Nutrient niche partitioning between tropical legumes and grasses strengthens under elevated CO_2

Amber C. Churchill[1,2*], Haiyang Zhang[1], Gil Won Kim[1,3], Karen L. M. Catunda[1], Ian C. Anderson[1], Forest Isbell[2], Ben Moore[1], Elise Pendall[1], Jonathan M. Plett[1], Jeff R. Powell[1], Sally A. Power[1]

* Corresponding author: ac.churchill5@gmail.com; c: (1) 207-576-8591

[1]Hawkesbury Institute for the Environment, Western Sydney University, Hawkesbury Campus, Ground Floor, Building R2, Locked Bag 1797, Penrith 2751, NSW Australia

[2]Department of Ecology, Evolution and Behavior, University of Minnesota, 140 Gortner Laboratory, 1479 Gortner Ave., St. Paul, MN 55108, USA

[3]Institute of Agriculture and Life Sciences, Gyeongsang National University, 501 Jinju Daero, Jinju, 52828, South Korea

Total Word Count:

Summary- 200

Introduction- 1192

Materials and Methods-1599

Results- 1439

Discussion- 2027

Figures (in colour)- 6

Tables- 4

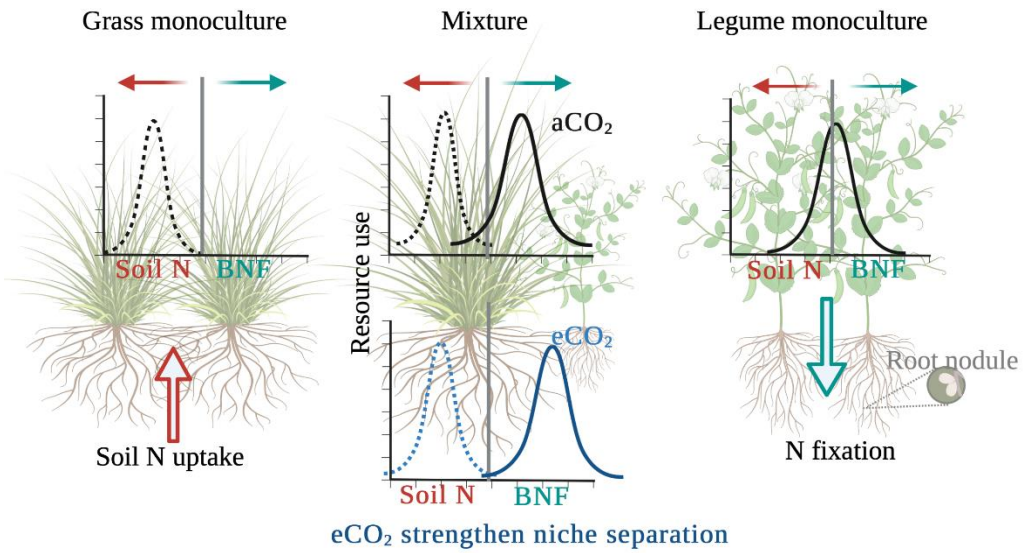
Supporting Information- 4 Figures, 3 Tables

Summary

- Plant community biodiversity can be maintained, at least partially, by shifts in species interactions between facilitation and competition for resources as environmental conditions change. These interactions also drive ecosystem functioning, including productivity, and can promote over-yielding- an ecosystem service prioritized in working landscapes that occurs when there is either less competition, more facilitation, or both, between species in a community than within species in a population. Importantly, shifts in species interactions relating to plant niche partitioning that often produce over-yielding is uncertain given rising CO_2 concentrations.
- We examined the relative performance of two species pairs of tropical pasture grasses and legumes growing in monoculture and mixtures in a glasshouse experiment manipulating CO_2. We investigated how over-yielding can arise from on nitrogen (N) niche partitioning and abiotic facilitation using stable isotopes to differentiate soil N from biological N fixation.
- We found that N niche partitioning drove species interactions in mixtures, and partitioning, though not necessarily overyielding, was generally enhanced under elevated CO_2. However, this finding was mixture-dependent based on biomass of dominant species in mixtures.
- Rising atmospheric CO_2 may alter niche partitioning between co-occurring species, with consequences for shifts in grassland composition and negative implications for the over-yielding benefits predicted for legume-grass mixtures.

Key Words *(5-8 in alphabetical order)***:**

Biological nitrogen fixation, complementarity effect, elevated carbon dioxide, interspecific competition, natural abundance ^{15}N, nutrient niche partitioning, tropical pastures



Graphical abstract (Image by H. Zhang): Among our tropical pasture species we found that grasses (dotted lines) grown in monoculture rely fully on soil nitrogen (N), while legumes (solid lines) grown in monoculture relied approximately equally on soil N and biological nitrogen fixation (BNF) to meet N requirements. When grown with tropical grasses, however, legumes shifted to rely more strongly on BNF, indicative of niche partitioning and decreased competition for soil nutrients with grasses. This separation of niche space was strengthened under elevated CO_2 conditions, ultimately reducing legume production.

Introduction

Interactions between plant species within communities and ecosystems vary over time and as environmental conditions change, thereby driving shifts between competition and facilitation for resources (Adler *et al.*, 2012; Arfin Khan *et al.*, 2014). Niche partitioning is a mechanism allowing the co-existence of numerous species that may overlap in fundamental niche space but differ in realized niche space to reduce impacts from competition (Jumpponen *et al.*, 2002; McKane *et al.*, 2002; Zuppinger-Dingley *et al.*, 2014). Additionally, changes in niche space associated with interspecific interactions are a common feature of complementarity whereby interactions among community members result in greater ecosystem functioning, relative to the sum of individual contributions (Loreau & Hector, 2001; Loreau *et al.*, 2008; Barry *et al.*, 2019); when applied to productivity this concept is often referred to as over-yielding (Vandermeer, 1981). Confirming mechanisms of complementarity that underpin interspecific interactions, such as niche partitioning, is fundamental to understanding relationships between biodiversity and ecosystem function.

Nitrogen (N) is a primary often-limiting nutrient that plays an important role in structuring community interactions and ecosystem function alongside phosphorus (P) and other mineral nutrients (Vitousek & Howarth, 1991; Fay *et al.*, 2015). Plants that host N-fixing bacteria (i.e. N-fixers), such as legumes, are often considered to have an advantage under low soil N conditions as the mutualism can provide an alternative route for accessing N. This biological N fixation (BNF), therefore, can promote biodiversity and ecosystem function, via resource complementarity (Mulder *et al.*, 2002; Lee *et al.*, 2003; Perring *et al.*, 2010; Barry *et al.*, 2019; Elias & Agrawal, 2021).

In communities containing legumes, BNF is often assumed to result in nutrient facilitation and an increase in the overall pool of ecosystem N available for neighbouring plants (Lee *et al.*, 2003) that contributes to resource niche complementarity among comprising species (Fornara & Tilman, 2008; von Felton *et al.*, 2009; Barry *et al.*, 2019). There are two main pathways enabling this outcome: 1) direct acquisition of N resources from legumes to non-legumes (i.e. grasses) and 2) via niche partitioning where grasses compete less with legumes for shared soil nitrogen, given that legumes have access to a non-soil pool of N (Barry *et al.*, 2019). There are also a number of direct biotic feedback-based mechanisms including decomposition of legume leaf litter (Makkonen *et al.*, 2012; Wei *et al.*, 2019), or roots (Fustec *et al.*, 2009; Mclaren & Turkington, 2010) that lead to higher soil N. Additionally,

some literature has highlighted the potential importance of root exudation of high N-containing compounds that impact N (or carbon: C) availability in the rhizosphere (Paynel *et al.*, 2001; Henneron *et al.*, 2020), or transfer through the activity of soil fauna (Gilarte *et al.*, 2020), thereby promoting increased N availability for surrounding community members. Alternatively, niche partitioning can support greater overall ecosystem N availability as legumes rely on BNF for N resources, leaving the soil N pool available to neighbouring community members (Ashworth *et al.*, 2018). These pathways offer numerous strategies that ultimately promote increased community productivity in the presence of legumes representing a potential for over-yielding.

Atmospheric CO_2 concentrations have increased by 50% since the industrial revolution (IPCC, 2022). There are many ecosystem consequences associated with this change, including an increase in plant productivity (Gill *et al.*, 2002; de Graaff *et al.*, 2006), altered plant community composition and diversity (Reich, 2009; Zelikova *et al.*, 2014), and modified plant interactions within a community that impact ecosystem scale processes (Blumenthal *et al.*, 2013; Gonçalves de Oliveira *et al.*, 2021; Maschler *et al.*, 2022). However, some of these patterns may only occur when water is limiting, nutrients are abundant (Soussana & Lüscher, 2007; Rogers *et al.*, 2009; Reich *et al.*, 2014), or when plant diversity is high (Reich *et al.*, 2001; Shaw *et al.*, 2002). Under well-watered conditions, eCO_2 generally benefits the C_3 photosynthetic pathway over the C_4 pathway (used by warm season or tropical grasses) due to differences in carbon capture (Ainsworth & Long, 2005; Reich *et al.*, 2014, but see Reich *et al.*, 2018). Furthermore, eCO_2 is reported to increase belowground biomass, especially for fine roots (Nie *et al.*, 2013), which may increase access to soil nutrients (Reich *et al.*, 2001; de Graaff *et al.*, 2006; Dieleman *et al.*, 2012).

Many studies have found that eCO_2 fertilization effects are larger for N fixers than grasses due to greater C resource availability to support the energetically demanding process of N-fixation (Liang *et al.*, 2016). However, N-fixer responses in natural ecosystems have been varied, as potential benefits from increased photosynthesis can become constrained by other limiting nutrients such as phosphorus (Coventry *et al.*, 1985; Hungate *et al.*, 2004; Soussana & Lüscher, 2007; Rogers *et al.*, 2009), or reduced due to temperature or drought conditions (Rogers *et al.*, 2009). Even so, most studies find decreased foliar N concentrations for non-N-fixers under eCO_2 with subsequent increases C:N ratios in plant tissues (Ainsworth & Long, 2005; Rogers *et al.*, 2009; Augustine *et al.*, 2018; Gojon *et al.*, 2022). However, within mixed

grass-legume grassland communities, leaf N and C:N ratios are less likely to be impacted by eCO_2 for the legume component of the sward (Winkler & Herbst, 2004; Rogers *et al.*, 2009). These findings suggest that grassland responses to eCO_2 may depend on the presence of legumes (Jones & Donnelly, 2004). Importantly, the perceived 'benefit' of eCO_2 for tropical and subtropical ecosystems is less well documented, as most studies have focused on temperate species, with C_3 photosynthetic pathways (Naudts *et al.*, 2013). The potential impacts of eCO_2 on nutrient dynamics in C_4 grasses, including those in mixed C_3-C_4 systems is of particular interest, due to the increasing abundance of warm season grasses in many areas of the world (McKeon *et al.*, 2009). Given the diverse predicted outcomes for ecosystems as eCO_2 concentrations increase (Cannell & Thornley, 1998; de Graaff *et al.*, 2006; Dijkstra *et al.*, 2010), there is a need for improved understanding of the impacts of rising CO_2 in managed and natural grasslands. These include ongoing shifts in grassland composition, driven by species differences in response to climate change (Sherry *et al.*, 2007; McKeon *et al.*, 2009; Wang *et al.*, 2013; Zelikova *et al.*, 2014; Munson & Long, 2017; Xie *et al.*, 2022). Increased understanding of the processes that underlie plant-plant interactions between tropical grasses and legumes will therefore increase the accuracy of predictions for how grassland species composition and productivity will be impacted as atmospheric CO_2 concentrations continue to rise and human-induced shifts in ecosystem biodiversity are realized.

To address this area of research, we examined potential mechanisms by which eCO_2 might benefit warm season (C_4) grasses grown in mixed swards with tropical legumes. Specifically, we evaluated the potential for nutrient niche complementarity between species, driven by facilitation whereby biologically fixed N (from rhizobia) is directly taken up by grasses. We hypothesized that 1) grasses grown with legumes benefit in terms of both nutrition and productivity, relative to those growing in monoculture, due to access to BNF from legume rhizobia; 2) tropical legumes grown under eCO_2 will increase production as a consequence of increased carbon-use-efficiency; 3) increased C availability under eCO_2 will increase legume N fixation and both legume shoot N and soil N content.

Materials and Methods

Experimental design

This experiment was conducted in a glasshouse at Western Sydney University's Hawkesbury Campus, Richmond, New South Wales (33°61'S,150°74'E), Australia. We used six separate

glasshouse chambers, with three randomly assigned to ambient conditions (410 - 430 ppm; aCO_2) and three an elevated regime (630 - 650 ppm; eCO_2). The ambient treatment matches contemporary atmospheric concentration, while the elevated regime was consistent with the upper-end of predicted end-of-century increase in global atmospheric [CO_2] (Szulejko *et al.*, 2017). We maintained a consistent temperature regime across all chambers at a daily average of 27°C (**Fig S1A**). This average included a temperature ramp to mimic diurnal patterns (00-06 hours: 22°C, 06-09 hrs: 27°C, 09-18 hrs: 32°C, 18-21 hrs: 27°C, 21-00 hrs: 22°C) for spring to summer temperatures for areas currently cultivating tropical pasture in northern NSW and southern Queensland (BOM, 2020). Additionally, all chambers were controlled at 50-60% relative humidity (**Fig S1B**) with natural lighting between 11 hours (August) and 14 hours (December).

The soil used in this experiment was collected adjacent to the Pastures and Climate Extremes field experimental facility (Churchill *et al.*, 2022). Soil was sieved to 5 mm before being air dried and mixed with quartz sand (soil:sand 7:3 v/v). Each pot (3.7L, 150 mm diameter, 240 mm height) was filled with 3.9 kg of the soil-sand mixture before planting (Zhang *et al.*, 2021). The final soil mixture was ~ 84% sand, with an average pH of 5.6, plant available N at 55 mg kg^{-1}, plant available (bray) P at 39 mg kg^{-1} and soil organic carbon at 1.1%. All pots were maintained under well-watered conditions using an automated irrigation system such that soil water capacity was brought to 100% every two days (Zhang *et al.*, 2021). In order to promote legume nodulation, no nutrients were added to existing.

Two tropical grasses and two tropical legumes were selected, based on current widespread use or consideration for future use by key pasture industries within Australia (**Table 1**). All species are commonly grown in multi-species improved pastures in southern Queensland and New South Wales, Australia, as well as among other sub-tropical grasslands world-wide (Thomas & Sumberg, 1995; Bell *et al.*, 2012; Miegoue *et al.*, 2016; Melesse *et al.*, 2017; DPI, 2018). Specific species-pairs were the legume *Macroptilium bracteatum* (Nees. & Marti.) growing with the grass *Chloris gayana* (Kunth), and the legume *Desmodium intortum* (Mill) Urb with grass *Panicum maximum* var. *trichoglume* (Robyns). All seeds were supplied by Heritage Seeds and contained AgriCote advanced seed coating technology; legume seed came pre-inoculated with appropriate rhizobia (Barenbrug Co., Heritage Seeds, New South Wales, Australia). Seeds were planted directly into seedling trays (~5 seeds per cell) and thinned to a single individual over time. After three weeks, two individuals for the

monoculture or one individual from each species for the mixture were then transplanted into the pots. The experimental design therefore included the two CO_2 treatments (aCO_2 and eCO_2), six types of plant combinations in pots ($grass_1$, $legume_1$, $mixed_1$, $grass_2$, $legume_2$, $mixed_2$), and twelve replicates per plant combination per treatment (four replicates per chamber).

Aboveground sampling

After transplanting the seedlings, all plants were grown for 11 weeks before a final harvest, just as individuals were reaching the flowering phenophase. All aboveground standing plant material (shoot; leaves and stems/tillers together) was cut at soil level and for mixed species pots, separated by species, then weighed. The fresh aboveground biomass was frozen at -18°C. Within one month all samples were then microwaved at medium power for 90 seconds to halt enzymic degradation of plant tissue compounds, and oven dried at 70°C for 48 hours then weighed (Landhäusser *et al.*, 2018). All dried plant material was homogenized, ground to pass through a 2-mm screen in a laboratory cyclone mill (Foss Cyclotec Mill, Denmark) then ball-milled to a fine powder (Retsch® MM200; Hann, Germany) for nitrogen (N) and isotopic (i.e. $\delta^{15}N$) measurements, which were determined using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer at the UC Davis Stable Isotope Facility (Davis, CA, USA).

Belowground biomass

After aboveground material was removed, belowground biomass was determined. All pot soils were carefully sieved using 2 mm sieves to retain root fragments and roots were oven dried to constant mass. We assessed legume nodule number and weight for a subset of fresh root material in each legume- or mixed species pot by randomly subsetting roots and nodules from different locations within the root system. From this subset, we separated the nodules from the roots and weighed the fresh biomass for each component separately. Fresh nodules were incubated with acetylene for 1 hour and ethylene evolution tested as a proxy to estimate the average instantaneous rate of nitrogenase activity of the bacteria within the nodules using acetylene reduction assays (ARA; Plett *et al.*, 2016). After ARA measurement, root nodules were oven dried and weighed as above. Final estimates of biological nitrogen fixation (BNF) activity were calculated as the rate of ethylene production per gram of incubated nodule dry biomass.

Soil nutrients

Nutrient availability was assessed using ion exchange resins for nitrate (NO_3^-), ammonia (NH_4^+) and phosphate (PO_4^{-3}). We enclosed 5 g of mixed bed resin in a 3 cm x 5 cm nylon bag (42 µm mesh) and placed one bag in each pot in the upper 10 cm. Resin bags were collected during the biomass harvest, briefly rinsed to remove attached soil and root particles before immediate storage at -70 °C prior to extraction with 0.5 M HCl solution. Extractable NO_3^-, NH_4^+ and PO_4^{-3} concentrations in the resin bag were determined on a SEAL AQ2 Discrete Analyser (SEAL Analytical Inc., USA).

Quantifying contributions of biological nitrogen fixation

The contribution of BNF to plant total N was calculated as described in Chalk *et al.* (2016) and Bell *et al.* (2017), where lower $\delta^{15}N$ is indicative of a higher N-fixation amount. The minimum observed values for $\delta^{15}N$ for both legume species in this experiment (*B*; *Macroptilium*: -2.7 ‰; *Desmodium*: -2.0 ‰) were used to represent N derived solely from BNF. Note that while this could overestimate the absolute estimate of BNF, the general patterns among different treatments should remain unaffected (Chalk *et al.*, 2016). Mean $\delta^{15}N$ values for companion grass species in monoculture at each treatment level of CO_2 were used as baselines for soil derived N (*Ref*; *Chloris*: 3.9 ‰, 3.7‰; *Panicum*: 4.9 ‰, 4.4 ‰; aCO_2, eCO_2 respectively), and we then calculated the relative contribution of BNF vs. soil N to plant aboveground N following Equation 1:

$$(Equation\ 1)\ BNF\ contribution\ to\ plant\ N = \frac{\left(Ref - Sample_{15_N}\right)}{Ref - B}$$

Calculations and statistical analyses

Effects of CO_2 treatment on aboveground plant responses measured on pot level totals (aboveground shoot biomass, total shoot N content, soil derived shoot N content, belowground biomass, resin extractable soil nutrients, total shoot P content, and shoot N:P) were analysed using linear mixed effects models. For these analyses, 'Species Pair', 'CO_2' and 'Plant Type' treatments were included as fixed effects with all two-way and three-way interactions; 'Chamber' was also included as a random effect in all models to account for non-independence among replicates within each chamber due to potential between-chamber environmental variation. Treatments of CO_2 included two levels (aCO_2 and eCO_2), 'Species Pair' included '*Macroptilium* & *Chloris*' and '*Desmodium* & *Panicum*', while the 'Plant

Type' category included 'Grass', 'Legume', and 'Mixed' pots. Nodule data were also analysed using linear mixed effects models, such that 'CO_2', 'Species Pair', and 'Plant Type' were included as interactive fixed effects and 'Chamber' as a random effect. For these analyses 'Plant Type' only included 'Legume' and 'Mixed' pots.

Over-yielding was determined as the increased productivity of a mixed pasture over the discrete contributions of the constituent species grown in monoculture. We calculated over-yielding for each species pair based on mixture total productivity (and total N content in aboveground plant material) and the associated productivity of the monoculture grass and legume species standardized by the number of individuals per pot, following Equation 2:

$$(Equation\ 2)\ Overyield\ effect\ of\ mixtures = \frac{\underline{Mixed}}{\frac{\underline{Grass}}{2} + \frac{\underline{Legume}}{2}} - 1$$

Effects of eCO_2 on over-yielding were examined by calculating over-yield estimates based on monoculture and mixed pots in each chamber (n = 6; 3 for each CO_2 level). We then ran a linear mixed effects model with over-yield as the response, 'CO_2' level and 'Species Pair' as the fixed effects and 'Chamber' as a random effect. Effect size of over-yielding was determined by calculating mean treatment values and 95% confidence intervals (CI) across all replicates (n = 12 for each plant type and CO_2 level) and over-yielding was deemed significant if CIs didn't overlap with 0.

The net biodiversity effect, and its constituents- the complementarity effect and selection effect- were calculated following Loreau & Hector (2001) based on averages for monoculture and mixed pots of each species pair in each chamber and analysed statistically as described for the over-yield metric above. Net biodiversity effect was therefore defined as the difference between the observed yield and the expected yield in the absence of complementarity or selection (Loreau & Hector, 2001). Complementarity effects are positive when mixture yields are higher than expected based on the constituent monoculture species, while selection effects are positive when species with greater than average yields dominate in mixture.

All analyses were conducted in R version 4.1.1 (R Core Team, 2020) using the package lme4 (Bates *et al.*, 2015) with statistical contrasts based on Kenward-Roger degrees of freedom calculated using the 'Anova' function from the 'car' package (Fox & Weisberg, 2019).

Pairwise comparisons to determine treatment differences in plant responses for all models were conducted using the R package emmeans (Length, 2020) and using the Tukey method for *P*-value adjustment.

Results

Shoot biomass and N content

Shoot biomass differed strongly between our species pairs, with greater productivity in pots with the *Macroptilium* & *Chloris* monocultures and mixture, compared with *Desmodium* & *Panicum* (**Fig 1**; **Table 2**). Elevated CO_2 (eCO_2) had limited effects on shoot biomass, with increased productivity only observed for *Macroptilium* monoculture pots (**Fig 1a**). There were no significant productivity differences among plant types (grass, legume, mixed) for the *Macroptilium* & *Chloris* pairing, while *Desmodium* in monoculture produced less shoot biomass than *Panicum* or the mixed plant type (**Fig 1b**). Despite little change in pot-level biomass among monocultures and mixed pots, the contribution of grasses and legumes to mixed pot total biomass was heavily weighted toward grass production in both species-pairs, with *Chloris* contributing 65% and 61% of total pot biomass under aCO_2 and eCO_2 and *Panicum* at 90% and 95%, respectively. Relative to growth by individual plants in monoculture pots, this change represented a 38%/52% gain in biomass under aCO_2 and eCO_2 by *Chloris* and 20%/28% reduction in growth by *Macroptilium* (**Fig 1a**). This trend was stronger for the *Desmodium* & *Panicum* species pair with 96%/87% gain for *Panicum* and 65%/86% loss for *Desmodium* between monoculture and mixed pots (**Fig 1b**).

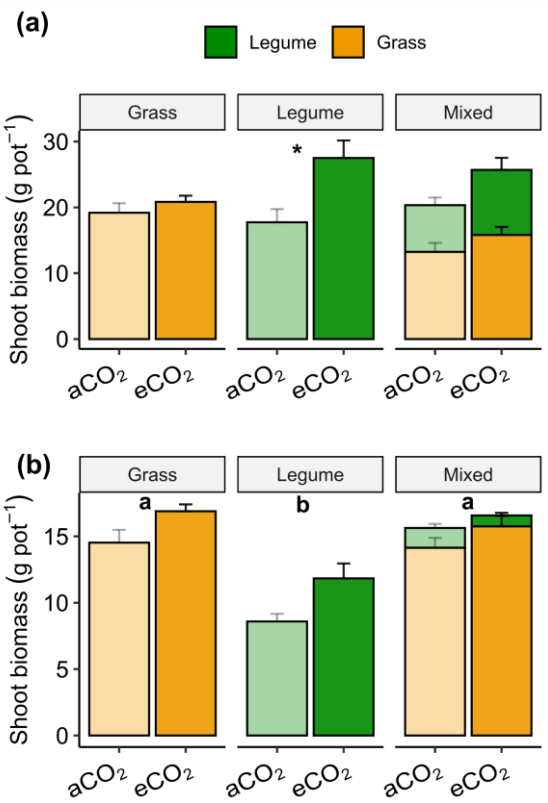


Figure 1. Shoot biomass of grass and legume grown as monoculture or mixtures for two species pairs under ambient (aCO_2) and elevated CO_2 (eCO_2) comprising (a) *Macroptilium* & *Chloris* and (b) *Desmodium* & *Panicum*. Bars indicate mean values, plus standard error. Significant differences ($p < 0.05$) between CO_2 treatments are indicated by '*', and significant differences among plant types (grass, legume, mixed) are indicated by differing letter designations.

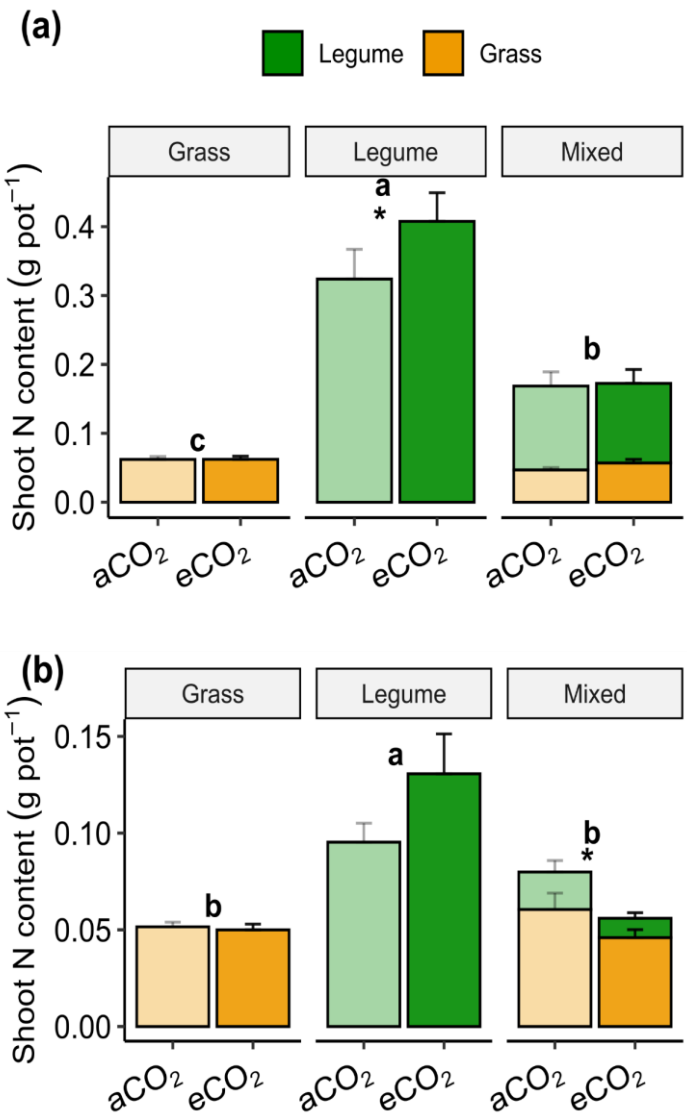


Figure 2. Shoot nitrogen (N) content (%N multiplied by shoot biomass) partitioned between grasses and legumes grown as monocultures or mixtures under ambient (aCO_2) and elevated CO_2 (eCO_2), for (a) *Macroptilium* & *Chloris* and (b) *Desmodium* & *Panicum*. Bars indicate mean values, plus standard error. Notations follow Fig. 1.

We found that legumes had a higher shoot %N than grasses, and this resulted in greater total shoot N content for the legumes in both species pairs (**Fig 2**; **Table 2**). Additionally, the *Macroptilium* & *Chloris* species pair generally had greater total shoot N content than *Desmodium* & *Panicum*, driven by the differences in aboveground biomass rather than shoot N concentrations. *Macroptilium* exhibited increased shoot N content under eCO_2 in monoculture, however there was no change in N content in the mixed pots that included this species (**Fig 2a**). In contrast, *Desmodium* N content was not significantly affected by eCO_2 in monoculture, however pot total N content in the mixtures with *Panicum* was reduced under eCO_2 (**Fig 2b**). Legume contributions to shoot N content in mixed pots also differed between the two species pairs, with *Macroptilium* contributing 72%/67% under aCO_2 and eCO_2 and

Desmodium contributing 24%/18% of the total. Importantly, in both species pairs the shoot N content per individual plant in grasses grown with legumes increased (*Chloris*: 51%/83%, *Panicum*: 135%/84%; aCO_2/eCO_2, respectively), while the corresponding shoot N content per individual in the mixed pot legumes declined compared with monoculture pots (*Macroptilium*: -25%/-43%, *Desmodium*: -59%/-85%). For the *Desmodium* & *Panicum* pair this change was large enough that the *Panicum* grown with *Desmodium* contained comparable shoot N content to the *Desmodium* grown in monoculture.

N uptake source partitioning: soil N uptake

Despite variability in shoot N contents, the portion of shoot N that was derived from soil nutrients (**Fig 3**) remained similar among plant types within each species pair (**Table 2**). There were no effects of eCO_2 on soil-derived shoot N for either species pair, however the fraction of soil-derived N in aboveground plant material in mixed pots was not equally divided between grasses and legumes. Instead, grasses dominated soil nutrient uptake, accounting for 74%/83% for aCO_2 and eCO_2 pots in the *Macroptilium* & *Chloris* species pair and 94%/95% in *Desmodium* & *Panicum*. Raw values for $\delta^{15}N$ for each plant type and CO_2 treatment are included in **Table S1**. Resin extractable soil nutrients indicated that eCO_2 did not impact availability (**Fig S2**) of NO_3^-, NH_4^+ or PO_4^{-3} ($p > 0.05$). We did, however, find differences in the soil availability of NO_3^- and NH_4^+ among plant types within the *Macroptilium* & *Chloris* species pair. *Chloris* monoculture pots had less NO_3^- than in mixed pots (**Fig S2a**), and *Macroptilium* monoculture pots had less NH_4^+ than mixed pots (**Fig S2c**).

The ability of plants to take up soil nutrients is contingent on belowground traits, and we found that there were key differences in the root biomass associated with eCO_2 (*Macroptilium* & *Chloris* **Fig S3a**; **Table S2**) and among plant types (legume/grass/mixed, *Desmodium* & *Panicum*; **Fig S3b**). In general, there was increased root biomass in pots under eCO_2, driven by significant increases in *Macroptilium* roots in monoculture and for mixed roots in the *Macroptilium* & *Chloris* pots (**Fig S3a**). In the *Desmodium* & *Panicum* species pair there was no impact of eCO_2, however *Panicum* had the greatest root biomass, followed by the mixed pots and then *Desmodium* in monoculture (**Fig S3b**). Despite these changes in belowground biomass, there were no shifts in the root mass fraction between species pairs, among plant types or associated with eCO_2 (**Fig S3c** & **d**; **Table S2**).

N uptake source partitioning: biological nitrogen fixation

We found that a large fraction of the total shoot N was derived from BNF in legume monoculture and mixed species pots, although this differed between legume species and between plant types (**Fig 4**; **Table 3**). *Macroptilium* derived more shoot N via BNF than *Desmodium* (*Macroptilium*: 77/82% of total shoot N in aCO_2 vs. eCO_2, *Desmodium*: 32/39%; **Table 3**) in monoculture. Pot level shoot N relied more strongly on BNF in mixtures, with *Macroptilium* & *Chloris* pots increasing reliance on BNF to 84/93% under aCO_2/eCO_2 and *Desmodium* & *Panicum* shoot N increasing to 72/48%. Importantly, although both legumes derived less shoot N from BNF by mass in mixture than monoculture (**Fig 4a** & **c**), BNF contributed a greater percentage of the total shoot N in contrast to soil N (**Fig 4d**) for *Desmodium* especially under aCO_2. Across both species pairs there were low levels of BNF-derived N uptake by grasses (*Chloris*: 3/1%, *Panicum*: 6/1%). However, by mass, the BNF derived N accounted for 29% of the shoot N in *Panicum* in mixtures under aCO_2, but only 2% under eCO_2 (**Fig 4b** & **d**) thereby providing evidence of direct facilitation under ambient conditions within this species pair that was disrupted under eCO_2.

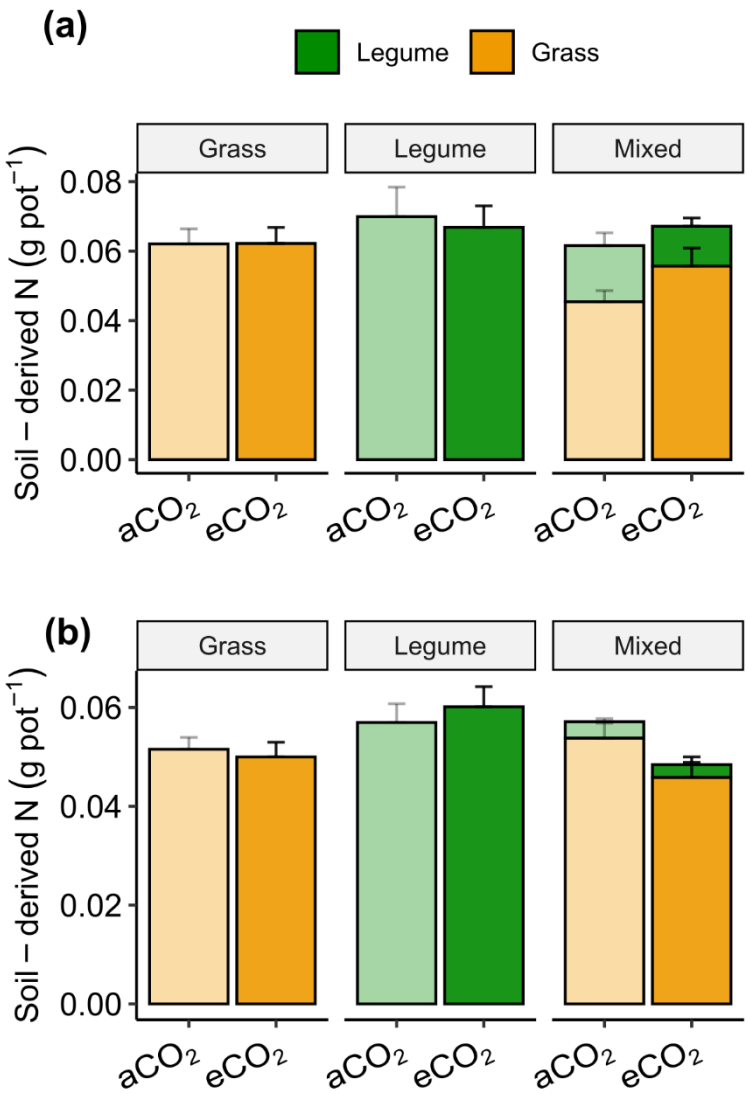


Fig 3. Shoot N content derived from soil for (a) *Macroptilium* & *Chloris* and (b) *Desmodium* & *Panicum* under ambient (aCO_2) and elevated (eCO_2) concentrations of CO_2. Values shown are pot-level means + 1 SE. Note no significant differences with CO_2 treatment or among plant types were observed.

365

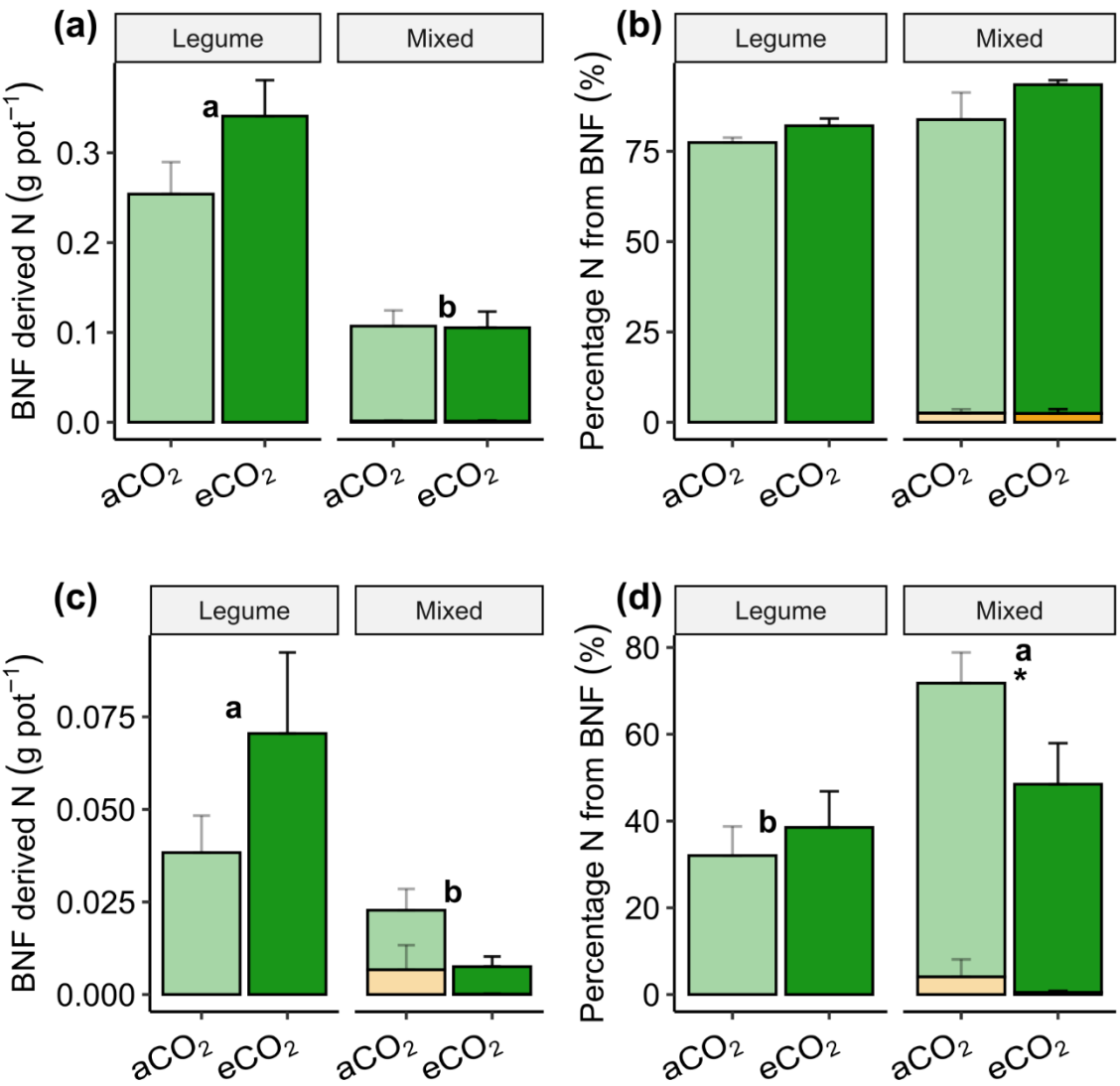


Fig. 4. Shoot N content derived from biological N fixation (BNF; a & c) and the proportion of aboveground N content derived from BNF (b & d) for *Macroptilium* & *Chloris* (a & b) and *Desmodium* & *Panicum* (c & d). Values shown are means ± 1 SE. Colours in panels match Fig 1, with green indicating N in legume shoots, and orange indicating N in grass shoots. Notations from Fig. 1.

The ability of legumes to support rhizobia in nodules is the main constraint on the potential role of BNF as a source for shoot N, and we found clear differences between species in terms of root nodules (**Fig 5**; **Table 4**). In parallel with total shoot N content, *Macroptilium* had greater nodule biomass (**Fig 5a**) and number of nodules (**Fig 5b**), compared to *Desmodium*, in monoculture. Additionally, both legumes had a significant reduction in nodule biomass and number of nodules when grown with grass, and there were no impacts of eCO_2 on these measurements. Despite the difference in nodule biomass and number between legume species, *Desmodium* had equivalent levels of instantaneous nodule activity to *Macroptilium*,

based on production of ethylene as a proxy for the rate of nitrogen fixation following harvest (**Fig 5c**) and in monoculture there was no impact of eCO_2 for either species. However, for *Desmodium* grown in mixture with *Panicum*, real-time nodule activity significantly increased for individuals grown under eCO_2 (**Fig 5c**).

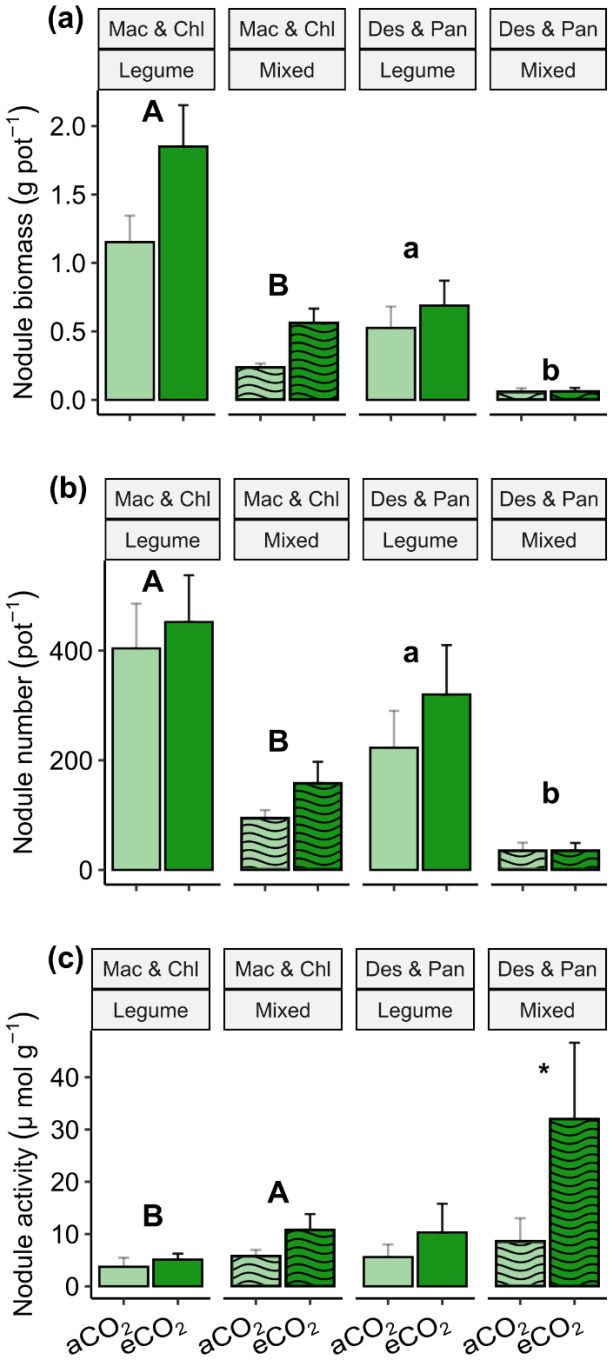


Fig 5. Effects of eCO_2 on metrics of nitrogen fixation capacity relating to root nodules for legumes grown in monoculture (Legume) and in mixtures with grasses (Mixed; *Macroptilium* and *Chloris*: Mac & Chl, *Desmodium* and *Panicum:* Des & Pan) including (a) the total biomass of nodules per pot, (b) the number of nodules per pot, and (c) root nodule activity as measured by the production of ethylene as a proxy for the rate of nitrogen fixation. Values shown are means ± 1 SE. Differences between plant types within

a species pair are indicated by letters (Mac & Chl uppercase, Des & Pan lowercase) and significant differences between CO_2 treatments are indicated by '*'.

Over-yielding and Complementarity vs. Selection Effects

We found a non-significant positive response of over-yielding for both species' pairs (**Fig. 6a**). In *Macroptilium* & *Chloris* mixture, over-yielding was mainly determined by the complementarity effect but not the selection effect (**Fig. 6bc**). By contrast, for the *Desmodium* & *Panicum* mixture, the over-yielding was driven more by selection compared with the complementarity effect (**Fig. 6bc**).

We also found eCO_2 significantly reduced over-yielding (**Fig. 6a**). In the *Macroptilium* & *Chloris* mixture, complementarity effects shifted from neutral under aCO_2 to significantly positive under eCO_2. Meanwhile, selection effects shifted from neutral under aCO_2 to significantly negative under eCO_2, meaning that the dominant species had less biomass than expected in the mixture (**Fig. 6c**). In the *Desmodium* & *Panicum* mixture, however, the complementarity effect shifted from significantly positive under aCO_2 to neutral under eCO_2, meaning that niche partitioning was weakened. At the same time, the selection effect was unaffected by CO_2 (**Fig. 6c**), although values were significantly positive under both CO_2 scenarios, meaning that the dominant species (*Panicum*) consistently had greater biomass in mixtures than proportional production would suggest from monocultures, regardless of CO_2 treatment. There were no significant effects of eCO_2 among the two species pairs for either complementarity (CO_2: $F_{1,4} = 0.3$, $p = 0.62$; Species pair: $F_{1,44} = 1.9$, $p = 0.18$; CO_2 x Species pair: $F_{1,44} = 2.1$, $p = 0.15$) or selection effects (CO_2: $F_{1,4} = 1.0$, $p = 0.37$; Species pair: $F_{1,44} = 94.9$, $p < 0.01$; CO_2 x Species pair: $F_{1,44} = 2.6$, $p = 0.11$). Summing these components of the net biodiversity effect supports the general outcomes of biomass over-yielding only in the *Desmodium* & *Panicum* mixture, with reduced over-yielding under eCO_2 (**Fig. 6e**). There were, however, no significant effects of CO_2 between the species pairs (CO_2: $F_{1,4} = 0.7$, $p = 0.44$; Species pair: $F_{1,44} = 3.0$, $p = 0.09$; CO_2 x Species pair: $F_{1,44} = 0.8$, $p = 0.37$).

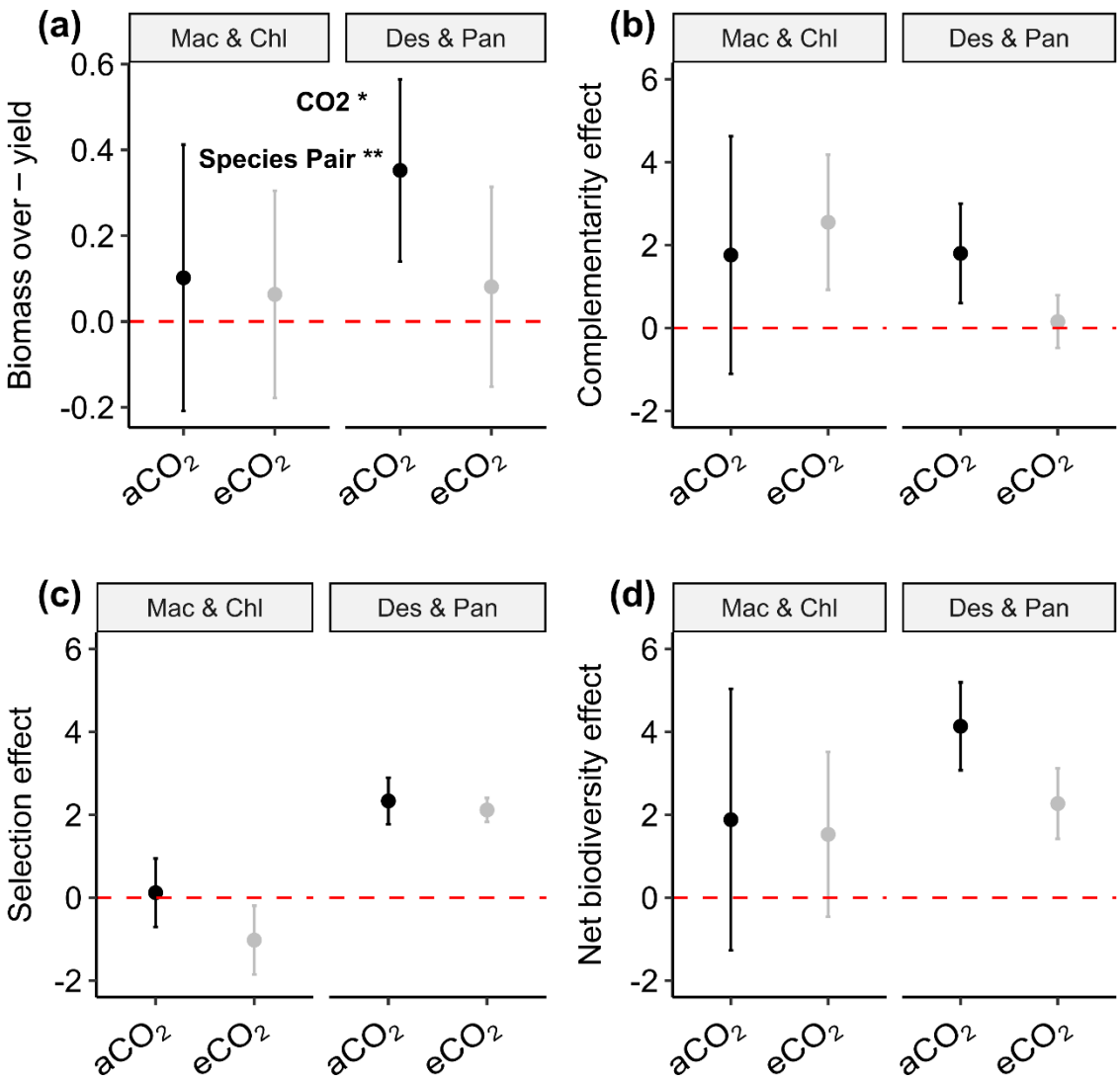


Fig. 6. Over-yield effect size for (a) aboveground productivity for pots grown with mixed grass-legume plant types in comparison with standardized summed totals for grasses and legumes grown in monoculture. Standardized species-pair level calculations for examining (b) complementarity effect, (c) selection effect and (d) the net biodiversity effect. Positive values indicate an increase relative to monoculture pots (a) or a net positive effect in interaction type between species (b-d). Non-overlapping bars indicate a significant difference from 0. Points shown are means values with 95% CI for *Macroptilium* & *Chloris* (Mac & Chl) mixtures and *Desmodium* & *Panicum* (Des & Pan) mixtures.

Discussion

In mixed grasslands or pastures containing both grasses and legumes resource niche partitioning among community members can promote species coexistence and ecosystem function, particularly by influencing resource (nutrient) availability (Fornara & Tilman, 2009; da Silveira Pontes *et al.*, 2015; Craven *et al.*, 2018). While direct-nutrient sharing is often predicted between community members (Pirhofer-Walzl *et al.*, 2012), nutrient niche partitioning is a more common finding in ecological and agricultural studies under field conditions (Nyfeler *et al.*, 2011; Pelzer *et al.*, 2014; Zuppinger-Dingley *et al.*, 2014). In these situations, community members access different pools of available resources, over time and/or space, enabling the persistence of many species by reducing competitive interactions (Adler *et al.*, 2012; Zuppinger-Dingley *et al.*, 2014). Our results found that (1) within tropical grass-legume mixtures, nutrient niche partitioning drove plant-plant interactions such that legumes increased reliance on biological nitrogen fixation (BNF) relative to soil N when grown with grasses; (2) elevated CO_2 (eCO_2) had varied impact on individual tropical forage species, with benefits in growth and total N content for one legume, but reductions in pot-level total N content for mixed pots containing a second legume species- and no direct benefit for tropical grasses; (3) community productivity and N content from tropical legume-grass mixtures were lower under eCO_2 than aCO_2, suggesting that legume-grass pairings may benefit ecosystem function to a lesser extent in the face of rising CO_2. Furthermore, the species-specific responses associated to over-yielding and responses to eCO_2, were linked with differences in complementarity and selection effects operating between the two mixed communities.

Tropical grass-legume mixtures

Relationships between plant functional group diversity and ecosystem function typically predict and find that increased diversity supports greater ecosystem productivity (Hector *et al.*, 1999; Loreau & Hector, 2001; Küchenmeister *et al.*, 2012; Tilman *et al.*, 2014; Mason *et al.*, 2017). In testing this hypothesis, many studies have included grass-forb-legume or grass-shrub-forb combinations experimentally to examine potential over-yielding benefits (Finn *et al.*, 2013), but fewer ecological studies have included C_4 grasses alongside legumes (Lilley *et al.*, 2001a; Lee *et al.*, 2003; Rasmussen *et al.*, 2013). This is despite early results suggesting that the high competitive ability of some C_4 grasses may functionally contribute to over-yielding (Lambers *et al.*, 2004) and the economic importance of such mixtures in many agricultural grasslands around the world (Butler *et al.*, 2013; Duchene *et al.*, 2017). Previous

results have shown that, at a global scale tropical grasses show lower productivity benefits from the addition of legumes, relative to temperate mixed-species systems (Ashworth *et al.*, 2018). Therefore, increased understanding of the mechanisms that may promote over-yielding are of broad interest. Our study found strong evidence for productivity gains by the grass component in tropical grass-legume mixtures, accompanied by declines in the legume partner, which translated into minimal changes at the pot level. This result aligns with earlier tropical mixed pasture trials that concluded that the relative abundance of grasses and legumes has an important impact on the transfer of N and sward productivity benefits of mixtures, compared to monocultures of the component species (Baba *et al.*, 2011).

Increased productivity in mixed species grasslands, relative to monocultures, is primarily attributed to increased access to resources, due to facilitation effects on light, water or nutrient availability, or niche partitioning allowing different species to access different resource pools in time or space to minimize competition (Jumpponen *et al.*, 2002; da Silveira Pontes *et al.*, 2015; Thilakarathna *et al.*, 2016). Biological or environmental conditions under which different complementarity mechanisms may dominate are still an area of active research (Brooker *et al.*, 2015), and our study addressed the potential for niche partitioning vs. direct facilitation for N between co-occurring grasses and legumes (Ajayi *et al.*, 2008; Baba *et al.*, 2011; Miegoue *et al.*, 2016; Bell *et al.*, 2017). Despite only limited gains in shoot N for mixed pots, relative to the grass monocultures, this indicated niche complementarity as the main mechanism promoting plant N access between the component species. Under these conditions, the proportion of shoot N derived from BNF increased for legumes even as the total mass of shoot N declined in comparison with monoculture pots. In agricultural-grassland settings mixtures of grasses and legumes are often used to specifically improve forage quality and production in the absence of fertilizer applications (Li *et al.*, 2015; Brooker *et al.*, 2016; Warwick *et al.*, 2016). This practice is predicted to play an important role in grassland management for grazed systems as C_4 grasses become more dominant under changing climate (McKeon *et al.*, 2009; Butler *et al.*, 2013; Still *et al.*, 2019).

There are a variety of mechanisms that enable N nutrient niche complementarity in mixed-species grasslands, and it is worth noting that the duration of species associations may play a role in the potential for direct-facilitation of BNF-derived N between for companion grasses in mixed grasslands (Heichel & Henjum, 1991; Thilakarathna *et al.*, 2016). Such a time lag may be associated with the decomposition of legume leaf and root litter that can provide

sources for increased ecosystem N availability on longer time scales (Cannell & Thornley, 1998; Kohmann *et al.*, 2019). While there was likely root turnover over the course of our glasshouse experiment, and any senesced leaves were retained in pots, these mechanisms are likely more substantial in field settings especially where soil disturbance may promote incorporation of plant materials directly into soils or where legume residues have sufficient time to undergo decomposition (Brooker *et al.*, 2015; Thilakarathna *et al.*, 2016). Furthermore, under field conditions, legumes often shift to BNF as plants mature (Edmeades & Goh, 1984; Wery *et al.*, 1986) and this can impact the potential facilitation provided to companion species (Bell *et al.*, 2017). In our study all plants were harvested immediately prior to the first flowering phenophase, and consequently observed patterns are from first-year perennial plants in a rapid growth phase when individuals may rely more strongly on available soil N pools. Grasses in mixed pots in our study did show some evidence for incorporation of BNF-derived N into aboveground plant tissue (up to 29% of shoot N for *Panicum* grown with *Desmodium*), a contribution that could increase as swards mature (Thilakarathna *et al.*, 2016).

Consequences of elevated CO_2

Previous work has shown that eCO_2 can increase grassland and crop productivity (Shaw *et al.*, 2002; Terrer *et al.*, 2021), or have no impact over differing time scales, and can result in shifts in the abundance of component species (Carroll *et al.*, 2003; Bloor *et al.*, 2010; Mueller *et al.*, 2016). Under well-watered conditions, C_4 grasses are not predicted to benefit directly from eCO_2 and our results follow those predictions (Ainsworth & Long, 2005; Soussana & Lüscher, 2007; Wang *et al.*, 2022; but see Reich *et al.*, 2018). On the other hand, we found some limited effects of eCO_2 on legume production, with increased growth in *Macroptilium* likely due to increases in shoot N and root biomass. In contrast, *Desmodium* did not respond to eCO_2, potentially due to a greater reliance on soil N rather than BNF in monoculture, that may have induced stoichiometric constraints in responding to eCO_2. This result is in line with research showing that the strongest predictor for an individual's or species' positive response to eCO_2 in legumes is the ability to form nodules (Cernusak *et al.*, 2011; Parvin *et al.*, 2020). For example, *Macroptilium* responded positively to eCO_2 and also had greater nodule production than *Desmodium*, although the number of nodules was unaffected by eCO_2. Indeed, despite the positive effect of eCO_2 on *Desmodium* nodule activity in mixtures, low nodule numbers and biomass overall resulted in a proportional reduction in legume biomass within the mixed sward.

At the sward level, despite some gain in productivity for legume biomass and total shoot N in monoculture (*Macroptilium* only), we didn't find an increase in productivity for mixed pots under eCO_2. While this result is in contrast to C_3 grass-legume mixtures for temperate pastures in Australia where eCO_2 has been shown to increase total shoot N yield (Lilley *et al.*, 2001b), it is consistent with evidence for limited responses to eCO_4 by C_4 grasses and legumes. One explanation may be related to shifts in the nutritional quality of component species under eCO_2, as reductions in quality for aboveground biomass have been reported across ecosystems (Augustine *et al.*, 2018; Bhargava & Mitra, 2021). Our species experienced limited shifts in quality based on shoot N%, including lower N in *Macroptilium* when grown with *Chloris* and in *Panicum* when grown with *Desmodium*. Even so, these patterns align with other studies measuring nutritional shifts in both tropical grasses and legumes under low soil nutrients where legume shoot N% declined in mixture under eCO_2 (Edwards *et al.*, 2006).

While the use of confined soil space to test mechanisms for the source of nutrient use and acquisition between grasses and legumes is typical in glasshouse settings, there are some key factors that are likely to impact the species interactions and dynamics in response to eCO_2 that are not captured here that are important for overarching messages in relation to nutrient complementarity. Our study found that eCO_2 conditions differentially altered the shoot N% between legumes in mixture but had no impact on legumes grown in monoculture. Under field settings, the effects of herbivory on legumes that maintain a higher nutritive quality relative to surrounding species may result in a comparative loss in abundance or persistence within the community, ultimately reducing the potential over-yield or net biodiversity effect (Rogers *et al.*, 2009). Additionally, literature reviews on field studies have generally found an increase in root length and biomass associated with eCO_2 in grasslands (Dieleman *et al.*, 2012; Nie *et al.*, 2013), a pattern that was seen in pots containing *Macroptilium* in this study. While our pots were not root-bound by the end of the experiment, other key differences between field and glasshouse conditions may have limited further responses, for example Nie et al. (2013) also found a shift in the depth distribution of roots that is not possible under non-field settings. Such spatial re-distribution of roots with depth can play a major role in complementarity effects between species within mixtures (Oram *et al.*, 2018).

Over-yield implications under future CO_2 scenarios

Reliance on different sources of N among species within mixed plant communities can ultimately reduce competition especially under low nutrient availability (Ball *et al.*, 2021; Elias & Agrawal, 2021). The increased niche partitioning between grasses and legumes grown in mixture found in this study support this conclusion. Furthermore, the species pair that had a substantial shift in N source use between monoculture and mixtures was also associated with a significant over-yielding benefit. This suggests that greater plasticity in niche space may promote species coexistence and enhance ecosystem production. Belowground, the idea of nutrient form (nitrate vs. ammonium) niche plasticity supporting coexistence has been well-established for cold-climate grasslands (Ashton *et al.*, 2010) and aboveground light-use plasticity has been shown to translate into increased production in experimental grasslands (Meilhac *et al.*, 2020).

Importantly, however, we found that eCO_2 reduced over-yielding for both aboveground biomass and total shoot N in our tropical grass-legume mixtures. While there were no statistical effects of eCO_2 on complementarity and selection effects directly, we found key differences in the relative importance of these effects in contributing to the over-yield/net biodiversity effect, with positive complementarity and selection together resulting in increased sward level over-yielding that was greatest under aCO_2.

Conclusions

Plant responses to eCO_2 are largely dependent on soil water and nutrient availability. Therefore, shifts in plant-plant interactions including competition (evidenced by niche partitioning) or facilitation among community members may drive diverse ecosystem responses to changes in CO_2 concentrations. Among our tropical pasture species, we found that legumes grown in monoculture relied approximately equally on available soil N pools and BNF for their nitrogen requirements. When grown with tropical grasses, however, legumes shifted to rely more strongly on BNF, indicative of stronger niche partitioning and less successful competition for soil nutrients with grasses. This separation of niche space was strengthened under elevated CO_2 conditions, ultimately reducing legume production and minimizing over-yielding benefits. Disentangling contributions of niche partitioning and abiotic facilitation, here quantified using stable isotopes, are key in understanding grass-legume contributions to ecosystem function under future climate conditions.

Acknowledgements

The authors would like to acknowledge individuals whose efforts supported the data collection of this work, including Kathryn Fuller, Alexandra Boyd, Shania Therese Didier Serre, Samantha Weller, Benjamin Capel, Minh Doan. We thank Neha Mohan Babu for comments on the final manuscript. Valuable input from PACE Advisory Board members - Doug McNicholl (Meat and Livestock Australia), Cath Lescun (Dairy Australia), Catherine Phelps (Dairy Australia), Tom Dickson (Heritage Seeds), Brendan Cullen (University Melbourne) and Suzanne Boschman (Department of Primary Industries) - is also gratefully acknowledged. This project was supported by funding from Meat and Livestock Australia's Donor Company (P.PSH. 0793), Dairy Australia (C100002357) and Western Sydney University. Authors ACC and HZ wrote the paper with input from all co-authors on draft iterations; ACC conducted statistical analyses with input from HZ, JRP and FII; ACC, HZ, and GWK designed the experiment with input from SAP, EP, JRP, JMP, and KLMC. A version of this manuscript will be included as a preprint on BioRxiv (to be submitted), and all associated data are available through a Dryad data repository: https://doi.org/10.5061/dryad.nk98sf7ww.

References

Adler PB, Dalgleish HJ, Ellner SP. **2012**. Forecasting plant community impacts of climate variability and change: when do competitive interactions matter? *Journal of Ecology* **100**: 478–487.

Ainsworth EA, Long SP. **2005**. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist* **165**: 351–372.

Ajayi FT, Babayemi OJ, Taiwo AA. **2008**. Effects of supplementation of *Panicum maximum* with four herbaceous forage legumes on performance, nutrient digestibility and nitrogen balance in West African dwarf goats. *Animal Science Journal* **79**: 673–679.

Arfin Khan MAS, Grant K, Beierkuhnlein C, Kreyling J, Jentsch A. **2014**. Climatic extremes lead to species-specific legume facilitation in an experimental temperate grassland. *Plant and Soil* **379**: 161–175.

Ashton IW, Miller AE, Bowman WD, Suding KN. **2010**. Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. *Ecology* **91**: 3252–60.

Ashworth AJ, Toler HD, Allen FL, Auge RM. **2018**. Global meta-analysis reveals agro-grassland productivity varies based on species diversity over time. *PLoS ONE* **13**: 1–20.

Augustine DJ, Blumenthal DM, Springer TL, LeCain DR, Gunter SA, Derner JD. **2018**. Elevated CO_2 induces substantial and persistent declines in forage quality irrespective of warming in mixedgrass prairie. *Ecological Applications* **28**: 721–735.

Baba M, Halim RA, Alimon AR, Abubakar I. **2011**. Grass-legume mixtures for enhanced forage production: Analysis of dry matter yield and competition indices. *African Journal of Agricultural Research* **6**: 5242–5250.

Ball KR, Woodin SJ, Power SA, Brien C, Berger B, Smith P, Pendall E. **2021**. Integrating

ecological stoichiometry to understand nutrient limitation and potential for competition in mixed pasture assemblages. *Journal of Soil Science and Plant Nutrition* **21**: 24989–2500.

Barry KE, Mommer L, Ruijven J Van, Wirth C, Wright AJ, Bai Y, Connolly J, Deyn GB De, Kroon H De, Isbell F, *et al.* 2019. The future of complementarity: disentangling causes from consequences. *Trends in Ecology & Evolution* **34**: 167–180.

Bates D, Maechler M, Bolker BM, Walker S. **2015**. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**: 1–48.

Bell M, Harch G, Moody P. **2012**. Diversification from cropping into mixed crop-livestock systems–the sustainability risks posed by hay removal from pasture or forage blocks. *Proceedings of 16th Agronomy Conference 2012* **18**: 75.

Bell LW, Lawrence J, Johnson B, Peoples MB. **2017**. New ley legumes increase nitrogen fixation and availability and grain crop yields in subtropical cropping systems. *Crop and Pasture Science* **68**: 11–26.

Bhargava S, Mitra S. **2021**. Elevated atmospheric CO_2 and the future of crop plants. *Plant Breeding* **140**: 1–11.

Bloor JMG, Pichon P, Falcimagne R, Leadley P, Soussana J-F. **2010**. Effects of warming, summer drought, and CO_2 enrichment on aboveground biomass production, flowering phenology, and community structure in an upland grassland ecosystem. *Ecosystems* **13**: 888–900.

Blumenthal DM, Resco V, Morgan JA, Williams DG, Lecain DR, Hardy EM, Pendall E, Bladyka E. **2013**. Invasive forb benefits from water savings by native plants and carbon fertilization under elevated CO_2 and warming. *New Phytologist* **200**: 1156–1165.

Brooker RW, Bennett AE, Cong WF, Daniell TJ, George TS, Hallett PD, Hawes C, Iannetta PPM, Jones HG, Karley AJ, *et al.* 2015. Improving intercropping: A synthesis of research in agronomy, plant physiology and ecology. *New Phytologist* **206**: 107–117.

Brooker RW, Karley AJ, Newton AC, Pakeman RJ, Schöb C. **2016**. Facilitation and sustainable agriculture: A mechanistic approach to reconciling crop production and conservation. *Functional Ecology* **30**: 98–107.

Butler TJ, Interrante SM, Foster JL. **2013**. Assessing the production and nutritive value of warm-season legumes in Oklahoma and Texas. *Forage & Grazinglands* **11**: 1–6.

Cannell MGR, Thornley JHM. **1998**. N-poor ecosystems may respond more to elevated [CO2] than N-rich ones in the long term. A model analysis of grassland. *Global Change Biology* **4**: 431–442.

Carroll JA, Caporn SJM, Johnson D, Morecroft MD, Lee JA. **2003**. The interactions between plant growth, vegetation structure and soil processes in semi-natural acidic and calcareous grasslands receiving long-term inputs of simulated pollutant nitrogen deposition. *Environmental Pollution* **121**: 363–376.

Cernusak LA, Winter K, Martínez C, Correa E, Aranda J, Garcia M, Jaramillo C, Turner BL. **2011**. Responses of legume versus nonlegume tropical tree seedlings to elevated CO_2 concentration. *Plant Physiology* **157**: 372–385.

Chalk PM, Inácio CT, Balieiro FC, Rouws JRC. **2016**. Do techniques based on ^{15}N enrichment and ^{15}N natural abundance give consistent estimates of the symbiotic dependence of N_2-fixing plants? *Plant and Soil* **399**: 415–426.

Churchill AC, Zhang H, Fuller KJ, Amiji B, Anderson IC, Barton CVM, Carrillo Y, Catunda KLM, Chandregowda MH, Igwenagu C, *et al.* 2022. Pastures and climate extremes: impacts of cool season warming and drought on the productivity of key pasture species in a field experiment. *Frontiers in Plant Science* **13**: 1–16.

Coventry DR, Hirth JR, Reeves TG, Burnett VF. **1985**. Growth and nitrogen fixation by subterranean clover in response to inoculation, molybdenum application and soil amendment with lime. *Soil Biology and Biochemistry* **17**: 791–796.

Craven D, Eisenhauer N, Pearse WD, Hautier Y, Isbell FI, Roscher C, Bahn M, Beierkuhnlein C, Bönisch G, Buchmann N, *et al.* 2018. Multiple facets of biodiversity drive the diversity–stability relationship. *Nature Ecology and Evolution* **2**: 1579–1587.

Dieleman WIJ, Vicca S, Dijkstra FA, Hagedorn F, Hovenden MJ, Larsen KS, Morgan JA, Volder A, Beier C, Dukes JS, *et al.* 2012. Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO_2 and temperature. *Global Change Biology* **18**: 2681–2693.

Dijkstra FA, Blumenthal D, Morgan JA, Pendall E, Carrillo Y, Follett RF. **2010**. Contrasting effects of elevated CO_2 and warming on nitrogen cycling in a semiarid grassland. *New Phytologist* **187**: 426–437.

Duchene O, Vian JF, Celette F. **2017**. Intercropping with legume for agroecological cropping systems: Complementarity and facilitation processes and the importance of soil microorganisms. A review. *Agriculture, Ecosystems and Environment* **240**: 148–161.

Edmeades DC, Goh KM. **1984**. Symbiotic nitrogen fixation in a sequence of pastures of increasing age measured by a ^{15}N dilution technique. *New Zealand Journal of Agricultural Research* **21**: 623–628.

Edwards EJ, McCaffery S, Evans JR. **2006**. Phosphorus availability and elevated CO_2 affect biological nitrogen fixation and nutrient fluxes in a clover-dominated sward. *New Phytologist* **169**: 157–167.

Elias JD, Agrawal AA. **2021**. A private channel of nitrogen alleviates interspecific competition for an annual legume. *Ecology* **102**: 1–10.

Fay PA, Prober SM, Harpole WS, Knops JMH, Bakker JD, Borer ET, Lind EM, MacDougall AS, Seabloom EW, Wragg PD, *et al.* 2015. Grassland productivity limited by multiple nutrients. *Nature Plants* **1**: 1–5.

von Felton S, Hector A, Buchmann N, Niklaus PA, Schmid B, Scherer-Lorenzen M. **2009**. Belowground nitrogen partitioning in experimental grassland plant communities of varying species richness. *Ecology* **90**: 1389–1399.

Finn JA, Kirwan L, Connolly J, Sebastià MT, Helgadottir A, Baadshaug OH, Bélanger G, Black A, Brophy C, Collins RP, *et al.* 2013. Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: A 3-year continental-scale field experiment. *Journal of Applied Ecology* **50**: 365–375.

Fornara DA, Tilman DG. **2008**. Plant functional composition influences rates of soil carbon and nitrogen accumulation. *Journal of Ecology* **96**: 314–322.

Fornara DA, Tilman DG. **2009**. Ecological mechanisms associated with the positive diversity-productivity relationship in an N-limited grassland. *Ecology* **90**: 408–418.

Fox J, Weisberg S. **2019**. *An R companion to applied regression*. Thousand Oaks, CA: Sage.

Fustec J, Lesuffleur F, Mahieu S, Cliquet JB. **2009**. Nitrogen rhizodeposition of legumes. *Sustainable Agriculture* **2**: 869–881.

Gilarte P, Plett J, Pendall E, Carrillo Y, Nielsen UN. **2020**. Direct and indirect trophic interactions of soil nematodes impact chickpea and oat nutrition. *Plant and Soil* **457**: 255–268.

Gill RA, Polley HW, Johnson HB, Anderson LJ, Maherali H, Jackson RB. **2002**. Nonlinear grassland responses to past and future atmospheric CO_2. *Nature* **417**: 279–282.

Gojon A, Cassan O, Bach L, Lejay L, Martin A. **2022**. The decline of plant mineral nutrition under rising CO_2 : physiological and molecular aspects of a bad deal. *Trends in Plant Science*: 1–14.

Gonçalves de Oliveira AC, Rios PM, Pereira EG, Souza JP. **2021**. Growth and competition between a native leguminous forb and an alien grass from the Cerrado under elevated CO_2. *Austral Ecology* **46**: 750–761.

de Graaff MA, van Groenigen KJ, Six J, Hungate B, van Kessel C. **2006**. Interactions

between plant growth and soil nutrient cycling under elevated CO_2: A meta-analysis. *Global Change Biology* **12**: 2077–2091.

Hector A, Schmid B, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, Finn JA, Freitas H, Giller PS, Good J, *et al.* 1999. Plant diversity and productivity experiments in European grasslands. *Science* **286**: 1123–1127.

Heichel GH, Henjum KI. **1991**. Dinitrogen fixation, nitrogen transfer, and productivity of forage legume-grass communities. *Crop Science* **31**: 202–208.

Henneron L, Kardol P, Wardle DA, Cros C, Fontaine S. **2020**. Rhizosphere control of soil nitrogen cycling: a key component of plant economic strategies. *New Phytologist* **228**: 1269–1282.

Hungate BA, Stiling PD, Dijkstra P, Johnson DW, Ketterer ME, Hymus GJ, Hinkle CR, Drake BG. **2004**. CO_2 elicits long-term decline in nitrogen fixation. *Science* **304**: 1291.

IPCC. **2022**. *Climate Change 2022 Impacts, Adaptation and Vulnerability*.

Jones MB, Donnelly A. **2004**. Carbon sequestration in temperate grassland ecosytems and the inlfuence of management, climate and elevated CO_2. *New Phytologist* **164**: 423–439.

Jumpponen A, Högberg P, Huss-Danell K, Mulder CPH. **2002**. Interspecific and spatial differences in nitrogen uptake in monocultures and two-species mixtures in north European grasslands. *Functional Ecology* **16**: 454–461.

Kohmann MM, Sollenberger LE, Dubeux JCB, Silveira ML, Moreno LSB. **2019**. Legume proportion in grassland litter affects decomposition dynamics and nutrient mineralization. *Agronomy Journal* **111**: 1079–1089.

Küchenmeister F, Küchenmeister K, Wrage N, Kayser M, Isselstein J. **2012**. Yield and yield stability in mixtures of productive grassland species: Does species number or functional group composition matter? *Grassland Science* **58**: 94–100.

Lambers JHR, Harpole WS, Tilman DG, Knops J, Reich PB. **2004**. Mechanisms responsible for the positive diversity-productivity relationship in Minnesota grasslands. *Ecology Letters* **7**: 661–668.

Landhäusser SM, Chow PS, Turin Dickman L, Furze ME, Kuhlman I, Schmid S, Wiesenbauer J, Wild B, Gleixner G, Hartmann H, *et al.* 2018. Standardized protocols and procedures can precisely and accurately quantify non-structural carbohydrates. *Tree Physiology* **38**: 1764–1778.

Lee TD, Reich PB, Tjoelker MG. **2003**. Legume presence increases photosynthesis and N concentrations of co-occurring non-fixers but does not modulate their responsiveness to carbon dioxide enrichment. *Oecologia* **137**: 22–31.

Length R. **2020**. emmeans: estimated marginal means, aka least-squares means. : https://cran.r-project.org/package=emmeans.

Li Q, Song Y, Li G, Yu P, Wang P, Zhou D. **2015**. Grass-legume mixtures impact soil N, species recruitment, and productivity in temperate steppe grassland. *Plant and Soil* **394**: 271–285.

Liang J, Qi X, Souza L, Luo Y. **2016**. Processes regulating progressive nitrogen limitation under elevated carbon dioxide: A meta-analysis. *Biogeosciences* **13**: 2689–2699.

Lilley JM, Bolger TP, Gifford RM. **2001a**. Productivity of *Trifolium subterraneum* and *Phalaris aquatica* under warmer, high CO_2 conditions. *New Phytologist* **150**: 371–383.

Lilley JM, Bolger TP, Peoples MB, Gifford RM. **2001b**. Nutritive value and the nitrogen dynamics of *Trifolium subterraneum* and *Phalaris aquatica* under warmer, high CO_2 conditions. *New Phytologist* **150**: 385–395.

Loreau M, Hector A. **2001**. Partitioning selection and complementarity in biodiversity experiments. *Nature* **412**: 72–76.

Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hooper DU, Huston MA, Raffaelli D, Schmid B, Tilman DG, *et al.* 2008. Biodiversity and ecosystem functioning:

current knowledge and future challenges. *Science* **804**: 804–809.
Makkonen M, Berg MP, Handa IT, Hättenschwiler S, van Ruijven J, van Bodegom PM, Aerts R. **2012**. Highly consistent effects of plant litter identity and functional traits on decomposition across a latitudinal gradient. *Ecology Letters* **15**: 1033–1041.
Maschler J, Bialic-Murphy L, Wan J, Andresen LC, Zohner CM, Reich PB, Lüscher A, Schneider MK, Müller C, Moser G, *et al.* 2022. Links across ecological scales: plant biomass responses to elevated CO_2. *Global Change Biology*: 1–20.
Mason NWH, Palmer DJ, Romera A, Waugh D, Mudge PL. **2017**. Combining field experiments and predictive models to assess potential for increased plant diversity to climate-proof intensive agriculture. *Ecology and Evolution* **7**: 4907–4918.
McKane RB, Johnson LC, Shaver GR, Nadelhoffer KJ, Rastetter EB, Fry B, Giblin AE, Kielland K, Kwlatkowski BL, Laundre JA, *et al.* 2002. Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature* **415**: 68–71.
McKeon GM, Stone GS, Syktus JI, Carter JO, Flood NR, Ahrens DG, Bruget DN, Chilcott CR, Cobon DH, Cowley RA, *et al.* 2009. Climate change impacts on northern Australian rangeland livestock carrying capacity: a review of issues. *Rangeland Journal* **31**: 1–29.
Mclaren JR, Turkington R. **2010**. Plant functional group identity differentially affects leaf and root decomposition. *Global Change Biology* **16**: 3075–3084.
Meilhac J, Deschamps L, Maire V, Flajoulot S, Litrico I. **2020**. Both selection and plasticity drive niche differentiation in experimental grasslands. *Nature Plants* **6**: 28–33.
Melesse A, Steingass H, Schollenberger M, Rodehutscord M. **2017**. Screening of common tropical grass and legume forages in Ethiopia for their nutrient composition and methane production profile in vitro. *Tropical Grasslands-Forrajes Tropicales* **5**: 163–175.
Meterology B of. **2020**. Bureau of Meterology. *Government, Australian*.
Miegoue E, Tendonkeng F, Lemoufouet J, Noumbissi MNB, Mweugang NN, Zougou TG, Nkouadjio MF, Boukila B, Tedonkeng EP. **2016**. Pre-weaning growth of cavies feed on *Panicum maximum* supplemented with diet containing *Arachis glabrata*, *Calliandra calothyrsus* or *Desmodium intortum*. *International Journal of Biological and Chemical Sciences* **10**: 313.
Mueller KE, Blumenthal DM, Pendall E, Carrillo Y, Dijkstra FA, Williams DG, Follett RF, Morgan JA. **2016**. Impacts of warming and elevated CO_2 on a semi-arid grassland are non-additive, shift with precipitation, and reverse over time. *Ecology letters* **19**: 956–966.
Mulder CPH, Jumpponen A, Högberg P, Huss-Danell K. **2002**. How plant diversity and legumes affect nitrogen dynamics in experimental grassland communities. *Oecologia* **133**: 412–421.
Munson SM, Long AL. **2017**. Climate drives shifts in grass reproductive phenology across the western USA. *New Phytologist* **213**: 1945–1955.
Naudts K, Van den Berge J, Farfan E, Rose P, AbdElgawad H, Ceulemans R, Janssens IA, Asard H, Nijs I. **2013**. Future climate alleviates stress impact on grassland productivity through altered antioxidant capacity. *Environmental and Experimental Botany* **99**: 150–158.
Nie M, Lu M, Bell J, Raut S, Pendall E. **2013**. Altered root traits due to elevated CO_2: a meta-analysis. *Global Ecology and Biogeography* **22**: 1095–1105.
Nyfeler D, Huguenin-Elie O, Suter M, Frossard E, Lüscher A. **2011**. Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture, Ecosystems and Environment* **140**: 155–163.
Oram NJ, Ravenek JM, Barry KE, Weigelt A, Chen H, Gessler A, Gockele A, de Kroon H, van der Paauw JW, Scherer-Lorenzen M, *et al.* 2018. Below-ground complementarity effects in a grassland biodiversity experiment are related to deep-rooting species. *Journal of*

Ecology **106**: 265–277.
Parvin S, Uddin S, Tausz-Posch S, Armstrong R, Tausz M. **2020**. Carbon sink strength of nodules but not other organs modulates photosynthesis of faba bean (*Vicia faba*) grown under elevated [CO_2] and different water supply. *New Phytologist* **227**: 132–145.
Paynel F, Murray PJ, Bernard Cliquet J. **2001**. Root exudates: A pathway for short-term N transfer from clover and ryegrass. *Plant and Soil* **229**: 235–243.
Pelzer E, Hombert N, Jeuffroy MH, Makowski D. **2014**. Meta-analysis of the effect of nitrogen fertilization on annual cereal–legume intercrop production. *Agronomy Journal* **106**: 1775–1786.
Perring MP, Cullen BR, Johnson IR, Hovenden MJ. **2010**. Modelled effects of rising CO_2 concentration and climate change on native perennial grass and sown grass-legume pastures. *Climate Research* **42**: 65–78.
Pirhofer-Walzl K, Rasmussen J, Høgh-Jensen H, Eriksen J, Søegaard K, Rasmussen J. **2012**. Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species grassland. *Plant and Soil* **350**: 71–84.
Plett JM, Plett KL, Bithell SL, Mitchell C, Moore K, Powell JR, Anderson IC. **2016**. Improved Phytophthora resistance in commercial chickpea (*Cicer arietinum*) varieties negatively impacts symbiotic gene signalling and symbiotic potential in some varieties. *Plant Cell and Environment* **39**: 1858–1869.
R Core Team. **2020**. R: A language and environment for statistical computing. : http://www.r-project.org/.
Rasmussen J, Gylfadóttir T, Loges R, Eriksen J, Helgadóttir Á. **2013**. Spatial and temporal variation in N transfer in grass-white clover mixtures at three Northern European field sites. *Soil Biology and Biochemistry* **57**: 654–662.
Reich PB. **2009**. Elevated CO_2 reduces losses of plant diversity caused by nitrogen deposition. *Science* **326**: 1399–1402.
Reich PB, Hobbie SE, Lee TD. **2014**. Plant growth enhancement by elevated CO_2 eliminated by joint water and nitrogen limitation. *Nature Geoscience* **7**: 920–924.
Reich PB, Hobbie SE, Lee TD, Pastore MA. **2018**. Unexpected reversal of C3 versus C4 grass response to elevated CO_2 during a 20-year field experiment. *Science* **361**: 317–320.
Reich PB, Knops J, Tilman DG, Craine JM, Ellsworth DS, Tjoelker MG, Lee T, Wedink D, Naeem S, Bahauddin D, *et al.* 2001. Plant diversity enhances ecosystem responses to elevated CO_2 and nitrogen deposition. *Nature* **410**: 809–812.
Rogers A, Ainsworth EA, Leakey ADB. **2009**. Will elevated carbon dioxide concentration amplify the benefits of nitrogen fixation in legumes? *Plant Physiology* **151**: 1009–1016.
Shaw MR, Zavaleta ES, Chiariello NR, Cleland EE, Mooney HA, Field CB. **2002**. Grassland responses to global environmental changes suppressed by elevated CO_2. *Science* **298**: 1987–1990.
Sherry RA, Zhou X, Gu S, Arnone JA, Schimel DS, Verburg PS, Wallace LL, Luo Y. **2007**. Divergence of reproductive phenology under climate warming. *PNAS* **104**: 198–202.
da Silveira Pontes L, Maire V, Schellberg J, Louault F. **2015**. Grass strategies and grassland community responses to environmental drivers: a review. *Agronomy for Sustainable Development* **35**: 1297–1318.
Soussana JF, Lüscher A. **2007**. Temperate grasslands and global atmospheric change: a review. *Grass and Forage Science* **62**: 127–134.
Still CJ, Cotton JM, Griffith DM. **2019**. Assessing earth system model predictions of C4 grass cover in North America: From the glacial era to the end of this century. *Global Ecology and Biogeography* **28**: 145–157.
Szulejko JE, Kumar P, Deep A, Kim KH. **2017**. Global warming projections to 2100 using simple CO_2 greenhouse gas modeling and comments on CO_2 climate sensitivity factor.

Atmospheric Pollution Research **8**: 136–140.
Terrer C, Phillips R, Hungate B, Rosende J, Pett-Ridge J, Craig M, van Groenigen K, Keenan T, Sulman B, Stocker B, *et al.* 2021. A trade-off between plant and soil carbon storage under elevated CO_2. *Nature* **591**.
Thilakarathna MS, McElroy MS, Chapagain T, Papadopoulos YA, Raizada MN. **2016**. Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. A review. *Agronomy for Sustainable Development* **36**.
Thomas D, Sumberg JE. **1995**. A review of the evaluation and use of tropical forage legumes in sub-Saharan Africa. *Agriculture, Ecosystems and Environment* **54**: 151–163.
Tilman DG, Isbell FI, Cowles JM. **2014**. Biodiversity and ecosystem functioning. *Annual Review of Ecology, Evolution, and Systematics* **45**: 471–493.
Vandermeer J. **1981**. The interference production principle: an ecological theory for agriculture. *BioScience* **31**: 361–364.
Vitousek PM, Howarth RW. **1991**. Nitrogen limitation on land and in the sea: how can it occur. *Biogeochemistry* **13**: 87–115.
Wales D of PINS. **2018**. Pasture species and varieties. *Pastures and rangelands*: https://www.dpi.nsw.gov.au/agriculture/pastures-an.
Wang C, Hunt ER, Zhang L, Guo H. **2013**. Phenology-assisted classification of C3 and C4 grasses in the U.S. Great Plains and their climate dependency with MODIS time series. *Remote Sensing of Environment* **138**: 90–101.
Wang Z, Wang C, Liu S. **2022**. Elevated CO_2 alleviates adverse effects of drought on plant water relations and photosynthesis: A global meta-analysis. *Journal of Ecology*: 1–14.
Warwick K, Allen FL, Keyser PD, Ashworth AJ, Bates GE, Tyler DD, Lambdin PL, Harper CA. **2016**. Biomass and integrated forage/biomass yields of switchgrass as affected by intercropped cool- and warm-season legumes. *Journal of Soil and Water Conservation* **71**: 21–28.
Wei X, Reich PB, Hobbie SE. **2019**. Legumes regulate grassland soil N cycling and its response to variation in species diversity and N supply but not CO_2. *Global Change Biology* **25**: 2396–2409.
Wery J, Turc O, Salsac L. **1986**. Relationship between growth, nitrogen fixation and assimilation in a legume (Medicago sativa L.). *Plant and Soil* **9629**: 17–29.
Winkler JB, Herbst M. **2004**. Do plants of a semi-natural grassland community benefit from long-term CO_2 enrichment? *Basic and Applied Ecology* **5**: 131–143.
Xie Q, Huete A, Hall CC, Medlyn BE, Power SA, Davies JM, Medek DE, Beggs PJ. **2022**. Satellite-observed shifts in C3/C4 abundance in Australian grasslands are associated with rainfall patterns. *Remote Sensing of Environment* **273**: 112983.
Zelikova TJ, Blumenthal DM, Williams DG, Souza L, LeCain DR, Morgan J, Pendall E. **2014**. Long-term exposure to elevated CO_2 enhances plant community stability by suppressing dominant plant species in a mixed-grass prairie. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 15456–15461.
Zhang H, Powell JR, Plett JM, Churchill AC, Power SA, Macdonald CA, Jacob V, Kim GW, Pendall E, Tissue D, *et al.* 2021. Climate warming negates arbuscular mycorrhizal fungal reductions in soil phosphorus leaching with tall fescue but not lucerne. *Soil Biology and Biochemistry* **152**: 108075.
Zuppinger-Dingley D, Schmid B, Petermann JS, Yadav V, De Deyn GB, Flynn DFB. **2014**. Selection for niche differentiation in plant communities increases biodiversity effects. *Nature* **515**: 108–111.

Table 1. Tropical species and associated mixtures for addressing legume-grass facilitation

Scientific Name*	Common Name	Variety	Growth form	Paired species
Chloris gayana	Rhodes grass	Katambora	Grass	Burgundy
Desmodium intortum	Greenleaf Desmodium	Greenleaf	Legume	Panic
Macroptilium bracteatum	Burgundy Bean	Presto	Legume	Rhodes
Panicum maximum var. trichoglume	Panic grass	Megamax 059	Grass	Desmodium

**Species are referenced by genera in text*

Table 2) Effects of CO_2 treatment (CO_2; ambient, elevated), species pair (SP; *Macroptilium*/*Chloris* and *Desmodium*/*Panicum*) and plant type (PT; grass, legume, mixed-species), for pot level total shoot biomass, total shoot N, total shoot N derived from soil (shoot N- soil), the percent of N derived from biologically fixed N (Shoot N- BNF %; legume and mixed plant types only) and total shoot N derived from BNF (Shoot N- BNF content; legume and mixed plant types only).

Response	**Factors**	***F* Statistic***	***P* Value**	r^2**-M**	r^2**-C**
Shoot biomass^	CO_2	$5.23_{1,4}$	0.08	0.40	0.45
	Species pair	$49.9_{1,143}$	<0.01		
	Plant type	$12.1_{2,143}$	<0.01		
	CO_2 x SP	$1.9_{1,143}$	0.17		
	CO_2 x PT	$2.5_{2,143}$	0.08		
	SP x PT	$5.6_{2,143}$	<0.01		
	CO_2 x SP x PT	$1.0_{2,143}$	0.38		
Shoot N content^	CO_2	$0.3_{1,4}$	0.60	0.65	0.66
	Species pair	$102.7_{1,143}$	<0.01		
	Plant type	$79.4_{2,143}$	<0.01		
	CO_2 x SP	$1.9_{1,143}$	0.18		
	CO_2 x PT	$3.7_{2,143}$	0.03		
	SP x PT	$14.7_{2,143}$	<0.01		
	CO_2 x SP x PT	$0.4_{2,143}$	0.64		
Shoot N-soil	CO_2	$0.1_{1,4}$	0.79	0.14	0.15
	Species pair	$17.8_{1,143}$	<0.01		
	Plant type	$2.7_{2,143}$	0.07		
	CO_2 x SP	$0.4_{1,143}$	0.54		
	CO_2 x PT	$0.1_{1,143}$	0.92		
	SP x PT	$0.04_{2,143}$	0.95		
	CO_2 x SP x PT	$1.3_{2,143}$	0.27		

**Subscripts indicate degrees of freedom, ^data were ln transformed to meet assumptions of normality. All models included growth chamber as a random effect.*

Table 3) Effects of CO_2 treatment (CO_2; ambient, elevated), species pair (SP; *Macroptilium*/*Chloris* and *Desmodium*/*Panicum*) and plant type (PT; legume and mixed-species only), for pot level percent of N derived from biologically fixed N (Shoot N- BNF %; legume and mixed plant types only) and total shoot N derived from BNF (Shoot N-BNF content).

Response	**Factors**	***F* Statistic***	***P* Value**	r^2**-M**	r^2**-C**
Shoot N- BNF %	CO_2	$0.03_{1,4}$	0.87	0.44	0.45
	Species pair	$59.0_{1,94}$	<0.01		
	Plant type	$13.8_{1,94}$	<0.01		
	CO_2 x SP	$2.5_{1,94}$	0.12		
	CO_2 x PT	$2.1_{1,94}$	0.15		
	SP x PT	$2.8_{1,94}$	0.10		
	CO_2 x SP x PT	$3.3_{1,94}$	0.07		
Shoot N- BNF content^	CO_2	$0.1_{1,4}$	0.80	0.59	0.63
	Species pair	$132.3_{1,94}$	<0.01		
	Plant type	$30.8_{1,94}$	<0.01		
	CO_2 x SP	$0.7_{1,94}$	0.39		
	CO_2 x PT	$3.5_{1,94}$	0.06		
	SP x PT	$1.0_{1,94}$	0.32		
	CO_2 x SP x PT	$0.2_{1,94}$	0.62		

**Subscripts indicate degrees of freedom, ^data were ln transformed to meet assumptions of normality. All models included growth chamber as a random effect.*

Table 4) Effects of CO_2 treatment (CO_2; ambient, elevated) for pot level total biomass of root nodules (Nodule biomass), number of root nodules present (Nodule number), and the activity of nodules at the time of harvest (Nodule activity) by species pair (SP; *Macroptilium*/*Chloris* and *Desmodium*/*Panicum*) and plant type (PT; legumes in monoculture vs mixed pots only)

Response	**Factors**	***F* Statistic***	***P* Value**	r^2**-M**	r^2**-C**
Nodule biomass^	CO_2	$4.0_{1,4}$	0.12	0.68	0.69
	Species pair	$65.6_{1,68}$	<0.01		
	Plant type	$97.2_{1,68}$	<0.01		
	CO_2 x SP	$0.6_{1,68}$	0.43		
	CO_2 x PT	$0.1_{1,68}$	0.80		
	SP x PT	$4.6_{1,68}$	0.04		
	CO_2 x SP x PT	$0.3_{1,68}$	0.58		
Nodule number^	CO_2	$0.4_{1,4}$	0.55	0.55	0.58
	Species pair	$28.8_{1,68}$	<0.01		
	Plant type	$72.9_{1,68}$	<0.01		
	CO_2 x SP	$0.02_{1,68}$	0.88		
	CO_2 x PT	$0.1_{1,68}$	0.8		
	SP x PT	$4.0_{1,68}$	0.05		
	CO_2 x SP x PT	$0.4_{1,68}$	0.52		
Nodule activity^	CO_2	$4.7_{1,4}$	0.10	0.16	0.16
	Species pair	$0.7_{1,68}$	0.42		
	Plant type	$7.0_{1,68}$	0.01		
	CO_2 x SP	$0.6_{1,68}$	0.44		
	CO_2 x PT	$1.0_{1,68}$	0.33		
	SP x PT	$0.01_{1,68}$	0.90		
	CO_2 x SP x PT	$1.9_{1,68}$	0.17		

**Subscripts indicate degrees of freedom, ^data were ln transformed to meet assumptions of normality. All models included growth chamber as a random effect.*