



Introducing climate change into the biochemistry and molecular biology curriculum

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Abstract

Our climate is changing due to anthropogenic emissions of greenhouse gases from the production and use of fossil fuels. Present atmospheric levels of CO₂ were last seen 3 million years ago, when planetary temperature sustained high Arctic camels. As scientists and educators, we should feel a professional responsibility to discuss major scientific issues like climate change, and its profound consequences for humanity, with students who look up to us for knowledge and leadership, and who will be most affected in the future. We offer simple to complex backgrounds and examples to enable and encourage biochemistry educators to routinely incorporate this most important topic into their classrooms.

KEYWORDS

curriculum design development and implementation, effective in-class problems, ethics in science and scientific research, learning and curriculum design, original models for teaching and learning, plant biochemistry

1 | WHY WE SHOULD CARE

Carbon dioxide 451. Metaphorically, those words are intended to bring the same alarm and urgency as its literary progenitor, Fahrenheit 451, the title of Ray Bradbury's dystopian book. In the world he created, literature is purposely burned as a mechanism of social control. We live in an actual world where scientists project that on our present course, CO₂ will reach that number around 2035.¹ Some would metaphorically burn the knowledge created by scientists to hide the effects of ever-increasing CO₂.

The 2018 Intergovernmental Panel on Climate Change (IPCC) reports suggest that if we wish to keep the average temperature rise since the start of the industrial revolution to 1.5°C, global emissions must peak in 2020,² an impossible feat. Even with significant climate

action, the IPCC 2018 report predicts that the planet will be on average 2°C warmer than preindustrial levels by 2040.³ Climate scientist have predicted that those numbers will cause a series of feedback responses amplifying the warming.⁴ Books will not burn but parts of the earth will become increasingly uninhabitable.

As scientists, what is expected of us in our professional lives as teachers and researchers when we know and trust the effects that so many climate scientists have spent their lives uncovering? As teachers we have curricular restraints determined by what we are expected to teach as we try to meet learning goals and competencies required of students who will apply to professional and graduate schools as well as those entering the work force. We already make choices based on those expectations. How much time do we spend on nitrogenase, for



example, compared to mitochondrial electron transport proteins that can be linked directly to human health and disease?

As scientists, we also have ethical obligations to our students as well as to the public. The American Society for Biochemistry and Molecular Biology (ASBMB) states that investigators will promote and follow practices that enhance the public interest or well-being.⁵

Less is written about what and how we should teach. The ASBMB states that “regular, explicit attention should also be devoted to the principles of ethical conduct of research and scholarship”.⁶ Thompson et al.⁷ have written that we should intentionally include issues of social justice in our science classes. They argue that in the classroom, we should

- Develop awareness of current and historical injustices and injuries promoted or perpetrated by science and scientists;
- Use science as a tool to improve the human condition and create more just communities; and
- Speak out in defense of sound science and against scientific abuses and unreasoned attacks on science.

What obligations do we have, as biochemists and molecular biologists, to teach the science of climate change and its effects? If we accept the climate science and can extend our ideas of professional ethics to include issues of social justice as well as human and biosphere health, perhaps a more appropriate question is how we cannot include it. Instead of arguing that our curricula are too full already, we could explore ideas to incorporate climate change examples that are similar or complementary to examples we already use in our courses.

This article provides background materials and examples (from simple to complex) that will allow biochemistry educators to bring the science of climate change into their classrooms. In addition, recent advances in our knowledge of the cause and effects of climate change are presented as most readers are probably not versed in that climate-change literature. A compendium of relevant climate change web links provides data and additional information.⁸

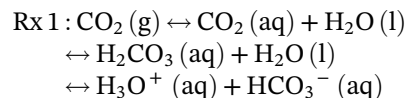
We hope our examples will move readers to introduce their own climate change examples for students, who most assuredly need to hear about the urgency of our climate crisis from trusted teachers.

2 | CO₂ AND THE CARBON CYCLE

As any student who has taken a background infrared spectrum to remove contributions from atmospheric CO₂ knows,

CO₂ absorbs infrared radiation and is a potent greenhouse gas. Its relevant chemistry for climate change must be understood in the context of the global carbon cycle, shown in Figure 1. Key players in the cycle are inorganic (CO₂, HCO₃⁻, H₂CO₃, and CO₃²⁻ and its insoluble salts) and organic carbon which is ultimately derived from life.

The carbonic acid/bicarbonate buffer system helps control blood and cellular pH and is a bit more complicated than other polyprotic acids since the equilibria must account for CO₂ solubility in aqueous solution. The overall chemical reactions look like this:



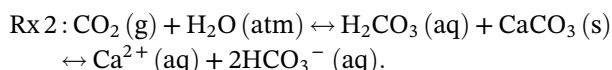
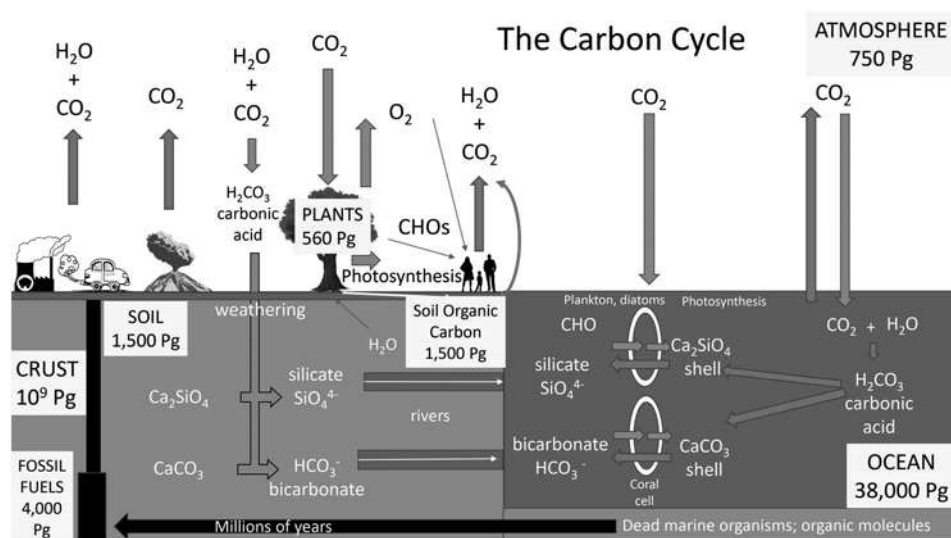
The relevant chemistry involves limited solubility of the nonpolar gas CO₂ and the resulting generation of a buffer solution with a weak acid, carbonic acid (pK_a = 3.6 or 6.3, if the hydration reaction is considered) and its conjugate base, bicarbonate (hydrogen carbonate). This buffering system is used to explain how shifting equilibria caused by excessive CO₂ release (from rapid deep breathing) or decreased CO₂ release (associated with pulmonary disease, shallow rapid breathing, and panic responses) can lead to respiratory alkalosis and acidosis, respectively.

To extend the breathing metaphor, consider the oceans (if not the whole biosphere) as a “breathing” system in which the same equilibria apply. With constant atmospheric CO₂, the system is superficially in equilibrium if the oceans are considered as a giant sink for CO₂. Data show that the atmosphere has absorbed about 30% of anthropogenic CO_{2 atm} emitted into the atmosphere from 1994 to 2007, similar to the absorption rate from 1800 to 1994 from all sources of released CO₂. Biosphere sinks however are not able to keep up with our ever-increasing emissions of CO_{2 atm}, so it has been increasing.⁹

Reaction 1 is reversible, which should give us concern as ocean inorganic carbon can return to the atmosphere as CO₂ and amplify warming. Regulated feedback mechanisms are critical in metabolic control as biology eschews runaway reactions. Unfortunately, the biosphere has not yet had time to adapt through evolution to the rapid pulse of CO₂ we have put into the atmosphere from burning fossil fuels.

As with the blood, increasing CO_{2 atm} leads to ocean acidification. In another equilibria reaction discussed in our courses, H₂CO₃(aq) can find a base other than water to react with in the oceans, namely CO₃²⁻ in the form of CaCO₃(s) in shells of marine organisms (phytoplankton, diatoms, coral) in the following reaction:

FIGURE 1 A simplified view of the carbon cycle. Carbon amounts are expressed in Pg (10^{15} g) [Color figure can be viewed at wileyonlinelibrary.com]



Ocean acidification is obviously not good for these organisms.

Biochemists probably have never studied biogeochemistry, but by simple extensions of blood carbonate chemistry, the geochemistry of “weathering” as it is linked to the carbon cycle can be understood, from the set of linked equations in Rx 2.

The land source of $\text{CaCO}_3(\text{s})$ in this reaction is limestone and marble. This production of $\text{HCO}_3^{-}(\text{aq})$ is called weathering and leads to a flow of HCO_3^{-} into rivers and then oceans. Consider it a soluble form of carbon (in comparison to either CO_2 or CO_3^{2-} , which is quite insoluble in the presence of divalent cations like Ca^{2+} and Mg^{2+}). It is this form of carbon that is imported through an anion transporter into species that make shells by a process analogous to the transport of HCO_3^{-} , a main regulator of cellular pH in animals, by membrane proteins, which couple exchange with Cl^{-} , Na^{+} , or K^{+} .

Rock weathering reactions also apply to noncarbon-based minerals/rocks composed of $\text{Ca}^{2+}/\text{Mg}^{2+}$ and silicates (SiO_4^{4-}), which occurs in chains of tetrahedrally linked silicates. As a simple and readily available carbon capture technique, pulverized basalt is being spread on agricultural fields to promote weathering and carbon uptake into the soil, concomitantly improving soil health.¹⁰ On weathering through the same process, silicates enter the ocean where they are taken up by shell-creating organisms. These weathering reactions are clearly slow compared to the anthropogenic release of CO_2 from fossil fuel burning. However, in geologic time, they are key players in carbon regulation. Perhaps this

example might help students understand slow, fast, steady state, and rate-limiting reaction steps.

3 | MASS BALANCES, FLUXES, POOLS, AND RATE CONSTANTS

Mathematical analyses are important to fully understand individual biochemical reactions. They are essential in understanding the complexities of multiple linked reactions that comprise metabolic and signal transduction pathways. Yet most students struggle with chemical equilibria and kinetic equations. They find it conceptually difficult to differentiate between equilibrium and steady state reactions, especially in metabolic pathways in which we talk about pools of metabolites and their fluxes through the pathway.¹¹ Many biochemistry textbooks do not adequately cover flux analyses through pathways.

Would a more macroscopic approach to flux analyses help students understand metabolite flow through pathways? If so, the fate of $\text{CO}_2(\text{atm})$ in the biosphere would be an excellent example. Figure 2 shows how pools of $\text{CO}_2 + \text{HCO}_3^{-} + \text{H}_2\text{CO}_3$ (but not CO_3^{2-}) distribute in the biosphere. In comparison, Figure 3 shows a “simplified diagram” of the chemical reaction diagrams for the cellular adenine nucleotide pools.¹²

4 | SOIL METABOLISM

The soil itself is a huge sink for carbon but it is not a topic for general discussion in biochemistry courses. It stores more carbon (1500 gigatons [Gt] = 1.5 Pt = 1500 Pg) than the atmosphere and vegetation combined. Soil

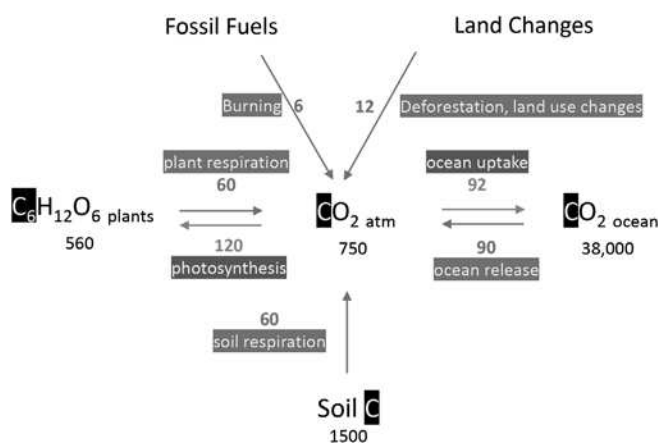


FIGURE 2 Simplified interconversion of biosphere carbon. Mass is expressed in Pg = 10^{15} g. The numbers above the reaction arrow represent fluxes in petagrams per year (Pg/yr) [Color figure can be viewed at wileyonlinelibrary.com]

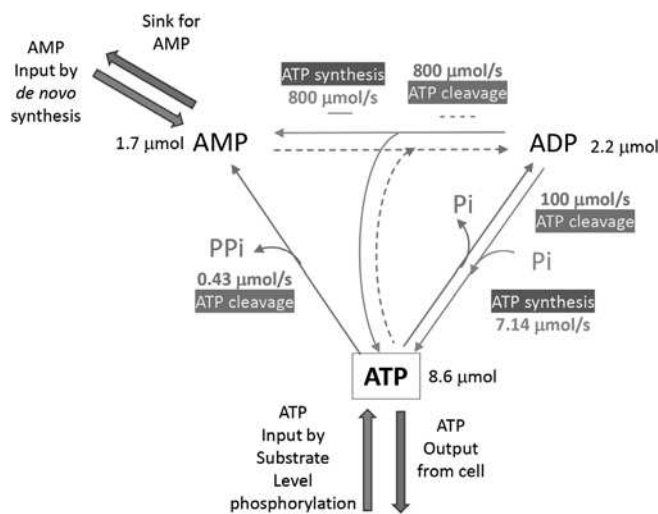


FIGURE 3 Interconversion of adenine nucleotide pools in cells. The rates shown ($\mu\text{M/s}$) are the maximal rates of the reactions, whose overall rates would depend on K_m values as well. Cellular concentrations are average values under normal conditions for ATP synthesis with a total pool average of $12.5 \mu\text{M}$. AMP, ADP, and ATP concentrations oscillate around the average values as does average the adenylate energy charge¹² [Color figure can be viewed at wileyonlinelibrary.com]

organic carbon (SOC) originates from photosynthetic organisms, which must build biomass from CO_2 atm. On plant death, decaying carbon-containing molecules enter the soil where they are used by heterotrophic (non-photosynthetic) organisms for energy and for conversion to more complex carbon molecules. Inorganic carbon (in the form of CO_2 , HCO_3^- , and CO_3^{2-}) can be released back to the atmosphere or complex with cations in soil in a process called mineralization.

On tilling of soil, a procedure used by modern mass agriculture, these buried carbon-containing molecules

become more exposed to O_2 and are more readily oxidized to reform CO_2 atm. Simple changes in procedures, such as the adoption of no-till agricultural processes and cover crops, may reduce the source of atmospheric carbon dioxide. The benefit of no-till for carbon sequestration has recently been shown to be less effective than many have expected.¹³

The organisms that live in and on the soil (bacteria, fungi, protist, animals) process the soil carbon sources and ultimately control plant growth and climate, so understanding this vast interconnected soil biome and their local and global environments is essential. Crowther et al. have recently reviewed the soil biome and its effects on our planet.¹⁴

The amount of SOC depends on their rate of synthesis and turnover. Photosynthesis and decay of plant and animal matter add carbon to the soil while plant and soil microbial respiration (yes plants oxidize their own carbon stocks and produce CO_2) remove it. The input rate is determined mostly by root biomass.¹⁵ Figure 4a,b shows the inverse relationship between SOC (high in northern latitudes) and heterotrophic respiration (high in the tropics). SOC accumulation (as well as mineralization) is higher in the warm and wet tropics (characterized by high photosynthetic rates) but it is depleted rapidly by respiration from heterotrophs, which are most abundant in the warm, wet tropics. In contrast, SOC is high in the northern latitudes where it has accumulated over time and where it undergoes less decomposition by heterotrophs under cold conditions.

Figure 5a,b also from Crowther et al.¹⁴ shows the remarkable distribution of terrestrial carbon stocks above and below ground at various latitudes (A) along with soil microbial mass (B). The two below ground curves in A and B have similar shapes, with the north having the most abundant SOC reserves as well as microbial biomass, which are less active under cold and dry conditions.

Underground organism biomass comprise part of the total 1500 Gt of soil carbon, with 12 Gt contributed by fungi, 7 by bacteria, and 2 by animals. Hence, soil metabolism is highly dependent on fungi, which engage in slow decomposition of complex organic molecules and in the process facilitate the establishment of slow-growing species like trees. In contrast, bacteria, with higher metabolic rates, are most abundant in fast-growing grasslands. Soil organisms are often characterized by soil function and reactions such as carbon mineralization, nitrification/denitrification, nitrogen fixation, carbon fixation, methanotrophy, methanogenesis). Archeal methanogens found abundantly in wetland soils (which represent 6% of land surface) contribute around one third of total methane emissions. Colocalized with these methanogens are the methanotrophs, which use the methane.

FIGURE 4 Inverse relationship between soil organic carbon (A) and heterotrophic respiration (B). From Crowther et al.¹⁴ with permission from AAAS [Color figure can be viewed at wileyonlinelibrary.com]

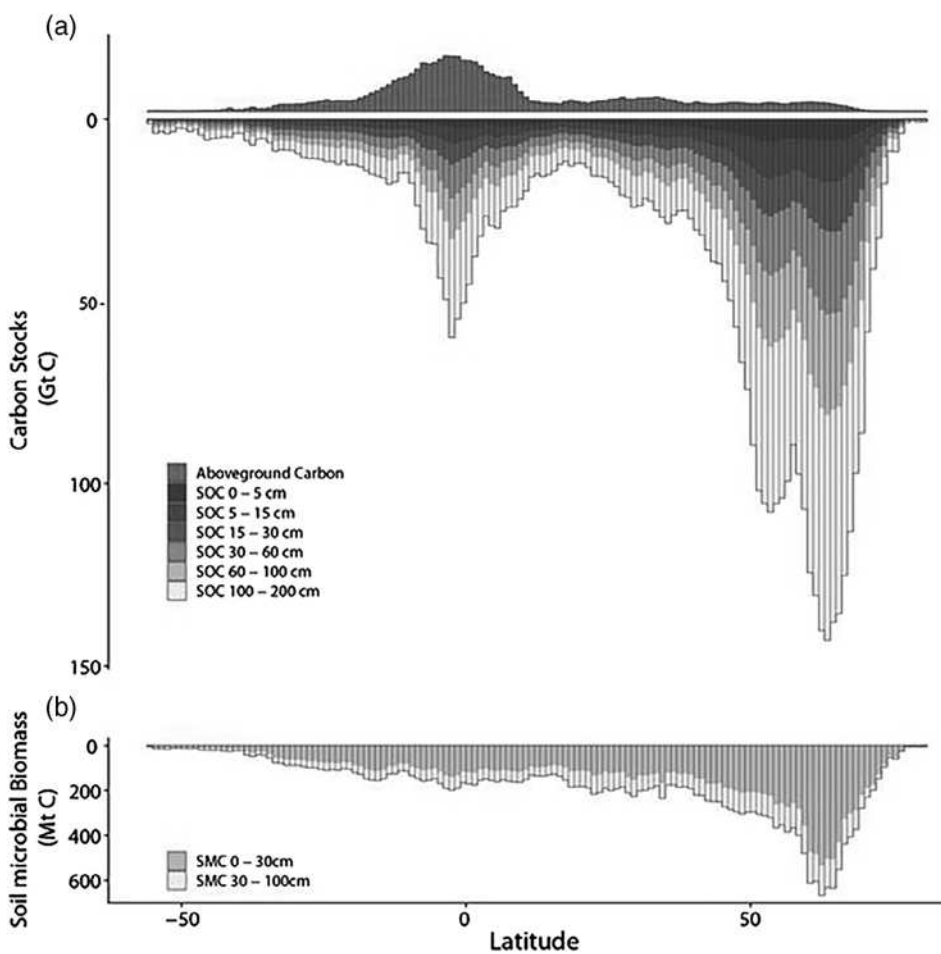
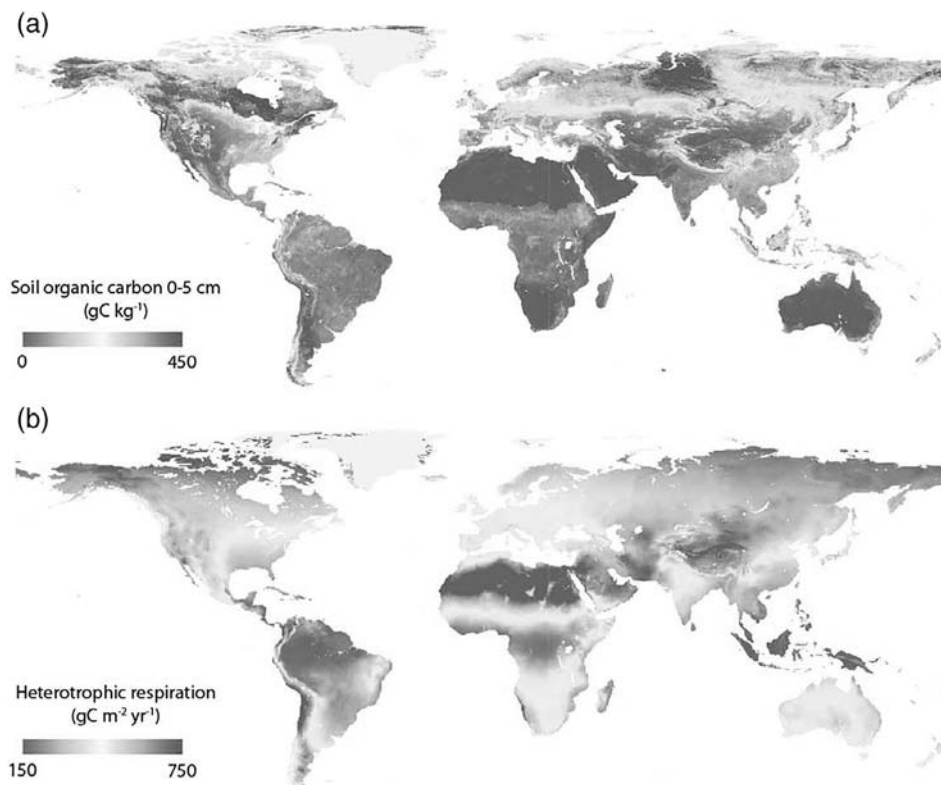


FIGURE 5 Distribution of terrestrial carbon stocks above and below ground at various latitudes (A) along with soil microbial mass (B). From Crowther et al.¹⁴ Reprinted with permission from AAAS [Color figure can be viewed at wileyonlinelibrary.com]

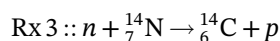


5 | ISOTOPIC ANALYSES

Isotopes have a long history of use in biochemistry to detect trace amounts of labeled materials, to follow intermediates in chemical reactions and pathways and to study reaction mechanisms (isotope effects). They are also used to date samples and as a proxy measure for temperature in climate studies. Most faculty probably do not use radioisotopes in undergraduate labs given safety issues and the need for specialized equipment. Perhaps we need more in-class examples of isotope use other than following carbon atoms in the tricarboxylic acid cycle. The following also offers a brief review for faculty who do not cover multiple methods for isotope use.

5.1 | Radioisotope dating

Most students should be familiar with carbon dating using radioactive ^{14}C decay, but few can probably explain its basis. Most isotopes were created by nucleosynthesis in stars. In contrast, ^{14}C ($t_{1/2} = 5730$ years) is constantly being made in the atmosphere on bombardment by high energy neutrons of nitrogen (^{14}N), leading to proton/neutron exchange and the formation of ^{14}C as shown in the nuclear reaction below (Rx 3):



The rate of synthesis is relatively constant over time but can be affected by the amount of CO_2 in the atmosphere and factors that would influence cosmic ray intensity (changes in solar activity, earth's magnetic field). Correction factors have been applied for these effects.

$^{14}\text{CO}_2$ is taken up by plants and other carbon fixing organisms (and by the organisms that consume them) until their death at which point no further exchange with atmospheric $^{14}\text{CO}_2$ would occur. Ratios of $^{14}\text{C}/^{12}\text{C}$ in a biological sample are measured to determine the age. Radioactive decay is not affected by temperature or physical processes so it cannot, by itself, be used as a direct measure for temperature. Given carbon's relatively short half-life, carbon dating can only go back to samples 40,000 years ago.

5.2 | Physical, chemical, and biological reaction mechanisms

In contrast to decay of radioisotopes, the ratio of the percentages of isotopes in compounds or complex samples such as an ice core or an organism can be used to make

inferences about mechanisms and conditions (temperature for example) of incorporation of the isotope into the sample.

Simple physical reactions play a part. Polar ice core samples (Greenland, Antarctica) are depleted in "heavier" water (H_2^{18}O and deuterated versions). Both evaporation and condensation play a role. As water evaporates at mid-latitudes and moves toward the poles, it becomes enriched in H_2^{16}O since it evaporates and is transported more easily than H_2^{18}O . In addition, H_2^{18}O condenses more readily at lower latitudes and is preferentially removed in rain. These factors lead to increases in H_2^{16}O in polar ices and increases in H_2^{18}O in liquid, more southern waters. Water (ice in Greenland and Antarctica) contains about 5% less H_2^{18}O than water (liquid) at locations at 20°C . Hence this ratio is a proxy for temperature.^{16,17}

As glacial ice during the Ice Ages was depleted in H_2^{18}O , the oceans became enriched in it during those times. When the ice shields melted, the proportion of H_2^{18}O in the oceans must fall, which concomitantly would also decrease the salinity of the oceans. In climate papers, the differences in delta (δ) value are often used to describe changes in isotopic composition of a sample. The delta value percent for $^{18}\text{O}/^{16}\text{O}$, which can be positive or negative, is defined as:

$$\delta^{18}\text{O} = \left[\frac{(^{18}\text{O}/^{16}\text{O})_{\text{sample}} - 1}{(^{18}\text{O}/^{16}\text{O})_{\text{reference}} - 1} \right] \times 1000$$

In cold conditions with large ice shields, $\delta^{18}\text{O}$ values from shells of planktic foraminifera (which live in the upper ocean surface) and benthic foraminifera (which live in deep seas) are more positive. On ice shield melting, $\delta^{18}\text{O}$ values become more negative. Benthic foraminifera give a global temperature estimate as deep waters are more homogeneous. Planktic foraminifera $\delta^{18}\text{O}$ values are proxies for more local temperatures as they are in a more changing, less mixed environment, and are more affected by evaporation and precipitation.

The ratio of $^{18}\text{O}/^{16}\text{O}$ in the carbonate shells of ocean foraminifera and mollusks, which die and deposit in the ocean sediment over time, can be used to study climate conditions over time. This ratio is also a proxy for temperature. Calcite (CaCO_3) in ocean sediments from shells is enriched in $\text{C}^{18}\text{O}_3^{2-}$ with the exact ratios determined by both equilibrium and kinetic factors. Kinetics are affected by the rate of ion transfer to the growing crystal and exchange with the total carbon pool. At equilibrium, Urey showed that calcite is enriched in $\text{C}^{18}\text{O}_3^{2-}$, probably due to the lower vibrational energy of the heavier form of

carbonate, favoring the formation of the solid.¹⁸ This enhancement is even more pronounced in colder water. Likewise, the ratio depends on the $^{18}\text{O}/^{16}\text{O}$ ratio in the water. Ring formation in shells, as in trees, is then correlated with temperature and other environmental factors.^{19,20}

In analyses of biochemical reactions, kinetic experiments can be performed with isotopically labeled substrates and the effects on kinetic constants (k_{cat}/K_M , k_{cat} , and K_M) can be determined.²¹ Isotope effects are especially useful in reactions involving the cleavage of C—H bonds in which isotopes of H (^2H or D, and ^3H or T) are used. The transition state for cleavage of a C—H, C—D, or C—T bonds are of similar energy, but the ground state vibrational energy for the heavier isotopes are proportionately lower. Hence the activation energy barrier is greater for the isotopically substituted molecule. This primary isotope effect would give rate limiting steps for C—D and C—T cleavage to be 7× and 16× slower compared to C—H cleavage, respectively. Smaller rate effects (secondary) are found if the distance between the reacting bond and the isotopically enriched bonds increases.²²

The $^{13}\text{C}/^{12}\text{C}$ ratios in plants are lower than that of the atmosphere, suggesting that $^{12}\text{CO}_2$ is preferentially taken up by plants and other photosynthetic organisms. The lower ratio in plants is a consequence of the isotope effect, as $^{12}\text{CO}_2$ is expected to be fixed (i.e. form a covalent bond) at a faster rate than $^{13}\text{CO}_2$ by ribulose-bisphosphate carboxylase/oxygenase. Also, diffusion of CO_2 atm across the stomata, regulated pores through which gases like CO_2 , O_2 and H_2O pass, contribute to the fractionation of $^{13}\text{C}/^{12}\text{C}$ in plants and hence organisms that consume them. $\delta^{13}\text{C}$ values are positive when photosynthetic organisms are productive, a process characterized by growth and robust photosynthesis which involves preferential uptake and incorporation of ^{12}C . This leaves more ^{13}C for shell formation. Increased production leads to increase burial of sediments in the seabed. Conversely, decreased $\delta^{13}\text{C}$ values are associated with lower production which leads to increased relative incorporation of ^{12}C in shells.

CO_2 from volcanic sources has a $\delta^{13}\text{C}$ value similar to that of the preindustrial era, so eruptions would have little effect on the value. However, since the beginning of the industrial revolution, the increase in CO_2 atm has been accompanied by a decrease in the $\delta^{13}\text{C}$ value for CO_2 . This can only be explained by fossil fuel emissions of $^{12}\text{CO}_2$ from oil or coal, which ultimately are of biological origin. Figure 6 shows the perfect negative relationship between CO_2 levels and $\delta^{13}\text{C}$ value, clearing implication anthropogenic origins of emissions.²³

6 | DATA, MODELS AND FEEDBACKS

Experimental science requires carefully acquired data and appropriate selection of independent and dependent variables to show correlation and more importantly causation. Theoretical science produces a variety of models based on data and different mechanisms that must be tested to determine which best fits the observed experimental data.

The complexity of the mathematical models used in systems biology to analyze cellular behavior seems daunting, but the development of free programs to conduct quantitative analyses has made modeling available to the nonmathematician. Using ordinary differential equations to calculate changes in concentrations, particle numbers or their fluxes, and using parameters derived from the study of individual reactions, complete quantitative models for cells have been developed. The programs allow the identification of reactions and parameters which control the flux and concentrations of key species. These predictions can then be used to design in vivo experiments to change expression of key enzymes or alter their kinetic parameter which helps validate the theoretical models.

The complexity of these analyses is rivaled by that of modeling earth's climatic system over time (going back millions of years ago) and space (geography). We have only recently acquired the tools and data (examples include discontinuous ice cores going back 4 million years²⁴ and modern satellite data to measure cryosphere ice loss) and computer capacity and modeling capability. One such model found that changes in the frequencies of periodic Northern Hemisphere ice ages from 40,000 to 100,000 years could be explained by declining CO_2 and regolith removal.²⁵

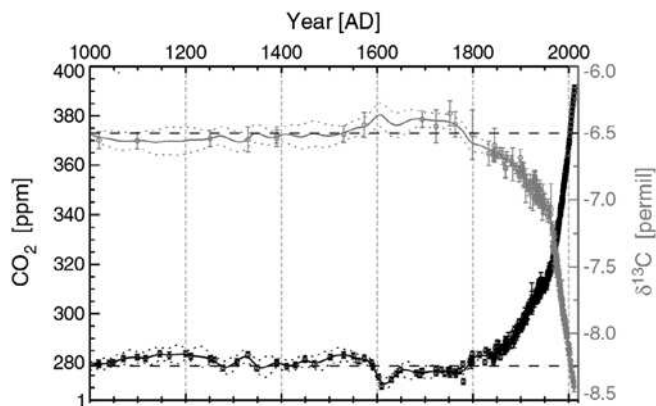


FIGURE 6 CO_2 concentration (black circles) and the $\delta^{13}\text{C}$ value (brown circles).²³ With permission [Color figure can be viewed at wileyonlinelibrary.com]



Figure 7 (top) shows the correlations between CO₂ from continuous Antarctic ice cores and proxy temperature variations over the last 500,000 years. Parameterized theoretical computer models fit ice core data going back 3 million years.²⁵ Note the clear correlation between the oscillatory CO₂ and temperature changes. This graph would easily be shown when discussing the cell cycle, which is driven by oscillating levels of active cyclic-dependent protein kinase 1.²⁶ An in-class activity to access and plot CO₂ and temperature over the last 450,000 years available.²⁷

Figure 7 (bottom) shows Fe and n-alkane deposition in the Southern Ocean over the last 500,000 years.^{24,28,29} Note that the nadir in ocean Fe and n-alkanes at around 120 K and 420 K years ago are correlated with peak temperature and atmospheric CO₂ levels. Atmospheric winds in the region lead to deep water upwelling, bringing both nutrients and deep ocean CO₂. Limiting Fe would limit uptake of atmospheric CO₂ by phytoplankton, further increasing it in the atmosphere. Our current high levels

of atmospheric CO₂ are leading to ocean acidification, which inhibits phytoplankton growth, causing a positive feedback loop to further increase in atmospheric CO₂, a process that should give us cause for concern.

Fe, needed for phytoplankton growth and n-alkanes, were delivered by dust. The long chain n-alkanes, which are significantly enriched in odd carbon numbers, are components of leaf wax layers designed to prevent water loss from the surface of terrestrial plants. In many plants, these are the most abundant lipid in the wax and hence in the biosphere.³⁰ The switch from a relatively warm Pliocene period to the Pleistocene period, characterized by recurrent northern hemisphere glaciation, was accompanied by the first records of dust deposition. Previously, the warmer earth was characterized by smaller atmospheric temperature gradients, lower wind speeds and more abundant rain, limiting dust formation and atmospheric transport. Weathering of the bedrock of the northern hemisphere eventually produced regolith (solid, broken

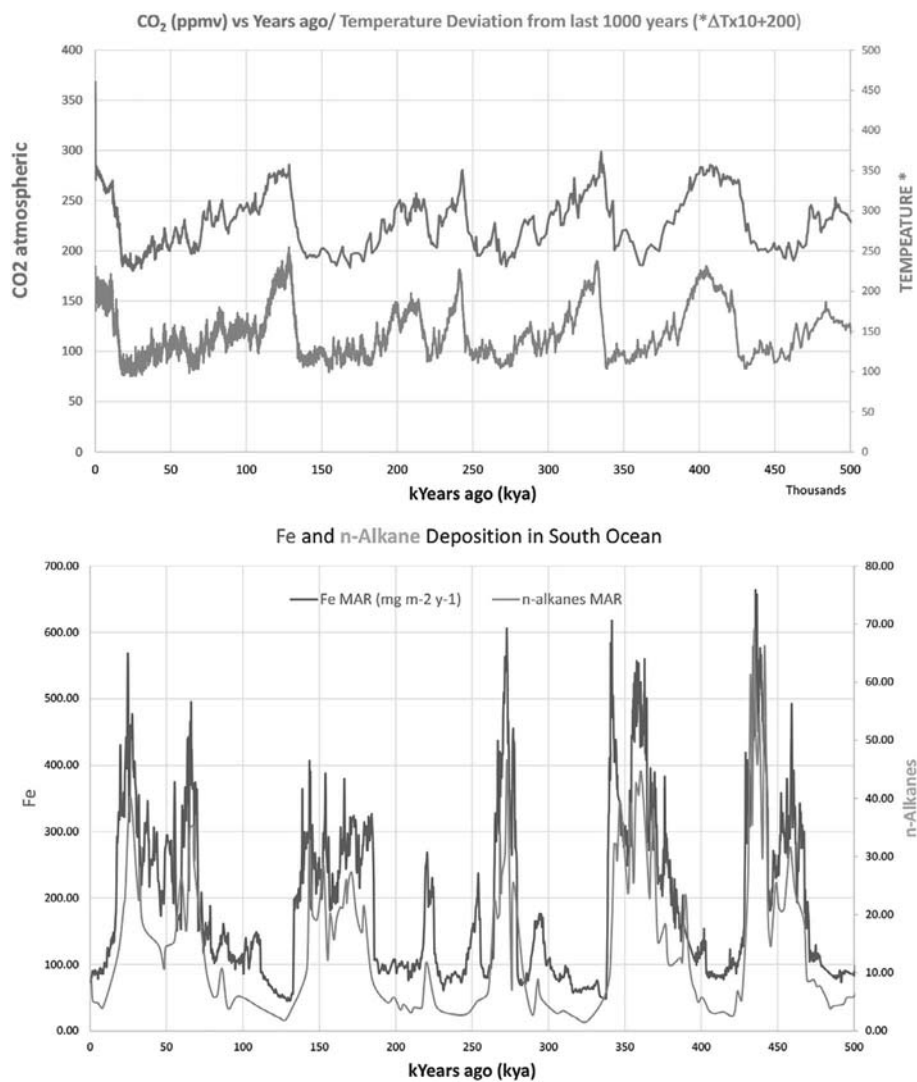


FIGURE 7 (Top) Correlations between CO₂ from continuous Antarctic ice cores and proxy temperature variations over the last 500,000 years.^{22,26,27} (Bottom) Fe and n-alkane deposition in the Southern Ocean over the last 500,000 year.^{22,26,27} [Color figure can be viewed at wileyonlinelibrary.com]

rock, dust) which would promote faster glacial advances and increased dust production and distribution.

Any science student should know that correlation does not imply causation. Many clear and even ludicrous examples of spurious correlations are available to illustrate this point.³¹ An astute viewer of Figure 7 should wonder if CO₂ causes temperature to rise, or if temperature rise precedes that of CO₂, or if hidden variables cause both to rise. Climate skeptics would argue that temperature rises first, and CO₂ follows, so we do not have to worry about fossil fuel emissions. An astute reader might also recognize that the CO₂ records are local (Antarctica) while the temperature indicators are global.

If we do use climate examples in our classes, we need to be ready for such questions. There are many causes for global temperature increases. Indeed, north latitudes warm based on the approximate 40,000-year precession of the earth axes around the sun (Milankovitch cycle) and in the long term with large releases of CO₂ by mega volcanic activity. In contrast, geological weathering of exposed silicate and carbonate rocks leads to long geologic time scale weathering and resulting CO₂ drawdown. This is particularly associated with tropical shelf exposure of carbonates in the warm, wet tropical latitude along “suture zones” where oceanic and continental plates meet. A recent study showed strong correlation with glaciation and exposure of silicate/carbonate rock in the Late Ordovician (455–440 Mya), Permo-Carboniferous (335–280 Mya), and the Cenozoic (35–0 Ma).³²

Analyses of data from our last ice age show a multifactorial cause of deglaciation with complex feedbacks. A recent study suggests that a global biphasic 0.3°C temperature rise occurred before an increase in CO₂. This occurred first in the north latitudes (75°–80° N) and was probably due to orbital “forcing” as the earth’s orbital axis led to greater northern hemisphere irradiance. This “forcing” led to localized melting and concomitant warming of the northern Atlantic Ocean. Warmer waters would lead to slowing of the major Atlantic meridional overturning circulation, which would have slowed upwelling of cold southern waters leading to increased tropical and Southern Ocean temperatures. This “interhemispheric see-saw transfer of heat” caused release of stored CO₂ in the

oceans. The majority of global warming then ensued and strongly correlates with CO₂ atm. For this study, a variety of marine, land and ice core temperature proxies were used, including relative concentration of variants of branched tetraethers, made by bacteria and archaea, which correlate with terrestrial temperatures.^{33,34}

7 | UNCERTAINTY AND BIAS

All data and experimental models have uncertainties, which we attempt to quantify through statistical error analyses and through conducting multiple simulations and examining the distribution of outcomes. Multiple mechanistic models are used to fit data, with the one offering the best fit chosen as the likely explanatory model. A classic case in biochemistry is the analysis of enzyme inhibition data. Multiple models (competitive, uncompetitive, mixed, and noncompetitive) are used to fit the data with statistical analyses (standard deviations, chi squared, correlation coefficients, standard errors) used to determine best fit.

The IPCC has developed and over the years refined different climate models which predict average global temperatures and sea level rise given various emission levels and future efforts at carbon capture. They grouped predictive models into four Relative Concentration Pathways (RCP) based on the notion that both the concentration of CO₂ in the atmosphere and the pathway to get there are important. The four pathways are called RCP 2.6, 4.5, 6, and 8.5, with higher numbers associated with higher temperatures and CO₂ levels. Each assumes a starting value and estimated emissions (which depend on technology, politics, economics, etc.). An RCP of 8.5 suggests that the radiative forces (excess heat energy/[m² s]) by 2100 would be stabilized at 8.5 watts/m² (or J/[s m²]). This would occur in the absence of climate action policies which is unlikely unless nondriven voices dominate. The RCP 2.6 scenario assumes that the peak radiative forcing would be 3 watts/m² which would decline through drastic socioeconomic means before 2100 (by 2030–2040) to reach 2.5. Table 1 shows a summary of the RCP and their meanings.³⁵

TABLE 1 Relative concentration pathways descriptions

RCP (W/m ²)	Time	CO ₂ atm equivalent (ppm)	Temp. increase (°C/°F)	Description
8.5	In 2100	1370	4.9/8.8	Rising
6.0	After 2100	850	3/5.4	Stabilizing without overshoot
4.5	After 2100	650	2.4/4.3	Stabilizing without overshoot
2.6	Decline from 3 before 2100	490	1.5/2.7	Peak and decline

Abbreviation: RCP, relative concentration pathways.



Given the complexity of modeling earth's global climate system, each model has inherent uncertainty. The Paris Accords gave us a goal of holding the average temperature rise to 1.5°C above preindustrial levels. Unfortunately, parts of the world have already surpassed that and given the present and predicted concentration and lifetime of CO₂ atm, we have no chance of holding warming to the RCP 2.6 level. We can cumulatively release about 30 Gt of CO₂ more if we wish to stay below the 1.5°C target. If we stay our present course, we will emit 60 Gt by 2030.³⁶

Uncertainties in the RCP are determined by two factors, *confidence* and *likelihood*. These are used in order to convey meaning to the general public. *Confidence* is determined by the quality of the evidence (limited, medium, robust) and agreement (low, medium, and high). *Likelihood* depends on the calculated uncertainty. Very likely or certain have likelihoods greater than 90%. Likely has a probability greater than 66%.³⁷

Disturbing recent data show that the climate predictions from almost all climate models underestimate the degree of warming and associated climate change that we have already experienced. Temperatures and sea levels are rising, and our cryosphere is melting at a faster rate than the models predict. That most models underestimate climate change could suggest that the models are incomplete and hidden or poorly understood variables have more deleterious effect on climate than are presently known. More likely, there is systematic bias in the reporting of predicted severity.³⁸ This is in contrast to skeptics who often say that climate scientists are scaring the public with outlandishly deleterious projections.

One reason for underestimating the risk is that scientists wish to present a consensus prediction. A more conservative prediction would have the support of the greatest number of scientists. Building scientific consensus on such a complicated topic as climate change is also slow, and scientists do not wish to support more deleterious conclusions and be proven wrong, which would damage their careers until the science becomes so clear that they cannot be shown to be wrong. Finally, scientists worry that if their consensus conclusions are not unanimous, lay people will see their pronouncements as mere opinions and not facts.

8 | C3/C4 PLANTS AND CLIMATE CHANGE

C3 and C4 plants represent 90% of plants on Earth, with C3 plants comprising almost all of the plants that are edible to humans and livestock. Unfortunately, C3 plants suffer from decreased photosynthetic rates at higher

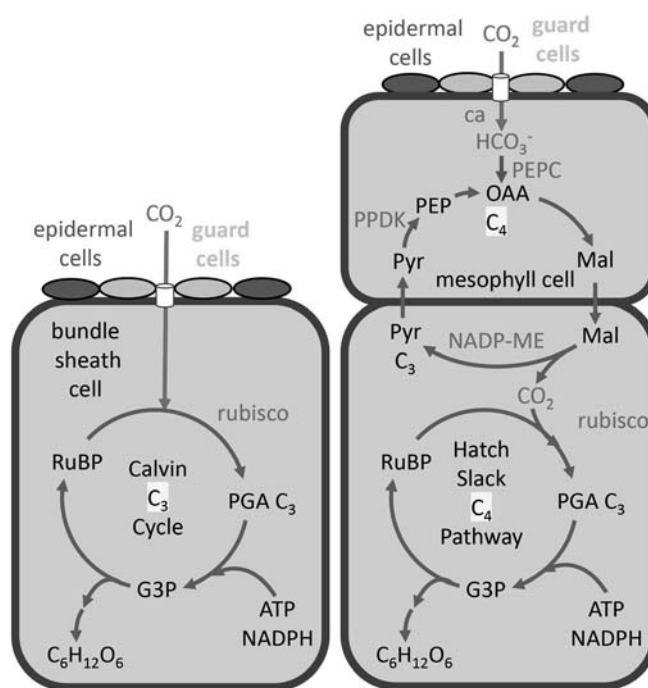
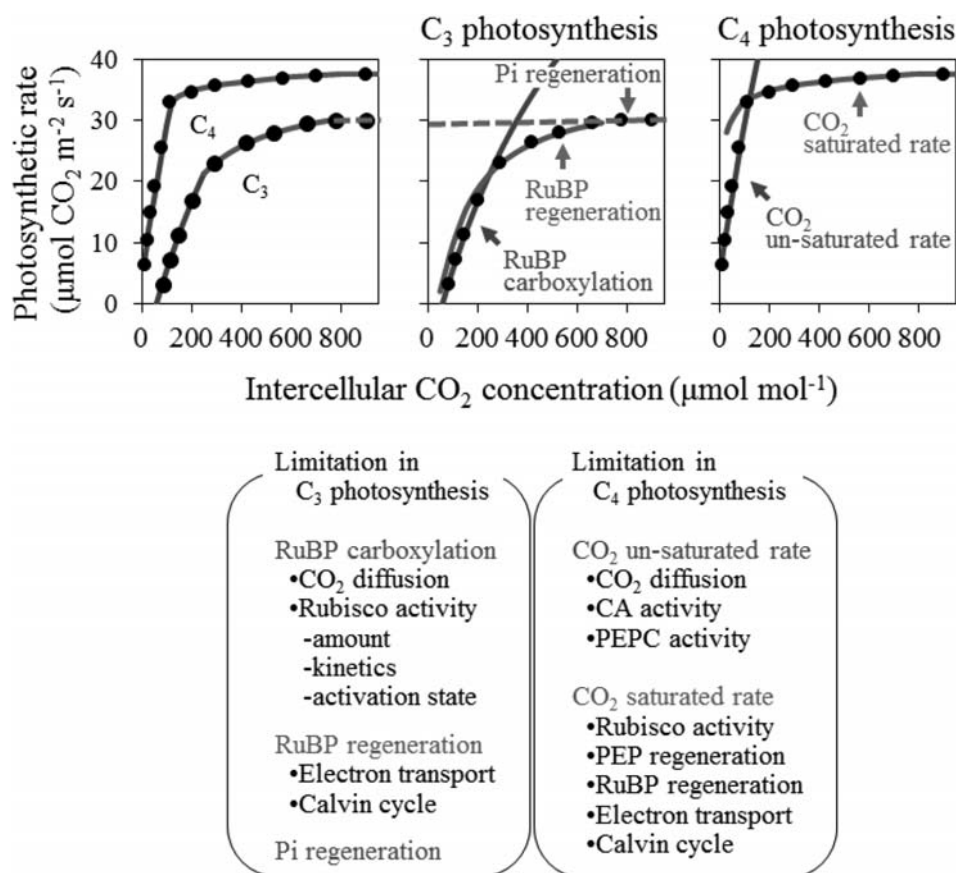


FIGURE 8 C3 and C4 carbon fixation pathways. Adapted from Yamori et al.³⁹ [Color figure can be viewed at wileyonlinelibrary.com]

temperatures, more sunlight and less water. Effectively, as C3 plants deteriorate with the effects of climate change so will we. C4 plants, however, have many evolutionary advantages over C3 plants in terms of temperature adaptation, water resistance, nitrogen assimilation, and carbon assimilation, thus making them promising subjects for biochemical research. What plant biochemical adaptations will impact photosynthesis at higher temperatures? Figure 8 offers a quick review of the C3 and C4 pathways (adapted from Yamori et al.).³⁹

In terms of temperature adaptation, scientists initially rationalized that changes to thylakoid membrane permeability would cause the most significant decrease in photosynthetic efficiency.^{40–43} The thylakoid membrane is of particular importance as this membrane houses the photosynthetic electron transport chain (ETC) with photosystem I (PSI) and photosystem II (PSII) enzymes responsible for generating the electrochemical gradient that ATP synthase harnesses to generate ATP. The photosynthetic ETC is also responsible for generating NADPH. The Calvin Cycle is powered by these ATP molecules and uses the electrons from NADPH to build sugar molecules. As temperature increases, the thylakoid membrane fluidity will also increase. This could lead to a greater number of protons diffusing out of the chloroplast and thus decreasing the efficiency of ATP synthase.^{40–43} Plants grown at higher temperatures overcome this leaky proton problem, and corresponding decrease in ATP, by increasing cyclic electron flow through PSI.^{40,42–44}

FIGURE 9 The top two right graphs deconstruct the left graph and show key pathway steps responsible for photosynthetic rates for C₃ and C₄ photosynthetic pathways at low and saturating CO₂ concentrations, with the lower boxes showing key rate controlling steps. Figure S1 freely available from Supporting Information³⁹ [Color figure can be viewed at wileyonlinelibrary.com]



More recently, however, Salvucci and Crafts-Brandner⁴⁵ and others have proposed that the Rubisco (ribulose biphosphate carboxylase oxygenase), the enzyme responsible for assimilating atmospheric CO₂ into sugar molecules, likely poses a greater limitation to temperature adaptation. The C₃, C₄ alphanumeric designation refers to how the plant assimilates CO₂ from the atmosphere into carbon intermediates that ultimately become dietary, structural, or signaling sugar molecules. As shown in Figure 8, C₃ plants attach, or “fix,” a CO₂ molecule to create the three-carbon intermediate 3-phosphoglyceric acid (PGA); whereas C₄ plants first fix a CO₂ molecule to yield the four-carbon intermediate oxaloacetate (OAA). OAA is then converted to malate or aspartate in order to facilitate transporting the carbon intermediate into the bundle sheath of the leaf. Here the four-carbon intermediate OAA is decarboxylated to release the originally assimilated CO₂. The carbon dioxide in C₄ bundle sheaths is then fixed to create the same three-carbon intermediate as C₃ plants, PGA. In both plants, Rubisco is responsible for catalyzing the reaction that yields PGA.

Rubisco is a slow enzyme with the capability to turnover about three CO₂ molecules per second. A recent study show a median k_{cat} for around 2500 enzymes from metabolic pathways with natural substrates of around 10 s^{-1} , which stands in stark contrast to one of the fastest

enzymes known, carbonic anhydrase (paradoxically for this manuscript), which can “hydrate” CO₂ with a k_{cat} of close to $1 \times 10^6 \text{ s}^{-1}$.⁴⁶ To overcome this deficiency, plants express an abundance of Rubisco inside of the chloroplast. C₄ plants express fewer Rubisco enzymes due to the small space of the bundle sheath, but these plants suffer less from the specificity issue with Rubisco using less O₂ as a substrate, and more CO₂.

The movement of CO₂ into the bundle sheath compartment provides a significant evolutionary advantage of C₄ plants. Here, the trapped CO₂ increases the local CO₂ concentration near Rubisco which increases the enzyme's efficiency 10- to 100-fold.^{47,48} In C₃ plants, a lower CO₂ concentration also means a higher O₂ concentration near Rubisco. This promiscuous enzyme can also attach an O₂ molecule to a sugar chain, creating an unwanted oxygenated product that the plant must correct. This decreases the efficiency of the carbon fixation pathway in C₃ plants by as much as 40% and is exacerbated by climate change stress conditions such as drought, high light, and high temperatures.

In many plants, as temperature increases, the activity of Rubisco decreases dramatically, and even moderate temperatures of 40°C can deactivate Rubisco.^{45,49} Compounding this problem is that the enzyme responsible for regulating Rubisco, Rubisco activase, is also thermolabile.^{45,50,51} However, in temperature adaption studies a second, heat stable



isozyme of Rubisco activase, is expressed to help stabilize Rubisco in some plants.^{52–54} In addition to Rubisco activase, some studies demonstrated that plants with adaptations for higher temperatures also express heat shock protein and chaperone proteins associated with the chloroplast.^{55–58}

It would seem that Rubisco could be a useful biochemical target for improving photosynthesis in C3 plants. However, when C3 and C4 plants are compared, they differ with respect to their limiting step, and even this can differ among plant species depending on growth temperatures.⁵⁹ Figure 9 charts the C3 and C4 plant response to CO₂ and proposed possible limiting steps.³⁹ It illustrates the difficulties in target selection and likely means that each plant will need to be independently studied to further investigate the limiting step, which would offer the highest gain in terms of bioengineering C3 plants that can withstand climate changes.

Of course, along with elevated temperatures, plants will also respond to elevated CO₂ levels. Theory and supportive observations would suggest that C4 species would be less affected by increasing CO₂. Recent experiments on experimental grassland plots show that the opposite might be the case.⁶⁰

9 | FIXING CARBON FIXATION

To avoid severe climate change and associated damages, we need not only to drastically reduce fossil fuel use but also develop truly carbon-neutral biofuels and economical and scalable carbon sequestration. Plants already make biofuels and capture carbon, but we need to do more than plant more trees and decrease burning of forests. Numerous other methods for sequestration and biofuel production have been proposed, but they are not presently cost effective or scalable. New approaches use genetic engineering and synthetic biology.

9.1 | Carbonic anhydrase

This enzyme is used to transport CO₂, a product of oxidative metabolism, away from respiring tissue by *reversibly* converting it to HCO₃[−] in red blood cells and releasing it back as CO₂ in the lungs. It is found in most organisms and works at essentially diffusion-controlled limits. Could a thermostable version be used to capture CO₂ released from fossil fuel burning in the form of HCO₃[−] in a mineralization process? This idea has been reviewed by Bose et al.⁶¹ Such a variant would have to overcome a problem inherent in typical form of the enzyme, namely product inhibition by bicarbonate. High concentrations of this enzyme allow it effectively to run in reverse to

produce high local concentration of CO₂ for carbon fixation by Rubisco in C4 plants.

In addition to thermostability, it would be ideal for the enzyme to be stable in alkaline conditions, which are needed for mineralization of the HCO₃[−] produced, as shown below (Rx 4):



The resulting carbonate forms insoluble salts with Ca²⁺, Mg²⁺, and Fe²⁺ divalent cations.

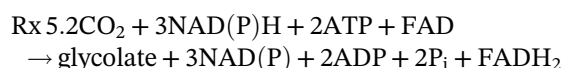
Capture of CO₂ in flue gases would have to occur at higher temperatures. Site directed mutagenesis⁶² and directed evolution⁶³ have been used to make variants of carbonic anhydrase which are stable at 90°C and 107°C, respectively. A mutant engineered to have a disulfide bond for stabilization⁶⁴ was kinetically more active and thermodynamically more stable at high temperatures. Carbonic anhydrases from extremophilic organisms have been isolated and examined for structure/activity relationships as well.⁶¹

9.2 | Novel in vitro carbon fixation pathways

Swander et al. have used synthetic biology to produce a new reaction pathway to fix CO₂ with the goal of producing feedstocks to create biofuels.⁶⁵ The Calvin cycle accounts for 90% of all autotrophic carbon fixation but it is catalytically slow and undergoes a competing photorespiration reaction with O₂. They identified an ideal (efficient) carboxylase, constructed a full pathway, calculated free energy and ATP/NADPH requirements, identified candidate enzymes from databases, and proceeded to optimize pathway.

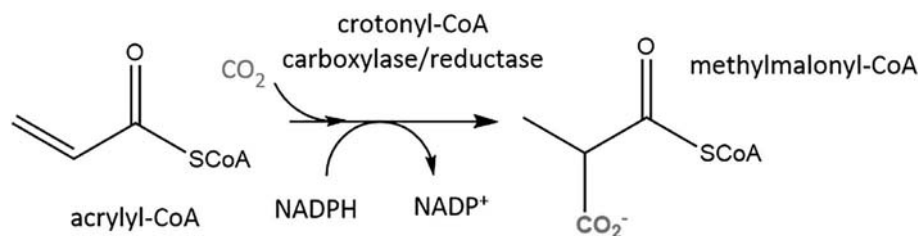
The carboxylases available in the biosphere include those familiar to most biochemistry students: acetyl-CoA carboxylase, Rubisco, propionyl-CoA carboxylase, PEP carboxykinase, 2-oxoglutarate carboxylase, and pyruvate carboxylase. They selected coenzyme A(CoA)-dependent carboxylases, and enoyl-CoA carboxylases/reductases enzymes. The carboxylation step for one of their synthetic pathways is shown in Figure 10.

They named the synthetic reactions the CETCH (Crotonyl-CoA-ETHylmalonyl-CoA-4Hydroxybutyl-CoA) pathway, which catalyzed the Rx 5 in vitro:



The rate of CO₂ fixation by this in vitro pathway was similar to Calvin cycle rates in cell lysates. The authors

FIGURE 10 The fixation of CO_2 by crotonyl-CoA carboxylase/reductase [Color figure can be viewed at wileyonlinelibrary.com]



speculate that this pathway may be transplanted into appropriate cells for future development of artificial photosynthetic systems.

9.3 | Synthetic biology carbon fixation pathways in *Escherichia coli*

Gleizer et al.⁶⁶ have made an experimental breakthrough in using synthetic biology and adaptive evolution to allow synthesis in a regulatable fashion of all of its biomass of *E. coli* from CO_2 . Formate was supplied as a source of energy and reduction power and Calvin cycle enzymes were used for carbon fixation. Cells were grown for 10 generations in $^{13}\text{CO}_2$ and ^{12}C -labeled formate to show the complete autotrophy from the synthetic pathway. Adaptive evolution was needed to rewire *E. coli* to convert it from a heterotroph to an autotroph. Alterations in growth media were made to prefer autophagy from the synthetic genes. Targeted genes were knocked out in main carbon metabolic pathways, including phosphofructokinase (glycolysis) and glucose-6-phosphate-dehydrogenase (pentose-phosphate pathway). New genes (Rubisco and phosphoribulokinase and carbonic anhydrase, needed for reversible conversion of CO_2 and HCO_3^-) were added. Xylose was utilized in the growth media as it would keep cells growing as it starves heterotrophic metabolic pathways.

Sequence analysis was also performed to determine the changes in genes required for conversion to autotrophy. One class of genes connected directly to the Calvin cycle function. Another class had members common to other adapted evolution experiments.

One drawback of their work was noted. Formate was ultimately converted to CO_2 so net production occurs. This could be relieved by electrochemical production of the formate as the substrate.⁶⁷

10 | OCEANS AND PHYTOPLANKTON

Phytoplankton are a diverse group of aquatic single-celled photosynthetic organisms ranging in size from 0.5 μm (cyanobacterium *Prochlorococcus*) to 2.0 mm (diatoms such as

Ethmodiscus rex). Because phytoplankton standing stock is replaced every 2–6 days,⁶⁸ they are responsible for roughly half of the earth's annual biological carbon fixation despite their biomass being diminutive compared to land plants.⁶⁹ While most phytoplankton primary production from CO_2 atm is respired relatively quickly and released to the atmosphere, a sizeable fraction is exported to greater depths, entering either long-term storage in ocean circulation or becoming buried in sediments at the seafloor.⁷⁰ Because this “biological carbon pump” represents one of the primary mechanisms for long-term storage of atmospheric carbon, understanding the biogeochemical variables affecting phytoplankton is central to anticipating the consequences of global climate change. Recent studies document changes that are already occurring in plankton populations due to increase temperature and ocean acidification.^{71–73} We damage them at our peril.

10.1 | Phytoplankton ecophysiology

At the cellular level, phytoplankton carbon fixation rates are determined by the availability of photosynthetically available radiation (PAR) and dissolved inorganic carbon (DIC, which include CO_2 , HCO_3^- , and CO_3^{2-}) to support photosynthesis. Phytoplankton carbon fixation rates generally increase with increases in PAR but are ultimately limited by the maximum reaction rates of Rubisco. Increases in PAR beyond this maximal rate may cause reductions in carbon fixation rates, as various photoprotective mechanisms begin directing energy away from chlorophyll reaction centers.⁷⁴ However, optimal PAR levels vary widely among phytoplankton taxa, reflecting acclimation to a range of aquatic environments.⁷⁵ Phytoplankton generally assimilate carbon in the form of dissolved bicarbonate ion, using carbonic anhydrase to interconvert HCO_3^- and CO_2 . However, assimilation mechanisms vary between different groups. Some taxa acquire DIC diffusively, while others utilize carbon-concentrating mechanisms (CCMs) to maintain high intracellular DIC concentrations near Rubisco.⁷⁶

Provided that PAR and DIC are available, phytoplankton primary production is limited by the availability of inorganic nutrients, particularly phosphorus, nitrogen, and iron, in addition to vitamins and micronutrients.



Additional nutrient requirements are taxon-specific: for example, cell wall construction requires sources of silica in the case of diatoms, and sources of carbonate in foraminifera and coccolithophores. Nutrients are typically acquired via active transport of dissolved inorganic forms via transmembrane channels, or by the enzymatic hydrolysis of dissolved organic substrates.⁷⁷ Over the last several decades, it has also become increasingly clear that many phytoplankton taxa obtain carbon, nutrients, or both via mixotrophy: the ingestion of other organisms via phagotrophy, or the ingestion of dissolved organic substrates via osmotrophy.⁷⁸

The availability of PAR and dissolved nutrients to phytoplankton is largely controlled by mixing. Vertical mixing is primarily controlled by seasonal temperature changes, with heating at the surface establishing a “mixed layer” (typically between 10 and 100 m in depth) of low-density surface water effectively separated from water masses at greater depth. This stratification of the water column has the simultaneous effect of enhancing phytoplankton growth (due to increased irradiance incident on phytoplankton cells mixed closer to the surface), while also preventing the equilibration of surface nutrients with those at depth. Horizontal mixing near the surface generally occurs as a result of interactions between the sea surface and the atmosphere and is responsible for both nutrient-rich upwelling zones at continental margins, as well as the expansive marine deserts at the center of ocean basins. Other nutrient inputs include continental runoff, wind deposition and, in the case of nitrogen, reduction of atmospheric dinitrogen gas to biologically available forms by nitrogen fixing phytoplankton.

Community-level primary production generally increases with increases in nutrient concentrations,⁷⁹ with phytoplankton biomass and primary production typically being greatest in coastal regions and upwelling zones. The efficiency of export of phytoplankton carbon to sediments is greatest in near coasts, with export flux being reported to be disproportionately greater in coastal regions compared to open ocean sites.⁸⁰ With the proportion of smaller phytoplankton cells (e.g., cyanobacteria) being inversely related to total phytoplankton biomass,⁸¹ this difference in export efficiency is generally attributed to faster sinking rates of the large cells characteristic of coastal waters. However, the extent to which this explanation applies is unclear.⁸²

10.2 | Anticipated impacts of climate change on phytoplankton physiology

Climate change is expected to have large effects on virtually all biogeochemical parameters affecting phytoplankton

physiology. However, owing to the diversity of phytoplankton and the interactive nature of changes in different biogeochemical variables, it is difficult to extrapolate experimental results to derive mechanistic explanations of how community-scale phytoplankton primary production will be affected by climate change.⁸³

For example, the large-scale effect of elevated CO₂ on phytoplankton communities is complicated by the diversity of CCMs present across different phytoplankton taxa. Moreover, CCM activity itself may be altered by DIC concentrations, in addition to temperature, PAR, and nutrient availability.⁸⁴ Species lacking CCMs might be expected to benefit the most from elevated CO₂ concentrations, resulting in a shift in community composition under high-CO₂ conditions. However, experimental studies have returned mixed results, with some studies reporting a change in community composition under elevated CO₂ with no effect on primary production⁸⁵ while another shows an increase in primary production under elevated CO₂ with no effect on community composition.⁸⁶

Similarly, elevated concentrations of dissolved CO₂ are expected to favor the formation of bicarbonate ion while reducing concentrations of carbonate ion. This reduction is expected to in turn favor the dissolution of calcite into calcium and carbonate ions (Rx 2). In theory, this would result in reduced calcification and/or growth rates of calcifying organisms such as coccolithophores or foraminiferans. However, the extent to which this can be expected to occur, and to what degree it might affect primary production by these organisms has been demonstrated to be species-specific.^{87,88}

Despite the challenges in generalizing the impacts of climate change on a cellular scale, the effects anticipated at the community level are less ambiguous. Increases in temperature might have especially deleterious consequences for phytoplankton communities, resulting in a greater degree of stratification in the photic zone and in turn reducing the average mixed layer depth.^{89,90} Because efficient mixing is essential to the redistribution of dissolved nutrients, increased stratification could increase the extent of nutrient depleted waters in the open ocean, causing phytoplankton community size structure to shift toward smaller taxa.⁹¹ By increasing the daily integrated UV radiation incident on phytoplankton cells, shallow mixed layer depths could additionally reduce primary production.⁹² In contrast to these reductions phytoplankton biomass, changes in temperature have also been reported to increase the frequency and duration of harmful algal blooms (HABs) over the last 40 years, especially in midlatitude regions⁹³. HABs may result in the bioaccumulation of toxins in food webs, impacting aquatic life and fisheries in both marine and freshwater environments while also posing public health hazards.⁹⁴

Numerous studies have drawn on remote sensed chlorophyll data to understand how these changes might affect phytoplankton at the global scale. Based on an analysis of chlorophyll data from the SeaWiFS satellite, Polovina et al. reported a 4% annual increase in the extent of low chlorophyll regions between the years 1998 and 2006.⁹⁵ Rousseaux and Gregg applied the NASA Ocean Biogeochemical model to 14 years of ocean color data from both the SeaWiFS and MODIS satellites, reporting a global decrease in diatom abundance correlated with a reduction in average mixed layer depth between 1998 and 2012.⁹⁶ Behrenfeld et al. used SeaWiFS data from the same period to estimate net primary production (defined as the difference between total carbon assimilation and the fraction subsequently respired), reporting an annual decrease of 190 Tg C/year.⁸⁹ If carbon export decreases more rapidly than primary production, these decreases in primary production could result in even larger changes in carbon export. In a modeling study of global diatom distributions, Bopp et al. concluded that a 15% decrease in primary production at the surface could correspond to up to a 25% reduction in carbon export from the photic zone.⁹⁷

10.3 | Using oceanographic data in the classroom

There is an abundance of freely available oceanographic data that can be used to teach phytoplankton community dynamics in the classroom. Most data sets consist of depth profiles of physical properties (salinity, temperature, density, PAR), in addition to concentrations of macronutrients ($\text{NO}_2 + \text{NO}_3$, PO_4^{3-} , Si), and abundances of cyanobacterial and picoeukaryotic cells. Data are generally collected either as part of a time series, or on individual research cruises to different ocean regions. The Hawaii Ocean Time Series provides data collected over the last 30 years, along with an online data-visualization tool. A similarly extensive data set is available for the Bermuda Atlantic Time Series, although online data visualization tools are not available⁹⁸; the `bats_bottle.txt` file provides a compilation of all parameter measurements for the duration of the program). The Lefe Cyber website hosts data from a number of cruises and time series, although the data may require compilation prior to use.⁹⁹

Ocean Data View is a freely available software package specifically designed for visualizing and analyzing oceanographic data sets.¹⁰⁰ Satellite chlorophyll data are easily accessible using NASA's SeaDAS software package. The SeaDAS website¹⁰¹ includes video tutorials on acquiring data and performing analyses. The Biogeochemical ARGO program maintains measurements of

biogeochemical parameters (including chlorophyll fluorescence) for a global array of autonomous profiling floats, available in netCDF or csv format.¹⁰²

11 | METABOLISM AND CLIMATE CHANGE: ECOLOGICAL CONTEXTS

Most biochemistry courses focus on core biochemical pathways that are shared across massive swaths of the tree of life. In contrast, advanced biochemistry courses often highlight pathways that are unique to specific branches of that tree as well as biochemical subdisciplines, including plant biochemistry, medicinal chemistry, metabolic evolution, or metabolic engineering. Student interest in these focus areas can be enhanced by placing lessons into a real-world context. Ecology is one such context, which not only enables real-world, tangible case studies and examples, but also links this array of biochemical subdisciplines to our changing climate.

We present a case study in an ecological context which links lineage-specific metabolic pathways, metabolic engineering, biodiversity, and climate change, all with a focus on the concepts of enzyme specificity and promiscuity—components in both basic and advanced biochemistry courses. This case study places the reader into a historically accurate chronology describing a 20-year span of research projects and publications aimed at understanding the medicinal natural product celastrol. This reference and the story they tell could be used as, for example, a framework for lecture, discussion-based or active learning classroom settings. This case study also includes a set of short answer homework or discussion questions designed to send students into primary literature, promote critical thinking, and reinforce links between biochemistry and climate change.

11.1 | Case study: Celastrol biosynthesis

Homework/discussion questions with answers that relate to climate change are found in Supporting Information. Though highly specific enzymes are ubiquitous in core metabolic processes, promiscuous enzymes that accept multiple substrates or generate multiple products are also widespread, though they generally occur in the periphery of metabolic spaces and in species-specific patterns. We present a case study on a species-specific metabolic pathway mediated by an enzyme that generates multiple products from a single substrate. Scientists are interested in the plant genus *Tripterygium*, members of which are used in Chinese traditional medicine. You want to understand the details of how *Tripterygium* acts as a medicine. A search of the scientific



literature reveals that *Tripterygium wilfordii* produces a unique compound called celastrol¹⁰³ that is active against HIV¹⁰⁴ and Crohn's disease.¹⁰⁵ Scientists have also developed a strain of *Tripterygium* cells that can be cultured to produce celastrol.¹⁰⁶ However, to obtain this compound in even larger quantities, they are working to find enzymes required for celastrol biosynthesis in vivo which could be transferred to other organisms for synthesis. One group found that *T. wilfordii* contains cyclases (OSCs) that can convert 2,3-oxidosqualene into a celastrol precursor called friedelin (Figure 11a). On binding of 2,3-oxidosqualene to OSC, the reaction proceeds through a carbocation intermediate, followed by deprotonation to generate its product. However, the deprotonation can occur in more than one way. In one way (Figure 11a, green path), deprotonation leads to a cascade of methyl and proton shifts to generate the celastrol precursor friedelin.¹⁰⁷ In another path, a different deprotonation (Figure 11a, blue path) leads to the product β -amyrin.

Zhou and colleagues discovered that *T. wilfordii* actually contains three OSC enzymes (TwOSC1–TwOSC3), each of which produces a different ratio of β -amyrin to friedelin (TwOSC1—1:3; TwOSC2—1:0, and TwOSC3—1:2; see Figure 11b). To study this product promiscuity in more detail, Zhou et al. also created several mutant enzymes. They found, for example, that TwOSC1-L486V produces a 4:1 product ratio of β -amyrin to friedelin and

that TwOSC3-L482V yields a 1:0 ratio (Figure 11b). Finally, in a separate study, another group has reported the discovery of several cytochrome P450 enzymes that catalyze additional oxidation steps on the pathway to celastrol¹⁰⁸; Figure 11a).

12 | TOOLS FOR CLIMATE CHANGE IN THE CLASSROOM

The field of biochemistry has the potential to address numerous threats to humanity, including water and food availability, emerging diseases and long-standing health concerns, as well as climate change. At the same time, both the controversy surrounding the topics and the lack of dedicated time for class innovation, prevent many instructors from adequately preparing students to address these issues. Continued advancements in biochemistry-dependent fields requires that students—who represent the next generation of professionals—develop deep understandings of key concepts and be able to apply the knowledge they gain to diverse scenarios.¹⁰⁹

Here, we attempt to reduce the difficulty of class innovation by providing two of the most commonly used formative assessment methods (clicker and homework questions). These are all provided as resources for instructors who are encouraged to modify them to fit

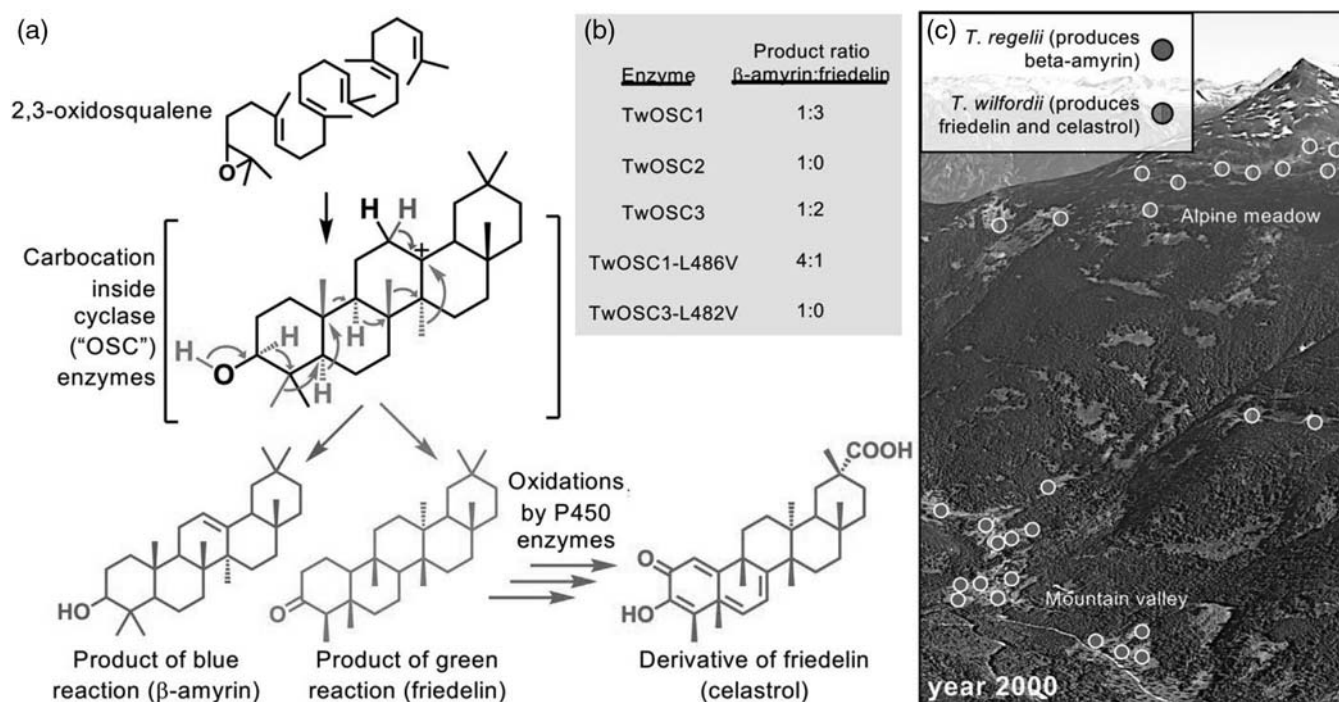


FIGURE 11 Data for a case study of celastrol biosynthesis. Known biosynthetic steps on the pathway to the medicinal compound celastrol. B product profiles of cyclase (OSC) enzymes from *Tripterygium wilfordii* as reported by Zhou et al. C fictitious locations of *Tripterygium regelii* and *T. wilfordii* plants on a mountainside, for use in short answer or discussion questions 3–5 in Supporting Information [Color figure can be viewed at wileyonlinelibrary.com]

their class needs. Together, the questions repeatedly incorporate the most basic facts about climate change, namely that CO_2 concentrations are increasing, and the average temperatures are climbing. Students are asked to solve various biochemical ramifications of the established facts in multiple scenarios. This provides repeated passive exposure to climate change and active thought about their consequences. It is our hope that these tools will inspire students to seek more information, even if it is not otherwise taught. Two Supporting Information files include solved homework problems and clicker questions.

In the Supporting Information (File—Homework), instructors will find a series of questions that address topics typically included in a biochemistry classroom, and the effects that increasing concentrations of CO_2 , or temperature have on these systems. Citations for potentially controversial ideas are included, as are complete answer keys with reasoning. The questions cover the following topics:

- Calculation of human blood pH, an early review for many biochemistry lecture and lab classes. It requires use of the Henderson-Hasselbalch equation and a solid knowledge of algebra.
- Protein binding and allostery, specifically hemoglobin binding of oxygen. It introduces the Bohr Effect on hemoglobin's binding of oxygen. It requires a mathematical understanding of fractional binding and allosteric effects.
- Gibbs free energy and enzyme effects on a reaction. It uses a reaction from glycolysis to illustrate the effect of temperature on equilibrium and protein folding. An in-depth understanding of glycolysis is not necessary.
- The effects of fatty acids, unsaturation, and cholesterol on membrane fluidity.
- How C_4 and C_3 photosynthesizing organisms would be affected by climate change. It requires a conceptual understanding of the differences between C_4 and C_3 photosynthesis.
- DNA sequence changes that would occur to offset temperature. It requires an understanding of how base pairs interact and DNA melting.

In the Supporting Information (File—Clicker Questions), each question has an introductory slide that introduces the biochemical topic and is meant to immediately precede the clicker question. Each question is focused on a change occurring due to the increasing concentrations of CO_2 or the increase in temperature. The questions cover the following topics: blood buffering, the Bohr effect on hemoglobin, enzyme kinetics— V_{max} , enzyme kinetics, K_m , membrane fluidity,

introductory photosynthesis/the carbon cycle, DNA base pairing, and DNA base stacking. An enhanced description of the prerequisite knowledge and teaching and discussion points for each topic are found in the Supporting Information.

13 | ENGAGING STUDENTS IN SCIENCE AND RESEARCH: NASA

The National Aeronautics and Space Administration (NASA) offers a large collection of free STEM and climate change resources designed for students, educators and citizen scientists across all grade levels for formal and informal educational institutions.¹¹⁰ These resources could and should be used across all STEM disciplines, including biochemistry. The Mission and Vision for the NASA Office of STEM Engagement is to engage the nation in NASA's mission and immerse the public in NASA's work.

Multiple studies and our own experience as STEM educators show that involving students in research is a high impact practice.¹¹¹ NASA offers the year-long Climate Change Research Initiative (CCRI), which provides research opportunities for STEM students and faculty at the NASA Goddard Institute for Space Studies. Participants are aligned in a multidisciplinary team. Graduate level students and high school STEM teachers are paired with NASA scientist and work on a designated climate change research project.¹¹² High school teachers who participate integrate what they learn into their classrooms by developing an Applied Research STEM Curriculum Unit Plan which translates a component of the teachers research project into a multi lesson unit plan aligned with the NGSS, state science and common core standards while integrating NASA education content, resources, platforms and missions. Programs for high school teachers are designed to meet both NASA science standards and Next Generation Science Standards.¹¹³

The program expands the research team in the summer to include both a high school and undergraduate students. At the end of the 8-week summer program, CCRI teams present scientific posters, publishable research papers and comprehensive project PowerPoint presentations at local, regional, and national symposiums, the NASA Goddard Institute for Space Studies (GISS) and the NASA Goddard Space Flight Center. Past thematic research projects, which are run at GISS and nearby universities include: Atmospheric Rivers in a Changing Climate, Characterizing the Urban Land Surface Temperature via an Innovative, Multi-Platformed Suite of Satellite and Ground-Based Remote Sensing



Technologies, Climate Change in the Hudson Estuary—Past, Present, and Future, and Earth Observation Applications for Resiliency: Assessing Climate Change Impacts in Urban, Agricultural, and Natural Environments. The CCRI program substantially improves STEM and climate change education while using NASA's unique resources. The time has come for biochemist to become involved.

Additional examples of NASA education content resources related to climate change include:

*The GLOBE Program*¹¹⁴: The Global Learning and Observations to Benefit the Environment (GLOBE) Program is an international science and education program that provides students and the public worldwide with the opportunity to participate in data collection and the scientific process, and contribute meaningfully to our understanding of the Earth system and global environment.

*My NASA Data*¹¹⁵: NASA offers petabytes of global Earth science data collected from satellites but accessing these data in a traditional science classroom can be tricky. After nearly 15 years of offering Earth science data to educators and students, NASA continues to refine the My NASA Data program to better suit the needs of teachers and students in engaging students in authentic data analysis.

*Global Climate Change—Vital Signs of the Planet*¹¹⁶: A broad collection of articles, mitigation strategies, exploration opportunities, imagery, and education resources on the sciences related to climate change.

*NASA's Scientific Visualization Studio*¹¹⁷: The SVS works closely with scientists in the creation of visualizations, animations, and images in order to promote a greater understanding of Earth and Space Science research activities at NASA and within the academic research community supported by NASA.

*GISS Surface Temperature Analysis (GISTEMP v4)*¹¹⁸: The GISS Surface Temperature Analysis (GISTEMP v4) is an estimate of global surface temperature change. Graphs and tables are updated around the middle of every month using current data files from NOAA GHCN v4 (meteorological stations), and ERSST v5 (ocean areas).

14 | SUMMARY

It is difficult to argue against the proposition that biochemistry educators have a professional and ethical obligation to discuss climate change and its consequences in their classes. In that light, we have offered a variety of biochemistry-related examples, from simple to complex, to show how this can be accomplished. In addition, we have provided background information for those who are not versed in the climate change literature. We hope that

these examples and background help move educators to routinely discuss climate change and its consequences with their students, who look up to them as role models and who will be impacted most by their climate futures. In addition, we hope this manuscript leads to future submissions on this most important topic. Two themes immediately come to mind. One would deal with the biochemistry and ethics of genetically-modified crops that would help humankind feed the world's people in a changing climate. Another would discuss the links between the COVID-19 and other pandemics and climate change.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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