Science of the Total Environment 776 (2021) 145764

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

An attributional life cycle assessment of microbial protein production: A case study on using hydrogen-oxidizing bacteria

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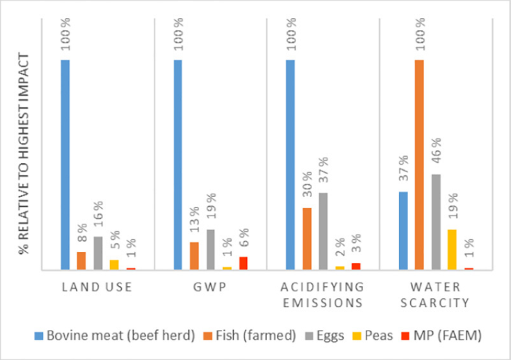
HIGHLIGHTS

- MP had 53–100% lower environmental impacts than animal-based food protein sources.
- Compared to peas and nuts, impacts were 47–99% lower when using hydropower.
- Compared to feed protein sources, MP had a low to average impact.
- However, energy demand for MP is 0.03–25 times that of other feed protein.
- Using renewable energy increased the decoupling of MP from planetary resources.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 December 2020
Received in revised form 30 January 2021
Accepted 6 February 2021
Available online 11 February 2021

Editor: Deyi Hou

Keywords:
Microbial protein
Cellular agriculture
LCA
Food
Hydrogen-oxidizing bacteria

ABSTRACT

Novel food production technologies are being developed to address the challenges of securing sustainable and healthy nutrition for the growing global population. This study assessed the environmental impacts of microbial protein (MP) produced by autotrophic hydrogen-oxidizing bacteria (HOB). Data was collected from a company currently producing MP using HOB (hereafter simply referred to as MP) on a small-scale. Earlier studies have performed an environmental assessment of MP on a theoretical basis but no study yet has used empirical data. An attributional life cycle assessment (LCA) with a cradle-to-gate approach was used to quantify global warming potential (GWP), land use, freshwater and marine eutrophication potential, water scarcity, human (non-)carcinogenic toxicity, and the cumulative energy demand (CED) of MP production in Finland. A Monte Carlo analysis was performed to assess uncertainties while a sensitivity analysis was used to explore the impacts of alternative production options and locations. The results were compared with animal- and plant-based protein sources for human consumption as well as protein sources for feed. Electricity consumption had the highest contribution to environmental impacts. Therefore, the source of energy had a substantial impact on the results. MP production using hydropower as an energy source yielded 87.5% lower GWP compared to using the average Finnish electricity mix. In comparison with animal-based protein sources for food production, MP had 53–100% lower environmental impacts depending on the reference product and the source of energy assumed for MP production. When compared with plant-based protein sources for food production, MP had lower land and water use requirements, and eutrophication potential but GWP was reduced only if low-emission energy sources were used. Compared to

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https://doi.org/10.1016/j.scitotenv.2021.145764

protein sources for feed production, MP production often resulted in lower environmental impact for GWP (FHE), land use, and eutrophication and acidification potential, but generally caused high water scarcity and required more energy.

1. Introduction

Food production is the main contributor to environmental change, such as climate change, land degradation, water scarcity and biodiversity losses (Campbell et al., 2017). Studies have shown that reduction in consumption of animal-based foods is required for improving the sustainability of food systems (Roe et al., 2019; Willett et al., 2019). Novel food production technologies are one way to support this shift (Parodi et al., 2018). The emerging field of cellular agriculture, which uses cell-culturing technologies for food production, has potential to contribute to the supply of sustainable alternatives to animal-based foods (Tuomisto, 2019; Rischer et al., 2020).

Cellular agriculture includes technologies for cultivating animal, microbial, or plant cells in closed conditions, usually utilizing bioreactors with the objective to reduce resource use and environmental impacts, as closed production systems allow efficient recycling and control of emissions. In addition, cellular agriculture may improve the resilience of food production towards environmental changes, as the production systems are not directly impacted by weather conditions and contamination by chemicals and microbes (Rischer et al., 2020). However, the application of cellular agriculture is not completely independent of crop production, as heterotrophic organisms require glucose that is generally sourced from grain or sugar crops (Tuomisto, 2019). The use of autotrophic microbes that are able to obtain carbon from carbon dioxide (CO_2) or methane (CH_4) gas provides advantages as the production process is completely independent of outdoor agriculture. Methanotrophic bacteria obtain energy and carbon from methane, whereas hydrogen-oxidizing bacteria (HOB) utilize hydrogen and carbon dioxide; therefore, crops are not needed as a source of carbon. Technologies for producing methanotrophic bacteria for protein feed are already at commercial-level production, while the development of feed and food ingredients from HOB is currently under development (Ritala et al., 2017; Pikaar et al., 2018a). One promising example of a HOB for the purpose of feed and food production includes the *Cupriavidus necator* (formerly *Ralstonia eutropha*) (Yu, 2014; Liu et al., 2016).

The interest in producing microbial protein (MP) from autotrophic bacteria as a protein replacement for human consumption has grown in recent years (Pikaar et al., 2018b). The inputs for HOB production consist of CO_2 gas, hydrogen, oxygen, nitrogen, and other nutrients. Hydrogen is extracted from water molecules through electrolysis and nutrients are added as a form of fertilizers. Earlier studies have indicated the potential of MP through HOB (hereafter simply referred to as MP unless otherwise specified) to contribute to a sustainable supply of food, particularly through saving of land and water resources as well as by reducing the global warming potential (GWP) and eutrophication potential (Pikaar et al., 2018a, 2018b; Sillman et al., 2019).

However, the performed comparisons in the previous studies were mostly focused on feed replacement and were limited to crops, mycoprotein and microbial protein produced using methanotrophic bacteria (Pikaar et al., 2018b; Sillman et al., 2020). More importantly, the results of both studies were based on theoretical assumption using currently available but limited literature values. Because of that, the system boundaries were limited with many nutrient inputs, the cleaning processes, and wastewater treatment excluded from the studies. Also direct land use for facilities was not taken into account. In addition, the previous studies are limited to a small number of impact categories, which are mostly relevant for conventional crop- or animal-based protein sources, such as GWP, eutrophication, and water use. Due to the high energy requirements, the environmental analysis of the production of MP requires the impact categories to include also those relevant for products that are produced in an industrial setting rather than agriculturally. Additionally, although the former mentioned studies had included water use in the analysis of MP, none of the studies looked at water scarcity using AWARE — the latest consensus characterization model to assess the impacts of water use (Boulay et al., 2018). Due to these limitations, there is a need to estimate the environmental impact of MP production using empirical data and to expand both the environmental impact categories as well as the comparison to other protein sources for food and feed.

This study aimed to assess the environmental impacts of MP production for the first time on an empirical basis while expanding the system boundary and impact categories compared to the previous studies. This was necessary to increase the knowledge on the environmental impact of MP production and fill up the existing knowledge gaps described earlier. The required inputs were calculated based on data from a currently existing test-scale production process. As MP can potentially be consumed by humans in addition to being used as a novel feed ingredient, this study, additionally, aimed to compare the impacts of MP production with protein sources used for both feed and food; these include animal- and plant-based protein sources, as well as protein produced with insects and algae. An attributional life cycle assessment (LCA) was used for the assessment. Performing a LCA quantifies the environmental impacts throughout the entire life cycle of the product along the selected system boundaries and allows for a trade-off comparison of multiple impact categories (Henriksson et al., 2011; Dijkman et al., 2017). Uncertainties were calculated using a Monte Carlo analysis. As large-scale MP production has a high reliance on electricity, this study also included an assessment of the impacts of using alternative energy sources in various production locations using a sensitivity analysis.

2. Materials and methods

2.1. Scope of the study

The goal of the study was to assess the cradle-to-gate environmental impacts of MP production and compare the impacts with other protein sources. In the base scenarios, it was assumed that MP production takes place in the Helsinki metropolitan area, as production of MP is currently being developed in Finland. In the sensitivity analysis, different production options were considered, including a change of the production location with Morocco and Iceland as alternatives. These locations were chosen as a possible best representative to optimize the corresponding renewable electricity sources —geothermal energy and solar energy— as these are not sensible options within Finland.

The assessment was performed using SimaPro 9.1.0.11 PhD LCA software package (PRé Consultants, 2020). The ReCiPe 2016 v1.1 Midpoint (H) method was selected to calculate the GWP100, land use, freshwater and marine eutrophication potential, terrestrial acidification, and human carcinogenic and non-carcinogenic toxicity (Huijbregts et al., 2017). The impact of water use was assessed in terms of the water scarcity using the AWARE method that is part of the LCA_{water} assessment (Boulay et al., 2018). The AWARE yearly aggregated non-agriculture characterization factor (CF) (WULCA, 2015) was selected to calculate the water scarcity based on the water use of the product. Both direct and indirect water usage were considered but specific local AWARE factors could only be applied for direct water usage owing to the uncertainty of the origin of water usage in the background activities. The life cycle industrial energy use was calculated with the CED V1.11 method by ecoinvent (Althaus et al., 2007).

The high electricity consumption differentiates cellular agricultural products, including MP, from typical agricultural food and feed items. Electricity production can results in environmental impacts that are otherwise less relevant for agricultural products. This article therefore aims to extent the environmental impact analysis from previous studies (Pikaar et al., 2018b; Sillman et al., 2019, 2020) by including the impact categories that belong to the $\text{LCA}_{\text{water}}$ degradation category (Boulay et al., 2018) and the CED.

The functional unit (FU) of the system was 1 kg of MP product prior to packing with a 5% moisture content at factory gate. The nutritional content was 65% protein, 6% fat, 2.2% carbohydrates, and 11% fiber, although higher protein concentrations are also possible by increasing the nitrogen inputs (Sillman et al., 2020). It was assumed that there are no byproducts, although the wastewater of the separation and drying phase could potentially be used as a fertilizer due to the amount of nutrients present. However, this was outside of the scope of our research.

With the exception of the impact on land use, facilities were excluded from the scope of this study. This was due to the minor contribution to the total environmental impacts of MP and to be consistent with the methodology used in the quantification of the impacts for the other protein sources that MP was compared with (Poore and Nemecek, 2018a). More details regarding the environmental impacts of facilities are shown in SI1, Section 8.

2.2. System description

2.2.1. System boundaries of microbial protein production

The production of single-cell protein starts by propagation of the HOB for fermentation by increasing the cultivation volume in 10-fold increments until a production volume of 200 m^3 is reached. The production occurs in a continuous stirred-tank bioreactor where the bacteria grow continuously in steady-state conditions. Hydrogen, oxygen, and CO_2 gases are the main inputs into the fermentation. Hydrogen and oxygen are produced from water and electricity in water electrolysis.

Water-based liquid mineral medium is supplied continuously to the cultivation through filter sterilization. The medium contains ammonium as a nitrogen source and inorganic salts containing sulfur, phosphorus, magnesium, sodium, potassium, iron, and calcium. Manganese, zinc, vanadium, boron, molybdenum, cobalt, nickel, and copper are present in minor amounts. Phosphoric acid (H_3PO_4) and sodium hydroxide (NaOH) are used to control pH. In addition to water electrolysis, electricity is also needed for reactor mixing and pumping of the medium feeds. The CO_2 fed to the microbes as a carbon source is assumed to be released back to the atmosphere during the consumption of MP and therefore will have no net effect on the GWP. It is common practice in LCA not to account for carbon assimilated into the body. This is mostly because there would be many assumptions to be made on whether or not the carbon is assimilated in the body and for how long. Liquid CO_2 was supplied to the factory and stored outside. CO_2 was modeled as a waste gas of chemical production processes in the ecoinvent database (Hischier, 2019). The SI1 section 1 provides a full list of details on assumptions per ingredient and possible transportation distances for the base model.

After fermentation, the broth is pasteurized by heating with low-pressure (LP) steam to 120 °C, after which the broth proceeds to the separation stage. In the separation unit, the supernatant is separated from the biomass through continuous centrifugal separation. While the supernatant is sent to the municipal wastewater treatment plant (WWTP), the concentrated cell slurry proceeds to the drying unit, where a drum dryer is used to remove the remaining excess water from the product. The drum dryer cylinders are heated with low-pressure steam to 120 °C. The final single-cell protein product then comes out as a flour-like powder. The final packaging of the product is beyond the scope of this article. A flowchart of the process is shown in Fig. 1.

The bioreactor, inoculum reactors, media preparation line, and downstream processing equipment all require regular cleaning. All cleaning occurs 4 times a year through the cleaning in place (CIP) method. CIP involves washing the equipment and connecting pipes with NaOH and nitric acid solutions and flushing with water (Eide et al., 2003). Exact details on the inventory of CIP are given in SI1, section 3 and 8.

2.2.2. Scenarios

Two scenarios, named Finnish average energy mix (FAEM) and Finnish hydropower energy (FHE), were compared to explore the impacts of different conditions under which MP could be produced. The scenarios had differences in energy sources, production of steam and CO_2 inputs, and recycling of wastewater (Table 1).

2.2.3. Life cycle inventory data

Data for the MP production processes were gathered from current pilot-scale production settings performed by the company Solar Foods Oy located in Finland, expert interviews, and the literature. The ecoinvent 3 database was used for data for background processes (Wernet et al., 2016). The total plant area was 1580 m^2. Emissions for direct land-use change (LUC) were assumed to be zero as it was assumed that the facilities are occupying land that was previously land for farm facilities. This was based on the assumption that MP could replace protein sources that require a substantial amount of land, such as beef production (Poore and Nemecek, 2018a). Inventory data for the production of MP provided by Solar Foods is provided in SI1, section 8 per FU.

Regarding wastewater recycling, the freshwater balance (in the form of tap water) was calculated for each process step as the difference between the water inputs and the water outputs (Pfister et al., 2016). For the centralized WWTP, operational energy and chemical consumptions were estimated based on a report published by a local authority (HSY, 2017). For the on-site wastewater treatment system, reverse osmosis (RO) with ultrafiltration as pretreatment was considered. It was assumed that reject water from the treatment system was sent to the centralized WWTP.

Inventory data for these wastewater treatment processes were taken from published literature (Muñoz and Fernández-Alba, 2008; Vince et al., 2008; Greenlee et al., 2009). In the Helsinki metropolitan area, almost all tap water is extracted from a nearby lake and treated wastewater is released to the sea and thus considered as no longer available for use at the source of extraction (HSY, 2019). Wastewater pollutants are listed in SI1, section 8, where phosphorus emissions are based on 80% uptake of phosphorus in the production process. Further information about wastewater treatment is provided in SI2.

The production of MP also requires cooling water. However, as a closed circulation system is utilized, it was assumed that water was extracted once during the construction of the plant. The amount of cooling water is therefore considered negligible in the $\text{LCA}_{\text{water}}$ analysis.

2.3. Uncertainty analysis and sensitivity analysis

A Monte Carlo analysis (MC) with 1000 iterations was performed with a 95% confidence interval. The pedigree matrix was used to calculate uncertainty ranges in SimaPro (Wernet et al., 2016) (SI2 provides uncertainty ranges). Ranges were conservatively overestimated rather than underestimated. In addition to the MC analysis, the bootstrap method was used to handle extremely large uncertainty ranges that normally result from MC analysis of water scarcity results. These are due to the incorrect estimation of probability distribution of the AWARE characterization factors (Lee et al., 2018). The bootstrap

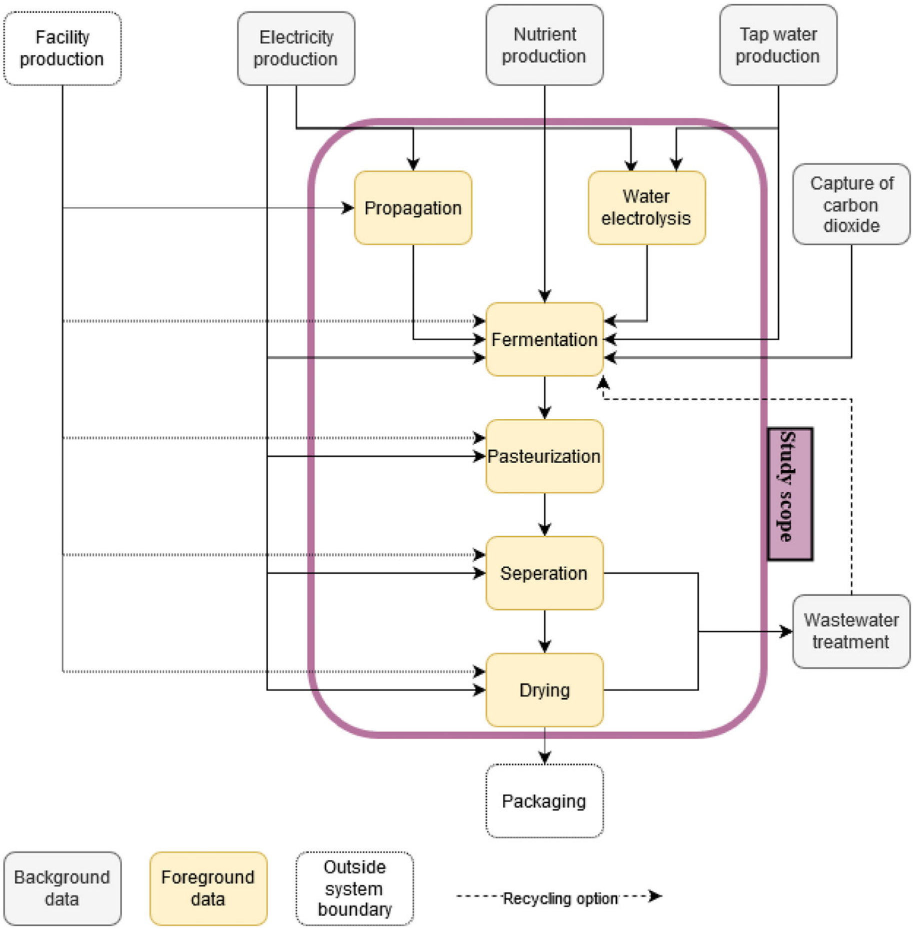

Fig. 1. Flow chart and system boundaries of MP production as studies here.

analysis was performed with Python 3.0, running 1000 simulations with a sample size of 300 allowing for replacements.

A sensitivity analysis was used to assess the impacts of different assumptions on the results. The following two separate sensitivity tests were performed: i) to test the sensitivity of the production inputs using the FAEM scenario, and ii) to test the sensitivity of the environmental impacts of MP production resulting from the choice in low carbon energy sources. The FHE scenario was used for this purpose. As not all low carbon energy sources are suitable for Finland, the production location was changed accordingly. Morocco and Iceland were selected for the alternative production locations due to their special characteristics enabling feasible renewable energy production (photovoltaic cells (PV) in Morocco and geothermal energy in Iceland). Nuclear power was selected as an alternative for Finland due to the country's current high reliability on nuclear power and its role in the Finnish Climate and Energy Strategy (Ministry of Economic affairs and employment in Finland, 2020). Table 2 shows the tests used in the sensitivity analysis.

Table 1
Scenarios for MP production.

Variables	Scenarios	
	Finnish average energy mix (FAEM)	Finnish hydropower energy (FHE)
Location	Helsinki, Finland	Helsinki, Finland
Electricity	Finland average electricity mix[a]	100% hydropower
Steam	Supplied	On-site using electricity[b]
CO_2	Supplied	On-site using electricity[b]
Wastewater	Sent to central municipality wastewater treatment plant	Recycling of 80% of the supernatant on-site using reverse osmosis and combined with ultrafiltration.

[a] SI1, section 5 lists the mix of energy sources for the Finnish electricity mix as modeled in this article.

[b] SI1, section 2 provides details on calculations for water and electricity requirements for on-site production.

2.4. Comparison to existing and novel protein sources

The environmental impacts of MP according to the two baseline scenarios were compared with other protein sources traditionally used for human consumption based on data from Poore and Nemecek (2018a). In the comparison, 100 g of protein was used as a FU with a 65% assumed protein content of MP. Europe-specific results from the study by Poore and Nemecek (2018a) were used and adjusted to match the cradle-to-gate system boundary of this study (SI2, 'comparison') (Personal communication with Poore and Nemecek, 2018b). To allow for comparison, LCA results for MP production have been recalculated using the methods applied by Poore and Nemecek (2018a).

In addition, there are alternative protein sources that are either novel and/or used as a feed ingredient (some of which can also be used for human consumption). The environmental impact results from MP production were therefore also compared to those listed in Table 2A from the study by Smetana et al. (2019). The results for mycoprotein from the study by Smetana et al. (2015) as well as GWP results from MP calculated by Sillman et al. (2020) were added to the comparison in the SI2, 'comparison'. Other impacts calculated by Sillman et al. (2020) were not included as units were different from the results published by Smetana et al. (2019). Most of the results in

Table 2
Variables for the sensitivity analyses.

Sensitivity analysis 1: Finnish average energy mix (FAEM)

Test name	Changed parameter	Baseline	Alternative	Explanation
Ingredients				
FAEM - steam	Steam	Supplied	On-site production	All ingredients are supplied in the baseline model. However, steam and CO_2 could be produced on-site. The impact of producing steam and CO_2 on-site by using the Finnish average electricity mix was tested.
FAEM - CO_2 on-site	CO_2	Supplied	On-site production	
FAEM - electrolyzer	Electricity (kWh)	14.13 (79%)	18.6 (60%)	The efficiency of electrolysis is in the range of 60%–80% (Hydrogen Europe, 2021)
FAEM - nutrients 85% utilization	Utilization of CO_2, H_2, O_2, and NH_3	99%	85%	In an earlier set-up performed by the company producing MP, the utilization of these nutrients in the bioreactor was tested at 85–90%.
Transportation				
FAEM - transport[c]	**Lorry (tkm):**	0.0571	0.0171	Transportation distances for the baseline scenarios were calculated based on the location of the potential European supplies in relation to the Helsinki metropolitan area. However, these were approximations as it is unknown where supplies come from. In the alternative scenario, we assumed that suppliers are located in China.
	Ammonia water (km)	400	150	
	Iron sulfate (km)	100	100	
	Sodium sulfate (km)	400	150	
	Plane (tkm):	0.0488	0.8723	
	Ammonia water (km)	–	7365	
	Iron sulfate (km)	2250	7365	
	Sodium sulfate (km)	1500	7365	
Wastewater				
FAEM – 80% water recycling	Recycling of supernatant	No recycling	80% recycling	The impact of recycling of wastewater versus no recycling concerning eutrophication and water consumption. This was expected to decrease water scarcity results.
FAEM – 50% water recycling	Recycling of supernatant	No recycling	50% recycling	

Sensitivity analysis 2: Finnish hydropower energy (FHE)

Test name	Changed parameters	Baseline	Alternative	Explanation
Energy source within Finland				
FHE – wind (FI)	Wind[a]	100% hydropower	100% wind	
FHE – nuclear (FI)	Nuclear[b]	100% hydropower	100% nuclear	
Location and energy source				
FHE - solar (MR)	Location	Helsinki, Finland	Morocco	Morocco could be a potential candidate for MP production based on solar energy.
	Energy source	100% hydropower	100% solar power[d]	Most sensible renewable energy source will vary per location.
	PV yield (kWh/kWp)	–	1826 (World bank group, 2020)	Approximation. The land requirements vary depending on the location of the PV cells.
	Land requirements ($m^2a\ kwh^{-1}$)	–	0.0065 (Martín-Chivelet, 2016)	Approximation. The land requirements vary depending on the location of the PV cells.
	Land occupation (type)	Grassland	Sparsely vegetated	The land occupation for Morocco was set to sparsely vegetated.
	Transportation, lorry (tkm)	0.0571	0.0336	See SI2 for further details on assumptions.
	Transportation, plane (tkm)	0.0488	0.2967	See SI2 for further details on assumptions.
	AWARE factor	2.2	54.031 (WULCA, 2015)	AWARE scarcity factor is location dependent.
	Water source	Lake	River (SEMIDE, 2005)	Most drinking water comes from rivers.
	Water recycling	Yes	Yes	Recycling water in water-scarce areas is preferred.
FHE – geothermal (IS)	Location	Helsinki, Finland	Iceland	Iceland could be a potential candidate for MP production based on geothermal energy.
	Energy source	100% hydropower	100% geo-thermal[e]	Most sensible renewable energy source will vary per location.
	Transportation, lorry (tkm)	0.0571	0.0336	See SI2 for further details on assumptions.
	Transportation, plane (tkm)	0.0488	0.2862	See SI2 for further details on assumptions.
	AWARE factor	2.2	1.083 (WULCA, 2015)	
	Water source	Lake	Ground (Gunnarsdottir et al., 2016)	In Iceland, 95% of drinking water comes from groundwater and does not require treatment.
	Water recycling	Yes	Yes	

[a] Adjusted ecoinvent 3.6 database 'Market for electricity, medium voltage | Cut-off {FI}' to include only the wind energy in the ratio that was already there (Wernet et al., 2016).
[b] Adjusted ecoinvent 3.6 database 'Market for electricity, medium voltage | Cut-off {FI}' to include only the nuclear energy in the ratio that was already there (Wernet et al., 2016).
[c] See SI, section 1 for details on travel distance assumptions.
[d] Ecoinvent only contains rooftop-installed PV cells. To model for ground-installed PV cells, it was therefore decided to use the rooftop-installed PV cells from the ecoinvent database and add the required 0.0065 $m^2\ a^{-1}\ kwh^{-1}$ land use in the Simapro model as 'land occupation, industrial area'. The ecoinvent equation was used to recalculate the total amount of installed units required to produce 1 kWh for the Moroccan conditions (Treyer, 2019; Jungbluth et al., 2009)
[e] Adjusted ecoinvent 3.6 database 'Market for electricity, medium voltage | Cut-off {IS}' to include only geothermal energy in the ratio that was already there (Wernet et al., 2016).

the article by Smetana et al. (2019, 2015) were calculated using the IMPACT 2002+ impact method (Jolliet et al., 2003). The environmental impact of MP production was therefore additionally calculated for the corresponding methods when necessary. As the system boundary in the study by Smetana et al. (2015) included also transport and cooking after processing, final results of the study were reduced to match the system boundary used in this study; this was done by using the results of the contribution analysis.

3. Results

3.1. Results and contribution analysis

Fig. 2 shows the results and contributions per scenario for each impact category with standard deviations (SD) from the MC test indicated with a black line. The results show that the FAEM scenario had a higher environmental impact than the FHE scenario on all evaluated categories.

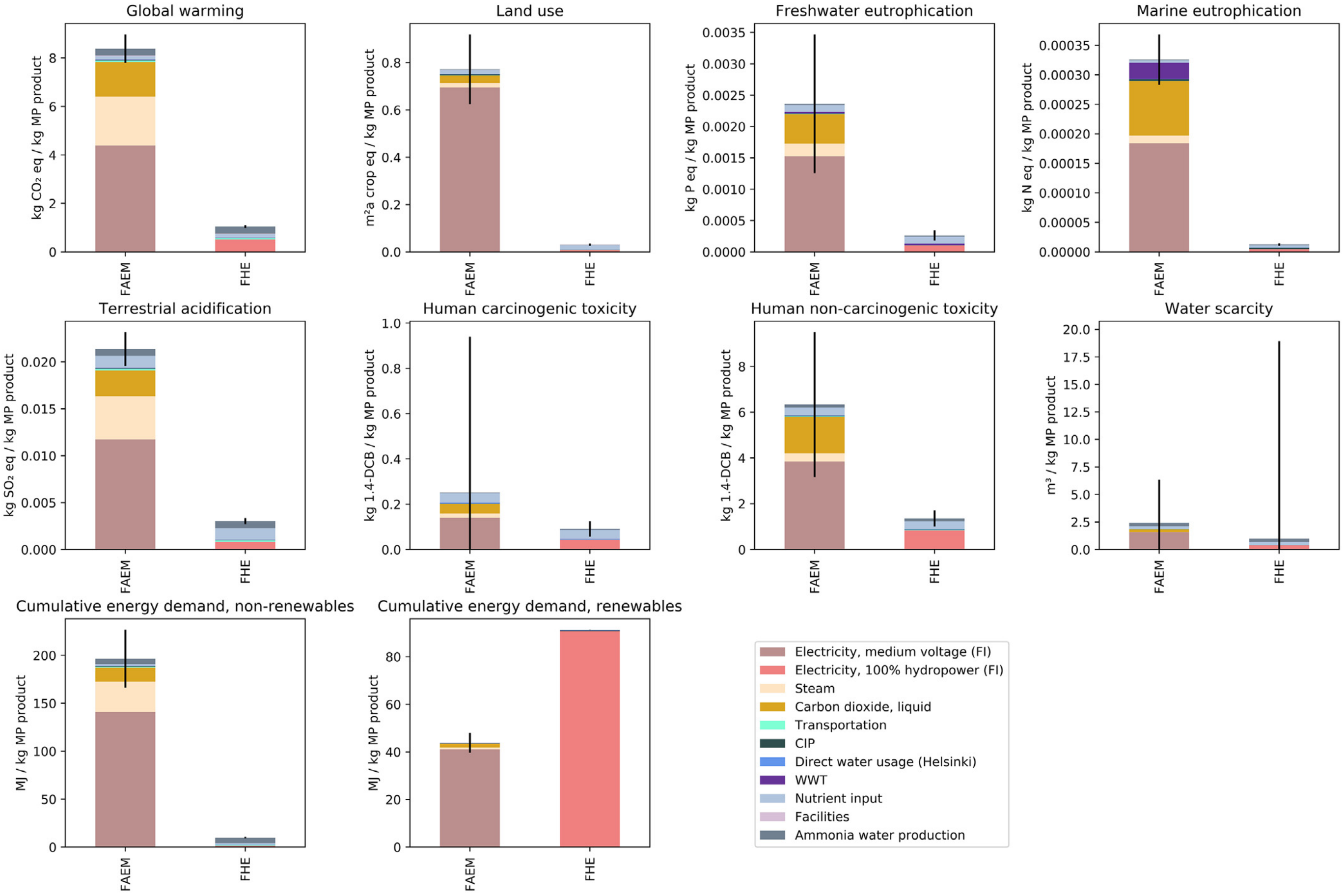

Fig. 2. Results and contributions for different impact categories for all scenarios per kg of MP product with Monte Carlo standard deviation results indicated with a black line, and where FAEM refers to the 'Finnish average electricity mix' scenario and FHE refers to the 'Finnish hydropower energy' scenario.

The results show a high contribution of electricity production for both scenarios and across all impact categories. Most of this electricity was consumed during fermentation in the electrolyzer block (SI1, section 8). A detailed description of the results and the relative contributions are shown in SI1 section 6.

Total land use for the FAEM scenario is over 25 times higher than that of the FHE scenario, despite the higher direct electricity consumption in the latter (see SI2, contribution analysis). This can be explained by the small reliance of hydropower on land use (0.003 m^{2}a crop eq/kWh) in comparison to that of the Finnish average electricity mix (0.046 m^{2}a crop eq/kWh). This means that producing CO_2 on-site using hydropower would further reduce the land requirements for MP production, because the land use requirements of hydropower are low in comparison to the land requirements of the supplied CO_2 (0.047 m^{2}a crop eq/kg MP).

The FHE scenario had eight times lower GWP per FU than the FAEM scenario. This large difference can be explained by the high contribution of electricity production. The total emissions caused by direct electricity consumption were 4.38 kg CO_2 eq/FU in the FAEM scenario and 0.52 CO_2 eq/FU in the FHE scenario. GWP caused by the supply of CO_2 and steam resulted in additional emissions of 1.43 kg CO_2 eq/FU and 2.02 kg CO_2 eq/FU, respectively, in the FAEM scenario. However, in the FHE scenario these were both produced on-site using renewable energy.

The FAEM scenario had the highest CED score, with 240.2 MJ (SD 21.65) of energy consumed. The share of renewables was 18%, which was explained by the relatively high reliance on renewable energy within the Finnish electricity mix (Statistics Finland, 2018). The CED for the FHE scenario was 101.2 MJ (SD 0.52), with the majority coming from renewables (90%). Most of the CED is related to electricity consumption, with 182 MJ (76% of the total contributions) and 92 MJ (91%) for the FAEM and FHE scenario, respectively. This was despite the fact that the direct electricity consumption was higher for the FHE scenario as both steam and CO_2 are produced on-site. This is explained by the lower impact factor resulting from energy use through hydropower than that of the average electricity mix in Finland. The on-site production of CO_2 and steam also ensures that these inputs were produced with renewables, thereby further reducing the reliance on fossil energy sources.

Results for water scarcity shows high uncertainty ranges for water use, with a SD of 3.9 and 18.0 m^3 for the FAEM and FHE, respectively, even after the bootstrapping analysis. The larger uncertainty range of the FHE scenario could potentially be explained by the large water requirements for electricity generation (using hydropower at 0.0167 m^3/kWh) (Wernet et al., 2016). Although most water only passes through the system and thereby remains available for the ecosystem, some water is lost. When a large amount of electricity is needed, as for the production of MP, the uncertainty related to total water lost in the throughput of water during electricity production could therefore contribute to a high uncertainty in the water scarcity results. The direct water usage and wastewater treatment had a minor contribution to water scarcity. Although the water demand for recycling water increased due to a high increase in electricity used for a RO unit, the combined water scarcity for direct water usage and water used for wastewater treatment options was smaller when the supernatant was recycled.

Fig. 2 shows that the FHE scenario also had a substantially lower impact for eutrophication, acidification, and human toxicity than the FAEM scenario. This is mostly explained by the different electricity sources and the use of renewable electricity for the production of steam and CO_2 on-site. The freshwater eutrophication of the FHE scenario was approximately a tenth of the FAEM scenario. In addition, recycling and treating the supernatant on-site before sending it to the WWTP reduced the contribution of wastewater treatment to marine eutrophication potential by 99.7%. This reduction in the water degradation scores was mostly explained by the switch in electricity from the average Finnish electricity mix in the FAEM scenario to hydropower in the FHE scenario.

3.2. Sensitivity analysis

3.2.1. Sensitivity analysis of the FAEM scenario

Fig. 3 shows the effects of various assumptions related to the production of MP on the results and the trade-off between these assumptions. For example, the choice to produce steam on-site rather than having it supplied reduced the GWP and terrestrial acidification but increased the impact on all other categories. An assumed increase in supply distances increased the GWP with 17.8% despite the relatively small contribution of transport in the initial results of the FAEM scenario. Another increase in the results that could be found from the sensitivity analysis was the increase in the environmental burden when the assumed efficiency of the electrolyzer was lowered. The assumption that CO_2 would be produced on-site rather than supplied reduced the overall impact of MP production. The biggest change was visible when the utilization of the main nutrients in the bioreactor changed from 99% to 85%. This was especially true for marine eutrophication due to the 13 fold increase in the amount of ammonia in the wastewater. Water scarcity decreased when wastewater was recycled. However, the results show high uncertainty ranges, even after bootstrapping. Uncertainty ranges for water scarcity between tests also overlapped. This limits the possibility to make conclusions about the effect of different assumptions on water scarcity.

3.2.2. Sensitivity analysis for the FHE scenario

The results for producing MP with various sources of energy and for different production sites are shown in Fig. 3. Producing MP with 100% hydropower generally resulted in the lowest environmental impact. For water scarcity, however, the advantage of using hydropower was less clear and uncertainties were high. MP production with Finnish nuclear power had the lowest GWP but had the highest contribution to water scarcity. This was the case even though Finland had a relatively low water scarcity impact factor compared to i.e. Morocco, where the water scarcity impact factor is high (WULCA, 2015). This can be explained by the fact that most water for MP production if produced in Morocco was used indirectly during electricity generation, meaning that the Moroccan local impact factor had a minor relevance. Only 20.3% of the contribution to water scarcity in the FHE-solar (Morocco) test was caused by direct water use. However, uncertainty ranges for water scarcity were generally large and the relative difference between the various tests were relatively small in comparison. Therefore, conclusions related to the impact of electricity source and production site on the water scarcity need to be drawn with care.

The environmental impact of MP produced with solar energy was mostly related to silicon production, which contributed approximately 23.2% to the total GWP of solar panel production. MP produced with solar energy in Morocco had the highest impacts in many impact categories. However, in comparison to the FAEM scenario, all different varieties of the FHE scenario generally resulted in lower environmental impacts.

3.3. Comparison with alternative protein sources

Fig. 4 shows the results for the comparison between the production of MP and the alternative protein sources for human consumption. The results show that MP production had lower environmental impacts compared to animal-based protein sources. The GWP from MP in the FAEM scenario was 6.2% and 7.3% of that when producing the same amount of protein from bovine meat from beef herd and dairy herd, respectively. For the plant-based proteins that were included, peas had a lower GWP compared to MP produced in the FAEM scenario. The mean acidification potential for peas was also lower.

Fig. 4 also presents the environmental impacts of protein sources for feed including MP results for both the FAEM and FHE scenario (SI2 provides a more detailed overview including original data sources, including the comparison to mycoprotein from a study by Smetana et al. (2015)). The comparison shows that the production of MP in the FAEM scenario results in a similar GWP as most other protein sources for feed. However, only soybean meal and rapeseed cake had a lower GWP when MP is produced with conditions in the FHE scenario. For acidification, eutrophication, ozone depletion and land use, MP production in both scenarios resulted in mostly lower scores compared to the other protein sources whereas its production caused mostly more water scarcity and required a higher energy demand.

4. Discussion

The environmental analysis performed in this study was based on an attributional LCA. However, an alternative option would have been to perform a consequential LCA, which would be in accordance with the ISO 14049 (Weidema, 2014). One argument for this would be that the attributional system is often described as modeling a system that *has contributed* to an environmental impact, whereas a consequential system would examine what *is expected to change* when the product is produced (Weidema, 2014). As MP is not yet on the market, it would be recommended for future research to analyze the environmental impacts based on the consequential approach. The biggest expected difference in results would relate to electricity consumption, as consequential LCA would model the marginal electricity source rather than choosing a preferred supplier, as in this study (Consequential-LCA, 2015).

As electricity consumption contributed most to the environmental impact of MP production, the choice and availability of the electricity sources will influence results. The electricity mix used in the FAEM scenario consists of 17.9% renewable energy and 29.1% nuclear power (Treyer, 2014). When producing MP in a country with an electricity mix that relies more heavily on fossil fuels, the environmental impact would likely be higher and vice versa. In addition, the high reliance on industrial energy might also result in other sustainability conflicts. For example, as different sectors rely increasingly on renewable energy sources, issues such as a shortage of rare earth metals required for production of solar panels or wind turbines may limit the scale of these technologies (Smith Stegen, 2015).

However, MP production is more flexible than the most protein sources as it does not require agricultural land. The low reliance on land for MP production enables possibilities to use land for other purposes, something that can also be referred to as *land opportunity costs*. A potential future shift towards protein consumption from MP (FAEM) instead of from dairy herd or bovine meat produced in Europe will save about 15.9 (7.7–26.8) m^2 and 35.6 (23.9–44.69) m^2 land per 100 g of protein, respectively. This is a relevant difference, as land use pressure increases with a growing world population and a potential increase in biofuel production. This could also open up the possibility to restore land to forest areas. The current most effective way of storing carbon is through (re)plantation of forests across the earth (Bastin et al., 2019).

Although this study has increased the number of impact categories included in the LCA study of MP production in comparison to previously published articles, there are still impact categories that were excluded from the assessment. One example is biodiversity. This is especially relevant when comparing the impact of MP production to other protein sources. For example, Torres-Miralles et al. (2019) have looked at the High Nature Value (HNV) farming systems using semi-natural grassland

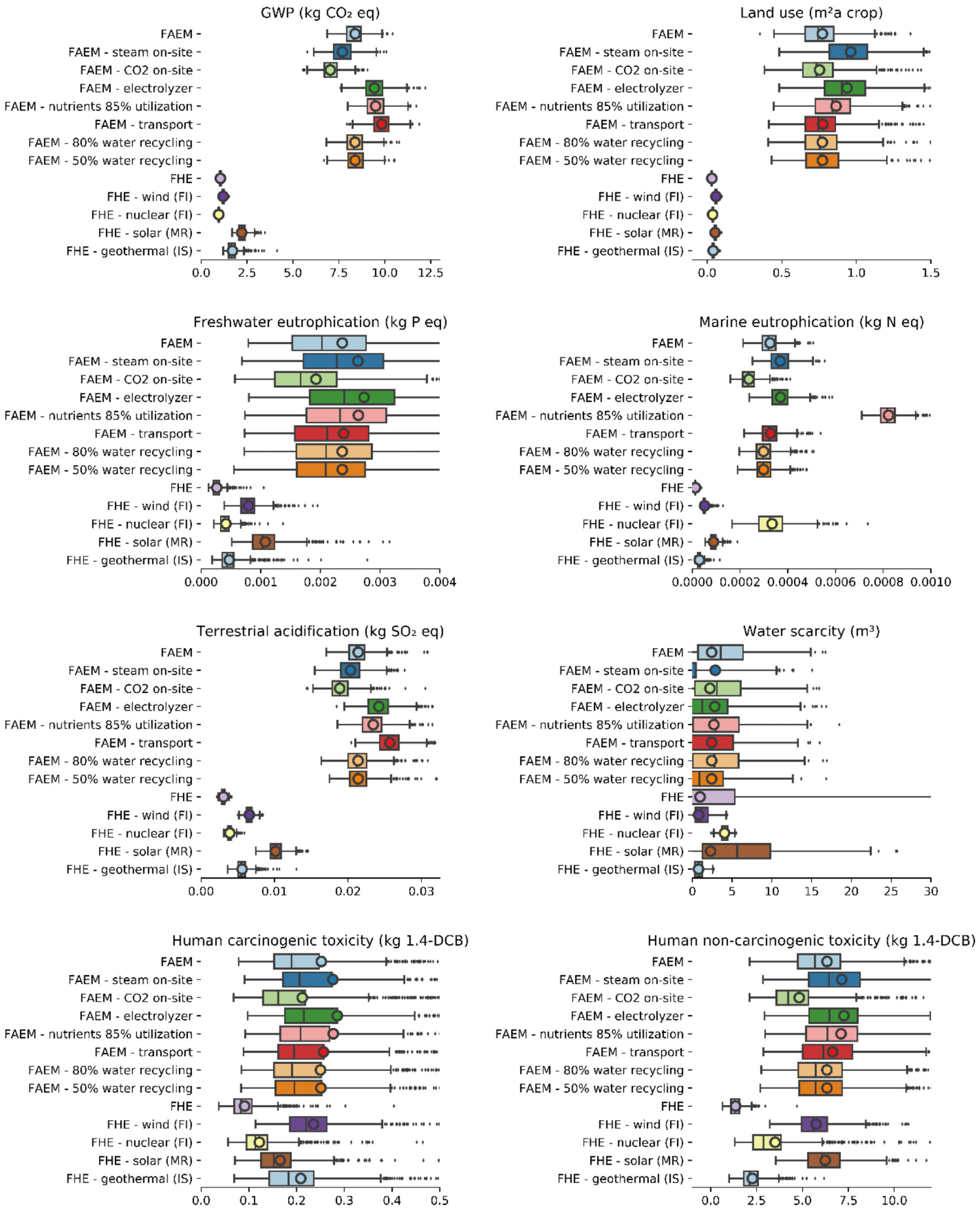

Fig. 3. Results of the sensitivity analysis per 1 kg of product in boxplots and outliers for the Finnish average energy mix (FAEM) scenario and the Finnish hydropower energy (FHE) scenario, with baseline results shown in circles for Finland (FI), Morocco (MR), and Iceland (IS).

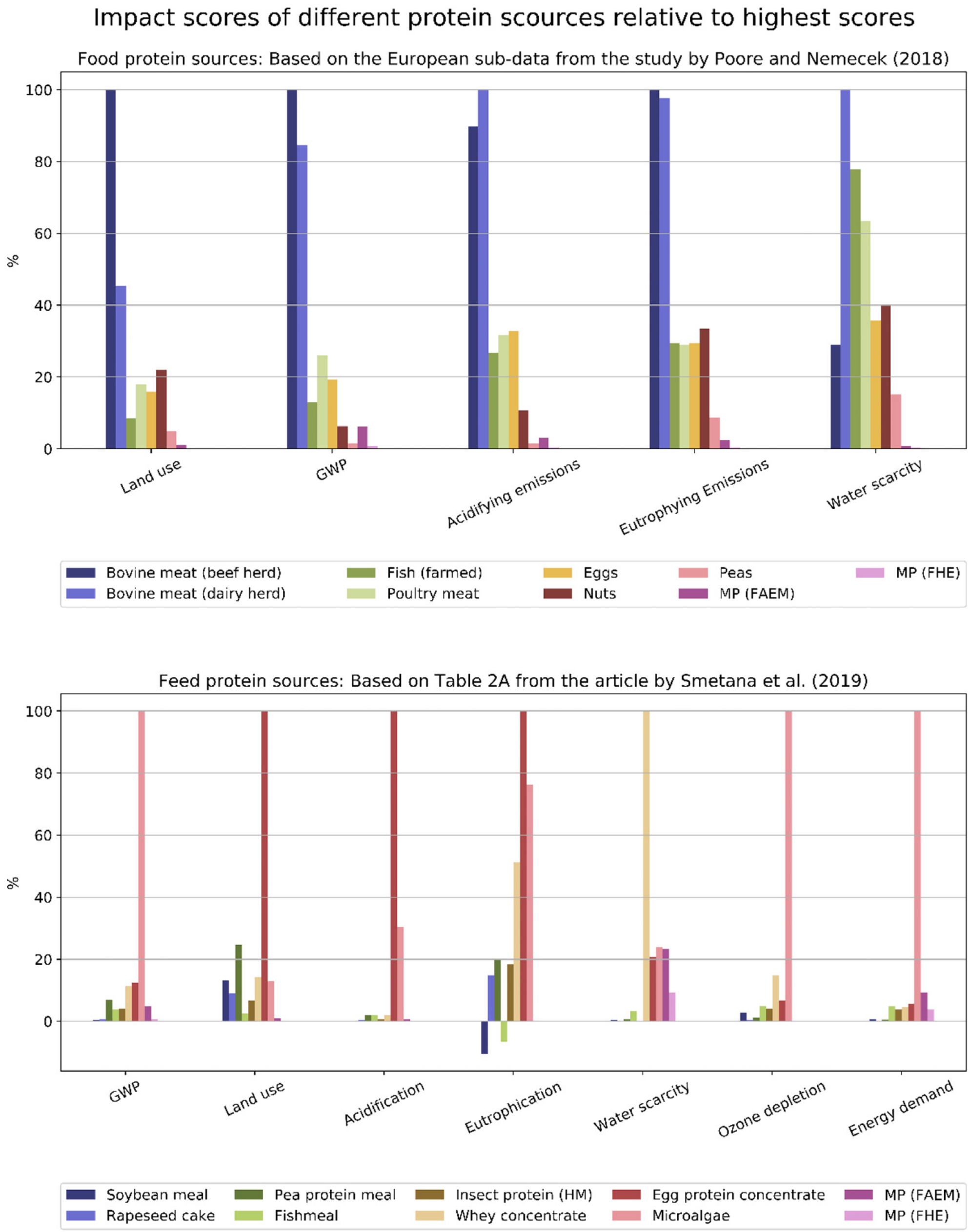

Fig. 4. Comparison of the environmental impact results of MP production with other protein sources for food and feed production.

in Finland producing animal products. Although animal products from the NHV generally have a higher GWP than that of MP products, the HNV system does contribute to the maintenance of biodiversity within Finland (Torres-Miralles et al., 2019). Since MP is an industrial product, it would not have positive impact on biodiversity. On the other hand, the production of MP requires a small amount of land, and land use and land-use change have been shown to have severe effects on both biodiversity as well as ecosystem services that land provides (Koellner and Geyer, 2013). As biodiversity loss plays an important aspect when looking at food production systems, further research is needed to compare different cellular protein sources with agricultural protein sources (Crenna et al., 2019).

A limitation to our results is the functional unit (100 g of protein) that was used for comparing the results of MP with other protein sources. Although comparing results in units of protein is a common practice (Poore and Nemecek, 2018a; Smetana et al., 2019; Sillman et al., 2020), there are limitations to this as the nutritional content of different protein sources vary. Some studies have suggested the use of functional units based on nutritional indexes that consider multiple nutrients (Saarinen et al., 2017; Sonesson et al., 2019). Another way to compare food products would be to use a balanced meals delivering approximately the same nutrition to the consumer (Virtanen et al., 2011). We recommend for future research to take this into account.

About one third of the MC results of the AWARE method gave negative values, and in some cases, human carcinogenic toxicity results were also negative. These values were ignored, as it is not logical for the production process of MP to have negative results in these impact categories. Negative results for any impact category with MC can be explained by the fact that the computational matrix of LCIs can result in inverted operators where numbers flip from positive to negative or vice versa due to random sampling (Henriksson et al., 2015). Additionally, for water scarcity, the negative values were a result of how MC iterations are performed. Both water input and output are first calculated independently and then subtracted from each other. This sometimes leads to a situation where the sampled output is larger than the sampled inputs. This is a known problem with water use (*Communication Within the Pré Sustainability LCA Discussion List With the Topic "AWARE Water Scarcity, Negative Outcomes for Monte Carlo"*, 2020).

In addition, Heijungs (2020) demonstrated that the application of MC leads to overly precise estimated parameters. This is typical for cases with a limited amount of samples, which is often the case in LCA studies. This is also a limitation of this study as the results are based on a single case study. Heijungs (2020) further states that when using the popular pedigree approach, large-scale MC should not be used. However, the paper also states that there are currently no means to address these types of uncertainty in LCA. Despite our acknowledgement and agreement with this limitation, it was decided to perform a MC while not reporting central values due to the lack of an alternative way to report uncertainties. Instead, ranges (with box-and-whisker plots) of the MC iterations were reported to show the uncertainties of the results. This was recommended by Henriksson et al. (2015) to address the aforementioned inaccurate MC results. As Fig. 3 shows, with the exception of human carcinogenic toxicity, all reported MC ranges fell around the baseline results. To decrease the uncertainty and increase the accuracy of the results, more LCA studies should be performed in the future when more case studies of MP production are available.

Our study has increased the current understanding of the impacts of MP production gained from previous studies (Pikaar et al., 2018b; Sillman et al., 2019, 2020). It has done so by accessing the environmental impacts on an empirical basis and by expanding the system boundaries previously used (Pikaar et al., 2018b; Sillman et al., 2019, 2020) to include all nutrients required for the process and related transportation, CIP, and the impacts of wastewater treatment. This study also expanded the environmental impact categories as MP production relies heavily on electricity, arguably making the product more industrial than agricultural. Additionally the impact of water use for MP production was, for the first time, measured in terms of contribution to water scarcity, as currently recommended (Boulay et al., 2018).

The biggest difference between the current study and the only previously published LCA study of MP available (Sillman et al., 2020) is the electricity requirement. Whereas this study assumed an electricity requirement of 18 kWh per 1 kg product produced, Sillman et al. (2020) estimated 10.96 kWh per 1 kg product produced. This difference could mostly be explained by the fact that the estimate of Sillman et al. (2020) was based on literature values whereas this study was based on empirical data. This could also partly explain why the GWP results of the FAEM scenario in this study were two times larger per 100 g protein than for the somewhat corresponding FImix scenario in the study by Sillman et al. (2020). GWP results of the Base scenario in the study from Sillman et al. (2020) were also smaller than the somewhat comparable results of the FHE – solar (MR) sensitivity test of this study, but larger than the GWP of the FHE scenario. This was despite the larger energy requirements and extended system boundaries of this study. This could be explained by the fact that Sillman et al. (2020) assumed the use of solar energy in the base scenario in Finland versus the use of hydropower in the FHE scenario. We argue, that when producing MP using renewable energy, solar energy is not an optimal or logical choice due to the high latitude of Finland (World bank group, 2020). In this study renewable energy sources were chosen on the basis of their potential at the particular location, which is why solar energy was used only in Morocco.

In addition, the results by Sillman et al. (2020) were based on the impact methods by Gabi 6.0 which is different from the impact categories used in this study which also could explain partly some of the differences found between the studies. On the other hand, three different impact categories were used in this study to calculate GWP for MP production. Variances in results were within a limited range of 1.16–1.3 kg CO_2-eq per 100 protein for the FAEM scenario. Another difference between the studies was the assumed protein content. Sillman et al. (2020) assumed a theoretical 60% protein content while in this study a 65% protein content was used based on nutritional measurements of the product. The comparison also shows a relatively large contribution of nutrients to the total greenhouse gas (GHG) in the study by Sillman et al. (2020), compared to the results here. Even though electricity consumption constitutes the largest contributor any impact category in this study (between 26% and 90% depending on the scenario and impact category, excluding CED), the contribution analysis has shown that for some impact categories the above-mentioned inputs previously excluded by Sillman et al. (2020) can be of relevance. For example, in the FHE scenario wastewater treatment accounts for 8% of all impacts on freshwater eutrophication whereas CIP is responsible for 17% of marine eutrophication.

As the production of MP is still in their infancy and the number of studies in limited, more research on the topic is needed. The technology of MP production can vary per producer and higher number of LCA studies of different system designs would improve the understanding of the environmental impacts of the technology.

The results of this study showed that MP production has substantially lower environmental impacts per unit of protein when compared to other protein sources for human consumption. The study showed that the environmental impact of MP production would be even lower when renewable energy sources are used. On the other hand, when compared to protein sources for feed production, trade-offs can be found between the different protein options. MP production generally causes lower environmental impact in terms of GWP (in the FHE scenario), land use, and eutrophication and acidification potential, but caused high water scarcity and a higher energy demand in comparison. However, despite having a higher energy demand, MP production had a low to average GWP. This could partly be explained by the use of renewable energy in the FHE scenario and the overall lower carbon emissions per kWh for the Finnish electricity mix due to the relatively high reliance on nuclear power and renewable energy (Statistics Finland, 2018). Another reason is that for agricultural products the industrial energy demand is not the main source of GHG emissions (Poore and Nemecek, 2018a). With MP production agricultural emissions are avoided, such as N_2O emissions from soils and CH_4 emissions from ruminant enteric fermentation. Caution has to be taken as impact categories differed between studies used in the comparison as in the original table by Smetana et al. (2019), although units were harmonized. This is unfortunately a common problem when comparing LCA studies. Further research is needed to understand the wider environmental impacts that may be caused as a consequence of replacing animal- or plant-based protein sources with MP, such as changes in land use, energy generation, and diets. The total environmental impacts of MP production also depend on how MP powder will be processed to food products. Therefore, future research should also consider also post-factory gate processes. Ultimately, the environmental benefits gained through MP will be determined by how much and what type of products consumers choose to replace with MP.

Ethical standards

Compliance with ethical standards.

CRediT authorship contribution statement

Natasha Järviö: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Netta-Leena Maljanen:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Yumi Kobayashi:** Methodology, Validation, Investigation, Writing – review & editing. **Toni Ryynänen:** Writing – review & editing. **Hanna L. Tuomisto:** Conceptualization, Methodology, Investigation, Validation, Resources, Supervision, Project administration, Funding acquisition, Writing – review & editing. N.J., N-L.M., and H.L.T designed the research. N.J., N-L.M., Y.K., and H.L.T gathered the data. N.J. performed most of the analysis with assistance from Y.K. for the water recycling model. N.J. wrote the manuscript with input from all authors. H.L.T. supervised the project. All authors have given approval to the final version of the manuscript.

Declaration of competing interest

The authors declare that the process details and data regarding the production of MP has been given by Solar Foods Oy. The company financed Netta-Leena Maljanen for 2 months for consultation prior to the research conducted for this article. This was done with the purpose of calculating the environmental impact of MP. However, during the research performed for this article was performed as a separate and independent project led by the main author of the article. The role of Solar Foods Oy was limited to data provision and the research was funded by the independent bodies listed under 'Acknowledgements'. The authors also declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was part of the "Cultured meat in the post-animal bioeconomy" project (201802185) funded by the KONE foundation and the "Transforming agriculture with agroecological symbiosis combined with cellular agriculture-Environmental impacts and perceptions of farmers and consumers" project funded by the Finnish Cultural Foundation.

The authors would like to thank Solar Foods Oy for providing data about MP production process. In addition, the authors would like to acknowledge Joseph Poore for providing European-level data about environmental impacts of animal- and plant-based protein sources.

Appendix A. Supplementary data

This material is available free of charge via the Internet at http://pubs.acs.org. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.145764.

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