

The oxygen and carbon dioxide compensation points of C₃ plants: Possible role in regulating atmospheric oxygen

(photosynthetic carbon/O₂ inhibition/photorespiration/*Nicotiana tobacum*/*Spinacea oleracea*)

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ABSTRACT The O₂ and CO₂ compensation points (O₂ [and CO₂ [) of plants in a closed system depend on the ratio of CO₂ and O₂ concentrations in air and in the chloroplast and the specificities of ribulose biphosphate carboxylase/oxygenase (Rubisco). The photosynthetic O₂ [is defined as the atmospheric O₂ level, with a given CO₂ level and temperature, at which net O₂ exchange is zero. In experiments with C₃ plants, the O₂ [with 220 ppm CO₂ is 23% O₂; O₂ [increases to 27% with 350 ppm CO₂ and to 35% with 700 ppm CO₂. At O₂ levels below the O₂ [, CO₂ uptake and reduction are accompanied by net O₂ evolution. At O₂ levels above the O₂ [, net O₂ uptake occurs with a reduced rate of CO₂ fixation, more carbohydrates are oxidized by photorespiration to products of the C₂ oxidative photosynthetic carbon cycle, and plants senesce prematurely. The CO₂ [increases from 50 ppm CO₂ with 21% O₂ to 220 ppm with 100% O₂. At a low CO₂/high O₂ ratio that inhibits the carboxylase activity of Rubisco, much malate accumulates, which suggests that the oxygen-insensitive phosphoenolpyruvate carboxylase becomes a significant component of the lower CO₂ fixation rate. Because of low global levels of CO₂ and a Rubisco specificity that favors the carboxylase activity, relatively rapid changes in the atmospheric CO₂ level should control the permissive O₂ [that could lead to slow changes in the immense O₂ pool.

In contrast to the attention that regulation of atmospheric CO₂ has attracted, a photosynthetic O₂ compensation point (O₂ [) has not been described or considered as part of the global O₂ cycle that has equilibrated the atmospheric O₂ level at 21%. Although O₂ inhibition of photosynthesis has been known for 75 yr (1) and its biochemical process has been recognized as photorespiration (2–8), the existence of an O₂ [was not described because at high O₂ levels, ¹⁸O₂ exchange and a lower rate of CO₂ fixation continue, and plants senesce only slowly. High CO₂ alleviates O₂ inhibition and low CO₂ intensifies it, as expected from the dual activities of ribulose biphosphate carboxylase/oxygenase (Rubisco) (9). In the absence of O₂ the K_m (CO₂) is ≈12 μM, which increases to 26–42 μM between 20° and 30°C with 21% O₂. Reported K_m (O₂) values for the oxygenase activity are between 250 and 400 μM O₂ at 20°–30°C in the presence of low levels of CO₂ (9).

Photosynthesis contributes to the atmospheric O₂ balance by oxygen production from water during CO₂ assimilation in the C₃ reductive photosynthetic carbon cycle. Net CO₂ fixation by the carboxylase activity of Rubisco and subsequent reduction are illustrated on the left part of Fig. 1. The oxygenase activity of Rubisco initiates photorespiration via the C₂ oxidative photosynthetic carbon cycle that composes both parts of Fig. 1. The C₂ and C₃ carbon cycles coexist and together constitute photosynthetic carbon metabolism (10, 11). In the complete C₂ cycle the CO₂ released is refixed to regenerate the ribulose

biphosphate to sustain the C₂ cycle. Refixation of CO₂ generates the same amount of O₂ as taken up during the C₂ cycle. There is no net CO₂ and O₂ gas exchange during photorespiration (11) unless the complete C₂ cycle is blocked or metabolically interrupted by accumulation or removal of products such as glycine or serine. Photorespiration dissipates excess photosynthetic capacity (ATP and NADPH) without CO₂ reduction or net O₂ change. Photosynthetic carbon metabolism is a competition between CO₂ and O₂ for the dual activities of Rubisco, based on the ratio of CO₂ and O₂ concentrations in the chloroplast and the specificities of Rubisco for its gaseous substrates. As a consequence, the distribution of carbon flow around the C₃ and C₂ cycles is proportional to the ratio of atmospheric CO₂ and O₂ and to processes for CO₂ import and O₂ export.

The CO₂ compensation point (CO₂ [) is defined as the CO₂ concentration at which net CO₂ fixation is zero at a given O₂ level and temperature (12, 13). It has been assumed that at the CO₂ [respiratory and photorespiratory processes oxidize carbohydrate to CO₂ as fast as CO₂ is photosynthetically fixed. This concept may have to be modified to include CO₂ fixation by phosphoenolpyruvate carboxylase at low ratios of CO₂ to O₂ (see Discussion). The CO₂ [is ≈50 ppm CO₂ for an isolated C₃ plant in a closed chamber at 21% O₂ and 20°C. A minimum atmospheric CO₂ equilibrium, resulting from the capacity of plants for CO₂ uptake and counteracted by abiotic and biotic CO₂-generating processes of the global carbon cycle (14), was probably reached millions of years ago. Ice cores from the past 165,000 yr (15) show that such an equilibrium has been ≈235 ± 45 ppm CO₂ until this last century.

Much attention has been devoted over the past 50 yr to the increased atmospheric CO₂ and its regulation and to the CO₂ [with 21% O₂, but the role of Rubisco in regulating the atmospheric O₂ has not been considered. Because of the dual activities of Rubisco, an O₂ [should exist in addition to a CO₂ [. In correspondence with the CO₂ [, the O₂ [is defined as the O₂ concentration at which net O₂ exchange is zero at a given CO₂ level and temperature. A photosynthetic O₂ [should be expected as a part of the global O₂ cycle with a given level of CO₂ and should establish upper limits on the O₂ concentration at which a positive carbon balance allows plant growth. Studies with ¹⁸O₂ revealed a rapid exchange of atmospheric O₂ in plants during photosynthesis that conformed with a significant O₂ uptake by photorespiration (16–18). Based on net O₂ exchange rather than on CO₂ fixation, we have found that there is an O₂ [for C₃ plants (tobacco and spinach) that is not far above the current concentration of atmospheric O₂. At O₂ levels above the O₂ [there is measurable net oxygen uptake by these plants, while CO₂ fixation continues at reduced rates with concomitant malate accumulation. With the lowest past recorded levels of CO₂ (≈220 ppm), the O₂ [with an isolated C₃ plant is ≈23% near current atmospheric levels of O₂, and with

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Abbreviations: Rubisco, ribulose biphosphate carboxylase/oxygenase; [, compensation point.

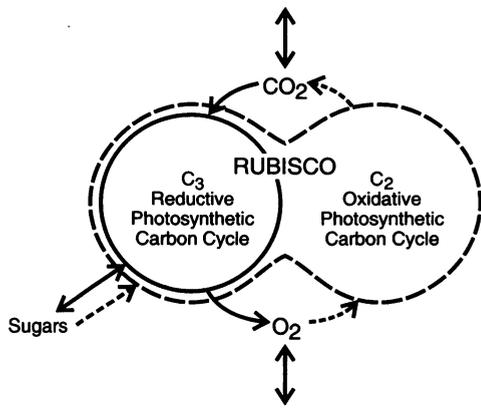


FIG. 1. Scheme for photosynthetic carbon metabolism that consists of the C₃ reductive cycle (solid line on left) and the C₂ oxidative cycle (dashed lines around both right and left sides).

increased CO₂ to <350 ppm today the O₂ ↑ increases to ≈27%. From the global carbon and oxygen cycles and to allow plant growth, atmospheric CO₂ levels must be >CO₂ ↑ of a C₃ plant, and the O₂ level must be <O₂ ↑. A lower limit for atmospheric CO₂ (≈235 ± 45 ppm) and an upper limit of O₂ (≈21%) would appear to be the global equilibria that are set by the average specificities of the abundant Rubisco for CO₂ and O₂ and the corresponding C₃ and C₂ photosynthetic carbon cycles.

MATERIALS AND METHODS

A closed photosynthetic chamber for simultaneous measurements of changes in the atmospheric O₂ and CO₂ was constructed for these tests. It is essential that the system does not leak O₂. Six- to seven-week-old whole tobacco (*Nicotiana tabacum* cv. Samsun) or spinach (*Spinacea oleracea*) plants in pots, which were enclosed in a gas-tight cover just before the experiment to prevent gas exchange with the soil, were put in an air-tight, 19-liter, glass chamber at a controlled temperature, usually of 20°C. The atmosphere in the chamber was stirred with a fan. The CO₂ and O₂ concentrations were continuously measured by pumping a stream of the air through a closed, oxygen-light, circuit with an IR gas analyzer (Binos 1.1, Leybold-Heraeus, Hanau, Germany) for CO₂ measurements and an oxygen electrode (Hansatech Instruments, Pentney King's Lynn, U.K.) covered with 2 ml of water. The CO₂ and O₂ contents of the atmosphere in the chamber could be arbitrarily set by aerating the chamber with oxygen or nitrogen from pressurized steel cylinders and by injecting CO₂ with a calibrated syringe through a small rubber plug in the jar lid. According to the IR gas analyzer recordings, additional volumes of CO₂ were repeatedly supplied to maintain a desired constant CO₂ concentration within ±5%. Experiments were run in atmospheres ranging from 220 to 1000 ppm CO₂ and oxygen concentrations from 2 to 100%. The O₂ level during an experiment was not supplemented because the percentage changes were relatively small. All experiments were run for a length of time that resulted in the same amount of total CO₂ uptake. Time periods of up to 8 hr were required at low CO₂ (220 ppm) or high O₂ levels (40–90%), when the rates of CO₂ fixation were reduced. Each point on the figures represents one experiment. Individual plants could be used for the experiments of 1 day. At the end of a day, the leaves were removed, and their areas were determined with an areometer. Upon change of atmosphere the plants were allowed to acclimatize to the new conditions for 1 hr. Usually the rates of gas exchange became constant after 30 min.

Three physiological variables that would alter photorespiration were kept constant—temperature, light intensity, and previous growth conditions. The effect of altering these conditions on the O₂ ↑ has not been studied in detail. Temperature

alters the differential solubility of O₂ to CO₂, as increased temperature decreases the solubility of CO₂ more than O₂ (19). The relative amount of photorespiration increases more at higher light intensity than net CO₂ fixation (20), perhaps because more photosynthetic assimilatory capacity needs to be dissipated. A constant light intensity of 300 μmol·m⁻²·s⁻¹ from fluorescent and incandescent bulbs was used in the current experiments. When the light intensity was decreased, the magnitude of O₂ uptake above the O₂ ↑ decreased. Plants were used immediately after growth in greenhouses or growth chambers. Plants held more than 12 hr in the dark had lower levels of O₂ uptake when over the O₂ ↑, presumably from depletion of the carbohydrate, needed for photorespiration.

RESULTS

The CO₂ ↑ has been measured in the past by placing a plant in a closed chamber in the light with air and determining the CO₂ equilibrium (12, 13), and the CO₂ ↑ has been reported at ≈50 ± 10 ppm CO₂ with 21% O₂ at 20°C. In Fig. 2 different CO₂ ↑s are shown for atmospheres with different O₂ levels. The rates of CO₂ removal (left side) at the beginning of the experiment indicate the inhibition of CO₂ fixation by changing O₂ levels. The constant CO₂ levels reached after ≈50 min, are the CO₂ ↑s. There is a linear dependence of the CO₂ ↑ on the oxygen concentration between 10% and 42% O₂ (curve not shown), indicating the competition between O₂ and CO₂ for the oxygenase or carboxylase activity of Rubisco. At 100% O₂, the CO₂ ↑ had risen to 220 ppm CO₂. The similarity of this high CO₂ ↑ with minimal past atmospheric CO₂ levels may be coincidental but from the chloroplast thylakoid where O₂ is evolved, oxygen diffusion outward may start at concentrations well above air level (21).

When explaining the O₂ ↑, net O₂ evolution occurs with net photosynthetic CO₂ reduction, and net O₂ uptake should occur when the rate of photorespiration exceeds the rate of net O₂ evolution from reduction of fixed CO₂ to carbohydrate. The O₂ ↑ is the O₂ level when net O₂ change becomes zero in the presence of a given level of CO₂ (Fig. 3). The maximum rate of O₂ release in the plant chamber, at a constant CO₂ level of 350 ppm and oxygen concentrations below the O₂ ↑, declined

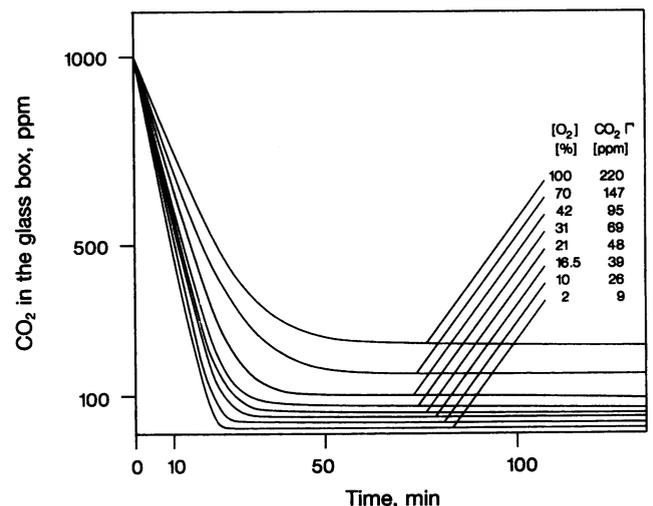


FIG. 2. Time course of net CO₂ uptake at 20°C by a tobacco plant at various oxygen concentrations in a closed chamber. The rate of CO₂ fixation is the initial slope on the left, and the CO₂ ↑ is the equilibrium on the right, when there is no further change in the CO₂ level in the closed chamber. Corresponding curves for oxygen were not measured because the contribution of the plant to the large O₂ volume in the jar (19 liters) is relatively small in the short experimental time. Therefore, the O₂ ↑ was determined from a plot of the rates of oxygen release or uptake vs. O₂ concentration (Fig. 3).

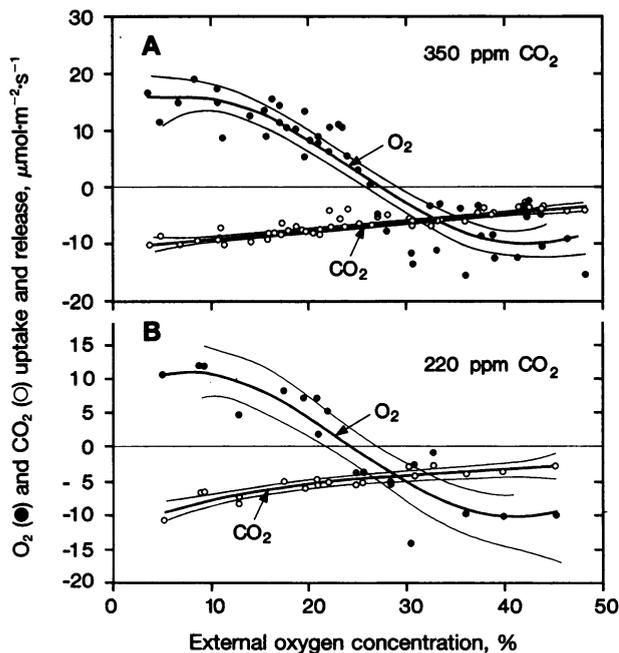


FIG. 3 (A). Photosynthetic CO_2 and O_2 gas exchange of tobacco plants depending on the oxygen concentration in a closed chamber. The main line represents the regression curves from the data, while the thin lines include the areas for a 5% statistical error. The temperature was 20°C , and the CO_2 concentration was maintained at 350 ppm. $\text{O}_2 \uparrow$ is defined as the atmospheric oxygen concentration at which the change in O_2 level in a closed system was zero. At an O_2 concentration $< \text{O}_2 \uparrow$, net O_2 evolution occurred (to the left); at O_2 levels $> \text{O}_2 \uparrow$, net O_2 uptake was recorded. O_2 inhibition of CO_2 fixation is indicated by the decrease in rate of net CO_2 uptake. (B) Influence of atmospheric oxygen concentration on photosynthetic CO_2 and O_2 gas exchange by tobacco plants at a constant CO_2 level of 220 ppm and at 20°C .

as the atmospheric O_2 was increased from 20% to the $\text{O}_2 \uparrow$ at 27% O_2 (Fig. 3A). Similarly, the $\text{O}_2 \uparrow$ was $\approx 23\%$ with a constant lower level of 220 ppm CO_2 (Fig. 3B). At O_2 levels above the $\text{O}_2 \uparrow$, net O_2 uptake presumably from photorespiration, increased to levels approaching rates of O_2 evolution at low oxygen concentrations (Fig. 3). The results are consistent with the main use of photosynthetic energy below the $\text{O}_2 \uparrow$ for CO_2 fixation with O_2 evolution. At O_2 levels above the $\text{O}_2 \uparrow$ a high rate of O_2 uptake seems to imply that much light energy was consumed by increased photorespiration with oxygen uptake serving as the acceptor of photosynthetic energy rather than CO_2 . As discussed later, CO_2 fixation without reduction to carbohydrate, as by phosphoenolpyruvate carboxylase, would evolve less O_2 evolution. Low rates of O_2 uptake by dark respiration increased from $\approx 1.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 8% O_2 to $3 \mu\text{mol}$ at 54% O_2 .

Inevitable variations when working with many plants, even when raised under controlled conditions, resulted in some scattering of the data (Fig. 3). However, a consistent trend in all the results indicated that the $\text{O}_2 \uparrow$ values could be measured within $< 1\%$. Similar results were obtained with tobacco and spinach plants. The $\text{O}_2 \uparrow$ decreased with a decrease in the CO_2 level to maintain an apparently similar CO_2/O_2 ratio for the two competitive activities of Rubisco. Data in Table 1 plot as a straight line for the $\text{O}_2 \uparrow$ vs. the CO_2 concentrations.

Plants survive only at oxygen concentrations below the $\text{O}_2 \uparrow$ and CO_2 concentrations above the $\text{CO}_2 \uparrow$. C_3 plants held below the $\text{CO}_2 \uparrow$ evolve CO_2 and senesce in 5 to 6 days in continuous light due to carbohydrate depletion by photorespiration (12, 13). The rate of senescence depends on the rate of photorespiration, which is faster at higher temperatures, high light

Table 1. Oxygen \uparrow at 20° with increased CO_2 concentrations for a tobacco plant

CO_2 concentration, ppm	$\text{O}_2 \uparrow$, % O_2
220	23
350	27*
700	35

*The corresponding $\text{O}_2 \uparrow$ of spinach was 28% O_2 .

intensity, and higher oxygen and lower CO_2 (19, 20, 22). Above the $\text{O}_2 \uparrow$ the C_3 plants exhibited negative O_2 balance with high rates of net O_2 uptake. Whereas below the $\text{CO}_2 \uparrow$ there is net CO_2 loss from photorespiration, above the $\text{O}_2 \uparrow$ with net O_2 uptake there was still continuous net CO_2 fixation at 20–50% of that at O_2 levels below the $\text{O}_2 \uparrow$ (Fig. 3). In subsequent papers we will present a review of the literature and additional data showing that C_3 plants oxidize their carbohydrates by increased rates of photorespiration at higher O_2 to form large amounts of oxidized products (glycolate, glycine, serine, glycinate) of the C_2 oxidative photosynthetic carbon cycle (6, 10, 11). At O_2 levels above the $\text{O}_2 \uparrow$, growth decreased and our tobacco plants senesced within ≈ 10 days in continuous light or within ≈ 14 –16 days on a 16:8 hr day–night regime.

Whereas below the $\text{CO}_2 \uparrow$ there is net CO_2 loss from photorespiration, above the $\text{O}_2 \uparrow$ with net O_2 uptake there was still continuous net CO_2 fixation at 20–50% of that at O_2 levels below the $\text{O}_2 \uparrow$ (Fig. 3). Net O_2 uptake in the light, but with continued CO_2 fixation, invokes several hypotheses. (i) Above the $\text{O}_2 \uparrow$, increased O_2 uptake from photorespiration could exceed the lower rates of CO_2 reduction to carbohydrate and of O_2 evolution. This hypothesis is supported by continuous $^{18}\text{O}_2$ exchange measurements (17). (ii) Another hypothesis, consistent with oxygen inhibition of CO_2 fixation by Rubisco, would be a partial substitution of CO_2 fixation by Rubisco for bicarbonate fixation by the oxygen-insensitive phosphoenolpyruvate carboxylase, which results in malate formation with less O_2 evolution, as occurs in the mesophyll cells of a C_4 plant. CO_2 fixation from new CO_2 , or from photorespiration without net reduction to carbohydrate, would greatly reduce O_2 evolution. GC-MS analyses found that the malate content of our tobacco leaves from plants placed in an atmosphere of 350 ppm CO_2 and 40% O_2 was five times higher than that of leaves from plants maintained at 350 ppm CO_2 and 21% O_2 . C_3 leaves have substantial amounts of phosphoenolpyruvate carboxylase (23), and phosphoenolpyruvate could be formed from 3-phosphoglycerate produced by the ribulose biphosphate oxygenase reaction. This malate pool can be gluconeogenic in the dark (24) and might explain why the leaves of tobacco plants survived in high O_2 for 2 weeks on a 16:8 hr light–dark day with 30% O_2 and 350 ppm CO_2 . However, in continuous light with 40% O_2 and 350 ppm CO_2 the plants senesced substantially faster, probably because of continuous net degradation of carbohydrates by photorespiration. Dual photosynthetic processes for fixing inorganic carbon (CO_2 and HCO_3^-), one of which is not competitive with O_2 uptake, explain the noncoincidence of the CO_2 and $\text{O}_2 \uparrow$ s.

DISCUSSION

The $\text{O}_2 \uparrow$ represents an upper limit on the atmospheric O_2 (with a given CO_2 level) above which plants cannot survive. Thus, a minimum atmospheric CO_2 concentration of 220 ppm CO_2 restricts the atmospheric O_2 level to some value less than the 23% $\text{O}_2 \uparrow$. A lower atmospheric oxygen concentration than the $\text{O}_2 \uparrow$ is required for a plant to grow. In the global O_2 cycle (14) atmospheric O_2 is lowered by oxidation of minerals, pyrite, and biological materials. The difference between our current global O_2 levels at 21% and the measured 23% $\text{O}_2 \uparrow$ of a C_3 plant with 220 ppm CO_2 (at 20°C) seems small, suggesting that the

global O₂ level is close to the O₂ ⌈ for plant photosynthesis. With the past minimum CO₂ level, the global O₂ level could not have risen further because that would have limited the survival of C₃ plants, the major source of atmospheric oxygen. Higher O₂ levels could only have existed if the CO₂ levels were higher than in the recent past. The global atmospheric 0.03% CO₂ and 21% O₂ equilibria seem to be limits set by the average specificity properties of Rubisco from plants and algae. Since the global CO₂ level has risen to 350 ppm CO₂ in this century, the potential O₂ ⌈ for C₃ plants has risen to 27% at 20°C (Fig. 3A), and the permissive global O₂ equilibrium could also rise. However, a higher permissive O₂ level may be offset by accelerated O₂ uptake at present times from combustion of fossil photosynthate (25).

The specificity of Rubisco seems to establish both a CO₂ ⌈ and O₂ ⌈, which depend on the ratio of CO₂ to O₂ concentration. These two photosynthetic ⌈s, in turn, are rapid parts of the global carbon and oxygen cycles, which had equilibrated with the atmosphere, at least over the past 165,000 yr between 180 and 280 ppm (average 235 ± 45 ppm) CO₂ (15) and 21% O₂ (26). Earlier when the CO₂ level was >1000–1500 ppm CO₂ and/or the O₂ level was lower, Rubisco functioned primarily only as a carboxylase, and the CO₂/O₂ ratio for the dual activities of Rubisco was not a controlling factor on plant growth. However, once the level of O₂ increased and that of CO₂ decreased, the oxygenase activity of Rubisco limited CO₂ removal and the CO₂/O₂ ratio became a governing factor on net photosynthesis, plant growth, and the atmospheric composition. O₂ peaked at ≈35% about 300 million yr ago for a period of millions of years (26). This condition was mimicked in our growth chamber by using 700 ppm CO₂, which allowed an O₂ ⌈ of 35% for a C₃ plant (Table 1). In the past century, the CO₂ level has risen from ≈250 ppm, where the O₂ ⌈ was 23–24% to 350 ppm CO₂ with a permissive O₂ ⌈ of 27%. The potential O₂ ⌈ can rise with increased CO₂ concentration to maintain a CO₂/O₂ ratio based on the average Rubisco specificity for these two substrates. Because there has been ≈700 times more O₂ in the atmosphere than CO₂, any permissive increase in atmospheric O₂ will be very slow. Because of the very low level of CO₂ and a specificity of Rubisco that favors the carboxylase activity, it is changes in the level of atmospheric CO₂ that quickly change the CO₂/O₂ ratio relatively to slow changes in the immense O₂ pool.

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