



Sustainability assessment of waste and wastewater recovery for edible mushroom production through an integrated nexus. A case study in Lazio

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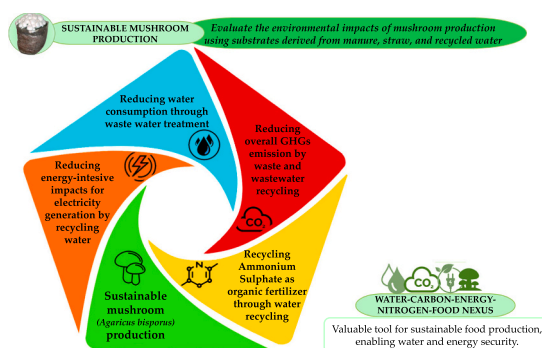
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HIGHLIGHTS

- A Life cycle assessment of fungal production from recycled materials is studied.
- The water-energy-nitrogen-carbon-food nexus is considered.
- The production of 1 kg of mushrooms emits about 2.28 kg CO₂ eq.
- Wastewater recovery reduces environmental impacts without compromising water and energy security.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Jacopo Bacenetti

Keywords:

Life cycle assessment
Circular economy
Mushroom production
Water recycling
Ammonium Sulphate

ABSTRACT

With a global population of eight billion people, improving the sustainability and nutritional quality of diets has become critical. Mushrooms offer a promising solution because of their nutritional value and ability to be grown from agricultural residues, in line with the circular economy. This study, therefore, focuses on assessing the environmental compatibility of *Agaricus bisporus* mushroom production in Italy, the world's third largest per capita consumer, by using a Life Cycle Assessment (LCA) and an integrated Water-Energy-Nitrogen-Carbon-Food (WENCF) nexus analysis. The LCA results reveal that for a functional unit of 23,000 kg of the substrate, the production process emits 2.55×10^4 kg of CO₂ eq. Sensitivity analysis shows that changing input quantities can reduce environmental impacts by about 5%. In addition, one scenario evaluates the environmental effects of recycling resources by introducing water and ammonium sulfate from scratch instead of continuous recycling, along with water purification. The study shows that sustainable food production can mitigate resource depletion, climate-altering emissions, and intersectoral competition. Using agro residues for mushroom cultivation and optimizing resource management contribute to environmental sustainability. This approach could not only improve the resilience and efficiency of the food system but could also improve the sustainability of diets. In conclusion, this study highlights the importance of adopting sustainable and circular approaches in mushroom production to address global challenges related to food sustainability.

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1. Introduction

The agri-food sector contributes around 17.3 billion tons of carbon dioxide (CO₂) each year, accounting for 35 % of anthropogenic greenhouse gas (GHGs) emissions (Xu et al., 2021). However, these rates will most probably increase even more, in part due to the continuing growth of the population, which reached 8 billion in November 2022 and is estimated to reach 9 billion by 2037. This would result in an increasing demand for food (+40 %) (Gouel and Guimbard, 2019), leading to greater pressure on natural resources. One solution to these challenges could be providing more food with fewer inputs for its production, as well as reducing unavoidable food waste, developing circular economy (CE) systems, and reusing and recycling organic waste materials (Grimm and Wösten, 2018). CE, whose relevance in current research and policies is demonstrated by its central role in the European Green Deal and cities' action plans (Mairie de Paris, 2017; European Commission, 2020), calls for a move away from linear economy models and focuses on closing the circuits of raw materials, energy sources, and nutrients. Through this philosophy, it might be possible to optimize and reduce the production of raw materials by replacing them with waste products and consequently lower polluting emissions by avoiding waste and making products more competitive.

In the agri-food sector, due to the high amount of organic waste it produces, CE could be implemented quite efficiently, as some organic agro-wastes (livestock manure and lignocellulosic biomass) can be bio-converted for food production, through their use as organic fertilizers and soil improvers, instead of synthetic ones (Bai et al., 2018; Grimm and Wösten, 2018). One sector that could particularly benefit from the bioconversion of agro-residues such as straws, leaves, stems, bagasse, and manure, is the production of mushrooms. This is because, although they normally grow in the wild on moist, nutrient-rich forest soils, they can also be grown effectively on organic waste and plant-derived materials, such as spent wood from various agro-industrial waste products containing lignocellulose and/or in association with manure (Dorr et al., 2021). Currently, among the approximately 2000 edible mushroom species in the world (Soto, 2019), 85 % of the total production is covered by five species: *Lentinus edodes* (Shiitake), *Pleurotus ostreatus* (Oyster), *Boletus edulis* (Porcini), *Agaricus bisporus* (Champignon) and *Flammulina velutipes* (Royse et al., 2017). These five species contribute about \$16.7 billion (Research and Market, 2021) to the mushroom market, by global production of about 11.8 million tons (Faostat, 2021), +57 % within 2010–2020 (Faostat, 2021), thus demonstrating their considerable importance. In particular, this is due to increased awareness about their nutritional qualities, such as crude fiber (19 % of total dry weight), vitamins (A, C, E, K), carbohydrates and minerals (potassium and selenium), antioxidant compounds (flavonoids, tannins, etc.), monounsaturated fatty acids (ω -3 and ω -6, etc.) and their protein value making them a good plant source of essential amino acids (Cheung, 2010; Valverde et al., 2015; Thakur, 2020). Furthermore, the cultivation of edible mushrooms offers an opportunity to address the issue of unsustainable disposal or burning of agro-residues like manure and agricultural biomass (Chen et al., 2022). By integrating mushroom cultivation, we can achieve multiple benefits simultaneously, including the recovery of raw materials, waste reduction, and the bioconversion of these residues into high-quality agro-food products. Therefore, mushroom cultivation could be a potential alternative for balancing nutritional deficiencies and food insecurity, as well as tackling the decline of natural resources, and climate change. In addition, since a fungal spore germinates, the fungus is highly dependent on water for its growth (which is needed for all stages of its life cycle, even because fungi consist of about 90 % water and degrade organic matter by secreting enzymes, which need water to break down the substrate) (Herman and Bleichrodt, 2022). Then, in a CE context, proper water resource management must be included to avoid the multiple impacts associated with its extraction (Tarpani and Azapagic, 2023) and preserve the conservation of that resource. In this regard, a widely used and valid tool for studying the

environmental compatibility of products or processes is the Life Cycle Assessment (LCA), especially in the agri-food sector (Zingale et al., 2022). In recent years few LCA studies have been conducted in relation to mushroom production, mostly related to the cultivation of *Agaricus bisporus*. Starting with the pioneering work of Gunady et al. (2012), showing that for every kg of *Agaricus bisporus*, 2.76 kg CO₂ eq is produced. Dissimilar results from the study by Leiva et al. (2015) (Spain), who, for 1 kg of *Agaricus bisporus* found about 4.41 kg CO₂ eq. Or even Robinson et al. (2019) studied the production of 1 kg of *Agaricus bisporus* in the U.S. from compost, showing how the results ranged from 2.13 to 2.19 kg CO₂ eq. Until the contribution of Dorr et al. (2021), who analyze the environmental impacts of a circular mushroom farm in France, showing how 1 kg of *Pleurotus ostreatus* emits about 2.99 to 3.18 kg CO₂ eq. Differences in results are a function of variability in processes, background inventory assumptions, and the methodological approach underlying LCA studies. But also, background systems, cultural practices, and soil and climate conditions. In addition to *Agaricus bisporus* and *Pleurotus ostreatus*, an additional study related to *Shiitake* mushroom cultivation (Rungnapa Tongpool and Pongpat, 2013) in Thailand was found in the literature. The main finding made by the two authors is that 1 kg of mushrooms emits about 1.87 kg CO₂ eq, although *Shiitakes* are cultivated differently than *Agaricus*. The literature related to the analysis of the environmental compatibility of mushroom production is rather modest and covers only a few countries, including the USA, Australia, China and Spain. However, no study has focused on identifying impacts on the mushroom production process in Italy, which on the basis of market research appears to be the world's third largest consumer per capita (4.87 kg per person) (Research and Market, 2021). Considering the nutritional qualities of mushrooms and given the great opportunities their production offers to close the cycles of matter and energy it may be important to lead efforts to increase their consumption as part of a healthier and more sustainable diet. Therefore, to help fill knowledge gaps on the environmental impact of the CE and mushroom cultivation, a Life Cycle Thinking (LCT) approach was used in this research to assess the sustainability of mushroom production. The study involved the Italian company "Funghitex S.S," which is active in the production of substrates for growing *Champignon* located in Giulianello (Latina). The aim of the study is: i) to quantify the impacts of this type of activity; ii) to identify the most impactful production steps, and iii) to investigate CE aspects of the company and adaptable improvement opportunities. Consistently with the twofold objectives of this study, LCA was coupled with a Water-Energy-Nitrogen-Carbon-Food Nexus analysis (ISO, 2006a, 2006b; Frischknecht et al., 2004; IPCC, 2006; Aldaya et al., 2011). The research was complemented by a sensitivity analysis that was carried out with the aim to identify some possible scenarios for reducing environmental impact in *Agaricus bisporus* production and in the field of agriculture and food. Our study aims to contribute to the existing body of knowledge in different ways. First, by exploring the environmental impacts of mushroom production using specific substrates derived from manure, straw, and recycled water. This approach is in line with the principles of waste reduction, resource efficiency, and sustainable practices. By focusing on this particular production method, we aim to highlight the potential of using agricultural by-products and recycled water as inputs for mushroom cultivation, thus promoting the principles of the circular economy. Furthermore, another highlight lies in the possibility of proposing sustainable food production from recycled raw materials in an integrated nexus perspective, in which energy use and water system were continuously interchanged, thus generating a reduction in total CF and GHG emissions. The results could then provide knowledge to the scientific community, practitioners, policymakers, and other stakeholders involved in mushroom production and sustainability, contributing to a broader understanding of the environmental impacts associated with different production methods and promoting informed decision-making for more sustainable practices. Finally, although the study focuses on specific case studies, the results and methodologies could provide valuable insights for similar production systems. The

challenges faced by the mushroom industry in terms of waste management, resource efficiency, and greenhouse gas emissions are not unique to our case study but are relevant to many regions globally.

2. Materials and methods

2.1. Case study description

Funghitex operates in the production of substrates for the cultivation of Champignon in Giulianello (41°40'20.648" N, 12°52'59.499" E) (Lazio, Italy). It produces almost exclusively with raw materials from previous agricultural production of other neighboring farms (i.e., manure and straw) according to a logic of industrial symbiosis, while water and ammonium sulfate are continually recycled and reintroduced into subsequent production cycles, as shown in S1 (paragraph 1.1) (Supplementary). The case study of *Funghitex* was selected for three orders of reasons: first, as a matter of relevance to the research objectives and the specific focus of the study. Indeed, the selected case study provided an opportunity to evaluate the environmental performances of mushroom production using substrates derived from manure, straw, and recycled water. This specific production method was considered attractive because of its potential for waste reduction, resource efficiency, and sustainable practices. Next, because of the question of data availability. In particular, the availability of comprehensive information for this case study has a key role in its selection, as access to detailed data on inputs, processes, and emissions from the company allowed for in-depth analysis and accurate assessment of the environmental impacts associated with mushroom production. And finally, as a matter of feasibility since data accessibility and cooperation from the company made it possible to conduct the study in an acceptable time frame.

2.2. Life cycle assessment

2.2.1. Goal and scope

The study aimed to analyze the resources consumed and substances emitted to produce the substrate for mushroom cultivation through different quantities of raw materials used. The FU is 23,000 kg of finished bulk product, i.e., the quantity of a mushroom cultivation room. The system boundaries considered the entire substrate production process, from the procurement of raw materials to the finished product (Fig. 1).

2.2.2. Life cycle inventory (LCI)

All inventory data are primary (2018 production) and described in Table 1 and Table A1 (Supplementary). In this study, the current model used by the company was selected as it represents the standard operational practices implemented in their *Agaricus bisporus* mushroom production. The production data used in the analysis was obtained by considering average operational parameters, including input quantities, energy consumption, and waste generation, based on the company's historical records and production logs.

As for water, its management follows the principle of reuse. Specifically, there are four water collection tanks and three silos intended for recovery in the processing cycle. All tanks are covered and equipped with both an aeration system to avoid anaerobic phenomena and an air intake system sent to the Scrubber treatment system. Water used in the production process is classified into black and clean water (Fig. 2). The first is the wastewater from the leaching of material within the production areas, from the collection at the bottom of the production areas of rainwater, and from the occasional washing of the yards and the trucks (Fig. 2A). Clean water comes from the company's three wells and is stored in silos, and used to cool the hatchery unit, control temperature, toilets, and wash yards and trucks (Fig. 2B). Rainwater, on the other hand, is stored in three cisterns and flowed into special channels.

All water is sent to the production cycle and continuously recycled. A total of 23,000 kg of substrate is obtained from the production process, and transportation was also considered for the study (Table 1). Data were modeled through databases in SimaPro 9.2.2. and adapted to Italian conditions.

2.2.3. Allocation procedures

Impact allocation to define the percentage contribution of the environmental burden to each processing by-product according to the simple cut-off method (Ekvall and Tillman, 1997), also known as one of the most used methods for modeling recycling process in LCA and recommended by the international system for Environmental Product Declarations (EPD).

The method is applicable when the environmental impacts of recycling are lower than the combined impacts of virgin material production and final waste management and thus promotes the use of recycled materials throughout the life cycle. This cut-off method means the LCA does not include activities that are avoided due to, for example, the recovery of materials or energy in waste-management processes. In particular, the method gives incentives to use recycled material as long

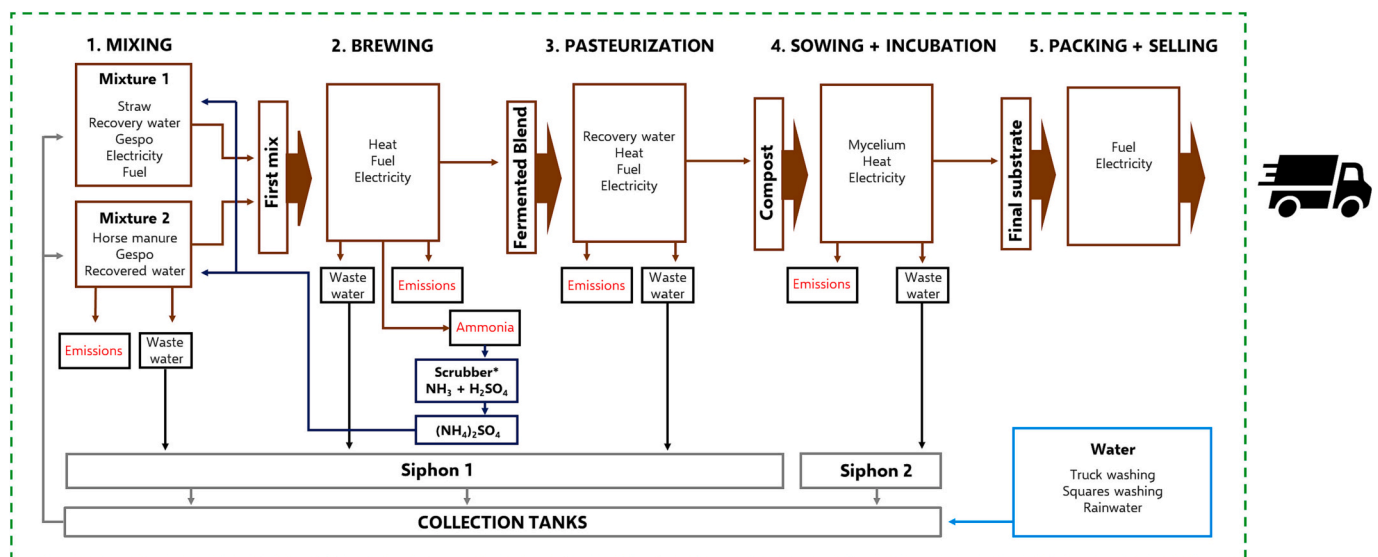


Fig. 1. System boundaries of mushroom production.

Table 1
LCI of the production process of Funghitex.

Input/Output	Unit	Quantity	Provenance	Means of transportation	Tkm	Source
Input						
Horse manure		18,000	Lazio, Tuscany, Campania		90	Agribalyse
Wheat straw		3500	Puglia	Truck	300	
Poultry manure		2700	Campania, Molise		250	Ecoinvent v3.8
Agricultural Gypsum (Calcium Sulfate)	kg	1200	Tuscany	Tanker truck	296	
Ammonium sulfate (solid)		800	Lazio	Truck	93	WFLDB
Mycelium (<i>Agaricus bisporus</i>)		980	France		1600	
Ammonium sulfate (liquid)		0.11	By-product	Production Plant	–	–
Diesel	m ³	50				Ecoinvent v3.8
Water		168.3				–
Electricity	kWh	2016				Ecoinvent v3.8
Output						
Substrate	kg	23,000				
Atmospherical emissions						
CO ₂		22,822				
CH ₄		2425				
N ₂ O	kg CO ₂ eq	261				IPPC, 2006
SF ₆		18.5				
FCs		0.9				
Emissions to water (Freshwater)						
Phosphate	kg P eq	0.3736				IPPC, 2006
Phosphorus		0.0379				
Emissions to water (marine)						
Ammonia		0.0565				
Ammonium, ion		0.0449				
Nitrate	kg N eq	0.6469				IPPC, 2006
Nitrite		0.0003				
Nitrogen		0.0067				

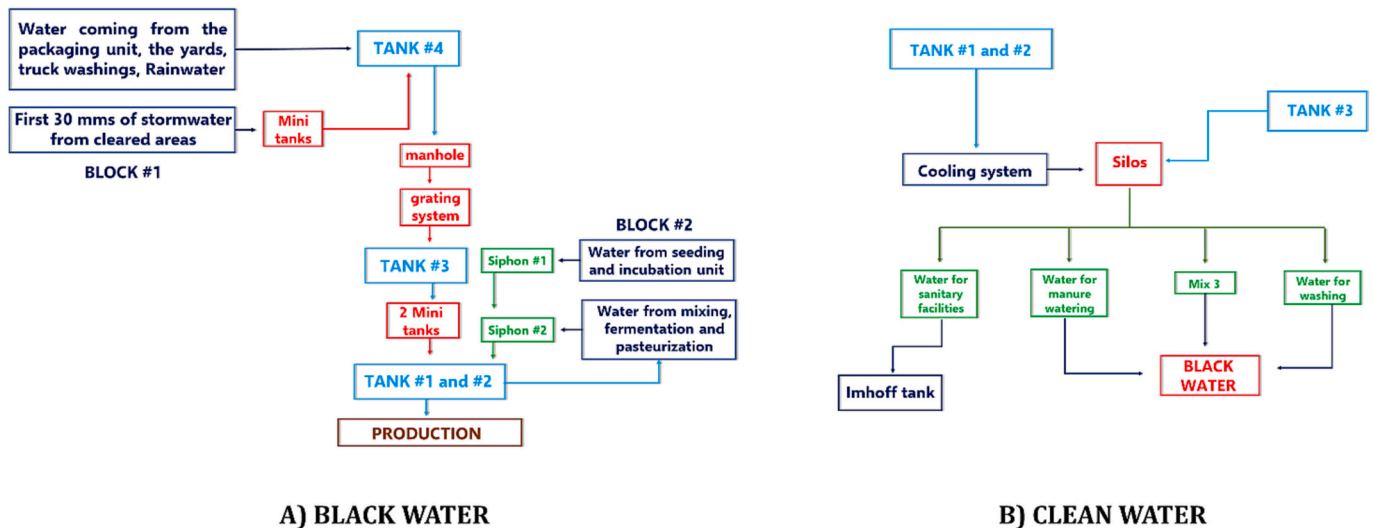


Fig. 2. Description of the cycle of Funghitex black water (A) and clean water (B).

as the recycling has less environmental impact than virgin materials production ($E_V > E_R$). Furthermore, the recycling process of a product after use is emphasized when the final disposal has a negative net impact on the environment ($E_D > 0$). According to Ekvall et al. (2020), each product should be assigned all the environmental impacts (E) directly attributable to the production process, as in Eq. (1):

$$E = (1-R_1) \times E_V + R_1 \times E_R + (1-R_2) \times E_D \quad (1)$$

where R_1 is the share of recycled material in the product; R_2 is the rate of

recycling of material after use in the product, and E_V , E_R , and E_D correspond to the environmental burdens of the virgin raw material.

2.2.4. Life cycle impact assessment (LCIA)

To ensure the robustness of our study, we followed the ReCiPe 2016 MidPoint (I), a recognized LCA methodology that provides a systematic and transparent approach for selecting impact categories. SimaPro 9.2.2. software was used, and the 18 impact categories were grouped into four macro areas.

- i. *Atmospheric Effects*: Global Warming Potential (GWP); Stratospheric Ozone Depletion (SOD); Ionizing radiation (IR); Ozone Formation, Human Health (OFHH); Fine Particulate Matter Formation (FPMP); Ozone formation, Terrestrial ecosystems (OFTE); Terrestrial acidification Potential (TAP),
- ii. *Eutrophication*: Freshwater Eutrophication Potential (FEP) and Marine Eutrophication Potential (MEP),
- iii. *Toxicity*: Terrestrial Ecotoxicity (TEC); Freshwater Ecotoxicity (FEC); Marine Ecotoxicity (MEC); Human Carcinogenic Toxicity (HCT); Human Non-Carcinogenic Toxicity (HNCT),
- iv. *Abiotic Resources*: Land Use (LU); Mineral Resources Scarcity (MRS); Fossil Resources Scarcity (FRS), Water Consumption (WC).

We considered several factors when selecting impact categories, including scientific relevance, stakeholder concerns, and environmental context. The ReCiPe 2016 MidPoint (I) was chosen and preferred over other calculation methods such as ILCD 2011, CML 2001, or TRACI because having eighteen impact categories (compared to 16 in ILCD 2011 Midpoint, 15 in IMPACT 2002 +, 11 in CML -IA Baseline, and 9 in TRACI) it can provide more comprehensive, articulate, and specific results on the environmental impacts of mushroom production. Therefore, ReCiPe could provide a broader picture with a greater degree of detail on the environmental impacts of production. In adopting the ReCiPe method, we aimed to capture a full range of potential impacts associated with mushroom production, although some impact categories might seem less obvious in an internal context. For example, marine eutrophication and marine ecotoxicity may seem far removed from our LCA concerns, but in conducting our LCA study, we aimed to take a holistic approach that considers direct and indirect contributions from specific processes or inputs within the life cycle of mushroom production. While our focus was on direct environmental impacts, we recognize that these impacts can have downstream consequences, and for this reason, it may be important to consider a broader environmental context.

2.2.5. Sensitivity analysis (SA)

Because it is not regulated by ISO, SA is an optional step, deferred to the voluntariness of the authors, who create alternative scenarios to demonstrate possible examples of improvement (Ferretti et al., 2016). However, because SA measures how variability in inventory data can affect results, it could be useful because it improves model prediction by qualitatively and quantitatively studying the study's response to varying input variables. Moreover, although interpretation is voluntary, it remains a key step in LCA because, in addition to ensuring the reliability and robustness of the study, the transition to more sustainable and circular economy models requires various hotspot improvement and management options, which can be examined precisely through an SA. Therefore, based on these assumptions, SA was conducted to assess how, by changing certain input parameters, the company's environmental performance may change. Specifically, three scenarios were created:

1. Scenario 1 (S1) (2018): The process with the initial inputs.
2. Scenario 2 (S2) (2019): horse manure, compared with S1, increased by +11 %, while wheat straw, poultry manure, and agricultural gypsum decreased by -9 %, -11 %, and -20 %, respectively. Solid ammonium sulfate increased by +13 %. Net electricity consumption decreased by -13 %.
3. Scenario 3 (S3) (2020): horse manure, compared with S2 increased by +5 %, while wheat straw decreased by -13 %. Poultry manure, agricultural gypsum, and solid ammonium sulfate increased by +17 %, +20 %, and +22 % respectively. Electricity consumption decreased by -7 %.
4. Scenario 4 (S4) (2021): horse manure increased compared to S3 by +15 %, while wheat straw was reduced by -29 %. Poultry manure was unchanged, while calcium sulfate (-13 %) and solid ammonium sulfate (-36 %) decreased. In contrast to S2, liquid ammonium

sulfate increased by +45 %. In the latter case, mycelium was also reduced by -8 % as well as electricity by -2 %.

The four scenarios were established through a combination of expert knowledge and model simulations. Expert knowledge from on-farm experts helped determine changes in quantity based on the cost of raw materials used, thus preferring to increase manure and reduce straw, keeping the final quality of the compost unaltered. Then, to verify the environmental as well as economic feasibility of this change, experimental tests and simulations were done using Simapro 9.5 software, which allowed the data collected on resource use and emission factors to be entered, thus calculating environmental footprints. Therefore, the scenarios were first modeled based on production costs, which allowed the amount of raw material to be determined, which was then verified from an environmental perspective arriving at the various production scenarios over the years.

2.3. Water-energy-nitrogen-carbon-food nexus

Subsequently, an alternative scenario was created, in which the company, instead of continuously recycling water and ammonium sulfate, adds them from time to time. In addition, it was also assumed that the wastewater, instead of being fed back into the production cycle, is treated in a wastewater treatment system. So, to highlight potential synergies and identify critical hotspots in the mushroom production system a Water-Energy-Nitrogen-Carbon-Food nexus was assessed. The objective was to quantify the potential savings of resources and emissions, thus identifying possible interactions to improve energy, water, food, and environmental issues.

2.3.1. Carbon footprint (CF)

Next, to estimate how much the company could save in GHG emissions from the use of recycled water and ammonium sulfate, an additional scenario was created in which these two inputs were replaced with non-recycled inputs, and the CF was calculated according to Forster and Artaxo (2007) as in Eq. (2)

$$CF = \sum G_i G_i \times k_i \quad (2)$$

where G_i is the amount of GHG produced and k_i is the CO₂ equivalent coefficient for that gas.

2.3.2. Water footprint (WF)

WF was calculated based on the business water footprint model proposed by Aldaya et al. (2011). This methodology has been chosen because in this way, as opposed to focusing on water use in business operations, taking into consideration the entire supply chain could explore far larger water footprints than the normal operational water footprint. WF is the total volume of freshwater used directly or indirectly for an industrial production expressed in m³. It is calculated as the sum of the operational (direct) WF (the volume of freshwater consumed or polluted due to business operations), and the supply chain (indirect) WF (the volume of freshwater consumed or polluted to produce all goods and services that constitute inputs, as shown in Eq. (3).

$$WF_{bus [u]} = WF_{bus,oper} + WF_{bus,sup} \quad (3)$$

where $WF_{bus [u]}$ is the water footprint of the business unit, $WF_{bus,oper}$ is the operational water footprint of that unit (water incorporated into the product, water consumed or polluted through a process), and $WF_{bus,sup}$ is the supply chain water footprint (water footprint of product ingredients purchased by the company, water footprint of other elements of the company for product processing, water footprint of materials and energy for general use). Therefore, to calculate the WF associated with output production, assuming a schematization of the enterprise into multiple business units through which the process is articulated, the total water

footprint ($WF_{bus,tot}$) is calculated by aggregating the WFs of the various business processes. To avoid double counting, virtual water flows between the various business units within the enterprise must be subtracted. The calculation of the output water footprint is shown in Eq. (4).

$$WF_{bus,tot} = \sum_u WF_{bus [u]} - \sum_u \sum_p (WF_{prod [u, p]} \times Pk[u, p]) \quad (4)$$

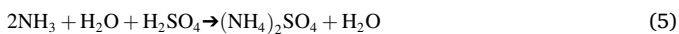
where $\sum_u WF_{bus [u]}$ is the WF of business unit u , calculated as in Eq. (3), $WF_{prod [u, p]}$ represents the product WF of product p exiting business unit u and $Pk[u, p]$ represents the volume of product output p from business unit u . The calculations were performed with SimaPro Software 9.2.2.

2.3.3. Energy footprint (EF)

In this study EF was calculated based on the Cumulative Energy Demand (CED) approach, which is an impact indicator that expresses the consumption of fossil energy, from hard coal, lignite, natural gas and crude oil during the entire product life cycle (Boldrin et al., 2022). In this study, the authors calculated it according to Frischknecht et al. (2004), using the 'Cumulative Energy Demand' method (v 1.11) described in Ecoinvent database v. 3.8. Specifically, the energy removed from nature was calculated for each input involved in the production process and multiplied by each production factor. The method was used because, as stated by Huijbregts et al. (2006), it considers the energy consumed directly and indirectly for each step in the life cycle of a process, distinguishing between renewable and nonrenewable energy, which in this study were summed and considered total energy.

2.3.4. Nitrogen footprint (NF)

During the mushroom fermentation phase, ammonia-rich air is released, the emission of which is prohibited by Legislative Decree 152/06. Thus, to comply with these obligations, the air is captured by extraction systems and transferred into the Scrubber working with sulfuric acid (H_2SO_4). This system converts the ammonia (NH_3) into ammonium sulphate ($(NH_4)_2SO_4$), as follows (Eq. (5)):



In this study, NF was used to assess the total N emissions and related losses along the ammonium sulfate production process for mushroom production (Eq. (6))

$$NF = \frac{NE_{total}}{U} \quad (6)$$

where NF is nitrogen footprint (kg N eq), NE_{total} is the total amount of N emissions throughout the entire process of mushroom production from cradle to gate, and U is the functional unit, considering Eqs. (7)–(9).

$$NE_{total} = NE_{inputs} + NV_{NH_3} \quad (7)$$

$$NE_{inputs} = \sum NE_{straw} + NE_{manure} \times U \quad (8)$$

where NE_{inputs} is the cumulative amount of N emissions associated using straw and manure as input per unit of the process (U). The nitrogen emissions include the volatilization loss (NV) of NH_3 (kg N eq), mainly allocated to the use of fertilizers (49 %), manure distribution (30 %), livestock and liquid manure storage (21 %). The NV_{NH_3} was calculated as in Eq. (9) from Arunrat et al. (2022).

$$NV_{NH_3} = N \times \varphi \times \frac{17}{14} \times 0.833 \quad (9)$$

where N corresponds to the pure amount of nitrogen emissions (kg N eq), φ is the coefficient of NH_3 volatilization loss (0.338), $\frac{17}{14}$ corresponds to the molecular weight ratios of NH_3/N , and 0.833 is the eutrophication potential factor of NH_3 (kg N eq).

3. Results and discussions

Considering LCIA results (Table 2), diesel showed the highest environmental values ($50 \text{ m}^3/\text{FU}$ mainly for heating production departments and operating mechanical shovels), which causes major impacts in 16 out of 18 categories. It mainly affects:

- For 98 % and 97 % on IR and FRS, respectively;
- For 92 % of HCT;
- For 91 % of SOD, FMPF, TAP, and FEC;
- For 90 % of MEC.

For the remaining impact categories, such as GWP, OFHH, OFTE, FEP, TEC, HCNT, and MRS, diesel impacts ranged between 46 and 82 %. The only two categories in which diesel does not have higher impacts than the others are MEP and WC. In both cases, wheat straw is responsible for 50 % of the total impacts. Also, mycelium showed about 38.3 % of the MEP impacts. It is worth noting also the FEP category, where ammonium sulfate accounts for 36 % of the total impacts (1.50×10^{-1} kg P eq out of 4.20×10^{-1} kg P eq total). As for IR, for which diesel fuel has the greatest relative impact (98 % of total IR), it is produced by mining and offshore oil and gas platforms (Chambers et al., 2008). Diesel has also a significant impact on the category of Human Carcinogenic Toxicity, primarily due to the release and inhalation of heavy metals from exhaust gases during combustion (Mohammadi et al., 2019). Additionally, poor waste disposal practices during the oil mining phase can lead to heavy metals entering groundwater near the mining sites (Wang et al., 2005), subsequently accumulating in human organisms. Likewise, heavy metals released from the diesel process/combustion, also create damage to ecosystems, as they are emitted into water and air, and this is reflected in a high value (91 % of the total impact) in FEC (Costa-Böddeker et al., 2020), confirming the value for HCT. Values around 91 %, are related to SOD (4.20×10^{-2} kg CFC11 eq), FPMF (5.78×10^1 kg $PM_{2.5}$ eq), and TAP (1.71×10^{-2} kg SO_2 eq), which express the release of GHGs.

These values can be attributed to the fact that fossil fuels used emit GHGs such as CO , CO_2 , SO_2 , NO_x (Dincer and Ratlamwala, 2013). Although trichlorofluoromethane has been chosen as a reference substance for SOD in LCA studies, the Montreal Protocol prohibits its production. Therefore, the values related to SOD, although expressed in CFC₁₁ eq, most likely refer to NO_x emissions (one of the main causes of ozone depletion) due to diesel combustion, precisely because CFCs and other ozone-depleting gases (among which NO_x is not included) have been banned by the Montreal Protocol (Above and Bankole, 2018) and currently their concentrations in the atmosphere have been reduced. Regarding MEC, diesel accounts for 90 % (1.73×10^{-2} kg 1.4-DCB), very similar percentages to FEC (91 %) although the values are more than three times as high. Again, as with MEC, probably particularly affecting this impact category is the release of heavy metals in wastewater because of the oil extraction and diesel refining process (Pulles et al., 2012). Concerning GWP, OFTE, OFHH, FEP, TEC, HCNT, MRS, diesel affects values between 69 and 82 %. In GWP, 79 % of the contribution is from the direct combustion of diesel in the production process (Above and Bankole, 2018), and this is also confirmed by the fact that 11 % is also due to the transportation of the various inputs, which is done by endothermic-engine vehicles. Finally, an additional 6 % is due to the ammonium sulfate production process, which involves the synthesis of pure NH_3 and sulfuric acid at $60^\circ C$. In the case of OFTE and OFHH, they are both expressed as NO_x eq, which is produced during diesel combustion, and in these two categories, as with GWP, these data are confirmed by the transport phase (15 % for OFTE and 14 % for OFHH) during which NO_x is produced. In the case of TEC and HCNT, again diesel is the main culprit, causing the emission of 6.75×10^4 , and 3.43×10^3 kg 1.4-DCB eq, respectively due to heavy metals released during the combustion process. For TEC, an additional 9 % is generated from the ammonium sulfate production and an additional 9 % from input

Table 2
Results from life cycle impact assessment.

Impact categories	Unit	Mycelium		Horse Manure	Poultry Manure	Agricultural Gypsum (Calcium Sulfate)		Diesel	Ammonium Sulfate		Wheat Straw		Electricity		Transport		Total	
		Value	%			Value	%		Value	%	Value	%	Value	%	Value	%		
Atmospheric effects																		
GWP	kg CO ₂ eq	1.01 × 10 ²	0.40 %	–	–	8.98 × 10 ⁰	0.04 %	2.01 × 10 ⁴	79 %	1.60 × 10 ³	6 %	2.07 × 10 ²	1 %	8.67 × 10 ²	3 %	2.68 × 10 ³	11 %	2.55 × 10 ⁴
SOD	kg CFC11 eq	8.07 × 10 ⁻⁴	2.10 %	–	–	8.52 × 10 ⁻⁶	0.02 %	3.78 × 10 ⁻²	91 %	4.97 × 10 ⁻⁴	1 %	1.24 × 10 ⁻³	3 %	6.66 × 10 ⁻⁴	2 %	1.00 × 10 ⁻³	1 %	4.17 × 10 ⁻²
IR	kBq Co-60 eq	5.59 × 10 ⁻¹	0.10 %	–	–	9.04 × 10 ⁻²	0.01 %	1.08 × 10 ³	98 %	6.12 × 10 ⁰	1 %	5.14 × 10 ⁻¹	0 %	6.71 × 10 ⁰	1 %	3.26 × 10 ⁰	0 %	1.09 × 10 ³
OFHH	kg NO _x eq	4.91 × 10 ⁻¹	0.50 %	–	–	9.60 × 10 ⁻²	0.09 %	8.58 × 10 ¹	81 %	2.33 × 10 ⁰	2 %	4.09 × 10 ⁰	0 %	1.56 × 10 ⁰	1 %	1.54 × 10 ¹	15 %	1.06 × 10 ²
FPMP	kg PM _{2.5} eq	1.90 × 10 ⁻¹	0.30 %	–	–	1.17 × 10 ⁻¹	0.19 %	5.78 × 10 ¹	91 %	1.75 × 10 ⁰	3 %	2.97 × 10 ⁻¹	0 %	9.34 × 10 ⁻¹	1 %	2.08 × 10 ⁰	3 %	6.31 × 10 ¹
OFTE	kg NO _x eq	4.99 × 10 ⁻¹	0.40 %	–	–	9.77 × 10 ⁻²	0.09 %	9.10 × 10 ¹	82 %	2.42 × 10 ⁰	2 %	4.17 × 10 ⁻¹	0 %	1.59 × 10 ⁰	1 %	1.55 × 10 ¹	14 %	1.11 × 10 ²
TAP	kg SO ₂ eq	5.20 × 10 ⁻¹	0.30 %	–	–	5.75 × 10 ⁻²	0.03 %	1.71 × 10 ²	91 %	4.54 × 10 ⁰	2 %	1.24 × 10 ⁰	1 %	2.72 × 10 ⁰	1 %	6.87 × 10 ⁰	4 %	1.87 × 10 ²
Eutrophication																		
FEP	kg P eq	1.26 × 10 ⁻²	3.00 %	–	–	1.05 × 10 ⁻⁴	0.02 %	1.95 × 10 ⁻¹	46 %	1.52 × 10 ⁻¹	36 %	3.47 × 10 ⁻²	8 %	2.59 × 10 ⁻²	6 %	1.00 × 10 ⁻³	0 %	4.22 × 10 ⁻¹
MEP	kg N eq	2.68 × 10 ⁻¹	38.30 %	–	–	4.15 × 10 ⁻⁵	0.01 %	7.22 × 10 ⁻²	10 %	5.63 × 10 ⁻³	1 %	3.40 × 10 ⁻¹	49 %	9.34 × 10 ⁻³	1 %	4.00 × 10 ⁻³	1 %	6.99 × 10 ⁻¹
Toxicity																		
TEC	kg 1,4-DCB	2.56 × 10 ²	0.30 %	–	–	1.08 × 10 ²	0.13 %	6.75 × 10 ⁴	80 %	7.90 × 10 ³	9 %	4.24 × 10 ²	1 %	1.02 × 10 ³	1 %	7.54 × 10 ³	9 %	8.47 × 10 ⁴
FEC	kg 1,4-DCB	2.23 × 10 ⁻¹	0.40 %	–	–	2.02 × 10 ⁻²	0.04 %	5.05 × 10 ¹	91 %	6.90 × 10 ⁻¹	1 %	3.96 × 10 ⁻¹	1 %	3.78 × 10 ⁻¹	1 %	3.57 × 10 ⁰	6 %	5.57 × 10 ¹
MEC	kg 1,4-DCB	3.28 × 10 ⁻¹	0.20 %	–	–	8.64 × 10 ⁻²	0.04 %	1.73 × 10 ²	90 %	6.63 × 10 ⁰	3 %	5.76 × 10 ⁻¹	0 %	1.41 × 10 ⁰	1 %	1.01 × 10 ¹	5 %	1.92 × 10 ²
HCT	kg 1,4-DCB	6.89 × 10 ⁻¹	0.20 %	–	–	2.09 × 10 ⁻¹	0.06 %	3.12 × 10 ²	92 %	1.36 × 10 ¹	4 %	1.04 × 10 ⁰	0 %	1.27 × 10 ¹	4 %	5.50 × 10 ⁻¹	0 %	3.41 × 10 ²
HNCT	kg 1,4-DCB	2.49 × 10 ²	5.60 %	–	–	2.18 × 10 ⁰	0.05 %	3.43 × 10 ³	77 %	4.12 × 10 ²	9 %	3.45 × 10 ¹	1 %	1.56 × 10 ²	3 %	1.70 × 10 ²	4 %	4.46 × 10 ³
Abiotic resources																		
LU	m ² a crop eq	1.64 × 10 ²	14.60 %	–	–	7.10 × 10 ⁻¹	0.06 %	3.92 × 10 ²	35 %	6.57 × 10 ¹	6 %	3.12 × 10 ²	28 %	1.92 × 10 ²	17 %	–	0 %	1.13 × 10 ³
MRS	kg Cu eq	4.80 × 10 ⁻¹	1.00 %	–	–	3.42 × 10 ⁰	6.92 %	3.41 × 10 ¹	69 %	9.01 × 10 ⁰	18 %	6.20 × 10 ⁻¹	1 %	1.63 × 10 ⁰	3 %	9.00 × 10 ⁻²	0 %	4.94 × 10 ¹
FRS	kg oil eq	2.44 × 10 ¹	0.00 %	–	–	2.91 × 10 ⁰	0.01 %	4.85 × 10 ⁴	97 %	5.31 × 10 ²	1 %	2.60 × 10 ¹	0 %	2.68 × 10 ²	1 %	8.22 × 10 ²	2 %	5.02 × 10 ⁴
WC	m ³	7.22 × 10 ⁰	7.10 %	–	–	3.47 × 10 ⁻²	0.03 %	2.11 × 10 ¹	21 %	5.74 × 10 ⁰	6 %	5.06 × 10 ¹	50 %	1.70 × 10 ¹	17 %	2.40 × 10 ⁻¹	0 %	1.02 × 10 ²

transport. Also, for HNCT 9 % (4.12×10^2 kg 1,4-DCB) is generated from the ammonium sulfate production process while a 4 % (1.70×10^2 kg 1,4-DCB) from transportation and a 3 % (1.56×10^2 kg 1,4-DCB) from electricity. Among these data, it is interesting to note how ammonium sulfate affects TEC and HNCT, thus showing adverse effects on ecosystems and human health. In fact, in the first case, although ammonium sulfate, is not harmful to aquatic fauna in the long term, it may be harmful to fish in the short term. In the second case, on the other hand, several studies, have reported that the effects of ammonium sulfate inhalation could include noncancer effects such as asthma (de Vooght et al., 2010), inflammation (Last et al., 1982), and damage to reproductive functions (Bae et al., 2020). Finally, the last category in which diesel has the greatest impact is MRS (69 %) but it is noteworthy that ammonium sulfate also has a nonnegligible impact (9.01×10^0 kg Cu eq, or 18 % of total MRS). MRS is most likely due to the depletion of fossil resources used for diesel and ammonium sulfate production, as this could lead to a general increase in prices, which results in a consequent increase in the extraction of mineral raw materials. Finally, in FEP, MEP, and WC, a major contribution is made by Ammonium Sulfate (36 % in FEP), Mycelium and Wheat Straw (38.3 % and 49 % in MEP), and Wheat (50 % in WC), respectively. Especially, in the case of FEP, 36 % of the contribution (1.50×10^{-1} kg P eq) comes from ammonium sulfate. This is because its production generates a large amount of wastewater with a high concentration of ammonia nitrogen, which, although it can be used as a nutrient of microalgae, high levels could inhibit their growth, as also noted by Guo et al. (2021) and Qin et al. (2021). Regarding MEP, the contribution to the impact categories is divided between Mycelium (2.70×10^{-1} kg N eq, or 38.3 % of the total) and Wheat Straw (3.40×10^{-1} kg N eq, or 49 %). In the former case, the impacts are probably due to the mycelium production that begins with the selection of the growth medium (or coating layer), which is generally represented by sorghum, wheat, or rye seeds (Leiva et al., 2015). Indeed, the physical structure of the seeds is a favorable element for the growth and development of mycelium as a lignocellulosic source. Therefore, MEP for mycelium production could be affected by rye preparation, which requires fertilizers. Likewise, even in the case of straw production, MEP is most likely influenced by the fertilizers used in its formation. Again, for reasons related to grain growth, straw also affects the WC (50 % of the total impacts). Regarding the latter impact category, a residual share of impacts is related to diesel (2.12×10^1 m³ of water, or 21 % of total WC) and electricity mix production (1.70×10^1 m³, or 17 %). In this regard, literature studies (Wang et al., 2018) show how some of the depletion of water resources in the electric mix production chain could also be due to the water supply for the cooling towers of power plants, which are fueled by fossil fuels.

3.1. Sensitivity analysis

Since the amount of diesel cannot be changed for production reasons (i.e., drive of machinery), the improvement options focused especially on the use of manure, wheat straw, calcium, ammonium sulfate, and electricity (Table 3), thus indicating three alternative improvement options.

The results of the SA are expressed in Fig. 3. Regarding atmospheric effects (Fig. 3A), the preferred option is S4, as it induces improvements in all 7 impact categories. For example, compared with S1, GWP goes from 2.55×10^4 to 2.50×10^4 , or SOD goes from 4.17×10^2 to 4.15×10^2 , in both cases with a -2 % reduction, while for the remaining there is a -1 % reduction. The observed improvements in environmental performance are most likely attributed to the reduction in electricity consumption from fossil fuels, achieved through the installation of photovoltaic panels, as well as the decreased use of mycelium. Regarding eutrophication and toxicity on the other hand (Fig. 3B), even in these cases, S4 is the preferred option over the other three. In this case, these reductions are most likely due to a lower use of straw, the amount of which was almost halved in three years (-43 %) as well as

Table 3

Variation of input parameters for sensitivity analysis.

Input/Output	Unit	(S1)	(S2)	(S3)	(S4)
		2018	2019	2020	2021
Input					
Horse manure		18,000	20,000	20,900	24,000
Wheat straw		3500	3200	2800	2000
Poultry manure		2700	2400	2800	2800
Agricultural Gypsum (Calcium Sulfate)	kg	1200	960	1150	1000
Ammonium sulfate (solid)		800	900	1100	700
Ammonium sulfate (liquid)	m ³	0.11	0.11	0.11	0.16
Mycelium (<i>Agaricus bisporus</i>)	kg	980	980	980	900
Diesel	m ³	50	50	50	50
Electricity	kWh	2016	1761	1633	1600
Water	m ³	168	168	168	168
Output					
Substrate	kg	23,000	23,000	23,000	23,000

due to a reduction in the mycelium. As for toxicity, on the other hand, again there are reductions, although more pronounced, between S1 and S4, reductions that are -2 % (TEC), -1 % (FEC, MEC and HCT) and -3 % (HNCT). Finally, even in the case of abiotic resource depletion, S4 is the preferred option over the other three (Fig. 3C), with lower land consumption (from 1.13×10^3 to 9.31×10^2 m²a) due to less area for straw cultivation, which therefore also induces water savings of -26 % (from 1.02×10^2 to 7.55×10^2 m³). The preferred option is S4, as it induces the greatest reduction in impacts compared to the other three. Therefore, the company was able to devise a new recipe, which involves a lower consumption of straw that is balanced by a higher consumption of horse manure, which turns out to have a minimal environmental impact. To ensure product quality, however, it was necessary to keep the compost structure unchanged, and it was, therefore, essential to also change the amounts of poultry manure and gypsum.

The company has also recently installed meters for greater control over electricity consumption as well as photovoltaic systems, so as to reduce electricity consumption, significantly lowering the production of CO₂, resulting in economic and environmental benefits.

3.2. Comparison with other LCAs for mushrooms production

The results of our study show that a 23,000 kg substrate emits about 25,049 kg CO₂ eq, and since the yield is 11,000 kg of mushrooms per substrate, it is possible to consider how 1 kg of mushrooms emits 2.28 kg CO₂ eq. In order to assess the validity and consistency of our findings, we conducted a comparative analysis by examining data from other studies that investigated the environmental impacts of various mushroom species. It is worth noting that the existing literature on the assessment of the environmental impacts of mushroom production is relatively limited, and only a few studies have been previously published. Therefore, it was possible to compare and verify our results with a small number of articles available in the literature. For instance, Gunady et al. (2012) examined the carbon emissions of *Agaricus bisporus* in Australia and reported emissions of around 2.72 kg of CO₂ eq per 1 kg of mushrooms. Similarly, Leiva et al. (2015) found emissions of approximately 4.41 kg of CO₂ equivalent per 1 kg of *Agaricus bisporus* in their study. Furthermore, Robinson et al. (2019) investigated the carbon footprint of *Agaricus bisporus* and reported a range of emissions between 2.13 kg and 2.19 kg of CO₂ eq per 1 kg of mushrooms. Additionally, Dorr et al. (2021) explored the emissions of *Pleurotus* and found a range of 2.99 kg to 3.18 kg of CO₂ eq per 1 kg of mushrooms. Comparing our study results with these findings, it is possible to note how our emission estimates for mushroom production are in line with the previous body of research. In these regards, it is worth highlighting those differences in the farm-

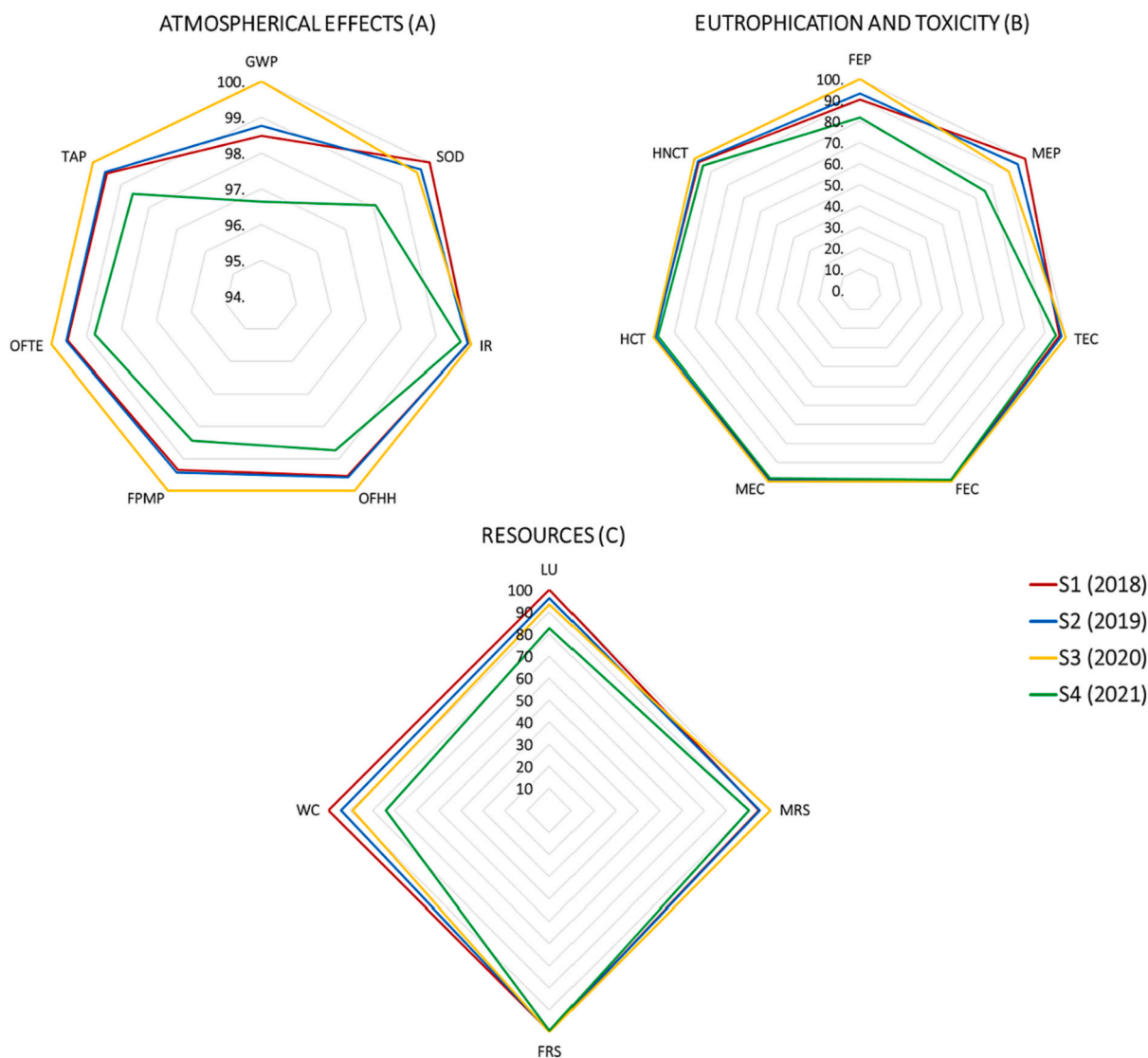


Fig. 3. Sensitivity analysis results.

specific cultivation practices, substrate materials as well as climate conditions, background systems, and modeling choices can represent a source of variability in LCA results. Despite these variations, the overall range of emissions per kilogram of mushrooms remains relatively similar. These results, therefore, are consistent with and reinforce the findings of previous research, arriving at comparable estimates of emissions for mushroom production, contributing to a more comprehensive understanding of the environmental impact of this industry. In addition, it is also important to underline that also pedo-climatic conditions (especially in terms of temperature and humidity) were regarded as key meteorological variables for growing straw mushrooms. In particular, the study of [Robinson et al. \(2019\)](#) observed a relation between GWP intensity and regional variance, mainly influenced by grid electricity generation, fuel, and upstream water consumption. Different requirements in terms of energy demand in certain climate zones could lead to an increase in greenhouse gas emissions, as well as fuel consumption for transport and logistics. For example, considering different energy mixes at the regional level between literature cited countries for mushroom production, mainly Spain, France, Australia, and the U.S.A., it was possible to highlight that regions consuming more renewables (such as Spain: 28 %, and Australia: 29 %) and less coal resulted into lower GWP per megajoule generated ([Gunady et al., 2012](#); [Leiva et al., 2015](#)). In this sense, renewable energy technologies, particularly solar

and wind power, have a key role in the reduction of GWP, when used as an alternative to conventional fossil fuel-based energy sources. The energy use for the Funghitex production process proves to be markedly lower (0.087 kWh/kg mushrooms) compared to other studies in the literature, on a per kilogram of mushroom basis, reporting energy requirements ranging from 0.26 kWh to 0.56 kWh/kg mushrooms ([Robinson et al., 2019](#); [Leiva et al., 2015](#)). The differences observed in emissions between our study and other research are mainly due to two factors: i) different modeling choices, such as data sources, and the selection of GWP conversion factors used for carbon accounting, which lead each study to make specific assumptions or use different methodologies to assess environmental impacts, generating variations in the reported results; ii) regional and geographic factors, which affect the final energy mix. Regions with greater integration of renewables and less reliance on coal-based electricity generally induce a lower GWP per megajoule generated, resulting in lower overall environmental impacts. In [Robinson et al. \(2019\)](#), for example, the location of the production site influences the electricity mix. Producing within a western rather than an eastern region allows more or less renewable energy produced on-site through a biomass gasification unit to be used, thus helping to reduce the electricity demand of the grid, in turn reducing emissions. The above factors, therefore, help to better understand the nuances in emission estimates across studies and regions, emphasizing the importance of

adopting location-specific data, uniform calculation methodologies, and considering regional energy mixes. Furthermore, the availability and use of renewable energy sources (i.e., solar and wind energy), could positively influence the mitigation of impacts associated with GWP. In this sense, Italy has been actively promoting the use of renewables to reduce its reliance on fossil fuels, and in 2021, the Italian electricity system relied on 42.3 % of on-site renewable energy production (GSE, 2021), representing the third-largest producer of renewable energy in Europe. However, the results of our study could also change by considering regional differences in terms of on-site renewable energy production. For example, it is useful to note that northern Italy generally receives less solar irradiance (about 3.6 k kWh/m²/day) than central and southern Italy (about 5.4 k kWh/m²/day) (ENEA, 2023), mainly due to higher latitude and the presence of mountainous terrain that can cause shading and reduce available sunlight. Therefore, considering the above, taking into consideration a production of 416 kWh of electricity from photovoltaics by the company in question, should it be in northern Italy it could benefit from slightly less irradiation (by 10–20 % less), producing solar energy of about 332–374 kWh. Conversely, if the Funghitex farming company would be in southern Italy, it could receive slightly more irradiance (by 10–20 % more), producing an energy of 457–499 kWh. In both cases then, there would be an increase or decrease in the amount of net energy, as shown in Table 4, which would affect, albeit slightly, the number of emissions generated (Table 4).

Therefore, by selecting a suitable location with optimal solar irradiation, mushroom farms could maximize their potential for on-site renewable energy production. This can lead to greater energy self-sufficiency and less dependence on external energy sources, which often have associated environmental impacts. However, it is important to note that the solar irradiance values presented are estimates and may vary depending on the specific location and prevailing weather conditions. Factors such as latitude, cloud cover, and shading from surrounding structures or topography can influence the actual solar energy received. Therefore, it is important for mushroom farm operators to conduct site-specific assessments and consider local climate data to accurately determine the expected solar irradiance.

3.3. WENCF nexus: recycling scenario vs non-recycling scenario

To quantify the savings in resources and avoided emissions, an additional scenario (S5) was created in which water and ammonium sulfate are added from time to time, assuming also that the same amount of water that is used as input in S4 is purified in S5 as it should actually be. This scenario contrasts with S4, where water and ammonium sulfate are constantly recycled and re-injected. The WENCF nexus between S4 and S5 was then calculated (Fig. 4).

In S4 (Fig. 4A), through wastewater recovery, it might be possible to reduce the overall WF of the entire production process, which is associated with the consequent reduction of the energy used for its extraction as well as the total CF and Nitrogen emissions. This could be attributable to the following reasons.

1. First, the water supply chain is highly energy intensive and has a high environmental impact due to the process of generating electricity (Giron, 2014), which is required for its extraction (wells), treatment

(processes and sludge removal), transmission and distribution, cooling and heating of power towers, resulting in climate-changing emissions (0.3–0.7 kg CO₂ per 1 kWh of energy) (Alresheedi et al., 2022). Moreover, in S5, if the water was not recycled and used as a production input, it would have to be purified as prescribed by legislative decrees 152/1999, 152/2006, and Law 167/2017, and the most surprising finding in our study is about the CF of the final process, which increases from 2.43×10^4 kg CO₂ eq in S4 to 1.46×10^6 kg CO₂ in S5. This is because, as also reported in literature (Alresheedi et al., 2022; Cieri et al., 2022; Zhang and Liu, 2022), wastewater treatment is also associated with high energy and water consumption, leading to a significant amount of GHG emissions. Within this study, on the other hand, a more sustainable wastewater recycling approach is shown to reduce water withdrawals from natural water systems as well as their pollution, due to indiscriminate discharge of untreated wastewater, as shown by WF (7.83×10^1 m³ in S4 vs 1.76×10^2 m³ in S5, with a reduction of –46 %). Consequently, direct emissions (generated at wastewater collection and discharge points) and indirect emissions (electricity consumption, use and transfer of chemicals during in-process sludge treatment) are reduced, avoiding at least 1.44×10^6 kg CO₂ eq per FU, recycling water for food production and saving energy (–1.64 MJ eq) for water extraction.

2. Secondly, water recycling also induces the recycling of ammonium sulfate, which is commercially used as fertilizer (21 % N and 24 % S). In the case of the company in question, ammonium sulfate is obtained by the direct reaction between H₂SO₄ and NH₄, the latter being retained and converted by means of a scrubber. Without in-house water recycling (S5), it would need to be supplied externally, whereas, through water recycling (S4), there is a reduction in WF, as well as in energy and climate-changing emissions. In fact, by recycling (NH₄)₂SO₄, a dispersion of nitrogen (5.29×10^{-1} kg N eq for S4 vs 7.57×10^{-1} kg N eq for S5, with an N reduction of –43 %) and ammonia into the air would be avoided, also in view of the fact that atmospheric NH₃ levels in Italy were 334.59 kt in 2020, 95 % of which came from the agricultural sector (ISPRA, 2021). By recycling ammonium sulfate instead, the company could contribute to achieving the targets set by the NEC Directive (2016/2284), namely –5 % reduction in ammonia emissions.

Therefore, the results of the scenario analysis show how a circular approach to wastewater reuse could potentially be effective in promoting sustainable resource use, thus establishing an interesting nexus. In fact, it is shown how for food production through wastewater recycling, it might be possible to reduce water to produce energy and to produce food, as well as to reduce energy to produce water and food, ultimately reducing energy use by the water system and water use by the energy system for food production, associated with a reduction in total CF and emissions. It is therefore shown how the recovery of wastewater that is destined for food production could be a way to maximize its economic, environmental, and social value, oriented towards the need to manage water resources in a circular way, rather than transforming it entirely into drinking water. Instead, through a recycling approach, it might be possible to reduce the generation of energy, the use of water that is destined for other food production, as well as to recover ammonium sulfate, thus providing a useful way to contribute to food security, without compromising water and energy security.

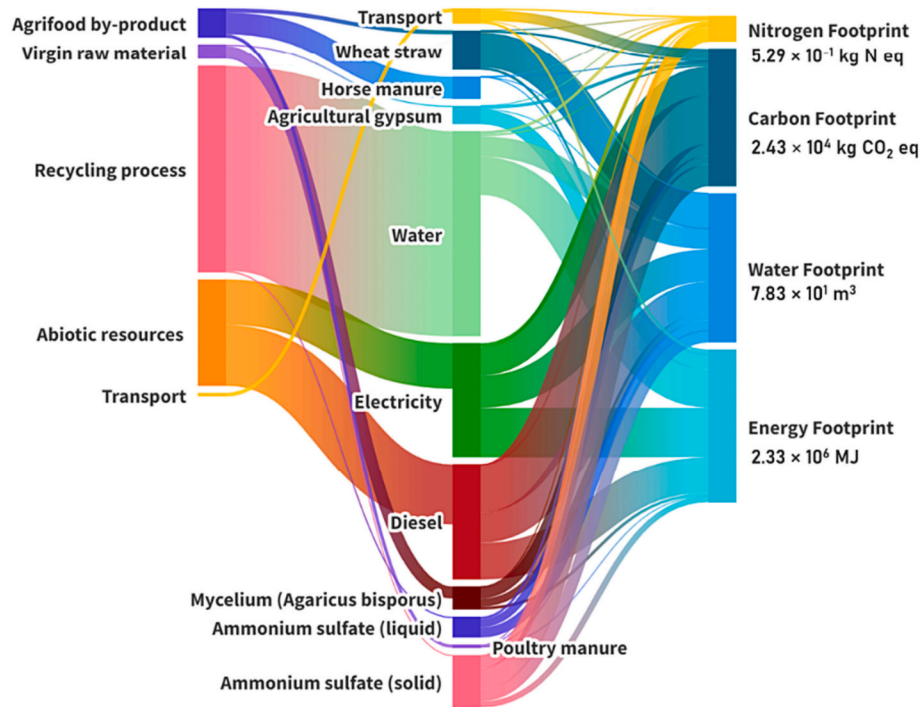
4. Conclusions, limitations, and future perspectives

This study assessed the environmental compatibility of *Agaricus bisporus* production in central Italy through an integrated Water-Energy-Nitrogen-Carbon-Food (WENCF) footprint assessment, considering an LCA approach. The results of the analysis show that, per functional unit (23,000 kg of the substrate), diesel impacted more in 16 of the 18 categories considered, highlighting IR and FRS as the largest contributors.

Table 4
Energy and GWP changes by irradiance in southern and northern Italy.

Location	Required energy	Energy from solar	Net energy	GWP (FU)
Central Italy		416 kWh	1600 kWh	25,049 kg CO ₂ eq
North Italy	2016 kWh	332–374 kWh	1684–1642 kWh	25,067–25,085 kg CO ₂ eq
South Italy		457–499 kWh	1559–1517 kWh	25,013–25,031 kg CO ₂ eq

A) SCENARIO 4: RECYCLING



B) SCENARIO 5: NON RECYCLING

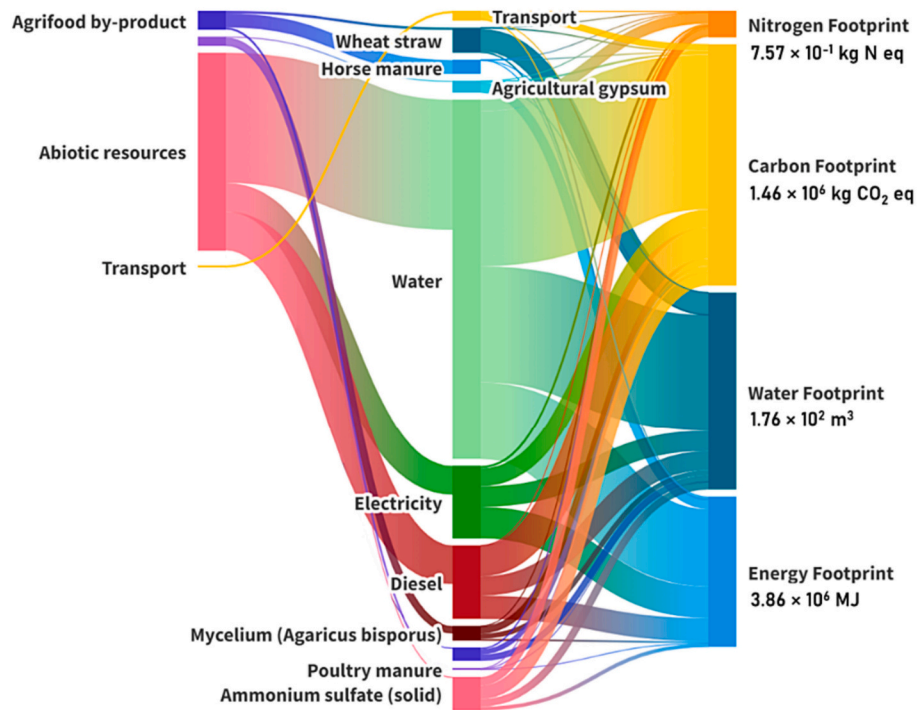


Fig. 4. Difference between the two scenarios. S4: Recycled water and ammonium sulfate; S5: Non-recycled water and ammonium sulfate.

The amounts of straw and ammonium sulfate also showed higher impacts on WC (50 %), LU (28 %) for straw and FEP (36 %), and MRS (18 %) for ammonium sulfate. Considering the environmental responses of mushroom production, a sensitivity analysis was carried out for four scenarios, thus showing that lower consumption of straw (−43 %), agricultural gypsum (−17 %), solid mycelium (−13 %), ammonium sulfate (−8 %) and electricity from fossil fuels (−21 %), offset by higher consumption of horse manure (+33 %) and poultry manure (+4 %), could allow the farm to obtain the same quality of mushrooms, balancing the final compost structure. This resulted in a decrease in environmental impacts of −5 % on average, with values ranging from a low of −1 % to a high of −26 %. Finally, to evaluate the environmental effects of the water and ammonium sulfate recycling strategy, an additional scenario (S5) was created in which these two elements, instead of being continuously recycled, are externally supplied each time ex novo for each production cycle, including that water is dispersed into the environment and purified. Thus, the holistic water-energy-nitrogen-carbon-food nexus was considered. The results show that the recovery strategy (S4) could lead to a reduction of WF by −56 %, which induces a reduction of EF by −40 % and NF by −30 %. Thus, there were three main contributions of the study: it is shown how sustainable food production can result from efficiency improvements within the system rather than from further integration of circular principles. A comprehensive life cycle inventory of mushroom production in Italy is provided so that it can also be used for other LCA studies. In particular, understanding these results could help create useful comparisons to reduce the impacts of the agri-food system. Finally, recommendations are provided to suggest where improvements can be made within the commercial mushroom production system, from cradle to gate. These recommendations include reducing the consumption of straw, gypsum, mycelium, ammonium sulfate, and energy, as well as increasing manure, also being able to open paths of industrial symbiosis in this way. However, it is important to note that although this case study provides valuable insights, its selection is not intended to represent a comprehensive example of mushroom production. In this regard, it is indeed important to recognize the limitations and scope of the study. Indeed, the case study represents a specific production system and may not necessarily be representative of all mushroom production practices worldwide. The findings and conclusions of this study contribute to the existing body of knowledge on environmental impacts in mushroom production, but further research is needed to capture the diversity of production systems in different regions and countries.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.166044>.

CRediT authorship contribution statement

Giuliana Vinci: Conceptualization, Methodology, Software.
Marco Ruggeri and Sabrina Antonia Prencipe: Data curation, Writing- Original draft preparation.
Federica Perrotta: Visualization, Investigation.
Luigi Pucinischi: Supervision.
Giuliana Vinci: Software, Validation.
Marco Ruggeri and Sabrina Antonia Prencipe: Writing- Reviewing and Editing.

Funding

The authors declare they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

Data will be made available on request.

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