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Phytosequestration: Carbon Biosequestration by Plants and the Prospects of Genetic Engineering

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Photosynthetic assimilation of atmospheric carbon dioxide by land plants offers the underpinnings for terrestrial carbon (C) sequestration. A proportion of the C captured in plant biomass is partitioned to roots, where it enters the pools of soil organic C and soil inorganic C and can be sequestered for millennia. Bioenergy crops serve the dual role of providing biofuel that offsets fossil-fuel greenhouse gas (GHG) emissions and sequestering C in the soil through extensive root systems. Carbon captured in plant biomass can also contribute to C sequestration through the deliberate addition of biochar to soil, wood burial, or the use of durable plant products. Increasing our understanding of plant, microbial, and soil biology, and harnessing the benefits of traditional genetics and genetic engineering, will help us fully realize the GHG mitigation potential of phytosequestration.

Keywords: bioenergy crops, carbon sequestration, genetic engineering, phytosequestration

Global carbon (C) cycling depends largely on the photosynthetic uptake of atmospheric carbon dioxide (CO₂). The total C stock (i.e., organic and inorganic C) in terrestrial systems is estimated to be around 3170 gigatons (GT; 1 GT = 1 petagram = 1 billion metric tons)—2500 GT in the soil and 560 GT and 110 GT in plant and microbial biomass, respectively (figure 1). Total C in the oceans is 38,000 GT (Tuskan and Walsh 2001, Lal 2004, 2008a, Houghton 2007, Graber et al. 2008). The soil C pool, which is 3.3 times the size of the atmospheric C pool of 760 GT, includes about 1550 GT of soil organic carbon (SOC) and 950 GT of soil inorganic carbon (SIC) (Lal 2004, 2008a). Of the C present in the world's biota, 99.9% is contributed by vegetation and microbial biomass; animals constitute a negligible C reservoir. The annual fluxes of C between the atmosphere and land, and atmosphere and oceans, are 123 and 92 GT, respectively. Therefore, 123 GT represents the photosynthetic C uptake, or the gross primary productivity (GPP), of the global terrestrial system (see box 1 for definitions and symbols used throughout this article). Approximately 60 GT of the GPP captured by plants through photosynthesis is returned to the atmosphere almost immediately through plant respiration. The remaining amount is the net primary productivity (NPP). Following subsequent allocation and processing, such as allocation of C to roots and plant metabolism of root C, most of this C is subject to heterotrophic metabolism and is lost to the atmosphere through microbial respiration. The rest, around 10 GT per year, is

defined as the net ecosystem productivity (NEP). Depending on the nature of preservation, this C has the potential to persist in the ecosystem for decades to centuries to millennia. In reality, however, most of it is lost because of land use, biotic stresses, fires, and other disturbances. Accounting for these factors, long-term C (bio)sequestration in a terrestrial system is calculated to be a fraction of NEP and is referred to as the net biome productivity (NBP). Global annual values for NBP have varied considerably during the last decades, between 0.3 and 5.0 GT. The current global NBP is around 3 GT per year. The majority of this is believed to be contained in forests in the Northern Hemisphere, but plants in all biomes capture and sequester measurable amounts of CO₂ each year.

Human activities (mainly fossil-fuel consumption and cement production) are currently responsible for an annual emission of 9 GT C (33 GT CO₂). Terrestrial and oceanic systems manage to absorb 3 and 2 GT of this anthropogenic C release, respectively, but the rest, 4 GT, remains in the atmosphere. To remove excess CO₂ from the atmosphere, we will have to enhance the NBP by increasing and applying our understanding of plant and rhizosphere biology and exploring how advanced genetic engineering approaches (see box 1) can help us achieve significant growth in NBP rates in different terrestrial biomes, such as forests, grasslands, bioenergy plantations, and agriculture. To quote the physicist and futurist Freeman Dyson (2008): “If we can control what the plants do with carbon, the fate of the carbon in the atmosphere is in our hands.”

Box 1: Definitions and explanations.

The terrestrial carbon cycle

GHG, greenhouse gases: Gases that absorb infrared radiation and trap the heat in the atmosphere. The most important GHG are water vapor, carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), and ozone. A major contributor to anthropogenic CO₂ emission is burning of fossil fuels, but CO₂ is also released to the atmosphere through processes such as deforestation.

GPP, gross primary productivity: The total amount of carbon (C) per year that enters an ecosystem through photosynthesis.

NPP, net primary productivity: The amount of C left after plant respiration; that is, $NPP = GPP - R_a$, where R_a is autotrophic (plant) respiration. NPP is a measure of the total annual production of organic matter in the system.

NEP, net ecosystem productivity: What remains of NPP after C is lost to the atmosphere through respiration by soil microorganisms; $NEP = GPP - [R_a + R_h]$, where R_h is heterotrophic (microbial) respiration. NEP consists of aboveground and belowground biomass, detritus, and soil organic carbon and soil inorganic carbon.

NBP, net biome productivity: What remains of NEP after C losses due to harvesting and disturbances such as fires, erosion, and so on.

SIC, soil inorganic C: Elemental C; carbonate minerals such as calcite, aragonite, and gypsum; gaseous CO₂; and an equilibrium of H₂CO₃, HCO₃⁻, and CO₃²⁻ in solution. The carbonates are formed either from weathering of limestone and other calcareous material or through reaction of CO₂ with Mg²⁺ or Ca²⁺.

SOC, soil organic C: The total inventory of organic C in the soil. SOC is a component of the soil organic matter. SOC represents a heterogeneous pool of C. Some materials such as fresh litter or released sugars represent a biologically highly active fraction of SOC with a residence time in the soil of a few years to decades. Other fractions contain humic substances or mechanically protected clay aggregates that are more or less inert and can reside in soils for up to millennia.

Plant biology

Calvin cycle: A sequence of biochemical reactions by which photosynthetic plants, algae, and cyanobacteria capture atmospheric CO₂ and reduce it to organic compounds. The energy and reducing power for the Calvin cycle comes from photophosphorylation, a process where solar energy is converted to cellular energy in the form of ATP and NADPH. The Calvin cycle is named after Melvin Calvin, a professor at the University of California, Berkeley. Calvin was awarded the 1961 Nobel Prize in Chemistry for his discoveries. In plants, rubisco and the other enzymes of the Calvin cycle are located in the chloroplast stroma of mesophyll cells.

C₃ photosynthesis: The type of photosynthesis in most plants. In these plants (C₃ plants) the first organic compound formed from the captured CO₂ is the 3-C molecule 3-phosphoglycerate (3-PGA) in the Calvin cycle. C₃ plants mostly occupy areas with moderate light intensity and temperatures. Examples are crops such as wheat, rice, and soybean.

C₄ photosynthesis: In C₄ plants, photosynthesis involves not only the mesophyll cells but also the bundle sheath (BS) cells. These two cell types occur as concentric rings around the vascular bundles, with the BS cells forming an inner ring and the mesophyll cells in the outer ring (a characteristic referred to as Kranz anatomy, after the German word Kranz for wreath). In C₄ plants, the first organic compound formed from the captured CO₂ is a 4-C acid, for example, malate. This CO₂ assimilation does not involve rubisco but is catalyzed by the enzyme phosphoenolpyruvate carboxylase and occurs in the mesophyll cells. The 4-C acid is transported to the BS cells where it is converted to pyruvate by splitting of CO₂, which is delivered to the Calvin cycle and rubisco in the chloroplasts. The effect is a “pumping” of CO₂ to the site of rubisco. This and other features of C₄ plants, for example, corn, sorghum, sugarcane, Miscanthus, and switchgrass, allow them to avoid or minimize photorespiration at high temperatures and thrive in tropical or subtropical climates. As a result of the high energy requirement for C₄ photosynthesis, C₄ plants are often less competitive than C₃ plants in temperate climates.

GE, genetic engineering: Here, we define GE as any modern strategy to modify the genetic composition of the targeted genotype or individual, including marker-assisted selection, transgenics, and induced mutagenesis. Plant GE is not a stand-alone application but works in concert with other aspects of breeding such as crossing and selection.

Light saturation point: The photosynthetic activity of a phototroph such as a plant increases with light intensity. However, eventually an intensity is reached above which light is no longer the factor limiting the overall rate of photosynthesis. This light intensity is called the light saturation point. Above the light saturation point, the factor that normally limits photosynthesis is the CO₂ concentration at the site of rubisco.

Photorespiration: In the condensation of CO₂ and the sugar ribulose 1,5-bisphosphate (RuBP), rubisco catalyzes the formation of two 3-PGA molecules. This is referred to as rubisco’s carboxylation reaction. In the oxygenation reaction, when rubisco instead of CO₂ binds oxygen (O₂), only one molecule of 3-PGA is formed from RuBP, together with one molecule of the 2-C compound phosphoglycolate (PG). PG is a dead end, and to reclaim the C in PG, and possibly to avoid toxic effects of PG accumulation, plants engage in a series of reactions that convert PG to 3-PGA for the Calvin cycle. This process is alternatively called the C2 cycle (for the 2-C PG molecule), or photorespiration, since just as in respiration, CO₂ is released and O₂ is taken up. Photorespiration is energetically costly, and at high temperatures, when rubisco’s oxygenation reaction is significant, plants would not survive without mechanisms such as C₄ photosynthesis by which the necessity for photorespiration is minimized.

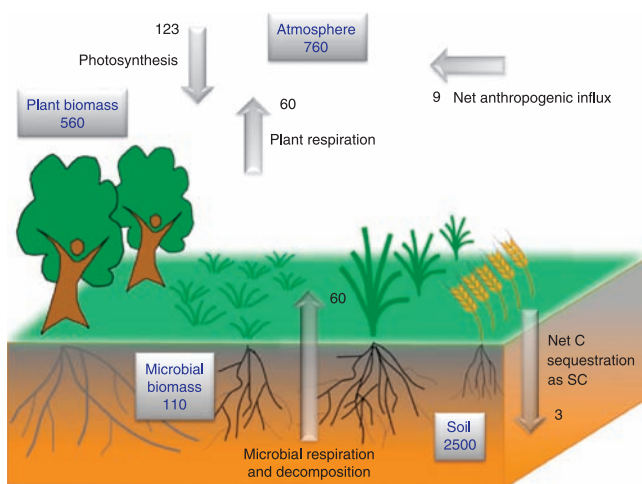


Figure 1. The terrestrial carbon (C) cycle. Carbon stocks (boxes) are shown as gigatons (GT), and fluxes (arrows) are shown in GT per year. Current net removal of atmospheric C by terrestrial systems amounts to around 3 GT per year. Values are from Lal (2004, 2008a), Houghton (2007), and Graber and colleagues (2008). Soil microbial biomass was calculated from data in Whitman and colleagues (1998).

With this encouraging prophecy in mind, we start our review of phytosequestration by describing how plants contribute to the mitigation of greenhouse gases (GHG). We follow up with a discussion on how plants can be further optimized for this task, and the role of genetic engineering in this process.

Plants as carbon sinks

Plants can play two fundamentally different roles as C sinks. By capturing atmospheric CO_2 through photosynthesis (figure 2) plants store large amounts of organic C in above- and belowground biomass. This is particularly relevant for perennial trees and herbaceous plants with extensive root systems. Storing C in living biomass represents a rather short-term (decades to centuries) sequestration; when the plants decay, C is returned to the atmosphere. However, if they are well maintained or undisturbed, plants in an ecosystem can continue to act as a C sink for several centuries. Plant biomass can also be harvested and converted to durable plant products, such as composites and fiber-cement materials, but again, the C storage capacity is relatively short lived. Long-term (millennia) C sequestration can be achieved when C from aboveground biomass transfers to the roots and enters the pool of SOC or SIC (hereafter SC, for soil C). Carbon can be incorporated in the soil by other means as well; for example, as biochar or phytoliths. A second way by which plants can act as C sinks, in addition to photoassimilation of CO_2 , is by use as bioenergy crops, thereby displacing GHG emissions from fossil fuels.

Below, we consider the different cases for terrestrial biosequestration of C in some detail, followed by a presentation of how genetic engineering approaches in plant breeding

can enhance phytosequestration; that is, the capacity for plants to serve as C sinks. This discussion is summarized in figure 3.

Biomass. A substantial amount of C can be sequestered in plant biomass. As about 90% of the world's terrestrial C is stored in forests, forest plantations and the preservation of old forests are of chief importance in controlling the size of the overall terrestrial C sink. For example, forests in the Northern Hemisphere have been estimated to sequester up to 0.7 GT of C annually, which accounts for almost 10% of current global fossil-fuel C emissions (Goodale et al. 2002). Of this, 0.2 GT per year was in living biomass, 0.15 GT per year in dead wood, and 0.13 GT per year in the forest floor and SC. The remainder occurred as forest products.

Root-derived soil carbon. Roots are the primary vector for most C entering the SC pool. In temperate and boreal forests, the amount of C stored in the soil is about four times as high as that stored in the vegetation, and 33% higher than the total C storage in tropical forests (IPCC 2000). Grasslands (broadly defined here as ecosystems with a dominant vegetation of herbaceous species), which cover 50% of Earth's surface, or roughly 1.2 billion hectares (ha), are another important ecosystem for SC sequestration. In grasslands, 98% of the total C store is sequestered belowground in roots and in soil. Globally, grassland soils store an estimated 194 GT of C, which accounts for around 8% of the world's SC.

The potential sequestration capacity in the total SC pool is at least as large as what has been lost by soil degradation and erosion during the preindustrial and industrial eras. The size of this loss is uncertain; for the SOC fraction, the estimates vary from 44 to 537 GT (Lal 2004). It has been suggested that between 80 and 130 GT could be sequestered as SOC over a 50- to 100-year span by implementing land-management changes such as reforestation, afforestation, and improved agricultural practices (Thomson et al. 2008).

Biochar. Charcoal is made by heating wood or other organic material with a limited supply of oxygen (pyrolysis). Depending on the nature of the raw material used and the process of pyrolysis, the end products vary; volatile hydrocarbons and most of the oxygen and hydrogen in the biomass are generally burned or driven off, leaving C-enriched black solids, called charcoal. Charcoal can be used as fuel for transportation, industry, or cooking, and has various other applications, such as water purification and filtration. Charcoal, which holds twice as much C than ordinary biomass, can also be applied to soil for long-term C sequestration—such charcoal is referred to as biochar. Partly because of its low hydrogen-to-carbon ratio and its aromatic nature, biochar is a poor microbial substrate, and the half-life of C in soil biochar is in the range of several hundred to several thousand years. Furthermore, biochar has several important impacts on soils: It (a) can increase the soil's capacity to

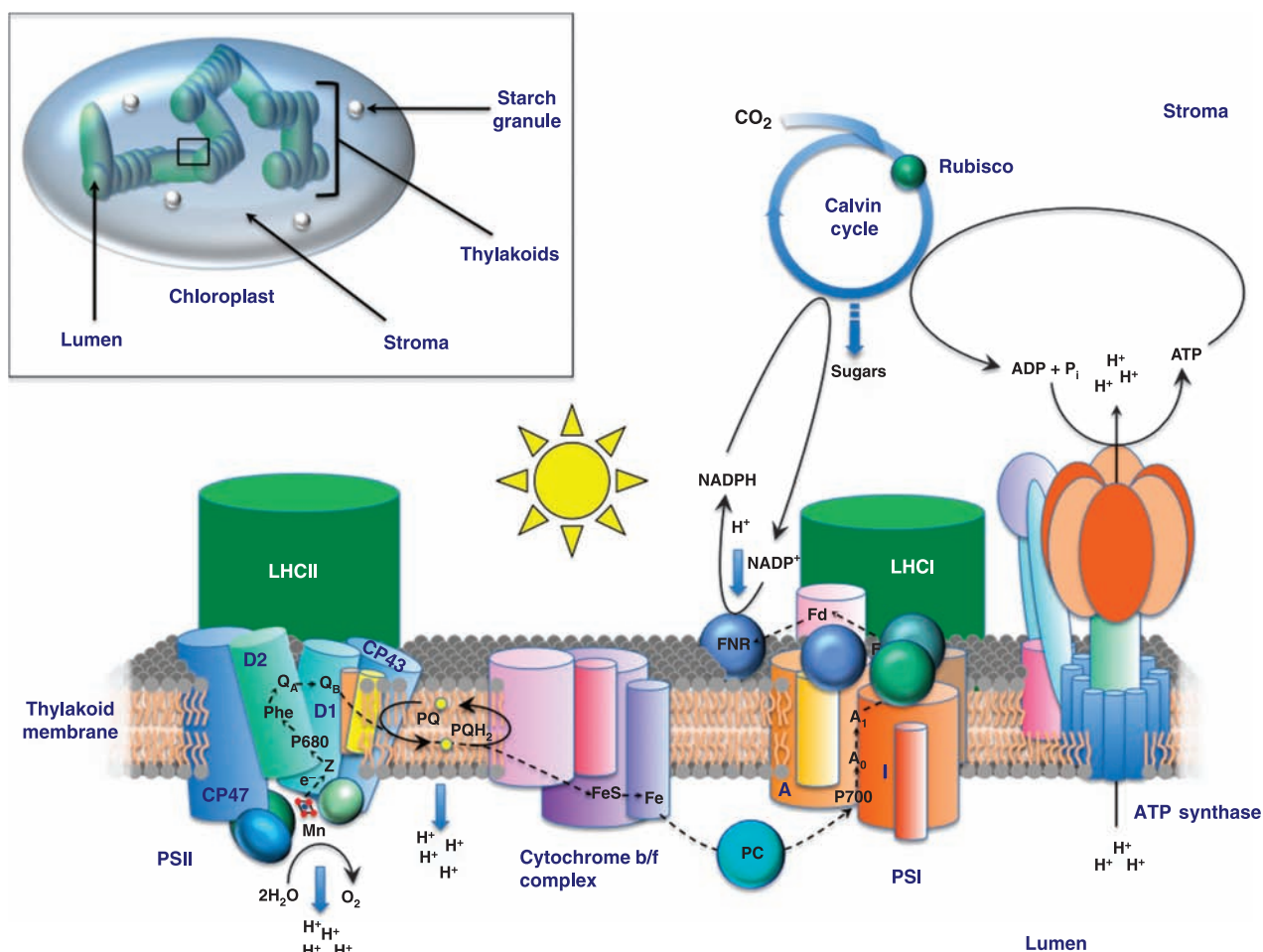


Figure 2. Photophosphorylation. Photosynthetic electron transport and adenosine triphosphate (ATP) synthesis in thylakoid membranes of plant chloroplasts. Light energy is harvested by the two photosystems, photosystem II (PSII) and photosystem I (PSI), associated with the light-harvesting complex II (LHCII) and I (LHCI), respectively. Light energy in PSII and PSI excites electrons, supporting an electron transport from water to NADP^+ through an electron transport chain involving a large number of redox components, including the two photosystems, plastoquinones (PQ), the cytochrome *b/f* complex, plastocyanin (PC), and ferredoxin-NADPH oxido-reductase (FNR). Some of the polypeptide subunits and electron carriers in the PSII, PSI and cytochrome *b/f* complexes are indicated, including the reaction center proteins D1 and D2 of PSII. The electron transport in the thylakoid membrane generates a proton gradient, which is the driving force for ATP synthesis by the ATP synthase. ATP and NADPH produced by photophosphorylation are used to fuel the Calvin cycle in the stroma, whereby atmospheric carbon dioxide is reduced to organic compounds by ribulose-1,5 bisphosphate carboxylase/oxygenase (rubisco) and other enzymes. Inset: chloroplast showing the thylakoid membranes, the stroma, and the intrathylakoid lumen. Starch granules in the stroma are indicated.

adsorb plant nutrients and agrochemicals; (b) contains most of the plant nutrients from the harvested biomass, and can slowly release those nutrients to the rhizosphere; and (c) has a low-density structure, and helps increase drainage, aeration, and root penetration in soils.

According to the “Charcoal Vision” (Laird 2008), a national system of distributed pyrolyzers for processing biomass into biofuel and biochar could reduce US demand for fossil fuel by 25%, reduce US GHG emissions by 10%, increase agricultural productivity, and enhance soil and water quality (Laird 2008). If renewable fuel needs in the future were met through pyrolysis, the global potential for C sequestration

as biochar would be close to 10 GT per year (Lehmann et al. 2006). Roberts and colleagues (2010) recently presented life-cycle assessments of several biochar systems.

Phytoliths. Phytoliths (plantstones, plant opals) are microscopic silica bodies that precipitate in or between plant cells. Silica in the soil is taken up by plant roots, and phytoliths are formed as a result of biomineralization within plants. Phytoliths are found in all parts of the plants that produce them and are released to the soil when plants are burned, digested, or decay. Many plants, and in particular, grasses, are prolific producers of phytoliths. In general, phytoliths constitute up

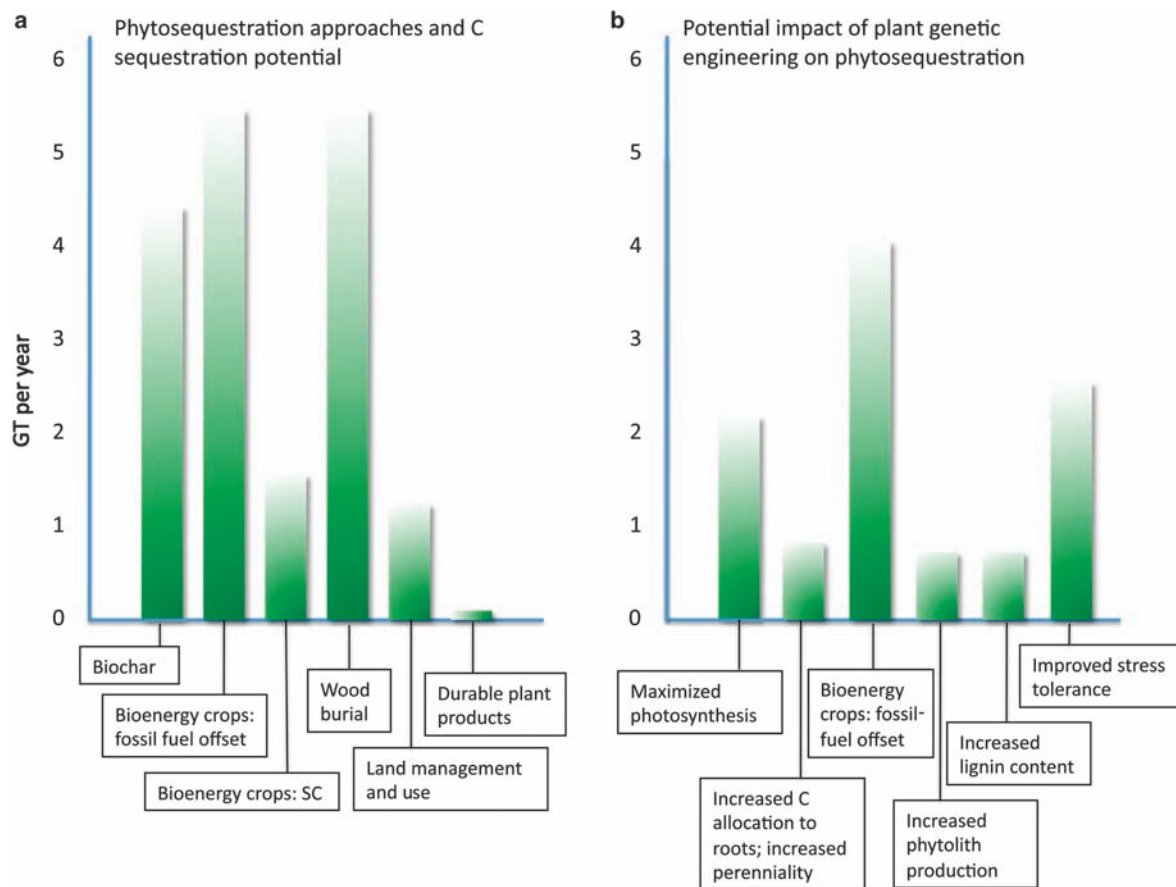


Figure 3. Phytosequestration, including fossil-fuel offset by bioenergy crops. (a) Potential strategies for phytosequestration and estimated carbon (C) sequestration rates by 2050. (b) Potential plant genetic engineering approaches in phytosequestration and estimated C sequestration rates by 2050. GT, gigatons; SC, soil carbon.

to 3% of total soil mass (Drees et al. 1989). Phytoliths in soil are very stable and insensitive to land-use changes such as deforestation, so organic C encapsulated in soil phytoliths can be a substantial component of the SC pool and is sequestered for centuries or millennia (Parr and Sullivan 2005). It has been estimated that the global potential for C sequestration as siliceous phytoliths is around 1.5 GT per year (Parr and Sullivan 2005, Parr et al. 2010). Another kind of phytolith is calcareous deposits such as calcium oxalates and calcium phosphates (Franceschi and Horner 1980). They occur mainly in succulents but also appear in some other plants. After plants have been degraded or burnt, calcareous phytoliths usually go through a series of chemical reactions and end up as calcium carbonates.

Durable plant products. Wood, including bamboo, can be incorporated into construction material for buildings, houses, furniture, and for other durable products, resulting in sequestration of forest C over years or even centuries. According to the US Forest Service, 90 megatons of sequestered C was estimated to be locked up in wood products worldwide in 2008 (Sedjo 2001).

In addition to sequestering C, durable plant products offer a potential advantage over other materials for two

reasons. First, they require less energy to produce; for example, the estimated embodied energy in a simple sawed wood product (14 gigajoules [GJ] per megagram [Mg]) is considerably less than in steel (10 to 25 GJ per Mg), aluminum (190 GJ per Mg), or plastic (60 to 80 GJ per Mg). Second, they are C-neutral feedstock replacements for petrochemical products—for example, the CO₂ released when starch-based bioplastics degrade was previously incorporated in the starch through photosynthesis.

Wood burial. A thought-provoking contribution to long-term C sequestration through tree burial was recently proposed by Scholz and Hasse (2008) and Zeng (2008). They suggested that dead or live trees be harvested and buried under anaerobic conditions in trenches, brown coal open pits, surface mining sites, the bottoms of selected lakes, or in aboveground shelters. It is estimated that the C sequestration potential for this wood burial would amount to around 10 GT C per year, with the largest share for tropical forests (Zeng 2008). However, these calculations do not account for the amount of CO₂ emitted during the harvest, transport, and burial of the timber. Scholz and Hasse (2008) concluded that to sequester the entire current annual CO₂ emission by tree planting and burial would require 1 billion ha. They also

made the interesting observation that this acreage roughly equals the area of primeval forests lost in the last century.

Bioenergy crops. Bioenergy crops can be defined as any plant used to produce bioenergy (i.e., renewable energy from biological sources). Today, sugarcane, oil crops, and cereals, particularly maize and wheat, make the largest contribution to bioenergy. However, it is widely believed that lignocellulosic biomass from perennial grasses such as *Miscanthus* and switchgrass, and from short-rotation woody crops (SRWC) such as poplar, represent a more sustainable bioenergy feedstock than grain. Compared with annual food and feed crops, the perennial biomass crops require fewer inputs, produce more energy, and contribute more toward reduction of GHG emissions.

Bioenergy crops provide a C-neutral energy source; the net CO₂ emitted from the use of biofuels comes from the fossil fuel spent in the production and processing of plant biomass and in the transportation of the refined products. The reductions in the emission of CO₂ equivalents that result from replacing fossil fuel with bioenergy crops vary from a low 8.1 grams (g) per megajoule (MJ), calculated as ethanol, for conventional tillage corn-soybean, to around 24 g per MJ for switchgrass and hybrid poplar (Adler et al. 2007). Assuming that in the near future, perennial grasses and SRWC will dominate the plant-based bioenergy crops (microalgae and cyanobacteria are likely to constitute another important group of bioenergy producers), it is likely that the decrease in net GHG emissions associated with bioenergy crops will be between 25 and 30 g CO₂ equivalents per MJ ethanol in the next 50 years. Further assuming that the projection by Berndes and colleagues (2003) of a renewable biomass energy supply of 180 to 310 exajoules per year is correct, we estimate that bioenergy cropping systems will have the potential to offset fossil GHG emissions by 5 to 8 GT per year by 2050. These calculations do not account for plant improvements through genetic engineering technologies.

Just like other plants, bioenergy crops can sequester C in roots and soil; this constitutes the second-largest C sink for bioenergy crops (after fossil-fuel displacement; Tuskan and Walsh 2001, Adler et al. 2007). Considering that 750 million ha of land are available worldwide for the growth of bioenergy crops, with a total biomass sequestration of 1.6 GT C per year (Lemus and Lal 2005), there is vast potential for C sequestration as SC from bioenergy crop cultivation, especially if economically marginal land is used for diversified agroecosystems. Such systems could provide a net ecosystem C sequestration of 4.4 million grams (Mg; 4.4×10^{-9} GT) per ha per year (Tilman et al. 2006).

Land management and use. Adoption of appropriate crop management practices can yield considerable enhancements of the SC pool. A model based on more than 50% of the US cropland predicted a 15% increase in SOC with reduced tillage practices, and 50% with no-till farming (Lemus and Lal 2005). Conversion from annual crops to perennials can result in enhanced SOC by increasing root biomass and reducing soil erosion. In

a three-year conversion study, Tolbert and colleagues (2002) reported a SOC increase of 0.4% in the upper soil layer after the replacement of annual agricultural crops with switchgrass.

A young, rapidly growing forest can sequester large volumes of C, whereas an old-growth forest acts more as a reservoir for C, not experiencing much net growth. Proper forest management can maintain a forest with an optimal balance of net C uptake and storage. In a pantropical study it was suggested that reforestation practices in 52 tropical countries could result in additional C sequestration of 56 GT by 2050 (Butcher et al. 1998). Globally, appropriate forest policies could increase the amount of C sequestered in terrestrial biomass by up to 100 GT, or up to 2 GT per year (Dahlman et al. 2001).

As demand for renewable energy increases, land in undisturbed rainforests and grasslands and agricultural ecosystems may be converted to biofuel production. The diversion of conventional agricultural land to bioenergy plantations leads to further occupation of native habitats as more land is cleared for production of food and feed crops. These conversions of native lands release C to the atmosphere through burning and plant biomass decomposition, the latter of which goes on for a prolonged period of time. Fargione and colleagues (2008) coined the term “carbon debts” to assess the amount of C being released as a result of a land conversion process for biofuel production. They calculated C debts for different cases and estimated how many years it would take the biofuel operation to repay the C debt through fossil-fuel displacement. As an example, conversion of tropical rainforest land for palm biodiesel production could incur a C debt of as much as 6000 Mg C per ha, with a payback time exceeding 840 years (Fargione et al. 2008). Therefore, C balance models should serve as an important decisionmaking tool for the adoption of land-management and land-use practices.

Genetic engineering approaches to enhance phytosequestration

Major objectives for enhancing terrestrial C biosequestration include improving photosynthetic incorporation of atmospheric CO₂ into plant biomass; increasing C shunting into cellular C pools with low turnover, such as cell walls; and enhancing the allocation of C as recalcitrant organic matter to deep roots for transfer to the SOC pool. Bioenergy crops occupy a distinctive position in future terrestrial C sequestration. The vast areas of bioenergy cultivation envisioned for sustainable biofuel production, especially from perennial grasses and woody species, offer the potential for substantial mitigation of GHG emissions both by displacing fossil fuels and through phytosequestration through extensive root systems.

We start this discussion by looking into ways that plant genetic engineering can be employed to enhance photosynthetic yield. In subsequent sections we briefly cover C allocation to roots, stress tolerance, biomass quality, perenniality, and bioenergy crops. We finish with a synthesis section in which we try to estimate the benefits of genetic engineering for phytosequestration (figure 3b).

Maximizing photosynthesis. Through photosynthesis (figure 2), plants convert atmospheric CO₂ to sugars, which are transported as sucrose from net sugar-exporting (source) sites—that is, mature leaves—to net sugar-importing (sink) sites (i.e., branches, stems, seeds, and roots for storage, meristematic growth, or cell-wall synthesis; note the use of “sink” here as a physiological term). Of the several factors that affect biomass productivity, the efficiency with which solar radiation is intercepted by the plant and the efficiency by which solar energy is converted into biomass are two of the most important.

1. Increasing light interception efficiency. For C₃ plants, the light saturation point is approximately 25% of maximum full sunlight, and the rate-limiting step in photosynthesis during moderate to high light intensities is the carboxylation reaction, catalyzed by the enzyme ribulose 1,5-bisphosphate (RuBP) carboxylase/oxygenase (rubisco; figure 2). C₄ plants, including the bioenergy crops switchgrass and *Miscanthus*, have considerably higher light saturation points and are more efficient than C₃ plants in converting light energy to biomass. However, all plants experience extended periods of non-light-saturated conditions; for example, in the morning and late afternoon, and in the subsurface levels of canopies. Mathematical models and transgenic studies suggest that significant improvement in light reception can be accomplished through genetic engineering aimed at modifying canopy structure (Reynolds et al. 2000, Richards 2000, Yamamuro et al. 2000, Tuskan et al. 2004, Sakamoto et al. 2006, Wang et al. 2006, Adler et al. 2007, Sakamoto and Matsuoka 2008).

When photosystem II (PSII) in the photosynthetic apparatus experiences more light energy than can be drained in useful photochemical reactions (photochemical quenching), the excess excitation is dissipated as harmless heat in various nonphotochemical quenching processes, protecting the reaction center from overexcitation and ensuing photoinhibition by reactive oxygen species (ROS). Nonphotochemical quenching covers a wide range of responses. One example is carotenoid quenching of excitation energy through the xanthophyll cycle (Niyogi 1999, Holt et al. 2005). This quenching controls the emission of light from the PSII light-harvesting antenna complex (LHCII) to the PSII reaction center. Another example is state transition quenching, which involves swapping part of the LHCII between PSII and photosystem I to balance the energy between the two photosystems. This is achieved by uncoupling LHCII from PSII through the activation of redox-regulated reversible phosphorylation of the outer, mobile LHCII (Allen 1992).

Photoinhibition of PSII generally describes the light-induced loss of photosynthetic efficiency resulting from photodamage to PSII, particularly to the reaction center protein D1, or photoprotective dissipation of excitation energy. Photoinhibition can be induced even at low or moderate light intensities, especially at chilling temperatures. Photoinhibited PSII reaction centers are continuously repaired by de novo D1 protein synthesis, and net

photoinhibition occurs if the processes of repair cannot keep pace with those of photoinhibition. The primary cause and sequence of events of photoinhibition in the PSII reaction center are still controversial, and many hypotheses have been presented about the mechanisms involved (Takahashi and Murata 2008). These modes of action are not mutually exclusive, and it is possible that different types of photoinhibition operate depending on environmental conditions.

Genetic engineering to render the D1 protein less sensitive to photooxidative damage is challenging, because the protein serves as a fuse in PSII, and D1 turnover prevents degradation of the entire PSII complex. Instead, efforts to enhance and speed up the photoprotection mechanisms may be a tractable strategy for improving biomass yield. For example, transgenic cotton with increased levels of ROS scavengers (ascorbate peroxidase and glutathione reductase) exhibited significantly greater PSII activity than wild-type plants (Korniyev et al. 2001). Work done in Krishna Niyogi’s lab (Li et al. 2002) showed that transgenic *Arabidopsis* with overproduction of the PsbS protein involved in nonphotochemical quenching had greater tolerance to high-light stress. Analysis of the super-rice hybrids and elite wheat cultivars revealed that, in addition to their optimized light reception as a result of altered canopy design, they are more resistant to photooxidative damage. For rice (Jiao and Ji 2001, Wang et al. 2002), this was traced to a higher rate of D1 synthesis, a larger pool of the ROS scavenger superoxide dismutase, and higher xanthophyll-cycle capacity, which also may explain the higher tolerance to photoinhibition for japonica rice as compared with indica rice. In wheat (Yang et al. 2006), and possibly to some extent in rice (Wang et al. 2006), high tolerance to photooxidative damage was correlated with greater CO₂ capture in the flag leaves, as a result of high activity of rubisco and other Calvin cycle enzymes (see further below).

2. Increasing solar energy conversion to biomass. A key factor in the greater conversion of solar energy to biomass is the activity of the Calvin cycle; in particular, the carboxylation step catalyzed by rubisco (figure 2). Because of its slow turnover rate, rubisco catalyzes the rate-limiting step in C₃ photosynthesis under optimal light conditions. To compensate for this inefficiency, rubisco makes up 40% to 80% of the leaves’ protein content, making it one of the most abundant proteins on Earth. Furthermore, rubisco is able to use not only CO₂ but also oxygen (O₂) as substrate. The latter would result in a metabolic terminus were it not for the energetically costly photorespiration process that returns C to the Calvin cycle (Foyer et al. 2009). Because solubility in the aqueous stroma decreases much more rapidly with rising temperatures for CO₂ than for O₂, photorespiration is most prominent for C₃ plants at high temperatures. C₄ plants, which thrive in subtropical and tropical areas, have developed enzymatic and anatomical features that concentrate CO₂ at the site of rubisco, eliminating the requirement for photorespiration. C₄ plants can therefore use light more efficiently to assimilate and

reduce CO₂ than can C₃ plants. C₄ photosynthesis comes with an extra cost, however, and at lower temperatures the overall productivity can be higher for C₃ than for C₄ plants. Despite this, theoretical models show that even at temperatures as low as 5 degrees Celsius, an advantage can be gained from C₄ photosynthesis (Long et al. 2006).

Several attempts have been made to enhance photosynthesis in C₃ plants such as rice through the introduction of maize or sorghum genes encoding C₄ metabolic enzymes; however, these efforts (Capell and Christou 2004, Roitsch and Gonzalez 2004, Ithemere et al. 2006, Bieniawska et al. 2007, Coleman et al. 2007, Smidansky et al. 2007, Jansson et al. 2009) have so far met with little success (Taniguchi et al. 2008). An alternative CO₂-concentrating mechanism (CCM) is found in cyanobacteria and microalgae (Jansson and Northen 2010), and prospects for introducing cyanobacterial CCM components in plants have been discussed (Price et al. 2008). Given the discussion above, which suggests an advantage of C₄ photosynthesis at lower temperatures, another feasible approach may be to improve cold tolerance in C₄ plants. An understanding of the mechanisms underpinning the high productivity of certain *Miscanthus* varieties at low to moderate temperatures (Long et al. 2006) should prove valuable for engineering other C₄ grasses for increased cold tolerance.

Engineering the active site of rubisco to increase its specificity for CO₂ seems, a priori, an obvious target for diminishing the need for photorespiration. However, as elaborated by Long and colleagues (2006), this approach may also negatively affect rubisco carboxylation. Alternatively, a large number and diversity of rubisco enzymes among plants, algae, dinoflagellates, cyanobacteria, proteobacteria, and archaea show RuBP-dependent CO₂-fixing capacity (Badger and Bek 2008); this holds preliminary promise for improving C₃ photosynthesis by engineering plants with novel rubisco types. A specific example worth mentioning is the rubisco enzyme from certain red algae that has an apparent Michaelis-Menten constant (K_m) for CO₂ that is significantly smaller and CO₂ and O₂ relative specificity that is around 2.5 times higher than that of rubisco from plants (Uemura et al. 1997).

The activity of rubisco depends on rubisco activase, an enzyme that seems to constrain photosynthesis at high temperatures and high CO₂ levels (Crafts-Brandner and Salvucci 2000). Understanding the temperature sensitivity of rubisco activase and how the enzyme can be modified to maintain a high activation state for rubisco over a wider temperature range merits further investigations.

The Calvin cycle is the bottleneck in photosynthetic reaction flux at light saturation, mainly because of the regeneration of RuBP; therefore, other Calvin cycle enzymes in addition to rubisco, as well as proteins in the photophosphorylation process, should also be considered when trying to engineer plants for higher photosynthetic performance. For example, transgenic plants overexpressing genes for sedulose-1,7-bisphosphatase had enhanced photosynthetic capacity (Raines 2003, 2006). It is unclear whether compo-

nents in the photosynthetic electron transport chain or in chloroplastic ATP (adenosine triphosphate) synthesis may be rate limiting for the overall photosynthetic activity under natural conditions, although there are indications from transgenic plants with antisense suppression of the cytochrome b/f complex that this might be the case (Price et al. 1998). The activity of the Calvin cycle is important also in preventing photoinhibition, since the Calvin cycle reactions constitute an electron sink for photosynthetic charge separation and electron transport.

There is ample evidence to suggest that sink strength has a dominant influence on source photosynthesis and carbon partitioning (Paul et al. 2001, McCormick et al. 2006). Sink strength is governed by sucrose metabolism channeling C into storage or structural components. Metabolic engineering targeting the activity of selected isoforms of enzymes such as sucrose synthase, invertase, and ADP-glucose pyrophosphorylase should provide a feasible means to increase sink strength (Capell and Christou 2004, Roitsch and Gonzalez 2004, Ithemere et al. 2006, Bieniawska et al. 2007, Coleman et al. 2007, Smidansky et al. 2007, Jansson et al. 2009). Alterations to sucrose metabolism also alter the turgor pressure of cells and levels of hexose that serve as signaling molecules, thus affecting cell growth and division and hence sink strength (Koch 2004).

In addition to metabolic enzymes, transcription factors and other regulatory proteins that influence source-sink interactions—for example, SnRK1 (McKibbin et al. 2006) and the SUSIBAs (Sun et al. 2003)—also need to be considered as an alternative strategy to increase sink strength. Furthermore, studies have shown that cellular levels of active phytohormones such as cytokinin and auxin are important determinants of xylem or wood development, biomass formation, and secondary metabolism (Pesquet et al. 2005, Andersson-Gunneras et al. 2006). These processes are central to driving the use of photosynthate in longer-term C pools within plant biomass, hence increasing the carbon sequestration potential of plants.

Increasing carbon allocation to roots. The sink strength of root systems has a number of implications for phytosequestration. First, soil deposition of C through allocation to deep roots and their slow turnover constitutes a means for substantial long-term C sequestration. Second, C loss through root exudates and soil respiration can negatively affect both C sequestration and biomass production. Third, sufficient C stores in the roots are necessary as carbohydrate reserves for perennial grasses. Fourth, extensive root growth and proliferation is an important determinant for efficient water uptake and drought resistance. Carbon partitioning to different sink sites is controlled by both sink demand and source control of photosynthate production, and is a heritable trait (Wullschleger et al. 2005). Thus, unraveling the genes and proteins behind source-sink regulation is critical for our understanding of plant growth and development, and for our efforts to engineer sink strength and C partitioning.

Improving tolerance to biotic and abiotic stress. Plant productivity and, therefore, the capacity for CO₂ uptake, are greatly affected by abiotic stresses. In fact, drought stress is already a major limiting factor in plant growth, and will become even more so as we face global scarcity of water resources and increased salinization of soil and water. To cope with environmental stresses, plants have evolved phytohormones such as jasmonic acid, salicylic acid, ethylene, and abscisic acid that regulate plant responses to both abiotic and biotic stresses, with considerable signaling crosstalk (Agarwal et al. 2006, Nakashima et al. 2009).

As we strive to claim more marginal land for bioenergy crop production, it will be particularly important to identify molecular genetic controls for tolerance of drought, heat, and salinity. Studies on transgenic plants overexpressing drought-induced transcription factors, leading to increased plant tolerance to dehydration and salt, are promising, and suggest that recruitment of transcription factors along with stress-induced promoters can be an effective way to produce stress-tolerant plants without compromising yield (Agarwal et al. 2006).

Improving biomass quality. Improving the biomass quality of bioenergy crops will broaden the employment of biofuels and, therefore, the amount of CO₂ emission from fossil fuels that can be offset. The main targets are cell-wall digestibility and reduction or modification of lignin synthesis as a means to reduce the needs for pretreatment processing. Although reduced recalcitrance is a desirable property in bioenergy feedstocks, the opposite is true for increasing the phytosequestration potential of plants. Since the residence time of C sequestered to soil from deep roots depends on the chemical form of the C (Tuskan and Walsh 2001), the more recalcitrant the soil organic matter, the longer it will escape microbial respiration and reentry into the atmosphere as CO₂. Therefore, engineering plants to synthesize lignin, tannins, and other aromatic compounds to a greater extent in roots and to a lesser extent in aboveground biomass will be useful for phytosequestration as well as bioenergy purposes. However, more research is needed to assess the fate and stability of these compounds in the soil and under different conditions.

Developing high-yielding perennials for agriculture. Because of their extensive root systems, which commonly exceed depths of two meters, perennial grasses and trees deliver large amounts of C to the SOC pool, and store a substantial quantity of C as root biomass. Also, because perennial grasses and perennial forage legumes (alfalfa) can be harvested and regrown in multiple growing seasons without being replanted, perennial cultivation avoids the soil disturbances associated with annual crops. For the same reasons, perennials require fewer passes of farm machinery and fewer inputs of agrochemicals as compared with annual cultivation, which translates to less fossil-fuel use.

About 85% of global harvested cropland is planted with annual crops. Wheat, corn, and rice encompass more than half of that area. A way to increase the contribution of peren-

ennial cultivation in agriculture is therefore to generate high-yielding perennial grain crops. Some work is in progress to obtain perennial cereals by domestication of wild perennial species, or by hybridization of annual cereals with perennial relatives (Glover et al. 2007). Genetic engineering should present a suitable means to introduce perennial traits in cereals or increase grain yield in perennial relatives. It becomes important to identify genes responsible for perenniality on one hand, and grain filling and seed shattering and dormancy on the other. Perennial habit is a highly complex suite of traits, most of which are quantitative in nature. Westerbergh and Doebley (2004) identified 38 quantitative trait loci (QTL) for traits associated with perenniality by studying crosses between an annual maize subspecies and teosinte. Because of the high degree of gene synteny between grasses, it is likely that map positions for perenniality-related traits in teosinte will help in finding corresponding QTL in other grasses.

Can plant genetic engineering make a difference, and is it sustainable? The loss of C from the terrestrial pool during the last 10,000 years has been approximated to a little more than 450 GT (Lal 2008b). If this entire amount could be resequenced during the next 50 years, it would translate to 9 GT per year. This is similar to the 10 GT per year predicted by Graber and colleagues (2008), provided scientific breakthroughs come into play. Even if only half of the historic loss could be recaptured and stored, it would constitute a major tap into the atmospheric C pool. This article has so far dealt with different strategies that are amenable to improvement by traditional breeding and genetic engineering approaches. We now speculate on the extent to which such measures can contribute to GHG mitigations. We want to emphasize that we do not view plant genetic engineering as a stand-alone procedure but rather as one feature of modern molecular plant breeding, where transgenics, “omics,” QTL mapping, and other molecular applications integrate with conventional breeding.

In the following paragraphs we make an attempt at estimating the contribution of plant genetic engineering in phytosequestration (figure 3). Our outlook is the year 2050; the implementation time for the different strategies will vary and we assume that they can be fully deployed by then. First, we assume that the ecosystems most likely to be affected by genetic engineering are agricultural croplands for food and fodder, agroforestry, and bioenergy plantations, whereas large areas of uncultivated natural forests and grasslands are less likely to benefit from these technologies. Second, it should be noted that most if not all of the options discussed are linked, so the effects are not additive.

Over the last 50 years, crop productivity in agriculture has grown nearly 100% (Long et al. 2006). Using maize as an example, half of this increase was due to genetic improvements, and half to improved management (Long et al. 2006). With these observations as a guideline, we postulate that continued scientific advancements will be able to boost biomass production in food and nonfood crops at least 50% in the coming 50 years, and that genetic-engineering-assisted breeding will

be progressively more instrumental in this achievement. For example, engineering plants with reduced photorespiration could theoretically increase the photosynthetic rate by 10% to 30% for most C_3 crops (Metting et al. 2001), resulting in a 6% yield increase (Sinclair et al. 2004). Collectively, maximizing photosynthesis could lead to a 50% increase in productivity (Long et al. 2006). If this potential is realized only for land under cultivation, currently 1.8 giga hectares (Gha) with an NPP of 6 GT per year, a 50% increase in NPP corresponds to 3 GT per year. Since most aboveground biomass in croplands has a fast turnover, the majority of the 3 GT C will return to the atmosphere on an annual basis, whereas less than 1 GT might find its way to the SC pool. If we allow for a scenario with plantations of engineered trees endowed with enhanced photosynthesis, the total sequestration potential in biomass and SC might reach 2 to 3 GT per year.

The potential for soil C sequestration in bioenergy plantations alone is 1.6 GT per year (Lemus and Lal 2005). This assumes that 750 million ha of land worldwide is claimed for bioenergy crops. If we speculate that half of this land would be under cultivation by 2050, sequestration equals 0.8 GT per year. It seems reasonable to assume that this amount could double in genetically improved perennial grasses and SRWC with increased C partitioning to roots. This reallocation of resources needs to be coupled with enhanced photosynthesis so as not to lower biomass yield for energy purposes, and be titrated against other cellular processes such as respiration, flowering, and seed set.

By increasing the contribution of transgenic perennial cereals in agriculture, we could expect further growth in the transfer of C to root biomass. When comparing corn and switchgrass, it was found that although there was no difference in SC sequestration per se; switchgrass was five times more efficient in sequestering C in root biomass (at a rate of 1.1 Mg per ha per year) than corn (0.2 Mg per ha per year; Lemus and Lal 2005). Annual cereals occupy more than 50% of the 1.5 Gha classified as arable and permanent cropland. If we hypothesize that 10% of that acreage will be devoted to high-yielding perennial cereals by 2050, total root C sequestration would grow by around 0.05 GT per year.

We estimate that bioenergy crops could conceivably offset fossil-fuel GHG emissions equivalent to 5 to 8 GT C by 2050 (see above). It is highly likely that plant genetic engineering will significantly increase this potential offset by generating bioenergy crops with enhanced photosynthesis, improved stress tolerance, and optimized metabolic pathways, including that of carbon partitioning and allocation. We suggest this increase to be around 4 GT.

The high variability in phytolith accumulation among plant species (Parr and Sullivan 2005) is a telltale for the potential to increase the C sequestration as phytoliths in selected genetically modified crops, once the mechanisms for this process are understood. Studies so far suggest that greater phytolith production does not compromise yield (Parr and Sullivan 2005), and we propose that C sequestration as phytoliths in agricultural croplands and grasslands could double or triple by

2050 to give an additional sequestration of around 0.5 to 1 GT per year.

Increasing the content of lignin in roots and leaves of crop plants including bioenergy grasses and SRWC through metabolic engineering may prolong the residence time for plant detritus in soil and hence slow microbial respiration and CO_2 release to the atmosphere. This could result in sequestration of another 0.5 to 1 GT C per year. Engineering plants with improved tolerance to drought and salinity will raise NPP and, consequently, increase C sequestration in arid and semiarid ecosystems, as well as boost fossil-fuel emission offset by bioenergy crops. We predict that the combined effects of such an approach correspond to 2 to 3 GT C per year.

The calculations outlined above are set against a backdrop of several issues that we overlook for the sake of simplicity. For example, ecosystems containing extensive transgenic plant populations might meet with societal resistance. Also, how global climate change—with increasing atmospheric CO_2 levels and higher temperatures—affects C sequestration is a complex question. In general, elevated CO_2 enhances photosynthesis and stimulates initial C sequestration. The sustainability of this CO_2 -fertilization effect depends partly on whether the plants acclimate to the higher CO_2 levels, and partly on ecosystem nitrogen and water availability and supply. The sensitivity of SC pools to global warming is another big uncertainty in the C cycle; according to many models, the overall terrestrial C sink is expected to weaken with global warming as the CO_2 fertilizing effect loses out to increased plant and soil respiration (Bonan 2008, Sokolov et al. 2008) but the extent by which the C pools will decrease is unclear (Canadell et al. 2007). Additionally, the feasibility of establishing extensive bioenergy plantations needs to be assessed in terms of land demands, nutrient requirements, wildlife use, and so on.

Conclusion

Our efforts to mitigate elevated levels of atmospheric CO_2 , of which phytosequestration is an important aspect, should be viewed as a continuing process, as the strategies and technologies employed will evolve over time depending on the nature of public and political will, economic incentives, and environmental sustainability projections. We have described examples by which plant genetic engineering can contribute to increased phytosequestration, and have made an effort to quantify these strategies. It is our intent for this article to stimulate further discussion and new research activities to explore plant genetic engineering as a means to enhance C sequestration in above- and belowground biomass and SC pools.

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