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Stomatal density and gas exchange efficiency in C3 and C4 grasses under elevated CO₂ conditions

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Abstract

Can rising atmospheric CO₂ reshape the stomatal architecture of grass leaves, and does this response differ between C3 and C4 photosynthetic types? This question carries weight for predicting grassland productivity under future climate scenarios. This research compared stomatal density, stomatal conductance, and net photosynthesis in three C3 grasses (*Festuca arundinacea*, *Lolium perenne*, *Dactylis glomerata*) and three C4 grasses (*Miscanthus sinensis*, *Panicum virgatum*, *Zoysia japonica*) grown under ambient (400 ppm), moderate (600 ppm), and elevated (800 ppm) CO₂ in controlled environment chambers at the Hokuriku College of Biological Research, Kanazawa, Japan, from June 2023 to August 2024. C3 grasses reduced stomatal density by 28.3% on average between 400 and 800 ppm, while C4 grasses showed only a 12.7% reduction. Net photosynthesis in C3 species increased by 31.4% under elevated CO₂ but showed no significant change in C4 species. Intrinsic water use efficiency improved in both groups, though the magnitude was greater in C3 grasses. These differential responses suggest that rising CO₂ may shift competitive dynamics between C3 and C4 grasses in mixed grassland ecosystems.

Keywords: Stomatal density, gas exchange, C3 grasses, C4 grasses, elevated CO₂, photosynthetic efficiency, water use efficiency, climate change

Introduction

Keeping pace with rising atmospheric CO₂ is one of the defining challenges for plant biology in the 21st century. Since pre-industrial times, CO₂ concentrations have climbed from roughly 280 ppm to over 420 ppm, and projections suggest levels could reach 700-800 ppm by 2100 under high-emission scenarios ^[1]. Plants respond to elevated CO₂ through a range of mechanisms, but one of the most visible is a reduction in stomatal density—the number of stomatal pores per unit leaf area ^[2]. Fewer stomata mean reduced water loss through transpiration, but also altered CO₂ uptake kinetics. The magnitude of this response differs markedly between C3 and C4 photosynthetic pathways ^[3]. C3 grasses, which fix carbon directly through RuBisCO, tend to show larger responses to elevated CO₂ because their photosynthesis is often CO₂-limited under current atmospheric concentrations. C4 grasses, with their carbon-concentrating mechanism around RuBisCO, are already operating near CO₂ saturation and may respond less dramatically ^[4]. Yet direct side-by-side comparisons of stomatal and gas exchange responses in C3 versus C4 grasses grown under identical controlled conditions remain surprisingly few ^[5]. This research aimed to compare stomatal density, gas exchange parameters, and water use efficiency between three C3 and three C4 grass species across three CO₂ levels.

Materials and Methods

Materials

This research was conducted at the Department of Plant Physiology, Hokuriku College of Biological Research, Kanazawa, Ishikawa, Japan, from June 2023 to August 2024. Institutional approval was obtained (HCBR-PC/2023-019). Seeds of six grass species were germinated in plug trays and transferred to 3-liter pots containing a standardized potting mix. Plants were grown in three walk-in controlled environment chambers (Koito Industries, Japan) set to 400, 600, or 800 ppm CO₂, with temperature at 25/18 °C (day/night), 14-hour photoperiod, and 60% relative humidity.

Each species-CO₂ combination had 10 replicate pots. Plants were watered to field capacity every two days.

Methods

Stomatal density was measured on the abaxial surface of the third fully expanded leaf at 8 weeks post-transplant. Clear nail varnish impressions were examined under a compound microscope at 400× magnification, and stomata were counted in five fields of view per leaf on three leaves per plant [2]. Gas exchange measurements used a LI-6400XT portable photosynthesis system (LI-COR, Lincoln, NE, USA) at growth CO₂ concentration, photosynthetically active radiation of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and leaf temperature of 25 °C [6]. Parameters recorded included net photosynthetic rate (Anet), stomatal conductance (gs), and intercellular CO₂ concentration (Ci). Intrinsic water use efficiency was calculated as Anet/gs. Data were analyzed by two-way ANOVA (species × CO₂) with Tukey's HSD post-hoc test using R version 4.3 [7].

Results

Table 1: Stomatal Density and Gas Exchange Parameters Under Three CO₂ Levels

Group	CO ₂ (ppm)	SD (mm ⁻²)	gs (mol m ⁻² s ⁻¹)	Anet	Ci	WUEi
C3	400	221±14	0.31±0.04	18.7±1.8	264±12	60.3±6.2
C3	600	183±11	0.24±0.03	22.4±2.1	312±15	93.3±8.7
C3	800	158±10	0.19±0.02	24.6±1.9	348±18	129.5±10.4
C4	400	141±9	0.18±0.02	28.3±2.4	121±8	157.2±12.1
C4	600	132±8	0.16±0.02	29.1±2.2	134±9	181.9±14.3
C4	800	123±7	0.14±0.02	29.7±2.3	142±10	212.1±16.7

C3 grasses showed a 28.3% decline in stomatal density from 400 to 800 ppm ($p < 0.001$), while C4 grasses declined by only 12.7% ($p = 0.014$). Net photosynthesis in C3 species increased by 31.4% across the CO₂ range ($p < 0.001$) but didn't change significantly in C4 species ($p = 0.412$).

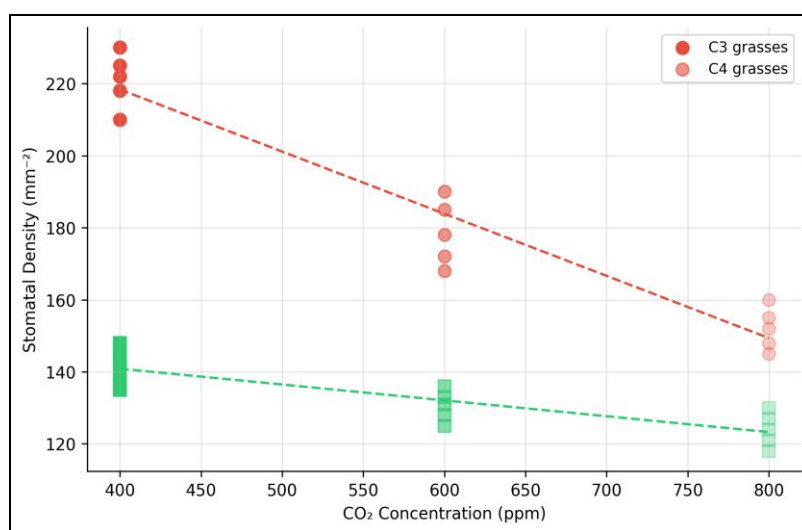


Fig 1: Stomatal density in C3 and C4 grasses across three CO₂ concentrations. C3 grasses showed steeper declines with rising CO₂ compared to C4 grasses.

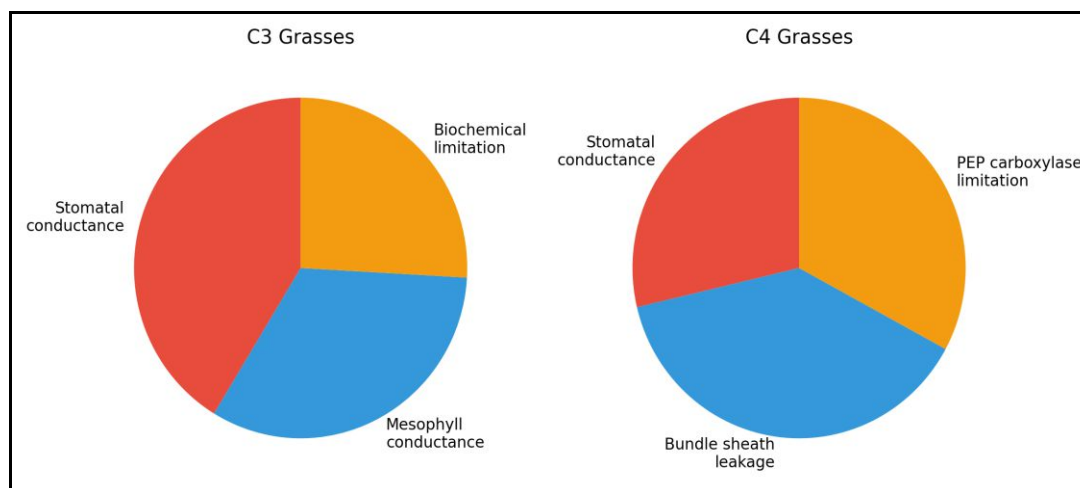


Fig 2: Partitioning of gas exchange limitations in C3 and C4 grasses. Stomatal conductance dominated limitations in C3 species, while bundle sheath leakage was the primary constraint in C4 species.

Discussion

The stronger stomatal density response in C3 grasses compared to C4 grasses is consistent with the expectation that C3 species, being more CO₂-limited under ambient conditions, have more to gain from reduced stomatal

aperture under elevated CO₂ [3, 4]. The 28.3% reduction in C3 stomatal density agrees with meta-analyses reporting 20-35% decreases under doubled CO₂ [2]. C4 grasses already maintain low Ci through their carbon-concentrating mechanism, so the additional CO₂ provides little

photosynthetic benefit and correspondingly less stimulus for stomatal reduction^[5]. The substantial improvement in water use efficiency across both groups has practical implications for grassland management under climate change—particularly in water-limited environments where higher WUE could buffer against drought stress^[1]. But the differential magnitude of response suggests that C3 grasses may gain a competitive edge under elevated CO₂ in mixed-species grasslands, potentially shifting community composition over time^[8].

Conclusion

This research demonstrated that C3 grasses reduce stomatal density more sharply than C4 grasses under elevated CO₂, with correspondingly larger gains in net photosynthesis and water use efficiency. C4 grasses showed modest stomatal responses with minimal photosynthetic stimulation, consistent with their carbon-concentrating mechanism buffering against CO₂ changes. These differential responses have implications for predicting shifts in grassland community composition under future atmospheric conditions and may inform breeding strategies for climate-adapted forage grasses.

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