Panicum milioides (C₃–C₄) does not have improved water or nitrogen economies relative to C₃ and C₄ congeners exposed to industrial-age climate change

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Abstract

The physiological implications of C₃–C₄ photosynthesis were investigated using closely related Panicum species exposed to industrial-age climate change. Panicum bisulcatum (C₃), P. milioides (C₃–C₄), and P. coloratum (C₄) were grown in a glasshouse at three CO₂ concentrations ([CO₂]: 280, 400, and 650 μl l⁻¹) and two air temperatures [ambient (27/19 °C day/night) and ambient + 4 °C] for 12 weeks. Under current ambient [CO₂] and temperature, the C₃–C₄ species had higher photosynthetic rates and lower stomatal limitation and electron cost of photosynthesis relative to the C₃ species. These photosynthetic advantages did not improve leaf- or plant-level water (WUE) or nitrogen (NUE) use efficiencies of the C₃–C₄ relative to the C₃ Panicum species. In contrast, the C₄ species had higher photosynthetic rates and WUE but similar NUE to the C₃ species. Increasing [CO₂] mainly stimulated photosynthesis of the C₃ and C₃–C₄ species, while high temperature had no or negative effects on photosynthesis of the Panicum species. Under ambient temperature, increasing [CO₂] enhanced the biomass of the C₃ species only. Under high temperature, increasing [CO₂] enhanced the biomass of the C₃ and C₃–C₄ species to the same extent, indicating increased CO₂ limitation in the C₃–C₄ intermediate at high temperature. Growth [CO₂] and temperature had complex interactive effects, but did not alter the ranking of key physiological parameters amongst the Panicum species. In conclusion, the ability of C₃–C₄ intermediate species partially to recycle photorespired CO₂ did not improve WUE or NUE relative to congeneric C₃ or C₄ species grown under varying [CO₂] and temperature conditions.

Key words: C₃, C₄, and C₃–C₄ photosynthesis, climate change, Panicum, water and nitrogen use efficiency.

Introduction

C₄ photosynthesis evolved from C₃ photosynthesis some 20–30 million years ago to overcome the inefficiencies of the primary CO₂-fixing enzyme in plants, Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), particularly at low atmospheric CO₂ concentration ([CO₂]) and warm temperatures (Sage, 2004). The key novelty of C₄ photosynthesis is the employment of spatially separated carboxylation and decarboxylation reactions. The initial carboxylation reaction is catalysed by phosphoenolpyruvate carboxylase (PEPC) and involves the transient incorporation of inorganic carbon into organic C₄ acids in the mesophyll cell (MC). This is followed by the decarboxylation of C₄ acids in the bundle sheath cell (BSC) and the release of CO₂ for permanent fixation into organic C₃ acids in a reaction catalysed by Rubisco. The fast delivery of CO₂ by the C₄ cycle leads to high [CO₂] in the BSC which serves to saturate the carboxylation and suppress the oxygenation reactions of Rubisco under current ambient [CO₂] (Hatch, 1987). The high BSC [CO₂] and the suppression of photorespiration have well-recognized effects on the physiology and ecophysiology of C₄ plants (Brown, 1978; Osmond et al., 1982; Ehleringer and Monson, 1993; Long, 1999; Ghannoum et al., 2011).

Importantly, high BSC [CO₂] saturates C₄ photosynthesis at relatively low intercellular [CO₂] (Ci), allowing C₄ plants to operate with lower stomatal conductance (gs). Thus, leaf-level photosynthetic water-use efficiency (PWUE, rate of
CO₂ assimilation/stomatal conductance) is usually higher in C₄ than in C₃ plants. Relative to C₃ plants, Rubisco of C₄ plants is faster (higher catalytic turnover rate) and operates under saturating [CO₂]. Thus, C₄ plants typically achieve higher photosynthetic rates with less Rubisco and leaf nitrogen (N). Hence, leaf-level photosynthetic N-use efficiency (PNUE, rate of CO₂ assimilation/leaf N concentration) is higher in C₄ than in C₃ plants (Brown, 1978; Taylor et al., 2010; Ghannoum et al., 2011). Although C₄ photosynthesis requires additional ATP for the regeneration of PEP, the energy cost of photorespiration exceeds that associated with overcycling of CO₂ into the BSC at temperatures above 25 °C (Ehleringer and Bjorkman, 1977).

The translation of PWUE and PNUE advantages conferred by C₄ photosynthesis at the whole-plant level is less consistent. Whole-plant WUE (plant dry mass/cumulative water use) and NUE (plant dry mass/total leaf N) depend on additional non-photosynthetic factors including biomass partitioning, and shoot and root respiration (Farquhar et al., 1989). Nevertheless, greater whole-plant WUE and NUE have been observed in C₄ relative to C₃ species (Brown, 1978; Osmond et al., 1982; Sage and Pearcy, 1987). Moreover, C₃ and C₄ plants have a distinct geographic distribution. C₄ grasses prevail in summer-dominated rainfall regions whereas C₃ grasses prevail in winter-dominated rainfall regions. The abundance of C₄ grasses increases with increasing growing season temperature and aridity (Hattersley, 1983; Edwards and Still, 2008). To date, variations in photosynthetic quantum yield provide the best theoretical framework for explaining differences in the geographic distribution between C₃ and C₄ grasses (Ehleringer, 1978; Ehleringer et al., 1997). Consequently, photosynthetic characteristics have demonstrable impacts on plant physiological and ecological functions.

A small number (20–30) of plant species have been identified as possessing intermediate C₃ and C₄ photosynthetic characteristics and CO₂ compensation points (Monson and Moore, 1989; Rawsthorne, 1992; Vogan et al., 2007). These intermediate species are probably remnants of the complex processes that led to the evolution of C₄ plants from C₃ ancestors. The small number of intermediate species found so far raises questions about their physiological and ecological fitness, and whether they represent living fossils of evolutionary paths or dead-ends (Monson and Moore, 1989). Leaves of all C₃–C₄ intermediates have partial or full Kranz anatomy. Biochemically, C₃–C₄ intermediates differ in both the way and the extent to which CO₂ is concentrated in the BSC (Brown, 1980; Brown et al., 1983; Ku et al., 1983, 1991; Brown and Hattersley, 1989). For the three intermediate Panicum species, including the one used in this study, CO₂ compensation points are lowered relative to C₃ leaves due to a weak photorespiratory pump. In these species, glycine decarboxylase activity is localized to BSCs, away from MCs. Photorespired CO₂ diffuses back through MCs, where it may be refixed by Rubisco in MCs. In addition, this system may weakly elevate CO₂ in BSCs under high photorespiration (Edwards and Hatch, 1982; Hylton et al., 1988; Sage, 2005). Early work conducted with these species yielded inconclusive evidence on the possible photosynthetic advantages of the C₃–C₄ relative to C₃ species, except for small improvements in PWUE and PNUE under low [CO₂] (Ku and Edwards, 1978; Brown and Simmons, 1979; Bolton and Brown, 1980). Therefore, it remains unclear whether the type of C₃–C₄ photosynthesis present in intermediate Panicum species leads to detectable improvements in PWUE and PNUE relative to closely related C₃ Panicum species.

In addition to the evolutionary aspect, there is a need to understand whether physiological advantages conferred by C₄ photosynthesis, and presumably conferred by C₃–C₄ photosynthesis, hold under predicted future elevated atmospheric [CO₂] and high air temperature scenarios. To our knowledge, there are no published studies investigating the combined effects of elevated [CO₂] and high temperature or the effects of subambient [CO₂] on the physiology of C₅–C₄ intermediate species. Consequently, the current study was undertaken to compare the photosynthetic and whole-plant WUE and NUE of closely related C₃, C₄, and C₃–C₄ Panicum species under pre-industrial, current ambient, and projected mid-21st century atmospheric [CO₂]. Plants were also exposed to ambient temperature or ambient temperature + 4 °C to investigate the combined effects of elevated [CO₂] and global warming on the physiology of the Panicum species.

**Materials and methods**

**Plant culture and water use measurements**

The experiment was conducted in a naturally lit glasshouse consisting of six adjacent chambers (~5 m² each). Three chambers were maintained at ambient temperature, and three chambers were maintained at ambient temperature + 4 °C. The average day/night temperatures for ambient and high temperature treatments were 27/19 °C and 31/22 °C, respectively. The average relative humidity of the glasshouse rooms was ~75%. The three ambient temperature rooms were maintained at three different [CO₂]: subambient (280 µl l⁻¹, pre-industrial), ambient (400 µl l⁻¹, current), and elevated (640 µl l⁻¹, predicted for the mid-21st century). The [CO₂] treatments were similar for ambient and high temperature treatments. A detailed description of the temperature and CO₂ control can be found in Ghannoum et al. (2010a).

Polyethylene bags were placed inside 3.5 l cylindrical pots to prevent water leakage. Pot mass was adjusted to 0.8 kg using pebbles. Air-dried and coarsely sieved soil (3.7 kg) was added to each pot. Soil water capacity was calculated as the difference between the mass of two non-watered pots and that of pots watered and then left to drain freely overnight. Pots were watered to 100% capacity then transferred to the six glasshouse chambers. Seeds for Panicum bisulcatum (C₃), Panicum milioides (C₃–C₄), and Panicum coloratum (C₄) were obtained from AusPGRIS (Australian Plant Genetic Resources Information System, Australia). Seeds were sown in germination trays. Three-week old seedlings were transplanted into the experimental pots. Pots were randomly rotated within the glasshouse chambers throughout the experiment. There were eight pots per species, [CO₂] and temperature treatment.

Three days after transplanting, a pre-weighed layer of fine stones was added to the soil surface to minimize direct soil evaporation. There were two control pots, which were filled with soil but had no plants, in each room; these were used to estimate water loss by direct soil evaporation. Pots were irrigated to 100% capacity every 1–2 d. Pot mass was recorded before watering, and masses of all the pots were maintained at 5.5 kg after watering. Extra care was taken to avoid overwatering and prevent water accumulation at...
the bottom of the pots. A commercial fertilizer (General Purpose, Thrive Professional, Yates, Australia) was used on two occasions (0.2 g N 1⁻¹). Total, cumulative water use was calculated by summing daily water use and subtracting the amount of water lost by control pots without plants (Ghannoum et al., 2002).

Gas exchange measurements

Concurrent leaf gas exchange and chlorophyll fluorescence were measured using a portable open gas exchange system (LI-6400XT, LI-COR, Lincoln, NE, USA) connected to a leaf chamber fluorometer (6400-40, LI-COR) to determine the light-saturated photosynthetic rate (A_sat), stomatal conductance for water vapour (g_s), ratio of intercellular to ambient [CO₂] (C_i/C_a), and F_i/F_m. These measurements were used to calculate the ratio of the quantum yield of photosystem II (PSII) activity to the CO₂ assimilation rate (ΦPSII/ΦCO₂). Measurements were taken between 10:00 h and 14:00 h on an attached last fully expanded leaf (LFEF) of the main stem, 7 weeks after transplanting. Leaves of P. bisulcatum and P. coloratum filled the 2 cm² chamber; for P. milioides, leaf area was estimated by measuring the length and width of leaf sections. Measurements were made at a light intensity of 1800 µmol m⁻² s⁻¹, leaf-to-air vapour pressure deficit of 2.3–2.8 kPa, target growth [CO₂] (280, 400, or 640 µl 1⁻¹) and target mid-day growth temperature (26 °C or 30 °C). Before each measurement, the leaf was allowed to stabilize for 10–20 min until it reached a steady state of CO₂ uptake. There were four replicate measurements per treatment. The A–C_i response curves were measured by raising cuvette [CO₂] in 10 steps (40, 70, 130, 230, 280, 400, 640, 900, 1200, and 1800 µl 1⁻¹). Relative stomatal limitation (Ls) was calculated from A–C_i curves as Ls=(A_sat–A)/A_sat, where A denotes the net rate of CO₂ assimilation at current ambient [CO₂] and subscript zero denotes potential A if stomatal resistance were zero (i.e. C_i=C_a) (Farquhar and Sharkey, 1982). There were 3–4 replicate leaves measured per species and treatment.

Results

Leaf anatomy, gas exchange, and chlorophyll fluorescence

Microscopic examination of leaf sections revealed well-developed BSCs for P. milioides, in accordance with previous observations (Brown and Brown, 1975; Brown et al., 1983). Panicum bisulcatum and P. coloratum had classical C₃ and C₄ leaf structure (data not shown). Average interveinal distances (mean ± SE of three sections) were 201 ± 3, 136 ± 3, and 98 ± 4 µm for P. bisulcatum, P. milioides, and P. coloratum, respectively. These data confirmed that P. milioides has intermediate C₃–C₄ leaf anatomy (Ohsugi and Murata, 1986; Dengler et al., 1994).

When measured under current ambient [CO₂] and temperature, the light-saturated photosynthetic rate (A_sat) was highest in C₄, intermediate in C₃–C₄, and lowest in C₃ leaves. The effects of [CO₂] on A_sat differed between species and growth temperature. For the C₃ and C₃–C₄ species, increasing [CO₂] stimulated A_sat similarly at both growth temperatures. C₃–C₄ plants grown at subambient and ambient [CO₂] had lower A_sat at high relative to ambient temperature. For the C₄ species, A_sat was lower at subambient [CO₂] and ambient temperature relative to the other treatments (Fig. 1A, B; Table 1). Under current ambient [CO₂] and temperature, stomatal conductance (g_s) was higher in C₃ and C₃–C₄ relative to C₄ leaves. Increasing [CO₂] reduced g_s in C₄ plants at both growth temperatures and in C₃ plants at ambient temperature. For C₃–C₄ and high temperature-grown C₃ plants, g_s decreased between ambient and elevated [CO₂]. High temperature increased g_s of the C₄ species at subambient and ambient [CO₂] and of the C₃ species at ambient [CO₂] (Fig. 1C, D; Table 1). Under current ambient [CO₂] and temperature, the ratio of intercellular to ambient [CO₂] (C_i/C_a) was similar between C₃ and C₃–C₄ plants and higher relative to C₄ plants. Growth [CO₂] had no significant effect
on $C_i/C_a$, while high temperature increased the average $C_i/C_a$ of the *Panicum* species (Fig. 1E, F; Table 1). The ratio of the quantum yields of PSII activity to CO$_2$ assimilation rate ($\Phi_{\text{PSII}}/\Phi_{\text{CO}_2}$) measured under current ambient [CO$_2$] and temperature was higher in C$_3$ plants relative to C$_4$ and C$_3$–C$_4$ plants, which had similar $\Phi_{\text{PSII}}/\Phi_{\text{CO}_2}$. At ambient temperature, $\Phi_{\text{PSII}}/\Phi_{\text{CO}_2}$ decreased with increasing [CO$_2$] in C$_3$ plants; at high temperature, $\Phi_{\text{PSII}}/\Phi_{\text{CO}_2}$ decreased between subambient and ambient
Table 1. Summary of three-way ANOVA for the effects of species, [CO₂], and temperature on various parameters collected for *P. bisulcatum* (C₃), *P. milioides* (C₃–C₄), and *P. coloratum* (C₄) grown at three atmospheric [CO₂] and two air temperatures. Significance levels are NS, not significant (*P > 0.05*); *P < 0.05; **P < 0.01; ***P < 0.001.

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[CO₂] in C₃ and C₃–C₄ plants. High temperature increased *Φ*ₚₚ₅ *Φ*₃₅ *Φ*₅₄ of C₃–C₄ plants at subambient [CO₂] (Fig. 1G, H; Table 1).

The response of CO₂ assimilation rate to increasing *C*₅ (*A*₅ curve) showed a similar pattern for the C₃ and C₃–C₄ leaves. Gross CO₂ compensation points were lower in C₄ leaves but not significantly different between C₃ and C₃–C₄ leaves. Elevated [CO₂] reduced maximal photosynthetic rates of C₃ leaves at high temperature. *Panicum coloratum* showed typical C₄ –*A*₅ curve which were not affected by growth [CO₂]. Maximal photosynthetic rates were generally lower at high temperature (Fig. 2). Relative stomatal limitation (*L*ₕ) was highest in C₃ (39%) leaves and not significantly different between C₃–C₄ (23%) and C₄ (17%) leaves under all growth conditions (Figs 2, 3). Growth [CO₂] and temperature had no effect on *L*ₕ of the *Panicum* species (Table 1). There was a linear relationship between *L*ₕ and *C*₄/*C*₃ measured for the C₃ and C₄ leaves only; *L*ₕ of C₃–C₄ leaves clustered outside this relationship (Fig. 3). The C₃–C₄ leaves had C₄-like *C*₄/*C*₃ values and C₃-like *L*ₕ values (Fig. 3).

Plant growth and water use

Under current ambient [CO₂] and temperature, plant dry mass and total leaf area were highest in C₃, intermediate in C₄, and lowest in C₃–C₄ plants; total water use was similar for C₄ and C₃–C₄ plants but smaller relative to C₃ plants (Fig. 4, Table 1). The C₃ plants also had the highest leaf area ratio (LAR, total leaf area/plant dry mass; *P < 0.001; data not shown). Under ambient temperature, increasing [CO₂] enhanced plant dry mass and leaf area in the C₃ species but not in the C₃–C₄ or C₄ species. Under high temperature, increasing [CO₂] stimulated plant dry mass and leaf area in the C₃ and C₃–C₄ species; C₄ plants grown at elevated [CO₂] and high temperature had greater plant dry mass and leaf area relative to other treatments. Growth [CO₂] had no significant effect on total water used by the *Panicum* species. High temperature increased plant dry mass, leaf area, and water use of all three species (Fig. 4; Table 1). Increased leaf area was correlated with greater leaf number rather than leaf size (data not shown).

Leaf [N] and mass per area

At ambient temperature, LMA was highest in C₃–C₄ and lowest in C₃ species. At high temperature, LMA was highest in C₄ and lowest in C₃ species. At elevated [CO₂], LMA was similar for all *Panicum* species at both growth temperatures (Fig. 5A, B; Table 1). Under current ambient [CO₂] and temperature, leaf N concentration per unit dry mass ([N]ₚₗ) was highest in C₃–C₄ relative to C₃ and C₄ plants. Increasing [CO₂] reduced leaf [N]ₚₗ in the C₃ species. At ambient [CO₂], high temperature-grown C₃ plants had higher leaf [N]ₚₗ relative to ambient temperature (Fig. 5C, D; Table 1). Under current ambient [CO₂] and temperature, leaf N concentration per unit leaf area ([N]ₚₗ) was highest in C₃–C₄ plants and lowest in C₃ plants. Increasing [CO₂] reduced leaf [N]ₚₗ mainly in C₄ plants at high temperature. Relative to ambient temperature, high temperature-grown plants had lower leaf [N]ₚₗ in the C₃–C₄ species at ambient [CO₂] and higher leaf [N]ₚₗ in the C₄ species at subambient [CO₂] (Fig. 5E, F; Table 1).

Leaf and whole-plant water and nitrogen use efficiencies

When measured under current ambient [CO₂] and temperature, PWUE was higher in C₄ relative to C₃ and C₃–C₄ plants, which had similar PWUE. For the *Panicum* species, increasing [CO₂] stimulated PWUE at both growth temperatures. PWUE
was lower at high relative to ambient temperature in the three Panicum species (Fig. 6A, B; Table 1). Under current ambient [CO₂] and temperature, whole-plant WUE was highest in C₄, intermediate in C₃, and lowest in C₃–C₄ plants. Increasing [CO₂] enhanced plant WUE in C₃–C₄ and C₄ plants more than in C₃ plants (P <0.1). High temperature reduced plant WUE of the Panicum species mainly at elevated [CO₂] (Fig. 6C, D; Table 1). When measured under current ambient [CO₂] and temperature, PNUE was lower in C₃–C₄ relative to C₃ and C₄ plants, which had similar PNUE. Increasing [CO₂] enhanced PNUE of the three Panicum species at both growth temperatures. High temperature had no significant effect on PNUE of the three species (Fig. 7A, B; Table 1). Under current ambient [CO₂] and temperature, plant NUE was similar between the three Panicum species. Under ambient temperature, plant NUE increased between ambient and elevated [CO₂]. Under high temperature, plant NUE increased from subambient to ambient [CO₂] in the C₃–C₄ and C₄ plants...
and between ambient and elevated \([\text{CO}_2]\) in the \(C_3\) plants. High temperature increased plant NUE of the \(Panicum\) species at ambient and elevated \([\text{CO}_2]\) (Fig. 7C, D; Table 1). Leaf- and plant-level WUE measured in the three species and under the six growth conditions were linearly related; PWUE explained 60% of the variation in plant WUE (Fig. 8A). No similar relationship was observed between PNUE and plant NUE (Fig. 8B). There was a strong, positive relationship between PWUE and PNUE measured in this study (Fig. 8C).

Discussion

Photosynthesis and growth of the \(C_3\), \(C_3-C_4\), and \(C_4\) \(Panicum\) species

Under current ambient \([\text{CO}_2]\) and temperature, \(P.\ milioides\) \((C_3-C_4)\) had intermediate \(A_{\text{sat}}\) relative to the \(C_3\) and \(C_4\) \(Panicum\) species (Fig. 1A, B). Interestingly, \(C_3-C_4\) photosynthesis reduced the overall stomatal limitation relative to \(C_3\) photosynthesis without affecting \(C_i/C_a\), as otherwise is the case for \(C_4\) photosynthesis (Fig. 3). Lower stomatal limitation may be due to reduced \(C_2\) limitation as a result of partial recycling of photorespired \(\text{CO}_2\) during \(C_3-C_4\) photosynthesis (Brown et al., 1991). The \(C_3-C_4\) photosynthetic advantage diminished under elevated \([\text{CO}_2]\). Hence, relative to \(C_3\) photosynthesis, the \(C_3-C_4\) pathway did not boost photosynthetic capacity but rather alleviated stomatal limitation (Figs 2, 3). The \(\Phi_{\text{PSII}}/\Phi_{\text{CO}_2}\) ratio provides an estimate of the photosynthetic electron cost. Under most conditions, \(\Phi_{\text{PSII}}/\Phi_{\text{CO}_2}\) was similar between the \(C_3-C_4\) and \(C_4\) species except for subambient \([\text{CO}_2]\) and high temperature, where \(\Phi_{\text{PSII}}/\Phi_{\text{CO}_2}\) was similar between \(C_3\) and \(C_3-C_4\) plants (Fig. 1G, H). These results suggest that the ratio of Rubisco
oxygenation to carboxylation was generally lower in C_3–C_4 relative to C_3 plants. This can be attributed to a lower [O_2]/[CO_2] ratio, i.e. a marginal CO_2-concentrating mechanism, in BSCs of C_3–C_4 leaves relative to MCs of C_3 leaves. A lower [O_2]/[CO_2] ratio may result from the local delivery of CO_2 by glycine decarboxylation (Hylton et al., 1988) or lower PSII activity in BSC chloroplasts of C_3–C_4 intermediates as usually observed in BSC chloroplasts of C_4 species (Ghannoum et al., 2005). The latter aspect has not been investigated in C_3–C_4 intermediates. Under subambient [CO_2] and high temperature, \( \Phi_{\text{PSII}}/\Phi_{\text{CO}_2} \) was similar between C_3 and C_3–C_4 leaves, indicating that with increasing photorespiration, the proportion of CO_2 that cannot be refixed in the C_3–C_4 leaves increases.

For the C_3 species, increasing [CO_2] stimulated \( A_{\text{sat}} \) and reduced energy partitioning to photorespiration as indicated by the similar \( \Phi_{\text{PSII}}/\Phi_{\text{CO}_2} \) between C_3 and C_4 leaves at elevated [CO_2]. These responses are commonly reported for C_3 plants exposed to elevated [CO_2] (Ainsworth and Rogers, 2007). As expected, \( A_{\text{sat}} \) showed a weak CO_2 response while \( \Phi_{\text{PSII}}/\Phi_{\text{CO}_2} \) did not change with increasing [CO_2] in the C_4 species (Ghannoum et al., 2000). At high temperature, \( A_{\text{sat}} \) was either unaffected (C_3 and C_4) or reduced (C_3–C_4) in the Panicum species, indicating strong thermal acclimation of photosynthesis. Most plant species acclimate to changes in growth temperature by shifting the photosynthetic thermal optimum closer to the new growth conditions (Berry and Björkman, 1980; Dwyer et al., 2007; Sage and Kubien, 2007; Ghannoum et al., 2010b). Reduced \( A_{\text{sat}} \) at high temperature in P. milioides may reflect lower photosynthetic thermal optima in this C_3–C_4 species relative to the C_3 and C_4 congeners.

Whole-plant biomass accumulation can provide a long-term integrative response of the instantaneous photosynthetic responses to growth [CO_2]. In line with the photosynthetic responses and the published literature, the C_3 Panicum species showed a consistent biomass response to increasing growth [CO_2] from subambient to elevated [CO_2] (Ghannoum et al., 1997, 2010a; Wand et al., 1999). Under high temperature, the C_3–C_4 plants had a similar growth responsiveness, and hence CO_2 limitation, to their C_3 counterparts. Under ambient temperature, the growth response of C_3–C_4 plants to increasing [CO_2] was weak (Fig. 4A, B). These results indirectly suggest that CO_2 recycling during C_3–C_4 photosynthesis improves the carbon budget of C_3–C_4 plants, thus reducing their CO_2 limitation. The relative significance of CO_2 recycling diminishes as photorespiration increases (e.g. due to high temperature) beyond the capacity of BSCs and MCs to refix increasing amounts of photorespired CO_2. In contrast to previous interpretations (Monson and Moore, 1989; Schuster and Monson, 1990), the present results (plant dry mass; \( \Phi_{\text{PSII}}/\Phi_{\text{CO}_2} \)) imply that, in relative terms, the contribution of CO_2 recycling to CO_2 sequestration by C_3–C_4 plants is reduced at low [CO_2] and high temperature, i.e. with increasing photorespiration. C_4 plants accumulated more dry mass under elevated [CO_2] and high temperature only, most probably due to reduced \( g_s \) and hence plant water use. This effect was more pronounced in the larger elevated [CO_2]- and high temperature-grown plants because they are more likely to reduce soil moisture substantially during the course of the day (Ghannoum et al., 2000; Seneweera et al., 2001).

Photosynthetic WUE in C_3, C_3–C_4, and C_4 Panicum species

In this study, C_3–C_4 and C_3 leaves did not have significantly different PWUE (Fig. 6A, B). During both C_3 and C_3–C_4
photosynthesis, delivery of CO₂ to Rubisco in the MC relies on diffusion down a narrow concentration gradient which necessitates greater stomatal opening relative to C₄ leaves. On average, gs, C₄/C₃, and the responsiveness of photosynthetic rates to [CO₂] were similar between C₃ and C₃–C₄ plants. Hence, C₃–C₄ photosynthesis in P. milioides offered no PWUE advantage over C₃ photosynthesis in P. bisulcatum, even at subambient [CO₂]. These results are in line with an early study using P. milioides (Brown and Simmons, 1979) but not with another (Ku and Edwards, 1978). In contrast, the C₄ species P. coloratum maintained higher PWUE than its C₃ and C₁–C₄ counterparts under all growth conditions. Improved PWUE is one of the most consistently reported advantages of C₄ photosynthesis. The CO₂-concentrating mechanism saturates Rubisco with CO₂ at a lower Cᵣ relative to C₃ species, allowing C₄ leaves to operate with lower gs relative to C₃ leaves (Long, 1999; Taylor et al., 2010; Ghannoum et al., 2011). Results obtained using the more widely studied Flaveria (dicot) intermediate species indicated that C₄-like PWUE depends on the establishment of significant C₄ cycle activity and a near-complete localization of the C₃ and C₄ cycle carboxylases and decarboxylases (Monson, 1989; Monson and Moore, 1989; Schuster and Monson, 1990; Apel, 1994). Accordingly, leaky C₃-like CO₂-concentrating systems (e.g. Flaveria spp.) and CO₂ refixation (e.g. Flaveria and Panicum spp.) may improve photosynthetic rates and reduce photorespiration relative to related C₃ species; however, these advantages do not necessarily improve PWUE to the same extent as in C₄ species with fully developed CO₂-concentrating mechanisms.

Increasing [CO₂] stimulated PWUE of all the three grasses similarly but not by the same mechanisms. In the C₄ species, increasing [CO₂] stimulated PWUE mainly by reducing gs. In the C₃ and C₃–C₄ species, increasing [CO₂] stimulated PWUE by enhancing Aₛₑₜ and reducing gs. These results demonstrate that gs was more sensitive to increasing [CO₂] in C₄ relative to C₃ and C₃–C₄ leaves. In agreement, Huxman and Monson (2003) showed that the slope of the relationship between gs and Ci was steeper in C₄ than in C₃ and C₃–C₄ intermediate Flaveria species. However, Morison and Gifford (1983) found no evidence that stomata were more sensitive to [CO₂] in C₄ relative to C₃ grasses. More work is needed to resolve this aspect of the CO₂ response. High temperature reduced PWUE of the Panicum species mainly by increasing gs and, in some instances, decreasing Aₛₑₜ.

Photosynthetic NUE in C₃, C₃–C₄, and C₄ Panicum species

In contrast to PWUE, variations in PNUE were not solely predictable based on the photosynthetic pathway (Fig. 7A, B). It is well established that relative to C₃, C₄ leaves operate with higher carboxylation efficiency due to the higher activity of PEPC relative to Rubisco and the CO₂ saturation of Rubisco. This, together with the higher catalytic turnover rate of C₄ relative to C₃ Rubisco, often leads to higher Aₛₑₜ per Rubisco active sites (Long, 1999; von Caemmerer and Quick, 2000; Sage, 2002). Ghannoum et al. (2005) demonstrated that Aₛₑₜ per Rubisco explains most of the variation in PNUE amongst C₄ grasses. Higher Aₛₑₜ per Rubisco is also expected to enhance PNUE in C₄ relative to C₃ leaves. While this has been often observed (Taylor et al., 2010), it was not the case in the current study. Panicum coloratum is a forage grass species which may explain its unusually higher leaf [N] relative to other C₃ and C₄ grasses (Ghannoum et al., 1997; Ghannoum and Conroy, 1998). Panicum milioides (C₃–C₄) had the highest leaf [N] and lowest PNUE in the current study. High leaf [N] may reflect higher photosynthetic or non-photosynthetic N investment. In our study, carboxylation efficiency, as determined from the initial slope of the A – Cᵣ response curves, was similar for C₃ and C₃–C₄ leaves. Similar carboxylation efficiencies and Rubisco activities were reported between P. bisulcatum (C₃) and P. milioides in an early study (Ku and Edwards, 1978). These results suggest that C₃ and C₃–C₄ species had similar Rubisco activity per leaf area. Hence, it can be concluded that P. milioides had higher Aₛₑₜ per Rubisco despite its lower PNUE relative to the C₃ Panicum species. Lower PNUE and higher leaf [N] in P. milioides may reflect the greater non-photosynthetic N requirements of the C₃–C₄ pathway, such as the N construction costs associated with the provision of BSCs in C₁–C₄ intermediates (Schuster and Monson, 1990). The trends in PNUE observed here need to be confirmed using more phylogenetically related species.

Increasing [CO₂] stimulated PNUE mainly by enhancing Aₛₑₜ of C₃ and C₃–C₄ plants, and reducing leaf [N]area of C₄ plants. Reduced leaf [N] is a commonly observed response at OUP site access on April 22, 2013 http://jxb.oxfordjournals.org/ Downloaded from
between leaf and plant WUE than between leaf and plant NUE (Fig. 8A, B). Remarkably, PWUE explained ~60% of the variation in plant WUE. The remaining 40% depends on biomass partitioning and plant respiration, which, in turn, depend on the relative growth rate (Farquhar et al., 1989). The counterbalancing effect of plant growth rate and PWUE was on display in this study. Panicum coloratum (C4) had the highest PWUE, while P. bisulcatum (C3) had the highest plant dry mass and LAR (i.e. the highest relative growth rate); both species achieved similar plant WUE. In a study using three wheat cultivars, van den Boogaard et al. (1996) reported that plants with the highest relative growth rate also had the highest plant WUE. Panicum milioides (C3-C4) had a similar PWUE to its C3 counterparts, but its low biomass exacerbated its whole-plant WUE. The present results highlight the importance of plant growth characteristics in moderating the photosynthetic pathway differences in PWUE. In the field, other parameters, including wind speed, humidity, temperature, and stomatal coupling, amongst others, may further dilute the significance of PWUE variations. The lack of relationship between PNUE and plant WUE was mainly due to the narrow range of variation for the latter parameter across species and growth conditions. In contrast to PWUE, the translation of PNUE to the whole-plant level is fraught with more complexities. Yet, some studies have demonstrated positive relationships between leaf and plant NUE in various species (Brown, 1978; Sage and Pearcy, 1987; Ghannoum et al., 2005). Plant NUE, as calculated in this study, depends on leaf [N]_mass, leaf mass fraction, and total plant dry mass, all of which are independent of photosynthetic capacity, except for leaf [N]_mass. However, as argued earlier, differences in photosynthetic capacity and Rubisco content may not be linearly related to leaf [N] due to variations in photosynthetic and non-photosynthetic N partitioning (Evans, 1989; Evans and Seemann, 1989). These aspects will be considered in our future research.

Conclusions

Results obtained in this study using Panicum intermediates and those previously reported using other types of C3–C4 intermediates demonstrate that, short of substantial C4 cycle activity and advanced cell-specific localization of C3 and C4 cycle enzymes between the MC and BSC, photosynthetic intermediacy does not improve WUE. In addition, it may reduce NUE at both the leaf and plant levels and under a wide range of growth conditions. Partial recycling of photorespired CO2 may reduce energy loss and stomatal limitation normally associated with C3 photosynthesis. Improvement in plant productivity, however, is also contingent on the species’ intrinsic relative growth rate. Overall, there was a positive relationship between PWUE and PNUE across species and growth conditions. However, only PWUE clearly translated into improved plant WUE. This may highlight the strong ecological nature of plant NUE. The type of C3–C4 photosynthesis present in Panicum species reflects early evolutionary changes on the path from C3 towards C4 photosynthesis; however, it does not present ecophysiological advantages under climatic scenarios of the recent past and future.

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