A Bioengineering Roadmap for Negative Emissions Technologies

by

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Submitted to the Program in Media Arts and Sciences, School of Architecture and Planning, in partial fulfillment of the requirements for the degree of

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Abstract:

Negative emissions technologies that can remove carbon dioxide from the atmosphere are a critical tool to limit global temperature rise and ocean acidification. Bioengineering capabilities have not been sufficiently assessed or utilized for the development of negative emissions technologies. Bioengineering holds the potential to improve the efficiency of some existing technologies and to create new methods of carbon removal. I review existing technologies to assess how bioengineering could improve them, focusing on technologies that could achieve at least 1 Gt of CO_2 removal per year. I also investigate and describe potential new methods of carbon removal that leverage bioengineering. Key questions for additional research are identified, as are key engineering targets for the development of improved negative emissions technologies. This evaluation of potential high-impact R&D work is intended to provide an initial roadmap for the development of bioengineered negative emissions technologies that are scalable, sustainable, and can remove gigatons of CO_2 from the atmosphere.

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Introduction

Human activities are increasing the atmospheric concentration of CO_2, which is changing the Earth's climate in ways that will be — and in some cases already are — catastrophic to the thriving of many people and ecosystems. Human activity since the start of the Industrial age (~1850) has increased the amount of CO_2, a powerful greenhouse gas that traps heat and warms the planet, in the atmosphere by about 50% (NASA, 2021). The atmospheric concentration of CO_2 has risen from ~285 ppm to ~415 ppm during this timeframe, and continues to rise at an accelerating rate, adding around 2.7 ppm per year (NASA, 2021). To stop climate change, humans must stop emitting greenhouse gases such as CO_2, a process known as mitigation. In addition to reducing CO_2 emissions to near-zero, however, hundreds of billions of tons of CO_2 must be removed from the atmosphere in order to have a reasonable chance of limiting global temperature rise to global targets of 1.5°C or 2°C. Removing CO_2 is generally referred to as carbon dioxide removal or CDR, and technologies that can achieve such removal are called negative emissions technologies (NETs).

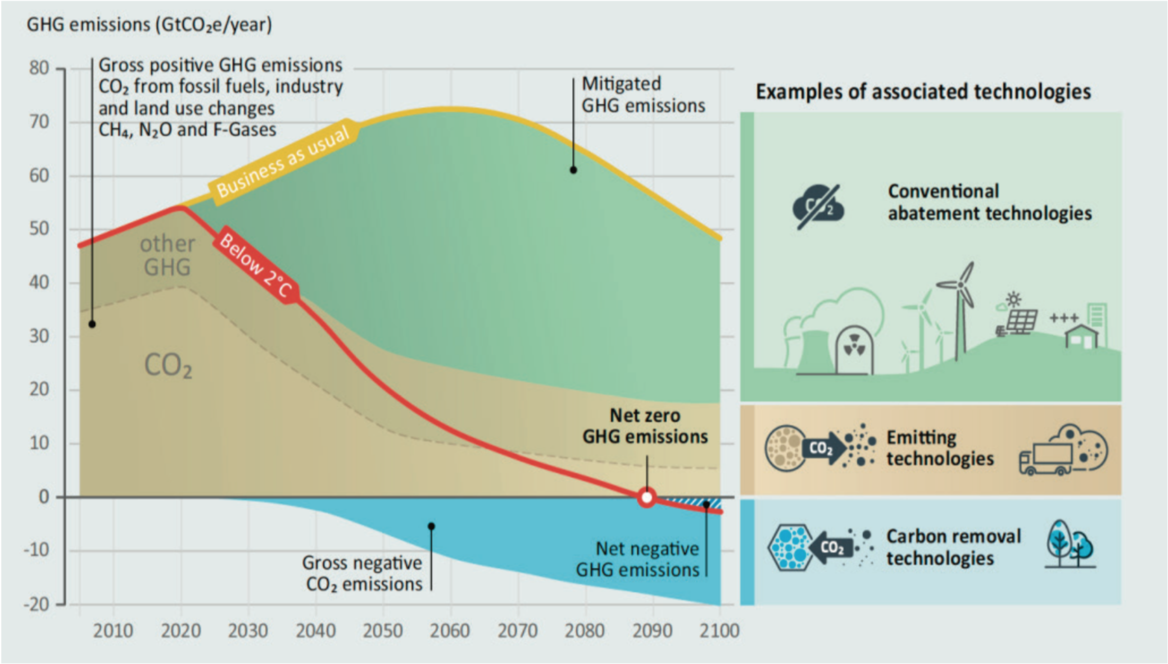


Image 1: Projected scenario of the significance of negative emissions relative to mitigation in reaching net zero emissions. Source: NASEM, 2019.

The Intergovernmental Panel on Climate Change (IPCC) estimates that we need to start removing large quantities of CO_2 this decade, ramping up to at least 10 Gt CO_2 or CO_2e/year in about 30 years in order to have a reasonable chance of limiting global temperature rise to 1.5°C (CO_2e means greenhouse gases with radiative forcing effect equivalent to that of CO_2) (IPCC, 2014). The majority of pathways for limiting temperature rise to 2°C rely on NETs (Fuss et al., 2014). This is a tremendous amount of material to capture and move: 10 Gt is about the size of the global cement industry, and atmospheric CO_2 is dilute: only 1 molecule in ~ 2500 of ambient air is CO_2. This scale of this challenge has been described as creating a new industry that can have the same effect as running the entire fossil fuel industry in reverse. It is important to note that CDR is the only option for both reducing global temperatures and reversing the destructive chemical changes to the world's oceans, whose acidity has increased 30% since pre-Industrial times and continues to increase, threatening the survival of coral reefs and a broad array of marine life (NOAA, 2020) (Intergovernmental Panel on Climate Change, 2018). Other technologies for reducing global temperatures, such as Solar Radiation Management, are likely to be a less expensive and faster way to reduce global temperatures than CDR, but they cannot solve the underlying problem of an excess of CO_2 in the atmosphere, or address ocean acidification. Thus, CDR is critical to any discussion of stabilizing the climate.

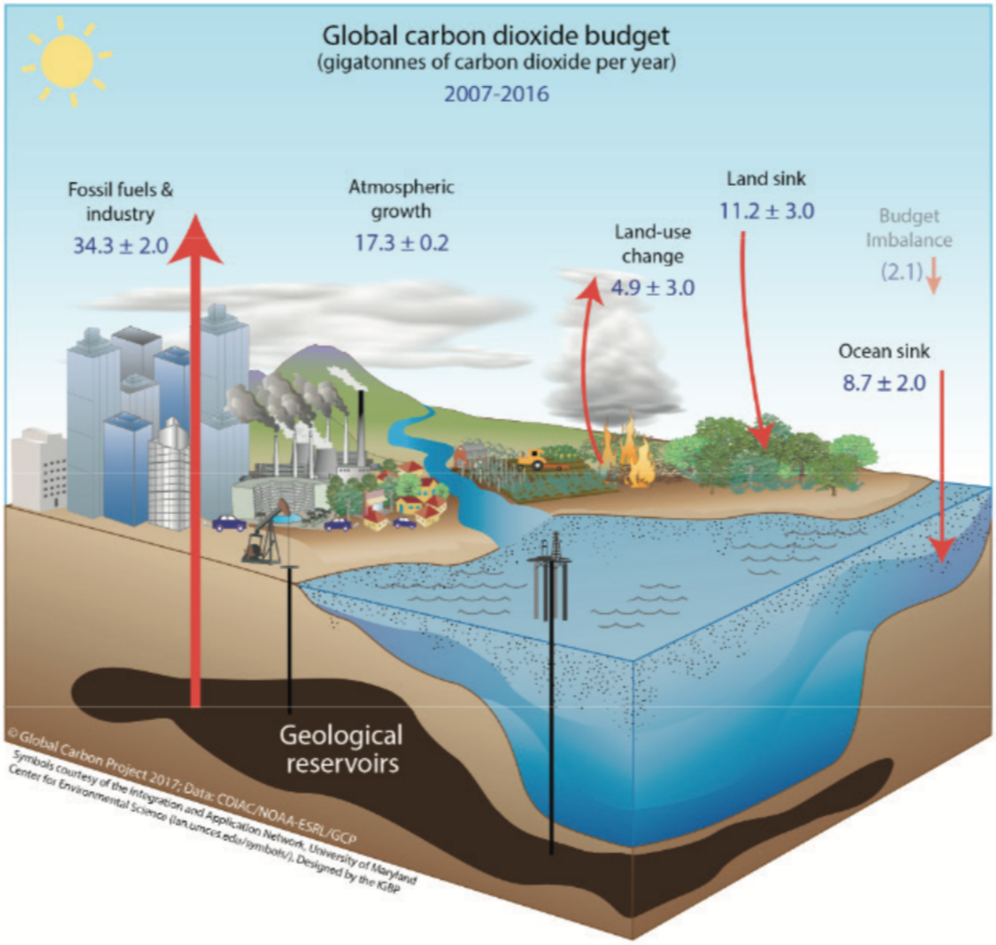
CDR is an area of increasing interest to climate change policymakers and researchers as the timeframe for acting to limit global temperature rise shortens. Much attention has been given to researching biological solutions for CDR, such as planting trees, and abiological technologies, such as direct air capture (DAC) machines. However, relatively little attention has been given to the field sitting at the intersection of these two concepts: bioengineering. Some exceptions of note are a recent series of reports from the Energy Futures Initiative highlights the potential for biotechnology-based contributions to CDR and an opinion piece from a group of researchers outlining broad strategies for synthetic biology led climate solutions including CDR and calling for more research into this topic (EFI, 2020) (DeLisi et al., 2020).

Purpose

Bioengineering has the potential to enhance the effectiveness of both biological and abiological solutions by lowering costs and energy requirements and increasing the speed and effectiveness of some CDR methods. This paper aims to survey the possible ways that bioengineering could be employed to develop NETs that can operate at the 1 Gt CO_2/yr scale or more, providing significant additional capabilities to efforts to stabilize the Earth's climate. This paper will discuss the main categories of NETs that can be achieved or enhanced by biotechnology, focusing heavily but not exclusively on plant engineering and enhanced rock weathering (ERW) due to their low energetic requirements, high scalability, and in the case of rock weathering, permanence. Each section begins with an explanation of why the proposed NETs strategy is promising and key facts, then discusses possible bioengineering improvements that could be developed, and ends with an outline of key research questions to answer and key engineering targets to pursue. A table summarizing the carbon-capturing potential, technological risk, and environmental risk of each technology is included at the end in order to provide a snapshot comparison of potential benefits and risks of each strategy (**Table 1**). The overall goal of this paper is to provide a roadmap for how bioengineering tools and techniques could be applied to help reduce atmospheric CO_2 levels.

Scope and Methodology

The carbon cycle and existing proposals for enhanced carbon removal were used as a starting point to assess possible routes of carbon removal. Major global negative carbon fluxes, also known as carbon sinks, that involve biology were assessed to understand how carbon removal processes could be enhanced or newly introduced. For terrestrial approaches using plants, focus was placed on solutions that could be deployed on lands that are already highly managed or underproductive (e.g. farmland and marginal lands), and intervention was ruled out for biodiverse, ecologically sensitive, and native ecosystems such as protected areas, parks, old growth forests, and rainforests.



© Global Carbon Project 2017; Data: CDIAC/NOAA-ESRL/GCP
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Image 2: The global carbon flux, illustrating major CO_2 sources and sinks. Source: NASEM, 2019.

In addition to existing biological carbon cycling pathways, consideration was also given to new pathways that could be created, such as ERW systems utilizing microbes for faster CO_2 uptake, or DAC machines that encompass biologically-derived components such as engineered enzymes. For each topic, I reviewed the literature to assess the state of knowledge in the field and the potential for improvements to CDR processes. I conducted a series of 1-on-1 discussions with researchers studying relevant aspects of the global carbon cycle, climate change, and biological processes that drive carbon removal. Finally, I held two workshops on new approaches to CDR, one in September 2019 at the MIT Media Lab and one held virtually in June 2020. Both workshops featured presentations by expert researchers and graduate students on new concepts for carbon removal, followed by discussion and ideation. The September workshop included both abiological and biological ideas, while the June workshop focused solely on biological approaches.

Historical context for biological change to atmospheric CO2 levels

In the big picture — encompassing geologic time going back several billion years — there is plenty of precedent for biologically-driven drawdown of CO_2 so extensive that it dramatically altered Earth's climate. Evidence for these shifts abounds in the geological record captured in sediments and ice cores, among other types of evidence. These changes took place over tens or hundreds of thousands of years: slowly from the perspective of humans, but relatively quickly from the perspective of geologic time. They provide insight into how biologically fixed carbon can become locked away from the atmosphere for long enough periods of time to affect the climate, pathways that could potentially be exploited today.

One major drawdown event occurred during the Carboniferous era, when newly evolved trees absorbed tremendous quantities of CO_2 and stored it in their biomass in the form of lignin over millions of years. The conventional explanation for the tremendous build-up of biomass is that fungi had not yet evolved the capability to break down lignin, however recent research challenge that notion and instead points to the highly productive ecosystem of warm tropics entombing dead trees in anoxic sediments, which were then buried during glacial periods (Nelsen et al., 2016). The immense deposits of Carboniferous biomass were eventually transformed by geological pressure into coal deposits. Today's rapid digging-up and combustion of coal, sending its stored carbon into the atmosphere, represents a rapid-fire undoing of millions of years of carbon fixation by photosynthesis. These two theories explain Carboniferous era CO_2 drawdown as the result of either the formation of difficult-to-degrade carbon in the form of lignin, or as the result of environmental conditions that physically sequestered carbon in sediments where it could not be degraded.

Another instructive drawdown is the Azolla event, an extended period during the Eocene era when a fast-growing freshwater aquatic plant known as *Azolla* (duckweed) grew in copious amounts in the Arctic. A large freshwater lake, stratified over a saline layer, formed where azolla could grow continuously, refreshed by nutrients from continental runoff (Speelman et al., 2009). A symbiotic relationship with nitrogen-fixing cyanobacteria provided Azolla with unlimited nitrogen to feed its growth. As individual *Azolla* plants died, they sank into an anoxic zone at the bottom of the lake and was trapped in sediments (Speelman et al., 2009)Over hundreds of thousands of years, this process is thought to have drastically changed the amount of the CO_2 in the atmosphere from about 3000ppm CO_2 to under 1000ppm CO_2 (Pearson & Palmer, 2000)Among other evidence for the Azolla event are the remains of 50M year old *Azolla* found in Arctic sediments (Speelman et al., 2009).

These prehistorical precedents illuminate how shifts in the balance of biomass fixation and degradation can result in large-scale carbon removal that can change the Earth's climate. As dramatic as they were for the climate, however, the Azolla event and Carboniferous drawdown occurred over hundreds of thousands and millions of years, respectively. IPCC targets for the amount of carbon removal needed to remove enough CO_2 to remain under 1.5°C of global heating, roughly on the order of ~10 gigatons/year for the next century, do not require matching the net amount of carbon removal from these events, but they would require a much faster rate of carbon removal. Meeting this target requires interventions that can work on the order of one to several orders of magnitude faster than prehistoric carbon removal events.

Resource constraints

Carbon removal schemes must be designed with the limitations of the Earth's resources in mind. Much of Earth's land area is already in use for human habitation or agriculture, is difficult to access (Arctic land, mountain ranges), or is set aside for conservation. Fresh water and nutrients such as phosphorous fertilizer, critically important for growing terrestrial plants, are already facing resource constraints. Use of land, fertilizers, and water to grow plants for purposes of carbon removal will eventually conflict with the use of those resources to grow food for an ever-increasing global population.

In a recent review article highlighting resource constraints, researchers calculated that implementing one of the most commonly pointed-to CDR strategies, Bioenergy Carbon Capture and Storage (BECCS), would conflict with the use of arable land for food or bioenergy production (P. Smith et al., 2016). BECCS is a process in which biomass is grown, harvests, and burned in a facility to generate energy and capture the resulting carbon emissions for storage off-site. To capture 10 Gt CO_2/yr in 2100 with BECCS would require 380-700 Mha, which represents 7-25% of all agricultural land and 25-46% of arable plus permanent crop land (P. Smith et al., 2016). The researchers found that afforestation and reforestation would require a similar amount of land to sequester the same amount of carbon as BECCS. This land requirement is 2 - 4 times the amount of land considered marginal, making the limitations of land use inescapable when contemplating NETs at the multi-gigaton scale (P. Smith et al., 2016). CDR concepts that require minimal amounts of land, freshwater and nutrients are preferable. An awareness of these constraints is necessary but also helpful, as it may direct innovation towards solutions like nutrient recycling, thought to have fueled the perpetual blooms of the Azolla event.

Permanence

Not all carbon removal is created equal. Removing carbon from the atmosphere and storing it in living biomass creates a flight risk: in the right situations, that carbon can be released almost instantaneously back into the atmosphere, undoing years of carbon removal work. Fires are one such way that biomass can quickly make its way back to the atmosphere. Another risk of storage in living biomass is land use change. A forest that is planted can always be cut down, and a field can always be paved over. Biomass that is locked away for only a few years or decades could start to return to the atmosphere while society is in still throwing its (proposed) CDR program into high-gear, thus making CDR targets even harder to achieve. However, some forms of carbon removal can last for eons. For example, carbon locked away in carbonate form, such as magnesium carbonate ($MgCO_3$) is highly stable and can sequester carbon effectively permanently, and so is being investigated for long-term carbon storage (Lackner et al., 1995). Permanence is an important consideration when valuing individual CDR solutions and when building an overall portfolio of solutions.

Increasing biomass

The growth of biomass represents an important pathway for carbon removal. Natural growing biomass — primarily in the form of forests, wetlands, grasslands, phytoplankton, and other photosynthetic things — is the primary driver of the carbon cycle on an annual basis. The amount of new biomass that is created is referred to as Net Primary Productivity and amounts to about 105 GtC/yr, roughly half of which is produced on land and half in the ocean (Field et al., 1998). Emissions from human activities amount to roughly 11 GtC/yr (equivalent to 40 Gt CO_2/yr), which means that biology fixes nearly ten times as much carbon each year as humans emit (Intergovernmental Panel on Climate Change, 2018). Most of the biomass created each year is however not sequestered, but rather is released back into the carbon cycle over time frames ranging from hours to months. Only about 2.5% of carbon fixed by photosynthesis becomes sequestered as soil carbon (ARPA-E, n.d.). As plants, microbes and other organisms respire, die and decompose, the carbon they removed from the atmosphere is returned.

This low sequestration rate is why increasing biomass production alone will not result in appreciable net amounts of carbon removal. A study by the United States Geological Survey of carbon fluxes in the US shows that forests have the largest annual negative carbon flux, yet they store only 0.28% of total forest biomass each year (Zhu et al., 2010). Annual net negative carbon flux is usually only a tiny proportion of an ecosystem's total biomass. Given the very large amounts of additional carbon storage needed to slow global temperature rise to under 2°C, and the limited availability of arable land for planting more forests and grasslands, it is more helpful to focus on increasing the *proportion* of total biomass carbon that is sequestered rather than on producing more biomass.

Many biomes should be avoided for NETs implementation, in order to preserve natural ecosystems and biodiversity. The best target areas for increased carbon storage are those which are already managed, which contain mainly non-native plants, or which do not represent ecologically rich and biodiverse landscapes. On land, this means croplands, pasturelands, commercial forests, marginal and degraded lands, and other non-native ecosystems should be considered for additional carbon storage. In addition to considering the carbon removal potential of an area, we must also consider its wider climate and ecological impact.

Increases in biomass can be effective at removing carbon if those increases are paired with strategies for sequestering this carbon for long periods of time. This can be accomplished through several strategies, including storing carbon in the form of molecules that are slow to degrade in environmental conditions (referred to as "recalcitrant" carbon), storing carbon in chemical environments where natural degradation processes are blocked, such as anoxic sediments, and storing carbon in physical locations where it is locked away from release back into the atmosphere, such as the deep ocean. We will examine the carbon removal potential of each of these strategies.

Growing new biomass is not always a clear win: sometimes it can even be counter-productive to climate stabilization goals. Land use change must consider the whole picture. For example, replacing a carbon-storing forest with crops might reduce the amount of carbon stored on that piece of land. Replacing desert with tree cover may increase carbon storage, but may also decrease the albedo of the surface. Albedo is an aspect of the Earth's climate system that acts as an important dampener to global warming by reflecting radiation back into space. Planting trees where there is not enough groundwater or rain to sustain them may further deplete scarce groundwater resources and harm both the trees and the native vegetation. Additional impacts of land use change include the effect of vegetation on transpiration and evaporation, rainwater and nutrient runoff, and local weather. With these concerns in mind, there is room to make significant headway in CDR by increasing land-based biomass storage. It is estimated that since the Industrial Revolution, land use change and soil carbon losses have results in the release of 136 ± 55 petagrams of carbon to the atmosphere, equivalent to 266 to 628 Gt CO_2 (Zomer et al., 2017).

In addition to planting more area with productive plants, strategies could be employed to increase the plant nutrient uptake. This would be effective in areas where nutrients are limiting factors to growth.

Improvements in Photosynthesis

A strategy that could be highly impactful for increasing biomass is increasing photosynthetic efficiency. Terrestrial plants only use about 1% of incoming sunlight. Other organisms, such as algae, have demonstrated the ability to capture ten times more energy from sunlight (Khan et al., 2018). Increasing photosynthetic efficiency would give plants more energy with which to fix carbon and build more biomass.

Historically, improvements in plant productivity have come from traditional techniques such as selective breeding, cross-breeding, irrigation and the addition of nutrients in the form of manure and synthetic fertilizer. Modern tools of genomics and synthetic biology offer the opportunity to not just fine-tune these traditional strategies, but to re-engineer photosynthesis itself. Multiple different strategies have been proposed and investigated for improving photosynthetic efficiency(Kubis & Bar-Even, 2019). These efforts are largely driven by a desire to increase the productivity of harvested crops such as food and biomass used for biofuels production, but they could also be used to increase carbon removal. The resulting biomass increases would need to be paired with strategies for producing carbon that is kept from returning to the atmosphere for decades or longer, such as recalcitrant carbon or carbon stored in deep soils. Several broad concepts for increasing photosynthetic efficiency are highlighted here. A recent review paper by Kubis & Bar-Even describes the photosynthetic changes most likely to result in increased carbon fixation (Kubis & Bar-Even, 2019).

At the heart of many approaches to improve photosynthesis is addressing the fundamental inefficiency of the key protein that fixes carbon dioxide from the atmosphere, Rubisco. Rubisco is a somewhat clumsy protein. Its job is to bind CO_2, but it has trouble differentiating CO_2 from O_2 and "misfires" about 20 - 25% of the time (Sharkey, 1988) (RIPE, 2020). This causes plants to waste a large amount of energy, first in binding the wrong molecule, and second in cleaning up the resulting mess from unwanted reactive oxygen inside the cell, a process called photorespiration. Many plants have evolved strategies to work around Rubisco's poor performance.

Convert plants from C3 to C4 metabolism

The type of photosynthesis used by most plants around the world is referred to as C3 photosynthesis, but in hot, dry climates, some plants have evolved a version of photosynthesis, called C4 photosynthesis, that reduces water loss involved in the uptake of CO_2 and increases photosynthetic efficiency. C4 plants use a carboxylase enzyme, PEP, to fix carbon into a four-chain carbon molecule (C4) called malate, which is then shuttled into anatomical structures called bundle sheaths that allow carbon to be concentrated around Rubisco. Plant breeding efforts such as the RIPE project, a partnership between universities, government agencies and nonprofits, are attempting to improve productivity by introducing C4 traits into C3 crop plants (RIPE, 2020).

Although no successful C3 to C4 plant conversions have yet been announced, it has been pointed out that such as conversion is likely to be achievable given that C4 metabolism has evolved at least 66 independent times and that C3 plants already contain many key C4 traits, such as PEP carboxylases and carbon transport capabilities (Kubis & Bar-Even, 2019).

Redesign Rubisco
The structure of Rubisco could be modified to improve its functionality, or Rubisco could be replaced with a faster variant found in nature. Researchers have successfully expressed cyanobacterial Rubisco in tobacco plants, although they saw a decrease in growth rate, likely because additional components such as carbon concentrating mechanisms were needed as well (Hanson et al., 2016).

Faster regeneration and clearing of Rubisco
Faster clearing of Rubisco that has mistakenly bound O_2 could increase the effective amount of Rubisco available for binding CO_2. Increased expression of the chaperone molecule, Rca, that clear sugar-phosphates interfering with Rubisco carbon binding sites, has been shown to increase Rubsico activity (Yamori et al., 2012).

Reduce kinetic bottlenecks in Calvin cycle (see above)
Kubis and Bar-Even point to modeling experiments that indicate which rate-limiting steps could be increased to improve the overall rate for regenerating RuBP, which binds to and fixes CO_2. They suggest that “higher levels of sedoheptulose-1,7- bisphosphatase and fructose-1,6-bisphosphate aldolase, as well as enzymes linked to sink capacity, could support higher productivity” (Kubis & Bar-Even, 2019).

Improving carbon concentrating mechanisms from algae
Importing carbon concentrating mechanisms from algae could allow plants to dramatically increase their photosynthetic efficiency. Researchers have investigated adding carbon transporters, to bring more carbon into the cell, and carboxysomes, to concentrate the carbon around Rubisco. An experiment to engineer algal carbon transporters and carbonic anhydrases in Arabidopsis plants showed stable expression, although not biomass increases (Atkinson et al., 2016). This result was expected, as it pointed to the anticipated need for additional components of the carbon concentrating system to be imported as well in order to achieve improved growth rates.

Improve photorespiration speed or bypass photorespiration
Increasing the photorespiration rate or bypassing some of its more inefficient steps are potential strategies, supported by experimental evidence, for saving plants energy and increasing the availability of CO_2 (Kubis & Bar-Even, 2019).

Many of these strategies could potentially be combined in the same plant to achieve efficiency gains, although components may need to be modified in order to create a whole system that works together with optimal efficiency. Researchers assessed a set of 6 specific photosynthetic improvements and estimated that they could be implemented within 10 - 15 years due to relative ease of genetic modification, and increase crop yields by 50% (Long et al., 2006). Recent work has identified specific genes that can be targeted to increase plant productivity (Nowicka, 2019).

Key research questions

- What is the theoretical maximum carbon-fixing efficiency increase for plants relevant to developing NETs?
- What are near-term and medium-term engineering targets of low to moderate difficulty that can achieve a large increase in carbon fixation?

- What additional fundamental research needs to be done to create useful models that can predict efficiency gains from stacking multiple different bioengineered improvements together?

Key engineering targets

- Successfully demonstrate each of the strategies described above in a model plant.
- Develop model that can successfully predict the relationship between expression level and maximum biomass increase for each of the photosynthetic improvements described above.

Soil Carbon Sequestration

In the past few years, increasing attention has been paid to the idea of storing additional carbon in soils. Due to modern agricultural practices that degrade soils, soils have lost a tremendous amount of carbon. One study estimates the total soil organic carbon (SOC) loss since the Industrial Revolution at 78 ± 12 GtC while another puts the total at 133 GtC lost from soil organic matter and erosion since the beginning of agriculture (Zomer et al., 2017) (UNEP, 2019). Soil can be degraded by agricultural practices such as tilling, which exposes dirt to erosion, and the replacement of native plants with modern crops such as wheat which have shorter roots that do not hold soils together as well as deeper-rooted plants. Agricultural practices can also result in dirt being removed from land by increased runoff via rainwater into streams and sediment basins. Soil organic carbon also oxides and returns to the atmosphere as CO_2. This all adds up. 24 billion tons of topsoil lost every year (UNEP, 2019).

Targeted interventions could increase the amount of carbon stored in soils. By one estimate, croplands worldwide could sequester 0.9 to 1.85PgC/yr, the equivalent of about 3 to 6Gt CO_2/yr (Zomer et al., 2017) A United Nations Environment Programme survey of literature or soil carbon estimates the total amount of carbon that can be sequestered on agricultural lands through improved practices at 10 Gt/year, with a “practically archivable” amount much lower at 1.5 -2 .5 GtC/yr (UNEP, 2019). The same review puts the theoretical sequestration potential of non-agricultural soils at 0.8 - 8 GtC/yr.

Carbon is added to the soil through natural processes through which plants transport carbon, fixed from the atmosphere via photosynthesis, to their roots in the form of carbohydrates, waxy molecules like suberin, and other carbon-rich molecules. This carbon is then transported to the soil as root exudates and as organic carbon that accumulates after a plant has died and its roots decompose. Carbon is also added to soils in the form of breakdown products from above-ground plant matter, also known as plant litter, such as stems, bark and leaves.

The main strategy being investigated for returning carbon to soils is so-called “regenerative agriculture” practices. These practices aim to essentially reverse the land-degrading farming practices that came before, and include no-till agriculture, the use of cover crops that build SOC, crop rotations to reduce degradation of soils, and silvopasture, a practice of planting trees throughout crop fields to retain moisture and build stronger root networks. These practices are promising, if not thoroughly understood, due to the absence of long-term studies and the understanding of the variance these practices may have in different climates and amongst different crops and soil types. The accumulation and long-term maintenance of

SOC is made more challenging because measuring SOC is difficult. SOC content can differ from meter to meter and at different soil depths even in the same field.

Biotechnology strategies for enhancing soil carbon

In addition to the gains in SOC that could be achieved through regenerative agriculture practices, there is strong potential for even further gains to be achieved through biotechnology applications. The National Academies of Sciences estimates that 6 $GtCO_2$/yr could be added to soils through the use of "frontier biotech" although this is the amount considered practical, not a theoretical maximum (NASEM, 2019).

Improvements to photosynthetic efficiency, explored in this paper in an earlier section, would allow plants to grow more overall biomass. This biomass increase could be used to boost the production of plant components that add to soil carbon. Additional strategies for adding to soil carbon, discussed in more detail in the following sections, include engineering plants to:

Store more carbon underground via a **higher root to shoot ratio**
Grow **recalcitrant forms of carbon** such as suberin and lignin
Shift from being **annual to perennial** so they develop deeper root structures
Slow the **decomposition** of biomass
Thrive on **marginal and degraded lands**

In addition to altering plants themselves, changing the plant root-soil ecosystem may provide a path to sequester more carbon. Fungi and microbes play critical roles in soil ecosystems. Both can be decomposers, breaking down carbon and respiring it back into the atmosphere or converting it into more or less stable forms of soil carbon. Some fungi also have a symbiotic relationship with plants, funneling nutrients to plants roots via long filamentous hyphae structures, and in return for this service plants transfer to fungi carbon-rich carbohydrates from their roots. Because these proposals are speculative and have not been studied thoroughly or deployed at scale, their potential impact on global carbon storage is not well understood, and the discussion of each of these solutions will be more qualitative than quantitative.

Increasing carbon stored underground via enhanced roots

It has been proposed by multiple scientists that increasing plant biomass production underground could be a means of drawing down and storing additional CO_2 from the atmosphere (Kell, 2012)(Salk, n.d.). Although it is known that roots are a major root by which carbon enters the soil, the relationship between global primary production and soil carbon accumulation from roots is not fully understood. Fewer than 10% of studies of terrestrial net primary production (NPP) look at below-ground production, although roots are thought to account for 30 - 40% of terrestrial NPP (Chapin & Eviner, 2013).

Despite this, there are multiple strategies that present appealing targets for increasing soil carbon from roots. For one, enhancing the overall below-ground biomass that plants produce should yield more soil carbon. Increasing attention is being paid to the development of plants with enhanced roots that are deeper, more branched, and express a greater proportion of forms of carbon that persist for a long time in soils, such as suberin. The ARPA-E Roots Program commissioned a study that modeled deeper roots with up to 100% more carbon allocation and found that even modest gains in these properties, if deployed across US crops, would "significantly offset" US GHG emissions, up to about 1.1 Gt CO_2 (ARPA-E, n.d.).

The Salk Institute's Harnessing Plants Initiative has done much to promote the idea of engineered crop plants that have deeper, more branched roots which express more suberin, a difficult-to-breakdown carbon-rich substance that is already produced by plants (Salk, n.d.) Much more fundamental work that needs to be done in this area: one recent paper bemoans that "[o]ur knowledge of the physiological basis underlying root growth and development and the relevant variations in belowground biomass production is poor"(Ogden et al., 2020). However, there is substantial research literature to support the idea that root architecture is a feasible engineering target. It is thought that most crops have flexibility to alter their root architecture (S. Smith & de Smet, 2012). The same paper that pointed out the lack of overall understanding of root growth was able to identify families of transcription factors that were expressed to different degrees during root growth stages in a model plant from the same temperate grass family as wheat and rice (Ogden et al., 2020). Traditional molecular biology techniques have been fruitful in identifying the function, expression, and localization of many gene products, and even in describing small genetic regulatory networks controlling root properties such as Casparian strip development (Wachsman et al., 2015). For further understanding of how small gene networks interact (a possible number of combinations too big to try out via plant engineering experiments), computer models can simulate interaction and compare scenarios to see what matches with experimental data, an approach that successfully predicted gene expression patterns that regulate root epidermal development.

In addition to exploring enhanced carbon storage traits, roots could also be improved in ways that increase a plant's ability to explore and exploit soil conditions, especially in poor quality soils. This would allow plants to grow deeper roots contributing to overall plant growth and carbon deposition. A 2014 review of the potential to create deeper rooted crops identified opportunities for regulating the genes that control plants' adaptability to soil conditions that limit root growth, including metal toxicity (particularly from Al and Mn), P and Ca deficiency, salinity, and suboptimal soil temperatures (Lynch & Wojciechowski, 2015). Many of these poor soil conditions will be exacerbated under climate change and will affect more agricultural lands. For example, as coastal areas flood, they will experience increased soil salinity. Adapting important agricultural plants now to these conditions can yield increased benefits for carbon storage and plant productivity, and may also help avoid decreases in agricultural yields and farmable land.

It is possible that modifying the root or soil microbiome could contribute to additional carbon transfer from roots to soil, or the slower breakdown of plant-derived carbon in soil. The effect of root-microbe interactions on soil carbon sequestration, while known to be important, are not fully understood. A minireview in 2009 of microbe-plant interactions and carbon sequestration concluded that "More long-term and standardized studies, under different environmental conditions, of belowground carbon fluxes, integrating models and measurements are needed." (Wu et al., 2009) While many factors are known to be influential, including temperature, microbial activity, and soil water content, the specific influence of these factors needs to be better characterized across different plant species and environments.

Challenges going forward will be to develop models that can predict overall plant root enhancements and soil carbon accumulation via pathways which won't harm other desirable plant traits, and models that can work with limited data. Researchers will also need to develop a better understanding of which genes influencing root development are shared among plants species of interest, and to what degree they interact with each other to control root development.

Key Research Questions:

- What are the key root-microbe-fungi interactions that drive the accumulation of stable SOC?
- What are the key genes governing plant partitioning of carbon to roots?

Key Engineering Opportunities:

- Better understanding of genes controlling root growth, branching, mass, depth, and carbon deposition over the lifetime of the plant, particularly with regard to recalcitrant forms of carbon like suberin and lignin.
- Development of models that can predict organism-wide modifications for optimization of root architecture and carbon storage
- Development and testing of engineered strains that greatly enhance SOC without sacrificing other desired traits such as crop productivity
- Embrace and integrate new tools such as synthetic plant gene promoters for fine-tuned control of gene expression and localization
- Create models of the gene regulatory networks governing plant adaptability to poor soil conditions
- Engineer roots with improved adaptability to poor soil conditions such as Al and Mn toxicity, suboptimal temperatures, and hypoxia

Recalcitrant molecules for longer term carbon storage

In addition to storing more carbon deeper underground in enhanced roots, researchers are focusing on increasing the proportion of carbon that organisms fix into long-lived forms of carbon. The Salk Institute Harnessing Plants Initiative is focused on increasing suberin production in crops and cover crops (Salk, n.d.). Lignin and glomalin should also be considered as targets for enhancement. Suberin, lignin and glomalin are all substances already produced in abundance by plants and fungi, and they are resistant to degradation, and can persist in the soil for many decades or even longer.

Suberin

Suberin is a tough, waxy substance that accumulates in the cell walls of plant roots and in some other plant features, such as the bark of cork trees. It is slow to degrade in the soil, and is a significant source of soil organic carbon. Suberin originating in roots is considered to be an important contributor to long-term soil organic carbon stores (Filley et al., 2008)

Suberin provides an important function in creating a barrier to moisture and to protect the plant from attack by pathogens. It is composed of long chains of hydrocarbons linked together: highly stable ring-forming aromatic molecules that are covalently linked in an alternating pattern to aliphatic molecules (Bernards, 2002). These molecules are cross-linked via ester bonds, increasing the stability of the overall structure. Because they are composed primarily of hydrogen and carbon, they are mostly composed of carbon by weight. While suberin subunits can be found in many variants, a study of suberin in soybean roots found the aliphatic domain composed of chains ranging in length from 16 to 24 carbons, with C:18 being the most common chain length, demonstrating the high proportion of carbon (Thomas et al., 2007). Because it contains a high proportion of carbon and is not rich in nitrogen and phosphorous, suberin is an excellent option for storing more organic carbon without locking up key nutrients which would degrade soils and put increased stress on global phosphorous production.

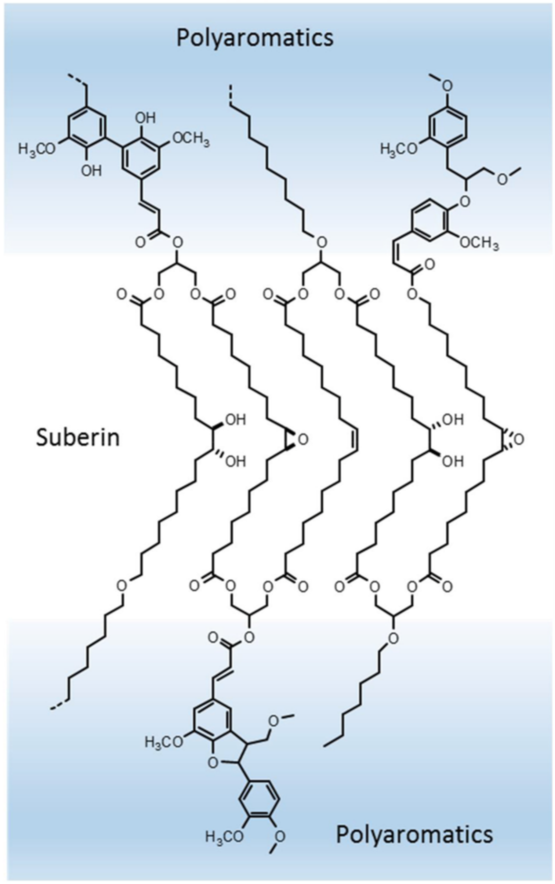

FIGURE 3 | Suberin hypothetical model in the context of suberized cell walls structure: core suberin, a regularly packed poly(acylglycerol) macromolecule, makes the translucent lamellae; this suberin polyester is covalently linked through esterification to ferulic acid to the neighboring lignin-like polyaromatics, which account for the dark lamellae.

Image 3: Source: (Graça, 2015)

Although the suberin biosynthesis pathway is not completely understood, experiments have shown that suberin production can be vastly increased. An *enhanced suberin 1* mutant Arabidopsis plant showed a two-fold increase in root suberin content, along with increased drought tolerance (Franke et al., 2012). A 2002 review described 85 enzymes involved in the biosynthesis pathway of suberin precursors (Bernards, 2002). Modern genomics techniques are quickly adding to our knowledge of suberin biosynthesis. A more detailed understanding of suberin biosynthesis will reveal opportunities for increasing suberin production. Beyond that, a more detailed understanding of how the structural properties of suberin (both subunits and macromolecular assembly) relate to its resistance to degradation may illuminate opportunities to engineer novel suberin structures to be even more degradation-resistant, or to enhance the expression of existing suberin structural elements and arrangements that confer resistance. For example, several studies have pointed to the aliphatic layer as critical in providing resistance to attack from dry-rot fungus and root diseases caused by the microbe *Phytophthora soja* (Thomas et al., 2007)

Inducible suberin upregulation

Formation of suberin lamellae in plant roots has been demonstrated to be dynamic, forming and reforming in response to changing soil conditions, a flexibility that allows plant roots to reroute around barriers in soils and towards nutrient-rich deposits (Ursache et al, 2020). This dynamism suggests that excess suberin production might be inducible at a desirable point in the plant life cycle, dramatically increasing the amount of suberin in the plant prior to its death, without impacting the majority of its life cycle. Such a property could be desirable in crop plants, triggered after harvest, as it would provide a way to increase suberin content without diverting the plant's energy needed to produce food crops. The trigger for ramping up suberin production could be an element timed to the harvest: for example, the age of the plant (many types of crops are engineered to be ready a set number of days after seed planting), the amount of daylight at the end of the planting season, colder weather, or perhaps even an exogenous chemical stimulant. Research on temperature-induced lignin deposition in several species, including post-harvest in at least one, points to possible genetic pathways for creating a responsive control for suberin expression (Q. Liu et al., 2018).

This is likely to be a technically challenging approach and would need to yield a significant increase in suberin production to justify the development effort. However, given the tremendous amount of land area occupied by crops, even a moderate increase in suberin growth and hence stored carbon is worth investigating. The development of new tools for inducing controllable plant behaviors may also yield other benefits in fighting crop disease, adapting crops to climate change, or increasing crop productivity.

Key research questions

- What are the key mechanisms by which suberin is broken down in soils, and what are the most promising points of intervention to slow degradation?
- How much, and in which locations inside a plant, can suberin content be increased before harming plant health?
- What are the key genetic controls in the biosynthesis and expression of suberin that could be exploited to increase production?
- Which genetic controls could be exploited to create inducible or time-dependent suberin overexpression?

Key engineering opportunities

- Preferentially express monomers that result in more stable suberin and SOC, and design and test new monomers with improved characteristics
- Enlarge existing suberin deposits in plants
- Create suberin deposits on more locations on plant

- Increase recalcitrance: Better understanding of macromolecular assembly may provide clues as to how to further strengthen these bonds from microbial and fungal attack after plant death
- Overexpression at end stages of crop plant life (waxy entombment)

Lignin

Lignin, like suberin, is a major component of plant biomass that provides structural stability and protects the plant from degradation and attack. Lignin is one of the most common organic polymers in the biosphere, comprising 30% of organic carbon (Ralph et al., 2004). It is particularly resistant to degradation in nature, and has been described as “the most recalcitrant component in biopolymers for physically impeding the accessibility of enzymes to polysaccharides substrate.”(Zhang et al., 2019). Lignin’s structure provides insight into its durability. It is composed of lignin monomers, which come in a wide variety of forms, that are added to a growing polymer chain in a semi-random fashion, depending on the availability of reactants and the local intracellular environment during polymer synthesis (Ralph et al., 2004). Because of this variability, there are an “astronomical” number of possible lignin macrostructures and a low probability of two lignin macromolecules being identical (Ralph et al., 2004). Lignin monomers are produced in the cytoplasm and transported to the cell wall, where they are assembled into macromolecules (Q. Liu et al., 2018).

The past few decades have brought a great increase in our understanding of lignin biosynthesis.
A 2017 mini-review identified 17 genes involved in lignin biosynthesis that had been identified in the preceding 5 years, many of which may be targets for increasing lignin expression or recalcitrance (Q. Liu et al., 2018). A general biosynthesis pathway has been proposed that describes the three key steps of monomer synthesis, transport, and polymerization and the key enabling enzymes (Q. Liu et al., 2018). While there is doubtless much more to learn, studies so far have shown many pathways for achieving double-digit increases in lignin biosynthesis, and increased lignin content has been linked to cold temperature exposure in some plants, such as in the leaves of *Rhodedendron* and the wood of the Norway spruce (Q. Liu et al., 2018). In roots, a positive feedback loop enhancing lignin biosynthesis was identified (Wachsman et al., 2015). In addition to increasing lignin content in plant biomass, pathways for increasing lignin content that result in increased SOC have also been identified. Single-gene mutants were demonstrated to have increased lignin biosynthesis and to result in increased carbon stored in soils due to decreased degradation of plant matter (Whalen et al., 2014).

Somewhat ironically, much of the climate-related engineering work on lignin has aimed to make it easier to degrade lignin, not harder. This is because of the extreme interest in the past several decades in using harvested biomass as a starting material for making biofuels. Repurposing this biomass requires first expending a large amount of energy in processing it to break down the lignin and separate out hydrocarbons that can be used as precursors for making fuels. Thus, a substantial amount of research has gone into furthering the breakdown of lignin. Much of this research could actually provide a starting point for strengthening lignin, by essentially doing the reverse of previous genetic engineering strategies.

There are many potential approaches to engineering plants to express more, and more durable, lignin. First, the extreme structural variability of lignin means it can tolerate a lot of interference in its form without sacrificing function. In natural lignin, many subunits are arranged in a fantastic array of forms, which “provides significant opportunities to engineer the structures of lignins beyond the limits explored to date.” (Ralph et al., 2004) It is known that the composition of monomers in the macromolecules of lignin greatly affects the degradation susceptibility (Ragauskas et al., 2006). Modification of the monomer composition to enhance degradation resistance represents a path forward to enhance the amount of recalcitrant carbon in soils.

The location of lignin accumulation within plants could also be controlled, allowing for example more lignin accumulation in areas of the plant (such as deep roots) that are particularly shielded from environmental degradation. Ferulates have been shown to be nucleation sites for beginning the lignification process in grasses (Ralph et al., 1995). Within cells, “[t]he binding of peroxidases to specific epitopes within the cell wall is potentially a means to control lignin deposition in space within the cell wall.” (Ralph et al., 2004)

Pathways linking lignin deposition to environmental stresses and triggers which could be exploited to create bioengineered, temporally-controlled means of lignin overexpression. In satsuma mandarin trees, high temperature post-harvest was found to correlate with increased lignin deposition in the tree pericarp (Yun et al., 2013). It is possible that this adaptation serves to protect the plant from heat stress (Q. Liu et al., 2018).

These and other engineering strategies describe credible ways to control the molecular structure, intra-cellular location, intraplant location, overall expression levels, and temporal expression of lignin synthesis in plants in ways that could substantially increase long-term carbon storage.

Deployment

There are multiple potential strategies for deploying lignin-enhanced plants to store more atmospheric carbon. As with suberin, lignin could be overexpressed in the roots of crops, grasses, and trees across many biomes. Lignin can also be enhanced in above-ground biomass, where it is already found in great abundance in plant stems, stalks, canes, and all types of woody biomass. The primary value of increased lignin concentrations would be for increasing the proportion that ends up in soils or other long-lived deposits. On its own, more lignin stored in a living plant does not necessarily mean more carbon drawn down from the atmosphere. Increased lignin content in wood has been correlated with decreased growth rates (Novaes et al., 2010). Attempts to engineer high-lignin containing trees may need to offset lower growth rates by introducing growth-enhancing traits such as improved photosynthesis, water retention or nutrient uptake in order to not see a decrease in overall biomass production. Lignin-enriched wood might increase the carbon flux in forests towards a higher SOC content, however this would need to be confirmed experimentally.

With regard to crops, there are potential paths for both increased and decreased lignin to be useful in environmental carbon removal. Much above-ground biomass, for example the stalks of corn and wheat, is not high-value material and is often considered waste (this and other low-value leftover biomass post-harvest is referred to as “crop residue”). Crop residue is often burned to clear fields for the next harvest, which releases tremendous amounts of CO_2 back into the atmosphere and contributes greatly to local air pollution. One proposal to store carbon from crop waste imagines harvesting it and throwing it in the ocean, weighed down by stone ballast to sink into the deep ocean (Strand & Benford, 2009). While likely to work, this proposal would remove crucial nutrients from soils and degrade them over time, so it is not likely to be sustainable.

It might be possible to engineer crops for planned degradation that enhances SOC, to avoid the current practices of burning and tilling while reaping the same benefits of clearing fields for the new harvest and returning nutrients and organic matter to the soil. One can imagine a scenario where crop residues from specially engineered crops, after harvest, begin to degrade quickly into smaller pieces, perhaps millimeters in size, that are more easily integrated into the soil as SOC. Lignin however could persist in small, stable aggregates in the soil, thus enhancing long-term SOC while facilitating biodegradation that clears the field for the next harvest. A different strategy envisions crop residues being harvested and burned in facilities that capture CO2. The residues would be combusted at high temperature to create carbon-rich biochar, which would be returned to the fields (BECCS with biochar). Biochar has beneficial qualities that make this strategy appealing: it can sequester carbon in the soil for hundreds of thousands of years (Spokas, 2010). It plays a role in stabilizing soil structure and increasing water retention, and it provides an avenue for the storage of carbon from crop residues that need to be cleared from their original fields quickly. To improve the efficiency of this strategy, crops could be engineered to overexpress lignin and other molecules that are highly retained as stable carbon in the process of forming biochar.

Key Research Questions:

- What are the genetic controls and environmental stimuli that could be exploited to cause controlled degradation of crop residue that clears fields quickly but allows lignin to persist in SOC?
- What is the relationship between enhanced lignin production and increases in SOC?
- What aspects of lignin structure and chemistry help it resist degradation, and how could these traits be enhanced?
- What new chemistry and structural arrangements, perhaps adapted from other degradation-resistant organic molecules, could be introduced to lignin to make it more degradation resistant?

Key Engineering Opportunity:

- Create a model of the genetic controls that could be exploited to create planned degradation for key crop species
- Design and test “super recalcitrant” forms of lignin
- Engineer a model plant to overexpress lignin at the end stages of its life cycle (woody entombment)

Enhancing soil carbon from fungi (Glomalin)

In addition to increasing recalcitrant soil carbon that is derived from plant biomass, we can consider increasing the amount of long-lived soil carbon that originates in fungi. Glomalin is a recently discovered (1996) and relatively understudied component of soils, associated with certain types of fungi, that is thought to store a significant percentage of long-term stable soil carbon (Wright & Upadhyaya, 1996) (Comis, 2002). By one estimate from a U.S. Department of Energy funded study, glomalin may account for as much as 27% of soil carbon (Comis, 2002). Glomalin was first identified by a harsh extraction process that exposed soils to high heat and acid, leaving a durable and carbon-rich substance remaining.

This substance is referred to as glomalin, although a specific molecular structure for glomalin has not been identified. More recent research has shown that glomalin is composed of a variety of different molecules, some of non-fungal origin (Holátko, 2020). Because glomalin is not identified by a specific structure, but rather is identified by its extraction process and reactivity to an assay, it is currently referred to as glomalin-related soil protein (GRSP).

Some aspects of GRSP are known: it is mainly a hydrophobic glycoprotein (composed of both sugar and protein) produced in association with the arbuscular mycorrhizal fungi (ARF) which live in symbiosis with plant roots on at least 70% of terrestrial plants worldwide (Brundrett & Tedersoo, 2018). ARF grow hyphae filaments that explore the soil for nutrients and trade them to plant roots in exchange for carbohydrates. Through this relationship, plants redirect 4 - 15% of their photosynthetically fixed carbon to ARFs in the form of sugars and fatty acids (Holátko, 2020). It is assumed that ARFs produce GRSP, but this has not been definitely proven: only a correlation has been established between AMF presence and glomalin (Holátko, 2020). At least some amount of the GSRP is the result of the activity of bacteria (Gillespie et al., 2011). This GSRP is either produced by the bacteria on their own or through the conversion of ARF-products (González-Chávez et al., 2008). GSRP has been shown to stabilize soil aggregates, which are critical to storing carbon in soils and protecting it from chemical and physical degradation (Rillig et al., 2010). Aggregates also help hold soils together, preventing loss of carbon derived from other sources (Comis, 2002). Several studies have found GRSP contains 28-45% C, 0.9 - 7.3% N, and 0.03 - 0.1% P, a range that indicates GSRP is composed of a variety of molecules that can be found in different proportions (Holátko, 2020).

GRSP is clearly a significant store of SOC globally. However, the mechanism by which it is produced, and the degree to which its production is the result of ARF and/or the relationship between ARF, plant roots, and soil microbes, needs to be further defined and validated experimentally in different ecosystems. Given the large percentage of soil carbon contained in GSRP, the question of how GSRP is created and maintained is critical for efforts to increase carbon content in soils. Importantly, this relationship may function differently in different biomes and climates. Strategies for enhancing GSRP or its most stable fractions may need to be tailored to different local ecosystems.

If ARF turns out to be the main driver of GSRP creation and subsequent stable SOC accumulation, then efforts to increase soil carbon should focus on enhancing ARF production of GSRP. This could take the form of engineering the fungus itself to overproduce key carbon-rich compounds; to engineer ARFs that can block the activity of microbes that consume and degrade GSRP, or to modify plants in ways that may increase ARF growth by for example feeding them more carbohydrates via plant roots.

Key research questions:

- How is GSRP produced? What role do ARF, plant roots, and microbes play in the production of GSRP components that contribute to long-term SOC?
- How could this relationship be exploited to enhance GSRP production and permanence?
- How could strains of ARF be cultivated in desired areas? How can modified strains be maintained on land without being outcompeted by native strains?
- Is there an ecological risk or negative soil health impact associated with soils that contain higher glomalin content?

- What is the risk of escape of this trait into natural strains of ARF or other types of fungi, and what might the consequences be?

Key Engineering directions:

- Overexpress in plant roots, ARF, or microbes the factors the contribute to GSRP creation and stability
- Experiment with strategies for making GSRP even more resistant to degradation
- Investigate expressing the stable carbon fraction of GSRP in faster-growing organisms that could be grown at scale, possible in above ground biomass
- Assess the structure of the most recalcitrant carbon-rich fraction of GSRP to learn how to engineer other plant or fungal derived proteins to be more resistant to degradation

Sporopollenin

Sporopollenin is the most durable known biopolymer, which can remain chemically intact for millions of years (Mackenzie et al., 2015). Sporopollenin is found in the outer walls of plant pollen of all plants, serving a protective function (Mackenzie et al., 2015). Despite its ubiquity, biological importance and impressive stability, relatively little is known about sporopollenin. Its structure was first published in 2019, and indeed the durability of the biopolymer made degrading it for analysis particularly difficult (Li et al., 2019). This work showed that pine sporopollenin is composed of aliphatic-polyketide-derived polyvinyl alcohol units cross-linked to 7-*O*-*p*-coumaroylated C16 aliphatic units via a dioxane moiety featuring an acetal(Li et al., 2019). Li et al suggest that the presence of two types of cross-linking, both ester and acetal, contributes to the polymer's durability, allowing it withstand degradation under both acidic and alkaline conditions.

Studies in *Arabidopsis* and rice have begun to identify the genes responsible for sporopollenin biosynthesis (Quilichini et al., 2015). The process of sporopollenin biosynthesis is highly conserved, and recent work has begun to identify genes responsible for synthesis, translocation, and regulation (Ariizumi & Toriyama, 2011)Further research should increase understanding of the controls of precursor production, transport and assembly into a wall structure(Quilichini et al., 2015).

Extremely durable "Super-tubers"

The extreme stability and degradation-resistance of sporopollenin might be repurposed for enhanced biological carbon storage. One hypothetical concept is to create a thin sporopollenin-composed shell around the tuberous portion of the roots of highly productive plants, such as cassava or potatoes. Cassava is one of the most productive root vegetables and is one of the world's major crops, with production capacity reaching 80 tons per hectare(FAO, 2013). Just as sporopollenin functions to protect the interior of seeds, the outer protective shell could protect a carbon-rich interior mass from attack by fungi, microbes, and environmental degradation. If such a shell could be expressed around tubers matching the productivity of cassava, and we assume tubers are 50% C by mass, then storing 1 GtC/yr, equivalent to 3.6 Gt CO_2/yr, would require 250,000 km^2, about twice the area taken up by lawns in the US (lawns occupy under 2% of the land area of the US). Improvements from photosynthetic efficiency, nutrient production and uptake (for example by co-cultivating w N fixing microbes), increase in root mass via engineering the genes controlling root/shoot partitioning, might substantially increase carbon storage capabilities per unit area. An experiment in increasing the carbon-fixing ability of potatoes by engineering just one enzyme to reduce photorespiration resulted in a 230% increase in tuber biomass (Nölke et al., 2014). Super-tubers could also be engineered to grow on marginal lands like agricultural lands taken out of crop production due to salt-water incursions.

Existing genetic pathways could potentially be repurposed for creating tubers encased by sporopollenin. It's been noted that sporopollenin is similar in structure and function to cutin, the material that makes up the aerial outer wall of plants, and there is evidence that biosynthesis s of both materials may share similar catalytic enzymes involved in the production of fatty acid derivatives (Ariizumi & Toriyama, 2011). This might provide a starting point for engineering by repurposing aspects of the cutin production pathway to make sporopollenin. Sporopollenin has been identified as a component of the outer cell wall in the microalgae *Chlorella protothecoides* (He et al., 2016). This observation provides a potential model organism for experiments in controlling sporopollenin production, localization and deposition.

One obvious downside to encasing roots in sporopollenin is that it would impede their ability to take up water and nutrients from the surrounding environment. This presents a significant challenge. A possible solution could be to engineer the sporopollenin layer to only surround the portion of roots storing carbon-rich molecules (starches, for example) and not express sporopollenin in the finer roots and roots hairs. Or, sporopollenin deposition could perhaps be temporally controlled, ramping up expression only when roots are at maturity.

Annual to Perennial Conversions

Perennial plants allocate more resources to below-ground biomass (root) growth than annuals, and invest in growing deeper roots (Vico et al., 2016). These larger, deeper roots enable more carbon to be stored deeper underground, where it can be more effectively locked away from being recycled back to the atmosphere. By switching croplands and other managed lands to grow perennials rather than annuals, tremendous additional amounts of carbon could be stored in soils. In particular, pairing an annual-to-perennial conversion with plant engineering that causes plant roots to express more recalcitrant forms of carbon, such as suberin, could have a double-whammy effect at increasing soil carbon. Perennial crops could also lead to less soil carbon loss from soil disturbance than annual plants, as the land doesn't need to be tilled or resown every year. A 100-year study conducted in Missouri compared the effects of an annual cropping system vs a rotation of 4 years of cropping alternating with 2 years of perennial grasses, and found the perennial system maintained 30% more topsoil and was 54 times more effective in controlling soil erosion (Glover, 2003).

There is good reason to believe that annual-to-perennial modifications could cause a large increase in underground biomass and resulting soil carbon. Perennial roots for grasses can contain orders of magnitude more biomass and extend meters deeper into the soil than their perennial cousins, as illustrated in a photo (**Image 4**, below) comparing the root structures of annual and perennial wheatgrasses. Converting pastureland to perennial C4 grasses has been shown to increase root biomass and soil carbon content (Bresciano et al., 2019). One study in Kansas compared land that had been used for annual production of wheat for 75 years to perennial grasslands, and found that the perennial grasslands had three to seven times greater root biomass and root lengths twice as long (DuPont et al., 2014). In the first meter of soil depth (the extent of the study), perennial grasslands were found to contain 33% more soil organic carbon (DuPont et al., 2014). A 10-year study in Kansas of perennial and annual biofuel crops found similar results: the perennials showed 3.7 to 7.8 times greater root biomass and increases in soil organic carbon of 0.8 to 1.3 MgC per hectare per year (McGowan et al., 2019).

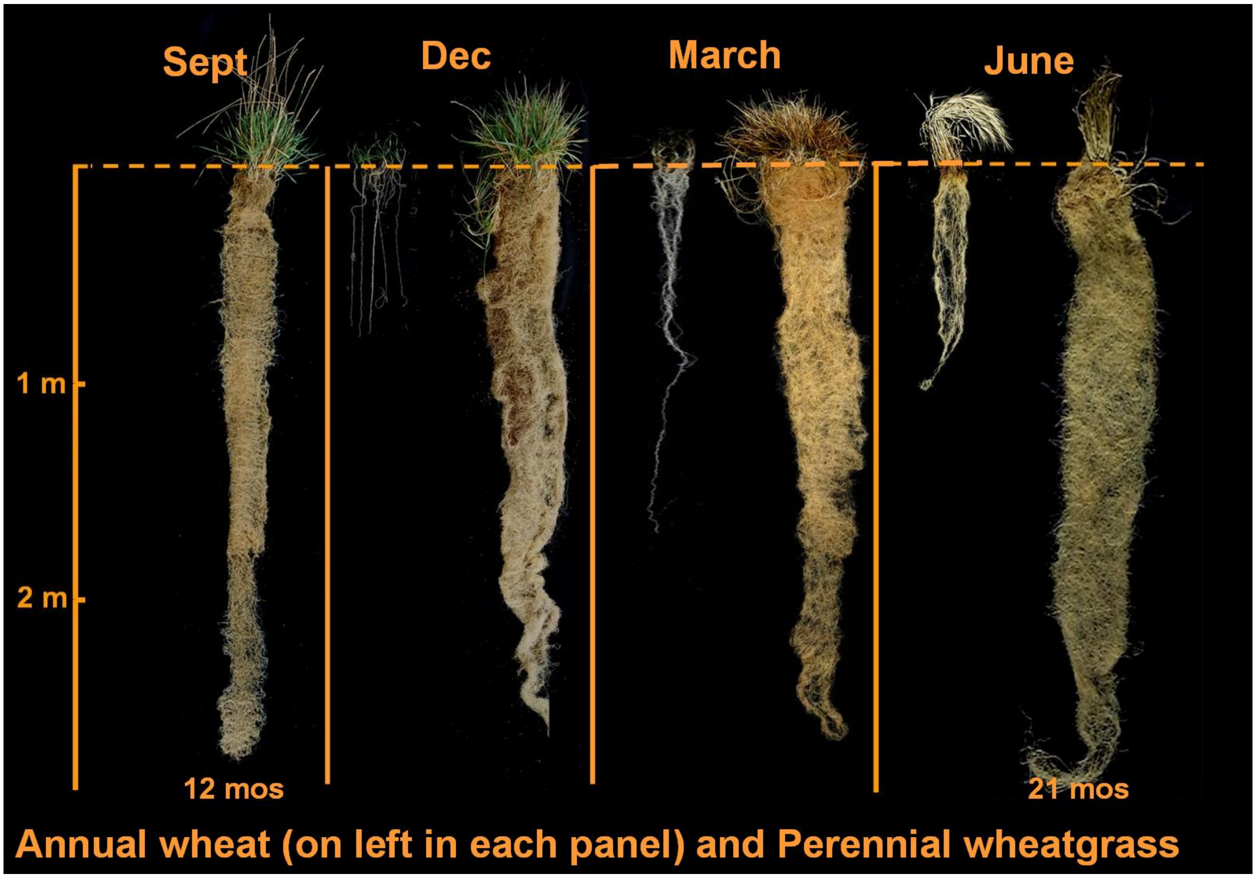

Image 4: Photo of the roots of perennial versus annual grasses. Source: Glover, 2008.

The Land Institute, a nonprofit organization dedicated to sustainable agriculture, is attempting to engineer new perennial crop plants to help restore the health of soils while continuing to feed a growing global population (The Land Institute, 2021). The Land Institute focuses on engineering perennial traits into annual crops, like corn and wheat, to restore the soil health and carbon content of the prairie lands that modern monoculture farms replaced. The organization's engineering strategy revolves around selective breeding of domestic strains to find perennial traits, and cross-breeding of wild perennials with domestic strains (The Land Institute, 2021). These efforts, however, could be supercharged with the use of synthetic biology and genetic engineering.

There is good reason to think that annual plants could be engineered to become perennials. Perennial plants have evolved naturally into annuals many times, and annuals have evolved into perennials as well (Heidel et al., 2016). This evolutionary history indicates that such annual-to-perennial switches can be introduced by a limited number of genetic changes, rather than requiring a full redesign of core metabolism and physiology. A 2008 study found that regulating just two genes related to flowering induces perennial-like traits in otherwise annual plants(Melzer et al., 2008). Another study compared perennial and annual varieties of four plants, and identified five gene families that may be responsible for the evolutionary switch from perennial to annual, encoding a kinase, an oxidoreductase, a lactoylglutathione lyase, a F-box protein and a zinc finger protein (Heidel et al., 2016). It is likely that modern genetic and molecular analysis techniques can identify the relevant genes and metabolic shifts needed to convert annuals to perennials. Such changes could be modeled in silico and optimized before being engineered into plants, speeding up the experimental cycle time.

Key research questions

- What are the key genetic controls governing the transformation from an annual to a perennial life cycle?
- Can crops be converted to perennials without harming productivity?

Key engineering targets

- Identify key genetic changes responsible for annual to perennial transitions
- Create models of annual-to-perennial conversion for species of interest to optimize and speed plant engineering efforts
- Create genetically modified perennial versions of plants that also include other carbon storage-enhancing traits (e.g. overexpressed suberin or lignin)

Slower decomposition

As plant matter decomposes, carbon is released back into the carbon cycle. Interventions that slow degradation could play a role in increasing the total amount of carbon stored in soils and the net annual carbon flux to soils. One potential source of organic carbon that could be preserved for longer is crop residues. Crop residues are the stalks, stems, and other low-value components of above-ground plant biomass that is leftover after the harvest of crops like corn and wheat.

Crop residues around the world are used for animal feed, building materials, or plowed back into the soil (a process that breaks up soil and can further erosion). Even plowing residues back into the soil will result in relatively rapid decomposition. One study of no-till wheat showed 66% of the carbon was respired within a year (Gale & Cambardella, 2002). However, in many areas crop residues are burned, releasing the contained carbon immediately back into the atmosphere as CO_2 and contributes heavily to local air pollution. The annual burning of crop waste after Harvest in Northern India has been linked to a 60% increase in harmful PM 2.5 particulate matter, impacting the health of millions (Jethva et al., 2019). Globally, there is an estimated 5 Gt of crop residue every year from the major crops, which contains about 40% carbon (Strand & Benford, 2009). If this material was reengineered or repurposed, that could translate to a substantial amount of additional carbon storage per year (functioning both as mitigation and as carbon removal). One proposal calls for dumping crop residues in the deep ocean as a means of long-term carbon storage. While technically this is likely feasible, removing even a third of global crop residues could “exacerbate soil erosion hazard, deplete the SOC pool, accentuate emission of CO_2 and other GHGs from soil to the atmosphere, and exacerbate the risks of global climate change” (Lal, 2005). Thus, we should look for other solutions that allow crop residues to return organic carbon and nutrients to the soil.

An option for stabilizing the carbon in crop residues is to turn it into biochar, which can then be returned to soils as an amendment. Biochar is a dense, carbon rich substance that results from the pyrolysis burning of plant matter, which is burning in the absence of oxygen, typically at temperatures of 350-800°C (Bridgwater, 2003). Biochar can have a different makeup and long-term stability in the soil depending on what type of plant matter was processed to make it, and at what temperature it was burned (Rehrah et al., 2014). Biochar is extremely stable in soils over long periods of time. One study found the carbon content in biochar to have a half-life ranging from 100 to 1 million years, with a 100-year loss of carbon ranging from 3-26%(Zimmerman, 2010). According to one estimate, transforming crop residues into biochar could sequester the equivalent of 0.7 GtC/yr (P. Smith, 2016).

Could it be possible to engineer crop plants such that the resulting pyrolyzed residues will result in greater amounts of biochar and more stable biochar? It has been suggested that the relative amount of aliphatic and more volatile components in biochar influences its long-term stability (Zimmerman, 2010). However, a review of the literature showed that no clear relationship has yet been proven, and pointed out that volatile components' impact on biochar stability may be an artifact of experimental techniques and lab storage conditions (Chantre Nongpiur et al., 2016). The study did find a strong correlation between biochar O:C ratios and long-term stability, with less oxygen corresponding to more stable biochar. Overall, across the studies examined, the review found that "biochar with an O:C molar ratio of less than 0.2 are typically the most stable, possessing an estimated half-life of more than 1000 years; biochar with an O:C ratio of 0.2–0.6 have intermediate half-lives (100–1000 years); and, finally biochar with an O:C ratio of greater than 0.6 possess a half-life in the order of over 100 years" (Spokas, 2010). This provides an intriguing starting point for plant engineering that can produce biomass which will yield biochar with a low O:C ratio. Other studies looking at biochar particle size have shown that larger particle sizes of biochar (above 0.25mm grain size) had greater durability in soils, potentially because larger particles lock away carbon more effectively from microbial attack and chemical degradation (Zimmerman, 2010). Potential engineering targets are thus to create crop residue that will result in biochar with a low O:C ratio and create greater than 0.25mm grain sizes. It is possible that grain size is mostly driven by processing conditions and only minimally by biomass feedstock type: this is an area for further study. An area that also deserves further study is whether it is feasible to modify plant stems, stalks, and other post-harvest "waste" such that processing it results in biochar with a low O:C ratio.

A different idea for how to handle crop residue is to design it for controlled degradation in a way that will clear fields relatively quickly for farmers, but return a high proportion of long-term stable carbon to the soil. While speculative, planned degradation could make use of existing known plant biology and exploit known degradation processes that occur on surface litter of agricultural lands (which will vary based on the local weather, soil conditions, soil microbiome, and so on). For example, corn stalks could be designed to have highly enriched pockets of overexpressed lignin, alternating with easy-to-degrade, sugar-rich pockets. When corn is harvested and stalks are pressed to the ground, the sugar-rich pockets would attract microbial and fungal decomposers, while the lignin-rich deposits would return to the soil as stable SOC. There are presumably much better strategies than this that could be envisioned by experts in crop biology and residue decomposition. Crop residues that break down into stable carbon-rich aggregates could allow crop residues to reside in fields and decompose in the for next harvest, while returning carbon and nutrients to the soil saving energy from not having to collect and process residues, and reducing the amount of CO_2 emitted from burning fields. The quest for the production of easy-to-breakdown biomass in order to extract precursors for making biofuels has yielded some insights that could be repurposed to create a controlled in-field degradation process. For example, mutant *Arabidopsis* plants were identified that made a form of xylan, an abundant plant polysaccharide, which lacked branching in its molecular structure. This made the cell wall easier to breakdown with fewer enzymes needed for hydrolysis, and although the plant stem was weakened the plants still grew to a normal size (Mortimer et al., 2010).

Engineering biomass to grow on marginal land and land degraded due to climate change

The carbon-storing bioengineering strategies discussed in this paper require land for deployment. Millions of square kilometers of land will unfortunately become disrupted due to climate change in ways that may jeopardize its previous use, either as managed land or wild land. Arctic permafrost is already melting and may be mostly thawed this century. One study projects that by 2100, of the 10.5M km^2 of global permafrost, 9.5M km^2 may experience near-surface melt (Lawrence & Slater, 2005). This melt will change the local ecosystem: from water and nutrient availability in soils, to the soil microbiome and the composition of vegetation. Melting permafrost is projected to shift local ecosystems towards becoming either wetter or drier biomes, depending on the underlying soil drainage (Jin et al., 2020). Bioengineering could be used to adapt carbon-storing terrestrial or aquatic plants to thrive in parts of these environments that may become degraded enough to be inhospitable to local ecosystems: for example, on landslides exposed by rapid melting. Here, engineered plants may help quickly cover and hold the soil together, providing some insulation from solar heating, and adding carbon to the soil. Any such interventions would cause a significant ecosystem disruption, so perhaps native plants could be adapted that can thrive in newly thawed lands while also storing extra carbon. One strategy to enhance the viability of plants could be to engineer cold and heat tolerance capabilities to handle large daily and seasonal temperature fluxes. This might take the form, for example, of modified Siberian grasses that can withstand extreme heat and cold stress, and which develop extra deep suberin-rich roots. Carbon-storing engineered plants that could be deployed on thawed permafrost is not an area that has received research attention, and may turn out to be impractical. It is being highlighted here as an area worthy of research, rather than as a viable strategy for a NETs that could be deployed near term.

In addition to melting permafrost, another large land use disruption will come in the form of rising ocean waters flooding coastal areas. As seawater floods agricultural lands, they may become unsuitable for growing crops. There has been significant research on the effects of salinity on crops, and how genetic engineering might help adapt plants to thrive in more saline environments. Some of the bioengineered carbon-storing plants discussed in this paper might have more capability than crop plants to upregulate or accept new salinity-tolerating genes and pathways, given that commercial food yield is not a concern. Multiple genes governing plant salt regulation have been identified, and genomics approaches have allowed researchers to make rapid progress in identifying and characterizes new candidate salt-tolerance genes (Formentin, 2017). Enhancing salt tolerance is not likely to be a simple matter of upregulating genes encoding transporters however: there is a trade-off between keeping salt out of roots and keeping out other nutrients the plant needs to thrive (Formentin, 2017). In addition to the complexities engineering plants to balance salt movement/barriers and growth, bio-enhanced plants designed to store excess carbon may face additional challenges. There may be competition between metabolic and regulatory pathways involved in additional root carbon fixation, production of recalcitrant carbon, and salt tolerance.

Algae for CDR

Algae are globally a major component of carbon cycle, found on freshwater and seawater. These photosynthetic organisms are significant drivers of global biological carbon fixation: marine primary producers are responsible for at least 50% of the world's annual fixed carbon and may be responsible for 71% of all carbon storage (Chung et al., 2011). They can utilize sunlight with ten times the efficiency of terrestrial plants. Single-celled microalgae are invisible to the naked eye and can be heterotrophic or autotrophic. Larger, macroscopic algae are easily visible and are commonly referred to as seaweed.

Macroalgae (seaweed)

Although the contributions of macroalgae to the global carbon cycle have been under-researched, they appear to be significant: it is estimated that 173 TgC/yr is sequestered by macroalgae, representing about 11% of all global net macroalgal primary production of fixed carbon (Krause-Jensen et al., 2018). Macroalgae are the main primary producers in coastal waters, but they do not usually grow in open waters as the roots need anchors, limiting the potential for increased production (Krause-Jensen & Duarte, 2016). They are considered to be one of the most efficient photosynthetic producers on the planet (Fernand et al., 2017). In offshore farms macroalgae can produce more biomass per acre than terrestrial crop farms, up to 57 dry tons/acre, compared to the highest producing crop, sugar cane, at 40 fresh tons/acre (Laurens et al., 2020). Macroalgae are thought to contribute to marine carbon sequestration through two pathways: marine biomass drifting into submarine canyons and sediments, or sinking to the deep ocean (Krause-Jensen & Duarte, 2016). From a Net Primary Production of 1.521 GtgC/yr, it is estimated that of the 0.173 GtC that is sequestered, 0.035 GtC is exported to the deep ocean directly and 0.117 GtC is exported as remineralized carbon (Krause-Jensen & Duarte, 2016).

It has been proposed that carbon sequestration from macroalgae could be increased by deliberate cultivation of macroalgae in the ocean, and that this could become a significant carbon removal pathway. A Korean seaweed farm demonstrated drawdown of 10 t CO_2/ha/yr and maximal production from one species, *Gracilaria*, has been estimated at 66 ash free dry tons/ha/yr (Chung et al., 2013) (N'Yeurt et al., 2012). One proposal for open ocean macroalgal farming calculates that farming 9% of the ocean's surface — a tremendous proposition — could sequester 53 Gt CO_2/yr (N'Yeurt et al., 2012). In this system, the biomass would be harvested and digested, with the carbon-containing gases stored permanently and the nutrient-containing portions of biomass returned to the ocean. Aside from this particular proposal, there is growing interest in developing off-shore macroalgae farming in the open ocean as a means of production for biofuels, food, and other materials, and potentially (though much less discussed) for carbon removal. There are several potential advantages to growing macroalgae in the deep ocean: these waters comprise a much greater total area than coastal waters, which are already heavily used by human activity and home to rich natural ecosystems such as wetlands; they do not use land area and thus are not competitive with other land uses, and macroalgae can grow quickly without requiring the intense fertilizers and freshwater irrigation that much land-based agriculture demands.

Open ocean seaweed farming is beginning to receive research support: the ARPA-E MARINER program is funding the research and testing of large-scale kelp farming, although for purposes of making biofuel, not sequestering carbon (ARPA-E, 2017). Established techniques already exist for farming seaweed far from shore that could be further optimized and automated, including using ropes and nets to provide anchoring points for seaweed and cultivating seedlings indoors and transplanting them to ropes in the ocean in order to establish specific cultivars (Fernand et al., 2017). Off-shore ocean farming could potentially take advantage of the nutrient runoff of fertilizers from farms into areas such as the Gulf of Mexico, which currently causes large dead zones. As rainwater causes runoff from farms to enter the ocean via streams and stormwaters, the flood of nutrients can cause toxic algal blooms and eutrophication that chokes out native marine life. Macroalgae cultivation in these areas could consume some of the excess nutrients (primarily N and P), thus serving an environmental remediation role in addition to a carbon removal role (Fernand et al., 2017).

In order to ensure that farmed macroalgae contributes meaningfully to carbon sequestration, all or most of the carbon in the biomass needs to be deliberately sequestered at the end of the macroalgae lifecycle. This could happen through harvest and storage, with recycling of nutrients and sequestering of carbon as proposed by N'Yeurt et al, or more efficiently through controlled sinking of the biomass to a depth of at least 1000 meters, the "carbon sequestration horizon," or "deep sea" in this context, beyond which the weight of the ocean's waters will physically sequester the biomass (and the carbon it contains) for a long timeframe, on the order 1000 years (Krause-Jensen & Duarte, 2016). Carbon which reaches this zone is effectively sequestered by the weight of the ocean and is prevented physically from mixing with the upper waters for a very long time. Carbon that reaches the sea floor may also be buried in sediments and become sequestered in this anoxic environment for geological time (millions of years). The carbon content of macroalgae varies but can comprise around half the biomass: a sample of macroalgae species (*Ulva, Sargassum, and Gracilaria*) was found to vary between 27-45% (Laurens et al., 2020).

The alternatives to sinking in the deep ocean are to harvest the biomass and store the carbon it contains in a long-term stable form and location. One solution is to use BECCS to create biochar which can be spread on agricultural lands. Another idea is to engineer macroalgae to produce much of its carbon in recalcitrant forms. Recalcitrant macroalgal carbon, though little studied in the context of carbon sequestration, is thought to play a role in the carbon cycle. One study on this topic pointed to "... the potentially significant role of refractory, taxon-specific compounds (carbonates, long-chain lipids, alginates, xylans, and sulfated polysaccharides) from macroalgae and seagrasses for their long-term sedimentary C storage... marine macroalgae do contain refractory compounds and thus may be more valuable to long-term carbon sequestration than we previously have considered (Trevathan-Tackett et al., 2015). While this recalcitrant carbon may play a key role in natural macroalgal carbon sinks, it is less likely to be important in a hypothetical engineered system in which all the macroalgal biomass is deliberately and quickly sunk to the deep ocean for sequestration. Given the ease and low cost with which all of the carbon could be sequestered via sinking, it does not appear that engineering more recalcitrant carbon would be a good target for increasing the overall amount of carbon removed from the atmosphere in this context. Additionally, there would be environmental concerns about adding lots of recalcitrant carbon to the ocean, substances which also might wash up on shore rather than stay in marine environments. Sequestering in place via sinking is feasible, low-cost, and would result in nearly 100% of fixed carbon being transported to the deep ocean for storage, so there seems to be little benefit in focusing on increasing recalcitrant carbon in combination with a strategy of sinking macroalgal biomass.

The most promising engineering targets for macroalgae intended to be sunk to the deep ocean are strategies that increase the amount of carbon-rich biomass which can be created and successfully reach the deep waters. This would include engineering faster-growing strains, strains that can thrive in open water conditions such as high waves and wind, strains that can efficiently take advantage of the nutrients available in these waters, strains that resist attack from common marine consumers (such as snails and mussels), and strains that produce a higher proportion of carbon in their biomass. In addition to directly taking up nutrients more efficiently, strains might be engineered that could be co-cultivated or even symbiotic with other organisms that can fix nitrogen from the air or concentrate carbon to make it more available for uptake. These capabilities are already widespread in marine microorganisms: cyanobacteria fix large amounts of atmospheric nitrogen, and some algae produce external carbonic anhydrase that can locally concentrate inorganic carbon in the form of HCO_3^-. Nitrogen is thought to be the most common limiting factor for macroalgae growth, although phosphorus may be the limiting factor in some places (Fernand et al., 2017). Thus, a focus on increasing nitrogen availability and uptake may be a significant boost to macroalgae farming efforts. Nitrogen availability could be increased by engineering a symbiotic relationship like the one between Azolla plants and Anabaena cyanobacteria, a highly successful partnership that can produce ~600 kg of fixed nitrogen per hectare per year in rice fields (Fernández Valiente et al., 2000).

Additional engineering efforts could reduce the amount of energy that macroalgae waste investing in forms of growth they won't need as purely hosts for carbon-fixing and storing, such as investing in reproduction. The switch from vegetative to sexual reproductive life cycle in macroalgae, or sporulation, can limit biomass growth and thus might be worth reducing or eliminating in engineered strains (Fernand et al., 2017). Engineering sterility could have the additional benefit of limiting the likelihood that engineered strains would escape and thrive in the wild.

Engineering fast-growing macroalgae could significantly increase the efficiency and lower the costs of macroalgae farming. The growth rate variation among natural strains of macroalgae demonstrates the potential scale of the efficiency gains. "One of the fastest growing species in the world, *Macrocystis pyrifera*, could contribute approximately 27% more production per hectare than the average species, and maximizing seaweed carbon content could potentially reduce costs by 38%," (Froehlich et al., 2019) While current cost estimates are high, ranging from $71 to over $1000 per ton CO2 sequestered, this is a nascent industry (Froehlich et al., 2019). Engineering, efficiencies of scale, and process improvements might drastically lower costs. Most current cost assumptions project harvesting, sale and use of the seaweed. If the seaweed instead were sequestered in place via controlled sinking, many costs would be saved (although the sale price of the seaweed product would also be lost, though it could presumably be replaced with a carbon removal credit). In addition to process improvements through automated seeding, monitoring, and sinking of algae grown on off-shore platforms, bioengineering strategies could also be employed to increase efficiency and lower costs.

Key research questions

- What are the main risks of open-ocean algal farming? What is the ecological impact?
- How much can costs be lowered and carbon uptake increased through biotechnological improvements?

- What genetic controls govern growth rate, carbon uptake, and (possible) symbiosis with nitrogen-fixing cyanobacteria?
- What is the maximum feasible amount of macroalgal-derived carbon sequestration in the deep ocean? At what rate of farming do nutrients become limiting?

Key Engineering targets

- Screen and identify strains that have the most efficiency growth characteristics (biomass production, nutrient uptake, photosynthetic efficiency, etc.) and identify the genes and regulatory elements responsible for these traits
- Increase C uptake by adding air CO2 uptake capability to macroalgae that don't have it, and improving the mechanisms for uptake of dissolved carbon such as enhancing carboxysome efficiency or production
- Increase N availability by creating macroalgae that can be easily co-cultured with nitrogen fixing cyanobacteria, creating macroalgae-cyanobacteria symbiosis
- Increase growth rate by engineering faster-growing strains (ones that for example utilize nutrients more effectively, invest less in long-term stability or reproductive capacity)
- Increase photosynthetic efficiency of macroalgae
- Increase the proportion of biomass that already fixes the most carbon (eg. cellulose or lignin-like structures).
- Create strains that are less susceptible to fouling by microorganisms and degradation (for example could make macroalgae variants that are toxic to common predators such as snails and blue mussels)
- Create strains that are optimized for enhanced carbon content for conversion to biochar, or planned degradation and separation of carbon-rich content for burial

Microalgae (Algae)

Microalgae are photosynthetic microorganisms that grow in a wide range of freshwater and saltwater environments, including lakes and the open ocean. They can thrive in a range of temperatures, pH values, and salinity, and live alone or in symbiosis with other organisms (Khan et al., 2018). They can accumulate 2 to 10 times more biomass per land area than terrestrial plants, because of their greater photosynthetic capabilities, including the ability to uptake carbon via carbon concentrating mechanisms, and lack of need to invest resources in building and maintaining supporting structures like stems, roots and leaves (Sayre, 2010). Because of these traits as well as their fast growth and relatively easy engineerability compared to plants, they are being investigated as a possible route for large-scale carbon sequestration. Large-scale microalgal cultivation can occur via several methods, such as in a closed, stirred tank reactor or in a high-rate pond (HRP). Microalgae are super-photosynthesizers able to capture light at 10 times the efficiency of terrestrial plants, amounting to a 9-10% capture of incoming sunlight, and can transfer solar energy into biomass with a theoretical yield of 77g biomass/m^2/day, or about 280 t/ha/yr (Khan et al., 2018).

Once microalgae have captured carbon in the form of biomass, how can it be sequestered? The simplest answer is to bury the biomass whole. However, just as with macroalgae burial, this would remove a large amount of nutrients (such as N and P), up to 7% by weight, and is therefore not sustainable (Sayre, 2010). As with macroalgae, the biomass could be processed to separate out the stable carbon fractions, or heated and converted to biochar. Any type of processing adds economic and energy costs. Additionally, these two processing routes considered only sequester less than 50% or 55% of the biomass carbon, respectively (Sayre, 2010).

To grow large amounts of fresh algal biomass continuously at the gigaton scale, key nutrients such as nitrogen and phosphorus must be recycled. One possible way to do this is to engineer algae to die in a way that enables carbon storage. Engineered algae could maximize production of recalcitrant carbon as they age, up until death. After death the algae would decompose, leaving stable carbon and returning their nutrients to the growth medium. The stable carbon could be siphoned off from the growth medium (a HRP, bioreactor, or pond) and stored, either as fresh biomass or after conversion to biochar. The death process could be engineered to speed decomposition while releasing recalcitrant carbon: for example, by storing recalcitrant carbon in dense macrostructures or vesicles that physically separate as the cell breaks apart. The recalcitrant carbon could then be physically abstracted from the medium by separation processes in a continuous batch process: skimmed off the top if it floats, or pumped from the bottom if heavier than the medium. One option for recalcitrant carbon might be lignin. It was until recently thought that algae do not make lignin, until an example, the red algae *Ulva lactucato*, was found to contain 1.56% lignin (Tsai et al., 2017). The percentage of lignin produced by algae would have to be drastically increased through genetic engineering, perhaps up to 25 - 50% by weight, to make this an efficient means of carbon sequestration. Perhaps a better target might be cellulose, which can make up 25% of the dry weight of microalgae (Baba et al., 2016).

A possible strategy for increasing the overall carbon concentration in microalgae is to use genetic controls for carbon partitioning. This is a process that controls the cell's allocation of the carbon it fixes towards different purposes within the cell. There is evidence that modifying carbon partitioning controls can yield substantial increases in output of a target product and increase overall biomass 180% (Oliver & Atsumi, 2015).

Growth rates and potentially carbon content could be increased by enhancing the cell's carbon uptake mechanisms. Cyanobacteria use organelles called carboxysomes to concentrate carbon around Rubisco and thus increase Rubisco's efficiency while bypassing the energy-wasting process of photorespiration, in which oxygen is erroneously fixed instead of carbon. Recent research has shown that 5% of carboxysomes within a cell are "ultra-effective" compared to the rest of the carboxysomes (Moore et al., 2020). Further research into the nature of this ultra-high efficiency could highlight engineering targets for expanding this carbon concentrating ability to all the carboxysomes in the cell. In semi-enclosed systems like HRPs, algae could potentially be engineered to exude carbonic anhydrase. This would allow the pond water to make CO_2 dissolved from the atmosphere more readily available for uptake by cells.

Cells' ability to harvest light might be increased through genetic modification, including modifications designed to maximum light harvesting in dense cell-culture conditions. An experiment that shortened the phycobilisome light-harvest antenna in cyanobacteria showed a doubled photosynthetic saturation and a 1.57-fold increase in biomass (Kirst et al., 2014).

Key Research Questions

- How much could photosynthesis in algae be improved?
- How much could carbon fixation be increased through the use of passive and active transport improvements, such as extracellular carbonic anhydrases, enhanced carboxysomes, or other strategies?
- Could algae be planned to decompose in a way that allows the majority of their carbon to be separated? What form of carbon most facilitate separation?

Key Engineering Targets

- Compare genome sequences from many strains to identify genes associated with fast growth and ultra-efficient carbon fixation
- Characterize the features, genetic and molecular, that distinguish highly efficient carboxysomes from regular carboxysomes within a given cell
- Make engineered microalgae with carbon-fixing improvements such as over-expressed carboxysomes or a high proportion of ultra-efficient carboxysomes

Bio-Enhanced Rock Weathering

Rock weathering is an important part of the global carbon cycle, in which carbon is removed from the atmosphere and permanently sequestered as rock. In this process, natural phenomena slowly degrade rocks, which then bind chemically to CO_2 in the atmosphere or to dissolved carbon in water in a spontaneous reaction. These natural phenomena that cause rock weathering include tectonic plate movements exposing fresh rock to the air and the action of weakly acidic rainwater and runoff dissolving rock. The types of rocks which are capable of naturally capturing CO_2 are silicate rocks rich in Mg or Ca, such as olivine, serpentine, wollastonite, and peridotite (NASEM, 2019). Ultramafic rocks have the most carbon sequestration capability by mass and the fastest dissolution rates among silicate rocks, and thus are a prime candidate for enhanced weathering(Moosdorf et al., 2014). When these silicate rocks erode, Mg and Ca form free cations that spontaneously bind CO_2 to form carbonate rocks (eg, $CaCO_3$, $MgCO_3$) (NASEM, 2019). The silicate rock weathering reactions being considered for ERW are energetically favored (they have a negative Gibbs free energy). For example, the carbonation of forsterite, Mg_2SiO_4 has a ΔG of -66.8 kJ/mol (Renforth et al., 2011).

Despite the overall energetic favorability of the reaction, natural rock weathering occurs slowly and so is only a small portion of the global carbon flux, in total drawing down only 0.22 to 0.29 GtC/yr (Hartmann et al., 2013). The main reason the reaction is slow is that the kinetics of carbon molecules interacting with and binding to exposed cations (usually Mg+ or Ca+) faces several significant constraints. Firstly, CO_2 is very dilute in the air (~0.04%), so the concentration of CO2 reacting with silicate rocks is low. Secondly, energy is required to break apart rocks to expose fresh surface on which reactions can occur. This energy can be supplied abiotically, as when weakly acidic rainwater dissolves rock, or biologically, as when microbes and fungi bind to rocks and physically and chemically dissolve them. Carbon bound in rock form as carbonates is very stable, and is a main driver of atmospheric CO_2 removal over geological time (Berner et al., 1983). Olivine is a focus of research efforts as it contains a relatively high proportion of CO_2-sequestering Mg, and is abundant in the Earth’s crust. The upper theoretical limit of olivine sequestration is 1.25 t CO_2 per ton of olivine (Köhler et al., 2010). In practice, carbonation rates for untreated silicate rocks range from 10-60%, and can reach 40-90% if pre-treated with mechanical crushing or heat (Renforth et al., 2011).

Increasing weathering rates would be greatly beneficial to the efficiency and economics of enhanced rock weathering schemes, saving energy costs, time, or both. A faster weathering rate corresponds to a decrease in the energy needed for rock grinding, since larger rock particles could be used to the same effect, or greater amounts of carbon removal per year could be achieved in a given system and land area by taking advantage of the faster weathering rate (Krevor & Lackner, 2011). Both of these outcomes should translate to reduced costs, since infrastructure requirements and energy needs are reduced per ton CO_2 sequestered. Particle size is a significant factor in weatherization rates: reducing particle size from 10 mm to 10 μm corresponds to a mineralization rate increase of 10^6 (Myers & Nakagaki, 2020).

Part of the appeal of enhanced rock weathering lies in its abundant and accessible raw materials: silicate rocks are inexpensive and are widely distributed throughout the world. In addition, technical infrastructure and know-how already exists: the mining industry has developed and commercialized machines for digging up and crushing vast amounts of rock. Indeed, many large piles of mining waste (called "mine tailings") already contain the appropriate types of silicate rocks for ERW. Potential drawbacks to ERW include the potential for trace toxic elements (such as lead) to contaminate water or soils, the tremendous quantities of rock and potentially land needed, and the significant amount of energy needed to crush rock into small pieces with a large enough total surface area for substantially more weathering to occur (Beerling et al., 2018).

Living organisms can play a significant role in rock weathering. In nature, microbes, plants, lichens and fungi contribute to rock weathering (Gadd, 2007). Organisms can employ both physical and chemical means to break down rock. Lichens extend finger-like projections called hyphae into tiny crevices in rocks, then exert physical force through the swelling of the hyphae to break apart the rocks. The roots of plants growing in rocky surfaces, and fungi, act similarly. Organisms may also exude chemicals to dissolve rock, including acids and cause other changes to surface chemistry that promote weathering (Gadd, 2007). Fungi can significantly accelerate rock weathering, although it is also hypothesized that under some circumstances, they can also slow it down. In laboratory experiments, the rock-inhabiting fungi *Knufia petricola* was shown to increase olivine dissolution rates by ~700% (Pokharel et al., 2019).

It is possible that a category of silicate-dissolving enzymes, silicases, might be repurposed for enhanced rock weathering systems. To understand how biology might be engineered to enhance rock weathering, it is necessary to take a closer look at the key reaction of silicate dissolution. As described by Schröer et al. in **Image 5 (**below), there are two main chemical reactions involved in the dissolution of silicate rocks by silicases (Schröer et al., 2003).

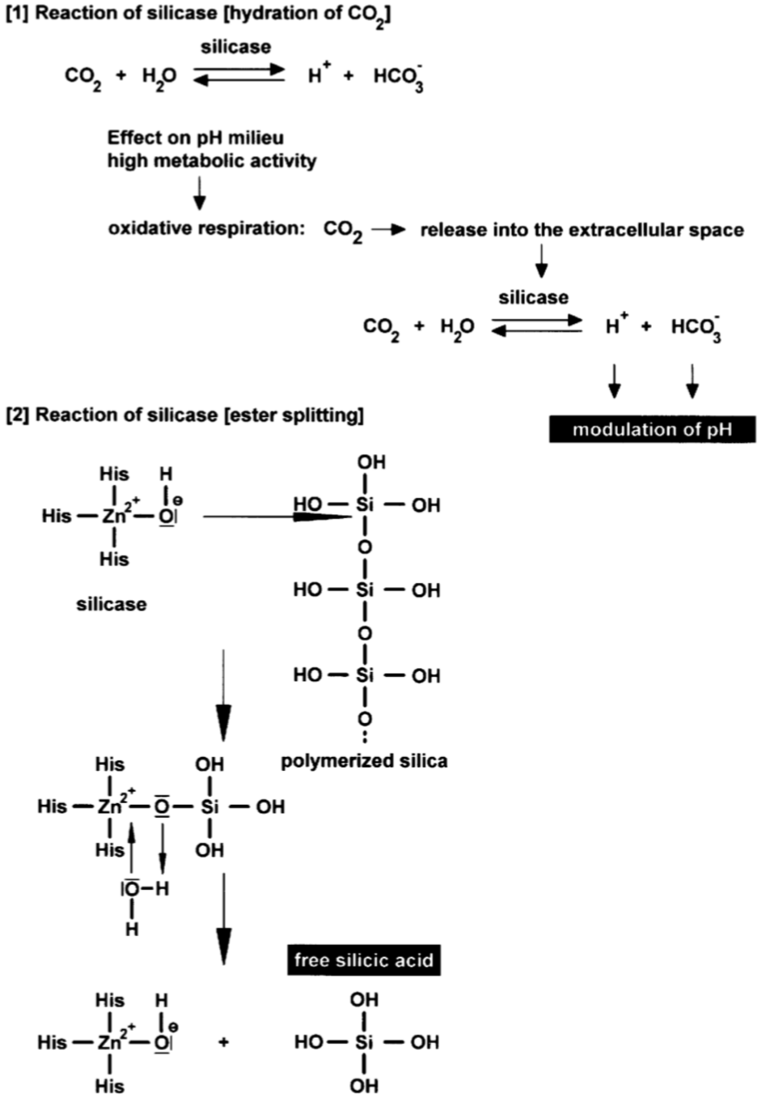

Image 5: Source: Schröer et al., 2003.

The first reaction involves hydrating CO_2. This reaction facilities the transition of dissolved CO_2 into a more reactive anionic form, bicarbonate. This reaction releases a proton, making the solution more acidic. HCO_3^- is then able to react with a divalent cation such as Ca^{2+}, creating $CaCO_3$. This $CaCO_3$ will precipitate out of solution, thus binding and removing C from the system. The same reaction also occurs with Mg^{2+} as the cation instead of Ca^{2+}. The second key chemical reaction involves splitting apart units of polymerized silica. Silicase is an enzyme composed of a Zinc ion surrounded by three histones, and when hydrated it can act as a Lewis acid and break an ester bond between silica subunits, thus releasing a silica as free silicic acid (Schröer et al., 2003).

Dissolving silicates with silicase enzymes

In nature, sponges produce silicases to dissolve and reform silicate to create silica-based spines that support the sponge's structure like a skeleton. Microbes or fungi could potentially be engineered to release silicases optimized for rock weathering, perhaps in a semi-enclosed system analogous to a raceway pond or a stirred tank bioreactor to allow enzymes to become concentrated in a liquid medium. Silicases in such a system, if successful, may enhance silicate rock dissolution.

Disrupting the silica amorphous layer

Once silica is released from rock however, it can linger as an amorphous silicon layer (SAL) on the rock surface, thus impeding further reactions (Pokharel et al., 2019). Some organisms disrupt the SAL through physical and chemical means that effectively creates holes through which dissolution reactions can continue to occur on fresh silicate. The fungus *K. petricola* is thought to attach to the silicon amorphous layer and exude protons, which penetrate the SAL and replace Mg+ in olivine, thus furthering olivine dissolution (Pokharel et al., 2019). It is also thought that *K. petricola* can remove iron from the SAL by protonating or chelating it, creating holes in the SAL and increasing dissolution rates (Pokharel et al., 2019).

Removing silicic acid

The result of silicate dissolution is the creation of free silicic acid (which also contributes to the formation of the SAL). Engineered processes for ERW should consider methods to remove the SAL layer as it is produced. This might be achieved be through physical or chemical means such as stirring the crushed silicate rock in a liquid medium to agitate the SAL, encouraging the growth of microbes that can physically or chemically disrupt the SAL, or by abstracting or precipitating amorphous silica out of solution. Organisms might be able to introduce a metabolic process that consumes or neutralizes the newly created silicic acid. Co-culture of organisms should also be explored: perhaps organisms could act in concert to create silicic acid (via silicate dissolution) and consume it (via absorption or neutralization). One study found that a co-culture of bacteria was more effective than each individually in dissolving the silicate Bauxite (Zhan et al., 2013).

There are multiple additional ways in which microbes or fungi could use physical and chemical means to enhance initial silicate dissolution or alter the surrounding environment, for example by changing the pH, to enhance weathering rates.

Increase CO_2 in solution

The availability of CO_2 appears to be the limiting factor in the rate of carbon sequestration for at least some mine tailings (Harrison et al., 2013). If reacting silicate rocks in a liquid medium, carbonic anhydrase could be added (either by itself, or exuded by microbes in the reactor) to increase the availability of HCO_3^- as a reactant. In experiments, a bovine-derived carbonic anhydrase was added to reactors to carbonate brucite, and was shown to increase carbonation rates by up to 240% (Power et al., 2016). This experimental result is also supported by modeling that shows HCO_3^- promotes dissolution accelerated brucite carbonation (Harrison et al., 2013).

Create micro-environments to speed both acidic and alkaline reactions

Very acidic environments favor silicate dissolution, but alkaline environments favor carbonate formation, and so a solution that can achieve both reactions must be at a near neutral pH. However, microbes can create specialized localized chemistry, either in the microenvironment near the cell's surface and/or at the reaction surface or in the interstitial space within a biofilm (Power et al., 2014). Theoretically, this could allow reactions that take place in very acidic solutions and reactions that take place in very alkaline solutions to both take place simultaneously, separated by biological membranes or biofilms. Cyanobacteria can use photosynthetic energy to drive active uptake of carbon dioxide, which creates an alkaline environment that favors carbonate precipitation (Riding, 2006). The ability of microbes to create both acidic and alkaline micro-environments could be harnessed in a co-culture to further increase weathering reaction rates.

Add neutral organic salts to enhance dissolution kinetics and carbonate precipitation

The chemistry at the surface of silicate rocks plays an important role in their dissolution rates. While acid helps dissolve the rocks, a solution that is too acidic will not allow carbonates to precipitate. A study looking at ways to improve dissolution rates suggests that "...[n]eutral organic salts such as sodium oxalate and citrate enhance dissolution kinetics of serpentine in the circum-neutral pH range appropriate for mineral carbonate precipitation and have potential for use in an enhanced carbonation process" (Krevor & Lackner, 2011). With regard to serpentine, citrate and oxalate were demonstrated to increase the dissolution rate by three orders of magnitude compared to proton-promoted dissolution rates, at a pH conducive to carbonate formation (Krevor & Lackner, 2011). Many previous studies as well have shown that organic acids and citrate solutions can enhance silicate dissolution rates (Krevor & Lackner, 2011). Oxalate and citrate can be produced by microbes, and should be investigated, along with other organic acids, as means for speeding dissolution reactions.

Create carbonate nucleation sites

There are two key steps in the formation of carbonates in solution: the first is the dissolution of cations (Mg, Ca, etc.) from silicates into solution; the second is the nucleation and growth of solid carbonates (Krevor & Lackner, 2011). Microbes can potentially enhance carbonate formation by facilitating nucleation. One study of cyanobacteria growing on serpentine mine tailings found carbonates forming directly on cyanobacterial filaments (McCutcheon et al., 2016). A wide variety of microorganisms, including cyanobacteria, algae, and sulfate-reducing bacteria, can cause calcification (Jansson & Northen, 2010). A cyanobacteria-dominated consortium of microbes has been demonstrated to precipitate $MgCO_3$ (McCutcheon et al., 2014). Carbonate precipitation in cyanobacteria may be closely tied to the action of carbon concentrating mechanisms (Riding, 2006). Existing microbial mechanisms for concentrating and nucleating carbonates could potentially be adapted and engineered to improve carbonate formation in ERW systems.

Enclosed engineered process (abiotic):

Recently, several proposals have been developed to speed up rock weathering in contained systems utilizing reactor beds or similar technologies. One proposal suggests directly binding atmospheric CO_2 to crushed rocks in a greenhouse where, to enhance mineralization rates, relative humidity is kept high through the use of salt water in trays (Myers & Nakagaki, 2020). This method employs passive techniques for maintaining airflow and humidity, thus minimizing energy requirements. Most of the energy involved is used for mining and rock crushing, averaging 1.5 GJ/ton CO_2 (Myers & Nakagaki, 2020). The authors estimate that this process, deployed in Japan over 726 sites, could sequester 7.6 Gt CO_2 per year with a land use of 1.1 km^2 per Mt CO_2 removed per year.

Enclosed engineered process (biotic)

While the strategy described above is abiotic, one can imagine a similar process in which lithotrophic microbes are encouraged to grow on rock surfaces and speed up weathering in a contained environment. This strategy might work best in a liquid medium, where the pH can be controlled for faster rock dissolution. Immersion in water could allow for faster molecular interactions and more accessibility for enzymes such as silicases and carbonic anhydrases to speedily dissolve rock. Given the low energy requirements, the abundance of raw input materials (silicate rocks, microbes, sunlight, water), it is reasonable to expect that such a system, if economically feasible, might be able to reach the scale of gigatons or tens of gigatons CO_2 per year.

Such a system might resemble a semi-enclosed bioreactor tank, perhaps a large pond into which crushed rocks are dumped and which is seeded with engineered microbes. While this strategy might seem speculative (and it is), it is actually quite similar in concept to the natural process of rock weathering in called karsts. Karsts are underground formations of limestone or limestone-like materials through which rivers and streams pass on their way to the ocean, carrying with them dissolved CO_2 from the atmosphere. Karst waters can achieve substantially concentrated levels of dissolved carbon. One study found that HCO_3^- concentrations in a karst spring were 10x that of nearby non-karst waters, and that the concentration of inorganic carbon flux was 23.8x greater (Cao, 2018).

Rock weathering in karsts is not well studied or understood, but it might in fact have a large effect on the global carbon cycle as a carbon sink. A recent study suggests that the global karst carbon sink is 0.53-0.58 PgC per year, equivalent to 31.18% - 34.41% of the global carbon sink from forests (Cao, 2018). It is noteworthy that the Carbfix project in Iceland is, in essence, creating an intensified mimic of a karst system by concentrating CO_2 in water and then streaming that water through rock which can bind the dissolved carbon. Whereas in nature the water is acidified through natural processes, such as water leaching through soils and absorbing CO2 being respired by plant roots, in the Carbfix project CO_2 is concentrated through the use of geothermal energy and filters, and then combined with water for injection underground (Ragnheidardottir et al., 2011).

Heap leach mining with microbes

Another option for utilizing microbes for CDR is to adapt heap leach mining, in which microbes colonize piles of crushed rock, through which a stream of acidic water is passed through repeatedly. Microbes have been used in industrial heap leach mining for over a hundred years to help break down rocks and extract precious metals such as copper and gold. As described by Brierley & Brierley, ore is placed in a large pile with a tarp underneath. A dilute sulfuric acid solution is leached through the piled ore, which enables inoculated or naturally occurring lithotrophic bacteria to grow on the ore (Brierley & Brierley, 2013). Solubilized valuable metals can they be recovered from the solution, and the solution recycled back to the heap. Today, heap bioleaching is used in about 15% of global copper production (Brierley & Brierley, 2013). Given the well demonstrated capability of microorganisms to break down crushed rock in industrial settings, it has been suggested that heap leaching or similar processes could be applied to dissolve silicate rocks for the purpose of atmospheric carbon dioxide removal (McCutcheon et al., 2016) (Jansson & Northen, 2010).

One estimate suggests that a heap leach mine of 7500 tons of ultramafic mine tailings could sequester 2800 tons of CO_2 per year in a 0.12km2 area (Siegrist et al., 2017). This is a lot of land area, but process improvements, like taller piles of ore, faster flow rates, and engineered microbes could potentially increase these rates significantly. About 2 B of tons of alkaline mine tailings are produced each year, and 90 billion tons of past years' waste is sitting in large heaps already (Service, 2020).

The use of bacteria for enhanced weathering of mine wastes is an underexplored area. One study at Gordon Southam of the University of Queensland, St. Lucia is currently testing whether the addition of cyanobacteria can enhance weathering rates (Service, 2020). Another study has shown that an alkalinity-generating cyanobacteria-dominated consortium of microbes growing on a leaching heap of serpentine mine tailings can accelerate rock dissolution, accumulating magnesium carbonates directly on the surface of cyanobacterial filaments (see **Image 6**, below) (McCutcheon et al., 2016). Laboratory experiments have been conducted to test the potential of cyanobacterial magnesium carbonate formation for carbon sequestration from mine tailings, and concluded that a wetland bioreactor system could store 238 t CO_2/ha/yr (McCutcheon et al., 2019).

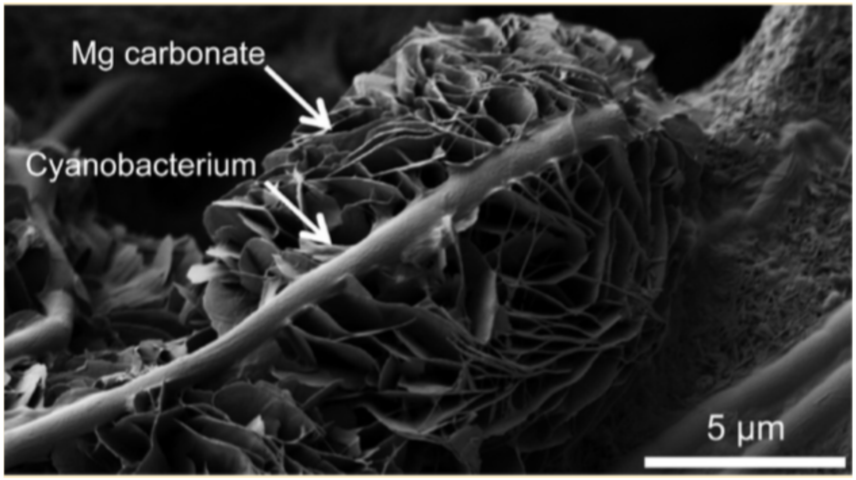

Image 6. Magnesium carbonate deposition on cyanobacteria. Source: McCutcheon et al., 2015.

The design of engineered microbes should take into account the weathering capacity of specific microbial processes discussed earlier in this section. With regard to heap leach mining specifically, it has been proposed that acidic leaching solutions could be created via the oxidation of sulfides/sulfur by *Acidithiobacillus spp* (Power et al., 2014). Experiments have demonstrated that the addition of sulfates and *Acidithiobacillus spp* to ultramafic mine tailings can increase the amount of Mg ions in leachates by a factor of ten, enhancing carbonate formation (Power et al., 2010). Co-cultures have been shown to be more effective than monocultures in at least some types of bioleaching, and so the design of a co-culture system should be investigated, particularly with regard to the design and use of engineered strains (Fathollahzadeh et al., 2018).

Microbes forming carbonate minerals at abandoned mine sites illustrate how the deposition and weathering processes that might be adapted to create a dedicated carbon removal system. **Image 7**, from Power et al., shows significant carbonate formations reaching 15cm high at the Clinton Creek mine in Canada, with electron microscope scans clearly showing the carbonate entombment of cyanobacteria (Power et al., 2014).

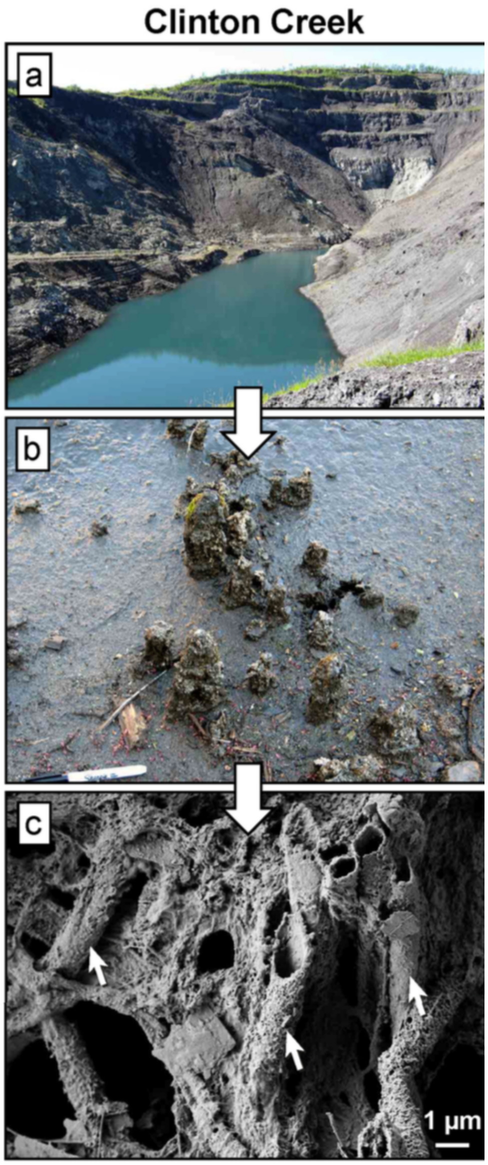

Image 7. Carbonate deposition by cyanobacteria at an abandoned mine. Source: Power et al, 2014.

The chemistry of the liquid medium for heap leaching has a strong effect on the reaction rate. Dissolution and precipitation can only happen in the same aqueous system if it is undersaturated with respect to silicate but oversaturated with respect to carbonate, which eliminates solutions that are strongly acidic or basic (Krevor & Lackner, 2011). In these systems, the kinetics of silicate dissolution are the rate-limiting step. It has been shown that the dissolution rate of serpentine, the most common of the mafic rocks, can be enhanced in the presence of citrate and acetate up to three orders of magnitude greater than by proton-promoted dissolution rates, in the pH range which allows stable carbonates to precipitate (Krevor & Lackner, 2011). Citrate and acetate production by cultivated or engineered microbes should be investigated as a potential method for increasing the kinetics of olivine and serpentine dissolution. Different versions of bio-based strategies could be developed that are optimized for the weathering of specific types of rock.

Spreading silicate rocks on land

Aside from proposals to do ERW in dedicated sites like mines or bioreactors, it has also been proposed that crushed silicate rocks could be weathered by spreading them over farmland (Beerling et al., 2018). Bioengineering may be able to contribute to this process. Using the same biological and chemical insights that would be employed in dedicated bio-ERW facilities, engineered microbes could be seeded alongside silicate rocks to enhance weathering rates. Fungi might also be a good option, as they have been shown in lab experiments to increase olivine weathering rates by 700% (Pokharel et al., 2019).

Microbes or fungi expected to perform unneeded (to the organism) extra rock weathering might be at a competitive disadvantage to native soil microbes. Potentially, during cultivation and before deployment, the microbes could be induced to create a stockpile of sugar within the cells to extend their lifespan or competitiveness, a strategy being employed by the biotech company Kula Bio, which manufactures nitrogen-fixing microbes (Sakimoto, n.d.). Whether applied microbes would last long enough in the soil to meaningfully enhance rock weathering is an open question.

A 2020 review paper details many ways in which biological activity can speed up rock weathering on farmlands (Ribiero, 2020). Root microorganisms can produce siderophores and organic acids, can produce acids to alter the pH, and can drive redox reactions (Ribiero, 2020). When designing interventions to be deployed alongside crop production, it is important to look at the entire process and where the minerals end up. Minerals adjacent to plant roots may be taken up by the plant, rather than end up as carbonates in the soil. Indeed, the majority of the interest in weathering rocks on farmland to date has been to increase nutrient availability to crops and thus increase productivity. These fundamental questions of how biological processes interact with silicate rocks on farmland require further investigation and experimentation. Answering these questions may point the way towards strategies for large-scale, low-cost ERW that utilizes living plants, microbes, or fungi.

Any bio-based ERW strategy that operates in an open environment must carefully assess potential ecological impacts, so that an informed decision about costs and benefits can be made before any deployment. Small scale tests may help inform these decisions. Such ecological impacts include, but are not limited to, the escape of bioengineered organisms into the wider environment, disruptions to the local ecology by the introduction of new species and the chemical changes associated with ERW, and the introduction of toxic trace metals such as lead from dissolved rocks.

Key research questions:

- What local conditions can optimize for rock weathering (pH, temperature, physical disruption/stirring, etc.)
- What mix of organisms can co-exist in a local ecology that maximizes rock weathering?
- What aspects of organisms' metabolism and life cycle can be modified to enhance rock weathering?
- What is the theoretical maximum ERW reaction rate that could be achieved employing biological processes?
- What is the anticipated effect on soils and on mineral uptake by plants, of enhancing rock weathering with microbes and fungi?

Key Engineering targets:

- Develop an organism-wide understanding of the constraints on enhancement of key metabolic functions that result in weathering: for example, how much can acidity, growth rate, etc. be improved without harming the organism or its rock weathering ability?
- Develop microbial strains with enhanced production of acidic and enzymatic weathering agents (potentially silicases, carbonic anhydrases, oxalates, citrate, acetate, etc.)

Bio-Enhanced Mineralization

CO_2 that is captured is commonly stored by injection into rock structures deep underground that can store it stably for long periods of time, due to their physical structures filled with pores and micro cracks as well as their favorable chemistry. One goal of such injection is to push CO_2 into contact with rocks, such as basalt, that can bind (mineralize) the CO_2, thus storing it for geologic time. This CO_2 may be injected as a stream of pure or highly enriched CO_2 gas, or dissolved into water. Strategies to enhance mineralization rates could bring several benefits: they could allow CO_2 to be injected and successfully mineralized over a lower surface area, in less time, at a lower concentration rate, or with a greater overall success rate for the proportion of injected CO_2 that ends up as carbonated rock.

There is increasing understanding that the microbes which inhabit subsurface rocks affect the rate of mineralization. A study was recently conducted to study such microbial impacts at the CO_2 injection site associated with the Hellisheidi geothermal power plant and the Carbfix CO_2 storage project in Iceland (Trias et al., 2017). Subsurface microbes can have both positive and negative effects on carbonation rates, which overall have not been thoroughly investigated and so are not well understood.

A potential benefit is that some organisms found at injection sites, such as the sulfate-reducing species of bacteria *Desulfotomaculum*, act as alkalinizing agents that could enhance carbonation rates (Trias et al., 2017). In other cases, microbes can take advantage of the minerals newly released from rock dissolved by the incoming acidic stream of CO_2 enriched water. These released minerals, such as Mg, Fe, and Ca cations, are critical in microbe metabolism and may be taken up by cells. However, this reduces their availability to serve as binders for CO_2, the other participant in the reaction that makes stable carbonates (Trias et al., 2017). Also, the iron redox state has an effect on the porosity of the dissolved silica layer at the rock-fluid interface, which can affect carbonation rates (Sissmann et al., 2013). The overall effects of microbes on mineralization needs further investigation. A better understanding could shed light on what balance of microbial activity will most enhance carbonation rates.

Some aspects of microbial metabolism could potentially be influenced in order to enhance carbonation rates. Possible targets could be increasing silicase activity, increasing alkalinization, and decreasing the production of iron redox states that interfere with carbonation. How exactly these microbial processes could be encouraged is an open question that has not been substantially explored.

One idea is to inject underground, along with CO_2-enriched water, packets of desired microbes (whether engineered for enhanced activity or not) that can speed mineralization. Whether these microbes would survive the journey, whether the amount needed is impractical, and other questions are significant and may pose insurmountable challenges. Potential other approaches are the direct injection of relevant enzymes (silicases, carbonic anhydrases) that facilitate mineralization. One could also consider injecting phages that would infect local microbes in the storage rock, causing them to express pro-mineralization traits. Injecting microbes or phages could carry significant ecological risk, which at present is poorly understood. Injection of enzymes, which do not replicate and are already widely found in nature, is unlikely to pose a risk. Much more detail of the theory, practice and risk of these strategies would need to be worked out before contemplating any deployment, and the benefits of enhanced mineralization weighed against the risks.

Some projects store CO_2 in underground saline aquifers, rather than rock formations. Here, the risk of CO_2 leakage can be a more significant concern. Microbes could potentially be used for monitoring of mineralization and injection sites, and enhancement of CO_2 mineralization at injection sites. It has been suggested that prokaryotes could be used as bioindicators, early warning signals for leakage from CO_2 that has been injected and stored in underground saline aquifers (Hicks et al., 2017). Samples taken from a site could be analyzed through high-throughput sequence to detect changes in the microbial community indicative of a CO_2 leak (Hicks et al., 2017). Microbes can also contribute to overall carbon storage. Ureolytic bacteria have been shown to increase $CaCO_3$ precipitation. This reaction doesn’t directly represent additional carbon uptake, since an equivalent amount of dissolved carbon is created by urea hydrolysis. However, the overall metabolic process increases the pH of the solution which drives a net influx of CO_2 into solution, thus storing more carbon (Mitchell et al., 2010).

Key research questions:

- What is the relationship of subsurface microbiota to mineralization rates? What are the key positive and negative drivers of mineralization?
- To what extent can microbial processes that drive mineralization be increased (chemically, biologically)?
- What are the ecological risks of modifying the underground microbiota at CO_2injection sites?

Key engineering targets:

- Create models of the key interactions between subsurface microbiota at injection sites that drive mineralization, taking into account the chemical changes that accompany CO_2 injection.
- Map out the genetic controls needed to upregulate microbial activity that can increase mineralization rates.
- Develop the theoretical understanding of whether natural or engineered versions of enzymes such as silicases and carbonic anhydrases could be effective enough to merit further investigation.
- Build and test these modified microbes and enzymes in a lab model of a subsurface injection site to see the effect they have on mineralization.

Bio-Enhanced Abiological Carbon Removal Systems (Hybrid approaches)

Industrial carbon capture systems generally take one of two approaches to carbon capture: the most commonly used method is to adsorb carbon dioxide (or one of its derivative forms). In this approach, a surface that is chemically highly likely to bind carbon dioxide is employed, and CO_2 in large quantities binds to this sorbent surface. The carbon must then be released and stored to regenerate the sorbent, and if not, vast amounts of new sorbent must be generated in direct proportion to the desired carbon to be captured. The other approach is to filter CO_2 from ambient air or liquid using a membrane that selects for CO_2. This method has not been industrially successful to date, as the challenge of filtering CO_2 from air is significant: CO_2 is a very small and inert molecule that is chemically similar to O_2, which overwhelms it by large quantities in ambient air (~21% O_2 to ~0.04% CO_2). Biotechnology has the potential to improve the efficiency and lower the costs of both categories of abiological carbon removal, membrane-based and sorbent-based.

Enzyme-enhanced membranes for carbon dioxide filtering:

Membranes for CO_2 capture face two key challenges: selectivity (the ability to distinguish and select for CO_2 over other gas molecules) and throughput (the rate at which air comes into contact with the membrane, and specifically the CO_2-selecting pores). There is growing interest in repurposing carbonic anhydrase for CO_2 capture. Carbonic anhydrase is an enzyme commonly found in nature across a wide spectrum of organisms. It interconverts CO_2 into bicarbonate in solution, thus making it more readily available for uptake and transport. In humans, carbonic anhydrase serves a critical function in red blood cells in CO_2 transport. The enzyme is extremely efficient and fast, able to carry out 1 million transformations per second without requiring any energy input (Fu et al., 2018).

Recent experiments have shown that membranes constructed with 8nm-diameter pores lined with carbonic anhydrase can effectively filter CO_2 in a way that can be competitive with amine-based solvent capture with regard to cost and energy consumed (Kentish, 2018). The carbonic anhydrase used in these experiments was taken from the extremophile bacteria *Desulfovibrio vulgaris*, which can remain stable at up to 90°C and at a pH range of 2 – 10 (Kentish, 2018). A key advantage of embedding carbonic anhydrase is a membrane is the ability to control its location around pores and its concentration: researchers were able to achieve ten times the concentration of carbonic anhydrase than that which would be possible in solution (Fu et al., 2018).

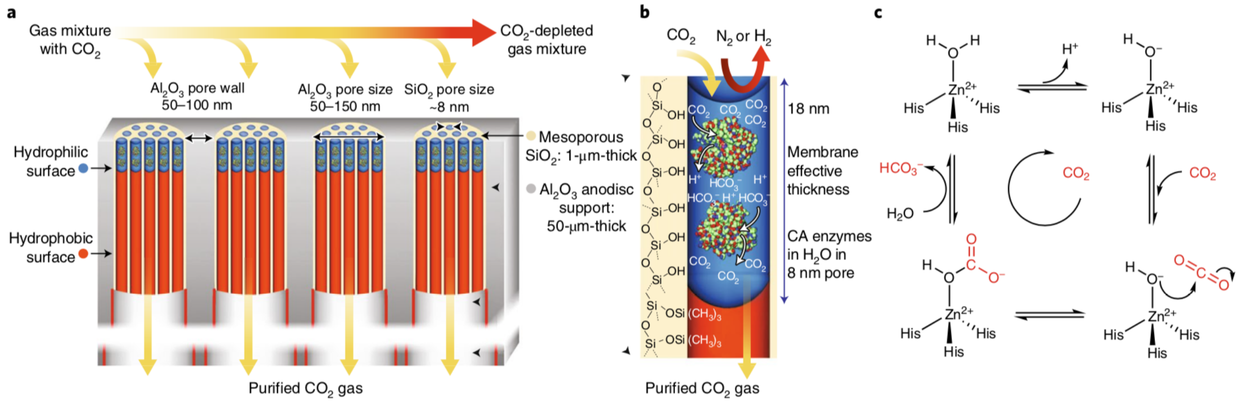

Image 8. *Carbon capture using embedded carbonic anhydrase. Source Kentish, 2018.*

This experiment was aimed at developing a solution for point-source capture from flue gas, which is highly concentrated in CO_2. It remains to be seen whether membrane-based capture could be efficient enough and low-cost enough to capture CO_2 from ambient air, which is necessary for CDR. Earlier work had demonstrated the ability to create recombinant versions of carbonic anhydrase that express elements of a cellulose-binding domain adapted from *Clostridium thermocellum*, which allows the carbonic anhydrase to be immobilized in a membrane (Z. Liu et al., 2009). The companies Carbozyme and CO_2 Solutions have developed and patented methods of immobilizing carbonic anhydrases on membranes for purposes of CO_2 capture (Z. Liu et al., 2009). Carbonic anhydrase has been immobilized onto various inorganic and polymer surfaces, in various shapes including membranes and nanoparticles, and produced from a range of sources including mammalian and extremophile bacterial versions (Boone & McKenna, 2015). Future engineering work could further optimize carbonic anhydrase by screens of naturally-occurring variants, rational design, and directed evolution for attachment to pores at high concentration, for stability in the temperature range, pH range, and other characteristics of ideal reaction conditions.

Enzymes for more efficient sorbent utilization

Enzymes could be used to facilitate sorbent-based carbon capture systems. The company CO_2 Solutions demonstrated the double-digit efficiency improvements in an amine-based carbon capture system through the use of a carbonic anhydrase engineered to be stable at extreme temperatures and pressures (Fradette et al., 2017). The enzyme, which was developed through a natural screen and followed by directed evolution, was dissolved in solvent and shown to be stable operating in industrial conditions in a demonstration plant capturing 10 t CO_2/day (Fradette et al., 2017). The enzyme improved the kinetics of CO_2 absorption, allowing inexpensive low-grade heat (<80°C) to be used to strip the CO_2 off and regenerate the solvent (Fradette et al., 2017). For ease of production and experimentation, carbonic anhydrases from extremophile bacteria, such as species living in deep-sea hydrothermal vents, have been expressed in *E. Coli*, a well-understood and easy to cultivate model organism (Jo et al., 2014).

Engineered carbonic anhydrase might also be used to increase CO_2 absorption and decrease energy requirements with other types of sorbents. Recombinant carbonic anhydrase expressed in *E.Coli* has been demonstrated to enhance $CaCO_3$ mineralization rates due to acceleration of CO_2 hydration and the transformation of initial vaterite phase crystals to calcite crystals (Kim et al., 2012). Recombinant carbonic anhydrase was also shown to be effective in speeding up the mineralization process of $CaCO_3$ from gaseous CO_2 at 30°C and 60°C, suggesting that it could be utilized in plans to sequester CO_2 as mineralized $CaCO_3$ (Jo et al., 2014). Another potential option is to utilize enzymes to accelerate mineralization of CO_2 in schemes that re-use sorbent materials, such as MgO or CaO looping, although the high heat currently used in these systems (~600°C) may present insurmountable barriers to using biologically active materials (MacDowell et al., 2010) (McQueen et al., 2020).

Method	Likely Impact	Technical Risk	Ecological Risk
Biomass Increase			
Photosynthetic improvements	High	Medium	Low to Medium
Soil Organic Carbon			
Larger and deeper roots	High	Low	Low
Overexpressed suberin	High	Low	Low
Overexpressed lignin	High	Low to Medium	Low
Annual to perennial conversion	High	Medium	Low
Glomalin increase	High	Medium to High	Low to Medium
Sporopollenin "super tubers"	High	High	Low to Medium
Engineering for Marginal and Degraded Lands			
Engineering plants to grow on melting permafrost	Medium to High	Low to Medium	Medium
Engineered grasses on salty soils	Medium to High	Medium to High	Medium
Marine engineered macroalgae farms	Medium	Low to Medium	Medium
Bio-enhanced ERW	High	Low to Medium	Low to Medium
Bio-enhanced Mineralization	High	Medium	Low to Medium
Biological/Abiological Hybrid technologies	Medium to High	Low to Medium	Low

Table 1: Bio-NETs Assessments. A qualitative assessment of the likely impact, technical risk, and ecological risk of each broad category of bio-enhanced NET.

Conclusion

There are many ways in which the tools and techniques of bioengineering can be applied to develop new and improved technologies for carbon removal. Research at the lab scale provides amble evidence that carbon removal rates could be enhanced by significant enough amounts, perhaps 1 to 3 orders of magnitude depending on the specific technology and CDR pathway being investigated, to justify substantial investment in R&D. Particular attention should be paid to developing systems for biologically enhanced rock weathering, due to its permanence, low energy requirements, and ability to scale. Substantial research investment should also go towards developing plants with increased ability to store recalcitrant carbon underground. Each of these concepts, as well as others explored in this paper, brings with it many technical challenges and sub-challenges. Solving these challenges could dramatically improve CDR technologies, and would have the added benefit of increasing knowledge and capabilities in the areas of plant genetics, photosynthesis modification, materials processing by microbes, and other important areas with wide applicability. Many fundamental questions still need to be answered on the way to develop new bio-based NETs. Answering these critical questions should be a significant focus for the field of bioengineering, and related fields, in the next 10 – 20 years in order to develop bio-based NETs that can be deployed by mid-century.

GLOSSARY

ARF: Arbuscular mycorrhizal fungi
BECCS: Bioenergy carbon capture and storage
CDR: Carbon dioxide removal
CW: CO_2 saturated water (usually at under 1% CO_2)
DAC: Direct air capture
ERW: Enhanced rock weathering
GRSP: Glomalin-related soil protein
HRP: High-rate pond (system for cultivating microalgae)
IPCC: Intergovernmental Panel on Climate Change
NET: Negative emissions technology
PPM: Parts per million
SAL: Silica amorphous layer
sCO2: Supercritical CO_2 (CO_2 under high pressure)
SOC: Soil organic carbon

CONVERSIONS AND UNITS

Carbon:
1 ton = 1000 kg
1 ton C = 3.6 ton CO_2
1 GtC = 1 billion tons carbon = 3.6 Gt CO_2

Area:
100 ha = 1 km^2

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