Convergence in phosphorus constraints to photosynthesis in forests around the world

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Tropical forests exchange more carbon with the atmosphere than any other biome, and absorb >35 Pg C per annum by photosynthesis despite phosphorus-depleted soils. Phosphorus (P) limitations to this C uptake are paramount for tropical and subtropical forests and in other forests around the globe. Yet low-P feedbacks to photosynthesis and productivity are still site-based, questioning their generality and their representation in terrestrial biosphere models. Here we demonstrate the dependence of photosynthesis and its constituent processes on both leaf N and P concentrations across an unprecedented pan-tropical domain, using photosynthetic CO2 response curves from over 450 woody species. There was similar regulation of photosynthetic capacity by both nutrients across four continents, arguing for generality. In a terrestrial model implementing P constraints, total photosynthesis was reduced by 36% across the tropics and subtropics relative to traditional N constraints with sufficient P. This demonstrates the power of joint regulation by nitrogen and phosphorus on carbon uptake in global models. There is scope for further understanding of the role of P, but we urge inclusion of a P dependence for photosynthesis in global terrestrial models.

Leaf phosphorus economics based on the partitioning of phosphorus among different functional fractions

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An essential theme in plant ecophysiology is to understand the adaptive mechanisms of plants to soil phosphorus (P) limitation, because the availability of P in soils is often constrained by leaching and/or conversion to biologically unavailable forms during pedogenesis. Plants on low-P soils have a range of strategies to cope with P stress which, in turn, relates to the patterning of vegetation and the functioning of terrestrial ecosystems. Leaf-level P-use efficiency (PUE, net lifetime photosynthetic gain of individual leaves per unit leaf P) is presumed highest among species with adaptations to low-P soils. Key mechanisms underpinning high PUE include those that increase the residence time of P in plant tissues (e.g., more complete nutrient resorption before leaf abscission, and extended leaf lifespans), and those that allow more effective use of leaf P in photosynthesis. Still, the physiology and biochemistry of these mechanisms remain unclear. In our work we are addressing these matters in relation to differential allocation of leaf P among functional fractions. Leaf P can be chemically separated into several fractions, these being inorganic P, metabolite P (ATP, ADP, photosynthetic reactants, etc), nucleic acid P (DNA and RNA), lipid P (phospholipids in cell membranes), and residual P fractions (phosphoproteins and unidentified residues). Among these fractions, an apparent trade-off in P allocation between photosynthetic-related P (e.g., metabolite P) versus structural P (including cell membrane

phospholipids) is proposed to be involved in the coordination/trade-off between the capacity for photosynthesis and leaf lifespan. Different costs among fractions for degradation and translocation may be involved in the variation of P resorption before leaf abscission. In this talk, we will discuss how leaf P fraction varies among species while optimising PUE, based on data collected from Bornean rain-forest species and woody species local to the Sydney region.

Ecological stoichiometry and fungal community turnover reveal variation among mycorrhizal partners in their responses to warming and drought

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Symbiotic fungi mediate important energy and nutrient transfers in terrestrial ecosystems. Environmental change can lead to shifts in diverse communities of symbiotic fungi, but the consequences of these shifts for nutrient dynamics among symbiotic partners are poorly understood. Here, we assessed variation in carbon (C), nitrogen (N) and phosphorus (P) in tissues of arbuscular mycorrhizal (AM) fungi and a host plant (*Medicago sativa*) in response to experimental warming and drought. We linked compositional shifts in AM fungal communities in roots and soil to variation in hyphal chemistry by using high-throughput DNA sequencing and joint species distribution modelling.Compared to plants, AM hyphae had 43% lower in [C] and 24% lower in [N] but more than nine times higher in [P], with significantly lower C:N, C:P and N:P ratios. Warming and drought resulted in increases in [P] and reduced C:P and N:P ratios in all tissues, indicating fungal P accumulation was exacerbated by climate-associated stress. Warming and drought modified the composition of AM fungal communities, and many of the AM fungal genera that were linked to shifts in mycelial chemistry were also negatively impacted by climate variation. Our study offers a unified framework to link climate change, fungal community composition, and community-level functional traits. Thus, our study provides insight into how environmental change can alter ecosystem functions via the promotion or reduction of fungal taxa with different stoichiometric characteristics and responses.

Closing the carbon cycle: ecological dynamics within a 5-year wood decomposition experiment

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Mechanisms of xylem hydraulic recovery after drought in Eucalyptus saligna

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The mechanisms by which woody plants recover xylem hydraulic capacity after drought stress are not well understood, particularly with regards to the role of embolism refilling. We evaluated the recovery of xylem hydraulic capacity in young *Eucalyptus saligna* plants exposed to cycles of drought stress and rewatering. Plants were exposed to moderate and severe drought stress treatments, with recovery monitored at time intervals from 24 hrs to 6 months after rewatering. The percentage loss of xylem vessels due to embolism (PLV) was quantified at each time point using micro-computed tomography with stem water potential (Ψ_x) and canopy transpiration (E_c) measured prior to scans. Plants exposed to severe drought stress suffered high levels of embolism (47.38 ± 10.97 % PLV) and almost complete canopy loss. No evidence of embolism refilling was observed at 24 hrs, one week, or three weeks after rewatering despite rapid recovery in Ψ_x. Recovery of hydraulic capacity was achieved over a 6-month period by growth of new xylem tissue, with canopy leaf area and E_c recovering over the same period. These findings indicate that *E. saligna* recovers slowly from severe drought stress, with potential for embolism to persist in the xylem for many months after rainfall events.

Surviving 50 °C – How hot is too hot for plant function?

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Tree mortality during global-change-type drought is usually attributed to xylem dysfunction, but as climate change increases the frequency of extreme heat events, it is necessary to better understand the interactive role of heat stress. We hypothesized that some drought-stressed plants paradoxically open stomata in heatwaves to prevent leaves from critically overheating. Using a combination of experimental and observational data, we determined the high-temperature limits for physiological function of different plant species. In a glasshouse study, we experimentally imposed heat (>40°C) and drought stress onto 20 broadleaf evergreen tree/shrub species. Most well-watered plants avoided lethal overheating, but drought exacerbated thermal damage during heatwaves. On average, well-watered plants closed stomata and decreased stomatal conductance (g_s) during the heatwave, but droughted plants did not. Plant species with low g_s, either due to isohydric stomatal behavior under water deficit or inherently low transpirational capacity, opened stomata and increased g_s under high temperatures. Our experimental data is consistent with observations from urban trees in Western Sydney, where we tracked the effect of extreme heat on leaf gas exchange during the record-breaking 2019-2020 Black Summer. We observed decoupling between photosynthesis and g_s at high leaf temperatures, although responses varied greatly among species growing in the urban environment. The current paradigm maintains that stomata close before hydraulic thresholds are surpassed, but our results suggest that some species

may dramatically increase g_s (over 6-fold increases) even past their leaf turgor loss point. By actively increasing water loss at high temperatures, plants can be driven towards mortality thresholds more rapidly than has been previously recognized. Inclusion of stomatal responses to heat stress could improve our ability to predict the vulnerability of different tree species to future droughts.

Antarctic terrestrial communities in a time of change

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Antarctica has experienced major changes in temperature, wind speed and stratospheric ozone levels over the last 50 years. Whilst West Antarctica and the peninsula have shown rapid warming and consequent ecosystem change, East Antarctica appeared to be little impacted by climate warming, thus biological changes were predicted to be relatively slow. Detecting the biological effects of Antarctic climate change has been hindered by the paucity of long-term data sets, particularly for organisms that have been exposed to these changes throughout their lives. Radiocarbon signals preserved along shoots of the dominant Antarctic moss flora can be used to determine accurate growth rates over a period of several decades, allowing us to explore the influence of environmental variables on growth and providing a dramatic demonstration of the effects of the recent climate change. Stable isotopes of carbon and nitrogen provide information about water availability and nutrient supplies. Together this data has revealed evidence of a drying trend in several extensive moss beds in the Windmill Islands region of East Antarctica. The implications of this potential ecosystem collapse will be discussed.

Pandora's box: How to constrain regional projections of the carbon cycle

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Australia plays an important role in the global terrestrial carbon cycle on inter-annual timescales. This was highlighted by the 2011 La Niña event where Australia contributed up to 60% to the

record global carbon sink, and studies suggest that the Australian terrestrial carbon sink may be enhanced due to more extreme rainfall events projected for future decades. Despite this known importance to the global carbon cycle, there is still significant uncertainty regarding the Australian carbon cycle, and its contributions to global and regional carbon budgets. In this study we explore different methods to constrain regional projections of the carbon cycle in Australia. We employ a dynamic global vegetation model (LPJ-GUESS) and force it with unconstrained CMIP6 data to assess the uncertainty associated with the choice of climate forcing. We then discuss different methods to either bias correct or calculate ensemble averages over the original forcing data to reduce the uncertainty in the regional projection of the Australian carbon cycle. Our results demonstrate the importance of the role of bias correction, as well as the ensemble averaging method, to accurately projecting future continental carbon fluxes.

The co-ordination of stomatal, hydraulic, and leaf browning responses of grass species to experimental drought

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There are growing concerns about the vulnerability of grasslands to the predicted increase in the frequency and severity of droughts across the globe. Plant responses to drought are governed by several mechanisms including stomatal regulation of transpirational water loss and carbon gain, hydraulic impairment, and leaf senescence. In woody plants, these mechanisms occur in a relatively consistent sequence, progressing from stomatal closure to hydraulic impairment and then leaf browning. There is a strong co-ordination among the traits describing these mechanisms, and between the traits and the species' climate of origin. However, it is not known whether similar patterns occur in grasses. We measured drought responses in 16 Australian grass species with different climatic origins and photosynthetic pathways (C3 and C4). We quantified the leaf water potentials that induce 12%, 50 % and, 88% loss of stomatal conductance, leaf hydraulic conductivity, effective plant hydraulic conductivity, and leaf greenness as well as turgor loss point. In our grasses, stomatal closure, hydraulic impairment and leaf browning occurred concurrently, in contrast to the progressive sequence observed in trees. In addition, the measured traits were not correlated with the species' climate of origin. The lack of correlation of these traits to climate of origin suggests that grasses might use different mechanisms to adapt to water availability than trees. Moreover, our results imply that early impairment of leaf hydraulic conductivity and leaf browning might be less costly to grasses than to trees because of their different life history strategy. Further, these traits can be used to parameterize the models to predict drought responses of grasslands.

Understanding admixture in eucalypt species: introgression, adaptive genetic variation and muddy taxonomy

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Eucalypts are notoriously hard to identify confidently in the field. Uncertainty is often due to apparent phenotypic plasticity and genetic variation within species, and the traits shared among species. Hybridisation, introgression and geographic clines are also a challenge in some cases, and the relative importance of hybridisation has been much discussed. We are utilising whole-genome sequencing to examine recent and ancient admixture in *Eucalyptus*, using tools such as landscape genetics and ancestry deconvolution. Results indicate admixture has contributed to spatial variation within species, but we have not found widespread evidence of introgressed alleles sweeping through species. Our results help to resolve the role of introgression in providing adaptive genetic variation in eucalypts and clarify the muddy taxonomy.

Spend it or save it: water budgets and hydraulic coordination to optimize photosynthetic gains

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Australia recently experienced the most intense drought we have seen in the past two centuries. During such dry conditions, xylem vessels in plants operate under high tension making them prone to cavitation thereby blocking water flow. This may lead to loss in capacity to conduct water in stems. Under extreme conditions, this may lead to hydraulic failure and canopy death. In order to avoid such cascading effects, many plants may store water in their tissues to be used when water demands rise. Internally-stored water can contribute up to 50% of daily transpired water but in one sense it also makes water more costly as plants presumably need to increase investment in tissues used for water storage, i.e., sapwood parenchyma, fibres, etc. We studied eighteen woody plant species at Castlereagh Nature Reserve in western Sydney and, among other traits, measured sapwood capacitance which quantifies the amount of stored water released into the hydraulic stream to supplement plant's water demand. We found that species with higher capacitance restricted loss in hydraulic conductivity to lower levels during daytime. These species were also more tolerant to prospective drought. They had more negative P50 – the water potential at which stem xylem loses half of its conductivity. We also calculated a "hydraulic time constant" for these species which represents how plants coordinate water storage and flow rates in stems to delay the effect of sudden changes in water demands. Species with a delayed effect and a high reliance on stored resources were able to achieve higher water use efficiency over seasonal time-scales (as inferred by leaf δ13C isotopic signature). Using cost-benefit analysis at multiple time scales this study highlights key water management strategies in plants, filling an

important gap in understanding of plant water dynamics and hydraulic-photosynthetic coordination.

Altering the primary carboxylase of C4 photosynthesis to improve crop resilience to future climates

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An escalating challenge facing Australia's grain crop production is sustaining the supply of nutritious food within water limited environments. Extreme climate events and decline in available water are threatening crop yield and quality necessitating new solutions to ensure optimal water use within future food systems. We have sought to identify natural biochemical solutions within C4 plants that confer improved responses to carbon assimilation and plant productivity under future climates. We have taken a transgenic approach to overexpress the primary carboxylase of C4 photosynthesis - Phosphoenolpyruvate Carboxylase (PEPC) in S. viridis and a biochemical approach to investigate the in vitro biochemistry of PEPC across biochemical subtypes within the Paniceae tribe of C4 grasses. We successfully expressed recombinant PEPC isoforms within E. coli and discovered that the enzyme predominated in its dimeric form. This raised new questions on the regulation of tetramer formation and catalytic competency of the enzyme. In addition, we discovered that PCK PEPC's were catalytically superior forms and contained an amino acid signature that corresponds to decreased malate inhibition which is crucial for increasing flux through the CO2 concentrating mechanism (CCM). Therefore, we have identified a number of new avenues to improve carbon in C4 plants particularly under abiotic stress conditions. An update on the current progress of this research will be presented.

Applications of low-field NMR to the ecophysiology of eucalypts

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The development of a precise, non-invasive method to measure leaf water status is an important step towards testing established theories and models of leaf hydraulic function. Stomatal responses to rapid changes in leaf water content (minutes to hours) are difficult to measure, in part due to destructive sampling methodologies required to measure leaf water content. Low-field NMR is a new technology that has the ability to dynamically measure *in vivo* leaf relative water content (RWC) and reveal new insights into how stomata respond to rapid changes in tissue water content. Using this new tool, coupled with leaf gas exchange, we investigated the relationship between the rate of stomatal closure during leaf dehydration and the home climate of nine eucalypt species grown in a common garden environment. We found that the RWC at 50%

stomatal conductance ($RWC_{gs\ 50}$) showed a strong linear relationship to mean annual temperature of seed locations of species from contrasting climates across Australia. Using the optical visualisation method to measure the cavitation progression of leaves from the same species, we confirm the results of other studies that cavitation metrics are closely linked with home climate precipitation. Our results demonstrate that mechanisms of adaption to home climate in eucalypt species occur at the leaf level, with stomatal adaptations helping protect species in hotter climates from rapid water loss and more resilient leaf vein networks helping to protect species in dryer climates from hydraulic failure. We consider $RWC_{gs\ 50}$ as a promising new trait which can guide future investigations comparing stomatal responses to water deficit and act as a useful phenotyping trait to delineate tolerance and adaption to hot temperatures and high leaf-to-air vapour pressure deficit.

Deciphering the ecological processes that underpin microbial colonization of plants

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The plant microbiome is considered an extension of the plant genome, and together they form the plant hologenome. While the importance of the plant microbiome for maintaining plant fitness is increasingly recognised, the ecological processes that underpin microbial colonization of the host remain unresolved. For example, we have little understanding of the relative contribution of host selection, environmental stresses, and soil microbial characteristics in determining the plant microbiome assembly. To address this knowledge gap, a full factorial experiment was established where we first manipulated the soil microbial community to obtain three levels of diversity. Then, five plants belonging to three functional groups - C3, C4, and Nitrogen-fixing - were grown in the three soil microbial dilutions as both monocultures and C3 and C4 combinations, to mimic changes in plant diversity. After establishment, the plants were subjected to a drought treatment to evaluate the effect of environmental stress. We then determined the microbial colonization in leaf, root, and soil using amplicon sequencing. We found that the host identity and plant functional group had the strongest effect on microbial colonization of leaves. while root and soil were comparatively less sensitive to host selection. The presence of another plant in a 2-species combination also impacted the microbiome assembly. Similarly, soil microbial dilution significantly impacted soil microbial diversity and structure whereas it significantly impacted the community structure but not the alpha diversity in root and leaf tissue. By contrast, drought treatment did not significantly impact the microbial diversity and structure in any of the host compartments. We concluded that leaf colonization is mainly driven by host species identity while colonization of root compartment is influenced by both species identity and soil microbial community.

Genetic analysis of phi thickening development in Brassica roots

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Phi thickenings are single bands of secondary cell wall material found in radial walls of cortical cells in plant roots, where only thin, primary cell walls normally occur. These bands, present in diverse species from angiosperms to gymnosperms, are coordinated between adjacent cells so that the thickenings viewed in cross-section appear similar to the Greek letter phi. Although first described in the 19th century, research into their development and functions has been minimal. We speculate that they stiffen the root tip, aiding in soil penetration, and are thus agriculturally important. We have developed a simple induction protocol for phi thickening development in primary roots of *Brassica*, and have demonstrated that both osmotic stress and mechanical effects trigger thickening induction through a jasmonic acid (JA)-dependent pathway. Different *Brassica* spp. cultivars also vary in their ability to form phi thickening in response to 1 µM JA and we have used this phenotypic variability to characterise the molecular development of phi thickenings. We performed a genome wide association study (GWAS) screening more than 230 *B. napus* lines from the BnASSYST diversity panel for phi thickening induction in response to JA. We have also screened several *Brassica* breeding populations after identifying crosses in which parental lines showed different phi thickening induction responses. In one particular *B. rapa* cross, the distribution of two separate phenotypes in the F2 progeny is consistent with the presence of multiple genetic differences, one in JA signalling and a second late in the induction pathway. We have identified major loci linked to phi thickening development on chromosomes A06 and A07 of the *B. rapa* genome. RT-qPCR analysis of candidate genes associated with this locus will be reported.

Control of plant development by cell-type boundaries

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In plants, the dorsoventral (DV) boundary of leaves defines an axis of symmetry separating the dorsal (top) and ventral (bottom) tissues. Intriguingly, cells located at this boundary appear to act as an organizer to control leaf orientation and morphology analogously to DV and AP boundaries in the Drosophila wing imaginal disk. A gap of one to two cells in width separating the dorsally expressed REVOLUTA (REV) and ventrally expressed KANADI (KAN) transcription factors is thought to define this boundary as a zone of localised auxin activity, potentially due to the

absence of repression by these transcription factors. However, our recent mosaic experiments reveal that REV clones promote growth and boundary identity non-cell autonomously, even without a gap between REV and KAN expression being present. This juxtaposition-dependent signalling and development parallels findings in the fly wing, demonstrating that a similar overall logic is at work. We are currently testing several candidate signals that may be downstream of REV function, but our experiments so far suggest that short-range signalling is unlikely to involve auxin, cytokinin or tasi-RNAs.