Title

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Abstract

C_3 carbon fixation has a bad reputation, primarily because it is associated with photorespiration, a biochemical pathway thought to waste a substantial amount of the carbohydrate produced in a plant. This review presents evidence collected over nearly a century that (1) Rubisco when associated with Mn^{2+} generates additional reductant during photorespiration, (2) this reductant participates in the assimilation of nitrate into protein, and (3) this nitrate assimilation facilitates the use of a nitrogen source that other organisms tend to avoid. This phenomenon explains the continued dominance of C_3 plants during the past 23 million years of low CO_2 atmospheres as well as the decline in plant protein concentrations as atmospheric CO_2 rises.

Keywords

photorespiration, C_3 carbon fixation, nitrate assimilation, photosynthesis, plant evolution, nitrogen sources

Premise

Plants, by most accounts, convert less than 6% of the incoming solar energy into useable chemical energy (Hall et al. 1999; Zhu et al. 2008). Efforts to improve this conversion rate have focused on the light-independent reactions of photosynthesis (e.g., Parry et al. 2013; Studer et al. 2014; Whitney et al. 2011; Zhu et al. 2010). "The light reactions are highly efficient, converting as much as 40% – 50% of the captured solar energy into energy carriers. The dark reactions are not developed for energy efficiency and it is here the energy is…*lost*" (Swedish Energy Agency 2003). In particular, Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase; EC 4.1.1.39), the enzyme which catalyzes the first reaction of the C_3 pathway and constitutes about half of the protein in leaves (Parry et al. 2003), has been identified as a target of opportunity.

Competing Reactions

Rubisco exhibits opposing tendencies in that it catalyzes two different chemical reactions: one reaction combines a five-carbon sugar RuBP (ribulose-1,5-bisphosphate) with CO_2 (carboxylation), and the other reaction combines this same sugar with O_2 (oxygenation).

- The carboxylation reaction of RuBP produces a six-carbon compound that quickly divides into two molecules of a three-carbon compound, PGA (3-phosphoglycerate), hence the name *C_3 carbon fixation*. Six of these PGA molecules pass through an elaborate pathway that expends the energy of 18 ATP and 12 NADPH molecules, forms one molecule of fructose-6-phosphate, a six-carbon sugar, and regenerates six molecules of RuBP.
- The oxygenation reaction splits the RuBP into one molecule of a three-carbon PGA and one molecule of a two-carbon PG (2-phosphoglycolate), hence the name *C_2 pathway* or, more commonly, photorespiration (Foyer et al. 2009). In total, photorespiration consumes 3.5 ATP and 2 NADPH per RuBP oxygenated and regenerated, but does not result in any net production of sugar (Bauwe et al. 2010; Tolbert 1994). Thus photorespiration seems to be largely a superfluous process, one thought to dissipate 76.3 kcal mol^{-1} as waste heat (Frank et al. 2000).

The balance between C_3 carbon fixation and photorespiration depends on the relative amounts of CO_2 and O_2 entering the active site of Rubisco and the specificity of the enzyme for each gas. Atmospheric concentrations of CO_2 and O_2 are currently 0.04% and 20.94%, respectively, yielding a CO_2:O_2 ratio of 0.0019. Gaseous CO_2, however, is much more soluble in water than O_2, and so the CO_2:O_2 ratio near the chloroplast, the part of a cell where these reactions occur, is about 0.026 at 25°C. Rubisco has about a 50-fold (cyanobacteria) to 100-fold (higher plants) greater specificity for CO_2 than O_2 (Galmes et al. 2005). Together, because of the relative concentrations of and specificity for CO_2 over O_2, Rubisco catalyzes about two to three cycles of C_3 carbon fixation for every cycle of photorespiration under current atmospheres (Sharkey 1988). Conditions that inhibit photorespiration—namely, high CO_2, or low O_2 atmospheric concentrations—stimulate carbon fixation in the short term by about 35%.

Temperature influences the balance between C_3 carbon fixation and photorespiration in two ways. First, as temperature rises, the solubility of CO_2 in water decreases more than the solubility of O_2, resulting in a lower CO_2:O_2 ratio. Second, the enzymatic properties of Rubisco shift with increasing temperature, stimulating the reaction with O_2 to a greater degree than the one with CO_2. Warmer temperatures, therefore, favor photorespiration over C_3 carbon fixation, and photosynthetic conversion of absorbed light into sugars becomes less efficient (Ehleringer et al. 1997). Based on the temperature response of Rubisco carboxylation and oxygenation, C_4 plants should be more competitive in regions where the mean monthly air temperature exceeds 22°C (Collatz et al. 1998).

Overall, Rubisco seems a vestige of the high CO_2 and low O_2 atmospheres under which plants first evolved (Wingler et al. 2000). To compensate for the shortcomings of Rubisco, some plants employ CO_2 pumping mechanisms such as C_4 carbon fixation that elevate CO_2 concentrations at the active site of the enzyme. The C_4 pathway is one of the most convergent evolutionary adaptations in life with at least 66 independent origins (Sage et al. 2012). Extensive efforts are underway to emulate Mother Nature and transfer the C_4 pathway into rice and other C_3 crops (von Caemmerer et al. 2012).

Several observations, however, are inconsistent with the presumption that Rubisco is poorly suited to modern times.

- Earth's atmosphere has contained relatively low CO_2 concentrations (lower than 0.04%) for the past 23 million years (Figure 1). During this period, the plant kingdom experienced major changes including the diversification of modern graminoids, especially grasses and sedges, and the appearance of many new C_4 species, especially when CO_2 concentrations fell below 0.02%, (Sage et al. 2012). In a relatively short period of time (6 or 7 million years) (Osborne and Beerling 2006), the kinetics of Rubisco diverged between C_3 and C_4 plants (Studer et al. 2014). Rubisco in C_4 plants operates under elevated CO_2 conditions, and so the C_4 enzyme has traded a lower specificity for CO_2 relative to O_2 ($S_{c/o}$) for a higher catalytic efficiency (k_{cat}^c) (Galmes et al. 2005; Sage 2002). Surprisingly, the kinetic properties of Rubisco do not differ greatly among higher C_3 plants (Kane et al. 1994; Tcherkez et al. 2006). Thus, the kinetic properties of Rubisco were able to change when a species adopted the C_4 pathway, but such changes were not warranted in C_3 plants because Rubisco may already be "nearly perfectly optimized" for C_3 carbon fixation (Tcherkez et al. 2006).
- Despite 23 million years of low atmospheric CO_2 concentrations, 96% of plant species depend solely on the C_3 carbon fixation pathway (Sage et al. 1999). C_3 species account for over 94% of the Earth's biomass (Still et al. 2003). Species using other carbon fixation pathways are dominant only in hot and dry environments.
- The response of C_3 species to elevated CO_2 atmospheres is highly variable and often depends on plant N status (Cavagnaro et al. 2011; Duval et al. 2012; Finzi et al. 2007; Norby et al. 2010; Reich et al. 2006). Initially, elevated CO_2 stimulates biomass accumulation by about 35% (Figure 2). This stimulation, however, tends to abate upon longer exposures in conjunction with a decline in plant protein concentrations (Cotrufo et al. 1998; Long et al. 2004).

Explanations for the decline in plant protein concentrations at elevated CO_2 include: (*a*) plants under elevated CO_2 grow larger, diluting the protein within their tissues (Ellsworth et al. 2004; Taub and Wang 2008); (*b*) carbohydrates accumulate within leaves, down-regulating the amount of the most prevalent protein Rubisco (Long et al. 2004); (*c*) carbon enrichment of the rhizosphere leads to progressively greater limitations in the soil N available to plants (Reich et al. 2006); and (*d*) elevated CO_2 directly inhibits plant N metabolism, especially the assimilation of NO_3^- into proteins in shoots of C_3 plants (Bloom et al. 2012b). Recently, several independent meta-analyses conclude that this last explanation is the one most consistent with observations from hundreds of studies (Cheng et al. 2012; Myers et al. 2014; Pleijel and Uddling 2012).

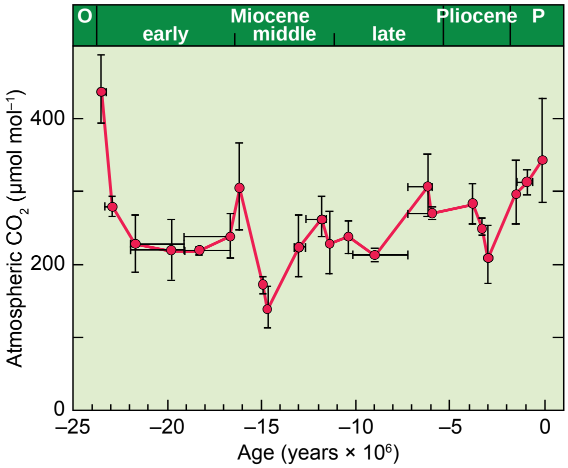


Fig. 1 A reconstruction of atmospheric CO_2 concentrations based on boron isotope ratios of ancient planktonic foraminifer shells. (Data from Pearson and Palmer 2000)

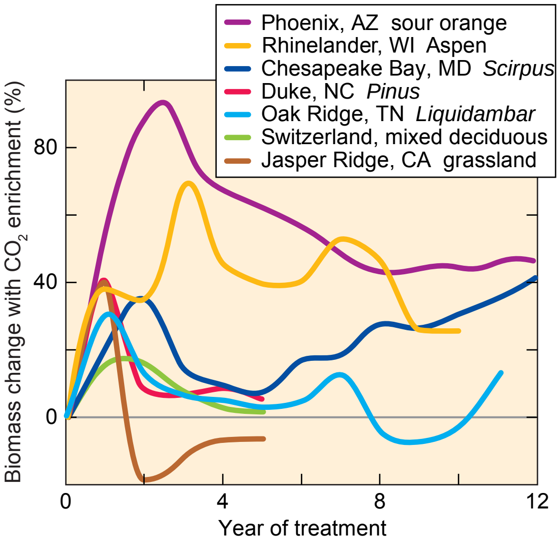


Fig. 2 Differences in biomass between elevated (≈ 567 ppm) and ambient (≈ 365 ppm) atmospheric CO_2 after years of treatment. Shown are the data from seven different studies using the designated types of plants. (Data from Dukes et al. 2005; Kimball et al. 2007; Korner 2006; Norby et al. 2010; Rasse et al. 2005; Talhelm et al. 2014).

CO_2 inhibits NO_3^- Assimilation

Many independent methods for estimating NO_3^- assimilation confirm that elevated CO_2 inhibits shoot NO_3^- assimilation in C_3 plants. These methods include:

1. **^{15}N-labeling**. Plants grown on NO_3^- containing N isotopes at natural abundance levels (≈ 0.366% ^{15}N) were exposed to a pulse of NO_3^- that was heavily enriched in ^{15}N. The difference between the ^{15}N enrichment of total N and that of free NO_3^- provided an estimate of ^{15}N-NO_3^- assimilation, which decreased under CO_2 enrichment (Bloom et al. 2010).
2. **^{14}N-labeling.** Plants grown on 99.9% enriched ^{15}N-NO_3^- were exposed to a pulse of NO_3^- containing N isotopes at natural abundance levels (≈ 0.366% ^{15}N); the difference between the ^{14}N enrichment of total N and that of free NO_3^- provided an estimate of ^{14}N-NO_3^- assimilation, which decreased under CO_2 enrichment (Bloom et al. 2010).
3. **Organic N accumulation.** Accumulation of organic N was followed in plants receiving NO_3^- as a sole N source, and this accumulation decreased under CO_2 enrichment (Aranjuelo et al. 2013; Bloom et al. 2010; Lekshmy et al. 2013; Pleijel and Uddling 2012; Rachmilevitch et al. 2004).
4. **NO_3^- depletion from a medium.** The decline of NO_3^- concentrations in a nutrient solution was monitored to calculate net plant NO_3^- absorption. The difference between this NO_3^- absorption and the accumulation of free NO_3^- within plant tissues estimated plant NO_3^- assimilation, which decreased under CO_2 enrichment (Bloom et al. 2010; Rachmilevitch et al. 2004).
5. **Plant growth.** C_3 species received either NO_3^- or NH_4^+ as their sole N source. CO_2 enrichment decreased growth of plants receiving NO_3^- (Figure 3) but increased growth of those receiving NH_4^+ (Bloom et al. 2012b; Bloom et al. 2002; Carlisle et al. 2012).

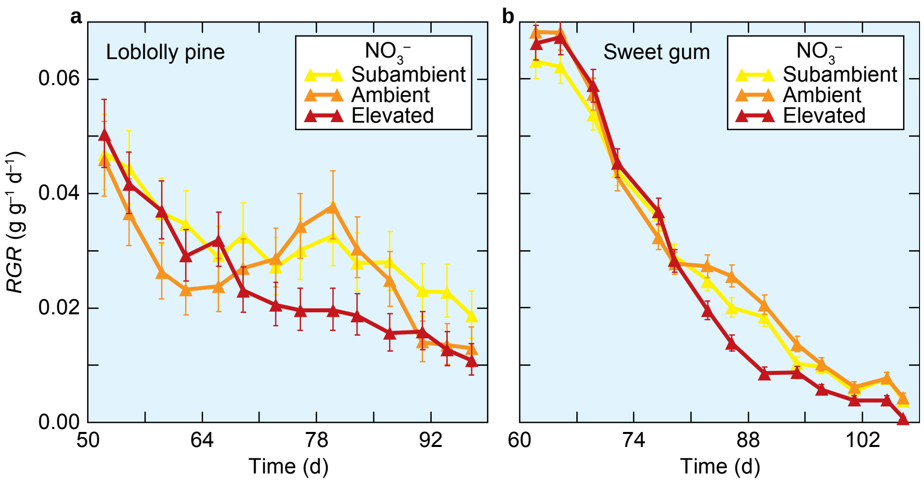


Fig. 3 Relative growth rate in $g\ g^{-1}\ d^{-1}$ of **(a)** loblolly pine *Pinus taeda* and **(b)** sweet gum *Liquidambar styraciflua* receiving NO_3^- nutrition in controlled environment chambers at subambient CO_2 (310 μmol mol^{-1}, the level of about 50 years ago), ambient CO_2 (400 μmol mol^{-1}, current level), or elevated CO_2 (720 μmol mol^{-1}, the level anticipated in about 50 years). CO_2 concentration had no significant effect on the growth of plants receiving NH_4^+ nutrition (data not shown). Time is in days after transplanting to a hydroponic solution. Shown are the predicted values and standard errors from mixed linear models with repeated measures on 6 to 10 individual plants. (Bloom et al. 2012b)

6. **Isotopic discrimination by NO_3^- reductase.** Plants were grown under NO_3^- containing N isotopes at natural abundance levels (≈ 0.366% ^{15}N). Under CO_2 enrichment, plant tissues became less enriched in ^{15}N-organic N compounds presumably because (*a*) CO_2 inhibited shoot NO_3^- assimilation, (*b*) NO_3^- availability became less limiting to assimilation, (*c*) NO_3^- reductase discriminated more against ^{15}N-NO_3^-, and (*d*) shoots assimilated relatively less ^{15}N-NO_3^- (Bloom et al. 2010; Bloom et al. 2014).

7. **Δ*AQ*.** Assimilatory quotient (*AQ*), the ratio of net CO_2 consumption to net O_2 evolution from shoots was measured in a plant receiving NH_4^+ or NO_3^- as its sole N source (Figure 4); *AQ* decreased as NO_3^- assimilation increased because additional electrons generated from the light-dependent reactions of photosynthesis were transferred first to NO_3^- and then to NO_2^-. This stimulated net O_2 evolution, but had

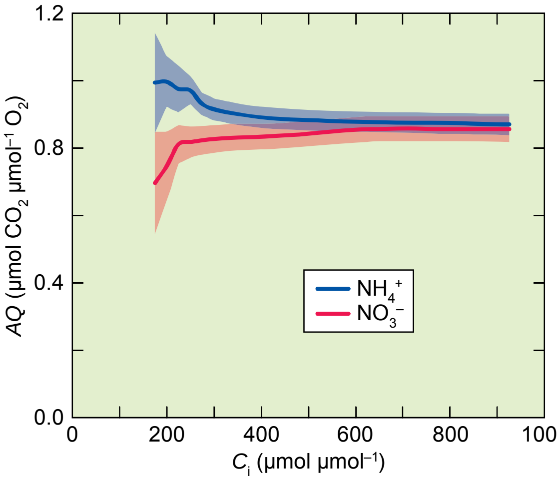


Fig. 4 Shoot *AQ* (net CO_2 consumed/net O_2 evolved) as a function of internal CO_2 concentrations (C_i) for the 9 C_3 species in Figure 4 when they received NH_4^+ or NO_3^- as a sole N source (mean ± SE; solid ± shaded area). (Bloom, unpublished data)

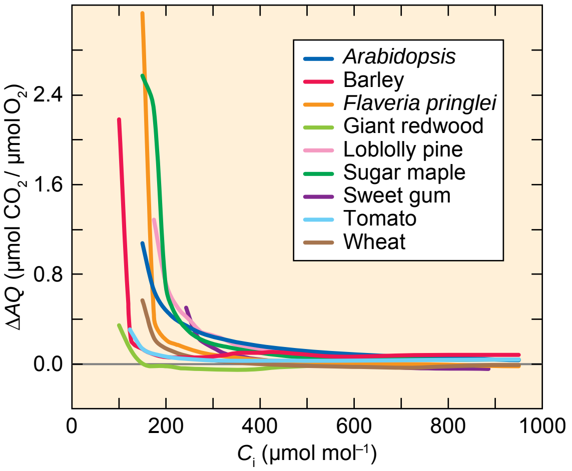


Fig. 5 Shoot NO_3^- assimilation as a function of shoot internal CO_2 concentration (C_i) for 9 C_3 species. Shoot NO_3^- assimilation is assessed by Δ*AQ* (change in the ratio of shoot CO_2 consumption to O_2 evolution with a shift from NO_3^- to NH_4^+ nutrition). (Bloom et al. 2012b; Searles and Bloom 2003)

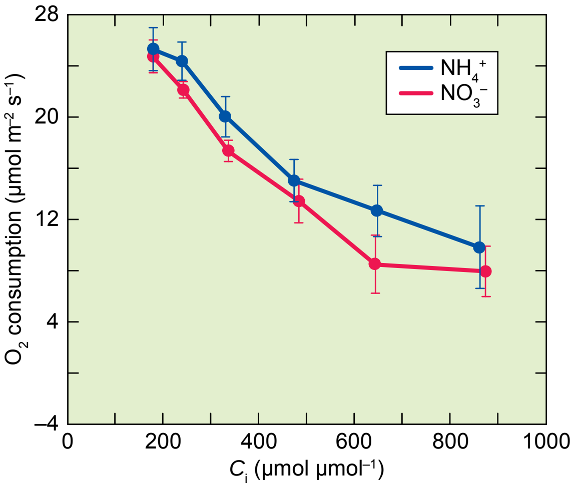


Fig. 6 Shoot O_2 consumption in the light (gross O_2 – net O_2) as a function of C_i for wheat receiving NH_4^+ or NO_3^- as a sole N source. Shown are the means ± SE for 5–7 replicates per treatment.(Cousins and Bloom 2004)

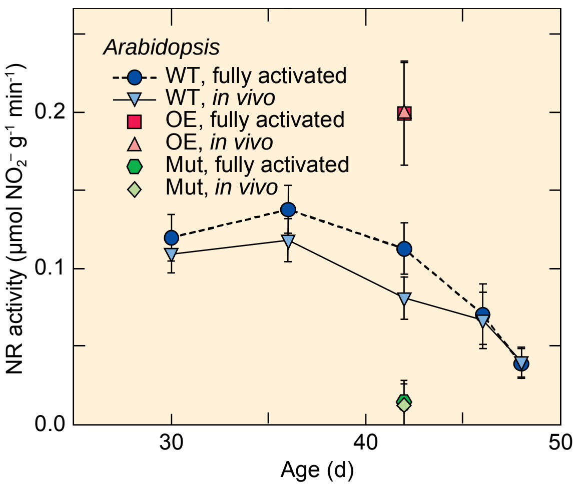


Fig. 7 NO_3^- reductase activity (μmol of NO_2^- generated per g fresh mass per min) as a function of plant age (d) in leaves of a wild-type *A. thaliana* cv. Columbia (WT), a transgenic line harboring the chimeric gene *Lhch1*3*::*Nia1*2* (OE), and a genotype (*nia1 nia2*) with mutations in both structural genes for NO_3^- reductase (Mut). Because NO_3^- reductase is regulated through phosphorylation, leaf tissue was assayed under conditions that either dephosphorylated the enzyme (fully activated) or did not change its phosphorylation (*in vivo*). Shown are the mean ± SE (*n* = 5–8 plants). (Rachmilevitch et al. 2004)

little effect on CO_2 consumption; therefore, the change in *AQ* when a plant received NH_4^+ instead of NO_3^- (Δ*AQ*) provided an estimate of shoot NO_3^- assimilation (Bloom et al. 1989; Bloom et al. 2002; Cen et al. 2001; Cramer and Myers 1948; Rachmilevitch et al. 2004; Van Niel et al. 1953; Warburg and Negelein 1920). In nine taxonomically diverse C_3 species, Δ*AQ* decreased as shoot internal CO_2 increased (Figure 5).

8. **O_2 consumption.** Shoot O_2 consumption in the light was estimated from the difference between gross O_2 evolution via chlorophyll fluorescence and net O_2 evolution via an O_2 analyzer (Figure 6). At ambient CO_2, O_2 consumption was lower when wheat plants received NO_3^- rather than NH_4^+ because NO_3^- and NO_2^- were serving as electron acceptors. At elevated CO_2, O_2 consumption was not significantly different under the two N sources presumably because NO_3^- assimilation was negligible.

9. **Altered NO_3^- reductase capacity.** Shoot CO_2 and O_2 fluxes at ambient and elevated CO_2 were contrasted between stages of plant development or genotypes that have greatly different NO_3^- reductase activities *in situ*. In particular, we contrasted 36- vs. 48-d old wild-type Arabidopsis, Arabidopsis NO_3^- reductase knockout mutants vs. transgenic Arabidopsis overexpressing NO_3^- reductase (Figure 7), and NO_3^- reductase-deficient barley mutants vs. wild-type barley. Δ*AQ* (change in the ratio of net CO_2 consumption to net O_2 evolution when a plant received NH_4^+ instead of NO_3^-) differed between these stages of development and genotypes under ambient CO_2, but not under elevated CO_2 (Figure 8).This indicates that none of the stages of development or genotypes were assimilating NO_3^- under elevated CO_2 (Bloom et al. 1989; Rachmilevitch et al. 2004).

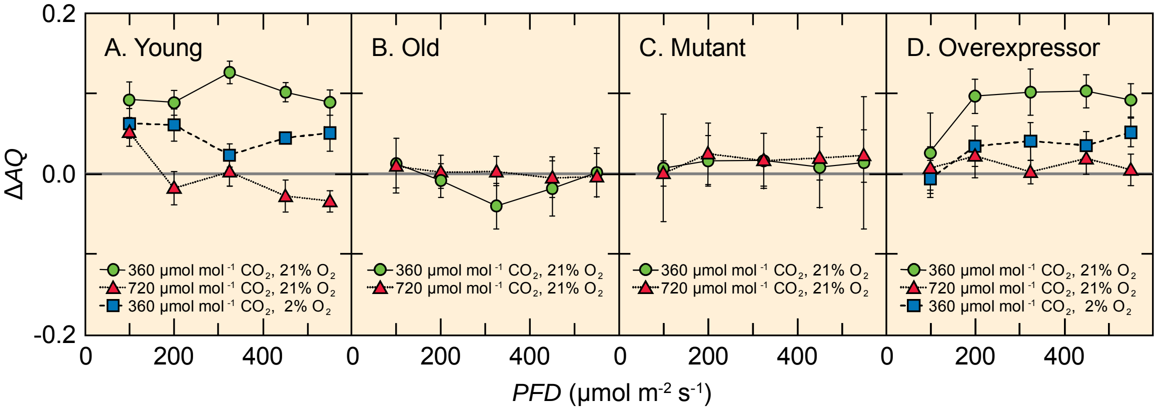


Fig. 8 Changes in assimilatory quotient with the shift from NO_3^- to NH_4^+ (ΔAQ) as a function of photosynthetic *PFD* (photon flux density) from shoots of *A. thaliana* cv. Columbia. (A) 36-day-old wild-type plants, (B) 48-d-old wild-type plants (C), genotype with null mutations, and (D) overexpressing line. The plants were grown under ambient CO_2 (360 μmol mol^{-1}) and measured under ambient CO_2 and O_2 (360 μmol mol^{-1} CO_2 and 21% O_2; circles), elevated CO_2 (720 μmol mol^{-1} CO_2 and 21% O_2; triangles), or low O_2 (360 μmol mol^{-1} CO_2 and 2% O_2; squares). Shown are the mean ± SE, $n = 5 – 8$ plants. (Rachmilevitch et al. 2004)

10. **NO_3^- reductase activity.** Maximum *in vitro* NO_3^- reductase activity generally declined under CO_2 enrichment (Lekshmy et al. 2013; Matt et al. 2001). Presumably, this reflected slower NO_3^- assimilation under CO_2 enrichment.

Physiological Mechanisms

Three physiological mechanisms may be responsible for CO_2 inhibition of shoot NO_3^- assimilation (Bloom et al. 2010).

- One mechanism is that elevated CO_2 inhibits nitrite (NO_2^-) transport into chloroplasts (Figure 9). A chloroplast NO_2^- transporter from higher plants has only recently been identified (Maeda et al. 2014), and so the nature of this inhibition has yet to be determined. Nevertheless, this mechanism can be independent of photosynthesis and, thus, is probably responsible for CO_2 inhibition of shoot NO_3^- assimilation in Arabidopsis and wheat during the nighttime (Rubio-Asensio, Rachmilevitch, and Bloom, unpublished data).

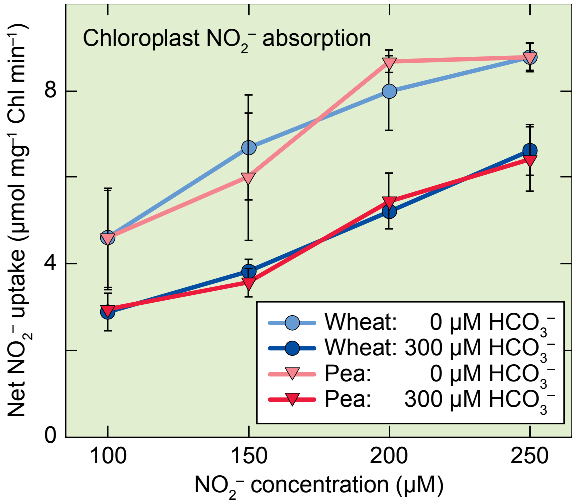


Fig. 9 Net NO_2^- uptake (μmol mg^{-1} chlorophyll min^{-1}) by isolated chloroplasts as a function of NO_2^- concentration when the medium contained 0 (light symbols) or 0.3 (dark symbols) μM HCO_3^-. Shown are the mean ± SE (n = 3) for wheat (circles) and pea (inverted triangles). (Bloom et al. 2002)

- Another mechanism is that processes in the chloroplast stroma compete for reduced ferredoxin (Fd_r). FNR (ferredoxin-NADP reductase) has a higher affinity for Fd_r than NiR (nitrite reductase) (Knaff 1996), and so NO_3^- assimilation proceeds only if the availability of Fd_r exceeds that needed for NADPH formation (Backhausen et al. 2000; Robinson 1987). For most plants, this occurs when CO_2 availability limits C_3 carbon fixation (Bloom et al. 2010).
- A third mechanism involves photorespiration. Multiple lines of evidence link photorespiration with shoot NO_3^- assimilation in C_3 plants. (*a*) Photorespiration stimulates the export of malate from chloroplasts (Backhausen et al. 1998; Taniguchi and Miyake 2012; Voss et al. 2013); this malate in the cytoplasm generates NADH (Igamberdiev et al. 2001; Taniguchi and Miyake 2012) that powers the first step of NO_3^- assimilation, the reduction of NO_3^- to NO_2^- (Quesada et al. 2000; Rathnam 1978; Robinson 1987). (*b*) Conditions that decrease photorespiration—namely, elevated CO_2 and low O_2—decrease shoot NO_3^- reduction (Bloom et al. 2010; Rachmilevitch et al. 2004). (*c*) Mutants that alter malate transport or metabolism also alter both photorespiration and NO_3^- assimilation (Dutilleul et al. 2005; Schneidereit et al. 2006).

The first carboxylation reaction in the C_4 carbon fixation pathway, by contrast, generates ample amounts of malate and NADH in the cytoplasm of mesophyll cells. This explains the CO_2 independence of shoot NO_3^- assimilation in C_4 plants (Bloom et al. 2010; Bloom et al. 2012b).

The Rubisco Complex

Information about the biochemistry of RuBP oxygenation is limited. The stroma of the chloroplast contains similar amounts of Mg^{2+} (2 mM, Ishijima et al. 2003) and Mn^{2+} (2 mM, Burnell 1988; Robinson and Gibbs 1982). Rubisco may form a complex with either Mg^{2+} or Mn^{2+} (Pierce and Reddy 1986) , but the affinity of Rubisco for Mn^{2+} is more than five time greater than that for Mg^{2+} (Christeller 1981). The stoichiometry of CO_2 trapping (Miziorko and Sealy 1980) and ^{31}P and ^{13}C NMR measurements (Pierce and Reddy 1986) indicate that Mn^{2+} and Mg^{2+} share a common binding site in the large subunit of Rubisco. Nearly all of the biochemistry of Rubisco has been conducted in the presence of Mg^{2+} and in the absence of Mn^{2+} because Rubisco when associated with Mn^{2+} strongly favors RuBP oxygenation, whereas Rubisco when associated with Mg^{2+} favors RuBP carboxylation (Chen and Spreitzer 1992; Christeller and Laing 1979; Houtz et al. 1988; Jordan and Ogren 1981; Raghavendra et al. 1981; Wildner and Henkel 1979).

Mg^{2+} has a pair of electrons in its outer shell, whereas Mn^{2+} has up to five unpaired electrons and thus participates more readily in redox reactions. In specific, Mn^{2+} participates in the catalytic process of RuBP oxygenation (Miziorko and Sealy 1984) during which it becomes excited and transfers an electron with every turnover (Lilley et al. 2003). One possibility is that Mn^{2+} transfers electrons to $NADP^+$ (Figure 11). The resultant NADPH activates Rubisco (Laing and Christeller 1976) and then converts OAA to malate for export to the cytoplasm. This malate in the cytoplasm generates NADH to convert NO_3^- to NO_2^-.

Several additional observations are consistent with this hypothesis. RuBP oxygenation releases 76.3 kcal mol^{-1} (Frank et al. 2000), substantially more than the 52 kcal mol^{-1} required to reduce $NADP^+$ to NADPH (Taiz and Zeiger 2010). NADPH complexes strongly with Rubisco and activates the enzyme, but only when CO_2 and Mg^{2+} are present in suboptimal concentrations (Chollet and Anderson 1976; Chu and Bassham 1974; Matsumura et al. 2012; McCurry et al. 1981). NADPH binds to the catalytic site of Rubisco through metal-coordinated water molecules (Matsumura et al. 2012).

If Rubisco generates NADPH during RuBP oxygenation, C_3 carbon fixation is more efficient than previously thought, and both C_3 and C_4 carbon fixation at moderate temperatures will expend the equivalent

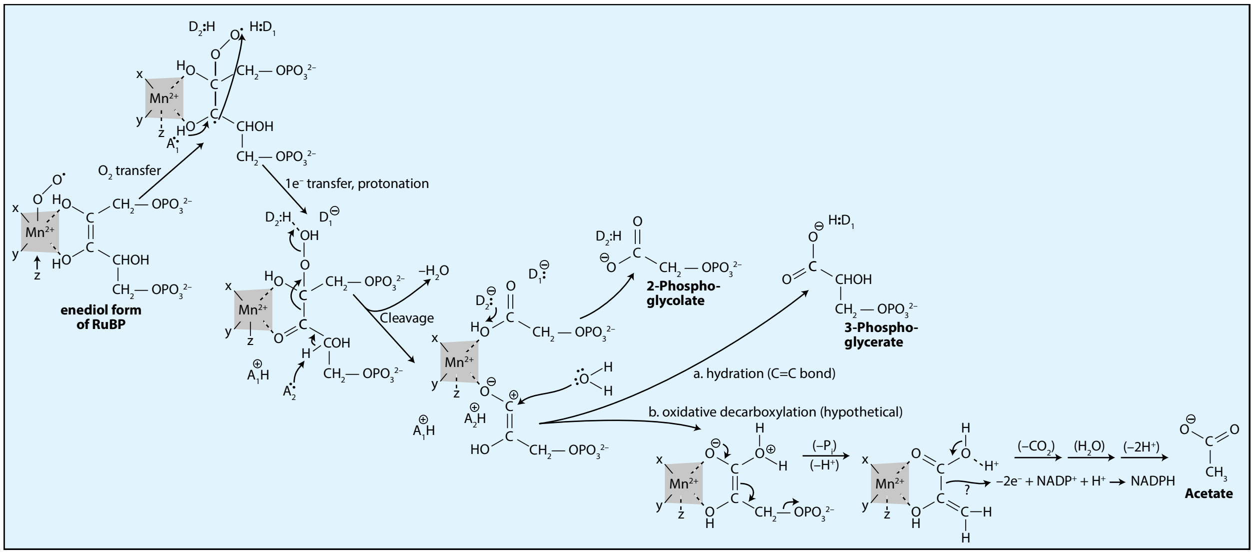


Fig. 10 One possible scenario for the intermediates formed during RuBP oxygenation (Chen and Spreitzer 1992; Cleland et al. 1998; Lilley et al. 2003; Oliva et al. 2001; Tapia and Andrés 1992; Tcherkez et al. 2006)

of about 11 ATPs per CO_2 fixed. Indeed, the quantum yield of photosynthesis in an ambient CO_2 and O_2 atmosphere does not differ significantly between C_3 and C_4 species at temperatures between 25° and 30°C (Skillman 2008). Only under hotter and drier conditions does C_4 carbon fixation become more efficient than C_3 fixation. Therefore, C_3 species continue to dominate in most locations.

Why is photorespiration still prevalent?

Several phenomena are responsible for the persistence of photorespiration through 23 million years of low atmospheric CO_2 concentrations.

- Rubisco oxygenation is inseparable from Rubisco carboxylation (Moroney et al. 2013; Tcherkez et al. 2006). Rubisco catalyzes the carboxylation reaction through stabilizing the formation of the enediol conformation of RuBP (Figure 10). This conformation, however, can react with either CO_2 or O_2. The specificity of Rubisco for CO_2 over O_2 derives from stabilizing the six carbon intermediate before it is cleaved to form two molecules of PGA. Consequently, any mutation that increases the specificity of Rubisco for CO_2 over O_2 slows the carboxylation reaction.
- Photorespiration maintains redox homeostasis within plant cells (Scheibe and Dietz 2012). Photosynthesis generates highly reactive compounds as it captures solar energy and converts it into energy-rich, but stable compounds such as carbohydrates. Metabolic pathways, especially under stressful conditions, may become unbalanced, and dangerous compounds such as reactive oxygen species (ROS) may accumulate (Voss et al. 2013). Photorespiration can dissipate many of these potentially dangerous compounds.
- Photorespiration produces H_2O_2 in the peroxisome and thus serves as a mechanism for rapidly transferring a signal of photosynthesis to the entire plant cell (Foyer et al. 2009). This signal is involved in photoperiod detection and pathogen defense as well as responses to abiotic stress.
- Photorespiration serves as a mechanism for plants to use NO_3^- as a nitrogen source without diverting energy from CO_2 fixation. The following provides details about this phenomenon.

Nitrate as a nitrogen source

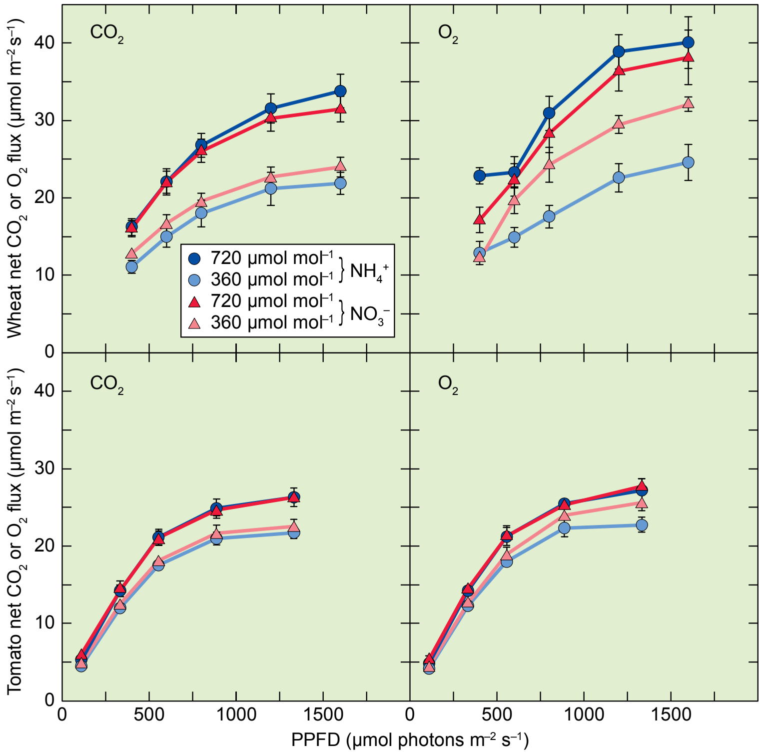
The element nitrogen is a constituent of many organic compounds including all amino acids and nucleic acids. As such, plants require a greater amount of nitrogen than any other mineral element, and its availability generally limits the productivity of natural and agricultural ecosystems (Epstein and Bloom 2005). Conversions among various nitrogen compounds are among the most energy-intensive reactions in life. Consider that plants are generally between 1 and 2% organic nitrogen on a percentage dry weight basis, but that the conversion of NO_3^- into organic nitrogen expends about 25% of the total energy in shoots (Bloom et al. 1989) and roots (Bloom et al. 1992). These processes expend the energy equivalent of 12 ATP per NO_3^- assimilated, whereas most biochemical reactions expend the energy equivalent of one or perhaps two ATP.

Most organisms prefer higher energy forms of nitrogen such as NH_4^+ or amino acids. Phytoplankton (Dortch 1990), fungi (Hodge et al. 2010), cyanobacteria (Ohashi et al. 2011), and bacteria (Luque-Almagro et al. 2011) absorb and assimilate NO_3^- only in the absence of NH_4^+. In many soils, microorganisms quickly absorb NH_4^+ and either assimilate it into amino acids or nitrify it to NO_3^-. NH_4^+ also becomes adsorbed on the soil cation exchange matrix. Because soil microorganisms often ignore NO_3^- and because NO_3^- as an anion moves relatively freely through the soil, NO_3^- is often the predominant form of nitrogen available to plants (Epstein and Bloom 2005).

Nitrogen nutrition, NH_4^+ vs. NO_3^-, neither influences net CO_2 consumption (Figure 11) nor cyclic electron flow around photosystem I at low light levels (Walker et al. 2014). This is consistent with the lack of competition for reductant between CO_2 fixation and NO_3^- assimilation (Robinson 1988) because, as discussed previously, FNR has a higher affinity for Fd_r than NiR. At high light levels and ambient CO_2 and O_2 concentrations, net O_2 evolution is faster (Figures 11 and 12) and cyclic electron flow around photosystem I is higher (Walker et al. 2014) when plants receive NO_3^- rather than NH_4^+ as a nitrogen source. Presumably, plants use reductant generated from the light dependent reactions rather than mitochondrial respiration to assimilate NO_3^- when CO_2 concentration limits CO_2 fixation.

When factors other than CO_2 limit CO_2 fixation, plants may delay assimilating the NO_3^- that they have absorbed. Free NO_3^- may comprise as much as 60% of the total nitrogen in a plant (Maynard et al. 1976). This NO_3^- serves as a metabolically benign osmoticant that balances other ions such as potassium in plant tissues and helps to maintain a favorable cellular water status (Bloom et al. 2012a; Burns et al. 2010; Hanson and Hitz 1983; McIntyre 1997; Veen and Kleinendorst 1986).

In summary, the linkage between photorespiration and NO_3^- assimilation provides higher plants with a relatively abundant nitrogen source that other organisms cannot afford to use, but that C_3 plants can use with little additional cost. Yes, photorespiration may sacrifice 20% to 35% of CO_2 fixation, but plants that are dependent on NO_3^- as a nitrogen source are spared the expense of either devoting 25% of their photosynthate to NO_3^- assimilation or suffering protein deprivation. Apparently, over the last 23 million years, 96% of higher plant species have adapted to this tradeoff.



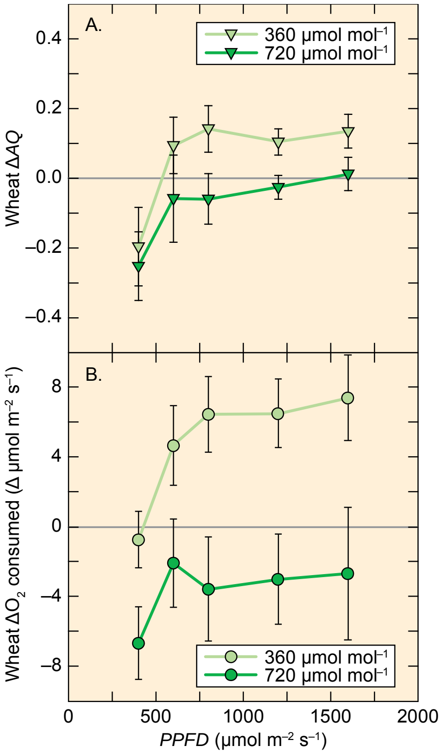


Fig. 11 Response of net CO_2 consumption (left panels) and net O_2 evolution (right panels) to photosynthetic photon flux density (PPFD) in wheat (upper panels) and tomato (lower panels) leaves when the plants received NH_4^+ (blue) or NO_3^- (red) nutrition and were exposed to an atmosphere containing 720 (dark colors) or 360 (light colors) µmol mol^{-1} CO_2. Shown are the means ± SE for 6 wheat plants and 6 to 9 tomato plants per treatment. Notice that in both species, CO_2 fluxes do not differ with N source, and that O_2 fluxes are faster under NO_3^- nutrition than NH_4^+ nutrition, but only at higher light levels and 360 µmol mol^{-1} CO_2. (Cousins and Bloom 2004; Searles and Bloom 2003)

Fig. 12 Responses of wheat shoots (mean ± SE, *n* = 6) to photosynthetic photon flux density (*PPFD*). (A) Changes in assimilatory quotient (*AQ* = net CO_2 consumed / net O_2 evolved) with the shift from NO_3^- to NH_4^+ as a N source. (B) Changes in the gross O_2 consumed (gross O_2 evolved minus net O_2 evolved) with the shift from NO_3^- to NH_4^+ as a N source. As light levels increased and 360 µmol mol^{-1} CO_2 limited carbon fixation, exposure to NO_3^- stimulated the light dependent reactions of photosynthesis to split water, evolve oxygen, and transfer electrons to NO_3^- and NO_2^- rather than to CO_2, and decreased gross O_2 consumption (Cousins and Bloom 2004).

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Conflicts of Interest

The author has no conflicts of interest with regards to this research.

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