Received: 22 August 2022 | Revised: 2 November 2022 | Accepted: 9 December 2022

DOI: 10.1002/moda.1

PERSPECTIVE

MODA WILEY

Why cutting respiratory CO_2 loss from crops is possible, practicable, and prudential

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Funding information

Office of Science, Grant/Award Number: DE-SC0020153; USDA National Institute of Food and Agriculture, Grant/Award Number: FLA-HOS-005796

Abstract

Plants release back to the atmosphere about half of the CO_2 they capture by photosynthesis. Decreasing the rate of crop respiration could therefore potentially increase yields, store more carbon in the soil and draw down atmospheric CO_2. However, decreasing respiration rate has had very little research effort compared to increasing photosynthesis, the historically dominant metabolic paradigm for crop improvement. Conceptual and technical advances, particularly in protein turnover and directed enzyme evolution, have now opened ways to trim the large fraction of respiration that fuels proteome maintenance by lowering the breakdown and resynthesis rates of enzymes and other proteins. In addition to being theoretically possible and practicable, exploring the reduction of respiration is prudential, given that it (i) has barely yet been tried and (ii) could help meet the challenges of sustaining crop productivity and managing atmospheric carbon.

KEYWORDS

carbon capture and storage, crop respiration, directed evolution, protein turnover, yield

Key points

- Because plants return to the atmosphere about half the CO_2 captured by photosynthesis, decreasing crop respiration could raise yields, improve soil carbon storage and draw down atmospheric CO_2.
- Relative to photosynthesis, decreasing respiration rate nevertheless remained a low research priority for decades.
- This situation is now changing, thanks particularly to advances in proteomics and directed evolution that make it possible to cut respiration rates by slowing breakdown and resynthesis rates of enzymes.

INTRODUCTION

Increasing photosynthetic CO_2 fixation has been the dominant metabolic paradigm for increasing crop yields for >40 years,[1–4] and has now also become a dominant paradigm for plant-based carbon sequestration strategies.[5,6] The alternative of reducing respiratory CO_2 production has—like plant respiration in general (Figure 1a)[7]—had far less research even though plants release roughly half the carbon fixed by photosynthesis back to the atmosphere via respiration (Figure 1b).[8,9] Plant respiration (often called 'dark' respiration) refers

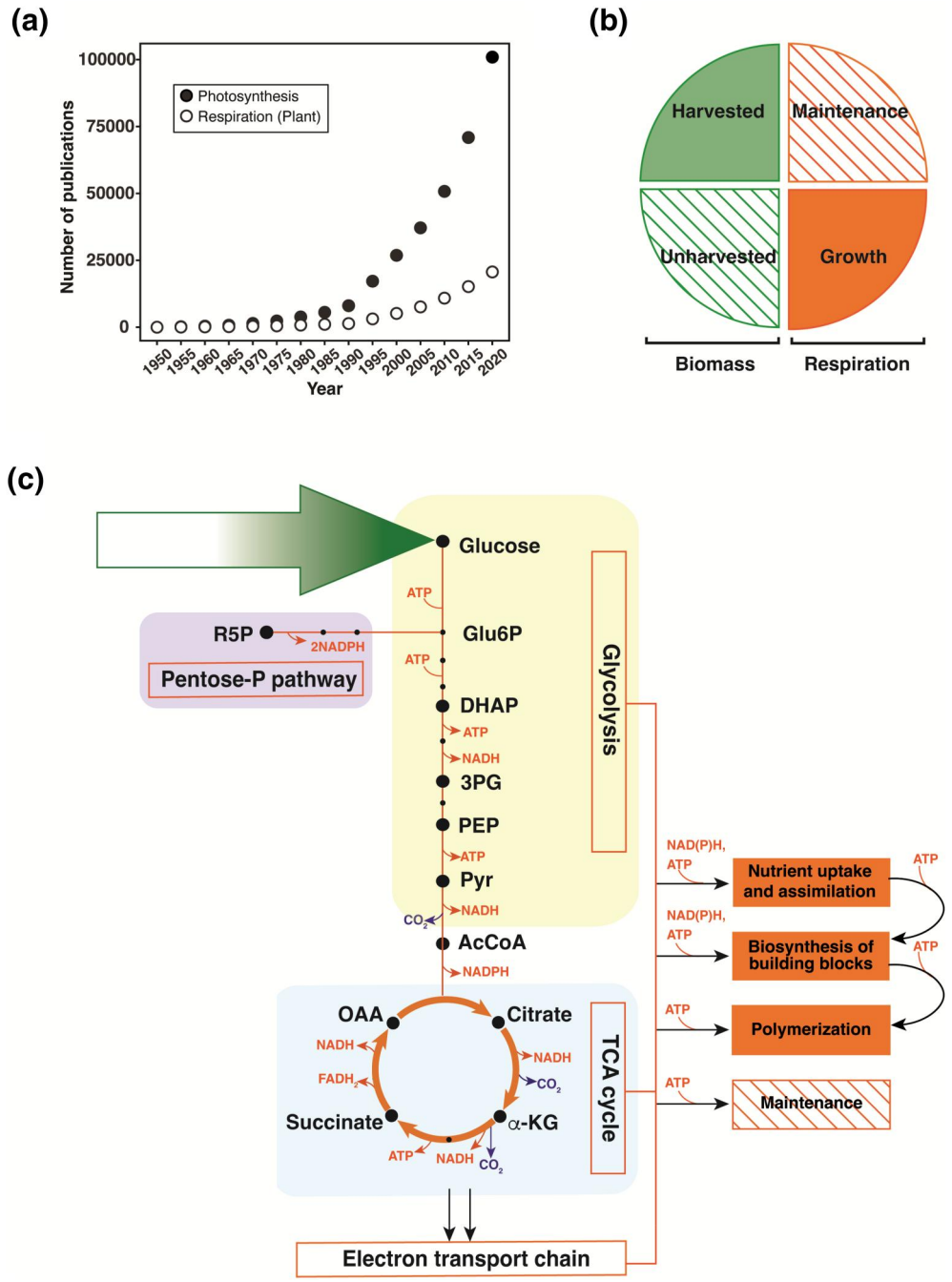

FIGURE 1 The disparity between research on respiration and photosynthesis relative to their contributions to plant carbon budgets, and the nature of growth and maintenance respiration. (a) Cumulative publications (1950–2020) in the Web of Science database with 'photosynthesis' or 'plant' plus 'respiration' in any search field. The disparity widened from 3.1:1 in 1950 (not visible due to superposed data points) to 4.9:1 in 2020. (b) A generalised carbon budget for an annual grain crop to show how much photosynthetically fixed carbon is respired or retained in harvested or non-harvested biomass. Quadrant areas are proportional to the amounts of carbon they represent. (c) Scheme showing how chemical energy generated by respiratory pathways is used to build new biomass or maintain existing biomass (inspired by[19]). Glucose is shown as a generic but not the only substrate for plant respiration; in leaves at night it is often glucose exported from chloroplasts as a product of starch degradation, in leaves in the day it is triose-phosphates like dihydroxyacetone phosphate exported from chloroplasts and entering glycolysis, and in sink tissues it is largely sucrose generated in source leaves for export and used to generate glucose, fructose and then glucose 6-phosphate in sink tissues.

to the CO_2 released when photosynthate, typically taken as hexose, is metabolised to provide carbon skeletons, ATP and reducing power for various processes. Plant respiration is a distinct process and has a different role from photorespiration, which recycles phosphoglycolate produced by the Rubisco oxygenation reaction. One reason that plant respiration has been sidelined up to now is that many different metabolic processes and reactions are involved, making it harder to define targets for engineered improvement than it is for photosynthesis.[9] This situation is changing, as we show below.

Respiration in all organisms can be conceptually split into 'growth' and 'maintenance' components that differ in how the ATP, reductant and carbon skeletons produced by respiratory pathways are used, not in the pathways themselves (Figure 1c).[10,11] Growth respiration supports the construction of new biomass, that is, processes such

as nitrate and sulphate uptake and reduction, amino acid synthesis and amino acid polymerisation into proteins. Maintenance respiration supports cyclic processes such as protein and mRNA turnover and countering ion leakage to sustain cellular ion pools and membrane potentials. Critically, these cyclic processes, like most other biochemical processes, run faster as temperature rises, roughly doubling in rate for each 10°C rise in temperature (i.e., $Q_{10} \approx 2$).[11,12] Photosynthesis is commonly an exception, reaching a plateau and then declining above ~30°C, whereas respiration rate increases until ~45°C.[13] For this reason, increased maintenance respiration is often a major contributor to yield losses in chronic, moderately high temperature conditions.[12] As global temperatures rise, maintenance respiration could thus become an even greater determinant of yield than it has been to date.[11–13]

The scope for reducing growth respiration is quite limited,[9] except by changing biomass composition to an energetically cheaper mix (e.g., more carbohydrate, less protein or lipid). This is because the pathways that plants use are generally efficient ones, and the ATP, reductant and carbon requirements of most of these pathways are set by chemical stoichiometries. There is more scope to reduce maintenance respiration, for which a particularly promising strategy is to reduce protein turnover rates.[9] Protein turnover is reckoned (within large uncertainty intervals) to account for ~50% of maintenance respiration (Figure 1b),[9,14] and perhaps more if the ATP yield of respiration is below that usually assumed.[15] As we explain in the next two sections, theory and evidence indicate that slowing protein turnover—and hence decreasing respiration—is possible, and advances in proteomics, directed evolution, metabolic modelling and genome editing make it a practical option to explore. The last section argues that it is agricultural and environmental common sense to start this exploration.

SLOWING PROTEIN TURNOVER TO CUT RESPIRATION IS POSSIBLE IN PRINCIPLE

Keeping the cost of the proteome down by maximising the use of energetically cheap amino acids, minimising protein size, matching protein levels to demand and tuning protein turnover rates has shaped evolution in plants and all other organisms.[16–19] However, unlike heterotrophic microbes and animals, plants fix their own carbon. Plants in natural environments often experience an excess of carbon (and hence energy) availability relative to nitrogen, phosphorus and other nutrients,[20–23] and this can also occur in crop situations.[24] Moreover, land plants evolved during the past 500 million years in conditions predisposing to even greater carbon excess because atmospheric CO_2 concentrations throughout much of this period were at least three-fold higher than today.[25] The availability of surplus carbon could logically weaken selection for thrift in carbon (i.e., energy) expenditure on protein turnover, leading to enzymes and other proteins with needlessly high turnover rates and respiratory costs. This would fit a general pattern in which plants apparently use excess carbon wastefully.[23,26] It would also fit with Arabidopsis having a higher proportion of enzymes with short working lifespans than yeast or a dairy bacterium,[27] and with a large variation in protein turnover rate among Arabidopsis accessions that correlates negatively with growth rate.[28]

If crop ancestors, and crops themselves until the 20th century, evolved in conditions of carbon excess imposed by nutrient limitations, it follows that crops today cannot take the best advantage of well-fertilised agricultural environments because they are geared to inefficiently use surplus carbon that is no longer present.[9] Put differently, the Haber–Bosch nitrogen fixation process and industrial agriculture in general[29] may have placed an unprecedented premium on efficient respiratory carbon use that breeding programmes can exploit. There are hints that conventional breeding has started to do this. For instance, a study of eight wheat genotypes showed that biomass accumulation and canopy respiration rate were strongly negatively correlated in a way consistent with variation in maintenance respiration (Figure 2a).[30] Similarly, a study of six commercial maize hybrids released between 1959 and 1995 found a significant downward time trend in leaf respiration rate (Figure 2b), and a strong negative correlation between respiration rate and biomass accumulation (Figure 2c).[31] If maintenance respiration were to fall by the maximum percentage seen in these studies (36% and 14%, respectively), the amount of carbon spared could contribute substantially to biomass increases.[32] Relatedly, a comparison of 30 maize hybrids, commercialised between 1930 and 2017, found that overall radiation use efficiency (RUE) rose from 1.5 to 2.0 g MJ^{-1} of intercepted radiation (Figure 2d).[33] The physiological basis for the increase is unknown and is surely multifactorial, not just a reduction in maintenance respiration. It is nonetheless notable that an RUE increase of this size is within the range that variation in maintenance respiration could theoretically account for, and that this is a likelier explanation than the alternatives, that is, changes in root to shoot allocation or increased photosynthesis.[34] It is noteworthy that the indicative studies summarised in Figure 2a–c are 20–30 years old, and that the lack of more recent follow-up work reflects ongoing underinvestment in respiration research (Figure 1a).[7]

To summarise, the conclusions are as follows: There is good cause to think (i) that protein turnover is a major drain on plant carbon budgets, (ii) that enzymes and other plant proteins have generally not been selected for the longest possible lifespan, and hence (iii) that it is biologically reasonable to expect gains in response to selection for enzyme lifespan and that longer lifespan will increase net carbon fixation and retention in biomass.

SLOWING PROTEIN TURNOVER SHOULD BE ACHIEVABLE IN PRACTICE

As just said, conventional breeding programmes may already have reduced the maintenance respiration associated with protein turnover by acting on natural

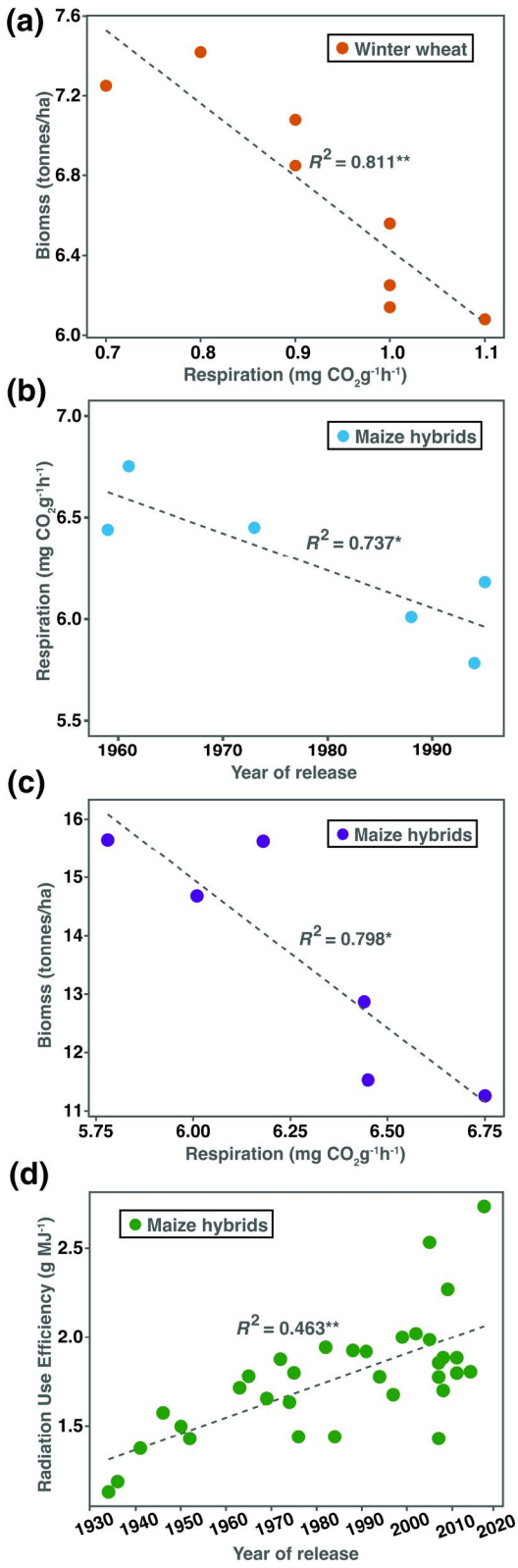

FIGURE 2 Crop breeding progress in relation to trends in respiration rate or radiation use efficiency. Data are replotted from the original publications. (a) Significant negative correlation among eight Canadian winter wheat genotypes between biomass accumulation and canopy respiration rate at 25°C.[30] Significant negative correlations among six commercial maize hybrids between (b) respiration rate of mature upper leaves at 25°C and release date and (c) biomass accumulation and respiration rate.[31] (d) Significant positive correlation among 30 commercial maize hybrids between radiation use efficiency and date of release.[33] The regression line in D is based simply on the data points, which are mean values; the original publication weighted the data points by their errors. R^2 significance: $^*p < 0.05$ and $^{**}p < 0.01$.

variation in turnover rates similar to the variation found among Arabidopsis accessions[28] and between species.[35] While effective, this approach is indirect, limited by the genetic variation available in the crop's primary gene pool and can take decades. Advances in proteomics, metabolic modelling and synthetic biology now enable an alternative approach that is direct, creates its own near-infinite genetic variation and takes only weeks to improve an enzyme. The basic scheme is to identify a short-lived crop enzyme, to rapidly evolve it *ex planta* for longer life, to identify the mutations responsible and to make these mutations in the crop itself by genome editing (Figure 3a).

Proteomics and the CCR concept

The proteomics advance is the combination of stable isotope labelling with mass spectrometry to measure protein turnover rates on a 'proteome-wide' basis.[36–38] In practice, this means assessments for hundreds of relatively abundant proteins rather than the whole proteome. However, since only abundant proteins can cause substantial turnover costs, this is not a limitation if the aim is to pinpoint high-cost enzyme targets. The turnover rate of an enzyme protein is necessary but not sufficient information to pick the best targets for lifespan extension. Also needed is the metabolic flux that the enzyme carries in vivo because this allows the enzyme's working life to be expressed as the number of catalytic cycles that each enzyme molecule mediates before being replaced.[27,39] The formal term for this is 'Catalytic-Cycles-till-Replacement' (CCR). Mathematically, it is given by the metabolic flux rate divided by the enzyme protein turnover rate:

$$\mathrm{CCR} = \frac{\text{Metabolic flux rate}}{\text{Enzyme replacement rate}} \quad (1)$$

Expressing enzyme working life as a CCR value is critical because many enzymes are subject to self-inactivation by damage from the very reaction that they catalyse, so that the more reactions an enzyme molecule performs, the likelier it is to die.[27,40] Thus, finding enzymes that often die young, that is, have unusually low CCRs, is a logical way to locate the most improvable targets; raw protein turnover rate is a less effective screen for enzyme lifespan improvability because enzymes that die young in CCR terms do not necessarily turn over especially fast.[27,40] It is the balance of metabolic flux and enzyme replacement rate that matters (Equation 1).

Metabolic modelling

The numerator—the in vivo metabolic flux through an enzyme step—is obtained from modelling.[39] Although fluxes can be measured using isotope labelling for small numbers of reactions, the global coverage needed to extract large numbers of CCR values is still

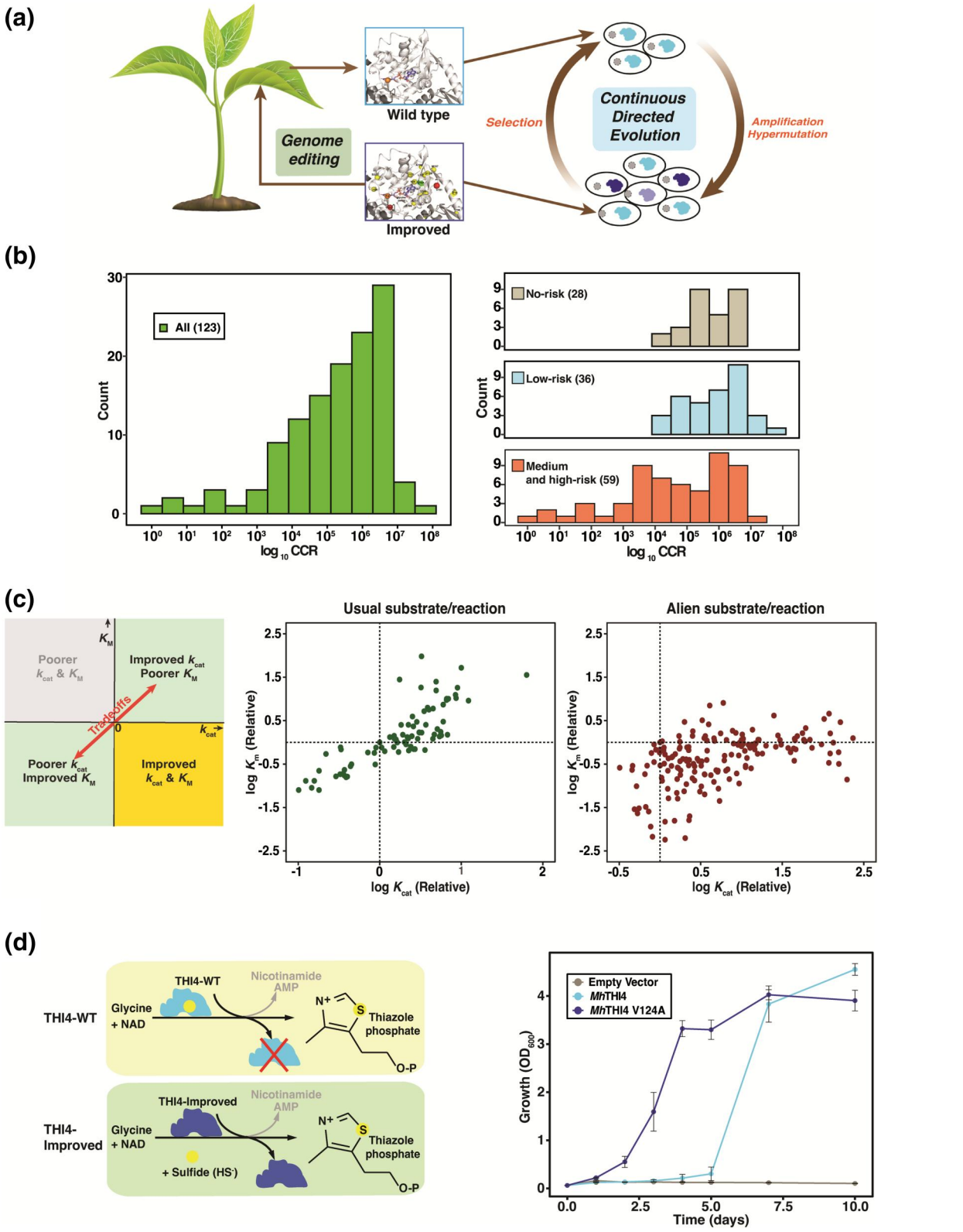

FIGURE 3 Directed evolution of enzyme lifespan. (a) The concept of taking a crop enzyme gene, lengthening the enzyme's life via continuous directed evolution of the gene in a microbe, and then making the life-lengthening mutations in the crop by genome editing or gene replacement. (b) The distribution of CCR values among 123 Arabidopsis metabolic enzymes (left) and the same data with enzymes classified by risk of damage from the reaction they mediate (right).[27] (c) K_M versus k_{cat} scatter plots for enzymes catalysing their canonical reaction on a substrate they are well adapted to (centre) or either catalysing a new reaction or acting on an alien substrate (right). See Supporting Information S1 for original data sources. The scheme (left) explains the quadrants in the plots. (d) The thiazole synthesis reaction mediated by non-suicidal bacterial THI4s and a typical outcome of a continuous directed evolution campaign.[60] A single mutation (V124A) in *Mucinivorans hirudinis* THI4 (MhTHI4) substantially improves the growth of the yeast *thi4*Δ platform strain compared to wildtype MhTHI4.

out of reach. Fortunately, genome-scale flux balance analysis (FBA) models, when constrained by measurements, can output robust flux estimates for all steps in major pathways[41,42]; fluxes through multiple isoforms can be allocated based on the isoforms' subcellular locations and relative abundances.[27] FBA models are

the preferred way to obtain CCRs, but if no satisfactory FBA model is available, an acceptable alternative is stoichiometric modelling based on biomass composition and growth rate, which gives reliable estimates of net forward fluxes.[27]

CCR values for Arabidopsis leaves span more than seven orders of magnitude, from a low of one (the suicide thiamin biosynthetic enzyme THI4) to $>10^7$, with a median value of 4×10^5 (Figure 3b).[27] Importantly, many low-CCR enzymes are at high risk of damage from their chemically reactive substrates, products or intermediates or from the catalytic mechanism per se (Figure 3b).[40] As the damage-proneness of such enzymes can potentially be reduced or eliminated by replacing the residues that are subject to damage, high-abundance enzymes of this type are the best targets for lifespan increase.[40]

Directed evolution

The advance in synthetic biology that makes it possible to go beyond natural variation and rapidly change enzyme characteristics is directed evolution, specifically continuous directed evolution rather than the classical type.[43,44] Classical directed evolution systems hypermutate the target gene in vitro, for example, via error-prone PCR, transform the resulting library into *Escherichia coli* or yeast cells, screen or select for improved variants, then isolate the improved gene.[44] This cycle is repeated until the desired level improvement is achieved or a plateau is reached. The flaws of classical directed evolution are (i) that its high labour requirement limits the scale of operations and (ii) that it can essentially access only evolutionary paths comprised of successive single mutations, each of which must improve fitness. It therefore cannot easily traverse fitness valleys to reach still higher fitness peaks, which limits the depth of an evolutionary search. Continuous directed evolution overcomes these scale and depth limitations by making hypermutation an in vivo process and coupling performance of the target gene to growth of the platform *E. coli* or yeast cells and then selecting for growth (Figure 3a).[43,44] Such coupling is in principle possible for the many core metabolic enzymes that plants share with *E. coli* and yeast.[44]

A straightforward way to evolve enzymes for longer life is simply to select for higher activity (i.e., higher growth rate). The rationale is as follows: Enzyme activity is determined by the rates of transcription, translation and enzyme inactivation. If the inactivation rate decreases (i.e., the enzyme remains active for more catalytic cycles), then overall activity will increase. An obvious alternative path to higher activity would be via improved kinetics (higher k_{cat}, lower K_M or both). However, such improvements are a priori unlikely, particularly for enzymes whose kinetic characteristics are already well adapted to their normal metabolic task (as most crop enzymes' kinetics would be). In the first place, K_M depends on k_{cat}; in the steady-state rate equation (Equation 2), the position of k_{cat} in the numerator of K_M requires K_M to increase as k_{cat} increases[45] (other factors remaining the same):

$$E + S \underset{k_r}{\overset{k_f}{\rightleftarrows}} E \cdot S \overset{k_{cat}}{\rightarrow} E + P$$
$$K_M = (k_{cat} + k_r)/k_f \quad (2)$$

Thus, if a mutation changes k_{cat}, then K_M will generally change in a directly proportional way, that is, these parameters are necessarily traded off against each other. Evidence from directed evolution experiments confirms this: a meta-analysis of the outcomes of >50 directed enzyme evolution campaigns (Figure 3c) shows a clear trade-off between k_{cat} and K_M when enzymes are acting on their normal substrates. Roughly speaking, making k_{cat} better in these cases makes K_M proportionally worse and vice versa so that k_{cat}/K_M—the catalytic potential—changes little. This contrasts sharply with the large, simultaneous changes in both k_{cat} and K_M often obtained when enzymes are selected to act on an alien substrate (e.g., $D \rightarrow L$ isomer) or to catalyse a non-native reaction (e.g., lyase → racemase) (Figure 3c). That the 1:1 correlation ($R^2 = 0.68$, $p < 0.001$) for the evolution of enzymes acting on normal substrates (Figure 3c) is far stronger than the global correlation between k_{cat} and K_M ($R^2 = 0.09$) for many natural enzymes together[46] presumably reflects the loss of resolution due to pooling data of variable quality[47] for very different enzymes. In the second place, the above strategy of selecting for higher in vivo activity seems more likely to select for longer life than for improved kinetics. This is because the lifespan of core angiosperm metabolic enzymes has been under natural selection that was probably weaker (see above) and of shorter duration than that for kinetic properties, which began evolving billions of years before angiosperms appeared.[48]

An alternative way to evolve longer-lived enzymes would be to sharply shut off expression of the target gene, thus locking subsequent growth to the number of catalytic cycles that the target mediates before inactivation.[27] The downsides of this conceptually appealing strategy are (i) that sharp and complete shutdown of gene expression is very difficult (suitable regulated promoters may not be available, and in any case practically all of them leak[49]), and (ii) that the strategy is highly vulnerable to 'cheater' mutations that subvert the selection by degrading the system that controls target gene expression (cheaters plague directed evolution campaigns,[44] as briefly explained below).

Genome editing

Another synthetic biology advance that brings enzyme lifespan improvement in crops within reach is genome editing, notably CRISPR/Cas9 (Figure 3a). This technology can in principle make multiple single-nucleotide mutations in a target gene in any crop via base-editing or gene replacement.[50,51] Directed evolution can substantially improve enzyme characteristics such as

substrate preference or thermostability after a half-dozen or fewer residue mutations,[52,53] and the same applies to in vitro lifespan.[54,55] This makes it reasonable to expect modest numbers of mutations obtained in fairly short evolution campaigns to deliver worthwhile increases in enzyme lifespan in vivo.

Progress towards evolving longer-life enzymes

A proof-of-concept project, still at an early stage, has produced encouraging results (Figure 3d). This project took on an extreme target: the plant THI4 thiamin thiazole synthase, which—as noted above—is a suicide enzyme, that is, it has a CCR value of one, the lowest possible.[56] THI4 is abundant (top 5% of proteins) and has the fastest turnover rate measured in barley and Arabidopsis leaves.[36,37] THI4 turnover costs are consequently high and can account for several percent of total maintenance respiration.[32] Replacing the suicidal THI4 with an enzyme capable of multiple catalytic cycles could substantially increase annual biomass accumulation; an increase of ~2% has been estimated for replacing THI4 plus the less costly enzyme THIC.[32] Although evolving plant THI4s is the long-term objective, pilot work used prokaryotic THI4s, which are not suicidal but apparently mediate very few turnovers before being inactivated,[57] besides being ill-adapted to work in plant-like conditions.[32,58–60] The directed evolution system used was yeast OrthoRep,[61] which is durable (i.e., runs indefinitely without losing hypermutation capacity) and target gene-specific (i.e., makes no unwanted mutations).[60] Markedly improved performance, due to single-residue changes, was obtained in 3-ml populations after ~20–30 passages under various selection regimes (Figure 3d), the simplest and most effective being total withdrawal of thiamin from the medium.[60] That various single-residue changes each conferred a benefit suggests that additional mutations will enable further advance under selection.[60]

What could go wrong when selecting plant enzyme for longer life in microbes?

There are foreseeable tactical challenges, which can be dealt with.[44] One is codon usage bias; this may differ widely between the target plant gene itself and the microbe used to evolve it, and wide differences can drastically reduce protein expression.[62] Suitable recoding of the target gene can mitigate the problem, as can looking out for the recurrence of a synonymous mutation in independent evolving populations, which flags a fitness advantage in the microbial host that is unlikely to apply in plants.[63] Another tactical challenge is cheaters, for example, strains carrying mutations that result in the desired phenotype but are not in the target gene's open reading frame. Off-target mutations in the host microbe's genome are one cheater class; they can be detected by transferring the target gene to fresh host cells. Mutations in the target gene promoter are another class; these can be identified by sequencing. An insidious class of cheaters in metabolic selection schemes are 'freeloaders'—cells in a population that may have no mutations at all but grow because they are cross-fed by other cells, perhaps a minority, that carry mutations that increase production of the desired metabolite, some of which is released to the medium.[60,64] Freeloaders can be eliminated by plating the evolving population on selective medium or—for metabolites that depend on a specific transporter to enter the host cell—by deleting this transporter from the host genome.[60,64]

There is also a more strategic or conceptual challenge: Whether longer enzyme life selected in a microbe will translate to longer life *in planta*. This is a priori likely to be a greater challenge if *E. coli* rather than yeast is used as host, given the many prokaryote–eukaryote differences in cytoplasmic redox and other conditions and in protein degradation systems.[60,65,66] Even with yeast as host, though, there is still a risk that the plant enzyme has merely been adapted to have a lower CCR only in yeast. However, this risk can be decreased by targeting enzymes with low CCRs attributable to catalysis-related damage.[27,40] This is because CCR for such an enzyme is inherently *portable*: as catalysis-related damage is done by an enzyme to itself, a significant proportion of its CCR is in principle independent of environment, that is, it is damage that occurs whether the enzyme is in its native plant host, a foreign microbial host or in vitro.[40] An increased CCR value of such an enzyme that has been evolved in a microbe should therefore be maintained when the enzyme is returned to its original plant host.

ATTEMPTING TO CUT RESPIRATION IS PRUDENTIAL BECAUSE OF THE POTENTIAL BENEFITS

As said above, it is estimated that reducing respiration by extending the life of the suicide enzyme THI4 could increase carbon conservation in biomass by somewhat under 2%.[32] Improved longevity traits for several enzymes could be stacked to give similar or larger increases. Modelling such stacking in a mid-latitude grain crop (increasing tenfold the CCRs of 15 abundant low-CCR enzymes to cut maintenance respiration by 6.5%, and otherwise making conservative assumptions) predicted a 2.4% biomass yield increase (Figure 4a) and a 2.9% grain yield increase.[67] Such extra carbon income could be channelled to contribute to three global imperatives: sustaining yields, retaining carbon in the soil and drawing down atmospheric CO_2, as discussed below and in a recent review[67] centred on respiratory energy budgets.

Because crop maintenance respiration releases about the same amount of carbon as harvested products contain (Figure 1b),[11,67] it follows that for each 1% reduction in photosynthate (sugar) used for maintenance respiration, yield could rise by roughly half to three-quarters that amount if all the sugar saved is partitioned to harvested parts, after accounting for

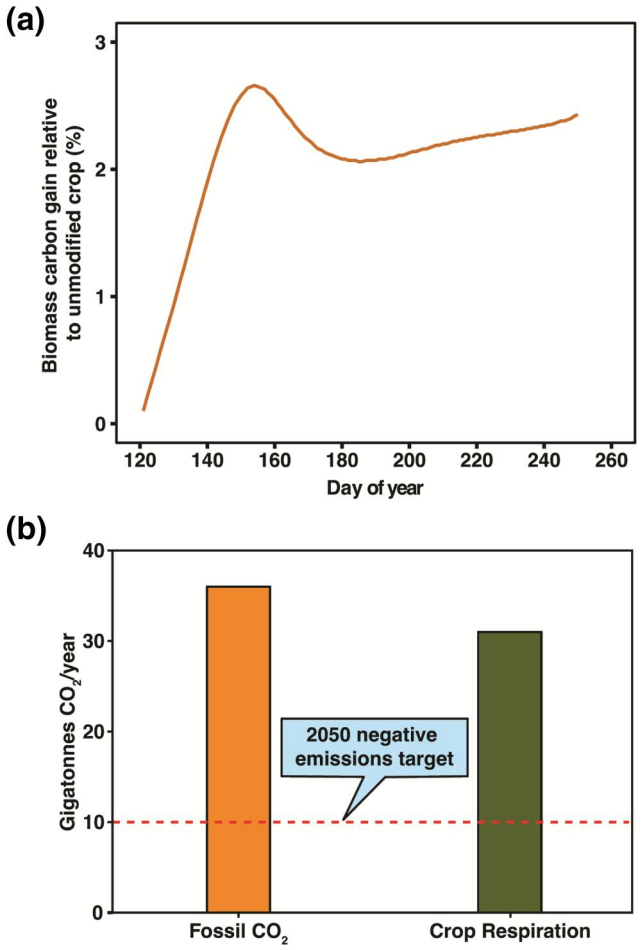

FIGURE 4 Cutting crop respiration in relation to sustaining crop yields and sequestering atmospheric CO_2. (a) Modelling for a generic grain crop to show the potential increase in biomass carbon accumulation, relative to the unmodified crop, given by a 6.5% reduction in maintenance respiration achieved by extending the lifespan of 15 abundant enzymes.[67] (b) The comparable global scales of anthropogenic CO_2 release to the atmosphere from fossil carbon sources and respiratory CO_2 release from crops, that is, the quantity of CO_2 they are estimated to output. Crop CO_2 release was estimated using a value of 60 Gt carbon y^{-1} for terrestrial plant respiration[78] and assuming 14% is from crops, based on the contribution of crops to terrestrial primary productivity.[79] The horizontal line is the 2050 target for negative CO_2 emissions.[70]

growth respiration and translocation cost. Annual yield advances in major crops have now fallen to ~1%, whereas about twice this is needed to meet the projected 2050 demands from rising population, diet changes and biofuel production.[68,69] This slowing of yield advance rate makes the increases that reduced respiration could deliver worthwhile (Figure 4a). Further, combining strategies that increase photosynthetic carbon fixation[1–4] with reduced respiration would have a multiplicative effect on net carbon gain.[9]

The above arguments rest on the premise that carbon spared by reducing respiratory loss, due to lower maintenance ATP demand, would be used directly to support growth and yield rather than being consumed by uncoupled respiration[70] or exuded from roots,[23] and that unused carbon fixation products would not build up sufficiently to inhibit photosynthesis.[71] While these factors could clearly come into play, it is reasonable to think, based on available evidence and on engineering solutions that could be enacted, that they need not nullify the benefit of cutting respiratory carbon loss. Firstly, evidence from CO_2 enrichment experiments shows that extra fixed carbon stimulates growth and yield provided that nutrition is adequate,[72] and research that aims to increase crop photosynthesis[73] assumes this is the case, that is, it rests on the same premise as ours above. Secondly, many plant breeding advances can be accounted for by efficient reallocation of carbon from one sink to another, for example, the increase in maize grain starch that parallels the decrease in grain protein.[74] The less protein produced, the more photosynthate becomes available for starch production because, weight-for-weight, protein synthesis uses ~50% more hexose than starch synthesis.[67] Finally, smart engineering solutions that avoid increases in uncoupled respiration by controlling alternative oxidase and NADH dehydrogenase gene expression[9] or that minimise exudation via transcriptional and post-translational regulation of organic acid transporters[75] could enable excess carbon fixation products to be shunted to internal sinks.

Removing CO_2 from the atmosphere in a permanent or long-term way, that is, carbon capture and storage (CCS), has shot to the top of engineering, agriculture/forestry and synthetic biology research agendas.[76,77] Increased net photosynthesis (i.e., photosynthesis minus respiration)[78] and increased deposition in crop roots of recalcitrant (i.e., biodegradation-resistant) forms of carbon such as suberin or sporopollenin[79,80] are seen as attainable, scalable and low-cost ways to support CCS. Moreover, implementing CCS in agricultural systems (carbon farming) is viewed as a promising new source of farm income from sale of carbon offsets.[81] Making more carbon available to partition to recalcitrant carbon-rich polymers through reduced maintenance respiration could thus be useful,[82] especially to mitigate yield reduction that could result from polymer synthesis and grain growth competing for photosynthate.[67] The theoretical global scope for a respiration reduction/recalcitrant polymer approach to CCS can be seen by comparing present CO_2 emissions (from fossil fuel burning and cement production) with the estimated CO_2 release by crop respiration and the 2050 target for atmospheric CO_2 removal (Figure 4b).

Fossil and crop CO_2 emissions are roughly comparable, and three to four times greater than the 10 Gt y^{-1} CO_2 removal target.[83–85] Thus, sequestering the carbon saved by a 1% reduction in crop emissions (0.3 Gt y^{-1}) could contribute a modest but useful 3% of the 2050 target and ancillary benefits from improved soil carbon stocks that favour crop productivity[86] could multiply sequestration. While agricultural soils are not bottomless sinks for carbon, the estimated global loss of soil organic carbon due to human land use—equivalent to 425 Gt of CO_2[87]—shows that 0.3 Gt of carbon (or much more) could be added to soil every year for a very long period before soil carbon levels exceed their pre-agricultural values.

We close by reprising the theme that reducing respiratory CO_2 production has been largely overlooked and—especially given research advances—really should be looked at again. A simple analysis of plant literature since 1950 (Figure 1a) showed how overwhelming the research emphasis on photosynthesis has been and how it has increased over time, an initial three-fold differential between photosynthesis and respiration research having grown to five-fold. But this disparity can—and indeed should—be seen not as a history of lost opportunities but as a forecast of future progress, that is, that respiration research still has actionable, 'low-hanging fruit' of the type that photosynthesis research picked long ago. We argue that needlessly fast turnover of enzyme proteins is in this category, that the potential to slow turnover is real, that slowing turnover could bring useful benefits and that slowing should be explored on the grounds that it is prudential, that is, a de-risked, good judgement and common sense approach.

AUTHOR CONTRIBUTIONS

Jaya Joshi: Conceptualization (Supporting); Visualization (Lead); Writing – original draft (Supporting). **Jeffrey S. Amthor**: Conceptualization (Supporting); Data curation (Lead); Writing – review & editing (Supporting). **Donald R. McCarty**: Conceptualization (Supporting); Writing – review & editing (Supporting). **Carlos D. Messina**: Conceptualization (Supporting); Data curation (Supporting); Writing – review & editing (Supporting). **Mark A. Wilson**: Conceptualization (Supporting); Writing – review & editing (Supporting). **A. Harvey Millar**: Conceptualization (Supporting); Writing – review & editing (Supporting). **Andrew D. Hanson**: Conceptualization (Lead); Data curation (Supporting); Funding acquisition (Lead); Investigation (Lead); Visualization (Supporting); Writing – original draft (Lead).

ACKNOWLEDGEMENT

This work was supported primarily by the U.S. Department of Energy, Office of Science, Basic Energy Sciences under Award DE-SC0020153, by the USDA National Institute of Food and Agriculture (Hatch project FLA-HOS-005796) and by an Endowment from the C. V. Griffin, Sr. Foundation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Joshi J, Amthor JS, McCarty DR, Messina CD, Wilson MA, Millar AH, et al. Why cutting respiratory CO_2 loss from crops is possible, practicable, and prudential. Modern Agriculture 2023;1(1):16–26. https://doi.org/10.1002/moda.1