

Photosynthesis in Quillworts, or why are some aquatic plants similar to Cacti?

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CAM photosynthesis involves the night-time uptake of carbon dioxide and incorporation into organic acids. These acids, typically malic acid, accumulate overnight in the cell vacuole. During the day these acids are broken down and the carbon dioxide released is used as the carbon substrate for photosynthesis. This pathway is found in two very different ecological situations: certain arid environments where daytime closure of stomatal pores, in order to conserve water, limits carbon dioxide uptake, and certain aquatic habitats where daytime ambient carbon dioxide levels are limiting to photosynthesis.

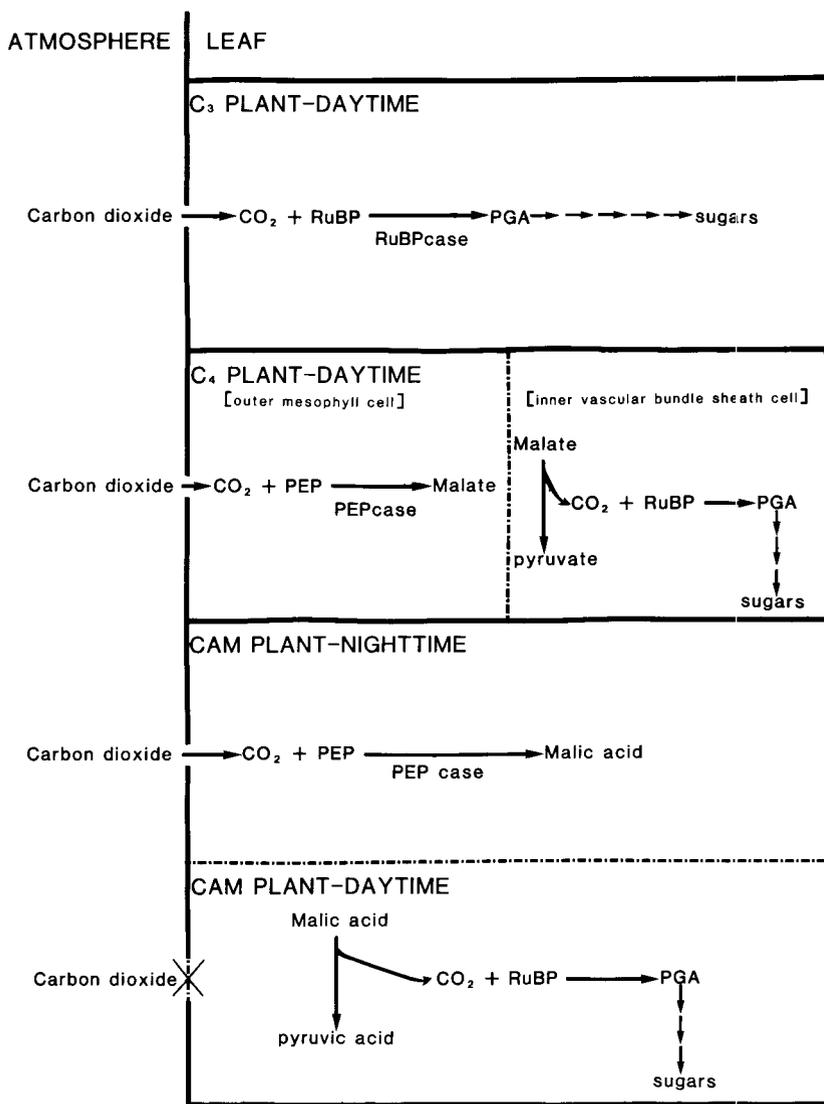
Photosynthesis is the physiological process by which plants 'fix' the sun's radiant energy into organic molecules such as sugar. The backbone of all such organic compounds is a skeleton composed of carbon atoms and plants utilize carbon dioxide from the atmosphere for their carbon source. Our present understanding of the biochemical steps involved in this process indicate that there are three routes or pathways utilized by different plant species. The vast majority of plants use a single reaction to attach carbon dioxide from the atmosphere on to an organic compound. The enzyme that catalyzes this conversion is ribulose biphosphate carboxylase (RuBPcase) and the first stable organic product is a 3-carbon organic acid which then enters a pathway leading to sugar formation (Fig. 1). Such plants are referred to as C₃ plants.

For nearly two decades, scientists took it as axiomatic that all plants photosynthesised in this manner, but in the early 1960's, researchers

studying sugarcane, discovered a pathway that involved incorporation of carbon dioxide into organic products at two different stages (Fig. 1). In the first stage, atmospheric carbon dioxide enters the sugarcane leaf and fixation by an entirely different enzyme, phosphoenolpyruvate carboxylase (PEPcase), yields a 4-carbon organic acid, either malate or aspartate: such plants are known as C₄ plants. The organic acid then moves to the centre of the leaf where it is broken down and carbon dioxide is released in specialized cells which differ from other photosynthetic cells by virtue of the fact that they alone contain the enzyme RuBPcase. This enzyme then fixes the carbon dioxide a second time,

into the 3-carbon organic acid which leads to sugar formation. C₄ plants, which also include corn (*Zea mays*), are thought to have evolved this double carbon-fixation as a means of enhancing the efficiency of photosynthesis. One factor is that the enzyme RuBPcase is very inefficient when exposed to atmospheric levels of oxygen and by sequestering this enzyme in the interior of the leaf, C₄ plants are capable of reducing this inhibitory effect. Increased CO₂ levels in the bundle sheath cells also enhance fixation by RuBP case. Thus it is not surprising that sugarcane and corn are among our more productive crops.

The third photosynthetic pathway, that had already been discovered in desert plants, is present in cacti and succulent species. These plants have the same two carbon-fixing steps as are present in C₄ plants, but, rather than being spatially separated in the leaf, the two carbon dioxide fixing enzymes occur within the same cell and are temporally separated in their activity (Fig. 1). The first carbon dioxide fixing step (catalyzed by PEPcase) occurs at night. The 4-carbon organic acid product (mostly malic acid) is then stored in the cell vacuole through the dark period. In such plants sizeable levels of acid accumulate overnight in their photosynthetic tissues. During the daylight period these acids are broken down, releasing carbon dioxide inside the cell, which is then fixed by RuBPcase into the 3-carbon precursor for sugar formation. Due to the substantial day/night change in acid levels (something noted for cactus hundreds of years ago), and the fact that the pathway was originally studied in plants of the plant family Crassulaceae, this is known as Crassulacean Acid Meta-



the organic acid pool. Thus, it is not surprising cactus and other succulent CAM plants are among the most efficient plants at conserving water.

In the light of this, the last place one might expect to find CAM photosynthesis would be in an aquatic environment. However, I discovered in 1979 that the aquatic plants known as quillworts, indeed possess a well-developed CAM pathway.

Quillworts

Also known as Merlin's-grass, quillworts are members of the genus *Isoetes* (family Isoetaceae). This family of spore-bearing plants are aligned with 'primitive' plant groups such as club mosses and horsetails. Species of *Isoetes* are found world-wide, ranging from near sea-level in temperate regions of Europe to over 5000 m in the Andes of South America. The name quillwort derives from the growth form which consists of a rosette of long cylindrical pointed leaves (Fig. 2), in some species terminating in a spiny tip. This growth form is common throughout the genus of several hundred species, regardless of habitat. The vast majority of the species are aquatic, occurring either as seasonal aquatics in temporary pools or perennial aquatics in lakes.

CAM photosynthesis was originally discovered in *Isoetes howellii*, a species common in seasonal pools in California, USA. In the mediterranean-type climate of this region, such pools flourish for a brief period in the

bolism or by the acronym CAM. The adaptive significance of this pathway is relatively straightforward: since sugar formation requires ATP and NADPH derived from light-dependant reactions, the enzyme RuBPcase is functional only in the light. Sugar formation also requires carbon dioxide, which ultimately enters the plant through stomatal pores on the leaf, and often the level of photosynthesis is dictated by the amount of carbon dioxide which enters the leaf. However, whenever the stomata are open, water vapour is lost into the atmosphere and plants often are faced with the risk of losing too much water and lethal desiccation. Nowhere is this more likely than in xeric environments such as deserts. CAM plants open their stomata at night, when it is cool, and the evaporative power of the atmosphere is less. The carbon dioxide in a sense is temporarily captured in organic acids. During the day, CAM plants close their stomatal pores, thus conserving water, and utilize the carbon dioxide source stored in

▲ Fig. 1. Scheme of carbon-fixation pathways for the three main photosynthetic plant types, C₃, C₄, and CAM.



Fig. 2. Quillwort *Isoetes howellii*.



◀ Fig. 3. Seasonal 'vernal' pool in southern California, USA.

spring and hence are known locally as vernal pools. The pools fill during the winter rains and most plants initiate their growth underwater in early spring and grow as submerged aquatics for a period of weeks or months, dependent on the year. As the summer drought sets in, the water level drops and many of the annual flowering species endemic to such habitats provide a beautiful floral display, in rings around the periphery of the pool (Fig. 3). During the summer the pool beds are bone-dry and the flora over-summer as dormant seeds or vegetative parts, e.g. corms in *Isoetes*.

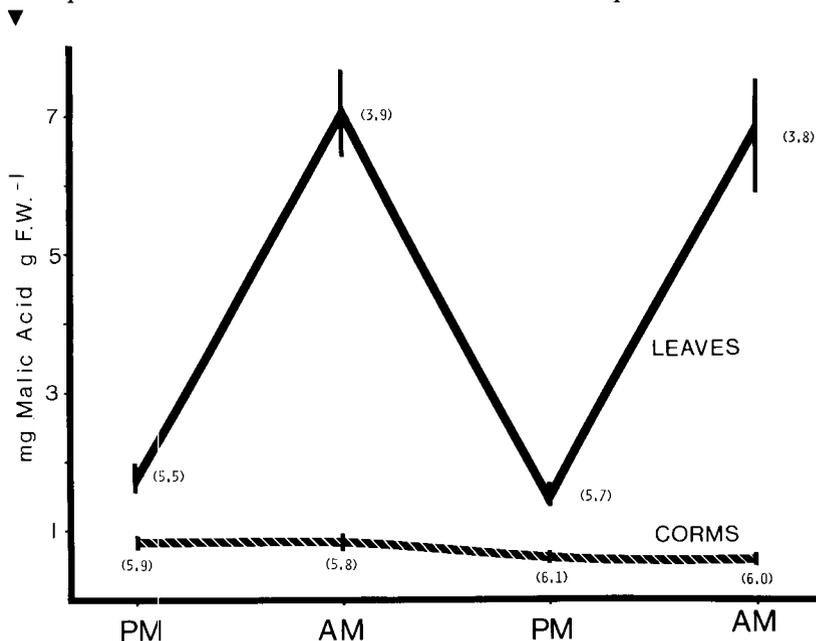
Discovery of CAM photosynthesis in an aquatic plant

Needless to say the discovery in an aquatic plant, of a photosynthetic pathway normally associated with water conservation in desert succulents, was unexpected. My focus at the time was to test a theory that the organic acid, malate, was an anaerobic respiration product, as ethanol is in alcoholic fermentation. This idea had been proposed a number of years earlier by Professor Crawford at St Andrews University and was a matter of some controversy. A colleague of mine, Robert Hays, pointed out to me that vernal pools underwent a daily change in oxygen levels; being high during the day (due to photosynthesis of the pool flora) and low at night (due to respiration). He suggested that per-

haps overnight, plants may be subject to oxygen deficits and thus be forced to use some form of anaerobic respiration. Thus, I hypothesized that we should find changes between day and night in malate levels in these aquatic plants. When I found that one species, *Isoetes howellii*, had very high malate levels in the end of the morning and very low levels at the end of the day, it seemed that the hypothesis had been proven. I now know that the hypothesis is incorrect and I often use this example in classes to illustrate why certain philosophers of science maintain that hypotheses can never be proven, only disproven.

The first clue that this substantial malate change was related to photosynthesis was the observation that diurnal changes in malate levels occurred only in leaves not in corms (Fig. 4). If malate had been an anaerobic end product, one would have expected oxygen deficits to be even more severe in underground corms than in leaves. One additional observation was that this overnight accumulation was of the organic acid, malic acid, not the anion form, malate. Indeed, failure to appreciate this fact led to some brief confusion in interpreting results of protein assays. We found no soluble protein in plant extracts taken in the morning but very high levels in afternoon samples. As it turns out, the extreme acidity of the leaf extracts (in which the cell vacuoles had been disrupted) in the morning (pH 3.5 or lower) caused the protein to precipitate out of solution and end up as the discarded 'pellet' at the bottom of our centrifuge tubes. Although somewhat embarrassed by not using a sufficient buffer, I take some solace in that the early CAM researchers had similar experiences.

Fig. 4. Malic acid concentration in the submerged leaves and underground corms of *Isoetes howellii* over a 24-hr period.



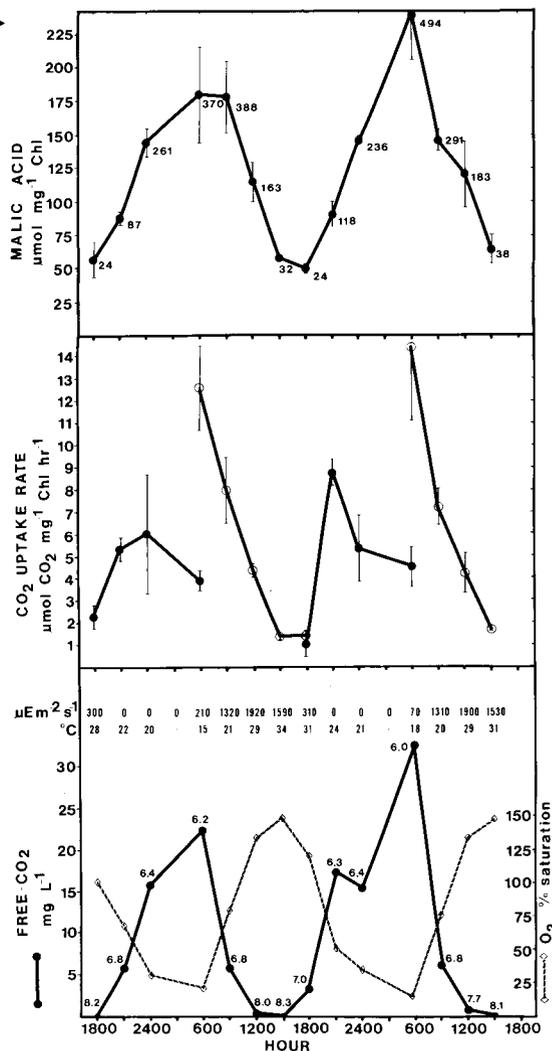
Subsequent studies have provided further evidence of Crassulacean Acid Metabolism or CAM in *Isoetes howellii*. This plant does take in carbon dioxide in the dark and fix it into the 4-carbon organic acid malic acid, and this fixation is catalyzed by the enzyme PEPcase. Malic acid accumulates overnight in the vacuole and during the day it is broken down and the carbon dioxide used as a source for sugar production, via RuBPcase.

Why CAM in an aquatic plant?

In terrestrial CAM plants carbon dioxide uptake is regulated by opening and closing of stomata. Aquatic plants lack functional stomata and movement of carbon dioxide into the plant occurs readily across the epidermis (which commonly lacks the well-developed cuticle typical of land plants) and carbon dioxide uptake is largely regulated by the ambient level of this gas. If in the laboratory, *I. howellii* is exposed to high carbon dioxide levels in the light and in the dark, it is capable of taking up carbon dioxide under both conditions; in the light it is fixed via the C₃ enzyme (RuBPcase) and in the dark via the C₄ enzyme (PEPcase). The same is true of terrestrial CAM plants if stomatal control is overcome, e.g. by removing the epidermis.

However, photosynthesis by *Isoetes howellii* under natural field conditions does not involve continuous carbon dioxide uptake during both day and night, the reason being that the water chemistry of these pools undergoes dynamic changes through the day. Figure 5 (bottom panel) illustrates the diurnal pattern typical for pools in southern California. Carbon dioxide levels are typically high early in the morning. These pools, however, are densely vegetated and relatively shallow, and commonly by noon of each day the photosynthetic demand has depleted the water of carbon dioxide. As carbon dioxide is removed from the water the pH of the pool rises and on some days I have observed the pH rise from pH 6 in the morning to pH 10 in the afternoon. Consequently, throughout much of the day these aquatic plants are subjected to ambient conditions depleted of carbon dioxide. Other inorganic forms of carbon such as bicarbonate are present, but not all aquatic plants have evolved the capacity for taking up bicarbonate. Since this compound is ionized, it does not move freely across membranes as does carbon dioxide. Hence, cells must utilize cellular energy in the form of ATP to bring in this form of carbon. Under some environmental conditions it is apparently 'worth' the cost to take up bicarbonate but under other environmental

Fig. 5. 24-hour cycle of: Lower panel: carbon dioxide levels and oxygen levels in the water of a vernal pool (pH is indicated adjacent to the carbon dioxide line). Middle panel: carbon dioxide uptake and fixation rates in the dark (●) and in the light (○) by leaves of *Isoetes howellii* (vertical lines indicate the standard deviation, $n = 3$). Top panel: malic acid concentration in *Isoetes howellii* leaves.



conditions it is not. In the case of *Isoetes*, there is little or no capacity for bicarbonate uptake. The uptake of carbon by these plants thus follows the changes in carbon dioxide levels in the water (Fig. 5, middle panel).

In summary, we can describe photosynthesis in *Isoetes howellii* as follows. Early in the morning, when ambient carbon dioxide levels are high, uptake and fixation occurs readily. At this time carbon dioxide is fixed by the C₃ enzyme RuBPcase and the carbon enters directly into the pathway to sugar production. Later in the day, uptake of carbon from the water is minimal, however, the RuBPcase enzyme continues catalyzing the fixation of carbon, but now it utilizes the carbon released from the overnight stores of malic acid (note the drop in acid levels in the top panel of Fig. 5). During the night, as carbon dioxide levels in the water rise, due to respiration by the pool flora, the *Isoetes* once again take up carbon from the water, but now it is fixed via the C₄ enzyme PEPcase into malic acid, which is stored until the following day.

When CAM was first discovered in *Isoetes*, colleagues suggested an alternative hypothesis for the adaptive significance of this photosynthetic pathway. Since these seasonal pools dry down in the late spring, as the summer drought approaches, perhaps CAM was selected for the same role as in desert cactus, i.e. to conserve water. This, however, is not the case, as we have discovered that overnight accumulation of malic acid disappears once the leaves are exposed to the air (Table 1). This is coupled with a loss of night-time carbon dioxide uptake and a switch to strictly daytime C₃ photosynthesis. This loss of CAM activity occurs on a cell-by-cell basis, as leaf bases still submerged maintain active acid accumulation whereas aerial tips do not (Table 1).

The precise physiological mechanism behind this switch from aquatic to aerial physiology is still unknown, but two hypothetical environmental cues responsible for this switch have been tested: the aerial leaves could respond to the higher ratio of red/far-red light or to the lower atmospheric water potential. To test between these hypotheses *Isoetes* plants were grown out of water under combinations of light filtered through water in glass bowls and under high and low humidity. It is clear from the results in Table 2 that filtering light through water (which acts as a filter to far-red light) has no influence on overnight acid accumulation. However, if atmospheric humidity is maintained near saturation, CAM is not lost despite the fact that the plants are growing in an aerial environment.

Conclusions

Studies from several laboratories have revealed that the phenomenon of CAM photosynthesis in aquatic plants is present in all aquatic species of the genus *Isoetes*. Outside of that it occurs in only a few other plants. In general, aquatic CAM plants are found only in potentially carbon-limited environments

	Malic acid ($\mu\text{mol per gram fresh weight}$) in leaves	
	AM $\bar{X} \pm \text{SD}^*$	PM $\bar{X} \pm \text{SD}$
Entirely submerged	138 \pm 12	4 \pm 1
Partially submerged		
Submerged bases	101 \pm 28	3 \pm 2
Aerial tips	32 \pm 7	3 \pm 2
Entirely aerial		
Growing in moist soil	47 \pm 31	31 \pm 2
Growing in dry soil	18 \pm 8	12 \pm 1

* Mean plus or minus one standard deviation ($n = 3$).

	Malic acid ($\mu\text{mol per gram fresh weight}$) in leaves	
	AM $\bar{X} \pm \text{SD}^*$	PM $\bar{X} \pm \text{SD}$
Light filtered through glass bowl filled with water		
Humidity near saturation	144 \pm 40	2 \pm 0
Humidity below 65% RH	32 \pm 3	1 \pm 1
Light filtered through empty glass bowl		
Humidity near saturation	128 \pm 8	5 \pm 3
Humidity below 65% RH	29 \pm 12	2 \pm 3
Plants submerged	208 \pm 19	15 \pm 4

* Mean plus or minus one standard deviation ($n = 3$).

Table 2. Malic acid concentrations in aerial leaves of the quillwort, *Isoetes howellii*, growing with light filtered through water versus light filtered only through glass, and growing under high atmospheric humidity versus low humidity.

such as shallow pools, with daytime depletion of carbon dioxide, or in very-low-productivity (oligotrophic) lakes that occur at high elevations or high latitudes. Aquatic CAM plants do not persist long under eutrophic conditions, as they are typically outcompeted by other faster growing species. A problem for future research is how do the other components of the aquatic flora, which co-exist with *Isoetes* but lack CAM, manage to compete successfully?

Suggestions for further reading

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The responses of stomata to changes in atmospheric levels of CO₂

F. I. WOODWARD

It is well established that for many plants, stomatal opening is decreased as CO₂ levels are increased. Recent observations on leaves grown in controlled environments and those stored for the last 200 years in a herbarium have shown, for the first time, that leaf stomatal density may also decrease with increasing CO₂. This response has been effective throughout the period of the industrial revolution, however the evidence suggests that it does not generally occur when the level of CO₂ is increased above the current value. Experiments have shown that the decrease in stomatal density may have been associated with increasing water-use efficiency and with leaf area expansion in well-watered plants.

The world's atmospheric concentration of CO₂ is rapidly escalating: of this we are sure as a result of 30 years of continuous measurements. It is now practically feasible to measure historical levels of CO₂ by analysing different-aged bubbles of air which have been locked in the ice caps of the world. Such measurements (Fig. 1) dovetail perfectly with present-day measurements and indicate the non-linear and ever-advancing levels of CO₂ since the onset of the industrial revolution in the 18th century. The increasing levels of CO₂ describe man's ever-increasing industrial activity, based on the burning of fossil fuels, and his need to extend the limits of controlled agriculture, by burning native forests. Estimates of CO₂ released to the

atmosphere by these two processes show that in 1980, for example, 10 million tonnes of CO₂ were released by deforestation and 26 million tonnes by burning fossil fuels. This total anthropogenic release may be four or five times greater than that released in 1950, and the annual releases are still increasing.

The effects of CO₂ on plants

Ecologists have not been slow to investigate the likely ecological impact of the elevated CO₂ levels expected in the near future. Many hundreds of experiments have investigated the effects on plants of doubling the present CO₂ level. An increase in the photosynthetic rate and a decrease in stomatal opening have been generally observed in these experiments and indeed these responses to increased CO₂ have been known for nearly 100 years. This prior knowledge, in addition to the more recent understanding of biochemical pathways of photosynthesis, has indicated that increasing CO₂ levels should increase photosynthetic rates in 'C₃' species, which depend on the enzyme ribulose biphosphate carboxylase/oxygenase as the sole enzyme for fixing CO₂. Partial stomatal closure with increasing CO₂ will reduce the volume of water vapour lost from the leaf during the fixation of CO₂. In combination these two responses lead to plants that are more 'water-use efficient', i.e. more efficient at gaining CO₂ per unit loss of water vapour. These responses are shown in Fig. 2 for *Acer*

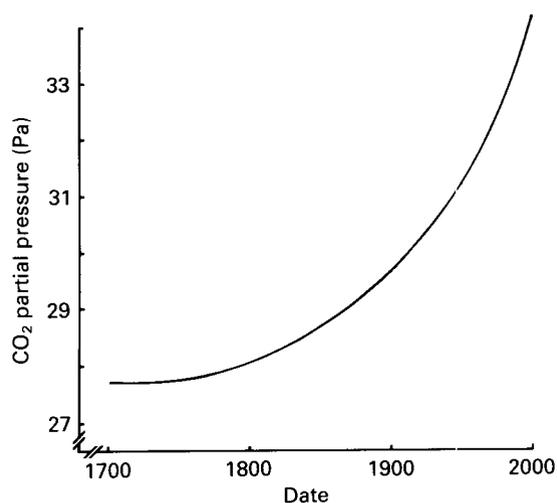


Fig. 1. Changes in atmospheric levels of CO₂ since the 18th century, as measured in bubbles of air encased in the Antarctic ice cap.