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The oxygen and carbon dioxide compensation points of C_3 plants: Possible role in regulating atmospheric oxygen

(photosynthetic carbon/O_2 inhibition/photorespiration/*Nicotiana tobacum*/*Spinacea oleracea*)

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ABSTRACT **The O_2 and CO_2 compensation points ($O_2\lceil$ and $CO_2\lceil$) of plants in a closed system depend on the ratio of CO_2 and O_2 concentrations in air and in the chloroplast and the specificities of ribulose bisphosphate carboxylase/oxygenase (Rubisco). The photosynthetic $O_2\lceil$ is defined as the atmospheric O_2 level, with a given CO_2 level and temperature, at which net O_2 exchange is zero. In experiments with C_3 plants, the $O_2\lceil$ with 220 ppm CO_2 is 23% O_2; $O_2\lceil$ increases to 27% with 350 ppm CO_2 and to 35% O_2 with 700 ppm CO_2. At O_2 levels below the $O_2\lceil$, CO_2 uptake and reduction are accompanied by net O_2 evolution. At O_2 levels above the $O_2\lceil$, net O_2 uptake occurs with a reduced rate of CO_2 fixation, more carbohydrates are oxidized by photorespiration to products of the C_2 oxidative photosynthetic carbon cycle, and plants senesce prematurely. The $CO_2\lceil$ increases from 50 ppm CO_2 with 21% O_2 to 220 ppm with 100% O_2. At a low CO_2/high O_2 ratio that inhibits the carboxylase activity of Rubisco, much malate accumulates, which suggests that the oxygen-insensitive phospho*enol*pyruvate carboxylase becomes a significant component of the lower CO_2 fixation rate. Because of low global levels of CO_2 and a Rubisco specificity that favors the carboxylase activity, relatively rapid changes in the atmospheric CO_2 level should control the permissive $O_2\lceil$ that could lead to slow changes in the immense O_2 pool.**

In contrast to the attention that regulation of atmospheric CO_2 has attracted, a photosynthetic O_2 compensation point ($O_2\lceil$) has not been described or considered as part of the global O_2 cycle that has equilibrated the atmospheric O_2 level at 21%. Although O_2 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 $O_2\lceil$ was not described because at high O_2 levels, $^{18}O_2$ exchange and a lower rate of CO_2 fixation continue, and plants senesce only slowly. High CO_2 alleviates O_2 inhibition and low CO_2 intensifies it, as expected from the dual activities of ribulose bisphosphate carboxylase/oxygenase (Rubisco) (9). In the absence of O_2 the K_m (CO_2) is $\approx$12 μM, which increases to 26–42 μM between 20° and 30°C with 21% O_2. Reported K_m (O_2) values for the oxygenase activity are between 250 and 400 μM O_2 at 20°–30°C in the presence of low levels of CO_2 (9).

Photosynthesis contributes to the atmospheric O_2 balance by oxygen production from water during CO_2 assimilation in the C_3 reductive photosynthetic carbon cycle. Net CO_2 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 C_2 oxidative photosynthetic carbon cycle that composes both parts of Fig. 1. The C_2 and C_3 carbon cycles coexist and together constitute photosynthetic carbon metabolism (10, 11). In the complete C_2 cycle the CO_2 released is refixed to regenerate the ribulose bisphosphate to sustain the C_2 cycle. Refixation of CO_2 generates the same amount of O_2 as taken up during the C_2 cycle. There is no net CO_2 and O_2 gas exchange during photorespiration (11) unless the complete C_2 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 CO_2 reduction or net O_2 change. Photosynthetic carbon metabolism is a competition between CO_2 and O_2 for the dual activities of Rubisco, based on the ratio of CO_2 and O_2 concentrations in the chloroplast and the specificities of Rubisco for its gaseous substrates. As a consequence, the distribution of carbon flow around the C_3 and C_2 cycles is proportional to the ratio of atmospheric CO_2 and O_2 and to processes for CO_2 import and O_2 export.

The CO_2 compensation point ($CO_2\lceil$) is defined as the CO_2 concentration at which net CO_2 fixation is zero at a given O_2 level and temperature (12, 13). It has been assumed that at the $CO_2\lceil$ respiratory and photorespiratory processes oxidize carbohydrate to CO_2 as fast as CO_2 is photosynthetically fixed. This concept may have to be modified to include CO_2 fixation by phospho*enol*pyruvate carboxylase at low ratios of CO_2 to O_2 (see *Discussion*). The $CO_2\lceil$ is $\approx$50 ppm CO_2 for an isolated C_3 plant in a closed chamber at 21% O_2 and 20°C. A minimum atmospheric CO_2 equilibrium, resulting from the capacity of plants for CO_2 uptake and counteracted by abiotic and biotic CO_2-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 $\approx$235 $\pm$ 45 ppm CO_2 until this last century.

Much attention has been devoted over the past 50 yr to the increased atmospheric CO_2 and its regulation and to the $CO_2\lceil$ with 21% O_2, but the role of Rubisco in regulating the atmospheric O_2 has not been considered. Because of the dual activities of Rubisco, an $O_2\lceil$ should exist in addition to a $CO_2\lceil$. In correspondence with the $CO_2\lceil$, the $O_2\lceil$ is defined as the O_2 concentration at which net O_2 exchange is zero at a given CO_2 level and temperature. A photosynthetic $O_2\lceil$ should be expected as a part of the global O_2 cycle with a given level of CO_2 and should establish upper limits on the O_2 concentration at which a positive carbon balance allows plant growth. Studies with $^{18}O_2$ revealed a rapid exchange of atmospheric O_2 in plants during photosynthesis that conformed with a significant O_2 uptake by photorespiration (16–18). Based on net O_2 exchange rather than on CO_2 fixation, we have found that there is an $O_2\lceil$ for C_3 plants (tobacco and spinach) that is not far above the current concentration of atmospheric O_2. At O_2 levels above the $O_2\lceil$ there is measurable net oxygen uptake by these plants, while CO_2 fixation continues at reduced rates with concomitant malate accumulation. With the lowest past recorded levels of CO_2 ($\approx$220 ppm), the $O_2\lceil$ with an isolated C_3 plant is $\approx$23% near current atmospheric levels of O_2, and with

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Abbreviations: Rubisco, ribulose bisphosphate carboxylase/oxygenase; $\lceil$, compensation point.

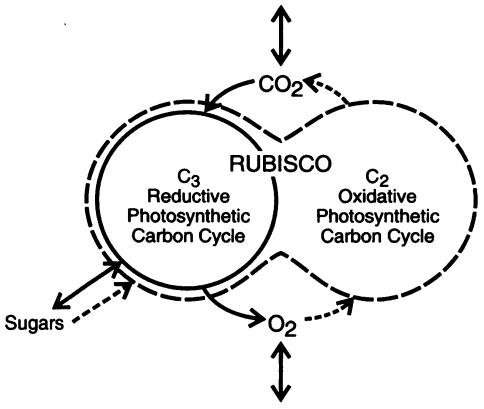

FIG. 1. Scheme for photosynthetic carbon metabolism that consists of the C_3 reductive cycle (solid line on left) and the C_2 oxidative cycle (dashed lines around both right and left sides).

increased CO_2 to <350 ppm today the $O_2\lceil$ increases to ≈27%. From the global carbon and oxygen cycles and to allow plant growth, atmospheric CO_2 levels must be $>CO_2\lceil$ of a C_3 plant, and the O_2 level must be $<O_2\lceil$. A lower limit for atmospheric CO_2 (≈235 ± 45 ppm) and an upper limit of O_2 (≈21%) would appear to be the global equilibria that are set by the average specificities of the abundant Rubisco for CO_2 and O_2 and the corresponding C_3 and C_2 photosynthetic carbon cycles.

MATERIALS AND METHODS

A closed photosynthetic chamber for simultaneous measurements of changes in the atmospheric O_2 and CO_2 was constructed for these tests. It is essential that the system does not leak O_2. 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 CO_2 and O_2 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–Heareus, Hanau, Germany) for CO_2 measurements and an oxygen electrode (Hansatech Instruments, Pentney King's Lynn, U.K.) covered with 2 ml of water. The CO_2 and O_2 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 CO_2 with a calibrated syringe through a small rubber plug in the jar lid. According to the IR gas analyzer recordings, additional volumes of CO_2 were repeatedly supplied to maintain a desired constant CO_2 concentration within ±5%. Experiments were run in atmospheres ranging from 220 to 1000 ppm CO_2 and oxygen concentrations from 2 to 100%. The O_2 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 CO_2 uptake. Time periods of up to 8 hr were required at low CO_2 (220 ppm) or high O_2 levels (40–90%), when the rates of CO_2 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 $O_2\lceil$ has not been studied in detail. Temperature alters the differential solubility of O_2 to CO_2, as increased temperature decreases the solubility of CO_2 more than O_2 (19). The relative amount of photorespiration increases more at higher light intensity than net CO_2 fixation (20), perhaps because more photosynthetic assimilatory capacity needs to be dissipated. A constant light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from fluorescent and incandescent bulbs was used in the current experiments. When the light intensity was decreased, the magnitude of O_2 uptake above the $O_2\lceil$ 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 O_2 uptake when over the $O_2\lceil$, presumably from depletion of the carbohydrate, needed for photorespiration.

RESULTS

The $CO_2\lceil$ has been measured in the past by placing a plant in a closed chamber in the light with air and determining the CO_2 equilibrium (12, 13), and the $CO_2\lceil$ has been reported at ≈50 ± 10 ppm CO_2 with 21% O_2 at 20°C. In Fig. 2 different $CO_2\lceil$s are shown for atmospheres with different O_2 levels. The rates of CO_2 removal (left side) at the beginning of the experiment indicate the inhibition of CO_2 fixation by changing O_2 levels. The constant CO_2 levels reached after ≈50 min, are the $CO_2\lceil$s. There is a linear dependence of the $CO_2\lceil$ on the oxygen concentration between 10% and 42% O_2 (curve not shown), indicating the competition between O_2 and CO_2 for the oxygenase or carboxylase activity of Rubisco. At 100% O_2, the $CO_2\lceil$ had risen to 220 ppm CO_2. The similarity of this high $CO_2\lceil$ with minimal past atmospheric CO_2 levels may be coincidental but from the chloroplast thylakoid where O_2 is evolved, oxygen diffusion outward may start at concentrations well above air level (21).

When explaining the $O_2\lceil$, net O_2 evolution occurs with net photosynthetic CO_2 reduction, and net O_2 uptake should occur when the rate of photorespiration exceeds the rate of net O_2 evolution from reduction of fixed CO_2 to carbohydrate. The $O_2\lceil$ is the O_2 level when net O_2 change becomes zero in the presence of a given level of CO_2 (Fig. 3). The maximum rate of O_2 release in the plant chamber, at a constant CO_2 level of 350 ppm and oxygen concentrations below the $O_2\lceil$, declined

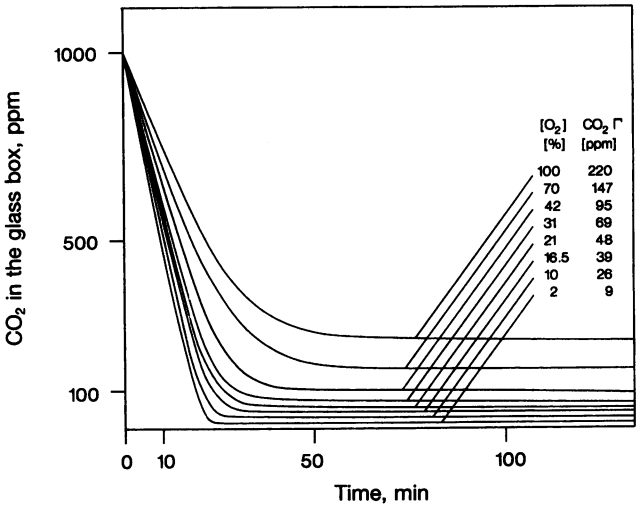

FIG. 2. Time course of net CO_2 uptake at 20°C by a tobacco plant at various oxygen concentrations in a closed chamber. The rate of CO_2 fixation is the initial slope on the left, and the $CO_2\lceil$ is the equilibrium on the right, when there is no further change in the CO_2 level in the closed chamber. Corresponding curves for oxygen were not measured because the contribution of the plant to the large O_2 volume in the jar (19 liters) is relatively small in the short experimental time. Therefore, the $O_2\lceil$ was determined from a plot of the rates of oxygen release or uptake vs. O_2 concentration (Fig. 3).

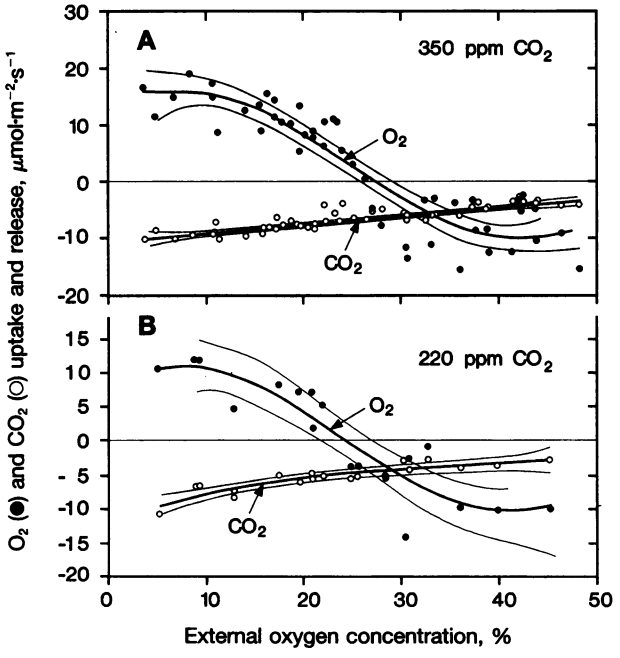

FIG. 3 (*A*). Photosynthetic CO_2 and O_2 gas exchange of tobacco plants depending on the oxygen concentration in a closed chamber. The main line represents the regression curves from the data, while the thin lines include the areas for a 5% statistical error. The temperature was 20°C, and the CO_2 concentration was maintained at 350 ppm. O_2 ⌈ 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 $<O_2$⌈, net O_2 evolution occurred (to the left); at O_2 levels $>O_2$ ⌈, 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 O_2⌈ at 27% O_2 (Fig. 3*A*). Similarly, the O_2 ⌈ was ≈23% with a constant lower level of 220 ppm CO_2 (Fig. 3*B*). At O_2 levels above the O_2⌈, 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 O_2⌈ for CO_2 fixation with O_2 evolution. At O_2 levels above the O_2⌈ 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 phospho*enol*pyruvate carboxylase, would evolve less O_2 evolution. Low rates of O_2 uptake by dark respiration increased from ≈1.5 μmol·m^{-2}·s^{-1} at 8% O_2 to 3 μmol 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 O_2⌈ values could be measured within <1%. Similar results were obtained with tobacco and spinach plants. The O_2⌈ 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 O_2⌈ vs. the CO_2 concentrations.

Plants survive only at oxygen concentrations below the O_2⌈ and CO_2 concentrations above the CO_2⌈. C_3 plants held below the CO_2⌈ 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 intensity, and higher oxygen and lower CO_2 (19, 20, 22). Above the O_2⌈ the C_3 plants exhibited negative O_2 balance with high rates of net O_2 uptake. Whereas below the CO_2⌈ there is net CO_2 loss from photorespiration, above the O_2⌈ with net O_2 uptake there was still continuous net CO_2 fixation at 20–50% of that at O_2 levels below the O_2⌈ (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, glycerate) of the C_2 oxidative photosynthetic carbon cycle (6, 10, 11). At O_2 levels above the O_2⌈, 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.

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

CO_2 concentration, ppm	O_2⌈, % O_2
220	23
350	27*
700	35

*The corresponding O_2⌈ of spinach was 28% O_2.

Whereas below the CO_2 ⌈ there is net CO_2 loss from photorespiration, above the O_2⌈ with net O_2 uptake there was still continuous net CO_2 fixation at 20–50% of that at O_2 levels below the O_2⌈ (Fig. 3). Net O_2 uptake in the light, but with continued CO_2 fixation, invokes several hypotheses. (*i*) Above the O_2 ⌈, 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 $^{18}O_2$ 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 phospho*enol*-pyruvate 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 phospho*enol*pyruvate carboxylase (23), and phospho*enol*pyruvate could be formed from 3-phosphoglycerate produced by the ribulose bisphosphate 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 O_2⌈s.

DISCUSSION

The O_2⌈ 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% O_2⌈. A lower atmospheric oxygen concentration than the O_2⌈ 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% O_2⌈ of a C_3 plant with 220 ppm CO_2 (at 20°C) seems small, suggesting that the

global O_2 level is close to the O_2 ⌈ for plant photosynthesis. With the past minimum CO_2 level, the global O_2 level could not have risen further because that would have limited the survival of C_3 plants, the major source of atmospheric oxygen. Higher O_2 levels could only have existed if the CO_2 levels were higher than in the recent past. The global atmospheric 0.03% CO_2 and 21% O_2 equilibria seem to be limits set by the average specificity properties of Rubisco from plants and algae. Since the global CO_2 level has risen to 350 ppm CO_2 in this century, the potential O_2 ⌈ for C_3 plants has risen to 27% at 20°C (Fig. 3*A*), and the permissive global O_2 equilibrium could also rise. However, a higher permissive O_2 level may be offset by accelerated O_2 uptake at present times from combustion of fossil photosynthate (25).

The specificity of Rubisco seems to establish both a CO_2 ⌈ and O_2 ⌈, which depend on the ratio of CO_2 to O_2 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) CO_2 (15) and 21% O_2 (26). Earlier when the CO_2 level was >1000–1500 ppm CO_2 and/or the O_2 level was lower, Rubisco functioned primarily only as a carboxylase, and the CO_2/O_2 ratio for the dual activities of Rubisco was not a controlling factor on plant growth. However, once the level of O_2 increased and that of CO_2 decreased, the oxygenase activity of Rubisco limited CO_2 removal and the CO_2/O_2 ratio became a governing factor on net photosynthesis, plant growth, and the atmospheric composition. O_2 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 CO_2, which allowed an O_2 ⌈ of 35% for a C_3 plant (Table 1). In the past century, the CO_2 level has risen from ≈250 ppm, where the O_2 ⌈ was 23–24% to 350 ppm CO_2 with a permissive O_2⌈ of 27%. The potential O_2 ⌈ can rise with increased CO_2 concentration to maintain a CO_2/O_2 ratio based on the average Rubisco specificity for these two substrates. Because there has been ≈700 times more O_2 in the atmosphere than CO_2, any permissive increase in atmospheric O_2 will be very slow. Because of the very low level of CO_2 and a specificity of Rubisco that favors the carboxylase activity, it is changes in the level of atmospheric CO_2 that quickly change the CO_2/O_2 ratio relatively to slow changes in the immense O_2 pool.

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