance: creation of analytical methods to quantify leaf proteins on a per leaf area basis across hundreds of samples—something that was not previously possible. Key elements are a quantitative leaf protein extraction method and a rapid mass spectrometry method.

Natural variation in leaf proteins on a continent scale. Leaves of native Australian plants were sampled from Tasmania to Far North Queensland and from very high rainfall to desert sites. The range of environmental conditions covered most of the rainfall and temperature combinations of Earth. This large-scale sampling was funded by an ongoing ARC Discovery Project that dramatically increased the scope of the SIEF project.

We have so far analysed >300 leaf samples from 35 species of *Eucalyptus* and are in process of analysing >700 samples from *Acacia* and *Proteaceae* species. We have identified significant changes in leaf protein composition over rainfall and temperature gradients and have also discovered that long-held assumptions about leaf protein composition may be false, at least for *Eucalyptus*.

Variation in leaf proteins in response to environmental stress. In collaboration with the Hawkesbury Institute for the Environment we quantified leaf protein changes among four *Eucalyptus* species in response to heat stress in a glasshouse experiment. In all four species photosynthesis decreased in response to stress. We measured a decline in photosynthesis-related proteins in response to stress, and an increase in stress-response proteins, that provide a possible mechanistic explanation for the observed decrease in photosynthesis. After the end of heat treatment, stress proteins returned to near normal levels and three of the four species showed normal levels of photosynthesis-related proteins. Photosynthesis-related proteins in the fourth species did not recover to normal levels, suggesting that some plant species might be less resilient to environmental stress than others because of long-lasting changes in leaf proteins.

Also significant was that photosynthesis-related proteins in wild *Eucalyptus* leaves had substantially different composition compared to glasshouse grown *Eucalyptus*. For example, the predominant protein complex in wild leaves was Photosystem II, the complex that uses sunlight energy to split water and make oxygen; whereas the predominant complex in glasshouse grown leaves was Rubisco—an enzyme involved in turning carbon dioxide into sugar. Rubisco is widely believed to be the most abundant protein on Earth because it is typically the most abundant protein in leaf biochemistry experiments. However, most experiments use well-watered, fertilized plants in controlled environments. Our results suggest that those artificial experimental systems may be producing results that do not represent how leaves are constructed in the wild.

Outputs and ongoing research. A manuscript describing the *Eucalyptus* heat stress experiment is nearly finished. We are in the process of analysing data from the >300 *Eucalyptus* leaf experiment, which will be the second major manuscript from this work. The Discovery Project is expected to end December 2017.