1	Carbon emission avoidance and capture by producing in-reactor microbial
2	biomass based food, feed and slow release fertilizer: potentials and
3	limitations
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### 22 Abstract

To adhere to the Paris Agreement of 2015, we need to store several gigatonnes (Gt) of carbon 23 annually. In the last years, a variety of technologies for carbon capture and storage (CCS) and 24 carbon capture and usage (CCU) have been demonstrated. While conventional CCS and CCU 25 are techno-economically feasible, their climate change mitigation potentials are limited, due to 26 limited amount of CO<sub>2</sub> that can be captured. Hence, there is an urgent need to explore other 27 CCS and CCU routes. Here we discuss an interesting alternative route for capture of carbon 28 dioxide from industrial point sources, using CO<sub>2</sub>-binding, so-called autotrophic aerobic bacteria 29 to produce microbial biomass as a C-storage product. The produced microbial biomass is often 30 31 referred to as microbial protein (MP) because it has a crude protein content of ~70-75%. Depending on the industrial production process and final quality of the produced MP, it can be 32 used for human consumption as meat replacement, protein supplement in animal diets, or slow-33 34 release organic fertilizer thus providing both organic nitrogen and carbon to agricultural soils. Here, we discuss the potentials and limitations of this so far unexplored CCU approach. A 35 preliminary assessment of the economic feasibility of the different routes for CO<sub>2</sub> carbon 36 avoidance, capture and utilization indicates that the value chain to food is becoming attractive 37 and that the other end-points warrant close monitoring over the coming years. 38

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#### 42 The need for and limitations of carbon capture and utilization methods

To meet the climate change mitigation challenge and adhere to the Paris Agreement of 2015, we need to store about 4-5 Gigatonnes (Gt) CO<sub>2</sub> per year (Mac Dowell et al. 2017). Several technologies for carbon capture and storage (CCS) and carbon capture and usage (CCU) exist and their technological feasibility has been demonstrated (Mac Dowell et al. 2017). Underground storage of CO<sub>2</sub> gas is the cheapest option (Service 2016), but beyond climate change abatement, this approach brings about no net benefits.

While conventional methods of CCS and CCU are techno-economically feasible, their 49 overall potential in terms of climate change mitigation is limited (Mac Dowell et al. 2017). For 50 example, the expectation of CO<sub>2</sub> injection into geological reservoirs to achieve enhanced oil 51 52 recovery (EOR-CCS), at the current prices of oil and of CO<sub>2</sub>, at best only cover 4-8% of the mitigation challenge by 2050 (Mac Dowell et al. 2017). The economic feasibility is directly 53 linked to the oil price, so with low oil prices, the economics of storage by the oil industry also 54 55 becomes less attractive. Another route is the use of carbon dioxide as a feedstock to produce chemicals (Aresta et al. 2013, Martens et al. 2017). The two chemicals which really represent 56 major CO<sub>2</sub> capture potential at present are urea (*i.e.*, 132 Mt CO<sub>2</sub> equivalent per year) through 57 an 2-step chemical process in which CO<sub>2</sub> first undergoes an exothermic reaction with liquid 58 ammonia to form (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> followed by endothermic decomposition and dehydration of 59 (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> into urea and methanol (*i.e.*, 10 Mt CO<sub>2</sub> equivalent per year) via catalytic 60 hydrogenation of CO<sub>2</sub> (Aresta et al. 2013, Boot-Handford et al. 2014). However, the entire CO<sub>2</sub>-61 to-chemical route can, at best, account for about 1% of required carbon storage, and will most 62 63 likely not play a major role in climate change mitigation in the years ahead (Mac Dowell et al. 2017). Hence, although these different CCS and CCU techniques allow efficient and 64 economically feasible carbon capture, their ability to decrease current CO<sub>2</sub> emission levels is, 65 66 at present, insufficient.

Considering the limitations of the available methods, and the urgency to deal with climate 67 change, there is a need to explore other routes that can (1) effectively avoid carbon emissions, 68 (2) capture and utilize carbon, and (3) offer the possibility of being implemented in the near 69 future. Obviously, such alternative routes must have a clear-cut positive impact on the global 70 economy, the environment and public health. An interesting alternative option that, so far, has 71 not been explored on industrial scale, is carbon capture coupled with storage in and utilization 72 as microbial biomass by using autotropic micro-organisms that rely on renewable hydrogen, 73 the so-called hydrogen oxidizing bacteria (HOB) (Figure 1) (Matassa et al. 2015, Matassa et al. 74 2016b, Pikaar et al. 2017a). The key feature of these bacteria is that they have a special capacity 75 to use the energy which becomes available when they enzymatically combine hydrogen gas 76 with oxygen gas to produce water; the renewable energy initially invested to electrolyse the 77 water to hydrogen and oxygen is thus recovered by the bacteria and used to build up  $CO_2$  and 78 79 minerals into their cellular components.





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Fig 1. Overall scheme of the production of Microbial Based Biomass from Haber-Bosch

- 83 nitrogen, CO<sub>2</sub> and H<sub>2</sub> and O<sub>2</sub> driven by renewable energy.
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The autotrophic microbial biomass that is formed from CO<sub>2</sub> under aerobic conditions 85 can, depending on the industrial production process and final quality of the microbial biomass, 86 be used for (1) human food as a protein source (as a meat substitute), (2) protein rich feed for 87 livestock and (3) slow-release organic fertilizer providing both nutrients to the crops, but also 88 serving as a means to store carbon in agricultural soils (Lal 2004a, b, 2008, Paustian et al. 1997, 89 Paustian et al. 2016, Smith 2016). Clearly, in all three cases, the microbial based biomass 90 91 represents a temporary storage, but this approach integrates possibilities to decrease the demand for fossil fuel through direct CO<sub>2</sub> usage by the autotrophic HOB. In this paper, we highlight the 92 potential and limitations of such an approach, and we assess the economic feasibility of the 93 94 different routes for CO<sub>2</sub> carbon avoidance, capture and utilization routes.

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#### 96 Carbon capture potential and its economics

97 Independent of the different end-use possibilities described above, the factor determining the practical feasibility of the carbon capture and storage potential, is the availability of CO<sub>2</sub> that 98 can be captured and upgraded to adequate quality at large scale from industrial point sources 99 (e.g. incinerators, cement ovens and steel plants) or could even be transformed into syngas at 100 economically competitive costs (Boot-Handford et al. 2014, Verbeeck et al. 2018). Assessments 101 102 by the Intergovernmental Panel on Climate Change (IPCC) have revealed that already in 2020, the annual amount CO<sub>2</sub> that can be captured at economically feasible costs from industrial point 103 sources will reach 0.7 - 1.3 Gt C/year (2.6 to 4.9 Gt CO<sub>2</sub>/year) (IPCC 2005). This is already in 104 the same order as the amount of carbon, *i.e.*, 4-5 Gt/year that needs to be stored. By 2050, the 105 carbon capture potential is estimated to reach 1.3-10 Gt C/year, which reflects 4.7 - 37.5 Gt 106 CO<sub>2</sub>/year. 107

The production costs of hydrogen-based microbial protein are estimated at US\$2800 per
 tonne product (*i.e.*, dry microbial based biomass at a crude protein content of 70-75%) (Pikaar

et al. 2018). The hydrogen production costs by means of water electrolysis comprise about 60%
of the total production costs for hydrogen based microbial protein (Pikaar et al. 2018). These
estimated costs are based on a cost of hydrogen of \$3/kg hydrogen through water electrolysis
using renewable energy as the energy source at a unit price of \$0.05 per kWh. In recent years,
considerable progress has been made in renewable energy generation, with costs for large scale
electricity generation using large scale-solar photovoltaics, with recent bids already reaching
prices as low as US\$0.03 per kWh generated (Haegel et al. 2017).

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## 118 Microbial protein for human consumption as a meat substitute

119 Microbial protein (MP) as a food product suitable for human consumption is not new with microorganisms in the form of fungi, yeast, bacteria and algae being used in food processing 120 for human consumption (e.g., bread, yoghurt, mushrooms and beer) for thousands of years 121 122 (Anupama and Ravindra 2000, Matassa et al. 2016a). In recent years, there has been an increasing interest in MP for human consumption as a meat substitute. Indeed, MP can already 123 be produced at commercially competitive prices, and is increasingly sold as meat substituents 124 in fungal based MP products, like Quorn<sup>(TM)</sup>. Yet, a more challenging issue is to opt for MP 125 produced not from carbohydrates (as in the case for Quorn<sup>(TM)</sup>), but from non-food CO<sub>2</sub> as a 126 127 carbon source, coupled with hydrogen as an energy source (Figure 1). Interestingly, the concept of using carbon dioxide and hydrogen to produce MP food is not completely new. Human trials 128 were already conducted by NASA in the 1960s in their quest to produce food for astronauts 129 130 (Waslien et al. 1969). The production of Spirulina platensis in the MELiSSA loop is an excellent example of how integrated nutrient recovery in space can be used to produce MP 131 (Gòdia et al. 2002). 132

133 Considering total production costs of about \$2800/tonne (dry microbial based biomass
134 at a crude protein content of 70-75%; all costs in terms of ingredients, mixing, pumping,

dewatering, drying, sterilization, processing, overhead, and CAPEX) (Pikaar et al. 2018), and 135 136 the current value of top-quality protein for human food in the market (such as, for instance, pea protein) of about US3500-5000/tonne, it appears that the capture and upgrading of CO<sub>2</sub> to 137 microbial protein has reached a stage of economic feasibility. However, when looking at 138 absolute values, carbohydrate-based products like Quorn <sup>(TM)</sup>, although increasing in produced 139 volumes, at present, represent only a very small fraction of the overall protein market with an 140 annual production of 25,000 tonnes per year (Matassa et al. 2016a). A comparison of the CO<sub>2</sub> 141 footprint of N-fixing crops, such as soy, reveals that it amounts 4-8 tonnes of CO<sub>2</sub> equivalents 142 per tonne soy dry matter produced (http://faostat.fao.org/). In contrast, the microbial route has, 143 144 in principle, a  $CO_2$  footprint that is negative, since anthropogenic  $CO_2$  is fixed, and the microbial biomass produced is generated through green energy, and can be harvested and dried 145 in an energy neutral way, by using natural drying processes. Hence, if the concept of hydrogen-146 147 oxidizing bacteria based food production could be implemented, it offers the potential to contribute to CO<sub>2</sub> avoidance relative to the conventional agro-supply line. Despite the enormous 148 149 market potential to feed 7.5 billion people worldwide with nutritious microbial protein, it seems unlikely that this microbial-based carbon capture and utilization route will directly influence 150 climate change. This is related to the fact that consumers would need to adapt rapidly in the 151 152 near future to this unusual food supply, provided also that it qualifies under the rigorous demands imposed by the regulator on novel foods. However, as the market is currently already 153 open to microbial products, such as Quorn<sup>(TM)</sup>, Spirulina, and other less obvious microbial 154 products, such as cheese and beer, the legislative and societal acceptance could fall within this 155 framework, making the transition to MP less troublesome. The onset of such a route in the 156 coming decades has the potential to decrease the pressure on agricultural land with some 9% 157 (Pikaar et al. 2017a), one of the key drivers of deforestation, biodiversity loss and land use 158

change induced greenhouse gas emissions (Crist et al. 2017, Maxwell et al. 2016, Newbold etal. 2015, Popp et al. 2014).

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# 162 Microbial based biomass for protein rich animal feed

The production of MP to produce livestock feed is well documented (Anupama and Ravindra 163 2000, Kihlberg 1972). It was already produced at industrial scale in the 1970s (Matassa et al. 164 2016a, Pikaar et al. 2017a), when MP was often referred to as single cell protein (SCP). In 1976, 165 the UNESCO science price was awarded to 'large-scale and low-cost production of single cell 166 proteins from oil' (Pikaar et al. 2017b). The bacterial protein product, called Pruteen<sup>®</sup>, produced 167 168 from methanol, was commercialized by Imperial Chemical Industries Ltd in 1980. Interestingly, the Soviet Union government was very active in achieving large-scale industrial 169 production of microbial protein. As described in a recent de-classified CIA report, the Soviet 170 171 Union had a state-wide research programme, entitled "The Soviet Hydrocarbon-Based Single Cell Protein Program", aiming to produce microbial protein in the form of yeast using n-paraffin 172 derived from oil as the carbon and energy source (CIA 1977). Despite these major international 173 efforts, MP never reached full market potential with most of these initiatives being ceased at 174 the end of the 1980s. 175

In recent years, the production of MP has regained significant interest, particularly in the aquaculture industry, with the production of natural gas based MP as a fish food, reaching industrial production at economically competitive prices (http://calystanutrition.com/). The fact that this process relies heavily on the use of natural gas implies that such a pathway will not provide an ultimate long-term sustainable solution. Recently, it was demonstrated that highquality MP with an amino-acid composition similar to fish meal can be produced using hydrogen as energy source coupled with carbon capture (Matassa et al. 2016b) (see Fig 2).



Figure 2. Comparison of essential amino acid composition of H<sub>2</sub>-oxidizing microbial protein by a *Sulfuricurvum spp*. dominated culture (red) with fish meal (orange) and soy bean meal (purple), adapted from Mattasa et al., (2016) (Matassa et al. 2016b).

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Currently, MP production costs appear to be substantially higher than conventional protein-rich 188 supplements, like soy bean meal and fish meal, with market prices in the last 5 years in the 189 order of US\$600-1100 per ton for soy bean meal and \$2000-3000 per ton for fish meal, both 190 191 expressed as 100% protein crude content. The hydrogen based MP production route cannot 192 compete yet with the soybean-for-feed route. It could be competitive with fish protein, though its demand will certainly remain high, due to its very valuable amino acid profile. Current 193 practice of supplying aquaculture with wild-catch fish protein harvested from the ocean, 194 however, is subject to severe environmental considerations, which creates possibilities for other 195 more sustainable opportunities, such as MP. The global aquaculture industry is, at present, 196 under enormous pressure to find alternative, more sustainable, protein sources. Microbial 197 protein production, driven by renewable energy, and coupled with carbon capture, could be an 198 interesting 'out of the box' solution that warrants further exploration. 199

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# Production of Microbial Based slow release Organic Nitrogen (MBB-SON) fertilizer for soil carbon sequestration

It is widely accepted that enhancing the soil organic carbon content in general improves the soil 203 204 health and is well known to increase crop yields (Diacono and Montemurro 2010, Lal 2006, 2009, 2010, 2011, Lal et al. 2007, Steiner et al. 2007). The increase in soil organic carbon also 205 enhances the water holding capacity (Emerson 1995, Rawls et al. 2003), cation exchange 206 capacity and aggregation, and reduces the occurrence of soil erosion (Lal 2006). Recently, 207 increasing the soil carbon of agricultural soils has been proposed as a climate change mitigation 208 tool (Minasny et al. 2017). Indeed, the global carbon currently stored in soils is about a factor 209 3.3 higher than the CO<sub>2</sub> levels in our atmosphere (Lal 2004a). In 2015, the '4 per mille Soils 210 for Food Security and Climate' (http://4p1000.org/) was launched at the COP21 in Paris, with 211 212 the aspirational goal to increase global soil organic matter stocks by 0.4 % per year, which could make a strong contribution to decreasing atmospheric CO<sub>2</sub> concentrations (Minasny et 213 al. 2017). Agricultural soils are of particular interest because these soils have been substantially 214 215 depleted in soil organic carbon since the introduction of intensive agricultural practices, so these have the highest potential to increase in carbon content (Lal 2004b). If the 0.4% increase were 216 217 restricted to agricultural soils, the carbon sequestration potential would be around 1.2 GtC/ year, which corresponds to about 4-5 Gt CO<sub>2</sub> per year (van Groenigen et al. 2017), and this should, 218 in theory, be sufficient to comply with Paris Agreement targets, if immediate and aggressive 219 mitigation is pursued. However, considering an average C/N ratio of 12 for soil organic carbon 220 (SOC) (Batjes 1996), this would require some 100 Teragram reactive nitrogen per year. This 221 value corresponds with the yearly supply of nitrogen from the entire global fertilizer industry 222 (Bodirsky et al. 2014). Hence, achieving the  $CO_2$  mitigating challenge in which the soils play 223 an important role seems unlikely, with the availability of nitrogen being the limiting factor. It 224 can be suggested to focus on 'over-exploited' soils, and try to return them to agricultural 225 practices that assure that the soil organic carbon is not decreasing, and at least remains constant. 226

This will not only prevent increasing soil-related CO<sub>2</sub> emissions, it may also sustain overall
physico-chemical stability of the soil, with higher biomass yields.

The addition of organic materials, such as compost, peat, sewage sludge, and manure, 229 to increase soil organic carbon levels and enhance crop yield are well-established methods 230 (Diacono and Montemurro 2010). However, the use of compost and sewage sludge is often 231 impaired by the fact that these can contain heavy metals and organic pollutants arising from 232 pesticides, pharmaceuticals and personal care products (Andrade et al. 2010, Lozano et al. 2013, 233 Tou et al. 2017, Westerhoff et al. 2015). Animal manure is largely free from such pollutants, 234 but there is increasing concern that manure addition could result in agricultural soils that 235 236 accumulate antibiotic resistant bacteria (McGrath et al. 1995, Singer et al. 2016, Tou et al. 2017, Udikovic-Kolic et al. 2014, Westerhoff et al. 2015, Zhu et al. 2013). Moreover, their overall 237 potential in terms of climate change mitigation is limited (Edenhofer 2014). Considering the above-238 239 mentioned stoichiometric constraints in terms of nutrient, especially nitrogen, availability, and limitations of conventional methods to increase carbon content of soils in the context of climate 240 241 mitigation, we suggest to use a novel approach in which MP is used as a slow-release organic nitrogen fertilizer (see Figure 1). The production process is almost identical to the MP based food 242 and feed production processes described in the sections above, but with some key differences in 243 244 process requirements. The fermentation conditions are less strict in terms of hygiene, there is no need for sterilization and consistent composition of the microbial biomass (i.e., no need for 245 strict, pure culture conditions), and the final product does not require a 100% dry form, reducing 246 the drying requirements. 247

The production of this MP for slow-release nitrogen supply to the soil would still rely on the use of Haber-Bosch process to produce the reactive nitrogen source (Figure 1). However, the inorganic Haber-Bosch nitrogen fertilizer is transformed into an organic nitrogen form. Indeed, it is integrated by the microbes into their cell biomass. The rationale behind this is that, worldwide,

inorganic nitrogen fertilizer has a very low use-efficiency of 40%, due to leaching, run-off, 252 denitrification and volatilization (Bodirsky et al. 2014). The concept is that upgrading this 253 mineral nitrogen to organic nitrogen in the form of MP increases the nitrogen use efficiency 254 with concomitant enrichment of the agricultural soil with organic matter. While many studies 255 highlight the positive impact of increasing the soil organic carbon on *e.g.*, agricultural yields, 256 carbon storage, nutrient and water retention as highlighted above, greenhouse gas fluxes from 257 agricultural soils are very large, complex and highly heterogeneous (Singh et al. 2010, Smith et 258 259 al. 2008, Xu et al. 2011). As such, under certain soil conditions, the increase in soil organic carbon and organic nitrogen levels could even increase carbon dioxide, methane and nitrogen 260 261 emissions from the soil. Long-term trials would be essential to verify whether the addition of MP results in increased storage of carbon in the soil organic matrix, coupled with low nitrogen 262 and highly potent greenhouse gas emissions. 263

264 A situation can be considered in which the total current global use of Haber-Bosch fertilizer N of ~100 Mt/year (Zhang et al. 2015) would first be upgraded to MP. Considering a 265 typical C/N ratio for microbial biomass of 5 (Pikaar et al. 2017a), the theoretical potential of 266 MP to capture and temporarily store carbon in the soils reaches 0.5 Gt C/year (1.83 Gt 267 CO<sub>2</sub>/year). This is substantially lower than the amount of carbon that has to be sequestrated per 268 269 annum in soils according to the Paris mitigation challenges (*i.e.*, 1.2 Gt C) (Minasny et al. 2017). Moreover, part of this MP carbon will be released from the soils over time, as it is biodegraded 270 to release nitrogen to the plants, thus decreasing the net carbon captured. 271

In addition to the limitations in carbon capture potential, this approach also comes with considerable economic constraints. Considering the production cost of about 2800 US\$/tonne HOB-based MP, which is equivalent to a cost of  $\sim$ US\$1500/tCO<sub>2</sub> incorporated, it is clear that these values are much higher than the economic costs for underground carbon storage of CO<sub>2</sub> or other available CCU routes (Service 2016). In contrast to the production of MP as human

food or animal feed, where the microbial biomass product has a high market value, MP for soil 277 278 application has to compete with alternative organic nitrogen fertilizers, such as (digested) manure, sludge, kelp, feathers, and horn meal, as well as with inorganic fertilizer. These have 279 280 a relatively low market value, especially inorganic fertilizers, with prices for urea below US\$500/t N (http://www.indexmundi.com/commodities/?commodity=urea&months=60). The 281 use of inorganic nitrogen is integrated in the MP production cost at a value of US\$112/tonne 282 MP, which is only a fraction of the overall production cost (4%). Even when considering high 283 carbon pricing schemes of US\$150–220/t CO<sub>2</sub> when implementing low stabilisation climate 284 targets such as the Representative Concentration Pathways (RCPs) (van Vuuren et al. 2011), at 285 286 best, a carbon capture benefit of about US\$ 400/tonne MP can be achieved. Even under these low stabilization climate targets, the organic nitrogen has a cost of about US\$2280 per 160 kg 287 N present in the MP, which is equivalent to 14000 US\$/tonne organic N. This is a factor 10 -288 289 20 higher than the current commodity prices for inorganic and organic nitrogen.

In addition to the economic limitations describe above, there are also substantial energyrelated constraints. The production of MP for soil application requires substantial amounts of renewable energy to produce hydrogen *via* water electrolysis. It would require about 3000 Gigawatt of renewable energy per Gt of MP produced (Pikaar et al. 2018). To put this amount into a global context; the current installed capacity of renewables worldwide is only 912 Gigawatt (REN21 2017).

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# 297 Concluding remarks

To deal with the climate change challenge, there is an urgent need to develop alternative routes that can be implemented in the near future, capable of effectively avoiding carbon emissions and/or capturing and utilizing carbon, that also have a positive impact on the environment and the global economy. In this short paper, we examined the potential of autotrophic hydrogen-

oxidizing bacteria to capture and utilize carbon in the form of human food, protein rich animal 302 feed and slow-release nitrogen fertilizer. The production of food via the route of microbial 303 protein has the current potential to decrease the use of fossil fuel, water, pesticides, and land 304 use, to provide the global population with nutritious protein, but there may be issues with public 305 acceptability/demand and would require further research concerning its composition and 306 potential side effects. The production of microbial protein as animal feed via autotrophic 307 microbial biomass is not yet economically competitive. At current hydrogen production costs 308 through water electrolysis, the overall production price of microbial protein exceeds the costs 309 of conventional soybean and fishmeal. Yet, if in the future or in specific geographic regions the 310 311 cost can be decreased substantially or the as costs of conventional soybean and fishmeal increase, this line of production of protein could become cost competitive. 312

The production of microbial protein for slow-release organic nitrogen fertilizer applications 313 314 is clearly of interest as a means to considerably increase the carbon content in agricultural soils, and in light of its potential to reduce global nitrogen pollution. For many reasons, the dynamics 315 316 of such an increase in soil organic carbon storage through this route are hard to predict and would – simply because MP is fully biodegradable – be reversible. Despite its theoretical 317 potential as a clean-tech solution to capture carbon and increase soil organic carbon content, 318 319 the current low market value of organic nitrogen fertilizer, the high-energy demands and current production costs, severely limit the practical feasibility and potential as a climate change 320 mitigation tool. Although MP does not seem immediately ready for practice, this concept opens 321 new long-term perspectives to serve as a food and feed source, combined with its potential to 322 contribute to carbon capture and climate change abatement. 323

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