Article Title: Crops, algae and carbon sinks

(Ruolo dei produttori primari nella formazione e mantenimento dei "carbon sinks")

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Abstract: The glasshouse effect is crucial in maintaining the average earth temperature at 15°C, well above the expected -30°C and is modulated by the relative abundance of atmospheric components including carbon dioxide (CO_2) and oxygen (O_2) whose concentration are controlled by the biological activity of primary producers, plants and algae, as well as consumers and saprophytes, fungi and bacteria. During the industrial era, following the perfection of the steam engine in 1776, substantial CO_2 emissions from combustion of fossil fuels have increased its abundance in atmosphere. This has been suggested to cause the observed enhancement of average temperature of the planet; a relation which is expected to yield into an explosive rise of both parameters following the population dynamics and economic development in the forthcoming decades to a dangerous planetary condition. In this work, we summarize the evidence and perspectives of using agriculture and biofuel production from algae in order to balance CO_2 absorption and emissions. We suggest that new concepts in engineering photosynthesis might allow for a 30% increase in the primary production from crops and unicellular algae. Implementing this technology will both increase food stocks and provide biomass for replacing fossil fuels with carbon-neutral biofuels. We conclude that present technology potentially allows for stabilization of CO_2 levels on Earth.

Sommario: L'effetto serra è fondamentale per mantenere la temperatura media della Terra a 15°C, ben al di sopra dei previsti -30°C, ed è modulata dalla abbondanza relativa dei componenti atmosferici tra cui anidride carbonica (CO_2) e ossigeno (O_2) la cui concentrazione è controllata dalle attività biologiche dei produttori primari, piante e alghe, così come dei consumatori e saprofiti, funghi e batteri. Durante l'epoca industriale, dopo il perfezionamento del motore a vapore nel 1776, le emissioni di CO_2 dalla combustione di combustibili fossili hanno aumentato la sua abbondanza nell'atmosfera. È stato suggerito che ciò sia causa dell'innalzamento della temperatura media del pianeta. Tale relazione, in seguito alla dinamica della popolazione e allo sviluppo economico nei prossimi decenni, si prevede produrrà un aumento esplosivo di entrambi i parametri a una condizione planetaria pericolosa. In questo lavoro, riassumiamo i risultati e le prospettive riguardo alla possibilità di utilizzare l'agricoltura e la produzione di biocarburanti da alghe per bilanciare l'assorbimento e le emissioni di CO_2. Suggeriamo che concetti recentemente sviluppati sulla efficienza dei produttori primari permettano un aumento del 30% della produzione fotosintetica da colture agrarie e alghe unicellulari. Ciò permetterà di ampliare sia le scorte alimentari che la biomassa disponibile per la sostituzione dei combustibili fossili con biocarburanti a emissioni zero. Concludiamo che la tecnologia attuale consente di stabilizzare i livelli di CO_2 planetari.

Main text

1.1 Atmospheric carbon dioxide, oxygen and their dynamics

The greenhouse effect is crucial in maintaining the average Earth temperature at 15°C, well above the expected -30°C, and is modulated by the relative abundance of atmospheric components, including CO_2 and O_2. The abundance of these two gasses dramatically evolved during Earth's life as a result of biological activity of primary producers, plants and algae, as well as consumers and saprophytes, fungi and bacteria. Pre-biotic CO_2 concentration was similar to the volcanic emission, i.e. 2-3% (Kasting, 1993) while photosynthetic activity coupled to mineralization acted as carbon sink to lithosphere (Raven and Falkowski, 1999) leading to the 275 parts per million (ppm) concentration in the pre-industrial era with small variations (Siegenthaler et al., 2005). Following the perfection of the steam engine in 1776, substantial CO_2 emissions from combustion of fossil fuels have increased CO_2 abundance yielding into 318 ppm at the initial instrumental determination in 1958 (Keeling et al., 2001). Current level is 403 ppm thus an increase by 85 ppm in 60 years, corresponding to 124 gigatonnes (GtC) (Heinze et al., 2015). Its effect in enhancing the earth temperature was documented in 1896 already (Arrhenius, 1896).

Modelling of CO_2 emission based on Gross Domestic Product (GDP) growth been suggested to cause the observed enhancement of average temperature of the planet; a relation which is expected to yield into an explosive enhancement of both parameters following the population dynamics and economic development in the forthcoming decades to a dangerous planetary condition (Wagner et al., 2016).

1.2 Oxygen dynamics end evolution

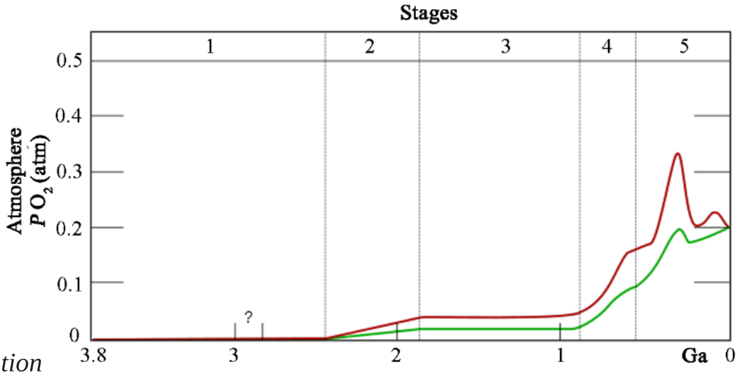

Figure 1. *Estimated evolution of atmospheric oxygen. The upper red and lower green lines represent the range of the estimates. The stages are: stage 1 (3.85–2.45 Ga), stage 2 (2.45–1.85 Ga), stage 3 (1.85–0.85 Ga), Stage 4 (0.85–0.54 Ga) and stage 5 (0.54 Ga–present; Ga:"Giga years ago"(By Oxygenation-atm.svg: Heinrich D. Hollandderivative work: Loudubewe (talk) - Oxygenation-atm.svg, CC BY-SA 3.0 https://commons.wikimedia.org/w/index.php?curid=12776502)*

The atmosphere is thought derived from volcanic outgassing from the differentiated interior and controlled by the redox state of the Earth's mantle, which appears to have been constant since 3.8 Ga (Delano, 2001). Pre-biotic atmosphere was almost completely anoxic (Farquhar et al., 2000) and first traces of O_2 were detected dating 2.33 years ago (ya) (Luo et al., 2016). This was followed by a slow oxygenation of the atmosphere and seas. O_2 was indeed produced by oxygenic photoautotrophs; yet, absorbption in oceans and seabed rock while substantial rise of oxygen was only detected starting from 850 million ya, allowing evolution of multicellular organisms and land colonization. Maximal O_2 level have likely occurred in the Carboniferous before returning to its present value (Holland, 2006). In the last 800.000 years, however, O_2 concentration has dropped by 0.7% (Stolper et al., 2016).

1.3 Effect of enhanced carbon dioxide on crops and vegetation

CO_2 and O_2 are, respectively, substrate and product of photosynthesis, suggesting changes in their concentration affect primary productivity in Earth and Sea. Modelling of future planetary evolution strongly depends on primary productivity while this factor is one of the few player in global analysis that is accessible to experimental verification in growth chambers with modified air composition and Free-Air Carbon dioxide Enrichment (FACE) experiments. In this procedure, plants are grown outdoors, and the CO_2 concentration of the surrounding air is artificially elevated by gas emitters in the perimeter of the experimental surface. FACE experiments, using generally lower CO_2 levels than the greenhouse studies, showed sensible productivity gains, although lower than reported in greenhouse studies. Large differences were observed depending heavily on the species under study. A 2005 review of 12 experiments at 475-600 ppm showed an average gain of 17% in crop yield, with legumes typically showing a greater response than other species, and C4 plants generally showing less (Ainsworth and Long, 2005). A 2017 article describes research showing that increased CO_2 levels have a negative impact on a variety of human food crops, by increasing the levels of carbohydrates, such as glucose, while decreasing the levels of important nutrients such as protein, iron, and zinc. Crops are experiencing a decrease in protein include rice, wheat, barley and potatoes (Evich, 2017).

1.4 Land and sea biological *vs* inorganic carbon sinks

Humans have a strong impact on land environment, less on seas. Thus, it is of interest to assess the relative size of the primary production in the two macro-environments. Fast turning over carbon fluxes include 109 Gt of carbon fixed by land plants per year, further increased by 14 Gt as a result of enhanced photosynthetic activity by human action (crop improvement plus consequence of enhanced CO_2 on Calvin-Benson-Bassham activity) while CO_2 evolution by respiration was sized at 107 (+ 11.6) Gt. Further emission from fossil fuels burning and cement production amount to 7.8 Gt (+ 1.1) from land use. Corresponding values for seas are 60.6 (+17.7) Gt emissions and 60 (+20) Gt fixation. However, most fixation (release) is due to reversible inorganic solubilization of CO_2 as HCO_3^- while primary photosynthetic productivity is low due to uncoupling between availability of photosynthetic active radiation in the surface layers of the waters and nutrient availability which is high in deep layers only (Heinze et al., 2015). It is concluded that land CO_2 exchanges are the most relevant for short term evolution of CO_2 levels. Sum of negative and positive carbon exchanges resulting from anthropogenic activity yields a positive emission of +3.5 Gt per year (Heinze et al., 2015). Further, it should be noticed that productivity of unicellular algae, the major primary producers in seas, is only low because of nutrient limitation while algae have potential productivity far better than crops, making them a possible source for food, fuels and an efficient sink for CO_2 when cultivated in nutrient replete conditions (Pinnola et al., 2017).

2.1 Appropriation of primary productivity by human activity

Analysis of land surface use by human activities including fires, woodland, built-up land and cropland from 1910 to 2012 almost doubled (from 6.9 to 14.8 Gt). The efficiency of biomass production per land surface has progressively improved overwhelmingly because of the rise in crop yields due to technological improvements including breeding, fertilization, irrigation (Krausmann et al., 2013). From this analysis it can be concluded that further improvement in crop yield to a significant extent has the potential for compensating emissions (see above) and carbon sinks. Further, substitution of (part) of fossil fuels with renewable energy including biofuels would bring atmospheric CO_2 levels towards pre-industrial levels.

2.2 Developing concepts on engineering photosynthesis

Algae can convert solar energy into chemical energy through the process of photosynthesis.
The whole process of oxygenic photosynthesis can be summarized by Equation 1.

1. $$6H_2O + 6CO_2 + 8\text{ photons} \rightarrow C_6H_{12}O_6 + 6O_2$$

Light energy is used to extract electrons from H_2O, thus generating oxygen (O_2). These electrons are transported through a linear electron transfer chain and finally reduce nicotinamide adenine dinucleotide phosphate ($NADP^+$) to NADPH. Photosynthetic electron transport is coupled to the generation of a transmembrane electrochemical gradient, whose stored energy is used to synthesize adenosine triphosphate (ATP). NADPH and ATP are then used to produce glyceraldehyde-3-phosphate (G3P) from CO_2 in a metabolic pathway: the Calvin–Benson–Bassham cycle (CBB cycle) (Benson and Calvin, 1950). Photosynthesis converts inorganic CO_2 into organic carbons and finally to biomass. A theoretical estimation of biomass yield can be assessed considering the efficiency of photosynthesis: a minimum of 8 photons of light energy are absorbed per each O_2 molecule evolved; the actual average measurement is 9.5 photons per O_2 (Melis, 2009). Per O_2 evolved, four electrons are channeled into the linear photosynthetic electron transport chain and two molecules of NADPH, and in addition, three of ATP are produced in the process using the transmembrane pH gradient produced as a by-product of the electron flow. In order to convert three CO_2 molecules into G3P, nine ATP and six NADPH are required. The average insolation, that is, the solar radiation energy on a surface area in a given time, is between 3 and 5 kWh m^{-2} day^{-1} (full solar spectrum) at temperate regions. However, only about 40% of solar radiation is photosynthetically active (PAR), because only photons with wavelengths between 400 and 700 nm (visible spectrum) can be absorbed by pigments serving photosystems, since these wavelengths carry an energy equal to the change in the energetic level between the ground state (S_0) and the first excited one (S_1^*).
A photon has an energy, E, that is directly proportional to its frequency and inversely proportional to its wavelength, following Equation 2.

2. $$\mathbf{E} = hv = c/\lambda$$

(h, Planck constant, $6.626 \cdot 10^{-34}$ J·s. c, light speed, $3 \cdot 10^8$ m/s. v, frequency. λ, wavelength)

For example, 1 mol photons (1 Einstein) at 440 nm has energy of 272 kJ, while 1 mol photons at 670 nm has energy of 178 kJ. Out of the full solar radiation of 5 kWh m^{-2} day^{-1}, the photosynthetically active radiation (PAR) is about 35 mol photons m^{-2} day^{-1}. Considering the energy requirement of 9.5 mol photons per 1 mol CO_2, with such average available light radiation, the cell could assimilate 3.68 mol CO_2 m^{-2} day^{-1}. Since biomass composition can be approximated to the formula CH_2O, the previous data would translate in the synthesis of about 110 g biomass m^{-2} day^{-1}. Nevertheless, accumulation of organic carbons as biomass is lower, due to respiration and other metabolic activities and energy losses are accounted to 30%. The resulting expected maximum biomass productivity would be of 77 g biomass m^{-2} day^{-1} (280 tons ha^{-1} $year^{-1}$), corresponding to a solar-to-biomass conversion efficiency of 8–10% (Melis, 2009).
However, real algal biomass productivity achieved so far in small-scale systems does not exceed the 73–146 t dry weight ha^{-1} a^{-1} (20–40 g dry weight m^{-2} day^{-1}) and 3% of solar-to-biomass conversion efficiency (Melis, 2009) in the best cases. Crop productivity is far low, generally ranging between 0.1-0.3% with a 0.6% figure in sugar cane cultivated in Brazil. Real productivity is far below theoretical estimations and record yields, especially in large-scale and over long-lasting periods, and a major future goal would be to reduce this gap.

Plant productivity is described by the following Equation 3 (Monteith, 1977, 1972).

3. $$\text{Yield} = 0.487 \cdot St \cdot \varepsilon i \cdot \varepsilon c \cdot \varepsilon p$$

(*St*, duration of the season growth season; *εi*, light interception efficiency; *εc*, photosynthesis efficiency; *εp*, harvest index)

Crop breeding coupled to fertilization and irrigation has successful provided a sustained increase in crop yield over the last century and yet this process has reached its saturation and even expensive breeding programs yield into marginal enhancements every year (Tilman et al., 2002; Zhu et al., 2010). Breeding programs have so far focused on increasing harvest index, enhancing mineral uptake efficiency and on plant architecture, while the possibility of improving photosynthetic light use efficiency has been disregarded. This was in part due to the difficulties of interfacing the traditional breeding based on genetic recombination and phenotyping with the mechanism-based design of programs for improving photosynthesis. Also, a misinterpretation of the high quantum yield values of primary photosynthetic reactions (0.83 for Photosystem II, PSII, and 0.96 for Photosystem I, PSI), suggest little space was left to improvement. This proved to be utterly inaccurate since downstream reactions and the time constants of the reactions composing the photosynthetic electron chains and CO_2 reduction cycle range from 10-12 (PSII, PSI charge separation) to 10-1 (Rubisco carboxylation/oxygenation). Because of the different time constants and temperature dependence of the photosynthetic reactions, high efficiency can only be found under constant light and temperature conditions, which is nowhere to be found in a rotating planet producing night/day sequences and, even worse, under canopies where sunflecks and rapid changes in photon supply are unavoidable.

2.3 Oxygen makes photosynthesis vulnerable

Changes in light supply to the system are fast and strong while downsteam metabolism cannot be regulated fast-enough in order to maintain efficiency of light use energy. Thus, energy, in excess with respect to the maximal rate of downstream reactions, is either dissipated into heat or transferred to oxygen. The latter, yielding into Reactive Oxygen Species (ROS) and damage to the chloroplast with loss of function (Photoinhibition). Heat dissipation is a photoprotective reaction and prevents photoinhibition. Yet, its activity does severely affect productivity by dissipating most of the energy associated with photon absorption. Over-excitation of photosystems yields into photodamage by at least two partially overlapping mechanisms: first is enhanced in conditions of excess light when NADPH is produced in excess with respect to the rate of its oxidation by the CBB cycle. Lack of $NADP^+$ acceptor leave a univalent strong reductant, i.e. PSI free to reduce non-physiological substrates such as O_2, producing superoxide. Although superoxide can be turned into the less dangerous H_2O_2 by superoxide dismutase enzyme (SOD), further reduction to water depends on physiological conditions and can be limited at low temperature. High light but also intermittent low light conditions under canopies caused over-reduction of the plastoquinone pool, the electron acceptor from PSII. PQH_2 cannot accept photo-activated electrons from Q_a in PSII, thus allowing for charge recombination from oxidized P680 ($P680^+$) to singlet-excited state of P680 ($^1P680^*$). In such condition $^1P680^*$ increases its lifetime, due to lack of photochemical quenching, and undergoes transition to triplet-excited state of P680 ($^3P680^*$) which readily reacts with $^3O_2$ into $^1O_2$, causing photodamage. The photosynthetic apparatus is rich in carotenoids which are active in triplet quenching and yet this is not the case of PSII acceptor side because the Oxygen Evolving Complex (OEC) generates oxidizing species up to *+1volt* which would easily destroy any carotenoid ligand located in vicinity. This makes PSII the most vulnerable point of photosynthesis and the site of photoinhibition. It might be asked why photosynthesis did not evolve into a more robust oxygen-resistant machinery and yet, it can be observed that evolution occurred in H_2O (water) and low O_2 levels were maintained for at least 2.5 billion years (Fig. 1), thus making irreversible the original oxygen-vulnerable design. Photo-oxidative stress is the underlying cause of most abiotic stresses which, in turn, are by far the strongest limit to crop productivity (Tester and Bacic, 2005; Bressan et al., 2009). Enhancing plants and algal photosynthetic efficiency will require elucidation and up-regulation of mechanisms preventing damage and increasing tolerance to photoxidation.

3.1 Strategies for improvement of photosynthetic efficiency in plants and algae

Awareness of feasibility of improving photosynthesis is recent. In the following, we will offer a few examples of successful attempts using as a guideline the well-known pattern of light response of photosynthetic activity where three steps can be clearly distinguished i.e. (a) a light limited range in which photosynthetic rate linearly increases with photon flux; (b) a light saturation range, at the maximum photosynthetic rate (constant P_{max}) where excess light is dissipated into heat and (c) a photodamage range at which activity is increasingly limited by photodamage (Fig. 2). Each of these steps can be engineered for increased efficiency. In the following, examples for each are shown and discussed in the case of microalgae and plants:

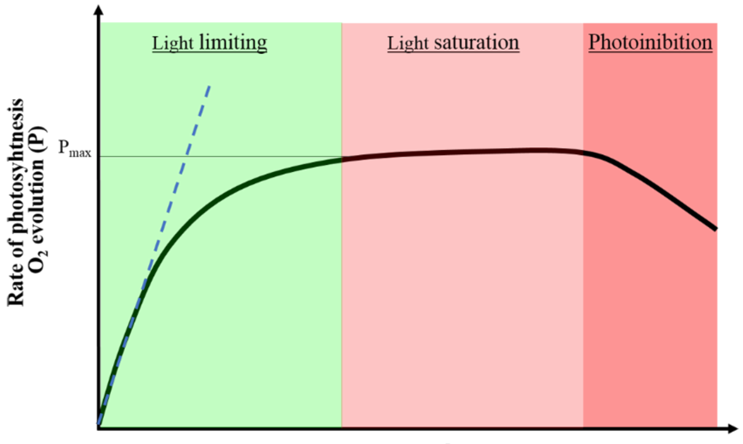

Figure 2. *Light dependence of photosynthetic activity. Photosynthetic oxygen evolution (P) increases linearly with the light intensity until saturation.* P_{max} *is the maximum photosynthetic rate. Upon saturation of photosynthesis excess absorbed energy is dissipated as heat. Upon saturation of heat dissipation capacity further light increase leads to photoinhibition of photosystem II. Dotted line shows an idealized light-response curve that would be obtained with unlimited photosynthetic capacity. The photosynthetic apparatus can be engineered for optimizing each of the three light steps, or combinations, for increased efficiency.*

3.2 Enhancing light distribution in the canopy or photobioreactor

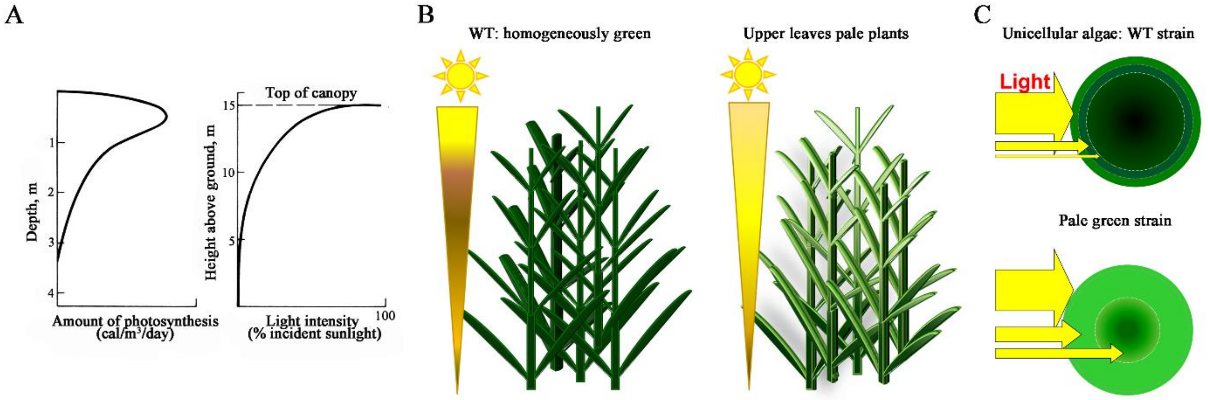

Figure 3*. Light distribution and photosynthetic activity in canopies and algal cultures* ***(A)****. Light distribution is limited by the high optical density of the tissue or cell layers in plant canopies* ***(B, on the left)*** *or photobioreactors* ***(C, upper panel)****. Mutations decreasing chlorophyll concentrations without disrupting photoprotection allow for increasing productivity of the shaded tissues* ***(B, on the right)*** */cells (****C, lower panel)*** *and decrease energy dissipation in the full light exposed tissues/cells, thus increasing overall light use efficiency.*

Light distribution in tall, dense, plant canopies is strongly inhomogeneous. In a corn field only the top leaves are exposed to saturating light (> 800 μE) while 85% of the photosynthetic tissues is light limited even at midday or photobioreactors (upper right) is limited by the high optical density of the tissue or full light exposed cell layers. Mutations decreasing chlorophyll concentrations without disrupting photoprotection allow for increasing productivity of the shaded tissues/cells and decrease energy dissipation in the full light exposed tissues/cells. Pale green mutants have been created and assayed for productivity in the green algae *Chlamydomonas reinhardtii* (Kirst et al., 2012; Bonente et al., 2011) and *Chlorella sorokiniana* (Cazzaniga et al., 2014) showing increased light efficiency in biomass accumulation in a wide range of medium and high light conditions. Recent experimentation with tobacco plants yielded similar results (Kirst et al., 2017) overall enhancement in biomass was between 20 and 30%. Notice that mutants might carry multiple lesions which decrease efficiency of random metabolic processes thus nullifying the advantage of increased light transmission. Thus, a preliminary physiological characterization is strongly recommended before proceeding to comparative growth analysis (Cazzaniga et al., 2014).

3.3 Modulating energy dissipation

The first evidence that energy dissipation limits photosynthetic productivity was obtained in *Arabidopsis thaliana* (Dall'Osto et al., 2005) from growth analysis of *A. thaliana* plants carrying mutations for constitutive activation of energy dissipation (*npq2 mutant*) or blocked in the mechanism (*npq4* mutant). Growth rate was reduced in the former and enhanced in the latters (Fig. 4).

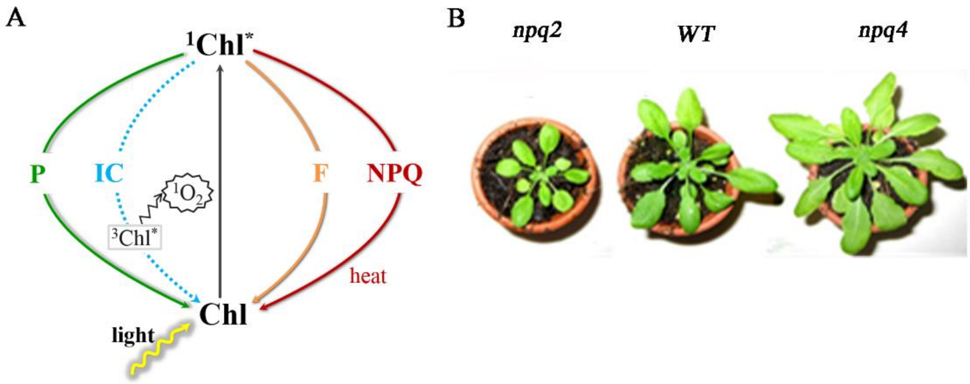

Figure 4. *Scheme of operation for the non-photochemical energy dissipation in plants and algae. Upon photon absorption, Chl is promoted to its first excited state ($^1Chl^*$). The energy of this state can be used to fuel photosynthetic reactions (P), or it can de-excite to ground state by dissipating heat (NPQ); both of these mechanisms reduce the fluorescence yield (F). In high light condition, the lifetime of $^1Chl^*$ increases because of limited photochemical quenching: $^1Chl^*$ undergoes intersystem crossing to triplet chlorophyll ($^3Chl^*$). $^3Chl^*$ readily reacts with O_2, forming singlet oxygen ($^1O_2{}^*$), a highly toxic/dangerous/unstable ROS. Redrawn from* (Müller et al., 2001) ***(A)****. Growth of A. thaliana plants carrying mutations for constitutive activation of energy dissipation (npq2 mutant) or blocked in the dissipative mechanism (npq4 mutant). From* (Dall'Osto et al., 2005)***(B)****.*

Non-Photochemical Quenching (NPQ) is a photoprotective process dissipating light energy absorbed in excess respect to the capacity for use by the downstream metabolic reactions. In excess light, saturation of CBB cycle leads to ADP and P_i depletion which inhibits ATPase and causes lumen acidification which leads to protonation of lumen-exposed acidic residues in both PSBS (plants) and LHCSR (algae) proteins which initiate energy dissipation which is obtaining by favoring de-excitation of Chlorophyll singlet excited states to the ground state. The relation between energy dissipation/conservation and growth is straight forward and was verified in 2016 (Berteotti et al., 2016) in *C. reinhardtii* by the observation that growth rate was strongly dependent on the *lhcsr* gene copy number and accumulation of the LHCSR gene products: the *npq4* mutant (Peers et al., 2009) with one *lhcsr* gene and reduced accumulation of the protein had significantly enhanced biomass productivity respect to Wild Type (WT). However, further deletion of the residual copy in the *npq4xlhcsr1* genotype yielded a reduced growth due to photooxidative stress (Berteotti et al., 2016). It was concluded that algae favor photoprotection over growth rate thus operating energy dissipation to rate far in excess respect to what needed for photoprotection. Thus, it is possible to increase productivity by decreasing photoprotective energy dissipation to a limited extent provided extreme stress is avoided.

Further evidence in plant was obtained by Kromdjik an collaborators (Kromdijk et al., 2016) which overexpressed violaxanthin epoxidase and PSBS, in order to increase NPQ, as well as zeaxanthin epoxidase in order to accelerate relaxation from the dissipative state in low light and enhancing integrated light harvesting. This resulted into a 20-30% increased yield in transgenic tobacco plants grown outdoor.

3.4 Increasing resistance to photoxidative stress

Although no evidence was yet published for enhanced growth rate of plants or algae with increased resistance for oxidative stress, several laboratories are working in this perspective based on preliminary results. Photoinhibition can significantly affect yield and fitness in crops and model plant species (Zhu et al., 2004; Frenkel et al., 2006; Raven, 2011). This may be particularly relevant for rice growing regions in South and Southeast Asia, which are positioned close to the equator where radiation can reach very high levels between periods of cloud cover. Genotypes with increased resistance to photoinhibition are expected to yield into a significant growth rate improvement under light stress conditions. Such result could be obtained either by increasing antioxidant metabolites or through reversible activation of energy dissipation processes such as described in monocots (Bergantino et al., 1995; Betterle et al., 2017, 2015; Mauro et al., 1997).

In summary, it appears that the combined effect of classical crop breeding and direct engineering of photosynthetic process will lead to a significant enhancement of light use efficiency for biomass production which imply enhanced CO2 uptake from the atmosphere. Assuming a 30% increase from combining enhancement, processes described in sections 3.2 to 3.5, the compensation of the fossil fuel emissions appears as a feasible perspective.

4.1 Limits of photo-autotrophic algal growth and mixothrophy

Although engineering photosynthesis in algae yielded strong improvement in productivity (Cazzaniga et al., 2014; Berteotti et al., 2016) which might open the way to industrial exploitation for production of high value products, the costly hardware needed for algae cultivation in photobioreactors is delaying large scale biofuel production of biofuels from algae due to the low oil price of in the last decade. Among innovations that enhance sustainability of large scale algal cultures is engineering for selective growth thus avoiding expensive procedure for sterilization of photobioreactors (Loera-Quezada et al., 2016; López-Arredondo and Herrera-Estrella, 2013). Yet, very large sun-exposed surfaces of photobioreactors are needed for photon harvesting which potentially competes with farming, suggesting that alternatives to direct light harvesting by unicellular algae might be of interest. Unicellular algae are either phototrophic, heterotrophic or both (Flynn et al., 2013). Mixotrophy is suggested to play an important ecological role when inorganic nutrients are low (e.g., in oligotrophic systems; e.g., (Arenovski et al., 1995)) or unbalanced (e.g., in eutrophied systems; e.g., (Nygaard and Tobiesen, 1993; Burkholder et al., 2008)), and when light is limiting. Mixotrophy may become of crucial importance in the production of biofuel because of their high lipid content and fast growth on organic substrates (Wang et al., 2013). Indeed, although light use efficiency by algae is low, harvest index values are often below 0.5 (Evans, 1993) thus leaving large energy-rich biomass stocks unused. Exploitation of lignocellulose biomasses through chemical or enzymatic degradation to simple sugars that can be either fermented to ethanol (Zabed et al., 2016) or fed to microalgae or yeasts with costs as low as 1.5$/L (Gomez et al., 2016). This technology appears as the closest to commercial exploitation and yet improvement are required in the efficiency and environmental sustainability of lignocellulosic degradation of biomasses to sugars and engineering of microalgal metabolism for sugar transport and lipid storage (Blifernez-Klassen et al., 2012).

5.1 Conclusions.

Biosphere sustainability is challenged by the increase of human population which exploits resources to a level previously unexperienced. Massive fossil fuels combustion increases atmospheric CO_2. The dilemma *food or fuels* has retarded action aimed to reduce fossil fuel use for sake of maintaining food price low for population in less developed countries (Lederer; UN Rapporteur, 2008; Oxfam, 2008). However, the increased CO_2 fixation by farming with crops with enhanced productivity has significantly contributed to the slow CO_2 accumulation in the atmosphere while substitution of fossil fuels with carbon-neutral biofuels appears as the only sustainable perspective for preserving carbon cycle balance, together with the increasing use of non-carbon energy vectors. The recent understanding that photosynthetic CO_2 fixation can be significantly improved both in unicellular algae and crops opens a perspective for providing both food and fuels while re-equilibrating carbon cycle on Earth. Neo-Luddism (Jones, 2006) is an important component of the present environment conservation activism and yet this perspective does not appear to indicate solution for problems associated to carbon cycle stability. Experimental plant biology and biotechnology might provide better answers to global challenges.

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