UNIVERSITY OF HELSINKI
RURALIA INSTITUTE

PUBLICATIONS 39

THE PLANET WE EAT

Comparing environmental impacts of protein-rich food and feed ingredients from cellular agriculture, agriculture and aquaculture

NATASHA JÄRVIÖ

Doctoral programme in Sustainable Use of Renewable
Natural Resources (AGFOREE)

Faculty of Agriculture and Forestry
University of Helsinki

THE PLANET WE EAT

Comparing environmental impacts of protein-rich food and feed ingredients from cellular agriculture, agriculture and aquaculture

NATASHA JÄRVIÖ

DOCTORAL DISSERTATION

To be presented for public examination with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, at Infokeskus K1088, Viikki, on the 8th of November 2022 at 9 o'clock.

2022

Publisher	University of Helsinki Ruralia Institute www.helsinki.fi/en/ruralia-institute
	Lönnrotinkatu 7 50100 FI-MIKKELI
	Kampusranta 9 C 60320 FI-SEINÄJOKI
Series	Publications 39
Cover Photo	VTT
ISBN	978-951-51-7345-4 978-951-51-7346-1 (pdf)
ISSN	1796-0649 1796-0657 (pdf)

The Faculty of Agriculture and Forestry uses the Ouriginal system (plagiarism recognition) to examine all doctoral dissertations.

Helsinki, 2022

CUSTOS

Associate Professor Hanna L. Tuomisto
University of Helsinki
Department of Agricultural Sciences
Helsinki, Finland

SUPERVISORS

Associate Professor Hanna L. Tuomisto
University of Helsinki
Department of Agricultural Sciences
Helsinki, Finland

Dr., Docent Toni Ryynänen
University of Helsinki
Ruralia Institute
Mikkeli, Finland

PRE-EXAMINERS

Deputy Leader Life Cycle Assessment Research Group
Dr. Thomas Nemecek
Agroscope
Agroecology and Environment
Zurich, Switzerland

Head of Food Data Group
Dr. Sergiy Smetana
German Institute of Food Technologies (DIL e.V.)
Food Data Group
Quakenbrück, Germany

OPPONENT

Professor Sarah McLaren
Massey University
New Zealand Life Cycle Management Centre
Palmerston North, New Zealand

MEMBERS OF THE THESIS ADVISORY COMMITTEE

Associate Professor Miguel Mendonca Reis Brandão
KTH Royal Institute of Technology
School of Architecture and the Build Environment (ABE)
Sustainable development, Environmental science and engineering
Stockholm, Sweden

University lecturer
Dr., Docent Iryna Herzon
University of Helsinki
Department of Agricultural Sciences
Helsinki, Finland

Head of Unit
Dr., Docent Sampo Soimakallio
Finnish Environment Institute (SYKE)
Centre for Sustainable Consumption and Production
Helsinki, Finland

ACKNOWLEDGEMENTS

Obtaining a doctoral degree is a long journey that one does not undertake alone, and I am very grateful to many people for their support. I would like to thank the entire Future Sustainable Food Systems research group, that I was proud to be a part of during my time at the University of Helsinki. This group of diverse talents, with different backgrounds and nationalities, surely enriched my working life. It was unfortunate that COVID-19 prevented us from spending more time together and celebrating important milestones, such as the publication of our articles. I also had the good fortune to work with a large group of people from outside my research group, not least the great group of co-authors I have collaborated with. Thank you all for your valuable contributions! Without your support, these articles would not exist. I would also like to thank my thesis committee members, who selflessly listened to me rambling on about PhD life on a yearly basis. For that patience and the advice received, I am thankful!

Although all those mentioned above have been important in my work, there are some I would like to thank separately. I would like to express my thanks to my main supervisor, Associate Professor Hanna L. Tuomisto, for always being supportive and open-minded to any ideas I had. You always treated me as an equal and trusted me to do my work well. This gave me freedom and confidence to explore and discuss different research methods, concepts, and ideas, thereby improving the quality of my work and my understanding of the environmental impacts of the food system. Whenever I needed advice, you were always available to discuss my work at length, and I appreciated that greatly. Your support made me a better and more confident researcher and for this I am very grateful.

I would also like to thank my supervisor, Senior Researcher Toni Ryynänen for his feedback and comments. These were especially helpful in guiding my work on this dissertation in the right direction. I would like to thank him and Hanna Tuomisto for putting their faith in me and giving me a chance to work with the KONE foundation's solid 4-year funding, which enabled me to focus on my work fully without the distractions of grant applications. On that account, I would also like to express my gratitude to the KONE foundation. I am also grateful to the Ruralia institute for their support during my PhD studies and the facilities they provided that enabled me to do my work.

And then I would like to express my gratitude to my colleagues, whose support has been invaluable during the past years. I would like to thank Dr. Yumi Kobayashi for her contributions to two of my papers, but more so for the great conversations and laughter we shared before COVID-19 banned us from the office. I would also like to express my thanks to Venla Kyttä for sharing her thoughts and LCA knowledge, but mostly for the mental support during the last stages of our PhD work. I would like to thank Laia Dalmau for supporting me during my first real teaching experiences and for always being so positive. I would also like to thank Tuure Parviainen. You have been such a great co-author and it has been both efficient and enjoyable to work together on our Nature Food publication. I would like to thank Rachel Mazac for our pleasant walks, great talks, and collaborations and Kari Koppelmäki for our much-appreciated little coffee breaks. To all the great people in our research group, Jana Moritz, Niko Räty, Miriam Torres Miralles, Marja Roitto, Netta-Leena Maljanen, Anniina Lehtilä, Elina Karimaa, Mohammad El-Wali, and all others, thank you for all the great meetings and conversations. I hope we will stay in touch.

I would like to thank Dr. Patrik Henriksson sincerely for all his help and guidance during the process of writing my first article. I was often positively amazed at the effort and time you put into it. And of course, Associate Professor Jeroen Guinée. It was an honor and pleasure to work with you. Thank you for the overall guidance, support and encouragement that led me to writing a scientific article. As always, writing the very first article is associated with a steep learning curve. I am grateful that I had both Patrik and Jeroen at my side.

I would also like to thank all my other co-authors, Emilia Nordlund, Lauri Kujanpää, Dilek Ercili-Cura, and Christopher Landowski. Thank you for all your valuable inputs, efforts and discussions. Without them this work would not have been possible. I would also like to thank Juha-Pekka Pitkänen and Pasi Vainikka for their data and valuable explanations on the production process of microbial protein production.

I would like to thank the pre-examiners for their time and devotion in reading my dissertation and for the useful comments they gave me during the pre-examination. Through their expertise in LCA they helped me improve my work even in the last stages. Additionally, I would like to thank my opponent in advance. Even though her work has yet to start at the time of writing this, I am grateful she has courageously accepted her role, and is willing to take the time out of her evening to attend my defense from the other side of the world.

I would also like to thank Jáchym Judl for introducing me to the Finnish LCA life and for co-organizing and co-hosting the Consequential LCA workshop with me. It was a pleasure to be colleagues again for a little while, but mostly I am grateful for the friendship we have established over the past years, and the interesting LCA discussions we have had together.

Last, but far from least, I would like to express my deep appreciation for my supportive family. To my sweetest daughters, who give me so much joy and laughter. You little girls are the best distraction from work anyone could have wished for. You kept me motivated to continue the fight for a better and greener future, especially at times when the research topic became depressing and overwhelming, and giving up hope seemed too easy. As you grow up, I will miss the little naps you took on my lap while I was working, and the curious glances you gave to the camera during remote meetings. And, to my best friend in life, my husband, who has had to listen endlessly to my complaints, frustrations and ideas along the way. I appreciate you (pretending) to listen and I appreciate your enthusiasm for celebrating every milestone along the way — even when the true reason for this might have less to do with my accomplishment and more with the celebratory sweets and drinks. But mostly, you have my fullest respect for remaining so cool now that we really are becoming "Dr. and Mr. Järviö".

This PhD thesis is hopefully just the beginning of my scientific career. I treated it as an opportunity to learn as much as I could, to acquire a deeper understanding of my own field of study and the research methods I have used, but also to learn new skills, meet new people and take new paths in life. I am eager to apply what I have learned in the next chapter of my life. I am looking forward to many more years of collaboration with all the great people that I have met during my PhD journey. Our job is far from over and much remains unstudied. Let us work towards a healthier food system, so we can pass our planet proudly on to our children!

Natasha Järviö
Helsinki, 25.09.2022

CONTENTS

ARTICLES

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I. **Järviö N**., Henriksson P.J.G., Guinée J.B. 2018. Including GHG emissions from mangrove forests LULUC in LCA: a case study on shrimp farming in the Mekong Delta, Vietnam. *Int J Life Cycle Assess* **23**, 1078-1090. https://doi.org/10.1007/s11367-017-1332-9

II. **Järviö N**., Maljanen N.L., Kobayashi Y., Ryynänen T., Tuomisto H.L. 2021. An attributional life cycle assessment of microbial protein production: A case study on using hydrogen-oxidizing bacteria. *Science of the Total Environment* **776**, https://doi.org/10.1016/j.scitotenv.2021.145764.

III. **Järvio N**., Parviainen T., Maljanen N.L., Kobayashi Y., Kujanpää L., Ercili-Cura D., Landowski C.P., Ryynänen T., Nordlund E., Tuomisto H.L. 2021. An anticipatory life cycle assessment of egg albumin production in a cell-cultured process using *Trichoderma reesei*. *Nature Food **2**, 1005-1013*, https://doi.org/10.1038/s43016-021-00418-2. Reproduced with permission from Springer Nature

The publications are referred to in the text by their roman numerals.

Article I was co-authored by Patrik Henriksson and Jeroen Guinée. All authors have contributed to the conceptualization of the article. Järviö had the main responsibility for developing the method introduced in the article and the case study calculations that were performed to test the method. Järviö also had the main responsibility for writing the article with guidance and additional input from Henriksson. Both Guinée and Henriksson were responsible for reviewing, editing and supervising the article. Guinée was responsible for the overall project.

Article II was co-authored by Netta-Leena Maljanen, Yumi Kobayashi, Toni Ryynänen and Hanna Tuomisto. Tuomisto was responsible for the idea and conceptualization of the article. Tuomisto, Maljanen and Järviö collected data. Järviö used the collected data to create the life cycle model in Simapro and calculated the environmental results for microbial protein production. Kobayashi performed wastewater treatment calculations. Järviö wrote the original draft. All others contributed to the editing and reviewing of the article. Tuomisto was responsible for the supervision of the project.

Article III was co-authored by Tuure Parviainen, Netta-Leena Maljanen, Yumi Kobayashi, Lauri Kujanpää, Dilek Ercili-Cura, Christopher Landowski, Toni Ryynänen, Emilia Nordlund and Hanna Tuomisto. Tuomisto, Landowski, and Nordlund were responsible for the initial idea and conceptualization of the article. Maljanen, Parviainen and Järviö took part in the conceptualization. Maljanen made an initial rough estimation of environmental impacts for Tr-OVA. Järviö created the Tr-OVA and chicken-based egg model in Simapro and performed the environmental calculations and statistical calculations presented in the article. Järviö was mainly responsible for writing the original draft, and the visualizations. Parviainen wrote the initial introduction and contributed a few minor sections throughout the text. He also performed further essential data validations. Maljanen drafted a minor part of the method section. Järviö, Parviainen, Kobayashi, Ercili-Cura, Landowski, Ryynänen, Nordlund and Tuomisto were responsible for reviewing and editing the text. Tuomisto and Nordlund led and supervised the project.

ABBREVIATIONS

CIP	-	cleaning-in-place
CH_4	-	methane
CO_2	-	carbon dioxide
DE	-	Germany
EC	-	European Commission
EEA	-	European Environmental Agency
EU	-	European Union
FAEM	-	Finnish average energy mix
FAO	-	Food and Agriculture Organization of the United Nations
FHE	-	Finnish hydropower energy
FI	-	Finland
FI-LC	-	Finland, low-carbon energy mix
FU	-	functional unit
GHG	-	greenhouse gas
GWP	-	global warming potential
H_3PO_4	-	phosphoric acid
HOB	-	hydrogen-oxidizing bacteria
IPCC	-	Intergovernmental Panel for Climate Change
IS	-	Iceland
ISO	-	International Organisation for Standardisation
LCA	-	life cycle assessment
LCI	-	life cycle inventory
LCIA	-	life cycle impact assessment
LP	-	low pressure
LU	-	land use
LUC	-	land-use change
LULUC	-	land use and land-use change
MC	-	Monte Carlo
MKP	-	monopotassium phosphate
MP	-	microbial protein
MR	-	Morocco
N	-	nitrogen

NaOH	-	sodium hydroxide
N_2O	-	nitrous oxide / dinitrogen oxide
P	-	phosphorus
PL	-	Poland
RO	-	reverse osmosis
TEA	-	techno-economic assessment
Tr-OVA	-	ovalbumin produced using the fungus *Trichoderma reesei*
UN	-	United Nations
US	-	United States
WWT	-	wastewater treatment
WWTP	-	wastewater treatment plant

ABSTRACT

Advancements in agriculture and increases in the human population have led to a surge in agricultural and aquacultural production. This increase in production has come at a cost. The consequences of current food production for the environment have never been so pronounced in human history, threatening the relatively stable state in which the Earth system has remained over the past 11,700 years of the Holocene epoch. Intensive farming systems often rely heavily on external inputs such as pesticides, herbicides and fertilizers to suppress the natural process of species diversification on the land, which lead to problems such as eutrophication, soil exhaustion and desertification. Other examples of environmental impacts resulting from current agricultural practices include deforestation and land degradation leading to the loss of valuable ecosystems and biodiversity, accelerated climate change, over-extraction of groundwater, terrestrial acidification, and biological crises such as the outbreak of COVID-19 pandemic.

To reduce the environmental impacts of the food system, the search for more sustainable protein alternatives to replace animal-based proteins is one of the foremost research topics in food science and biotechnology today. Cellular agriculture — the production of agricultural products using cell-culturing technologies — is an approach that seeks to decouple food production from conventional agricultural farming, and, therefore, has the potential to decrease the environmental burden of food production. Cell-culturing technologies usually utilize bioreactors, creating closed production processes that allow for efficient recycling of inputs, and control of emissions from the production process. Another benefit of cell-cultured products is increased resilience of the food production system towards environmental changes, due to reduced reliance on conventional agricultural inputs. However, estimates of the environmental impacts of cell-cultured foods are still mostly lacking due to the novelty of these products.

The aim of this dissertation is to improve the understanding of environmental impacts of protein-rich cellular agricultural products in comparison with those of existing protein-rich food and feed ingredients originating from agricultural and aquacultural systems. The environmental impacts of protein-rich cellular agricultural products were quantified to gain an understanding of the production processes contributing most to these impacts, and how these differ from agricultural and aquacultural products. Lastly, the work presented in this dissertation seeks to explore how the environmental impacts of cellular agricultural protein products can be reduced through alterations to their production processes. However, as GHG emissions resulting from aquacultural production in mangrove forests have been systematically excluded from environmental impacts assessments, a fair comparison between protein produced by cellular agriculture and aquaculture is compromised. The work in this dissertation, therefore, additionally focuses on the development and application of a method to quantify the GHG emissions caused by land use and land-use change (LULUC) of mangrove forest.

The protein-rich food and feed ingredients studied were microbial protein produced using hydrogen-oxidizing bacteria (HOB) (hereafter referred to as MP) and ovalbumin produced using the *Trichodora reesei* fungi (Tr-OVA). Shrimp was selected on the basis that aquaculture products are often underrepresented in environmental assessment studies in contrast to their importance as a protein source for many people (Gephart et al.,

2021). MP and Tr-OVA are recently developed cellular agricultural products that can be used either as food or feed ingredients and are examples of cellular (MP) and acellular (Tr-OVA) products.

To address the aims of the research, the life cycle assessment (LCA) method was used. LCA allows for the quantification of inputs and outputs at all production stages throughout a product's life cycle and the coupling of these to various environmental impact categories, such as global warming potential (GWP), land use, and eutrophication. This enables a fair comparison between different protein-rich food and feed products originating from distinctly different systems. The environmental impacts of the three protein-rich products studied were also compared to the environmental impacts of other protein-rich products found in literature.

GHG emissions caused by LULUC of mangrove forests are often overlooked in LCA studies, despite the large contribution of LULUC emissions to climate change (approximately 13% of global emissions in the year 2015). Article I, consequently, focuses on the introduction of a method to include this specific emissions source and on applying it to a case study of shrimp farming in mangrove areas. Article II quantifies the environmental impacts of MP production. MP is a single-cell protein in the form of a flour-like powder with a 65% protein content. Because MP production uses autotrophic HOB there is no reliance on any agricultural inputs. Article III investigates the environmental impacts of Tr-OVA production. Like MO, Tr-OVA is a protein-rich powder produced in bioreactors through a closed process and has a 92% protein content. However, unlike MP, its production relies on glucose from agriculture. Using modern biotechnological tools, the gene carrying the blueprint for ovalbumin (*SERPINB14)* is inserted into the fungus, which then starts to produce the same protein — ovalbumin — that is normally found in chicken eggs. Cell-cultured ovalbumin can be used as a direct replacement for the chicken-based egg white that is widely used in food processing.

The results of this dissertation showed great potential for MP and Tr-OVA to reduce the environmental impacts associated with protein production — especially when replacing protein from livestock sources — with the greatest reductions seen in land use and GWP compared to other protein-rich food and feed sources. The amount of land needed to produce MP and Tr-OVA was 0.1-1.3% of the land required for beef herds. Even by comparison to peas, which generally require little land compared to other animal and plant-based protein sources, land use requirements were 73-97% less. Both MP and Tr-OVA production also led to reductions in GWP when compared to other protein-rich foods, especially by comparison to animal-based protein sources. However, agricultural protein alternatives with a lower GWP were also identified, such as peas, rapeseed cake and soybean meal.

Differences in the impacts of MP and Tr-OVA production were mostly explained by the reliance of Tr-OVA on agricultural inputs. Depending on the impact category, up to 94% of the environmental impacts of Tr-OVA production were related to its use of agriculturally sourced glucose. For MP, environmental impacts were mainly caused by the use of electricity; up to 90% depending on the category. For Tr-OVA, the impacts caused by the use of electricity were between 0% and 56%. The results for shrimp clearly indicated the importance of the inclusion of LULUC emissions from mangrove deforestation, as GHG emissions were 14-60 times higher for shrimp farming systems located in former mangrove areas than for systems that were not. The GHG emissions of shrimp produced in mangrove areas far outweighed the GWP of other protein-rich food sources and were 4.5 times higher than that of beef production from beef herds.

Despite the great potential of cellular agriculture products to reduce the environmental impacts of protein production, minor trade-offs were found. For example, the potential for ozone depletion and water scarcity were higher for Tr-OVA in comparison to other feed protein alternatives. The environmental impacts of cellular agricultural products could be further reduced by using renewable or low-carbon energy sources. However, the electricity requirements for cellular agricultural products are higher than those of agriculture and aquaculture. As many sectors are looking to move away from fossil fuels towards low-carbon electricity sources, potential greater demand for electricity from the food sector will add further pressure to increase sustainable electricity production capacity.

The work in this dissertation focused solely on the environmental impacts of food production. Additional research is needed into the role of cell-cultured food within a sustainable food system. This means that the research begun in this dissertation should be expanded to include other environmental impact categories such as biodiversity impacts, and the loss of ecosystem services. Additionally, there is a need to increase understanding of the social and economic implications of the introduction of cell-cultured foods, such as MP and Tr-OVA, into the food systems in different regions of the world.

TIIVISTELMÄ

Ruoantuotannon ympäristövaikutukset eivät ole koskaan aikaisemmin olleet näin merkittäviä ihmiskunnan historiassa. Tehoviljelyjärjestelmät tukeutuvat usein voimakkaasti ulkopuolisiin tuotantopanoksiin, kuten torjunta-aineisiin ja lannoitteisiin, jotka tukahduttavat lajien luonnollisen monimuotoistumisprosessin maassa. Tämä johtaa rehevöitymisen, maaperän köyhtymisen ja aavikoitumisen kaltaisiin ongelmiin. Muita esimerkkejä nykyisten maatalouskäytäntöjen aiheuttamista ympäristövaikutuksista ovat metsäkato ja maaperän köyhtyminen, jotka johtavat arvokkaiden ekosysteemien ja biologisen monimuotoisuuden häviämiseen, ilmastonmuutoksen kiihtymiseen ja pohjaveden liialliseen käyttöön.

Solumaatalous on tuotantotapa, jolla pyritään irrottamaan ruoantuotanto tavanomaisesta maanviljelystä ja vähentämään ruoantuotannon aiheuttamaa ympäristökuormitusta. Soluviljelytekniikoissa käytetään yleensä bioreaktoreita, joissa luodaan suljettuja tuotantoprosesseja, jotka mahdollistavat raaka-aineiden tehokkaan kierrätyksen ja tuotantoprosessin päästöjen hallinnan. Soluviljeltyjen elintarvikkeiden ympäristövaikutuksista ei kuitenkaan ole vielä tehty juurikaan arvioita näiden tuotteiden uutuuden vuoksi.

Tämän väitöskirjan tavoitteena on syventää ymmärrystä kahden proteiinipitoisen soluviljellyn maataloustuotteen - vetyä hapettavien bakteerien avulla tuotetun mikrobiproteiinin (MP) ja *Trichodora reesei* -sienen avulla tuotetun ovalbumiinin (Tr-OVA) - ympäristövaikutuksista verrattuna nykyisten maatalous- ja vesiviljelyjärjestelmistä peräisin olevien proteiinipitoisten elintarvikkeiden ja rehujen ainesosien ympäristövaikutuksiin elinkaariarvioinnin (LCA) avulla. LCA:n soveltaminen mahdollistaa ympäristöstä hyödynnettävien ja ympäristöön päästettävien virtojen laskemisen tuotteen elinkaaren eri vaiheissa sekä niiden yhdistämisen erilaisiin ympäristövaikutusluokkiin, kuten ilmaston lämmityspotentiaaliin (GWP). Tämä mahdollistaa tasapuolisen vertailun eri proteiinipitoisten elintarvikkeiden ja rehujen ainesosien välillä.

Tämän väitöskirjan tulokset osoittivat, että MP:lla ja Tr-OVA:lla on potentiaalia vähentää proteiinintuotantoon liittyviä ympäristövaikutuksia, erityisesti silloin, kun niillä korvataan tuotantoeläimistä saatavia proteiineja. Suurimmat vähennykset havaittiin maankäytön ja GWP:n osalta. Esimerkiksi MP:n ja Tr-OVA:n tuottamiseen tarvittava maa-ala oli 0,1-1,3 prosenttia lihakarjan tarvitsemasta. Sekä MP:n että Tr-OVA:n tuotanto johti myös GWP:n vähenemiseen verrattuna muihin runsasproteiinisiin elintarvikkeisiin, erityisesti eläinperäisiin proteiinilähteisiin verrattuna. MP:a ja Tr-OVA:a vertailtiin myös maataloudessa käytettäviin proteiinin lähteisiin, joiden GWP on alhaisempi, kuten herneisiin, rapsikakkuun ja soijajauhoon.

Erot MP:n ja Tr-OVA:n tuotannon vaikutuksissa selittyivät pääasiassa sillä, että Tr-OVA:n tuotanto on riippuvainen maatalouden raaka-aineista. Jopa 94 prosenttia Tr-OVA:n tuotannon ympäristövaikutuksista liittyi maataloudesta peräisin olevan glukoosin käyttöön. MP:n ympäristövaikutukset johtuivat pääasiassa sähkön käytöstä, poiketen maatalous- ja vesiviljelysektorin tavallisesti aiheuttamista ympäristövaikutuksista. Esimerkiksi entisissä mangrovemetsissä tapahtuvasta katkarapujen tuotannosta aiheutuvista GWP-päästöistä suurin osa johtui mangrovemetsien hävittämisestä aiheutuneista LULUC-päästöistä. Päästöt olivat 14-60 kertaa suuremmat entisillä mangrovemetsäalu-

eilla sijaitsevissa katkarapujen kasvatusjärjestelmissä kuin sellaisissa, jotka eivät sijainneet mangrovemetsissä.

Huolimatta soluviljelytuotteiden suuresta potentiaalista vähentää proteiinintuotannon ympäristövaikutuksia, havaittiin myös joitakin haittapuolia. Esimerkiksi Tr-OVA johti muihin rehuproteiinivaihtoehtoihin verrattuna suurempaan otsonikatoon ja vesivarojen vähenemisestä johtuvaan ekosysteemien kuormitukseen. Solumaataloustuotteiden ympäristövaikutuksia voitaisiin edelleen vähentää käyttämällä uusiutuvia tai matalapäästöisiä energianlähteitä. Vaihtoehtoiset glukoosin lähteet, kuten maatalouden sivuvirroista saatava glukoosi, voisivat edelleen vähentää Tr-OVA:n ympäristövaikutuksia.

1. INTRODUCTION

1.1 ENVIRONMENTAL CHALLENGES RESULTING FROM AGRICULTURE

Advancements in agriculture and an increase in the human population have led to a surge in agricultural and aquacultural production. However, this growth has come at a cost. Environmental challenges caused by food production are now more severe than ever before in human history. In fact, current agricultural practices are threatening the relatively stable state in which the Earth system has remained over the past 11,700 years of the Holocene epoch, thereby threatening the success of agricultural harvests (Steffen et al., 2015).

Intensive farming systems often rely heavily on the input of pesticides, herbicides, and fertilizers (Shukla et al., 2019) to suppress the natural process of species diversification on the land, and lead to problems such as eutrophication, soil exhaustion and desertification. Other examples of environmental impacts resulting from agricultural practices include deforestation and land degradation, that have contributed to the loss of valuable ecosystems and biodiversity, climate change due to increased greenhouse gas (GHG) emissions, over-extraction of groundwater and terrestrial acidification (United Nations, 2021). At the same time, human presence, and the food systems that support us, have almost completely replaced mammalian wildlife. In 2018, human beings represent 36% of the total mammalian biomass and livestock made up a staggering 60%, meaning that wild biomass only accounted for 4% (Bar-On et al., 2018).

Pressures on the environment caused by the food system have also adversely affected agricultural production. As a recent article by FAO (2020b) pointed out, at no point in time have agricultural-food systems faced such a large amount of new threats, including megafires, extreme weather events, unusually large desert locust swarms, and biological crises like the outbreak of COVID-19 pandemic. The annual occurrence of disasters has increased three-fold since the 1970s (FAO, 2020b). This steep increase in natural disasters is the consequence of human actions constraining the planetary boundaries, to the point that a new geological epoch has been proposed: the Anthropocene. This era is said to have started in the latter part of the 18th century when increased concentrations of carbon dioxide (CO_2) and methane (CH_4) in the atmosphere were first recorded (Crutzen, 2002). Steffen et al. (2015) illustrated the status of the planetary boundaries, and the extent to which we operate inside or outside of the safety zones. For biogeochemical flows, and the loss of genetic diversity, we are in a high-risk zone, beyond uncertainties of what will happen to the planet consequently. For many other categories, such as climate change and freshwater use, we are in the zone of uncertainty. Campbell et al. (2017) assessed the role of agriculture in each of the planetary boundaries, and found that the agricultural sector plays a major role in overshooting all of the planetary safety zones.

Additionally, despite technological advancements in the agricultural sectors, famine and undernourishment are still a serious threat to many (Mazoyer & Roudart, 2006). The number of people that are affected by hunger has slowly increased since 2014 (FAO et al., 2020). Conversely, the average caloric intake per capita has increased by about one-third since 1961 (Shukla et al., 2019), and consumption of vegetable oils and meat has doubled between 1961 and 2019. This has led to increasing health problems such as diabetes and obesity (Willett et al., 2019).

Meat is a good source of nutrients, with high levels of protein, iron, zinc, and vitamin B12. However, a diet high in red and/or processed meat can also lead to adverse health effects such as colorectal cancer (Forouzanfar et al., 2015). Additionally, the production of meat leads to more greenhouse gas (GHG) emissions per unit of energy than any plant-based foods (Xu et al., 2021) and contributes greatly to land use and land-use change (LULUC) (Clark et al., 2020; Crippa et al., 2021). One third of global cereal production ends up being used for feed necessary for the production of livestock (FAO, 2020a). About 80% of soybeans are used for animal feed. The combination of a growing human population and an increase in meat consumption, related to an increase in wealth, will most likely result in further increases in meat consumption in the future. Different models have estimated that meat consumption will increase by somewhere between 62% and 144% by 2050 (Valin et al., 2013).

The Food and Agriculture organization (FAO), estimated that by 2050 we would need to produce 60% more food to feed the estimated 9.3 billion people that will inhabit this planet by then (United Nations, 2021). This means that the agricultural sector will likely increase the pressure it puts on our planet unless we radically change the way we produce our food. As the human population continues to grow, we are faced with the question of how to feed everybody.

1.2 ENVIRONMENTAL PRESSURE RESULTING FROM AQUACULTURE

Environmental pressures from food production are often considered taking agriculture as a starting point. However, humanity also depends on water as a growth medium to provide nutrition, or as a livelihood, or both (Pradeepkiran, 2019). Aquatic animals and plants are often highly nutritious and can serve as an essential source of protein, micronutrients, and minerals for poor people due to their affordability over land animals. They can, therefore, play an essential role in supplying food security (Pradeepkiran, 2019). In addition, research has shown that aquatic foods can play an important part in the transition to more sustainable diets (Gephart et al., 2016; Hallström et al., 2019). From the total production of fish in 2018, about 46% was produced in aquaculture systems, reaching a record amount of 114.5 million tons in live weight. The remaining 54% was captured wild stock. Approximately 87% of total global fish production ends in human consumption, while the remainder is mainly used to produce fishmeal and fish oil. The majority (69%) of all fish is produced in Asia, with China alone accounting for 35% of global production (FAO, 2020b).

Despite the continuous growth of fish production, and the importance of its products, wild-stock fish capture and aquaculture production are often overlooked in environmental assessments by comparison to the high diversity of production options. This means that the environmental impacts caused by such systems are not as well understood as the impacts of food produced on land (Gephart et al., 2021).

The environmental impacts of aquaculture food products can differ substantially from those produced on land because of the different medium in which the products are produced. For example, many fish species require brackish water — water that has more salinity than freshwater but less than seawater — leading to environmental impacts on coastal zone regions (Spalding & Leal, 2021). Mangrove forests are one of the ecosystems that have been under threat globally, due to increased demand for aquaculture products such as shrimp (Orchard et al., 2016).

Despite the contribution of aquaculture practices to mangrove deforestation, GHG emissions resulting from LULUC of mangrove forests have rarely been considered in quantification of the environmental impacts of aquaculture products like shrimp. This can be partly explained by a lack of guidelines on emissions from wetland systems, that were initially missing from the 2006 guidelines for national greenhouse gas inventories produced by the International Panel for Climate Change (IPCC). These guidelines were only introduced in 2013 (IPCC, 2014). When it comes to aquaculture practices, the supplementary guidelines are limited to CO_2 emissions resulting from land-use change and nitrous oxide (N_2O) emissions released during aquaculture use within mangrove forests. This means that both CH_4 emissions resulting from the disturbance of the mangrove soils and the missed potential carbon sequestration from continuous carbon burial into the mangrove soils are not considered. There is, therefore, a need to expand on the existing guidelines to include all emissions resulting from LULUC of mangrove forests. There is also a need to apply this method to a case study on aquacultural production of shrimps and/or other species that have been linked to mangrove deforestation to gain scientific understanding of the environmental impacts of aquaculture products.

1.3 POTENTIAL OF CELLULAR AGRICULTURAL TO LOWER THE ENVIRONMENTAL IMPACTS OF PROTEIN PRODUCTION

With the increasing pressures the agricultural sector places upon our planet's system boundaries, the search for alternative, more sustainable, protein sources to replace animal-based proteins has emerged. This is an expanding area of research in food science and biotechnology (Eibl et al., 2021; Rischer et al., 2020). Cellular agriculture relies on an approach that tries to decouple food production from conventional agriculture and animal farming (Ercili-Cura & Barth, 2021; Parodi et al., 2018). In doing so it has the potential to decrease the environmental burden associated with food production (Mattick, 2018; Pikaar, Matassa, et al., 2018; Sillman et al., 2019, 2020; Rischer, Szilvay and Oksman-Caldentey, 2020).

Cellular agriculture is the process of producing agricultural products through the means of cell-culturing technologies. Cellular agriculture is mainly used for producing alternatives or analogues to animal-based products, due to their relatively high environmental impact. Cells from micro-organisms, plants or animals are cultivated in bioreactors, in combination with the additional nutrients required for cell growth (Mattick, 2018; Parodi et al., 2018; Rischer et al., 2020; Tuomisto et al., 2022). The use of bioreactors creates a closed production process that allows for the efficient recycling of inputs and the control of emissions originating from the production process. Another benefit of cell-cultured products is increased resilience towards environmental challenges such as drought, due to reduced reliance on conventional agricultural inputs (Rischer et al., 2020).

Cell-cultured products are classified either as cellular or acellular. The distinction lies in whether the final product does or does not include the cultivated cells. The former is referred to as cellular products and the latter as acellular. Examples of cellular products include cultivated meat and microbial biomass (single-cell protein) (Rischer et al., 2020; Sillman et al., 2020), whereas acellular products consist of organic molecules such as proteins, lipids, vitamins, enzymes, hormones, and flavors synthesized by microbes and separated from the cell mass (New harvest, 2021).

Several studies have focused on the potential of cell-cultured products to reduce the environmental impacts of food production. Examples include studies on cultivated meat (Lynch & Pierrehumbert, 2019; Mattick et al., 2015; Tuomisto et al., 2022; Tuomisto & Teixeira de Mattos, 2011), microbial protein (Linder, 2019; Pikaar, Matassa, et al., 2018; Sillman et al., 2019, 2020), cell-cultured protein using genetically engineered fungi (Perfect Day Inc., 2021), and other cell-cultured future foods, such as mycoprotein and insect-based products (Asim et al., 2021; Linder, 2019; Parodi et al., 2018; Upcraft et al., 2021). These studies have shown the potential of such products to reduce the environmental impacts of production compared to conventional protein sources, especially when replacing protein from livestock production (Humpenöder et al., 2022).

Previous studies have estimated the land use, water use, global warming potential (GWP) and eutrophication potential of microbial protein production (Humpenöder et al., 2022; Pikaar, de Vrieze, et al., 2018; Pikaar, Matassa, et al., 2018; Sillman et al., 2020). However, cell-cultured food production, such as microbial protein production, relies heavily on industrial energy. Potentially important impact categories related to the production of industrial energy (i.e. ionizing radiation and the cumulative energy demand (CED)) were not included in these previous studies. Additionally, both food and energy production are major water users and this competitive situation has been referred to as the global food-energy-water nexus (D'Odorico et al., 2018). Although the water use of cell-cultured food and feed production have been quantified in previous analyses (Pikaar, de Vrieze, et al., 2018; Pikaar, Matassa, et al., 2018; Sillman et al., 2020), none have applied the latest consensus characterization method to assess the impacts of water use taking into account the local availability of water.

Not only are there limitations in the impact categories used, but the number of studies quantifying the environmental impact of cell-cultured food and feed ingredients is rather limited, as data about the production processes has been limited. Previous quantifications of the environmental impacts of microbial protein were based on the limited availability of theoretical estimates of the required inputs and outputs of microbial protein production. This meant that some process steps were not considered in quantification of the environmental impacts of microbial protein production, such as cleaning of equipment, sourcing of required nutrients, and the treatment of wastewater from the system. More studies are needed, as environmental impacts tend to differ between cell-cultured products, due to their varying dependency on conventional agriculture for inputs (Tuomisto, 2019).

To the best of this author's knowledge, peer reviewed articles reporting the environmental impacts of ovalbumin produced using the *Trichoderma reesei* (*T. reesei*) fungi (Tr-OVA) did not exist before 2021. This means that there is an urgent need for research that analyzes the environmental impacts of cell-cultured foods and compares these impacts to those associated with existing protein-rich food and feed ingredients.

2. OBJECTIVE OF THE DISSERTATION

2.1 AIM OF THE DISSERTATION

The aim of this dissertation is to improve the understanding of the environmental impacts of protein-rich cellular agricultural products in the context of the environmental impacts of existing protein-rich food and feed ingredients originating from agricultural and aquacultural systems. The work in this thesis focuses on quantification of the environmental impacts of two examples of protein production through cellular agriculture. Subsequentially, these environmental impacts are compared with those of existing protein-rich food and feed ingredients in order to understand how cellular agriculture could reduce the environmental impacts that are associated with protein-production. The quantification of environmental impacts at each production stage of the two cellular agricultural products helps to understand during which part of production impacts occur, and how these differ from existing protein-rich products that originate from agricultural and aquacultural systems. In addition, the quantification enables an understanding of how environmental impacts can be reduced through alterations to the production processes of cellular agricultural products.

However, as GHG emissions resulting from aquacultural production in mangrove forests have been systematically excluded from environmental impact assessments, a fair comparison between protein produced within cellular agriculture and aquaculture has, hitherto, not been possible. The work in this dissertation, therefore, also undertook the development and application of a method to quantify the GHG emissions caused by LULUC of mangrove forest.

The protein-rich food and feed ingredients studied are shrimp, microbial protein produced using hydrogen-oxidizing bacteria (HOB) (hereafter simply referred to as MP), and Tr-OVA. Shrimp was selected on the basis that aquaculture products are often underrepresented in environmental assessment studies in comparison to their importance as a protein source to many people (Gephart et al., 2021) and have been linked to mangrove deforestation (Orchard et al., 2016). MP and Tr-OVA are recently developed cellular agricultural products that can be used either as food or feed ingredients and are examples of cellular (MP) and acellular (Tr-OVA) products.

The research questions associated with the aims of this dissertation are:

RQ1: What are the product-level environmental impacts associated with the production of shrimp, MP and Tr-OVA?

RQ2: How does the environmental impact of MP and Tr-OVA produced through cell-culturing techniques compare to those of protein-rich food and feed ingredients originating from agriculture, such as beef, or aquaculture, such as shrimp?

RQ3: What are the associated emissions sources of shrimp, MP, and Tr-OVA production and during which production stage(s) do these emissions occur?

RQ4: How can the environmental impacts of MP and Tr-OVA be reduced through alterations of the production design.

Comparisons were performed using the life cycle assessment (LCA) method, that allows for the quantification of the inputs and outputs of all production stages throughout a product's life cycle, and the coupling of those to various environmental impact categories, such as GWP, land use, and eutrophication. This enables a justified comparison between different protein-rich food and feed products originating from distinctly different systems. The environmental impacts of three different protein-rich food and feed ingredients were quantified separately in the three articles that constitute this dissertation. The environmental impacts are compared on a protein basis. Additionally, the environmental impacts of three patties are compared using shrimp, MP, and Tr-OVA separately as the main source of protein in each patty.

2.2 ARTICLES INCLUDED IN THE DISSERTATION

This dissertation includes three articles that aim to answer the afore mentioned research questions (Table 1). Article I introduced a method for inclusion of GHG emissions from LULUC of mangrove forests. Quantification of GHG emissions from LULUC enables a fair comparison between products, by aligning the system boundaries of food and feed products. The alignment of system boundaries means that the production phases included in the studies are the same. In the case of food systems, the production process starts with the extraction of raw materials and includes any emissions that are associated with this first phase, such as deforestation to establish a production site. In order to put LULUC emissions originating from shrimp farming in mangrove areas into perspective, article I compared the results of a case study to that of the GHG emissions resulting from the entire life cycle of shrimp farming in semi-intensive and intensive production chains, using the LCA method (ISO, 2006).

Article II was concerned with the quantification of environmental impacts associated with MP production. The environmental impacts of MP production have been studied to an extent, but only based on the theoretical input requirements found in the literature (Pikaar, Matassa, et al., 2018; Sillman et al., 2019; 2020). Article II used empirical data on the actual inputs and outputs associated with MP production to build an LCA model that increases understanding of its environmental impacts. This research expanded on existing knowledge of the environmental impacts of MP, by including more environmental impact categories into the LCA model.

Article III provided a first estimation of the environmental impacts associated with Tr-OVA production. It performed a contribution analysis to identify the production stages that contributed most to the environmental impacts of Tr-OVA production. Different scenarios were created to explore the possibilities to reduce the found environmental impacts. Additionally, an LCA model of chicken-based egg white protein powder was created, and its environmental impacts were compared to that of Tr-OVA. The LCA model for Tr-OVA was based on empirical data on actual inputs and outputs, collected from a pilot-scale production site, and energy and mass balances.

Table 1: Contribution of articles I-III to answer the research questions of this dissertation.

Research question	Article I	Article II	Article III	Dissertation
RQ1: What are the product-level environmental impacts associated with the production of shrimp, MP, and Tr-OVA?	- Literature review on carbon stocks in mangrove forests and CH_4 and N_2O fluxes from aquaculture ponds - Development of method to estimate GHG emissions from LULUC in mangrove forests - Spatial analysis of the contribution of different types of shrimp farms on mangrove deforestation - Quantification of the GWP resulting from shrimp farming in mangrove areas	- Collection of data on inputs and outputs related to the production of MP - Performance of an LCA on MP production, based on empirical data - Performance of an uncertainty analysis	- Collection of data on inputs and outputs related to the production of Tr-OVA and chicken-based egg white protein powder - Performance of an LCA on Tr-OVA production, based on empirical data	
RQ2: How does the environmental impact of MP and Tr-OVA produced through cell-culturing techniques compare to those originating from agriculture or aquaculture?	- Comparison of the GHG emissions resulting from shrimp farming in mangrove areas to existing LCA results on shrimp farming	- Comparison of the environmental impacts of MP production with existing studies on MP and alternative protein-rich foods and feed ingredients	- Performance of an LCA for chicken-based egg white protein powder production, based on an existing model from literature - Performance of an uncertainty analysis for Tr-OVA and chicken-based egg white protein powder - Comparison of the environmental impacts of Tr-OVA production with those of chicken-based egg white protein powder	- Comparison of the environmental impacts of shrimp, MP and Tr-OVA with alternative protein-rich foods and feed ingredients. - Comparison of the environmental impacts from shrimp, MP and Tr-OVA as protein ingredients in an imagined patty
RQ3: What are the associated emissions sources of shrimp, MP, and Tr-OVA production, and during which production stage(s) do these emissions occur?	- Quantification of the LULUC emissions associated with different shrimp farming practices - Comparison of the contribution of LULUC emissions with emissions from other life cycle steps	- Performance of a contribution analysis of MP production to identify substantial production processes within the product system	- Performance of a contribution analysis of Tr-OVA production to identify substantial production processes within the product system	
RQ4: How can the environmental impacts of MP and Tr-OVA be reduced through alterations of the production design?		- Design of two main MP production scenarios - Performance of sensitivity analyses	- Design of four alternative Tr-OVA production scenarios - Performance of sensitivity analyses	

3. BACKGROUND

3.1 ENVIRONMENTAL IMPACTS

To understand the environmental impacts of cellular agriculture in the context of those of existing protein-rich food and feed ingredients originating from agricultural and aquacultural systems, it is necessary to first understand what are the commonly-identified environmental impacts associated with existing protein production. The following sub-sections will discuss and explain several environmental issues that are often associated with agricultural production. Campbell et al. (2017) identified the agricultural sector as the main activity driving destabilization of the planetary boundaries. These issues are also the main environmental impact categories that are often considered in LCA studies of food products (i.e. Poore & Nemecek, 2018b). After the overview of commonly-identified environmental impacts of protein production, this section continues by elaborating on the conflict between shrimp farming and mangrove forests and why mangrove deforestation is a pressing issue. Discussing these environmental issues in detail contributes to understanding of how the production of protein-rich food and feed ingredients are destabilizing the Earth systems current stable state, and where possible solutions can be found (Steffen et al., 2015). Through understanding of the impacts discussed below, it is possible to identify how cellular agricultural products could potentially decrease the environmental impacts associated with protein production.

3.1.1 GLOBAL WARMING

Global warming is being caused by increasing amounts of GHG in the atmosphere that are primarily a result of human activities (NASA, 2021). This has led to a global (land-ocean) mean surface temperature rise of about 1°C since the pre-industrial period (Shukla et al., 2019). A recent estimate suggested that about one third of all global anthropogenic GHG emissions are caused by our food system (Crippa et al., 2021). By far the largest contribution came from agricultural (including the cultivation of food and non-food crops, and livestock production) and LULUC activities (Crippa et al., 2021).

The three main GHG emissions resulting from agriculture and aquaculture are CO_2 , N_2O and CH_4 (Clark et al., 2020; Crippa et al., 2021). Clark et al. (2020) summarized the main sources of these emissions based on the historic data of the emission times series by Gütschow et al. (2019):

- the production and application of fertilizers and other agrichemicals are leading to CO_2, N_2O and CH_4 (N fertilizers alone contributed up to an estimated 21.5% of agricultural emissions in 2018 (GRAIN et al., 2021))
- major fluxes of CH_4 are caused by enteric fermentation in ruminants (such as cows, sheep and goats) (17% of agricultural related emissions (Crippa et al., 2021)) and by production of rice in paddies (3.5% of agricultural related emissions in 2015 (Ritchie et al., 2020))
- CH_4 and N_2O emissions result from livestock manure and its management (2% of agricultural related emissions in 2015 (Crippa et al., 2021))

- food production processes are causing CO_2 emissions (4% of agricultural related emissions in 2015 (Crippa et al., 2021)), and
- LULUCs mainly lead to fluxes of CO_2 and N_2O, and are one of the largest sources of emissions within the food-system (32% of agricultural related emissions in 2015 (Crippa et al., 2021)).

In addition, the natural processes of wetlands and the burning of biomass are major sources of CH_4 emissions (Forster et al., 2007). The total emissions from LULUC were 5.7 Gt CO_2-eq in the year 2015, approximately 13% of global GHG emissions (Crippa et al., 2021; The World Bank, 2022), which were mainly caused by deforestation and the degradation of organic soils. Although most of the LULUC emissions occur in developing countries (Crippa et al., 2021), input-output analyses have shown that most of the emissions are associated with food consumption in industrialized countries (Wood et al., 2018). Developing countries were responsible for 73% of all agriculture-related GHG emissions. Of these emissions, the land-based sector was the dominant contributor. Just over half of food-sector emissions were caused by downstream energy-related sectors in industrial countries. Despite the growth of food-system related GHG emissions overall, the emissions-intensity of food production has decreased during the period 1990-2015 (Crippa et al., 2021).

To limit global temperature increases to 1.5°C, or at least well below 2°C, GHG emissions will need to be reduced rapidly (Rogelj et al., 2018). In 2020, CO_2 levels were 412.5 parts per million (ppm), higher than at any other point in the past 800,000 years (Lindsey, 2020). The zone of uncertainty for the climate change planetary boundary is considered to be between 350-450 ppm CO_2 (IPCC, 2012). This means that humanity might end up in a high risk situation if we continue on a business-as-usual path (Steffen et al., 2015). Overshooting the planetary boundaries for climate change has been predicted to decrease crop yield (Asseng et al., 2014; Challinor, 2014). This will potentially lead to a destructive cycle in which lower yield will result in additional land-use changes, further increasing global GHG emissions and starting a new cycle. Crop yields have already started to decrease as a result of global temperature rises (D. K. Ray et al., 2019). Certain regions of the world will be more at risk, while others might benefit from global temperature rises (D. K. Ray et al., 2019). Southern Africa is one of these regions that has experienced reduced crop yield. Madagascar has been reported to be the world's first country experiencing a climate-change related famine (UN Madagascar, 2021).

One potential path to reducing the absolute amount of GHG emissions from the food sector is by changing food consumption patterns (Poore & Nemecek, 2018b): to replace food items with high emissions with those that cause generally low emissions, while keeping in mind nutritional requirements (Willett et al., 2019). This would mean moving away from animal-based products, as they result in a higher amount of GHG emissions compared to plant-based foods (Poore & Nemecek, 2018b; Xu et al., 2021). One of the main reasons that animal products contribute to GWP is the high amount of CH_4 emissions emitted by ruminants (Clark et al., 2020). Although having a low half-life in the atmosphere, CH_4 has a relatively high GWP in comparison to CO_2 (Eurostat, 2021). Another reason is the feed requirements of ruminants and the associated loss of energy at each trophic level (Xu et al., 2021). This also links back to emissions resulting from land clearance for feed production. A reduction in meat consumption seems especially effective in countries where meat-consumption is generally high (Godfray et al., 2018; Springmann et al., 2018) and would also lead to a reduction in adverse health effects related to a high intake of meat (Forouzanfar et al., 2015; Willett et al., 2019). To stay within the 1.5°C temperature limits and increase the overall health of the population, the EAT–Lancet Commission has concluded that global consumption of red-meat and sugars will need to be reduced by 50% while consumption of fruits, vegetables, nuts and legumes will need to double (Willett et al., 2019).

A further way to decrease emissions from the agricultural sector is by reducing food losses. Food losses at various stages of the life cycle contribute substantially to the total GWP caused by the food system and are estimated to make up 8% of the food systems GHG emissions (EC, 2012). About one third of all food produced is wasted every year, while up to 811 million people are still undernourished and are unable to live a healthy and active life in 2020 (FAO et al., 2021). Most of the food waste occurs within the developed nations (FAO, 2014). Reducing food waste would therefore not only help to reduce the GWP of food, but also reduce pressure on other planetary boundaries, and help in achieving the sustainable development goals of the United Nations (UN) (Lemaire & Limbourg, 2019).

3.1.2 WATER USE

Agriculture is the largest global user of freshwater, responsible for 70% of withdrawals, making humans the largest consumers of the earth's freshwater resources (Campbell et al., 2017; FAO, 2020a). Most freshwater consumption takes place through the transpiration of crops and the evaporation of water held in soils and irrigation structures (Campbell et al., 2017). During the past two decades, annual freshwater resources have declined by 20%. The two main reasons for increased water consumption for agricultural purposes are the growing population, and the shift towards more water-intensive food sources such as beef (Campbell et al., 2017; FAO, 2020a; Poore & Nemecek, 2018b). Sugar and coffee producers are also large consumers of water (Kassem et al., 2021).

The greater water consumption associated with animal products is mostly explained by the low agricultural efficiency of livestock farming: large amounts of crops are produced (and irrigated) for livestock feed production. Recent research has suggested that dietary changes, where animal products are replaced with increased consumption of pulses, nuts, fruits and vegetables, cereal products, and other meat replacements such as insects, could also result in decreased global water consumption (Kassem et al., 2021; Poore & Nemecek, 2018b; Vanham et al., 2018).

Growing thirst of water is having a detrimental effect on the environment, with 41% of current global water irrigation occurring at the expense of environmental flow requirements (FAO, 2020a). Both ground and surface water, as well as soil moisture, are extracted in favor of crop and animal production leaving less available for local ecosystems. In some regions, groundwater levels are experiencing a rapid depletion that is causing concern (Campbell et al., 2017). One such example is in California's Central Valley, where the dropping groundwater level is causing deformation of the surface area (Vasco et al., 2019). However, there are many other examples of agricultural areas worldwide that depend on groundwater for irrigation to the extent that groundwater levels are rapidly falling (Cotterman et al., 2018).

Groundwater is of the utmost importance to many ecosystem services, a fact that has often been neglected (Khorrami & Malekmohammadi, 2021). Dropping groundwater levels alter the flow of water from the groundwater source to streams, resulting in potentially devastating effects on aquatic ecosystems (de Graaf et al., 2019). The abstraction of water for food production can have severe impacts on the local ecosystems, especially in areas where water availability is generally low, such as arid areas where potential evapotranspiration outweighs water received through precipitation by five times (Boulay et al., 2015). At the same time, water use by the agricultural sector in these arid areas is generally high: ~87% on the African continent, and even ~90% in some of the Arab countries (Campbell et al., 2017). Human-induced climate change will put further stress on both the food system and natural ecosystems due to the predicted increase in extreme weather events, which increases the risk of drought that will, in turn, further increase reliance on groundwater resources (de Graaf et al., 2019).

Overuse of freshwater resources may affect not only nature conservation but also food security (Schyns et al., 2015). A recent analysis on the effects of groundwater depletion in India, the world's largest user of groundwater, suggests that crop production may decrease by 20% throughout the nation and up to 68% in groundwater depleted regions. This would mean an increase in dependency on irregular rainfall patterns (Jain et al., 2021). Direct rainwater consumption has increased steadily for agricultural purposes. This limits the water that is allowed to flow naturally, with potential adverse effects on valuable ecosystems (Schyns et al., 2019).

3.1.3 FERTILIZER USE AND EUTROPHICATION

Modern agriculture relies heavily upon external inputs such as industrially produced fertilizers, for which demand is expected to increase as global demand for crops will likely double by 2050 compared to 2005 (Tilman et al., 2011; X. Yu et al., 2021). Approximately 80% of current global consumption of phosphorus (P) is used in the production of fertilizer necessary to sustain current agricultural yields (Van Vuuren et al., 2010). Globally, soil P levels are low, increasing the need to apply P fertilizers manufactured from phosphate rocks (X. Yu et al., 2021). The potential depletion of P resources could inhibit the further intensification of agriculture necessary to meet predicted increases in agricultural demands (Van Vuuren et al., 2010). Changes in agricultural management are therefore essential to increase the efficient use of P fertilizers. P fertilizer use efficiency (PFUE) is further affected by regional climate and soil characteristics (X. Yu et al., 2021).

The production of ammonia (NH_3), the foundation of modern-day nitrogen (N) fertilizer, was invented as a response to the growing need for food, and the concomitant need to replace field N losses due to crop harvesting. It is produced in a process known as the Haber-Bosch process (Erisman et al., 2008). The invention of this process transformed the world and was necessary to support human population growth (Erisman et al., 2008; Smil, 2001). Approximately 80% of chemically-produced NH_3 (produced from N and hydrogen) is used as fertilizer to support the growth of crops and fiber (Erisman et al., 2007). However, the amount of N actually reaching humans is only 5-15% (Erisman et al., 2007).

Overuse of P fertilizers leads to large amounts of P entering the environment causing both freshwater and marine eutrophication. The additional input of N has now been recognized as the main contributor to coastal eutrophication with an estimated 24% of anthropogenic N released into coastal watersheds reaching coastal ecosystems. This excess input of nutrients leads to algal blooms and scum, toxic phytoplankton events and massive growth of macrophytes. This, in turn, leads to oxygen depletion in water, limited primary production in some systems, and the loss of biologically engineered habitats that together can lead to a potential development of dead zones (Howarth & Marino, 2006; Malone & Newton, 2020; Van Vuuren et al., 2010).

Furthermore, the production of N requires a high amount of energy often supplied by the consumption of natural gas and oil (Erisman et al., 2007). Agricultural emissions resulting from low nitrogen use efficiencies and the consequential partial release of N2 into the atmosphere contribute to GWP. An estimated 65% of global NH_3 emissions are linked to agriculture (Erisman et al., 2007). However, national governments have an opportunity to reduce the application of N and the consequent eutrophication effects, with only a minor increase in the yield gap (Wuepper et al., 2020).

3.1.4 LAND USE

Forest cover has been steadily disappearing on land areas suitable for agricultural purposes since before the start of the industrial era (Kaplan et al., 2009). Approximately three-quarters of total global ice-free land surface area was affected by human activity in the year 2015. Cropland covers approximately 12-14% of this ice-free surface. 60-85% of all forests and 70-90% of other natural ecosystems are currently impacted by human activities. This agricultural land use has resulted in an 11-14% decrease in global biodiversity (Shukla et al., 2019). An estimated 49% of global ice-free surface land is reserved for agriculture use. Approximately a quarter of this agricultural land area is used to grow crops while the rest is occupied by livestock for meat and dairy production. (Ritchie & Roser, 2019; Shukla et al., 2019). However, meat and dairy supply a minority of global calories and protein (Ritchie & Roser, 2019).

Land use for agricultural production not only causes GHG emissions through the loss of above-ground, below-ground, and soil-organic carbon storage, it also leaves less land available for natural vegetation and ecosystems, therefore contributing to biodiversity and ecosystem service losses (Marques et al., 2019; Metzger et al., 2006; Newbold et al., 2015). These losses are considered a loss of life forms and genetic variability, but they also present a threat to human prosperity as degraded ecosystems cannot provide the same goods and services that humans need to prosper (Cardinale et al., 2012).

The loss of wild areas is closely linked to both carbon stock and biodiversity losses, which means that both problems can potentially be addressed at the same time (Soto-Navarro et al., 2020). One solution would be to decouple agricultural activities from natural resource use and reduce the pressure on biodiversity-rich regions (Venter et al., 2016). Unfortunately, despite the observed decoupling of economic growth from resource use, many places in the world are faced with increasing pressures from human activities, of which agriculture is a major part. A mere 3% of biodiverse hotspots are currently free from human-induced pressure (Venter et al., 2016).

The widespread use of land for agricultural purposes and the overexploitation of the soil are negatively affecting both the earth and the agricultural system leading to reduced productivity and ultimately soil death. Soil organic matter — containing about 58% of soil organic carbon (SOC) — provides ecosystem services such as climate regulation, water filtration and purification, and support of biodiversity (Timmis & Ramos, 2021; Trivedi et al., 2018). Sustainable agricultural and land-use practices are therefore of huge importance in combating land degradation and desertification (Trivedi et al., 2018). After all, we are dependent on healthy, productive soil for nearly all the food we produce. Desertification — known as land degradation in arid, sub-arid, and dry sub-humid areas — has already reduced agricultural productivity and agriculture-associated income and led to an increase in biodiversity losses. An estimated 500 million people were affected by desertification in 2015. As desertification is most likely to hit regions where the human population is projected to increase the most in the coming years, changes in agricultural practices are required. (Mirzabaev et al., 2019).

3.1.5 LAND COMPETITION BETWEEN MANGROVE FORESTS AND SHRIMP FARMING

Mangrove forests are woody trees and shrubs that grow in coastal intertidal regions of the world between approximately 30°N and 30°S latitude (Giri et al., 2011; Kaiser et al., 2005). They are unique because they are able to live in waterlogged, salty, and often unstable and harsh conditions (Kaiser et al., 2005; Spalding & Leal, 2021). Mangrove forests are known for a vast variety of ecosystem services, such as coastal protection, providing support for life in estuaries and near-shore water, and the provision of nursing grounds and habitats for aquatic animals (Duarte et al., 2005; Eong, 1993; Kristensen et al., 2008; Mcleod et al., 2011; Nagelkerken et al., 2008; Spalding & Leal, 2021).

One ecosystem service that makes this habitat unique is the capacity of mangrove forests to sequester carbon from the atmosphere. Half the amount of carbon stored in the marine environment is stored in these forests (UNEP, 2009). This is because, unlike other terrestrial forests, mangrove forests do not saturate with carbon but continue to sequester carbon into their forest floor, accreting sediments vertically. This process of carbon sequestration into the depth of the soil has been estimated to continue over millennia (Duarte et al., 2005; Eong, 1993; Mcleod et al., 2011). Estimated sequestration rates can be as high as 3.53 t C ha^{-1} $year^{-1}$ (Sanders et al., 2010), compared to a carbon sequestration rate of 0.007-0.131 t C ha-1 $year^{-1}$ of terrestrial forests (Mcleod et al., 2011). This makes them one of the most productive natural tropical ecosystems in the world (Donato et al., 2011; Eong, 1993; Mcleod et al., 2011; Spalding & Leal, 2021; Twilley et al., 1992).

The mechanisms behind this high carbon sequestration rate are a combination of hypoxic conditions, the lack of other high-energy oxidants, and the paucity of fungi, which together limit the process of degradation (Middleton & McKee, 2001). The result is several meters of organic material, making up one of the largest carbon reserves in the terrestrial biosphere (Chmura et al., 2003; Lovelock, 2008). This is what makes mangrove forests a vital partner in our battle to decrease atmospheric CO_2 emissions and prevent global temperatures from rising further.

Mangrove forests have been under threat globally for many years (Spalding & Leal, 2021). Historic annual deforestation rates of mangrove forests have been between 0.7-2.4%, exceeding those of inland tropical forests (B. C. Murray et al., 2012). Between 1996 and 2016 there was a 4.3% net loss of mangrove forests worldwide. However, the rate of mangrove deforestation has slowed down in recent years, possibly because of the recognition of the importance of mangrove forests and their ecosystem services. Many organizations and countries have started collaboration to attempt to both slow further losses and rehabilitate former mangrove forests back to their original state (McNally et al., 2010;

Spalding & Leal, 2021). The reasons for mangrove deforestation have been demand for firewood and conflict of land use demands, such as urban and leisure coastal developments, and the establishment of aquaculture ponds (McNally et al., 2010; Spalding & Leal, 2021).

Shrimp are one of the many species that can naturally be found in mangrove forests. Production of the giant tiger prawn (*Penaeus monodon*), specifically, has grown substantially since the mid-1980s, driven by demand mainly from Europe, the USA and Japan (FAO, 2005; Lebel et al., 2002). This shrimp species is predominantly produced in aquaculture ponds in South East Asia (FAO, 2009). Vietnam is one of the main exporters of shrimps. The giant tiger prawns are produced mainly in the Mekong Delta area due to topographical and geographical features that make the region a perfect location for aquaculture production (Phan et al., 2011). Ca Mau province is especially suitable to shrimp production, and more than half of the land surface in this area is used for aquaculture production. It is also the area where one third of Vietnam's mangrove forests are found (Jonell & Henriksson, 2015). An estimated 74% of all mangrove forests in Ca Mau were lost, for the most part due to the growth of the aquaculture industry during the period 1979 – 2013 (Son et al., 2015).

3.2 CELLULAR AGRICULTURE

The following sub-sections give an introduction to the MP and Tr-OVA products. They briefly explain the characteristics of these cellular products and how they can be used as either protein-rich food or feed ingredients. Details of the production processes of the products can be found in article II (MP) and III (Tr-OVA).

3.2.1 MICROBIAL PROTEIN

Most cell-cultured products rely to some extent on agricultural input by using heterotrophic organisms that eat other plants and animals to acquire energy and nutrients. Often glucose is required in the production process as an energy source. Currently, glucose is typically extracted from agricultural crops such as grain, corn or sugar crops (Tuomisto, 2019). However, there are examples of organisms that are completely independent from agricultural inputs, and these are referred to as autotrophic microbes, such as methanotrophic bacteria that obtain energy and nutrients from methane and carbon (Cumberlege et al., 2016). Technologies utilizing methanotrophic bacteria to produce feed ingredients are already in commercial-scale production (Cumberlege et al., 2016).

Another example of autotrophic microbes is HOB that obtain their energy from hydrogen and CO_2. Technologies to bring HOB to market as food or feed ingredients are currently under development (Pikaar, de Vrieze, et al., 2018; Ritala et al., 2017). Cupriavidus necator (formerly Ralstonia eutropha) is one example of an HOB that can be utilized for this purpose (Liu et al., 2016; J. Yu, 2014), and it is used for the production of MP.

Just as many other cellular agricultural products, MP cannot replace an existing agricultural product one-to-one due to its powdery form. However, it can be used in meals (such as in the production of pancakes or patties) to replace animal-based ingredients due to its high-quality protein. For example, protein availability in proteolytic enzymes has been shown to be greater than that of wheat, and the composition of their essential amino acids is similar to that of animal protein (Matassa et al., 2016; Volova & Barashkov, 2010). Microbial protein can also be used as a feed ingredient (Pikaar, Matassa, et al., 2018), replacing other protein sources such as soybeans that are often associated with destruction of rainforests (Brown, 2009; M. H. Costa et al., 2007). As such, the impacts of microbial protein can be compared with alternative protein sources used in food and feed, based on the protein content of the ingredients.

3.2.2 CELL-CULTURED EGG OVALBUMIN

Yearly production of chicken eggs reached 86.7 million t in 2020 (FAOSTAT, 2021). Egg white, separated from egg yolk and processed into a powder, is a high-quality protein source that is widely used in the food industry. Ovalbumin makes up over 50% of the egg-white protein. However, egg production is associated with many environmental burdens (Crippa et al., 2021; Poore & Nemecek, 2018b; Smil, 2002; Van der Warf & Petit, 2002). Intensive chicken farming has also resulted in zoonotic disease outbreaks and has attracted ethical criticisms (C. K. Johnson et al., 2020). Regardless, the market for egg white protein powder is expected to expand (Markets & Markets, 2019). To meet this growing demand, while potentially decoupling ovalbumin production from the aforementioned issues, the development of alternatives to chicken-based egg white protein powder has started to gain interest within the food industry (Eibl et al., 2021).

The techniques used in cellular agriculture had already made it possible to produce ovalbumin in a bioreactor using genetically modified bacteria (Escherichia coli) by 1995 (Takahashi et al., 1995). Since then, technological advancements have made it possible to produce recombinant or cell-cultured ovalbumin in a large enough scale for it to be considered an economically feasible alternative to chicken-based egg white protein (Voutilainen et al., 2021). This novel process involves using a well-established and efficient production organism of the mesophilic filamentous ascomycete fungus *T. reesei*. *T. reesei* is also able to produce other proteins such as the milk protein β-lactoglobulin.

Tr-OVA is currently produced in bioreactors on a pilot scale. This is done by means of using biotechnological tools to insert the gene carrying the blueprint for ovalbumin (length: 386 amino acids) into the fungus. The coding gene SERPINB14 (https://www.uniprot.org/uniprot/P01012) used in the process comes from chickens (Gallus gallus domesticus). After the modification, the fungus starts secreting the same ovalbumin protein found in eggs produced by chickens. The process results in a protein powder with comparable functional properties to chicken-based egg white protein powder. The product can be used directly as a replacement ingredient in foods. Tr-OVA is an example of an acellular product as the fungal mass is removed from the final product.

3.3 LIFE CYCLE ASSESSMENT

LCA was applied in the environmental impact assessment of shrimp, MP, and Tr-OVA as a means to include emissions at all stages of production. The section below gives a general introduction to the LCA method and how it was applied in the research presented in this dissertation. The quantification of GHG emissions resulting from LULUC within an LCA is discussed after this.

3.3.1 OVERVIEW OF LIFE CYCLE ASSESSMENT

Environmental (LCA) was introduced in the 1980s as a method to quantify the environmental impact of products throughout their life cycle. It has been employed to address difficult questions that could only be answered by taking into account the entire production system behind each product alternative (Guinée et al., 2011).

As the application of LCA has increased, so has the need for standardization of the method. The International Organisation for Standardisation (ISO) developed guidelines for LCA standardization that resulted in the first international standards published in 2006 (ISO, 2006). ISO describes LCA as the activity of gathering an overview of all inputs, outputs and the potential environmental impacts related to a product or service through its lifetime (ISO, 2006).

However, ISO standards were never developed to guide LCA practitioners in the details of LCA, nor is there a common agreement among scientists on how to interpret the ISO rules (Heijungs et al., 2021; B. Weidema, 2014). For example, the ISO guidelines are ambiguous on the matters of system expansion and substitution, creating confusion over the definition of the two methods available for dealing with the multi-functionality problem (Heijungs et al., 2021). System expansion refers to the expansion of the FU by including all co-products of the system to the FU (e.g., both meat and milk become part of the FU of the system). Substitution refers to the process of solving the multi-functionality problem by subtracting avoided environmental burdens related to the co-product that are not included in the FU (Heijungs et al., 2021).

Despite the standardization of the LCA method there has been a divergence of approaches such as consequential versus attributional LCA (B. P. Weidema et al., 2019), the anticipatory LCA (Wender et al., 2014), prospective LCA for emerging technologies (G. Thomassen et al., 2019), hybrid LCA and environmental input and output based LCA (EIO-LCA) (Stadler et al., 2018), and specialized tools such as the carbon footprints (Pandey et al., 2011) and water footprints (Hoekstra et al., 2011). Additionally, the social LCA (SLCA) (Dreyer et al., 2010) and life cycle costing (LCC) were developed to expand on the sustainability assessment of a product system (Woodward, 1997).

One of the most common divisions between LCA methods is the attributional vs the consequential LCA. Attributional LCA calculates the average impact associated with different inputs while consequential LCA studies the impact of the marginal supplier that can respond to increased demand in the required inputs of the studied production process (Schaubroeck et al., 2021; M. A. Thomassen et al., 2008). Both approaches require modelling choices that will lead to potentially different results when applied to the same product. For example, a common practice within consequential modelling is to apply substitution to avoid allocation in the case of multi-functionality, while allocation is a commonly used way to divide emissions within the attributional LCA (Consequential-LCA, 2015a; Schaubroeck et al., 2021). Ecoinvent has developed a separate database that can be used for consequential LCA. While the attributional LCA databases focus on the average impact of a process (e.g., the average electricity mix of a country), the consequential LCA database focuses on the marginal supplier of an increase in demand for a product. This means, in the exemplary case of electricity, that only certain suppliers are able to meet the extra demand for electricity in the short-term (Consequential-LCA, 2015b; M. A. Thomassen et al., 2008).

One ongoing discussion within the LCA method concerns the choice of functional unit (FU) that should be used in the comparison of different products or services. The environmental impacts of a product are quantified based on the function a product provides (ISO, 2006). A FU can be as simple as '1 kg of product', but in the case of food can also be set as 'one meal', '1 kg of protein', or '1 kcal' depending on the main function of the item and the goal of the study (Cucurachi et al., 2019; Poore & Nemecek, 2018b). Of particular concern to LCA of food products has been discussion as to what the function of food is (e.g., the delivery of an individual nutrient or the alleviation of the feeling of hunger). Along these lines, questions remain about how to account for the differing nutritional aspects of food products as the nutritional content of two products usually vary considerably, thereby complicating comparison. (Mclaren et al., 2021; Saarinen et al., 2017; B. P. Weidema & Stylianou, 2020).

3.3.2 LAND USE AND LAND-USE CHANGE EMISSIONS IN LCA

Although LCA began primarily as a method to assess industrial production processes, concern over the impacts of the agricultural sector has led to the inclusion of food products (Roy et al., 2009). Eventually, the application of LCA extended to aquaculture products when the first assessment was performed in the early 2000s by Papatryphon et al. (2003) on salmon feed. In 2019, Cururachi et al. (2019) published an article on the principles of, and steps required for conducting an LCA study on food systems.

LULUC emissions are a major part of the life-cycle related emissions of food products (Clark et al., 2020; Crippa et al., 2021). Emissions can occur from changes in above-ground, below-ground, litter, and soil carbon stocks, or the application of fertilizers (IPCC, 2006a, 2014). Historically, these emissions have often been excluded from LCA and LCA databases due to lack of data, methodological debates on the correct estimations for LUC emissions, or a lack of understanding on the causal relationship between deforestation and land use (Donke et al., 2020; IPCC, 2006a; Schmidt et al., 2015). For example, land soil carbon stock fluxes are often overlooked within LCA food studies, which can partly be explained by the lack of a well-defined procedure to account for them (Goglio et al., 2015). The potential for agricultural soil worldwide to store carbon from the atmosphere has been estimated to be somewhere between 0.4-1.2 Gt of CO_2, the equivalent of 5-15% of global fossil fuel emissions by changing land management practices and decreasing LUC (Goglio et al., 2015). Additionally, indirect LUC is poorly represented in LCA studies, leading to potential underestimation of GHG emissions and poor decision making. Indirect LUC occurs when the demand for crops in one region causes changes such as LUC, intensification or reduced consumption in another (Schmidt et al., 2015).

Efforts are constantly being made towards better estimates of LULUC and the implementation of LULUC emissions into existing guidelines and databases (Donke et al., 2020; IPCC, 2014; Nemecek et al., 2016; Schmidt et al., 2015).

4 METHODS AND DATA

4.1 USE OF LCA IN ARTICLES I, II AND III

The LCA method has been applied in all three articles presented in this dissertation to quantify the environmental impacts of shrimp farming (article I), MP (article II), and Tr-OVA production (article III). The impact categories that were used in the analyses included those that are typically associated with the agriculture and aquaculture sector such as GWP, land use and water consumption. Because of the industrial nature of cellular agriculture, with a high reliance on electricity and natural gas, these impact categories where extended to include a number of categories generally associated with industrial production processes such as human carcinogenic and non-carcinogenic toxicity, and ionizing radiation (Gésan-Guiziou et al., 2019; Noya et al., 2018; Santos et al., 2017; Tsai et al., 2020; Zouaghi et al., 2019). For the same reason, the CED was also assessed using the CED V1.11 method by ecoinvent (Althaus et al., 2007). The impacts of water consumption were quantified using the AWARE method developed by Boulay et al. (2018). The AWARE method considers the general water scarcity of the region from which water is extracted when quantifying the environmental impacts of water consumption.

A cradle-to-gate system boundary was applied to all three studies. This includes all inputs and outputs related to the processes, from resource extraction up to the factory or farm gate. Land use of the cellular agricultural facilities was allocated over a 20-year production period. However, the production and construction of production facilities was excluded from the system boundaries due to their relatively small contribution to the environmental impacts of the products. Additionally, facilities has typically been excluded from LCA studies used in the comparison of protein-rich food and feed ingredients (Poore & Nemecek, 2018b). Similarly, packaging processes were excluded from the analyses as well.

Three FUs were applied in this dissertation. The first FU was based on the products' weight and was defined as 1 kg or 1 t of product. This was chosen to explore the environmental burden of the product. A second FU was based on the protein content of the products and allowed for the comparison of protein-rich products that each have a different protein concentration. The third FU, that was applied to study the environmental impacts of different protein sources, was based on a meal and defined as 1 patty using shrimp, MP, or Tr-OVA as the main protein source.

The main difference between the estimated environmental impacts of the shrimp farming system and the production of MP and Tr-OVA is that the latter two were based on data from one production facility while the former was based on multiple cases (N=11). The sensitivity and uncertainties of the MP and Tr-OVA model related to this limitation were captured using Monte Carlo (MC) analyses and sensitivity analyses. MC analyses were performed with the Simapro 9.1.0.11 PhD software package (PRé Consultants, 2020) using 100 iterations with a 95% confidence interval. A parametric bootstrap method was applied to handle the extremely large uncertainty ranges of the AWARE water scarcity method (Lee et al., 2018). For this purpose, Python 3.0 was used, applying 1,000 simulations using a sample size of 300 and allowing for replacements.

4.2 ARTICLE I: METHOD, DATA AND SYSTEM DESCRIPTION

Research performed for article I was divided into two parts. Part one describes a general method for accounting for LULUC emissions resulting from mangrove deforestation based on a literature review on carbon stocks in mangrove forests. This method is recommended when no other details are known about how the new land use has affected the mangrove carbon stocks. Part one also details the amounts of CH_4 and N_2O emitted during aquaculture farming. Part two quantifies the GHG emissions from mangrove LULUC caused by shrimp farming. It addresses the specifics of establishment LUC from mangrove forest areas to shrimp farms, and how this impacts the underlying assumptions of the general method described in part one.

4.2.1 ESTIMATING GHG EMISSIONS FROM LULUC OF MANGROVE FORESTS

LULUC emissions resulting from mangrove deforestation were calculated based on changes in the carbon stocks of mangrove forests where before and after states were compared. The method assumes that a default 1m soil depth is affected and that 96% of soil carbon is oxidized as a result of mangrove deforestation. This is in line with the guidelines of the IPCC (IPCC, 2006a). The effects of mangrove deforestation on carbon stocks are visualized in Figure 1 where $t = 0$ represents the moment of LUC and $t > 0$ refers to the period of LU. LUC emissions result from the loss of carbon stocks and are represented by β and δ, where β refers to the sum of the above-ground, below-ground, litter, and soil carbon stocks. The continuation of soil carbon oxidation at $t > 0$ is represented by δ. Although LUC emissions may in practice occur over several years, they are attributed to the event of mangrove deforestation at $t = 0$. The difference in carbon stocks (ΔC LUCi) caused by LUC of mangrove forests was calculated using equation 2 found in article I.

LU emissions were calculated based on the average soil carbon sequestration rate of mangrove forests and are represented by θ in Figure 1. These emissions are referred to as the missed potential carbon sequestration of the mangrove forest caused by LULUC. Additional potential emissions resulting from LU are in the form of anoxic CH_4 formation, and nitrogen volatilization originating from aquaculture practices (Astudillo et al., 2015; Hu et al., 2012). The differences in carbon stocks caused by the missed carbon sequestration (ΔC LUi) were calculated using equation 3 found in article I.

Total CO_2 emissions resulting from LULUC were calculated by summing the difference in carbon stocks from LULUC of mangrove forests and applying a conversion factor (44/12). This conversion factor is based on the molecular weight of the carbon in relation to CO_2. LULUC emissions are attributed annually to the activity leading to mangrove deforestation over a 20-year period, as recommended by the IPCC (IPCC, 2006a).

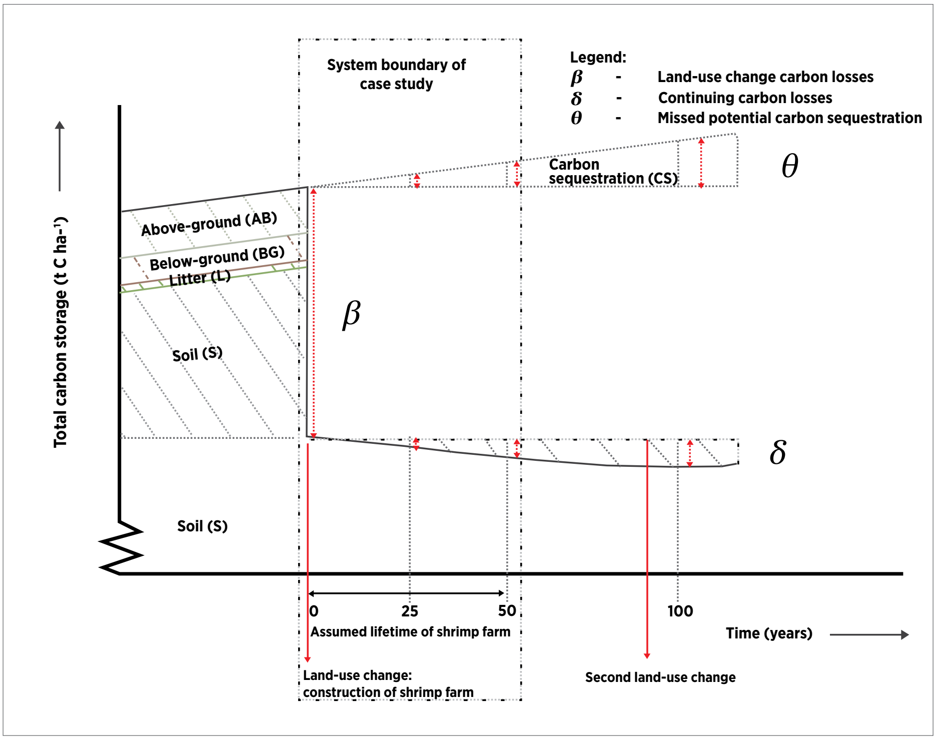


Figure 1: Graphical representation of the changes in carbon stocks resulting from mangrove deforestation. Carbon losses occur during land-use change (LUC) at point t=0 (β), and the continuous emissions during oxidation of soil carbon in the following years (δ). The LUC carbon losses are categorized into above-ground (AG), below-ground (BG), soil (S), and litter (L) carbon losses and are accounted for regardless of when in time they occur (i.e. the oxidization of carbon in the soil over time, δ) (Cederberg et al., 2011; IPCC, 2006a). The missed potential to store carbon in mangrove soils is referred to as 'missed potential carbon sequestration' (θ) and is the result of the land use (LU) of the former mangrove area. These LU carbon losses are potential sequestration rates based on historic data on carbon sequestration while the LUC carbon losses are factual losses than can be measured at the point of occurrence. Adapted from article I.

Estimates on average carbon stocks of mangrove forests and potential CH_4 and N_2O emissions were gathered through a literature review. Articles included in the review were found through Google Scholar by using a combination of the following keywords: "primary production", "mangrove forests", "carbon stocks", "methane", "dinitrogen oxide", "CH_4", "N_2O", "fluxes", "mangrove", "carbon", "emissions", "storage", "sequestration", "soil", and "land-use change". Estimates on CH_4 and N_2O emissions resulting from LU by aquaculture in mangrove forests are scarce (Astudillo et al., 2015; Hu et al., 2012). It was therefore necessary to rely on more general estimates reported for CH_4 and N_2O emissions from standing mangrove forests and aquaculture in general.

A MC analysis was performed to account for uncertainties of the calculated emissions from mangrove deforestation. It was based on the approximate distribution of carbon stock values found in the reviewed literature. The analysis was performed with the CMLCA v5.2 software, using 10,000 iterations.

4.2.2 CASE STUDY ARTICLE I: SHRIMP FARMING IN THE MEKONG DELTA, VIETNAM

There are five different types of shrimp farming practices found in the Mekong Delta area: intensive, semi-intensive, improved extensive, improved extensive alternate, and mixed mangrove concurrent farming. (Henriksson et al., 2015; F. J. Murray et al., 2013). The mixed mangrove concurrent practice was established as a means to protect mangrove areas by allowing farmer families to use up to 30% of the total surface area to generate an income with the intention of preventing complete deforestation (McNally et al., 2010). Mixed mangrove concurrent farms practice extensive shrimp farming and therefore require little external input (Phan et al., 2011). The shrimp yield of these farms' averages at 0.13 t shrimp ha^{-1} water area $year^{-1}$. To put this into perspective, semi-intensive shrimp farms produce 6.6 t shrimp ha^{-1} water area $year^{-1}$ and intense shrimp farms 7.6 t shrimp ha^{-1} water area $year^{-1}$ on average. (FAO, 2005; F. J. Murray et al., 2013; Phan et al., 2011).

To estimate LULUC emissions resulting from mangrove deforestation due to the establishment of shrimp farms, it was first necessary to identify which types of shrimp farming practices within the Mekong Delta have led to mangrove losses. This was achieved using the GPS coordinates of 200 randomly selected shrimp farms located in the area. Collected GPS coordinates were used to perform an evaluation of present and historic satellite images found from Google Earth (Google, 2013), the Global Land Cover by the National Mapping Organizations (ISCGM, 2003) and the Google Earth overlay map of the global distribution of mangrove forests of the world (Giri et al., 2011). The historic satellite images found, showed the land use of the affected areas before the establishment of the selected shrimp farms.

Shrimp farms located in mangrove areas are established by deforesting (part of) the mangrove forest by the removal of trees, including the roots. Forest litter is removed from the area and a pond hole is dug into the soil. The average pond depth is about 1.5m. (F. J. Murray et al., 2013; Phan et al., 2011). The amount of carbon in the soil that will eventually be exposed to oxygen is difficult to predict due to the use of part of the soil material during the construction of the pond walls, variation in pond depth, and the fate of sediments after abandonment of the shrimp farm (Jonell & Henriksson, 2015). A study by Eong (1993) conservatively estimated that about 50% of the carbon ends up being oxidized and this estimate was adopted into the method used in article I.

Resulting emissions were annualized over 50 years, based on the expected life time of a shrimp farm by Jonell and Henriksson (2015). As the predicted expected life time of a shrimp farm will have a considerable influence on the results, a sensitivity analysis was performed where a 20-year annualization was applied, as recommended by the IPCC (IPCC, 2006a). A FU of 1 t of live weight shrimp at the farm gate was applied.

4.3 ARTICLE II: METHOD, DATA AND SYSTEM DESCRIPTION

4.3.1 MODEL AND RELATED ASSUMPTIONS

The environmental impacts of MP production were estimated using an LCA. Sillman et al. (2020) conducted a similar study based on theoretical values found in existing literature, as empirical data was not available at the time. Because of the potential of MP to be used either as a protein-rich food or feed ingredient with lower environmental impacts than existing protein sources (Matassa et al., 2016; Pikaar, de Vrieze, et al., 2018; Pikaar, Matassa, et al., 2018; Sillman et al., 2020), there is scientific interest in the quantification of these impacts through the use of empirical data. The goal of the research was therefore to quantify the environmental impacts of MP production using empirical data collected from a pilot-scale production facility.

The final product is a single-cell protein powder suitable for both human consumption or as a feed ingredient for livestock. The environmental impacts were calculated using a FU of 1 kg of product with a 5% moisture content. The product contains 65% protein, 11% fiber, 6% fat and 2.2% carbohydrates. The system does not produce by-products to which allocation should be applied.

Aggregated inputs and outputs were linked to the impact categories GWP (kg CO_2 eq), LU (m^2 crop eq), freshwater eutrophication potential (kg P eq), marine eutrophication potential (kg N eq), terrestrial acidification (kg SO_2 eq), and human carcinogenic and non-carcinogenic toxicity (both in kg 1.4-DCB) using the ReCiPe 2016 v1.1 Midpoint (H) method (Huijbregts et al., 2017). Additionally, water scarcity and CEDs were calculated for the system. The LCA was performed using the Simapro 9.1.0.11 PhD software package (PRé Consultants, 2020).

As no commercial-scale production facility was yet operational at the time of writing article II, several production design choices existed, including the choice of electricity source used to produce MP and the production location. MP was developed by a Finnish start-up company within the Helsinki metropolitan area. Therefore, the baseline LCA model was created assuming this production location. This choice of location was relevant for both the assumed average electricity mix, and the AWARE factors used to measure water scarcity.

Two production scenarios were created and analyzed for MP. The first scenario was based on more conservative assumptions using the average Finnish electricity mix, while the second scenario was based on renewable energy sources. The latter is also more in line with general future expectations on the switch from fossil to renewable energy sources for electricity production (Ministry of Economic affairs and employment in Finland, 2018). Details on both scenarios can be found in Table 2.

Table 2: Two different scenarios for MP production (adapted from article II).

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 of article I, section 5 lists the mix of energy sources for the Finnish electricity mix as modeled in this article.

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

The effects of choosing different production locations, with differing renewable energy sources available, were tested in the sensitivity analyses applied to the model. Other sensitivity analyses included variations in the utilization of nutrients, transport distances and electrolyzer efficiency, the on-site production of CO_2 and steam, and the possibility to recycle water on-site. Table 3 gives a list of the various sensitivity analyses that were performed. Further details on these analyses can be found in article II.

To account for uncertainties in the system, an MC analysis was applied using 100 iterations and a 95% confidence interval. Uncertainties were estimated with the pedigree matrix available in Simapro. The bootstrap method was applied to deal with the naturally large uncertainty ranges the AWARE method produces in an MC (Lee et al., 2018). Bootstrapping is a statistical technique based on resampling the original sample with replacements. The bootstrapping method is recommended to reduce the large uncertainty ranges in water scarcity results that are a result of the use of discrete distributions with greater extremes (Lee et al., 2018). Bootstrapping was performed with Python 3.0. The bootstrap was implemented with 1000 simulations using a sample size of 300 while allowing for replacements.

Table 3: Changed parameters for the sensitivity analyses of MP production (adapted from article II).

Sensitivity analysis 1: Finnish average energy mix (FAEM)	
Name of analysis	**Changed parameter**
FAEM – steam	Steam production on site using electricity, rather than it being supplied
FAEM – CO_2 on-site	CO_2 production on-site using electricity, rather than it being supplied
FAEM – electrolyzer	Reduction in assumed efficiency of the electrolyzer
FAEM – nutrients 85% utilization	Reduction in the uptake of nutrients supplied to the process
FAEM – transport	Increase in air-based transport due to a change in the origin of the exporting country from Europe to China.
FAEM – 80% water recycling	80% of wastewater recycled on-site
FAEM – 50% water recycling	50% of wastewater recycled on-site
Sensitivity analysis 2: Finnish hydropower energy (FHE)	
Name of analysis	**Changed parameter**
FHE – wind	100% wind energy
FHE – nuclear	100% nuclear energy
FHE – solar (MR)	Production location changed to Morocco using PV cells to produce the required electricity
FHE – geothermal (IS)	Production location changed to Iceland using geothermal to produce the required electricity

4.3.2 DATA COLLECTION

The primary data for the LCA was received from the company Solar Foods Oy. Solar Foods developed the bacterial strain used in the production of MP and made data available on the inputs and outputs of a pilot scale production process (see SI of article II). The production of MP in this facility required the supply of oxygen and hydrogen through electrolysis, and CO_2 gases captured from the air to start the fermentation process. In addition, the cultivation is supplied continuously with water-based liquid minerals through filter sterilization. This mineral liquid contains a mixture of ammonium (for nitrogen) and inorganic salts made-up of sulfur, phosphorus, magnesium, sodium, potassium, iron, and calcium. The mineral liquid also contains traces of manganese, zinc, vanadium, boron, molybdenum, cobalt, nickel, and copper. The pH of the system is controlled by adding phosphoric acid (H_3PO_4) and sodium hydroxide (NaOH). Cleaning-in-place (CIP) of the bioreactors took place four times per year. The given inputs and outputs were measured and/or calculated through mass and energy balances.

Other data used in the LCA was collected from experts and through literature review. The ecoinvent 3 database was used to model background data. Data on the inputs and outputs of wastewater treatment in the centralized WWTP was taken from the Helsinki Region Environmental Services (HSY, 2019).

4.4 ARTICLE III: METHOD, DATA AND SYSTEM DESCRIPTION

4.4.1 MODEL AND RELATED ASSUMPTIONS

The goal of the study performed in article III was to quantify the expected environmental burden of Tr-OVA production on an industrial scale using data from a pilot study. An anticipatory LCA was conducted to consider uncertainties related to the model. The benefit of an anticipatory LCA is that it allows for the inclusion of an extensive amount of sensitivity analyses, as well as the use of large uncertainty ranges as data originating from a commercial scale production system was not yet available. The techno-economic assessment (TEA) of Tr-OVA produced by VTT was consulted in order to identify steps in the production process that could have a substantial effect on the environmental burdens resulting from its production. These steps were used as parameters for the sensitivity analyses. Details of the anticipatory LCA model and the sensitivity analyses can be found in article III.

The production and fermentation process of Tr-OVA was conducted in a pilot-scale project at the VTT during 2018-2019. The engineered *T. reesei* fungus was nourished in a bioreactor with glucose, water, and a salt mix containing magnesium sulfate, calcium chloride, monopotassium phosphate (MKP), ammonia sulfate, iron sulfate, manganese sulfate, zinc sulfate, and cobalt chloride. An antifoaming agent was added, and ammonia water for pH control. Bioreactors required up to 50 rounds of CIP per year. The resulting product is a protein powder containing 8% moisture and 92% protein. The protein powder contains the same functional properties as protein from egg white powder (Voutilainen et al., 2021).

Like MP production, the production of Tr-OVA requires a relatively high amount of industrial energy in comparison to protein sources coming from an agricultural field. The choice of electricity source was therefore also in this study of importance when considering the environmental impact of its production. Four different scenarios were made using the average electricity mix of Germany (DE), Poland (PL), and Finland (FI) and a low-carbon Finnish electricity mix (FI – LC). These country mixes were chosen based on their stepwise levels of carbon-intensity per kWh produced, where Poland has a carbon intensity of 911 g CO_2 eq kWh^{-1}, Germany of 588 g CO_2 eq kWh^{-1}, Finland of 204 g CO_2 eq kWh^{-1}, and the Finnish low-carbon mix of 50 g CO_2 eq kWh^{-1} (Moro & Lonza, 2018).

LCA of Tr-OVA was performed using the Simapro 9.1.0.11 PhD software package (PRé Consultants, 2020). Uncertainty ranges were based on a uniform distribution of inputs with a ± 20% margin. The environmental impacts included in the study were GWP (kg CO_2 eq), land use (m^2a crop eq), water scarcity (m^3), freshwater and marine eutrophication potential (kg P-eq; kg N-eq), terrestrial acidification (kg SO_2 eq), ionizing radiation (kBq Co_{60} eq), human carcinogenic and non-carcinogenic toxicity (kg 1,4-DCB; kg 1,4-DCB), stratospheric ozone depletion (kg CFC_{11} eq) and the CED (MJ) using the ReCiPe 2016 Midpoint (H) method, the AWARE method, and the CED V1.1 method by ecoinvent (Althaus et al., 2007; Boulay et al., 2018; Huijbregts et al., 2017).

4.4.2 COMPARISON WITH CHICKEN-BASED EGG WHITE PROTEIN POWDER

To understand how environmental impacts related to the production of ovalbumin would change when using fungi rather than chickens, a model for chicken-based egg white protein powder was created. The model was based on the model described in an article by Tsai et al. (2020) on the production of egg yolk powder. Uncertainties in the chicken-based egg white protein powder model were generated using the pedigree method as no original uncertainty ranges were given by the authors. Protein powder produced from chicken eggs is part of a multifunction product system with multiple outputs. A 55% allocation factor was applied to egg whites based on the model of Tsai et al. (2020).

The two products were compared on a protein basis as the functionality of egg white powder is often defined in terms of protein quality. One example is the use of egg white powder for the whisking ability adding structure to cakes. The protein content of chicken-based egg white protein powder is 79.8% (USDA, 2019b).

The significance of the differences between the environmental impacts of Tr-OVA and chicken-based egg white protein powder was tested using dependent modified null hypothesis significance testing (NHST) (Heijungs, 2021). The uncertainty ranges from the MC analyses were used for this purpose. A seed value of zero was applied to all MC analyses to simulate artificial dependency between the two models, which is necessary to perform statistical tests and to account for common uncertainties between the Tr-OVA and chicken-based egg white powder model (Mendoza Beltran et al., 2018).

The tested null hypothesis was that H_0: $S_{i,j,k} \leq \delta_0$, where S refers to the standardized difference of means, i and j refer to Tr-OVA and chicken-based egg white protein powder, and k refers to the impact. We used a difference threshold δ_0 of 0.2 and a significance level α of 0.05. A one-sided (right) cumulative distribution function was used to calculate the P value (Heijungs, 2021; Mendoza Beltran et al., 2018). A discernibility test was also conducted to explore the extent of the differences in environmental impacts resulting from the MC runs of the two product alternatives (Mendoza Beltran et al., 2018).

4.4.3 DATA COLLECTION

Data for the Tr-OVA model was adopted from a pilot study and the TEA of Tr-OVA produced by VTT (Voutilainen et al., 2021) and was used to estimate the environmental burden of of an assumed 100,000 kg annual industrial production scale. The industrial requirements of the processes were validated using an energy and material balance. Background data was taken from the ecoinvent database 3.6, cut-off system. Proxies were used for those minerals that could not be found from this database, using expert opinions on similarities of properties of functions. Inputs were adjusted to reflect the respective country location of each scenario as much as possible. These included water use, electricity mix, and the natural gas production mix. Emissions from the combustion of natural gas during the drying phase were calculated using the emissions factors published by the IPCC (IPCC, 2006b; Simmons, 2000). Inputs and outputs required for the CIP process were calculated based on the article by Eide et al. (2003).

The World Food LCA Database (WFLDB) was used to model chicken-based egg white protein powder as it included data on egg production in Germany and Poland. However, Finland is not listed in the database and the FI and FI – LC scenarios were consequently not considered in the comparison. As the WFLDB relies on the ecoinvent v.3.5 cut-off system in its background model, system boundaries should be similar between the chicken-based egg white protein powder model and the Tr-OVA model that was also build using ecoinvent for background processes. However, one major difference between the WFLDB and ecoinvent databases was that the former includes emissions from LULUC, while the latter does not. This is especially important for aquacultural and agricultural products as they generally require large amounts of land per output (Poore & Nemecek, 2018b). In light of this, the WFLDB was used to model glucose into the Tr-OVA model, in order to align the system boundaries of the background data.

4.5 COMPARISON BETWEEN PROTEIN-RICH FOOD AND FEED INGREDIENTS

4.5.1 COMPARISON ON A PROTEIN CONTENT BASIS

As all the products studied in articles I, II, and III have a relatively high protein content, the results of the environmental impact of MP production (both the FAEM and the FHE scenario), Tr-OVA (PL and FI – LC) and shrimp (mixed mangrove concurrent and intense farming) were compared to that of alternative food and feed protein sources. The production models selected for each of the three products were chosen because they represented the highest and lowest scores in terms of GWP. The environmental impacts of the other food and feed protein sources were taken from existing literature. These were protein-rich food products, taken from the article by Poore and Nemecek (2018b) and feed ingredients taken from the article by Smetana et al. (2019). In addition, the results on mycoprotein presented by Smetana et al. (2015) and the GWP results of MP taken from Sillman et al. (2020) were included in the comparison. The system boundaries used in all alternative products taken from literature were adapted to match the cradle-to-factory/farm gate approach used in the studies presented in the three articles of this dissertation. As article III only presents GHG emissions for shrimp farming, the comparison of shrimp to other protein sources was limited to GWP.

The environmental impacts for each product were calculated using a FU of 100 g protein. The reason for this is that protein intake plays an essential role in a healthy diet with a minimum requirement per day (Phillips, 2017). Additionally, protein content is a commonly used FU applied in LCA studies and allows for a comparison between products that otherwise have a distinctively different nutritional profile (Poore & Nemecek, 2018b). Because of the different nutritional profiles of the different protein-rich food and feed ingredients, there is still debate among LCA practitioners regarding the optimal FU to employ when comparing different products (Saarinen et al., 2017). Consequently, an additional comparison was made within this dissertation, based on an imaginary patty prepared with protein originating from shrimp, MP, or Tr-OVA.

To compare the environmental impacts of shrimp, MP, and Tr-OVA with the protein-rich products from the aforementioned studies, the impacts of shrimp, MP, and Tr-OVA were recalculated using the same LCIA method that was used by Poore and Nemecek (2018b) and Smetana et al. (2019). This was done to avoid differences in results caused by the application of the different impact factors used in the different life cycle impact assessment (LCIA) methods. Poore and Nemecek (2018b) applied the IPCC 2013 method that includes CC feedbacks, to estimate GWP, land use was calculated based on the sum of all land required in m^2 per FU, acidifying and eutrophying emissions were calculated using the CML2 baseline method, and the impact of water use was estimated using the AWARE method. The results for different feed options and mycoprotein by Smetana (2015, 2019) were calculated using the IMPACT 2002+ method.

4.5.2 COMPARISON ON A PATTY BASIS

The three products studied within this dissertation have a relatively high protein content and are considered protein-rich ingredients that can be used to constitute a meal. For this reason the FU in this analysis was a patty, in line with the idea that each ingredient is seldom eaten in isolation, but rather as part of a meal (Saarinen et al., 2012). Three types of patties with different protein sources were considered in the comparison: a patty made with shrimp, a patty made with MP, and a patty made with Tr-OVA. The average Finnish electricity mix was used in both the MP (FAEM scenario) and Tr-OVA (FI scenario) patty.

The patty recipe was adapted from two different recipes for patties that can be prepared at home by the consumer, using one of the three protein sources (Evans, 2021; Thomas, 2018). The ingredients list of the patties is reported in Table 4. The amount of the ingredients was altered for each patty in order to match the macronutrient content. Data from the USDA FoodData Central database was used to identify the nutritional content of the ingredients (e.g. USDA, 2019a). The nutritional content of microbial protein and ovalbumin was taken from the organizations that developed the products, Solar Foods Oy and VTT respectively. Spices were not included in the analysis for two reasons: they are a minor part of the patty in weight, and it was assumed that they would be similar in each patty.

Both MP and Tr-OVA can be added as is to the patty recipe. Harvested shrimp need to be processed. It was assumed that the shrimp were shelled and cleaned by hand by the consumer when preparing the ingredients. It was further assumed that edible shrimp lose 34% of their weight compared to live shrimp at the farm, as both the head and shell are removed (Louisiana Direct Seafood, 2011; Zirlotts Gulf Products, 2013). The protein content of the remaining product was assumed to be 20.1% (USDA, 2019a), while MP powder had a protein content of 65% and Tr-OVA powder 92%. The impacts of packaging or cooking were not considered.

The patties were modelled with Simapro 9.2.0.2 using data from ecoinvent 3.7.1 and the WFLDB 3.5 that uses the ecoinvent 3.5 database in background processes (i.e., the process for electricity production). The ReCiPe 2016 v1.05 impact factors were used to calculate the environmental impacts of each patty. Since only GHG emissions were considered for the case study on shrimp farming in mangrove forests, the comparison was restricted to GWP.

Table 4: Ingredient list for the different patty options and macronutrients of each patty based on data from the USDA (e.g. USDA, 2019a).

Ingredients (grams)	Shrimp patty	Microbial protein patty	Ovalbumin patty
Shrimp	140	-	-
Microbial protein	-	44	-
Ovalbumin using *T. reesei*	-	-	32
Bread, white	12.7	12.7	12.7
Onion	5.8	5.8	5.8
Breadcrumbs	13.2	13.2	13.2
Vegetable oil	14.3	12.5	14.3
Cooked kidney beans[1]	20	20	20
Sweet potato[2]	50	35	50
Water	-	25	25
Total weight	241	168	173
Nutritional content			
Energy (Kcal)	390.7	402.9	397.8
Protein (grams)	33.6	35.2	34.1
Fat (grams)	16.3	15.6	16.5
Carbohydrates (grams)	28.1	31.2	29.1
Fiber (grams)	3.6	4.1	3.6

1 Modelled as fava beans due to a lack of data availability in the databases. Fava beans were chosen as the Agribalyse database also modelled kidney beans using fava beans as a proxy.

2 Modelled using potato as a proxy.

5. RESULTS

5.1 RESULTS OF ARTICLE I

5.1.1 ESTIMATES OF MANGROVE CARBON STOCKS AND GHG EMISSIONS FROM MANGROVE DEFORESTATION

The results of the literature review on global average carbon stocks in mangrove forests are displayed in Table 5. Based on these values and a 20-year annualization period, as recommended by IPCC guidelines, deforestation of mangrove forests would lead to 129 t of CO_2 emissions per hectare per year (CV = 0.441, ln). Using a 50-year annualization period, based on the expected lifetime of a shrimp farm, lowers these emissions to 54 t CO_2 per hectare per year (CV = 0.424, ln). CH_4 emissions from aquaculture in mangrove areas were estimated as 533 kg CH_4 per hectare per year (CV = 0.4, ln), following the assumption of Astudillo et al. (2015). Despite the knowledge that most mangrove shrimp farming systems generally remove N from the atmosphere (Jonell & Henriksson, 2015), estimates of N emissions were conservatively set to 1.67 kg N_2O per hectare per year (CV = 0.575, ln), in line with the estimates from Allen et al. (2012).

Table 5: Literature review results of global average mangrove carbon stocks (adapted from article I)

Reference	Parameter	Unit	Median	CV (distribution)	Range	N
AG	Above-ground C stock[a]	t C ha^{-1}	131	0.462 (ln)	49.5–261	9
BG	Below-ground C stock[b]	t C ha^{-1}	80	1.525 (ln)	9.61–410	8
S	Soil C stock per 1.5 m of depth[c]	t C ha^{-1}	724	0.595 (ln)	186.15–1575	8
L	Litter C stocks[d]	t C ha^{-1}	4.03	0.477 (n)	0.15–7	12
CS	Missed potential C sequestration[e]	t C ha^{-1} yr^{-1}	1.25	0.936 (ln)	0.012–3.53	8

Distributions: *ln* = lognormal distribution, *n* = normal distribution

[a] Twilley et al. (1992); Eong (1993); Matsui (1998); Kauffman et al. (2011); Donato et al. (2011); Ray et al. (2011); Donato et al. (2012)

[b] Komiyama et al. (1987); Twilley et al. (1992); Matsui (1998); Kauffman et al. (2011); Ray et al. (2011); Donato et al. (2012)

[c] Eong (1993); Matsui (1998); Kauffman et al. (2011); Ray et al. (2011); Donato et al. (2012); Lundstrum and Chen (2014) "awareness of the high carbon (C"

[d] Twilley et al. (1992); Amarasinghe and Balasubramaniam (1992); Eong (1993); Day et al. (1996); Middleton and McKee (2001); Jennerjahn and Ittekkot (2004); Guzman et al. (2005); Ray et al. (2011)

[e] Twilley et al. (1992); Eong (1993); Duarte and Cabrián (1996); Chmura et al. (2003); Alongi (2008); Sanders et al. (2010); Ray et al. (2011); Mcleod et al. (2011)

Note: CV - coefficient of variation; N - number of observations.

5.1.2 ESTIMATED GHG EMISSIONS FROM LULUC OF SHRIMP FARMING

Analysis of historical data on land use identified only mixed mangrove concurrent shrimp farms as being located in areas previously occupied by pristine mangrove forests. The resulting LULUC emissions are shown in Table 6.

Table 6: GHG emissions from LULUC in mangrove areas due to mixed mangrove concurrent shrimp farming, annualized over 50 years, in t CO_2 equivalent ha^{-1} yr^{-1} (adapted from article I).

Reference	$\Delta\beta_{AG}$	$\Delta\beta_{BG}$	$\Delta\beta_{L}$	$\Delta\beta\delta_{S}$	$\Delta\theta_{MP}$	Total t CO_2	CH_4 emissions	N_2O emissions	Total GHG emissions
Average (t CO_2-eq ha^{-1} yr^{-1})	9.6	5.9	0.3	26.5	4.6	46.9	14.9	0.4	62.2
CV (t CO_2-eq ha^{-1} yr^{-1})	0.467	1.503	0.268	0.601	0.903	0.409	0.400	0.575	-

The average mixed mangrove concurrent farm produced about 6.5 t of live shrimp ha^{-1} over a period of 50 years. Other shrimp species and mud crabs were also produced in the same farm (Jonell & Henriksson, 2015; Vu et al., 2013) making up 60.8% of the total harvest by weight and 40.3% by value. Using mass allocation, the estimated GHG emissions from LULUC for the *Penaeus monodon* harvest resulted in an estimated 184 t CO_2-eq t^{-1} live shrimp at farm gate and 282 t CO_2-eq t^{-1} live shrimp based on economic allocation. Although these are high emissions per t of shrimp, mixed mangrove concurrent farms are the only shrimp farming practice associated with this particular source of emissions and make up less than 5% of all shrimp production in Vietnam. Globally, only 1.2% of shrimps originate from these type of farms (FAO - Fisheries division, 2021; Jonell & Henriksson, 2015). An additional analysis on the mangrove cover remaining within the property area of 25 mixed mangrove concurrent farms showed that 39% (CV = 0.322, range 16–69%) of the original mangrove forest remains standing.

To compare the results of the LULUC emission per t of live shrimp coming from the mixed mangrove concurrent system, a comparison was made with emissions coming from the intense shrimp farming systems analyzed in an article by Henriksson et al. (2015). This study analyzed the semi-intense and intense systems of the Mekong Delta. Given that neither of the other shrimp farming practices are in previous mangrove areas, the LULUC emissions of these systems are relatively small. The results of this study are presented in Table 7. The results show the clear impact of LULUC-related GHG emissions from mangrove forests on the overall GWP of shrimp production.

Table 7: Comparison of GHG emissions, annualized over 50 years, from three shrimp farming systems found in the Mekong delta of Vietnam (adapted from article I.)

System	Allocation factor	Prior land use	t shrimp ha^{-1} water surface area $year^{-1}$	LULUC t CO_2 t^{-1} shrimp	LU CH_4 emissions, t CO_2-eq t^{-1} shrimp	LU N_2O emissions, kg CO_2-eq t^{-1} shrimp	Lifecycle emissions, t CO_2-eq t^{-1} shrimp[a]	Total, CO_2-eq t^{-1} shrimp
Mixed mangrove	Mass (38.5%)	Mangrove	0.13	139	44.2	1.31	minimal	184
Semi-intensive	Mass (100%)	Aquaculture pond	6.6		2.3	Included in LCA	13.2	15.5
Semi-intensive	Mass (100%)	Rice paddy	6.6	2.4	2.3	Included in LCA	13.2	21.5
Intensive	Mass (100%)	Aquaculture pond	7.6		2.0	Included in LCA	13.2	15.2
Intensive	Mass (100%)	Rice paddy	7.6	2.1	2.0	Included in LCA	13.2	20.4
Mixed mangrove	Eco (58.8%)	Mangrove	0.13	212	67.5	2	minimal	282
Semi-intensive	Eco (100%)	Aquaculture pond	6.6		2.3	Included in LCA	4.7	7.0
Semi-intensive	Eco (100%)	Rice paddy	6.6	2.4	2.3	Included in LCA	4.7	13.0
Intensive	Eco (100%)	Aquaculture pond	7.6		2.0	Included in LCA	5.1	7.1
Intensive	Eco (100%)	Rice paddy	7.6	2.1	2.0	Included in LCA	5.1	12.3

[a] Values taken from Henriksson et al. (2015)

5.2 RESULTS OF ARTICLE II – ENVIRONMENTAL IMPACTS OF MP PRODUCTION

5.2.1 MAIN RESULTS FOR MP PRODUCTION

Article II focused on quantifying the environmental impacts of MP production using LCA. Figure 2 shows the results per kg of MP for both the FAEM and FHE scenario. Negative MC results for this production process were ignored as they would intuitively be illogical and can be explained by the computational manner of the MC leading to a potential flip of positive to negative values and vice versa, as explained by Henriksson et al. (2015).

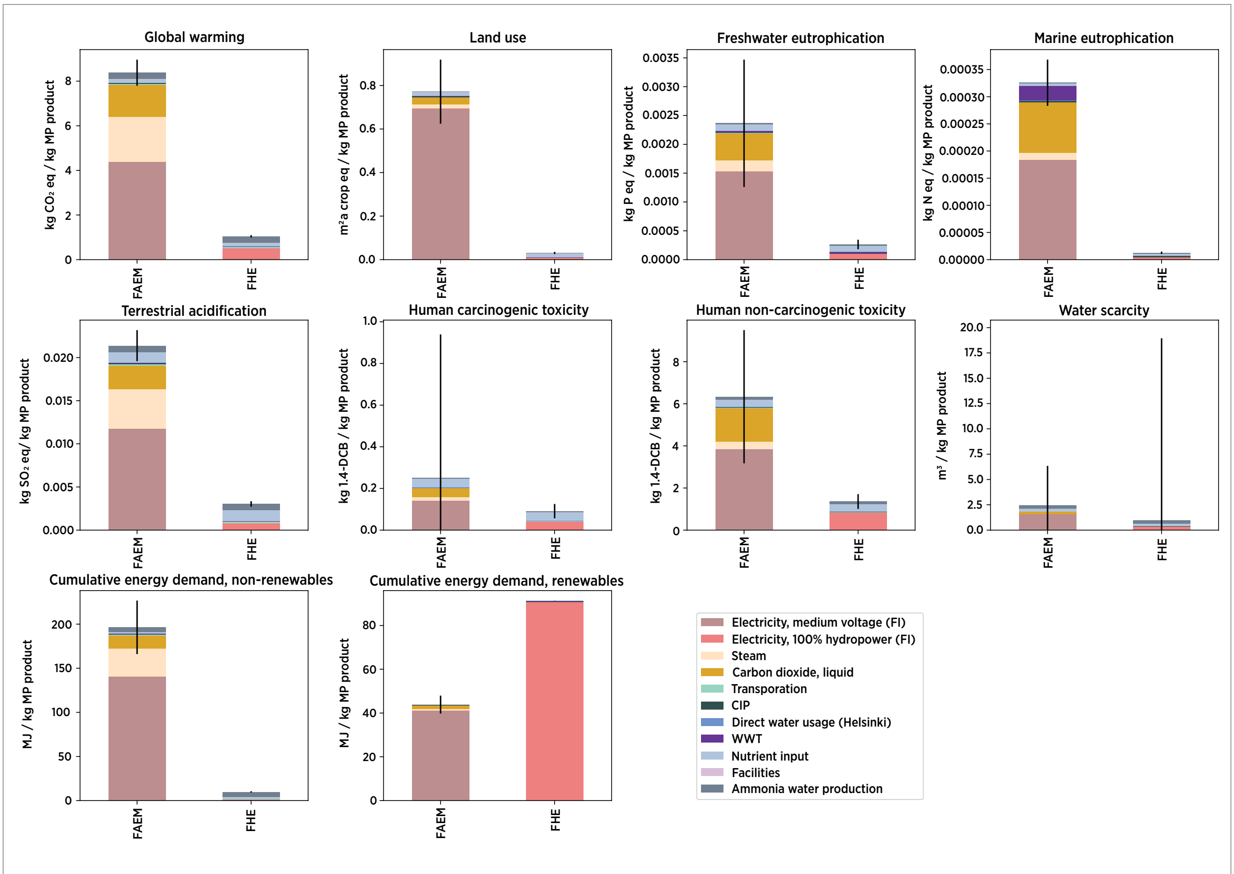


Figure 2: Results of the environmental impacts of 1 kg of microbial protein (MP) for the Finnish average electricity mix (FAEM) and the Finnish hydropower energy (FHE) scenarios, with black error bars representing standard deviations from the Monte Carlo (MC) analysis. Note: FI – Finland, CIP – cleaning-in-place, WWT – wastewater treatment. (Adapted from article II)

The results in Figure 2 show a substantial reduction in environmental impacts when using the FHE scenario for MP production compared to the FAEM scenario. For example, land use was 25 times higher for MP production in the FAEM scenario, and marine eutrophication for MP produced in the FHE scenario was just 4% of that produced in the FAEM scenario. The smallest difference between the environmental impacts of the two scenarios was in water scarcity impacts. Water scarcity for MP production in the FAEM scenario was 2.5 times higher than in the FHE. However, the results of the uncertainty analyses on water scarcity showed high uncertainty ranges. Even after the bootstrapping method, standard deviations were 4 m^3 for the FAEM and 16.9 m^3 for the FHE scenario. This indicates that the impacts of water use in the FHE scenario could potentially be higher than in the FAEM scenario.

As shown from the contributions in the figure, a switch in electricity source from the average Finnish electricity mix to hydropower explains most of the differences in environmental impacts between the two scenarios. The environmental impact of hydropower is much smaller than that of the average Finnish electricity mix. Although hydropower makes up about 16% of the Finnish average mix (Treyer, 2014), other sources in the Finnish mix, such as coal (5.0%) and imported Russian electricity (7.5%) that is mainly produced using natural gas, increase the average environmental impacts of this mix. Most of the electricity consumed in the production process was used during fermentation and for the operation of the electrolyzer used to split water into hydrogen and oxygen.

Electricity consumption contributed the most to the environmental impacts caused by the production of MP, except for terrestrial acidification in the FHE scenario that was mainly caused by the input of nutrients. In the FAEM scenario, electricity consumption was responsible for 52.3-93.7% of all environmental impacts while this fell to 13.9-61.5% in the FHE scenario. Nutrient inputs were the second largest contributor with ranges between 1.3-16.6% in the FAEM scenario and 14-59.5% in the FHE scenario.

5.2.2 RESULTS OF THE SENSITIVITY ANALYSES

Sensitivity analyses were performed to analyze the differences observed in the environmental impacts of MP production when different production choices were made. A distinction was made between: 1) changing the assumptions of the FAEM scenario related to the input requirements, and; 2) the choice of renewable energy sources in the FHE scenario. The production location of MP was changed from Finland to Morocco and Iceland for the use of solar and geothermal energy, respectively, as these are not optimal renewable energy options for Finland. The results of the sensitivity analyses of MP production are shown in Figure 3.

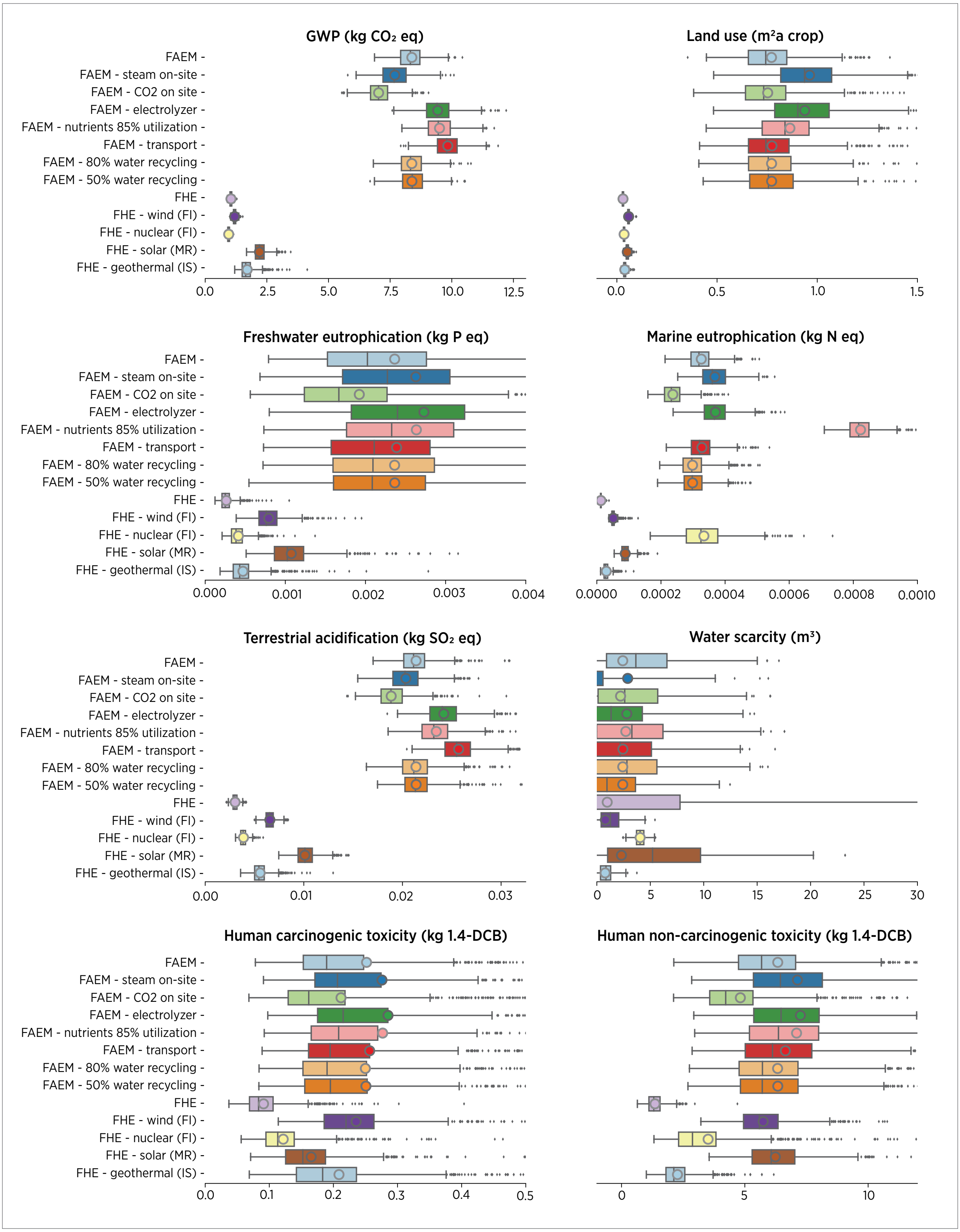


Figure 3: Results of sensitivity analyses per 1 kg of MP, in boxplot and whiskers, for both the Finnish average energy mix (FAEM) and Finnish hydropower energy (FHE) scenarios. Deterministic results are indicated by circles in the corresponding color and outliers with dots. Note: GWP – global warming potential, FI – Finland, MR – Morocco, IS – Iceland. (Adapted from article II)

Results for the FAEM scenario show that producing steam and CO_2 on-site reduced the GWP of MP production. This means that even when steam is produced using the average Finnish electricity mix, the GWP for steam production on-site is still smaller than when supplied externally. Using renewable energy sources further reduced the GWP for the steam and CO_2 requirements of the production process. This aligns with the results of the comparison between the FAEM and FHE scenarios presented in Figure 2. However, the production of steam on-site does require more land due to the high land use per kWh of the Finnish electricity mix in comparison to the land required for the external production of steam. The production of one kg of MP requires 5.65 kWh, which equates to 0.129 m^2a crop-eq versus 0.019 m^2a crop-eq for the supply of steam from an external source. Also, eutrophication potential and human toxicity levels were higher when steam was produced on-site. Switching to renewable energy could potentially lower these impacts. For CO_2, the production on-site resulted in a lower environmental impact for all categories, even when produced using the Finnish average electricity mix.

The sensitivity results furthermore showed that a decrease in the assumed efficiency of the electrolyzer resulted in a substantially higher environmental impact. This can be explained by the high contribution of electricity to the overall environmental impact of MP production. Approximately 74% of the electricity requirements of MP production in the FAEM scenario originate from the electrolyzer. This highlights the environmental potential of MP production when technological advancements increase the overall efficiency of the electrolyzer.

Results of the other sensitivity analyses also highlighted the importance of nutrient uptake efficiency in reducing marine eutrophication potential. This is because of the high level of nitrogen input required by the system. In cases of lower nutrient uptake, these nutrients leave the system in higher concentrations. Increased transportation distances, due to differences in the supply chain, mostly increased the GWP and terrestrial acidification. In fact, increased distances had the largest effect on both these impact categories of all the sensitivity analyses performed in this study. This can be explained by the reliance of MP production on industrial inputs in terms of mass that would then have to travel over a greater distance. The sensitivity results also showed that the potential recycling of wastewater on-site would have limited influence on the environmental impact of MP production.

The sensitivity results based on the FHE scenario show a potential to reduce the environmental impacts of MP production by changing the renewable electricity source when that source is appropriate to the location. The use of hydropower in Finland generally resulted in the smallest environmental impact in comparison to the other energy sources included in the analyses. However, all renewable energy options tested in the sensitivity analysis generally resulted in smaller environmental impacts than the FAEM scenario. This was mostly related to the use of the Finnish average electricity mix in the FAEM scenario. Land use requirements for MP production varied least between the different renewable energy sources. However, for other impact categories such as human toxicity and marine eutrophication, the choice of renewable energy source mattered more. This shows that the environmental impacts of MP production can be further reduced by optimizing production choices. However, trade-offs are visible between different sources of renewable energy.

5.3 RESULTS OF ARTICLE III – ENVIRONMENTAL IMPACTS OF TR-OVA PRODUCTION

5.3.1 MAIN RESULTS FOR TR-OVA

The environmental impacts of Tr-OVA production for the FI, DE, PL, FI – LC scenarios are presented in Figure 4. The results showed that the FI - LC scenario caused the least amount of environmental impact, except for water scarcity and ionizing radiation from the increased use of nuclear energy within the electricity mix. This suggests that using low-carbon energy sources would lower the overall environmental impact of Tr-OVA production. The extent of the potential reduction varied per impact category and some minor trade-offs were detected, such as for ionizing radiation. However, uncertainty ranges for the results on ionizing radiation were large thereby reducing the certainty of conclusions that could be drawn based on the deterministic results. This was also the case for several other impact categories as shown in Figure 4.

Based on the comparison of the scenarios, the production of Tr-OVA in Poland would generally lead to the highest environmental burden, explained by the relatively high reliance on fossil fuels such as coal (43.5%) within the country's average electricity mix (Treyer, 2021). Due to the relatively high input of electricity to the production of Tr-OVA, the difference in carbon intensity of the different electricity source is well reflected in the difference in the contribution to GWP: the FI – LC scenario's GWP is only 56.4% of that of the PL scenario.

The contribution analysis showed that glucose appears to contribute the most to many of the environmental impact categories considered. In the FI scenario, 86% of the land requirements were due to glucose production; 81.2% of all required land was used to grow corn outside of Finland. In addition, 62.5% of the impact from water use and 52.8% of marine eutrophication were attributed to corn production. However, electricity consumption was the largest contributor to freshwater eutrophication in the PL and DE scenarios (48% and 44% respectively) followed by CIP (34% and 32% respectively). The antifoaming agent was mainly responsible for stratospheric ozone depletion.

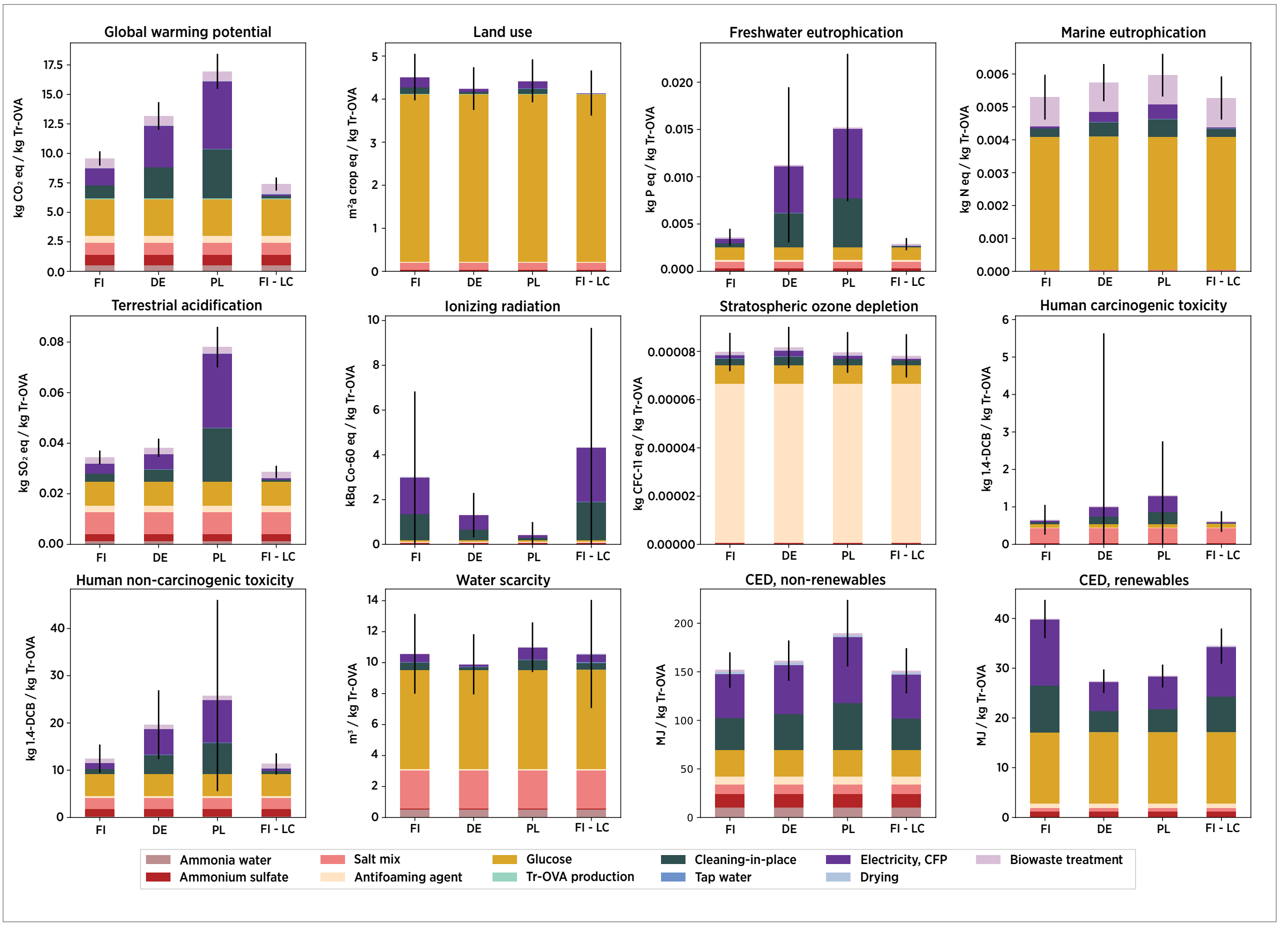


Figure 4: Deterministic results of 1 kg of ovalbumin produced using the fungi *T. reesei* (Tr-OVA) in the Finland (FI), Germany (DE), Poland (PL) and low carbon energy sources in Finland (FI-LC) scenarios. Standard deviations are displayed with black vertical lines based on the MC runs (n=100). Note: Tr-OVA production refers to direct emissions and land use taking place at the production site of Tr-OVA; CFP – cultivation, filtration and purification. (adapted from article III)

5.3.2 RESULTS COMPARISON BETWEEN TR-OVA AND CHICKEN-BASED EGG WHITE PROTEIN

The results presented in Figure 5 show that most of the environmental impacts of Tr-OVA were smaller than those of the chicken-based alternative. Despite relatively high uncertainty ranges for some impact categories, the dependent NHST led to the rejection of the null hypothesis for all alternatives and impact categories. This meant that the differences between the environmental impacts of Tr-OVA and chicken-based egg white protein powder were significant for each impact category. Those categories where Tr-OVA resulted in a larger environmental impact were mostly explained by the relatively high reliance on electricity to produce Tr-OVA compared to chicken-based egg white protein powder.

The difference in environmental impacts between the production of ovalbumin using fungi or chicken reflects the differences between the two production processes. The production process of chicken-based egg white protein powder is almost completely dependent on agricultural inputs, which results in high scores in the environmental impact categories typically associated with agricultural production, such as GWP, land use, and water use. The industrial production of Tr-OVA results in higher impacts in categories that are associated with the electricity consumption of Tr-OVA production, such as ionizing radiation and human toxicity.

Like the production of chicken-based egg white protein powder, Tr-OVA production depends on agricultural inputs. However, its production process seems to be more efficient in the use of agricultural products than egg white protein from chickens: 1 kg of Tr-OVA protein production only required 2.5 kg of glucose while agricultural inputs in the form of feed for chicken-based egg white protein powder were 27.5 kg. This is only partly compensated by the input of minerals in the salt mix and nitrogen in the production of Tr-OVA, with a combined total of 2.04 kg of salt mix per kg of protein produced. Most of the environmental impact from the minerals came from production of MKP. The salt mix contains 41% MKP by weight but, due to data limitations, this was modelled using sodium phosphate as a proxy.

The results of the model for chicken-based egg white protein powder show that the contribution of the processing of eggs to powder was generally minimal. Its impacts ranged from 0.1-22%, depending on the country and impact category. This means that the assumptions related to the production of chicken eggs, and especially feed production, are most essential in understanding the environmental impacts of chicken-based egg white protein powder production and building a reliable model for comparison with Tr-OVA production.

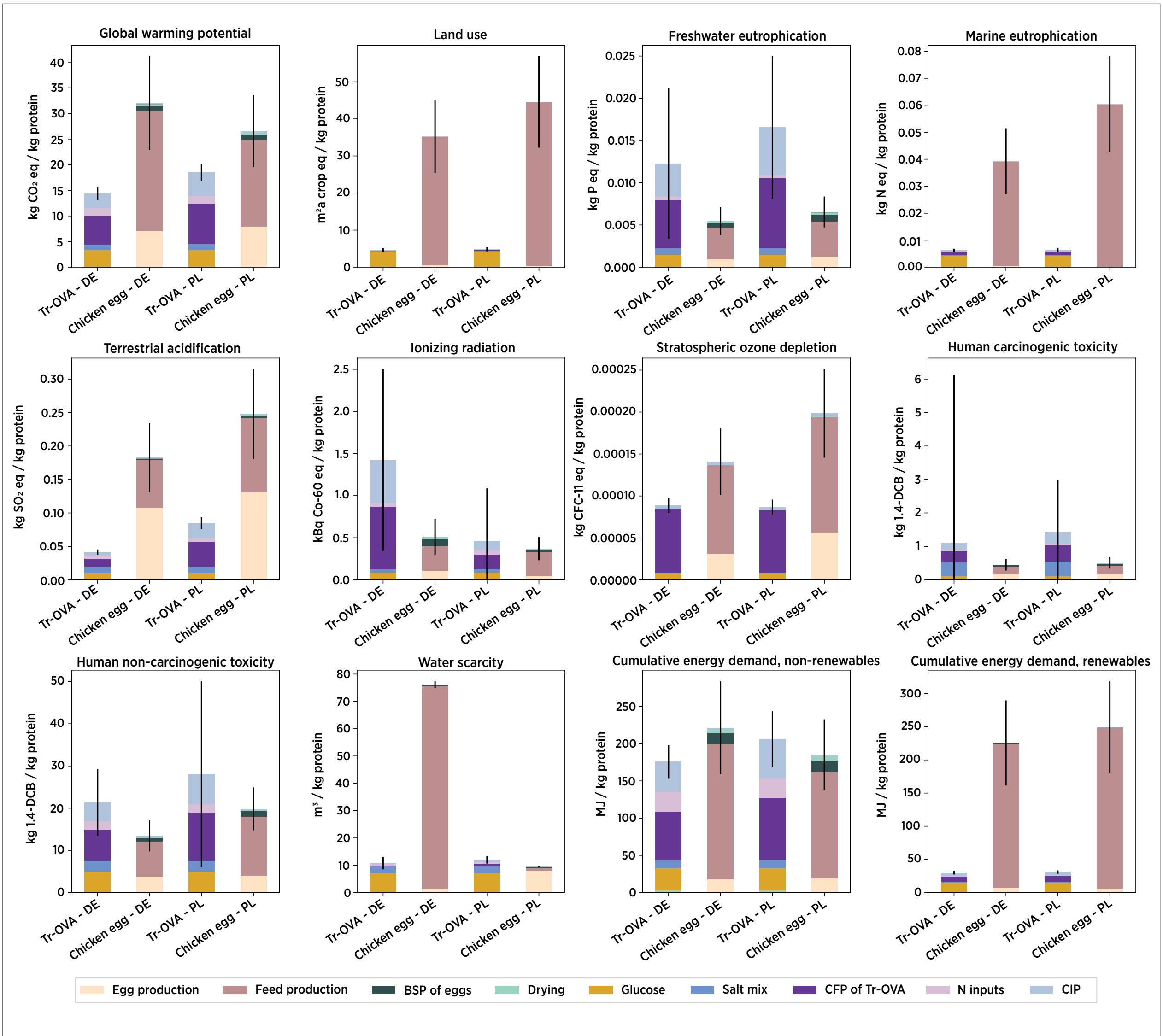


Figure 5: Deterministic results per kg of protein in the comparison of Tr-OVA produced in Germany (DE) and Poland (PL) with chicken-based egg white protein powder produced in Germany and Poland. Standard deviations are given with black vertical lines and are based on the MC analysis (n = 100). (Adapted from article III)

5.3.3 RESULTS OF THE SENSITIVITY RESULTS FOR TR-OVA PRODUCED IN FINLAND

Sensitivity analyses were performed using the FI scenario as a baseline, which included the use of the average Finnish electricity mix. They are displayed in Figure 6. The results show that the environmental impacts of Tr-OVA production decrease most when using the fungal biomass as a by-product and applying a protein-based allocation factor. The minimum product sales price (MPSP)-based allocation factor did not seem to substantially reduce the environmental impact for Tr-OVA production. This was because the allocation factor for MPSP was just over 5% for the fungal biomass and 33.8% when applying the protein-based allocation method.

The other methodological choice that led to a noticeable difference in environmental impact results was the choice of the background data source for glucose production. The original model used the WFLDB for the modelling of glucose because it included emissions from land-use change while the ecoinvent database did not. Because the WFLDB was also used in the model of chicken-based egg white protein powder, the system boundaries of the two product systems remained aligned. Additionally, the results of article I highlighted the potential role of GHG emissions from LULUC in comparison to other life cycle emissions. Nonetheless, excluding LULUC-related emissions associated with glucose production by using the ecoinvent database did not lead to a substantial difference in the GWP of Tr-OVA production. However, using the ecoinvent database rather than the WFLDB did lead to a substantially smaller land use impact and larger terrestrial acidification. This difference highlights the importance in aligning system boundaries when comparing two different product systems.

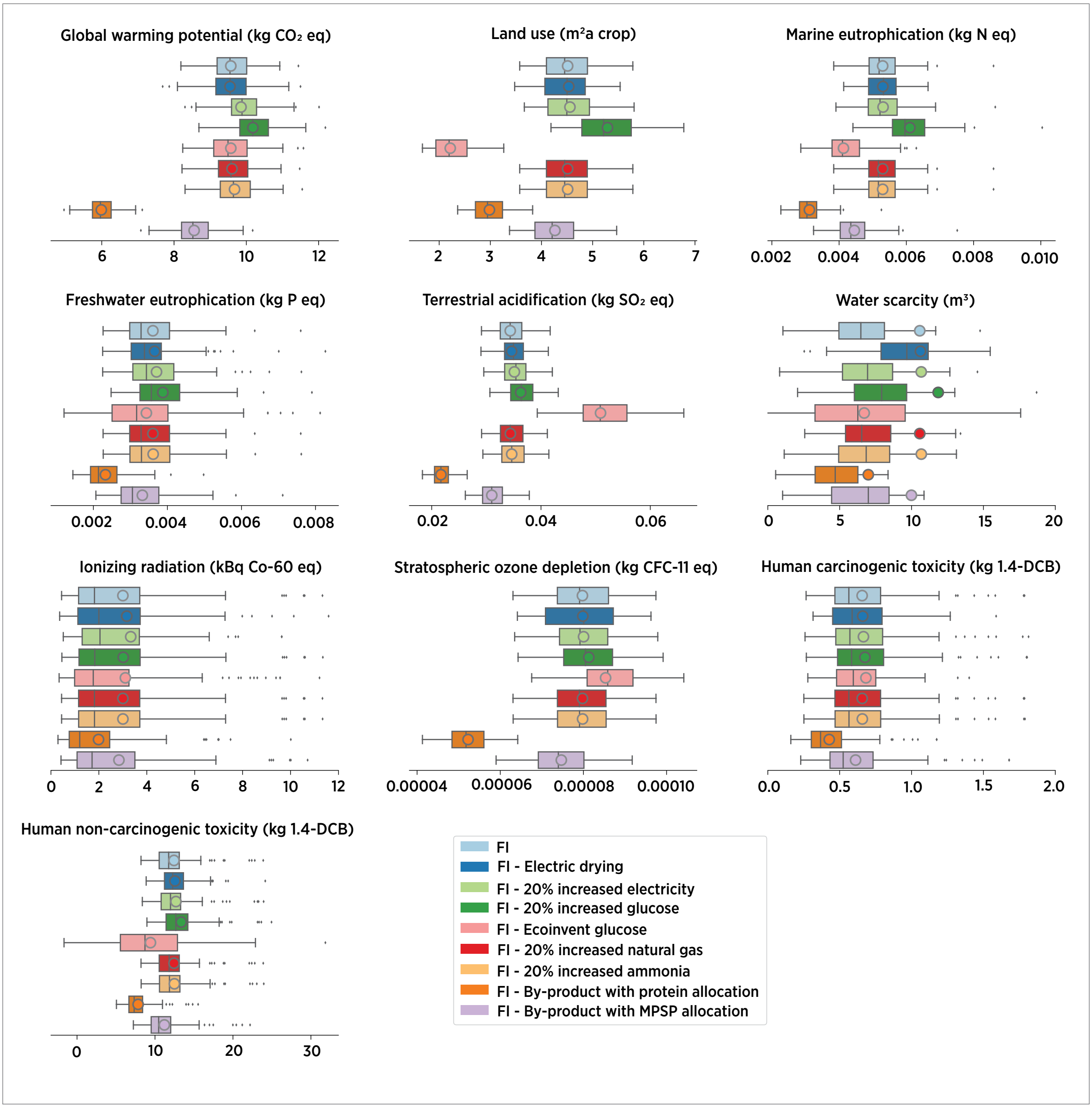


Figure 6: Sensitivity results for 1kg of Tr-OVA produced in the Finnish (FI) scenario. Uncertainty ranges of each sensitivity test are displayed with box and whiskers (0th, 25th, 50th, 75th and 100th percentile) based on the MC method (n = 100) while deterministic results are indicated with a circle in the corresponding color. (Adapted from article III)

5.4 RESULTS OF THE COMPARISON BETWEEN THE THREE PRODUCT OPTIONS

5.4.1 COMPARISON ON THE BASIS OF THE PRODUCTS' PROTEIN CONTENT

Figure 7 shows a comparison of the environmental impacts of different protein sources for livestock feed. It shows that the GWP of MP and Tr-OVA are relatively similar to the other protein-rich feed ingredients. However, only rapeseed cake and soybean meal have a lower GWP than MP and Tr-OVA. The land use requirement of MP was the smallest. Protein from microalgae resulted in the highest GWP and protein from egg protein concentrate resulted in the greatest land use requirements, as calculated by Smetana et al. (2019).

Tr-OVA production generally has a higher environmental impact than that of MP, suggesting that MP would be preferred over Tr-OVA when it comes to delivering protein to animal feed mix. For example, Tr-OVA production results in the highest ozone depletion potential of all the feed protein options compared due to the use of the anti-foaming agent polydimethylsiloxane. In addition, the impact of water use is relatively large for Tr-OVA production with only whey concentrate resulting in a higher score. However, (marine) eutrophication of Tr-OVA is relatively low.

Switching electricity sources from a Polish mix to a low-carbon Finnish mix for Tr-OVA production did not seem to make a large difference in the overall comparison of Tr-OVA with other protein-rich feed options. This was different for the scenarios used in MP production in which more than just the electricity sources were changed, such as the electrification of the process inputs and recycling of water. The reduction in the GWP of MP when changing assumptions about the production process made the product a more environmentally competitive protein-rich feed ingredient than other protein alternatives. A large decrease in the impact of water use could also be seen. However, the impact of water use, and especially overall energy demand, was relatively high due to the industrial nature of the product in comparison to other products that all to some extend depend on agricultural inputs.

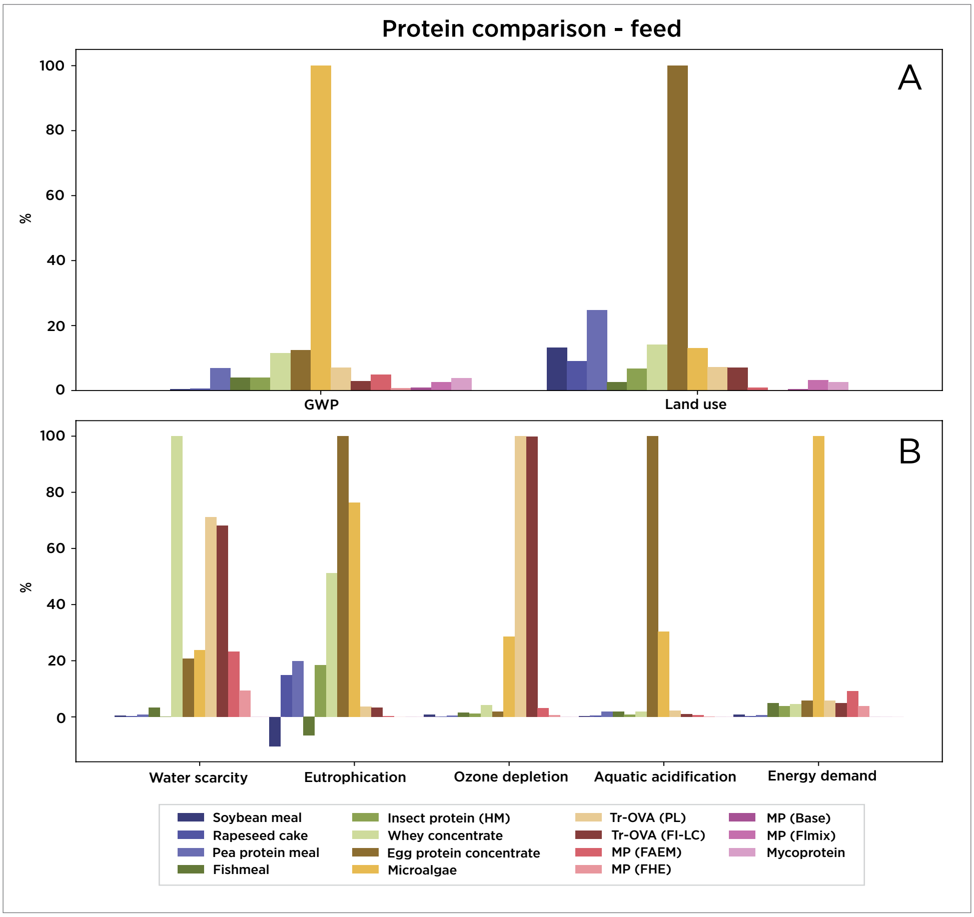


Figure 7: Environmental impacts of the different protein sources that can be used as feed for livestock calculated for a functional unit of 100g of protein. Data on alternative protein sources was based on Table 2A from Smetana et al. (2019) associated with upstream increase in feed (increase in commercial feed production and the article by Sillman et al.(2020). Figure A displays all product alternatives, while MP (Base), MP (Flmix) and mycoprotein were excluded from Figure B due to a lack of data on the respective impact of this sub-figure. Note: PL – Poland; FI-LC – Finland low carbon scenario; GWP – global warming potential; HM - *H. illucens* meal (defatted protein concentrate); MP – microbial protein; FAEM – Finnish average electricity mix; FHE – Finnish hydrogen energy; MP (Base) – baseline scenario used in the article by Sillman et al.(2020); MP (Flmix) – Finnish average electricity mix used in the article by Sillman et al. (2020). Water scarcity was calculated using the AWARE method, Energy demand using the CED method and all others using the IMPACT2002+ method, with the exception of eutrophication which was expressed in kg N in Table 2A and were calculated using the ReCiPe results presented in articles II and III.

Figure 8 shows a comparison of the environmental impacts of different protein sources for human consumption. Figure 8A includes all protein sources that were analyzed in articles I, II and III. Protein from shrimp farmed in mangrove forests led to the highest GWP. This far exceeded the emissions caused by protein sourced from beef herds, which is generally considered to have the highest carbon footprint among protein alternatives (Poore & Nemecek, 2018b). However, the GWP dropped substantially when shrimps were grown in an intensive farming practice and was then lower than that of both beef farming practices. Nonetheless, the impacts were still higher than all the other protein sources considered here.

The difference in GWP between the two shrimp production systems was solely explained by the LULUC emissions originating from mangrove forests and were astonishingly high in comparison to any other life cycle steps. The GWP of shrimp grown in a mangrove concurrent system was high despite the avoided emissions associated with intensive shrimp farming practices, highlighting the importance of avoidance of (mangrove) deforestation.

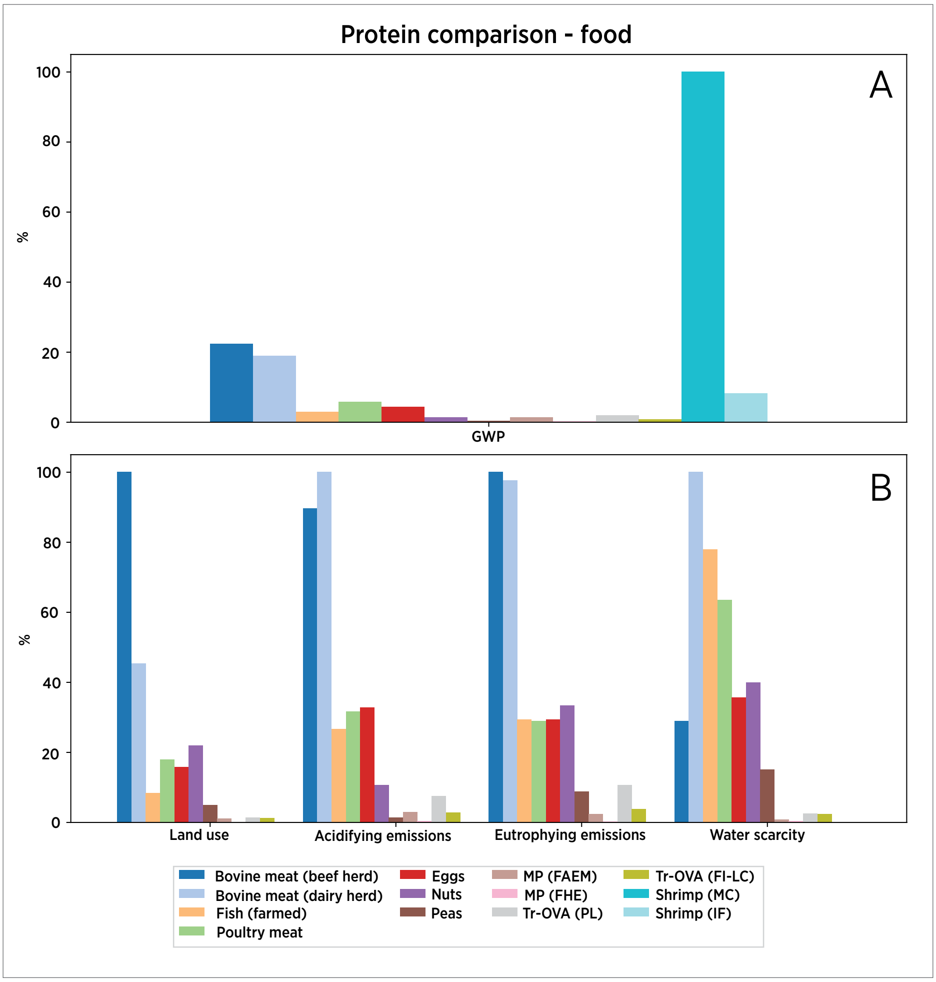


Figure 8: Impact scores of different protein sources that can be used as food for humans, based on the European sub-data set used in the article by Poore and Nemecek (2018a) and results from article I, II and III. Impacts were calculated using a functional unit of 100g of protein. Impacts from shrimp farming in mangrove concurrent systems (MC) and intense farming systems (IF) are included in Figure A but not in Figure B due to lack of data. Note: FAEM – Finnish average electricity mix; FHE – Finnish hydropower energy; PL – Poland; FI-LC – Finland low carbon scenario; GWP – global warming potential calculated using the IPCC 2013 including CC feedbacks method, land use was calculated in total m^2 per FU, eutrophication and acidifying emissions were estimated using the CML2 baseline method and water scarcity using the AWARE method.

Figure 8B displays the results in other impact categories for the different protein sources meant for human consumption, except for shrimp, which were not included due to a lack of data. MP production generally resulted in lower environmental impacts than the other protein-rich food alternatives. Although Tr-OVA generally had a higher impact compared to other feed protein sources, in comparison to other food protein sources its impact was relatively minor. Only peas and MP had a lower environmental impact than Tr-OVA (PL) for each impact category except land use. MP production had the least environmental impact, except for peas in the comparison of acidifying emissions.

5.4.2 COMPARISON OF SHRIMP, MP, AND TR-OVA AS PATTY INGREDIENTS

The patty prepared with shrimp grown in mixed mangrove concurrent systems resulted in the highest GWP. The GWP was 11 times higher than the patty produced with (semi-)intensively farmed shrimp and 90 times higher than MP-based patties. This was mostly due to the large GWP of these shrimps and, to a lesser extent, because of the lower concentration of protein in comparison to the other protein sources. This meant that more shrimps were required to obtain a similar protein content in the patty.

Using shrimp from the AGRIBALYSE shrimp systems not located in mangrove forests resulted in a similar GWP as for patties with MP and Tr-OVA. Another difference was that AGRIBALYSE shrimp model was based on data obtained from indoor shrimp farms in the U.S. (Asselin-Balençon et al., 2020). It was the only shrimp dataset available in the AGRIBALYSE database which raises concerns regarding the global representation of shrimp production and over the validity of the results presented in Figure 9

Approximately 75% of globally produced shrimp originate from Asia where shrimps are farmed outdoors and only a fraction originate from the U.S. (FAO - Fisheries division, 2021). Also, shrimp farmed in mixed mangrove concurrent shrimp farms would not be a good global representation as only about 5% of all shrimp originate from these systems. Most shrimps in Vietnam are farmed in (semi-)intensive farming systems. These systems therefore give the best representation of possible emissions from shrimp farming systems of the three shrimp farming systems compared here. Based on the GWP results for (semi-)intensive farming systems, the shrimp patty has about 8%-9% higher emissions than that of MP and Tr-OVA, respectively.

Unlike the results for GWP calculated on a protein content basis, the MP-based patty resulted in a higher GWP than a patty based on Tr-OVA. The difference is mainly due to the higher protein content of Tr-OVA, which required 1.4 times less of the product compared to MP. The contribution to GWP of all other non-protein ingredients were minimal.

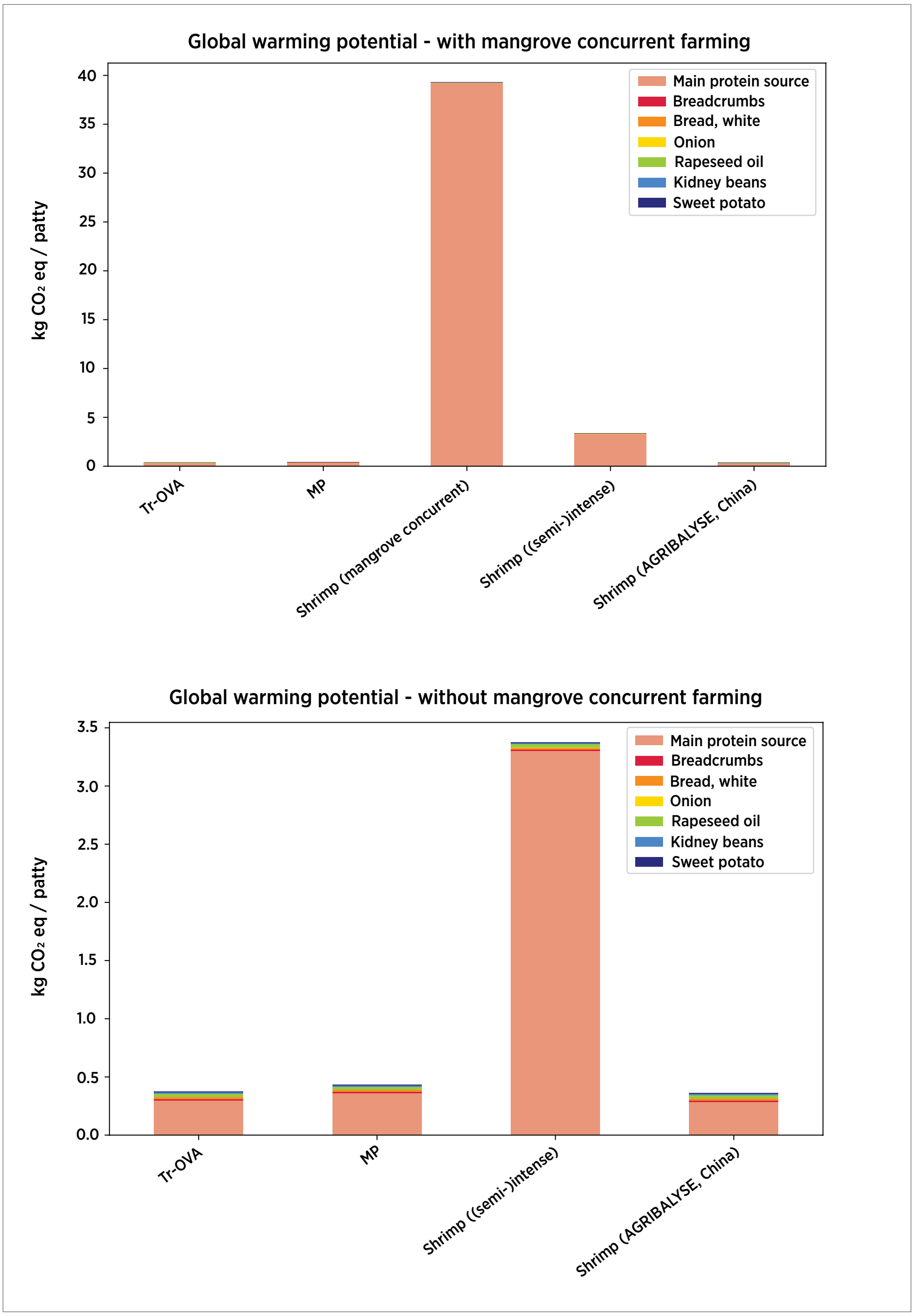


Figure 9: Results for GWP of the patties with Tr-OVA (FI), MP (FAEM) or shrimp (mass-allocation), where the above picture includes shrimp farmed in a mixed mangrove concurrent farm, (semi-)intensive shrimp farm or in shrimp farm in China from the AGRIBALYSE database.

6. DISCUSSION

6.1 DECOUPLING PROTEIN PRODUCTION FROM AGRICULTURAL LAND

6.1.1 HOW CELLULAR AGRICULTURAL PRODUCTS CAN REDUCE LAND USE REQUIREMENTS

The comparison of different protein sources showed that the environmental burden of protein production could potentially be lowered by a shift from protein-rich food and feed ingredients from agriculture and aquaculture to cellular agriculture, depending on the product. The largest difference was explained by the decoupling of cellular agricultural products from agricultural land use, achieved by decreased utilization of agricultural inputs during production. MP production requires no agricultural land and even the production of Tr-OVA required only 2.54 kg of glucose per kg of protein. By comparison, 27.5 kg of feed was required to produce 1 kg of chicken-based egg white protein powder. However, the required feed contains embedded energy collected naturally from the sun in the agricultural field. Although the same applies to glucose production, the main energy requirements for the growth of fungi biomass were supplied by industrial electricity. Replacing chicken on agricultural fields with fungi in bioreactors means a switch from energy obtained from the sun to energy supplied by industrial electricity in ovalbumin production. Nonetheless, the land use requirements for MP and Tr-OVA were smaller than most other protein-rich foods and feed ingredients.

Utilization of environmentally optimal energy sources could further reduce the land requirements of protein-rich cellular agricultural products. Land use requirements can vary substantially between different energy sources as was shown by the difference in land use between the FAEM and FHE scenarios to produce MP. This was partly explained by the difference in land use between renewable and non-renewable energy sources in Finland. For example, hydropower and nuclear power — responsible for 16% and 29% of the Finnish average electricity mix respectively — require relatively little land in comparison to electricity produced from hard coal and peat. However, the land requirements for heat and power co-generation fueled by wood chips (approximately 5% of the Finnish country mix) were the highest with 0.39 m^2a crop eq; almost 43 times larger than electricity from hard coal. (Treyer, 2014).

Land use requirements for the different Tr-OVA production scenarios were relatively similar despite the use of different energy mixes. Although there were substantial differences in GWP, the land use requirements of each country mix did not fluctuate much. Results for land use requirements when using different renewable energy sources for MP production were similar, as was shown from the sensitivity analyses. However, a comparison between different wind turbines showed that land use requirements can be reduced by three to four times when offshore wind turbines are used in comparison to those placed on land.

6.1.2 ADVANTAGES AND DISADVANTAGES OF DECOUPLING PROTEIN PRODUCTION FROM AGRICULTURAL FIELDS

Decreased reliance on agricultural land brings advantages. The increase of climate change-intensified weather events and issues such as soil degradation potentially leading to desertification of land, negatively affect agricultural yields. MP manufacturing could replace other protein production in regions with unfavorable growing conditions and thereby help to meet the United Nations (UN) sustainable development goals such as 'zero hunger' and 'responsible consumption and production'.

However, cellular agriculture also has disadvantages. Increased electricity demand from all sectors means that there will be an increased need for rare earth metals in order to supply electricity demands in the future (Smith Stegen, 2015). The question is whether all the required resources can be delivered in the upcoming decades to facilitate the production of required cellular proteins, such as MP and Tr-OVA, while also providing enough electricity for all other sectors.

The direct electricity demand to produce 1 kg of MP (FAEM) or 1 kg Tr-OVA protein was estimated to be 27.8 kWh and 10.5 kWh, respectively. Daily protein consumption in Finland in 2019 was 118.2 grams per person per day (FAOSTAT, 2022). With a population of 5.5 million people, this means a roughly estimated daily consumption of 650 100 kg protein. Producing just 10% of this daily protein demand with protein from both MP and Tr-OVA on a 50-50 basis would require 1.27 GWh of electricity. For an entire year, the electricity demand would be 462.7 GWh. The total electricity consumption of Finland's agricultural and horticultural sector was 1 883 GWh in 2020 (LUKE, 2021). This would mean that electricity demand from the food sector would significantly increase.

6.2 THE INTERACTION OF LAND USE, GLOBAL WARMING POTENTIAL, AND LOSSES IN ECOSYSTEM SERVICES

The comparison of cellular agricultural products with protein-rich food and feed ingredients showed that the largest reduction in environmental impacts could be achieved when protein production is decoupled from livestock. This was observed, for example, in the comparison of cellular agricultural products to shrimp (grown in mangrove areas) and beef. A large part of the GHG emissions associated with beef production originate from enteric fermentation in ruminants and to a lesser extent from manure (see section 3.1.1). Both result in the emission of CH_4. CH_4 is also emitted during the production of aquacultural products, such as shrimp (see article I). In addition, the production of protein using livestock requires more land both directly through the occupation of land by the livestock itself and the land required to produce its feed. When comparing the agricultural feed requirements of *T. reesei* to that of chickens for the production of albumin, microbes seemed to be more efficient in the conversion of agricultural feed to protein.

Land requirements are coupled with GWP as LULUC results in fluxes of GHG emissions. This connection was observed in the comparison of the five different patties, which emphasized the substantial contribution of LULUC emissions from mangrove deforestation to the GWP of the shrimp-based patty. Even though other sources of emissions were excluded from the analysis on shrimp farming due to a lack of information, the emissions caused solely by LULUC of mangroves exceeded those of any other protein source, including those of other shrimp farming practices. LULUC emissions are not only an issue in shrimp farming. Other agricultural products, such as the production of tofu and poultry meat, are known to result in LULUC-related GWP, which, in both cases, could be explained by the input of soybeans (Poore & Nemecek, 2018b). As LULUC is the largest source of emissions related to food production, it partly explains the difference in GWP between cellular agricultural production and protein production using livestock.

Emissions from LULUC are often excluded in LCA of food products, as discussed in articles I and III. However, the results presented in this dissertation showed that carbon fluxes from above-ground, below-ground, litter, and soil carbon stocks can be profound. Deforestation of mangrove areas for food production not only results in loss of carbon already stored in these ecosystems, but also the loss of the mangroves' potential to sequester carbon into their soil. Soil carbon sequestration is an important climate mitigation potential (Goglio et al., 2015). The LULUC of mangrove therefore means the release of carbon already stored in the soil and the loss of the potential of further carbon sequestering in the future. The results of article I emphasize the findings of previous studies on the importance of including LULUC emissions into LCA studies (Goglio et al., 2015)

Shrimp farming requires brackish water, which means that suitable locations, especially extensive farming practices like the mixed mangrove concurrent system, are limited to coastal areas potentially leading to land use conflicts with coastal ecosystems such as mangrove forests. The original intent of the mixed mangrove concurrent shrimp farming practice was that families were allowed to cut down only a limited amount (up to 30%) of the mangrove area in the Mekong Delta to generate an income. This would help preserve the fast-disappearing mangrove forest while meeting the needs of the people living in these rural areas (McNally et al., 2010). Based on the results of the analysis using satellite images, it seems that this aim only partly succeeded as most of the forests were cut down. The analysis of the mangrove cover remaining within the mixed mangrove concurrent shrimp farming showed that about 39% of the original mangrove cover was still intact, which undermined the effectiveness of these shrimp farming systems to protect the already vulnerable ecosystem. It is not only a biodiversity conservation problem, but also results in the loss of valuable ecosystem services provided by the mangrove forests (Friess et al., 2012).

The balance between economic aspirations and conservation of mangrove ecosystems is therefore difficult to achieve in Vietnam (Orchard et al., 2016). This problem extends to other aquaculture systems that have led to the demise of mangrove areas within Vietnam and deprived low-income households of their livelihood (Orchard et al., 2016). Additionally, mangrove losses make the coastline of these areas vulnerable to the forces of the sea (Tri et al., 1998). Increased protection and rehabilitation of the mangrove areas will both secure the livelihoods of low-income families and provide coastal protection — additionally allowing mangroves to contribute in the fight against climate change (Orchard et al., 2016; Tri et al., 1998).

The analysis of mangrove cover in Vietnam was based on satellite images of just 25 farms. The quality and nature of these satellite images only allowed for rough estimates. Additionally, the images only covered a period starting from 2000, while deforestation of mangroves in Vietnam mostly took place during the 1980s and 1990s (Richards & Friess, 2015; WWF, 2013). However, as mangrove deforestation has continued after this period, the results put into question support for these systems when the goal is to protect mangrove ecosystems.

6.3 WATER RESOURCE DEPLETION

The results of this dissertation suggest that the production of protein using cell-culturing technologies can substantially reduce water requirements by comparison to plant-based and especially animal-based alternatives. This was despite the fact that cell-cultured products relied more on the local electricity mixes. In comparison to other protein-rich foods, MP production resulted in the smallest impact of water use. This was especially true when hydropower was used rather than the average Finnish electricity mix. This suggests that a transition from fossil fuels to renewable energy sources could solve a range of problems, by lowering both the GWP and the water requirements of protein production. However, compared to other feed protein sources, MP did not result in a lower water scarcity impact. A similar situation was observed when comparing Tr-OVA with chicken-based egg white protein powder in the Polish scenario. These observations show that the switch from agriculturally produced protein to cell-cultured protein does not necessarily intensify the competition for water between food and energy production.

The water scarcity score of the chicken-based egg white protein powder was mostly influenced by the assumed composition and origin of chicken feed. The environmental impacts of chicken feed used in Germany and Poland, that were modelled with the WFLDB, differed substantially from each other even though both models relied on data from FAO. While the Polish feed basket was made specifically for Poland, a German feed basket was not available in the WFLDB meaning that an average European feed basket for laying hens was composed for the German laying hens in the WFLDB. Both the composition of the feed and the origin of the ingredients were therefore different for the German and Polish laying hens. The input of grain was higher in the European model (90% versus 81%) and was the major contributor of chicken feed to water scarcity. Most of this was due to the input of irrigated maize grain originating from Spain, with a total contribution of 93.6% of water impacts per 1 kg of chicken eggs.

In the Polish chicken feed model, maize was produced without irrigation in Poland. Instead, most of the water scarcity impact was related to seed production of wheat grain at a global level. The inputs of wheat grains for both the Polish and German systems were very similar, meaning that the difference in water scarcity could only be explained by the use of Spanish maize in the European feed mix for German egg production. This was confirmed by the fact that the AWARE factor for water use for irrigation in Spain is 80.76, which is high compared to an average global factor of 45.74.

As water use for irrigation is often a main contributor to the overall impact of water use, MP production has a clear advantage over Tr-OVA production by being independent from agricultural inputs. Irrigated maize-based glucose production was indeed the main contributor to water scarcity in the production of Tr-OVA. In fact, the irrigation of maize was responsible for 62.6% of water scarcity impacts of Tr-OVA production.

Because MP production is independent from agricultural inputs, its production site could also be selected more flexibly in order to optimize energy availability and minimize impacts of land use, such as using a solar energy-rich desert area that would otherwise be unsuitable for agricultural production. However, the energy source used to produce MP needs to have an overall small water requirement, in order to keep the impacts of water use low and to be competitive with alternative protein sources for feed production.

6.4 EUTROPHICATION POTENTIAL

Agriculture is responsible for an estimated 65% of global NH_3 emissions (Erisman et al., 2007). Both MP and Tr-OVA production require NH_3 as an input. However, MP and Tr-OVA have the benefit of being produced in a closed and controlled production system. The uptake of nutrients such as N is as high as 99% in the production of MP. This avoids the potential direct leakage of N inputs into local water bodies and coastal areas as well as emissions to air as a result of the application of fertilizers, as commonly occurs in agricultural and aquacultural systems. This explains the low eutrophication potential for MP and Tr-OVA compared to the protein-rich foods produced on agricultural fields. Even when the uptake of NH_3 was reduced to 85%, the increase in marine eutrophication was limited since wastewater from the process — containing the excess nutrients — was treated in the WWTP before being released into the environment.

Additionally, the production of ammonia is an energy-intensive process causing environmental burden, while the amount of N reaching humans through agricultural production is low (Erisman et al., 2007). This means that because of the high uptake of N in the production processes of MP and Tr-OVA, the overall demand for ammonia and its related environmental impacts could be decreased when producing proteins through cellular agriculture rather than agriculture.

6.5 POSSIBLE REDUCTION IN ENVIRONMENTAL IMPACTS THROUGH ALTERATIONS IN THE PRODUCT DESIGN

The environmental impacts of Tr-OVA and MP could further be reduced by changes in the production processes and the choice of inputs. Results on the environmental impacts of MP and Tr-OVA showed that switching from the average country energy mixes to low carbon energy mixes reduced the environmental burdens of their production processes (Figure 2 and Figure 4). However, these reductions only slightly improved, depending on the impact category, the environmental competitiveness of MP and Tr-OVA compared to alternative protein-rich food and feed ingredients (Figure 7 and Figure 8). For example, the relative difference in land use between different MP and Tr-OVA production scenarios or electricity sources was small compared to the difference with alternative protein sources. This means that, although the environmental impact of MP and Tr-OVA can be lowered using low-carbon electricity options, the choice of which protein source to use makes a greater difference to overall environmental impacts than the energy choices of an individual product. The importance of the choice in protein source was also observed during the comparison of patties with different protein ingredients. The contribution of the protein sources to GWP outweighed the contribution of all other ingredients combined, underlining the importance of the protein choice in reducing the GWP of patty production.

Another way to reduce the environmental impacts of cellular agricultural products is through the use of co-products. The potential utilization of by-products, such as the fungal biomass in Tr-OVA production, would result in co-production and could reduce the environmental impacts of Tr-OVA substantially. The extent of the reduction depends on the method chosen to share the environmental impacts between the co-products (Figure 6). The use of fungal biomass would be problematic within the EU due to legislation concerning GMO products (European Commission, 2019). However, the legislation is more flexible towards GMO food products in the United States (US), which would allow for the use of fungal biomass for feed (FDA, 2022), meaning that fungal biomass would share the environmental burden of Tr-OVA production.

In comparison to other protein-rich feed ingredients, results showed that Tr-Ova production resulted in a larger ionizing radiation potential due to the use of polydimethylsiloxane (see Figure 7). Although polydimethylsiloxane is a common anti-foaming agent, other agents such as insoluble oils or other silicones can deliver the same function (Gochev et al., 2016). Their use could potentially decrease the ionizing radiation caused by the production of Tr-OVA.

The various sensitivity analyses applied to the case studies of MP and Tr-OVA showed that reductions in process efficiencies increased the environmental impacts of the products slightly, ranging from an increase of 0-17% for Tr-OVA, for example. The largest deviation was observed when efficiency in the utilization of glucose was reduced during the production of Tr-OVA, emphasizing the potential environmental benefits of decoupling cellular agriculture protein production from agricultural inputs. The sensitivity analyses of MP again mostly emphasized the potential reduction in environmental impacts of cellular agricultural products through the choice of energy source. The analyses also showed that the choice of electrifying CO_2 production could decrease the environmental impacts of MP production, while producing steam with electricity generally increased these impacts. However, for both the production of steam and CO_2, the Finnish average electricity mix was utilized in the respective sensitivity analyses. Using renewable energy sources could potentially also reduce the environmental impacts of steam. This should be explored in future research.

6.6 METHODOLOGICAL DISCUSSION

6.6.1 TIMEFRAME TO ALLOCATE LULUC EMISSIONS

The results presented in article I illustrated the effect of using different annualization periods, the period over which emissions are allocated, in calculating annual LULUC emissions. The Mekong Delta case study used a 50-year annualization method, which is in line with other studies on the expected lifetime of shrimp farms (Jonell & Henriksson, 2015). However, when using the 20-year period recommended in the default guidelines of the IPCC, emissions are allocated over a much shorter period, thereby increasing the already high emissions of shrimp farming in mangrove areas. Arguments against both of these choices could be made: the 20-year time frame can be called random and 50-year optimistic.

The impact of the choice of timeframe is rather large, which is illustrated by the annualization of LULUC GHG emissions resulting from shrimp farming in previous mangrove areas: using a 50-year period GHG were estimated at 184 and 282 t CO_2 -eq per t of live shrimp at the farm gate using mass and economic allocation, respectively. When applying the default timeframe of 20 years, emissions are allocated over a smaller harvest of shrimp and the emissions for one t of live shrimp are 372.2 and 568.5 t CO_2 -eq using mass and economic allocation, respectively. This is roughly a doubling of emissions using the same FU. This difference emphasizes the need to carefully choose a timeframe based on the best data available, which would support argumentation on the choice made.

6.6.2 THE EFFECT OF ALLOCATION CHOICES

Another methodological choice that led to variation in the GWP of shrimp farming was the decision to allocate based on either a physical or economic relationship. In article I, emissions were allocated based on either mass or income, and a 50% increase in GWP was observed when applying the latter. This large difference was explained by the relatively large income obtained from the *P. monodon* shrimp species in relation to its weight, in comparison to the wild shrimp that grow in the same area (Jonell & Henriksson, 2015).

Both allocation methods were applied in article I to explore the differences in the results between physical and economic allocation. No explicit choice was made when presenting the results, leaving room for future research to take either approach, depending on the allocation method applied in the analysis of an alternative protein source. ISO standards favor allocation based on a physical relationship over those that reflect another, such as economic values (ISO, 2006). However, it can be argued that the income regenerated from wild shrimp is relatively low by comparison to its weight. The main income for shrimp farmers comes from the sales of the *P. monodon* shrimps and mud crab, together accounting for 85% of the overall income (Jonell & Henriksson, 2015). It is this prospective income that eventually led families living in mangrove areas to establish aquacultural ponds in the mangrove area. This means that the economic allocation factor perhaps more accurately reflects the impact of this decision.

A similar discussion applies to the allocation of the potential by-products of Tr-OVA production. The results of the sensitivity analyses (article III) illustrated the difference that methodological choices could make to the results. The application of the MPSP-based allocation factor — reflecting an economic relationship between the co-products — led to a minor reduction in environmental impacts of Tr-OVA compared to when the protein-based allocation method was applied. However, current legislation within the EU (EC, 2019) prevents the use of fungal mass, as discussed previously. In addition, the potential revenue from the sale of the fungal biomass would be marginal (Voutilainen et al., 2021) and it could again be argued that that revenue from the biomass — that was considered waste in the main scenarios — would likely not lead to the production of Tr-OVA. Therefore, choosing protein-based allocation could make the product appear to have lower environmental impacts than in the case of applying the MPSP-based allocation factor.

6.6.3 SENSITIVITY ANALYSIS AND MONTE CARLO

Visual representations of the results of the MC analyses were based on the uncertainty data of both the background systems of the LCA databases and the foreground data gathered for the models. The uncertainty ranges in article II were estimated using the pedigree method implemented in Simapro (Ciroth, 2012). A uniform uncertainty range of +- 20% for the foreground data was used in article III as the model was based on data collected from a pilot-level production scale. The same uncertainty ranges were applied in the sensitivity analyses. This meant that a combination of uncertainty analyses with sensitivity analyses was applied, resulting in both deterministic results and uncertainty ranges for the results. The rationale for including the potential uncertainties of the system when presenting the sensitivity results was to simplify judgement on whether the difference between the sensitivity analyses and those of the main scenario were substantial, considering the uncertainties of the model.

To illustrate this the following example can be used: comparing the deterministic results of the "FHE – nuclear" sensitivity test and the deterministic results of the "FHE – solar" test shows that using nuclear energy results in a higher impact from water scarcity (see Figure 3). However, the opposite interpretation emerges when considering the uncertainty of the system, as the medium water scarcity of MP produced with solar energy was higher than the medium water scarcity score when using nuclear energy. In addition, the uncertainty range for the "FHE – solar" test is much larger than that of the "FHE – nuclear" test.

When a sensitivity analysis is applied, assuming, for example, that the system uses a different electricity source, it does not mean that the uncertainties regarding the amount of electricity are not there anymore or are not relevant anymore. Neither does the change in electricity source alter anything about the other uncertainties that are already in the foreground or the background model. Ignoring these uncertainties could therefore lead to a potential misinterpretation of the results as illustrated by the example above. Consideration of uncertainty ranges in sensitivity analyses could therefore lead to better interpretation of the results.

Interpretation of uncertainty ranges is challenging. Heijungs (2020) argued in his article on the number of MC runs that, partly due to the increased computing power of laptops, the number of runs used is typically getting larger. This consequently leads to overconfidence in the results: precision increases but accuracy does not (Heijungs, 2020). In theory, the number of MC runs should not exceed the number of sample points upon which the model is built but this is often impractical or impossible due to time and resource limitations (Heijungs, 2020).

So, the question remains: how many runs should be implemented for the cases presented in articles II and III? Or is MC an appropriate uncertainty analysis method at all in these case studies? However, alternative methods are currently not available in the LCA software, which is why estimates on the uncertainty ranges were used. This MC method still provides useful insights into the possible ranges within which results could fall. Without any uncertainty ranges there is a risk that deterministic results are interpreted as an ultimate truth. However, the amount of MC runs should be kept to a minimum to avoid this false sense of accuracy.

6.6.4 USE OF PROXIES

Due to limitations in the availability of background data, proxies were sometimes used. In the production of Tr-OVA sodium phosphate was used as a proxy to model the environmental impacts of MKP. MKP made up most of the salt mix by weight. The use of the proxy increased the uncertainty of the results. However, the environmental impacts of the salt mix were generally relatively small in comparison to those of the other required inputs. An exception to this was the impact on water scarcity, where the salt mix was the second highest contributor. An increase in data availability would, therefore, increase the certainty of the results presented in article III.

The model for fava beans from the ecoinvent database was used as a proxy for red kidney beans in the patty recipe, due to a lack of background models for red kidney beans. This decision was justified as the same proxy was used in the AGRIBALYSE database for kidney bean production. However, the model for fava beans from ecoinvent does not include potential LULUC emissions, although it did include changes in land use. In accordance with the analyses of shrimp production, this means that the GHG emissions from fava bean production could potentially have been underestimated in the GWP results of the patties. However, all patty models have the same underestimation of LULUC emissions as all recipes used the same amount of kidney beans. The same applied to the input of breadcrumbs that were also modelled using ecoinvent.

6.6.5 MIDPOINT VERSUS ENDPOINT

The environmental impacts of MP and Tr-OVA production were quantified using the ReCiPe 2016 v1.1 Midpoint (H) impact method. Environmental impacts could have been assessed using an endpoint level instead. However, the aim of this dissertation was to provide more insight into how the environmental impacts of cellular agricultural products differed from those of existing protein sources from agriculture and aquaculture. Midpoint level assessment allows for the comparison of single environmental flows and identify trade-offs between different environmental impact categories (Huijbregts et al., 2017). Additionally, midpoint assessments are commonly applied within LCA and therefore allows for a comparison with other protein-rich food and feed ingredients. Using an endpoint level assessment means that all environmental impacts are assessed through so-called impact pathways into an endpoint area of protection, such as damage to ecosystems. This means that endpoint levels are more aggregated, although the endpoint results can be broken-down into the contributions of individual environmental impacts. (Huijbregts et al., 2017)

One concern with the assessment on an endpoint level, is the increased uncertainties that result from data gaps and assumptions that are introduced throughout the case-effect chain. In addition, the aggregation of environmental impacts into endpoint levels requires a certain level of value choices. (Bare et al., 2000) However, the benefit of applying endpoint levels is that results are more easily interpreted by decision-makers. Additionally, although value choices are applied to the endpoint levels, they are based, at least to some extent, on informed weighting in contrast to letting decision makers make their own subjective choices. Midpoint and endpoint levels therefore both have their merits and limitations. Applying both could be one way to present all useful information. However, it is important to keep in mind the target audience and the goal of the LCA study when making the choice between midpoint and endpoint categories. (Bare et al., 2000; Kägi et al., 2015)

6.7 LIMITATIONS OF THE RESEARCH

The studies included in this dissertation have several limitations. The first is the exclusion of biodiversity impacts caused by the production of shrimp, MP, and Tr-OVA. Many environmental pressures that contribute to biodiversity loss, such as GWP, land use, water use, acidification and eutrophication are already included in the LCA method. However, many other pressures are not currently included in LCIA that are relevant when assessing biodiversity impacts, such as noise, overexploitation of resources, and invasive species. (Winter et al., 2017).

Two of the most important contributors to biodiversity losses are land use (approximately 30% for terrestrial ecosystems) and climate change (approximately 15% for terrestrial ecosystems) (IPBES, 2019). The results of article II showed that particularly MP production required only limited land use — most of it was related to the production of electricity. Similarly, Tr-OVA production required limited land use, scoring second best in comparison to other protein-rich food alternatives. Using renewable energy or low carbon energy sources reduced the GWP of both Tr-OVA and MP production, making them more environmentally competitive with the other protein sources examined. This especially applied in comparison to feed products as protein-rich feed ingredients generally had a lower GWP than food protein sources. Both Tr-OVA and especially MP production would potentially put less pressure on biodiversity than the alternatives through reductions in land use and GWP, which are two of the main drivers of biodiversity loss (IPBES, 2019).

The global food system is a major driver of biodiversity losses, and these losses are accelerating (Benton et al., 2021). The biodiversity impacts of cellular agricultural proteins should be assessed in order to advance understanding of their potential environmental benefits and drawbacks when replacing protein-rich products from agriculture and aquaculture systems. Although biodiversity impacts are best understood when all potential pressures are included in the LCA assessment, the current lack of a consensus-based LCA method measuring biodiversity impacts prohibits this. Therefore, biodiversity was not considered in articles I, II and III. However, because of the urgency of biodiversity losses (IPBES, 2019), future studies should use available methods that provide a better understanding of the biodiversity impacts of cellular agricultural products. In addition, future research would need to investigate what the transition from conventional agricultural foods to more industrial food products would mean for the fragmentation of the landscape, which has been shown to be positively correlated to biodiversity losses (Krauss et al., 2010).

A second limitation that was not considered in article I regarding LULUC emissions, is the potential impact of indirect land use changes. Article I showed that many semi-intensive and intensive shrimp farms were found in areas previously occupied by rice farming, meaning that there was no direct deforestation event associated with their establishment. However, the establishment of rice-paddies has been linked to mangrove deforestation. An area of up to 55.4 ha in total was lost between 2000 and 2012 due to rice-paddy farming (Richards & Friess, 2015). As the rice-paddy area increased by 1.2%, so too did the production of shrimps by 265%, both contributing to the LUCs. It can therefore be speculated that land-use change from rice-paddies to shrimp farms resulted in deforestation elsewhere, as the demand for rice did not decrease and former rice-paddy farms probably just relocated. These complex interactions were not considered in the analysis performed in article I and could potentially have led to an underestimation of total LULUC emissions by the exclusion of these indirect LULUC emissions.

A third limitation is the exclusion of an assessment of food losses and waste in the comparison of protein-rich products from cellular agriculture with agriculture and aquaculture. Current agriculture already produces enough food to meet expected demand from the growing human population in the coming decades. However, a substantial share of this food (~30%) is lost or wasted at the moment (Lal, 2020). This means that the means of achieving a more sustainable food system will not only be found by focusing on the production of less polluting food products but also in reducing loss and waste in the current system. One benefit of the novel food products discussed in this dissertation is that they are not fresh and can therefore be stored more easily and for a longer time. Tr-OVA also has the benefit of being less fragile compared to chicken eggs. Approximately 7% of eggs are disregarded during the production process of chicken egg yolk powder (Tsai et al., 2020). These losses, considered in the model of chicken-based egg white protein powder, could explain part of the difference in environmental impacts between Tr-OVA and egg white powder. Although the production inputs of Tr-OVA are not dependent on fragile eggs, food losses that may occur as a consequence of large-scale production are as yet unknown.

The food waste caused by shrimp consumption is relatively large as both the head and shell are removed and discarded before consumption. This constitutes a loss of approximately 34% of the weight of the produced shrimp. In addition, aquaculture products have a limited lifetime in comparison to powders that can be stored over a longer period of time. (Louisiana Direct Seafood, 2011; Zirlotts Gulf Products, 2013).

Fresh fruit and vegetables are more susceptible to being lost during the production phase than processed fruits and vegetables (Cui et al., 2018). Also, loss of animal-based products, such as beef, contribute to a higher GWP mostly at the stage of distribution and retail. Food waste at consumer are often not included in LCA studies (Poore & Nemecek, 2018b). As MP and Tr-OVA are both processed foods in a powder form, food losses and waste throughout their life cycle could potentially be low. Including food losses would give a better understanding of the potential of MP and Tr-OVA to replace other protein-rich food and feed ingredients. This reduction in food losses would also contribute to the Target 12.3 of the Sustainable Development Goal by the UN to reduce food losses and waste by half by 2030 (United Nations Department of Economic and Social Affairs, 2015). Future research should therefore include food loss and waste reductions in sustainability assessments of MP and Tr-OVA.

A fourth limitation was the choice of LCA method applied in articles II and III. Although the environmental impacts of both MP and Tr-OVA were analyzed using a variety of scenarios in articles II and III, the parameters included within the scenarios were limited. Sensitivity analyses were used to further explore the effects of changing assumptions related to the production processes. Both the use of scenarios and the application of sensitivity analyses were performed because the exact inputs and outputs of the production processes are uncertain and based on pilot-scale data. However, given the early developmental stage of cellular products, the application of a prospective LCA could have resulted in a more systematic impact analysis. A prospective approach is therefore recommended for future research on similar products. Additionally, the use of a consequential approach could potentially lead to different conclusions than those made on the basis of the attributional approach. The consequential approach utilizes marginal supplies rather than the average supplier. This would mean, for example, that instead of a country's average electricity sources, the providers that are able to respond to the increased demand for electricity will be taken into account in the model of cell-cultured products. As both MP and Tr-OVA require high levels of electricity, the electricity mix has a large role on the environmental impacts of their production processes.

A fifth limitation was the accuracy of modelled inputs. Several ingredients could not be accurately modelled in both the LCA of Tr-OVA and the patty models due to limitations in currently available databases. This problem also occurred in modelling the environmental impacts of MP production. Expert opinions and estimations were used in selecting proxies that represented similar production processes. Although the impact of individual minerals was generally limited in the overall impact of the products studied, it is recommended that these research gaps should be closed in future studies. Improvements in data availability would increase the accuracy of estimates of the environmental impacts of protein-rich food products and feed ingredients.

Lastly, Although the production of MP and Tr-OVA might reduce environmental impacts, this dissertation did not include any social, cultural or economic aspects that might have an impact on the overall judgement of whether a product is sustainable (Guinée, 2016). There are a number of articles available on the economic and social implications of the introduction of cellular agriculture, and the social acceptability of these products (Bryant & Barnett, 2020; Matassa et al., 2016; Newton & Blaustein-Rejto, 2021; Siegrist & Hartmann, 2020; Voutilainen et al., 2021). In addition, the production of Tr-OVA may not face the same issues with regard to ethical considerations and the potential outbreak of zoonotic diseases as is the case with the production of chicken eggs for egg white powder (C. K. Johnson et al., 2020). However, both MP and Tr-OVA are relatively new products, not yet available on the market in 2022, and relatively little is known about the economic, cultural and social implications of these specific products. Future research needs to address this in order to gain an understanding of the sustainability of replacing protein-rich products from agriculture and aquaculture with MP and Tr-OVA in different regions of the world.

6.8 FUTURE RESEARCH RECOMMENDATIONS

In addition to future research recommendations related to the limitations of this dissertation, presented in the previous section, there are several other avenues for further research. Article I presented an overview of global averages for mangrove carbon stocks. The data sources used in this literature review were mostly in line with the estimates of the IPCC report as there was a generally limited amount of data available on global mangrove carbon stocks. This also meant that local emissions from mangrove deforestation could only be estimated using global estimates on mangrove stocks. However, since the publication of the IPCC's (2014) supplemental report and article I, more research on carbon stocks in mangrove forests has been conducted. In addition, events that possibly contribute to mangrove carbon storage have been further explored. (Alimbon & Manseguiao, 2021; Belliard et al., 2022; M. T. Costa et al., 2019; J. L. Johnson et al., 2020) Future development of guidelines for estimating GHG emissions related to aquaculture practices in mangrove areas should include regionally specific estimates and pathways that lead to changes in carbon stocks, be it the presence of crabs, natural changes (such as age or natural spread of mangrove forests) or human interference (Andreetta et al., 2014; e.g. Charles et al., 2020; Walcker et al., 2018; Xiong et al., 2018). In addition, more knowledge on the exact CH_4 and N_2O emissions is needed to make more accurate estimates.

There is only a limited amount of literature available on the environmental impacts of cellular agricultural products. The comparison of protein-rich products from cellular agriculture with those from agricultural and aquacultural systems was limited to the MP and Tr-OVA production in this dissertation. Additionally, the studies presented in this dissertation are based on empirical data originating from just one organization per product. Sensitivity analyses were performed for each product to capture the potential operational differences that could occur between companies (Groen et al., 2017), but these assumptions were of a speculative nature. When these products reach the market, more research can and should be performed as replication is important for the scientific buildup of evidence (Makel & Plucker, 2014). Additionally, more research is needed on the environmental impacts of other protein-rich cellular agricultural products. An increase in scientific evidence on the environmental impacts of cellular agricultural products would also aid in understanding how these products can decrease the burden on the planet caused by protein production.

One key aspect of Tr-OVA production, that generally contributed the most to its environmental impact, was the source of glucose production. It was assumed that glucose was produced from cornstarch for three reasons. The first was due to data limitations as the databases only contained data on glucose production from cornstarch. The second was that cornstarch is the main source of current glucose production, although other sources can be used too (Voutilainen et al., 2021). The third reason is that we chose to err on the conservative side and rather overestimated the environmental impact of Tr-OVA production, as it is unclear what the actual source of glucose will be. Alternatively, glucose could be obtained from waste sources such as forestry waste or the side-streams of straw and cereal production (Asim et al., 2021; Upcraft et al., 2021). Using these glucose sources could potentially lower the environmental impacts of Tr-OVA production, but these options could not be further analyzed due to the lack of available data. It is therefore recommended that future research focuses on the environmental potential of the use of agricultural side-streams for Tr-OVA production. Side-streams of agricultural production processes could be used as an input to other cellular production processes as well, thereby creating a potential symbiotic agricultural relationship (Asim et al., 2021; Newton & Blaustein-Rejto, 2021; Upcraft et al., 2021).

The use of side-stream would also allow for Tr-OVA production to be part of other sustainable agricultural practices such as agroecological symbiosis that would potentially lead to additional environmental benefits. Research has shown the potential environmental gains of agroecological symbioses such as the increase in agricultural yields, substantial reduction in GWP and eutrophication potential, and a net-positive energy production (Koppelmäki et al., 2019). Future research would need to assess potential environmental gains when including cellular agricultural products into agroecological systems. This would mean that the research should be expanded from a product-based level, as was performed in articles I, II and III, to a food system level that not only analyzes the environmental benefits of a single protein source but explores the benefits of integrating production into a wider sustainable agricultural practice.

Despite the relatively high GWP and land requirements of many agricultural protein sources, the nature of production processes does allow for the possibility of improvement in terms of land management and its effect on carbon stocks (Goglio et al., 2015). For example, regenerative agricultural practices have shown the potential to reduce environmental impacts and even restore degraded sites with very little input, while maintaining high yields (Lal, 2020). Regenerative agricultural practices have even been shown to be a potential pathway to storing more carbon in the soil, thereby activating a potential carbon pool in the fight against climate change (Lal, 2020). These examples suggest that agricultural production does not have to have a large environmental impact by definition. However, LCA research has rarely been conducted on these regenerative production systems, let alone on the impact of these systems on biodiversity. To understand how the environmental impacts of the food system can be reduced and the extent of the role cellular agricultural products can play in achieving this goal, it is necessary to increase scientific knowledge on all available types of production system. This includes more studies on aquaculture production, as well as agricultural practices such as regenerative agriculture and agroecological farming and the potential role of integrating aquaculture, agriculture and cellular agriculture.

7. CONCLUSIONS

The aim of this dissertation was to understand the environmental impacts of protein-rich cellular agricultural products in comparison with those of protein-rich food and feed ingredients originating from agriculture and aquaculture. The results of article I showed an example of how current food production systems contribute to the degradation of the environment. The potential flow of GHG emissions released into the atmosphere, when natural ecosystems such as mangrove forests are replaced with food production, was analyzed. These emissions contribute significantly to GWP and will potentially increase climate-intensified extreme weather events such as drought and floods — events that are already challenging food production. Additionally, the production of shrimp in mangrove forests contributes to the further degradation of the valuable mangrove ecosystems and thus the loss of its many useful and critical ecosystem services. Emissions from shrimp farming in mangrove forest areas emphasize the need for the transition of our food system from one that harms nature to one that works with it.

The cell-cultured products that were analyzed in this dissertation have shown potential to reduce the environmental impacts of the food systems. Land use, GWP, and water use could be significantly reduced when a part of human protein demand is met by these cellular agricultural products. This was mostly true in comparison to animal-based protein sources for human consumption, such as shrimp and beef, but a similar conclusion was drawn by comparison to many protein-rich feed ingredients and the few plant-based protein options included in this study.

Despite the environmental benefits that the replacement of protein-rich foods with cellular agricultural products such as MP and Tr-OVA could offer, several minor trade-offs were identified. These trade-offs were mostly related to an increase in impact categories related to industrial production systems, such as ionizing radiation. These were related to the relatively high inputs of industrial electricity within cellular agricultural production processes compared to those from agricultural and aquacultural systems. This also meant that environmental impacts could be further reduced using renewable energy and improving efficiencies in the production process, such as producing inputs on-site using electricity, or the utilization of waste products.

MP production was shown to have greater potential in reducing environmental impacts when replacing protein-rich foods and feed ingredients, compared to Tr-OVA production. This was related to the independence of the system from agricultural inputs. Glucose was the largest contributor to almost all impact categories in Tr-OVA production. This means that the environmental burden of cellular agricultural products could potentially be reduced further by eliminating or reducing agricultural inputs or by finding environmentally friendlier options — such as the utilization of agricultural side-streams.

An additional way to reduce the environmental impacts of cellular agricultural products was by using renewable or low-carbon energy sources. Electricity consumption was the second largest contributor to the environmental impacts of Tr-OVA, and the largest of MP production. The GWP of shrimp was most reduced when avoiding the deforestation of mangrove forests.

We are faced with many environmental, social, and economic challenges of which a large proportion are associated with the ways food is produced. Challenges such as the growing demand for rare earth metals related to renewable energy production and those that arose at the time of writing this dissertation — the COVID-19 crisis and the war in Ukraine — are increasing pressure on the current food system to transform to a more sustainable one. They underline the need for self-sufficiency of food production as well as a need for creative solutions. The results of this dissertation have shown that cellular agriculture can play a role in reducing the environmental impacts of protein production. However, the full extent of their potential contribution to the transition of the food system can only be fully understood when combining the environmental data presented here with the social and economic pillars of sustainability. There is no silver bullet when it comes to transforming our food system to a more sustainable one. Multiple solutions that originate from cellular agriculture and the transformation of the current agricultural sector into more regenerative systems are needed. Future research will need to take a holistic approach to understanding the ways in which we can combine the variety of available solutions and create a healthy, sustainable, and possibly thriving food system and planet.

8. REFERENCES

Alimbon, J. A., & Manseguiao, M. R. S. (2021). Species composition, stand characteristics, aboveground biomass, and carbon stock of mangroves in Panabo Mangrove Park, Philippines. *Biodiversitas Journal of Biological Diversity*, *22*(6), 3130–3137. https://doi.org/10.13057/BIODIV/D220615

Alongi, D. M. (2008). Mangrove forests: Resilience, protection from tsunamis, and responses to global climate change. *Estuarine, Coastal and Shelf Science*, *76*(1), 1–13. https://doi.org/10.1016/j.ecss.2007.08.024

Althaus, H.-J., Bauer, C., Doka, G., Dones, R., Frischknecht, R., Hellweg, S., Humbert, S., Jungbluth, N., Köllner, T., Loerincik, Y., Margni, M., & Nemecek, T. (2007). Implementation of Life Cycle Impact Assessment Methods. Data v2.2 (2010). *Ecoinvent Report No. 3*, *3*.

Amarasinghe, M. D., & Balasubramaniam, S. (1992). Net primary productivity of two mangrove forest stands on the northwestern coast of Sri Lanka. *Hydrobiologia*, *247*(1–3), 37–47. https://doi.org/10.1007/BF00008203

Andreetta, A., Fusi, M., Cameldi, I., Cimò, F., Carnicelli, S., & Cannicci, S. (2014). Mangrove carbon sink. Do burrowing crabs contribute to sediment carbon storage? Evidence from a Kenyan mangrove system. *Journal of Sea Research*, *85*, 524–533. https://doi.org/10.1016/J.SEARES.2013.08.010

Asim, A. M., Uroos, M., Muhammad, N., & Hallett, J. P. (2021). Production of Food-Grade Glucose from Rice and Wheat Residues Using a Biocompatible Ionic Liquid. *ACS Sustainable Chemistry and Engineering*, *9*(24), 8080–8089. https://doi.org/10.1021/ACSSUSCHEMENG.1C00022/SUPPL_FILE/SC1C00022_SI_001.PDF

Asselin-Balençon, A., Broekema, R., Teulon, H., Gastaldi, G., Houssier, J., Moutia, A., Rousseau, V., Wermeille, A., & Colomb, V. (2020). *AGRIBALYSE v3.0 : the French agricultural and food LCI database. Methodology for the food products.*

Asseng, S., Ewert, F., Martre, P., Rötter, R. P., Lobell, D. B., Cammarano, D., Kimball, B. A., Ottman, M. J., Wall, G. W., White, J. W., Reynolds, M. P., Alderman, P. D., Prasad, P. V. V., Aggarwal, P. K., Anothai, J., Basso, B., Biernath, C., Challinor, A. J., De Sanctis, G., … Zhu, Y. (2014). Rising temperatures reduce global wheat production. *Nature Climate Change 2014 5:2*, *5*(2), 143–147. https://doi.org/10.1038/nclimate2470

Astudillo, M. F., Thalwitz, G., & Vollrath, F. (2015). Modern analysis of an ancient integrated farming arrangement: life cycle assessment of a mulberry dyke and pond system. *International Journal of Life Cycle Assessment*, *20*(10), 1387–1398. https://doi.org/10.1007/s11367-015-0950-3

Bar-On, Y. M., Phillips, R., & Milo, R. (2018). The biomass distribution on Earth. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(25), 6506–6511. https://doi.org/10.1073/PNAS.1711842115/SUPPL_FILE/1711842115.SAPP.PDF

Bare, J. C., Hofstetter, P., Pennington, D. W., & Udo de Haes, H. A. (2000). Life cycle impact assessment workshop summary. Midpoints versus endpoints: The sacrifices and benefits. *International Journal of Life Cycle Assessment*, *5*(6), 319–326.

Belliard, J.-P., Hernandez, S., Temmerman, S., Suello, R. H., Dominguez-Granda, L. E., Rosado-Moncayo, A. M., Ramos-Veliz, J. A., Parra-Narera, R. N., Pollete-Ramirez, K., Govers, G., Borges, A. V., & Bouillon, S. (2022). Carbon dynamics and CO_2 and CH_4 exchange in the mangrove dominated Guayas river delta, Ecuador. *Estuarine, Coastal and Shelf Science*, *267*, 107766. https://doi.org/10.1016/J.ECSS.2022.107766

Benton, T., Bieg, C., Harwatt, H., Pudasaini, R., & Wellesley, L. (2021). *Food system impacts on biodiversity loss.*

Boulay, A.-M., Bare, J., Benini, L., Berger, M., Lathuillière, M. J., Manzardo, A., Margni, M., Motoshita, M., Núñez, M., Pastor, A. V., Ridoutt, B., Oki, T., Worbe, S., & Pfister, S. (2018). The WULCA consensus characterization model for water scarcity footprints: assessing impacts of water consumption based on available water remaining (AWARE). *The International Journal of Life Cycle Assessment*, *23*(2), 368–378. https://doi.org/10.1007/s11367-017-1333-8

Boulay, A.-M., Bare, J., De Camillis, C., Döll, P., Gassert, F., Gerten, D., Humbert, S., Inaba, A., Itsubo, N., Lemoine, Y., Margni, M., Motoshita, M., Núñez, M., Pastor, A. V., Ridoutt, B., Schencker, U., Shirakawa, N., Vionnet, S., Worbe, S., … Pfister, S. (2015). Consensus building on the development of a stress-based indicator for LCA-based impact assessment of water consumption: outcome of the expert workshops. *International Journal of Life Cycle Assessment*, *20*(5), 577–583. https://doi.org/10.1007/S11367-015-0869-8/FIGURES/1

Brown, L. (2009). *Growing Demand for Soybeans Threatens Amazon Rainforest*. Earth Policy Institute. http://www.earth-policy.org/plan_b_updates/2009/update86

Bryant, C., & Barnett, J. (2020). Consumer Acceptance of Cultured Meat: An Updated Review (2018–2020). *Applied Sciences 2020, Vol. 10, Page 5201*, *10*(15), 5201. https://doi.org/10.3390/APP10155201

Campbell, B. M., Beare, D. J., Bennett, E. M., Hall-Spencer, J. M., Ingram, J. S. I., Jaramillo, F., Ortiz, R., Ramankutty, N., Sayer, J. A., & Shindell, D. (2017). Agriculture production as a major driver of the earth system exceeding planetary boundaries. *Ecology and Society*, *22*(4). https://doi.org/10.5751/ES-09595-220408

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., Narwani, A., MacE, G. M., Tilman, D., Wardle, D. A., Kinzig, A. P., Daily, G. C., Loreau, M., Grace, J. B., Larigauderie, A., Srivastava, D. S., & Naeem, S. (2012). Biodiversity loss and its impact on humanity. *Nature 2012 486:7401*, *486*(7401), 59–67. https://doi.org/10.1038/nature11148

Cederberg, C., Persson, U. M., Neovius, K., Molander, S., & Clift, R. (2011). Including carbon emissions from deforestation in the carbon footprint of Brazilian beef. *Environmental Science & Technology*, *45*(5), 1773–1779. https://doi.org/10.1021/es103240z

Challinor, A. (2014). A meta-analysis of crop yield under climate change and adaptation. *Nature Clim. Change*, *4*(4), 287–291. https://doi.org/10.1038/nclimate2153

Charles, S. P., Kominoski, J. S., Armitage, A. R., Guo, H., Weaver, C. A., Pennings, S. C., Charles, C. :, Kominoski, J. S., Armitage, A. R., Guo, H., Weaver, C. A., & Pennings, S. C. (2020). Quantifying how changing mangrove cover affects ecosystem carbon storage in coastal wetlands. *Ecology*, *101*(2), e02916. https://doi.org/10.1002/ECY.2916

Chmura, G. L., Anisfeld, S. C., Cahoon, D. R., & Lynch, J. C. (2003). Global carbon sequestration in tidal, saline wetland soils. *Global Biogeochemical Cycles*, *17*(4). https://doi.org/10.1029/2002GB001917

Ciroth, A. (2012). *Refining the pedigree matrix approach in ecoinvent*. https://lca-net.com/files/Pedigree_report_final_May2012.pdf

Clark, M. A., Domingo, N. G. G., Colgan, K., Thakrar, S. K., Tilman, D., Lynch, J., Azevedo, I. L., & Hill, J. D. (2020). Global food system emissions could preclude achieving the 1.5° and 2°C climate change targets. *Science*, *370*(6517), 705–708. https://doi.org/10.1126/SCIENCE.ABA7357

Consequential-LCA. (2015a). *By-products, recycling and waste*. https://consequential-lca.org/clca/by-products-recycling-and-waste/

Consequential-LCA. (2015b). *The special case of electricity*. https://consequential-lca.org/clca/marginal-suppliers/the-special-case-of-electricity/

Costa, M. H., Yanagi, S. N. M., Souza, P. J. O. P., Ribeiro, A., & Rocha, E. J. P. (2007). Climate change in Amazonia caused by soybean cropland expansion, as compared to caused by pastureland expansion. *Geophysical Research Letters*, *34*(7). https://doi.org/10.1029/2007GL029271

Costa, M. T., Salinas-de-León, P., & Aburto-Oropeza, O. (2019). Storage of blue carbon in isolated mangrove forests of the Galapagos' rocky coast. *Wetlands Ecology and Management*, *27*(4), 455–463. https://doi.org/10.1007/S11273-019-09653-8/FIGURES/4

Cotterman, K. A., Kendall, A. D., Basso, B., & Hyndman, D. W. (2018). Groundwater depletion and climate change: future prospects of crop production in the Central High Plains Aquifer. *Climatic Change*, *146*(1–2), 187–200. https://doi.org/10.1007/S10584-017-1947-7/FIGURES/4

Crippa, M., Solazzo, E., Guizzardi, D., Monforti-Ferrario, F., Tubiello, F. N., & Leip, A. (2021). Food systems are responsible for a third of global anthropogenic GHG emissions. *Nature Food*, *2*(3), 198–209. https://doi.org/10.1038/s43016-021-00225-9

Crutzen, P. J. (2002). Geology of mankind. *Nature 2002 415:6867*, *415*(6867), 23–23. https://doi.org/10.1038/415023a

Cucurachi, S., Scherer, L., Guinée, J., & Tukker, A. (2019). Life Cycle Assessment of Food Systems. *One Earth*, *1*(3), 292–297. https://doi.org/10.1016/J.ONEEAR.2019.10.014

Cui, Z., Zhang, H., Chen, X., Zhang, C., Ma, W., Huang, C., Zhang, W., Mi, G., Miao, Y., Li, X., Gao, Q., Yang, J., Wang, Z., Ye, Y., Guo, S., Lu, J., Huang, J., Lv, S., Sun, Y., … Dou, Z. (2018). Pursuing sustainable productivity with millions of smallholder farmers. *Nature 2018 555:7696*, *555*(7696), 363–366. https://doi.org/10.1038/nature25785

Cumberlege, T., Blenkinsopp, T., & Clark, J. (2016). *Assessment of environmental impact of FeedKind protein*.

D'Odorico, P., Davis, K. F., Rosa, L., Carr, J. A., Chiarelli, D., Dell'Angelo, J., Gephart, J., MacDonald, G. K., Seekell, D. A., Suweis, S., & Rulli, M. C. (2018). The Global Food-Energy-Water Nexus. *Reviews of Geophysics*, *56*(3), 456–531. https://doi.org/10.1029/2017RG000591

Day Jr., J. W., Coronado-Molina, C., Vera-Herrera, F. R., Twilley, R. R., Rivera-Monroy, V. H., Alvarez-Guillen, H., Day, R., & Conner, W. (1996). A 7 year record of above-ground net primary production in a southeastern Mexican mangrove forest. *Aquatic Botany*, *55*, 39–60.

de Graaf, I. E. M., Gleeson, T., (Rens) van Beek, L. P. H., Sutanudjaja, E. H., & Bierkens, M. F. P. (2019). Environmental flow limits to global groundwater pumping. *Nature 2019 574:7776*, *574*(7776), 90–94. https://doi.org/10.1038/s41586-019-1594-4

Donato, D. C., Kauffman, J. B., Mackenzie, R. A., Ainsworth, A., Pfleeger, A. Z., & Pfleeger., A. Z. (2012). Whole-island carbon stocks in the tropical pacific: implications for mangrove conservation and upland restoration. *Journal of Environmental Management*, *97*, 89–96.

Donato, D. C., Kauffman, J. B., Murdiyarso, D., Kurnianto, S., Stidham, M., & Kanninen, M. (2011). Mangroves among the most carbon-rich forests in the tropics. *Nature Geoscience*, *4*(5), 293–297. https://doi.org/10.1038/ngeo1123

Donke, A. C. G., Novaes, R. M. L., Pazianotto, R. A. A., Moreno-Ruiz, E., Reinhard, J., Picoli, J. F., & Folegatti-Matsuura, M. I. da S. (2020). Integrating regionalized Brazilian land use change datasets into the ecoinvent database: new data, premises and uncertainties have large effects in the results. *International Journal of Life Cycle Assessment*, *25*(6), 1027–1042. https://doi.org/10.1007/S11367-020-01763-3/FIGURES/5

Dreyer, L. C., Hauschild, M. Z., & Schierbeck, J. (2010). Characterisation of social impacts in LCA: Part 1: Development of indicators for labour rights. *International Journal of Life Cycle Assessment*, *15*(3), 247–259. https://doi.org/10.1007/S11367-009-0148-7/TABLES/4

Duarte, C. M., & Cabrián, J. (1996). The fate of marine autotrophic production. *Limnology and Oceanography*, *41*(8), 1758–1766.

Duarte, C. M., Middelburg, J. J., & Caraco, N. (2005). Major role of marine vegetation on the oceanic carbon cycle. *Biogeosciences*, *2*, 1–8.

EC, J. (2012). *Emission Database for Global Atmospheric Research*.

Eibl, R., Senn, Y., Gubser, G., Jossen, V., Van Den Bos, C., & Eibl, D. (2021). Cellular Agriculture: Opportunities and Challenges. *Annual Review of Food Science and Technology*, *12*, 51–73. https://doi.org/10.1146/annurev-food-063020-123940

Eide, M. ., Homleid, J. ., & Mattsson, B. (2003). Life cycle assessment (LCA) of cleaning-in-place processes in dairies. *LWT - Food Science and Technology*, *36*(3), 303–314. https://doi.org/10.1016/S0023-6438(02)00211-6

Eong, O. J. (1993). Mangroves - a carbon source and sink. *Chemosphere*, *27*(6), 1097–1107.

Ercili-Cura, D., & Barth, D. (2021). *Cellular Agriculture: Lab Grown Foods*. ACS Publications. https://pubs.acs.org/doi/10.1021/acs.infocus.7e4007

Erisman, J. W., Bleeker, A., Galloway, J., & Sutton, M. S. (2007). Reduced nitrogen in ecology and the environment. *Environmental Pollution*, *150*(1), 140–149. https://doi.org/10.1016/J.ENVPOL.2007.06.033

Erisman, J. W., Sutton, M. A., Galloway, J., Klimont, Z., & Winiwarter, W. (2008). How a century of ammonia synthesis changed the world. *Nature Geoscience 2008 1:10*, *1*(10), 636–639. https://doi.org/10.1038/ngeo325

European Commission. (2019). *GMO legislation*. https://ec.europa.eu/food/plants/genetically-modified-organisms/gmo-legislation_fi

Eurostat. (2021). *Glossary:Carbon dioxide equivalent*. https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Glossary:Carbon_dioxide_equivalent

Evans, N. (2021). *Homemade shrimp burger*. Macheesmo. https://www.macheesmo.com/shrimp-burger-recipe/

FAO. (2005). *Cultured aquatic species information programme: Penaeus monodon*. http://www.fao.org/fishery/culturedspecies/Penaeus_monodon/en

FAO. (2009). *Penaeus monodon. In Cultured aquatic species fact sheets. Text by Kongkeo, H. Edited and compiled by Valerio Crespi and Michael New*. http://www.fao.org/fishery/docs/CDrom/aquaculture/I1129m/file/en/en_gianttigerprawn.htm

FAO. (2014). *Food Wastage Footprint*. https://www.fao.org/nr/sustainability/food-loss-and-waste

FAO. (2020a). *The State of Food and Agriculture 2020. Overcoming water challenges in agriculture*.

FAO. (2020b). *The state of the world fisheries and aquaculture. Sustainability in action*. https://doi.org/10.4060/ca9229en

FAO - Fisheries division. (2021). *FishstatJ - software for fishery statistical time series*. http://www.fao.org/fishery/statistics/software/fishstatj/en

FAO, IFAD, UNICEF, WFP, & WHO. (2020). *The State of Food Security and Nutrition in the World 2020. Transforming food systems for affordable healthy diets*. https://www.fao.org/documents/card/en/c/ca9692en

FAO, IFAD, UNICEF, WFP, & WHO. (2021). *The State of Food Security and Nutrition in the World 2021. Transforming food systems for food security, improved nutrition and affordable healthy diets for all.*

FAOSTAT. (2021). *Crops and livestock products*. https://www.fao.org/faostat/en/#data/QCL

FAOSTAT. (2022). *Food Balances (2010-)*. https://www.fao.org/faostat/en/#data/FBS

FDA. (2022). *How GMOs Are Regulated for Food and Plant Safety in the United States*. https://www.fda.gov/food/agricultural-biotechnology/how-gmos-are-regulated-food-and-plant-safety-united-states

Forouzanfar, M. H., Alexander, L., Anderson, H. R., Bachman, V. F., Biryukov, S., Brauer, M., Burnett, R., Casey, D., Coates, M. M., Cohen, A., Delwiche, K., Estep, K., Frostad, J. J., KC, A., Kyu, H. H., Moradi-Lakeh, M., Ng, M., Slepak, E. L., Thomas, B. A., … Murray, C. J. (2015). Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. In *The Lancet* (Vol. 386, Issue 10010). Elsevier. https://doi.org/10.1016/S0140-6736(15)00128-2

Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D. W., Haywood, J., Lean, J., Lowe, D. C., Myhre, G., Nganga, J., Prinn, R., Raga, G., Schulz, M., & Van Dorland, R. (2007). *Changes in Atmospheric Constituents and in Radiative Forcing. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Ma.*

Friess, D. A., Phelps, J., Leong, R., Lee, W. K., Wee, A. K. S., Sivasothi, N., Oh, R. R. Y., & Webb, E. L. (2012). Mandai mangrove, Singapore: Lessons for the conservation of Southeast Asia's mangroves. *The Raffles Bulleting of Zoology*, *25*, 55–65. https://www.researchgate.net/publication/258312755

Gephart, J. A., Davis, K. F., Emery, K. A., Leach, A. M., Galloway, J. N., & Pace, M. L. (2016). The environmental cost of subsistence: Optimizing diets to minimize footprints. *Science of The Total Environment*, *553*, 120–127. https://doi.org/10.1016/J.SCITOTENV.2016.02.050

Gephart, J. A., Henriksson, P. J. G., Parker, R. W. R., Shepon, A., Gorospe, K. D., Bergman, K., Eshel, G., Golden, C. D., Halpern, B. S., Hornborg, S., Jonell, M., Metian, M., Mifflin, K., Newton, R., Tyedmers, P., Zhang, W., Ziegler, F., & Troell, M. (2021). Environmental performance of blue foods. *Nature 2021 597:7876*, *597*(7876), 360–365. https://doi.org/10.1038/s41586-021-03889-2

Gésan-Guiziou, G., Sobańtka, A. P., Omont, S., Froelich, D., Rabiller-Baudry, M., Thueux, F., Beudon, D., Tregret, L., Buson, C., & Auffret, D. (2019). Life Cycle Assessment of a milk protein fractionation process: Contribution of the production and the cleaning stages at unit process level. *Separation and Purification Technology*, *224*, 591–610. https://doi.org/10.1016/j.seppur.2019.05.008

Giri, C., Ochieng, E., Tieszen, L. L., Zhu, Z., Singh, a., Loveland, T., Masek, J., & Duke, N. (2011). Status and distribution of mangrove forests of the world using earth observation satellite data. *Global Ecology and Biogeography*, *20*(1), 154–159. https://doi.org/10.1111/j.1466-8238.2010.00584.x

Gochev, G., Ulaganathan, V., & Miller, R. (2016). Foams. *Ullmann's Encyclopedia of Industrial Chemistry*, 1–31. https://doi.org/10.1002/14356007.A11_465.PUB2

Godfray, H. C. J., Aveyard, P., Garnett, T., Hall, J. W., Key, T. J., Lorimer, J., Pierrehumbert, R. T., Scarborough, P., Springmann, M., & Jebb, S. A. (2018). Meat consumption, health, and the environment. In *Science (New York, N.Y.)* (Vol. 361, Issue 6399). https://doi.org/10.1126/science.aam5324

Goglio, P., Smith, W. N., Grant, B. B., Desjardins, R. L., McConkey, B. G., Campbell, C. A., & Nemecek, T. (2015). Accounting for soil carbon changes in agricultural life cycle assessment (LCA): A review. In *Journal of Cleaner Production* (Vol. 104, pp. 23–39). Elsevier Ltd. https://doi.org/10.1016/j.jclepro.2015.05.040

Google. (2013). *Google Earth*. http://www.google.com/earth/index.html

GRAIN, Greenpeace, & IATP. (2021). *New research shows 50 year binge on chemical fertilisers must end to address the climate crisis*. https://grain.org/en/article/6761-new-research-shows-50-year-binge-on-chemical-fertilisers-must-end-to-address-the-climate-crisis#_ftn3

Groen, E. A., Bokkers, E. A. M., Heijungs, R., & de Boer, I. J. M. (2017). Methods for global sensitivity analysis in life cycle assessment. *International Journal of Life Cycle Assessment*, *22*(7), 1125–1137. https://doi.org/10.1007/S11367-016-1217-3/FIGURES/8

Guinée, J. B. (2016). Life Cycle Sustainability Assessment: What Is It and What Are Its Challenges? In Clift, R. & A. Druckman (Eds.), *Taking Stock of Industrial Ecology*. Springer, Cham.

Guinée, J. B., Heijungs, R., Huppes, G., Zamagni, A., Masoni, P., Buonamici, R., Ekvall, T., & Rydberg, T. (2011). Life Cycle Assessment: Past, Present, and Future. *Environmental Science & Technology*, *45*(1), 90–96. https://doi.org/10.1021/es101316v

Gütschow, J., Jeffery, L., & Gieseke, R. (2019). *The PRIMAP-hist national historical emissions time series (1850-2016), version 2.0., GFZ Data Services*. https://doi.org/10.5880/pik.2019.001

Guzman, H. M., Barnes, P. A. G., Lovelock, C. E., & Feller, I. C. (2005). A site description of the CARICOMP mangrove, seagrass and Coral Reef Sites in the Bocas del Toro, Panama. *Caribbean Journal of Science*, *41*(3), 430–440.

Hallström, E., Bergman, K., Mifflin, K., Parker, R., Tyedmers, P., Troell, M., & Ziegler, F. (2019). Combined climate and nutritional performance of seafoods. *Journal of Cleaner Production*, *230*, 402–411. https://doi.org/10.1016/J.JCLEPRO.2019.04.229

Heijungs, R. (2020). On the number of Monte Carlo runs in comparative probabilistic LCA. *International Journal of Life Cycle Assessment*, *25*(2), 394–402. https://doi.org/10.1007/s11367-019-01698-4

Heijungs, R. (2021). Selecting the best product alternative in a sea of uncertainty. *International Journal of Life Cycle Assessment*, *26*(3), 616–632. https://doi.org/10.1007/s11367-020-01851-4

Heijungs, R., Allacker, K., Benetto, E., Brandão, M., Guinée, J., Schaubroeck, S., Schaubroeck, T., & Zamagni, A. (2021). System Expansion and Substitution in LCA: A Lost Opportunity of ISO 14044 Amendment 2. *Frontiers in Sustainability*, *2*. https://doi.org/10.3389/frsus.2021.692055

Henriksson, P. J. G., Rico, A., Zhang, W., Ahmad-Al-Nahid, S., Newton, R., Phan, L. T., Zhang, Z., Jaithiang, J., Dao, H. M., Phu, T. M., Little, D. C., Murray, F. J., Satapornvanit, K., Liu, L., Liu, Q., Haque, M. M., Kruijssen, F., de Snoo, G. R., Heijungs, R., … Guinée, J. B. (2015). Comparison of Asian Aquaculture Products by Use of Statistically Supported Life Cycle Assessment. *Environmental Science & Technology*, *49*(24), 14176–14183. https://doi.org/10.1021/acs.est.5b04634

Hoekstra, A. Y., Chapagain, A. K., Aldaya, M. M., & Mekonnen, M. M. (2011). *The water footprint assessment manual : setting the global standard*. Earthscan. https://books.google.fi/books?hl=fi&lr=&id=BCUNT_wLnScC&oi=fnd&pg=PR3&ots=M-SB2geVC4&sig=8yPz0Wbu6nEfRnV7y01UY7PxziA&redir_esc=y#v=onepage&q&f=false

Howarth, R. W., & Marino, R. (2006). Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. *Limnology and Oceanography*, *51*(1part2), 364–376. https://doi.org/10.4319/lo.2006.51.1_part_2.0364

HSY. (2019). *Where does your drinking water come from?* Helsinki Region Environmental Services Authority. https://hsyk01mstrxfa10prod.dxcloud.episerver.net/en/water-and-sewers/how-the-water-supply-works/#Veden hankinta ja puhdistus

Hu, Z., Lee, J. W., Chandran, K., Kim, S., & Khanal, S. K. (2012). Nitrous oxide (N_2O) emission from aquaculture: a review. *Environmental Science & Technology*, *46*(12), 6470–6480. https://doi.org/10.1021/es300110x

Huijbregts, M. A. J., Steinmann, Z. J. N., Elshout, P. M. F., Stam, G., Verones, F., Vieira, M., Zijp, M., Hollander, A., & van Zelm, R. (2017). ReCiPe2016: a harmonised life cycle impact assessment method at midpoint and endpoint level. *International Journal of Life Cycle Assessment*, *22*(2), 138–147. https://doi.org/10.1007/s11367-016-1246-y

Humpenöder, F., Bodirsky, B. L., Weindl, I., Lotze-Campen, H., Linder, T., & Popp, A. (2022). Projected environmental benefits of replacing beef with microbial protein. *Nature 2022 605:7908*, *605*(7908), 90–96. https://doi.org/10.1038/s41586-022-04629-w

IPBES. (2019). *Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services.*

IPCC. (2006a). *2006 IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme, Eggleston H.S., Buendia L., Miwa K., Ngara T. and Tanabe K. (eds).* http://www.ipcc-nggip.iges.or.jp/public/2006gl/

IPCC. (2006b). *Emission factor database*. https://www.ipcc-nggip.iges.or.jp/EFDB/find_ef.php

IPCC. (2012). *Managing the risks of extreme events and disasters to advance climate change adaptation. A special report of Working Groups I and II of the IPCC (Intergovernmental Panel on Climate Change). C.B. Field et al., Eds. (Cambridge University Press, Cambridge, U.*

IPCC. (2014). *2013 Supplement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories: Wetlands. Hiraishi, T, Krug, T, Tanabe, K, Srivastava, N, Baasansuren, J, Fukuda, M and Troxler, TG (eds)*. http://www.ipcc-nggip.iges.or.jp/public/wetlands/pdf/Wetlands_separate_files/WS_Chp4_Coastal_Wetlands.pdf

ISCGM. (2003). *Land Cover (GLCNMO) - Global version*. http://www.iscgm.org/gm/glcnmo.html

ISO. (2006). *ISO 14044; Environmental management—Life cycle assessment— Requirements and guidelines*.

Jain, M., Fishman, R., Mondal, P., Galford, G. L., Bhattarai, N., Naeem, S., Lall, U., Balwinder-Singh, & DeFries, R. S. (2021). Groundwater depletion will reduce cropping intensity in India. *Science Advances*, *7*(9). https://doi.org/10.1126/SCIADV.ABD2849/SUPPL_FILE/ABD2849_SM.PDF

Jennerjahn, T. C., & Ittekkot, V. (2004). Relevance of mangroves for the production and deposition of organic matter along tropical continental margins. *Naturwissenschaften*, *89*(1), 23–30. https://doi.org/10.1007/s00114-001-0283-x

Johnson, C. K., Hitchens, P. L., Pandit, P. S., Rushmore, J., Evans, T. S., Young, C. C. W., & Doyle, M. M. (2020). Global shifts in mammalian population trends reveal key predictors of virus spillover risk. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1924). https://doi.org/10.1098/rspb.2019.2736

Johnson, J. L., Raw, J. L., & Adams, J. B. (2020). First report on carbon storage in a warm-temperate mangrove forest in South Africa. *Estuarine, Coastal and Shelf Science*, *235*, 106566. https://doi.org/10.1016/J.ECSS.2019.106566

Jonell, M., & Henriksson, P. J. G. (2015). Mangrove-shrimp farms in Vietnam - comparing organic and conventional systems using life cycle assessment. *Aquaculture*, *447*, 66–75. https://doi.org/10.1016/j.aquaculture.2014.11.001

Kägi, T., Dinkel, F., Frischknecht, R., Humbert, S., Lindberg, J., De Mester, S., Ponsioen, T., Sala, S., & Schenker, U. W. (2015). Session "Midpoint, endpoint or single score for decision-making?"—SETAC Europe 25th Annual Meeting, May 5th, 2015. *The International Journal of Life Cycle Assessment 2015 21:1*, *21*(1), 129–132. https://doi.org/10.1007/S11367-015-0998-0

Kaiser, M. J., Attrill, M. J., Jennings, S., Thomas, D. N., Barnes, D. K. A., Brierley, A. S., Hiddink, J. G., Kaartokallio, H., Polunin, N. V. C., & Raffaelli, D. G. (2005). *Marine ecology* (Second). Oxford University Press.

Kaplan, J. O., Krumhardt, K. M., & Zimmermann, N. (2009). The prehistoric and preindustrial deforestation of Europe. *Quanternary Science Reviews*, *28*, 3016–3034. https://doi.org/10.1016/j.quascirev.2009.09.028

Kassem, R., Jepsen, M. R., & Salhofer, S. P. (2021). The water consumption of different diets in Denmark. *Journal of Cleaner Production*, *286*, 124938. https://doi.org/10.1016/J.JCLEPRO.2020.124938

Kauffman, J. B., Heider, C., Cole, T. G., Dwire, K. a., & Donato, D. C. (2011). Ecosystem Carbon Stocks of Micronesian Mangrove Forests. *Wetlands*, *31*(2), 343–352. https://doi.org/10.1007/s13157-011-0148-9

Khorrami, M., & Malekmohammadi, B. (2021). Effects of excessive water extraction on groundwater ecosystem services: Vulnerability assessments using biophysical approaches. *Science of The Total Environment*, *799*, 149304. https://doi.org/10.1016/J.SCITOTENV.2021.149304

Komiyama, A., Ogina, K., Aksornkoae, S., & Sabhasri, S. (1987). Root biomass of a mangrove forest in southern Thailand. 1. Estimation by the trench method and the zonal structure of root biomass. *Journal of Tropical Ecology*, *3*, 97–108.

Koppelmäki, K., Parviainen, T., Virkkunen, E., Winquist, E., Schulte, R. P. O., & Helenius, J. (2019). Ecological intensification by integrating biogas production into nutrient cycling: Modeling the case of Agroecological Symbiosis. *Agricultural Systems*, *170*, 39–48. https://doi.org/10.1016/J.AGSY.2018.12.007

Krauss, J., Bommarco, R., Guardiola, M., Heikkinen, R. K., Helm, A., Kuussaari, M., Lindborg, R., Öckinger, E., Pärtel, M., Pino, J., Pöyry, J., Raatikainen, K. M., Sang, A., Stefanescu, C., Teder, T., Zobel, M., & Steffan-Dewenter, I. (2010). Habitat fragmentation causes immediate and time-delayed biodiversity loss at different trophic levels. *Ecology Letters*, *13*(5), 597–605. https://doi.org/10.1111/J.1461-0248.2010.01457.X

Kristensen, E., Bouillon, S., Dittmar, T., & Marchand, C. (2008). Organic carbon dynamics in mangrove ecosystems: A review. *Aquatic Botany*, *89*(2), 201–219. https://doi.org/10.1016/j.aquabot.2007.12.005

Lal, R. (2020). Regenerative agriculture for food and climate. *Journal of Soil and Water Conservation*, *75*(5), 123A-124A. https://doi.org/10.2489/JSWC.2020.0620A

Lebel, L., Tri, N. H., Saengnoree, A., Pasong, S., Buatama, U., & Thoa, L. K. (2002). Industrial transformation and shrimp aquaculture in Thailand and Vietnam: pathways to ecological, social, and economic sustainability? *Ambio*, *31*(4), 311–323. http://www.ncbi.nlm.nih.gov/pubmed/12174602

Lee, J. S., Lee, M. H., Chun, Y. Y., & Lee, K. M. (2018). Uncertainty analysis of the water scarcity footprint based on the AWARE model considering temporal variations. *Water (Switzerland)*, *10*(3), 1–13. https://doi.org/10.3390/w10030341

Lemaire, A., & Limbourg, S. (2019). How can food loss and waste management achieve sustainable development goals? *Journal of Cleaner Production*, *234*, 1221–1234. https://doi.org/10.1016/J.JCLEPRO.2019.06.226

Linder, T. (2019). Making the case for edible microorganisms as an integral part of a more sustainable and resilient food production system. *Food Security*, *11*(2), 265–278. https://doi.org/10.1007/S12571-019-00912-3/FIGURES/2

Lindsey, R. (2020). *Climate Change: Atmospheric Carbon Dioxide*. NOAA. https://www.climate.gov/news-features/understanding-climate/climate-change-atmospheric-carbon-dioxide

Liu, C., Colón, B. C., Ziesack, M., Silver, P. A., & Nocera, D. G. (2016). Water splitting-biosynthetic system with CO_2 reduction efficiencies exceeding photosynthesis. *Science*, *352*(6290), 1210–1213. https://doi.org/10.1126/science.aaf5039

Louisiana Direct Seafood. (2011). *Seafood handbook: Shrimp story*. http://louisianadirectseafood.com/handbook

Lovelock, C. E. (2008). Soil Respiration and Belowground Carbon Allocation in Mangrove Forests. *Ecosystems*, *11*(2), 342–354. https://doi.org/10.1007/s10021-008-9125-4

LUKE. (2021). *Energy consumption of agriculture and horticulture*. https://statdb.luke.fi/PXWeb/pxweb/en/LUKE/LUKE__02 Maatalous__02 Rakenne__08 Maa- ja puutarhatalouden energiankulutus/01_Maa_ja_puutarhatal_energiankulutus.px/table/tableViewLayout2/

Lunstrum, A., & Chen, L. (2014). Soil carbon stocks and accumulation in young mangrove forests. *Soil Biology and Biochemistry*, *75*, 223–232. https://doi.org/10.1016/j.soilbio.2014.04.008

Lynch, J., & Pierrehumbert, R. (2019). Climate Impacts of Cultured Meat and Beef Cattle. *Frontiers in Sustainable Food Systems*, *3*, 5. https://doi.org/10.3389/FSUFS.2019.00005/BIBTEX

Makel, M. C., & Plucker, J. A. (2014). Facts Are More Important Than Novelty: Replication in the Education Sciences. *Http://Dx.Doi.Org/10.3102/0013189X14545513*, *43*(6), 304–316. https://doi.org/10.3102/0013189X14545513

Malone, T. C., & Newton, A. (2020). The Globalization of Cultural Eutrophication in the Coastal Ocean: Causes and Consequences. In *Frontiers in Marine Science* (Vol. 7, p. 670). Frontiers Media S.A. https://doi.org/10.3389/fmars.2020.00670

Markets & Markets. (2019). *Egg Powder Market: Global Industry Trends, Share, Size, Growth, Opportunity and Forecast 2021-2026*. https://www.researchandmarkets.com/reports/5264115/

Marques, A., Martins, I. S., Kastner, T., Plutzar, C., Theurl, M. C., Eisenmenger, N., Huijbregts, M. A. J., Wood, R., Stadler, K., Bruckner, M., Canelas, J., Hilbers, J. P., Tukker, A., Erb, K., & Pereira, H. M. (2019). Increasing impacts of land use on biodiversity and carbon sequestration driven by population and economic growth. *Nature Ecology & Evolution 2019 3:4*, *3*(4), 628–637. https://doi.org/10.1038/s41559-019-0824-3

Matassa, S., Boon, N., Pikaar, I., & Verstraete, W. (2016). Microbial protein: future sustainable food supply route with low environmental footprint. *Microbial Biotechnology*, *9*(5), 568–575. https://doi.org/10.1111/1751-7915.12369

Matsui, N. (1998). Estimated stocks of organic carbon in mangrove roots and sediments in Hinchinbrook channel, Australia. *Mangroves and Salt Marshes*, *2*, 199–204.

Mattick, C. S. (2018). Cellular agriculture: The coming revolution in food production. *Bulletin of the Atomic Scientists*, *74*(1), 32–35. https://doi.org/10.1080/00963402.2017.1413059

Mattick, C. S., Landis, A. E., Allenby, B. R., & Genovese, N. J. (2015). Anticipatory Life Cycle Analysis of In Vitro Biomass Cultivation for Cultured Meat Production in the United States. *Environmental Science and Technology*, *49*(19), 11941–11949. https://doi.org/10.1021/acs.est.5b01614

Mazoyer, M., & Roudart, L. (2006). *A History of World Agriculture: From the Neolithic Age to the Current Crisis* (English ed). Monthly review press.

Mclaren, S., Berardy, A., Henderson, A., Holden, N., Huppertz, T., Jolliet, O., De Camillis, C., Renouf, M., Ru-Gani, B., Saarinen, M., Van Der Pols, J., Vázquez-Rowe, I., Antón Vallejo, A., Bianchi, M., Chaudhary, A., Chen, C., Cooreman-Algo-Ed, M., Dong, H., Grant, T., … Van Zanten, H. (2021). *Integration of environment and nutrition in life cycle assessment of food items: opportunities and challenges*. www.fao.org/

Mcleod, E., Chmura, G. L., Bouillon, S., Salm, R., Björk, M., Duarte, C. M., Lovelock, C. E., Schlesinger, W. H., & Silliman, B. R. (2011). A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO 2. *Frontiers in Ecology and the Environment*, *9*(10), 552–560. https://doi.org/10.1890/110004

McNally, R., McEwin, A., & Holland, T. (2010). *The potential for mangrove carbon projects in Vietnam*. http://www.bibalex.org/search4dev/files/419125/442099.pdf

Mendoza Beltran, A., Prado, V., Font Vivanco, D., Henriksson, P. J. G., Guinée, J. B., & Heijungs, R. (2018). Quantified Uncertainties in Comparative Life Cycle Assessment: What Can Be Concluded? *Environmental Science and Technology*, *52*(4), 2152–2161. https://doi.org/10.1021/acs.est.7b06365

Metzger, M. J., Rounsevell, M. D. A., Acosta-Michlik, L., Leemans, R., & Schröter, D. (2006). The vulnerability of ecosystem services to land use change. *Agriculture, Ecosystems & Environment*, *114*(1), 69–85. https://doi.org/10.1016/J.AGEE.2005.11.025

Middleton, B. a., & McKee, K. L. (2001). Degradation of mangrove tissues and implications for peat formation in Belizean island forests. *Journal of Ecology*, *89*(5), 818–828. https://doi.org/10.1046/j.0022-0477.2001.00602.x

Ministry of Economic affairs and employment in Finland. (2018). *Renewable Energy in Finland*. https://tem.fi/en/renewable-energy

Mirzabaev, A., Wu, J., Evans, J., García-Oliva, F., Hussein, I. A. G., Iqbal, M. H., Kimutai, J., Knowles, T., Meza, F., Nedjraoui, D., Tena, F., Türkeş, M., Vázquez, R. J., & Welt, M. (2019). *Desertification. In: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems.*

Moro, A., & Lonza, L. (2018). Electricity carbon intensity in European Member States: Impacts on GHG emissions of electric vehicles. *Transportation Research Part D: Transport and Environment*, *64*, 5–14. https://doi.org/10.1016/j.trd.2017.07.012

Murray, B. C., Watt, C. E., Cooley, D. M., & Pendleton, L. H. (2012). *Coastal Blue Carbon and the United Nations Current Status and Future Directions; Policy brief* (Issue September). http://www.nicholasinstitute.duke.edu/

Murray, F. J., Haque, M. M., Zhang, W., Thanh, L. P., Nietes-Satapornvanit, A., & Little, D. C. (2013). *Defining boundaries towards understanding sustainable ethical aquaculture trade between Asia and Europe. SEAT Project Report 2.8.*

Nagelkerken, I., Blaber, S. J. M., Bouillon, S., Green, P., Haywood, M., Kirton, L. G., Meynecke, J. O., Pawlik, J., Penrose, H. M., Sasekumar, A., & Somerfield, P. J. (2008). The habitat function of mangroves for terrestrial and marine fauna: A review. *Aquatic Botany*, *89*(2), 155–185. https://doi.org/10.1016/J.AQUABOT.2007.12.007

NASA. (2021). *Overview: Weather, Global Warming and Climate Change*. https://climate.nasa.gov/resources/global-warming-vs-climate-change/

Nemecek, T., Schnetzer, J., & Reinhard, J. (2016). Updated and harmonised greenhouse gas emissions for crop inventories. *International Journal of Life Cycle Assessment*, *21*(9), 1361–1378. https://doi.org/10.1007/s11367-014-0712-7

New harvest. (2021). *What is cellular agriculture?* https://new-harvest.org/what-is-cellular-agriculture/

Newbold, T., Hudson, L. N., Hill, S. L. L., Contu, S., Lysenko, I., Senior, R. A., Börger, L., Bennett, D. J., Choimes, A., Collen, B., Day, J., De Palma, A., Díaz, S., Echeverria-Londoño, S., Edgar, M. J., Feldman, A., Garon, M., Harrison, M. L. K., Alhusseini, T., … Purvis, A. (2015). Global effects of land use on local terrestrial biodiversity. *Nature 2015 520:7545*, *520*(7545), 45–50. https://doi.org/10.1038/nature14324

Newton, P., & Blaustein-Rejto, D. (2021). Social and Economic Opportunities and Challenges of Plant-Based and Cultured Meat for Rural Producers in the US. *Frontiers in Sustainable Food Systems*, *5*, 10. https://doi.org/10.3389/FSUFS.2021.624270/BIBTEX

Noya, L. I., Vasilaki, V., Stojceska, V., González-García, S., Kleynhans, C., Tassou, S., Moreira, M. T., & Katsou, E. (2018). An environmental evaluation of food supply chain using life cycle assessment: A case study on gluten free biscuit products. *Journal of Cleaner Production*, *170*, 451–461. https://doi.org/10.1016/j.jclepro.2017.08.226

Orchard, S. E., Stringer, L. C., & Quinn, C. H. (2016). Mangrove system dynamics in Southeast Asia: linking livelihoods and ecosystem services in Vietnam. *Regional Environmental Change*, *16*(3), 865–879. https://doi.org/10.1007/S10113-015-0802-5/TABLES/6

Pandey, D., Agrawal, M., & Pandey, J. S. (2011). Carbon footprint: current methods of estimation. *Environmental Monitoring and Assessment*, *178*(1–4), 135–160. https://doi.org/10.1007/s10661-010-1678-y

Papatryphon, E., Petit, J., van der Werf, H. M. G., & Kaushik, S. J. (2003). *Life Cycle Assessment of trout farming in France: a farm level approach.*

Parodi, A., Leip, A., De Boer, I. J. M., Slegers, P. M., Ziegler, F., Temme, E. H. M., Herrero, M., Tuomisto, H., Valin, H., Van Middelaar, C. E., Van Loon, J. J. A., & Van Zanten, H. H. E. (2018). The potential of future foods for sustainable and healthy diets. *Nature Sustainability*, *1*(12), 782–789. https://doi.org/10.1038/s41893-018-0189-7

Perfect Day Inc. (2021). *ISO-conformant report: Comparatove GHG emissions assessment of Perfect Day whey protein production to dairy protein.*

Phan, L. T., Nguyen, P. T., Murray, F. J., & Little, C. D. (2011). *Development trends and local sustainability perceptions for the international trade in seafood farmed in Vietnam. Deliverable 2.1c of the SEAT project.*

Phillips, S. M. (2017). Current Concepts and Unresolved Questions in Dietary Protein Requirements and Supplements in Adults. *Frontiers in Nutrition*, *4*, 13. https://doi.org/10.3389/FNUT.2017.00013/BIBTEX

Pikaar, I., de Vrieze, J., Rabaey, K., Herrero, M., Smith, P., & Verstraete, W. (2018). Carbon emission avoidance and capture by producing in-reactor microbial biomass based food, feed and slow release fertilizer: Potentials and limitations. *Science of the Total Environment*, *644*, 1525–1530. https://doi.org/10.1016/j.scitotenv.2018.07.089

Pikaar, I., Matassa, S., Bodirsky, B. L., Weindl, I., Humpenöder, F., Rabaey, K., Boon, N., Bruschi, M., Yuan, Z., Van Zanten, H., Herrero, M., Verstraete, W., & Popp, A. (2018). Decoupling Livestock from Land Use through Industrial Feed Production Pathways. *Environmental Science and Technology*, *52*(13), 7351–7359. https://doi.org/10.1021/acs.est.8b00216

Poore, J., & Nemecek, T. (2018a). *European data subset from the study described in the article 'Reducing Food's Environmental Impacts through Producers and Consumers.' Published in 2018 in Science. Obtained by J Poore in June 2020.*

Poore, J., & Nemecek, T. (2018b). Reducing food's environmental impacts through producers and consumers. *Science*, *360*(6392), 987–992. https://doi.org/10.1126/science.aaq0216

Pradeepkiran, J. A. (2019). Aquaculture role in global food security with nutritional value: a review. *Translational Animal Science*, *3*(2), 903–910. https://doi.org/10.1093/TAS/TXZ012

PRé Consultants. (2020). *Simapro*. https://www.pre-sustainability.com/sustainability-consulting/sustainable-practices/custom-sustainability-software

Ray, D. K., West, P. C., Clark, M., Gerber, J. S., Prishchepov, A. V., & Chatterjee, S. (2019). Climate change has likely already affected global food production. *PLOS ONE*, *14*(5), e0217148. https://doi.org/10.1371/JOURNAL.PONE.0217148

Ray, R., Ganguly, D., Chowdhury, C., Dey, M., Das, S., Dutta, M. K., Mandal, S. K., Majumder, N., De, T. K., Mukhopadhyay, S. K., & Jana, T. K. (2011). Carbon sequestration and annual increase of carbon stock in a mangrove forest. *Atmospheric Environment*, *45*(28), 5016–5024. https://doi.org/10.1016/j.atmosenv.2011.04.074

Richards, D. R., & Friess, D. A. (2015). Rates and drivers of mangrove deforestation in Southeast Asia, 2000-2012. *Proceedings of the National Academy of Sciences of the United States of America*, *Early Edit*, 1–6. https://doi.org/10.1073/pnas.1510272113

Rischer, H., Szilvay, G. R., & Oksman-Caldentey, K. M. (2020). Cellular agriculture — industrial biotechnology for food and materials. In *Current Opinion in Biotechnology* (Vol. 61, pp. 128–134). Elsevier Ltd. https://doi.org/10.1016/j.copbio.2019.12.003

Ritala, A., Häkkinen, S. T., Toivari, M., & Wiebe, M. G. (2017). Single Cell Protein—State-of-the-Art, Industrial Landscape and Patents 2001–2016. *Frontiers in Microbiology*, *8*(OCT), 2009. https://doi.org/10.3389/fmicb.2017.02009

Ritchie, H., & Roser, M. (2019). *Half of the world's habitbale land is used for agriculture*. OurWorldinData.Org. https://ourworldindata.org/global-land-for-agriculture#licence

Ritchie, H., Roser, M., & Rosado, P. (2020). *CO2 and Greenhouse Gas Emissions*. Published Online at OurWorldInData.Org. https://ourworldindata.org/emissions-by-sector

Rogelj, J., Shindell, D., Jiang, K., Fifita, S., Forster, P., Ginzburg, V., Handa, C., Kheshgi, H., Kobayashi, S., Kriegler, E., Mundaca, L., Séférian, R., & Vilariño, M. . (2018). *Mitigation Pathways Compatible with 1.5°C in the Context of Sustainable Development. In: Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathw.*

Roy, P., Nei, D., Orikasa, T., Xu, Q., Okadome, H., Nakamura, N., & Shiina, T. (2009). A review of life cycle assessment (LCA) on some food products. *Journal of Food Engineering*, *90*(1), 1–10. https://doi.org/10.1016/j.jfoodeng.2008.06.016

Saarinen, M., Fogelholm, M., Tahvonen, R., & Kurppa, S. (2017). Taking nutrition into account within the life cycle assessment of food products. *Journal of Cleaner Production*, *149*, 828–844. https://doi.org/10.1016/j.jclepro.2017.02.062

Saarinen, M., Kurppa, S., Virtanen, Y., Usva, K., Mäkelä, J., & Nissinen, A. (2012). Life cycle assessment approach to the impact of home-made, ready-to-eat and school lunches on climate and eutrophication. *Journal of Cleaner Production*, *28*, 177–186. https://doi.org/10.1016/J.JCLEPRO.2011.11.038

Sanders, C. J., Smoak, J. M., Naidu, A. S., Sanders, L. M., & Patchineelam, S. R. (2010). Organic carbon burial in a mangrove forest, margin and intertial mud flat. *Esturine, Coastal and Shelf Science*, *30*(3), 168–172.

Santos, H. C. M., Maranduba, H. L., de Almeida Neto, J. A., & Rodrigues, L. B. (2017). Life cycle assessment of cheese production process in a small-sized dairy industry in Brazil. *Environmental Science and Pollution Research*, *24*(4), 3470–3482. https://doi.org/10.1007/s11356-016-8084-0

Schaubroeck, T., Schaubroeck, S., Heijungs, R., Zamagni, A., Brandão, M., & Benetto, E. (2021). Attributional & Consequential Life Cycle Assessment: Definitions, Conceptual Characteristics and Modelling Restrictions. *Sustainability 2021, Vol. 13, Page 7386*, *13*(13), 7386. https://doi.org/10.3390/SU13137386

Schmidt, J. H., Weidema, B. P., & Brandão, M. (2015). A framework for modelling indirect land use changes in Life Cycle Assessment. *Journal of Cleaner Production*, *99*, 230–238. https://doi.org/10.1016/J.JCLEPRO.2015.03.013

Schyns, J. F., Hoekstra, A. Y., & Booij, M. J. (2015). Review and classification of indicators of green water availability and scarcity. *Hydrology and Earth System Sciences*, *19*(11), 4581–4608. https://doi.org/10.5194/HESS-19-4581-2015

Schyns, J. F., Hoekstra, A. Y., Booij, M. J., Hogeboom, R. J., & Mekonnen, M. M. (2019). Limits to the world's green water resources for food, feed, fiber, timber, and bioenergy. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(11), 4893–4898. https://doi.org/10.1073/PNAS.1817380116/-/DCSUPPLEMENTAL

Shukla, P. R., Skea, J., Slade, R., Diemen, R. van, Haughey, E., Malley, J., Pathak, M., Pereira, J. P., & (eds.). (2019). *Technical Summary, 2019. In: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems [P.R. Shukla, J. Skea, E. Ca.*

Siegrist, M., & Hartmann, C. (2020). Consumer acceptance of novel food technologies. *Nature Food 2020 1:6*, *1*(6), 343–350. https://doi.org/10.1038/s43016-020-0094-x

Sillman, J., Nygren, L., Kahiluoto, H., Ruuskanen, V., Tamminen, A., Bajamundi, C., Nappa, M., Wuokko, M., Lindh, T., Vainikka, P., Pitkänen, J. P., & Ahola, J. (2019). Bacterial protein for food and feed generated via renewable energy and direct air capture of CO_2: Can it reduce land and water use? In *Global Food Security* (Vol. 22, pp. 25–32). Elsevier B.V. https://doi.org/10.1016/j.gfs.2019.09.007

Sillman, J., Uusitalo, V., Ruuskanen, V., Ojala, L., Kahiluoto, H., Soukka, R., & Ahola, J. (2020). A life cycle environmental sustainability analysis of microbial protein production via power-to-food approaches. *International Journal of Life Cycle Assessment*, 1–14. https://doi.org/10.1007/s11367-020-01771-3

Simmons, T. (2000). *Good Practice Guidance and Uncertainty Management in National Greenhouse Gas Inventories: CO_2 Emissions from Stationary Combustion of Fossil Fuels*. https://www.ipcc-nggip.iges.or.jp/public/gp/bgp/2_1_CO2_Stationary_Combustion.pdf

Smetana, S., Mathys, A., Knoch, A., & Heinz, V. (2015). Meat alternatives: life cycle assessment of most known meat substitutes. *International Journal of Life Cycle Assessment*, *20*(9), 1254–1267. https://doi.org/10.1007/s11367-015-0931-6

Smetana, S., Palanisamy, M., Mathys, A., & Heinz, V. (2016). Sustainability of insect use for feed and food: Life Cycle Assessment perspective. *Journal of Cleaner Production*, *137*, 741–751. https://doi.org/10.1016/j.jclepro.2016.07.148

Smetana, S., Schmitt, E., & Mathys, A. (2019). Sustainable use of Hermetia illucens insect biomass for feed and food: Attributional and consequential life cycle assessment. *Resources, Conservation and Recycling*, *144*, 285–296. https://doi.org/10.1016/j.resconrec.2019.01.042

Smil, V. (2001). *Enriching the Earth: Fritz Haber, Carl Bosch, and the Transformation of the World Food Production*. MassachusettsInstitute of Technology. https://books.google.fi/books?hl=fi&lr=&id=G9FljcEASycC&oi=fnd&pg=PP15&ots=qQVSNM0NTH&sig=09Rwp6eNOOw6xVRn1WiCedHO6do&redir_esc=y#v=onepage&q&f=false

Smil, V. (2002). Nitrogen and Food Production: Proteins for Human Diets. *AMBIO: A Journal of the Human Environment*, *31*(2), 126–131. https://doi.org/10.1579/0044-7447-31.2.126

Smith Stegen, K. (2015). Heavy rare earths, permanent magnets, and renewable energies: An imminent crisis. *Energy Policy*, *79*, 1–8. https://doi.org/10.1016/j.enpol.2014.12.015

Son, N. T., Chen, C. F., Chang, N. Bin, Chen, C. R., Chang, L. Y., & Thanh, B. X. (2015). Mangrove mapping and change detection in ca mau peninsula, vietnam, using landsat data and object-based image analysis. *IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing*, *8*(2), 503–510. https://doi.org/10.1109/JSTARS.2014.2360691

Soto-Navarro, C., Ravilious, C., Arnell, A., De Lamo, X., Harfoot, M., Hill, S. L. L., Wearn, O. R., Santoro, M., Bouvet, A., Mermoz, S., Le Toan, T., Xia, J., Liu, S., Yuan, W., Spawn, S. A., Gibbs, H. K., Ferrier, S., Harwood, T., Alkemade, R., … Kapos, V. (2020). Mapping co-benefits for carbon storage and biodiversity to inform conservation policy and action. *Philosophical Transactions of the Royal Society B*, *375*(1794). https://doi.org/10.1098/RSTB.2019.0128

Spalding, M. D., & Leal, M. (2021). *The state of the world's mangroves 2021. Global mangrove alliance.*

Springmann, M., Wiebe, K., Mason-D'Croz, D., Sulser, T. B., Rayner, M., & Scarborough, P. (2018). Health and nutritional aspects of sustainable diet strategies and their association with environmental impacts: a global modelling analysis with country-level detail. *The Lancet. Planetary Health*, *2*(10), e451–e461. https://doi.org/10.1016/S2542-5196(18)30206-7

Stadler, K., Wood, R., Bulavskaya, T., Södersten, C. J., Simas, M., Schmidt, S., Usubiaga, A., Acosta-Fernández, J., Kuenen, J., Bruckner, M., Giljum, S., Lutter, S., Merciai, S., Schmidt, J. H., Theurl, M. C., Plutzar, C., Kastner, T., Eisenmenger, N., Erb, K. H., … Tukker, A. (2018). EXIOBASE 3: Developing a Time Series of Detailed Environmentally Extended Multi-Regional Input-Output Tables. *Journal of Industrial Ecology*, *22*(3), 502–515. https://doi.org/10.1111/JIEC.12715

Steffen, W., Richardson, K., Rockstrom, J., Cornell, S. E., Fetzer, I., Bennett, E. M., Biggs, R., Carpenter, S. R., de Vries, W., de Wit, C. A., Folke, C., Gerten, D., Heinke, J., Mace, G. M., Persson, L. M., Ramanathan, V., Reyers, B., & Sorlin, S. (2015). Planetary boundaries: Guiding human development on a changing planet. *Science*, *347*(6223), 1259855–1259855. https://doi.org/10.1126/science.1259855

Takahashi, N., Orita, T., & Hirose, M. (1995). Production of chicken ovalbumin in Escherichia coli. *Gene*, *161*(2), 211–216. https://doi.org/10.1016/0378-1119(95)00234-W

The World Bank. (2022). *Total greenhouse gas emissions (kt of CO_2 equivalent)*. https://data.worldbank.org/indicator/EN.ATM.GHGT.KT.CE

Thomas. (2018). *High-protein vegan burgers*. Full of Plants. https://fullofplants.com/high-protein-vegan-burgers/

Thomassen, G., Van Dael, M., Van Passel, S., & You, F. (2019). How to assess the potential of emerging green technologies? Towards a prospective environmental and techno-economic assessment framework. *Green Chemistry*, *21*(18), 4868–4886. https://doi.org/10.1039/C9GC02223F

Thomassen, M. A., Dalgaard, R., Heijungs, R., & De Boer, I. (2008). Attributional and consequential LCA of milk production. *International Journal of Life Cycle Assessment*, *13*(4), 339–349. https://doi.org/10.1007/s11367-008-0007-y

Tilman, D., Balzer, C., Hill, J., & Befort, B. L. (2011). Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(50), 20260–20264. https://doi.org/10.1073/pnas.1116437108

Timmis, K., & Ramos, J. L. (2021). The soil crisis: the need to treat as a global health problem and the pivotal role of microbes in prophylaxis and therapy. *Microbial Biotechnology*, *14*(3), 769–797. https://doi.org/10.1111/1751-7915.13771

Treyer, K. (2014). *Market for electricity, high voltage {FI}, ecoinvent 3.6.*

Treyer, K. (2021). *Market for electricity, high voltage {PL}, ecoinvent 3.6.*

Tri, N. H., Adger, W. N., & Kelly, P. M. (1998). Natural resource management in mitigating climate impacts: the example of mangrove restoration in Vietnam. *Global Environmental Change*, *8*(1), 49–61. https://doi.org/10.1016/S0959-3780(97)00023-X

Trivedi, P., Singh, B. P., & Singh, B. K. (2018). Soil Carbon: Introduction, Importance, Status, Threat, and Mitigation. *Soil Carbon Storage: Modulators, Mechanisms and Modeling*, 1–28. https://doi.org/10.1016/B978-0-12-812766-7.00001-9

Tsai, J.-H., Huang, J.-Y., & Wilson, D. I. (2020). Life cycle assessment of cleaning-in-place operations in egg yolk powder production. *Journal of Cleaner Production*, 123936. https://doi.org/10.1016/j.jclepro.2020.123936

Tuomisto, H. L. (2019). The eco-friendly burger. *EMBO Reports*, *20*(1). https://doi.org/10.15252/embr.201847395

Tuomisto, H. L., Allan, S. J., & Ellis, M. J. (2022). Prospective life cycle assessment of a bioprocess design for cultured meat production in hollow fiber bioreactors. *Science of The Total Environment*, *851*, 158051. https://doi.org/10.1016/J.SCITOTENV.2022.158051

Tuomisto, H. L., & Teixeira de Mattos, M. J. (2011). Environmental Impacts of Cultured Meat Production. *Environmental Science & Technology*, *45*(14), 6117–6123. https://doi.org/10.1021/es200130u

Twilley, R. R., Chen, R. H., & Hargis, T. (1992). Carbon sinks in mangroves and their implications to carbon budget of tropical coastal ecosystems. *Water, Air, and Soil Pollution*, *64*, 265–288.

UN Madagascar. (2021). *Climate change and hunger in Madagascar: a UN Resident Coordinator blog*. https://news.un.org/en/story/2021/08/1098042

UNEP. (2009). *Blue carbon. A rapid response assessment.*

United Nations. (2021). *Feeding the world sustainably*. https://www.un.org/en/chronicle/article/feeding-world-sustainably

United Nations Department of Economic and Social Affairs. (2015). *Sustainable consumption and production*. https://sdgs.un.org/topics/sustainable-consumption-and-production

Upcraft, T., Tu, W. C., Johnson, R., Finnigan, T., Van Hung, N., Hallett, J., & Guo, M. (2021). Protein from renewable resources: mycoprotein production from agricultural residues. *Green Chemistry*, *23*(14), 5150–5165. https://doi.org/10.1039/D1GC01021B

USDA. (2019a). *FoodData Central: Crustaceans, shrimp, raw*. https://fdc.nal.usda.gov/fdc-app.html#/food-details/175179/nutrients

USDA. (2019b). *FoodData Central: Egg, white, dried*. https://fdc.nal.usda.gov/fdc-app.html#/food-details/323793/nutrients

Valin, H., Sands, R. D., Mensbrugghe, D. van der, Nelson, G. C., Ahammad, H., Blanc, E., Bodirsky, B., Fujimori, S., Hasegawa, T., Havlik, P., Heyhoe, E., Kyle, P., Mason-D'Croz, D., Paltsev, S., Rolinski, S., Tabeau, A., Meijl, H. van, Lampe, M. von, & Willenbockel, D. (2013). The future of food demand: understanding differences in global economic models. *Agricultural Economics*, *45*(1), 51–67. https://doi.org/10.1111/AGEC.12089

Van der Warf, H., & Petit, J. (2002). Evaluation of the environmental impact of agriculture at the farm level: a comparison and analysis of 12 indicator-based methods. *Agriculture, Ecosystems and Environment*, *93*(1–3), 131–145.

Van Vuuren, D. P., Bouwman, A. F., & Beusen, A. H. W. (2010). Phosphorus demand for the 1970-2100 period: A scenario analysis of resource depletion. *Global Environmental Change*, *20*(3), 428–439. https://doi.org/10.1016/j.gloenvcha.2010.04.004

Vanham, D., Comero, S., Gawlik, B. M., & Bidoglio, G. (2018). The water footprint of different diets within European sub-national geographical entities. *Nature Sustainability 2018 1:9*, *1*(9), 518–525. https://doi.org/10.1038/s41893-018-0133-x

Vasco, D. W., Farr, T. G., Jeanne, P., Doughty, C., & Nico, P. (2019). Satellite-based monitoring of groundwater depletion in California's Central Valley. *Scientific Reports 2019 9:1*, *9*(1), 1–14. https://doi.org/10.1038/s41598-019-52371-7

Venter, O., Sanderson, E. W., Magrach, A., Allan, J. R., Beher, J., Jones, K. R., Possingham, H. P., Laurance, W. F., Wood, P., Fekete, B. M., Levy, M. A., & Watson, J. E. M. (2016). Sixteen years of change in the global terrestrial human footprint and implications for biodiversity conservation. *Nature Communications 2016 7:1*, *7*(1), 1–11. https://doi.org/10.1038/ncomms12558

Volova, T. G., & Barashkov, V. A. (2010). Characteristics of proteins synthesized by hydrogen-oxidizing microorganisms. *Applied Biochemistry and Microbiology 2010 46:6*, *46*(6), 574–579. https://doi.org/10.1134/S0003683810060037

Voutilainen, E., Pihlajaniemi, V., & Parviainen, T. (2021). Economic comparison of food protein production with single-cell organisms from lignocellulose side-streams. *Bioresource Technology Reports*, 100683. https://doi.org/10.1016/j.biteb.2021.100683

Vu, T. A., Phan, L. T., Do, H. Van, Ngo, T. T. N., Nguyen, K. D., Phan, V. Q., & Nguyen, H. T. (2013). *Status of small-scale environmentally friendly shrimp production in Ca Mau Province, Viet Nam* (Issue June).

Walcker, R., Gandois, L., Proisy, C., Corenblit, D., Mougin, É., Laplanche, C., Ray, R., & Fromard, F. (2018). Control of "blue carbon" storage by mangrove ageing: Evidence from a 66-year chronosequence in French Guiana. *Global Change Biology*, *24*(6), 2325–2338. https://doi.org/10.1111/GCB.14100

Weidema, B. (2014). Has ISO 14040/44 Failed Its Role as a Standard for Life Cycle Assessment? *Journal of Industrial Ecology*, *18*(3), 324–326. https://doi.org/10.1111/jiec.12139

Weidema, B. P., Simas, M. S., Schmidt, J., Pizzol, M., Løkke, S., & Brancoli, P. L. (2019). Relevance of attributional and consequential information for environmental product labelling. *The International Journal of Life Cycle Assessment*, 1–5. https://doi.org/10.1007/s11367-019-01628-4

Weidema, B. P., & Stylianou, K. S. (2020). Nutrition in the life cycle assessment of foods—function or impact? *International Journal of Life Cycle Assessment*, *25*(7), 1210–1216. https://doi.org/10.1007/s11367-019-01658-y

Wender, B. A., Foley, R. W., Hottle, T. A., Sadowski, J., Prado-Lopez, V., Eisenberg, D. A., Laurin, L., & Seager, T. P. (2014). Anticipatory life-cycle assessment for responsible research and innovation. *Journal of Responsible Innovation*, *1*(2), 200–207. https://doi.org/10.1080/23299460.2014.920121

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., Garnett, T., Tilman, D., DeClerck, F., Wood, A., Jonell, M., Clark, M., Gordon, L. J., Fanzo, J., Hawkes, C., Zurayk, R., Rivera, J. A., De Vries, W., Majele Sibanda, L., … Murray, C. J. L. (2019). Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. In *The Lancet* (Vol. 393, Issue 10170). Lancet Publishing Group. https://doi.org/10.1016/S0140-6736(18)31788-4

Winter, L., Lehmann, A., Finogenova, N., & Finkbeiner, M. (2017). Including biodiversity in life cycle assessment – State of the art, gaps and research needs. *Environmental Impact Assessment Review*, *67*, 88–100. https://doi.org/10.1016/J.EIAR.2017.08.006

Wood, R., Stadler, K., Simas, M., Bulavskaya, T., Giljum, S., Lutter, S., & Tukker, A. (2018). Growth in Environmental Footprints and Environmental Impacts Embodied in Trade: Resource Efficiency Indicators from EXIOBASE3. *Journal of Industrial Ecology*, *22*(3), 553–564. https://doi.org/10.1111/JIEC.12735

Woodward, D. G. (1997). Life cycle costing—Theory, information acquisition and application. *International Journal of Project Management*, *15*(6), 335–344. https://doi.org/10.1016/S0263-7863(96)00089-0

Wuepper, D., Le Clech, S., Zilberman, D., Mueller, N., & Finger, R. (2020). Countries influence the trade-off between crop yields and nitrogen pollution. *Nature Food 2020 1:11*, *1*(11), 713–719. https://doi.org/10.1038/s43016-020-00185-6

WWF. (2013). Ecosystems in the Greater Mekong. Past trends, current status, possible futures; WWF report Greater Mekong. In *WWF report Greater Mekong 2013*. awsassets.panda.org/downloads/greater_mekong_ecosystems_report_020513.pdf

Xiong, Y., Liao, B., & Wang, F. (2018). Mangrove vegetation enhances soil carbon storage primarily through in situ inputs rather than increasing allochthonous sediments. *Marine Pollution Bulletin*, *131*, 378–385. https://doi.org/10.1016/J.MARPOLBUL.2018.04.043

Xu, X., Sharma, P., Shu, S., Lin, T.-S., Ciais, P., Tubiello, F. N., Smith, P., Campbell, N., & Jain, A. K. (2021). Global greenhouse gas emissions from animal-based foods are twice those of plant-based foods. *Nature Food 2021*, 1–9. https://doi.org/10.1038/s43016-021-00358-x

Yu, J. (2014). Bio-based products from solar energy and carbon dioxide. In *Trends in Biotechnology* (Vol. 32, Issue 1, pp. 5–10). Elsevier. https://doi.org/10.1016/j.tibtech.2013.11.001

Yu, X., Keitel, C., & Dijkstra, F. A. (2021). Global analysis of phosphorus fertilizer use efficiency in cereal crops. In *Global Food Security* (Vol. 29, p. 100545). Elsevier B.V. https://doi.org/10.1016/j.gfs.2021.100545

Zirlotts Gulf Products. (2013). *Shrimp*. http://www.zirlottseafood.com/store/shrimp.html

Zouaghi, S., Frémiot, J., André, C., Grunlan, M. A., Gruescu, C., Delaplace, G., Duquesne, S., & Jimenez, M. (2019). Investigating the Effect of an Antifouling Surface Modification on the Environmental Impact of a Pasteurization Process: An LCA Study. *ACS Sustainable Chemistry and Engineering*, *7*(10), 9133–9142. https://doi.org/10.1021/acssuschemeng.8b05835

9. APPENDICES

Appendix Table 1: Environmental impacts of protein alternatives for feed relative to the highest scores, belonging to Figure 7

Protein	GWP	Land use	Water scarcity	Eutrophication	Ozone depletion	Aquatic acidification	Energy demand
Soybean Meal	0.5 %	13.2 %	0.5 %	-10.5 %	0.8 %	0.3 %	0.8 %
Rapeseed Cake	0.6 %	9.0 %	0.3 %	14.9 %	0.1 %	0.4 %	0.3 %
Pea Protein Meal	6.8 %	24.8 %	0.8 %	19.9 %	0.4 %	1.9 %	0.6 %
Fishmeal	3.9 %	2.6 %	3.3 %	-6.7 %	1.4 %	1.9 %	4.9 %
Insect Protein (HM)	4.0 %	6.8 %	0.0 %	18.4 %	1.2 %	0.8 %	3.8 %
Whey Concentrate	11.4 %	14.2 %	100.0 %	51.3 %	4.3 %	1.9 %	4.5 %
Egg Protein Concentrate	12.4 %	100.0 %	20.7 %	100.0 %	1.9 %	100.0 %	5.7 %
Microalgae	100.0 %	13.0 %	23.8 %	76.3 %	28.7 %	30.3 %	100.0 %
Tr-OVA (PL)	7.0 %	7.2 %	71.2 %	3.7 %	100.0 %	2.2 %	5.8 %
Tr-OVA (FI-LC)	2.9 %	7.0 %	68.2 %	3.3 %	99.8 %	0.9 %	5.0 %
MP (FAEM)	4.9 %	0.9 %	23.3 %	0.3 %	3.0 %	0.7 %	9.2 %
MP (FHE)	0.7 %	0.1 %	9.3 %	0.0 %	0.6 %	0.1 %	3.9 %
MP (Base)	0.9 %	0.4 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %
MP (FImix)	2.5 %	3.2 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %
Mycoprotein	3.9 %	2.6 %	0.0 %	0.0 %	0.0 %	0.0 %	0.0 %

Results for protein alternatives from Smetana et al.(2016) and Sillman et al.(2020). Note: PL – Poland; FI-LC – Finland low carbon scenario; GWP – global warming potential; HM - *H. Illucens* meal (defatted protein concentrate); MP – microbial protein; FAEM – Finnish average electricity mix; FHE – Finnish hydrogen-based electricity; MP (base) – baseline scenario used in the article by Sillman et al.(2020); MP – FImix – Finnish average electricity mix used in the article by Sillman et al. (2020). Water scarcity was calculated using the aware method, energy demand using the CED method and all others using the IMPACT2002+ method, with the exception of eutrophication which was expressed in kg N in table 2a and were calculated using the ReCiPe results presented in articles II and III.

Appendix Table 2: Absolute values for the environmental impact of different protein alternatives for feed

Protein	GWP, kg CO_2-eq	land use, m^2a	water scarcity, m^3	eutrophication, g N eq.	ozone depletion, mg CFC11 eq.	acidification, g SO_2 eq.	ED, MJ
Soybean meal	0.11	0.66	0.0081	-1.82	0.050916497	1.255	3.14
Rapeseed cake	0.14	0.45	0.0045	2.59	0.007758621	2.05	1.02
Pea protein meal	1.615	1.24	0.013	3.45	0.024782609	9.48	2.28
Fishmeal	0.92	0.13	0.0527	-1.16	0.094090909	9.71	19.64
Insect protein (HM)	0.95	0.34	0.0005	3.2	0.076785714	3.8	15.03
Whey concentrate	2.7	0.71	1.6025	8.91	0.280416667	9.47	18.06
Egg protein concentrate	2.93	5.01	0.3313	17.38	0.12625	500	22.88
Microalgae	23.62	0.65	0.3818	13.26	1.881818182	151.64	399.85
Mycoprotein	0.91	0.13					
MP (Base)	0.21	0.023					
MP (FImix)	0.6	0.160					
MP (FAEM)	1.155979692	0.047028615	0.372886769	0.050153846	0.200016692	3.289384615	36.95975385
MP (FHE)	0.156947077	0.004280923	0.149160769	1.93696E-06	0.042358917	0.466923077	15.56773846
Tr-OVA (PL)	1.663043478	0.360257	1.140217391	0.648486478	6.56238337	10.86892435	23.00829891
Tr-OVA (FI-LC)	0.673588565	0.351733739	1.092654457	0.572994315	6.55090413	4.602566413	19.796725

Results for protein alternatives from Smetana et al.(2016) and Sillman et al.(2020). Note: PL – Poland; FI-LC – Finland low carbon scenario; GWP – global warming potential; HM - *H. Illucens* meal (defatted protein concentrate); MP – microbial protein; FAEM – Finnish average electricity mix; FHE – Finnish hydrogen-based electricity; MP (base) – baseline scenario used in the article by Sillman et al.(2020); MP – FImix – Finnish average electricity mix used in the article by Sillman et al. (2020). Water scarcity was calculated using the aware method, energy demand using the CED method and all others using the IMPACT2002+ method, with the exception of eutrophication which was expressed in kg N in table 2a and were calculated using the ReCiPe results presented in articles II and III.

Appendix Table 3: Impact scores of protein alternatives for food relative to the highest scores, belonging to Figure 8

Protein	Land use	GWP	Acidifying emissions	Eutrophying emissions	Water scarcity
Bovine meat (beef herd)	100 %	22 %	90 %	100 %	29 %
Bovine meat (dairy herd)	45 %	19 %	100 %	98 %	100 %
Fish (farmed)	8 %	3 %	27 %	29 %	78 %
Poultry meat	18 %	6 %	32 %	29 %	63 %
Eggs	16 %	4 %	33 %	29 %	36 %
Nuts	22 %	1 %	11 %	33 %	40 %
Peas	5 %	0 %	1 %	9 %	15 %
MP	1 %	1 %	3 %	2 %	1 %
MP FHE	0 %	0 %	0 %	0 %	0 %
Tr-OVA (PL)	1 %	2 %	8 %	11 %	3 %
Tr-OVA (FI-LC)	1 %	1 %	3 %	4 %	2 %
Shrimp (MC)	0 %	100 %	0 %	0 %	0 %
Shrimp (IF)	0 %	8 %	0 %	0 %	0 %

Results for protein alternatives from the European dataset produced as part of Poore and Nemecek (2018a). Note: FI-LC - Finland low-carbon; PL – Poland; Tr-OVA – ovalbumin produced using *Trichoderma reesei*; MP – microbial protein; FAEM – Finnish average energy mix; FHE – Finnish hydropower energy; MC – mangrove concurrent system; IF – intense farming system.

Appendix Table 4: Absolute values for the environmental impact of different protein alternatives for food

	Land Use (m^2/ nutritional unit)	GHG Emissions (kg CO2eq/NU, IPCC 2013 incl CC feedbacks)	Acidifying Emissions (kg SO_2eq/ NU, CML2 Baseline)	Eutrophying Emissions (kg PO_{43}-eq/ NU, CML2 Baseline)	Water scarcity (L/NU)
Bovine eat (beef herd)	35.95787362	20.4332999	0.120882648	0.064829488	13060.68205
Bovine meat (dairy herd)	16.31205674	17.28723404	0.134761905	0.063333333	45044.27558
Fish (farmed)	3.025652269	2.667397501	0.035957027	0.019057224	35090.76957
Poultry meat	6.466512702	5.32448037	0.042678984	0.018799076	28596.13164
Eggs	5.677721702	3.93925739	0.044286229	0.019033886	16064.07714
Nuts	7.899571341	1.293325168	0.014384568	0.021641151	17998.53031
Peas	1.755175518	0.295229523	0.001917192	0.005670567	6805.355536
MP (FAEM)	0.386426308	1.258804154	0.004004615	0.001504154	372.8867692
MP (FHE)	0.051914615	0.156923077	0.000569538	0.000189538	149.1607692
Tr-OVA (PL)	0.481024815	1.814542076	0.010154914	0.006913325	1140.149239
Tr-OVA (FI-LC)	0.450243272	0.776492391	0.003691848	0.002472754	1092.654457
Shrimp (MC)		91.54228856			
Shrimp (IF)		7.562189055			

Results for protein alternatives from the European dataset produced as part of Poore and Nemecek (2018a). Note: FI-LC - Finland low-carbon; PL – Poland; Tr-OVA – ovalbumin produced using *Trichoderma reesei*; MP – microbial protein; FAEM – Finnish average energy mix; FHE – Finnish hydropower energy; MC – mangrove concurrent system; IF – intense farming system.

Appendix Table 5: Results for GWP in CO_2-eq of a patty using 5 different protein sources.

Patty	Main protein source	Breadcrumbs	Bread, white	Onion	Rapeseed oil	Kidney beans	Sweet potato
Tr-OVA	0.295903	0.014384	0.014747	0.001437	0.029015	0.01087	0.007144
MP	0.360323	0.014384	0.014747	0.001437	0.025363	0.01087	0.005001
Shrimp (mangrove concurrent)	39.22	0.014384	0.014747	0.001437	0.029015	0.01087	0.007144
Shrimp ((semi-)intense)	3.3	0.014384	0.014747	0.001437	0.029015	0.01087	0.007144
Shrimp (AGRIBALYSE, china)	0.283871	0.014384	0.014747	0.001437	0.029015	0.01087	0.007144

ARTICLE I

Including GHG emissions from mangrove forests LULUC in LCA: a case study on shrimp farming in the Mekong Delta, Vietnam

Int J Life Cycle Assess (2018) 23:1078–1090
DOI 10.1007/s11367-017-1332-9

CrossMark

CHALLENGES AND BEST PRACTICE IN LCAS OF SEAFOOD AND OTHER AQUATIC PRODUCTS

Including GHG emissions from mangrove forests LULUC in LCA: a case study on shrimp farming in the Mekong Delta, Vietnam

Natasha Järviö[1,2] · **Patrik J. G. Henriksson**[3,4] · **Jeroen B. Guinée**[1]

Received: 6 March 2016 / Accepted: 18 April 2017 / Published online: 23 May 2017

Abstract

Purpose Mangrove forests have been recognized as important regulators of greenhouse gases (GHGs), yet the resulting land use and land-use change (LULUC) emissions have rarely been accounted for in life cycle assessment (LCA) studies. The present study therefore presents up-to-date estimates for GHG emissions from mangrove LULUC and applies them to a case study of shrimp farming in Vietnam.

Methods To estimate the global warming impacts of mangrove LULUC, a combination of the International Panel for Climate Change (IPCC) guidelines, the Net Committed Emissions, and the Missed Potential Carbon Sink method were used. A literature review was then conducted to characterize the most critical parameters for calculating carbon losses, missed sequestration, methane fluxes, and dinitrogen monoxide emissions.

Results and discussion Our estimated LUC emissions from mangrove deforestation resulted in 124 t CO_2 ha^{-1} $year^{-1}$, assuming IPCC's recommendations of 1 m of soil loss, and 96% carbon oxidation. In addition to this, 1.25 t of carbon would no longer be sequestered annually. Discounted over 20 years, this resulted in total LULUC emissions of 129 t CO_2 ha^{-1} $year^{-1}$ (CV = 0.441, lognormal distribution (ln)). Shrimp farms in the Mekong Delta, however, can today operate for 50 years or more, but are 1.5 m deep (50% oxidation). In addition to this, Asian tiger shrimp farming in mixed mangrove concurrent farms (the only type of shrimp farm that resulted in mangrove deforestation since 2000 in our case study) resulted in 533 kg methane and 1.67 kg dinitrogen monoxide per hectare annually. Consequently, the LULUC GHG emissions resulted in 184 and 282 t CO_2-eq t^{-1} live shrimp at farm gate, using mass and economic allocation, respectively. These GHG emissions are about an order of magnitude higher than from semi-intensive or intensive shrimp farming systems. Limitations in data quality and quantity also led us to quantify the uncertainties around our emission estimates, resulting in a CV of between 0.4 and 0.5.

Conclusions Our results reinforce the urgency of conserving mangrove forests and the need to quantify uncertainties around LULUC emissions. It also questions mixed mangrove concurrent shrimp farming, where partial removal of mangrove forests is endorsed based upon the benefits of partial mangrove conservation and maintenance of certain ecosystem services. While we recognize that these activities limit the chances of complete removal, our estimates show that large GHG emissions from mangrove LULUC question the sustainability of this type of shrimp farming, especially since mixed mangrove farming only provide 5% of all farmed shrimp produced in Vietnam.

Keywords Aquaculture · Carbon footprint · Deforestation · Land use · Land-use change · LCA · Mangrove · Shrimp · Vietnam

Responsible editor: Matthias Finkbeiner

Electronic supplementary material The online version of this article (doi:10.1007/s11367-017-1332-9) contains supplementary material, which is available to authorized users.

✉ Jeroen B. Guinée
guinee@cml.leidenuniv.nl

[1] Institute of Environmental Science CML, Leiden University, Einsteinweg 2, 2333 CC Leiden, Netherlands

[2] Finnish Environment Institute (SYKE), Mechelininkatu 34a, FI-00260 Helsinki, Finland

[3] Stockholm Resilience Centre, Stockholm University, Kräftriket 2B, 114 19 Stockholm, Sweden

[4] WorldFish, Jalan Batu Maung, 11960 Penang, Malaysia

1 Introduction

Mangrove forests are among the most productive tropical ecosystems in the world, with net annual production exceeding that of most terrestrial forests (Twilley et al. 1992; Eong 1993; Kauffman et al. 2011; Mcleod et al. 2011). They have also been recognized for their importance in providing valuable ecosystem services (Rönnbäck 1999; Hong and Dao 2004; Bouillon et al. 2008; Kristensen et al. 2008). These include coastline protection from storms, erosion, saline intrusion, and pollution (Rönnbäck 1999; Hong and Dao 2004), supporting and maintaining biodiversity, and provision of energy to adjacent ecosystems (Rönnbäck 1999; Kristensen et al. 2008). More recently, the important role of mangrove forests in the capture and sequestration of carbon dioxide (CO_2) from the atmosphere has gained increasing recognition, as mangrove forests, unlike terrestrial forests, do not become saturated with carbon, and sediments accrete vertically (Mcleod et al. 2011). The sequestration of carbon in sediments and the depth of the soil may therefore continue to increase over millennia (Eong 1993; Duarte et al. 2005; Mcleod et al. 2011). Their organically rich soils, typically extending downward over several meters, make up one of the largest organic carbon reserves in the terrestrial biosphere (Chmura et al. 2003; Lovelock 2008). Sequestration rates were estimated to be as high as 3.53 t C ha^{-1} yr^{-1} (Sanders et al., 2010). Hypoxic conditions and the lack of other high-energy oxidants, in combination withh a paucity of fungi, limit the opportunity for degradation, thereby providing good conditions for long-term storage of carbon (Middleton and McKee 2001). This, together with high biomass burial rates, the high potential age mangrove trees, and a slow turnover rate, results in carbon storage rates relevant at global scales (Duarte et al. 2005; FAO 2007).

Despite the recognition of their ecological value, mangrove forests worldwide are under threat from land-use change, with annual deforestation rates between 0.7 and 2.1%, far exceeding those of inland tropical forests (Murray et al. 2012). For example, countries like Thailand and Vietnam, which harbor large shares of the global mangrove forests, have been reported to have lost 43% of their mangrove forests since 1980 and are to be at risk of losing an additional third of the remaining forests over the next two decades unless their governments improve the protection of mangrove areas (WWF 2013). Expansions of aquaculture and especially shrimp farming have been held accountable for 30% of the mangrove loss in SE Asia (Richards and Friess 2015). This as most shrimp species are most productive when farmed in brackish water, which often results in the establishment of shrimp ponds in coastal regions where they compete with mangrove forests (Béland et al. 2006; Murray et al. 2012). Given an annual growth rate of the shrimp farming in Asia of 8% over the last decade, alongside continued agricultural and urban growth, the future will surely pose additional threats to the mangrove ecosystems in the region (FAO FishstatJ 2014; Richards and Friess 2015).

Already in 2007, Milà i Canals called for more papers on dealing with land use-related greenhouse gas emissions (GHG) in life cycle assessment (LCA), while growing scientific concerns about mangrove deforestation have been accumulating with regards to aquaculture and mangrove deforestation. Despite this, few aquaculture LCAs have included mangrove land use and land-use change (LULUC) emissions to date (Henriksson et al. 2012). The International Panel for Climate Change (IPCC) published guidelines for the estimation of carbon dioxide emissions from land-use change of mangrove forests caused by aquaculture in 2013 (IPCC 2014). These guidelines, however, have their limitations, as they provide no guidance on carbon or methane (CH_4) or dinitrogen monoxide (N_2O) emissions regarding land use for aquaculture purposes. This means that the continuous high rate of carbon burial into the soil of mangrove forests is not considered, although this is what distinguishes these forests from other terrestrial forests (Duarte et al. 2005; FAO 2007; Mcleod et al. 2011), and emissions of two other potent GHGs often go unaccounted for.

Following criticism, shrimp farming practices have been improved in many countries: new farms are now established outside mangrove areas, productivity has increased, and better farm management has allowed farms to continue operations over longer time periods without having to relocate due to sediment build-up (Lebel et al. 2002, 2010). Despite this, concerns about the conversion of mangrove forests into shrimp farms were again raised by Prof. JB Kauffman during the 2012 meeting of the American Association for the Advancement of Science (Stokstad 2012). During this meeting, Kauffman highlighted that the carbon dioxide emissions resulting from mangrove deforestation amounted to 198 kg CO_2 per 100 g of shrimp tails. While this definitely raises concerns, some assumptions regarding the location of newly established shrimp farms, pond productivity, and pond lifespan were later called into question by the Global Aquaculture Alliance (Global Aquaculture Alliance 2012).

Given the controversies surrounding the emissions resulting from LULUC due to mangrove deforestation for shrimp farming and the clear lack of its resulting emissions in LCAs, the present study aimed to present up-to-date GHG emission estimates for mangrove LULUC. We also demonstrate our accounting methods using shrimp farming as a case study. To put these emissions into context, we also used the LCA framework (ISO 2006), allowing GHG emissions from semi-intensive and intensive shrimp production chains to be considered as a reference. Using the LULUC emission factors and production data for shrimp farming in the Mekong Delta of Vietnam, we estimated the contributions of mangrove

LULUC emissions relative to the overall carbon footprint of shrimp farming.

In order to address our identified shortcoming in literature, each section first aims to quantify the GHG emission from generic mangrove deforestation using IPCC assumptions, followed by its application to the shrimp case study. Section 2 thus first identifies the relevant parameters for calculating the GHG emissions from mangrove deforestation, including changes in carbon stocks as visualized in Fig. 1, and characteristics of CH_4 and N_2O-fluxes (Section 2.3). This is followed by details about the case study in Section 2.3. Section 3 subsequently presents the ranges of results derived from literature for each parameter and summarizes these as easy-to-use LCA parameters including uncertainty estimates. These values were also modified for the case study in Section 3.3 to quantify the impact of shrimp farming in previous mangrove areas in terms of CO_2-equivalents (CO_2-eq). Finally, in the discussion and conclusion, we expand on the implications of our findings.

2 Materials and methods

The carbon stock dynamics resulting from mangrove deforestation are illustrated in Fig. 1 with each, before (time < 0) and after (time > 0) the establishment of shrimp ponds. Before land-use change (LUC), above- and below-ground and litter C stocks remain approximately constant over time. It is assumed that, in general, primary mangrove forests are in equilibrium and are therefore not storing more biomass over time (IPCC 2006). However, the carbon in the mangrove soil will increase over time due to a continuous carbon burial rate, as seen in Fig. 1. LUC is a consequence of the establishment of the shrimp farm during its first year by removing the above- and below-ground vegetation and excavation of ponds, resulting in the loss and oxidization of all carbon stored in the above- and below-ground parts of the mangrove, in the litter and in part of the soil. The resulting emissions are therefore allocated to the LUC activity (β). This also means that all emissions that occur during later years, i.e., oxidization of the

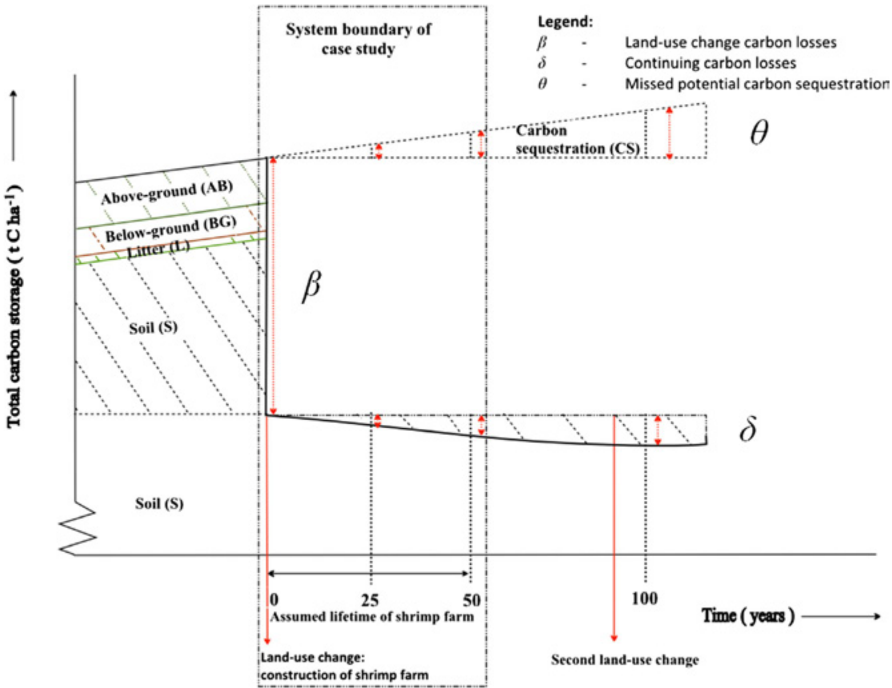


Fig. 1 To calculate CO_2 emissions resulting from mangrove LULUC, this figure depicts the important processes behind LUC (β and δ) and LU (θ) CO_2 emissions from mangrove forests. The LUC carbon losses consist of the above-ground (AG), below-ground (BG), soil (S), and litter (L) CO_2 emissions, regardless of when in time they occur (i.e. the oxidization of carbon in the soil over time, δ) (IPCC 2006; Cederberg et al. 2011). LU CO_2 emissions are based on the mangrove's potential carbon sequestration rates. The LUC carbon losses (β and δ) are factual losses that can be measured at the point when they occur. The emissions resulting from LU (θ), on the other hand, are based on potential sequestration rates

carbon in the soil over time (δ), are considered to be a consequence of the initial LUC and assigned to the activity and year of the LUC (IPCC 2006; Cederberg et al. 2011). During the time in which the farmer uses the land for shrimp farming, the area originally comprising the mangrove forests no longer takes up any carbon. Aquaculture ponds and other land uses will also result in altered methane and dinitrogen oxide emissions. These are the LU emissions and they are the result of the missed carbon sequestration (θ), anoxic methane formation, and nitrogen volatilization.

2.1 Carbon dioxide LULUC emissions from mangrove deforestation

In the present study, CO_2 emissions due to LUC of coastal wetlands with mangrove as vegetation, to a new land use were calculated using a combination of the Net Committed Emissions (NCE) method by Cederberg et al. 2011 and the IPCC guidelines (2006, 2014). The NCE method (Cederberg et al. 2011) offered us a way to include carbon stock changes resulting from LUC by comparing the carbon stocks before LUC with those after LUC, while we used the reference land use and guidelines provided by the IPCC (2006), such as a 1-m default soil depth and 96% of carbon oxidized (IPCC 2014). We used the Missed Potential Carbon Sink method proposed by Schmidinger and Stehfest (2012) as inspiration to evaluate the potential carbon storage missed as a result of land use (LU) [see Electronic Supplementary Material (ESM 1) for a full analysis of all methods]. The "missed potential carbon sequestration" is important to take into account, as mangrove forests left standing would continue to sequestrate substantial amounts of carbon in the soil (Eong 1993; Duarte et al. 2005; Mcleod et al. 2011). The missed potential carbon sequestration is therefore based on the mangrove carbon sequestration rate.

The total emissions were annualized and not amortized, in line with the IPCC guidelines. Many guidelines set a default value of the particular number of years, or timeframe, over which the LULUC CO_2 emissions should be annualized, usually ranging from 20 to 30 years (ISO 2006; FAO 2007; Cederberg et al. 2011). The IPCC (IPCC 2006) uses a default value of 20 years, based on the argument that this is the time required for carbon stocks to reach equilibrium. Noteworthy is that the assumed timeframe greatly influences estimated LULUC emissions.

The total change in the carbon balance resulting from mangrove LULUC can thus be summarized as follows:

$$\Delta C_{\mathrm{TOTAL}i} = \Delta C_{LUCi} + \Delta C_{LUi} \qquad (1)$$

where

ΔC_{TOTALi} is the total carbon loss resulting from the establishment of land use i during the timeframe (t C ha^{-1}) (multiply C with 3.667 for CO_2)

ΔC_{LUCi} is the carbon loss caused by land-use change for land use i (regardless of when in time they occur) (t C ha^{-1})

ΔC_{LUi} is the total carbon loss caused by land use i (t C ha^{-1})

i is the new land use (i.e., shrimp farming)

The total carbon loss caused by land-use change (ΔC_{LUCi}) can, in turn, be calculated using Eq. (1) (initial letters β, δ, or θ, refer to Fig. 1):

$$\Delta C_{LUCi} = \Delta\beta_{AGi} + \Delta\beta_{BGi} + \Delta\beta_{Li} + \left(\Delta\beta\delta_{Si}{}^{*}\, SO_i\right) \qquad (2)$$

where

$\Delta\beta_{AGi}$ is the change in the above-ground carbon stock caused by land-use change (t C ha^{-1})

$\Delta\beta_{BGi}$ is the change in the below-ground carbon stock caused by land-use change (t C ha^{-1})

$\Delta\beta_{Li}$ is the change in the litter carbon stock caused by land-use change (t C ha^{-1})

$\Delta\beta\delta_{Si}$ is the change in the soil carbon stock caused by land use change (t C ha^{-1}) (default depth of 1 m; IPCC 2014)

SO_i is the amount of carbon in soil exposed to oxidation, in percentage (t C ha^{-1}) (default value of 96%; IPCC 2014)

The total missed carbon sequestration caused by land use (ΔC_{LUi}) could, in turn, be calculated using Eq. (3):

$$\Delta C_{LUi} = \Delta\theta_{MPi}{}^{*}\, T \qquad (3)$$

where

θ_{MPi} is the missed potential of carbon that would have been sequestered if the mangrove was left standing (t C ha^{-1} year^{-1})

T is the timeframe, in years (default of 20 years; IPCC 2006)

The parameters identified for calculating the CO_2 emissions in Eqs. (2) and (3) were identified through a literature review (see Table 1 for the definitions). Articles were selected by searching for the keywords "mangrove", "carbon", "emissions", "storage", "soil", "land-use change", and "primary production" in Google Scholar (search carried out on April 21, 2015). To establish uncertainty parameters for the resulting CO_2 emissions from mangrove LULUC, ranges of results were produced over 10,000 Monte Carlo iterations. The Monte Carlo results were generated using CMLCA v5.2.

2.2 Methane and dinitrogen monoxide emissions from aquaculture farming

Only recently have estimates been made about methane (CH_4) and nitrous oxide (N_2O) emissions resulting from aquaculture (Hu et al. 2012; Astudillo et al. 2015). No study to our knowledge has, however, measured these emissions from aquaculture activities on converted mangrove forest. Instead, we therefore collected a range of available values reported for both standing mangrove and conventional aquaculture.

1082 Int J Life Cycle Assess (2018) 23:1078–1090

Table 1 Definition of parameters relevant to the calculation of the carbon footprint of mangrove LULUC

Parameter	Alternative names	Description
AG	Above-ground carbon	The above-ground carbon includes all carbon found in the live biomass located above the ground, which includes the stems of the trees, their branches, and their leaves.
BG	Below-ground carbon	Below-ground carbon includes all carbon found in live biomass located below the ground, which not only includes the carbon in actual below-ground root biomass but also the prop roots which are in fact located above the ground. It does not include the carbon found in soil.
S	Soil/sediments	Soil carbon is the carbon stored in the soil. Soil contains dead organic material derived from decomposed plants and animals, and inorganic matter that have built up over time. Per IPCC, a soil depth of 1 m should be considered, but for the shrimp case study, 1.5 m was adopted based upon the average depth reported by farmers.
L	Litter	Litter is all dead biomass including material that was previously part of the bulk of biomass in the net primary production. Litter C stocks include both above- and below-ground litter stocks. Litter can include just leaves, but also slash, stumps, dead trees, stipulates, reproductive parts, branches, and debris.
CS	(Missed potential for) carbon sequestration	Carbon burial to soil refers to the process of the carbon being buried in the sediments. This is caused by the production of carbon-containing litter in the ecosystem and the import of carbon from adjacent ecosystems. Part of this carbon gets trapped into the sediments, where it can remain in the soil for centuries. In the literature, carbon sequestration is often used interchangeably with carbon burial rates (Mcleod et al. 2011). Both refer to the long-term storage of CO_2 from the atmosphere and its deposition in reservoirs, where long-term refers to centuries to millennia (Mcleod et al. 2011). Missed potential carbon sequestration refers to the amount of carbon sequestration not realized due to mangrove forest being converted to, i.e., shrimp ponds.

2.3 Case study: shrimp farming in the Mekong Delta, Vietnam.

To illustrate the potential GHG emissions from mangrove LULUC, we applied the proposed method in combination with data collected for 200 randomly selected shrimp farmers in Vietnam between 2011 and 2012 as part of the EU FP7 SEAT project (European Commission within the Seventh Framework Programme, Sustainable Ethical Aquaculture Trade) (www.seatglobal.eu) (Murray et al. 2013; Henriksson et al. 2015). Vietnam is one of the world's leading exporters of shrimp, with a significant growth in production since the mid-1980s. Most of the shrimp farms are in the Mekong Delta, and specifically the province of Ca Mau. This area also harbors about one third of the remaining mangrove forests of Vietnam (Jonell and Henriksson 2014). These mangrove forests also support mixed mangrove concurrent farming of Asian tiger shrimp (*Penaeus monodon*), an extensive farming practice with minimal to no external inputs that takes place within the mangrove forest. The original intent of this practice was to provide partial protection of mangrove forests by allowing families to use up to 30% of the land and surface water to generate income, thereby preventing complete deforestation (McNally et al. 2010). Besides the mixed mangrove farms, four other kinds of shrimp farming practices have been identified in Mekong delta; these and their main difference are listed in Table 2 (FAO 2005; Phan et al. 2011). For a more elaborate explanation about the differences in farming practices identified during the SEAT project, we refer to Phan et al. (2011) and Henriksson et al. (2015).

Shrimp farms are established by removing the vegetation (or part of it) or transforming the land from alternative land uses (commonly rice paddies), followed by digging of ponds. Although the depth of ponds can vary slightly between farming practices, an average pond depth of 1.5 m was assumed (Phan et al. 2011; Murray et al. 2013) meaning that 1.5 m of soil carbon was assumed to be lost. Mangrove forests have shallow root systems, which means that most of the roots are found in the upper 0.7 m of the soil (National Oceanic and Atmospheric Administration-Earth System 2010). All mangrove roots were therefore considered lost during pond construction. The exact amount of carbon that is removed as soil is more difficult to predict, as some of the sediments normally are used to construct pond walls, where the carbon would

Table 2 Five common Asian tiger shrimp farming practices identified in Vietnam, with data from Murray et al. (2013), and in brackets from Phan et al. (2011), and the Food and Agriculture Organization of the United Nations (FAO 2005)

System	Crops year^{-1}	t shrimp crop^{-1} ha^{-1} water surface area	t shrimp ha^{-1} water surface area year^{-1}
Intensive	1	7.6 ± 7.0	7.6 (10–17.5)
Semi-intensive	1.15	4.4 ± 4.5	6.6 (2–4)
Improved extensive	1.25	0.25 ± 0.29	0.3 (1–1.2)
Mixed mangrove concurrent	1	0.13 ± 0.12	0.13 (0.25–0.30)

degrade under hypoxic conditions (Mungkung 2005). Carbon loss due to soil removal consequently depends on many factors, including the depth of the pond, the age of the shrimp farm and wall, the size and surface area of the wall, and the fate of the sediments after the shrimp farm has been abandoned. It has been conservatively assumed in previous studies that about half of the carbon is oxidized (Eong 1993), and this assumption was adopted in the calculations for the present study.

To identify which types of shrimp farms result in mangrove LULUC, the GPS coordinates from the SEAT survey were evaluated using satellite images, comparing land use before and after the establishment of the farms, and categorized based upon prior land use and farming practices. While the SEAT dataset also includes farmer testimonies on prior land use, these responses were sometimes biased due to shifts in farm ownership and to Vietnamese shrimp farmers being aware of the controversy surrounding mangrove deforestation. Satellite images from Google Earth (Google 2013), the Global Land Cover by National Mapping Organizations (ISCGM 2003), and a map showing global mangrove distribution developed by Giri et al. (2011) were therefore used to supplement the farmers' responses.

The combination of three sources of satellite imagery provided information about the historic land use going back to the year 2000, before any of the analyzed shrimp farms had been established. Present mangrove cover was estimated by locating the farms on Google Earth, drawing boundary lines around the pond, and estimating the percentage of mangrove within this boundary. All mangrove located within the boundary of the shrimp pond were considered to be part of the pond (for an example and description of mixed mangrove concurrent shrimp farm, see the study by Jonell and Henriksson 2014). Consequently, the difference in mangrove cover before and after pond establishment could be determined for the different types of shrimp farming (for more details on the method used, see the ESM 1 of this article). Low-resolution images were excluded from further analyses.

After the shrimp farming systems that resulted in mangrove deforestation had been identified, the resulting GHG emissions from LULUC were calculated. The functional unit of the shrimp production was defined as 1 t of live weight shrimp at farm. The average lifetime of shrimp farms was deemed as the most suitable timeframe, as all shrimp produced contribute equally to the mangrove forest LULUC. A timeframe of 50 years, the current life expectancy of a shrimp farm, was therefore used to annualize emissions (Jonell and Henriksson 2014) (see ESM2 for calculations).

3 Results

3.1 Carbon dioxide emissions per hectare of deforested mangrove

Parameters for Eqs. (2) and (3) are presented in Table 3. Due to the limited literature values, studies describing globally diverse mangrove forests were used. Our estimated emissions can therefore be used as proxies for mangrove LULUC emissions worldwide, but also entail large uncertainties (see ESM 1).

Given the presented ranges, all distributions except litter C (assumed as normally distributed) were assumed to be lognormal (ln). For default mangrove removal annualized over 20 years, as recommended by the IPCC, the resulting CO_2 emissions were 129 t CO_2 ha^{-1} year^{-1} (CV = 0.441, ln), while if the emissions were annualized over 50 years, the annual emission was estimated to 54 t CO_2 ha^{-1} year^{-1} (CV = 0.424, ln). The ESM 2 gives a more detailed report on the results of the literature study and the calculated results.

3.2 Methane and dinitrogen monoxide emissions per hectare of mangrove converted to aquaculture pond

Studies on intact mangrove suggest that methane fluxes in estuarine wetlands, including mangrove forests, are remarkably low due to the inhibition of methanogenesis by sulfates (Kristensen et al. 2008; Howe et al. 2009). Deforestation and fish farming undoubtedly increase these gas fluxes, an assumption also supported by Astudillo et al. (2015) (Table 4). We consequently adopted Astudillo et al.'s (2015) estimate (533 kg CH_4 ha^{-1} year^{-1}; CV = 0.4, ln) as a worst-case scenario. As for dinitrogen monoxide, emissions are more dependent

Table 3 Overview of average values of mangrove forest carbon stocks

Reference	Parameter	Median	CV (distribution)	Range	*n*
AG	Above-ground C stock (t C ha^{-1})[a]	131	0.462 (ln)	49.5–261	9
BG	Below-ground C stock (t C ha^{-1})[b]	80	1.525 (ln)	9.61–410	8
S	Soil C stock per 1.5 m of depth (t C ha^{-1})[c]	724	0.595 (ln)	186.15–1575	8
L	Litter loss C stocks (t C ha^{-1})[d]	4.03	0.477 (n)	0.15–7	12
CS	C Missed potential (t C ha^{-1} $year^{-1}$)[e]	1.25	0.936 (ln)	0.012–3.53	8

ln lognormal distribution, *n* normal distribution

[a] Twilley et al. 1992; Eong 1993; Matsui 1998; Kauffman et al. 2011; Donato et al. 2011; Ray et al. 2011; Donato et al. 2012

[b] Komiyama et al. 1987; Twilley et al. 1992; Matsui 1998; Kauffman et al. 2011; Ray et al. 2011; Donato et al. 2012

[c] Eong 1993; Matsui 1998; Kauffman et al. 2011; Ray et al. 2011; Donato et al. 2012; Lundstrum and Chen 2014

[d] Twilley et al. 1992; Amarasinghe and Balasubramaniam 1992; Eong 1993; Day et al. 1996; Middleton and McKee 2001; Jennerjahn and Ittekkot 2004; Guzman et al. 2005; Ray et al. 2011

[e] Twilley et al. 1992; Eong 1993; Duarte and Cabrián 1996; Chmura et al. 2003; Alongi 2008; Sanders et al. 2010; Ray et al. 2011; Mcleod et al. 2011

on the inputs of nitrogen into the ponds as feed or fertilizer. Hu et al. (2012), for example, assumed that 1.8% of the nitrogen input was converted to dinitrogen monoxide (as N_2O-N, or 1.15% as N_2O). The IPCC (2014) also adopted the generic estimate of 1.69 kg N_2O-N t^{-1} fish by Hu et al. (2012) for mangrove-integrated aquaculture. Since we know that most mixed mangrove shrimp farming systems are net removers of nitrogen (Jonell and Henriksson 2014), and that standing mangrove even potentially could be a net inhibitor of dinitrogen monoxide emissions (Allen et al. 2007), we here assume a precautions scenario of 1.67 kg N_2O ha^{-1} $year^{-1}$ (CV = 0.575, ln).

3.3 Greenhouse gas emissions from shrimp farming LULUC case study

The analysis of historic land use identified only "mixed mangrove concurrent" shrimp farms being established in former mangrove areas (Table 5). It was assumed that these shrimp farms were the primary cause for the mangrove deforestation, as there were no indications of other activities on this land. Considering that only "mixed mangrove concurrent" shrimp farms were identified as causing mangrove deforestation, the related GHG emissions from mangrove LULUC were only calculated for this type of farming practice. Table 6 lists the GHG emissions per hectare per year for mixed mangrove concurrent shrimp farms.

The average mixed mangrove concurrent shrimp farm produces about 6.5 t of shrimp by water area over 50 years. However, Asian tiger shrimp are not the only commodity produced in these ponds (Vu et al. 2013; Jonell and Henriksson 2014), with only about 39.2% of the total output being Asian tiger shrimp by volume and 59.7% by value. The rest of the harvest consists of a mix of other shrimps, mud crabs, and other aquaculture products (Vu et al. 2013; Jonell and Henriksson 2014). The GHG emissions from mangrove LULUC from shrimp farming were subsequently estimated to be 184 t CO_2-eq t^{-1} live shrimp at farm gate using mass allocation and 282 t CO_2-eq t^{-1} live shrimp using economic allocation (see ESM 2 for details on the calculations and

Table 4 Literature values

System	Emission	Mean	Uncertainty estimate	Reference
Intact mangrove forest	kg CH_4 ha^{-2} $year^{-1}$	342	CV = 1.448 (ln)	Allen et al. 2007
	kg N_2O ha^{-2} $year^{-1}$	1.67	CV = 0.575 (ln)	Allen et al. 2007
Open aquaculture ponds	kg CH_4 ha^{-1} $year^{-1}$	533	CV = 0.40 (ln)	Astrudillo et al. 2015
	N_2O-N	1.8% of N input	–	Hu et al. 2012
Rewetted land, previously vegetated by mangrove, salinity <18 ppm	kg CH_4 ha^{-1} $year^{-1}$	194	CV = 2.290 (ln)	IPCC 2014
Rewetted land, previously vegetated by mangrove, salinity >18 ppm	kg CH_4 ha^{-1} $year^{-1}$	0	Range = 0–40 (uniform)	IPCC 2014

Table 5 Prior land uses affected by LUC in the case study, in combination with data derived from Google Earth (Google 2013), Global Land Cover by National Mapping Organizations (GLCNMO) (ISCGM 2003), Giri et al. (2011), and Murray et al. (2013)

	Intensive monoculture	Semi-intensive monoculture	Improved extensive	Improved extensive alternate	Mixed mangrove concurrent
n of farms	17	51	20	6	11
Total number of hectares	250	105	26.8	10.9	35.5
Mangrove	0%	0%	0%	0%	100%
Aquaculture pond	0%	0%	0%	0%	0%
Rice paddies	18.4%	41.4%	100%	76.1%	0%
Forest land	21.5%	16.9%	0%	23.8%	0%
Grassland	48.1%	24.8%	0%	0%	0%
Cropland	12.0%	0%	0%	0%	0%
Settlement	0%	17.0%	0%	0%	0%
Total	100%	100%	100%	100%	100%

assumptions behind them). Noteworthy is that these LULUC emissions only apply to “mixed mangrove concurrent” shrimp farms, a farming practice that makes up less than 5% of the total shrimp production of Vietnam, and only about 1.2% global production (FAO FishstatJ 2014; Jonell and Henriksson 2014). Additionally, the analysis of 25 farms regarding their current land cover showed that this type of farming leaves an average of 39% (CV = 0.322, range 16–69%) of the original mangrove forest intact within the farming premises, thus removing 61%.

To put these LULUC emissions into proportion, they were compared with LCA emissions calculated by Henriksson et al. (2015). In their study, semi-intensive and intensive conventional shrimp farming both had global warming impacts of 13.2 t CO_2-eq t^{-1} shrimp using mass allocation, and 4.7 and 5.1 t CO_2-eq t^{-1} shrimp, respectively, using economic allocation (including N_2O emissions from ponds). Given that these ponds had been aquaculture ponds for a long time, or converted from rice paddies, we can compare the importance of LULUC emissions from mixed mangrove shrimp (excluding LCA emissions) with those of a set of different system combinations (Table 7). As rice conversion of rice farms included the removal of sediments during pond construction, LUC emissions from $\Delta\beta\delta_S$ were therefor included. Comparing the different systems and prior land uses highlight the magnitude of LULUC emissions from mixed mangrove farms. Using mass allocation, a ton of Asian tiger shrimp from mixed mangrove systems would emit an order of magnitude more GHG emissions than from any of the other systems, a difference that is even starker using economic allocation.

4 Discussion and conclusions

4.1 Methods and data for quantifying carbon dioxide emissions per hectare of mangrove deforested

Despite the importance of mangrove forests for carbon capture and sequestration, there are still only a few available studies quantifying its carbon content and burial rates. This naturally induces uncertainty when calculating GHG emissions caused by LULUC. Part of the difference in the outcomes can be explained by the natural variation among different types of mangrove forests. Reported values for soil carbon content, for example, range from 186 to 1575 t C ha^{-1} per 1.5 m depth. To be able to calculate more reliable global averages, there is a need for more data, as is emphasized by the large variances in the sensitivity results.

Relatively large differences in GHG emissions also arise from the assumptions on the depth and percentage of carbon in mangrove soils that is affected, although to a lesser degree. Following the IPCC (IPCC 2014) guidelines of 20-year annualization time and soil carbon loss of 1 m depth and 96% of carbon being oxidized would lead to estimates of 129 t CO_2 ha^{-1} $year^{-1}$ deforested mangrove forest. However, in case of shrimp farming,

Table 6 GHG emissions as tons of CO_2-equivalent including coefficients of variance from mangrove LULUC due to “mixed mangrove concurrent” shrimp farming per hectare and year annualized over 50 years

Reference	$\Delta\beta_{AG}$	$\Delta\beta_{BG}$	$\Delta\beta_L$	$\Delta\beta\delta_S$	$\Delta\theta_{MP}$	Total CO_2	CH_4 emissions	N_2O emissions	Total CO_2-eq ha^{-1} $year^{-1}$
Average	9.6	5.9	0.3	26.5	4.6	46.9	14.9	0.4	62.2
CV	0.467	1.503	0.268	0.601	0.903	0.409	0.400	0.575	

Table 7 LULUC, methane, and dinitrogen oxide emissions from mixed mangrove, semi-intensive, and intensive farms for farming Asian tiger shrimps during 50 years compared to LCA

System	Allocation factor	Prior land use	t shrimp ha^{-1} water surface area $year^{-1}$	LULUC t CO_2 t^{-1} shrimp	LU CH_4 emissions, t CO_2-eq t^{-1} shrimp	LU N_2O emissions, kg CO_2-eq t^{-1} shrimp	Lifecycle emissions, t CO_2-eq t^{-1} shrimp[a]	Total, CO_2-eq t^{-1} shrimp
Mixed mangrove	Mass (38.5%)	Mangrove	0.13	139	44.2	1.31		184
Semi-intensive	Mass (100%)	Aquaculture pond	6.6		2.3	Including in LCA	13.2	15.5
Semi-intensive	Mass (100%)	Rice paddy	6.6	2.4	2.3	Including in LCA	13.2	21.5
Intensive	Mass (100%)	Aquaculture pond	7.6		2.0	Including in LCA	13.2	15.2
Intensive	Mass (100%)	Rice paddy	7.6	2.1	2.0	Including in LCA	13.2	20.4
Mixed mangrove	Eco (58.8%)	Mangrove	0.13	212	67.5	2		282
Semi-intensive	Eco (100%)	Aquaculture pond	6.6		2.3	Including in LCA	4.7	7.0
Semi-intensive	Eco (100%)	Rice paddy	6.6	2.4	2.3	Including in LCA	4.7	13.0
Intensive	Eco (100%)	Aquaculture pond	7.6		2.0	Including in LCA	5.1	7.1
Intensive	Eco (100%)	Rice paddy	7.6	2.1	2.0	Including in LCA	5.1	12.3

[a] From Henriksson et al. (2015)

the soil is affected up to 1.5 m of depth while only 50% is oxidized and emissions are annualized over 50 years instead. This would in turn lead to 47 t CO_2 ha^{-1} $year^{-1}$ deforested mangrove forest. This shows how important it is to understand the consequences of different land uses on carbon stocks in mangrove soils. It also suggests that emissions from oxidized soil materials might also be relevant to consider when evaluating, i.e., the construction of Pangasius ponds (freshwater species not located in mangrove areas), as these normally are up to 4 m deep.

While carbon sequestration rates by mangrove forests nowadays are better understood and documented (Eong 1993; Duarte et al. 2005; Kauffman et al. 2011; Mcleod et al. 2011), they constituted another parameter with large data discrepancies. However, estimates of missed potential carbon sequestration were based on carbon build-up in the sediments that occurred over the past decades or even centuries (Eong 1993; Duarte et al. 2005; Mcleod et al. 2011). Choosing to ignore this unique property of mangrove forest would underestimate the global warming impact of mangrove LULUC with about 8%. We therefore encourage further research to better understand the drivers behind carbon sequestration in mangrove forests and the influence of sea-level rise on these. We also stress the importance of quantifying uncertainties when considering LULUC emissions, as it is the only way to provide a level of confidence behind comparisons and it indicates that emissions can differ widely among locations, managements, and species of mangroves.

4.2 Case study results

The case study revealed the importance of including LULUC emissions when doing LCAs of systems that result in mangrove deforestation, to understand the full global warming impacts. Within the temporal frame of this study (2000–present), only mixed mangrove concurrent shrimp farming resulted in direct mangrove deforestation. Our calculations resulted in LULUC GHG emission estimations of 184 t CO_2-eq t^{-1} live shrimp at farm gate using mass allocation and 282 t CO_2-eq t^{-1} live shrimp at farm gate using economic allocation of a mixed mangrove concurrent farm, with 68.0% originating from land-use change and 32.0% from land use. Amortized over 50 years, the emissions from mangrove LULUC were 24 to 37 t CO_2-eq ha^{-1} $year^{-1}$, which is far greater than other LCA emissions estimated for Vietnamese shrimp farms. It should be added that the calculations were based on the shrimp yield that was given per hectare of water area. As the patches of mangrove located within the pond area were therefore excluded from this value, we assumed 100% mangrove deforestation. However, it was noticed during the analysis of satellite data that many farmers had included these patches of mangrove land when reporting their total water area. This would thus mean that the emissions from mangrove deforestation resulting from shrimp farming would in practice be lower than those presented above. Moreover, the estimated single annual yield among intensive shrimp farmers were likely influenced by a disease outbreak, as shrimp farmers normally yield two, up to three, harvests per year.

Table 8 shows the extent to which this choice of timeframe directly influenced the results by varying the number of years over which the emissions were annualized. The present study based the timeframe within which the GHG emissions from mangrove LULUC were assigned to shrimp farming on the assumed life expectancy of the shrimp farm, which was 50 years. This resulted in 184 to 282 t CO_2-eq t^{-1} of shrimp for mass and economic allocation, respectively, with CO_2 from LUC accounting for 68.0% of the total emissions, CO_2 from LU for 7.4%, CH_4 emissions for 24.0%, and N_2O emissions for 0.7%. Fifty years seemed like a logical choice to consider all shrimp produced as equal contributors to the deforestation, based on the data on the life expectancy of farms. However, it is common practice to set the timeframe to a prefixed number of years. Following, i.e., the IPCC (2006) default recommendation of 20 years would lead to 372.2 to 568.5 t CO_2-eq t^{-1} of shrimp for mass and economic allocation, respectively. In this case, CO_2 from LUC would account for 81.4% of the total emissions, CO_2 from LU for 3.6%, CH_4 for 11.9%, and N_2O for 0.4%. Naturally, the shorter the timeframe, the higher the GHG emissions per ton of shrimp and the higher the contribution of LUC CO_2 emissions, which is why it is important to understand these implications and choose a realistic timeframe corresponding to current knowledge on shrimp farming.

Noteworthy is that the displacement of some prior land uses, such as agricultural fields, might result in these farms relocating elsewhere and consequently causing additional forest lost. Richards and Friess (2015), for example, allocated the loss of 55.4 ha of mangrove towards new rice farms between 2000 and 2012, while aquaculture farms were responsible for roughly the double (111.8 ha). The overall rice paddy area, in the main time, increased by 95,000 ha, an increase of 1.2% of the overall rice paddy area (FAO 2016), compared to a 265% increase in the farmed shrimp production during the same period. Thus, the interplay of marginal demands for land is complex and could not be explored within the context of the present research.

According to the Protection Forest Management Boards, families practicing mixed mangrove concurrent shrimp farming are allowed to use up to 30% of the land and surface water for their own purposes to generate income (McNally et al. 2010). According to satellite photos, however, an average of 61% of the mangrove forest was removed from pond areas. It is therefore debatable if mixed mangrove concurrent shrimp farming protects mangrove forests as effectively as originally intended. On the other hand, these farms only make up a small share of overall shrimp production in Vietnam, and only 25 farms in two provinces were evaluated. Moreover, satellite imagery only dated back to the year 2000, and the images only provide rough estimates of vegetation types, while most

Table 8 Results for GHG emissions as CO_2-equivalent from mangrove LULUC due to shrimp farming in the "mixed mangrove concurrent" farming system, per ton of live shrimp over different time frames

	t CO_2-eq t^{-1} shrimp	Years				
		10	20	50	100	200
Mass allocation						
LU	CO_2	13.6 (2.0%)	13.6 (3.6%)	13.6 (7.4%)	13.6 (11.1%)	13.6 (15.0%)
	CH_4	44.2 (6.4%)	44.2 (11.9%)	44.2 (24.0%)	44.2 (36.3%)	44.2 (48.9%)
	N_2O	1.3 (0.2%)	1.3 (0.4%)	1.3 (0.7%)	1.3 (1.1%)	1.3 (1.4%)
LUC	CO_2	626.3 (91.4%)	313.2 (84.1%)	125.3 (68.0%)	62.6 (51.5%)	31.3 (34.6%)
LULUC	CO_2-eq	685.4	372.2	184.3	121.7	90.4
Economic allocation						
LU	CO_2	20.7 (2.0%)	20.7 (3.6%)	20.7 (7.4%)	20.7 (11.1%)	20.7 (15.0%)
	CH_4	67.5 (6.4%)	67.5 (11.9%)	67.5 (24.0%)	67.5 (36.3%)	67.5 (48.9%)
	N_2O	2.0 (0.2%)	2.0 (0.4%)	2.0 (0.7%)	2.0 (1.1%)	2.0 (1.4%)
LUC	CO_2	956.6 (91.4%)	478.3 (84.1%)	191.3 (68.0%)	95.7 (51.5%)	47.8 (34.6%)
LULUC	CO_2-eq	1046.8	568.5	281.5	185.9	138.1

deforestation in Vietnam happened during the 1980s and 1990s (WWF 2013; Richards and Friess 2015). Despite this, agencies promoting these systems (i.e., Naturland) need to reevaluate their environmental sustainability, especially in countries where this type of shrimp farming is more common, including Indonesia (DasGupta and Shaw 2013; Richards and Friess 2015).

As mentioned before, research on methane emissions resulting from land use of mangrove area is rather limited and most research on methane fluxes focus on integrated rice-fish ponds (Frei and Becker 2005; Datta et al. 2009) or other human disturbances of mangroves (i.e., Konnerup et al. 2014). The pond conditions in the IAA systems, which were used as a proxy for methane emissions in this study, came closest to those found in the mixed mangrove concurrent systems (Astudillo et al. 2015). However, emissions are highly influenced by farming practices (including aeration, feed use, co-stocked species, and fertilization) and environmental conditions (including salinity, oxygen levels, and temperature) (Alongi 2005; Howe et al. 2009; Penha-Lopes et al. 2010; Astudillo et al. 2015). For example, the presence of sulfates in mangrove systems would limit the activity of methanogenesis (Howe et al. 2009), thereby lowering the methane emissions for the mixed mangrove system compared to the IAA system. It is therefore important to remember that our results for methane emissions are only a first proxy of the potential magnitude of the emissions, and we therefore urge for more research on this topic.

Despite the limitations highlighted above, our worst-case estimates are not even close to those presented by Kauffman (Stokstad 2012), who estimated the LULUC emissions due to shrimp farming to be 198 kg CO_2 per 100 g shrimp tail or 1307 t CO_2 t^{-1} shrimp (assuming an edible yield of 34%; Louisiana Direct Seafood 2011; Zirlotts Gulf Products 2013), more than four times higher than our highest estimate. Kauffman's calculations differ from ours mainly in different assumptions regarding the percentage of shrimp farms constructed in former mangrove areas (50–60%), early abandonment of farms (after 3–9 years), and an annual production of just 50 to 500 kg ha^{-1} for the average shrimp farm (Stokstad 2012). Kauffman's assumptions on early abandonment of farms can lead to the high climate warming impact, as the emissions are attributed to only a few years of shrimp farming yields. In contrast, our study found that a mere fraction of shrimp come from farms located in former mangrove areas, that management has improved to a point where farming can be maintained for 50 years or more (Jonell and Henriksson 2014), that only 39.2% of the pond mass output of extensive mixed mangrove farming is shrimp, and that even these farms produce 0.13 t shrimp ha^{-1} $year^{-1}$ on average (Murray et al. 2013).

Important to highlight is that GHG emissions are only one of the many environmental concerns associated with shrimp farming. Others include loss of biodiversity, eutrophication, freshwater ecotoxicity, overexploitation of juveniles, acidification, and photochemical oxidation (Jonell and Henriksson 2014). LCAs including LULUC therefore also consider such environmental impacts. In the process of doing so, the structure of the mangrove removal should be taken into consideration, as it could be argued that partial removal, as is done in mixed mangrove farms, may leave enough mangrove to buffer eutrophying emissions (Jonell and Henriksson 2014). Moreover, ecosystem services maintained by preserving part of the forest should not be neglected, as phenomena like tsunamis and typhoons are commonplace in SE Asia. Securing a livelihood in return for partial protection of the mangrove may therefore help to conserve the remaining mangrove forests, if properly managed. Such socio-ecological trade-offs surely need to be considered before making any policy decisions since long-term protection of the remaining mangroves in Asia is of the utmost importance.

Nonetheless, the results of our case study of shrimp farming in mangrove areas show how huge mangrove LULUC emissions can be compared with similar emissions from other activities in the shrimp farming value chain (i.e., feed provision and electricity generation). Despite uncertainties and limitations in the underlying data, the sheer magnitude of the emissions shows that excluding mangrove LULUC emissions from LCA studies most certainly leads to a severe underestimation of the actual GHG emissions.

Acknowledgements In memoriam of Prof. Dr. Ir. Gerard PJ Dijkema who was central to the early stages of shaping this research.

This work has been done as part of the Master Industrial Ecology of Leiden University/Technical University Delft and has built on results of the Sustaining Ethical Aquaculture Trade (SEAT) project, which was co-funded by the European Commission within the Seventh Framework Programme—Sustainable Development Global Change and Ecosystem (Project 222889); www.seatglobal.eu.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Allen DE, Dalal RC, Rennenberg H, Meyer RL, Reeves S, Schmidt S (2007) Spatial and temporal variation of nitrous oxide and methane flux between subtropical mangrove sediments and the atmosphere. Soil Biol Biochem 39(2):622–631

Alongi DM (2005) Mangrove–microbe–soil relations. In: Interactions between macro- and microorganisms in marine sediments. American Geophysical Union, pp 85–103

Alongi DM (2008) Mangrove forests: resilience, protection from tsunamis, and responses to global climate change. Estuar Coast Shelf Sci 76:1–13

Amarasinghe MD, Balasubramaniam S (1992) Net primary productivity of two mangrove forest stands on the northwestern coast of Sri Lanka. Hydrobiologia 247:37–47

Astudillo MF, Thalwitz G, Vollrath F (2015) Modern analysis of an ancient integrated farming arrangement: life cycle assessment of a mulberry dyke and pond system. Int J Life Cycle Assess 20:1387–1398

Béland M, Goïta K, Bonn F, Pham TTH (2006) Assessment of land-cover changes related to shrimp aquaculture using remote sensing data: a case study in the Giao Thuy District, Vietnam. Int J Remote Sens 27: 1491–1510

Bouillon S, Borges AV, Castañeda-Moya E et al (2008) Mangrove production and carbon sinks: a revision of global budget estimates. Glob Biogeochem Cycles. doi:10.1029/2007GB003052

Cederberg C, Persson UM, Neovius K et al (2011) Including carbon emissions from deforestation in the carbon footprint of Brazilian beef. Environ Sci Technol 45:1773–1779

Chmura GL, Anisfeld SC, Cahoon DR, Lynch JC (2003) Global carbon sequestration in tidal, saline wetland soils. Glob Biogeochem Cycles. doi:10.1029/2002GB001917

DasGupta R, Shaw R (2013) Cumulative impacts of human interventions and climate change on mangrove ecosystems of south and Southeast Asia: an overview. J Ecosyst 2013:1–15

Datta A, Nayak DR, Sinhababu DP, Adhya TK (2009) Methane and nitrous oxide emissions from an integrated rainfed rice-fish farming system of eastern India. Agric Ecosyst Environ 129:228–237

Day JW Jr, Coronado-Molina C, Vera-Herrera FR et al (1996) A 7 year record of above-ground net primary production in a southeastern Mexican mangrove forest. Aquat Bot 55:39–60

Donato DC, Kauffman JB, Murdiyarso D et al (2011) Mangroves among the most carbon-rich forests in the tropics. Nat Geosci 4:293–297

Donato DC, Kauffman JB, Mackenzie RA et al (2012) Whole-island carbon stocks in the tropical pacific: implications for mangrove conservation and upland restoration. J Environ Manag 97:89–96

Duarte CM, Cabrián J (1996) The fate of marine autotrophic production. Limnol Oceanogr 41:1758–1766

Duarte CM, Middelburg JJ, Caraco N (2005) Major role of marine vegetation on the oceanic carbon cycle. Biogeosciences 2:1–8

Eong OJ (1993) Mangroves—a carbon source and sink. Chemosphere 27:1097–1107

FAO (2005) Cultured aquatic species information programme: *Penaeus monodon*. http://www.fao.org/fishery/culturedspecies/Penaeus_monodon/en. Accessed 17 Jul 2013

FAO (2007) Mangrove trees and shrubs of the Maldives. Bangkok, Thailand

FAO (2016) FAOSTAT database. http://www.fao.org/faostat/en/#home. Accessed 1 Dec 2016

FAO FishstatJ (2014) FishstatJ—software for fishery statistical time series. http://www.fao.org/fishery/statistics/software/fishstatj/en. Accessed 10 Sep 2015

Frei M, Becker K (2005) Integrated rice-fish production and methane emission under greenhouse conditions. Agric Ecosyst Environ 107:51–56

Giri C, Ochieng E, Tieszen LL et al (2011) Status and distribution of mangrove forests of the world using earth observation satellite data. Glob Ecol Biogeogr 20:154–159

Global Aquaculture Alliance (2012) Dated, erroneous assumptions yield misleading "Carbon Footprint" for farmed shrimp. http://www.gaalliance.org/newsroom/news.php?Dated-Erroneous-Assumptions-Yield-Misleading-Carbon-Footprint-For-Farmed-Shrimp-59. Accessed 1 Aug 2013

Google (2013) Google Earth. http://www.google.com/earth/index.html. Accessed 15 Dec 2015

Guzman HM, Barnes PAG, Lovelock CE, Feller IC (2005) A site description of the CARICOMP mangrove, seagrass and coral reef sites in the Bocas del Toro, Panama. Caribb J Sci 41:430–440

Henriksson PJG, Guinée JB, Kleijn R, de Snoo GR (2012) Life cycle assessment of aquaculture systems—a review of methodologies. Int J Life Cycle Assess 17:304–313

Henriksson PJG, Rico A, Zhang W et al (2015) Comparison of Asian aquaculture products by use of statistically supported life cycle assessment. Environ Sci Technol 49:14176–14183

Hong PN, Dao QTQ (2004) Environmental impact of shrimp culture in the mangrove areas of Vietnam. Annual Report of FY 2003, The Core University Program between Japan Society for the Promotion of Science (JSPS) and National Centre for Natural Science and Technology (NCST). Hanoi, Vietnam

Howe AJ, Rodríguez JF, Saco PM (2009) Surface evolution and carbon sequestration in disturbed and undisturbed wetland soils of the hunter estuary, southeast Australia. Estuar Coast Shelf Sci 84:75–83

Hu Z, Lee JW, Chandran K et al (2012) Nitrous oxide (N_2O) emission from aquaculture: a review. Environ Sci Technol 46:6470–6480

IPCC (2006) 2006 IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme. Eggleston HS, Buendia L, Miwa K, Ngara T, Tanabe K (eds), Japan

IPCC (2014) 2013 Supplement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories: Wetlands. Hiraishi, T, Krug, T, Tanabe, K, Srivastava, N, Baasansuren, J, Fukuda, M and Troxler, TG (eds). Switzerland

ISCGM (2003) Land Cover (GLCNMO)—global version. http://www.iscgm.org/gm/glcnmo.html. Accessed 24 Jun 2013

ISO (2006) Environmental management—Life cycle assessment—Requirements and guidelines. International Standard ISO 14044; International Organisation for Standardisation: Geneva, Switzerland

Jennerjahn TC, Ittekkot V (2004) Relevance of mangroves for the production and deposition of organic matter along tropical continental margins. Naturwissenschaften 89:23–30

Jonell M, Henriksson PJG (2014) Mangrove-shrimp farms in Vietnam—comparing organic and conventional systems using life cycle assessment. Aquaculture. doi:10.1016/j.aquaculture.2014.11.001

Kauffman JB, Heider C, Cole TG et al (2011) Ecosystem carbon stocks of Micronesian mangrove forests. Wetlands 31:343–352

Komiyama A, Ogina K, Aksornkoae S, Sabhasri S (1987) Root biomass of a mangrove forest in southern Thailand. 1. Estimation by the trench method and the zonal structure of root biomass. J Trop Ecol 3:97–108

Konnerup D, Betancourt-Portela JM, Villamil C, Parra JP (2014) Nitrous oxide and methane emissions from the restored mangrove ecosystem of the Ciénaga Grande de Santa Marta, Colombia. Estuar Coast Shelf Sci 140:43–51

Kristensen E, Bouillon S, Dittmar T, Marchand C (2008) Organic carbon dynamics in mangrove ecosystems: a review. Aquat Bot 89:201–219

Lebel L, Tri NH, Saengnoree A et al (2002) Industrial transformation and shrimp aquaculture in Thailand and Vietnam: pathways to ecological, social, and economic sustainability? Ambio 31:311–323

Lebel L, Mungkung RT, Gheewala SH, Lebel P (2010) Innovation cycles, niches and sustainability in the shrimp aquaculture industry in Thailand. Environ Sci Pol 13:291–302

Louisiana Direct Seafood (2011) Seafood handbook: Shrimp story. http://louisianadirectseafood.com/handbook. Accessed 22 Jun 2013

Lovelock CE (2008) Soil respiration and belowground carbon allocation in mangrove forests. Ecosystems 11:342–354

Lundstrum A, Chen L (2014) Soil carbon stocks and accumulation in young mangrove forests. Soil Biol Biochem 75:223–232

Matsui N (1998) Estimated stocks of organic carbon in mangrove roots and sediments in Hinchinbrook channel, Australia. Mangroves salt marshes 2:199–204
Mcleod E, Chmura GL, Bouillon S et al (2011) A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO_2. Front Ecol Environ 9:552–560
McNally R, McEwin A, Holland T (2010) The potential for mangrove carbon projects in Vietnam. Netherlands
Middleton BA, McKee KL (2001) Degradation of mangrove tissues and implications for peat formation in Belizean island forests. J Ecol 89: 818–828
Mungkung RT (2005) Shrimp aquaculture in Thailand: application of life cycle assessment to support sustainable development, PhD thesis
Murray BC, Watt CE, Cooley DM, Pendleton LH (2012) Coastal Blue Carbon and the United Nations Current Status and Future Directions; Policy brief
Murray FJ, Haque MM, Zhang W et al (2013) Defining boundaries towards understanding sustainable ethical aquaculture trade between Asia and Europe. SEAT Project Report 2.8. Stirling, UK
National Oceanic and Atmospheric Administration-Earth System (2010) Mangrove ecology. In: Encycl. Earth. http://www.eoearth.org/article/Mangrove_ecology?topic=49514. Accessed 28 Jun 2013
Penha-Lopes G, Kristensen E, Flindt M et al (2010) The role of biogenic structures on the biogeochemical functioning of mangrove constructed wetlands sediments—a mesocosm approach. Mar Pollut Bull 60:560–572
Phan LT, Nguyen PT, Murray FJ, Little CD (2011) Development trends and local sustainability perceptions for the international trade in seafood farmed in Vietnam. Deliverable 2.1c of the SEAT project. Stirling, UK
Ray R, Ganguly D, Chowdhury C et al (2011) Carbon sequestration and annual increase of carbon stock in a mangrove forest. Atmos Environ 45:5016–5024
Richards DR, Friess DA (2015) Rates and drivers of mangrove deforestation in Southeast Asia, 2000–2012. Proc Natl Acad Sci USA Early Edit:1–6. doi: 10.1073/pnas.1510272113
Rönnbäck P (1999) The ecological basis for economic value of seafood production supported by mangrove ecosystems. Ecol Econ 29:235–252
Sanders CJ, Smoak JM, Naidu AS et al (2010) Organic carbon burial in a mangrove forest, margin and intertial mud flat. Esturine, Coast Shelf Sci 30:168–172
Schmidinger K, Stehfest E (2012) Including CO_2 implications of land occupation in LCAs—method and example for livestock products. Int J Life Cycle Assess 17:962–972
Stokstad E (2012) The carbon footprint of a shrimp cocktail. In: AAAS, Sci. News. http://news.sciencemag.org/earth/2012/02/carbon-footprint-shrimp-cocktail. Accessed 7 Sep 2015
Twilley RR, Chen RH, Hargis T (1992) Carbon sinks in mangroves and their implications to carbon budget of tropical coastal ecosystems. Water Air Soil Pollut 64:265–288
Vu TA, Phan LT, Do H Van et al (2013) Status of small-scale environmentally friendly shrimp production in Ca Mau Province, Viet Nam
WWF (2013) Ecosystems in the Greater Mekong. Past trends, current status, possible futures; WWF report Greater Mekong
Zirlotts Gulf Products (2013) Shrimp. http://www.zirlottseafood.com/store/shrimp.html. Accessed 22 Jun 2013

ARTICLE II

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

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

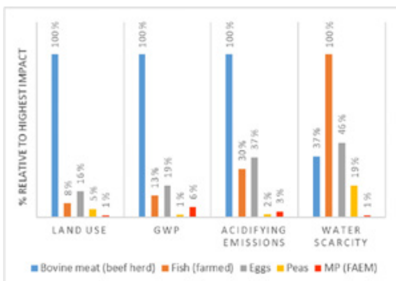
Natasha Järviö [a,b,c,*], Netta-Leena Maljanen [a,b,c], Yumi Kobayashi [a,b,d], Toni Ryynänen [a,b,c], Hanna L. Tuomisto [a,b,d,e]

[a] Future Sustainable Food Systems-Research Group, Faculty of Agriculture and Forestry, University of Helsinki, P.O. Box 27, 00014 University of Helsinki, Finland
[b] Helsinki Institute of Sustainability Science (HELSUS), University of Helsinki, P.O. Box 4, 00014 University of Helsinki, Finland
[c] Ruralia Institute, Faculty of Agriculture and Forestry, University of Helsinki, Lönnrotinkatu 7, 50100 Mikkeli, Finland
[d] Department of Agricultural Sciences, Faculty of Agriculture and Forestry, University of Helsinki, P.O. Box 27, 00014 University of Helsinki, Finland
[e] Natural Resources Institute Finland, P.O.Box 2, 00790 Helsinki, Finland

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

* Corresponding author at: Faculty of Agriculture and Forestry, University of Helsinki, P.O. Box 27, 00014 University of Helsinki, Finland.
E-mail address: natasha.jarvio@helsinki.fi (N. Järviö).

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 LCA_{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 LCA_{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

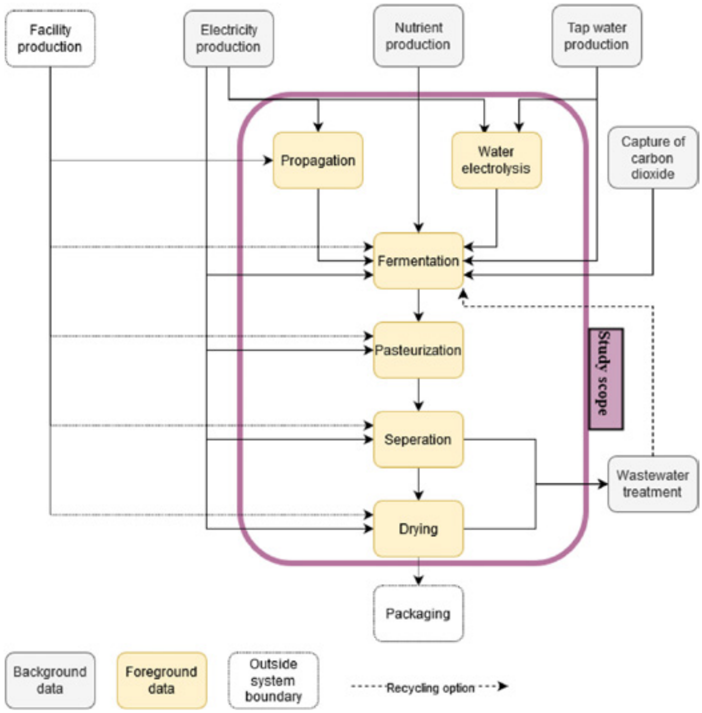


Fig. 1. Flow chart and system boundaries of MP production as studies here.

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.

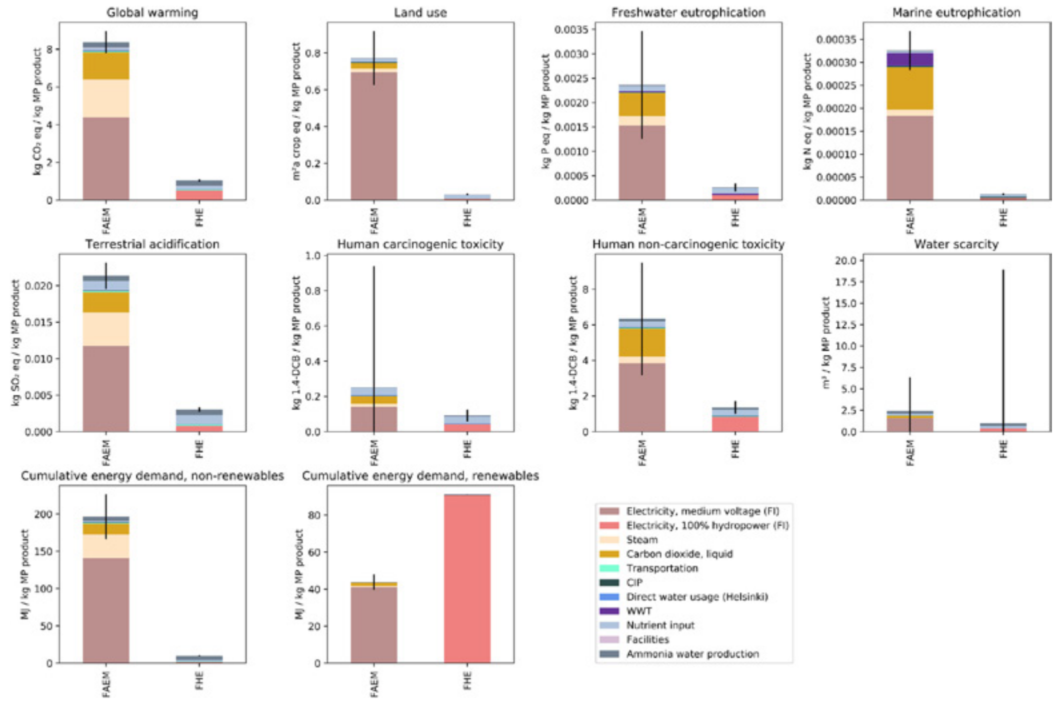


Fig. 2. Results and contributions for different impact categories for all scenarios per kg of MP product with Monte Carlo standard deviation results indicated with a black line, and where FAEM refers to the 'Finnish average electricity mix' scenario and FHE refers to the 'Finnish hydropower energy' scenario.

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^2a crop eq/kWh) in comparison to that of the Finnish average electricity mix (0.046 m^2a 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

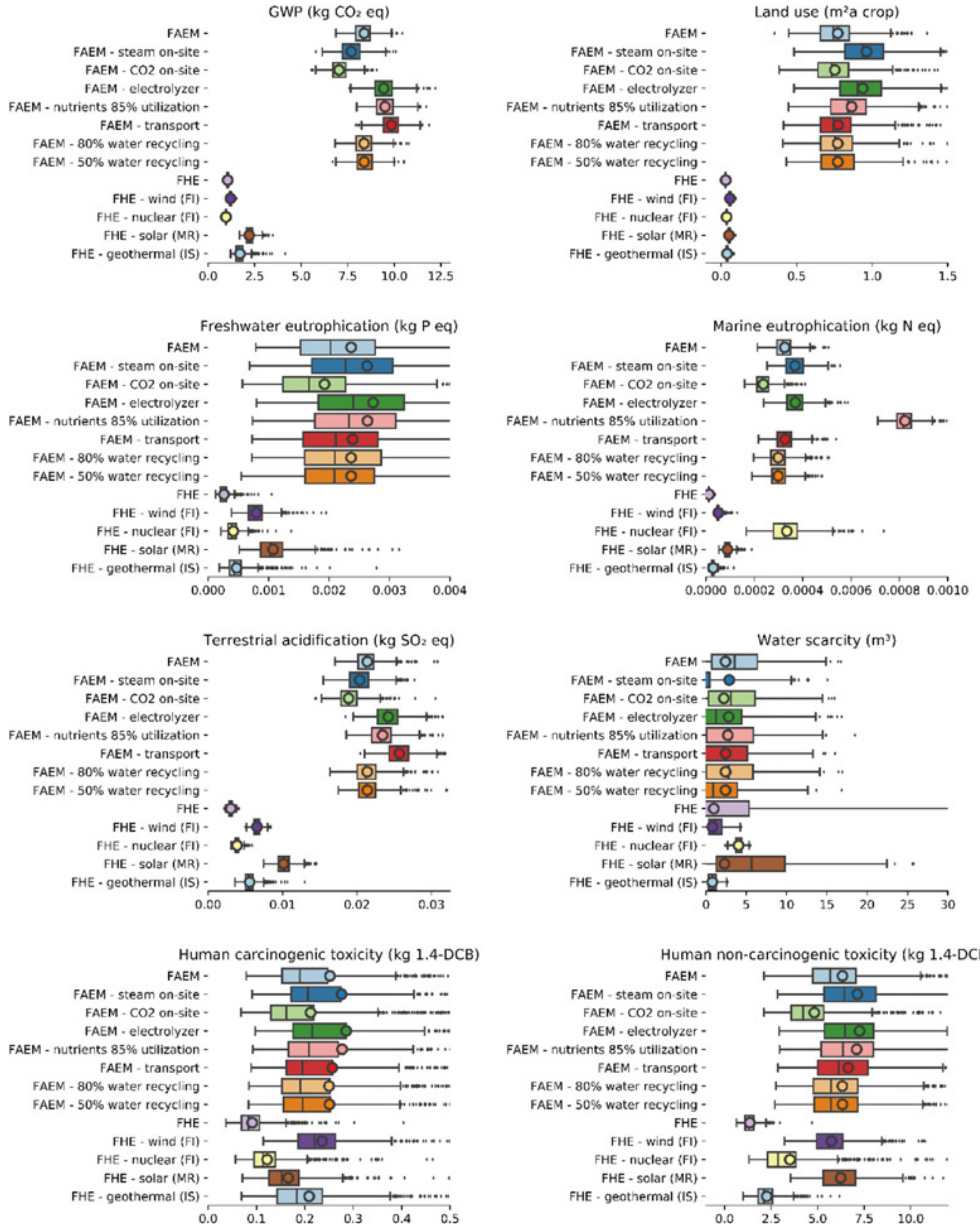


Fig. 3. Results of the sensitivity analysis per 1 kg of product in boxplots and outliers for the Finnish average energy mix (FAEM) scenario and the Finnish hydropower energy (FHE) scenario, with baseline results shown in circles for Finland (FI), Morocco (MR), and Iceland (IS).

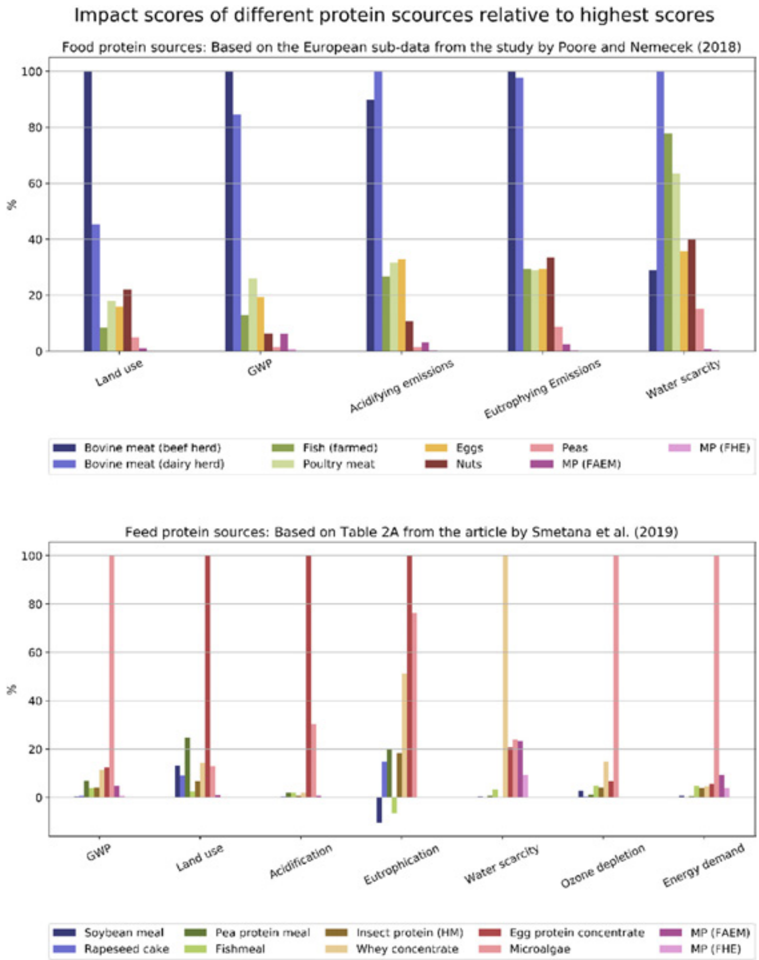


Fig. 4. Comparison of the environmental impact results of MP production with other protein sources for food and feed production.

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.

References

Althaus, H.-J., Bauer, C., Doka, G., et al., 2007. Implementation of Life Cycle Impact Assessment Methods. Data v2.2 (2010) (ecoinvent Rep No 3).

Bastin, J.-F., Finegold, Y., Garcia, C., et al., 2019. The global tree restoration potential. Science 365, 76–79. https://doi.org/10.1126/science.aax0848.

Boulay, A.-M., Bare, J., Benini, L., et al., 2018. The WULCA consensus characterization model for water scarcity footprints: assessing impacts of water consumption based on available water remaining (AWARE). Int. J. Life Cycle Assess. 23, 368–378. https://doi.org/10.1007/s11367-017-1333-8.

Campbell, B.M., Beare, D.J., Bennett, E.M., et al., 2017. Agriculture production as a major driver of the earth system exceeding planetary boundaries. Ecol. Soc. 22. https://doi.org/10.5751/ES-09595-220408.

Communication Within the Pré Sustainability LCA Discussion List With the Topic "AWARE Water Scarcity, Negative Outcomes for Monte Carlo".

Consequential-LCA, 2015. The special case of electricity. https://consequential-lca.org/clca/marginal-suppliers/the-special-case-of-electricity/. (Accessed 14 August 2020).

Crenna, E., Sinkko, T., Sala, S., 2019. Biodiversity impacts due to food consumption in Europe. J. Clean. Prod. 227, 378–391. https://doi.org/10.1016/j.jclepro.2019.04.054.

Dijkman, T.J., Basset-Mens, C., Antón, Assumpció, Núñez, Montserrat, 2017. LCA of food and agriculture. Life Cycle Assessment: Theory and Practice. Springer International Publishing, pp. 723–754.

Eide, M., Homleid, J., Mattsson, B., 2003. Life cycle assessment (LCA) of cleaning-in-place processes in dairies. LWT Food Sci. Technol. 36, 303–314. https://doi.org/10.1016/S0023-6438(02)00211-6.

Greenlee, L.F., Lawler, D.F., Freeman, B.D., et al., 2009. Reverse osmosis desalination: water sources, technology, and today's challenges. Water Res. 43, 2317–2348.

Gunnarsdottir, M.J., Gardarsson, S.M., St Gunnar, J., Bartramc, J., 2016. Chemical quality and regulatory compliance of drinking water in Iceland. Int. J. Hyg. Environ. Health 219, 724–733.

Heijungs, R., 2020. On the number of Monte Carlo runs in comparative probabilistic LCA. Int. J. Life Cycle Assess. 25, 394–402. https://doi.org/10.1007/s11367-019-01698-4.

Henriksson, P.J.G., Guinée, J.B., Kleijn, R., Snoo, G.R., 2011. Life cycle assessment of aquaculture systems—a review of methodologies. Int. J. Life Cycle Assess. 17, 304–313. https://doi.org/10.1007/s11367-011-0369-4.

Henriksson, P.J.G., Zhang, W., Guinée, J.B., 2015. Updated unit process data for coal-based energy in China including parameters for overall dispersions. Int. J. Life Cycle Assess. 20, 185–195. https://doi.org/10.1007/s11367-014-0816-0.

Hischier, R., 2019. Carbon dioxide Production, Liquid, RER, Cut-off (Ecoinvent database version 3.6).

HSY, 2017. Viikinmäki wastewater treatment plant. Helsinki Region Environmental Services Authority. HSY 1/2017 https://www.hsy.fi/sites/Esitteet/EsitteetKatalogi/viikinmaki_tekninenesite_en.pdf. (Accessed 15 November 2019).

HSY, 2019. Where Does your Drinking Water Come From? Helsinki Reg. Environ. Serv. Auth https://www.hsy.fi/en/residents/water/where-does-your-drinking-water-come-from/Pages/default.aspx. (Accessed 9 September 2019)

Huijbregts, M.A.J., Steinmann, Z.J.N., Elshout, P.M.F., et al., 2017. ReCiPe2016: a harmonised life cycle impact assessment method at midpoint and endpoint level. Int. J. Life Cycle Assess. 22, 138–147. https://doi.org/10.1007/s11367-016-1246-y.

Hydrogen Europe, 2021. Electrolysers. https://hydrogeneurope.eu/electrolysers. (Accessed 22 January 2021).

Jolliet, O., Margni, M., Charles, R., et al., 2003. IMPACT 2002+: a new life cycle impact assessment methodology. Int. J. Life Cycle Assess. 8, 324–330.

Jungbluth, N., Stucki, M., Frischknecht, R., 2009. *Photovoltaics. In Dones, R. (Ed.) et Al., Sachbilanzen von Energiesystemen: Grundlagen Für Den Ökologischen Vergleich von Energiesystemen Und Den Einbezug von Energiesystemen in Oekobilanzen Für Die Schweiz.* Ecoinvent Report No. 6-XII, Swiss Centre for Li.

Koellner, T., Geyer, R., 2013. Global land use impact assessment on biodiversity and ecosystem services in LCA. Int. J. Life Cycle Assess. 18, 1185–1187. https://doi.org/10.1007/s11367-013-0580-6.

Lee, J.S., Lee, M.H., Chun, Y.Y., Lee, K.M., 2018. Uncertainty analysis of the water scarcity footprint based on the AWARE model considering temporal variations. Water (Switzerland) 10, 1–13. https://doi.org/10.3390/w10030341.

Liu, C., Colón, B.C., Ziesack, M., et al., 2016. Water splitting-biosynthetic system with CO_2 reduction efficiencies exceeding photosynthesis. Science (80) 352, 1210–1213. https://doi.org/10.1126/science.aaf5039.

Martín-Chivelet, N., 2016. Photovoltaic potential and land-use estimation methodology. Energy 94, 233–242. https://doi.org/10.1016/J.ENERGY.2015.10.108.

Ministry of Economic affairs and employment in Finland, 2020. Nuclear energy is the foundation of electricity production in Finland. https://tem.fi/en/nuclear-energy. (Accessed 13 August 2020).

Muñoz, I., Fernández-Alba, A.R., 2008. Reducing the environmental impacts of reverse osmosis desalination by using brackish groundwater resources. Water Res. 42, 801–811.

Parodi, A., Leip, A., De Boer, I.J.M., et al., 2018. The potential of future foods for sustainable and healthy diets. Nat. Sustain. 1, 782–789. https://doi.org/10.1038/s41893-018-0189-7.

Pfister, S., Vionnet, S., Levova, T., Humbert, S., 2016. Ecoinvent 3: assessing water use in LCA and facilitating water footprinting. Int. J. Life Cycle Assess. 21, 1349–1360. https://doi.org/10.1007/s11367-015-0937-0.

Pikaar, I., de Vrieze, J., Rabaey, K., et al., 2018a. Carbon emission avoidance and capture by producing in-reactor microbial biomass based food, feed and slow release fertilizer: potentials and limitations. Sci. Total Environ. 644, 1525–1530. https://doi.org/10.1016/j.scitotenv.2018.07.089.

Pikaar, I., Matassa, S., Bodirsky, B.L., et al., 2018b. Decoupling livestock from land use through industrial feed production pathways. Environ. Sci. Technol. 52, 7351–7359. https://doi.org/10.1021/acs.est.8b00216.

Poore, J., Nemecek, T., 2018a. Reducing food's environmental impacts through producers and consumers. Science (80-) 360, 987–992. https://doi.org/10.1126/science.aaq0216.

Poore, J., Nemecek, T., 2018b. European Data Subset From the Study Described in the Article 'Reducing Food's Environmental Impacts Through Producers and Consumers' (Published in 2018 in Science. Obtained by J Poore in June 2020).

PRé Consultants, 2020. Simapro. https://www.pre-sustainability.com/sustainability-consulting/sustainable-practices/custom-sustainability-software. (Accessed 3 July 2020).

Rischer, H., Szilvay, G.R., Oksman-Caldentey, K.M., 2020. Cellular agriculture — industrial biotechnology for food and materials. Curr. Opin. Biotechnol. 61, 128–134.

Ritala, A., Häkkinen, S.T., Toivari, M., Wiebe, M.G., 2017. Single cell protein—state-of-the-art, industrial landscape and patents 2001–2016. Front. Microbiol. 8, 2009. https://doi.org/10.3389/fmicb.2017.02009.

Roe, S., Streck, C., Obersteiner, M., et al., 2019. Contribution of the land sector to a 1.5 °C world. Nat. Clim. Chang. 9, 817–828. https://doi.org/10.1038/s41558-019-0591-9.

Saarinen, M., Fogelholm, M., Tahvonen, R., Kurppa, S., 2017. Taking nutrition into account within the life cycle assessment of food products. J. Clean. Prod. 149, 828–844. https://doi.org/10.1016/j.jclepro.2017.02.062.

SEMIDE, 2005. Local Water Supply, Sanitation and Sewage: Country Report Morocco.

Sillman, J., Nygren, L., Kahiluoto, H., et al., 2019. Bacterial protein for food and feed generated via renewable energy and direct air capture of CO_2: can it reduce land and water use? Glob. Food Sec. 22, 25–32.

Sillman, J., Uusitalo, V., Ruuskanen, V., et al., 2020. A life cycle environmental sustainability analysis of microbial protein production via power-to-food approaches. Int. J. Life Cycle Assess., 1–14 https://doi.org/10.1007/s11367-020-01771-3.

Smetana, S., Mathys, A., Knoch, A., Heinz, V., 2015. Meat alternatives: life cycle assessment of most known meat substitutes. Int. J. Life Cycle Assess. 20, 1254–1267. https://doi.org/10.1007/s11367-015-0931-6.

Smetana, S., Schmitt, E., Mathys, A., 2019. Sustainable use of *Hermetia illucens* insect biomass for feed and food: attributional and consequential life cycle assessment. Resour. Conserv. Recycl. 144, 285–296. https://doi.org/10.1016/j.resconrec.2019.01.042.

Smith Stegen, K., 2015. Heavy rare earths, permanent magnets, and renewable energies: an imminent crisis. Energy Policy 79, 1–8. https://doi.org/10.1016/j.enpol.2014.12.015.

Sonesson, U., Davis, J., Hallström, E., Woodhouse, A., 2019. Dietary-dependent nutrient quality indexes as a complementary functional unit in LCA: a feasible option? J. Clean. Prod. 211, 620–627. https://doi.org/10.1016/j.jclepro.2018.11.171.

Statistics Finland, 2018. Official Statistics of Finland (OSF): Production of Electricity and Heat [e-publication]. ISSN=1798-5099. Statistics Finland, Helsinki, p. 53 (Accessed 16 Dec 2019).

Torres-Miralles, M., Särkelä, K., Koppelmäki, K., et al., 2019. Contribution of high nature value farming areas to sustainable livestock production: pilot case in Finland. Grass and Forage Science (Accepted abstract).

Treyer, K., 2014. Market for Electricity, High Voltage {FI}.

Treyer, K., 2019. Electricity Production, Photovoltaic, 3kWp Slanted-Roof Installation, Single-Si, Panel, Mounted, FI, Unit, Cut-off by Classification. Ecoinvent Database Version 3.3.

Tuomisto, H.L., 2019. The eco-friendly burger. EMBO Rep. 20. https://doi.org/10.15252/embr.201847395.

Vince, F., Aoustin, E., Bréant, P., Marechal, F., 2008. LCA tool for the environmental evaluation of potable water production. Desalination 220, 37–56.

Virtanen, Y., Kurppa, S., Saarinen, M., et al., 2011. Carbon footprint of food - approaches from national input-output statistics and a LCA of a food portion. J. Clean. Prod. 19, 1849–1856. https://doi.org/10.1016/j.jclepro.2011.07.001.

Weidema, B., 2014. Has ISO 14040/44 failed its role as a standard for life cycle assessment? J. Ind. Ecol. 18, 324–326. https://doi.org/10.1111/jiec.12139.

Wernet, G., Bauer, C., Steubing, B., et al., 2016. The ecoinvent database version 3 (part I): overview and methodology. Int. J. Life Cycle Assess. 21 (9), 1218–1230 online. Available at:. http://link.springer.com/10.1007/s11367-016-1087-8.

Willett, W., Rockström, J., Loken, B., et al., 2019. Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. Lancet 393, 447–492.

World bank group, 2020. Global solar atlas v2.2. https://globalsolaratlas.info/map. (Accessed 2 April 2020).

WULCA, 2015. AWARE. http://www.wulca-waterlca.org/aware.html. (Accessed 2 March 2020).

Yu, J., 2014. Bio-based products from solar energy and carbon dioxide. Trends Biotechnol. 32, 5–10.

ARTICLE III

Ovalbumin production using *Trichoderma reesei* culture and low-carbon energy could mitigate the environmental impacts of chicken-egg-derived ovalbumin.

nature food

ARTICLES

https://doi.org/10.1038/s43016-021-00418-2

Check for updates

Ovalbumin production using *Trichoderma reesei* culture and low-carbon energy could mitigate the environmental impacts of chicken-egg-derived ovalbumin

Natasha Järviö[1,2] ✉, Tuure Parviainen[3,4], Netta-Leena Maljanen[1,2], Yumi Kobayashi[2,4], Lauri Kujanpää[3], Dilek Ercili-Cura[5], Christopher P. Landowski[3], Toni Ryynänen[1,2], Emilia Nordlund[3] and Hanna L. Tuomisto[2,4,6]

Ovalbumin (OVA) produced using the fungus *Trichoderma reesei* (Tr-OVA) could become a sustainable replacement for chicken egg white protein powder—a widely used ingredient in the food industry. Although the approach can generate OVA at pilot scale, the environmental impacts of industrial-scale production have not been explored. Here, we conducted an anticipatory life cycle assessment using data from a pilot study to compare the impacts of Tr-OVA production with an equivalent functional unit of dried chicken egg white protein produced in Finland, Germany and Poland. Tr-OVA production reduced most agriculture-associated impacts, such as global warming and land use. Increased impacts were mostly related to industrial inputs, such as electricity production, but were also associated with glucose consumption. Switching to low-carbon energy sources could further reduce environmental impact, demonstrating the potential benefits of cellular agriculture over livestock agriculture for OVA production.

The global growing demand for chicken egg white protein production results in many environmental impacts, such as land use, climate change, water scarcity, resource depletion and eutrophication[1–4]. Ovalbumin (OVA) is the most abundant protein in egg whites, consisting of over 50% of egg white proteins. It has been expressed in several host organisms, including *Escherichia coli* and *Pichia pastoris*, mainly in the lab[5,6]. Advances in cellular agriculture concepts have made it possible to produce recombinant or cell-cultured OVA on a large enough scale to consider it an economically feasible option to chicken-based egg white powder[7]. Using the filamentous ascomycete fungus *Trichoderma reesei*, a well-established and efficient production organism, cell-cultured OVA is now produced in a bioreactor at a pilot scale. The process is a form of acellular production where microorganisms are grown to produce an extracellular recombinant protein, in this case OVA (length: 386 amino acids)[6,8]. The coding gene in chickens (*Gallus gallus domesticus*) is *SERPINB14* (https://www.uniprot.org/uniprot/P01012). The final product of cell-based production is a protein powder that typically shows comparable functional properties to chicken egg white protein powder and can be used as a replacement in food formulations.

The purpose of this study was to assess the environmental impacts of cell-cultured OVA production in comparison to chicken-based egg white protein powder (hereafter referred to as egg white powder, unless otherwise specified) production using an anticipatory life cycle assessment (LCA) method[9,10]. Using an LCA quantifies the environmental impact of *T. reesei*-produced OVA throughout all production steps and allows for the trade-off comparison between different impact categories[11,12]. The impacts of the production process were estimated for that of an industrial level of 100,000 kg, using data from a production-scale pilot and a techno-economic assessment (TEA) produced by VTT[7]. Uncertainties were calculated using Monte Carlo (MC) analysis, while the sensitivities of the results were estimated with various sensitivity analyses. Since production of *T. reesei* OVA (Tr-OVA) mainly relies on the provision of electricity and the carbon intensity of countries varies[13], we also assess the production of Tr-OVA in various countries. The flow chart in Fig. 1 shows the assumed process steps, including the most notable inputs and outputs, and indicates the main focus of this study.

Results

Impact of Tr-OVA for different scenarios. Figure 2 shows the environmental impact of Tr-OVA production per kg of product and contribution per process for four scenarios—Finland (FI), Germany (DE), Poland (PL) and Finland using a low-carbon electricity mix (FI-LC) that includes both renewable energy sources and nuclear power (the Supplementary Data shows the full inputs of this model), which were chosen to reflect different carbon intensity levels of country electricity mixes within the European Union[13]. The largest contributor for most impact categories comes from the input of glucose with a share of 2–94%, depending on the impact category and country. For land use, the contribution of glucose most clearly dominates (86–92%), illustrating the reliance of land use of agricultural products. In addition, for water scarcity —also

[1]Ruralia Institute, Faculty of Agriculture and Forestry, University of Helsinki, Mikkeli, Finland. [2]Helsinki Institute of Sustainability Science, University of Helsinki, Helsinki, Finland. [3]VTT Technical Research Centre of Finland Ltd, Espoo, Finland. [4]Department of Agricultural Sciences, Faculty of Agriculture and Forestry, University of Helsinki, Helsinki, Finland. [5]Solar Foods Ltd, Lappeenranta, Finland. [6]Natural Resources Institute Finland, Helsinki, Finland. ✉e-mail: Natasha.jarvio@helsinki.fi

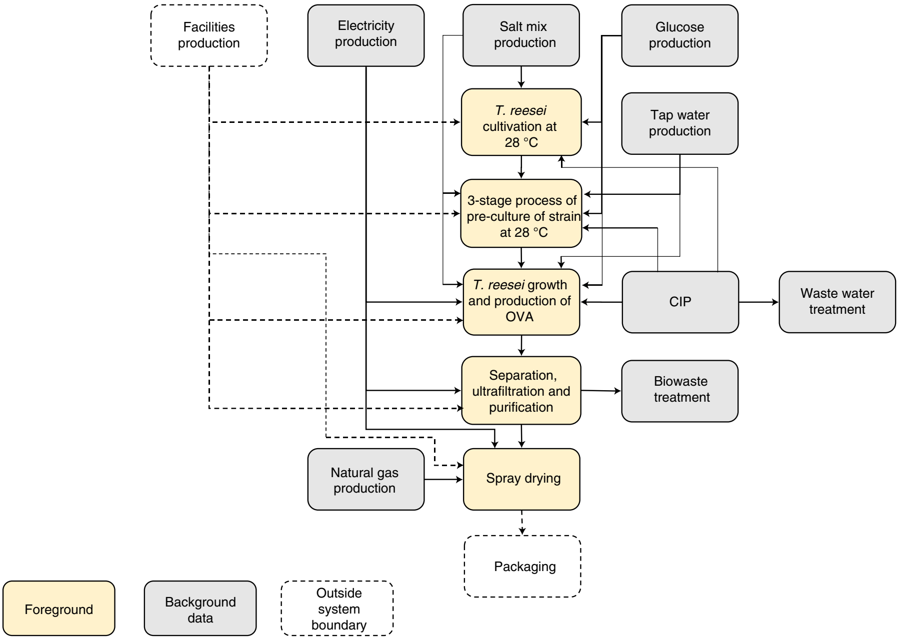


Fig. 1 | Flow chart of the processes involved in the production of Tr-OVA. Flow diagram of the input and outputs related to the production of Tr-OVA where the focus of this study was on the modelling of the environmental impacts of the foreground data (indicated with yellow boxes) while existing LCA databases were used for the background processes (in grey). Processes excluded are indicated with a dotted line.

considered a relevant impact category for agricultural products[4]—glucose had a contribution of 58–65%. The second largest contributor to water scarcity is the industrially produced salt mix (22–25%). However, the overall weight of the salt mix (0.85 kg kg^{-1} of product) was also 63% lower than the glucose inputs (2.34 kg kg^{-1} of product) per kg of Tr-OVA. Overall, the antifoaming agent had a minor contribution. An exception to this was the contribution of the agent to stratospheric ozone depletion with a range of 81–84%, depending on the scenario.

Differences in country-specific results are partly explained by the different electricity mixes for each country, where the Finnish electricity mix is dominated by nuclear power (29.1%) and has a high contribution from renewable energy (17.9%)[14], whereas Poland relies mostly on coal (72%)[15]. For example, the total contribution of electricity to the global warming potential (GWP) is 34% using the Polish mix but just 2% in the low-carbon scenario in Finland. The impacts of freshwater eutrophication and human carcinogenic and non-carcinogenic toxicity show a similar pattern. The results for ionizing radiation, on the other hand, are lowest in Poland. The results clearly show an overall reduction in environmental impact when producing Tr-OVA using the FI-LC. An exception to this is ionizing radiation, which is explained by the heavy reliance on nuclear power (55.5%) in this particular mix.

Comparison of Tr-OVA with egg white powder. The calculated *P* value with the dependent modified null hypothesis significance testing (NHST) led to the rejection of the null hypothesis for all alternatives and impact categories, meaning that the impact of Tr-OVA and egg white powder were significantly different from each other. (the Supplementary Data contains more information on the statistical test.) However, the *P* value of human carcinogenic toxicity for the comparison of the German alternatives was 0.046, meaning that the result would not have been significantly different at a lower α.

Figure 3 shows the deterministic results of our comparison between Tr-OVA produced in Germany and Poland and egg white powder produced in the respective countries per kg of protein. The results show that for most impact categories typically used for agricultural products (GWP, land use, water scarcity impact, terrestrial acidification and eutrophication potentials), Tr-OVA generally resulted in lower environmental impacts, with the exception of the impacts of freshwater eutrophication and water scarcity when produced in Poland. For example, the discernibility results showed that 91% and 97% of the MC runs of Tr-OVA production for freshwater eutrophication were larger than those of egg white powder for Germany and Poland, respectively. However, there is a trade-off; for some impact categories more typically burdened by industrial products (ionizing radiation and human carcinogenic and non-carcinogenic toxicity), the impact of Tr-OVA was higher than that of egg white powder. An exception were the results for ionizing radiation in Poland, where only 49% of the MC runs for Tr-OVA were larger. This partial shift in the environmental burden from the typical agricultural impacts to those impacts typically caused by industry could be explained by the high reliance on industrial processes for Tr-OVA production on the one hand and the agricultural inputs for egg production on the other. One example of the high reliance of industrial inputs for Tr-OVA production is the salt mix, which has a high overall contribution ranging from 0.3% to 50.5% depending on the impact category. Most of the impact is almost completely attributed to the input of monopotassium phosphate (MKP), which makes up 41% of the total salt mix by weight.

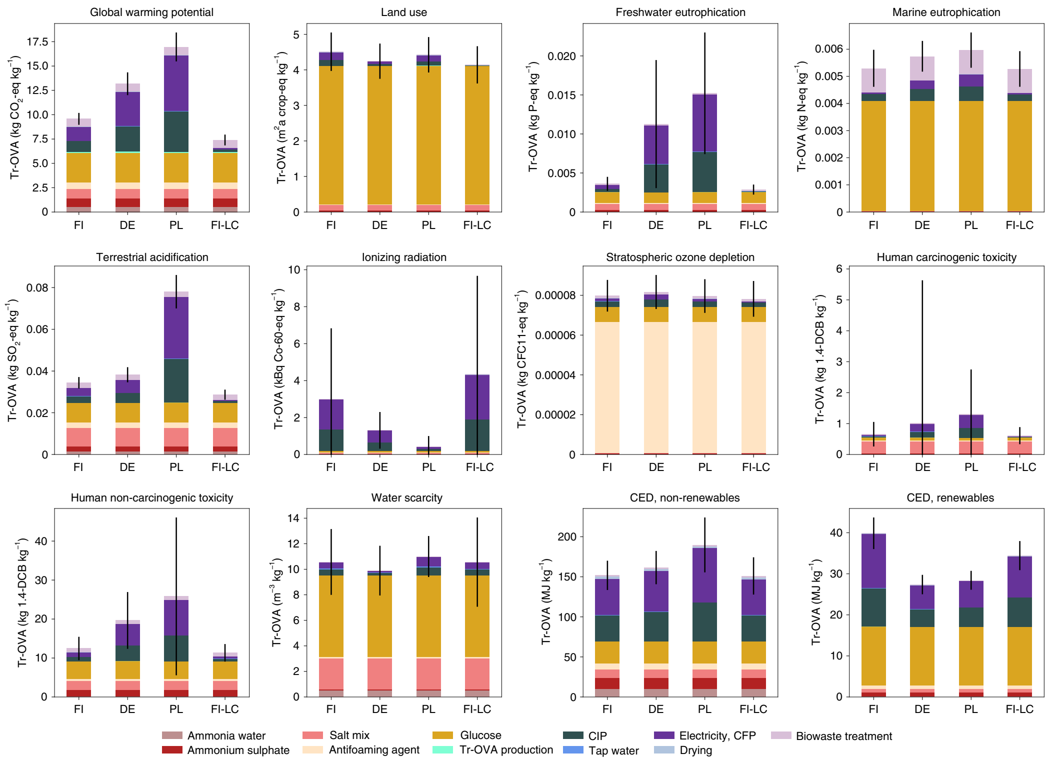


Fig. 2 | Environmental impact of Tr-OVA production per scenario. Deterministic results and process contributions in FI, DE, PL and FI-LC per kg of Tr-OVA product. Standard deviations from the MC runs ($n=100$) are indicated by the black line. Tr-OVA production refers to direct emissions and land use caused by the Tr-OVA production system. CFP, cultivation, filtration and purification.

However, MKP was modelled using sodium phosphate as a proxy due to limitations in data availability, making the results for the contribution of the salt mix uncertain.

The substantially lower reliance on land for Tr-OVA production compared to egg white powder—the discernibility results showed that 100% of the MC runs resulted in lower land use requirements—can be explained by the difference in the total required agricultural resources per kg of protein for each product. According to the World Food LCA Database (WFLDB), chickens require 2.4 kg of feed per kg of egg[16]. This means that the feed requirements per kg of protein are 27.5 kg, considering the amount of eggs required and the protein content of egg white produced by eggs. The production of Tr-OVA, on the other hand, requires only 2.54 kg of glucose per kg of protein, supplied with 2.04 kg of minerals and nitrogen. Therefore, the production of Tr-OVA has a greater agricultural material efficiency in the transformation process of agricultural products to egg white powder than when using chickens.

Although overall the results of the discernibility test showed a similar direction for the production of both alternatives in Germany and Poland, the outcome for water scarcity was very different. The results for Germany showed that 100% of the MC runs for egg white powder are larger than that of Tr-OVA, while in Poland 99% of the MC runs were larger for Tr-OVA per kg of protein. Most of this seems to be caused by a difference in the impact of feed production on water scarcity between Poland and Germany. In the WFLDB model, feed inputs for German eggs are modelled using a generic European average mix where corn produced in Spain causes 93.1% of the water scarcity impact for egg white powder. In the Polish model, chickens are fed mainly with grains originating from Poland. The water scarcity impact factors for Poland and Spain are very different, namely 1.962 and 77.7, respectively. Differences in these water scarcity impact factors explain most of the differences between the Polish and German egg white powder results. This difference in results highlights the need for more specific inputs for German egg production to make conclusions that are more reliable on the impact of Tr-OVA production versus egg white powder for water scarcity.

Although both the production of Tr-OVA and egg white powder require cleaning-in-place (CIP), Fig. 3 shows that the environmental impact of CIP for the former is 0.7–106 times that of the latter, depending on the impact category and country. This is partly explained by the use of bioreactors for Tr-OVA production that require regular cleaning.

Despite limitations in our model regarding the processing of eggs to egg white powder, our results show that the overall contribution of the processing of eggs is minor compared to egg production, with a total contribution of 0.1–22% for egg processing depending on the impact category and country. This means that the assumptions related to egg production are more important, as shown by the large difference between the impacts resulting from egg

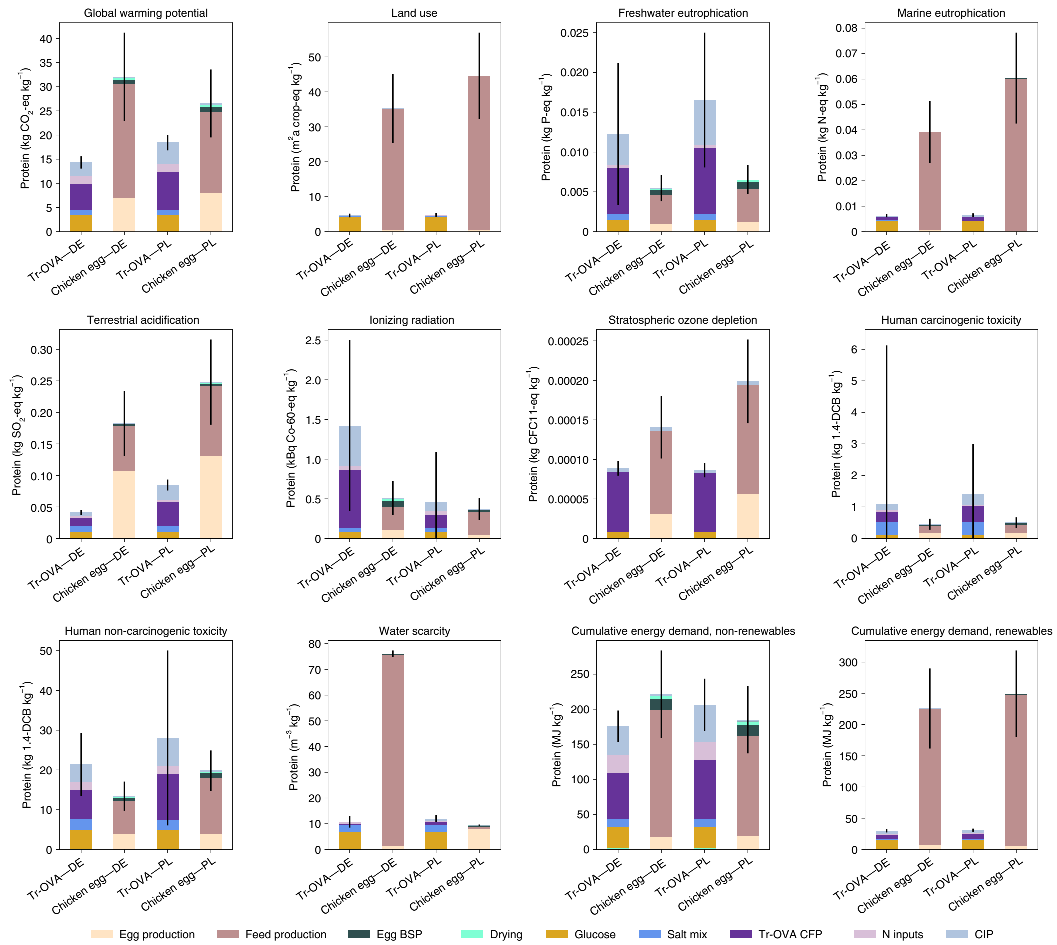


Fig. 3 | Comparison of the environmental impact of Tr-OVA with egg white powder. Deterministic results and process contributions for the production of Tr-OVA in DE and PL versus egg white powder production using chicken eggs in DE and PL per kg of protein. Standard deviations from the MC runs ($n = 100$) are indicated by the black lines.

production in Germany versus Poland. Limitations of the egg white powder model were mostly related to a lack of land use requirements for the processing of eggs to powder in the original study[17] and the replacement of chlorodifluoromethane with ammonia to cool egg white powder production due to compliance with EU regulations[18]. This replacement lowered the overall GWP of egg white powder.

Sensitivity analyses of the Tr-OVA model results. The sensitivity of our results was tested by varying the most relevant inputs of the Finnish model, for example, by increasing 1 particular input by 20%, changing the background dataset for glucose production or replacing natural gas in the drying step with electricity. Doing so allowed us to identify which changes in inputs resulted in most substantial variations of the results and to what extent. (Further background information on the changed parameters of the model can be found in Supplementary Table 2.) Figure 4 shows the results of the sensitivity analyses in kg per product. There was relatively limited variation in the results for most of the sensitivity tests, meaning that most changes in input had a minimal effect on the overall estimated impact of Tr-OVA production. For example, despite the high contribution of electricity consumption to the overall environmental impact of Tr-OVA production, an assumed 20% increase in electricity only increased the environmental impact by 0.2–10.9%, depending on the impact category. However, two of the sensitivity tests showed a larger effect on the results. The first was caused by a change in the background database used to model glucose production from the WFLDB used in the original Finnish scenario to the ecoinvent database used in the sensitivity test named 'FI–ecoinvent glucose'. Differences in the results were most noticeable for land use and terrestrial acidification. Although both datasets used corn starch as an input for glucose production, the assumed amounts differed noticeably with ecoinvent assuming 0.9 kg of corn starch

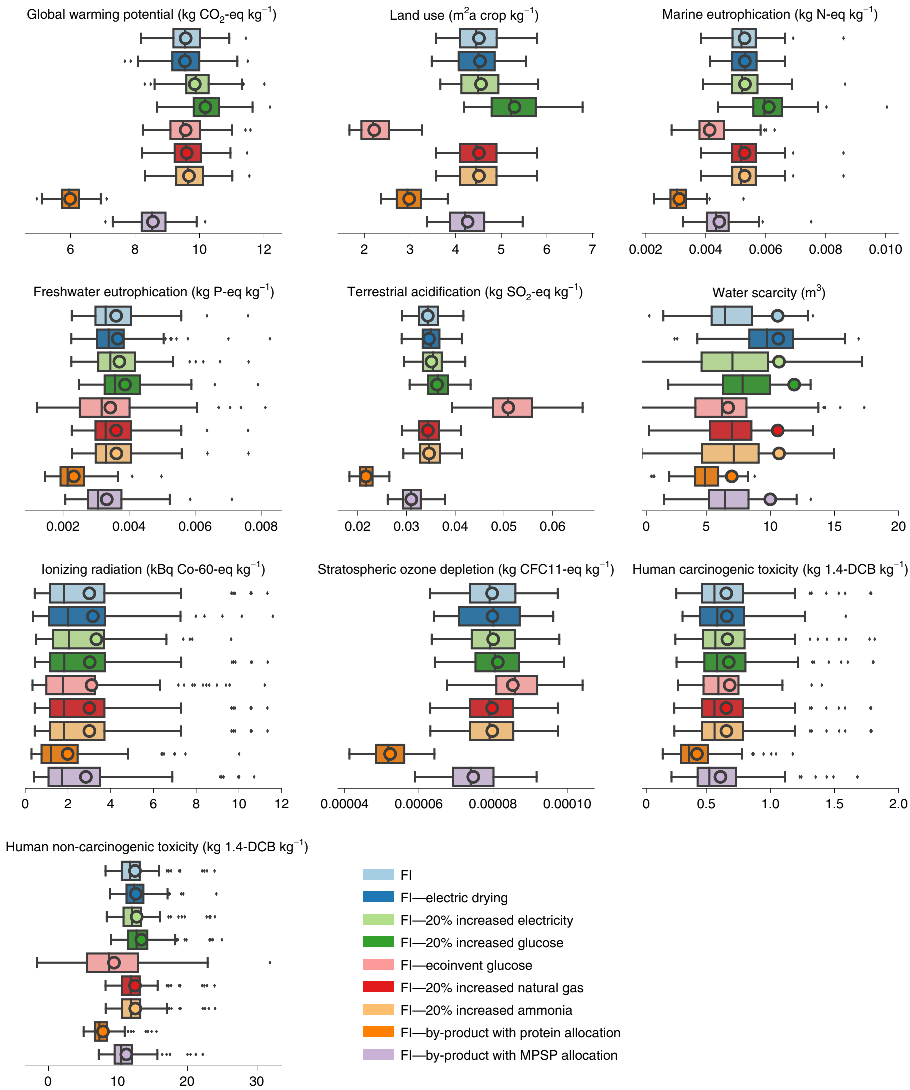


Fig. 4 | Sensitivity analyses of the FI Tr-OVA model per kg of Tr-OVA product. Results of the sensitivity analyses based on changed inputs of the FI scenario per 1 kg of Tr-OVA product. A change in input from natural gas to electricity for the drying step (FI—electric drying) was tested; a 20% increase of the main inputs (electricity, natural gas, ammonia) and a change in the database use of glucose production (FI—ecoinvent glucose) were also tested. Finally, an allocation of environmental impact from by-products (fungal biomass) based on protein content or MPSP (FI—by-product) was tested. Results of the MC runs (n = 100), to estimate the uncertainties of the analyses, are displayed using a box-and-whisker plot to indicate the 0th, 25th, 50th, 75th and 100th percentiles; the dots indicate outliers while the deterministic results of the sensitivity analyses are shown using circles.

per kg of glucose, whereas WFLDB had an input of 3.48 kg of corn starch per kg of glucose. Nevertheless, the GWP of both systems were the same (1.31 kg CO_2-eq kg^{-1} glucose).

The other notable sensitivity of the results was due to the assumptions relating to the potential use of the waste product, that is, genetically modified *T. reesei* fungal biomass containing some 40–60% moisture, as a feed ingredient. This was analysed using multiple impact allocation methods. The genetically modified *T. reesei* fungal biomass is not yet approved in the EU for feed use; it was thus considered as biowaste in the main scenarios at this stage. This is likely to change in the future since other by-products from the food and beverage industry are currently used as feed. This is the case, for example, for brewer's yeast which is a widely used by-product from the fermentation of beer.

The valorization of fungal biomass—as opposed to treating it as waste—reduced the overall environmental impact of Tr-OVA production in two ways: the reduction of waste for biowaste treatment and the sharing of the burden among products. The results in Fig. 4 show how the choice of allocation based on a physical or an economic relationship led to different outcomes for the environmental impact of Tr-OVA production. The protein-based allocation method resulted in a 33.8–41.2% decrease in impacts, depending on the impact category, for Tr-OVA production compared to a 5.5–15.9% decrease using a minimum product sales price (MPSP)-based allocation. The large decrease in the environmental impact of Tr-OVA production is explained by the relatively large amount of fungal mass that contains 45% protein and resulted in a 33.8% allocation factor for a product that was originally considered as waste in the system. Therefore, we would argue that MPSP allocation should be the preferred allocation method over protein-based allocation. The main argument for this is that whether or not the waste fungal mass is used does not affect the decision to produce Tr-OVA or not. Rather, its use would be an additional benefit that could improve the environmental impact of Tr-OVA production by reducing the need for waste treatment. Therefore, this relationship is better reflected using the MPSP-based allocation as a basis since it results in a higher allocation factor for Tr-OVA (94.6% compared to 66.2% with protein-based allocation). This is also reflected in the preferred use of economic allocation for other agricultural waste products, such as manure[19,20].

Discussion

The anticipatory LCA of cell-cultured egg white protein suggested that production of Tr-OVA as a host organism instead of chickens could reduce environmental impacts across a range of different impact categories, such as GWP, land use, marine eutrophication, terrestrial acidification and stratospheric ozone depletion. Most impacts and trade-offs between impact categories could potentially be further reduced using a low-carbon energy source. Using alternative and possible waste sources, such as forestry waste, straw or cereal side streams, instead of corn-based glucose, could potentially further reduce the environmental impact of Tr-OVA production[7]. However, because of data availability issues regarding the production process and an increased level of uncertainty, this could not be explored within the scope of this article. For example, the use of lignocellulosic side streams requires additional processing steps, such as preprocessing by steam explosion or diluted acid hydrolysis, processes that are yet to be used in food production. Since glucose from corn starch was identified as one of the main contributors to the environmental impact of Tr-OVA in the present study, we encourage future research to explore these possibilities.

The uncertainty of the results is high since the process is not yet in industrial use. For example, the purification step of Tr-OVA has not yet been tested on a commercial scale. Other uncertainties were caused by the lack of life cycle inventory data on some inputs, such as MKP, and the lack of more accurate information on CIP requirements. We tried to capture most of the uncertainties and sensitivities of the model buy using high uncertainty ranges and a sensitivity analysis. This increased the robustness of the results across the different scenarios. Therefore, the results provided a good initial overview of the possible ranges within which the impact of Tr-OVA production would likely fall and how these related to the production of egg white powder. Additionally—although not peer-reviewed—similar results for non-allocated GWP were found in a recent report by Perfect Day on the production of animal-free whey protein containing 90% protein and using the same host organism, *T. reesei*, for its production process, in the USA.

Nonetheless, more attention to practical measurements in industrial production is required to improve the accuracy of the results from an anticipatory study to a commercial process LCA in the future. As identified by the sensitivity test, a relevant modelling choice for future research would be the potential to use its by-products in the future for feed production or other added value applications. Additionally, we identified the impact of database choice and quality on the results for both Tr-OVA and egg white powder and recommend further development and accuracy of product systems in the different databases.

Methods

Goal and scope of the LCA study. The goal of this study was to estimate the environmental impacts of industrial-scale production of Tr-OVA. We applied an anticipatory LCA with a cradle-to-gate system boundary, based on current data gathered and estimated from a functioning production-scale pilot. Additionally, we used a TEA of Tr-OVA production performed to assess the process engineering requirements and device capabilities[7]. The TEA results were used to identify substantial steps in the production chain that would influence environmental load[7].

The environmental analysis of Tr-OVA production was modelled using the SimaPro v.9.1.0.11 software package[21] using the ecoinvent v.3.6 database. We used the ReCiPe 2016 midpoint (H) method to calculate the GWP (kg CO_2-eq), land use (m^2a crop eq), freshwater and marine eutrophication potential (kg P-eq and kg N-eq), terrestrial acidification (kg SO_2-eq), ionizing radiation (kBq Co-60-eq), human carcinogenic and non-carcinogenic toxicity (kg 1.4-DCB) and stratospheric ozone depletion (kg CFC11-eq) (ref. [22]). Water scarcity was assessed using the AWARE method[23]. Because the production of Tr-OVA is an industrial food manufacturing process that relies on electricity and natural gas, we included impact categories that are commonly used for both agricultural and industrial food manufacturing LCA studies[17,24–27]. Because of the industrial nature of the product, the life cycle industrial energy use was also assessed using the cumulative energy demand (CED) v.1.1 method by ecoinvent[28].

Two functional units were used in this study. The first functional unit (FU) is expressed as 1 kg of Tr-OVA product with an 8% moisture content and a 92% protein content and serves to reflect the environmental impact of the product. The second FU used is that of 1 kg of protein. Since Tr-OVA is a drop-in substitute that can replace protein from egg white powder[7], the second FU was used to compare the environmental impacts of both products. The cradle-to-gate system boundaries of this model start at the extraction of raw materials, includes the production of Tr-OVA and the cleaning of the facilities and ends at the factory gate. The flow chart of the system is shown in Fig. 1. The inoculum preparation phase, packaging and the materials and construction of facilities were excluded. However, land use for facilities was included in the model.

System description. The production of Tr-OVA started with the cultivation of fungal spores of engineered fungus *T. reesei* at 28 °C. The process then moved on to the preculture of the strain. This was a three-stage process where the fungi were fed with a continuous supply of water mixed with chemicals and nutrients for growth at 28 °C. After that, the mycelium was collected with a two-stage process performed at 28 °C and inoculated in a bioreactor where fermentation took place. During fermentation, the *T. reesei* fungus was supplied with glucose as the carbon source and other nutrients needed for growth in the fermentation process (Supplementary Table 1). Because the fermentation process produces heat, the fermented suspension needed to be cooled, sparged and mixed throughout the process. An assumed production of 100,000 kg Tr-OVA requires the use of 5 bioreactors for cultivation at the 0.06, 0.6, 9, 63 and 125 m^3 sizes. These bioreactors were cleaned using the CIP method after each fermentation cycle, which amounted to an estimated 50 cleaning operations per year.

After fermentation, the growth medium moved on to the filter press where the fungal biomass (solids) was separated from the produced proteins (liquid). This rejected fungal biomass left the system with a 58.3% moisture level. The filtrate with the OVA protein moved on to an ultrafiltration step, where 35.6 kg of water per kg of OVA product was removed as permeate. The retentate then entered the spray drying phase where it was heated and dried to generate an end product in powder format that was ready to be packed. The fermentation process was piloted at VTT during 2018 and 2019. The main fermentation parameters, such as feedstock and fermentation temperatures, were based on these test results. Energy consumption and mass flows were based on modelling[7]. To verify the model, the process was compared to the most similar existing processes, such as the NREL *T. reesei* process[29].

Scenarios. Industrial fermentation processes use substantial amounts of energy; thus, we decided to create four different production scenarios based on different production locations. We compared Tr-OVA production using the average electricity mix of Finland, Germany and Poland. The locations were selected based on stepwise levels of carbon intensity per kWh. In Finland, the carbon intensity is 204 g C kWh^{-1}, Germany 588 g C kWh^{-1} and Poland 911 g C kWh^{-1} (ref. [13]).

In addition, we created a scenario using a low-carbon intensity electricity mix within Finland, which consisted of non-combustion-based energy technologies[30]. This electricity mix was modelled conserving the ratios of the low-carbon energy sources listed in the original Finnish electricity mix based on data provided in the ecoivent database. Low-carbon electricity has a carbon intensity of less than 50 g C kWh^{-1} (ref. [30]).

Water use was modelled by adjusting the ecoinvent tap water process for Europe without Switzerland. In Finland, 65% of tap water is extracted from groundwater sources[31]. We assumed that the remaining tap water was sourced from lakes[32]. Tap water in Poland mostly comes from surface water (75%) and 25% is from groundwater[33]. Groundwater is the most important water source in Germany, providing more than 69% of the delivered tap water, while 15% comes from surface water and the remaining 16% from other resources, such as artificially recharged groundwater[34].

Data collection. The assessment of the environmental impact of Tr-OVA production at an industrial scale was based on the production-scale pilot and TEA produced by VTT[7]. Our LCA model was based on the input and output requirements of the pilot production and scaled to an industrial production level with an assumed 100,000 kg annual output. For more information on the model behind the assumed inputs and outputs required for industrial production level, we refer to the article and supporting information by Voutilainen et al.[7]. Supplementary Table 1 provides an overview of all inputs and outputs of the system per FU. Production of Tr-OVA at an industrial scale uses standard industrial fermentation and some downstream processing machinery used in large-scale production of single-cell proteins such as Quorn[35]. A major difference in downstream processing is the separation phase since OVA needs to be purified from the *T. reesei* biomass, other coproduced proteins and growth media.

Industrial requirements, including steam, electricity, chemicals and process water, were based on material and energy balance calculations. Due to limitations in the ecoinvent database, some of the nutrient inputs of the system were modelled using a proxy. These proxies were selected based on expert opinion of similarities of properties or functions. The use of natural gas in the spray dryer was modelled by adjusting the market for low-pressure natural gas from the ecoinvent database to the country-specific natural gas mix. Emissions from the combustion of natural gas were modelled according to the guidelines and emission factors published by the International Panel on Climate Change[36,37].

Direct land use requirements were roughly estimated to be 1,000 m^2 for all facilities based on the assumed production scale and were modelled as land occupation[7]. We assumed that the factory would be in operation for about 20 years, meaning that the transformation of 1,000 m^2 were allocated over 2,000,000 kg of Tr-OVA (see Supplementary Table 1 for details).

Waste coming from the system was mainly in the form of fungus mass, with a 40–60% moisture level and waste water from CIP. Treatment of the ultra-filtered waste water flow from the production process itself was excluded. Fungus mass was treated as biowaste in a biowaste treatment facility. The Supplementary Information provides details on the exact assumptions behind this part of the model.

The CIP requirements were estimated based on the water and detergent requirements for the typical cleaning of bioreactors used in industrial-scale food production. We assumed a CIP system that uses a partial reuse system where water and detergent requirements are reduced[38]. The electricity requirements, as well as the emissions related to the effluent of CIP, were estimated using the system by Eide et al.[39] on CIP methods for dairies. This was decided on the basis that both the production of Tr-OVA and milk result in proteinaceous deposits.

Treatment of waste water from the CIP of the five bioreactors was modelled using the process of average waste water treatment in Europe without Switzerland from the ecoinvent database. Additionally, we conservatively assumed that treated water did not return to the original source and ecosystem of water abstraction. This is, for example, the case of waste water treated in the Helsinki area in Finland[40]. Additionally, this avoids potential negative numbers for water scarcity (this has to do with mathematics behind the model calculations and is further addressed in Järviö et al.[41]). Therefore, we adjusted the original ecoinvent process so that any water outputs (that is, representing the return of water to its original source) were set to zero. The Supplementary Information provides details on the exact assumptions behind this part of the model.

Comparison to egg white production. The results of the environmental impacts of Tr-OVA production were compared to that of egg white powder production. We used the inventory data published in an article by Tsai et al.[17] on the production of egg yolk powder including CIP using continuous flow to remodel the emissions for egg white powder production. However, the moisture content of egg white is much higher than egg yolk, with 88% versus 48%, respectively[42,43]. Where Tsai et al.[17] assumed 2.18 kg of liquid egg yolk per 1 kg of egg yolk powder, we assumed 5 kg of liquid egg white to produce 1 kg of egg white powder with an 8% moisture content. Combining these data with the input of eggs as 1 kg of liquid egg white reported by Tsai et al.[17] meant that the total amount of eggs needed per 1 kg of egg white powder was 9.15 kg. Because of the higher moisture content in liquid egg white than egg yolk, we also adjusted the input requirements for the drying step. We assumed that the process of drying egg white would be similar to that of drying Tr-OVA. Because the moisture content of the unfinished wet products before the drying step is quite similar—12% and 13.3% for liquid egg white and Tr-OVA production, respectively—we used the same inputs per kg of product. This meant that the kWh for drying liquid egg white was less than originally listed in the article by Tsai et al.[17]. However, since drying inputs are highly dependent on the assumed efficiency of the system, comparing the two products would be fairer if based on the same assumptions.

The emissions resulting from egg production and breaking, storage and pasteurization were allocated based on the mass of the output products, where egg white makes up 55% of all outputs. This was based on the assumption that eggshells and residue are a by-product of the system[44]. We used data for egg production from the WFLDB since it relies on the ecoinvent v.3.5 cut-off system in its background model. Egg production for several countries was given, including Germany and Poland but not Finland. Therefore, we decided to compare egg white powder and Tr-OVA for only these two countries. Furthermore, we assumed that eggs travel about 100 km by truck from the farm to the egg white production plant. See Supplementary Data for the full model based on the inventory data of Tsai et al.[17].

We validated our model on egg white powder production by constructing a model for egg yolk powder production using the inventory data given in the article by Tsai et al.[17]. The results of this egg yolk powder model were compared to the results reported by the authors. The GWP results for our model were initially much higher. By far, most of the GWP was caused by the use of chlorodifluoromethane, which Tsai et al.[17] reported to be 0.079 kg per 1 kg of egg yolk powder. Because of the discrepancy in results and because chlorodifluoromethane cannot be used as a refrigerant within European Union countries due to its high ozone depletion potential and GWP[18], we decided to replace chlorodifluoromethane with ammonia in our egg white powder production model. Ammonia is a natural refrigerant that can be used for cooling in commercial refrigeration[45].

One major difference between the ecoinvent and WFLDB databases is that the latter includes the emissions from land use change. Since this can be a major source of emission contribution to the total GWP of food products[1,46], we decided to model glucose in the Tr-OVA production model using the WFLDB. This was to avoid unaligned system boundaries of the two product systems and a subsequent underestimation of the GWP of glucose used in Tr-OVA production. However, glucose in the WFLDB was modelled 'at plant'. To transform this into an 'at market' product, we included the estimated transportation distances used in the ecoinvent database.

Both products were compared based on the protein content using the second FU since the functionality of the end product is determined by the protein. For example, proteins are used to add texture in a cake-making application. Egg white powder contains 79.8% protein[47].

Uncertainty analysis and statistical tests. The environmental assessment of Tr-OVA production was based on the estimated inputs and outputs for Tr-OVA production at an industrial scale, using gathered and estimated data of Tr-OVA production at a pilot scale. Data uncertainties were high; therefore, the uncertainties of the results were analysed using an MC analysis modelled using the SimaPro v.9.1.0.11 software. The result of an MC analysis is a probability distribution within which the results are likely to fall, based on calculating the environmental impact repetitively[48]. It is a commonly used tool to capture uncertainty within LCA studies[49]. To perform the MC analysis, uncertainties were captured using a uniform distribution of inputs with a ±20% margin for the production of Tr-OVA (SI2 provides more details). Since the article by Tsai et al.[17] did not provide uncertainty ranges, we applied the pedigree method to add uncertainties to the egg white powder production. The MC simulation was performed in SimaPro using a limited number of 100 iterations[49]. We used a seed value of zero for all MC simulations to simulate dependent sampling. Doing so allowed us to account for common uncertainties between the Tr-OVA and chicken egg-based egg white powder and enabled a statistical comparison of the results[50]. In addition, we applied the parametric bootstrap method to handle the large uncertainty range of water scarcity that results from the incorrect estimation of probability distributions of the AWARE characterization factors[51]. We used Python 3.0 to run the bootstrap method, running 1,000 simulations with a sample size of 300 allowing for replacements. Any possible negative values that might naturally result from the MC analysis but were not sensible were ignored during the analysis (discussed in Järviö et al.[41]). Both a discernibility test[50] and dependent modified NHST[52] were used to explore differences in impacts between Tr-OVA and chicken-based egg white powder and confirm which alternative was significantly different. Dependent modified NHST testing was performed using a significance level α of 0.05 and a difference threshold δ_0 of 0.2. The null hypothesis was H_0: $S_{i,j,k} \leq \delta_0$, where S refers to the standardized difference of means, i and j refer to the different alternatives and k refers to the impact. The P value was calculated using a one-sided (right) cumulative distribution function[50,52]. Both statistical tests were performed on a per kg of protein basis.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

To the best of our ability, we have provided the data supporting the findings in this paper and its Supplementary Information files. Any additional data, particularly related to adjustments made in the background processes of our model, are available on request from the corresponding author.

Code availability

The code that was used to generate results for this study is freely available on request from the corresponding author.

Received: 2 June 2021; Accepted: 1 November 2021;
Published online: 16 December 2021

References

1. Crippa, M. et al. Food systems are responsible for a third of global anthropogenic GHG emissions. *Nat. Food* **2**, 198–209 (2021).
2. Smil, V. Nitrogen and food production: proteins for human diets. *Ambio* **31**, 126–131 (2002).
3. Van der Warf, H. & Petit, J. Evaluation of the environmental impact of agriculture at the farm level: a comparison and analysis of 12 indicator-based methods. *Agric. Ecosyst. Environ.* **93**, 131–145 (2002).
4. Poore, J. & Nemecek, T. Reducing food's environmental impacts through producers and consumers. *Science* **360**, 987–992 (2018).
5. Takahashi, N., Orita, T. & Hirose, M. Production of chicken ovalbumin in *Escherichia coli*. *Gene* **161**, 211–216 (1995).
6. Ercili-Cura, D. & Barth, D. *Cellular Agriculture: Lab-Grown Foods* (American Chemical Society, 2021).
7. Voutilainen, E., Pihlajaniemi, V. & Parviainen, T. Economic comparison of food protein production with single-cell organisms from lignocellulose side-streams. *Bioresour. Technol. Rep.* **14**, 100683 (2021).
8. U. S. Food and Drug Administration. *GRAS Notice for Non-Animal Whey Protein from Fermentation by Trichoderma reesei* https://www.fda.gov/media/136754/download (2019).
9. Zhou, X. et al. Promotion of novel plant-based dishes among older consumers using the 'dish of the day' as a nudging strategy in 4 EU countries. *Food Qual. Prefer.* **75**, 260–272 (2019).
10. Wender, B. A. et al. Anticipatory life-cycle assessment for responsible research and innovation. *J. Responsible Innov.* **1**, 200–207 (2014).
11. Dijkman, T. J., Basset-Mens, C., Antón A. & Núñez, M. in *Life Cycle Assessment: Theory and Practice* (eds Hauschild, M. Z., Rosenbaum, R. K. & Olsen, S. I.) 723–754 (Springer International Publishing, 2017).
12. Guinée, J. B. et al. in *Handbook on Life Cycle Assessment: Operational Guide to the ISO Standards* (eds Guinée, J. B. et al.) 525–634 (Kluwer Academic Publishers, 2002).
13. Moro, A. & Lonza, L. Electricity carbon intensity in European Member States: impacts on GHG emissions of electric vehicles. *Transp. Res. D Transp. Environ.* **64**, 5–14 (2018).
14. Treyer, K. *Electricity Production, High Voltage, at Market, Finland, FI, Allocation, Cut-Off by Classification.* Ecoinvent Database, version 3.6 (2014).
15. Treyer, K. *Electricity Production, High Voltage, at Market, Poland, PL, Allocation, Cut-Off Classification.* Ecoinvent Database, version 3.6 (2014).
16. *World Food LCA Database.* Feed basket archetype, poultry industrial laying system, as DM (WFLDB)/RER, U https://quantis-intl.com/metrics/databases/wfldb-food/ (Quantis, 2018).
17. Tsai, J.-H., Huang, J.-Y. & Wilson, D. Life cycle assessment of cleaning-in-place operations in egg yolk powder production. *J. Clean. Prod.* **278**, 123936 (2021).
18. European Commission. *Regulation (EU) No 517/2014 of the European Parliament and of the Council of 16 April 2014 on fluorinated greenhouse gases and repealing Regulation (EC) No 842/2006* https://www.eea.europa.eu/policy-documents/regulation-eu-no-517-2014 (European Environment Agency, 2014).
19. Van Paassen, M., Braconi, N., Kuling, L., Durlinger, B. & Gual, P. *Agri-footprint 5.0 Part 1: Methodology and Basic Principles* https://www.agri-footprint.com/wp-content/uploads/2019/11/Agri-Footprint-5.0-Part-1-Methodology-and-basic-principles-17-7-2019.pdf (Agri-footprint, 2019).
20. *Product Environmental Footprint Category 2 Rules Guidance, version 6.3, December 2017* https://ec.europa.eu/environment/eussd/smgp/pdf/PEFCR_guidance_v6.3.pdf (European Commission, 2017).
21. SimaPro. *LCA Software to Help You Drive Change* https://www.pre-sustainability.com/sustainability-consulting/sustainable-practices/custom-sustainability-software (2020).
22. Huijbregts, M. A. J. et al. ReCiPe2016: a harmonised life cycle impact assessment method at midpoint and endpoint level. *Int. J. Life Cycle Assess.* **22**, 138–147 (2017).
23. Boulay, A.-M. et al. The WULCA consensus characterization model for water scarcity footprints: assessing impacts of water consumption based on available water remaining (AWARE). *Int. J. Life Cycle Assess.* **23**, 368–378 (2018).
24. Gésan-Guiziou, G. et al. Life cycle assessment of a milk protein fractionation process: contribution of the production and the cleaning stages at unit process level. *Sep. Purif. Technol.* **224**, 591–610 (2019).
25. Santos, H. C. M. Jr., Maranduba, H. L., de Almeida Neto, J. A. & Rodrigues, L. B. Life cycle assessment of cheese production process in a small-sized dairy industry in Brazil. *Environ. Sci. Pollut. Res. Int.* **24**, 3470–3482 (2017).
26. Zouaghi, S. et al. Investigating the effect of an antifouling surface modification on the environmental impact of a pasteurization process: an LCA study. *ACS Sustain. Chem. Eng.* **7**, 9133–9142 (2019).
27. Noya, L. I. et al. An environmental evaluation of food supply chain using life cycle assessment: a case study on gluten free biscuit products. *J. Clean. Prod.* **170**, 451–461 (2018).
28. Althaus, H.-J. et al. *Implementation of Life Cycle Impact Assessment Methods. Data v.2.2 (2010)* https://docplayer.net/14249358-Implementation-of-life-cycle-impact-assessment-methods.html (Swiss Centre for Life Cycle Inventories, 2007).
29. Humbird, D. et al. *Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol: Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover* https://www.nrel.gov/docs/fy11osti/47764.pdf (NREL, 2011).
30. Harjanne, A. & Korhonen, J. M. Abandoning the concept of renewable energy. *Energy Policy* **127**, 330–340 (2019).
31. Ahkola, H. et al. Presence of active pharmaceutical ingredients in the continuum of surface and ground water used in drinking water production. *Environ. Sci. Pollut. Res.* **24**, 26778–26791 (2017).
32. Helsinki Region Environmental Services. Where does your drinking water come from? https://hsyk01mstrxfa10prod.dxcloud.episerver.net/en/water-and-sewers/how-the-water-supply-works/#Veden%20hankinta%20ja%20puhdistus (HSY, 2021).
33. Eurostat. *Annual Freshwater Abstraction by Source and Sector* https://ec.europa.eu/eurostat/databrowser/view/ENV_WAT_ABS__custom_344624/default/table?lang=en (Eurostat, 2020).
34. Umweltbundesamt Hauptsitz. *Drinking Water in Germany Again Rated 'Very Good'* https://www.umweltbundesamt.de/en/press/pressinformation/drinking-water-in-germany-again-rated-very-good (UBA, 2018).
35. Wiebe, M. Myco-protein from *Fusarium venenatum*: a well-established product for human consumption. *Appl. Microbiol. Biotechnol.* **58**, 421–427 (2002).
36. Simmons, T. *Good Practice Guidance and Uncertainty Management in National Greenhouse Gas Inventories: CO_2 Emissions from Stationary Combustion of Fossil Fuels* (IPCC, 2000).
37. *Emission Factor Database 2006* https://www.ipcc-nggip.iges.or.jp/EFDB/find_ef.php (IPCC, 2020).
38. WRAP. *Case Study: UK Drinks Sector. Clean-in-Place* www.wrap.org.uk (WRAP, 2006).
39. Eide, M. H., Homleid, J. P. & Mattsson, B. Life cycle assessment (LCA) of cleaning-in-place processes in dairies. *Lebensm. Wiss. Technol.* **36**, 303–314 (2003).
40. Helsinki Region Environmental Services Authority. *Viikinmäki Wastewater Treatment Plant. HSY 1/2017* https://www.hsy.fi/sites/Esitteet/EsitteetKatalogi/viikinmaki_tekninenesite_en.pdf (HSY, 2017).
41. Järviö, N., Maljanen, N.-L., Kobayashi, Y., Ryynänen, T. & Tuomisto, H. L. An attributional life cycle assessment of microbial protein production: a case study on using hydrogen-oxidizing bacteria. *Sci. Total Environ.* **776**, 145764 (2021).
42. U.S. Department of Agriculture. *FoodData Central: Egg, Yolk Only, Raw* https://fdc.nal.usda.gov/fdc-app.html#/food-details/1100203/nutrients (USDA, 2020).
43. U.S. Department of Agriculture. *FoodData Central: Egg, White Only, Raw* https://fdc.nal.usda.gov/fdc-app.html#/food-details/1100199/nutrients (USDA, 2020).
44. Daengprok, W., Garnjanagoonchorn, W. & Mine, Y. Fermented pork sausage fortified with commercial or hen eggshell calcium lactate. *Meat Sci.* **62**, 199–204 (2002).
45. European Commission. *Climate-friendly Alternatives to HFCs* https://ec.europa.eu/clima/policies/f-gas/alternatives_en (European Commission, 2014).
46. Järviö, N., Henriksson, P. J. G. & Guinée, J. B. Including GHG emissions from mangrove forests LULUC in LCA: a case study on shrimp farming in the Mekong Delta, Vietnam. *Int. J. Life Cycle Assess.* **23**, 1078–1090 (2018).
47. U.S. Department of Agriculture. *FoodData Central: Egg, White, Dried* https://fdc.nal.usda.gov/fdc-app.html#/food-details/323793/nutrients (USDA, 2019).
48. Helton, J. C., Johnson, J. D., Sallaberry, C. J. & Storlie, C. B. Survey of sampling-based methods for uncertainty and sensitivity analysis. *Reliab. Eng. Syst. Saf.* **91**, 1175–1209 (2006).
49. Heijungs, R. On the number of Monte Carlo runs in comparative probabilistic LCA. *Int. J. Life Cycle Assess.* **25**, 394–402 (2020).
50. Mendoza Beltran, A. et al. Quantified uncertainties in comparative life cycle assessment: what can be concluded? *Environ. Sci. Technol.* **52**, 2152–2161 (2018).

51. Lee, J. S., Lee, M. H., Chun, Y.-Y. & Lee, K. M. Uncertainty analysis of the water scarcity footprint based on the AWARE model considering temporal variations. *Water* **10**, 341 (2018).
52. Heijungs, R. Selecting the best product alternative in a sea of uncertainty. *Int. J. Life Cycle Assess.* **26**, 616–632 (2021).

Acknowledgements

This work was part of the 'Cultured meat in the post-animal bioeconomy' project (no. 201802185) funded by the KONE foundation (N.J. and T.R.) 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 (N.-L.M.). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. We thank A. M. Whipp (MPH Epidemiology) for her assistance with language editing and R. Heijungs for his instructions on how to perform a dependent modified null hypothesis significance test.

Author contributions

N.J., T.P., N.-L.M., Y.K., C.P.L., E.N. and H.L.T. designed the work. N.J., T.P., N.-L.M., L.K., C.P.L., E.N. and H.L.T. collected the data. N.J., T.P. and N.-L.M. created the model. N.J. and T.P. performed the interpretation and drafted the manuscript with valuable input from N.-L.M., Y.K., L.K., D.E.-C., C.P.L., T.R., E.N. and H.L.T. All authors reviewed and approved the final manuscript.

Competing interests

T.P. is a co-founder, shareholder and, from 20 April 2021, an employee of the start-up company Volare Solutions (Finland), which aims to commercialize the production of *Hermetia illucens* L. from industrial side streams and its use as feed (non-food) protein ingredient. This process, however, is unrelated to this article. All other authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s43016-021-00418-2.

Correspondence and requests for materials should be addressed to Natasha Järviö.

Peer review statement *Nature Food* thanks Thomas Brück, Vijai Kumar Gupta and Giuseppe Vignali for their contribution to the peer review of this work.

nature research

Corresponding author(s): Natasha Järviö
Last updated by author(s): Oct 19, 2021

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Microsoft Excel (Professional Plus 2016), Simapro 9.1.0.11 PhD software package was used to access Ecoinvent 3.6 and the World Food LCA databases
Data analysis	Commercial: Simapro 9.1.0.11 PhD software package, Microsoft Excel (Professional Plus 2016) Open source: Python 3.0

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The authors declare that to the best of our ability we have provided the data supporting the findings in this paper and its supplementary information files. Any additional data, particularly related to adjustments made in the background processes of our model, are available upon request from the corresponding author.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	This study performed a participatory life cycle assessment (LCA) on the production of ovalbumin using Trichoderma reesei produced in Finland, Germany, Poland and Finland using a low carbon electricity mix. Data on the inputs and environmental outputs of the production were modeled in Simapro (a LCA software). Uncertainties were calptured using a Monte Carlo analysis (100 runs) while sensitivity of the results were tested with various sensitivity tests.
Research sample	The research sample consisted out of a dataset on the inputs and outputs for the pilot-scale production of ovalbumin using Trichoderma reesei coming from VTT. This sample was chosen as it is currently the only available dataset we have on this.
Sampling strategy	Not applicable. We used the data that was available. As production will increase (and perhaps more companies will produce the product), more research can be done on the topic thereby increasing the robustness of the findings.
Data collection	Data was collected from VTT. Data was recorded and collected by Lauri Kujanpää, Christopher Landowski, Tuure Parviainen in VTT. Data was recorded / modeled in the Simapro software by Natasha Järviö.
Timing and spatial scale	Data was collected during 2017-2021 for production on a pilot scale.
Data exclusions	No data exclusions.
Reproducibility	Rerunning of the calculations in Simapro were performed to confirm that the same results are given. A seed value was used in the Monte Carlo analysis to be able to reproduce the same value each time and create an artifical dependency necessary for preforming statistical analyses.
Randomization	Not applicable as we did not work with organisms/participants in this particular study.
Blinding	Not relevant to the study as we were not handling living organisms or participants.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study	
☒	☐	Antibodies
☒	☐	Eukaryotic cell lines
☒	☐	Palaeontology and archaeology
☒	☐	Animals and other organisms
☒	☐	Human research participants
☒	☐	Clinical data
☒	☐	Dual use research of concern

Methods

n/a	Involved in the study	
☒	☐	ChIP-seq
☒	☐	Flow cytometry
☒	☐	MRI-based neuroimaging

UNIVERSITY OF HELSINKI
RURALIA INSTITUTE