Oyster mushroom cultivation on straw: aspects of productivity, sustainability and adaptability to the case of Uganda

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Thünen Working Paper 271

Oyster mushroom cultivation on straw: aspects of productivity, sustainability and adaptability to the case of Uganda

Thesis submitted for the degree:

Doktor der Agrarwissenschaften

(Dr. agr.)

Submitted to the Faculty of

Organic Agricultural Sciences (FB 11)

at Universität Kassel; Witzenhausen

by Daniel Grimm

Date of disputation: 16.01.2025

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List of abbreviations

- A: autoclaving
- ANOVA: analysis of variance
- BE: biological efficiency
- BCR: biomass conversion rate
- C: carbon
- cm: centimetre
- DF: digestible fibre
- DM: dry matter
- DL: digestible lipids
- DOM: digestible organic matter
- DP: digestible protein
- EU: European Union
- FAO: Food and Agricultural Organization of the United Nations
- FAOSTAT: Food and Agriculture Organization Corporate Statistical Database
- FM: fresh matter
- g: gram
- ha: hectare
- HAP: hot air pasteurization
- HI: harvest index
- HLP: hydrated lime pasteurization
- HWP: hot water pasteurization
- IPCC: Intergovernmental Panel on Climate Change
- K: potassium
- kcal: kilo calories
- kg: kilogram
- kJ: kilo Joule
- MDM: mushroom dry matter
- ME: metabolizable energy
- MJ: Megajoule
- N: nitrogen
- NDF: neutral detergent fibre
- P: phosphorous
- pH: potential of hydrogen
- SDM: stover dry matter
- SMS: spent mushroom substrate
- t: tons
- UN: United Nations
- UN DESA: United Nations Department of Economic and Social Affairs
- UNPD: United Nations Population Division
- USD: United States dollar
- XF: crude fibre
- XP: crude protein

1 Introduction

1.1 Background

The current world population of about 8 billion people is projected to grow to 9.7 billion by 2050 and 10.4 billion by 2100, according to medium estimates (United Nations, 2022b). More than half of the growth until the middle of the century is expected to take place in Africa, particularly sub-Saharan Africa, due to a youthful age structure and high fertility rates (Vollset et al., 2020). By the end of the century, the continent's current population of 1.4 billion will at least double, but more likely triple (United Nations, 2022a).

In view of the pressure on existing cropland and the expansion of agriculture into natural ecosystems that can be expected as a result of this demographic development, landless forms of food production, such as mushroom cultivation should be researched and assessed with regard to their possible contribution to food security. Mushroom cultivation is not only a form of food production, but also of composting and recycling (Stamets, 2000), which could make an important contribution to improving the 33 % of the world's agricultural soils which are moderately or severely degraded (FAO, 2021). Species like the grey oyster mushroom (*Pleurotus ostreatus*), which naturally grow on dead wood, can be cultivated on agricultural residues such as straw to produce large amounts of protein-rich food on a small surface area. In addition, the mushroom can provide various products for other agricultural and industrial purposes (Grimm, Wösten, 2018). Consequently, mushroom cultivation has the potential to play an important role in the framework of an innovative agricultural system. This system will enable densely populated regions of the world to address future challenges related to food security (Rahmann et al., 2021). However, mushroom cultivation, as it is practiced today, has many aspects that are unsustainable, especially when it comes to water and energy usage (Kurtzman, 2010; Wei et al., 2020). Therefore, the development of technologies that mitigate the environmental impact of mushroom cultivation should be a priority. Furthermore, these technologies should be easy to apply in densely populated developing countries where the majority of farmers are of low socio-economic status and cannot afford expensive equipment.

The present research investigates, develops and discusses sustainable means of mushroom production and evaluates their applicability in a case study in Uganda. Uganda is a country that is on one hand very fertile, with 72 % of the land being used for agricultural production, of which two thirds is cropland (FAOSTAT, 2024). On the other hand, it is a country whose population is projected to grow from 46 million today to 132 million in 2100, according to medium estimates (United Nations, 2022a). Mushroom cultivation, as in other African countries, is currently only a small industry in Uganda (Royse et al., 2017) but mushrooms are a traditional and highly valued food in the country (Nabubuya et al., 2010; Mayanja, Tipi, 2018). The combination of these factors makes Uganda an ideal candidate for investigating the potential and barriers to mushroom cultivation in a food-insecure system in tropical Africa.

1.2 Research aims

This work evaluates different methods for sustainable oyster mushroom cultivation and assesses their production potential on different substrates, their adaptability and their potential contribution to food security in tropical African countries, using Uganda as an example.

Production:

To assess the mushroom production potential of different substrates, an experiment was carried out with four different types of straw (wheat, maize, soy and faba bean). These two cereals and two legumes were chosen for their global relevance and their applicability in sustainable crop rotation systems in temperate and tropical zones. The mushroom production potential on the straw, in terms of fresh and dry mushroom

yield was determined and discussed in relation to alternative use-cases, especially the feeding of ruminants. To avoid competing use-cases, relatively nutrient poor substrates were chosen. Next to production potential, the mass flow and carbon (C) and nitrogen (N) cycling during mushroom production were investigated.

Sustainability

In order to potentially improve the sustainability of oyster mushroom production, an investigation of different methods for the sanitization (pasteurization or sterilization) of oyster mushroom substrates was undertaken. Substrate sanitization is necessary to let the mushroom mycelium grow with less, or even without competition from other microbes, and uses a lot of energy and water (Kurtzman, 2010). There are many different commonly used methods for sanitizing mushroom substrates, such as hot water pasteurization, hydrated lime pasteurization and autoclaving (Stamets, 2000), as well as less common methods like hot air pasteurization, which has only been scientifically studied for shiitake mushrooms (Wei et al., 2020). In this work, a comparison of these methods in terms of energy and water usage, as well as the experimentally determined effect on mushroom yields is undertaken. The hot air pasteurization technique was tested in different configurations to further develop this technology.

Adaptability

To evaluate the potential and adaptability of sustainable oyster mushroom cultivation beyond the theoretical and experimental research in this work, a case study in Uganda was conducted. This study included fieldwork, interviews with maize and mushroom farmers, and an experiment to investigate oyster mushroom production on local maize straw. With this mixed methodological approach, the experimentally determined mushroom production potential can be discussed in the backdrop of the socio-economic circumstances of farmers and of the Ugandan mushroom value chain. In this way, possible obstacles and pitfalls for upscaling mushroom production in a sustainable manner in Uganda were identified.

1.3 Methodology and structure

The research presented in these pages was mostly carried out at the Thünen-Institute of Organic Farming, as part of the LandLessFood project and as a PhD student at the University of Kassel, Faculty of Organic Agricultural Sciences. Part of the research was also carried out in Uganda, since a focus of the research was the creation of solutions for food security in sub-Saharan African countries.

Following the introductory section in chapter 1, two literature reviews are presented in chapter 2, which establish the theoretical foundations of the practical research which is presented in chapter 3, before discussing and synthesizing the findings to draw conclusions in chapter 4. In chapter 5, the findings are summarized, in both English and in German. The sources that are cited in the different chapters, are listed separately in chapter 6. Basic data used in the research papers in included in the annex in chapter 7. As Chapters 2 and 3 present independent scientific papers, two of which have been published in reputable peer-reviewed journals and two of which were under review for publication at the time of submission of this thesis, there may be some redundancy, particularly in the introductory sections of these different papers. Also, the research papers contain separate sub-chapters on methodology that go beyond the scope of this methodology section.

Chapter 2 starts with an analysis of the challenges facing the world's food systems in the future, focusing on the African continent and particularly on sub-Saharan Africa (sub-chapter 2.1.). This literature review was written to provide context and details for later chapters and to re-examine in the light of new data, topics I have worked on previously as a co-author in publications including Rahmann et al. (2020) and Rahmann et al. (2021). In order to outline the broader set of problems which motivate the research presented in later chapters, the historical development of modern agricultural production, demographic data and forecasts from different sources, as well as literature on the availability of land, resources and

agricultural inputs are analysed. The impact of unsustainable agricultural practices and climate change is also included in this analysis and the concept of circular agriculture and landless food production is introduced.

To describe how sustainable mushroom cultivation could play a role for future food security and for the improved sustainability of food systems, a literature review is presented in the next sub-chapter (2.2.), which was published in the Organic Agriculture journal. This peer-reviewed paper (Grimm et al., 2021) details the concept of mushroom cultivation as part of circular food systems in depth and lays out several ways of creating synergies with other agricultural sectors. Through a review of the sometimes-sparse literature in this niche-subject, the review lays the theoretical foundation of the practical research presented in the next chapters and is cited there at various points.

Chapter 3 is divided into three sub-chapters, on production (3.1), sustainability (3.2) and adaptability (3.3), as outlined in the research aims (1.2). Sub-chapter 3.1. presents a paper which has been submitted to a peer-reviewed journal, titled “Oyster mushroom cultivation on cereal and legume straw of poor feed quality”.

In sub-chapter 3.2., which focuses on sustainability within the mushroom production process, an experimental study published in the Journal of Microbiology, Biotechnology and Food Science, is presented (Grimm et al., 2024). This paper, titled “Evaluation of different pasteurization and sterilization methods for oyster mushroom production”, examines the energy and water usage of different methods for substrate sanitization and assesses their impact on mushroom yields.

Chapter 3.3. on adaptability, comprises a paper that has been submitted to a peer-reviewed journal, titled “A case study of oyster mushroom cultivation (*P. ostreatus*) on maize stover: potentials and challenges for reducing food insecurity in Uganda”. This study brings together different aspects of productivity and sustainability with the socio-economic realities within the mushroom value chain in Uganda.

The main results from chapters 2 and 3 are discussed and synthesized in chapter 4. In the first three sub-chapters of this section, the main findings on productivity (4.1.), sustainability (4.2.) and adaptability (4.3.) are discussed, before drawing conclusions and making recommendations for future research in the final sub-chapter (4.4.).

2 Literature review

This chapter reviews scientific literature to analyse the current state and future expectations of global food security (2.1.) and discusses how sustainable mushroom cultivation could contribute to this complex set of problems by creating synergies with other sectors within the food system (2.2.).

2.1 The future of food security

2.1.1 Limitations of discussing future food security

Many past predictions about the future prevalence of hunger in the world have luckily been too pessimistic. In 1798, the economist Robert Malthus published the book "An Essay on the Principle of Population", in which he argued that the world's population would grow exponentially, while agricultural production could only be increased linearly, which would lead to famine (Malthus, 1798). His prediction was so influential that such a constellation is today called a Malthusian trap.

One hundred years later, the population was indeed growing exponentially, but so was agricultural productivity (Giovanni, 2004), thanks in large part to the scientific contributions of the chemist Julius von Liebig. Liebig had discovered the importance of nitrogen for plant nutrition and described the growth-enhancing effect of guano (Liebig, 1861). These findings led to increases in agricultural productivity that Malthus could not have foreseen. However, it now became a widespread notion among scientists that food security was largely dependent on the guano reserves from small tropical islands, which were providing much of the nitrogen to fertilize fields in the "western world" (Crookes et al., 1900). By around 1918, so the argument, demand for guano would outpace supply and the agricultural output would therefore soon be insufficient to feed everyone. The problem however was technologically solved via the Haber-Bosch process, using natural gas, pressure and an iron catalyst to turn atmospheric nitrogen into ammonia (Humphreys et al., 2021). The explosion of the world's population from less than 2 billion in the year 1900 to more than 8 billion today (Roser, Ritchie, 2023) is often credited to this innovation, and it is a common trope that about half of the nitrogen in our bodies "started out in a fertilizer factory" (Charles, 2013).

While it would be easy to say that history has proved the food security pessimists wrong, it could also be argued that their predictions helped to create the urgency which motivated scientists and engineers to develop the necessary technological solutions.

So, when we speculate about planetary boundaries (Rockström et al., 2023) and discuss the current data on demography, resource availability, land availability and agricultural productivity in order to make predictions about future food security, we need to keep in mind that some of our assumptions might not be entirely accurate and that new technologies and unforeseen events could completely change the picture. But this is no reason not to engage in the debate.

2.1.2 Demography

One of the most important variables to look at when discussing food security is the number of people to feed. Important factors in predicting population growth are fertility, mortality, population size and age distribution. Global fertility rates have more than halved since 1950 and stood at 2.3 live births per woman in 2021 (United Nations, 2022). It is expected to fall to 2.1 births by 2050, according to the latest edition of the UN's World Population Prospects. Despite these low fertility rates, the population is projected to grow from 8.1 billion today to 9.7 billion in 2050, due to the growth of previous generations, the report says:

"Two-thirds of the projected increase in global population through 2050 will be driven by the momentum of past growth that is embedded in the youthful age structure of the current population. Such growth would

occur even if childbearing in today's high-fertility countries were to fall immediately to around two births per woman." (United Nations, 2022)

After the middle of the century, the certainty of growth projections becomes much less, as uncertainties about fertility and mortality rates accumulate. The UN Population Division's (UNPD) projections of this long-term growth have been revised sharply downwards in recent years. While the official projections of 2017 gave a medium estimate of 11.2 billion people in the year 2100 (United Nations, 2017), the projections in the current version predict a world population of 10.4 billion (United Nations, 2022). In the old report, the lowest projection for 2100 within a 95 % confidence interval was 9.6 billion and the highest 13.2 billion. In the new report, these minimum and maximum projections have been changed to 8.9 and 12.4 billion. This would be the difference between a 55 % increase and an 11 % increase in the current world population.

The estimates put forward by the UNPD are not uncontested. In 2023, a study which stated that the population would peak below 9 billion in the 2040s before starting to decline (Callegari, Stoknes, 2023) was published. The authors argue that fertility rates will fall faster than predicted by the UNPD due to economic growth and women's education. This non-peer reviewed study, commissioned by the Club of Rome and receiving much media attention, is however an outlier, has been criticized as unrealistic (O'Sullivan, 2023) and is not transparent about its methodology. A study in the Lancet concluded that the population would peak at 9.7 billion in 2064 (Vollset et al., 2020), which would be within the low range of the UN-predictions. Similar projections are made by the Wittgenstein Centre for Demography and Global Human Capital, projecting the population to peak around 2070 at 9.7 billion people (Wittgenstein Centre, 2024). In summary, there is a broad scientific consensus that the world population will peak between 9.7 and 11 billion people within the next 75 years, if there is no large natural catastrophe, pandemic or war (or a sudden, dramatic increase in fertility).

Perhaps as important for food security as the total population is the distribution of people in different regions. The various studies discussed above agree that sub-Saharan Africa will grow most out of all world regions. According to the UNPD, sub-Saharan Africa, with its young age structure and high fertility rate of around 4.6 children per woman, will contribute more than 50 % of total growth until 2050 and will continue to grow until the end of the century. Other regions of the world, such as Europe, East Asia and North America, will shrink (United Nations, 2022). If the medium projections of the UNPD turn out to be true, the African population will go from 1.5 billion today to 3.9 billion in 2100. The tropical countries in western and eastern Africa, including Uganda, will have the largest populations. While migration to other regions could disperse the population to some extent, it is nevertheless important to consider whether enough food can be produced on the continent to feed so many people.

2.1.3 Land availability

The share of the Earth's land surface used for agricultural production in 2021 stood at 36.8 %, having increased linearly from 34 % in 1960 to 37.5 % in 2000 and slowly stagnating since then (FAOSTAT, 2024). Leaving aside the space needed for farm buildings, agricultural land can broadly be divided into permanent pastures (including cultivated and non-cultivated meadows and rangelands) and cropland (including permanent crops, arable land and temporary fallows and pastures which are part of crop rotation). According to the statistics of the FAO, the share of global land that is covered by permanent pastures has been decreasing in the last two decades, from 26 % to 24.7 %. On the other hand, cropland has increased from 11.5 % to 12.1 over the same period. Despite this increase, the cropland available per person has gone down from 0.18 to 0.16 ha from 2003 to 2019 due to population growth (Potapov et al., 2022). This has created pressure for the expansion of cropland into natural ecosystems. As Potapov et al. (2022) show, half of the cropland gains in the world come from the transformation of permanent pastures, while the other half comes from the replacement of natural woody and herbaceous vegetation, which is most prevalent in Africa and South America. Past studies have clearly shown that most potential for cropland expansion in Africa lies in the transformation of the tropical rainforest in Central Africa (Chamberlin et al., 2014; Eitelberg

et al., 2015). Africa has more cropland per person (0.226 ha) than Asia (0.132 ha) but only about half as much as Europe (0.395 ha) or the Americas (0.402 ha) (Rahmann, Grimm, 2021). However, if cropland is not further expanded and population growth continues as predicted by the medium projections of the UNPD, the cropland per person in Africa will be only 0.062 ha and the global average cropland per person will be 0.146 ha. It is therefore likely that deforestation for cropland expansion will continue, especially in Africa. Due to the Sahara and Namib deserts, as well as large arid and semi-arid areas in the Sahel zone and in southern Africa, large swaths of land on the continent are ill-suited for conversion to cropland (Chamberlin et al., 2014). To avoid deforestation of more fertile areas as much as possible, productivity gains on existing cropland are necessary. Countries like Uganda, which have a lot of existing fertile cropland, are thus of particular importance. Resource restrictions and the impact of climate change are important considerations for increasing Africa's agricultural productivity per hectare in the future.

2.1.4 Agricultural inputs, resources and climate

Until the industrial revolution, the most important agricultural inputs were human and animal labour, which are internal inputs because they are part of the farming system, as opposed to external inputs, which are imported from outside the system. In modern, conventional agriculture, where external inputs have become increasingly important, animal labour no longer plays a role and human labour plays a much smaller role than it used to, as illustrated by the following statistic: In the year 2000, only 4 % of the population in industrial countries was involved in agriculture, while in sub-Saharan Africa 61 % were (Toenniessen et al., 2008).

The most important resources for crop production are water, fertile soil, and sunlight. However, in industrialized agriculture, mineral fertilizers have reduced the dependency on fertile soils and irrigation has reduced the dependence on rainfall. Even sunlight can be replaced with artificial light today. Arguably, therefore, the most important resource in today's agricultural system is fossil fuels, which provide most of the energy for the production of these inputs. In theory, only renewable energy could provide the energy, but such a goal is far from being reached. Since nitrogen for fertilizers can be synthesized from air, and is hardly present in rock formations, only phosphorous (P) and potassium (K), which are also crucial fertilizer ingredients (Bonilla-Cedrez et al., 2021), must be mined or extracted from waste-streams such as sewage. Global mineral K reserves are massive and will be able to meet increasing demand for centuries (Roberts, 2008). In addition, K can be extracted from seawater as a by-product of desalination (turning seawater into fresh water) and is therefore virtually unlimited (Sharkh et al., 2022). P, on the other hand, is less abundant in the Earth's crust and in the oceans (Sardans, Peñuelas, 2015). Even though phosphate rock is definitely a finite resource, the reserves are expected to meet demand for at least 300 years (van Kauwenbergh, 2010). However, it is foreseeable that the quality of the reserves will decline, and thus the price of the P-concentrates and the amount of toxic waste from production will increase (Cordell, White, 2011). Also, it is likely that the country with the largest reserves, Morocco, which produced only 15 % of P-concentrate in 2010, will take an increasing amount of the market share, to the point where it could control 80 % of production in 2100 (Cooper et al., 2011). Access to P for fertilizers might therefore become more restricted, especially for poorer countries, despite the existence of sufficient reserves for the next centuries.

The combination of agricultural inputs like fertilizers, pesticides, improved seed varieties and irrigation is often summarized under the umbrella term of the "green revolution", which helped countries like India to become one of the world's largest crop producers during a time of explosive population growth in the second half of the 20th century (John, Babu, 2021). Despite this achievement, this form of agriculture is today seen more critically due to negative effects on soils, ground water and climate which are now posing new threats to India's food security and undermining the foundation of the agricultural system (Singh, 2000; Toenniessen et al., 2008). In sub-Saharan Africa, the green revolution took less hold than in the rest of the world (Toenniessen et al., 2008). Nevertheless, soil degradation and climate change are affecting the continent to an alarming extent. The IPCC forecasts that East Africa will experience more droughts but also

floods in the coming decades and highlights that Africa in general will be one of the most affected continents, while having the least capital resources for meeting the challenge (IPCC, 2023).

Better access to fertilizers could help reduce the substantial yield gap in sub-Saharan Africa compared to the rest of the world, and thus reduce the need for cropland expansion (Bonilla-Cedrez et al., 2021). African agricultural soils are on average very deficient in P and K (Sardans, Peñuelas, 2015).

Another approach for improving agricultural productivity in Africa is organic agriculture, which offers a solution that does not rely on mineral fertilizers and pesticides. Though organic agriculture has positive effects on soil health, it is not always more climate friendly than conventional agriculture and on average produces 20 % lower yields than conventional agriculture (Ponti et al., 2012). Some therefore argue for a middle-of-the-road approach, suggesting the use of minimal amounts of fertilizers and pesticides while using sustainable methods from organic agriculture (Neuhoff, Kwesiga, 2021).

Access to water is of particular importance in Africa, where climate change threatens to change rainfall patterns and lead to prolonged droughts. This could widen the already significant yield gap between Africa and the rest of the world: for example, the average global maize yield in 2022 is 5.7 t/ha, while the average African maize yield is 2.2 t/ha (FAO, 2021). Increasing the area of existing cropland under irrigation could be one of the most effective ways to increase yields in Africa (You et al., 2010). This would require widespread construction of dams and other water retention projects. The competition over water resources has the potential to cause wars between and within countries in Africa.

In summary, there is substantial potential to increase crop productivity through increased use of agricultural inputs without overexploiting critical resources. However, the impact of unsustainable farming and of climate change threaten to impact the African continent in ways that make crop production more difficult.

2.1.5 Discussion

Today's predictions for global population growth are slightly lower than a few years ago, but the overall picture regarding future food security has not changed much. Globally, it can be concluded that there will be enough cropland available per person to produce sufficient food for everybody in 2100, but regionally, especially in sub-Saharan Africa, food security is far from guaranteed (Rahmann, Grimm, 2021; Rahmann et al., 2020). If less cropland area was assigned to producing animal feed and if food was distributed more fairly, hunger could become a thing of the past, most likely even in the face of extreme climate change. However, it would be naive to count on such a development in the current system, not least because food can be used as a "geostrategic weapon" (Sommerville et al., 2014). African countries should therefore strive to achieve food sovereignty in their agricultural production as much as possible, while adapting to the impact of climate change and increased fertilizer prices. Countries like Uganda, which already have a large share of cropland, are strategically important for increasing food production in the region and reducing the pressure to expand cropland through deforestation in sub-Saharan Africa.

Agricultural productivity gains could be achieved by "building an alliance for a green revolution in Africa" (Toenniessen et al., 2008). This would be a resource-intensive (high-input, high-output) approach, that relies on resources, such as phosphorous, that are likely to become more expensive over time (Cordell, White, 2011). Adopting circular agricultural approaches, where nutrients for fertilizing crops are recovered through recycling, is an attractive alternative to dependence on mineral fertilizers and could at least ameliorate such a dependence. However, organic agriculture, which heavily on circular approaches, is on average less productive than conventional agriculture (Ponti et al., 2012), which is why one can also argue for a "para-organic" path that incorporates methods from both sides (Neuhoff, Kwesiga, 2021). In addition to productivity gains in crop production, circular, landless food production methods, such as the cultivation of algae and mushrooms, could be an important part of the solution (Rahmann et al., 2020).

While nutrient cycling and landless food production are important for reducing the dependence on expensive and finite resources (phosphorous and land) and making agricultural production more sustainable, perhaps even more important for future food security will be the availability of water and energy resources. Since the impact of climate change is likely to lead to more droughts, the conservation and efficient use of water is very important. Technologies proposed to increase food production, such as mushroom production, should therefore not only be part of a sustainable recycling system, but should also use as little water and energy as possible. As will be shown in the following chapter, there are many different ways to integrate mushroom production with other agricultural sectors in order to conserve resources and increase productivity.

2.2 Integration of mushroom production into circular food chains

This chapter contains the following peer-reviewed literature review paper:

Grimm, Daniel; Kuenz, Anja; Rahmann, Gerold (2021). Integration of mushroom production into circular food chains. Organic Agriculture, 11(2), 309-317. DOI: 10.1007/s13165-020-00318-y

2.2.1 Abstract

Edible mushrooms are cultivated mainly on ligno-cellulosic plant materials, thereby turning agricultural wastes to high quality products. In this review, several ways in which mushroom cultivation could help in the transition towards a circular agricultural economy are discussed, including food, feed and compost production. The production processes of different mushroom species are also described and an overview of the global mushroom market and its history are given. Resource use efficiency could be maximized by using spent mushroom substrate as feed for invertebrates, such as insects or earthworms, which produce high-quality compost and can serve as food or feed for other animals. In the context of an increasing world population as well as limited resources and agricultural land, as described in the LandLessFood project, mushroom cultivation could fulfil the need for protein-rich food and for the recycling of nutrient-poor agricultural wastes.

2.2.2 Introduction

Population growth, climate change and the depletion of non-renewable and limited resources like phosphate and fossil fuels are some of the major challenges to the global agricultural system and threaten food security, especially in densely populated and less developed regions of the world (Rahmann et al., 2017). One of the most promising strategies for tackling these challenges is the improved usage and recycling of non-consumable biomass (Hamm et al., 2017). Among these wastes are large quantities of nutrient-poor plant residues from cropping, which are of little value as food, feed or fertilizer and are therefore often burned, disposed of in other unsustainable ways (Arai et al., 2015; Feng et al., 2011), or left on the fields as organic matter to keep or improve soil fertility (Rahmann et al., 2017). These materials include straw, various husks, leaves and stems, corncobs and all other parts of plants which are rich in the cell wall components cellulose, hemicellulose and lignin. Since fungi are the most efficient decomposers of such materials and especially of lignin (Stamets, 2000), a cleverer integration of edible mushrooms into the food and biomass chain could be the most sustainable way of utilizing this biomass. To realize this potential, it is however necessary to look at mushroom cultivation in a different way than is the case today: not primarily as a method of food production, but rather as the first step in a value-adding composting process which also provides feed for animals and nutrients for plants.

2.2.3 Mushroom production

Mushrooms have been cultivated by humans for more than a millennium (Stamets, 2000). However, in recent decades the scope and methods of cultivation have changed dramatically.

2.2.3.1 Market

According to Royse et al. (2017) the global consumption of mushrooms increased from 1993 to 2013 from 1 kg to 4.7 kg (fresh weight) per person and year. The mushroom market was valued at around 63 billion USD in 2013, only 8 % of which was accounted for by wild mushrooms. The global production of cultivated edible mushrooms has increased around 30-fold since 1978 to around 34 million tons annually. China is by far the largest mushroom producer in the world, accounting for around 87 % of the global production in 2013.

2.2.3.2 Mushroom production methods

Most cultivated species of mushrooms naturally grow on dead wood, while others are found on compost-like materials and nutrient-rich soils, often in association with manure (Stamets, 2000). Virtually all cultivated species of mushrooms have a saprotroph lifestyle, meaning they are decomposers of organic matter. Some wild edible mushrooms, like truffles (*Tuber melanosporum*), porcini (*Belotus edulis*) and chanterelles (*Cantharellus cibarius*), are mycorrhizal fungi which require a symbiotic tree partner to grow and can therefore not easily be mass-produced. The world's leading cultivated mushroom is shiitake (*Lentinula edodes*), followed by oyster (*Pleurotus spec.*) and wood ear mushrooms (*Auricularia spec*.) of different species and button mushrooms (*Agaricus bisporus*) which are the most popular mushroom species in western countries (Royse et al., 2017).

Stamets (2000) describes the production of shiitake, oyster and wood ear mushrooms. They are categorized as primary decomposers, all of them inhabiting wood in the wild. The traditional cultivation method of these fungi is simply to transfer mushroom mycelium onto logs of wood. These are kept outside, in a sufficiently moist and temperate environment (or in some cases buried) until mushrooms can be harvested. Nowadays they are usually cultivated in plastic bags filled with sterilized sawdust or other ligno-cellulosic materials like straw. To optimize yields, it is important to keep these bags at the right temperature and moisture conditions for the chosen species of fungus. Fruiting will often occur by itself but can be induced through changes in the temperature or light conditions, depending on the mushroom species (most of them do not require any light). Usually, two to three flushes can be harvested at intervals of around a week before the substrate has been depleted. To increase yields, nitrogen-rich supplements are often added to the substrates. However, even without supplements high yields can be achieved. The common measure for efficiency in mushroom cultivation is biological efficiency (BE). A BE of 100 % means that the mass of fresh mushrooms harvested is equal to the dry weight of the substrate. Given the water content of mushrooms of around 90 %, the conversion ratio in this example would be 10:1. Skilled cultivators produce mushrooms with a BE of between 75 % and 125 %.

The button mushroom is usually categorized as a secondary decomposer (Stamets, 2000). Such organisms depend on the prior activity of other microorganism and their metabolites to grow. However, it has been demonstrated that the button mushroom can also be cultivated on non-fermented substrates (Till 1962). The basis for substrate-formulation depends on local availability of substrates but most often a combination of straw and animal manure is used (Royse, Beelman, 2007). The most common substrates are compost-like materials which are prepared in a two-phase fermentation process. A complete production cycle for button mushroom production takes roughly 14 weeks, according to Royse and Beelman (2007) from whom the information of the following short summary was taken.

Phase 1: The composting takes about 6 to 14 days, depending on materials and facilities, such as the availability of forced aeration. The substrate materials are gathered in a large heap and mixed to achieve homogeneity. Water is added as well as gypsum to stabilize pH. During this phase, very high temperatures are reached due to microbial activity. Since temperatures should not rise above (but also not fall substantially below) 80 °C in the centre of the pile, it is necessary to turn and water the compost at intervals of about 2 to 3 days. The metabolic activity of the thermophilic microflora helps to create a more selective substrate for *A. bisporus*. When the compost has a chocolate brown colour, a strong smell of ammonia, soft, pliable straws and a water content between 68 and 74 %, it is ready for phase 2.

Phase 2: The purposes of this phase are to assimilate ammonium, to stimulate the growth of beneficial thermophilic microflora and to kill nematodes, insects, moulds and other possible pathogens of the button mushroom. The thermophilic microorganisms which thrive in this phase and help to metabolize the ammonium will not be competitive at the lower temperatures during cultivation and will serve as a food and nitrogen source to the mushroom. The optimal temperature range of the substrate during phase 2 is between 50 to 55 °C. Unlike in phase 1, it is very common for cultivators to use a climate-controlled chamber during this phase, instead of relying purely on self-heating of the substrate and on turning and watering to

decrease substrate temperatures. Once the phase is completed, after roughly 5 days (Gerrits, 1988), it is necessary to let the substrate cool down to room temperature (ca. 23 °C) before mixing the substrate with mushroom spawn.

A typical spawning rate for button mushrooms (as for many other species) is about 2 % (spawn to substrate, dry weight). The mushroom will colonize the substrate in 13 to 20 days and is then filled into trays and covered with casing soil (although it is also possible to cover the substrate with casing soil directly after spawning). Although hygienic conditions are important in button mushroom cultivation, the "semi-sterile" process described above is sufficient for very effective cultivation. The activity of some bacteria in the casing soil even seems to be beneficial, as it removes volatiles from the button mushroom which suppress fruiting (Noble et al., 2009). In general, the button mushroom prefers temperate over hot climates. China has therefore set up most of its button mushroom production in the northern parts of the country (Royse, 2017). In tropical countries, heat-resistant oyster mushroom species or paddy straw mushrooms (*Volvariella volvacea*) are particularly suited for cultivation (Stamets, 2000).

2.2.4 Mushrooms as food and feed

While the quality of mushrooms as food has received increased recognition and is reflected in rapidly increasing consumer demand, the potential of mushrooms as feed is largely unknown and unexploited. Mushrooms can be marketed fresh for roughly five days after harvest. If they are to be sold afterwards, they have to be conserved, for example by drying them. Dried mushrooms are very popular in Asia, while in western countries mainly fresh mushrooms are in demand (Stamets, 2000). Due to spoilage, overproduction or simply "low visual quality", many mushrooms never reach the market for human consumption. While it would make little economic sense to cultivate mushrooms primarily for animal feed, usage of "unmarketable" mushrooms in such a way would be a sustainable solution.

2.2.4.1 Mushrooms as human food

Edible mushrooms are calorie-poor but rich in protein, minerals and vitamins. Due to the high water content (ca. 90 %) of fresh mushrooms, their energy density is relatively low, with only ca. 30 kcal per 100 g fresh mushrooms (Mattila et al., 2002). Leaving aside the water content, the most common cultivated mushrooms – shiitake, various oyster mushrooms and button mushroom – have a protein-content of approximately 20 % and are a good source of all essential amino acids for human diets (Mattila et al., 2002). Mushrooms consist of around 50 % carbohydrates, around a third to a half of which is dietary fiber, while the fat-content is usually low, with around 3 – 4 % of the dry weight (Mattila et al., 2002). Mushrooms are a good source of the vitamins B2, B3, B9 and contain vitamin D, C and trace elements of vitamin B12 (Mattila et al., 2001), which is often lacking in vegetarian and vegan diets. Additionally, many mushrooms contain macromolecules with anti-carcinogenic, immuno-stimulating or other medical effects, such as enhanced neurogenesis (Rop et al., 2009; Ryu et al., 2018; Stamets, 2000).

Mushrooms are often equated to vegetables, even in the scientific literature, although they are more closely related and more similar to animals in their metabolism and nutrient composition. Supplementing mushrooms for meat can have significant health benefits for obese people, including weight loss, improved systolic and diastolic pressure, improved lipid profile and a decrease of inflammatory markers in their blood (Poddar et al., 2013). Studies such as this, as well as their dietary profile, show that mushrooms are a healthy food and especially suitable as meat substitutes. In sensoric tests, meat-analogues made from fungi were found to taste better than those made from vegetables. Additionally, the concentration of proteins and essential amino acids was found to be higher (Kumar et al., 2017). These meat analogues are most commonly produced from the mycelium of fungi such as *Fusarium graminearu*, which do not form mushrooms and are cultivated in liquid medium rather than on solid substrates. Given the increasing world-

wide need for protein, mushrooms and fungal meat analogues could play an important role in the future of the agricultural system, where high animal numbers might not be supportable.

2.2.4.2 Mushrooms as animal feed

Very few studies have been carried out on mushroom as feed. Slightly more literature is available on the use of spent mushroom substrate as feed (see 5.1).

Supplementation of 2 % shiitake mushroom extract in the diet of the rainbow trout Oncorhynchus mykiss significantly improved their immunological parameters and survival rate during exposure to the bacterial pathogen *Lactococcus garvieae* (Baba et al., 2015). A positive impact of mushrooms on weight gain of fish was found in a study where the feed of the fingerlings *Labeo rohita* and *Hemigrammus caudovittatus* was partly replaced with mushrooms. This study looked at the effect of replacing half of the fish meal with shiitake or earthworm meal in a regular feed composed of 18 % fish meal, 32 % ground nut oil cake, 28 % tapioca and 22 % rice bran. The diet with earth worm meal showed an approximately 2-fold higher growth rate compared to the fish meal diet, while the diet with mushrooms showed a 1.2 to 1.7-fold increase, depending on species of fish (Paripuranam et al., 2011). Similarly, shiitake extracts had positive effects on health parameters of chicken (Willis et al., 2007). Feeding button mushrooms to chicken at the rate of 20 g per kg of feed led to significant growth promotion and improved antioxidant-protective activity (Giannenas et al., 2010).

It is interesting to note that there are no published feeding trials with animals that are known to be fungivores. Insects and other invertebrates have so far received very little attention from scientists, even though they are the largest group of fungivores in nature, and often depend on wood-inhabiting fungi to complete their life cycle (Vega, Blackwell 2005; Boddy, Jones, 2008). Some mammals, such as squirrels and chipmunks, also have a strong reliance on fungi as a primary food (Fogel, Trappe, 1978), and wild boars are known to consume truffles and other types of mushrooms. Nevertheless, the only feeding trials with mushrooms found for this review were conducted on chicken and fish.

2.2.5 Mushroom compost

On average, roughly 5 kg of spent mushroom substrate are produced per kg of mushrooms (Finney et al., 2009). Therefore, since 34 million tons of mushrooms are produced globally per year (Royse et al., 2017), the amount of spent substrate might be roughly 170 million tons. However, Stamets (2000) speaks of a 2:1 ratio of spent substrate to (oyster) mushrooms, without specifying if this is on a dry weight or fresh weight basis (just as Finney et al. (2009) fail to specify this). The lack of clarity on this subject in the mushroom literature is altogether surprising.

The amount and quality of spent mushroom substrate as compost is dependent on the substrate ingredients, species of cultivated mushroom and method of cultivation. The cultivation of a single mushroom species will not result in complete decomposition of the materials. The cultivation of several species of mushroom in succession on the same substrate or further composting of spent mushroom substrate will however result in the production of rich topsoil (Stamets, 2000). It is also possible to use spent mushroom substrate as animal feed – and use the manure as fertilizer. In the following paragraphs both recycling pathways are discussed.

2.2.5.1 Mushroom compost as feed

As with mushrooms themselves, spent mushroom substrates have mainly been investigated as feed for common production animals – cows and pigs – rather than as feed for animals that naturally rely on fungal biomass as a primary food, which are mostly invertebrates.

Spent mushroom substrates as feed for pigs and cows have produced mixed results. Song et al. (2007) measured a negative effect on body weight gain of pigs with addition of 5 % fermented spent oyster mushroom substrate, while 3 % had no effect. Chu et al. (2012) also found negative to neutral effect of spent mushrooms substrate on growth. However, they describe an improvement in meat quality. Also, spent mushroom substrate could be a good bedding material for pigs. Durrell et al. (1997) found that enriching sow pens with spent (button) mushroom compost reduced aggressive behaviour, injuries, floor sniffing and lying down with open eyes.

Even though chemical analyses have shown that the cultivation of mushrooms should increase the digestibility of straw by reducing the amount of lignin and cellulose (Nasehi et al., 2017), feeding trials showed that cows refuse eating more than 17 % of straw-based spent oyster mushroom substrate in a maize and hay-based diet (Adamovic et al., 1998). The same study showed that supplementation above 10 % had negative effects on weight gain. However, in another study, the growth performance of post weaning calves was improved by 8 % by supplementing their feed with 10 % fermented sawdust-based spent oyster mushroom substrate (Kim et al., 2010).

Only one study (Lee et al., 2018) which examined the use of spent mushroom substrate as insect feed was found when writing this review. The paper is written in Korean (except for the abstract and tables in English) and was therefore analyzed using Google Translate (https://translate.google.com/) for this review. The study looked at a beetle (*Protaetia brevitarsis seulensis*) reared for medicinal purposes, fed with either fermented oak saw dust (control) or spent oyster mushroom and shiitake substrates. The results suggest that the beetle larvae grew fastest and gained most weight when reared on spent oyster mushroom (*P. eryngii*) substrate. Spent shiitake substrate on the other hand seems not to have fared better than the control. Aside from this source, only anecdotal evidence, as well as related studies which looked at the use of straw fermented by fungi rather than spent mushroom substrate, can serve as evidence that mushroom cultivation could produce insect feed as a side product. Gao et al. (2019) showed that black soldier fly larvae can be fed with maize straw fermented with *Aspergillus oryzae* (a mould which is usually used to produce soy sauce, sake or vinegar). In the most successful treatment, where straw was fermented for 24 hours, the success was similar (slightly but not significantly lower) than in the control treatment, where the insects were fed wheat bran. Qi et al. (2019) showed that fermentation of corn and wheat straw by the mould *Trichoderma viride* and the yeast *Saccharomyces cerevisiae* increased the bioconversion of these substrates by house fly larvae.

Several studies on vermicomposting of spent mushroom substrates have been carried out. However, while the quality of vermicompost from spent mushroom substrates was analyzed, there has been no investigation of the feed conversion ratios. Nevertheless, there is good reason to assume that conversion ratios are high. Edwards (2010) writes that earthworms convert cow dung with an efficiency of 10 %. In an experiment in which cow dung and spent oyster mushroom substrate were vermi-composted together, the treatment where earthworms grew fastest consisted of 60 % spent mushroom substrate and 40 % cow dung (Nik Nor Izyan et al., 2009). Therefore, the feed conversion ratio for spent mushroom substrate might also be 10 % or higher. This assumption is supported by another experiment: in a vermicompost consisting of 25 % sewage sludge and 75 % spent oyster mushroom substrate, the earthworm biomass increased by 896 % in only 70 days (Bakar et al., 2011).

2.2.5.2 Mushroom compost as fertilizer

Many studies have found mushroom composts to be of excellent quality and rich in nitrogen (N), phosphorous (P) and potassium (K). Nevertheless, the production of great amounts of spent mushroom substrate can lead to similar disposal problems as other kinds of organic wastes (Grimm, Wösten, 2018). This is especially the case for substrates which contain animal manure, such as used for the button mushroom. Spent button mushroom substrate is used for crop production in horticulture and agriculture. However, some authors recommend that, to convert this spent substrate to high-quality compost, it should

be subjected to a weathering period of at least 6 months, during which it is spread in heaps of roughly 1.5 m height and subjected to the elements. In this way salts and minerals which reduce the quality of the compost (Courtney, Mullen, 2008), are washed away and the decomposition of the material continues. In a comparison of spent button mushroom substrate, forced aeration compost and mineral "NPK" fertilizer, it was shown that of all treatments, a 100 t per ha application of spent substrate had the strongest positive effect on grain yield (59 % increase compared to no-fertilizer control), and that even 50t/ha came close to producing the same yields as the mineral fertilizer treatment. Also, the amount of soil phosphorous, potassium and nitrogen, as well as soil organic matter were greatly increased. The authors of this study remark that salinity problems are unlikely to occur "as the P content of soil and compost would limit further large applications" (Courtney, Mullen, 2008). The application of mushroom compost, as for any other compost or fertilizer, should nevertheless be case-depended. For example, magnesium-deficiency could arise at high application rates due to antagonism with potassium, which is abundant in mushroom compost (Uzun, 2004).

Spent substrates of oyster or shiitake mushrooms have been shown to not only improve plant growth but also their health status and to be able to suppress plant pathogens in soils. In a bio-essay experiment with cucumbers and the fungal pathogen *Colletotrichum lagenarium*, it was shown that spent shiitake substrate greatly reduced anthracnose symptoms (Di Piero et al., 2006). The effect was largest in unsterilized spent substrate. Fresh (unused) shiitake substrate showed a much slighter reduction in these symptoms. Therefore, metabolites from shiitake mushroom cultivation must be responsible for the positive effect. Spent oyster mushroom substrate, as well as extracts and live mycelium from the oyster mushroom, were shown to suppress the sugar beet nematode *Heterodera schatii*: the addition of 100 g and 200 g of spent substrate per 3 kg of soil reduced the numbers of nematode cysts by 85 % (Palizi et al., 2008). In another study, spent oyster mushroom substrate suppressed root-knot nematodes in field conditions, though not as effectively as other organic wastes (El-Sherbiny, Awd Allah, 2014).

Even though these results show that further composting is not strictly necessary for spent shiitake or oyster substrate, it can be very beneficial to do so. Through co-composting, it is also possible to recycle other organic wastes such as pig manure or sewage-sludge. This was shown in the context of vermicomposting, where high-quality composts were produced from sewage sludge and spent mushroom substrate (Bakar et al., 2011).

2.2.6 Discussion

This review showed the potential of mushrooms and spent mushroom substrate for food, feed and compost preparation from crude fiber and lignin-rich biomass. An improved integration of mushroom production into the food production chain could make important contributions to food security and human health, to soil fertility and carbon sequestration, as well as to animal and plant health, which could even help to reduce the use of antibiotics and pesticides. Other usages of mushrooms and spent mushroom substrates which were not discussed in this review include bioremediation and the production of materials and enzymes (Grimm, Wösten, 2018). The application of mushroom compost could also be used to increase biodiversity in agricultural landscapes and in forests. However, no studies on this have yet been conducted.

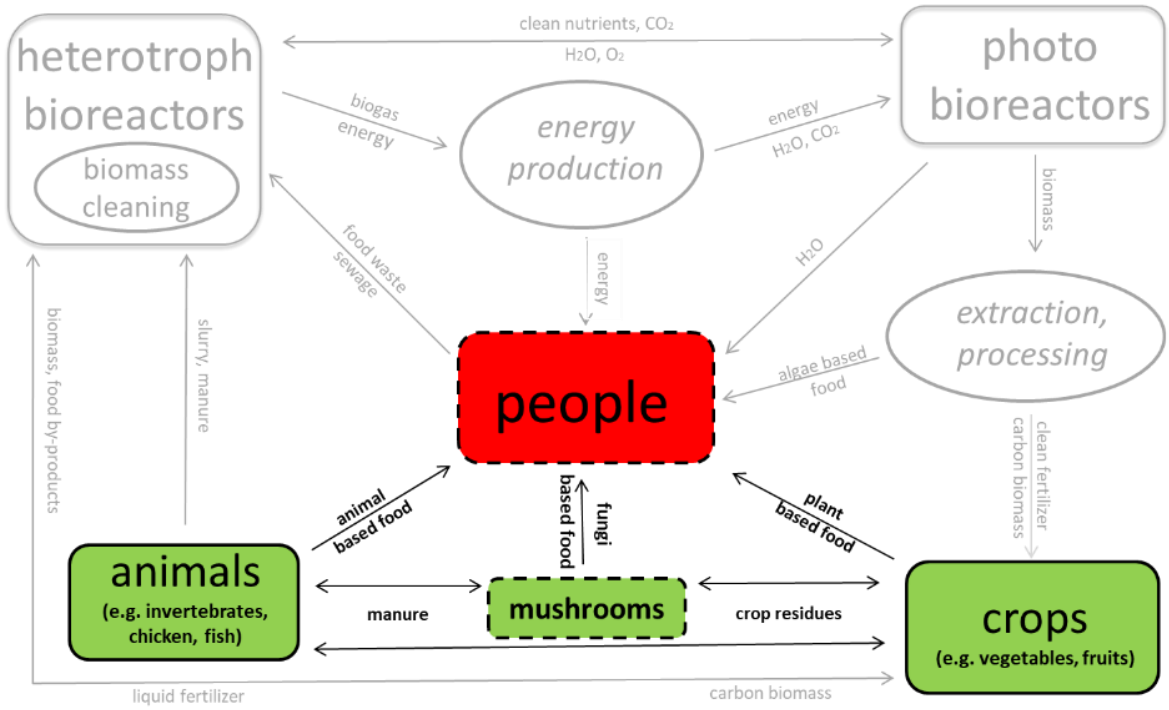
Mushroom cultivation can be integrated into many different agricultural systems, due to the sheer number of different ways in which mushrooms and mushroom compost ca be used. Industrial nations could include this in strategies for reaching their self-set climate and sustainable development goals, while the main incentive for developing nations to promote mushroom cultivation in circular food chains is likely to be food security and public health. Small scale farmers could profit most, as they have all the materials necessary for cultivation and need few materials to get started. Mushroom cultivation would be an additional source of income and food. The limited access to fertilizers in most African nations would be less of a problem if

high quality compost was available (Rahmann et al., 2019). Also, the feeding of chicken or fish with mushrooms and earthworms would reduce the need for other unsustainable feed.

The contribution to food security can best be visualized in an example. We assume that on a field of 1 hectare 4 t of wheat and 4 t of straw are produced (on a dry weight basis). 5 % of the grains are used for the production of oyster mushroom spawn, while all of the straw is used as substrate. Fanadzo et al. (2010) produced oyster mushrooms on un-supplemented wheat straw with a biological efficiency of 71 %. If we assume such a low efficiency, the amount of mushrooms produced from the 4 t of straw (dry) would be 2.8 t (fresh) – and therefore 280 kg in dry weight. If we assume a 20 % mass reduction from in the substrate during colonization, as Nasehi et al. (2017) found with an oyster mushroom, then the amount of spent mushroom substrate would be 3.2 t. If this spent substrate was vermicomposted, and if the conversion efficiency of mushroom compost by earthworms is indeed 10 % then 320 kg of earthworms could be produced. If the mass reduction of the compost during vermicomposting is again 20 %, and we also subtract the weight of the earthworms themselves, the amount of compost would be roughly 2.2 t. The calculations for the amount of earthworms and compost are by necessity inaccurate, as no literature on this exists. However, it might not be unrealistic to produce 280 kg of dried mushrooms, 320 kg of earthworms and 2200 kg of compost from one hectare of wheat straw. Given that fungal biomass is one of the main food sources for earthworms (Schönholzer et al., 1999) and that most of the fungal biomass is mycelium rather than mushrooms, it is possible that the mass of earthworms might exceed that of mushrooms. Nevertheless, it is also possible that the amount of earthworms here is exaggerated. To make a more accurate assessment of the potential of such a mycological recycling pathway, experiments will have to be conducted. These should investigate mushroom cultivation in the context of agricultural systems, rather than as an isolated industry. In this way, a great contribution to the agricultural system could be made.

2.2.7 Conclusion

Figure 1: The position of mushroom cultivation in the circular LandLessFood model.



Source: Gerold Rahmann, Anja Kuenz, Daniel Grimm

Mushrooms could be used to recycle byproducts and biomass from animal husbandry and especially crop production, as depicted in Figure 1. The integration of mushroom cultivation between these two systems could lead to more productivity and improved resource use efficiency. Mushrooms themselves are “quality” rather than “energy” food. Since they are particularly suited as a meat alternative, they could be used to create more sustainable agricultural ecosystems with relatively low animal densities. In such a system, ligno-cellulosic plant waste would be used for mushroom production instead of as ruminant feed. This would not only be more effective but also avoid methane emissions. Since animals such as fish and chicken have much better feed conversion ratios than most other livestock, and since mushrooms are a healthy feed supplement for them, these species would be the ideal animals in such a “mycological” agricultural system. By vermicomposting of spent mushroom substrate this system could be improved even further by providing earthworms as feed as well as compost for plant production in one step. If large amounts of spent mushroom substrates are produced, this could be an ideal bulking agent for the composting of sewage sludge, thus also offering a pathway for the recycling of nutrients that would otherwise be lost from the agricultural system.

3 Research

3.1 Production: Oyster mushroom cultivation on cereal and legume straw of poor feed quality

This chapter contains the following peer-reviewed research paper:

Grimm, Daniel; Sonntag, Enno; Rahmann, Gerold (2024). Oyster mushroom cultivation on cereal and legume straw of poor feed quality. Studies in Fungi, 9(1). DOI: 10.48130/sif-0024-0010

3.1.1 Abstract:

This study explores the viability of cultivating oyster mushrooms on cereal and legume straw of poor feed quality, investigating oyster mushroom productivity and the implications for mass, nitrogen and carbon flows. Four types of straw (wheat, maize, faba bean, and soybean) were utilized as substrates for mushroom cultivation. Fresh yields varied widely, from 114 % biological efficiency on maize straw to 58 % on wheat straw, while dry yields ranged from 9.2 % biomass conversion rate on maize straw to 3.8 % on wheat straw. Protein content of mushrooms varied between 16.8 % on wheat straw and 23.2 % on faba bean straw, correlating with the nitrogen content of the straw. Furthermore, results revealed significant variations in carbon emissions, ranging from an estimated 3.5 kg (on wheat straw) to 2.6 kg (on soy straw) emitted per kg of dry mushroom produced. These findings underscore the importance of substrate selection in mushroom cultivation, with implications for both agricultural resource management and protein-rich food production.

3.1.2 Introduction

Oyster mushrooms are the second most cultivated type of mushroom in the world, with about 19 % of market share (Royse et al., 2017). The grey oyster mushroom (*Pleurotus ostreatus*) is among the most versatile and robust mushroom species, as it can be cultivated on a very wide range of agricultural residues without the need for complete substrate sterilization (Stamets, 2000; Grimm et al., 2024).

Even though it is possible to grow oyster mushrooms on wood or straw, many mushroom farmers use substrate ingredients which could alternatively be used as food or feed, like cotton seed hulls or bran, which is a commonly recommended substrate supplement (Yang et al., 2013; Mayanja, Tipi, 2018). While supplementation with nitrogen-rich, edible ingredients can increase mushroom yields (Stamets, 2000), the use of substrates which cannot be used as food or feed is more sustainable in the context of food security and agroecology (Grimm et al., 2021).

Another aspect of sustainability in mushroom production is the crop rotation system from which the substrates are sourced. Legumes are an important part of crop rotation due to their ability to increase soil nitrogen and can increase the productivity of cereal crops that are cultivated on the same plot afterwards (Reckling et al., 2016; Aschi et al., 2017). Cereal crops are rich in carbohydrates and provide a lot of calories, while legumes are rich in proteins and fats, so that a combination of the two can provide most of the macronutrients needed for human nourishment (Rahmann, Grimm, 2021). Oyster mushrooms are rich in vitamins and minerals (Mattila et al., 2001) and are a good meat substitute due to their amino acid profile and high protein content (Mattila et al., 2002; Poddar et al., 2013; Kumar et al., 2017), which makes them a valuable addition to a diet mostly based on cereals and legumes.

Mushroom production does not compete with other food production on farmland, as it is nearly “landless” (Rahmann et al., 2020). However, it can compete with animal husbandry if feed-grade substrates are used. We stipulate that the ideal straw for oyster mushroom cultivation is so nutrient-poor that it provides too little metabolic energy (ME) and digestible protein for feeding a 60 kg goat. A 60 kg goat needs 9.7 MJ of

metabolizable energy (ME) and 70 g of digestible protein for its maintenance needs, which it has to draw from a maximum of 1.4 kg feed (dry matter = DM) which it can eat per day (Rahmann, 2007). Therefore, 6.93 MJ of ME and 50 g of digestible protein must be available per kg of feed DM. With this baseline, it is guaranteed that the substrate has very little value as feed and for most other agricultural use-cases apart from organic matter in the soil.

The crops providing the straws for our study were chosen partly due to their high relevance as staple crops and partly to represent typical legume and cereal crops from temperate climate zones (faba bean and wheat) and from more tropical regions (soy bean and maize). With the experiment we conducted, using these straws to cultivate oyster mushrooms, we want to answer the following research questions:

1. What is the nutrient composition and feed quality of the different straws?
2. What is the oyster mushroom production potential of the different straws?
3. How much carbon and nitrogen are retained in the spent mushroom substrate and what are the implications for the need of fertilizer or compost use on the field?
4. How much carbon is emitted in the process of mushroom cultivation?

3.1.3 Materials and methods

3.1.3.1 Substrates and preparation

Straw from two cereal crops and two legumes was used in the experiment: maize (*Zea mays*, variety Saludo), wheat (*Triticum aestivum*, variety Faustus), soy bean (*Glycine max,* a mix of varieties Merlin, GL Melanie, Marquise, Aurelina, ES Favor, RGT Sphinxa, ES Comandor, Amarok and Arcadia) and faba bean (*Vicia faba*, variety Tiffany). All straws were produced with certified organic farming practices (EU Regulation 848/2018), which ensures that there are no remains of fungicides on the material which could influence mushroom growth. Maize, faba bean and wheat were cultivated in 2019 under scientific controlled conditions on the experimental station of the Thünen-Institute of Organic Farming in northern Germany. To get a nutrient poor maize straw despite using a feed-variety of this crop (sweet maize varieties cannot be cultivated in cold, Northern German climate), the maize was left standing in the field for four months after harvest season before cutting it, allowing nutrients to leach back into the soil. Soy was grown at the organic experimental station Gladbacher Hof of the University Giessen in central Germany. The different straws included all the above-ground parts of the plant except the grain, and the cobs in the case of maize. All the straws were chopped and dried for five days at 40 °C for storability.

Grain spawn with mycelium of *P. ostreatus* ((Jacq.: Fr.) P. Kumm, strain number: P10001, type of grain: wheat) was used to inoculate the straws for mushroom cultivation.

3.1.3.2 Experimental design

The experiment consisted of four different treatments (Wheat, Maize, Soy, and Faba) with eight replicates. Each replicate consisted of a polypropylen mushroom grow bag with a micropore filter (100*16 cm, Hemoton brand ®) filled with 800 g of moist pasteurized substrate (200 g dry matter and 600 g water) and 20 g of mushroom grain spawn (6.6 g dry matter). Hot air pasteurization at 100 °C for three hours was used to pasteurize the substrate. Grain spawn was added to the substrate after letting it cool down, ensuring that the spawn was well distributed by twisting and shaking the bags. The mushrooms were cultivated under controlled conditions in the laboratories of the Thünen-Institute, at 21 °C and 90 % humidity in a grow-box (HOMEbox Vista Medium), as depicted in Figure 2.

Figure 2: Photo of experimental set-up. Different substrates are used for mushroom cultivation in a random replication approach in grow chambers

Source: Daniel Grimm

Since we wanted to use the same amount of dry matter and water in the different treatments, but the straws had different water holding capacities, we decided to hang the bags in a way that allowed excess water to dripple from a small opening at the bottom.

3.1.3.3 Data collection

Up to three flushes were harvested from each replicate. The fresh weight of the harvested mushrooms was determined immediately after harvest. The dry yield was determined after drying at 105 °C for 24 hours. From this data, the biological efficiency (BE), percentage of dry matter of substrate converted to fresh matter of mushrooms (Stamets, 2000) and the biomass conversion rate (BCR, percentage of dry matter of substrate converted to dry matter of mushroom) were calculated. The visually discernible occurrence of bacteria or moulds in each replicate was checked and the mycelial growth (from 0 % of substrate colonized to 100 %) was estimated weekly during the first three weeks of the experiment. After the cultivation period, the replicates were dried at 105 °C for 24 hours to determine the dry weight of the spent mushroom substrate and to take samples for chemical analyses.

3.1.3.4 Chemical analyses

The nitrogen (N) and carbon (C) content of the spawn, straw, spent mushroom substrate (SMS) and mushrooms were analysed with the DUMAS-method (Naumann, Bassler, 2004). The protein content (XP) of the straw and mushroom was estimated by multiplying the nitrogen content with the factor 6.25, which

is commonly used in the analyses of feed (Müller, 2014). Carbon emissions through respiration of the mushroom mycelium are estimated by subtracting the amount of carbon found in the SMS and the mushrooms from the amount present in the straw before cultivation. The crude fibre (XF) of the straw was determined with the Weender-van Soest analysis (Naumann, Bassler, 1976). The number of analyses was focused on SMS. While only one collective sample from the straw, spawn and mushrooms was taken, a separate sample from the SMS of each replicate was analysed. To assess whether the type of straw influences protein composition of the harvested mushrooms, three more samples from mushrooms cultivated on the same straw under the same conditions were analysed a year later and are included in the results of this study. All analyses were carried out in the laboratory of the Thünen-Institute of Organic Farming.

3.1.3.5 Estimation of metabolizable energy and digestible protein

To calculate the metabolizable energy (ME), of the different straws for ruminants, the following formula was used (DLG, 1997):

$$ME\ (MJ) = 0{,}0312 * \text{digestible fat (g)} + 0{,}0136 * \text{digestible fiber (g)} + 0{,}0147 * (\text{digestible organic matter (g)} - \text{digestible fat (g)} - \text{digestible fiber (g)}) + 0{,}00234 * \text{raw protein (g)}$$

The data on the digestibility of the different fractions of the maize and wheat straw was taken from the publications of the German association for agriculture (DLG, 1997), while the data on faba bean straw was taken from the Dutch central feedstuff databank (CVB, 2024). For soybean straw, only incomplete data could be found. Information for digestibility of organic matter and of protein of soy straw was found on the feedipedia databak (feedipedia, 2024). To fill in the missing values on the digestibility of lipids and fibers in soy straw we used the data on straw from a similar legume, namely the pea, *Pisum sativum*, from the DLG (1997). The compiled digestibility data can be found in Table 1.

Table 1: Digestibility of the different macronutrient fraction in the different straw types: Digestible organic matter (DOM), digestible protein (DP), digestible lipids (DL) and digestible fibre (DF).

Straw Type	DOM (%)	DP (%)	DL (%)	DF (%)
Wheat	47	20	49	53
Faba bean	52	46	53	42
Maize	72	50	64	68
Soy bean*	52	54	55	42

3.1.3.6 Statistical analysis

For statistical analysis, Microsoft Excel and the freeware R-studio (version 4.0.3) were used. One-way analysis of variance (ANOVA) and the post-hoc Tukey's test were used to compare different treatments.

3.1.4 Results

3.1.4.1 Nutrient composition and feed value of straws

The chemical analysis (Table 2) showed large differences between the types of straw with regards to the nitrogen content, while the carbon content was almost the same. The wheat straw contained the least nitrogen and faba bean the most. The carbon/nitrogen-ration (C/N-ratio) of the straws varied from 45 to 130. The content of crude fiber content (XF) was lowest in maize straw, followed by soy, faba bean and

wheat. The mushroom spawn had a notably higher nitrogen content than the straws with a C/N-ration of 15.6.

Table 2: Chemical composition of the dry matter (DM) of different straws and mushroom spawn used for oyster mushroom cultivation.

Sample	C (% DM)	N (% DM)	C/N-ratio	XF (% DM)
Wheat straw	47.12	0.36	130.37	47.97
Faba bean straw	47.12	1.05	44.75	49.86
Maize straw	47.07	0.68	69.72	38.03
Soy bean straw	47.18	0.59	79.91	43.86
Mushroom spawn	46.33	2.96	15.63	

In combination with the data in Table 1, the digestible protein and metabolizable energy was calculated. As Table 3 shows, wheat straw had the least digestible protein, while faba bean had the most. Maize straw, according to these calculations, had the most metabolizable energy.

Table 3: Protein, digestible protein (DP) and metabolizable energy (ME) of the different straws for 1 kg dry matter and 1,4 kg dry matter (estimated daily feed intake of a goat).

	Protein	Digestible protein		ME	
Sample	g/kg DM	g/kg DM	g/1,4 kg DM	MJ/kg DM	MJ/1,4 kg DM
Wheat straw	19.4	3.9	5.4	6.3	8.8
Faba bean straw	63.0	29	40.5	7	9.8
Maize straw	38.9	19.5	27.2	9.8	13.7
Soy bean straw	33.7	18.2	27.4	7.2	10.1

3.1.4.2 Mushroom production potential

In the 56 days of the experiments, most replicates produced two mushroom harvests, with a few replicates producing only one or even three harvests. No clear pattern was discernible between treatments in terms of number of harvests. The occurrence of green mould was limited to one replicate of the wheat straw treatment and two replicates of the maize straw treatment. Since these replicates failed to produce mushrooms, they were taken out of the experiment, reducing the number of replicates in wheat straw to seven and in maize straw to six. Mycelial growth was fastest in the soy bean straw treatment, where all replicates were fully colonized after 13 days, while the replicates in other treatments were fully colonized after 21 days.

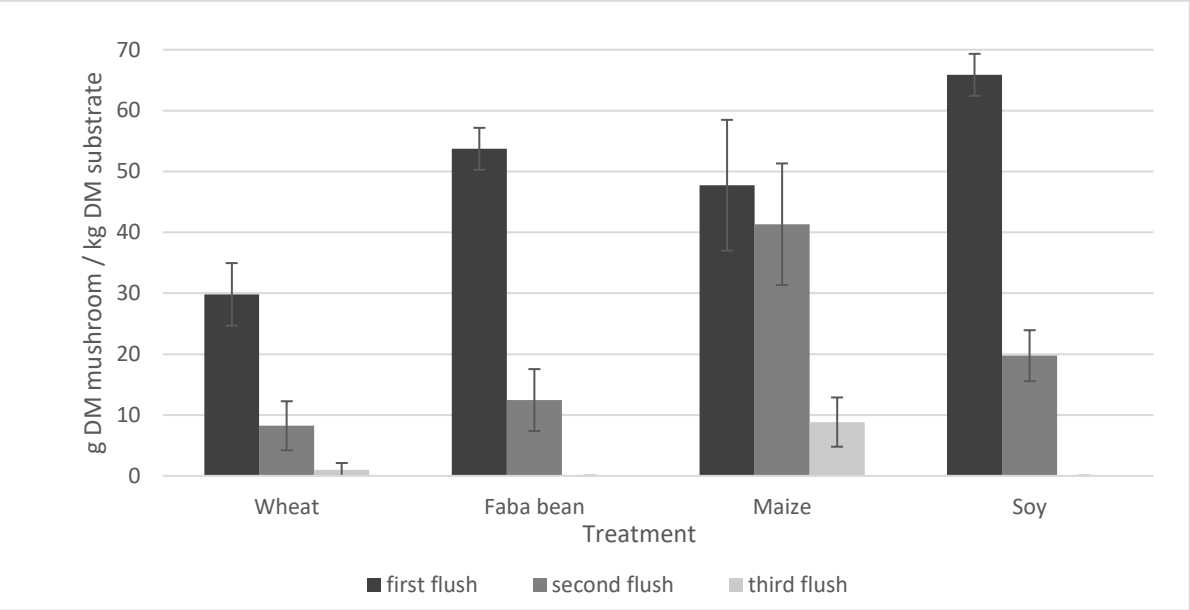
Wheat straw produced significantly less yields than all other treatments, both in terms of fresh matter (BE) and dry matter (BCR). Maize straw produced significantly more mushrooms than all other treatments in terms of fresh yield but not significantly more than soy straw in terms of dry yield (Table 4).

Table 4: Fresh and dry yield. Biological efficiency (BE) and biomass conversion rate (BCR) of the different treatments. Significant differences between treatments signified by letters. Standard deviation given in brackets behind the mean.

Treatment	Fresh matter BE (%)	Dry matter BCR (%)
Wheat straw	58^{b} (12.5)	3.8^{c} (0.8)
Faba bean straw	76^{bc} (17.3)	6.6^{b} (1.4)
Maize straw	114^{a} (10.2)	9.2^{a} (0.9)
Soy bean straw	89.1^{b} (14.7)	8.6^{a} (1.3)

While wheat, faba bean and soy bean straw produced more than 75 % of the total dry yield in the first harvest, maize straw on average produced more than 50 % of the mushrooms in the second and third harvest (Figure 3).

Figure 3: Average dry yield per dry substrate distributed over different harvest flushes in the different treatments. Error bars show standard deviation.



Source: Daniel Grimm

The composition of mushrooms from the different treatments is presented in Table 5. Mushrooms cultivated on faba bean straw contained significantly more nitrogen and thus protein than mushrooms from the other treatment.

Table 5: Chemical composition of the mushrooms from different treatments. Standard deviation given in brackets behind the mean. Significant differences between treatments signified by letters.

Treatment	C (%)	N (%)	C/N	Protein (%)
Wheat straw	44.7^{a} (1.1)	2.7^{b} (0.3)	16.8^{b}	16.8 (1.6)
Faba bean straw	45.4^{a} (1.2)	3.7^{a} (0.2)	12.2^{a}	23.2 (1.3)
Maize straw	45.1^{a} (0.8)	3^{b} (0.2)	14.9^{b}	19 (1.4)
Soy bean straw	45.6^{a} (0.6)	2.8^{b} (0.2)	16.2^{b}	17.7 (1.6)

By synthesizing the yield data and the chemical analysis, one can roughly estimate the amount of protein produced per kg of straw. On wheat straw, 6.4 g protein were produced per kg of straw, on soy bean straw 15.2 g, on faba bean straw 15.4 g and on maize straw 17.4 g.

3.1.4.3 Nitrogen and carbon flow

The change in nitrogen and carbon content of the straws after cultivation differed notably between the different treatments. While the C/N ratio in wheat, maize and soy bean straw was decreased by mushroom cultivation (Table 6). In the faba straw treatment, the C/N ratio of the SMS was slightly than that of straw before cultivation.

Table 6: Chemical composition of the spent mushroom substrate (SMS). Standard deviation of carbon and nitrogen content given in brackets after the mean. C/N change is the difference in the C/N ratio in comparison to the ratio of the straw before mushroom cultivation.

SMS from …	C (% DM)	N (% DM)	C/N	C/N ratio change
Wheat straw	45.5 (0.9)	0.4 (0)	106.0	-24.4
Faba bean straw	45.6 (0.4)	1 (0)	47.3	2.6
Maize straw	43.8 (0.4)	0.7 (0)	62.7	-7.0
Soy bean straw	43.9 (0.4)	0.6 (0)	71.7	-8.3

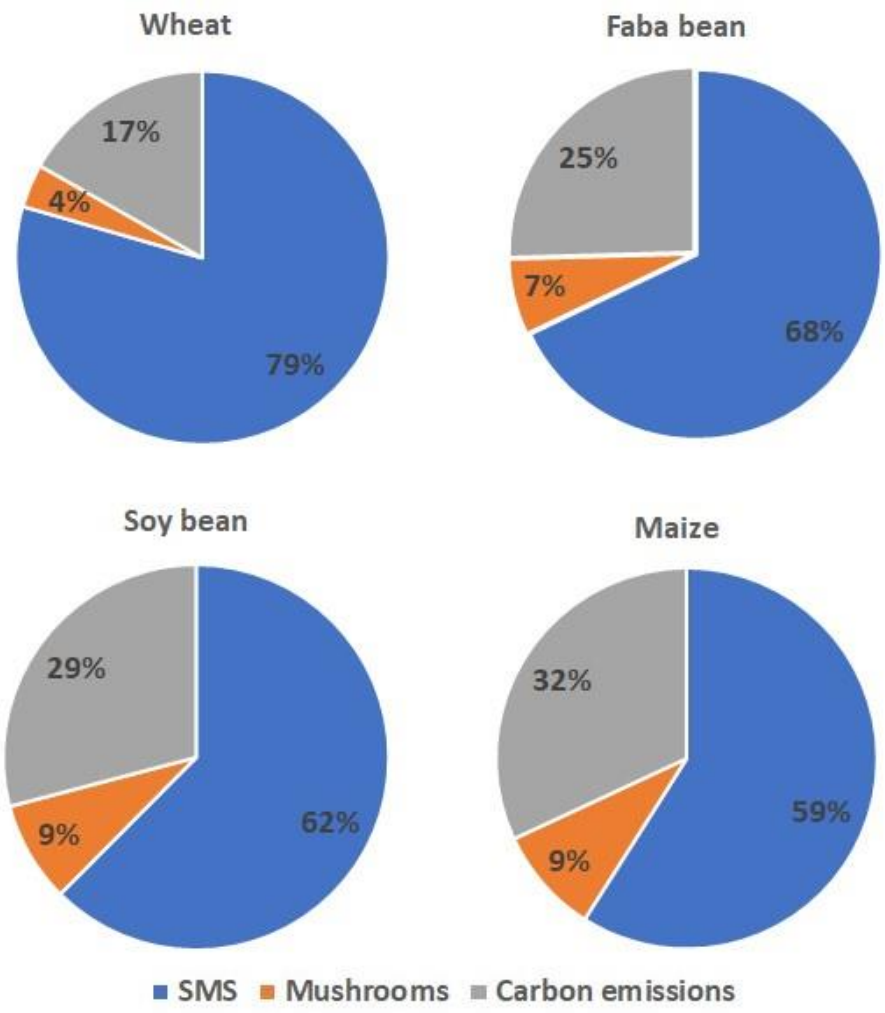
The amount of dry matter, carbon and nitrogen from the straw that remained in the spent mushroom substrate SMS after cultivation is presented in Table 7. Dry matter, carbon and nitrogen reduction was notably lower in wheat straw than in the other treatments. The strongest reduction in all these metrics occurred in the treatment with maize straw.

Table 7: Mass transfer from straw to spent mushroom substrate in the different treatments. Standard deviation given in brackets behind the mean.

Treatment	dry matter (%)	C (%)	N (%)
Wheat straw	82.2 (6.4)	79.5 (5.6)	79.3 (12.3)
Faba bean straw	70.1 (5)	67.9 (4.6)	60.5 (3.9)
Maize straw	63.4 (1.9)	59 (1.7)	59 (3.9)
Soy bean straw	67.1 (1.9)	62.4 (1.6)	61.5 (4)

By subtracting the amount of dry matter in the SMS (Table 7) and in the mushrooms (Table 5) from the amount of dry matter in each replicate at the beginning of the experiment (200 g straw + 6.6 g spawn), the unaccounted rest was calculated. This rest is the amount of carbon that was lost to respiration by the mushroom. As can be seen in Figure 4, in the wheat treatment, 17 % of the dry matter are estimated to be carbon emissions, 25 % in the faba bean treatment, 32 % in the maize treatment and 29 % in the soy treatment.

Figure 4: Dry matter transfer from the straw to different fraction (spent mushroom substrate, mushrooms and carbon emissions) during mushroom cultivation



Source: Daniel Grimm

3.1.5 Discussion

Nutrient composition and feed value of straws

None of the straws used for mushroom cultivation can be considered as good feed for goats or other ruminants, since none would provide both enough energy and protein for metabolic basic needs. Therefore, the use for mushroom production as supplementary food for human is wise, as long as the spent mushroom substrate is brought back to the soil for organic matter.

Mushroom production potential

The best performing straws in the experiment were maize and soy straw, which were almost evenly matched in terms of dry yield. There were however interesting differences between these two treatments which could be relevant to mushroom producers. On one hand, maize straw produced more fresh yield than soy straw, which may be related to differences in crude fibre contents and physical structure of the two materials. While soy straw is coarse and hard, maize straw is very soft and sponge-like and could thus better provide the mycelium with water and air, as for example Stamets (2000) discusses. Interestingly, soy produced more mushrooms than maize in the first flush, indicating that the nutrients were more accessible

to the mushroom. While maize produced more than 50 % of its total dry yield in the second and third harvest, all other straws produced more than 75 % of the mushrooms in the first harvest. From an economic and space-use efficiency standpoint, it could thus be beneficial for mushroom producers to harvest only once from all straws except from maize. Maize yields were high in comparison to other studies (Fanadzo et al., 2010; Musara et al., 2018; Obodai et al., 2003). Soy bean straw produced comparable results to another study which used different species of oyster mushrooms (Deshmukh, Deshmukh, 2016). No studies were found utilizing faba bean straw. The results in this study concerning yield from wheat straw, where in the range of other studies, with some having higher yields (Zhang et al., 2002) and some lower (Girmay et al., 2016). The low nitrogen content of the wheat straw used in this study surely contributed to the low productivity.

If we take the view of Stamets (2000) that an oyster mushroom cultivator should operate at around 100 % BE or more to be economical, the faba bean and wheat straw can be said to be un-economical if used as pure substrates. However, the validity of this view may differ on different markets and depending on the profit made through the use of spent mushroom substrate. Looking at the nutrient composition of the different straws, it is notable that faba bean straw produced relatively few mushrooms despite its high nitrogen content. This is supported by the fact that the C/N ratio increased in faba bean straw as a result of mushroom cultivation, indicating that there was more nitrogen available than necessary for the mushroom. The significantly increased protein content of mushrooms produced on faba bean straw in comparison to the other treatments is also most likely a result of this effect. This substrate may be more efficiently utilized in oyster mushrooms production if mixed with a more carbon rich material.

Mass, nitrogen and carbon flow

With a removal of 17 – 32 % of dry biomass from the different straws, the loss of mass was relatively low, though in the range of other studies (Zhang et al., 2002). Of the biomass that was not lost, between 4 % and 9 % was turned into mushroom biomass and between 59 % an 79 % were turned into SMS. This distribution is roughly equivalent, to that presented by Stamets (2000), who says that around 10 % of the biomass of a substrate is turned into mushrooms and 70 % into SMS.

Significant amounts of nitrogen were removed from the straw by the mushrooms in all treatments, ranging from 21 % in wheat to 41 % in maize. While the value of the spent mushroom substrate as fertilizer is higher than that of straw due to the decreased C/N ratio, this nevertheless means that more nitrogen would be removed from the field than if the straw was used as mulch. Therefore, the removal of straw from fields for mushroom cultivation might make it necessary for farmers to return nitrogen through application of fertilizers. It would also be possible to mix SMS with other materials like dung and compost them to solve this problem.

Carbon removal from the straw by the respiration of oyster mushroom mycelium was also significant and roughly in the same range as nitrogen. Large differences were found between the treatments in terms of the carbon that was emitted during mushroom cultivation. Wheat straw emitted the least CO_2. However, in terms of emissions per unit of mushrooms, wheat straw emitted most CO_2, with 3.5 kg per 1 kg of mushrooms, while soy straw emitted least, with 2.6 kg. In terms of greenhouse gas emissions, mushroom production on legume straw was more sustainable than on cereal straw. However, to get a complete picture, the emissions caused by pasteurizing the substrate and during the cultivation phase also have to be accounted for. Nevertheless, the carbon emissions associated to mushroom cultivation will most likely be quite low compared to other protein sources such as goat meat or other animal products (Poore, Nemecek, 2018). Further research into this topic should be conducted.

3.1.6 Conclusion

Using nutrient-poor straw from cereals and legumes for oyster mushroom cultivation is an efficient and sustainable way of producing protein-rich food on very little space. Even substrates which have almost no value as feed can be used in this way, which can increase the economic return of crop land if unused straw will be used for mushroom production. Maize stover was found to be the best straw, followed by soy straw, which also produced high yields. While wheat straw produced inferior yield, faba bean straw had mediocre yields, but produced mushrooms with a higher protein content. Since significant amounts of carbon and nitrogen are removed from the straw through mushroom production, it is necessary to increase fertilizer or compost application to the field where straw was removed, even if the spent mushroom substrate is returned as an organic amendment.

3.2 Sustainability: Evaluation of different pasteurization and sterilization methods for oyster mushroom substrates

This chapter contains the following, peer-reviewed and published research paper:

Grimm, Daniel; Sonntag, Enno; Rahmann, Gerold (2024). Evaluation of different pasteurization and sterilization methods for oyster mushroom substrates. Journal of microbiology, biotechnology and food sciences, e10428-e10428. DOI: 10.55251/jmbfs.10428

3.2.1 Abstract

Oyster mushrooms can be cultivated with great spatial efficiency, on nutrient-poor plant materials, without light and under diverse climatic conditions. Their production therefore has a great potential for improving food security, especially in impoverished and overpopulated areas. However, the pasteurization or sterilization of mushroom substrates uses a lot of energy and water. This study investigates the impact of different pasteurization and sterilization techniques on the growth and yield of oyster mushrooms, and evaluates their water and energy usage. The efficacy of heat-based methods, including hot water, hot air and pressurized steam, as well as a chemical method utilizing hydrated lime ($Ca(OH)_2$) were assessed. The results show that sterilizing mushroom substrates through autoclaving can significantly increase the dry yields, up to 50 % compared to pasteurization methods. However, pasteurization methods also achieved excellent results compared to untreated substrates, with good harvests and low pest occurrence. The mushroom water content was significantly higher in pasteurization methods where the substrate is submerged in water. In terms of fresh yield, hot water pasteurization was as good as autoclaving and significantly better than the other pasteurization methods. Hot air pasteurization has, on balance, a better water and energy efficiency than autoclave sterilization (about 75 % less energy) or hot water pasteurization (about 85 % less water). When performed at an air temperature of 75° C, which was found to be sufficient for successful mushroom cultivation, as little as 1068 kJ was needed to pasteurize one kg of dry substrate (e.g. maize straw). While hydrated lime pasteurization could use as little as 270 kJ per kg of dry substrate, it is very wasteful of water, as is hot water pasteurization and could lead to nutrient leaking. The success of mushroom cultivation, especially with hot air pasteurization, could also be influenced by the duration of substrate soaking before treatment. The study provides slight evidence, although inconclusive, for a positive effect of prolonged soaking periods on yield. Lastly, the study discusses the applicability of different disinfection techniques at varying production scales and for different mushroom species, focusing on African countries where comparably small mushroom economies are growing rapidly, but often through the use of unsustainable pasteurization technology.

3.2.2 Introduction

Grey oyster mushrooms (*Pleurotus ostreatus)* are easier to produce than most other edible mushrooms and can be cultivated on almost any agricultural plant waste (Stamets, 2000). Moreover, they are a rich source of proteins, vitamins, minerals and dietary fibre (Mattila et al., 2001; Alam et al., 2008) and a potent tool for nutrient cycling in agricultural systems (Grimm et al., 2021; Cunha Zied et al., 2020). Given the increasingly restricted availability of cropland, mushrooms, which can be cultivated without light or soil, could play an important role for guaranteeing food security in future agricultural systems (Rahmann et al., 2020). However, the environmental impact in terms of energy and water usage during the pasteurization or sterilization of mushroom substrates is considerable (Kurtzman, 2010; Dorr et al., 2021). This impact could be mitigated by implementing more efficient technologies.

Mushroom producers who have enough investment capital often choose to buy an expensive large-scale autoclave to sterilize substrates. This allows for the cultivation of less competitive mushroom species than the oyster mushroom, as virtually no living microorganism remains in a substrate after autoclaving. Mushroom farmers with less capital have to choose between different forms of pasteurization, which do

not eradicate the spores of green moulds, such as species from the genus of *Aspergillus* and *Penicillum*, and some other microbes as efficiently (Swenson et al., 2018; González et al., 2022), but usually reduce the microbe count to a sufficient degree for mushroom cultivation. Hot water pasteurization (HWP) and calcium hydroxide (also known as hydrated lime or slaked lime) pasteurization (HLP) are among the most common methods (Stamets, 2000). A less common method is hot air pasteurization (HAP), which requires an oven rather than an ordinary steel drum and is therefore more expensive than HWP and HLP, while still being cheaper than autoclaving. Also, according to Wei et al. (2020), HAP at 85 °C requires an estimated 2.75 times less energy than autoclaving (A) while producing the same mushroom yields. Since their study is limited to shiitake mushrooms on a birchwood-based substrate, which could have had a relatively low microbial load (no control treatments in the experiment to confirm that pasteurization was necessary), we chose to conduct further studies using oyster mushrooms and straw-based substrates and comparing HAP not only to A but also to HWP and HLP. Kurtzman (2010) notes that the function of pasteurization and sterilization is not only germ-reduction but also the soaking of the substrate. Since many plants have a thick cuticula, it can take several days to thoroughly soak a straw-based substrate in cold water (Kurtzman, 2010). While HWP fulfils the soaking-function very well, HAP does not. It is therefore possible that soaking the substrate for a long period before HAP would improve mushroom growth and yield and decrease pest occurrence. While it could be that microbes proliferate during soaking time, it could also be that this induces spores to germinate, which would make the microorganisms more susceptible to heat.

To investigate these issues, we carried out two experiments. First, we looked at the soaking effect and air temperature in hot air pasteurization. Secondly, we compared the different pasteurization methods and autoclaving. In both experiments we measured mycelial growth, pest occurrence, fresh yield and dry yield. Apart from looking at the success of cultivation, we also make estimates on the energy and water usage of the different germ reduction techniques and discuss their applicability in different settings.

3.2.3 Materials and methods

3.2.3.1 Substrates

All straws used in the experiment were from certified organic agriculture (EU Regulation 834/2007). Therefore, no fungicides or other chemical treatments that could potentially influence microbial growth and affect the experimental results were applied to the plants during their lifecycle. The maize, faba bean and wheat were cultivated on the land of the Thuenen Institute of Organic Agriculture in northern Germany, while the soy was cultivated at the Gladbacher Hof of the University Giessen in central Germany. After harvesting, the straws were chopped, dried in an oven at 40 °C and subsequently stored.

In the first experiment, maize straw (variety Saludo) was utilized. In the second experiment, a mixture consisting of equal parts of maize straw, faba bean straw (Tiffany variety), wheat straw (Faustus variety), and soy straw (a mix of varieties: Merlin, GL Melanie, Marquise, Aurelina, ES Favor, RGT Sphinxa, ES Comandor, Amarok and Arcadia) was employed. This diverse mixture of straws aimed to create a substrate with a broader range of microbial communities.

3.2.3.2 Model mushroom species

The grey oyster mushroom, *Pleurotus ostreatus*, was used as a model species. Grain spawn (strain number: P10001, type of grain: wheat) was acquired from Mushrooms & Equipment Shop, Münster Germany, and then used to produce more grain spawn (using wheat grain). The spawn used in the experiments was self-produced G3 (third generation) spawn from the culture that had been originally acquired.

3.2.3.3 Experimental design

The first experiment, as shown in Table 8, examined the influence of air temperature during HAP and the effect of soaking period length on mushroom growth and yield. The second experiment (Table 9) compared different germ reduction methods: hot air pasteurization (HAP), hot water pasteurization (HWP), calcium hydroxide —hydrated lime— pasteurization (HLP) and autoclaving (A) (see section 2.3. Pasteurization and Sterilization Methods).

For both experiments, a 25x50 cm PVC mushroom grow bag with a micropore filter (EgBert brand) was utilized. The bags were filled with 800 g of moist substrate (200 g dry matter and 600 g water) to which oyster mushroom spawn was added following pasteurization/sterilization (see section 2.2, Substrates). The control treatments in both experiments did not undergo pasteurization or sterilization, but the mushroom spawn was added simultaneously with the other treatments. This approach allowed for the assessment of whether successful substrate colonization by the mushroom was possible without pasteurization or sterilization. In the first experiment, water was added to the dry substrate of the various treatments either immediately before or four days prior to pasteurization (0 hours vs. 96 hours of soaking time). The replicates were then placed in an oven for three hours at temperatures of 75 °C, 85 °C, or 100 °C, excluding the control treatment replicates. After the substrate had cooled to room temperature, 20 g of fresh oyster mushroom spawn (equivalent to 5 g dry matter) was added to each replicate. The bags were sealed and transferred to the designated growing room (see section 2.4, Cultivation Conditions).

Table 8: Design of experiment 1 with different treatments of air temperature and soaking time. Each treatment with six replicates (n = 48).

Treatment name	Air Temperature	Soaking time
1 (Ctrl A)	-	0 h
2 (Ctrl B)	-	96 h
3 (75A)	75 °C	0 h
4 (85A)	85 °C	0 h
5 (100A)	100 °C	0 h
6 (75B)	75 °C	96 h
7 (85B)	85 °C	96 h
8 (100B)	100 °C	96 h

In the second experiment (Table 9), it was necessary to include two hot air treatments in order to account for differences in substrate moisture between treatments where the substrate was submerged in water (HWP and HLP) and treatments where water was added in the right amount to the substrate (HAP and A). Sterilized water was added to the HAP-heavy treatment so that it matched the 80 % moisture content of HWP and HLP, rather than the moisture of 75 % of the other treatments.

Table 9: Design of experiment 2 with different treatments of disinfection and substrate moisture. Each treatment with six replicates (n = 36).

Treatment name	Disinfection method	Substrate moisture
1 (Ctrl)	None	75 %
2 (HAP-1)	Hot air pasteurization	75 %
3 (HAP-2)	Hot air pasteurization	80 %
4 (HWP)	Hot water pasteurization	80 %
5 (HLP)	Hydrated lime ($Ca(OH)_2$) pasteurization	80 %
6 (A)	Autoclaving	75 %

3.2.3.4 Pasteurization and sterilization methods

Prior to pasteurization or sterilization, the moisture content of the substrate ingredients was determined. For the HAP+A treatments, the substrate was moistened to 75 %, as recommended by Stamets (2020) for straw-base mushroom substrates using tap water. The substrate was then filled into plastic mushroom bags and sealed with reusable zip-ties beneath the microfilter to prevent water from evaporating or entering during the pasteurization or autoclaving process. In the case of the HWP and HLP treatments, a different approach was followed since pasteurization inside plastic bags was not feasible. Instead, a self-made bag of gauze fabric was used and the substrate was submerged in water. This allowed the substrate to be fully immersed without losing smaller particles of the chopped straw. The substrate was filled into mushroom grow bags only after the pasteurizing and drenching the substrate.

For the HWP treatment, a clean oil barrel was set up with a propane-gas fire underneath. The water temperature was monitored using a thermometer placed 20 cm beneath the surface. Once the water temperature reached 63 °C, the substrate was submerged and kept inside the barrel for one and a half hours, with the temperature maintained between 63 °C and 70 °C.

In the HLP treatment, calcium hydroxide was added to the water at a rate of approximately 5 g per litre to achieve a pH of 9.5 before submerging the substrate. The substrate remained in the barrel for 8 hours. After pasteurization, the substrate of HLP and HWP was drenched overnight. To minimize spore entry during this period, the substrate was transferred to large plastic bags while still hot. To allow water to drip off, holes were punctured in the undersides of the bags, which were then hung from the ceiling using strings.

At this stage, samples were taken and the bags were weighed. The samples were dried in an oven at 105 °C overnight to determine the moisture content. The following day the bags were re-weighed to calculate the water loss overnight. This information was used to calculate the precise amount of substrate required for each mushroom bag, ensuring that each bag contained exactly 200 g of dry matter, as in all other treatments. Additionally, this approach enabled the calculation of the amount of water needed for the HAP-2 treatment, which was adjusted to achieve a moisture content of 80 % to match that of the HWP and HLP treatments.

By carefully monitoring and adjusting the moisture content of the substrate, the study aimed to ensure consistent conditions across the different germ reduction treatments.

3.2.3.5 Mushroom cultivation conditions

The mushroom cultivation period followed the parameters described in Stamets (2000) for the grey oyster mushroom, as outlined in Table 10. Once the substrate was sterilized or pasteurized and distributed into mushroom filter bags, mushroom spawn was added to the top of the substrate. The spawn was spread by shaking and twisting the bag between the hands for approximately ten seconds. In the first experiment, 20 g (fresh weight) of spawn were added to each bag, while in the second experiment only 10 g of spawn were added. The cultivation took place in growing boxes equipped with automatic humidity control within a temperature-controlled room. To ensure randomness, the replicates were rotated twice weekly. When the mushroom mycelium had fully colonized the substrates in all treatments except the control group, the bags were opened and the climate settings were adjusted to induce primordia formation. Once the mushrooms were harvested, the climate settings were reverted to those used during the initial spawn run for a period of three days. Afterward, the settings were adjusted again to promote primordia formation for subsequent flushes of mushrooms. This cycle of adjusting climate settings for fruiting and returning to spawn run conditions was followed throughout the cultivation period.

Table 10: Cultivation parameters for *Pleurotus ostreatus* according to Stamets (2000)

Spawn run (colonization phase):	**Primordia formation:**	**Fruitbody development:**
Duration: 12 – 21 days	Duration: 3 – 5 days	Duration: 4 – 7 days
Temperature: 24 °C	Temperature: 15 °C	Temperature: 20 °C
Relative humidity: 85 %	Relative humidity: 95 %	Relative humidity: 90 %

After opening the bags, yellow traps were set up, to reduce the number of flies laying eggs into the substrate – a problem that tends to increase with time. We used yellow sticky traps to mitigate this problem. In the first experiment, several flushes were harvested. In the second experiment, only the first flush was harvested due to time constraints and because the first harvest most accurately reflects the success of germ reduction, as germs can easily enter the substrate once the bags have been opened.

3.2.3.6 Data collection

Grey oyster mushrooms were harvested at the stage of fully maturity (Figure 5), following (Stamets, 2000).

Upon harvest, the fresh weight of the mushrooms was measured using a scale to determine their weight before any moisture loss occurred. Then the dry weight was determined, by drying for 24 hours in an oven at 105 °C. From the yield data, the biological efficiency and the biomass conversion rate were calculated. The biological efficiency is a commonly used expression of yield in mushroom cultivation, which gives the amount of fresh mushroom harvested per dry substrate (Stamets, 2000). According to the biological efficiency formula, a 100 % biological efficiency is achieved when one pound of fresh mushroom is harvested from one pound of dry substrate. The biomass conversion rate is a similar measure which gives the amount of dry yield as a percentage of the dry substrate. According to this formula, a 10 % conversion rate is achieved when 10 g of dry mushrooms are harvested from 100 g of dry substrate. The visually discernible occurrence of pests (e.g. bacteria, moulds and other fungi) in each mushroom bag was documented weekly during the cultivation process. Mycelial growth was regularly checked and estimated (from 0 % of substrate colonized to 100 %). The weight of the bags was measured once a week. Substrate pH was measured before and after sterilization/pasteurization, and after cultivation was completed.

Figure 5: Photo of fully mature grey oyster mushroom P. ostreatus, ready for being harvested

Source: Daniel Grimm

3.2.3.7 Chemical analysis

The straws used as mushroom substrates in the experiments were chemically analysed in the laboratory at the Thünen Institute of Organic Farming (Table 11). Most of the analyses were carried out as described in the Commission Regulation No 152/2009, Annex III (EC, 2009) and method numbers are given below. The dry matter content was determined by oven-drying at 103 °C (Annex III, A). Ash, crude fat and starch content were determined using methods M, H and L. Phosphorous content was determined photometrically (Annex III, P). The nitrogen and carbon-content were determined with the DUMAS-method (Naumann, Bassler, 2004).

Table 11: Chemical composition of the straws used for substrate formulation in the experiments.

	Straw type				
Parameter	Maize	Wheat	Faba bean	Soy	Mixture
Ash (% dm)	6.64	7.02	7.50	6.21	6.84
C (% dm)	47.07	47.12	47.12	47.18	47.12
N (% dm)	0.68	0.36	1.05	0.59	0.67
C/N ratio	69.2	130.9	44.9	80	70.3
K (% dm)	1.50	0.52	2.32	1,61	1.49
P (% dm)	0.40	0.22	0.14	0.07	0.21
ADF (% dm)	45.29	53.84	60.91	53.18	53.30
NDF (% dm)	74.82	75.74	71.24	66.65	72.11

Legend: The mixture was an equal-parts mix of the four different straws. All values are given as percentage of dry matter (% dm). C is carbon, N is nitrogen, K is potassium, P is phosphorous, ADF stands for acid detergent fibre (lignin and cellulose) and NDF stands for neutral detergent fibre (lignin, cellulose and hemicellulose).

3.2.3.8 Data analysis

For statistical analysis, Microsoft “Excel” and the freeware “R” were used. One-way analysis of variance (ANOVA) and the post-hoc Tukey’s test were used to compare different treatments.

3.2.3.9 Estimation of energy and water efficiency

The water usage of the different pasteurization and sterilization techniques was determined by considering the percentage of water in the substrate (75 %) and the water needed for transferring that heat and/or submerging the substrate. We measured that 18 kg of water were needed to submerge 1 kg of the dry substrate used in experiment 2. With a substrate that is more finely chopped, less water might be needed, but it could also lead to higher nutrient losses, which are a general disadvantage of HWP (González et al., 2022). The amount of water needed to transfer heat via steam, during autoclaving depends on the energy content of saturated steam at 2 bar atmospheric pressure, which is 2202 kJ/kg (Wei et al., 2020).

To estimate the energy usage per kg of substrate and kg of produced mushroom, we used thermodynamic equations and literature. The energy (Qt) that is needed to heat a given mass (m) from starting temperature (Ti) to final temperature (Tf) depends on the specific heat capacity (c) of the substances that are heated and can be calculated with the following formula: Qt = c *m * (Tf–Ti). The specific heat capacity of water is ca. 4.19 kJ/kg. For the straw, we assumed a specific heat capacity of 1.4 kJ/kg, as did Wei et al (2020) for their ligno-cellulosic raw materials. The specific heat capacity of the substrate (75 % water, 25 % straw) was therefore assumed to be 3.49 kJ/kg and the specific heat capacity of 18 kg of water and 1 kg of straw was assumed to be 4.04 kJ/kg. For autoclaving, we assumed that 2706 kJ/kg are needed to turn water in to saturated steam (Wei et al., 2020). Assuming a starting temperature of 10 °C, it takes 1823 kJ to heat 1 kg of the substrate to 121 °C. Therefore 828 g of steam would be needed to transfer that energy. In the HLP method, 5 g of calcium hydroxide were added per kg of water. The amount needed per kg of substrate was therefore 90 g. The amount of energy needed to produce calcium hydroxide varies from 3000 – 9000 kJ/kg, depending on such factors as the quality of raw materials and the type of fuel and kiln used for production (European Commission, 2013; Laveglia et al., 2022). For example, parallel flow regenerative kilns generally use less energy than annular shaft kilns, and larger ones are more efficient than small ones (European Commission, 2013).

3.2.4 Results and discussion

3.2.4.1 Effect of air temperature and soaking time (Experiment 1)

The duration of the experiment was 72 days. In this time, most of the pasteurized replicates produced two or three harvests and one replicate in treatment 100B produced four harvests, while replicates in non-pasteurized treatments (CtrlA and CtrlB) produced no or one harvest. The pasteurized treatments took on average 24 to 25 days until the first harvest. Those bags which produced mushrooms in the non-pasteurized treatments (5 of 6 in CtrlA and 3 of 6 in CtrlB) took on average 48 days (CtrlA) and 52 days (CtrlB) until the first harvest. Relevant harvest data is given in Table 12.

Table 12: Mean fresh yield (biological efficiency), dry yield (biomass conversion rate), water content and days until first harvest in the different treatments of experiment 1.

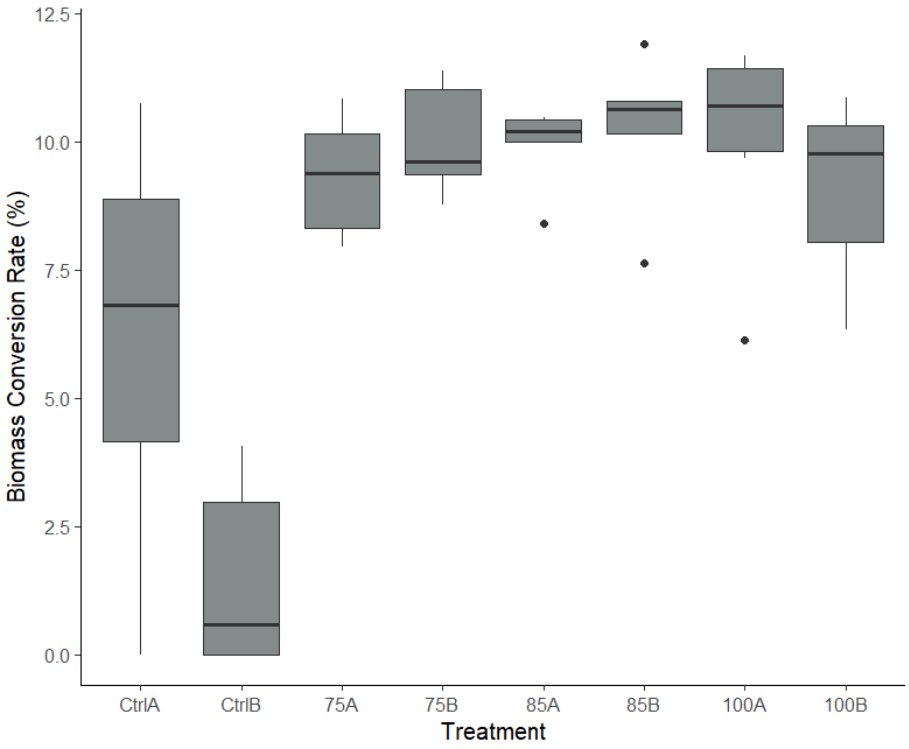
Treatment	Biological Efficiency (%)	Biomass Conversion Rate (%)	Water content (%)	Days to first harvest
CtrlA	63.6 (+/- 19.9)	6.2 (+/- 3.6)	90.2 (+/- 0.8)	47.8 (+/- 4)
CtrlB	15.4 (+/- 9.1)	1.5 (+/- 1.7)	90.4 (+/- 1.2)	52.3 (+/- 9.3)
75A	98.5 (+/- 5.7)	9.3 (+/- 1.1)	90.5 (+/- 0.5)	25 (+/- 1.5)
75B	110.9 (+/- 4)	10.5 (+/- 1.3)	90.5 (+/- 0.8)	25.3 (+/- 1.5)
85A	114.6 (+/- 5.8)	10 (+/- 0.7)	91.3 (+/- 0.4)	24.7 (+/- 2)
85B	116 (+/- 8.5)	10.6 (+/- 1.5)	90.9 (+/- 0.3)	24.3 (+/- 2.1)
100A	111.3 (+/- 9)	10.1 (+/- 1.9)	91 (+/- 0.3)	24.7 (+/- 0.9)
100B	115.1 (+/- 12)	10.9 (+/- 2.3)	90.5 (+/- 0.9)	25.2 (+/- 3.5)

Legend: The standard deviation is given in brackets behind the mean. Biological efficiency refers to the percentage of substrate dry matter converted to mushroom fresh matter. Biomass conversion rate refers to the percentage of substrate dry matter converted to mushroom dry matter.

Growth of green mould (*Aspergillus spec*.) was found in 5 out of 6 of the replicates of both CtrlA and CtrlB after two weeks and in all replicates of these treatments after three weeks. Fruit bodies of the snowy inkcap mushroom *Coprinopsis nivea* were observed in two replicates of CtrlA and four replicates of CtrlB after three weeks. This mushroom is very common on the fields of our research station, which explains its appearance in the control treatments, though it is remarkable, as it is usually associated with cow dung, which was not present in our substrates. No competitor microbes were found in any of the pasteurized treatments, except for a small occurrence of green mould on the replicate 100B1, which however did not spread or grow in size. The mycelial growth (spawn run) of the oyster mushroom was similar in all treatments except CtrlA and CtrlB, where it was much slower. After two weeks, all replicates in all treatments were fully colonized, except for the non-pasteurized treatments, where none were fully colonized. Only one replicate of the non-pasteurized treatment (replicate CtrlA1) was fully colonized during the experiment.

The total dry yields that were obtained from the different treatments during the experiment are shown in Figure 6.

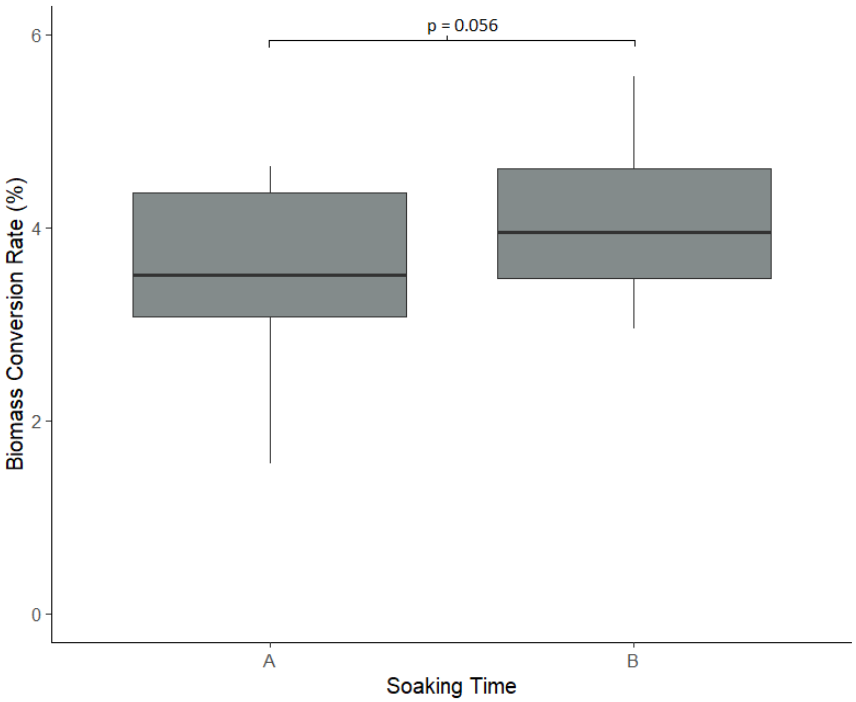
Figure 6: Total dry yield, given as the biomass conversion rate (the percentage of substrate dry matter converted to mushroom dry matter), per different treatments of air temperature and soaking time (Experiment 1). N = 48 (8 treatments with six replicates each).



Source: Daniel Grimm

The non-parametric Kruskal Wallis test revealed a significant difference between treatments in terms of dry yield ($p = 0.001$). The post-hoc Dunn's test revealed that this difference was only between the non-pasteurized and pasteurized treatments, with the former (CtrlA and CtrlB) producing significantly less mushrooms than the latter. Also, CtrlA produced significantly more mushrooms than CtrlB. When removing these two treatments from the data pool and only looking at the pasteurized treatments, it was possible to perform parametric tests since the assumptions of normal distribution of data and variation were met. ANOVA revealed no significant difference in terms of total dry ($p = 0.7$) and fresh yield ($p = 0.54$) between the pasteurized treatments. Likewise, no statistical significance was found for the factors temperature ($p = 0.45$) and soaking ($p = 0.12$). When looking only at the first harvest, no difference between pasteurized treatments was found either but, as shown in Figure 7, a trend ($p = 0.056$) toward higher dry yields in treatments soaked for 96 hours than in treatments soaked directly before pasteurization could be detected.

Figure 7: First harvest dry yield, given as the biomass conversion rate (the percentage of substrate dry matter converted to mushroom dry matter), in treatments soaked directly before (A) or 96 hours before hot air pasteurization (B) in experiment 1. N = 36 (18 replicates in group A and 18 in group B).



Source: Daniel Grimm

The first experiment confirmed that hot air pasteurization is an efficient form of disinfecting mushroom substrates. While non-pasteurized replicates often failed to produce any mushrooms or took twice as long until the first harvest, the pasteurized replicates produced very good yields in a short time, with a biological efficiency of more than 110 % in most treatments and biomass conversion rates of more than 10 %. This compares well to the oyster mushroom yields reported by González et al. (2022) using various pasteurization techniques on straw and wood-based substrates. In an experiment where maize straw was used, like in the one we conducted, a lower biological efficiency of only 97 % was reported after using the hot water bath method (Fanadzo et al., 2010). This result agrees with Stamets (2000) that a “good grower” should operate in the range of 75 % -125 % range of biological efficiency.

We did not find statistically significant differences between the different temperatures, so that 75 °C might be as good as 100 °C, although when looking at Figure 6 there seems to a small trend towards higher yields at higher temperatures. In an experiment with larger sample sizes, statistically significant differences might be found, but this remains unclear.

Interesting results were found regarding the effect of soaking time. As was expected, in the control treatments (CtrlA and CtrlB), a longer soaking time led to smaller yields because the microbiota in the substrate was able to grow before the mushroom spawn was added, which is a competitive disadvantage for the mushroom. But looking at the pasteurized treatments, the effect was reversed, with a trend towards higher first harvest yields in the treatments that where soaked for a longer time. Since this effect was just barely below statistical significance, more experiments, with larger sample sizes should be conducted. Also, the difference between treatments with different soaking times became smaller when looking at total yields, rather than just the first harvest. This could be explained by the fact that the mushroom faces less microbial competition once it has colonized the entire substrate. The first harvest, which occurs shortly after full colonization, is thus more likely to be affected by microbial competition than the second harvest, which occurs several weeks after the mushroom has “taken control” of the entire substrate. However,

whether the observed beneficial effect of soaking on the first harvest is due to better moisture distribution, as Kurtzman (2010) describes, or due to a better elimination of microorganisms, remains unclear.

3.2.4.2 Effect of different techniques of pasteurization and sterilization (Experiment 2).

The duration of the experiment was 45 days. When it ended, only the autoclaved mushroom bags had produced two harvests, while all pasteurized treatments had produced just one harvest and the non-pasteurized control treatment had produced no harvest because the oyster mushroom had failed to colonize the substrate. The occurrence of pests was higher in the control treatment, with green mould (*Apergillus spec*.) and other species such as *Coprinopsis nivea* and slime moulds, occurring in all replicates. In the other treatments, no pests were observed, except for the HLP treatment where a slime mould was found in one replicate and green mould in another one, although both pest occurrences remained small and contained. Relevant harvest data is depicted in Table 13.

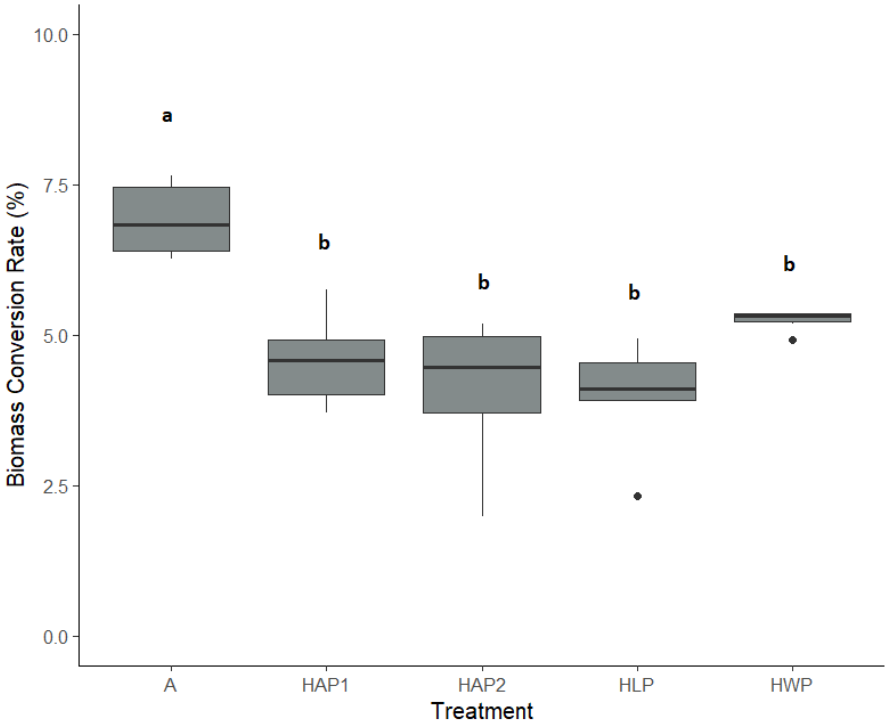
Table 13: First harvest fresh yield (biological efficiency), dry yield (biomass conversion rate), water content and days until first harvest in the different treatments of experiment 2.

Treatment	Biological Efficiency (%)	Biomass Conversion Rate (%)	Mushroom Water content (%)	Days to first harvest
A	66.8 (+/- 4.5) a	6.9 (+/- 0.6) a	89.6 (+/- 0.9) b	23.3 (+/- 0.5) a
HAP1	45.4 (+/- 3.9) b	4.6 (+/- 0.7) b	89.9 (+/- 1.4) b	27.5 (+/-2.3) b
HAP2	43.9 (+/- 11.1) b	4.1 (+/- 1.1) b	90.6 (+/- 0.8) b	28.8 (+/-4.2) b
HLP	52 (+/- 11.4) b	4 (+/- 0.8) b	92.3 (+/- 0.3) a	28.2 (+/- 1.1) b
HWP	75.7 (+/- 4.3) a	5.2 (+/- 0.2) b	93.1 (+/-0.3) a	26.8 (+/-0.9) ab

Legend: The standard deviation is given in brackets behind the mean. The letters a and b display significant differences between the treatments found with Tukey's test. Biological efficiency refers to the percentage of substrate dry matter converted to mushroom fresh matter. Biomass conversion rate refers to the percentage of substrate dry matter converted to mushroom dry matter.

The autoclaved replicates (treatment A) colonized the substrate faster and produced significantly more mushroom dry yield in the first harvest in significantly less time than all other treatments except HWP ($p < 0.05$), as ANOVA and Tukey's test revealed. The average first harvest dry yield of the sterilized treatment (A) was 54 % higher than the combined average of the pasteurized treatments. This difference is visible in Figure 8. No significant differences were detected in terms of dry yield or time to harvest between other treatments, though there was a trend towards higher dry yields in HWP than in HLP ($p = 0.09$).

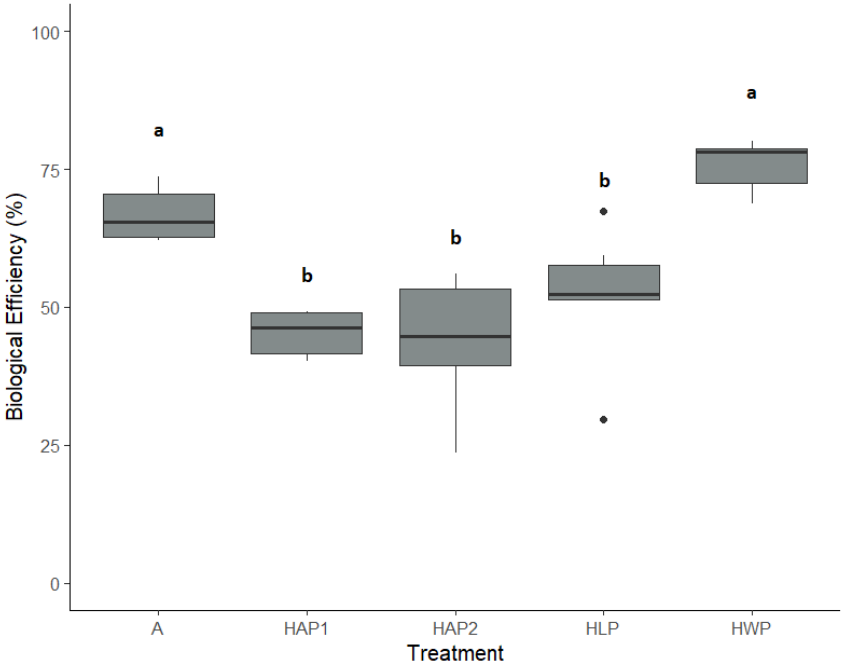
Figure 8: First harvest dry yield of experiment 2, given as the biomass conversion rate (the percentage of substrate dry matter converted to mushroom dry matter). The letters above the boxplots display significant differences found with Tukey's test. N = 30 (five treatments with six replicates each. Ctrl treatment not included as it produced no yields).



Source: Daniel Grimm

The picture was different when looking at the fresh yields, where the biological efficiency of the HWP treatment was the same as of the A treatment and significantly higher than the other treatments. This can be seen in Figure 9. Also, as can be seen in Table 13, the mushrooms in HWP and HLP contained significantly more water than those of the other treatments.

Figure 9: First harvest fresh yield of experiment 2, given as the biological efficiency (the percentage of substrate dry matter converted to mushroom fresh matter). The letters above the boxplots display significant differences found with Tukey's test. N = 30 (five treatments with six replicates each. Ctrl treatment not included as it produced no yields).



Source: Daniel Grimm

The second experiment revealed significant differences in mycelial growth and in yields between autoclaving and different pasteurization techniques. The autoclaved treatment produced significantly higher dry yields than the other treatments, as reflected by the biomass conversion rate. The results therefore contradict the findings of Wei et al. (2020), where hot air pasteurization yielded the same results as autoclaving. This could be because we used a substrate with a higher microbial load than they did – we deliberately mixed four different types of straw to get a broad spectrum of microorganism, while they used sawdust from birchwood. Another contributing factor could have been that we used a very low spawn rate. If a higher spawn rate had been used, the difference might have been less dramatic. A less likely explanation for the different findings is that we used a different mushroom species, since the oyster mushroom is more competitive and resilient than shiitake, which Wei et al. (2020) cultivated. In our experiment, autoclaved replicates also manifested significantly faster mycelial growth and needed less time until the first and second harvest than pasteurized replicates. Only the HWP treatment was comparable to autoclaving in terms of biological efficiency. This was because the mushroom water content of HWP was significantly higher (93.1 % vs. 89.6 %). HLP also had a higher water content than the other treatments. This suggests that methods where the substrate is submerged lead to higher mushroom water content. Interestingly, the HAP2 treatment, where the substrate water content was adjusted to that of HWP and HLP, had a significantly lower water content than those two treatments. This suggests that the water which we added after pasteurization, did not soak into the substrate and was less available to the mushroom. While the biological efficiency might be economically interesting for farmers, since mushroom producers mostly sell fresh rather than dry mushrooms, it plays less of a role in terms of food security and nutrition. For this reason, researchers should not only focus on biological efficiency, but also the biomass conversion rate. In terms of this measure, the experiment clearly showed that autoclaving was better than pasteurization, while the three different pasteurization techniques that we investigated were equally good. This also counters the hypothesis that pasteurization could have an advantage over sterilization by letting "beneficial" microbes survive, which is sometimes made (Kurtzman, 2010; González et al., 2022). While we

are aware of the existence of beneficial bacteria in button mushroom production (Cochet et al., 1992), we have not seen convincing evidence for the existence of beneficial bacteria in oyster mushroom cultivation.

3.2.4.3 Energy and water efficiency of different methods

The amount of energy and water needed for the different pasteurization and sterilization methods are presented in Table 14. The energy and water usage per kg of produced mushrooms takes into account the mushrooms produced in the different treatments in experiment 2, where only the first harvest was taken into account. HLP uses the least energy of all methods, even though the range of energy use is quite large due to differences in the production process of calcium hydroxide (European Commission, 2013). HAP uses the second least energy, even when using the most energy intensive treatment. Autoclaving is slightly less energy intensive than HWP, which is the most energy intensive treatment. In terms of water use, HWP and HLP use the most water. HAP has the lowest water footprint of all treatments.

Table 14: Estimations of energy and water efficiency of different Pasteurization and Sterilization techniques used in the experiments

Method	Energy usage per kg of substrate (dm)	Energy per kg of mushroom (dm)	Water usage per kg substrate (dm)
Hot Air Pasteurization			
100 °C	1478 kJ	32220 kJ	3 kg
85 °C	1232 kJ	26858 kJ	3 kg
75 °C	1068 kJ	23282 kJ	3 kg
Autoclaving	4050 kJ	58523 kJ	4 kg
Hot Water Pasteurization	4148 kJ	78812 kJ	18 kg
Hydrated Lime ($Ca(OH)_2$) Pasteurization	270 - 810 kJ	6750 – 20250 kJ	18 kg

In terms of environmental impact (energy and water usage), those pasteurization and sterilization methods which produced the highest yields in our experiments are not the most sustainable ones. Especially hot water pasteurization has a very high energy and water usage. Though the water usage could be reduced by using a substrate that takes up less volume, e.g. by chopping it more finely, this could also lead to increased leaching of nutrients from the substrate. Autoclaving needs only marginally more water than hot air pasteurization and much less than hot water or hydrated lime pasteurization. But since autoclaves are very expensive, they are not a solution for many farmers, especially in developing nations. Hot air pasteurization has, on balance, a better water and energy efficiency than autoclave sterilization (about 75 % less energy) or hot water pasteurization (about 85 % less water). When performed at an air temperature of 75° C, which was found to be sufficient for successful mushroom cultivation, as little as 1068 kJ was needed to pasteurize one kg of dry substrate (e.g. maize straw). While hydrated lime pasteurization could use as little as 270 kJ per kg of dry substrate, it is uses as much water as hot water pasteurization and could have the same problems of nutrient leaking. In addition, water that is mixed with hydrated lime ($Ca(OH)_2$) should not be released into the environment in large amount, especially in urban areas, as it could lead to water pollution (Laveglia et al., 2022).

Oyster mushroom cultivation has tremendous potential for sustainable food production. Especially densely populated regions of the world which have too little available farm land to achieve self-sufficiency, could profit greatly from increasing mushroom production (Grimm et al., 2021). This is especially the case in many African countries, where the mushroom economy is still very small compared to Europe, America and above all Asia (Royse et al., 2017). But if unsustainable pasteurization methods are used, a scale-up of production could also have very negative environmental consequences. Since oyster mushroom production in Africa is

often carried out by small-scale farmers (Atikpo et al., 2008; Fanadzo et al., 2010), simple methods such as hot water pasteurization or similar scalding techniques are the most common. During a trip to Uganda, we interviewed several women in and around Kampala, who produced oyster mushrooms. Four out of five of them used a variety of the hot water method, as shown in

Source: Daniel Grimm

, with wood as a fuel source. Only one of them performed hot air pasteurization, utilizing the same oven in which she prepares meals for her family (Figure 11). While the oven is also heated with firewood, it is built to use the heat more efficiently.

Figure 10: Photo of a mushroom farmer in Uganda using firewood for pasteurization of substrates.

Source: Daniel Grimm

Figure 11: Photo of an oven used for meal cooking and for hot air pasteurization of mushroom substrate in Uganda. Firewood is used to heat the metal drum, which is embedded in a clay structure.

Source: Daniel Grimm

While countries such as Uganda could greatly profit from an upscaling of mushroom production, since protein-rich food is much needed and agricultural residues are underutilised, it would be unsustainable to do so without providing the mushroom farmers with the means for water- and energy-efficient pasteurization methods. The sustainable development goals by the United Nations (UN DESA, 2023) provide a useful framework for what to focus on. While the goals number 1 (no poverty) and 2 (no hunger) would be positively impacted by increased mushroom production, one needs to be more careful to be in full concordance with the goals 6 (clean water and sanitation), 12 (responsible production and consumption), 13 (climate action) and 15 (life on land). Choosing which pasteurization or sterilization techniques to use for mushroom production touches on all of these different areas. In our view, to maximize sustainability, electrical devises should be used so that solar energy could be used for substrate disinfection, whether by hot air pasteurization (which would be most sustainable) or autoclaving (which would produce the highest yields). Autoclaving would require more investment capital, but would also enable the cultivation of less competitive mushrooms than the grey oyster mushroom. For oyster mushroom cultivation however, hot air pasteurization would probably be the best choice.

3.2.5 Conclusion

Hot air pasteurization is a sustainable method of substrate pasteurization for oyster mushroom production. It uses less water and energy than most other methods of substrate pasteurization while producing the same amount of dry yield. Soaking the substrate for several days before hot air pasteurization could increase yields. Sterilization by means of autoclaving, while requiring about four times as much energy as hot air pasteurization, can lead to more than 50 % better dry yields in the first harvest and to faster mycelial growth.

3.3 Adaptability: A case study of oyster mushroom cultivation on maize stover: Potentials and Challenges for reducing food insecurity in Uganda

3.3.1 Abstract

This case study investigates the potential of utilizing maize stover for oyster mushroom (*Pleurotus ostreatus*) cultivation in Uganda, applying an interdisciplinary approach which combines field work, a laboratory experiment and key informant interviews. Seasonal variations in maize grain and stover yields were observed, with higher yields in the second harvest season compared to the first. Maize grain yields averaged 2.62 t DM/ha in the second season and 1.63 t DM/ha in the first season. Similarly, maize stover yields were higher in the second season, averaging 2.94 t DM/ha compared to 1.74 t DM/ha in the first season. Oyster mushroom yields on the stover were high in comparison to similar studies, with an average of 12.1 g of dry mushrooms harvested per 100 g of dry stover, corresponding to a mean biological efficiency of 139.7 %. Based on these findings, around 0.56 t DM of oyster mushrooms could be produced annually per hectare of a maize field by utilizing the stover. Realizing this potential at scale would make a significant contribution to Ugandan food security, especially with regard to protein supply. Mushroom farmers, which currently mostly use cotton seed hulls as a substrate, reported a high motivation for using maize stover instead to reduce costs. Our results show that maize stover has potential to produce higher yields per kg of substrate than cotton seed hulls. However, milling and transporting stover from rural farming areas to urban centers, where most mushrooms are cultivated and sold, is a logistical challenge. Also, upscaling mushroom production could have negative environmental consequences unless more sustainable pasteurization methods are used to prepare the substrate. Better access to affordable, high-quality mushroom spawn was identified as a prerequisite for increasing mushroom production in Uganda. The study also discusses socioeconomic and gender considerations for ensuring equitable development of the mushroom industry and points out opportunities for government and private sector action.

3.3.2 Introduction

The majority of population growth until the year 2100 is predicted to take place in Africa. Today, approximately 1.4 billion people live in Africa and the population is expected to at least double until the end of the century (United Nations, 2022b). In consequence, the area of farmland available per person will be drastically reduced and food security increasingly threatened (Rahmann et al., 2020). Climate change and environmental degradation will further jeopardize agricultural productivity in Africa (Thompson et al., 2010), which is often low in small-holder systems prevalent across the continent (Jayne et al., 2022). Imports of fertilizers or staple crops are not a viable strategy to offset these challenges for most African countries due to lack of financial resources and increasing unreliability of global supply chains (Cooper et al., 2011; Cordell, White, 2011; van Kauwenbergh, 2010). A key challenge for these countries in the 21st century is therefore to sustainably increase local production of healthy food for their populations without expanding the agricultural frontier.

Circular food systems offer a promising solution to the challenge of increasing food production while alleviating pressure on limited resources, especially farmland (Rahmann et al., 2020). One avenue within these systems involves the farming of detrivorous organisms, such as edible mushrooms, earthworms or insects. This approach contributes to circularity by converting organic residual streams into protein-rich food or feed and high-quality organic amendments such as composts or fertilizers (Sonntag et al., 2023, Grimm et al., 2021). Notably, these systems require no additional farmland, rendering them essentially landless. Moreover, detrivorous organisms can be cultivated in controlled environments, potentially bolstering food system resilience in the face of increasingly erratic weather conditions.

Uganda, a landlocked country facing land scarcity, could benefit from adopting circular, landless food systems to improve food security. Around 80 % of Ugandan households are involved in farming activities

(Uganda Bureau of Statistics, 2019) and cultivate maize, beans, sweet potatoes, cassava and plantain as major staple crops (Uganda Bureau of Statistics, 2020). Around 30 % of Ugandans live in absolute poverty (World Bank, 2022) and the country is struggling to adjust to a rapidly increasing population. While the country had roughly 5 million inhabitants in 1950, it now has more than 46 million and is projected to reach 132 million in 2100, according to medium estimates (United Nations, 2022a). Despite this population pressure, the area used for crop production and other agricultural activities in Uganda has not increased in the last ten years (FAOSTAT, 2024). If land-use distribution in Uganda does not change and the population grows as predicted by medium estimates, the cropland available per person will decrease from currently 2260 m^2 per person to 1240 m^2 in the year 2050 and 830 m^2 in 2100. This makes additional landless food production from crop residues an interesting option. Maize farming is the most widely available source of crop residues in Uganda, where it is grown by 48 – 69 % of farming households (Uganda Bureau of Statistics, 2020). Maize stover is currently used in various ways, including for mulching, fodder, construction, selling, and cooking, but large amounts are also burned in the field (Lwasa et al., 2023; Roobroeck et al., 2019; Duncan et al., 2016; Swidiq et al., 2012). Another potentially superior choice from a nutritional and economic standpoint, is the use of maize stover for mushroom production.

Oyster mushrooms (*Pleurotus spec.*) grow vigorously on lignocellulose-rich substrates such as maize stover (Fanadzo et al., 2010) and can be cultivated with a low-tech approach that involves milling, pasteurization, inoculation with mycelium and packing into air-permeable plastic bags or other containers (Stamets, 2000). Within three weeks, oyster mushrooms can be harvested for the first of up to five times. The spent mushroom substrate (SMS) can be used as an organic soil amendment or for other activities such as vermicomposting (Grimm et al., 2021). Oyster mushrooms are rich in protein, vitamins and minerals (Mattila et al., 2001; Mattila et al., 2002) and could help to counteract malnutrition (Fernandes et al., 2021). Traditional appreciation of wild mushrooms in Uganda diets (Nabubuya et al., 2010) likely contributed to the eager adoption of oyster mushroom cultivation technology since it was introduced in 1989 (Mayanja, Tipi, 2018). While oyster mushrooms account for most of the mushroom market in Uganda, production is still limited to small-scale growers and faces several challenges. Measures that promote upscaling of the oyster mushroom sector could benefit the local economy and help to improve nutrition and land-use efficiency. However, such an intervention should be informed by a detailed understanding of the challenges currently facing the Ugandan oyster mushroom value chain.

The aim of this study is to assess potentials and challenges of using maize stover for oyster mushroom production for reducing food insecurity in Uganda. Our research addresses three objectives: a) to quantify the seasonally available amount of maize stover per hectare in Uganda, b) to determine seasonal oyster mushroom production per hectare on Ugandan maize stover, and c) to describe the oyster mushroom value chain and identify potential challenges for upscaling production in Uganda. To address these objectives, we chose an inter-disciplinary approach combining field measurements, laboratory cultivation of oyster mushrooms and key informant interviews.

3.3.3 Materials and methods

We used a combination of qualitative and quantitative research methods to address the three research objectives in the present study. First, we conducted fieldwork to quantify the amount of annually available maize stover per hectare, collect samples of this material and interview corresponding farmers. Second, we cultivated oyster mushrooms on the collected maize stover to assess their potential for mushroom production. Finally, we conducted interviews to investigate the Ugandan oyster mushroom value chain and identify potential bottle necks and pitfalls for upscaling.

3.3.3.1 Fieldwork

We conducted fieldwork at Strategic Farm Kabaskende, situated near Kibende in Kibaale district, Uganda. Aims of the fieldwork were to quantify maize grain and stover yields per hectare and season, take stover samples for subsequent oyster mushroom cultivation, and interview farmers about their crop management. The study area is exposed to a bimodal rainfall pattern resulting in two harvest seasons per year. We visited the farm twice and sampled during both harvest seasons in August 2022 and January 2023 to represent annual production. Access to the various fields and farmers was facilitated by a personal contact with the landowner. In August 2022, we also conducted semi-structured interviews with the farmers for additional information to help us interpret our findings. Questions covered family demographics, education, planting time, seed selection, input use and crop management practices. It was not possible to conduct farmer interviews in January 2023 due to the absence of our contact person.

3.3.3.2 Sampling

We selected three fields for sampling of whole maize plants during a transect walk across the farm in company of the landowner and local extension officer. Three maize fields were selected to represent high, medium and low yields across the farm, based on visual impression which was later confirmed by grain yields. GPS coordinates of field boundaries were recorded, to allow returning to the same field. However, maize was only grown in both seasons in field one. For fields two and three we sampled adjacent fields four and five in the second season. Samples were taken at three sites along a diagonal crossing each field. At each site we counted the maize plants within a 25 m^2 circle using a tape measure to delineate a 2.82 m radius. 20 of these maize plants were randomly selected and cut off at 5 cm above the soil line. Sampled maize plants were ready for harvest and almost completely dry at the time of sampling.

3.3.3.3 Sample preparation and analysis

We recorded the number of cobs in each sample of 20 maize plants and manually separated the grain from the cob. Maize grain (G) and stover (S), including stems, leaves, empty cob, ear wings, silks and tassels, were weighed to determine fresh matter (FM). The samples were then shredded using meat cutter (SM 65 STL, K+G Wetter GmbH), dried in a ventilated oven (CD150/75/150-15/S, Caldatrac Industrieofenbau GmbH & Co. KG) at 40 °C for 5 days and weighed again to determine dry matter (DM). Representative sub-samples of dry stover were taken for laboratory analyses. Nitrogen and carbon-contents were determined with the DUMAS-method (Naumann, Bassler, 2004). Protein content was determined by multiplying the nitrogen content with the commonly used factor 6.25 (Müller, 2014). Ash content was determined using a muffle furnace at 525 °C. Neutral detergent fibre (NDF), which includes hemiclulose, cellulose and lignin, and acid detergent fibre (ADF), which includes cellulose and lignin, were determined by Weender analysis (Naumann, Bassler, 1976).

3.3.3.4 Mushroom cultivation experiment

Grey oyster mushrooms (*Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm.) were cultivated on the Ugandan maize stover in a laboratory in Germany. A bulk sample was prepared for each field and season (n = 6), milled to < 4 mm using a granulator (Retsch SM 2000 Hochleistungs-Schneidmühle) and adjusted to 75 % moisture content. Six mushroom grow bags (25 x 50 cm PVC EgBert brand) with micropore filters per treatment were filled with 1000 g of moist maize stover (250 g DM). The bags were sterilized in an autoclave at 121 °C and 15 psi, and 25 g of fresh oyster mushroom spawn (10.67 g DM) was added to each bag after cooling to room temperature.

The mushrooms were cultivated in grow boxes for 113 days (February – June 2023) under controlled conditions. Relative humidity was automatically regulated by a sensor (Inkbird IHC-200) inside a grow box

(HOMEbox Vista Medium) and temperature in the room was controlled using an oil radiator (Klarstein Thermaxx 2500). The parameters recommended for oyster mushroom cultivation at different stages (Table 15) were followed until after the first harvest. Afterwards, high variability in developmental stages between treatments would have necessitated two growing environments, one for fruiting and one for primordia formation. Since only one controlled environment was available, growing conditions were optimized for fruitbody development (20 °C, 90 % relative humidity).

Table 15: Parameters for the cultivation of oyster mushrooms as proposed by Stamets (2000).

Colonization phase:	**Primordia formation:**	**Fruitbody development:**
Duration: 12 – 21 days	Duration: 3 – 5 days	Duration: 4 – 7 days
Temperature: 24 °C	Temperature: 15 °C	Temperature: 20 °C
Relative humidity: 85 %	Relative humidity: 95 %	Relative humidity: 90 %

After 21 days primordia formation was induced (see Table 15) as the replicates were all at least 80 % colonized (mean: 95.5 % colonized). The first harvest occurred on average on day 32.

When adjusting the climate settings for primordia formation for the first harvest, the mushroom bags were opened and after harvest they were closed again. For the harvests afterwards, the bags were regularly mustered (at least twice a week) and re-opened if new primordia were visible. This was done to minimize evaporation losses. Mushroom bags were examined weekly to record weight, colonization by the oyster mushroom mycelium and the occurrence of green moulds, both of which were visually estimated in percentage of the total substrate surface.

Mushrooms were harvested when fully mature and the fresh weight of the mushrooms and the mushroom bag with the remaining substrate were noted. Afterwards the mushrooms were oven dried at 40 °C for five days to determine mushroom dry weight (MDM). The biomass conversion rate (BCR) was calculated from stover (SDM) as follows:

$$BCR = \frac{M_{DM}}{S_{DM}}$$

$$S_{DW} = CR_{DM} + MS_{DM}$$

The biological efficiency (BE), a commonly used expression of yield in mushroom cultivation (Stamets, 2000), was calculated as follows:

$$BE = \frac{M_{FM}}{S_{DM}}$$

A bulk sample of mushrooms from each harvest was analysed for nitrogen content (DUMAS).

3.3.3.5 Calculations and statistical analyses

Grain and stover yields per hectare were calculated by multiplying the yield per 25 m^2 sampling point by 400. Seasonal means were calculated for nine data points, three in three fields each. Mushroom yield per hectare was then calculated by multiplying mean stover yield per field and season with the mushroom harvest-specific BCR per mushroom cultivation bag (n = 6) and summing up over the number of harvests per bag. Mushroom protein content per bag and harvest was calculated by multiplying the treatment-specific mushroom nitrogen content per mushroom harvest with the nitrogen to protein conversion ratio of 6.25 and dividing by 100. These values were then multiplied with mushroom yield per cultivation bag and mushroom harvest, and summed up to calculate mushroom protein yield per hectare.

To provide a reference for our results, we show minimum, maximum and mean maize yield per season based on data from the Annual Agricultural Survey 2019 Report (Uganda Bureau of Statistics, 2020). From these values, national stover yield range and means per season were calculated by dividing by the average African HI of 0.38 (Ludemann et al., 2022) and subtracting maize yield. These values were multiplied with minimum, maximum and mean BCR from our mushroom cultivation experiment to calculate national mushroom yield potential range and means per season. Similarly, these values were multiplied with minimum, maximum and mean protein yields to calculate national mushroom protein yield potential range and means per season.

Statistical analyses were performed using R and R-studio version 4.0.3. One-way analysis of variance (ANOVA) and the post-hoc Tukey's test were used to compare different treatments (e.g. grain and stover yield on different fields and seasons or mushroom yields from these different charges of stover).

3.3.3.6 Interviews

We conducted semi-structured interviews with five mushroom growers and three key informants in Kampala and its surrounding urban areas. The goal was to understand production techniques and identify challenges currently facing the oyster mushroom value chain in Uganda. Bullet points and audio recordings were taken during the interviews and later transcribed verbatim. The interview was structured along the following questions:

- What is the mushroom culture and market in Uganda like and how has it developed?
- What is the history of your own mushroom business?
- How do you produce mushrooms/spawn?
- Where do you get your substrates and what do you do with spent mushroom substrate?
- How do you see the future of mushroom production in Uganda?

Where possible, we also tried to get precise numbers on the costs and profits of production, as well as the amounts of raw materials used. All interviews except for one were mediated by a personal contact who works as a spawn producer in Kampala. The interviews took between fifteen minutes and an hour and were conducted in English. During our interviews with mushroom farmers, we visited their mushroom production facilities, which allowed us to observe, photograph and ask specific questions.

3.3.4 Results

3.3.4.1 Seasonal maize grain and stover yields

We observed that maize grain yields at the end of the second rainy season (Jan. 2023) were significantly higher than at the end of the first season (Aug. 2022), with means of 2.62 t DM/ha and 1.63 t DM/ha, respectively (Figure 12). This reflects the general pattern of seasonal maize yield differences on the national level in Uganda (Uganda Bureau of Statistics, 2020). All collected datapoints were within the national yield range, except for two samples taken in field three in August 2022, which were higher. Seasonal means of our field study exceeded national means by 16.5 % and 59.7 % in the first and second season, respectively. Variation within fields was greater in the second rainy season, resulting in three outliers. This may be linked to topographic heterogeneity at field level. Variation between fields was best explained by differing crop management reported by the farmers, particularly replanting of hybrid seeds, application of nitrogen fertilizer and timing of planting and weeding.

Maize stover yields in the second rainy season also exceeded yields in the first season, with means of 2.94 t DM/ha and 1.74 t DM/ha respectively. This reflects the pattern found in grain yields, although with a more pronounced difference between seasons. Mean stover yield was lower than national mean in the first

season and greater in the second. Variation within and between fields was greater in the second season, reflecting grain yields. The average harvest index (HI) of 0.47 was relatively high compared to African average HI of 0.38 (Ludemann et al., 2022).

Overall, chemical composition of maize stover varied little between fields and seasons, with the exception of nitrogen content in the first season (Aug. 2022) being more variable compared to the second season, with an exceptionally low nitrogen content in field one (

Table 16). In August 2022, stover nitrogen content was notably lower and higher than seasonal average in

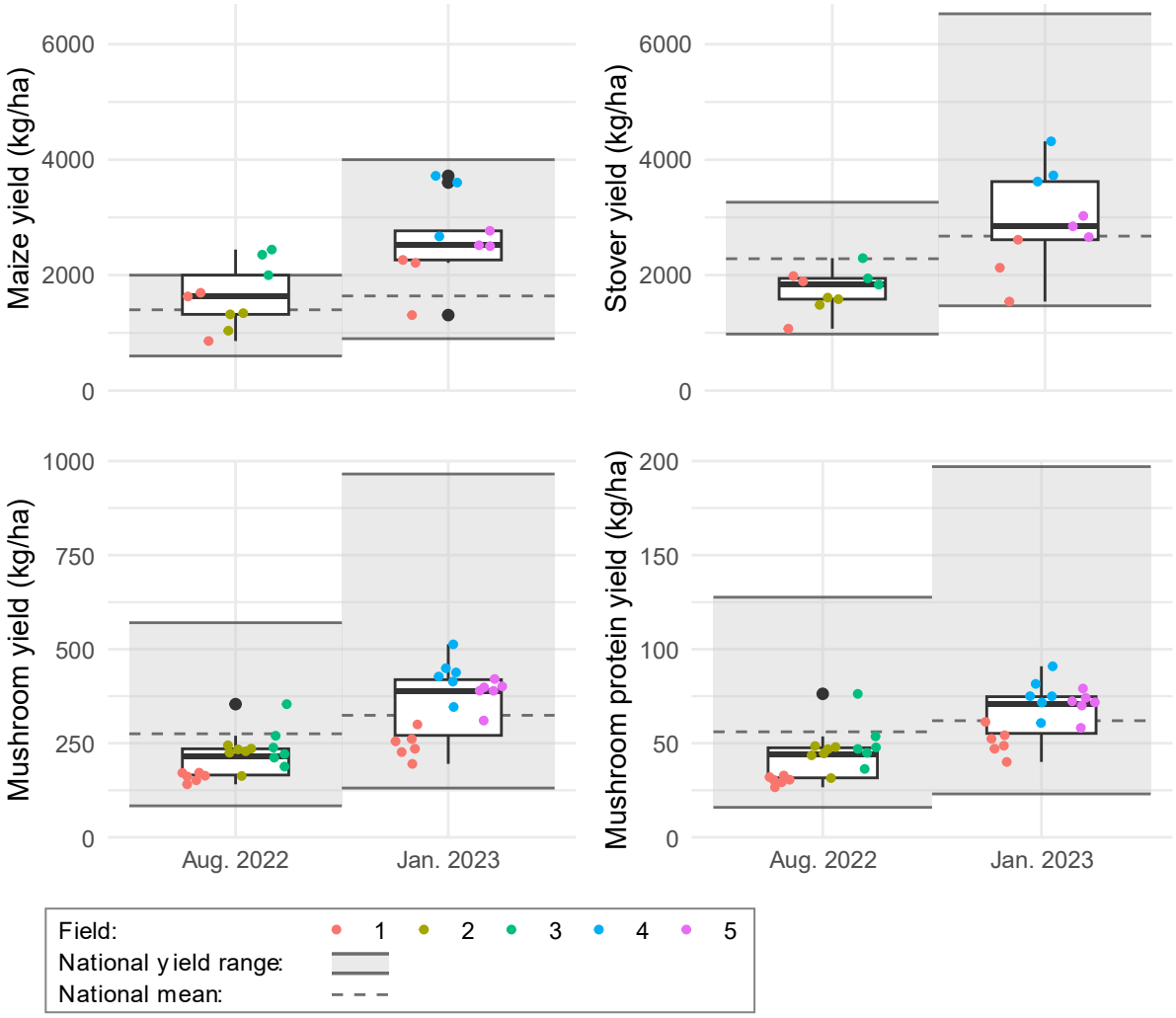
	Field	NDF	ADF	Ash	C	N	C/N
Season 1 Aug-22	1	74.43	43.38	4.04	49.59	0.48	102.86
	2	74.17	42.22	4.89	48.92	0.62	78.97
	3	73.97	41.58	4.50	49.03	0.68	72.28
	mean	**74.19**	**42.39**	**4.48**	**49.18**	**0.59**	**84.70**
Season 2 Jan-23	1	72.34	41.83	5.92	48.11	0.63	76.16
	4	77.73	45.97	5.09	48.35	0.61	79.72
	5	74.73	42.66	5.70	48.82	0.63	77.15
	mean	**74.93**	**43.49**	**5.57**	**48.43**	**0.62**	**77.68**

field one and field three, respectively. This leads to increased or decreased C/N ratios for these two fields, which may affect oyster mushroom yields.

Table 16: Chemical composition of maize stover from different fields across two seasons at Strategic Farm Kabaskende, Kibaale district, Uganda. All values are given as percentage of dry weight

	Field	NDF	ADF	Ash	C	N	C/N
Season 1 Aug-22	1	74.43	43.38	4.04	49.59	0.48	102.86
	2	74.17	42.22	4.89	48.92	0.62	78.97
	3	73.97	41.58	4.50	49.03	0.68	72.28
	mean	**74.19**	**42.39**	**4.48**	**49.18**	**0.59**	**84.70**
Season 2 Jan-23	1	72.34	41.83	5.92	48.11	0.63	76.16
	4	77.73	45.97	5.09	48.35	0.61	79.72
	5	74.73	42.66	5.70	48.82	0.63	77.15
	mean	**74.93**	**43.49**	**5.57**	**48.43**	**0.62**	**77.68**

Figure 12: Boxplots show data for maize grain (top left) and maize stover yields (top right) from five fields and two seasons based on a case study in Uganda. Boxplots for oyster mushroom (bottom left) and mushroom protein yields (bottom right) show data of laboratory cultivation on Ugandan maize stover with six replicates from each field. For reference, national yield ranges for Uganda are shown for both seasons as grey rectangles with dashed lines indicating means. Reference grain yields are based on national survey data for 2018/19 (Ugandan Bureau of Statistics 2020). Reference stover yields were calculated from reference grain yields using the mean harvest index of 0.38 for Africa (Ludemann et al., 2022). Reference mushroom yields were calculated using minimum, maximum and mean mushroom yields from our laboratory experiments. Reference mushroom protein yields were calculated using minimum, maximum and mean mushroom nitrogen contents found in our experiments and the standard nitrogen-to-protein conversion factor of 6.25.



Source: Enno Sonntag

3.3.4.2 Potential for oyster mushroom and mushroom protein production

Oyster mushroom mycelium colonized maize stover from all fields at a similar pace. Contamination with green mould occurred across treatments and was limited to 22 % of samples with 10 % or less of affected surface area. Three to five mushroom harvests were produced during 113 days of cultivation, with a higher mean number of total harvests produced on stover from the second season (Table 17). Mean BCR per field ranged from 9.7 % to 13.5 %, with means of 12.0 % and 12.1 % for first and second seasons, respectively. The overall mean biological efficiency was 139.7 %. This implies that on average 12.1 g of dry or 139.7 g of

fresh mushrooms were harvested per 100 g of dry stover. Mean total BCR was notably lower and higher than average in field one (Aug. 2022) and fields two (Aug. 2022) and five (Jan. 2023) respectively. This appears to be linked to stover C/N-ratio for field one and two, but the same correlation was not observed for field five.

Table 17: Minimum, maximum and total number harvests, as well as mean biomass conversion rate (BCR) and nitrogen content per field and season for oyster mushrooms grown on Ugandan maize stover. Statistically significant differences in BCR, determined with Tukey's test, are signified by the letters a and b.

	Field	min. Nr. harvests	max Nr. harvests	total Nr. harvests	mean total BCR (%)	mean protein content (% DM)
Season 1 Aug-22	1	3 (5)	4 (1)	19	9.7[b]	19
	2	4 (5)	5 (1)	25	14.2[a]	20.9
	3	3 (3)	4 (3)	21	12.2[ab]	22.4
	mean			**21.6**	**12.0**	**20.7**
Season 2 Jan-23	1	4 (3)	5 (3)	27	11.7[ab]	20.4
	4	3 (3)	4 (3)	21	11.1[ab]	18.7
	5	4 (5)	5 (1)	25	13.5[a]	18.8
	mean			**24.3**	**12.1**	**19.3**

Dry mushroom yields were calculated by multiplication of BCR per mushroom bag and mushroom harvest with the mean stover yield per field (Figure 12). Results show that the second season (Jan. 2023) exceeded the first (Aug. 2022) in mushroom yields, with means of 0.35 t DM/ha and 0.21 t DM/ha respectively, reflecting the difference in stover availability. Corresponding to mushroom yields, mushroom protein yields were also greater in the second (Jan. 2023) compared to the first season (Aug. 2022), with means of 0.066 t DM/ha and 0.042 t DM/ha, respectively.

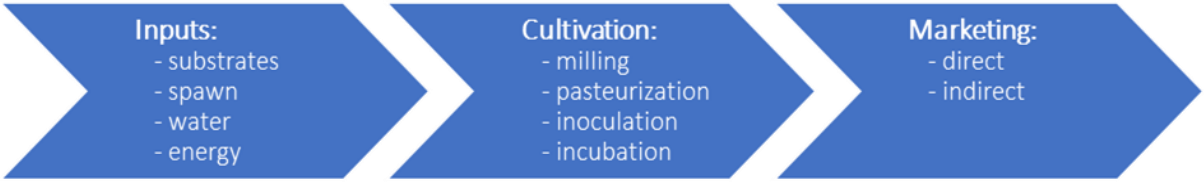
The data presented indicates that dry oyster mushrooms equivalent to 13 % of the yield of dry maize grain can be cultivated as supplementary food from maize stover per hectare in Uganda. Additionally, mushroom protein production could reach 32-33 % of the protein yield of maize grain, assuming a maize protein content of 7.7 % (USDA 2019). These findings underpin that oyster mushroom cultivation on maize stover has considerable potential for addressing food insecurity in Uganda.

3.3.4.3 The Ugandan oyster mushroom value chain

The Ugandan oyster mushroom sector benefits from the cultural appreciation of edible mushrooms, but faces significant challenges in realizing its potential to help address food insecurity. Wild mushrooms like *Termitomyces microcarpus*, locally known as Obutiko obubaala, are collected during rainy seasons and highly valued in Ugandan cuisine. Their decreasing availability due to habitat loss has created a new demand. This development has supported the expansion of oyster mushroom production since its introduction to Uganda in 1989. However, the sector is still in an early stage of technological and economic development and faces specific challenges at various steps of the value chain, which we investigate in the following section based on key informant interviews.

The Ugandan oyster mushroom value chain consists of inputs, cultivation and marketing (Figure 13). Inputs include mycelium starter culture (spawn), lignocellulose-rich substrates, water and energy. Cultivation involves pasteurization, substrate milling (in some cases), spawn inoculation, packing into cultivation bags and incubation in a humid, shaded room. After harvest, oyster mushrooms are either marketed directly, indirectly or consumed by the producer's family.

Figure 13: The Ugandan oyster mushroom value chain.



Source: Enno Sonntag, Daniel Grimm

Inputs

Fresh oyster mushroom mycelium culture is imported from Europe and then propagated in Uganda. These mother cultures can be stored refrigerated for up to three years and used for the production of many batches of spawn. For this purpose, millet or sorghum grains are sterilized using small autoclaves or pressure cookers and inoculated with fresh mycelium after cooling. For many years, spawn production was limited to two or three experts. Recently, as new spawn producers enter the market, mushroom growers increasingly encounter low-quality spawn affected by mould or slow growth.

Cotton seed hull is by far the most commonly used substrate for oyster mushroom production in Uganda. Recent price increases have however stimulated the motivation to use alternative substrates. Cotton seed hulls are a by-product from cotton production in Uganda, Kenya and Tanzania and has ideal properties for oyster mushroom cultivation. However, prices have tripled between 2020 and 2023 due to transport restrictions during the COVID-19 pandemic, inflation and increasing demand for competing uses such as animal feed. This has reduced profit margins and motivated mushroom farmers to experiment with alternative substrates, including coffee husks, sugar cane bagasse, corn cobs and wood chips. However, positive results were limited to substrate mixtures with cotton seed hulls as the main component.

Cultivation

The majority of oyster mushroom producers are women in peri-urban areas aiming to improve their income. Mushrooms are produced in small sheds near the homestead, allowing this activity to be combined with other household tasks. Mushroom production requires little space and starting capital, making it an easy entry economic activity. The revenue provides a degree of economic independence and contributes to household food security.

Oyster mushroom cultivation starts with pasteurization of the substrate, which is commonly done using steam. A plastic mesh bag containing substrate is placed above boiling water inside a metal barrel, which is covered and heated by a wood fuelled fire for around five hours. This method requires large amounts of fuel wood. Less fuel wood is required for hot air pasteurization, but only one informant used this method. Here, cultivation bags are filled with moist substrate and heated for up to three hours in an oven. After pasteurization with either method, substrates are left to cool before spawn is added. Filled cultivation bags are closed and placed on shelves in a cultivation shed. When fully colonized by mycelium, the bags are reopened and sprayed with water one to three times daily, depending on whether it is dry or rainy season. Mature mushrooms are usually harvested two to three, but up to five times per bag over a period of two to three months. After cultivation, the spent substrate is applied as mulch in urban gardens.

Marketing

Oyster mushrooms are primarily sold fresh, while dried mushrooms only make up a small share of the market. In the warm Ugandan climate and in absence of cooling infrastructure, oyster mushrooms need to be within a few days after harvest. This is either done directly by the mushroom farmer in the neighbourhood or indirectly via intermediaries and one of the central market places. The return on a kg of fresh mushrooms is greater in the neighbourhood, where it sells for 2.72 USD, compared to 1.90 USD per kg if sold via the central market. However, the demand in the neighbourhood is small compared to that on the central market. Oyster mushrooms are sometimes dried to extend their shelf life, but then generally used for home consumption. Some mushroom growers are experimenting with products such as dry mushroom powder or broth, but there is no established market for these products yet.

The production of one bag of mushroom spawn, which weighs 400 g and is sufficient for inoculating four mushroom bags costs about 0.27 USD. This includes the cost of grains and mother culture. It sells for 1.08 USD or 0.95 USD, if the customer buys more than a hundred. The profit per bag for the spawn producer is therefore 0.68 – 0.81 USD per spawn bag.

Based on this, the mushroom producers spend 0.24 - 0.27 USD on spawn per mushroom bag. Using on average 600 g (dm) of substrate per bag, the producer spends 0.2 USD on substrate per bag. The cost of firewood per pasteurization is roughly 5.44 USD. Since 45 bags can be pasteurized per run, this is 0.12 USD per bag. The price for producing one mushroom bag is therefore roughly 0.56 - 0.59 USD. A single bag produces between 0.6 and 1 kg of mushrooms. The profit per bag is therefore between 0.55 and 2.13 USD. One pasteurization run can therefore yield a profit of 24.75 – 94.5 USD. A typical Ugandan mushroom cultivator, who produces and harvests from 2000 bags per year will generate an annual profit of 1100 to 4200 USD and produce 1200 – 2000 kg of fresh mushrooms (roughly 200 kg dry) from 50 kg of (dry) spawn and 1200 kg of (dry) substrate.

3.3.5 Discussion

Our study demonstrated a substantial potential for oyster mushroom production on maize residues in Uganda. The amount of maize stover potentially available for oyster mushroom production in Uganda can be roughly estimated by dividing the country's average maize production of 3.29 mio. t for 2012-2022 (FAOSTAT, 2024) by the harvest index (HI) for maize. While our case study determined an HI of 0.47, broader studies have found an average HI of 0.38 for maize in Africa (Ludemann et al., 2022). Neither of these values might be representative for Uganda as a whole, however it seems more sensible to use our value as it will lead to more conservative estimates. This would amount to 7 mio. t of maize stover produced in Uganda annually, which could potentially yield 9.78 mio. t of fresh or 0.85 mio. t of dry oyster mushrooms and 0.17 mio. t of oyster mushroom protein when applying mean yields from our experiment, which were high in comparison with other studies using African maize straw (Fanadzo et al., 2010; Musara et al., 2018) or cotton seed hulls (Girmay et al., 2016; Islam, Riaz, 2017). This would be 212.6 kg of fresh or 18.5 kg of dry oyster mushrooms annually per person living in Uganda today. Three factors could have contributed to the high mushroom yields we recorded in our experiment: firstly, the substrate was sterilized instead of pasteurized, which can lead to significant yield increases (Grimm et al., 2024). Secondly, the straw was milled to very fine fragments (< 4 mm) which creates a very favourable matrix for a mycelium to grow on. Thirdly, the temperature and humidity were kept at an optimum. But even if smaller yields than we recorded should be the rule, realizing just a fraction of the mushroom production potential on maize stover would considerably contribute to food security in Uganda, considering global average consumption of 4.7 kg of fresh mushrooms per year (Royse et al., 2017). However, there are important infrastructural barriers, bottlenecks, as well as environmental and social risks associated to upscaling oyster mushroom production.

Spawn is an essential ingredient for oyster mushroom production and its supply an important bottleneck in the local value chain. Access to spawn, which perishes quickly if not kept cool, is especially limited in rural areas and more remote towns. Since spawn makes up almost half of the costs of producing a mushroom bag, a higher number of spawn producers could be advantageous for mushroom cultivators, since it could lower prices. However, as the past has shown, there is also a risk that bad-quality spawn enters the market, since the consistent production of high-quality spawn requires biotechnological expertise and good equipment, as well as high quality stem cultures, which currently need to be imported.

Apart from spawn, affordable substrates are the main limiting factor for profitability of oyster mushroom production in Uganda. Prior to our study, the high prices for cotton seed hulls, by far the most commonly used substrate in the country, have negatively impacted the returns of mushroom growers (Mayanja, Tipi, 2018). This effect was further exacerbated by the economic turmoil resulting from the COVID-19 pandemic and led some mushroom growers to give up their business. This highlights the importance of easily available, low-cost substrates. Our study demonstrates that maize stover has good potential for oyster mushroom production and is readily available in Uganda. At our study site, maize stover was typically burned in the fields, but it could be utilized more economically as a substrate for oyster mushroom cultivation. However, in other regions, crop residues are also used for mulching, fodder, construction, sale and cooking (Duncan et al., 2016; Swidiq et al., 2012). A study in eastern Uganda found that 53.7 % of maize stover were used as mulch, 19.5 % ploughed into the soil, 7.9 % used as fodder, and 16.8 % were burned (Lwasa et al., 2023). Another study in a nearby area found that 15.6 % of maize stover were used as mulch, 29.7 % as fodder and that 51.6 % may potentially be available for other uses (Roobroeck et al., 2019). Depending on current uses, removing maize stover for oyster mushroom production may result in economic or environmental trade-offs. For example, removing crop residues from the field instead of using them as mulch would result in nutrient and carbon losses in the soil. This risk could be mitigated by returning spent mushroom substrate to the field directly or after vermicomposting to produce high-quality compost and earthworms as food or feed (Sonntag et al., 2023).

Transportation of maize stover to the centres of mushroom production is the next important step. Currently, production is situated near the markets in urban centres. Collecting maize stover in rural areas and transporting it to production centres may present a considerable logistical challenge, which requires large investments. Milling of maize stover before transportation would reduce the volume and transport costs. Milling also facilitates rapid colonization of the material by oyster mushroom mycelium and is therefore a crucial processing step. However, it requires appropriate machinery which is costly. In the current system, middlemen with the needed capital could facilitate milling, transportation and delivery to mushroom growers. Alternatively, mushroom production centres could be promoted in rural areas, which would reduce the need to transport large volumes of maize stover. It would also facilitate return of spent mushroom substrate to the fields where stover was removed and thus improve nutrient cycling. However, this approach would require swift and/or cooled transportation of fresh oyster mushrooms to urban centres.

Energy and water use during substrate pasteurization present significant sustainability challenges associated to upscaling oyster mushroom production. Steaming, the most widely used pasteurization method in Uganda requires large volumes of water, firewood and time (Kurtzman, 2010). Expansion of hot air pasteurization, as used by one of our informants, would reduce the use of water and firewood (Grimm et al., 2024) without requiring complex technology as in the case of using solar power or other renewables. Another sustainability challenge is the plastic waste resulting from used mushroom cultivation bags, which would require proper recycling or incineration.

The capital required at different steps of the value chain in the effort for upscaling oyster mushroom production may favour wealthy individuals over current mushroom growers, who are mostly women with limited financial resources. Socioeconomic and gender aspects should therefore not be overlooked in any effort to modernize the Ugandan mushroom sector. Such efforts can be effectively supported by

government policy, as illustrated by China, which grew from producing less than 5 % to more than 70 % of global edible mushroom from the 1980s to today (Chang, 2005). By ensuring reliable and cheap access to high-quality spawn beyond Kampala and other urban centers, and by supporting the instalment of infrastructure for collecting, milling and transporting maize stover and other substrates, a large, viable and sustainable Ugandan mushroom economy, which could also benefit small farmers, could be fostered.

3.3.6 Conclusion

Based on our findings we conclude that oyster mushroom production has a considerable potential for addressing food insecurity in Uganda and that maize stover is an ideal substrate for increasing national mushroom production. Maize stover is a readily available by-product from farming that is currently under-utilized, but trade-offs with competing uses, especially mulching, should be considered when removing it for oyster mushroom production. In our study, oyster mushroom yields on maize stover were higher than results reported in other studies using cotton waste substrates. Maize stover is therefore an interesting alternative to cotton seed hull, the most commonly used substrate. Utilizing even a fraction of the annually available maize stover for oyster mushroom production could make a valuable addition to Ugandan diets, particularly in protein. However, we identified important socioeconomic, environmental and logistic challenges along the value chain which should be considered by policy makers and other stake holders in any effort to scale up oyster mushroom production in Uganda.

4 General discussion

The different research papers presented in chapter 3 were focused on production (3.1.), sustainability (3.2.) and adaptability to the sub-Saharan African context (3.3.). Despite the different focus points of these papers, there were also overlaps between the subject, so that each paper contains some information on all three topics. In the following discussion, these overlaps are highlighted, the main findings presented and finally recommendations for policy as well as future research are made.

4.1 Production

The production of food is the main purpose of mushroom cultivation. However, it is not, or at least should not be, the only purpose. The research on production presented in chapter 3.1. includes not only data on the mushroom yields that can be obtained from different types of straw, but also on the nutrient flows, since mushroom cultivation also creates other products than the fruitbodies that we eat. These other products are often referred to as “waste” or, less derogatively, as “by-products”. As chapter 2.2. elaborates, virtually all side products of mushroom production, even including the carbon emissions, can be used for other production processes within a circular economy. The research presented in chapter 3.1. provides hard data on the amounts and qualities of these different products from mushroom cultivation, filling a gap in the sparse available literature. This data could for example be used to calculate the nutrient balance on a field if the crop residues are removed for mushroom cultivation and the spent mushroom substrate is later returned as a soil amendment. This will help to estimate the amount of fertilizer or compost that needs to be added in addition to the SMS. From this perspective, one might recommend the use of maize or soy straw for mushroom production due to relatively high yields (114 % and 89 % BE) and low carbon emissions per kg of mushroom, while discouraging the use of faba bean straw due to relatively mediocre yields (76 % BE) and the high loss of nitrogen in the production process. However, if the main production objective was a high protein content of the mushrooms, as an indicator of high quality, faba bean straw would be the most recommendable substrate. Whichever focus one choses in the production process, wheat straw would be the worst substrate for mushroom cultivation, due to low yields (58 % BE) and high carbon emissions. Of course, it could be possible to increase yields by mixing straws, which was beyond the scope of this study, but is an interesting topic for further research.

Other chapters also touched on the production processes, despite their focus on other aspects of mushroom cultivation. For example, the research in chapter 3.2. shows that autoclaving can lead to yield increases of up to 50 % in comparison to pasteurization. The case study in Uganda presented in chapter 3.3. Like in chapter 3.1., maize straw produced high mushroom yields (139.7 % BE), but more importantly, the paper provides data which helps to put those yields in relation to the field size and the amount of maize and stover that were harvested. For example, the data indicates that dry oyster mushrooms, equivalent to 13 % of the yield of dry maize were harvested and that the amount of mushroom protein was around 32 - 33 % of maize protein. This shows that mushroom cultivation can make a significant contribution to food production per hectare and even to food security.

4.2 Sustainability

As was shown in chapter 3.2., there is a big difference in the energy and water use efficiency of different sterilization and pasteurization methods for oyster mushroom cultivation. Onn balance, hot air pasteurization is the most sustainable method as it requires about 75 % less energy than autoclaving and 85 % less water than the other two pasteurization methods. Hot water pasteurization, which is most common for small-scale producers, is the least sustainable method. The use of hydrated lime (CaO) is as wasteful in terms of water, but requires the least energy of any method and very little equipment. Hot air pasteurization could help to reduce the environmental impact of oyster mushroom production and would

be an easily scalable and only moderately expensive method. Autoclaving, on the other hand, can increase yields and enable the cultivation of less competitive mushroom species or be used for spawn production.

While efficient resource use in production processes is an important aspect of sustainability, other factors are important too. For example, the choice of substrates plays an important role, as was discussed at length in chapter 3.1. Comparing the use of substrates for mushroom cultivation to other use-cases, such as mulching or feeding ruminants, is important for the assessment of sustainability. The result of such an assessment may vary from case to case, but the results suggests that mushroom cultivation produces more food than using the substrate as feed while producing relatively low carbon emissions. Other aspects, such as the high amount of plastic waste that is typically produced fin mushroom production, are however not included in this analysis. The research in chapter 3.3. shows that mushroom cultivation also can have unsustainable consequences, especially due to inefficient pasteurization methods, but also because of the transportation of substrates and a resulting nutrient and carbon drainage from agricultural fields. This shows that the overall sustainability of mushroom production is very dependent on the surrounding agricultural system. Therefore, an important factor is also the usage of spent mushroom substrates (SMS). As the literature review in chapter 2.2. showed, there are many ways of utilizing SMS. Perhaps the most sustainable way of production would be a local approach where mushrooms are produced not far from the fields and the SMS, along with other waste products like dung, is used for vermicomposting.

4.3 Adaptability

The research in chapter 3.3. focuses on Uganda, but the study is likely to also contain information applicable to other developing countries in Africa and around the world. One important aspect that will vary from case to case is the culinary culture of a country. In Uganda, where wild mushrooms are a traditional food often eaten on special occasions, oyster mushrooms are also seen as a valuable, healthy and tasty product, driving demand. In other countries, the marketing of mushrooms might be less easy. Oyster mushrooms seem to be the mushroom species the cultivation of which can most easily be adapted to developing countries, at least judging by the fact that it is the most common mushroom in Uganda and many other African countries. The inability of mushroom farmers to afford large facilities and expensive equipment limits them to this robust species, which can grow on almost any substrate as long as it is chopped up and put in a bag.

There is no lack of entrepreneurial culture in Uganda, but an unfortunate lack of capital and infrastructure which present the main barriers for the development of a large, sustainable mushroom economy. As the previous two chapters in this discussion have laid out, mushroom cultivation would make a significant contribution to food security as well as the sustainability and productivity of the overall agricultural system. An effort to help Ugandan mushroom farmers, and those in other developing countries, overcome these economic barriers, seems to be a logical priority of agricultural government policies, as well as for private investors who are interested in sustainable, long-term growth. Access to high-quality spawn at low prices is one of the two most important factors for growing the mushroom economy. The other factor is access to substrates from crop residues. A village-based approach could work, where farming communities share machinery to chop the crop residues directly on the field. This significantly reduces the volume and makes transport easier. The farmers could use the residues for mushroom cultivation themselves or sell to larger production centres. Farmer's education programs, for example in the form of workshops, would be necessary to spread knowledge about mushroom cultivation. In Uganda, there is already a governmental network of agricultural consultants, which could aid in such project. In order to train the trainers, Universities should include mushroom production in their agricultural faculties and offer courses to students.

As was discussed in chapter 3.3., in the Ugandan context, low-tech methods that utilize cheap equipment are most likely to work. Unfortunately, the most low-tech solution in the case of substrate pasteurization is also the least sustainable one, as the research in chapter 3.2. shows. However, hot air pasteurization, which is much more sustainable than hot water pasteurization, can easily be adapted by Ugandan mushroom

farmers. After all, we found that one of the poorest mushroom farmers we visited already performed hot air pasteurization, using the same oven she used for cooking. This small oven made from clay and heated with fire wood, may not be the most efficient oven in the world, but it would certainly use much less fuel than a barrel filled with water and substrate which is placed over an open fire. If the knowledge about this is not spread via educational programs, the adaptation of this technology might nevertheless become more common, simply because of rising prices of firewood and other fuels.

4.4 Conclusions

The research presented in this work highlights the complex nature of mushroom cultivation with regards to production and sustainability, as well as adaptability within the sub-Saharan African context. Across the various studies it becomes evident that mushroom cultivation holds promise not only as a means of food production, but also as a driver of sustainable agricultural practices and economic development. Given the rapid population growth in many African countries and the pressure to expand cropland into forests and other natural habitats, mushroom cultivation as a landless form of food production might be an important solution.

In terms of productivity, the choice of substrate emerges as a critical factor influencing yield and quality of mushrooms, with implications for nutrient cycling and carbon emissions. While certain substrates, such as maize and soy straw, may offer higher yields or better nutritional profiles, in the case of faba bean straw, considerations of sustainability must also be factored in. The integration of mushroom cultivation into circular economic models (see chapter 2.2.), wherein side products are repurposed, would maximize resource efficiency. But even in non-circular models, the productivity of mushroom cultivation could make significant contributions to food security. This is exemplified by the data from the case study in Uganda (3.3.), where 13 % more food and 33 % more protein could be produced from the same area, if maize stover was used for mushroom cultivation rather than burning it.

Concerning the sustainability of mushroom production, energy and water use efficiency, as well as the management of side products like spent mushroom substrate, emerge as key areas for improvement. This is especially important in the African context, where water resources are often scarce and might become even more so, due to climate change (see chapter 2.1.4). Even though mushrooms are a more sustainable protein source than most meat, without the use of techniques for substrate pasteurization which use water and energy efficiently, an increase of mushroom production is likely to have negative consequences. Hot air pasteurization is on balance, the most sustainable method of substrate sanitization (see chapter 3.2.), but the use of hydrated lime can also be recommended, if water is not a limiting resource. Autoclaving on the other hand, while using more energy, can lead to increased yields and enables mushroom farmers to produce spawn, as well as a more diverse set of mushroom species. Hot water pasteurization or the steaming method, as carried out in Uganda, are however not to be recommended.

Hot air pasteurization also has the benefit, that it is easily adaptable to the sub-Saharan African context. Relatively inexpensive, low-tech ovens can be constructed by mushroom farmers themselves to help them burn less fuel in their production process. Ugandan farmers were also found to be interested in using maize straw and other crop residues as mushroom substrates, since the cotton seed hulls, which they currently use, have become increasingly expensive. However, realizing the potential of using crop residues requires concerted efforts to overcome barriers such as limited access to capital and infrastructure. Embracing low-tech solutions, coupled with targeted education and support programs, can empower communities to harness the benefits of mushroom cultivation while mitigating its environmental footprint.

More research is needed, not only on the use of crop residues or mixtures of different substrates for mushroom cultivation, but also on the productivity associated to the use of spent mushroom substrates in other agricultural activities, such as vermicomposting. If the focus in mushroom cultivation is shifted to include not only food production but also compost production, mushroom yield becomes only one of

several measures of productivity. More resources should be allocated to the research of mushroom cultivation, especially in sub-Saharan Africa. Creating solutions for sustainable mushroom value chains which are adapted to the context of developing countries, has the potential to bolster food security and improve the overall health and economic well-being of populations in these regions.

5 Summary

5.1 Summary (in English)

The research presented in this PhD thesis examines various aspects of oyster mushroom cultivation and food security, focusing on production, sustainability and adaptation in the case of Uganda. Due to population growth, decreasing crop land availability, depletion of agricultural resources and climate change, landless food production and circular agricultural systems could play a more important role in the future. It is particularly important to develop sustainable production techniques adapted to the context of sub-Saharan Africa, where the challenges are the greatest. Oyster mushrooms are protein-rich, high-yielding, can by cultivated on a wide range of crop residues and are the most commonly cultivated mushroom in Uganda and many other African countries. This makes them an important subject to study in the context of food security.

A review of the scientific literature on mushroom cultivation in the context of recycling discusses several pathways in which mushroom cultivation can contribute to the agricultural system as a whole, in addition to the primary objective of producing mushrooms. The use of spent mushroom substrate for vermicomposting to produce high quality compost and earthworms that can be used as animal feed may be the most promising circular model. However, there are many other options, such as using the mushrooms themselves as animal feed or producing several mushroom species in succession on the same substrate.

Since sustainable mushroom production requires an integration with crop and livestock production within a circular system, it is an important question which substrates to use for cultivation. The use of nutrient-poor straw from cereals and legumes for oyster mushroom cultivation is a good option, as these substrates only have very limited value as animal feed. The productivity of four different types of straw was determined experimentally. Maize and soy straw were particularly productive, yielding 9.2 and 8.6 g of dry mushrooms per 100 g of dry substrate. Faba bean straw was significantly less productive, with only 6.6 % of the substrate being converted into mushrooms. However, faba bean straw, which had the highest nitrogen content of the four straw types that were compared, also produced mushrooms with a higher protein content. Wheat straw, on the other hand, was found to be an inferior substrate, yielding only 3.8 g of dry mushrooms per 100 g of dry substrate. Approximately 60 – 80 % of the dry matter, carbon and nitrogen is retained in the spent mushroom substrate after cultivation and between 3.5 kg (on wheat straw) and 2.6 kg (on soy straw) of carbon is emitted per kg of mushroom produced.

Despite promising prospects, some aspects of current mushroom production are not sustainable. In particular, the pasteurization or sterilization of mushroom substrates uses a lot of energy and water. In an experimental comparison of four different methods, hot air pasteurization emerges as the most sustainable option. However, it was also found that sterilization can significantly increase oyster mushroom yields compared to pasteurization. The first harvest was up to 50 % higher when the substrate was autoclaved, while no significant difference could be found between the different pasteurization methods.

Adapting the use of sustainably sourced substrates and of resource-efficient pasteurization or sterilization methods to Uganda, was found to be challenging but ultimately have great potential for improving local food security. In a case-study, including field work, key-informant interviews and a mushroom cultivation experiment, maize stover was found to be an underutilized resource. 13 % more food and 33 % more protein could be produced on the same land if maize stover was used for mushroom cultivation instead of being burned, which is currently a common practice in Uganda. The main challenges to realizing this potential are infrastructural barriers for collecting and preparing maize straw for mushroom cultivation and for distributing cheap, high-quality mushroom spawn. It is also important to enable Ugandan mushroom farmers to use more sustainable pasteurization practices if mushroom production is to be promoted in the country. Given the great potential of mushroom production to increase food security and improve the

sustainability of the agricultural production, more resources should be devoted to researching mushroom cultivation in circular food systems and developing solutions that are applicable to the sub-Saharan African context.

5.2 Zusammenfassung (in German)

Die in dieser Dissertation vorgestellte Forschung untersucht verschiedene Aspekte des Austernpilzanbaus und der Ernährungssicherheit mit Schwerpunkt auf Produktion, Nachhaltigkeit und Anpassung am Beispiel Ugandas. Aufgrund des Bevölkerungswachstums, der abnehmenden Verfügbarkeit von Ackerland, der Erschöpfung landwirtschaftlicher Ressourcen und des Klimawandels könnten landlose Nahrungsmittelproduktion und zirkuläre landwirtschaftliche Systeme in Zukunft eine wichtigere Rolle spielen. Es ist besonders wichtig, nachhaltige Produktionstechniken zu entwickeln, die an den Kontext sub-Sahara Afrikas angepasst sind, wo die Herausforderungen am größten sind. Austernpilze sind proteinreich, ertragreich, können auf einer Vielzahl von Ernterückständen angebaut werden und sind die am häufigsten angebauten Pilze in Uganda und vielen anderen afrikanischen Ländern. Dies macht sie zu einem wichtigen Studienobjekt im Kontext der Ernährungssicherheit.

Bei der Analyse der wissenschaftlicher Literatur zum Pilzanbau in Recycling-Kontexten wurden mehrere Möglichkeiten diskutiert wie der Pilzanbau neben dem primären Ziel der Pilzproduktion zum landwirtschaftlichen System insgesamt beitragen kann. Die Verwendung von verbrauchtem Pilzsubstrat für die Wurmkompostierung zur Erzeugung von hochwertigem Kompost und Würmern, die als Futter verwendet werden können, könnte das vielversprechendste Kreislaufmodell sein. Aber auch viele andere Optionen, wie die Verwendung der Pilze selbst als Tierfutter oder die Produktion mehrerer Pilzarten nacheinander auf demselben Substrat, sind erwähnenswert.

Da eine nachhaltige Pilzproduktion eine Integration mit Pflanzen- und Tierproduktion innerhalb eines zirkulären Systems erfordert, ist es eine wichtige Frage, welche Substrate für den Anbau verwendet werden sollen. Die Verwendung von nährstoffarmem Getreide- und Leguminosenstroh ist für den Austernpilzanbau ist eine gute Option, da diese Substrate nur einen sehr begrenzten Wert als Tierfutter haben. Die Produktivität von vier verschiedenen Strohsorten wurde experimentell ermittelt. Mais- und Sojastroh waren besonders produktiv und lieferten 9,2 bzw. 8,6 g Trockenpilz pro 100 g Trockensubstrat. Ackerbohnenstroh war signifikant weniger produktiv, wobei nur 6,6 % des Substrats in Pilze umgewandelt wurden. Ackerbohnenstroh, das den höchsten Stickstoffgehalt der vier verglichenen Strohtypen hatte, produzierte jedoch auch Pilze mit einem höheren Proteingehalt. Weizenstroh hingegen erwies sich mit nur 3,8 g Trockenpilz pro 100 g Trockensubstrat als minderwertiges Substrat. Zwischen 60 – 80 % der Trockenmasse, des Kohlenstoffs und Stickstoffs blieben im verbrauchten Pilzsubstrat nach dem Anbau zurück, und zwischen 3,5 kg (bei Weizenstroh) und 2,6 kg (bei Sojastroh) Kohlenstoff wurden pro kg produzierter Pilze emittiert.

Trotz vielversprechender Aussichten sind einige Aspekte der derzeitigen Pilzproduktion nicht nachhaltig. Insbesondere die Pasteurisierung oder Sterilisierung von Pilzsubstraten verbraucht viel Energie und Wasser. Im experimentellen Vergleich von vier verschiedenen Methoden erwies sich die Heißluftpasteurisierung als die nachhaltigste Option. Es wurde jedoch auch festgestellt, dass Sterilisierung mit einem Autoklaven die Erträge von Austernpilzen im Vergleich zur Pasteurisierung signifikant erhöhen kann. Die erste Ernte war bis zu 50 % größer, wenn das Substrat autoklaviert wurde, während zwischen den verschiedenen Pasteurisierungsmethoden kein signifikanter Unterschied festgestellt werden konnte.

Die Verwendung nachhaltig produzierter Substrate und ressourceneffizienter Pasteurisierungs- oder Sterilisierungsmethoden erwies sich im ugandischen Kontext als herausfordernd, birgt jedoch großes Potenzial zur Verbesserung der Ernährungssicherheit. In einer Fallstudie, die Feldarbeit, Interviews und ein Experiment zur Pilzzucht umfasste, wurde festgestellt, dass Maisstroh häufig unproduktiv genutzt wird. 13 % mehr Nahrungsmittel und 33 % mehr Protein könnten auf der gleichen Fläche produziert werden, wenn

Maisstroh für den Pilzanbau verwendet würde, anstatt es zu verbrennen, was derzeit in Uganda weit verbreitet ist. Die Hauptprobleme bei der Realisierung dieses Potenzials sind infrastrukturelle Barrieren bei der Sammlung und Aufbereitung von Maisstroh für den Pilzanbau und bei der Bereitstellung von preiswerter, hochwertiger Pilzbrut. Es ist auch wichtig, ugandische Pilzbauer in die Lage zu versetzen, nachhaltigere Pasteurisierungspraktiken anzuwenden, wenn die Pilzproduktion im Land gefördert werden soll. Angesichts des großen Potenzials der Pilzproduktion die Ernährungssicherheit zu erhöhen und die Nachhaltigkeit der landwirtschaftlichen Produktion zu verbessern, sollten mehr Mittel für die Erforschung des Pilzanbaus in zirkulären Ernährungssystemen und für die Entwicklung von Lösungen die auf den Kontext in Subsahara-Afrika anwendbar sind, bereitgestellt werden.

6 Literature

6.1 Literature for chapter 1

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7 Annex

7.1 Basic data for 3.1.

Table 18: Basic data from mushroom cultivation experiment on four different straws. Replicates are from wheat straw (W), faba bean straw (F), soy bean straw (S) and maize (M). Biological efficiency (BE), biomass conversion rate (BCR) and dry matter transfer to spent mushroom substrate (SMS) are given as percentages of the amount of straw in each replicate at the beginning of the experiment (200 g, DM). The data on the transfer of N and C from straw to SMS is based on the C and N concentrations in the different straws at the beginning of the experiment, vs. the concentrations found in SMS.

Replicate	BE (%)	BCR (%)	Harvest 1 BCR (%)	Harvest 2 BCR (%)	Harvest 3 BCR (%)	Dry matter transfer to SMS (%)	N in SMS (%)	N transfer to SMS (%)	C in SMS (%)	C transfer to SMS (%)
W1	60,21	3,59	2,72	0,87	0,00	84,19	0,50	94,28	43,76	78,24
W2	49,27	3,22	3,22	0,00	0,00	81,99	0,42	76,56	46,28	80,57
W3	76,48	5,36	3,11	2,25	0,69	90,48	0,42	85,50	45,58	87,58
W5	64,09	3,93	2,47	1,46	0,00	74,66	0,37	61,51	46,61	73,89
W6	40,66	2,72	2,72	0,00	0,00	71,99	0,38	61,66	46,15	70,55
W7	70,67	4,57	3,38	1,18	0,00	89,40	0,42	84,40	45,34	86,08
W8	44,53	3,24	3,24	0,00	0,00	82,74	0,49	91,29	45,10	79,24
F1	69,41	5,54	5,54	0,00	0,00	74,59	0,97	64,84	45,68	72,35
F2	70,72	6,57	5,14	1,43	0,00	67,15	1,01	60,89	45,95	65,52
F3	104,84	8,60	6,25	2,35	0,00	66,24	0,98	58,33	46,04	64,77
F4	80,54	7,48	5,90	1,58	0,00	71,89	0,93	59,89	44,96	68,64
F5	60,07	5,69	5,69	0,00	0,00	77,73	0,88	61,20	45,51	75,12
F6	84,37	7,34	4,90	2,45	0,00	66,13	1,02	60,40	45,24	63,53
F7	46,08	3,87	3,87	0,00	0,00	74,68	0,99	65,99	45,51	72,16
F8	91,63	7,84	5,69	2,15	0,00	62,48	0,93	52,29	46,06	61,11
S1	85,58	8,58	6,28	2,30	0,00	70,21	0,61	63,57	43,97	65,47
S2	54,45	5,82	5,82	0,00	0,00	67,16	0,63	63,68	43,57	62,05
S3	95,84	9,15	6,19	2,96	0,00	65,75	0,62	61,41	43,71	60,95
S4	93,51	8,04	6,26	1,78	0,00	69,86	0,67	70,13	43,34	64,21
S5	85,53	7,53	5,90	1,64	0,00	64,50	0,62	59,53	43,94	60,11
S6	107,16	10,22	7,85	2,37	0,00	66,70	0,59	58,72	43,82	61,99
S7	99,32	9,71	7,32	2,39	0,00	65,72	0,59	58,38	44,29	61,74
S8	91,53	9,45	7,08	2,37	0,00	66,61	0,57	56,49	44,65	63,07
M1	110,79	9,56	3,97	5,18	0,41	61,06	0,67	54,63	44,06	57,18
M2	114,76	9,73	3,68	4,93	1,13	65,79	0,74	64,80	43,18	60,38
M3	132,82	10,23	5,36	4,86	1,54	62,18	0,72	59,71	43,82	57,91
M4	99,27	7,42	5,22	2,19	0,00	65,64	0,64	55,85	44,30	61,81
M7	108,71	8,75	5,16	3,60	0,00	61,42	0,68	55,95	44,02	57,47
M8	117,81	9,29	5,25	4,04	2,22	64,23	0,74	63,07	43,34	59,18

7.2 Basic data for 3.2.

Table 19: Basic experimental data of experiment 1. Biological efficiency (BE), biomass conversion rate (BCR) are given as percentages of the amount of straw in each replicate at the beginning of the experiment (200 g, DM).

The colonization of the substrate after 7 and 14 days is given as a percentage of visible substrate on which oyster mushroom mycelium was visible.

Replicate	BE (%)	BCR (%)	SMS (g, DM)	Harvest 1 BCR (%)	Harvest 2 BCR (%)	Harvest 3 (BCR)	7-day colonization (%)	14-day colonization (%)
CtrlA1	117,90	10,74	101,82	5,84	4,90	0,00	60	70
CtrlA2	74,95	7,38	115,69	5,24	2,14	0,00	80	40
CtrlA3	0,00	0,00	x	0,00	0,00	0,00	70	40
CtrlA4	98,50	9,39	134,34	6,23	3,16	0,00	60	60
CtrlA5	59,90	6,26	118,56	5,90	0,36	0,00	70	70
CtrlA6	30,35	3,46	150,06	3,46	0,00	0,00	60	40
CtrlB1	0,00	0,00	x	0,00	0,00	0,00	70	50
CtrlB2	36,70	4,07	x	4,07	0,00	0,00	70	80
CtrlB3	0,00	0,00	x	0,00	0,00	0,00	60	50
CtrlB4	43,80	3,58	x	3,58	0,00	0,00	20	40
CtrlB5	12,15	1,17	150,62	1,17	0,00	0,00	50	30
CtrlB6	0,00	0,00	x	0,00	0,00	0,00	30	10
75A1	120,85	10,85	124,56	4,63	4,01	2,21	90	100
75A2	94,20	9,34	154,93	3,51	5,84	0,00	90	100
75A3	103,60	10,39	123,62	3,84	4,99	1,56	90	100
75A4	95,70	9,44	126,76	4,42	2,67	2,35	80	100
75A5	90,25	7,98	164,99	4,64	3,34	0,00	90	100
75A6	86,40	7,96	144,81	1,55	3,56	2,85	80	100
75B1	100,75	8,78	145,42	4,21	4,56	0,00	95	100
75B2	118,40	12,86	104,28	3,68	4,31	4,86	95	100
75B3	99,40	9,37	138,15	4,61	3,54	1,22	90	100
75B4	116,95	9,62	146,42	4,02	2,51	3,09	90	100
75B5	117,88	11,39	133,03	3,51	3,73	4,15	90	100
75B6	111,85	11,02	124,82	3,37	5,24	2,42	95	100
85A1	125,10	10,48	135,56	3,51	3,47	3,51	95	100
85A2	115,55	9,98	148,90	4,24	3,63	2,11	95	100
85A3	118,65	10,42	143,53	4,44	3,67	2,32	90	100
85A4	128,35	10,41	153,25	3,52	3,67	3,23	90	100
85A5	104,75	10,01	152,14	3,04	4,25	2,73	90	100
85A6	95,00	8,40	158,77	2,24	4,19	1,97	90	100
85B1	85,15	7,63	160,85	3,13	3,62	0,89	90	100
85B2	137,85	11,91	122,99	3,79	4,86	3,26	90	100
85B3	132,60	12,34	115,81	3,95	6,11	2,28	90	100
85B4	110,65	10,16	122,70	4,74	3,14	2,28	100	100
85B5	113,20	10,80	110,22	5,57	4,54	0,69	90	100
85B6	116,45	10,62	113,88	4,68	4,26	1,69	85	100
100A1	123,50	11,50	130,93	3,05	4,61	3,84	90	100
100A2	128,90	11,67	143,53	3,30	4,99	3,38	90	100
100A3	74,00	6,14	132,59	3,59	2,55	0,00	95	100
100A4	120,00	11,20	139,67	2,92	3,49	4,78	95	100
100A5	107,75	9,67	139,61	3,16	5,99	0,53	90	100
100A6	113,75	10,23	139,86	4,40	5,82	0,00	95	100
100B1	116,00	9,76	131,56	4,80	4,96	0,00	95	100

100B2	129,55	10,86	135,74	4,58	4,16	2,12	95	100
100B3	109,90	12,28	120,90	6,10	6,17	0,00	95	100
100B4	138,95	13,41	114,69	2,95	4,28	4,28	95	100
100B5	130,35	12,50	118,11	3,47	5,44	3,60	90	100
100B6	65,75	6,34	154,56	3,00	3,34	0,00	95	100

Table 20: Basic data of experiment 2. Biological efficiency (BE), biomass conversion rate (BCR) are given as percentages of the amount of straw in each replicate at the beginning of the experiment (200 g, DM). The colonization of the substrate after 7 and 14 days is given as a percentage of visible substrate on which oyster mushroom mycelium was visible.

Repliate	BE (%)	BCR (%)	SMS (g, DW)	6-day colonization (%)	16-day colonization (%)
C1	0	0	148,44	10	20
C2	0	0	127,29	30	30
C3	0	0	151,19	10	50
C4	0	0	155,30	10	20
C5	0	0	151,33	20	10
C6	0	0	141,20	20	10
HAP1.1	49,15	4,98	151,72	40	100
HAP1.2	49,00	4,40	159,17	70	100
HAP1.3	49,25	4,77	161,53	40	100
HAP1.4	41,05	3,90	159,17	70	100
HAP1.5	43,60	5,75	158,89	70	100
HAP1.6	40,30	3,70	152,30	60	100
HAP2.1	23,65	1,98	141,15	30	90
HAP2.2	48,05	5,02	160,74	40	100
HAP2.3	56,05	4,89	161,46	60	100
HAP2.4	55,15	5,20	150,71	40	100
HAP2.5	38,75	4,07	147,52	30	100
HAP2.6	41,50	3,60	151,45	30	100
HLP1	59,35	4,63	142,82	30	90
HLP2	52,50	3,92	140,78	20	100
HLP3	51,15	4,25	140,70	30	90
HLP4	52,10	3,95	145,86	20	100
HLP5	29,70	2,32	137,59	20	100
HLP6	67,30	4,95	135,19	40	100
HWP1	78,60	5,35	139,31	30	100
HWP2	80,00	5,36	162,81	50	100
HWP3	68,80	4,92	153,58	20	100
HWP4	78,70	5,19	151,31	30	100
HWP5	77,50	5,34	134,78	40	100
HWP6	70,75	5,31	144,47	30	100
A1	63,35	6,27	150,70	90	100
A2	73,60	7,65	158,37	80	100
A3	62,20	7,59	158,35	90	100
A4	67,60	7,06	155,57	80	100
A5	71,40	6,62	155,95	80	100

A6	62,55	6,32	161,27	80	100

7.3 Basic data for 3.3.

Table 21: Basic data from mushroom cultivation experiment on Ugandan maize stover. Biological efficiency (BE), biomass conversion rate (BCR) are given as percentages of the amount of straw in each replicate at the beginning of the experiment (250 g, DM).

Replicate	BE (%)	BCR (%)	Harvest 1 BCR (%)	Harvest 2 BCR (%)	Harvest 3 BCR (%)	Harvest 4 BCR (%)	Harvest 5 BCR (%)	SMS (g, DM)
F1S1.1	101,97	9,19	4,23	2,06	2,90	0,00	0,00	126,31
F1S1.2	117,24	10,37	5,67	1,82	1,84	1,05	0,00	130,86
F1S1.3	112,56	9,91	3,68	3,99	2,25	0,00	0,00	129,24
F1S1.4	103,12	8,54	5,52	2,15	0,87	0,00	0,00	130,10
F1S1.5	114,36	10,41	4,52	3,98	1,91	0,00	0,00	130,85
F1S1.6	121,15	9,80	5,15	2,77	1,88	0,00	0,00	127,90
F2S1.1	160,62	15,68	6,05	4,06	2,49	3,08	0,00	110,11
F2S1.2	134,50	14,67	2,52	5,08	2,33	2,88	1,86	89,50
F2S1.3	164,35	15,10	5,43	4,60	2,57	2,51	0,00	109,89
F2S1.4	163,92	10,44	5,03	0,64	3,26	1,51	0,00	118,02
F2S1.5	160,28	14,98	7,03	3,77	2,52	1,66	0,00	96,10
F2S1.6	149,73	14,42	5,64	5,60	1,88	1,30	0,00	91,99
F3S1.1	147,47	13,34	4,96	5,14	3,23	0,00	0,00	118,89
F3S1.2	150,15	17,48	2,89	5,55	6,71	2,33	0,00	115,82
F3S1.3	102,70	9,28	5,41	3,58	0,29	0,00	0,00	101,33
F3S1.4	132,47	11,77	6,10	2,72	2,96	0,00	0,00	109,30
F3S1.5	124,14	10,46	2,46	3,60	3,13	1,27	0,00	98,98
F3S1.6	119,69	10,93	2,58	4,32	2,37	1,66	0,00	100,67
F1S2.1	139,76	12,18	3,26	2,98	2,21	2,43	1,30	105,64
F1S2.2	120,77	11,23	4,58	2,71	1,18	0,58	2,19	115,33
F1S2.3	102,97	9,32	3,44	1,51	2,11	2,26	0,00	134,12
F1S2.4	152,11	12,46	4,30	4,54	1,29	2,34	0,00	123,32
F1S2.5	158,40	14,31	3,20	5,24	3,06	2,81	0,00	121,97
F1S2.6	115,82	10,81	3,67	3,19	0,83	1,83	1,30	112,58
F3S2.1	149,12	13,19	3,57	4,74	3,26	1,62	0,00	105,25
F3S2.2	126,64	11,26	3,12	4,91	3,24	0,00	0,00	112,15
F3S2.3	136,95	11,55	4,17	2,77	2,17	2,45	0,00	109,40
F3S2.4	97,33	8,91	2,88	3,13	2,90	0,00	0,00	120,63
F3S2.5	125,02	10,99	2,62	4,37	1,62	2,38	0,00	116,42
F3S2.6	112,56	10,64	3,90	3,69	3,05	0,00	0,00	111,95
F4S2.1	111,83	10,92	3,66	3,24	1,69	2,32	0,00	100,50
F4S2.2	153,72	13,69	5,77	3,79	2,49	1,63	0,00	88,43
F4S2.3	136,57	14,79	7,60	3,49	2,21	1,49	0,00	86,36
F4S2.4	155,25	14,01	3,04	6,22	2,49	0,39	1,88	96,28
F4S2.5	162,89	13,70	5,00	4,07	2,95	1,67	0,00	93,57
F4S2.6	154,22	14,10	3,64	4,82	2,64	2,99	0,00	92,23

Bibliografische Information:
Die Deutsche Nationalbibliothek verzeichnet diese Publikationen in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet unter www.dnb.de abrufbar.

Bibliographic information:
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Grimm, D. (2025). Oyster mushroom cultivation on straw: aspects of productivity, sustainability and adaptability to the case of Uganda. Thünen Working Paper 271. Johann Heinrich von Thünen-Institut, Braunschweig. https://doi.org/10.3220/253-2025-68

THÜNEN

Thünen Working Paper 271

Herausgeber/Redaktionsanschrift – *Editor/address*
Johann Heinrich von Thünen-Institut
Bundesallee 50
38116 Braunschweig
Germany

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www.thuenen.de

DOI:10.3220/253-2025-68
urn:nbn:de:gbv:253-2025-000095-6