

Agave: a promising feedstock for biofuels in the water-energy-food- environment (WEFE) nexus

Xiaoyu Yan^{*, ‡, a, b}, Kendall R. Corbin^{‡, c, ^}, Rachel A. Burton^c, and Daniel K. Y. Tan^d

^a. Environment and Sustainability Institute, University of Exeter, UK.

E-mail: Xiaoyu.Yan@exeter.ac.uk; Telephone: 01326 259485

^b. College of Engineering, Mathematics and Physical Sciences, University of Exeter, UK

^c. ARC Centre of Excellence in Plant Cell Walls, University of Adelaide

^d. The University of Sydney, Sydney Institute of Agriculture, School of Life and Environmental Sciences, Faculty of Science, Sydney, NSW 2006, Australia

* Corresponding author

‡ These authors contributed equally

^ Current address: Environmental Epigenetics and Genetics Group, Department of Horticulture, College of Agriculture, Food and Environment, University of Kentucky, Lexington, Kentucky, USA

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Abstract

The aim of this study was to conduct the first comprehensive life cycle assessment and economic analysis on ethanol produced from agave. Compositional and field data from a field experiment in Queensland, Australia was used. Our study shows that ethanol yields from agave (7414 L/ha/year) are comparable to Brazilian sugarcane (9900/L/ha/year) and higher than US corn ethanol (3800/L/ha/year). Furthermore, agave outperforms current first generation biofuel crops in water-related impacts, including Freshwater Eutrophication (96% lower than corn and 88% lower than sugarcane), Marine Ecotoxicity (59% lower than corn and 53% lower than sugarcane) and Water Consumption (46% lower than corn and 69% lower than sugarcane). The life cycle fossil energy use (Fossil Resource Scarcity) for agave is 58% lower than corn and 6% higher than sugarcane. The Global Warming impact for agave is also 62% and 30% lower than that of corn and sugarcane, respectively. Although its Land Use impact, measured by land occupied per unit ethanol output, is 98% higher than corn and 2% higher than sugarcane, agave can be grown on arid land that is not suitable for food crops. The economic analysis suggests that first generation ethanol production from agave is not commercially viable without government support. Overall, the results show that agave is promising for biofuel production in the water-energy-food-environment context.

Key words: Agave; biofuel; environmental impact; life cycle analysis (LCA); water-energy-food-environment (WEFE)

Abbreviations: 1G, 1st generation biofuel; 2G, 2nd generation biofuel; 3y, 2.5-year-old plants; 5y, 4.5-year-old plants; AusLCI, Australian National Life Cycle Inventory

Database; GHG, greenhouse gas; LCA, life cycle analysis; LCIA, life cycle impact assessment; LCI, life cycle inventory; L/ha/y, litres/hectare/year; NPV, net present value; N₂O, nitrous oxide; WEF, water-energy-food; WEFEE, water-energy-food-environment; WSC, water soluble carbohydrate

1 **1. Introduction**

2 The water-energy-food-environment (WEFE) nexus is a huge challenge for the
3 transition from a fossil fuel-dominated energy system to a more renewable and clean
4 energy-based one. Although biomass is a renewable energy source that can potentially
5 contribute to energy security goals, there are growing concerns over the sustainability
6 of large-scale use of bioenergy (Popp et al., 2014). Its impacts on food security and food
7 prices (Naylor et al., 2007), fresh water resources (Gerbens-Leenes et al., 2009) and
8 many ecosystem services (Holland et al., 2015) have all been under increasing scrutiny
9 recently while its net climate effects in many cases are still disputed mainly due to
10 significant uncertainties in the associated indirect effects (e.g., potential changes in land
11 systems (Searchinger et al., 2008) and food markets (Searchinger et al., 2015)) and
12 nitrous oxide (N₂O) emissions from nitrogen fertiliser use (Crutzen et al., 2016).

13 Agave could be a promising bioenergy feedstock (Somerville et al., 2010) given its
14 potentially high productivities, ability to thrive in semiarid regions, high water-use
15 efficiency and low requirements for nitrogen fertilisers (Davis et al., 2011).
16 Furthermore, its high sugar and low-lignin content make it an attractive crop from a
17 bioprocessing perspective (Aleman-Nava et al., 2018). A seminal life cycle analysis
18 (LCA) shows that ethanol derived from agave could offer higher land-use efficiencies
19 and greenhouse gas (GHG) savings than ethanol produced from corn and switchgrass
20 (Yan et al., 2011). However, this LCA study, the only one on agave-derived biofuels to
21 date, is based on a hypothetical ethanol plant in Mexico using 1st generation (1G)
22 conversion technology only (i.e., hydrolysis and fermentation of simple sugars extracted
23 from the stem and leaves) and the agave and sugar yield data was sourced from literature

24 on tequila production. Moreover, it focused only on energy and GHG analysis. In fact,
25 comprehensive reviews (Davis et al., 2015, Cushman et al., 2015) on the use of
26 bioenergy feedstocks including agave have confirmed that Yan et al., (2001) is currently
27 the only LCA available on agave-derived biofuels and there is a need for a more
28 comprehensive study. Building on Yan et al. (2011), an LCA was conducted for the
29 possibility of integrating solar panels and annual agave production with synergies
30 provided by water inputs for cleaning solar panels being similar to the water
31 requirements for agave (Ravi et al., 2014). This LCA suggested that the hypothetical co-
32 location of solar panels provided higher returns per m³ of water used than either system
33 alone. Preliminary economic studies were also conducted on agave for bioenergy
34 production in Mexico (Nunez et al., 2011) and Australia (Subedi et al., 2017) based on
35 hypothetical scenarios. To better understand the environmental and economic
36 performance of agave-derived biofuels a comprehensive study using production and
37 compositional data from long-term field experiments is required.

38 The aim of this paper was to conduct the first comprehensive LCA and economic
39 analysis of 1st and 2nd generation (2G) ethanol produced from agave grown in Australia,
40 using data collected from a 5-year field experiment in Queensland. The key novelties of
41 our study therefore include the use of agave yield and sugar content data collected from
42 a field experiment as well as the consideration of 2nd generation ethanol production.
43 Australia has the largest proportion of semiarid land in the world (Davis et al., 2011).
44 These areas do not support the growth of common agricultural crops but are suited for
45 plants that thrive on marginal and dry lands, such as agave. Results from the LCA will
46 be discussed in the context of the water-energy-food-environment (WEFE) nexus. The
47 finding from this paper is expected to inform large-scale development of agave-based

48 ethanol in Australia and other countries with significant amounts of semiarid land.

49 2. Materials and Methods

50 2.1 Agave field experiment in Australia

51 This LCA study was based on data from a pilot agave field experimental site at Kalamia
52 Estate in the Burdekin River Irrigation System, near Ayr, Queensland (see Figure 1).

53 The site is in a region with tropical savanna climate. The annual average temperature,
54 based on recordings from the nearest weather station (Ayr DPI Research Station 33002),
55 is 23.9 °C and precipitation is 947 mm dominated by summer rainfall with very little
56 rain in the winter (Australian Government Bureau of Meteorology, 2018). For the field
57 experiment 3500 plants were planted in June 2009 from tissue cultured agave (*Agave*
58 *tequilana* Weber cv. *azul*) imported from Mexico by Mr Don Chambers of AusAgave
59 (Holtum et al., 2011).



60

61 **Figure 1** Pilot agave field experimental site at Kalamia Estate

62 As the experimental site was previously used for sugarcane and fruit (trees) production,
63 land preparation only included pre-planting operations such as laser levelling, deep
64 ripping, disc harrowing, rotary hoeing, bed forming and mulching. Nitrogen (250 kg ha⁻¹)
65 ¹) fertiliser and herbicides [Treflan[®] (trifluralin), Atradex[®] (atrazine), Gramoxone[®]
66 (paraquat) and Roundup[®] (glyphosate)] were applied before planting. The plants were
67 watered once before transplanting to aid establishment and no irrigation was used during
68 the experiment.

69 The agave plants were established on 15 cm raised beds at a density of 4000 plants ha⁻¹
70 at a row spacing of 1.8 m x 1.6 m. Every second row was thinned out (harvested) at
71 Year 2 (2011), leaving a density of 2000 plants ha⁻¹. Treflan[®], Atradex[®] and Roundup[®]
72 at recommended registered rates were applied once a year during the growing season.
73 Pruning was performed manually twice a year. The periodic removal of offshoots (also
74 referred to as suckers or pups) is required to encourage piña (stem) growth. The process
75 of removal can be mechanised in a commercial operation.

76 **2.2 Measurements of agave yield and sugar content**

77 Three individual agave plants were harvested from Kalamia Estate in 2012 and 2014.
78 Harvesting was carried out semi-mechanically in this experiment but can be mechanised
79 using a modified cane harvester and two haul-out trailers. At the time of harvest plants
80 were 2.5-year-old (2012), referred to hereafter as 3y plants and 4.5-year-old (2014),
81 referred to hereafter as 5y plants. Immediately upon harvest the roots were washed with
82 pressurized water to remove excess dirt. The weight of the whole plant and the
83 individual vegetative parts (leaves, roots, stem and offshoots) were recorded. A
84 commercial shredder (Cutter-Grinder CG03; South Australia, Australia) was used to
85 extract the juice from both the agave leaves and stems. Following shredding, the wet

86 fibrous bagasse fraction was collected, and the residual juice was removed by crushing
87 subsets (300 g) of the bagasse using a press metal cylinder. After crushing the 3y
88 samples were placed in a 65 °C oven for one week and the final weight recorded. The
89 bagasse pellets from the 5y plants were placed directly in the freezer (-20 °C) and later
90 lyophilized (Labconco-Freezone, Missouri, United States).

91 The dried bagasse material (3y and 5y samples) was homogenized and particle size
92 reduced using a 25 mL stainless steel grinding jar with one 7 mm steel ball. The grinding
93 jars were shaken at 30 Hz for 3 min (Retsch mill MM400, Retsch GmbH; Haan,
94 Germany). The ball-milled samples were extracted following a small-scale extraction
95 method (Corbin et al., 2015). Briefly, the bagasse samples were extracted sequentially in
96 water, 95% v/v ethanol and 70% v/v ethanol at 80°C for 15 min using a 1:5 ratio of biomass to
97 extraction liquid and dried to a constant weight.

98 The water extracts were incubated with fructanase (Fructan HK-Megazyme: AOAC
99 Method 999.03; International Ireland Ltd., Wicklow, Ireland) to hydrolyse fructan
100 polymers, as previously described (Corbin et al., 2015). The glucose, fructose and
101 sucrose in the extracts were quantified by hydrophilic interaction chromatography, using
102 a Prevail Carbohydrate ES column (150 × 4.6 mm) on an Agilent 1200 series liquid
103 chromatography instrument equipped with an evaporative light scattering detector
104 (Alltech ELSD 800) (Corbin et al., 2015). Sample peak areas were compared to
105 calibration curves of standard solutions.

106 For compositional analysis of agave bagasse samples, standardized National Renewable
107 Energy Laboratory (NREL) analytical methods were followed (Sluiter et al., 2004, Sluiter et
108 al., 2006, Sluiter et al., 2008) with minor changes, as previously described (Corbin et al., 2015).

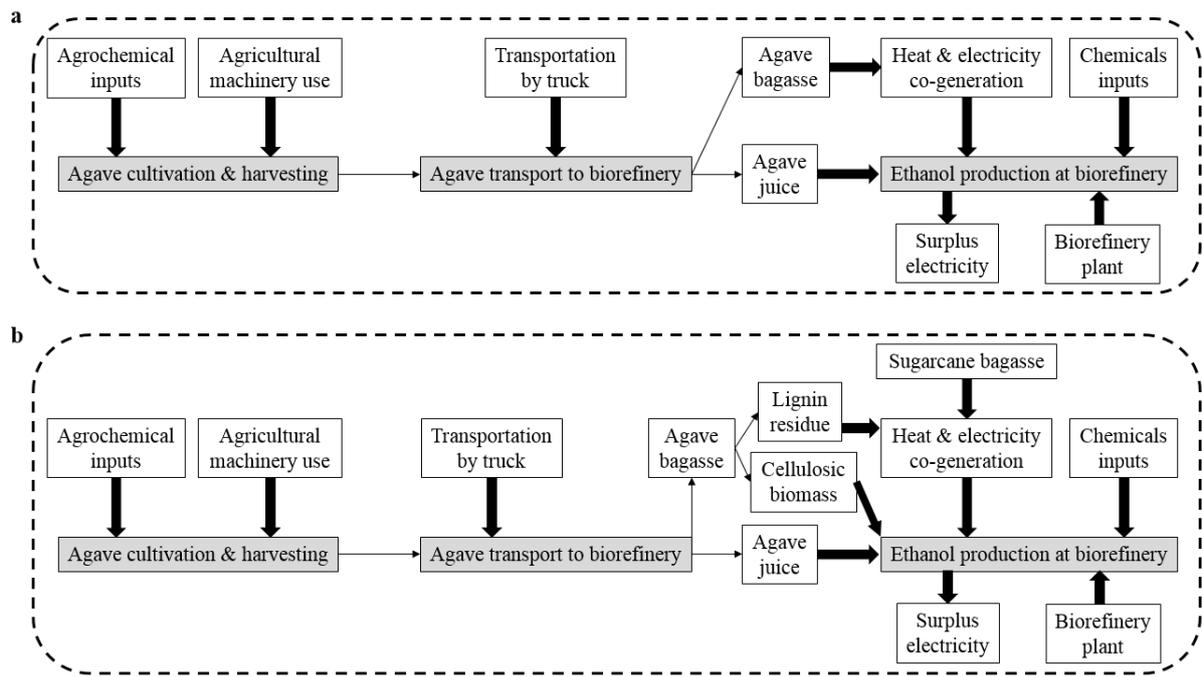
109 **2.3 Goal and scope of the life cycle assessment**

110 The goal of the LCA is to assess the environmental impacts and economic costs of
111 ethanol produced from agave grown in Australia in comparison with US corn ethanol
112 and Brazilian sugarcane ethanol. The system boundary considered is field-to-gate,
113 which includes the following main stages: crop cultivation and harvesting, feedstock
114 transportation and ethanol production at biorefinery. Transportation of the agave plants
115 from Mexico to Queensland is not included as this is only the case for the experiment
116 rather than potential industrial scale production in the future. The functional unit is 1 GJ
117 of fuel ethanol produced. LCA software SimaPro 8.4 (2018) was used to perform the
118 calculations.

119 To compare different key production options, 4 scenarios are evaluated considering the
120 use of either 3y or 5y plants for 1G or 1G+2G ethanol production. As agave has a
121 naturally long growth cycle (5-8 years), there are significant financial risks for growers
122 due to changing environmental and market conditions (Yan et al., 2011). Therefore, it
123 might be desirable to harvest agave plants early to reduce investment risks. In scenarios
124 1 and 3, the agave plants are harvested at the end of the third year. All the agrochemical
125 inputs, farm machinery use, and sugar yields are based on the 3y plants harvested in the
126 field experiment. Similarly, in scenarios 2 and 4, the agave plants are harvested at the
127 end of the fifth year and all data used correspond to the 5y plants harvested in the
128 experiment.

129 As the technology for 2G ethanol production from cellulosic biomass is still not mature,
130 we evaluate and compare scenarios that only involve 1G technology with those
131 involving both 1G and 2G technologies. In all scenarios juice is assumed to be extracted
132 from the agave stems and leaves using a diffuser system, following a previous study

133 (Yan et al., 2011). In the 1G ethanol scenarios (1 and 2), the juice is used to produce
 134 ethanol through enzymatic hydrolysis and fermentation. The bagasse generated from the
 135 extraction process is used as fuel for a cogeneration system to provide the process
 136 energy. In the 1G+2G ethanol scenarios (3 and 4), both the juice and bagasse are used
 137 to produce ethanol while the lignin residue is used as fuel. The system boundaries for
 138 the 1G only scenarios and 1G+2G scenarios are illustrated in Figure 2.



139
 140 **Figure 2** System boundaries for the 1G only scenarios (a) and 1G+2G scenarios (b)

141 **2.4 Life cycle inventory and environmental impact assessment**

142 The life cycle inventory (LCI) datasets for agave ethanol production are developed by
 143 modifying an existing dataset for Brazilian sugarcane ethanol production in the
 144 Ecoinvent LCI database within SimaPro. Key foreground inputs and their data sources
 145 are presented in the Supporting Information. The background datasets used are mainly
 146 from the Australian National Life Cycle Inventory Database (AusLCI, 2016) where
 147 possible, supplemented by the Ecoinvent database. Existing datasets for US corn ethanol

148 and Brazilian sugarcane ethanol in the Ecoinvent database are used for comparison. The
149 life cycle impact assessment method used is the ReCiPe 2016 Midpoint, which includes
150 17 impact categories (Huijbregts et al., 2016).

151 **2.5 Economic analysis**

152 The economic analysis used the same physical inputs in the LCA with unit costs in US\$.
153 The costing approach was similar to that of Subedi et al. (2017) except that actual field
154 production costs for Kalamia Estate, Queensland were used in our study rather than a
155 hypothetical farm of 37,000 ha over a 40-year investment cycle in Subedi et al. (2017).
156 All input costings were provided by the farm manager on a per hectare basis and
157 converted to the functional unit accordingly.

158 Ethanol plant operating cost was assumed to be US\$0.4/L from an existing ethanol plant
159 in the USA (Hofstrand, 2018). The base case for ethanol price was US\$0.50/L based on
160 the current ethanol price on the global market (Trading Economics, 2018). The
161 production costs were calculated for 3y and 5y agave under the 1G scenario since 2G
162 conversion technology is not yet mature in Australia (and costs are difficult to estimate).
163 Net present value (NPV) was calculated using a 5% discount rate following Subedi et
164 al. (2017).

165 **3. Results and Discussion**

166 **3.1 Chemical analysis of agave**

167 In this study, agave plants were harvested and characterized at two developmental stages, 3y
168 and 5y. The different anatomic fractions of the plants were separated and crushed following
169 harvest, yielding bagasse and juice fractions from the leaves, stem and offshoots (5y only). The
170 offshoots were not separated from the leaf fraction of the 3y plants. The average above-ground

171 fresh weight of 3y plants was 205 kg of which 88% was leaves and 12% stem biomass. For 5y
 172 agave plants the mass distribution was 45% leaves, 17% stem and 38% offshoots, averaging
 173 361 kg (Corbin et al., 2016). Unlike other feedstocks which have been considered as dedicated
 174 biofuel crops, agaves are water-dense (85-95%) (Corbin et al., 2015, Li et al., 2012). Using a
 175 press metal cylinder 68%, 43% and 27% of the starting mass (% w/w) from leaf, stem
 176 and offshoot tissues, respectively, was collected as juice. Stem bagasse was found to
 177 accumulate non-structural sugars (free sugars, water soluble carbohydrates (WSC) and
 178 fructans) at a higher rate than leaf tissue. Furthermore, the amount of lignin in 5y plants was
 179 lower than 3y plants in both leaf and stem bagasse. Over a two-year period, there was a 35%
 180 increase in sugar accumulation in the leaf juice and a 64% increase measured in the stem juice
 181 (Corbin et al., 2016, Corbin et al., 2015). This finding indicates that the type and amount of
 182 sugar in agave juice is both origin (leaf vs stem) and age dependent. A detailed mass balance
 183 of the bagasse fractions is summarized in Table 1.

184 **Table 1** Mass balance of *A. tequiliana* bagasse (% w/w)

Biomass	Extractives		Composition of raw biomass				Total sugar (%)	Total mass accounted for
	WSC*	Total extractives [^]	Cellulose	NCPs [§]	Lignin	Ash		
Leaf bagasse (3y)	4.3 ± 0.5	16.4 ± 4.2	26.1 ± 0.7	16.6 ± 0.7	17.0 ± 1.5	10.5 ± 0.8	47.0	86.6
Stem bagasse (3y)	8.9 ± 0.8	18.2 ± 1.8	22.3 ± 2.2	16.5 ± 2.5	12.7 ± 3.9	16.2 ± 2.0	47.7	85.9
Leaf bagasse (5y)	12.2 ± 2.3	27.9 ± 2.0	26.5 ± 2.9	17.8 ± 5.9	13.4 ± 1.0	9.7 ± 1.0	56.5	95.3
Stem bagasse (5y)	42.7 ± 5.2	53.3 ± 5.5	10.6 ± 2.2	10.6 ± 1.3	6.2 ± 1.4	8.0 ± 1.1	63.9	88.7
Offshoot bagasse (5y)	15.7 ± 1.7	33.2 ± 3.1	19.8 ± 2.1	13.8 ± 1.7	13.1 ± 1.6	11.1 ± 0.8	49.3	91.0

185
 186 [^] Combined water and ethanol extractions (includes soluble sugar, lignin, protein and ash).
 187 * Water extracts were hydrolysed with fructanase prior to analysis
 188 [§]NCPs: Non-cellulosic polysaccharide
 189 Results for cellulose, NCPs, lignin and ash are reported as percentage of non-extracted
 190 biomass.

191 *Italicized values are derived from calculation rather than direct measurement. Total sugar*
 192 *calculation includes WSC, cellulose and NCPs. Total mass is the sum of total extractives,*
 193 *cellulose, NCPs, lignin and ash. Data reported are the mean values of three replicates.*
 194

195 **3.2 Estimated ethanol yield**

196 We estimate potential ethanol yield based on data collected from the agave experiment,
 197 including weight of an agave plant and sugar contents presented above, as well as
 198 assumptions on the ethanol production process. The overall sugar utilisation efficiency
 199 is assumed to be 90% for 1G ethanol production (Yan et al., 2011) and 60% for 2G
 200 ethanol production as a range of 30% to 90% can be found in the literature (Kang et al.,
 201 2014, Limayem et al., 2012, Hamelinck et al., 2005). The yields of 1G ethanol from
 202 agave juice would be 4854 and 6673 L/ha/y for 3y and 5y agave plants, respectively
 203 (Table 2). These are higher than the ethanol yield of 3809 L/ha/y (81 GJ/ha/y) from 5y
 204 agave plants estimated in a previous study based on data from the Mexican tequila
 205 industry (Yan et al., 2011). If the bagasse is also used to produce 2G ethanol, yields
 206 would increase by 490 and 741 L/ha/y for 3y and 5y agave plants, respectively. Overall,
 207 the yields for 5y agave estimated in this study are comparable to sugarcane (6900 L/ha/y
 208 of 1G ethanol from juice and an additional 3000 L/ha/y of 2G ethanol from bagasse)
 209 and much higher than corn (2900 L/ha/y of 1G ethanol from grain and an additional 900
 210 L/ha/y of 2G ethanol from stover) (Somerville et al., 2010).

211 **Table 2** Estimates for ethanol production from agave

Measured data		Age of plant	
		3y	5y
Fresh weight of agave plant	kg/plant	205	361
1G sugar yield	kg/plant	10.4	25.8
2G sugar yield	kg/plant	1.6	4.3
Assumptions			
Overall 1G sugar utilisation efficiency		90%	90%

Overall 2G sugar utilisation efficiency		60%	60%
Theoretical ethanol yield from sugar	kg/kg sugar	0.51	0.51
Calculated data			
agave yield (fresh biomass)	t/ha	410	721
1G ethanol yield	L/ha/year	4854	6673
2G ethanol yield	L/ha/year	490	741

212

213 3.3 Life cycle assessment results

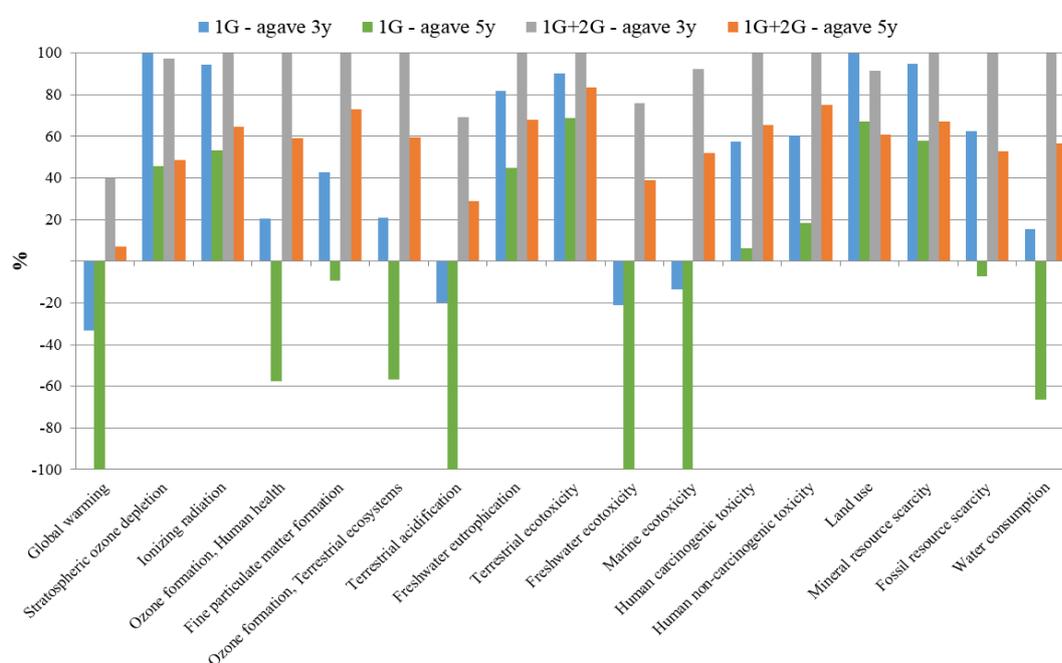
214 The LCA results for agave ethanol under the four production scenarios are shown in
215 Table 3 (absolute values) and Figure 3 (relative values). Ethanol that is produced from
216 5y agave plants has lower impacts for all categories compared to that from 3y plants.
217 This is mainly because of the relatively higher amounts of sugar and hence ethanol
218 produced from the 5y plants in proportion to the inputs needed. In general, ethanol
219 produced from the 1G only options have lower impacts than that from the 1G+2G
220 options for all impact categories except Land Use. This is mainly because the 1G option
221 produces significant amounts of surplus electricity from the bagasse and displaces grid
222 electricity in Queensland, which is mainly generated from coal. For many categories
223 such as Global Warming, Acidification and Ecotoxicity this even resulted in negative
224 impacts (i.e. net benefits). The lower Land Use impacts of the 1G+2G options were
225 because of their moderately higher ethanol yields per unit of land used.

226 **Table 3** LCA results for ethanol produced from agave in Australia under different production
227 options

Impact category	Unit	1G	1G	1G+2G	1G+2G
		agave 3y	agave 5y	agave 3y	agave 5y
Global warming	kg CO ₂ eq	-23.4	-70.0	28.0	5.1
Stratospheric ozone depletion	kg CFC11 eq	0.0002	0.0001	0.0002	0.0001
Ionizing radiation	kBq Co-60 eq	0.02	0.01	0.02	0.01
Ozone formation, Human health	kg NO _x eq	0.05	-0.14	0.25	0.15
Fine particulate matter formation	kg PM _{2.5} eq	0.04	-0.01	0.08	0.06

Ozone formation, Terrestrial ecosystems	kg NO _x eq	0.05	-0.14	0.25	0.15
Terrestrial acidification	kg SO ₂ eq	-0.05	-0.25	0.18	0.07
Freshwater eutrophication	kg P eq	0.001	0.001	0.002	0.001
Terrestrial ecotoxicity	kg 1,4-DCB e	0.03	0.02	0.03	0.03
Freshwater ecotoxicity	kg 1,4-DCB e	-0.10	-0.48	0.36	0.19
Marine ecotoxicity	kg 1,4-DBC e	-0.08	-0.60	0.55	0.31
Human carcinogenic toxicity	kg 1,4-DBC e	0.31	0.03	0.54	0.35
Human non-carcinogenic toxicity	kg 1,4-DBC e	681	208	1129	848
Land use	m ² a crop eq	117	79	107	71
Mineral resource scarcity	kg Cu eq	0.10	0.06	0.11	0.07
Fossil resource scarcity	kg oil eq	4.5	-0.5	7.2	3.8
Water consumption	m ³	1.6	-6.7	10.0	5.7

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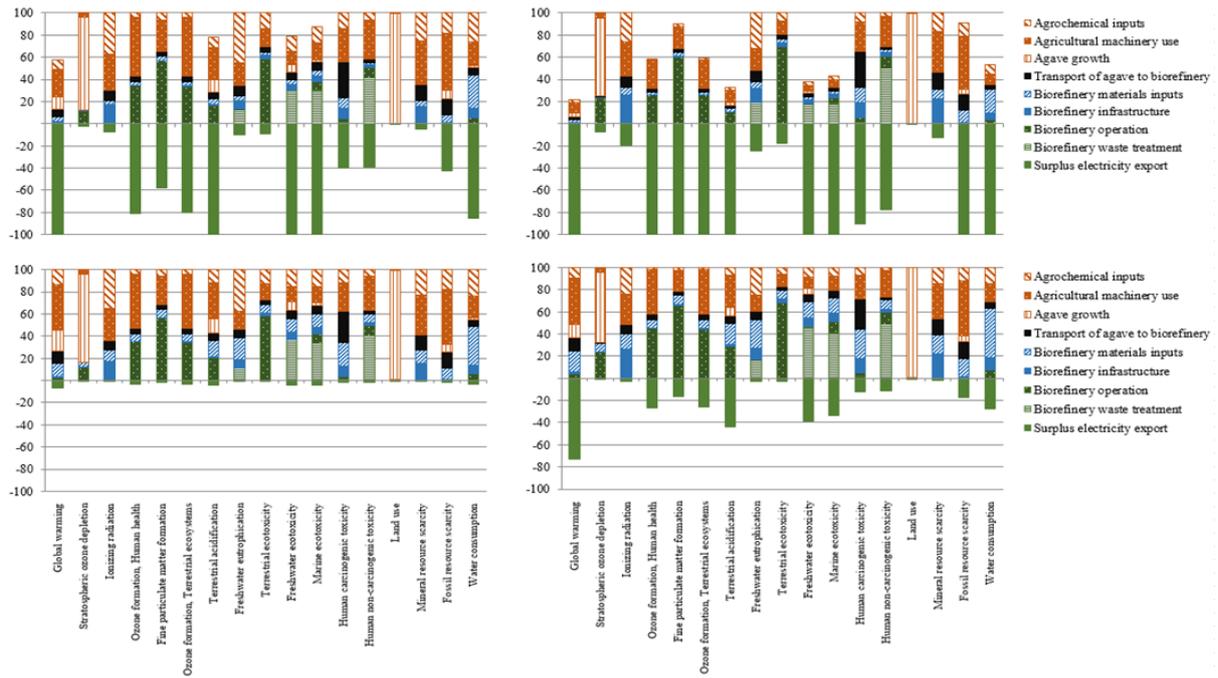


229

230 **Figure 3** Normalised LCA results for ethanol produced from agave in Australia under different
 231 production options

232 Examining the contributions of main life cycle stages to different categories of impacts
 233 reveals interesting insights (Figure 4). The manufacturing and transport of agrochemical
 234 inputs such as fertilisers and pesticides contribute noticeably to Ionizing Radiation,
 235 Freshwater Eutrophication, Mineral Resource Scarcity and Water Consumption. An

236 LCA of the herbicide, diuron on agave farms in Mexico showed that the most
237 environmentally friendly option was the one with the shortest transportation distance
238 (Tirametoakkhara and Lerkkasemsan, 2019). Agricultural machinery use makes
239 significant or noticeable contributions to most impact categories primarily because of
240 diesel fuel consumption and emissions and metals used in machinery production. Agave
241 growth dominates the Stratospheric Ozone Depletion (because of N₂O emissions from
242 nitrogen fertiliser used) and Land Use impacts. Transport of agave to biorefinery,
243 manufacturing and transport of biorefinery material inputs, and biorefinery
244 infrastructure together contribute noticeably to Ionizing Radiation, Freshwater
245 Eutrophication, Human Carcinogenic Toxicity, Mineral Resource Scarcity, Fossil
246 Resource Scarcity and Water Consumption. Biorefinery operation contributes
247 significantly to Ozone Formation, Fine Particulate Matter Formation and Terrestrial
248 Ecotoxicity primarily because of the burning of bagasse for energy generation. Sizable
249 contributions by biorefinery waste treatment can be seen for Freshwater Eutrophication,
250 Freshwater Ecotoxicity, Marine Ecotoxicity and Human Non-carcinogenic Toxicity.
251 Surplus electricity export can offset significant impacts for most categories in the case
252 of the 1G ethanol options. However, the potential offsets in the case of the 1G+2G
253 options are limited for 5y agave and insignificant for 3y agave because of much lower
254 electricity generation.



255

256 **Figure 4** Contributions of main life cycle stages to the different categories of environmental
 257 impacts for ethanol produced from agave in Australia under different production options
 258 (unit:%): upper left- 1G agave 3y; upper right- 1G agave 5y; lower left- 1G+2G agave 3 y; and
 259 lower right- 1G+2G agave 5y

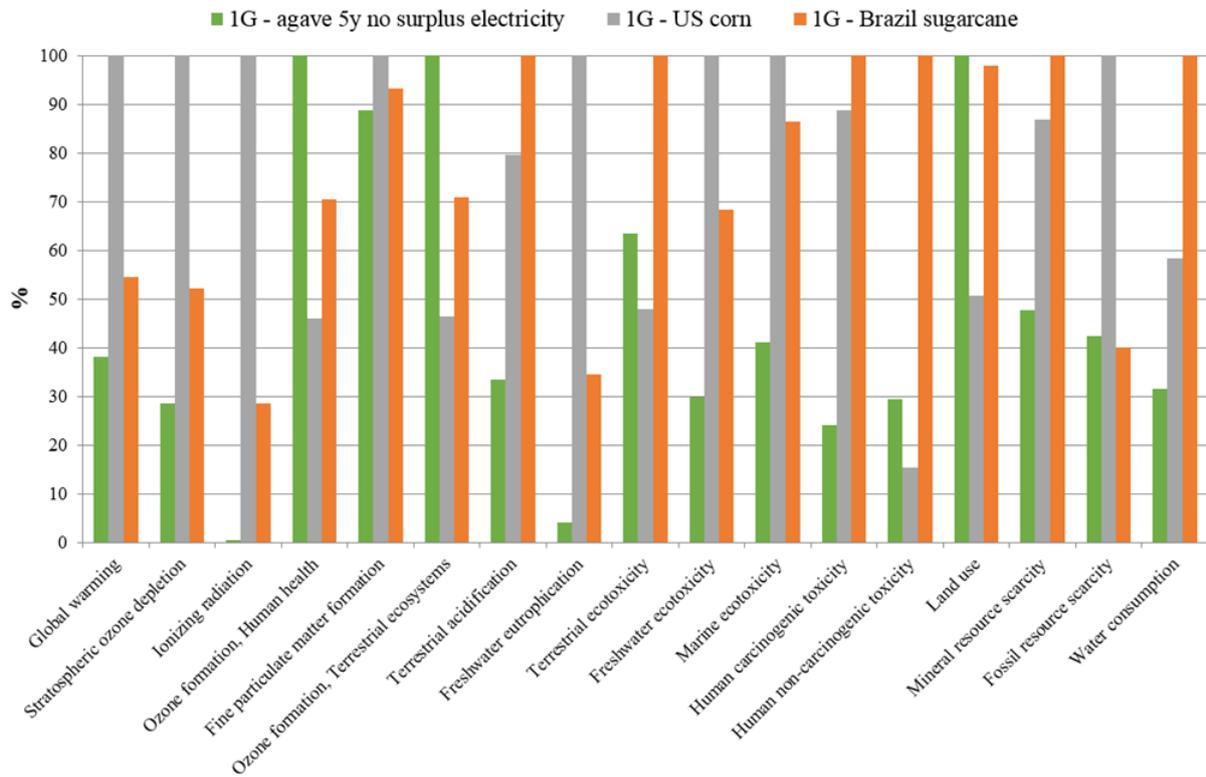
260 Comparison between ethanol produced from Australian agave, US corn and Brazilian
 261 sugarcane is shown in Table 4 and Figure 5. As existing datasets for US corn and
 262 Brazilian sugarcane ethanol available in the Ecoinvent LCI database only cover 1G
 263 ethanol production and do not consider surplus electricity generation, we compare them
 264 with 1G ethanol produced from 5y agave plants with surplus electricity generated from
 265 bagasse disregarded. The findings show that agave is the lowest in most impact
 266 categories except for Ozone Formation (higher than both corn and sugarcane),
 267 Terrestrial Ecotoxicity (higher than corn), Human Carcinogenic Toxicity (higher than
 268 corn), Land Use (higher than both corn and sugarcane) and Fossil Resource Scarcity
 269 (higher than sugarcane).

270

271 **Table 4** LCA results for ethanol produced from agave in Australia (5y old plants with no
 272 surplus electricity generated from bagasse), corn in US and sugarcane in Brazil

Impact category	Unit	1G agave (5y) <i>no surplus electricity</i>	1G US corn	1G Brazil sugarcane
Global warming	kg CO ₂ eq	19.1	50.0	27.2
Stratospheric ozone depletion	kg CFC11 eq	0.0001	0.0004	0.0002
Ionizing radiation	kBq Co-60 eq	0.01	2.49	0.71
Ozone formation, Human health	kg NO _x eq	0.20	0.09	0.14
Fine particulate matter formation	kg PM _{2.5} eq	0.07	0.08	0.07
Ozone formation, Terrestrial ecosystems	kg NO _x eq	0.21	0.10	0.15
Terrestrial acidification	kg SO ₂ eq	0.13	0.30	0.38
Freshwater eutrophication	kg P eq	0.001	0.021	0.007
Terrestrial ecotoxicity	kg 1,4-DCB e	0.03	0.02	0.04
Freshwater ecotoxicity	kg 1,4-DCB e	0.29	0.96	0.66
Marine ecotoxicity	kg 1,4-DBC e	0.45	1.10	0.95
Human carcinogenic toxicity	kg 1,4-DBC e	0.37	1.35	1.52
Human non-carcinogenic toxicity	kg 1,4-DBC e	928	484	3141
Land use	m ² a crop eq	79.3	40.1	77.6
Mineral resource scarcity	kg Cu eq	0.07	0.13	0.15
Fossil resource scarcity	kg oil eq	4.8	11.4	4.6
Water consumption	m ³	7.7	14.3	24.5

273

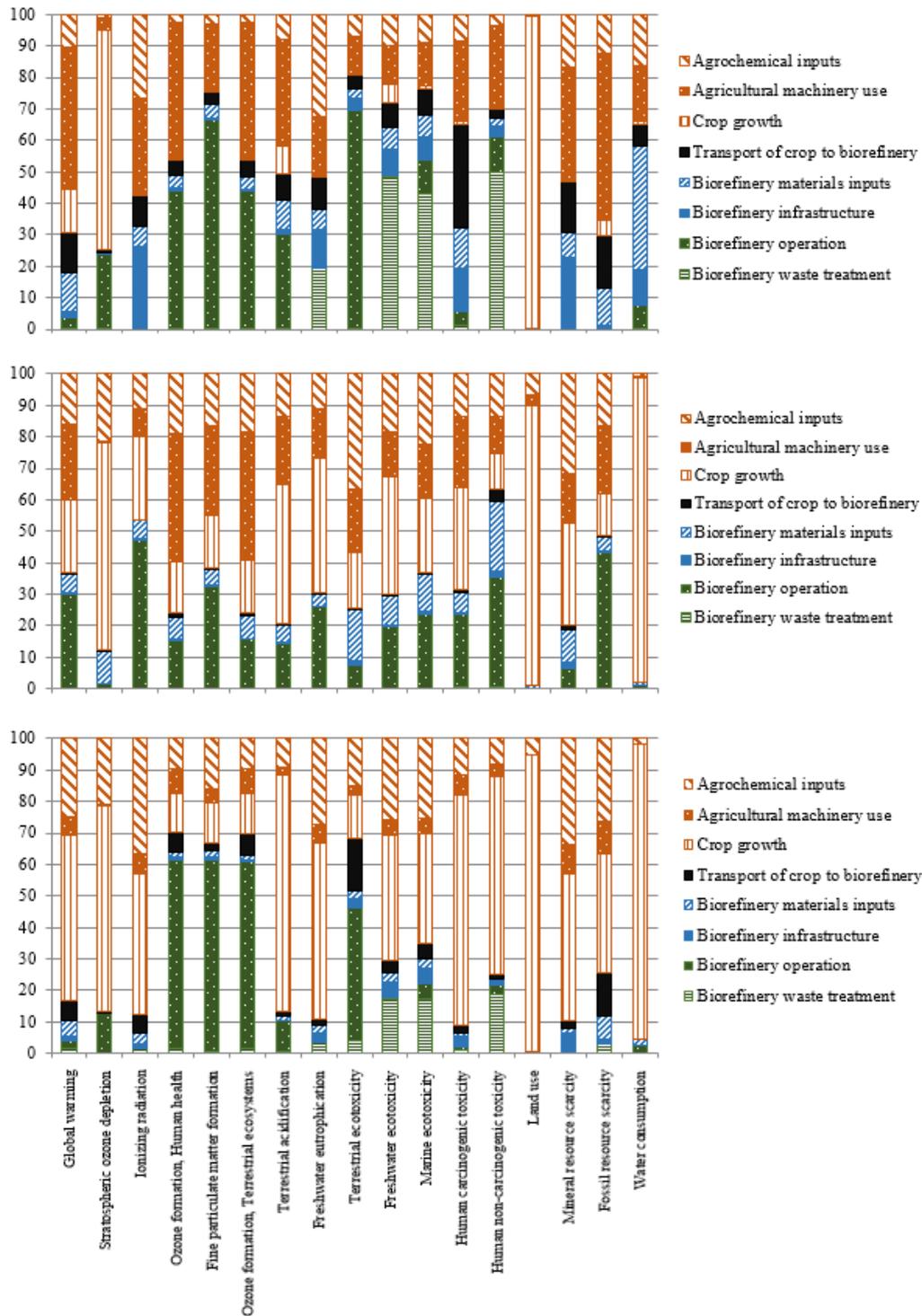


274

275 **Figure 5** Normalised LCA results for ethanol produced from agave in Australia (5-year
 276 old plants with no surplus electricity generated from bagasse), corn in US and sugarcane
 277 in Brazil

278 The contributions of main life cycle stages to different categories of impacts vary
 279 significantly between agave, corn and sugarcane except for Land Use (Figure 6). For
 280 example, the contribution of biorefinery operation to Global Warming is sizable for corn
 281 as natural gas is used as the main energy source in US corn ethanol plants. This
 282 contribution is insignificant for agave and sugarcane as bagasse is used as an energy
 283 source. However, the combustion of bagasse makes the contribution of biorefinery
 284 operation to air pollution (i.e., Ozone Formation and Fine Particulate Matter Formation)
 285 much higher for agave and sugarcane than for corn. Land Use is dominated by crop
 286 growth for all three crops. The lower Land Use for corn is due to the assumed crop
 287 rotation in the Ecoinvent dataset for corn ethanol as well as the credit gained from the

288 protein-rich coproduct used for animal feed. The yield of ethanol achieved from using
289 agave biomass (6673 L/ha/y) and sugarcane (6900 L/ha/y) are still higher than corn
290 (2900 L/ha/y). Water Consumption is dominated by irrigation during crop growth for
291 corn and sugarcane. On the other hand, crop growth accounts for only 1% of agave's
292 water consumption as the field was only irrigated once (first year) to facilitate plant
293 establishment in the agave field experiment. In fact, the majority of life cycle water
294 consumption for agave is due to the generation of hydropower consumed in various
295 industrial processes.



296

297 **Figure 6** Contributions of main life cycle stages to the different categories of environmental
 298 impacts for ethanol produced from (unit: %) top- agave in Australia (5-year old plants with no
 299 surplus electricity generated from bagasse); middle- corn in US; and bottom- sugarcane in
 300 Brazil.

301 **3.4 Economic assessment**

302 The cash flow for 1G ethanol production from 3y (Table 5) and 5y (Table 6) agave were
 303 projected and net present value (NPV) calculated using a 5% discount rate. The agave
 304 feedstock production costs were US\$0.94/L (70% of total costs) and US\$0.46/L (53%
 305 of total costs), for 3y and 5y agave, respectively. Assuming 1G ethanol processing costs
 306 of US\$0.40/L (Hofstrand, 2018), the overall production costs were US\$1.34/L and
 307 US\$0.86/L for ethanol produced from 3y and 5y agave, respectively. Hence, agave
 308 feedstock costs in Australia are lower than the US\$3/L agave feedstock costs estimated
 309 for Mexico, which is 6 times higher than the current price of ethanol (US\$0.50/L)
 310 (Nunez et al., 2011).

311 **Table 5** Cash flow projection and net present value calculation for 3-year agave (1G)

Production stage (\$/ha)	Year			Total
	1st	2nd	3rd	
Land preparation	3,373	0	0	3,373
Planting	3,692	0	0	3,692
Maintenance (chemical application, and pruning) (Including diesel use for tractors and the cost of labour)	1,313	1,313	1,313	3,938
Harvesting	0	0	2,635	2,635
Total cost for each year	8,377	1,313	3,948	13,637
Average production cost (\$/ha/y)	4,546			
Ethanol processing stage				
Cost of processing 4854 L/ha/year x 3y @ \$0.4/L	0	0	5825	5,825
Total production and processing costs per year				
	8,377	1,313	9,772	19,462
Average total production and processing costs (\$/ha/y)	6,487			
Average total production and processing costs (\$/L)	1.34			
Revenue from ethanol 4854 L/ha/year x 3y @ \$0.5/L				
	0	0	14,562	
Total cost of production and processing costs	8,377	1,313	9,772	
Cash flow	-8,377	-1,313	4,790	

Cumulative cash flow	-8,377	-9,690	-4,900	
Net Present Value (NPV) @5% discount rate	-\$5,031			

312

313

314 **Table 6** Cash flow projection and net present value calculation for 5-year agave (1G)

315

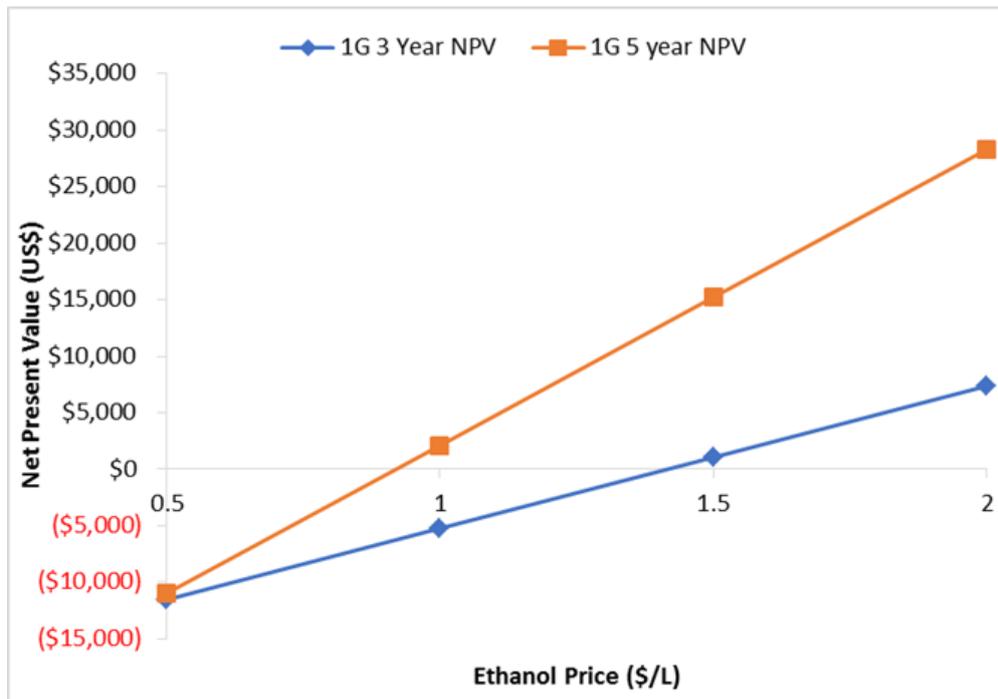
Production stage (\$/ha)	Year					Total
	1st	2nd	3rd	4th	5th	
Land preparation	3,373	0	0	0	0	3,373
Planting	3,692	0	0	0	0	3,692
Maintenance (chemical application, and pruning) (Including diesel use for tractors and the cost of labour)	959	959	959	959	959	4,794
Harvesting	0	0	0	0	3,444	3,444
Total cost for each year	8,023	959	959	959	4,402	15,302
Average production cost (\$/ha/y)	3,060					
Ethanol processing stage						
Cost of processing 6673 L/ha/year x 5 y @ \$0.4/L	0	0	0	0	13,346	13,346
Total production and processing costs per year						
Average total production and processing costs (\$/ha/y)	8,023	959	959	959	17,748	28,648
Average total production and processing costs (\$/L)	5,730					
	0.86					
Revenue from ethanol 6673 L/ha/y x 5 y @ \$0.5/L						
Total cost of production and processing costs	0	0	0	0	16,683	
Cash flow	8,023	959	959	959	17,748	
Cumulative cashflow	-8,023	-959	-959	-959	-1,066	
Net Present Value (NPV) @5% discount rate	-8,023	-8,982	-9,941	-10,900	-11,965	
	-\$10,963					

316

317 The sensitivity analysis of the NPV with changes in ethanol price (Figure 7) shows that

318 the NPV will only become positive at US\$1.5/L and US\$1/L ethanol price for 3y and

319 5y agave, respectively. This means that 1G ethanol produced from agave is currently
 320 not profitable until the ethanol price is >US\$1/L (double the current price of
 321 US\$0.50/L), consistent with the conclusion by Subedi et al. (2017). This suggests that
 322 government support is necessary for agave-based ethanol, as is true for most biofuels in
 323 production.



324

325 **Figure 7** Net present value (US\$) projection for 1G ethanol produced from 3y and 5y agave

326 **3.5 Limitations and further research needs**

327 The Water Consumption values calculated here include only blue water (i.e., freshwater
 328 from lakes, dams, rivers and aquifers) but not green water (i.e., soil moisture from
 329 precipitation), which was not covered by current LCI databases (Salmoral et al., 2018)
 330 Water Consumption would be even higher for sugarcane in comparison to agave and
 331 corn if green water is taken into account. This is attributed to the higher average rainfall
 332 in the sugarcane growing regions in Brazil (1407 mm; Marin et al., 2015) than in the

333 Corn Belt in the US (940 mm; Ort et al., 2014) and the agave field at Kalamia Estate in
334 Australia (947 mm; Australian Government Bureau of Meteorology, 2018).

335 Our study is based on agave yield measured at a field experiment in Queensland on land
336 that was previously used for agricultural purposes. Future studies should explore the
337 effects of potential yield variation across Australia, particularly in areas that receive less
338 rainfall, on the life cycle impacts.

339 Another limitation is the uncertainty in 2G ethanol conversion efficiency as the
340 technology is still not mature. This is also the reason why economic analysis was not
341 performed for the 1G+2G production options. Therefore, the analysis on 2G ethanol
342 production in our study can be improved when better data is available in the future.
343 Direct land use change emission is a key parameter that is not considered in our study
344 due to lack of data. However, there is potential for carbon sequestration when agave is
345 grown in arid regions and this should be accounted for in future LCA studies.
346 Experimental research is needed to determine the scale of this potential.

347 **4. Conclusion**

348 This is the first comprehensive LCA and economic analysis of ethanol produced from
349 a 5-year agave field experiment. Overall, agave performs better than current 1G biofuel
350 crops such as corn and sugarcane in water-related environmental impact categories and
351 produces competitive ethanol yields (L/ha/y). Although its Land Use impact is high,
352 agave can be grown in unfavourable conditions which do not support food crop
353 production. Overall, our results show that agave is a promising feedstock for biofuel
354 production in arid regions that should be supported in the context of the WEFÉ nexus.

355 We present the first comprehensive LCA and economic analysis of ethanol produced
356 from agave grown in Australia using data collected from a 5-year field experiment in
357 Queensland. Our analysis shows that an ethanol yield of 7414 L/ha/y (6673 from the
358 juice using 1st generation ethanol technology and 741 from the bagasse using 2nd
359 generation technology) is achievable with agave plants harvested at 5-years old. The
360 economic analysis suggests that 1G ethanol production from agave is not commercially
361 viable without government support, as with most biofuels in production.

362 The LCA results suggest that ethanol production using 1G technology only performs
363 better than 1G+2G technologies mainly because of the significant surplus electricity
364 generated from the 1G only production option. In addition, agave performs much better
365 than current 1G biofuel crops such as corn and sugarcane in water-related impacts,
366 including Freshwater Eutrophication, Freshwater Ecotoxicity, Marine Ecotoxicity and
367 Water Consumption. The life cycle fossil energy use (Fossil Resource Scarcity) for
368 agave is significantly lower than corn and only slightly higher than sugarcane. Agave is
369 not a major food crop and therefore will not have a direct impact on the global or local
370 food market. Although its Land Use impact, measured by land occupied per unit ethanol
371 output, is higher than corn and sugarcane, it can potentially be grown on arid land that
372 is not suitable for food crops. Therefore, competition with food production for land can
373 largely be avoided. The environmental performance of agave is also favourable when
374 compared with corn and sugarcane.

375

376 Overall, our analysis suggests that agave is a highly beneficial feedstock for biofuel
377 production in arid regions such as Australia that should be supported in the context of
378 the WEFE nexus.

379

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382 AusAgave in allowing access to the Kalamia site in Queensland and experimental use
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385 **6. Appendix: Electronic Supplementary Data**

386 Supplementary data of this work can be found in online version of the paper.

387 **Table S1.** Different production scenarios considered

388 **Table S2.** Agricultural inputs and their active ingredients for agave plants harvested at 3 and
389 5 years old based on information from the field trial at Kalamia Estate

390 **Table S3.** Agricultural machinery activities (number of times used) based on information
391 from the field trial at Kalamia Estate

392 **Table S4.** Biorefinery material inputs (kg/kg ethanol produced) based on the sugarcane
393 ethanol production dataset in Ecoinvent database

394 **Table S5.** Estimated biorefinery energy inputs and outputs (per kg ethanol produced) based
395 on the modelling work of Dias et al. (2012 and 2013) on sugarcane ethanol production

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399 **8. Author Contributions**

400 The manuscript was written through contributions of all authors. All authors have given
401 approval to the final version of the manuscript. ‡These authors contributed equally.

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