Biodiversity impacts of bioenergy production: Microalgae vs. first generation biofuels

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ABSTRACT

Energy and fuel demands, which are currently met primarily using fossil fuels, are expected to increase substantially in the coming decades. Burning fossil fuels results in the increase of net atmospheric CO_2 and climate change, hence there is widespread interest in identifying sustainable alternative fuel sources. Biofuels are one such alternative involving the production of different fuels which include biodiesel and bioethanol from plants. However, the environmental impacts of biofuels are not well understood. First generation biofuels (i.e. biofuels derived from edible biomass including crops such as maize and sugarcane) require extensive agricultural areas to produce sufficient quantities to replace fossil fuels, resulting in competition with food production, increased land clearing and pollution associated with agricultural production and harvesting. Microalgal production systems are a promising alternative that suffer from fewer environmental impacts. Here, we evaluate the potential impacts of microalgal production systems on biodiversity compared to first generation biofuels, through a review of studies and a comparison of environmental pressures that directly or indirectly impact biodiversity. We also compare the area of cultivation land required to meet gasoline and distillate fuel oil demands globally, accounting for spatial variation in productivity and energy consumption. We conclude that microalgal systems exert fewer pressures on biodiversity per unit of fuel generated compared to first generation biofuels, mainly because of reductions in direct and indirect land-use change, water consumption if water is recycled, and no application of pesticides. Further improvements of technologies and production methods, including optimization of productivities per unit area, colocation with wastewater systems and industrial CO_2 sources, nutrient and water recycling and use of coproducts for internal energy generation, would

further increase CO_2 savings. Overall pollution reductions can be achieved through increased energy efficiencies, along with nutrient and water recycling. Microalgal systems provide strong potential for meeting global energy demand sustainably.

Keywords: biofuel crops, ecological footprint, land-use change, life cycle assessment, tropic, vertebrate

1. INTRODUCTION

Future energy demands are expected to increase as a result of ongoing population and economic growth. According to the IEA [1], energy consumption could increase between 17% and 50% by 2040 relative to 2012, reaching around 15,629 and 20,039 million tons of oil equivalent (Mtoe) respectively. Meeting these demands under current levels of fossil fuels exploitation—with coal, oil and gas accounting for 82% of total primary energy consumption in 2012—is likely to drive increases in global atmospheric temperature above 3.6°C by 2100 in comparison to pre-industrial levels [1], leading to widespread changes in ecological communities and increases in extinction risks for species [2, 3]

Although a system that combines energy derived from the wind, water and sunlight has been proposed for supplying global energy demands [4], fuels with their high energy density will still be a major component in the future to power large machinery, planes, and ships. Biofuels, defined as high-density energy carriers derived from biomass transformation, could be a sustainable alternative to replace fossil fuels [5-7], especially for the transport sector [1, 8], which in 2012 accounted for around 23% of total CO_2 emissions [1]. Burning biofuel releases carbon that has been already fixed by plants through photosynthesis and thus, in theory, should not increase the net atmospheric CO_2 content [9, 10]. However, there are concerns about the environmental impacts that a widespread adoption of biofuels could exert at a global scale, which could lead to further environmental degradation depending on the production system and initial land-use [11, 12]. Furthermore, environmental impacts are a function of differences in energy demands per country and regional variation in biofuels' productivities.

Currently, biofuels are primarily produced in the forms of bioethanol and biodiesel derived from food crops (i.e. first generation biofuels). It is estimated that between 2013 and 2015 around 77% of produced bioethanol was based on the processing of maize and sugarcane; while around 81% of biodiesel was produced from vegetable oils [13]. Because first generation biofuels compete with agricultural lands, environmental degradation—including biodiversity losses due to land clearing of biodiverse systems—has been associated with biofuels' expansion [12, 14-19]. Furthermore, biofuel production can increase the magnitude of other pressures that directly or indirectly affect biodiversity, including CO_2 emissions from land-use change [20-22] and production systems [23-25], emission of pollutants [7, 17] and depletion of water [26-28].

Microalgal production systems, which include open ponds and closed photobioreactors [29-33] could overcome several drawbacks of first generation biofuels, because they offer higher biomass yields than terrestrial crops per unit area, can be grown on non-arable lands, can make use of brackish or seawater, and can be coupled with wastewater systems and industrial CO_2 sources, helping in water remediation and in CO_2 emission reductions [29, 30, 32, 34-36].

Previous work on microalgal production systems has addressed several environmental impacts of microalgal biofuel production, including resource consumption and pollution [37-42], water consumption [43] and potential impacts of genetically modified strains [44]. However, no study has focused on biodiversity or compared the potential impacts of microalgal systems on biodiversity in relation to first generation biofuels.

Here, we review the potential impacts of microalgal systems for biofuel production on biodiversity in contrast to first generation biofuels, focusing particularly on vertebrates in tropical and subtropical biodiverse regions of the world [45, 46] where the potential for agricultural expansion, including first generation biofuels, is greatest [47, 48]. We classify the different factors that affect biodiversity as due biofuel production, using the DPSIR framework which, based on Driving forces, Pressures, States, Impacts and Responses, is useful for describing the interactions between society and the environment [49, 50]. Then, we identify and compare the different pressures—defined as anthropogenic factors that induce environmental impacts [51]—that directly and indirectly impact biodiversity, when using microalgal systems or first generation biofuels. Accounting for spatial variation in productivity and energy consumption, we estimate the area of cultivation land required to meet gasoline and distillate fuel oil for each country using either microalgal systems or first generation biofuels, to investigate the relative feasibility of adopting biofuels as a substitute energy source.

2. MATERIALS AND METHODS

Relevant literature was identified in April 2016 using the Science Citation Index Expanded (SCI-EXPANDED) and the Emerging Sources Citation Index (*ESCI*) in Web of Science, with the following combinations of keywords: (biofuel OR bioenergy) AND (biodiversity OR wildlife), (biofuel OR bioenergy) AND (fish* OR bird* OR avian OR mammal* OR reptil* OR amphibian*). A citation report was made using Web of Science in order to show the progress in the field. Papers were screened to identify those that relate first generation biofuels or microalgae with impacts in tropical and subtropical areas of the world (i.e. between parallels 38°N and 38°S). We used these studies to identify the impacts that biofuel production has on biodiversity, the anthropogenic factors that induce impacts on biodiversity (i.e. pressures), as well as the mechanisms and processes by which those impacts occur.

Further comparisons between microalgal systems and first generation biofuels were based on pressures that directly or indirectly have shown to impact biodiversity. Environmental pressures were schematized based on the DPSIR causal framework [49, 50]. The DPSIR framework has been adopted by the European Environmental Agency [50] and has been widely applied for understanding relationships between factors that drive impacts on the environment and society responses [12], for allowing communication between scientists [52] as well as a tool for decision making [53]. For this comparison, life cycle assessments for microalgal production systems were reviewed.

An estimate of the area of cultivation land required by microalgal systems and first generation biofuels and microalgal systems to meet each country’s 2010 gasoline and distillate fuel oil demands [54] was developed. The average yield of crops that could be used for ethanol and biodiesel production between 2005 and 2014 was calculated using the "FAOSTAT" database [55] for each country. Average ethanol yields were then estimated using conversion efficiencies from feedstocks [56-59] and average biodiesel yields were estimated using reported lipid contents and oil-specific densities per crop [56, 60], assuming lipid extraction efficiencies of 90% and lipid conversion efficiencies of 90%. For microalgal systems, lipid yields were obtained using the global map developed by Moody, McGinty [61]. The most frequent value of lipid yield per country was obtained based on an area weighted average. The total area of cultivation land required to meet each country's gasoline and distillate fuel oil needs was then calculated by dividing their annual consumption in 2010 (GJ $year^{-1}$) by the average biofuel yield per country (GJ ha^{-1} $year^{-1}$) (see Appendix A for details about calculations).

3. RESULTS AND DISCUSSION

We identified 898 papers addressing the impacts of biofuels on biodiversity, 101 of which related first generation biofuels or microalgal systems to biodiversity in tropical and subtropical regions of the world. From this only three studies focused specifically on microalgal systems [62-64] (Tables A1 and A2, Appendix A). A citation report generated in Web of Science shows the increasing trend in number of citations for recent years, from five citations in 1993 to 5036 citations in 2015 and 4243 citations in 2016 (Fig. 1).

Increases in population growth, energy and food demands, and replacement of fossil fuels were identified as the main drivers for biodiversity changes arising from biofuel expansion.

A wide range of pressures that affect biodiversity were identified (Fig. 2). Because first generation biofuels make use of food crops, the pressures that impact biodiversity are closely related to those found for agricultural systems [65, 66]. These pressures corresponded to changes in land-use, overexploitation of resources, pollution and changes in environmental conditions that directly or indirectly impact biodiversity: land-use change (direct, indirect) and land-use intensification, increases in greenhouse gas emissions (leading to global warming), pesticide and fertilizer pollution, water depletion, overexploitation of soils (including soil erosion), increases in invasive species and genetic pollution, emissions of air pollutants and changes in environmental conditions that affect regional climate.

These pressures alter the state of ecosystems, resulting in a series of impacts on biodiversity (Fig. 2). Responses of society to these impacts may increase or decrease their magnitude. For instance, adaptation measures to climate change may drive further environmental degradation without an adequate planning for biodiversity conservation [67, 68], which outlines the importance of defining priorities that satisfy societal needs at the minimum costs for biodiversity [69].

These pressures can directly or indirectly impact biodiversity through several mechanisms. For instance, land-use change directly decreases available habitat, but can also lead to fragmentation that further increases potential extinction risks in the remaining habitat patches [70, 71]. Furthermore, the magnitude of biodiversity impacts resulting from biofuel crop expansion was found to be a function of initial land-use, type of biofuel system and its associated management practices and production technologies, and landscape configurations between biofuel crops and native ecosystems [12, 72]. We examine each category of pressure in detail in the following sections.

3.1 Direct land-use change and land-use intensification

Land-use change and land-use intensification were reported as the main pressures negatively impacting biodiversity due to the expansion of first generation biofuel systems [12, 73]. Replacement of native ecosystems and cropping intensification has been linked to habitat loss and degradation, decreases in richness and abundance of native vertebrates, affecting species of high conservation concern [12, 18, 74-78]. Furthermore, species that make use of biofuel plantations are mostly considered generalists and of low conservation value [15, 18, 72, 79-84].

Impacts on biodiversity depended upon the initial land-use, the type of biofuel production system and the landscape configuration. Reductions of species diversity are larger when transforming very biodiverse ecosystems [19] such as tropical forests [47, 75] and savannas [22, 77] and when using biofuel production systems that require a larger area per unit of energy produced [85]. In some circumstances, where biofuel crops recreate ecological conditions needed for the survival of native species, vertebrate diversity could increase. For instance, it has been proposed that the replacement of degraded lands by several biofuel crops could increase biodiversity values. In the Indonesian tropics, if degraded *Imperata* grasslands are replaced with oil palm plantations, which are structurally and functionally more complex than pastures, diversity of forest dependent vertebrates is expected to increase [84]—though mostly for low conservation value species—and lead to less pressure on forests [86]. In the USA, large patches of perennial crops (e.g. switchgrass, *Miscanthus*, mixed-grass prairies) are expected to be better than annual crops (e.g. maize) for maintaining populations of grassland specialists including endangered vertebrates (e.g. the Henslow's sparrow), provided that management practices (e.g. application of pesticides and harvesting) do not negatively affect the fitness of species [14, 18, 87].

If large patches of forests remain near to biofuel plantations, several forest species can use oil palm plantations, even endangered vertebrates. For instance, it has been shown that chimpanzees can make use of oil palm plantations, eating young leaves, flowers and fruits when other sources of food are scarce [88]. Populations of large and medium sized felids can make use of oil palm plantations if native forest tracts remain [82]. However, the benefits may be diminished by negative interactions between humans and wildlife where species are perceived as pests, or where they are systematically hunted [89-91].

Microalgal cultivation systems need less land than first generation biofuels in order to produce the same amount of energy, and thus it is expected that their widespread adoption would lead to less direct land-use changes and lower relative habitat losses for native species. However, estimates for lipid productivities are very wide, ranging between 2.3 and 136.9 kl $ha^{-1}year^{-1}$ [92]. Thus, we compare potential land savings based on a more conservative worldwide lipid estimation developed by Moody, McGinty [61], which closely resembles calculated productivities in experimental outdoor raceway ponds [93].

Our calculations show that microalgal cultivation systems consistently need less land than first generation biofuels (Fig. 3). For instance, in order to meet the USA gasoline and distillate fuel oil demands, microalgal systems would need 23.7% the area needed by olives and 40.8% the area needed by sugar beets cropped within the country (Tables A.5 and A.6, Appendix A). This is an optimistic scenario for first generation biofuels because yields are based on areas where crops grow well, and it is assumed that these crops can be readily used for biofuel production. For microalgal systems, conservative yields are assumed, based on the area-weighted average of average lipid yields within each country based on the Moody, McGinty [61] global estimates.

Furthermore, microalgae can be grown in areas not suitable for other crops (i.e. in poor soils and in regions with low precipitation values) [30, 32]. If microalgal production proves to be feasible in these areas, less land-use change and intensification in highly biodiverse regions is expected, although marginal or degraded lands can still retain considerable biodiversity values [74, 94], and construction of microalgal facilities will inevitably decrease available habitat for native species [63].

3.2 Indirect land-use change

Leakage effects result when economic activities are displaced into different regions where biofuels are grown [95]. Indirect land-use change occurs when agricultural lands are displaced into regions previously occupied by native ecosystems or non-intensive production systems including extensive pastures and agroforestry systems [17, 96-99]. For example, the European Union biofuel policies are expected to lead to increased land-use changes outside Europe and transfer environmental impacts to more biodiverse regions [100-102]. Biofuel cropping has also been related to indirect land-use change as a result of complex interactions between economic factors, including increases in food prices and economic incentives for biofuel production [103-105].

Infrastructure development associated with agricultural expansion can further drive land-use changes, as shown in tropical remote regions, where deforestation increases due to higher accessibility and cropping profitability when roads are constructed or paved [48, 106]. In fact, oil palm and soybean expansion are related to road expansion and further deforestation in Southeast Asia and South America [84, 107]. For instance, in the Brazilian Cerrado, increased accessibility to forests around soybean plantations has led to further deforestation for fueling the steel industry, which not only decreases the area of remaining forests but also generates profits for further soybean expansion [107, 108].

Microalgal systems are not considered to drive indirect land-use change [109]. This is because if they are produced in degraded, dry or marginal lands that are less suitable for food production, less competition with agricultural lands would occur, which is expected to lead to fewer leakage effects, land clearing and transformation of biodiverse systems. However, this assumption is contingent upon the feasibility of microalgal biofuel production in areas not suitable for agriculture production.

3.3 Increases in greenhouse gas emissions

Biofuel expansion affects the emission of greenhouse gasses via land-use change and energy intensive production systems [110], while coproducts can help in decreasing greenhouse gas emissions. These emissions of greenhouse gasses have been linked to local extinction and habitat shifts for native species through global warming [2, 3].

3.3.1 Greenhouse gas emissions as a result of land-use change

Clearing of rich carbon systems releases CO_2 when plant biomass is burnt and soil organic carbon is lost [111-113]. Fargione, Hill [20] estimated that 17 years would be needed by sugarcane ethanol production systems to recapture the CO_2 emitted after replacing Cerrado grasslands in Brazil. Oil palm production systems replacing peatland rainforests in Indonesia would need 423 years to recapture the emitted CO_2 [20]. [15] estimated that in Southeast Asia the replacement of native forests into oil palm can emit between 163 and 1,550 tons ha^{-1} of stored carbon. If peatland forests are transformed into oil palm crops, it could take up to 692 years by oil palm production systems to recapture this released carbon. Based on satellite images, Koh, Miettinen [16] estimated that between 2000 and 2010 conversion of forests into oil palm plantations in Malaysia, Borneo and Sumatra led to the loss of around 140 million Mg of aboveground biomass carbon. Even in tropical grasslands, significant carbon emissions are expected to occur if replaced by biofuel production systems [22]. Furthermore, first generation biofuel production can lead to indirect land-use changes, which would further drive clearing of native ecosystems for crop production, and thus increases greenhouse emissions [21].

Initial land-use is expected to alter the magnitude of CO_2 emissions under the construction of microalgal farms [114]. Because microalgal production systems need less land for producing the same amount of energy than terrestrial crops, and their production can be achieved in places with naturally lower carbon stocks (i.e. degraded and dry areas), it would be expected that much less CO_2 would be released following direct land-use changes when using microalgal systems compared to first generation biofuels. If degraded areas, dry areas and marginal lands are used for microalgal biofuel production, or even for the production of microalgal animal feed, less competition with crops is expected to occur, leading to less indirect land-use changes and lower CO_2 emissions. In fact, it has been estimated that the global expansion of microalgae as a feedstock for animal feed, in areas not suitable for agricultural production, could free almost 2 billion hectares of pastures and feed crops, where forest plantations can be established for bioenergy production and habitat restoration, leading to net atmospheric CO_2 reductions [115]. However, if rich carbon systems are used for microalgal production, CO_2 emissions may become substantial. For instance, Quiroz-Arita, Yilmaz [116] estimate that within the USA the CO_2 savings of microalgal systems may decrease between 3% and 85% as a result of losses in aboveground biomass and soil carbon associated to land-use changes.

3.3.2 Production technologies and greenhouse gas emissions

Biofuel production systems and their associated cropping management practices and conversion technologies affect the balance of greenhouse gas emissions [110]. In agriculture, greenhouse gas emissions come from energy consumed along the production chain (CO_2 emissions), fertilizer use (liberation of N_2O and CO_2), cultivation in flooding conditions (CH_4 emissions) and several soil management practices including tillage, addition of lime and irrigation frequency [117].

As a result, crops with lower fertilizer requirements, coupled with management practices that optimize nutrient uptake and soil carbon storage, and less energy-intensive production technologies, would lead to lower greenhouse gas emissions. For instance, in the USA biodiesel production from soybeans captures more greenhouse gasses than bioethanol production from maize (41% vs. 12% respectively; taking into account energy used for crop cultivation, biofuel production and transport), mainly becasue of lower agricultural inputs and less intensive processes for biofuel production [7].

In comparison to terrestrial crops, microalgal systems can offer higher CO_2 savings when using efficient technologies under optimal production conditions [118]. However, a consensus in an optimal production technology—that maximizes both cost-effectiveness and reductions in CO_2 emissions—has not been reached. This is because large commercial microalgal farms for biofuel production have not been deployed, and because of difficulties in comparing studies that have different system boundaries, sources of electrical energy, functional units, influence of coproducts and model parameters [92, 118, 119] (Table 1).

Open raceway ponds are estimated to be energetically more efficient than photobioreactors [42, 120], leading to higher CO_2 savings [121]. Using open ponds, carbon savings can increase due to higher productivities per unit area [40, 114, 122, 123], colocation of microalgal systems with CO_2 sources (e.g. use of flue gas) [38-40, 43, 124, 125] or wastewater systems [38, 39], use of technologies that allow nutrient recycling (e.g. water recycling) [43, 121, 124] and production of energy (e.g. anaerobic digestion for producing methane which can be used for electricity generation) [41, 92, 120, 122, 126-128]. However, Clarens, Nassau [38] suggest that anaerobic digestion for nutrient recycling and energy production is not the best approach for reducing greenhouse gas emissions compared to direct combustion of algal biomass, although increases in digestibility, methane production and nutrient recovery could increase the environmental benefits of this technology.

Increasing the energy efficiency of production methods is also important for reducing emissions, such as through improved water pumping methods and more efficient lipid extraction processes [42, 123]. In fact, wet extraction routes have potential for decreasing energy inputs and increase CO_2 savings [37, 42, 122], especially through hydrothermal liquefaction [126, 129]. Overall, increasing low-carbon energy sources for microalgal production systems, including heat, electricity grid, fertilizers, transport and building materials not derived from fossil fuels, would lead to further carbon savings [125].

Thus, substantial increased carbon savings in comparison to first generation biofuels are feasible. For instance, Lardon, Helias [37] estimated that assuming biomass productivities between 20 and 30 g m^{-2} d^{-1} for *Chlorella vulgaris* grown in in open raceway ponds under Mediterranean conditions, and using wet extraction lipid routes, microalgal production could lead to less global warming potential when compared to soybean and conventional diesel, but not to oil palm or rapeseed. However, this study did not take into account nutrient recycling through anaerobic digestion or culture medium recycling. Stephenson, Kazamia [114] estimated that the production of *C. vulgaris* in open raceway ponds under U.K. conditions could lead to higher carbon savings than biofuel obtained from soybean, sunflower and rapeseed grown in South Africa or from oil palm in Malaysia; assuming higher lipid productivities, production in degraded lands, use of flue gas from power stations, nutrient recycling and energy production through anaerobic digestion, and lower velocities for microalgal cultivation media. Clarens, Nassau [38] found that, assuming biomass yields of 91.1 Mg ha^{-1} $year^{-1}$ for brackish water species grown in Southwestern

USA conditions and lipid contents of 19.6%, greenhouse gas emissions per kilometer travelled would be lower compared to rapeseed.

3.3.3 Influence of coproducts in greenhouse gas emissions

Coproducts of biofuel production help in increasing CO_2 savings. These include dried distillers grains, feed products, CO_2, starch, syrup and oils (e.g. corn oil) in the case of bioethanol production from sugar and starch crops, as well as protein meal and glycerol from biodiesel production [9]. Microalgal systems can be designed to produce not only biodiesel or bioethanol as main biofuel products but also a wide arrange of coproducts that can be used for energy production, food and animal feed [35, 130]. For instance, using wet conversion routes it is possible to produce biodiesel, carbon monoxide, hydrogen, methane, ethane and propane, while through dry conversion, biodiesel, glycerol, pyrolysis oil and biogas can be produced [131].

Coproducts are considered fundamental for increasing the cost-effectiveness and sustainability of microalgal biofuel production systems [118, 130]. In particular, methane production has been identified as a key coproduct that increases carbon savings when it is combusted for replacing external energy requirements [41, 92, 120, 122, 128, 132].

3.4 Pesticide and fertilizer pollution

Pesticides and fertilizers can impact vertebrate biodiversity in biofuel crops and non-target areas, negatively affecting native ecosystems. Pesticides can directly and indirectly lead to the collapse of vertebrate populations as a result of several mechanisms, including direct poisoning, reduced amounts of prey, increases in occurrence of diseases [133-136] and decreases in fruit productivities when pollinator biodiversity is negatively affected [137].

Overuse of fertilizers can pollute soils with heavy metals that bioaccumulate in vertebrates [138] and indirectly alter biodiversity through increases in greenhouse gas emissions [117]. Eutrophication of aquatic systems as a consequence of runoff can lead to oxygen depletion and bioaccumulation of toxins produced by toxic algae blooms [139] and occurrence of diseases (e.g. nitrate accumulation in vertebrates) [140]. Besides the global warming potential of NO_x, increases in nitrogen compounds in the atmosphere have been suggested to reduce plant diversity and alter ecosystem functioning [141, 142].

The release of pollutants depends upon the type of biofuel production system and its associated management practices. For example, soybean cropping in the USA uses lower amounts of fertilizers and pesticides when compared to maize, leading to the release of 1% of the N, 8.3% of the P, and 13% of the pesticides, per net energy gain, used for maize ethanol production [7].

Unlike first generation biofuels, microalgal cultivation does not require the use of pesticides [35, 37, 143]. When grown in photobioreactors, contamination of cultures by pathogens and algae grazers does not often occur [32, 144]. In open ponds, methods other than pesticide addition help to decrease the incidence of undesired organisms, such as increases in pH and free ammonia concentrations [64, 93, 145, 146].

Microalgal systems make use of fertilizers mainly in the forms of nitrates, ammonium and phosphate [42]. It has been estimated that the production of 1 kg of biodiesel from *C.*

vulgaris grown in open raceway ponds under California conditions, needs 0.33, 0.71, 0.58, 0.27, and 0.15 kg of nitrogen, phosphorous, potassium, magnesium, and sulfur respectively [147]. At Pinjarra Hills (Brisbane, Australia) the production of 1 kg of biodiesel from *Scenedesmus dimorphus* requires 0.04 kg of monoammonium phosphate, 0.02 kg of magnesium sulfate, 0.2 kg of ammonium sulfate, plus 0.004 kg of micronutrients [93]. However, microalgal systems have lower eutrophication potential than first generation biofuels [37, 39, 41, 127], primarily because runoff can be controlled in contrast to terrestrial crops [39]. In fact, if cultivation wastewater is recycled, fertilizers would not reach aquatic systems, eliminating gray water footprints [131], and reducing nutrient requirements [43, 147]. For instance Yang, Xu [147] estimate that water recycling in open ponds using *C. vulgaris* could reduce fertilizers use by around 55%; and if using seawater or wastewater the use of nitrogen would decrease by 94%. Using sea/waste-water for algal culture can reduce nitrogen usage by 94% and eliminate the need for potassium, magnesium, and sulfur. However, if wastewater reaches aquatic systems negative impacts on biodiversity are expected due to eutrophication [42, 63, 64, 131].

3.5 Water depletion

Water depletion can affect biodiversity associated with water systems, because of direct withdrawals and changes in water quality, including increases in salinity and concentrations of minerals [148]. The water footprint (WF) can be divided into green WF (volume of rainwater consumed), blue WF (volume of surface and groundwater consumed) and gray WF (volume of polluted water) [149]. Microalgal systems have a green and blue WF as a result of evaporative losses in raceway open ponds, evaporative cooling in photobioreactors and evaporation from dry biodiesel conversion routes, while if wastewater is recycled or treated the gray WF should be zero [131]. As a consequence, for open ponds in California, the water footprint is expected to be reduced by around 84% if water is recycled, and by around 90% if seawater or wastewater are used [147].

Green and blue WFs using wet conversion routes and recycling water are between 2.7 and 32.6 kL per GJ of produced green diesel [131] (Table A.12, Appendix A). These values are lower than those obtained for terrestrial biofuel crops such as soybean, sugarcane, maize, rapeseed and sugar beet (Fig. 4). The variation in water requirements is a function of lipid productivity, local weather conditions and the architecture of the microalgal production system (photobioreactors or open ponds), being highest when using open ponds in places with high evaporation rates [43] and low lipid productivities [131]. Other factors that affect water consumption are the medium preference of microalgal strains (fresh, brackish or saline water) and the conversion technologies for biodiesel production (thermal drying and pyrolysis in dry conversion route vs. water reuse in wet conversion route), being higher when using freshwater species and when using dry conversion routes [131]. However, water use would be higher if it is not recycled. For instance, Clarens, Resurreccion [39] show that open raceway ponds in Virginia, Iowa, and California conditions, would need more water than rapeseed and maize cropped in the same locations, provided that there is not water recycling.

3.6 Overexploitation of soils

Soils are considered a renewable resource when managed in a sustainable way, by avoiding erosion and maintaining or increasing fertility and soil biodiversity [150]. Fertile soils increase food security, decrease desertification, help in climate change mitigation and increase biodiversity [151-153]. Biofuel production systems may negatively affect in-situ soil productivity, when using management practices that increase soil erosion and affect physical, chemical and biological properties in soils (e.g. indiscriminate tillage) [154, 155]. Additionally, soil erosion can negatively affect aquatic biodiversity due to eutrophication, sedimentation and the alteration of physical and chemical properties in aquatic systems [152].

Soils are not used directly for microalgal production systems. However, construction of open ponds could increase soil erosion, soil compaction and alter soil properties including texture and fertility [62, 63], if soil conservation practices are not implemented. The construction of elevated ponds (e.g. using bricks) could decrease soil removal (which could be around 225,000 and 450,000 tons ha^{-1}, assuming pond depths between 15 and 30 cm and soil bulk densities at 1.5 g/cm^3), although at higher economic costs. After ponds are constructed, soil erosion is expected to be lower than in agricultural production systems, which have reported erosion rates between 0.5 and 400 tons ha^{-1} $year^{-1}$ [151, 156].

3.7 Increases in invasive species and genetic pollution

Invasive species are a major threat to biodiversity [157, 158]. Biofuel crops can increase the occurrence of invasive species within and outside plantations, creating more favorable environmental conditions for the arrival and persistence of invasive organisms [159].

Furthermore, some species may become invasive as a result of their increased propagule production, dispersal and/or persistence abilities [160, 161]. Crops like sugarcane, soybean, sugar beet and maize are not considered invasive, while others have traits that increase their invasive potential (e.g. rapeseed produces large seed quantities that can be dispersed by a wide arrange of agents, and can hybridize with wild native varieties) [161, 162] (Table 2). As a consequence, it has been estimated that terrestrial plants suitable for biofuel production have two to four times higher potential than other crops to become naturalized or become invasive [161, 163].

In relation to microalgal production systems, the potential invasion of water systems could happen if leakage of growth medium, which may include genetically engineered species, occurs [14, 42, 62]. This is because the same traits that allow them to grow in a wide range of environmental conditions predispose them for invasiveness potential [164]. If toxic species are released (e.g. *Anabaena circinalis, Oscillatoria agardhii, Cylindrospermopsis raciborskii*) unexpected changes in ecosystem function could occur under favorable environmental conditions (e.g. expansion of toxic algae blooms in eutrophic aquatic systems) [161, 165]. However, if native or local microalgal strains are used for biofuel production, or if water is recycled, invasion potential is expected to decrease.

3.8 Emissions of air pollutants and changes in factors that affect regional climate

In addition to greenhouse gasses (section 3.2), the production and use of biofuels generate toxic substances that are released into the air, and that can negatively impact ecosystem functions and biodiversity. These pollutants include nitrogen oxides (NO_x), ammonia

(NH_3), carbon monoxide (CO), volatile organic compounds (VOC), particulate matter (PM), oxides of sulfur (SOx) [166], methyl bromide (CH_3Br) [167] and nitrous oxide (N_2O) [168]. These pollutants can beproduced during cropping (including fuel combustion for machinery operation during cropping practices, chemical applications and soil disturbance), biofuel production and combustion [166], and during the construction of facilities, extraction and shipping of resources [41]. They lead to increases in acidification (i.e. acid rain), ozone layer depletion, and photochemical oxidation, among other environmental impacts [169]. Their effects include changes in the structure and function of both terrestrial and aquatic ecosystems, including alterations in species composition [170-172].

Pollutant release differs among biofuel production systems. For instance, taking into account total life-cycle emissions, it has been shown that soybean biodiesel produced in the USA releases less air pollutants when compared to corn ethanol per net energy gain [7], while corn grain ethanol blended with gasoline (E-85) increases the amount of emitted pollutants (CO, VOC, PM10, NOx, SOx) in comparison to gasoline per unit of energy released upon combustion [173]. After accounting for cultivation and harvesting, it is estimated that in the USA corn grain ethanol would emit more pollutants per produced gallon than ethanol from switchgrass, corn stover, wheat straw and forest residues [166].

Compared to first generation biofuels, emission of air pollutants can be lower for microalgal systems [41, 127]. Collet, Helias [41] estimated that biodiesel produced from *C. vulgaris* grown in open raceway ponds in Mediterranean conditions coupled with anaerobic digestion and assuming low-energy cultivation systems, led to lower potential acidification, ozone layer depletion and photochemical oxidation per MJ of combusted fuel than first generation biofuels after accounting for extraction and shipping of resources, cultivation and biofuel production, and construction and dismantling of facilities. Using the same species and open raceway ponds in Mediterranean conditions, Lardon, Helias [37] found lower acidification potential in comparison to rapeseed, but higher ozone layer depletion and photochemical oxidation when compared to first generation biofuels. However, they did not account for nutrient recycling (e.g. using anaerobic digestion), which would lead to lower energetic burdens and decrease air pollutants.

Air pollution may also impact biodiversity via changes in atmospheric temperature and weather patterns: the release of substances that increase tropospheric ozone (CO, NO_x, VOC, CH_4) exacerbates global warming potential, while the release of aerosol particles (including sulfate, organic carbon, black carbon, biomass burning, nitrate and mineral dust aerosols) increase albedo and thus exert an atmospheric cooling effect [174]. Furthermore, it has been shown that aerosols affect not only cloud albedo but also the size and number of droplets in clouds, which can alter precipitation regimes worldwide depending on meteorological conditions [175-177]. Changes in surface albedo (that result from land-use change), coupled with increases in tropospheric ozone and aerosols, can alter atmospheric temperature and precipitation patterns, with potential impacts on ecosystems. While deforestation for biofuel production would decrease regional humidity and increase atmospheric temperature, evaporation from microalgal ponds could have the opposite effect [64], with potential increases in regional precipitation and additional cooling effects as water evaporates [177].

3.9 Considerations for the adoption of sustainable biofuel production systems

Transforming biodiverse landscapes into biofuel cropping systems is a detrimental practice for the short and long-term conservation of biodiversity. Biofuel production should only be promoted where few direct and indirect impacts on biodiverse systems are expected; implying that crops with low biofuel yields or crops that compete with available lands for agriculture or for the conservation of biodiversity should be avoided. Currently, biofuel is primarily produced from suboptimal crops that do not have the highest biofuel yields (Figs. A.1 to A.5, Tables A.7 to A.11, Appendix A) and that compete with agricultural lands or highly biodiverse landscapes. Thus, biofuel production systems, management practices and production technologies that have lower environmental footprints should be encouraged. This means that only systems with low potential to cause direct and indirect land-use change of agricultural lands and biodiverse regions and that offer higher carbon savings should be deployed, and also those systems with high freshwater consumption, high potential for pollution, soil degradation, and high invasiveness should be avoided.

4. CONCLUSIONS

The main pressures negatively impacting biodiversity due to biofuel production are direct and indirect land-use change, particularly when ecosystems with high biodiversity values (e.g. tropical and subtropical forests, native grasslands) are transformed into biofuel crops. Several other pressures that negatively impact biodiversity include greenhouse gas emissions, pesticide and fertilizer pollution, water depletion, overexploitation of soils, invasive species and genetic pollution, emissions of air pollutants and changes in factors that affect regional climate (e.g. alterations in albedo and evapotranspiration patterns), which directly or indirectly impact biodiversity.

Biofuel production systems and their associated management practices influence the magnitude of the impacts on biodiversity. In relation to land-use change, systems with higher productivity per unit area are expected to lead to less direct and indirect land-use changes, especially if their cultivation does not occupy fertile agricultural lands and does not compete with areas of high biodiversity value. Higher greenhouse gas savings would be achieved both when transforming low carbon systems (e.g. eroded lands) into biofuel crops and when using biofuel systems with lower energy intensive processes. Pollution would be reduced using systems with lower fertilizer and pesticide inputs, combined with less energy intensive processes that are currently powered by fossil fuels. Furthermore, biofuel and their associated management practices can be designed to achieve better water efficiency, less soil degradation (e.g. low soil erosion), and reduced invasive species and genes potential.

We estimated that microalgal production systems would need substantially less cultivation land compared to first generation biofuels per unit of produced energy, making them the most feasible option worldwide in term of reduced land needs, especially within tropical and subtropical regions of the world where they achieve higher productivities. Open ponds are the preferred option for increasing carbon savings, because of their lower energy-intensive production processes compared to photobioreactors. Increased carbon savings in microalgal systems can be achieved with the optimization of productivities per unit area, colocation with industrial CO_2 sources or wastewater systems, nutrient recycling and energy production (e.g. using anaerobic digestion and recycling water), and use of coproducts for reducing external energy sources (e.g. combustion of methane for internal electricity generation). Increasing energy efficiencies (e.g. using wet conversion routes for biodiesel production) and replacement of external fossil energy sources is expected to reduce greenhouse gas emissions. Increased energy efficiencies and nutrient recycling are expected to decrease emissions of air pollutants (NO_x, NH_3, CO, VOC, PM, SO_x, N_2O). Water recycling is also essential in order to reduce the gray water footprint, avoid pollution derived from the release of growth medium and decrease the chances of spreading invasive and potentially harmful microalgal strains.

Finally, we call for a better inclusion of biodiversity in future studies on environmental impacts of biofuel production systems as it is currently underrepresented, particularly in life cycle assessments [178-180].

ACKNOWLEDGEMENTS

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Table 1. Comparison between several life cycle assessments developed to date, in relation to system boundaries, main processing technologies, measured environmental impacts and main results. Open raceway pond (OP), photobioreactor (PB), open raceway pond integrated with photobioreactor (OP-PB), Not Stated (N.S.)

Species	**Growing**	**System boundaries**	**Main processing technologies**	**First gen. biofuels**	**Measured environmental impacts**	**Main results**	**Notes**	**Ref.**
Chlorella vulgaris	OP	Cradle-to-combustion analysis for the fuel, cradle-to-grave analysis for the facility. Includes extraction and production of raw materials, facility construction and dismantling, biofuel production and use in the engine.	1) Advanced drying followed by hexane extraction. 2) Direct extraction from the wet algal paste.	Rapeseed, soybean, oil palm	Abiotic depletion, potential acidification, eutrophication, global warming potential, ozone layer depletion, marine toxicity, human toxicity, land competition, emission of ionizing radiation, photochemical oxidation.	Lower land competition and eutrophication compared to first generation biofuels. Lower acidification potential in comparison to rapeseed and lower human toxicity in comparison to rapeseed and oil palm. Lower global warming potential in comparison to soybean. Higher abiotic depletion, ozone layer depletion, marine toxicity, ionizing radiation and photochemical oxidation compared to first generation biofuels.	Assumed biomass productivities at 20-30 g m^{-2} day^{-1} in Mediterranean conditions. Functional unit as the combustion of 1 MJ of fuel in a diesel engine.	[37]
Chlorella vulgaris	OP	Microalgal cultivation to downstream fuel production. Includes cultivation, harvesting, dewatering, oil extraction, oil upgrading and nutrient recycling.	Harvesting by flocculation and centrifugation, followed by dry conversion routes for lipids (transesterification) or wet conversion routes lipids (hydrogenation).	Several vegetable oils and sugar crops	Land use	Large positive energy balance in comparison to first generation biofuels can be achieved. Potential to increase productivity and decrease nutrient usage by nitrogen deprivation during growing. Larger land savings when increasing productivity per unit area.	Assumed lipid contents between 19.7-43% and 15% of nutrient recycling for wet processing route.	[123]
Chlorella vulgaris	OP	Production, harvesting and concentration of algae, methane extraction and combustion, facility construction and dismantling, extraction and shipping of resources.	Harvesting by settling and centrifugation followed by injection in anaerobic digesters, biogas burning and production, CO_2 reinjection into cultures.	Rapeseed, oil palm	Abiotic depletion, potential acidification, eutrophication, global warming potential, ozone layer depletion, human toxicity, land competition, emission of ionizing radiation, photochemical oxidation.	Lower impacts compared to first generation biofuels for acidification, eutrophication, ozone layer depletion and photochemical oxidation, when assuming low energy consumption by paddlewheels and pumping water [39]. Global warming potential decreases when assuming low energy consumption.	Assumed biomass productivities of 25 g m^{-2} day^{-1} in Mediterranean conditions (Narbonne, France). Low energy consumption of paddlewheels and pumping water is assumed based on Clarens, Resurreccion [39]. Functional unit as the combustion of 1 MJ of fuel in an internal combustion engine.	[41]
Chlorella vulgaris	OP	Cultivation, harvesting, lipid extraction, fuel distribution and combustion by end users.	Harvesting by flocculation, drying and algae oil extraction.	N.S.	Greenhouse gas emissions.	Higher CO_2 emissions compared to conventional diesel for most scenarios.	Assumed biomass productivities of 75 tons ha^{-1} $year^{-1}$ and average algae oil content of 30-70%. Explicit analyses in U.K., France, Brazil, China, Nigeria and Saudi Arabia. Assumes use of CO_2 from nearby power plants (12.5%). Includes three options for coproduct use: co-firing of biomass residues, direct combustion in a biomass/heating system or a biomass combined heat and power unit. Functional unit set as 1 MJ of biodiesel produced from algae oil.	[125]

Chlorella vulgaris	OP	Cradle-to-gate, including processes upstream of dried biomass production.	Harvesting by centrifugation or chamber filter press followed by two algal drying options (natural gas based drying or waste heat drying).	N.S.	Greenhouse gas emissions, direct water demands.	Greenhouse gas savings for 5 out of 8 scenarios analyzed. Water demands were related to geographic locations and their local evaporation rates.	Assumed algae composition of 20% lipids, 25% carbohydrates and 50% protein at 21 geographic locations in the contiguous USA. Includes colocation with natural gas power plant and water recycling. Functional unit as 1 MJ of dried algal biomass.	[43]
Chlorella vulgaris	OP	Culture, harvest, drying, extraction and esterification.	Drying and lipid extraction.	Maize, potato, sugarcane, sugar beet, sorghum, soybean	Water footprint, nutrient depletion.	The water footprint is in general lower compared to first generation biofuels, and lowest if recycling water or using wastewater/seawater. Nutrient usage is lower when recycling water and when using wastewater or seawater.	Assumed use of freshwater, seawater, and wastewater in California conditions.	[147]
Chlorella vulgaris	OP, PB	Cultivation, harvesting and lipid extraction, anaerobic digestion, oil extraction, esterification, transport of oil and final combustion in vehicles.	Harvesting by flocculation, followed by centrifugation (for open raceway ponds), cell disruption by homogenization, hexane lipid extraction, anaerobic digestion for onsite electricity use.	Rapeseed, sunflower, soybean, oil palm	Global warming potential (CO_2, NO_2, CH_4), water depletion.	Lower global warming potential for open raceway ponds and compared to rapeseed, sunflower and soybean biodiesel grown in arable lands in South Africa and compared to oil palm grown in Malaysia. Higher water requirements for photobioreactors under U.K conditions.	Assumed oil productivities at 40 tons ha^{-1} $year^{-1}$ and production in degraded lands in U.K. Assumes nitrogen deprivation, coproduct allocation, use of flue gas from power stations (12.5% CO_2). Functional unit as the combustion of 1 ton of biodiesel in a car engine filled at a U.K. station.	[114]
N.S.	N.S.	Well-to-wheel. Includes cultivation, processing and biofuel production, transport and final use of biodiesel.	Harvesting, and extraction followed by transesterification and excess methanol recycling.	Soybean	Abiotic depletion potential, global warming potential, ozone depletion potential, photochemical oxidation potential, acidification potential, eutrophication potential, human toxicity potential, freshwater aquatic ecotoxicity potential, marine aquatic ecotoxicity potential, terrestrial ecotoxicity potential.	Lower impacts in comparison to first generation biofuels for most assessed impacts.	Assumed biomass productivities between 5-50 g m^{-2} day^{-1} and lipid contents between 15-80% in China conditions. Includes coproduct allocation and analyses for water recycling. Functional unit as 1 MJ of energy from biodiesel well-to-wheel.	[127]
N.S.	OP	Cradle-to-gate, including the processes upstream of dry biomass production.	Harvesting through flocculation and centrifugation.	Rapeseed, maize	Water use, greenhouse gas emissions, eutrophication potential, land use.	Higher impacts than first generation biofuels in terms of energy use, greenhouse gas emissions and water use, mainly driven by demand for CO_2 and fertilizer. Lower impacts for land use and eutrophication potential compared to first generation biofuels. Using wastewater leads to CO_2 savings and decreases water footprint.	Th model was run for Virginia, Iowa, and California, USA. Included scenarios for colocation with wastewater and industrial CO_2 sources. Functional unit as 317 GJ of biomass-derived energy.	[39]
N.S.	OP-PB	Well-to-pump. From cultivation to biofuel final use at refueling stations.	1) Filtration through chamber filter press followed by drying and hexane extraction. 2) Centrifugation followed by drying and hexane extraction.	Soybean	CO_2 emissions, emissions of air pollutants (VOC, CO, NO_x, particulate matter, SO_x, CH_4).	Higher CO_2 savings in comparison to soybeans when using filter press and coproduct allocation. High energy consumption for thermal algae dewatering.	Assumed 30% lipids, 31% carbohydrates and 37.5% proteins. Includes recycling of water and addition of external CO_2 sources. Includes coproduct allocations. Functional unit as 1,000 MJ of energy at a refueling station.	[124]

N.S.	OP-PB	Well-to-wheel. Includes cultivation, harvesting and dewatering, lipid extraction, lipid conversion to a liquid transportation fuel, and coproducts from defatted algae.	1) Harvesting by best filter press followed by wet lipid extraction and hydrothermal liquefaction, hydrotreatment for lipid conversion and use of anaerobic digestion or animal feed. 2) Harvesting by centrifugation followed by hexane lipid extraction, lipid conversion by transesterification and use of anaerobic digestion or animal feed.	N.S.	Global warming potential.	Lower global warming potential for wet lipid extraction routes compared to dry extraction and for high productivity scenarios.	Estimated ranges of expected values of life cycle assessment metrics based on Monte Carlo simulations. Assumed 1210-ha microalgal facility using seawater and three ranges in biomass productivities: low (2.4−16 g m^{-2} day^{-1}), base (17−33 g m^{-2} day^{-1}), and high (34−50 g m^{-2} day^{-1}). Functional unit defined as 1 MJ of liquid biofuel (biodiesel or “green” diesel).	[122]
N.S.	OP, PB	Cradle-to-wheel. From upstream of the delivered energy product to consumer use (passenger automobile).	Harvesting by auto-flocculation followed by thickening, homogenization, lipid extraction, solvent recovery and anaerobic digestion, belt-filter pressing and transesterification for biodiesel production.	N.S.	Climate change (global warming potential from greenhouse gas emissions), net water use, net eutrophication potential.	Open ponds that use brackish water are the preferred option for decreasing global warming potential.	Assumed biomass yields between 41.6-95.7 Mg ha^{-1} $year^{-1}$, and lipid contents between 13.4-32.4% using freshwater and brackish water species. Assumes use of virgin CO_2 from commercial sources. Includes production of biodiesel and methane-derived bioelectricity.	[120]
N.S.	OP, PB	Several system boundaries based on reviewed studies.	Several processing technologies based on reviewed studies.	Sugarcane	CO_2 emissions.	Higher variability in CO_2 emissions in comparison to sugarcane. Emissions decrease when using open raceway ponds and when recycling water.	Reviews different studies that relate CO_2 emissions with production technologies.	[121]
N.S.	OP	Cradle-to-grave, excluding the production facilities and its construction	Addition of flocculants for algae concentration followed by heating, centrifugation and lipid extraction using methanol and a catalyst. Anaerobic digestion for electricity generation.	Rapeseed	Greenhouse gas emissions (CO_2, CH_4, NO_2).	Higher CO_2 savings in comparison to rapeseed, highest when assuming high algae productivities and when using CO_2 from an ammonia plant.	Assumed biomass productivities at 15-30 g m^{-2} day^{-1} and use of salt water in Australian conditions. Includes three options for CO_2 feeding: in pure form from an ammonia plant, from flue gas (15% concentration) or delivered by truck in liquefied form. Functional unit as combustion of enough fuel in an articulated truck diesel engine to transport one ton of freight one kilometer.	[40]

Nannochloropsis salina	OP-PB	Well-to-pump, including microalgal cultivation through the delivery of fuel to the filling station. Well-to-wheel for comparison with conventional diesel.	Harvesting by settling, dissolved air flotation and centrifugation, followed by pressure homogenization, hexane extraction and nutrient recovery through anaerobic digestion.	N.S.	Greenhouse gas emissions (CO_2, CH_4, NO_2).	Lower CO_2 emissions for the scenario that includes improved algae productivity and anaerobic digestion.	Assumed biomass productivities at 25 g m^{-2} day^{-1} and lipid concentrations between 25-50%. Four scenarios were taken into account: baseline, improved algal productivity, supercritical CO_2 extraction, no nutrient recycling (lipid extracted biomass used as cattle feed). Functional unit as 1MJ of biofuel produced.	[128]
Nannochloropsis sp.	OP-PB	Cradle-to-gate, including microalgal cultivation through biodiesel production.	Dewatering and drying through the use of flocculants and centrifugation, followed hexane extraction and transesterification.	N.S.	CO_2 emissions.	High energy consumption for lipid extraction and biodiesel production. CO_2 savings were not found.	Assumed biomass productivities of 25 g m^{-2} day^{-1} in Singapore conditions and using seawater. Lipid contents between 25-45%. Functional unit as 1 MJ biofuel.	[181]
Phaeodactylum sp., *Tetraselmis* sp.	OP	Well-to-wheel. Includes extraction of raw materials, cultivation and lipid extraction, conversion and use of biofuel in vehicles.	Cultivation followed by harvesting through auto-flocculation, thickening and homogenization. Several scenarios for biomass processing: 1) Anaerobic digestion of bulk algae biomass for production of electricity from methane. 2) Production of biodiesel from algae lipids coupled with anaerobic digestion for producing electricity. 3) Production of biodiesel from lipids and direct combustion for electricity production from residual algae biomass. 4) Direct combustion of algae biomass for producing electricity.	Rapeseed, maize	Net energy use, water use, and greenhouse gas emissions.	Highest energy efficiencies when using direct combustion of algae biomass for producing electricity, and lowest when producing biodiesel from algae lipids coupled with anaerobic digestion for producing electricity. Use of wastewater and flue gas increases energy efficiencies. Algae systems are better than rapeseed and maize in relation to vehicle kilometers traveled per ha. Greenhouse gas emissions and water used per kilometer traveled are lower compared to rapeseed	Assumed biomass yields of 91.1 Mg ha^{-1} $year^{-1}$ and lipid contents at 19.6% using brackish water species in Southwestern USA conditions and in marginal lands. Includes scenarios for CO_2 sources: virgin CO_2, carbon capture from coal-fired using chemical sorption, use of flue gas 12.5% CO_2 power plant. Includes one scenario for wastewater use. Makes use of stochastic inputs to capture uncertainty in processes. Functional unit as usable energy production per unit land area (vehicle kilometers traveled per ha) and environmental burdens (net energy use, water use, and greenhouse gas emissions per vehicle kilometers traveled**).**	[38]
Scenedesmus dimorphus	OP	Well-to-pump. Cultivation, dewatering, thermochemical bio-oil recovery, bio-oil stabilization, conversion to renewable diesel, and transport to the pump.	Harvesting by membrane filtration and centrifugation, followed by thermochemical conversion (hydrothermal liquefaction vs. pyrolysis)	Maize, soybean	Net energy ratio, greenhouse gas emissions.	Hydrothermal liquefaction leads to carbon savings in contrast to pyrolysis. Carbon savings are higher in comparison to maize bioethanol.	Biomass productivities at 6.5 g m^{-2} day^{-1} in Arizona conditions.	[129]

Several species	OP	Upstream resources, cultivation, conversion into biodiesel followed by anaerobic digestion.	Normalization of studies based on cultivation in open ponds, conversion into biodiesel and use of anaerobic digestion.	Maize, soybean	Greenhouse gas emissions	Energy consumption and greenhouse gas emissions would be similar to those obtained for terrestrial alternatives.	Meta-analysis based on six life cycle assessments for microalgal biofuel production. Functional unit set as 1,000 L biodiesel.	[118]
Several species	OP	Several system boundaries based on reviewed studies.	Several technologies based on the reviewed studies.	Rapeseed	Greenhouse gas emissions. Overview for water use, land use, nutrient and fertilizer use, carbon fertilization, fossil fuel inputs, eutrophication, genetically modified algae, algal toxicity.	Decreases in CO_2 for raceway ponds compared to photobioreactors, reaching similar values to those obtained for rapeseed. Major energy inputs are associated with pumping and mixing during cultivation and to the provision of heat for algae drying.	Review of seven life cycle assessments.	[42]
Several species	OP, PB	Several system boundaries based on reviewed studies.	Several biomass processing methods including solvent extraction, hydrothermal liquefaction, secretion, pyrolysis, supercritical water, in-situ transesterification.	N.S.	Global warming potential.	Global warming potential varies between production technologies and system boundaries. Thermochemical conversion and anaerobic digestion seem promising alternatives that reduce energy inputs.	Review that includes information about global warming potential for a set of microalgal production technologies.	[92]
Tetraselmis chui	PB	Cradle-to-grave, including cultivation, harvesting, processing and products (utilization and consumption).	Harvesting through primary to tertiary dewatering and spray drying, followed by slow pyrolysis, oil extraction by solvent and production of biogas, bio-oil, biodiesel and biochar.	Rapeseed, soybean	Global warming, abiotic resource depletion (excluding water), land transformation and use, water resource depletion, eutrophication, acidification, eco-toxicity, human toxicity, photochemical smog, ozone depletion, ionizing radiation, respiratory effects.	Lower global warming and land use in comparison to first generation biofuels. Higher eutrophication, water use, ecotoxicity, acidification, photochemical smog and respiratory effects in comparison to first generation biofuels. Improvements are expected if using hydrothermal liquefaction.	The system was modeled in Queensland conditions, Australia. Includes coproduct allocation, CO_2 addition from power plant station (13%), water use for evaporative cooling and water recycling. Functional units defined as 1 MJ of pyrolysis biogas combusted for electricity and 1 MJ of pyrolysis bio-oil combusted for electricity or extracted lipid refined for transport fuel.	[126]

Table 2. Comparison of widely used first generation biofuel crops in relation to their potential for genetic pollution and invasiveness.

Biofuel crop	**Center of origin**	**Dispersal units**	**Non-human effective dispersal vectors**	**Reported genetic pollution**	**Reported invasiveness**
Oil palm (*Elaeis guineensis*)	Tropical Africa [182]	Seeds	Animals	No	Yes [183, 184]
Maize (*Zea mays*)	Americas	Seeds	N.A.	Yes [185, 186]	No
Rapeseed (Brassica napus)	Mediterranean region [187]	Seeds	Autochory, wind, water, animals [188]	Yes [189, 190]	Yes [191, 192]
Sugarcane (*Saccharum* sp.)	Tropical region [193]	Cuts, seeds (low viability of seeds)	N.A.	No	No
Soybeans (*Glycine max*)	China	Seeds	Autochory [194]	Yes [195, 196]	No

Figure 1. Citation report using the Science Citation Index Expanded (SCI-EXPANDED) and the Emerging Sources Citation Index (*ESCI*) in Web of Science and the following combination of keywords: (biofuel OR bioenergy) AND (biodiversity OR wildlife), (biofuel OR bioenergy) AND (fish* OR bird* OR avian OR mammal* OR reptil* OR amphibian*).

Figure 2. Drivers, Pressures, States, Impacts and Responses of biofuel production on biodiversity, based on the reviewed literature and following the DPSIR framework.

Figure 3. Superimposed circles showing the area of cultivation land (km^2) required to meet gasoline and distillate fuel oil demands for each country in 2010, when comparing microalgal systems with the most productive biodiesel and bioethanol crop per country. For first generation biofuels, yields are based on areas where crops possibly grow best (average yields between 2005 and 2014) [55], while the most frequent value (area-weighted average) of average lipid yield within countries is used for microalgal systems. Microalgal lipid estimations are based on Moody, McGinty [61] .

Figure 4. Water consumption per unit of produced energy (GJ) derived from biodiesel (soybean, oil palm, microalgae) and bioethanol (maize, sugarcane). Based on calculations by Gerbens-Leenes, Xu [131] for wet conversion of microalgal biodiesel and assuming water recycling. Available water footprints for first generation biofuels were obtained from Mekonnen and Hoekstra [149]. Microalgal systems in New Mexico and Perth consist of open ponds using salty water (OPS), in Hawaii correspond to a combination of open ponds and photobioreactors using fresh water (OPF-PBF), in Italy consist of photobioreactors

using salty water (PBS), and in the Netherlands, France and Algeria consist of photobioreactors using fresh water (PBF).

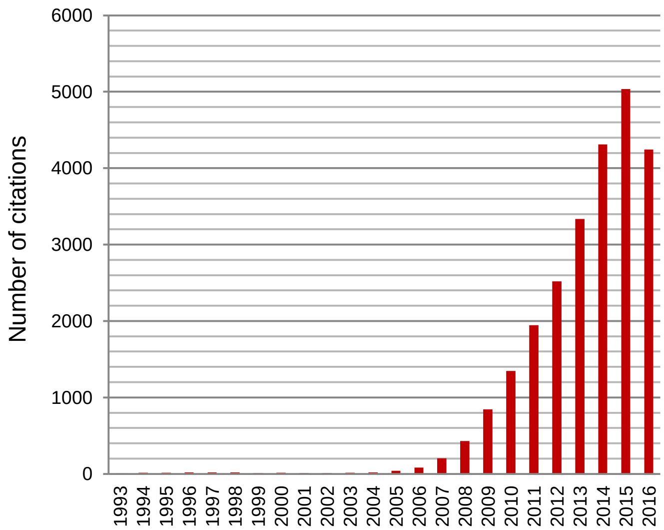
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Figure 1.

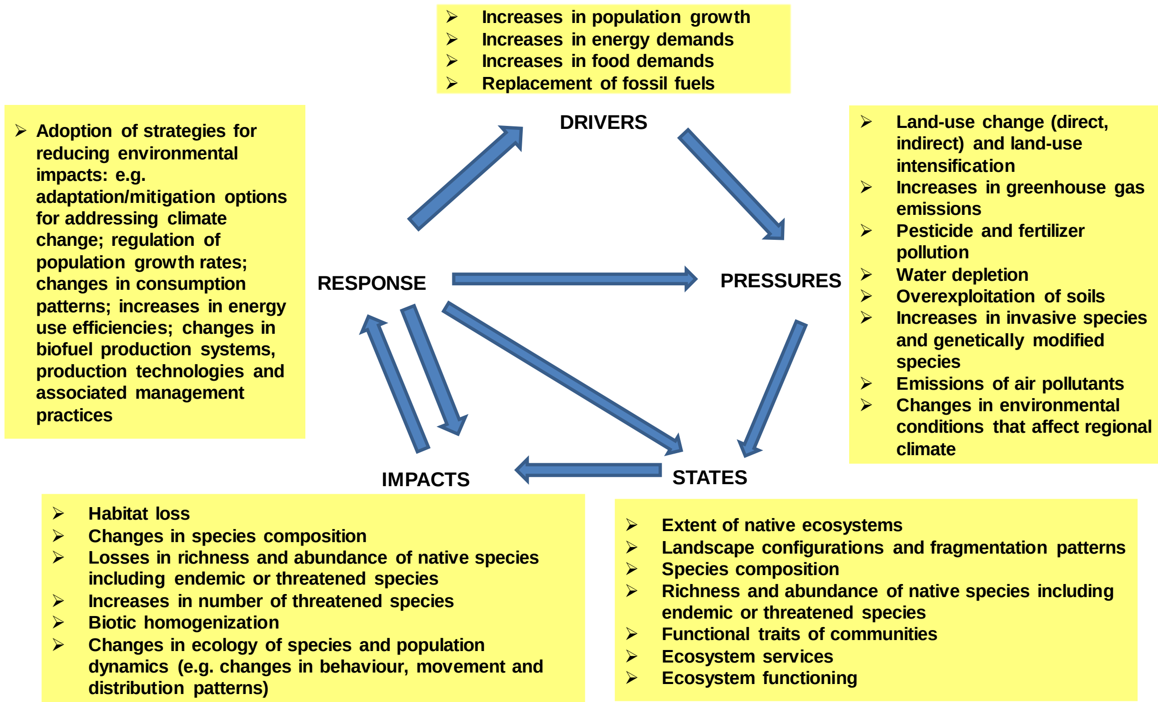

Increases in population growth
Increases in energy demands
Increases in food demands
Replacement of fossil fuels
DRIVERS
Adoption of strategies for reducing environmental impacts: e.g. adaptation/mitigation options for addressing climate change; regulation of population growth rates; changes in consumption patterns; increases in energy use efficiencies; changes in biofuel production systems, production technologies and associated management practices
RESPONSE
PRESSURES
Land-use change (direct, indirect) and land-use intensification
Increases in greenhouse gas emissions
Pesticide and fertilizer pollution
Water depletion
Overexploitation of soils
Increases in invasive species and genetically modified species
Emissions of air pollutants
Changes in environmental conditions that affect regional climate
IMPACTS
STATES
Habitat loss
Changes in species composition
Losses in richness and abundance of native species including endemic or threatened species
Increases in number of threatened species
Biotic homogenization
Changes in ecology of species and population dynamics (e.g. changes in behaviour, movement and distribution patterns)
Extent of native ecosystems
Landscape configurations and fragmentation patterns
Species composition
Richness and abundance of native species including endemic or threatened species
Functional traits of communities
Ecosystem services
Ecosystem functioning

Figure 2.

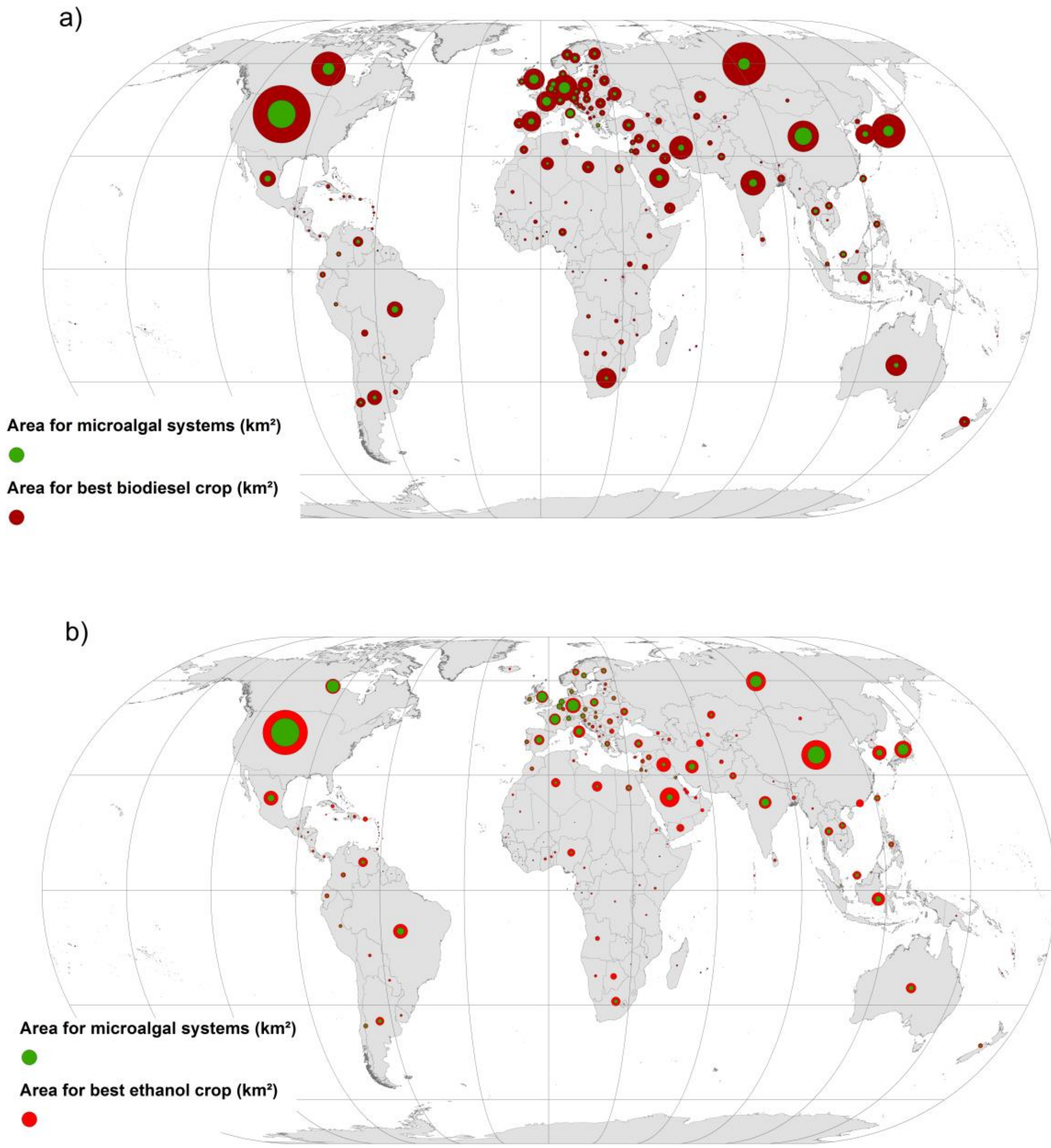
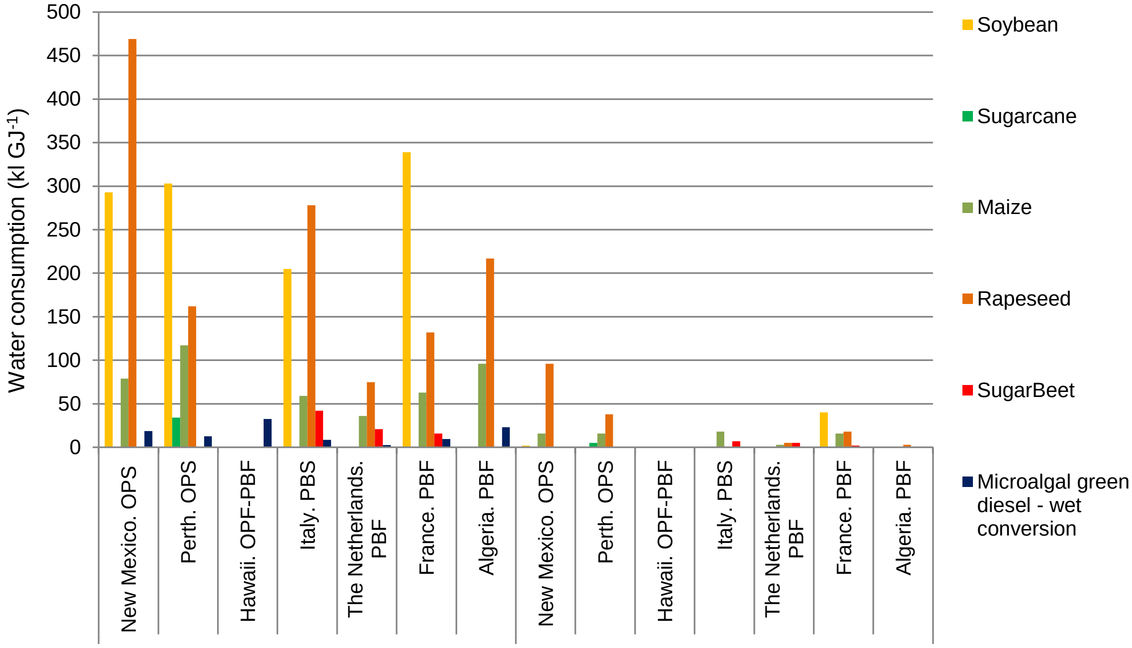

a)
Area for microalgal systems (km²)
Area for best biodiesel crop (km²)
b)
Area for microalgal systems (km²)
Area for best ethanol crop (km²)

Figure 3.



Water consumption (kl GJ-1)
500
450
400
350
300
250
200
150
100
50
0
New Mexico. OPS
Perth. OPS
Hawaii. OPF-PBF
Italy. PBS
The Netherlands. PBF
France. PBF
Algeria. PBF
New Mexico. OPS
Perth. OPS
Hawaii. OPF-PBF
Italy. PBS
The Netherlands. PBF
France. PBF
Algeria. PBF
Soybean
Sugarcane
Maize
Rapeseed
SugarBeet
Microalgal green diesel - wet conversion

Figure 4.

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