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Life cycle assessment of microbial plant biostimulant production for application in sustainable agricultural systems

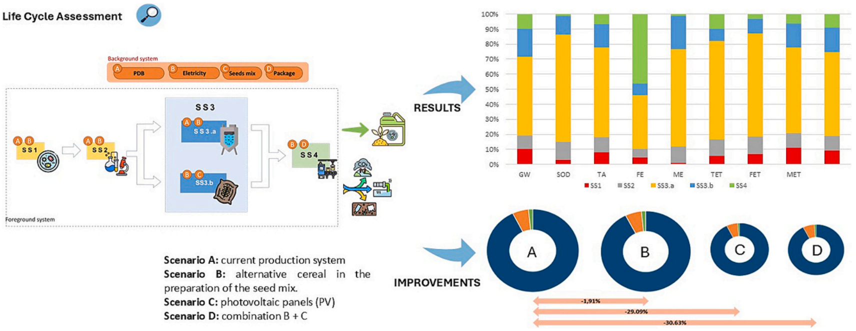
Claudio Calia [a], Sara González García [b], Carlo Ingrao [e,*], Giovanni Lagioia [c,e], Claudia Ruta [a], Nicola Secchi [d], Giuseppe De Mastro [a]

[a] Department of Soil, Plant and Food Science, University of Bari Aldo Moro, Via Amendola, 165/A, 70126 Bari, Italy
[b] CRETUS, Department of Chemical Engineering, School of Engineering, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain
[c] Faculty of Economic, Political and Social Sciences, Catholic University "Our Lady of Good Counsel", Tirana, Albania
[d] EUROVIX S.p.A., Viale Enrico Mattei, 17, 24060 Entratico, BG, Italy.
[e] Department of Economics, Management and Business Law, University of Bari Aldo Moro, Via Amendola, 165/A, 70126 Bari, Italy

HIGHLIGHTS

- The field of biostimulants was investigated in this study.
- Life Cycle Assessment was applied for the purpose.
- Primary data were used and were combined with secondary ones.
- Potato Dextrose Broth and electricity consumption are key impact contributors.
- Improvement solutions were proposed accordingly.

GRAPHICAL ABSTRACT



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ABSTRACT

By 2050, the global population will face increasing food demand due to population growth, prompting the need for sustainable agricultural practices. The European Union aims to address these challenges by reducing fertilizer and pesticide use, expanding organic farming, and limiting global warming to 1.5 °C. Biostimulants have emerged as promising natural tools to boost agricultural productivity by 8–30 % with minimal application per unit area. This study was aimed at assessing the environmental impact of producing Trichoderma-based biostimulants using the life cycle assessment (LCA) methodology, to identify environmental hotspots and propose improvements The research reconstructed the biostimulant production cycle and inventoried inputs and outputs for each step. Environmental impacts were evaluated using the ReCiPe 2016 Midpoint method for the current process and the Endpoint method for proposed improvements. From the study, it was found that the liquid substrate production phase had the highest environmental impact, contributing 368 kg CO_2 eq emissions in the global warming category. Key contributors were Potato Dextrose Broth (40 % of emissions) and electricity consumption (44 %). To mitigate these impacts, two measures were proposed by the authors: replacing Potato Dextrose Broth with winter cereals and transitioning to self-generated electricity. These optimizations reduced the overall environmental footprint by approximately one-third, from 16.26 Pt to 11.28 Pt.

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* Corresponding author.
E-mail address: carlo.ingrao@uniba.it (C. Ingrao).

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The study contributed to understanding the potential of biostimulants as sustainable alternatives in green agriculture, offering insights into optimizing production processes to balance increased productivity with environmental sustainability. Biostimulants thus represent a key tool in addressing future agricultural challenges while minimizing ecological impacts.

1. Introduction

Climate change and the growing global population are the two major problems that humankind will have to face in the foreseeable future. The world population is projected to reach 9.7 billion people in 2050 (Lago-Olveira et al., 2024), posing the big global challenge of how to produce enough food for all sustainably. Rapid population growth increases resource depletion, to produce the food and non-food commodities that are increasingly demanded and consumed by people all over the world. In addition to this, the raw material preparation and transformation processes are highly energy consuming and greenhouse gas (GHG) emitting, with the consequence of enhancing the climate change impact and exposing more people to climate-related risks (Washington and Kopnina, 2022).

The global average surface temperatures are expected to increase by 0.3 to 4.8 °C by the end of this century. The imbalance caused by the carbon and nitrogen (N) cycles on the planet is a major driver of GHG fluxes and contributes to more than 90 % of anthropogenic climate warming (Ma et al., 2025). As temperatures continue to rise, more soil desertification and salinization will be observed, thereby affecting agricultural productivity and plant growth (Hassani et al., 2021). As incomes rise, people will increasingly consume more resource-intensive and animal-based foods and, so, animal-centred diets will put increasing pressure on crop productivity worldwide (Henchion et al., 2014; Kuzmanovski et al., 2019; Nunes, 2023). Global demand for animal-derived food products is expected to increase by more than 70 % by 2050, following global population growth and animal welfare standards: the result of this will be the increased production of protein-rich forages (Deolu-Ajayi et al., 2022). As animal welfare improves, the proportion of animal-based food products in daily diets also increases. For example, in Western diets, the proportion of animal-protein intake has increased from 40 % in the 1960s to over 60 % today (Deolu-Ajayi et al., 2022; Jalil et al., 2020). Consequently, the demand for animal feed is expected to increase, since, up to 6 kg of plant protein is required to produce 1 kg of animal proteins (Deolu-Ajayi et al., 2022). At the same time, it is urgent to adopt climate change mitigation strategies in the agrifood sector, considering that one-quarter of the world's GHG emissions come from agriculture, forestry, and land-use change (Ahmed et al., 2020). Agricultural production largely contributes to – and is significantly influenced by - climate change. In this sense, agrochemicals have been at the centre of the agricultural sector in recent decades to improve growth and productivity, but their excessive use represents a threat to the environment and the ecosystem (Hamedani et al., 2020; Zingale et al., 2024). Solutions are, therefore, needed to address the key challenges of agricultural production in an environmentally friendly, cost-effective and food security-oriented manner for future generations. In the last years, various solutions have been proposed to improve the sustainability of production systems using innovative techniques that allow for agrochemical use reduction. In 2020, the European Commission (EC) introduced the 'Farmto Fork Strategy', setting a set of ambitious targets for the agrifood sector, that include: reducing the use of chemical pesticides by 50 % by 2030; reducing nutrient losses by at least 50 %; cutting fertilizer use by 20 %; and allocating 25 % of the agricultural area to organic cultivation (EC, 2020). The use of biostimulants, especially when combined with organic farming, can be an effective sustainability measure in agriculture (Kheiralipour et al., 2024). Biostimulants represent an innovative and promising strategy to reduce the use of chemicals in agricultural production by improving the physiological and metabolic responses of plants to biotic and abiotic stresses (Magnabosco et al., 2023; Owen et al., 2015; Vishwakarma et al., 2022). Plant or agricultural biostimulants can be produced from different sources, even waste material. In this regard, biostimulants derived from algae, microbes, and other natural sources are gaining interest as a sustainable strategy that, when applied to plants or the rhizosphere, allow:

- improve the innate capacity of treated plants to cope with tolerance to abiotic stress and increase productivity;
- make more efficient use of available resources nutrients (Romagnoli et al., 2024; Shukla and Prithiviraj, 2021; Singh et al., 2018).

However, the definition and concept of agricultural biostimulants are still under development, which is partly due to the diversity of inputs that can be considered biostimulants. With regard to this point, it is important to clarify that biostimulants do not have direct action against pests, so they do not fall within the regulatory framework of pesticides (Calvo et al., 2014). The term biostimulant was first coined by Zhang and Schmidt (1997) to describe substances that, in small quantities, improve plant growth. The European Regulation EU 2019/1009 (EC, 2009) defines biostimulants as a product that stimulates plant nutrition processes intending to improve efficiency in terms of nutrient use, tolerance to abiotic stress, quality characteristics and the availability of nutrients in the soil or rhizosphere. According to the literature (marketsandmarkets, 2024), the biostimulants market is estimated to be worth $4.3 billion by 2024 at a compound annual growth rate of 12 % and will continue to grow to $7.6 billion by 2029 (marketsandmarkets, 2023). The use of biostimulants could contribute to expanding the organic food industry, with a projection of reaching a value of $27.9 billion by 2028.

Biostimulants may contain microorganisms and/or hormone-like substances, whose function is to stimulate processes of nutrients uptake, tolerance to biotic and abiotic stress, and improve crop efficiency and quality (Franzoni et al., 2022). It is almost impossible to isolate and study a solitary compound, which makes it highly improbable that the efficacy of a biostimulant can be attributed to a single component. Instead, it is believed that synergistic interactions between various bioactive molecules are responsible for its efficacy (Romagnoli et al., 2024; Rouphael and Colla, 2018; Vijay Anand et al., 2018). Algae biostimulants offer a great plant-growth potential, mainly due to the high content of bioactive metabolites, such as peptides and amino acids, and plant hormones (e.g., cytokinins). Microalgae are single-celled, phototrophic organisms that are characterized by a high photosynthetic efficiency and a high adaptation capacity, so much so that they can grow in non-potable waters, such as industrial ones. They have an enhancing impact on plant growth and soil health, probably due to the presence of N-fixing enzymes, soluble amino acids, bio-mineral conjugates, polysaccharides and phytohormones. The specialised literature has confirmed that microalgae metabolites improve soil fertility, provide plant resiliency against biotic and abiotic stresses and, at the same time, improve the uptake of soil nutrients such as phosphorus, potassium and nitrogen (Parmar et al., 2023; Rojo et al., 2024). The application of microalgae biostimulants has increased significantly in recent years, with the aim of replacing commercial chemical fertilizers with biologically based molecules (Rojo et al., 2023; Singh et al., 2018). The cultivation of algae in the open sea eliminates the need for land use in competition with food production and may help to mitigate climate change (Deolu-Ajayi et al., 2022; Duarte et al., 2017; Singh et al., 2018). In addition, algae extracts have demonstrated the ability to increase resilience against abiotic stresses in numerous crops (Baum et al., 2015; Khan et al., 2009). Moreover, the application of seaweed biostimulants has been documented as effectively increasing protein production in protein-rich crops (Deolu-Ajayi et al., 2022; Petropoulos, 2020). Open-field applications of biostimulants can lead to average yield increases ranging from 8.5 % to 30.8 %: in particular, single foliar sprays proved effective in increasing yield, with a substantial increase of 14.9 %, while soil applications of biostimulants resulted in a yield increase of about 10 % (Niu et al., 2021; Li et al., 2022). Vegetables and legumes were documented in the literature to be the crops that respond best to biostimulant applications, with an increase in yield per hectare between 8.5 and 30.8 % (Hafeez et al., 2006; Li et al., 2022).

All that stated, despite the capacity to replace or complement chemical fertilizers and, at the same time, improve agricultural productivity, the relevant environmental sustainability issues connected with biostimulants need to be considered. This starts with assessing the major environmental impacts of biostimulants and the improvements that can be made in their production and application, thereby contributing to making them quality- and sustainability-sound: Life Cycle Assessment (LCA) can be valid and effective for such a purpose. It is a powerful environmental knowledge tool, that is generally used for communicating ecological information on products and services, and for comparing a set of pre-designed environmental-performance improvement scenarios (Ingrao and Wojnarowska, 2023; Zingale et al., 2024). LCA is a globally-recognized scientific method, that allows for identifying and quantifying environmental impacts and damages from resource exploitation and material emissions, throughout the entire life cycle of a given product (Guarnaccia et al., 2025).

To the authors' knowledge, though LCA has already been applied to explore and improve the relevant environmental-sustainability issues of algae-based biostimulants (Vijay Anand et al., 2018; Rojo et al., 2024; Rojo et al., 2023; Rouphael and Colla, 2018), there are no LCA applications with the same purpose in the field of microbial-based biostimulants. To contribute to filling this gap in the literature, this team of authors performed an environmental LCA of a *Trichoderma* spp. biostimulant production, with the twofold research question of understanding where the critical points are and what improvements can be made. It is expected that the consumption of electricity for the whole process is one major environmental impact and that improvements can be achieved using renewable energy sources. An Italian company was involved in the study development and provided all technical support needed from the early stages of study setting and system modelling to data collection and inventory. Finally, studies such as this can help to increase the knowledge and awareness of farmers, producers, policy and decision makers and other stakeholders about the environmental profile of biostimulants, considering they are increasingly being used to improve quality and yield aspects of agricultural systems.

2. Materials and methods

An attributional LCA was applied in this study, following the ISO 14040 and 14,044 standards (ISO, 2006a, 2006b), which means that it was developed through the phases of goal and scope definition, life cycle inventory (LCI), life cycle impact assessment (LCIA), and life cycle interpretation, which were described in the following sections.

2.1. Goal and scope definition

This LCA was aimed at identifying environmental hotspots and improvements in the production of microbial-based biostimulants, to support micro-level decision making on the company scale: to this end, following Ingrao et al. (2017), an attributional approach was used by this team of authors.

As an essential part of this LCA development step, both the functional unit (FU) and the system boundaries were defined, in a way that was consistent with the aim and scope of the study and was most representative of the system service The FU was set at the industrial scale to be a1m^3 batch, corresponding to one thousand containers of 1 l ready-to-use biostimulant. The choice of this FU was made to optimise the inventory phase, by more easily relating inputs to outputs and to the associated environmental impacts, and to favour comparisons with previously published LCAs on biostimulants, given the widespread practice of using volume-based FUs (Egas et al., 2023; Rojo et al., 2024). As far as the system boundaries are concerned, a cradle-to-gate approach was used for the assessment, meaning that the downstream phases of application and final fate of the biostimulants at issue were cut off from the system. To be excluded were also the transport activities for raw material supply to the factory gate and delivery of the biostimulants to the place of use for farming purposes. The reasons behind this exclusion stay in the authors' decision to focus only on the production cycle, to make the created system model reproducible anywhere in the world. On the contrary, the system studied covered the whole process from the preparation of the raw materials to the production of the ready-to-use biostimulant, including the management of the generated waste.

For this LCA development, the authors considered the case of EUROVIX S.p.A., a leading company in the biostimulant production sector. They did it, considering the wide range of microbial-based plant biostimulants on the market and the limited availability of data on the environmental impact of their production.

The production process has been analysed in detail, allowing the system to be modelled to have access to all inventory data. Fig. 1 depicts the system boundaries as well as the flow chart of the microbial-based plant biostimulant production system.

The overall foreground production line has been divided into four main steps:

- **Pre-culture (SS1)**: this is the first step of the production process, in which a starter culture of microorganisms is added within a sterilized solution of water and potato dextrose broth (PDB). PDB is a growth medium that is widely used in the growth of microorganisms and fungi. It has been used because it is one of the most common and efficient substrates in microbiology (Karpagavalli et al., 2024; Obiedallah et al., 2024). The solution is set at 28 °C for 5 days in an incubator;
- **(2) Pre-fermentation step (SS2),** which takes place in a 150 L fermenter to which 7 L of pre-culture (two cycles) is added and subsequently brought up to volume with a previously sterilized solution of water and PDB. The culture inside the fermenter remains for 5 days at a temperature of 28 °C.
- **(3) Microorganisms' multiplication (SS3)**: it is characterized by the separation of the pre-fermented stream from SS2, in which a part of the product (5 L) is used as inoculum for the solid culture and the rest (145 L) is used as inoculum for the 1000 L fermenter. This step is developed through two parallel sub-steps, namely:

o **Liquid fermentation (SS3.a),** which is based on the addition and filling of the 145 L of pre-fermented stream with a previously sterilized solution of water and PDB. The growth times and temperatures are the same as in the previous step; and

o **Solid substrate (SS3.b),** aiming at achieving the multiplication of those microorganisms that are characterized by the need for a normal level of oxygen. The substrate used is a mixture of grains chosen and used by the company, which is previously sterilized in an autoclave and then inoculated under a laminar flow hood.

- **Biostimulant final preparation (SS4)**: this is the last step before obtaining the finished product and is structured as follows: the liquid substrate, colonized by microorganisms, 'washes' the solid substrate and cleans the seeds of the spores present in them. The resulting liquid is mixed to homogenize the microbial colonies present in the solution and is subsequently used to prepare the final product: 1 L biostimulant packages. The washed seeds are composted in a plant that is close to the farm.

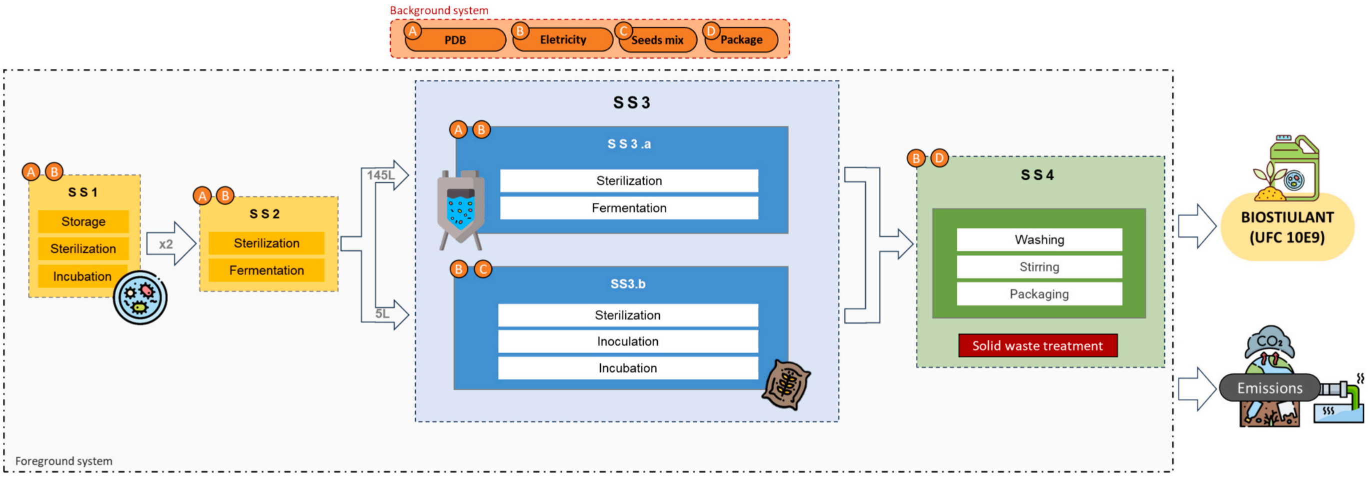

Fig. 1. Flow chart of the microbial biostimulant production system under study.

2.2. Life cycle inventory

This phase provides the combined qualification and quantification of the most important process inputs and outputs within the system boundaries. To that end, both primary and secondary data are generally used by LCA practitioners, to describe the system both technically and environmentally. Primary data are specific and accurate raw first-hand data that are collected and/or measured from the foreground part of the system, that is, the ensemble of processes directly related to the product or service being assessed Whereas, secondary data are existing information on the environmental performance of materials and processes that are drawn from reference literature sources and databases, and serve as the background part of the system (Ingrao et al., 2021). Putting it in simple words, the foreground portions of the system are designed by practitioners, whereas the background processes are selected and adapted (Kuczenski et al., 2018). So, overall, it can be said that using primary data is important as it:

- contributes to making LCAs more credible, through accurate raw data that are specific to a given footprint study, instead of relying on averages from third-party LCI databases; and
- provides practitioners with more reliable, authentic and objective footprint insights, thereby allowing for more focussed and effective sustainable efforts and giving companies more ownership over their LCAs.

In this study, the primary data listed in Table 1 were collected on site, using specifically designed questionnaires and conducting interviews with the EUROVIX operators. During data collection and allocation, this article's authors made sure that all step-by-step mass balances were satisfied, thereby correctly accounting for the mass flow throughout the investigated system. In each step of the biostimulant production process, electricity consumption was computed through direct measurement tools: the obtained values were reported in Table 1. The authors did so following Ingrao et al. (2021) findings, to avoid overestimation compared to theoretical calculations. Secondary data, as defined above, regarded the production of electricity, water, rice grains used in the solid substrate, packaging products and all the input requirements for PDB production, and were extracted from the Ecoinvent® v3.8 database, as also recommended by Wernet et al. (2016). On the other hand, the PDB production process was modelled through the SuperPro designer® software, due to the lack of data in the Ecoinvent® database, using information that was gathered from the available literature (Rojo et al., 2024). In particular, the PDB process was modelled following indications by the ATCC medium company (ATCC Medium 336, 2024). For each litre of PDB, it was considered that 300 g of potatoes were cut and subsequently boiled (100 °C) in 0.5 L of water for one hour. The resulting broth is then filtered and brought to a volume of 1 L with the addition of 20 g glucose and finally sterilized in an autoclave at 121 °C. Table 2 provides information on the processes considered in the Ecoinvent® v3.8 to produce the biostimulant (secondary data for background processes).

Table 1
Inventory of the production system related to the production of 1 m^3 of product.

Material	Amount	Unit
PDB (Potato Dextrose broth) production		
Water	1.00	kg
Electricity	0.16	kWh
Glucose	0.02	kg
Potatoes	0.30	kg
SS1: Pre-culture production		
Electricity	47.85	kWh
PDB	3.50	L
SS2: Pre-fermentation step		
Electricity	30.35	kWh
Pre-culture (from SS1)	7.00	L
PDB	143.00	L
SS3.a: Liquid substrate		
Electricity	173.12	kWh
Pre-fermented liquid (from SS2)	145.00	L
PDB	850	L
SS3.b: Solid substrate		
Electricity	124.68	kWh
Pre-fermented liquid (from SS2)	5.00	L
Seeds company mix	14.25	kg
SS4: Biostimulant final preparation		
Liquid substrate (from SS3.a)	955	L
Solid substrate (from SS3.b)	5.00	L
Electricity	2.28	kWh
Packaging	98.70	kg

2.3. Life cycle impact assessment

This phase was developed by aggregating material and energy inventories into a limited set of intermediate impact categories. The latter were quantified considering the characterisation factors of the ReCiPe 2016 V1.06 Hierarchist Midpoint World (2010) method (Huijbregts et al., 2017), which is included in the Simapro v9.4 software (Huijbregts et al., 2017). The inventory data were translated into environmental impacts and classified into nine impact categories according to their relevance in the agriculture sector (Costa et al., 2020). Those impact categories were selected by the authors to be Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA),

Table 2
Processes considered from the Ecoinvent® v3.8 database for biostimulant production.

Process name
PDB (Potato Dextrose broth) production
Glucose {GLO}| market for glucose | Cut-off, S
Potatoes, cooled, market mix, at regional storage {IT} Economic, U
Tap water {RER}| market group for | Cut-off, U
SS1: Pre-culture production
Electricity, low voltage {IT}| market for | Cut-off, U
PDB
SS2: Pre-fermentation step
Electricity, low voltage {IT}| market for | Cut-off, U
PDB
SS3.a: Liquid substrate
Electricity, low voltage {IT}| market for | Cut-off, U
PDB
SS3.b: Solid substrate
Rice, non-basmati {GLO}| market for rice, non-basmati | Cut-off, U
Wheat bran {RoW}| market for wheat bran | Cut-off, U
Electricity, low voltage {IT}| market for | Cut-off, U
SS4: Biostimulant final preparation
Electricity, low voltage {IT}| market for | Cut-off, U
Packaging, for pesticides {GLO}| market for packaging, for pesticides | Cut-off, U
Transport, freight, lorry 7.5–16 metric ton, euro4 {RER}| market for transport, freight, lorry 7.5–16 metric ton, EURO4 | Cut-off, U
Compost {RoW}| treatment of biowaste, industrial composting | Cut-off,U

Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS).

Consistently the objectives of this study, attention was be paid not only to identifying environmental burdens, but also to suggest alternatives in the production cycle, in order to reduce the environmental impact of microbial biostimulant production by comparing different scenarios.

3. Results and discussions

3.1. Environmental impact assessment

Results from the contribution assessment of the production of *Trichoderma*-based biostimulant were detailed in Fig. 2. The values of each midpoint category can be found in Table 3, along with contributing output inventories. Given it has an impact of more than 50 % in almost all categories, SS3.a can be considered as the most impactful subsystem. The category in which the value is highest is SOD (71 %). Except in FE,

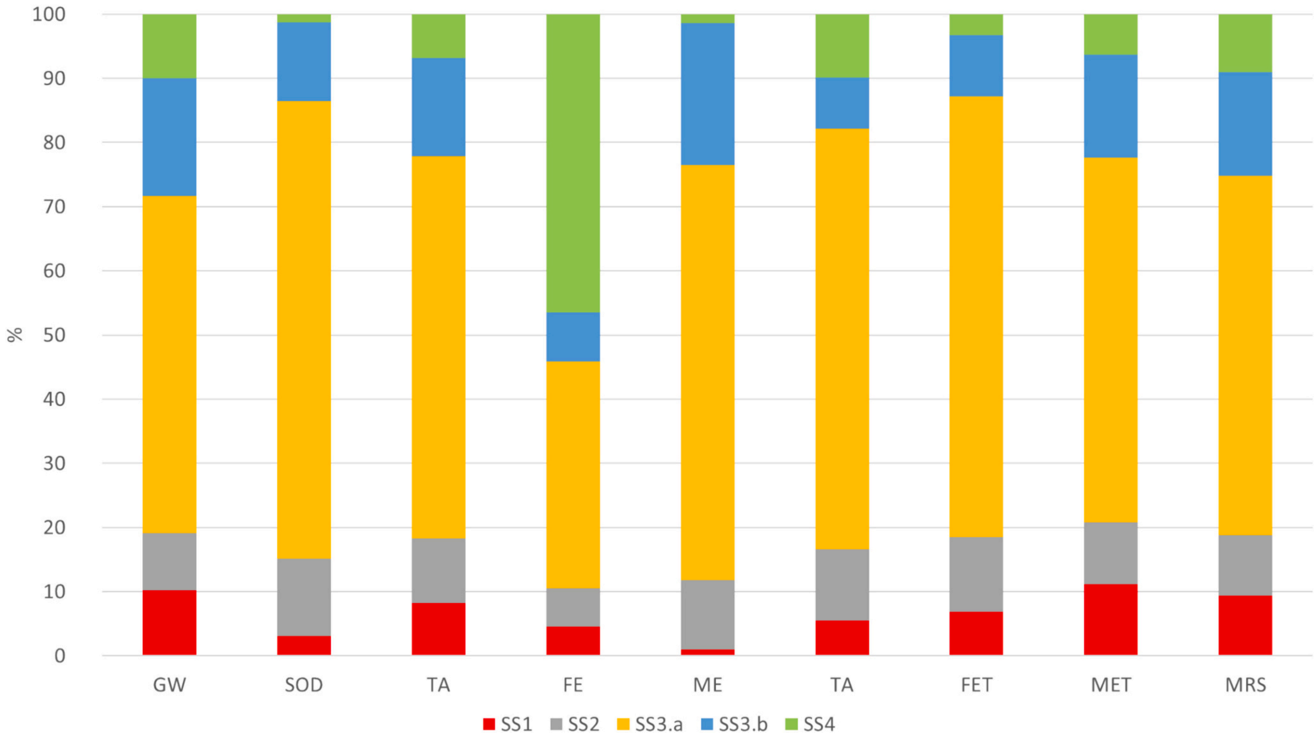

Fig. 2. Environmental profile of the *Trichoderma*-based biostimulant production. Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS). SS1–4 are the steps in which the biostimulant production system has been broken down into, as depicted in Fig. 1.

Table 3
Characterisation results for the different impact categories analysed and most relevant emitted substances and consumed resources. Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS).

Life cycle (ouput) inventories (i.e. emitted substances or consumed resources	Emission compartment	Units	Impact value	Contribution by phase					Belonging midpoint category
				SS1	SS2	SS3.a	SS3.b	SS4	
Carbon dioxide, fossil	Air	kg CO2 eq	286.032	32.931	25.258	148.210	48.046	31.587	GW - Global warming (368.22 kg CO2 eq)
Methane, fossil			28.628	3.282	2.349	13.765	4.614	4.618	
Dinitrogen monoxide			23.192	0.611	2.798	16.690	2.862	0.232	
Dinitrogen monoxide	Air	g CFC11 eq	0.856	0.023	0.103	0.616	0.106	0.009	SOD - Stratospheric ozone depletion (0.93 g CFC11 eq)
Methane, bromochlorodifluoro-, Halon 1211			0.034	0.005	0.003	0.019	0.007	0.000	
Dinitrogen monoxide, peat oxidation			0.021	0.000	0.003	0.018	0.000	0.000	
Sulfur dioxide	Air	kg SO2 eq	0.836	0.098	0.077	0.450	0.143	0.068	TA - Terrestrial acidification (1.57 kg SO2 eq)
Nitrogen oxides			0.302	0.023	0.029	0.172	0.040	0.038	
Ammonia			0.224	0.006	0.023	0.137	0.057	0.001	
Chemical Oxygen Demand	Water	kg P eq	0.090	0.001	0.001	0.005	0.001	0.083	FE - Freshwater eutrophication (0.236 kg P eq)
Phosphate			0.079	0.010	0.007	0.041	0.015	0.007	
Phosphorus, IT			0.029	0.000	0.004	0.025	0.000	0.000	
Nitrate	Water	kg N eq	0.063	0.001	0.004	0.023	0.035	0.001	ME - Marine eutrophication (0.158 kg N eq)
Nitrate, IT			0.053	0.000	0.008	0.046	0.000	0.000	
Copper	Air	t 1,4-DCB	1.370	0.079	0.149	0.887	0.116	0.138	TET - Terrestrial ecotoxicity (2.30 t 1,4-DCB)
Mancozeb			0.205	0.001	0.029	0.174	0.000	0.000	
Chlorothalonil	Soil		0.151	0.001	0.022	0.129	0.000	0.000	
Copper	Water	kg 1,4-DCB	16.983	2.953	1.377	7.978	4.262	0.413	FET - Freshwater ecotoxicity (53.29 kg 1,4-DCB)
Chlorpyrifos			12.815	0.089	1.821	10.890	0.014	0.000	
Metolachlor (S)	Soil		5.624	0.039	0.800	4.785	0.000	0.000	
Copper	Water	kg 1,4-DCB	20.251	3.521	1.642	9.514	5.081	0.493	MET - Marine ecotoxicity (39.13 kg 1,4-DCB)
Zinc			5.339	0.561	0.447	2.625	0.849	0.857	
Lambda-cyhalothrin	Air		2.846	0.020	0.405	2.421	0.000	0.000	
Iron	Raw material	kg Cu eq	0.173	0.015	0.016	0.096	0.025	0.020	MRS - Mineral resource scarcity (0.81 kg Cu eq)
Copper			0.158	0.021	0.015	0.085	0.031	0.007	
Nickel			0.111	0.009	0.011	0.063	0.018	0.012	

this value drops below the average to 35 %, because in this specific case, SS4 is the most impactful process with 46 % (Huijbregts et al., 2017). In the SS4 process, the impact is caused by plastic packaging, which significantly affects the FE. SS4 in the other impact categories is not a relevant cause. The second most impactful process in the production chain is SS3.b, particularly in impact categories ME (22 %) and GW (18 %). The other production processes appear less impactful, with a constant trend and percentage values generally not exceeding 10 %. As demonstrated by Rojo et al., 2024, in the production of algae-based biostimulants, the cultivation process turns out to be the most impactful, the same applies to the analysed biostimulant, where the multiplication of microorganisms appears to be the most relevant process. The primary contributor to environmental impacts is fossil-derived CO_2 (286.03 kg CO_2 eq), followed by fossil methane and nitrous oxide. Nitrous oxide and certain fluorinated gases chiefly drive the impact on stratospheric ozone depletion, resulting in a total effect of 0.93 g CFC11 eq. Terrestrial acidification is predominantly influenced by sulfur dioxide and nitrogen oxides, with a combined contribution of 1.57 kg SO_2 eq. Terrestrial ecotoxicity is also considerable, amounting to 2.30 t 1,4-DCB, largely attributable to copper and mancozeb. The most significant impacts of water emissions are linked to eutrophication and toxicity. Freshwater eutrophication is primarily driven by Chemical Oxygen Demand and phosphates, with a total contribution of 0.236 kg P eq. Marine eutrophication is also substantial, with a total impact of 0.158 kg N eq, mainly associated with nitrates. Freshwater ecotoxicity reaches notably high levels (53.29 kg 1,4-DCB), primarily due to copper and chlorpyrifos, whereas marine ecotoxicity (39.13 kg 1,4-DCB) is mainly driven by copper and zinc. Soil emissions have a relatively smaller influence compared to air and water emissions but still contribute to terrestrial ecotoxicity and mineral resource scarcity. Terrestrial ecotoxicity amounts to 2.30 t 1,4-DCB, primarily due to chlorothalonil (Table 3). The most impactful process is liquid fermentation, particularly in relation to global warming, terrestrial acidification, and marine ecotoxicity.

To identify production alternatives and propose improvements, it was crucial to identify the processes or raw materials that have the greatest impact on the production system. Confirming the expected outcome, electricity was one major cause of impact, though the assessment revealed others worth being mentioned, namely the PDB and the seed mix (Fig. 3). GW is mainly influenced by PDB (40 %) and electricity (44 %), mainly due to the amount of energy used and potato cultivation in PDB. The remaining minor processes, labelled as ‘others’ in the histogram of Fig. 3, contribute a total 46 % to the FE impact. However, PDB is the most impactful element of the production process, especially in the main SOD, ME, TET and FET categories, though it is a necessary substrate for the multiplication of microorganisms (Karpagavalli et al., 2024; Obiedallah et al., 2024). Results are overall in line with other studies on algae-based biostimulants, highlighting electricity as the greatest source of environmental impact (Arias et al., 2024; Rojo et al., 2024).

From Fig. 4, there is evidence that the two largest contributions to the environmental impact associated with SS3.a are made by the produced PDB and electricity. PDB appears to have the greatest impact in all midpoint categories. For the ME and SOD categories, PDB is responsible for more than 90 %, which is related to the use of fertilizers used in potato cultivation (Lago-Olveira et al., 2024).

The reason why the production of the PDB is so remarkable in terms of environmental impact is related to the production process of the PDB itself, mainly due to the production of the potato requirements (Fig. 5). In this respect, it should be noted that potato production is highly inventory-intensive, including the fertilizers, that are responsible for the high level of SOD and ME (González-García et al., 2021). Potato use is the main contributor, ranging from 38 % to 91 % of the impact. Electricity was found to be impacting 44 % of the GW and glucose use in the MRS was 40 %.

The GW is split between the consumption of electricity and the PDB due to the consumption of fuel for agricultural processing or the use of fossil fuels for energy. The PDB was modelled with the help of the Superpro software (Arias et al., 2024; Rojo et al., 2024), and potatoes were considered as the main source of carbohydrates, it would be useful to

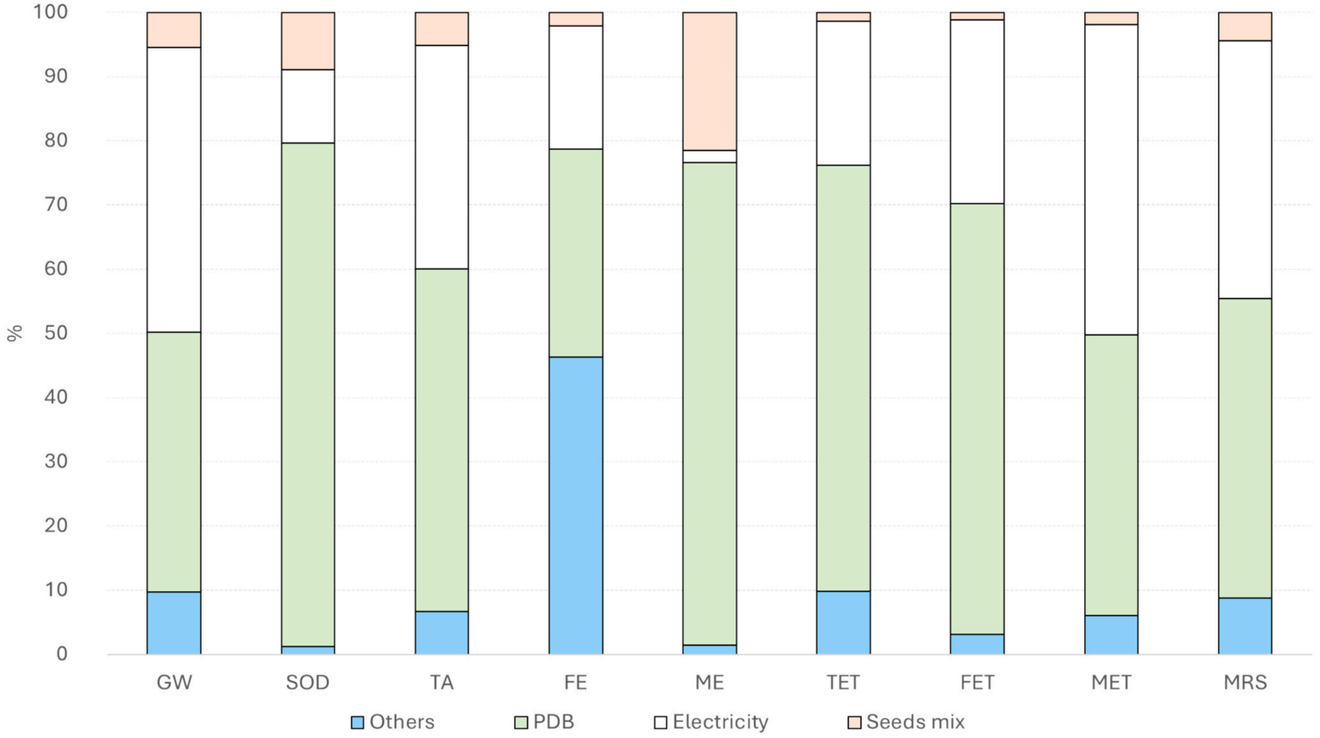

Fig. 3. Production factors most impacting the environmental profile of the *Trichoderma*-based biostimulant production. Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS).

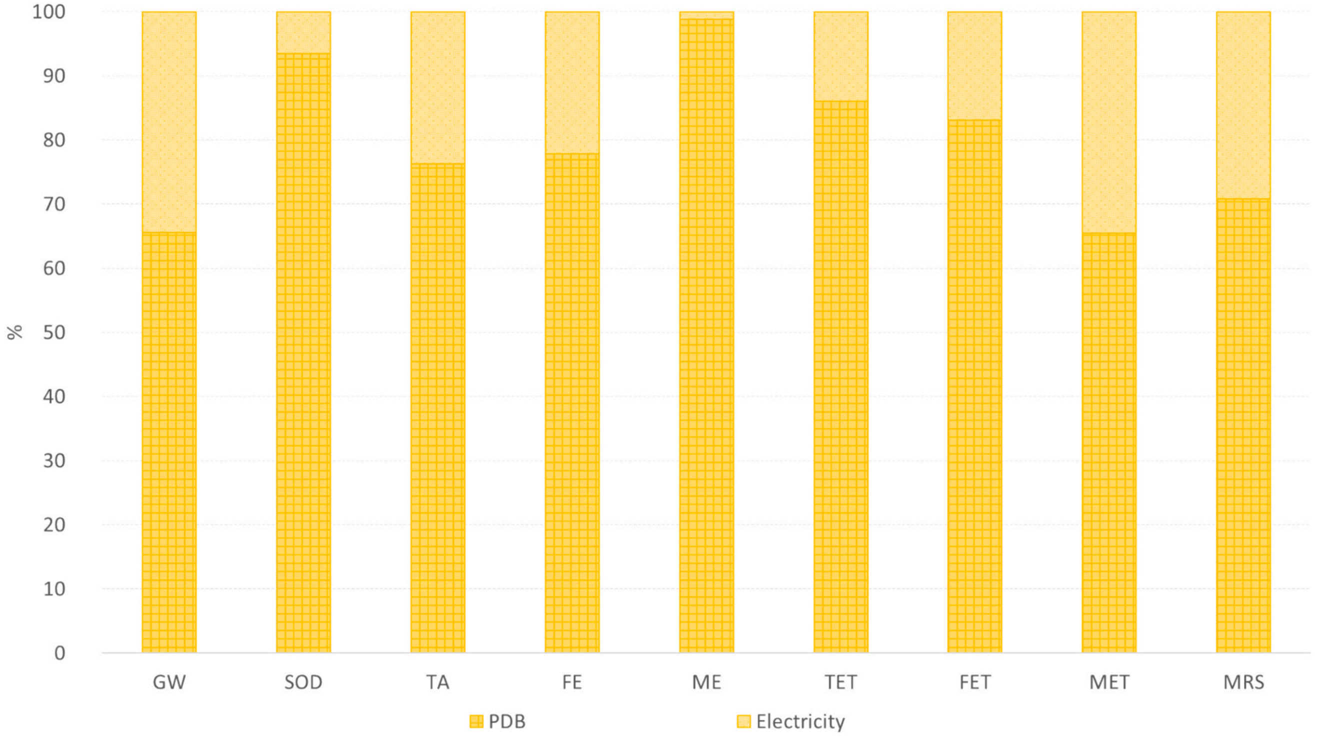

Fig. 4. Distribution of the environmental impacts of SS3.a. Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS).

investigate this further and replace potatoes with another carbohydrate-rich plant matrix.

As shown in Table 1, the main components required to produce the solid substrate (SS3.b) are the seeds mixture used and the energy consumed. The production of the energy is the main contributor to the impacts in almost all impact categories with values usually above 70 %.

Fig. 5. Environmental impact distribution of PDB production. Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS).

While the impact of the use of rice is highest in terms of SOD (62 %) and ME (91 %), where the production of the seed mixture is the hot spot (Fig. 6). As with potato cultivation in the case of PDB, the impacts of rice production are evident in the cultivation of this cereal.

The last of the highlighted processes is SS4, which is among the least impactful, and in which the packaging and transport stages for waste

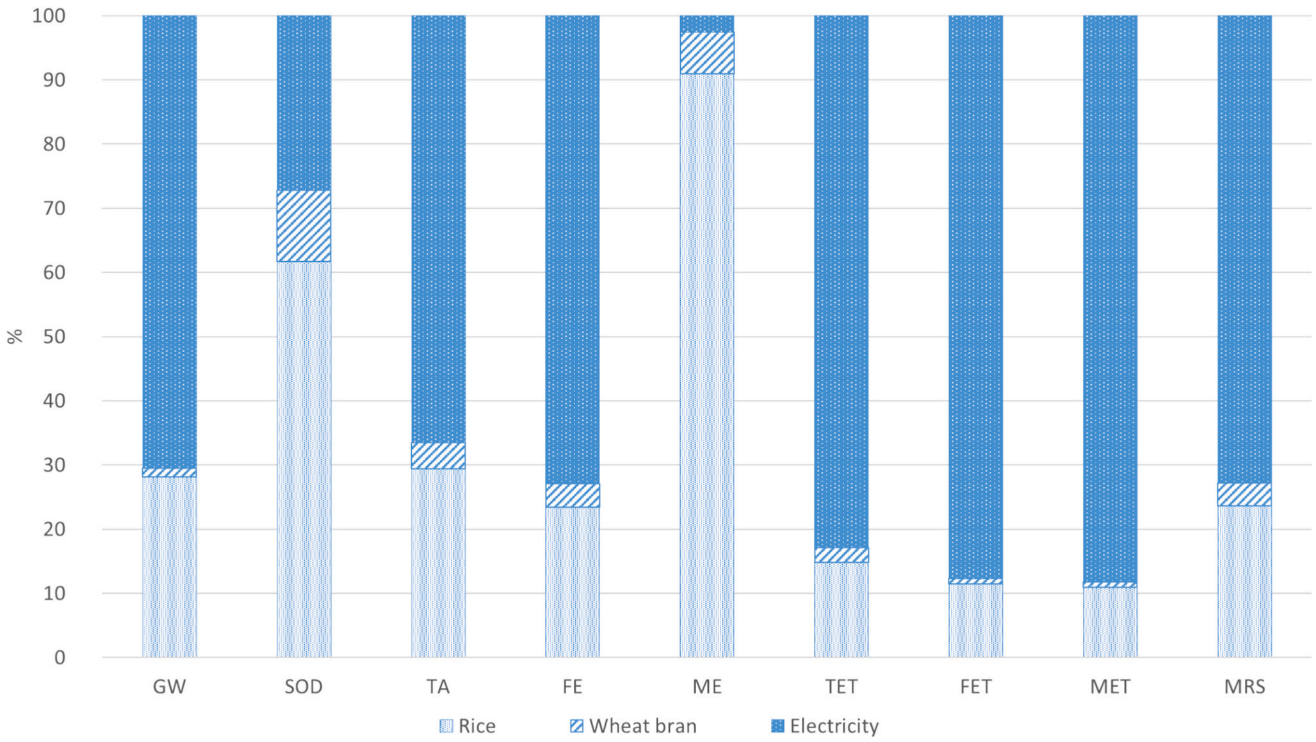

Fig. 6. Distribution of the environmental impacts of SS3.b. Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS).

disposal are present. As shown in Fig. 7, packaging is the most impactful stage. Packaging is affecting more than 90 % of all impact categories. Mainly for the following reasons, raw material used, and quantity of plastic used.

3.2. *Production process improvement*

Following the second, twelve, and thirteen sustainable development goals, there is increasing interest in making agricultural practices more sustainable, while preserving their production yields. Biostimulants can make a relevant contribution in this regard, as they can be an alternative or a valuable contribution to the reduction of chemically synthesised products (Egas et al., 2023; Magnabosco et al., 2023).

As specified by the company and scientific studies, PDB is essential for the growth of microorganisms, thus no alternatives were technically feasible (Karpagavalli et al., 2024). Bearing in mind the environmental hotspots (seeds mix and electricity) of the system investigated, improvements have been proposed to identify potential alternatives. There are three scenarios to compare with the current production system (henceforth Scenario A):

Scenario B: this alternative production system considers the use of an alternative cereal (e.g. wheat) instead of rice in the preparation of the seed mix.

Scenario C: the production of electricity requirements has been identified as an environmental hotspot, which is directly taken from the Italian electricity mix and considerably depends on fossil sources. Therefore, the use of photovoltaic panels (PV) has been considered to produce electricity requirements.

Scenario D: is a combination of Scenario B and C.

To compare the different alternatives proposed in the study, an endpoint level methodology was used, which assigns a single environmental score for each production process (Pt). For this purpose, normalisation and weighting factors taken from the ReCiPe 2016 Endpoint method were considered.

The endpoint approach was used by the authors, because it allows for holistic environmental evaluation and ranking on the same point scale, thereby favouring both system-level decision and larger-scale planning (Ingrao et al., 2025; Ingrao et al., 2024).

The analysis at endpoint level provides results through three main indicators representing the severity of damage in the following areas: Human Health Impact (HH), Ecosystem Quality (EQ) and Resource Scarcity (RS). Aggregating the midpoint categories and characterising them towards specific endpoints results in the three main endpoint (Fig. 8).

The analysis focused on the influence of the change of two key variables identified and highlighted in Fig. 3, representative of two of the system's environmental hotspots: the seeds mix and the self-production of electricity through photovoltaic panels (instead of drawing electricity directly from the grid). The main objective is to predict the possible environmental benefits of these potential changes. Comparing the midpoints of the proposed alternatives, the best scenario appears to be the one where the seeds mix alternative, and the self-production of photovoltaic panels are combined. The GW of biostimulant BS is reduced by 52 % (Table 4) compared to classical biostimulants (Table 3). The impact category TA is also reduced by 30 % compared to the classic biostimulant, though the self-generation of electricity with photovoltaic panels reduces several midpoints considerably.

Vavrova et al. (2022) reported a 200 kg CO_2eq GW to produce biostimulants from chicken feathers; this value is comparable to the production of the biostimulant PV. The percentages of the three endpoints are as follows in all 4 scenarios: 93 % human health, 5 % ecosystems, and 2 % resources. Fig. 9 shows the endpoints of the different alternatives proposed. With the use of photovoltaic systems and the use of winter cereal, the 'best case scenario' is reduced to 31 % for human health, 18 % for ecosystems and 52 % for resources. It can also be seen that the application of photovoltaic panels alone drastically reduces the score compared to changing the seed mix. The authors proposed the use of autumn-winter cereals to produce the solid substrate, which requires

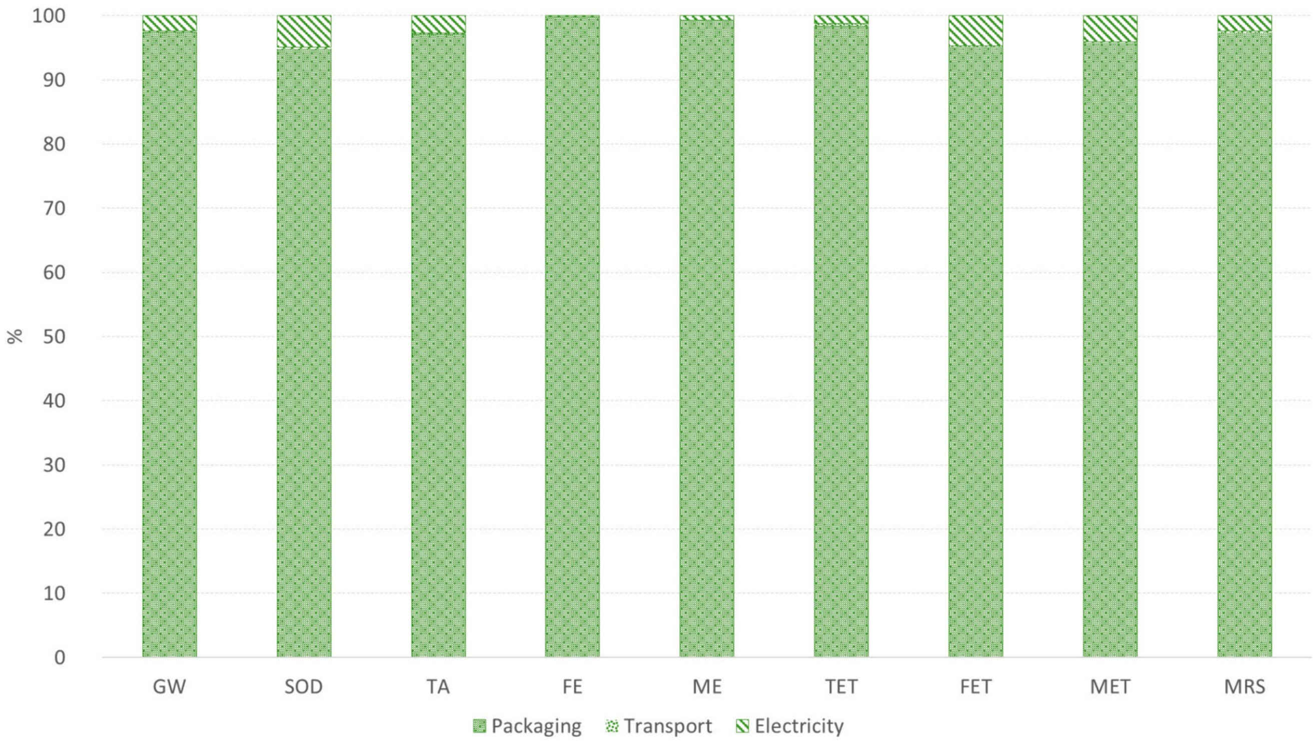

Fig. 7. Impact of the final process of production (SS4). Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS).

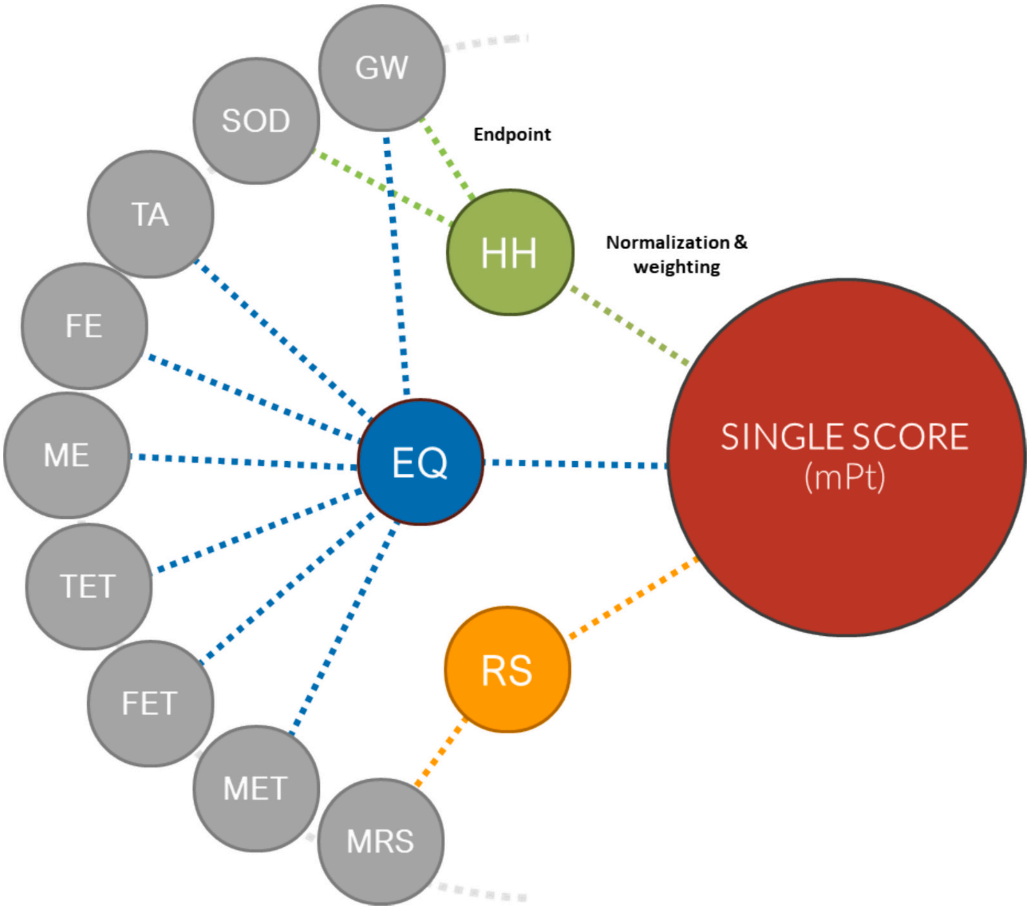

Fig. 8. Schematization for estimating individual endpoints. Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS).

Table 4
Characterisation results for the different impact categories following the proposed improvement Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS).

Impact category	Unit	Scenario B	Scenario C	Scenario D
GW	kg CO_2 eq	358.12	184.51	175.22
SOD	g CFC11 eq	1.02	0.80	0.90
TA	kg SO_2 eq	1.62	1.06	1.12
FE	kg P eq	0.24	0.21	0.21
ME	kg N eq	0.28	0.16	0.28
TET	t 1,4-DCB	2.30	3.22	3.24
FET	kg 1,4-DCB	53.19	56.98	57.22
MET	kg 1,4-DCB	39.08	44.21	44.47
MRS	kg Cu eq	0.82	1.14	1.15

less input than rice. However, the change compared to the original biostimulant is only 2 %.

4. Conclusions

In recent years, to ensure the food security of a growing world population, chemical fertilizers have been used intensively to increase agricultural productivity (Rose et al., 2014). However, this overuse poses a serious anthropogenic threat to global terrestrial and aquatic ecosystems. To solve this problem, it is imperative to develop sustainable strategies that increase agricultural production while reducing the use of chemicals (Shukla and Prithiviraj, 2021). The main causes of emissions in agriculture include nitrogen-based chemical fertilization (Hafeez et al., 2006; Rebolledo-Leiva et al., 2022). In this study, the modelling of a *Trichoderma*-based biostimulant production was investigated in depth. Biostimulants could contribute to reducing the use of chemical fertilizers by 2050, as requested by the European Commission (Li et al., 2022). The research has achieved the proposed objective, that is, to model the entire production process of *Trichoderma*-based biostimulant production and to evaluate the environmental issues arising from the production of these products. Few articles in the literature were foundt by the authors as dealing with this topic. Most of the articles consulted focused on the environmental impact assessment of products based on algae but not on microorganisms. It was also difficult to find articles that modelled these processes. In recent years, the use of biostimulants and the attention to reducing environmental impacts have grown. It is important to understand the real advantage of these products compared to the traditional fertilizers used in agriculture. From the assessment, it was found that the production of liquid substrate is the most impactful process, mainly due to the amount of PDB used. Alternative or waste materials could be used to reduce the environmental impact of the *Trichoderma*-based biostimulant production process. In addition, self electricity-production was effective in reducing much of the environmental impact, whereas the variation of cereal proposed as an alternative in the preparation of the solid substrate reduces the impact by 2 %. In addition, a further topic that could be explored is the use of packaging capable of holding larger volumes or eco-friendly packaging that can be a viable alternative to reduce environmental

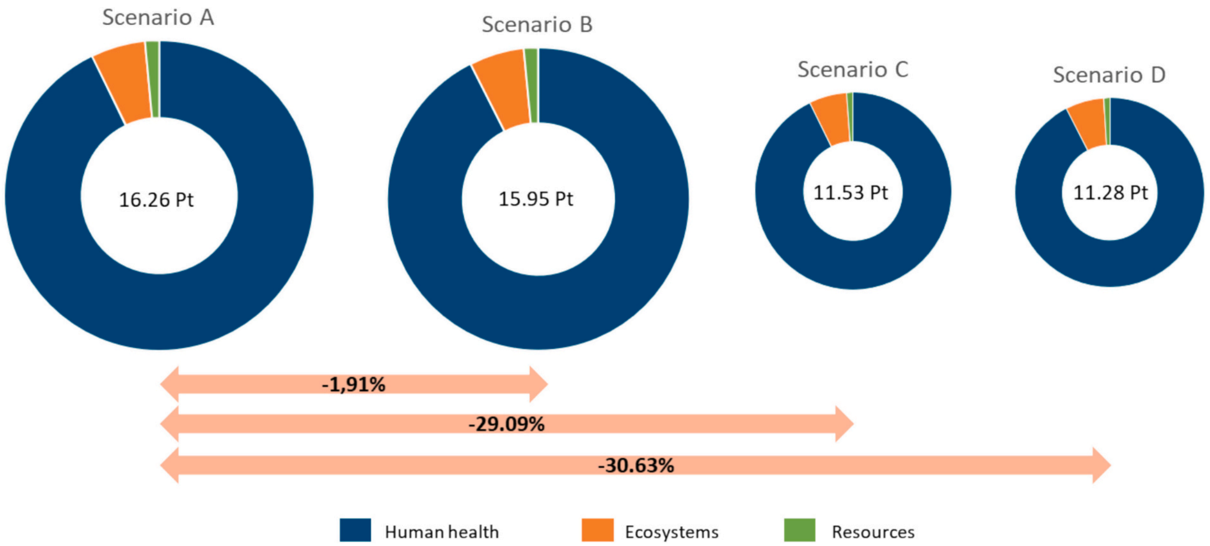

Fig. 9. Impact of the *Trichoderma*-based biostimulant production process in alternative scenarios compared to the baseline scenario (A): autumn-winter cereals (Scenario B), photovoltaic panels (Scenario C) and a combination of Scenarios B and C (Scenario D).

impact. The authors believe that this article can form the basis for evaluating other aspects of cultivation and environmental impact assessment in the agricultural sector to make agriculture more sustainable and, above all, to have effective tools to show the real sustainability of agricultural practices and products used.

AbbreviationsThe following abbreviations are used in this manuscript:

GW	Global warming
SOD	Stratospheric ozone depletion
TA	Terrestrial acidification
FE	Freshwater eutrophication
ME	Marine eutrophication
TET	Terrestrial ecotoxicity
FET	Freshwater ecotoxicity
MET	Marine ecotoxicity
MRS	Mineral resource scarcity
PDB	Potato Dextrose broth
HH	Human Health Impact
EQ	Ecosystem Quality
RS	Resource Scarcity
PV	Photovoltaic Panels

CRediT authorship contribution statement

Claudio Calia: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Sara González García:** Writing – review & editing, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Carlo Ingrao:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Giovanni Lagioia:** Writing – review & editing, Validation. **Claudia Ruta:** Writing – review & editing, Validation, Supervision, Investigation. **Nicola Secchi:** Validation, Investigation, Data curation. **Giuseppe De Mastro:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Giuseppe De Mastro reports financial support was provided by European Agricultural Fund for Rural Development. Giuseppe De Mastro reports financial support, administrative support, and equipment, drugs, or supplies were provided by University of Bari. Sara González-García reports financial support, administrative support, and equipment, drugs, or supplies were provided by European Commission. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Most of the primary data used were shared in the article, whilst a few others were not as confidential

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