# Ecophysiological traits of grasses: resolving the effects of photosynthetic pathway and phylogeny

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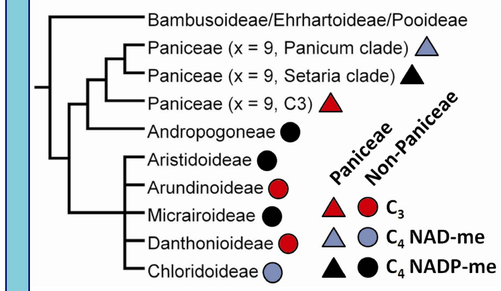
## Introduction:

$C_4$ photosynthesis is an important example of convergent evolution in plants, having arisen in eudicots, monocots and diatoms[1]. Comparisons between such diverse groups are confounded by phylogenetic and ecological differences, so that only broad generalisations can be made about the role of $C_4$ photosynthesis in determining ecophysiological traits. However, 60% of $C_4$ species occur in the grasses (Poaceae) and molecular phylogenetic techniques confirm that there are between 8 and 17 independent origins of $C_4$ photosynthesis in the Poaceae[2]. In a screening experiment, we compared leaf physiology and growth traits across several major independent $C_3$ & $C_4$ groups within the Poaceae, asking 1) which traits differ consistently between photosynthetic types and 2) which traits differ consistently between clades within each photosynthetic type.




## Design:

Species were picked at random from $C_3$, $C_4$ NAD-me and $C_4$ NADP-me clades (left). Plants were grown in 4 l. pots in a glasshouse, kept well-watered & unfertilised (right) Gas exchange and water potentials were measured. For a subset of species, sequential destructive harvests and curve-fitting were used to estimate growth traits at a common, small size.

## Leaf physiology:

$C_4$ photosynthesis is characterised by $CO_2$ uptake at low concentrations (via PEPc) and saturation of Rubisco with $CO_2$, minimizing photorespiration and leading to improvements in Rubisco carboxylation efficiency. $C_4$ plants are thus expected to show higher net $CO_2$ assimilation rates ($A$), lower stomatal conductance ($g_s$), lower leaf nitrogen ($N_{mass}$, $N_{area}$) and improved photosynthetic nitrogen and intrinsic water use efficiencies ($PNUE = A/N_{area}$, and $iWUE = A/g_s$ respectively) under a range of conditions[3].

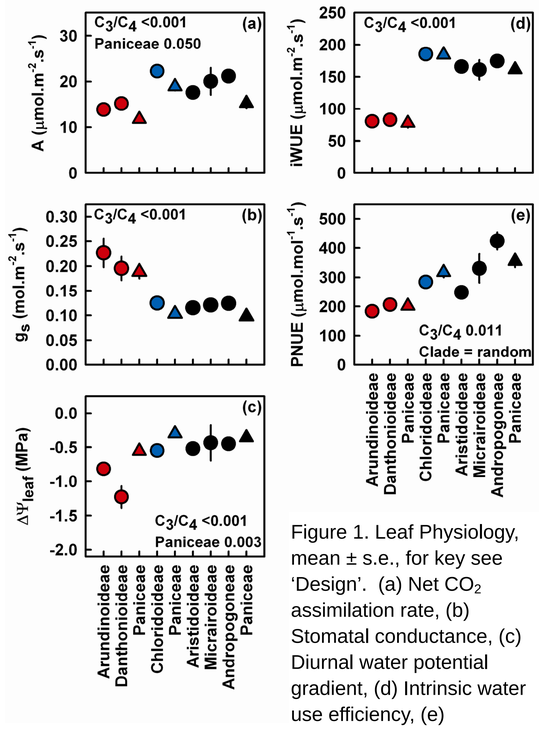



Figure 1. Leaf Physiology, mean ± s.e., for key see 'Design'. (a) Net $CO_2$ assimilation rate, (b) Stomatal conductance, (c) Diurnal water potential gradient, (d) Intrinsic water use efficiency, (e) Photosynthetic nitrogen use efficiency

We found

- Contrasts between $C_3$ and $C_4$ photosynthetic types in $A$, $g_s$, $\Delta\Psi_{leaf}$, $iWUE$ and $PNUE$ (Fig. 1a - e). Within either type, $g_s$ and $iWUE$ showed strong convergence.
- $PNUE$ showed divergence between clades and $\Delta\Psi_{leaf}$ showed divergence between Paniceae and other clades.

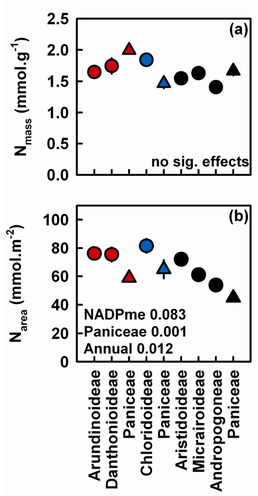



Figure 2. Foliar nitrogen, mean ± s.e., for key see 'Design'. (a) N per unit mass, (b) N per unit leaf area

- **Unexpectedly,** $N_{mass}$ showed no significant patterns (Fig. 2a). Paniceae had lower $N_{area}$ within each type (Fig. 2b), indicating that leaf area per unit leaf mass is high in this group. $N_{area}$ was similar between $C_3$ and $C_4$ NAD-me.

## Growth analysis:

Improved resource use efficiency is expected to correlate with differences in growth allocation between $C_3$ & $C_4$ plants[3]. If higher $A$ is translated into improved growth rate per unit canopy area (unit leaf rate, $ULR$), $C_4$ plants may show either higher relative growth rates per unit total mass ($RGR$), or a greater range of allocation strategies mediated via changes in the leaf mass ratio ($LMR$) and root mass ratio ($RMR$)[3]. The resulting effect on canopy leaf area ratio ($LAR$) is influenced by the specific leaf area ($SLA$) ($LAR = SLA \times LMR$).

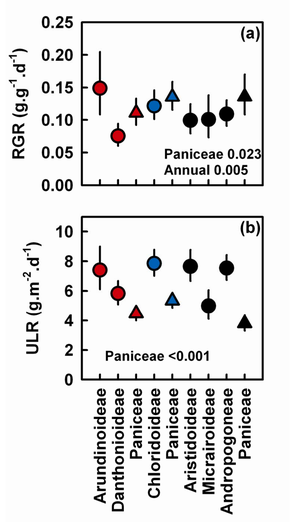



Figure 3. Growth efficiency, mean ± s.e., for key see 'Design'. (a) growth per whole plant mass, (b) growth per leaf area

We found

- No increase in $ULR$ and $RGR$ in $C_4$ types (Fig. 3a & b), despite higher $A$ and greater resource use efficiencies at the leaf level.
- Consistent, significant effects on growth rates and biomass allocation due to:

  1) Classification as perennial vs. annual/ weak perennial (annuals showed greater $SLA$, $LAR$ *and* $LMR$, data not shown).

  2) Paniceae, which showed increased $RGR$, $LAR$ & $LMR$, and reduced $RMR$ & $ULR$ (Figs. 3 & 4).
- $SLA$ (Fig. 4a) was the only growth trait to show a random effect of clade, indicating divergence between phylogenetic groups.
- The $C_4$ NADP-me subtype was associated with more extreme differences in allocation.

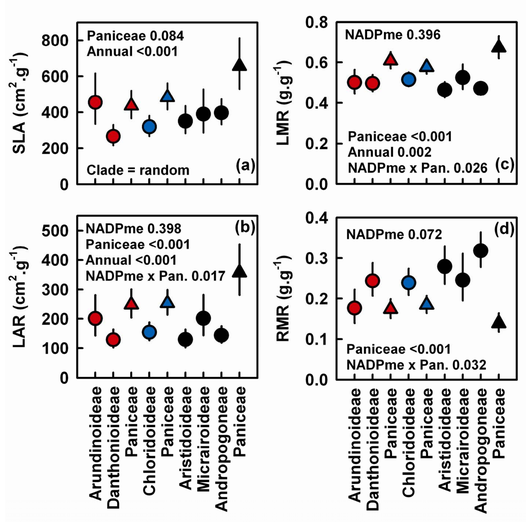



Figure 4. Whole plant allocation of growth, mean ± s.e., for key see 'Design'. (a) Specific Leaf Area, (b) Leaf Area Ratio, (c) Leaf Mass Ratio, (d) Root Mass Ratio

## Conclusions:

- Some traits associated with $C_3$ and $C_4$ photosynthesis show strong convergence across independent lineages (low $g_s$, high $iWUE$), whilst others vary substantially between lineages (high $A$, high $PNUE$). $C_3$ and $C_4$ photosynthesis were not distinguished by leaf N, but low $g_s$ in $C_4$ types relative to $C_3$ was associated with reduced diurnal water potential gradients.
- Within photosynthetic types, clades differed in allocation of resources at the leaf ($SLA$, $PNUE$ & $N_{area}$) and whole plant (LAR, LMR) levels (esp. between Paniceae and others); there was some evidence that this divergence was most extreme in the $C_4$ NADP-me type.
- Ecophysiological traits linked to $C_4$ photosynthesis in grasses are influenced by phylogeny and may show substantial divergence between independent $C_4$ lineages.

**References:**

[1] Sage, R.F. (2004) The Evolution of $C_4$ Photosynthesis. *New Phyt. 161*: 341-370.

[2] Christin, P. et al. (2009) Integrating Phylogeny into Studies of C4 Variation in the Grasses. *Plant Physiol. 149*: 82-87.

[3] Long, S.P. (1999) Environmental Responses. In, eds. Sage R.F. & Monson, R.K. *$C_4$ Plant Biology*. AP San Diego. pp. 215-250.

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