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Environmental assessment of undercurrent agricultural technologies towards circular nutrient management

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Preface

The following PhD thesis assesses full-scale technologies that have proven to close C, N, and P loops regarding their overall environmental impact by using an LCA approach. The technologies studied are presented as study cases unfolded in three main chapters, and another two chapters represented a brief general introduction, and last, the global conclusions of the work presented.

Chapter 1 gives a brief introduction explaining the context of the work, namely concepts of environmental impact, nutrient management, and LCA. The main goal, scope and contribution that encloses this work is presented as well.

Chapter 2 is embodied by a published work about sustainable microalgae production by using recovered waste streams.

Chapter 3 is characterized by a submitted work comparing the production and use of recovered fertilizers, in contrast with synthetic fertilizers regarding their environmental impact.

Chapter 4 is characterized by a submitted work connected with the previous chapter, related to the agronomic performance of digestate regarding its effects on soil, environment, and yield production.

Chapter 5 is embodied by the global conclusions of this study.

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Chapter I

Introduction.

1 Introduction

1.1 Fossil fuels era to break global boundaries

Thanks to extensive work resting on over more than 30 years of research, a quantification of the complex dynamics governing Earth's system has been ever done. Nine critical indicators constitute the planet boundaries framework for maintaining our planet in a stable stated.[1] The boundaries can be organized into three groups-systems, according to how they operate (Table 1). The "big three" are the processes with defined global thresholds representing a significant threat of melting the ice sheets and shifting from one state to another with no returning points. The second group are the "slow boundaries" associated with a local-to-regional scale. They are the variables that contribute to the core resilience of the Earth, with no evidence that exceeding these will lead to planetary no returning points. The last group consists of human-created threats, "the two aliens": air pollution from soot (black carbon), nitrates, sulphates, and other particles; and pollution of the biosphere by chemicals and heavy metals.[2]

At present, we have transgressed four of the nine boundaries: climate, land-system change, biodiversity, and the use of Nitrogen (N) and Phosphorus (P). The leading cause has been the exploitation of fossil fuels as a cheap and effective energy source since the industrial revolution.[3] This rapid expansion of fossil-fuel usage has slowly raised CO_2 concentrations in the atmosphere in the last century.[4] Climate change measured as "carbon dioxide equivalents: CO_2 eq" is represented mainly by Greenhouse gas emissions (GHG), which are accountable for some gases that capture more heat than carbon dioxide, such as CH_4 and NO_2. At present, the world's annual emission in CO_2 equivalent has reached 51 billion, where how we produce things (i.e. cement, steel and plastic), and energy-electricity are responsible for 31% and 27%, respectively.[5]

For instance, the last IPCC (Intergovernmental Panel on Climate change) Assessment Report (AR6) (2021)[6] have provided an unprecedented clarity of the future of our planet, and ultimately the need to reduce/eliminate our emissions of GHG. Consolidating data from previous reports; IPCC states the undeniable human influence in warming the atmosphere, ocean and land; concentrations have continued to increase in the atmosphere, reaching annual averages of 410 ppm for carbon dioxide (CO_2), 1866 ppb for methane (CH_4), and 332 ppb for nitrous oxide (N_2O) in 2019. Over the past six decades, land and ocean have taken up a near-constant proportion (globally about 56% per year) of CO_2 emissions from human activities. Therefore, there is an urgency to get a net-zero emission to avoid climate adversity by reducing fossil fuel sources and deploying technologies to produce clean, renewable energy.

Table 1. *Planetary boundaries framework; 9 Control variables and their current values. Adapted from Steffen et al., (2015)*[7]

Main groups	Earth system process	Control variable	Unit	Planetary boundary	Current value
Critical - The big three	Climate change	Atmospheric CO_2	ppm	350-450	398.5
	Stratospheric ozone depletion	Stratospheric O_3	DU	290	~ 200[a]
	Ocean acidification	Carbonate ion concentration[b]	% pre-industrial aragonite saturation state	>80%	~ 84%
Slow boundaries	Rate of biodiversity loss	Bll[c]	%	90%	84%[d]
	Freshwater consumption[e]	-	$km^3\ yr^{-1}$	4000	~2600
	Land-use change[f]	-	% Original forest cover	75%	62%
	Nitrogen and phosphorus pollution	P Global[g]	$Tg\ P\ yr^{-1}$	11–100	~22
		N Global[h]	$Tg\ N\ yr^{-1}$	62–82[i]	~150
The two aliens	Atmospheric aerosol loading	Aerosol Optical Depth (AOD)[j]	-	0.25–0.50[k]	0.3[l]
	Chemical pollution	No control variable defined	-	-	-

[a]Over Antarctica in Austral spring
[b]Saturation state with respect to aragonite
[c]Bll referes to biodiversity intactness index, where assessed as biomes/large regional areas
[d]Applied to southern Africa only
[e]Maximum globally amount of consumptive water use
[f]Area of forested land globally
[g]P flow from freshwater system into the ocean
[h]Industrial and intentional biological fixation of N
[i]Boundary acts as a global 'valve' limiting introduction of new reactive N to Earth System
[j]Much regional variation in AOD
[k]Over Indian subcontinent
[l]South Asian region

1.2 Agriculture and nutrient management

With an expected growing population (toward 10 billion people by the end of the century),[5] food security has become a challenge, mainly because how we produce food is one of the largest threats to transgressing boundaries such as land use, biodiversity, climate and nutrient flows as mentioned above.[7,8] How we grow plants and animals is responsible for 19% of the global GHGs,[5] counting that up to 60% of nitrogen pollution into groundwater and rivers comes from manure and fertilizers.[9] The fertilizer industry accounts for about 2-3% of the total global energy consumption,[10] becoming affordable adds more reactive nitrogen (Nr) into the biosphere than the natural nitrogen cycle. This linear approach accompanied by poor management creates a cascade of threats affecting the quality and health of the soil, water, air and ecosystems, and potentially human health.[11] For instance, a correlation between nitrogen vulnerable zones with intensified livestock production and high use of synthetic fertilizers has been found.[12] Although the European Union has developed necessary regulations on reactive Nr flows by limiting its intensive use (Nitrate Vulnerable Zones (NVZs) – application of N from animal manure must not exceed 170 kg N ha−1y−).[13,14] There is still a large part of N often imported from other continents that comes as feed for animals and fertilizers and in the case of P, where precisely the Europe Union imports >90% of it from one source in Finland.[15]

As a non-renewable nutrient, phosphorus's extraction to reach food can lead to up to 80% losses.[16] Excess losses are associated with water pollution, eutrophication, i.e. excessing algal growth and a fall in oxygen in waters.[17] On the other hand, management of carbon, as organic matter, plays an essential role in the structure of the soil and its fertility, besides that improves water infiltration and holding capacity.[18] Soils from croplands have lost up to 60% of their organic carbon and are reaching a point to become desertic areas.[19,20] Therefore, proper management should be given to reduce losses of these nutrients in the environment. Nutrient management can be defined by a group of articulate actions to achieve both agronomic and environmental goals,[21] by also considering the finite natural sources (e.g. phosphorus, potassium, magnesium) to produce fertilizer and nutrient compounds that can be depleted over time.[22,23] Some of the practical areas of action that are central to improving nutrient efficiency, and therefore, food and energy production while reducing the impact in the environment are presented in Figure 1.[8]

One of the key actions is to transition to a production practice where all N and P surpluses return into the agro-system, including manure and waste from areas where food is consumed. So, circular production is an indispensable practice for nutrient management by the recycling and reusing of waste.[3] Therefore, recently Biorefinery, i.e. the refining of chemicals, materials, energy and products from bio (waste) streams, has become a field that is gaining importance by deploying new technologies for nutrient recovery.[24]

Undercurrent technologies and practices for recovery and reuse in agriculture are dominated by manure as input substrate.[25] However, many of these are also now applied to work with sludge from wastewater treatment plants. Some technologies include; solid-liquid manure separation, drying, anaerobic digestion, biogas production, struvite precipitation, ammonia stripping, membrane filtration, composting, algal cultivation, among others.[15]

Reusing manure and other types of processed wastes also requires attention in its use efficiency to see improvements. The use of recovered fertilizer focuses on the N content, and the challenge is to apply enough N and P to match the crop's requirements. Since bio-products are needed to be stored prior the reuse, losing part of the N content (via ammonia and nitrate losses), resulting in lower N:P ratios that do not meet crop requirements, thus, in trying to match the N's crop requirement, excessive amounts of P ended loss in the field.[26] Nitrates (NO_3), as are more mobile than P in soil, finds their way into groundwater, causing pollution problems in marine systems which are N-limited.[27]

The reuse of waste materials from agriculture comes with advantages and disadvantages, depending on the technology/practice and whether the receiving water/soil system is P or N limited. This also implies that nutrient use efficiency (N and P) is another crucial factor that demand focus to reduce soil nutrient surpluses,[28] a part of lowering livestock density, giving a better manage by counting soil P reserves is essential.[29]

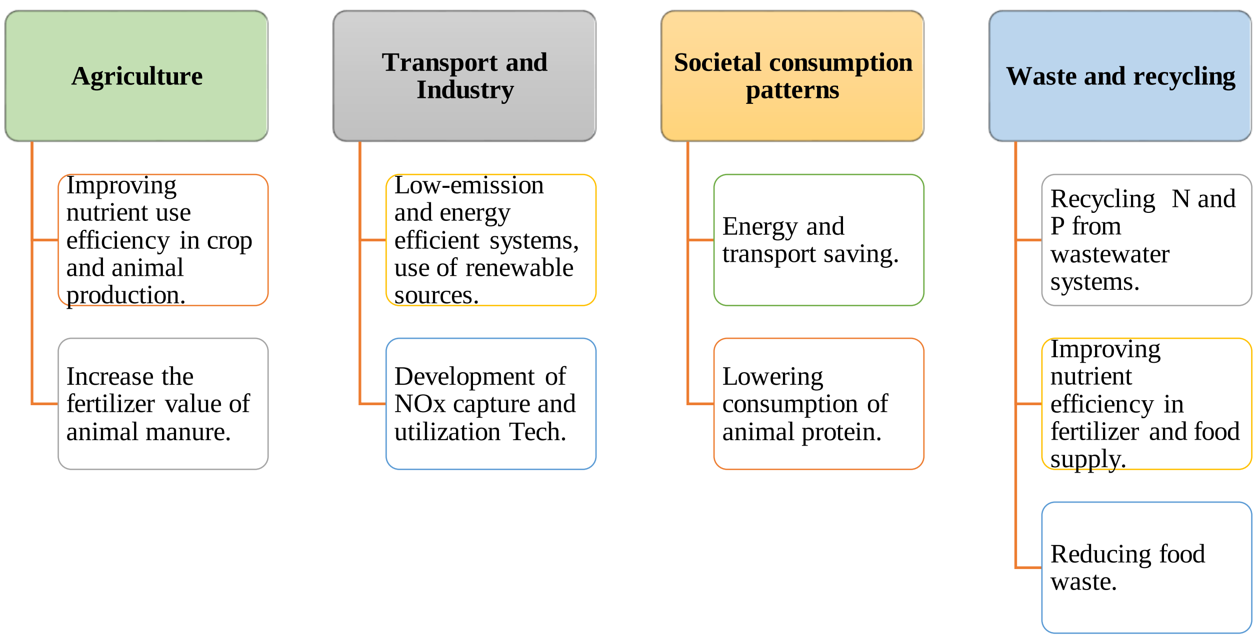

Figure 1. *Critical areas of action to produce more food and energy with less pollution. Adapted from Sutton et al., (2018)*[8]

Other important aspects are decarbonizing agricultural energy use for all energy inputs and using conservative agriculture for carbon storage, i.e. minimum tillage and mulch farming. Last but not least, landscape planning should provide ecosystems services, e.g. soil health, protecting pollinators and biodiversity, storing carbon and water management.[30]

1.3 LCA as a tool for assessing the environmental impact

To acquire a complete understanding of the environmental impacts of emerging treatment technologies, including the post-use phase of its products, requires an approach that can capture the full system effects, focusing on a defined range of environmental indicators.[31] To do so, Life Cycle Assessment (LCA) has become a widely applied methodology to analyze the impacts of processes producing biobased fertilizers.[32] LCA is distinguished from other types of analysis because it covers the whole life cycle of a product or service and attempts to include all-natural resources needed by and all emissions associated with them.[33]

Based on ISO standards, LCA covers main four stages: goal and scope, inventory analysis, impact assessment, and interpretation.[34] The inventory analysis is carried out using LCA software, making the calculations more accessible and provides a database with inventory data for some of the most common processes. Some of the widely used LCA software is GaBi,[35] and SimaPro,[36] commercially available, and openLCA,[37] which is open-access. In the impact assessment case, the most applied methods include EDIP 2003, IMPACT 2002+,[38] Eco-indicator 99,[39] ReCiPe,[40] and the ILCD method,[34] which are often included in the LCA software. ReCiPe, as a method, has the goal to transform the long list of the life cycle inventory into define indicator scores. However, ReCiPe has gained widespread use over other approaches because it has a broader set of midpoint categories and three endpoint categories, often covering a global scope in their impact calculations (Table 2).[36]

The environmental midpoint indicators gain more integrity and value when you realize that they correlate with our planetary boundaries. Although they could differ in the measuring method because of the scales of magnitude, they point in the same direction to cover the main elementary flows but are more elaborated (Table 2). Midpoint indicators are more precise to a point in the cause-effect impact chain than the endpoint indicators (three main areas of protection); therefore, depending on the analysis scope, both are important to get different perspectives in the assessment.

Table 2. *Overview of the midpoint impact categories and related indicators. Adapted from Recipe 2016*[40]

Areas of protection	Midpoint impact category	Indicator	Unit
Ecosystems	Climate change	Infrared radiative forcing increase	kg CO_2-eq to air
	Ozone depletion	Stratospheric ozone decrease	kg CFC-11-eq to air
	Ionising radiation	Absorbed dose increase	kBq Co-60-eq to air
	Fine particulate matter formation	PM2.5 population intake	kg PM2.5-eq to air
	Photochemical oxidant formation: terrestrial ecosystems	Tropospheric ozone increase	kg NOx-eq to air
	Photochemical oxidant formation: human health	Tropospheric ozone population intake increase	kg NOx-eq to air
	Terrestrial acidification	Proton increase in natural soils	kg SO_2-eq to air
	Freshwater eutrophication	Phosphorus increase in freshwater	kg P-eq to freshwater
Human health (Toxicity)	Human toxicity: cancer	Risk increase of cancer disease incidence	kg 1,4-DCB-eq to urban air
	Human toxicity: non-cancer	Risk increase of non-cancer disease incidence	kg 1,4-DCB-eq to urban air
	Terrestrial ecotoxicity	Hazard-weighted increase in natural soils	kg 1,4-DCB-eq to industrial soil
	Freshwater ecotoxicity	Hazard-weighted increase in freshwaters	kg 1,4-DCB-eq to freshwater
	Marine ecotoxicity	Hazard-weighted increase in marine water	kg 1,4-DCB-eq to marine water
Resources	Land use	Occupation and time-integrated land transformation	$m^2 \times$ yr annual cropland-eq
	Water use	Increase of water consumed	m^3 water-eq consumed
	Mineral resource scarcity	Increase of ore extracted Upper heating value	kg Cu-eq
	Fossil resource scarcity	Upper heating value	kg oil-eq

For LCA modelling, there are also two types of approaches to be chosen that depend on the LCA's scope and goal. One, the consequential, with a broader approach that reaches out the consequences that the target by-product could have by putting it into the market, by also identifying the product that could replace on the market by introducing it.[41] While the second one, the attributional, is presented more like a "descriptive" approach, with the main distinction that is limited and centred only on the environmental impacts of a product, process or system, rather than described the changes in production within the economic system as the consequential does.[42]

Beyond the impact assessment methods and the selection of modelling to follow, other essential aspects to consider when defining the scope definition are how to handle multiple outputs by one process (co-products). For instance, anaerobic digestion, in addition to the production of digestate, also produce biogas, and thus, energy. Compared with another bio-fertilizer, for example, compost production, under the assumption of "1 kg of N from biofertilizer" as a functional unit, they will differ as the latter does not provide the service in the production of biogas.[32] This multifunctionality issue (to be dealt with when different products systems share a process), according to ISO 14044:2006,[43] should be solved using a three-level hierarchy as follows:

1. Avoid allocation by subdivision (dividing the unit process into two or more sub-processes) or system expansion ("expanding the product system to include the additional functions related to the co-products").

2. Allocation following underlying physical relationships (an allocation that quantitatively reflects how the inputs and outputs are changed by changes in the amount of each system product).

3. Allocation (partitioning) based on other relationships (e.g., economic value).

The first one on the hierarchy, system expansion as one of the most common ways of solving the multifunctionality concern, has two possible approaches by expanding the boundaries: enlargement and substitution. Substitution is done by identifying which product the co-product would replace and then modelling the impact avoided (a negative addition) by the production of the replaced product. In the case of enlargement, it works by the addition of co-functions.[44]

Although many LCA studies agree with ISO standards, there are still differences in the allocation approaches applied when evaluating the same or similar products.[41] This is somehow because there is still a lack of a shared view among LCA practitioners to follow the ISO standard.[45] This is partly because there is no further specification regarding the differences between enlargement and substitution and its implementation in attributional or consequential LCAs.[44] Meanwhile, other authors follow a commonly or conservative allocation method applied in similar cases found in the literature.[45,46] Since the choice of allocation method affects the LCA outcome significantly, this has led to a cutback in the reliability and robustness of LCA results.[47,48]

1.4 Goal, scope and contribution

As introduced above, there is a need to move quickly to a more sustainable energy era, where our production processes should be redesigned to reduce wastes and losses in the environment and be more connected in harmony within the natural cycling processes as nature does. However, our current knowledge of applied circular practices is still in the early stages of consolidation as it comes from a multidisciplinary concept offering broad applications.

Therefore, to find valuable insights from the application of circular practices, the main objective of this study was to assess diverse proven full-scale technologies in recovering and reusing nutrients and carbon from different waste streams regarding their overall environmental impact. To verify the role of these technologies, two study cases are proposed in producing fertilizers to be used in the production of algae biomass and crops (maize). To identify and evaluate it, an LCA tool is used for performing environmental impact measurements of these practices.

Other specific objectives were:

- To use the LCA approach to get a "bigger picture" approach for system analysis by performing product perspective.
- To assess integrated resource recovery by combining individual pathways and technologies through an LCA evaluation.
- To integrate up-to-date cross-disciplinary knowledge (e.g. agriculture, soil science, environment) into LCA models to better quantify the environmental impacts using recycled nutrient products.

This study may contribute towards a better understanding of the environmental footprint on undercurrent technologies that can fit our current need to provide ecosystem services by recycling and reusing nutrient sources in agro-systems. In doing so, we can reduce pressure in the planet boundaries under the current emergency and find out what challenges come from circular management implementation.

Chapter II

Sustainable production of microalgae in raceways: nutrients and water management as key factors influencing environmental impacts.

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2 Sustainable production of microalgae in raceways: nutrients and water management as key factors influencing environmental impacts.

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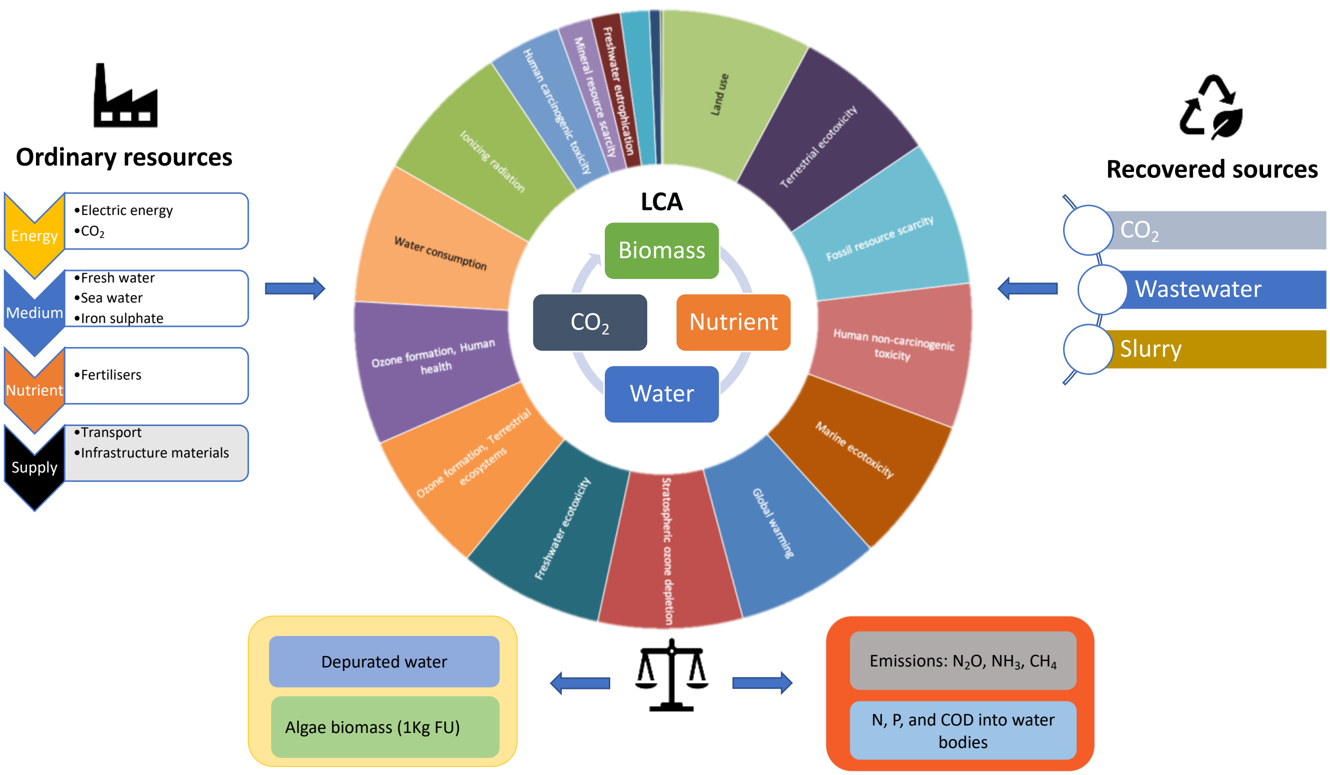
ABSTRACT ART



Abstract

Microalgae production has taken on importance for its ability to be more energy efficient than land crops, with low input requirements and a wide number of possible applications. This work aimed to evaluate the environmental impacts of the production of microalgae for use as bio-stimulants and aquaculture feeds. Inventory data from a real production facility of 1 ha located in Almería (Spain) were acquired, and LCA was applied to compare nine scenarios with alternative water bases (fresh, sea and waste), and nutrient sources (fertilizers, manure and wastewater), and the alternatives were also compared using a CO_2 supply (commercial liquid) versus a default scenario (recovered flue gas). The LCA results outlined that the main inputs affecting environmental performance were electricity use, chemical fertilizer demand (N and P) and transport. Scenarios using recovered nutrients from slurry and wastewater showed reductions in the climate change category (kg CO_2 eq.) of 80% and 20% respectively, compared to standard fertilizer use. The threshold of distance for manure transport was 40 km, beyond that value the scenarios using recovered nutrients performed worse than scenarios using chemical fertilizers. The multifunctionality of the process which included wastewater depuration, permitted compensation in most of the impact categories, yielding negative values in some (all of the toxicity categories).

Keywords: CO_2 source; Environmental impact; Life Cycle Assessment (LCA); Microalgae production; Raceway reactors; Wastewater.

2.1 Introduction

Population growth and the increase in the standard of living and consumption, place the search for new sustainable sources for the production of food, feed and feedstock at the centre of the development focus. To respond to these needs, issues such as the sustainable intensification of agriculture and the increase of high-performance forms of production, such as aquaculture, are becoming the focus of attention. Sustainable intensification of agriculture for food, feed and feedstock is the recurring mantra in the 2050 forecast for economic and social scenarios. In the same search for environmental sustainability, it has become mandatory to develop production models that minimise waste and that exploit the waste from other processes as raw materials, in a logic of industrial symbiosis and the circular economy. Within this framework, production of microalgae has been notable for traits like high photosynthetic efficiency reflected in productivity, its capacity to produce a wide range of active compounds, and the possibility of using alternative resources such as land not classed as fertile soil, sea water, and recovered streams (e.g. wastewater).[49–51]

At present, microalgae are actively being investigated for their ability to produce active substances (bio-stimulants) which applied in small quantities, can stimulate the growth of several crops, enhance nutrition efficiency and provide protection against abiotic and biotic stresses.[52–54] Amino acids already contained in proteins from microalgae must be adequately hydrolysed to obtain valuable bio-stimulants.[55] However, microalgae biomass also provides valuable phytohormones such as auxin-like and cytokinin-like molecules, stimulating the growth and root development of plants.[56] Microalgae biomass has been also reported as a source of valuable biopesticides, in this case the nature of the molecules involved being less known.[57]

Microalgae also have interesting applications in animal nutrition and aquaculture as highly nutritional dietary supplements, for their high content of proteins, high quality essential amino acids (methionine, threonine, and tryptophane, scarce and valuable in animal diets), vitamins, carotenoids, antioxidants, and other substances beneficial to animal health.[58,5960,61] This is a relevant issue, as the demand for animal protein will almost double by 2050 and marine based-proteins can contribute significantly to the global food supply.

Although microalgae production does not require arable land, it demands high water use and fertilizers. Microalgae can exploit slurry and wastewater nutrients, turning a problem into resources. In fact, nutrients in slurry are often mismanaged, and may be provided to crops in excess (due to the need to discharge them) and not effectively taken up by plants[62] causing problems related to pollution of surface waters and air. Various studies show that microalgae have a high capacity for the efficient removal of nutrients from wastewater and slurry, so that microalgae production could be an appropriate way for nutrient removal and recovery from a liquid stream, producing valuable biomass at the same time.[63,64] The possibility of using wastewater to satisfy nutrient demands could be beneficial for water treatment and reduce costs in the chain of production.[65]

When producing microalgae biomass using wastewater, a consortia of microalgae and biomass is established, quality of the biomass being a function of operation conditions: thus, if adequately managed, more than 95% of the produced biomass will be microalgae.[66] In this consortium the microalgae provide oxygen (O_2) for aerobic bacteria to biodegrade organic pollutants and in turn take up the CO_2 that is released by the bacteria via respiration[67] Organic compounds are thus mineralized, the released inorganic nutrients, such as N and P, being consumed by microalgae to produce microalgae biomass. Complete treatment, if possible in these systems, releases water fulfilling EU regulations, and the cost of wastewater treatment becomes lower than using conventional technologies.[66]

In domestic wastewaters, most of the nitrogen is present as ammonium (NH_4^+), with low concentrations of nitrite and nitrate. This feature favours nitrogen consumption by microalgae since NH_4^+ assimilation requires less energy than NO_3^- and NO_2^- conversion into structural nitrogen.[68] Slurry, on the other hand, holds higher concentrations of organic matter, nitrogen, and phosphorus in comparison with domestic wastewaters, the amounts present depending on animal nutrition and farming practices.[69,70] Although pig slurry can be rich in ammonium that is the favoured form of nitrogen for microalgae growth, NH_4^+ concentrations exceeding 100 mg L^{-1} could decrease microalgae growth in some species because of free ammonia toxicity.[71] Therefore slurry must be supplied at low loading rates to microalgae,[72] while wastewater can be used directly.[66]

To increase the productivity of microalgae related systems, CO_2, which is an essential macronutrient and maybe limiting in ambient air, should be provided. When only CO_2 from the atmosphere is available the biomass productivity is limited, whereas by providing additional CO_2 the biomass productivity has been reported to increase significantly.[66] The total amount of CO_2 required is a function of overall production capacity, theoretically up to 1.8 kg of CO_2 are required per kg of biomass to be produced. Its supply from a concentrated source has proved to effectively increase the availability of carbon for the growth of microalgae, and also to improve the recovery of nutrients by assimilation in their biomass.[68,73] As compressed CO_2 is costly, both from the economic and environmental points of view, it may be supplied from a recovered source in place of compressed CO_2 gas, by using flue gas from power plants fired with fossil fuels.[74–76] [75–77]

In order to assess the sustainability of a product in a new production chain, it is essential to rely on a standardised approach, by proceeding with complete validated evaluations. One of the tools used is the life cycle assessment (LCA). This procedure includes the calculation of all the inputs (energy and resources) and outputs (emissions) for each production steps of the life cycle of the study. Using the Life

Cycle Assessment (LCA) methodology has become increasingly widespread for the evaluation of products and services, with several studies evaluating the production of microalgae as food and energy outcomes. The LCA tool allows to precisely quantify emissions to the environment (physical quantities) highlight critical hot spots in the production process, compare production processes, and finally evaluate the opportunity to adopt an innovative production process with respect to the already existing options.

Many LCA studies have modelled virtual microalgae facilities with downstream processing, arranging different available technologies and reporting a widely available data on microalgae productivity.[78–81] Some references focus on the synergy of different production chain elements such as energy and feedstock or depuration and energy,[82] while others have an in-depth look at the emissions related to the microalgae growth steps such as ammonia and N_2O.[83] Some of the LCA works are quite optimistic in the future applicability and convenience of microalgae cultivation for commodities purpose i.e. energy and feedstock[84–86] while some others are more cautious in delineating and delimiting the role that microalgae production may have in the production systems of the future.[87–90]

However, many of the evaluations, however accurate, are made on virtual production plants, extrapolated from small pilots and laboratory data. The contribution this article intends to provide is the evaluation of sets of different working conditions, by including recovery practices that can highlight the environmental outcomes, all based on solid data from a full-scale facility.

2.2 Materials and Methods

2.2.1 Goal and Scope definition

The aim of this work is to provide a reliable attributional LCA of the production of microalgae for agriculture and aquaculture related applications, using primary data monitored from a full-scale production facility for microalgae based on raceway ponds and using different recovered inputs for water and nutrient sources, through the evaluation of different alternative scenarios. Some inquiries that this study will try to answer are: what is the most effective production model (including recovery of waste streams) for microalgae production? What are the differences between a production model using primary sources compared with a production model using recovered streams? What are the shifts in environmental impacts due to the use of recovered resources?

2.2.2 *System description*

Data to perform the LCA were collected from a demonstration facility at IFAPA (Investigación y Formación Agraria y Pesquera de Andalucia) Research Centre in Almería, Spain. On this location different reactors were available. The specific data included in this work were obtained from a raceway reactor of 1,000 m^2, which operated in a continuous mode for over one year. These data provided the basis for modelling a 5 ha model plant composed of nine large open raceway ponds for producing microalgae biomass (5,000 m^2 $reactor^{-1}$), 3 photobioreactors used for producing inoculum (1,500 m^2 $reactor^{-1}$, made of PVC linear with a length to width ratio value of 10), and a 1,000 m^2 surface area used for the auxiliary equipment (biomass harvesting and processing). The real unit has a biomass productivity of 20 g $m^{-2}\cdot day^{-1}$, the reactors operated in continuous mode at 0.2 day^{-1}, during an 8-hour day^{-1} for 300 days $year^{-1}$, and ten years was the lifespan assumed for the structure and equipment. The location has an average annual solar radiation in a daylight period of 815 µE m^{-2} s^{-1} and of 1630 µE m^{-2} s^{-1} at noon, with a temperature range from 9°C to 29°C, and an average value of 18°C. System substitution was included for considering the service provided by the management and depuration of wastewaters, which is described in the inventory analysis.

2.2.2.1 Production process

The production process started with producing a strain of microalgae in dedicated reactors to prepare inoculum. The inoculum was used as a seed culture for the ponds. Open ponds comprise a lined, shallow raceway in which water containing microalgae is circulated by paddle wheels. The culture medium was prepared from three different sources of water: freshwater, seawater and wastewater (Table 1). The fertilizers added as N and P sources were calcium nitrate and triple superphosphate respectively, quantities of water demanded and its partial recovering with wastewater, are summarised in Table 2, as is CO_2. Slurry was supplied by providing the same quantity of N supplied by the commercial fertilizer, an average value of 1.5 g kg^{-1} content of N was used for manure (average data from measurements). The energy demand was mainly due to the electricity supply for water pumping, mixing devices and gas injection (ambient air and CO_2 or flue gas, see scenarios); the input considered was the Spanish energy mix at the grid, medium voltage. An index of CO_2 absorption equal to 2 has been taken into account for each functional unit (FU) of produced microalgae biomass, based on measurement performed on the demoplant and consistent with previous references.[91,92]

2.2.2.2 Harvesting

The harvesting was performed in a two-step process, including pre-concentration by Dissolved Air Flotation (DAF) and a dewatering step using a nozzle separator (GEA Westfalia). At the end of the dewatering steps, the biomass sludge achieved a final biomass concentration of 100 g L^{-1} dry matter (dw), ready to be processed, and $FeSO_4$ was applied as a flocculant. We wish to underline that this two-stage harvesting is an optimised design, capable of stable operation and with a total energy demand of 0.2 kWh $kg_{biomass}^{-1}$, i.e. the lowest value within the range of 0.2-5 kWh $kg_{biomass}^{-1}$, reported in recent literature [93] for dilute solutions from open production systems. As for the growth, the input data of this phase are not from lab scale or theoretical consumption using equipment adapted for microalgae functioning, but data from equipment working at full-scale to harvest microalgae.

Table 1. List of scenarios related to water and nutrient use.

Water type	Nutrients supplied by	Recirculation	Scenario Code	Carbon supply
Freshwater	Fertilizers	Recirculation	W1	R/C
Freshwater	Fertilizers	Non-recirculation	W2	R/C
Freshwater	Manure	Recirculation	W3	R/C
Freshwater	Manure	Non-recirculation	W4	R/C
Seawater	Fertilizers	Recirculation	W5	R/C
Seawater	Fertilizers	Non-recirculation	W6	R/C
Seawater	Manure	Recirculation	W7	R/C
Seawater	Manure	Non-recirculation	W8	R/C
Wastewater	None	Non-recirculation	W9	R/C

2.2.2.3 Processing

After centrifugation, the paste biomass underwent cell disruption by a High-Pressure Homogeniser (HPH) (Niro-GEA Westfalia) to be finally processed by enzymatic hydrolysis (commercial Alcalase and Flavourizyme). Base and acid supply in addition to heating was applied in this phase to control reactor pH and temperature to the optimum values imposed by the enzymes used.

2.2.3 Functional unit and boundaries

The Functional Unit (FU) provides the reference to which all data in the assessment were normalised. In this study, the FU is 10 kg of produced microalgae paste after hydrolysis, containing 1 kg of dry weight biomass. The system boundaries included “cradle to gate”, starting from producing a strain of microalgae in a dedicated reactor to prepare inoculum, and the correlated processes for producing at large scale the biomass at the farm gate. Details of the main processes (Figure 1) considered in the LCA include the inputs and outputs of material and energy such as the construction of facilities, production of inoculum and biomass, harvesting, cell disruption and hydrolysis of microalgae, the supply of CO_2 and nutrients, transport of all the materials to the facility, and emissions to soil, water and air due to the managing of the microalgae production structure.

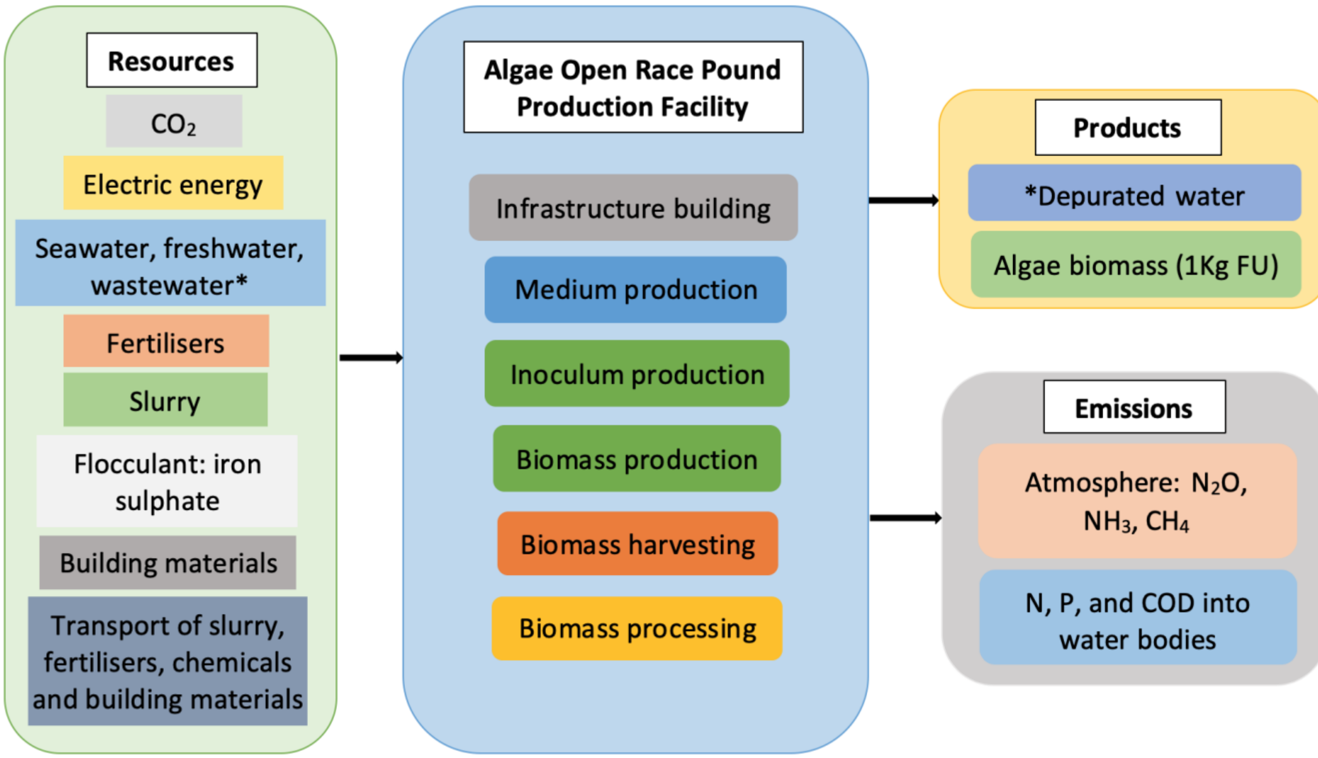


Figure 1. *System boundary considered in the LCA for the production of 1 kg of microalgae biomass.*

2.2.4 Scenarios' inventories

The managing of the facility relies on three inputs (water, nutrients and CO_2) supplied as follows. The water can come from three different sources: fresh, sea and wastewater (sewage). The management of water is also considered as a factor, i.e. with a recirculation or non-recirculation mode, where the recirculation has the advantage of being more efficient in the sense of nutrient uptake and of requiring both less water and energy consumption because of water pumped from the network. Nutrients are supplied either by chemical fertilizers or slurry, and with the wastewater scenario, its counted nutrients are provided by the stream itself. Nutrients in both sources are dosed according to microalgae growth, thus the release of N and P in discharged water is minimal for the non-recirculation mode and equal to zero for water within recirculation. For the case of CO_2 supply, two sources for the set of scenarios are considered:

(i) recovery (used by default), i.e. CO_2 was recovered while heat is provided by methane burning, and (ii) external supply (C), i.e. CO_2 was provided as compressed purified gas from external providers while heat for the hydrolysis step was provided by methane combustion. For CO_2 scenarios, it was assumed that the productivity yield should not be sensitive to the source of supply, but the environmental burdens change. CO_2, apart from when supplied as a purified compressed gas, was assumed to be recovered from the burning of gas used for other purposes. The CO_2 produced in the burning of natural gas in a boiler for the quota of heat needed in the microalgae processing (hydrolysis) was accounted as "recovered". In fact, more CO_2 than this supply is needed, and thus it was assumed that combustion was performed for some other purposes (industrial processing, heating, production of electricity), and flue gas was used in the microalgae facility with no burden accounted for it. Table 1 lists the total scenarios (nine) evaluated in this study. A system substitution is used to solve the multifunctionality in relation to wastewater treatment, as the production of microalgae also delivered depurated water as a product. In this case, the system boundaries of the wastewater scenario are subtracted from the inventory of wastewater treatment: energy, chemicals, structure for delivering depurated water. The process used in the Ecoinvent database for the wastewater treatment was *treatment, sewage, unpolluted, class 3*. Transport of goods, handcraft and commodities to the plant (chemicals, fertilizers, equipment) was assumed to be performed by a 32 Mg transport lorry, Euro 5. For an average 100 km each transport distance is expected to be with empty return. Slurry was assumed to have 20 km transport, while water and wastewater were presumed to be on site (water and wastewater networks).

About ammonia emission, a conservative approach was used, and average of 30% of the total N supplied was assumed to be lost via ammonia stripping when nitrogen is provided to microalgae into the medium via slurry[94,95] and 20% when wastewater is used, according to correlation with initial ammonia concentrations.[96] When commercial fertilizer was applied (nitrate salts) no ammonia emission was considered. As in each agricultural activity which involves the use of nitrogen and the availability of carbon, N_2O emission may occur. The proper mixing allowed high oxygen content during the entire cycle, that should prevent N_2O formation and emission.[97] The time of emission remains at night, when oxygen formation from photosynthesis stops and concentration of oxygen in the ponds decreases. Even if the N_2O emission is contained by adequate conditions, up-to-date literature stresses the importance of considering the N_2O metrics in LCA calculation[78] so as not to underestimate the real potential of CO_2 equivalent emissions. In the facility described, mixing was optimised and continuously monitored, thus the best conditions to reduce N_2O emission were guaranteed. The N_2O emission was assumed to be of 0.002% N input (for well-mixed ponds).[97] Lastly, methane emissions were considered to be of 0.01592 g CH_4 kg^{-1} microalgae, when biomass was calculated according to Ferrón et al., (2012) on the base of water-air interface in the Almería facility. The basic concept recently discovered is that CH_4 may be produced aerobically through bacterial uptake or degradation of algal products such as methyl-phosphonate.[98] The primary data compiled for the inventory denoted to the FU are presented in Table 2. All the inputs from trials were inserted including uncertainty and type of distribution.

2.2.5 Impact assessment

In the Life Cycle Impact Assessment (LCIA) phase, emissions and resource data identified during the LCI (Life Cycle Inventory) are translated into indicators that reflect environment pressures and resource scarcity. The software SimaPro® Analyst 9.0.0.41 was used for the computational implementation of the inventories,[36] and the set of libraries covered by Ecoinvent databases v3.5, 2018 to analyse the environmental impacts. Because of its representativeness at a global scale, the ReCiPe 2016[40] mid-point method (hierarchist approach) (version 1.13) was used to assess the environmental performance of microalgae production. Robustness of the LCA results was assessed by Montecarlo analysis, setting 10.000 runs.[99]

Table 2. *Data inventory used for each scenario for the calculation of impacts. When not indicated primary data measured on the plant, data from literature and assumption are indicated and critically discussed in the text.*

Parameter	***Unit***	***W1***	***W2***	***W3***	***W4***	***W5***	***W6***	***W7***	***W8***	***W9***
					Natural resources					
Soil occupation	m^2 kg algae^{-1}	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Freshwater demand	m^3 kg algae^{-1}	0.54	2.333	0.54	2.33	0.00	0.00	0.00	0.00	0.00
Seawater demand	m^3 kg algae^{-1}	0.00	0.00	0.00	0.00	0.54	2.33	0.54	2.33	0.00
Wastewater demand	m^3 kg algae^{-1}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.33
Water Release in the environment	m^3 kg algae^{-1}	0.24	2.00	0.24	2.00	0.24	2.00	0.24	2.00	2.00
					Nutrients supply					
N input	kg kg^{-1} algae	0.1	0.1	0	0	01	0.1	0	0	0
P input	kg kg^{-1} algae	0.016	0.016	0	0	0.016	0.016	0	0	0
Slurry	m^3 kg algae^{-1}	0	0	0.1	0.1	0	0	0.1	0.1	0
					Other chemicals					
Enzyme	g kg^{-1} algae	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
NaOH	g kg^{-1} algae	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Flocculant	kg kg^{-1} algae	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
					Energy					
Energy demand for culture medium preparation	kwh kg^{-1} algae	0.27	0.39	0.27	0.39	0.27	0.39	0.27	0.39	0.39
Energy demand for algae growth	kwh kg^{-1} algae	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57
Energy demand for Harvesting (DAF unit)	kwh kg^{-1} algae	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
Energy demand for Harvesting (Noozle concentrator)	kwh kg^{-1} algae	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Cell disruption (HPH)	kwh kg^{-1} algae	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024
Electricity for hydrolysis	kwh kg^{-1} algae	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Heat for hydrolysis	MJ kg^{-1} algae	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
					Emissions					

Nitrogen released in water	kg kg^{-1} algae	0	0.015	0	0.015	0	0.015	0	0.015	0.015
Phosphorus released in water	kg kg^{-1} algae	0.00	0.0017	0.00	0.0017	0.00	0.0017	0.00	0.0017	0
Organic carbon released in water (TOC)	kg kg^{-1} algae	0.01	0.07	0.01	0.07	0.01	0.07	0.01	0.07	0.07
Flocculant released in water	kg kg^{-1} algae	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002
N_2O released in atmosphere	mg kg^{-1} algae	2.03	2.033	2.85	2.85	2.03	2.03	2.85	2.85	2.85
NH_3 released in atmosphere	kg kg^{-1} algae	0	0.00	0.045	0.045	0.000	0.000	0.045	0.045	0.030
CH4 in the atmosphere	mg kg^{-1} algae	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05

2.2.6 Sensitivity analysis

To evaluate the influence of relevant parameters involved in using of recovered nutrients (slurry), i.e. the transport and the productivity, a sensitivity analysis was performed for scenario W3 considering the slurry transportation distance (10 and 30 km as a minimum and maximum value, i.e. 20 km as default value), and the productivity loss for the use of recovered nutrients (72 and 48 ton ha^{-1} as a minimum and maximum, i.e. 60 ton ha^{-1} as default). The sensitivity coefficient is calculated using the Equation 1.

$$S = \frac{\frac{(\mathrm{IC}\ high - \mathrm{IC}\ low)}{\mathrm{IC}\ default}}{\frac{(\mathrm{I}\ high - \mathrm{I}\ low)}{\mathrm{I}\ default}} \qquad \text{(Eq.1)}$$

Where IC is the value of the environmental Impact Category (max, min and default) and I is the value of the input considered for the analysis. Later, simulation provided threshold values for a maximum distance of transport and acceptable production losses due to the use of recovered nutrients.

2.3 Results and discussion

2.3.1 Environmental Impact assessment

The potential environmental impact associated with the nine scenarios at mid-point level is indicated in Table 3 and represented in Figure 2, results are reported as a relative value (%) achieved, assuming that the highest values for each impact would be equal to 100%. Results show that the scenarios with nutrient recovery (from both slurry and wastewater) are the most environmentally friendly alternatives with noticeable differences regarding the others, in areas that concern climate change, freshwater eutrophication, water depletion, terrestrial acidification and human toxicity. The other scenarios studied showed similar pattern-response in the considered categories.

Robustness of the LCA results was assessed by Montecarlo analysis. When comparing scenarios using recovered resources vs not using, for 11 categories, the scenarios including the use of fertilizers displaced higher results than the scenarios with recovered nutrients for more than 90% of the runs. For three categories (Human carcinogenic, Ozone depletion and Land use) results of scenarios including fertilizer use were higher in 60% of the runs, while for ozone formation (terrestrial and human) was the opposite: scenario with fertilizers displaced lower results than the scenario with recovered nutrients in 62% of the runs. For the last two categories (Particulate matter and Terrestrial acidification) the results of scenarios including recovered nutrients were higher than that of fertilizer use in 100% of the runs. The obtained results are consistent with other studies.[100,101] In particular, from those studies it was clear that the inputs of interchangeable factors such as the nutrient source, water type, and recirculation have a sharp effect in how they can impact the environment, thus supporting the re-use of resources (recovered nutrients) and the full exhaustion (water recirculation to exploit slurry nutrients). The negative bars reported in Figure 2 represent the avoided impacts in the related categories such as ecotoxicity (in the entire compartment, i.e. terrestrial, marine and freshwater), human carcinogenic toxicity, and mineral scarcity. These prevented impacts are attributed to Scenario W9, justified for the depuration of wastewater and for the avoided impacts linked to these processes (i.e. the saving of energy for depuration performed in a standard wastewater depuration plant). The removal of nutrients from wastewater has been previously reported with positive effects[102] in reducing the impact of eutrophication, and terrestrial - freshwater ecotoxicity. The contribution of the process for the different impact categories is reported in the following paragraphs.

2.3.1.1 Climate change.

Global Warming Potential (GWP), which represents the amount of additional emission pressure combined over 100 years because of an emission of 1 kg of CO_2 (expressed as CO_2 eq.)[40], is the Impact Category broadly used for climate change. For the scenarios considered its value was mainly dependent (Figure 3) on the use of energy and the production of fertilizers used to produce microalgae (still linked to the energy use), according to previous works.[74,103,104]

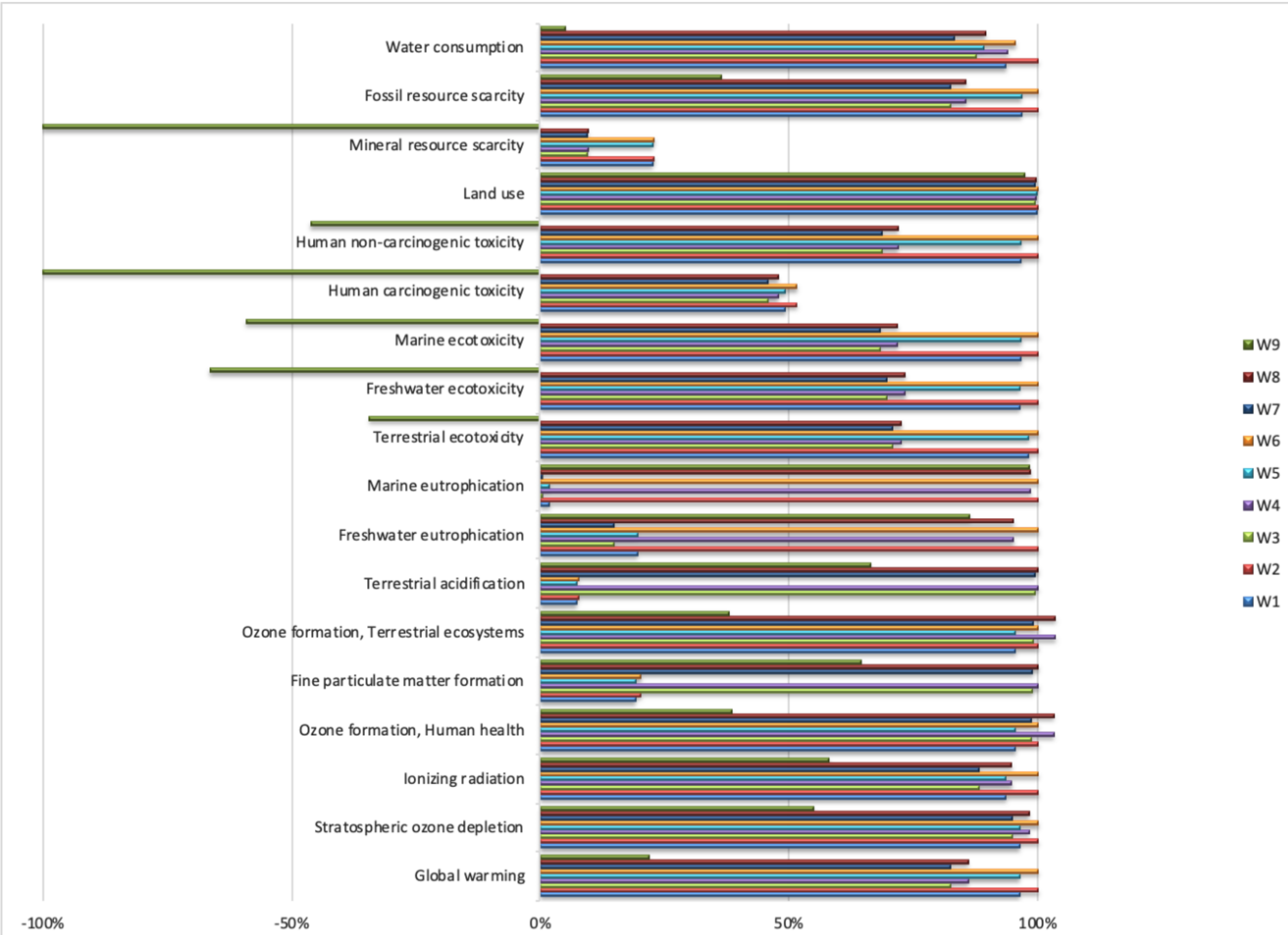


Figure 2. *Comparative environmental results for the nine scenarios considered. Impacts assessment calculated according to ReCiPe 2016 midpoint (H) V 1.03 method.*

For Scenario W1, for instance, $Ca(NO_3)_2$ addition caused 24% of the GWP, and electricity use, 38%. Recirculation of growth medium, in the corresponding Scenarios W1, W3, W5 and W7, allowed pumping less water, causing a slightly lower impact in this category (Table 3). It should be noted that transport caused higher impacts in the scenarios in which manure was used, because of manure transport: i.e. for each unit of fertilizer a large amount of water was also moved. The use of recovered fertilizer saved 0.39 kg CO_2 eq. in comparison with the chemical fertilizers' use, giving a better result (13%) for the GWP category (Table 3). The transport of materials for the facility construction was equal for all scenarios studied, so that it did not affect environmental impacts (Figure 3, GWP category).

Table 3. *Characterization of the 9 scenarios at the midpoint level impact categories according to ReCipe 2016 Midpoint (H) V.1.03.*

Impact category	***Unit***	***W1***	***W2***	***W3***	***W4***	***W5***	***W6***	***W7***	***W8***	***W9***
Global warming	kg CO_2 eq	1.45	1.50	1.24	1.29	1.45	1.50	1.24	1.29	0.33
Stratospheric ozone depletion	kg CFC11 eq	5.53E-07	5.73E-07	5.44E-07	5.64E-07	5.53E-07	5.73E-07	5.44E-07	5.64E-07	3.15E-07
Ionizing radiation	kBq Co-60 eq	0.41	0.43	0.38	0.41	0.41	0.43	0.38	0.41	0.25
Ozone formation, Human health	kg NOx eq	3.67E-03	3.84E-03	3.80E-03	3.97E-03	3.67E-03	3.84E-03	3.80E-03	3.97E-03	1.48E-03
Fine particulate matter formation	kg PM2.5 eq	2.49E-03	2.63E-03	1.29E-02	1.30E-02	2.49E-03	2.63E-03	1.29E-02	1.30E-02	1.20E-02
Ozone formation, Terrestrial ecosystems	kg NOx eq	3.72E-03	3.89E-03	3.86E-03	4.03E-03	3.72E-03	3.89E-03	3.86E-03	4.03E-03	1.48E-03
Terrestrial acidification	kg SO_2 eq	6.93E-03	7.32E-03	9.42E-02	9.46E-02	6.93E-03	7.32E-03	9.42E-02	9.46E-02	9.23E-02
Freshwater eutrophication	kg P eq	4.29E-04	2.18E-03	3.24E-04	2.08E-03	4.29E-04	2.18E-03	3.24E-04	2.08E-03	1.88E-03
Marine eutrophication	kg N eq	8.51E-05	4.54E-03	2.38E-05	4.48E-03	8.51E-05	4.54E-03	2.38E-05	4.48E-03	4.47E-03
Terrestrial ecotoxicity	kg 1,4-DCB	2.77	2.82	1.99	2.05	2.77	2.82	1.99	2.05	-0.97
Freshwater ecotoxicity	kg 1,4-DCB	2.02E-02	2.10E-02	1.46E-02	1.54E-02	2.02E-02	2.10E-02	1.46E-02	1.54E-02	-1.39E-02
Marine ecotoxicity	kg 1,4-DCB	3.11E-02	3.21E-02	2.20E-02	2.30E-02	3.11E-02	3.21E-02	2.20E-02	2.30E-02	-1.90E-02
Human carcinogenic toxicity	kg 1,4-DCB	4.15E-02	4.33E-02	3.85E-02	4.03E-02	4.15E-02	4.33E-02	3.85E-02	4.03E-02	-8.42E-02
Human non-carcinogenic toxicity	kg 1,4-DCB	0.66	0.68	0.47	0.49	0.66	0.68	0.47	0.49	-0.31
Land use	m^2 a crop eq	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.72
Mineral resource scarcity	kg Cu eq	5.97E-03	6.02E-03	2.50E-03	2.56E-03	5.97E-03	6.02E-03	2.50E-03	2.56E-03	-2.63E-02
Fossil resource scarcity	kg oil eq	0.44	0.45	0.37	0.39	0.44	0.45	0.37	0.39	0.16
Water consumption	m^3	6.35	6.78	5.95	6.37	6.05	6.47	5.65	6.06	0.34

In the production model under study, the energy input was optimised for both microalgae production (0.84-0.94 kWh $kg_{biomass}^{-1}$) and harvesting (0.19 kWh $kg_{biomass}^{-1}$). In the first case, optimisation was due to optimised reactor design and to the optimised fluid movement and the slow speed of the liquid adopted; in the second case, it was obtained via two-stage harvesting. Thus, the large gain that can be considered within scenarios is completely due to the inputs of recovered nutrients and the corresponding emissions for production and use. The lowest impact measured was registered for Scenario W9 (Figure 2, Table 3), where the service provided by wastewater depuration (system substitution accounting for the function of wastewater depuration) compensated for part of the CO_2 emissions. In Figure 2 are reported both positive and negative (credits) impacts. The values reported are similar to those presented by Collotta et al. (2018)[102], in which the use of wastewater, in addition to the injection of CO_2 recovered from a cement plant flue gas, gave the lowest impact emission, i.e. 0.306 kg CO_2 eq. for each kg of biomass, that is quite comparable with that reported for Scenario W9 scenarios, i.e. 0.47 kg CO_2 eq.

2.3.1.2 Stratospheric ozone depletion.

Emissions of ozone-depleting substances (ODSs) (expressed in kg CFC 11 eq.) which leads to the increase in UVB radiation,[105,106] are relatively small, with the lowest value reported for Scenario W9. As it was seen in GWP, the impact category was mainly due to the use of electricity, $Ca(NO_3)_2$ and fuel combustion (transport and building of infrastructure).

2.3.1.3 Ionising radiation.

The ionising radiation potential (IRP) reported as a Cobalt-60 eq. to air, quantifies radionucleotides emitted not only during nuclear activity but also in ordinary activities such as fuel burning and phosphate rock extraction. The process contribution that mainly explains its appearance was electricity, with 87% of contribution in Scenarios W1-W2-W5-W6 (in which fertilizers were used), followed by $Ca(NO_3)_2$ with 6.5%. In the other scenarios, the contribution of electricity was higher than 90%.

__Figure 3.__ Contribution of the main inputs to the different impact categories. ReCiPe 2016 midpoint (H) V 1.03 method.

2.3.1.4 *Photochemical Ozone Formation.*

The category quantifies, as NOx equivalent, the potential molecules leading to the formation of ozone, i.e. the photochemical reactions of NOx and Non-Methane Volatile Organic Compounds (NMVOCs) [40]. Many of the same processes mentioned in the previous categories explained the intensification of EOFP, where the impact was low and similar for Scenario W1-W8, and smaller for Scenario W9. The impact categories: ozone formation, human health, ozone formation and terrestrial ecosystem, despite low differences in absolute values, displayed the same pattern.

2.3.1.5 *Terrestrial Acidification.*

The category is linked to the atmospheric deposition of sulphates, nitrates and phosphates that cause a change in the acidity of soils. The highest values were linked to the scenarios using recovered nutrients, slurry and wastewater, that contain nitrogen in the form of ammonia which undergoes volatilisation during the production of microalgae. Once having considered direct ammonia emission, the other source of acidifying substances, far less relevant, is the use of electricity, i.e. the burning of fuel and corresponding NOx production. Electricity accounts for 5% of this category in the scenarios in which ammonia volatilisation occurred, while it was 70% for the others (Figure 2). For Scenario W9 the profile of impacts was analogous to the scenarios with recovered nutrients (Scenarios W3-4-7-8) due to less volatilisation of ammonia and the lowest electricity demand, the latter because there is no burden of transport of nutrients (i.e. manure) and there was a small avoided impact for wastewater depuration.

2.3.1.6 *Eutrophication.*

Eutrophication is due to the release of nutrients in water bodies; with freshwater eutrophication potentials (FEP) the impact is quantified as kg P eq. In the scenarios that considered recirculation, the main contribution to this category was the release of phosphorus (P) in the discharged water, i.e. 80% of the contribution, even if moderate (see Table 2). Other minor contributions are because of the use of electricity, production of $Ca(NO_3)_2$ and P discharged as waste during the production steps of triple phosphate typical of the scenarios using chemical fertilizers. When recirculation was performed, P discharge disappeared (Table 2) reducing the impact, and instead electricity became the main contributor to the footprint. In all the scenarios the impact encompassed both local (P released on site) and global emissions (P released globally in the process of tailing management). For Marine Eutrophication, expressed as N equivalent, the impact is completely due to the release of water in the non-recirculation scenarios (Figure 3), other contributions are negligible. Collotta et al., (2018), showed a significant favourable effect (negative value of impact categories) in the eutrophication impact when wastewater is used, because of the credit of avoided emissions for nutrients uptake and removal from wastewater. In this work, the system substitution took into consideration the avoided wastewater treatment, i.e. the credit was not relative to N and P, but it was relative to the energy demand for the wastewater treatment that was avoided, thus the eutrophication category for Scenario W9 was analogous to all the scenarios with a non-recirculation mode.

2.3.1.7 Ecotoxicity and human toxicity.

The emissions of 1,4-dichlorobenzene-equivalents (1,4DCB-eq) expressed in kg is used as characterisation factor of ecotoxicity in freshwater, marine and terrestrial ecosystems [40]. Ecotoxicity showed the same pattern described above for the other categories, i.e. a remarkable decrease of emission in the scenarios using recovered nutrients (-27%). The main factors affecting ecotoxicity were the use of both electricity and synthetic fertilizers (when supplied), and the processes related to transport all along the lifecycle (i.e. the use and the disposal of vehicles). As outlined in the discussion for other categories, the impacts of transport rose in the scenarios using slurry, with a very marked difference compared to scenarios not including slurries, this being particularly shown in the categories of freshwater and marine ecotoxicity.

In Scenario W9 the use of recovered nutrients within wastewater and the added service of water depuration, resulted in this scenario having a negative impact, i.e. the impact of the energy used for the production was "counterbalanced" by the service of waste depuration. This is easily understood if we consider that the direct electricity use for 1 m^3 of wastewater depurated by the microalgae system is 0.49 kWh m^{-3}, encompassing the energy for biomass production, while the depuration of wastewater by a conventional system costs on average 0.3-2.1 kWh m^{-3} of $wastewater^{-1}$. [107]

For terrestrial ecotoxicity (Figure 3) the role of transport was higher than that played in freshwater and marine ecotoxicity, and it was comparable to the share attributable to electricity and fertilizer use. Similarly, human non-carcinogenic toxicity was mainly due to electricity and fertilizers and presented a significant reduction in the scenarios with recovered nutrients (Figure 2), while the human carcinogenic category was explained by electricity use as first contributor and, fertilizers and transport related process (building of vehicles and roads) as a second one. The decrease in impact due to the non-use of synthetic fertilizers was eliminated by the greater impact related to the transport of slurry. This led to an equality of this category in the various scenarios considered, excluding, as before, Scenario 9, where the crediting for wastewater treatment brings a big decrease in the impact.

2.3.1.8 Use of resources: Land use, Fossil, Mineral and Water depletion

Land use expressed as the area occupied by the facility was almost equal in all scenarios. Fossil exploitation, quantified as kg oil eq., was explained by electricity use, $Ca(NO_3)_2$ (30% in the scenarios using fertilizer) and heating (natural gas). Due to the high contribution of $Ca(NO_3)_2$, the scenarios with recovered nutrients displayed a 20% decrease in this category, the remaining "credit" being compensated by the transport of slurry. Outside this array is Scenario W9, in which electricity and heat processes mainly provide the contribution, presenting a reduction of 61% in comparison with the scenarios using fertilizer.

Considering the category of mineral resource scarcity, Scenario W9, again, was the only one that displayed negative values, in the sense of preventing environmental impacts in the long term. By contrast, scenarios (Scenario W1-2-5-6) using an external artificial nutrient source presented the higher impact, this was mostly due to $Ca(NO_3)_2$ and triple superphosphate, respectively 42% and 24% of the category value. Water depletion level, expressed as m^3 of water consumed over water extracted, depended mostly on the upstream process of energy production. Thus, it was influenced by the use of electricity which is higher when there is constant pumping of water (recirculation off), and where in fact water consumption increases. The wastewater scenario displaced the lowest impact due to the release of depurated freshwater (2 m^3 kg^{-1} microalgae). When seawater and wastewater were used the impact on this process was lower (Figure 2).

2.3.2 Recovered fertilisers and sustainable transport: threshold distance

Markedly, using wastewater or recovered fertilizer from slurry, carried substantial environmental benefits compared with the external supply of macronutrients, this finding being backed up by recent literature .[88,102] Although the management of recovered nutrients showed a net environmental gain, it is appropriate to dwell on an item often cited as a significant component of some impact categories: the transport of recovered fertilizers. The concentrations of nutrients in slurry was not comparable with the concentration of nutrients in synthetic fertilizers; in addition, much water was involved in transport. In the illustrated scenarios, the slurry used had a concentration in N and P of 1.5 g kg^{-1} w/w and 0.16 g kg^{-1} w/w, from data in Table 2. Therefore, it becomes essential for proper programming, to understand the distances for sustainable transport of slurry, or the distance at which the emissions balance is still acceptable compared to the use of synthetic fertilizers. Moreover, LCA studies using wastewater as the alternative culture medium in growing microalgae[88] showed higher growth resulted by using NPK synthetic fertilizers, since the growth medium supplied was less turbid, allowing higher radiation infiltration compared to wastewater or slurry, both of them rich in suspended organic matter.

For these reasons it is clearly important to carefully evaluate nutrient source and slurry, not only to outline the benefits of slurry as an alternative nutrient source, but also because of the high variability that it could carry in real contexts (productivity), then it becomes reasonable to determine the effect that they could have in a production system. Table 4 shows the results of the sensitivity analysis for transport and productivity in scenario W3. For transport, the category mainly affected was mineral resource scarcity, since it was directly linked to the use of resources for the road infrastructure and maintenance. The other categories involved were all those related to toxicity (human and ecosystem), the effect on ozone (both stratospheric depletion and atmospheric formation) and the GWP. The simulation of different transport scenarios showed that 40 km was the limit because up to that distance, the solution with nutrient recovery was still sustainable and comparable to the scenario with synthetic fertilizers (W1) for the categories most affected. Beyond this threshold, the solution with recovered fertilizer was not advantageous. Concerning a possible decrease in production due to any issue related to recovered nutrients, the production system with recovered fertilizers was advantageous up to a productivity loss of 20%, i.e. at almost 48 Mg ha^{-1} of slurry the environmental benefits due to the recovery of nutrients were cancelled out by the drop in production.

2.3.3 CO_2 recovery

An additional scenario was taken into consideration for compressed and transported CO_2, instead of recovered CO_2 produced by the combustion of fuel. This approach is important because of the role that commodities such as the recovered CO_2 will play in the immediate future,[108] and to show how on site recovery, without the need for storage and compression, is an essential option for the sustainability of microalgae-related production chains. Using compressed CO_2 caused an impact increase of about 2-3 times, of the most relevant impact categories, i.e. global warming, eutrophication and toxicity. Terrestrial ecotoxicity, which is heavily influenced by the use of fuels (CO_2 transport, compression and purification) reported an increase of impact of as much as six times. Indeed, literature outlined that CO_2 injection was the primary factor affecting the environmental impact in the entire chain,[101] followed by both the nutrient supply and energy consumption,[102] indicating the relevance of the use of recovered sources. In the two years experimentation that provided data for this work, CO_2 from flue gas (methane combustion) was used routinely, demonstrating how productivity is not damaged by any NOx compounds produced during combustion, which actually work as a micronutrient. The topic was already addressed in literature,[109] with the recommendation of more detailed investigations in full-scale systems.

Table 4. *Sensitivity indices for transportation distance and productivity in a nutrient recovered source (Scenario W3: Slurry).*

Impact category	***Sensitivity coefficient transport***	***Sensitivity coefficient Productivity***
Global warming	0.157	-0.48
Stratospheric ozone depletion	0.189	-0.21
Ionizing radiation	0.038	-0.23
Ozone formation, Human health	0.193	-0.22
Fine particulate matter formation	0.080	-0.22
Ozone formation, Terrestrial ecosystems	0.197	-0.22
Terrestrial acidification	0.073	-0.22
Freshwater eutrophication	0.056	-0.22
Marine eutrophication	0.050	-0.22
Terrestrial ecotoxicity	0.298	-0.20
Freshwater ecotoxicity	0.216	-0.24
Marine ecotoxicity	0.221	-0.24
Human carcinogenic toxicity	0.230	-0.23
Human non-carcinogenic toxicity	0.245	-0.24
Land use	0.003	-0.40
Mineral resource scarcity	0.332	-0.20
Fossil resource scarcity	0.185	-0.19
Water consumption	0.037	-0.23

2.3.4 Evaluation of impacts and perspectives of microalgae cultivation.

To compare and discuss LCA results of microalgae cultivation -including the use of recovered resources- with existing literature, we can use the Global Warming Potential (GWP) category, as it is a robust indicator, widely used and common to different LCA assessment methods.

Schneider et al. 2018[88] performed cultivation trials in open raceways, with continuous working, using wastewater as one of the culture media, and reported values of GWP, equal to 5.34 and 2.69 when using fertilizers and wastewater respectively.

Completely different findings came from Porcelli et al. 2020,[109] that found values of 257 and 298 CO2 eq kgbiomass-1 using fertilizers and recovered CO_2 vs synthetic CO2. In this case, the production involved the use of artificial light, sterilization steps and energy-intensive practices for biomass treatment. So even if only 50% of the impacts were due to cultivation (around 120 CO2 eq kgbiomass-1) nonetheless the use of artificial light completely shifted the orders of magnitude of the impact respect to a field-based production based on sunlight Other studies that exploited recovered resources,[78,79,110] reported values of 1.4, 1.03, and 1.26 CO2eq for each kg of algae biomass. These studies modelled inputs from the scale-up of lab data, thus some uncertainty is present in the estimation of inputs or in the evaluation of other factors that, in the continuous operation, may reduce productivity, and provide a low and optimistic value of impacts for GWP category. The values obtained in this study, as regards to the scenarios with recovered nutrients (W3 W4, 1.24. and 1.29 kg CO2 eq kgbiomass-1 respectively), are close to the lowest values reported in literature, even if performed on a full scale, thus considering the actual measured productivity and consumption of inputs.

Different studies[49,111,112] outlined how, in microalgae production, fertilizers consumption, harvesting, and downstream processing, risk to nullify the benefits of the efficient photosynthetic yield of microalgae. As the critical work of Ketzer[113] highlights, the energy consumption for the cultivation of algae varies from the most optimistic 4 MJ / kg up to the value of two orders of magnitude higher (800MJkg). In this study the consumption is lower than 10 MJ kgbiomass-1, thus together with recovered resource valorization, it allows to obtain low GWP values, still the inputs of energy and the need of equipment are the main drawbacks respect to land plant production.

The results obtained in this work depict the state-of-the-art technology, at present, and report, in the best scenarios, in fact the lowest GWP scores, confirmed previous studies that scaled up lab production data in an industrial frame. Based on these data it is interesting to understand the effective potential and role of microalgae production, and thus to compare the environmental burden of microalgae grown in this industrial setting (open raceway, optimized equipment) with the production of land-based commodities plants, e.g. maize and soy, on dry matter basis. Values of GWP for silage maize biomass or soy range from 0.1 to 0.7 CO2 eq kg-1[114,115], thus steadily below that of microalgae production according to the state of the art technologies and circular recovery concept.

Considering both the inputs necessary for microalgae production and the environmental impact measured by LCA, the peculiarity of microalgae production systems becomes clear in comparison with traditional agricultural production. Microalgae production allows a higher productivity than "traditional agriculture" and it can limit some emissions due to the management of nutrients in the plant-soil system, i.e. N_2O emission and nutrients leaching in both surface and deep waters. The microalgae production systems are well isolated from the soil, carefully monitored and managed, and they have high technological input. Thus, microalgae systems allow an optimised management of the recovered nutrients, while traditional agro-systems present more critical issues in an open field, as they involve complex natural systems such as soil and water bodies. The main drivers of these advantages are: (i) the possibility of adding nutrients step by step following the microalgae uptake curve, thus ensuring a great efficiency in using both chemical and recovered fertilizers; (ii) the possibility of water recirculation, that allows the water to be discharged only once all the nutrients have been used; (iii) the closed and waterproof system of tanks used to produce microalgae prevents any leaching of nutrients before the water used for the production is discharged; (iv) the possibility of controlling the pH, together with the correct delivery of nutrients on demand and the high dissolved oxygen saturation of the media (greater than 100% of air saturation), which allows the reduction of both ammonia and N_2O emissions.

On the other hand, the production of microalgae requires an amount of energy (in the form of electricity) higher than that for traditional agricultural activities, i.e. the direct electricity demand to produce microalgae biomass is of 8.6 MJ kg biomass^{-1}, as primary energy, much higher than that reported for a crop, i.e. 1 MJ kg biomass^{-1}.[116] As outlined by the LCA analysis, the use of electricity is the most significant input in determining almost all the impact categories.

Another important element that differentiates microalgae from crop production is the use of the soil, not only, as previously discussed, in terms of the amount of soil required per unit of biomass produced but considering other soil services. The soil reserved for microalgae production is a sealed soil that cannot offer any eco-systemic services to the environment, such as, for example, draining and filtering of rainwater, habitat for entomofauna, capture of carbon in the soil etc.

Thus, the LCA numbers outline that microalgae production, even if performed at large scale, and according to circular approach and using low energy process, it is not, by now, for commodity purpose, nor energy nor food. Still, the microalgae production has a role for specific functions achieved thanks to its valuable and unique components: e.g. hormone-like molecules with bio stimulating activity on land plants, (assay performed using microalgae grown in the reported trials of this work[54,117] and PUFA, for the production of aquafeed [118], which replaces fish oils and decreases the pressure on marine ecosystems (trials performed in the frame of the same project). These kinds of products can provide functions not comparable to that of land-based commodities, and thus justify productions that display higher impacts.

Moreover, the evaluation of environmental pros and cons of microalgae production depends on contextual conditions, by not entirely captured by all the LCA approaches, such as the possibility to use seawater and wastewater for production, mainly in the areas where pressure on freshwater resources is high, or the opportunity to use non-arable land, soils poor and low in quality, not suitable for land crop production. For this reason, as often underlined in strategic studies for the location of microalgae plants, it is important to dedicate only industrial or low environmental value soils to a microalgae facility, while valorising high quality soils in proper crop agroecosystems.[119,120]

Beyond the added value of microalgae components and the valorisation of low-grade resource in place of scarce resources (seawater and non-arable soils), another critical issue in the environmental evaluation of microalga production is if other services are achieved. In scenario W9 the depuration of wastewater is performed, and the global sustainability of microalgae production changes completely and turns better respect to the production of land-based commodities.

2.4 Conclusions

Data inventory from an actual operating facility outlined that the main relevant inputs affecting environmental performances were electricity consumption, chemical fertilizer demand (N and P) and transport. Scenarios with recovered nutrients from slurry and the use of wastewater led to the better environmental performances. The threshold of distance for manure transport was 40 km, beyond that value the scenarios using recovered nutrients performed worse than the scenarios using chemical fertilizers. The maximum drop in productivity that the system with recovered nutrients could withstand was 20%, in addition to the environmental performance which was worse than in the scenario with chemical fertilizers. The multifunctionality of the process including wastewater depuration, allowed this scenario to compensate most of the impact categories, yielding negative values in some (e.g. all the toxicity categories). If CO_2 used is not from a recovered source, impacts are 2-3 times higher. Finally, state of the art technology by now justify the role of microalgae not for commodities production but for specific functions achievable thanks to microalgae metabolism, unless wastewater depuration is included.

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Chapter III

Environmental performance in the production and use of recovered fertilizers from organic wastes treated by anaerobic digestion vs. synthetic mineral fertilizers.

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3 Environmental performance in the production and use of recovered fertilizers from organic wastes treated by anaerobic digestion vs. synthetic mineral fertilizers.

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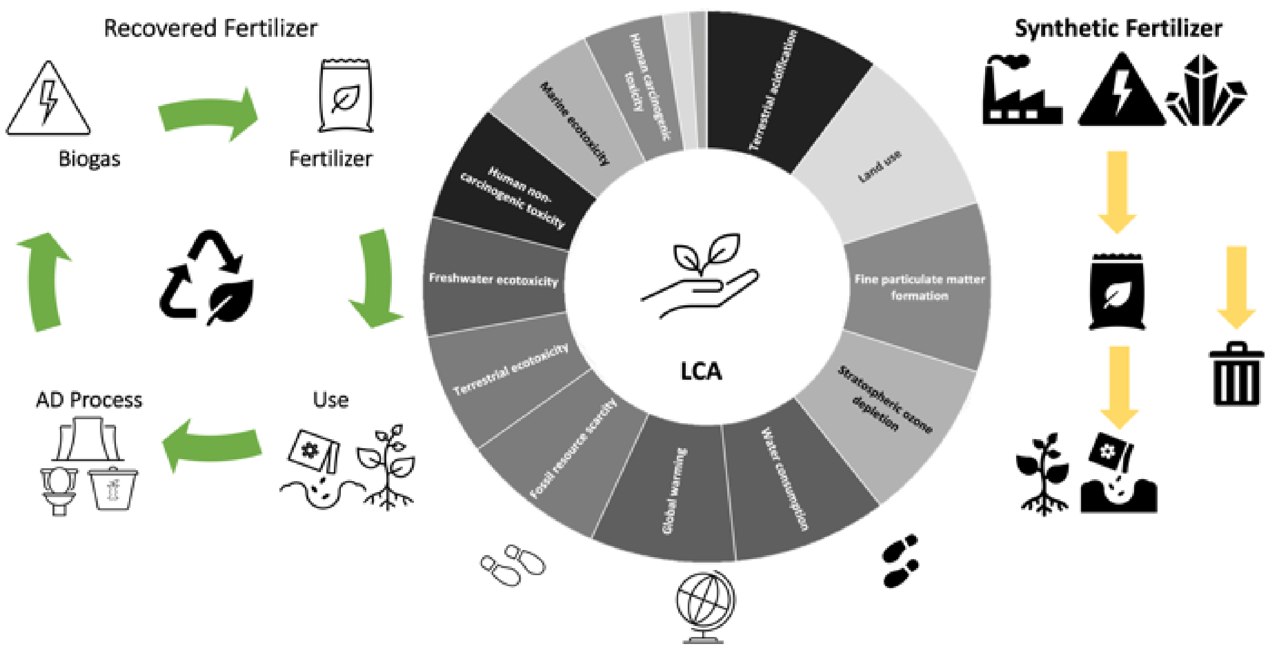
ABSTRACT ART



Abstract

Recovered fertilizers (RF), in the form of digestate and digestate-derived ammonium sulphate, were produced from organic wastes by thermophilic anaerobic digestion (AD) at full scale. RFs were then used for crop production (maize), substituting synthetic mineral fertilizers (SF). Environmental impacts due to both RF and SF production and use were studied by a Life Cycle Assessment (LCA) approach using, as much as possible, data directly measured at full-scale. The functional unit chosen was referred to as the fertilization of 1 Ha of maize, as this paper intends to investigate the impacts of the use of RF (Scenario RF) for crop fertilization compared to SF (Scenario SF).

(Scenario SF). Scenario RF showed better environmental performances than the system encompassing the production and use of urea and synthetic fertilizers (Scenario SF). For the Scenario RF, eleven of the eighteen categories showed a lower impact than Scenario SF, and four of the categories (*Ionizing radiation*, *Terrestrial ecotoxicity*, *Fossil resource scarcity* and *Water consumption*) showed net negative impacts in Scenario RF, getting the benefits from the credit for renewable energy production by AD. The LCA approach also allowed, proposing precautions able to reduce further fertilizer impacts, resulting in total negative impacts in using RF for crop production. Anaerobic digestion represents the key to propose a sustainable approach in producing renewable fertilizers, thanks to both energy production and to the modification which occurs to waste during a biological process, leaving a substrate (digestate) with high amending and fertilizing properties.

Keywords: Ammonium sulphate; Anaerobic digestion; Environmental impacts; Life Cycle Assessment (LCA); Digestate; Recovered fertilizers.

3.1 Introduction

The linear economy model based on the use of fossil fuel and raw sources has led our planet to encounter major environmental problems such as climate change, land degradation, and alteration of biochemical cycles.[3] With particular reference to N and P global flows, it has been reported that the current uses of these two elements is over Earth's boundaries because of anthropogenic perturbation due, mainly, to fertilizer application.[7] The use of chemically produced N and mined P is modifying and misbalancing not only the agroecosystem but also the natural ecosystems, putting biodiversity at risk.[1]

The regular production and use of mineral fertilizers in agriculture has a long track record of negative impacts in the environment[121] beyond the mere addition of nutrients to the soil. Fertilizer industry production and use causes about 2.5% (1203 Tg CO_2 eq.) of the global GHG emissions,[122] and N fertilizers account for 33% of the total annual creation of reactive N, i.e. 170 Tg N y^{-1} (fertilizers and livestock manure),[123,124] generating big environmental problems. In addition, the production of P and K fertilizers relies upon non-renewable and extracted resources that are becoming depleted[125] and are concentrated (e.g. P) in only a few countries.[126] The consequence of that is the need for new management strategies to reduce the additions of N and P into the ecosystem with particular reference to agriculture. The Circular Economy has been indicated as a new productive paradigm to produce goods, and it consists in the re-design of productive processes to allow the successive recovering of wastes for new productive processes, avoiding the use of new resources.[127]

Organic wastes can be explored as raw materials to recover nutrients and organic matter, representing an example of Circular Economy. To do so, wastes should be accurately chosen so that nutrient recovery can be made by applying suitable technologies,[128] producing fertilizers to replace synthetic ones.[129] Anaerobic digestion (AD) is a suitable biotechnology for producing biofertilizers, thanks to the process that modifies organic matter and the nutrients it contains, resulting in good amendment and fertilizer properties of the end-product, i.e. digestate.[130–132] In addition, the AD process renders the digestate more suitable for subsequent biological/physical/chemical treatments allowing organic matter (OM) and N and P to be separated, producing both an organic amendment, and N and P fertilizers.[128,133–135]

The recovery of nutrients allows the production of fertilizers able to substitute for synthetic ones, thus reducing the necessity to produce fertilizers using fossil energy (N and P) and fossil resources (P and K),[8] and closing the nutrient cycles. In addition, the recovery, also, of the organic matter represents a solution to the problem of low organic matter (OM) content (<1%) of soils,[136] which are attributed to the high carbon dioxide emissions which result from the intensification of agricultural practices.[137]

Despite the clear need to better manage nutrients already present in the ecosystem without adding new ones, a significant obstacle to this is the low efficiency and environmental performance which have been attributed to recovered nutrients.[123,138] Synthetic fertilizers contain concentrated nutrients under available forms, and so they are easy to apply to meet crop requirements. By contrast, the recovered wastes (sewage, manure, digestates etc.) contain nutrients with low efficiency and low concentration, and which also require good practices to be used to avoid environmental impacts.[139,140] Low Nutrient Use Efficiency (NUE) of recovered fertilizers might be due to their non-appropriate chemical form (mineral vs. organic forms), loss as NH_3 volatilization (10-65%), NO_3^- leaching and runoff (1-20%), and nitrification-denitrification (1-30%).[10,141] Therefore, the increase of NUE and environmental outcomes of recovered fertilizers represent challenges for modern agriculture.[142]

Recently, a scientific paper described,[128] at full scale, a plant producing recovered fertilizers (renewable fertilizers - RF) by anaerobic digestion, proposing that these fertilizers be used to substitute completely for fertilization by synthetic mineral fertilizers (SF).

This work aims to complete the path of the proposed Circular Economy in agriculture by recovering organic wastes by AD, measuring the environmental performances of the recovered fertilizers (digestate and ammonium sulphate) produced from organic wastes (mainly sewage sludge) by anaerobic digestion, to produce candidates to substitute completely for synthetic mineral fertilizers for crop production. To do so, Life Cycle Assessments (LCA) fed with both full-scale plant and agronomic data coming from crop trials performed at full scale have been carried out.

3.2 Materials and methods

3.2.1 Goal and scope

LCA analysis aims to measure the environmental impacts related to both production and to subsequent agronomic use of digestate and ammonium sulphate (Recovered Fertilizer) (RF) produced by the anaerobic digestion process using a mix of organic wastes (Scenario RF), compared to the production and use of synthetic fertilizers (SF), i.e. urea, triple phosphate and potassium sulphate (Scenario SF). This study covered the entire production and use of fertilizers, i.e. "from cradle to grave"[48] as it analysed a large full-scale anaerobic digestion plant used to transform organic wastes into bio-fertilizers (production phase),[128] and the subsequent full field application of the recovered bio-fertilizers (digestate and ammonia sulphate).

3.2.2 System description

3.2.2.1 Anaerobic digestion plant

The AD-plant (1 MWe power) for the combined production of fertilizers and energy is situated in the Lombardy Region (North Italy).[128] The plant exploits anaerobic digestion (AD) to transform different organic wastes (sewage sludges produced by municipal WWTP, agri-food factories, and liquid pulp-fraction of source-separated domestic food wastes) into organic-mineral fertilizers, i.e. digestate, mineral

N-fertilizer (i.e. ammonium sulphate) and energy (thermal and electrical). The plant is composed by two main sections comprising the AD plant and the ammonia-stripping unit (Figure 1a).

The AD plant produces biogas that is exploited to produce electrical energy delivered to the national grid and is also used for plant auto-consumption, and heat that is used for digester heating by steam injection and in the ammonia-stripping unit. During the process, several data were continuously monitored: digestate, pH (daily), digestate temperature, produced biogas and biogas composition (CH_4, CO_2 and H_2S, this latter 4 measurement per day).

Anaerobic digestion takes place in three reactors, working in series, of 4,500 m^3 each, made in carbon steel, with an average Hydraulic Retention Time (HRT) of 45-50 days to ensure good biological stability and sanitation.[128] The AD process is performed in thermophilic conditions (55°C), where the temperature is kept stable by using the heat produced from the Combined Heat and Power (CHP) unit. Reactor tanks have no mechanical mobile parts inside, with digestate mixing guaranteed by a system of external pumps. The tanks are covered with a gasometric dome membrane and maintained at constant pressure.

The system withdraws digestate from the second digester tank (DT 2) (Figure 1a) to the thin layer extractor, where ammonia is stripped from digestate by using the biogas or air.[128,143] The thin layer extractor consists of a cylindrical tank having inside a rotor with radial paddles, which by rotating at high speed keeps the digestate spread in a thin layer (few millimetres thick) on the internal walls of the cylinder.

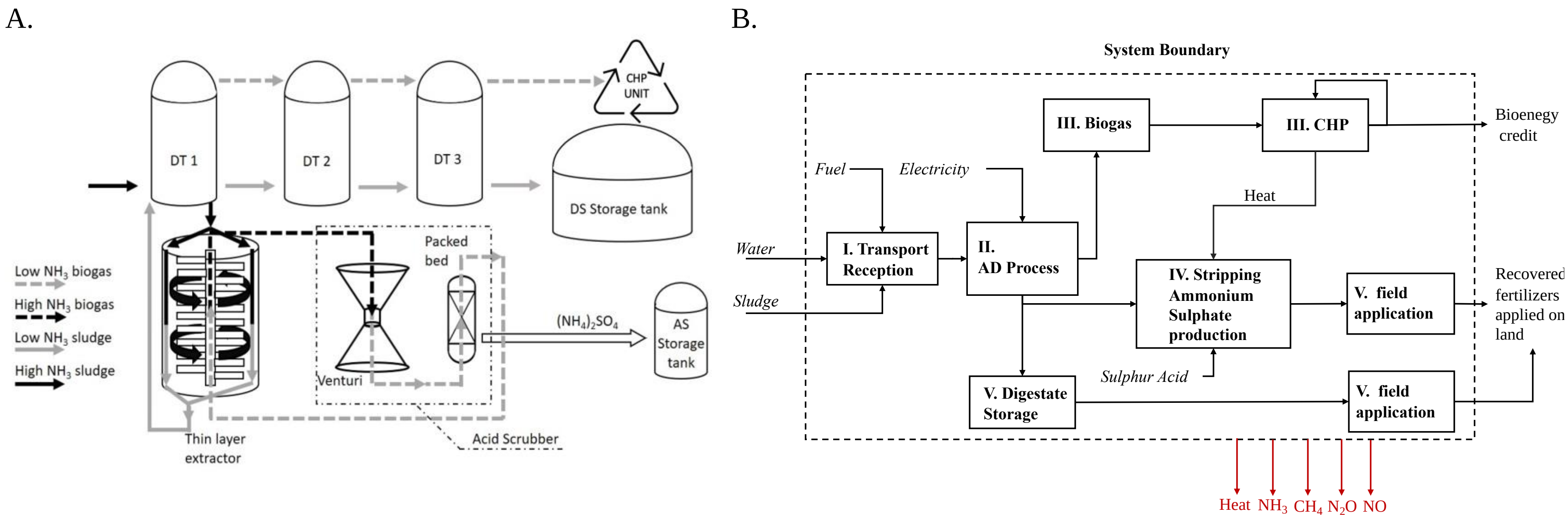


Figure 1. *Anaerobic Digestion (AD) plant and Nitrogen-stripping unit layouts (a); system boundaries and main processes for the Recovered Fertilizers (RF) (b).*

Meanwhile, the rotor keeps biogas at high turbulence to enhance the exchange of ammonia from the digestate to the gas. The transfer of ammonia occurs in a counter current; the digestate is pumped into the top of the cylinder, and it goes down by gravity in a thin layer while gas flux is from the bottom to the top. The walls of the cylinder are warmed at 80°C to increase the exchange from the digestate to the gas which is injected at 70°C. After the stripping in the thin layer, the low-content ammonia digestate is pumped back to the first digester (DT 1) while carrier gas in a closed loop cycle goes to the acid scrubber unit, where ammonia reacts with sulphuric acid generating ammonium sulphate. Both recovered fertilizers produced were used in substitution for synthetic fertilizers, both at pre-sowing (digestate) and as top-dressing (ammonium sulphate).

3.2.2.2 Recovered fertilizers produced

Recovered fertilizers (renewable fertilizers) characteristics are listed in Tables S1-S2; a complete description can be found in Pigoli et al. (2021).[128] The previous characterization made also included organic contaminants and target emerging organic contaminants (Table S1).

3.2.2.3 Full field agronomic use of renewable fertilizers in substitution of synthetic mineral fertilizers.

Full field agronomic performance and impact measurements, i.e. air emissions (NH_3, N_2O, CH_4 and CO_2) and nitrate leaching were carried out on soil plots distributed randomly close to the AD plant. Digestate was injected into the soil at a depth of 15 cm at the dose required assuming an N efficiency of 0.5, as suggested by the Regional Plan for Water Protection from Nitrate from Agriculture.[144] For the SF Scenario, urea was spread onto the soil surface following a routine agricultural procedure. Fertilizers used, doses applied and spreading methodology are reported in detail in Table S3 in Supporting Information and summarised in Table 1.

3.2.2.4 Emissions

GHG emissions (N_2O, CH_4 and CO_2) were measured in 2020, following the entire agronomic season of maize: from May (sowing) to October (harvest). The determination of emissions was conducted through the use of non-steady-state chambers.[145] Sampling chambers were placed in each of the experimental plots, furthermore, to obtain a background measurement, another 3 chambers were placed on non-fertilized plots. The air sampling inside the chamber was carried out with a frequency of 1 to 8 times a month, depending on the season and the state of the crop. The air taken was then analysed in the laboratory using a gas chromatograph, according to the method reported by Piccini and colleagues.[146] The cumulative emissions were obtained by estimating the flows in the non-sampling days, by linear interpolation.[147]

The concentration of NH_3 was monitored by the exposure of ALPHA passive samplers.[139,148] For each plot, the ALPHA samplers were installed in sets of three. To obtain background environmental concentration values, an additional sampling point was placed at a distance of about 1,000 meters away from the fertilized fields and other possible point sources of NH_3 emissions.

3.2.3 System boundaries and data inventory

3.2.3.1 System boundaries

The system boundary starts from the organic waste collection and transport, encompasses the production of digestate/bio-fertilizer and ammonia sulphate, the correlated processes for producing biogas which is transformed into electric energy and thermal energy and finally the use of the digestate in the field. The system boundary was represented by the dashed line in Figure 1b and comprised five main processes for Scenario RF (Recovered Fertilizer): i. the transport of sludge and organic wastes to the AD plant (assuming 100 km on average), ii. the AD process, iii. the biogas combustion and electricity production in CHP, iv. the digestate stripping process and ammonium sulphate production and v. the digestate storage, handling, and distribution into fields. Capital goods were included in the system, considering a lifespan of the structure of 20 years. The Scenario SF (Synthetic Fertilizer) encompassed the production of urea, triple phosphate, and potassium sulphate fertilizers (including logistics and transportation) and the timely distribution on fields. This Scenario was modelled using data coming from the literature and databases (Ecoinvent 3.6).[149]

The main data inventory is reported in Table 1, inputs and output of production were all taken directly from the plant facility. Air emission of the two systems, i.e. ammonia, methane, nitrous oxide and carbon dioxide were measured directly on monitored field plots as previously reported (Table 1) (Table S4). Indirect dinitrogen monoxide and NOx were estimated according to IPCC (2006).[150] Nitrate leaching was calculated according to IPCC (2006)[150] for Scenario SF, based on the N distributed, and assumed to be equal for Scenario RF, as the monitoring of nitrate content in deep soil layers during the year showed no differences (Table S4). Phosphorus in soil, leaching and run off was modelled according to Ecoinvent report 15.[151] Heavy metals supplied were included in the model according to the characterization data of digestate, plant uptake and accumulation rate in the soil system.[152,153] The input of organic pollutants was considered for PCDD/F, DEHP, PAH contained in digestate, as a proper numerical quantification was workable (see Table S1).

3.2.3.2 Functional Unit

The Functional Unit (FU) provided a reference to which all data in the assessment were normalized. Because this study considered the impacts derived from the production and use of fertilizers on crop maize, the functional unit chosen was referred to the fertilization (fertilizers production and use) of 1 Ha of maize, i.e. for Scenario SF: 402 kg of Urea (185 kg of N), 476 kg of chemical ammonium sulphate (100 kg N), 195 kg of triple phosphate (89 kg of P_2O_5) and 165 kg of potassium sulphate (82.5 kg of K_2O), and for Scenario RF: 48 Mg of digestate, i.e. 370 kg of total N, i.e.185 kg of effective N, 317 kg of P_2O_5 and 43 kg of K_2O, 1.38 Mg of recovered ammonium sulphate (100 kg of N), and 80 kg of potassium sulphate (40 kg of K_2O) (see Table 1).

3.2.3.3 Modelling framework and approach to multi-functionality

The modelling framework of this study was attributional, i.e. digestate and ammonium sulphate were considered as the target products of the production chain. Biogas was produced and valorised in the CHP module to generate electricity and heat. In order to consider these outputs and to make the two systems (Scenario RF and Scenario SF) comparable, the approach of system substitution, i.e. crediting for the avoided burden - was chosen. The option of system substitution was not exploited to include the service of waste treatment (i.e. incineration or landfill) that is performed, as it would have introduced great variability in the credits of the service. This approach was very prudential, as it did not consider the alternatives for disposal of organic wastes that in any case would be necessary and impacting. However, the credits for renewable electricity were accounted for and considered for substituting the electricity mix distributed in the national grid.

Table 1. *Inventory data of the considered scenario.*

INPUT	UNIT	QUANTITY	DATA SOURCE
Waste input (total)	Mg y^{-1}	81,886	Provided by facility
Methane (from national grid)	sm^3 y^{-1}	228,177	Provided by facility
Water (from aqueduct)	m^3 y^{-1}	19,744	Provided by facility
Water (from well)	m^3 y^{-1}	14,044.	Provided by facility
Water (total)	m^3 y^{-1}	33,788	Provided by facility
Electricity consumed from the grid	kWh y^{-1}	7,189	Provided by facility
Sulphur acid	Mg y^{-1}	316	Provided by facility
OUTPUT			
Digestate produced	Mg y^{-1}	112,322	Provided by facility
Electricity produced and fed to the grid	kWh y^{-1}	5,349,468	Provided by facility
Electricity produced and reused in the process	kWh y^{-1}	2,395,215	Provided by facility
Total electricity produced	kWh y^{-1}	7,737,494	Provided by facility
Ammonium sulphate	Mg y^{-1}	571	Provided by facility
Wastes from sieving sent to landfill	Mg y^{-1}	2.5	Provided by facility
Biogas produced	Mg y^{-1}	3,842	Provided by facility
Thermal energy produced (by CHP)	MWh_{th} y^{-1}	5,976	Provided by facility
EMISSIONS (from distribution)			
Digestate			
Ammonia ($N\text{-}NH_4$)	kg ha^{-1}	25.2	Detected on-site by the authors (Table S5)
Direct dinitrogen monoxide ($N\text{-}N_2O$)	kg ha^{-1}	9[a]	Detected on-site by the authors (Table S5)
Indirect dinitrogen monoxide ($N\text{-}N_2O$)	kg ha^{-1}	0.8	IPCC 2006
Nitrate leaching ($N\text{-}NO_3$)	kg ha^{-1}	83[b]	IPCC 2006
NOx (N-NOx)	kg ha^{-1}	0.5	IPCC 2006
P surface run-off (P)	kg ha^{-1}	1.4	EDIP 2003
Urea			
Ammonia ($N\text{-}NH_4$)	kg ha^{-1}	25.2	Detected on-site by the authors (Table S5)
Direct dinitrogen monoxide ($N\text{-}N_2O$)	kg ha^{-1}	9[a]	Detected on-site by the authors (Table S5)
Indirect dinitrogen monoxide ($N\text{-}N_2O$)	kg ha^{-1}	0.8	IPCC 2006
Nitrate leaching ($N\text{-}NO_3$)	kg ha^{-1}	83[b]	IPCC 2006
NOx (N-NOx)	kg ha^{-1}	0.3	IPCC 2006
Carbon dioxide ($C\text{-}CO_2$)	kg ha^{-1}	80.2	IPCC 2006
P surface run-off (P)	kg ha^{-1}	0.2	Nemecek & Kägi 2007
USE OF NUTRIENTS			
RF[c]			
Digestate	Mg ha^{-1}	48	Data from authors
TN supplied by digestate	kg ha^{-1}	370	Data from authors
TN delivered by ammonium sulphate	kg ha^{-1}	100	Data from authors
P supplied by digestate	kg ha^{-1}	138	Data from authors
K supplied by digestate	kg ha^{-1}	36	Data from authors
K delivered as potassium sulphate	kg ha^{-1}	34	Data from authors
SF[c]	kg ha^{-1}		

TN supplied by urea	kg ha^{-1}	185	Data from authors
TN delivered by ammonium sulphate	kg ha^{-1}	100	Data from authors
P provided by triple phosphate	kg ha^{-1}	39	Data from authors
K supplied as potassium sulphate	kg ha^{-1}	70	Data from authors

[a]N_2O emissions were considered similar (calculated on 1ha surface) for the two scenarios as revealed by full-field measurements made after digestate and urea distribution (see Table S4).
[b]N leaching was assumed similar (calculated on 1ha surface) for the two scenarios as revealed by soil sampling made at 1 m soil depth in full-field trials (see Table S4).
[c]RF: Recovered Fertilizer scenario, and SF: Synthetic Fertilizer scenario.

3.2.4 Life Cycle Impact Assessment

The Life Cycle Impact Assessment (LCIA) was based on the emissions and resource inputs identified during the data inventory, which was processed into indicators that reflect resource shortage and environmental burdens. The software SimaPro® Analyst 9.1.1.7[36] was used for the computational implementation of the inventories and the set of libraries covered by Ecoinvent databases v3.6, 2019 in order to analyse the environmental impacts. Because of its representativeness at the global scale, the ReCiPe 2016 method (version 1.13),[154] which contains midpoint impact indicators and endpoint areas of protection, was used to assess the environmental performance of bio-fertilizer and energy production. Global normalization factors from the same method were used.[31] Robustness of the LCA results was assessed by Montecarlo analysis, setting 10,000 runs.[99]

3.3 Results And Discussion

The results of the two scenarios reported as mid-point indicators and split for fertilizers production and use, as well as the impact deviations taking as reference the Scenario RF, are shown in Table 2. The Scenario RF showed better environmental performances than the system encompassing the production and use of urea and commercial fertilizers (Scenario SF). In particular, for the Scenario RF, eleven of the eighteen categories showed a lower impact than in Scenario SF, and four of the categories (*Ionizing radiation, Terrestrial ecotoxicity, Fossil resource scarcity and Water consumption*) showed net negative impacts in the Scenario RF, getting the benefits from the credit of renewable energy production by AD. The final end-point single score ranked 48 and 215 points for the Scenario RF and Scenario SF, respectively, which summarises the globally better outcome of the Scenario RF (Figure 2). Analysis and contributions of the processes to the categories are discussed below.

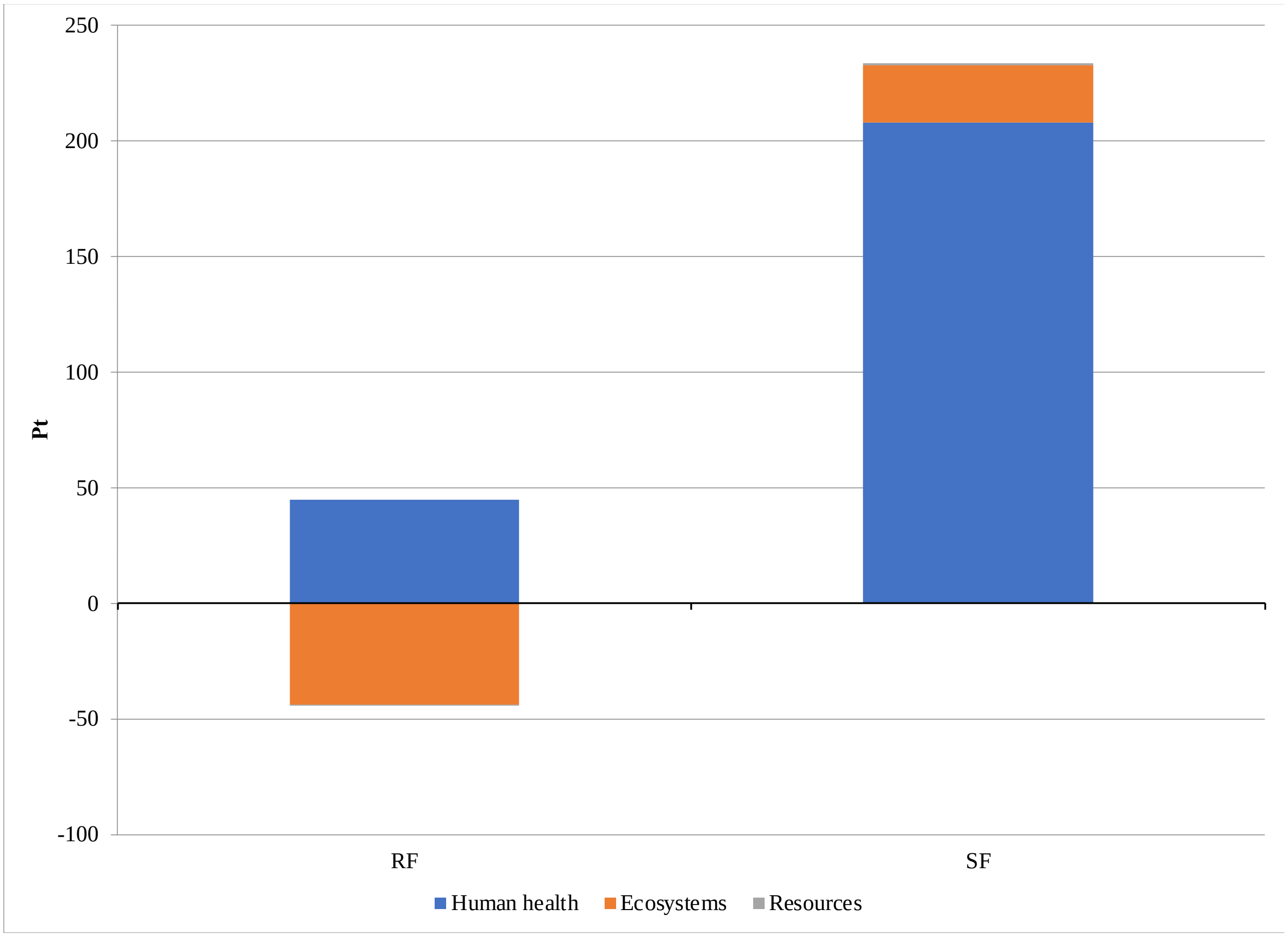


Figure 2. *Comparative environmental results for Scenarios Recovered Fertilisers (RF) and Synthetic Fertilisers (SF). Impacts assessment calculated according to ReCiPe 2016 endpoint (H) V 1.03 impact assessment method.*

3.3.1 Midpoint results of impact categories related to Ecosystem quality

Global Warming impact category; The production of the recovered fertilizers (Scenario RF), which included sludge transport and handling, the AD process, ammonia stripping and biogas burning, without considering the electricity credits, caused the emission of 669 $kgCO_{2eq}$., lower than the data reported for the production of synthetic mineral fertilizers, i.e. 834 $kgCO_{2eq}$. Beyond, thanks to the credits (avoided CO_2 emissions) due to the production of renewable energy (biogas), the value of the fertilizers production was negative, i.e. – 646 $kgCO_{2eq}$. With reference to the fertilizers use, which was reported to be the critical point in terms of emissions and environmental impacts for the recovered fertilizers,[155] the impact for the Scenario RF (i.e. 3,999 $kgCO_{2eq}$), was only slightly higher than that for the Scenario SF (i.e. 3,966 $kgCO_{2eq}$)

because of the higher energy consumption needed for digestate distribution into the soil than that required for urea and other mineral fertilizers distribution (Scenario SF).

From the data reported above, it was derived that the total net impact measured for the production and use of RF was of 3,354 $kgCO_{2eq}$, with this figure being lower (-30%) than that calculated for the Scenario SF, i.e. 4,800 $kgCO_{2eq}$ (Table 2). GHG impacts were due above all to direct emission of N_2O coming from nitrogen dosed to the soil as fertilizers, with the GHG coming from biogas burning and mass transportation playing only a minor role. The impacts measured for this gas were the same for the two scenarios studied, since the measured N_2O emissions were statistically identical to each other (Table S4).

Table 2. *Impact category values for the two compared systems SF and RF with their respective contribution due production and use (field emission and distribution), and credit-related for the electricity generated (CRE). Impact assessment calculated according to ReCiPe 2016 Midpoint (H) V.1.1. FU: 1ha Maize.*

Impact category	**Unit**	**RF**				**SF**		
		Production	**Use**	**CRE**	**Total**	**Production**	**Use**	**Total**
Global warming	kg CO_2 eq	669	3,999	-1,315	3,354	834	3,966	4,800
Stratospheric ozone depletion	kg CFC11 eq	0	0.1	0	0.1	0	0.1	0.1
Ionizing radiation	kBq Co-60 eq	38	10	-204	-156	82	4.5	86
Ozone formation, Human health	kg NOx eq	5	2	-3	4	1	1.0	2
Fine particulate matter formation	kg PM2.5 eq	2	6	-2	7	1	6.2	8
Ozone formation, Terrestrial ecosystems	kg NOx eq	5	2	-3	4	1	1.0	2
Terrestrial acidification	kg SO2 eq	6	50	-5	51	4	50	54
Freshwater eutrophication	kg P eq	0.1	8.4	-0.3	8.2	0.3	0.2	0.5
Marine eutrophication	kg N eq	0	17	0	17	0.0	17	17
Terrestrial ecotoxicity	kg 1,4-DCB	1,247	240	-1,370	117	2,550	114.8	2,664
Freshwater ecotoxicity	kg 1,4-DCB	8	351	-11	348	13	0.6	14
Marine ecotoxicity	kg 1,4-DCB	12	492	-16	488	23	0.9	24
Human carcinogenic toxicity	kg 1,4-DCB	35	9	-25	19	19	1.4	20
Human non-carcinogenic toxicity	kg 1,4-DCB	266	54,585	-330	54,521	458	88.8	547
Land use	m^2 a crop eq	7	3	-4	6	6	1.1	7
Mineral resource scarcity	kg Cu eq	3	1	-1	4	9	0.4	9
Fossil resource scarcity	kg oil eq	134	27	-384	-224	313	16	329
Water consumption	m^3	631	189	-8,575	-7,755	1,196	86	1,282

Results of this work appear more interesting if it is considered that much more N was added to the soil in the Scenario RF, i.e. total N of 470 kg ha^{-1} (Table S3) than in Scenario SF, i.e. 285 kg Ha^{-1} of N, suggesting that only the efficient (mineral) fraction of total N was responsible for N_2O emission, since these two figures were identical for the two scenarios studied (i.e. total mineral N dosed of 285 kg Ha^{-1} and 285 kg Ha^{-1} of N for Scenarios RF and SF, respectively) and that organic N (contained in the digestate) appeared not to additionally contribute at to emissions.

This result was consistent with the high biological stability of the digestate, measured by potential biogas production (BMP) (Table S1), that was even lower (i.e. with higher biological stability) than those reported for well-matured composts,[156] leading to null or a very low rate of mineralization of the organic N in short-medium time. Biological stability of the organic matter has recently been reported to play an important role in defining N mineralization in the soil. Tambone and Adani (2017)[157] reported that mineral N produced during organic substrate incubation correlated negatively with CO_2 evolved during soil incubation, i.e. the more stable was the substrate, the less C (and N) mineralization occurred. In this work, the CO_2 and CH_4 measurements carried out directly on plots during the cropping season (Table S4) indicated the absence of differences in C emission for soil fertilized with synthetic fertilizers and digestate, but also with the control (no fertilizers added) confirming that organic matter added with digestate was stable, contributing to restore soil organic matter. The increase of total organic carbon (TOC) in soil treated with digestate after three years of fertilization, compared to soil fertilized with mineral soil, seems to confirm this fact (TOC increased after three years from 10.3 ± 0.6 g kg^{-1} dry weight (dw) to 12.3 ± 0.4 g kg^{-1} dw, differently from the mineral fertilized and the unfertilized plots that did not show any increase) (unpublished data).

Results obtained in this work differed from those of previous studies that reported higher emissions of N_2O when recovered fertilizers (digestate) replaced mineral fertilizers.[158]

Nonetheless in that case, N_2O emissions were assumed (not measured directly) to be of 1% of the total N from mineralization, mineral fertilizers, digestate and existing crop residues; in addition, no data regarding the OM quality of digestate (potential N mineralization) i.e. biological stability, were reported. It can be concluded that N_2O emissions depended on available N (mineral) plus the easily mineralizable fraction of the organic N, which depended, in the first instance, on the biological stability of the organic substrate, so that this parameter becomes important for a rough estimation of the potential N_2O emission. This result was in contrast with that reported in the literature which indicated a direct proportionality between the total amount of nitrogen supplied and N_2O emissions,[150,159] without any specification of N type, i.e. organic vs. mineral N and organic matter stability responsible for potential N mineralization. We consider that this approach could lead to a misinterpretation of the real impacts of recovered organic fertilizers that need, as already discussed, to be better characterized.

Ammonia emissions represent another important issue in determining environmental impacts when using fertilizers. The full field approach indicated that there were no differences in ammonia emissions between Scenario RF and Scenario SF (Table S4) thanks to the digestate injection that resulted in a strong mitigation in ammonia emissions in comparison with superficial spreading,[140] as confirmed also by the literature.[139] The low ammonia emissions did not increase N_2O emission, as already discussed, in contrast with what has been reported in the literature, i.e. that ammonia emissions abatement led to an increase in N_2O emissions,[160] indicating that a well stabilized organic substrate and the adoption of an efficient distribution technique allowed containment of both NH_3 and N_2O emissions. The high biological stability of the digestate, providing for low organic matter mineralization, limited, also, the NO_3^- leaching for the Scenario RF, which was, according to the data measured directly at full field during the crop season, analogous to that measured for the Scenario SF (Table S4).

The identical N_2O emissions reported for the two scenarios studied led, also, to similar *Stratospheric ozone depletion* impact, since the emissions of ozone-depleting substances (ODSs) are mainly due to the direct N_2O emissions from fields.

Ionizing radiation quantified the emission of radionuclides in the environment that may be due to nuclear activity, but also to fuel burning. The Scenario RF achieved a total negative impact because of the production of renewable electricity that compensated for the other emissions caused by transport (transport of sludge to the AD facility), digestate handling and distribution. Considering just the fertilizer use, the measured impact was higher for the Scenario RF than that for Scenario SF, i.e. 9.7 vs 4.5 kBq Co-60_{eq}, (Table 2). High water content and low nutrient concentration for digestate, leading to more energy consumption for its distribution than for synthetic mineral fertilizers, were responsible for the higher impact.

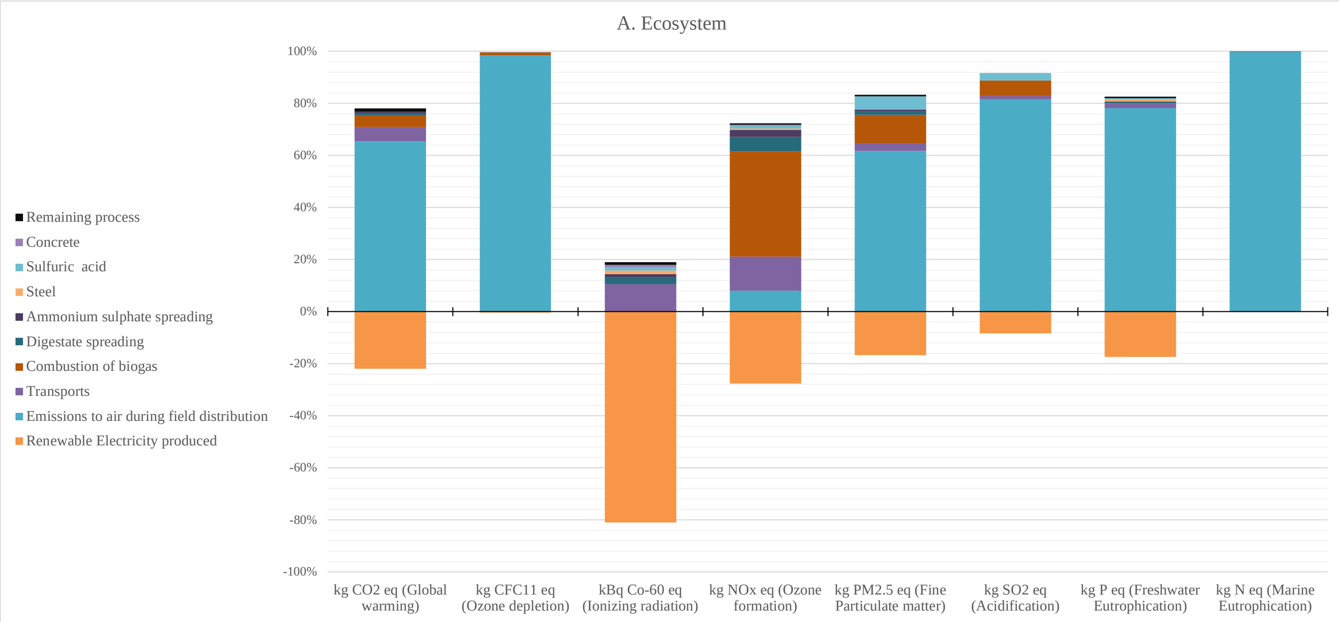
The categories *Ozone formation* (Human health and terrestrial ecosystem) that quantified the potential molecules leading to the formation of ozone as NOx equivalent[154] were two of the six categories reported to be higher for the Scenario RF than Scenario SF, the main contributor to this category being the biogas combustion for electricity production (Figure 3a). Less important, i.e. about 10%, was the impact due to direct emissions in the field, i.e. distribution of digestate (fuel machinery) and distribution of ammonium sulphate and NO_X direct emissions from land.

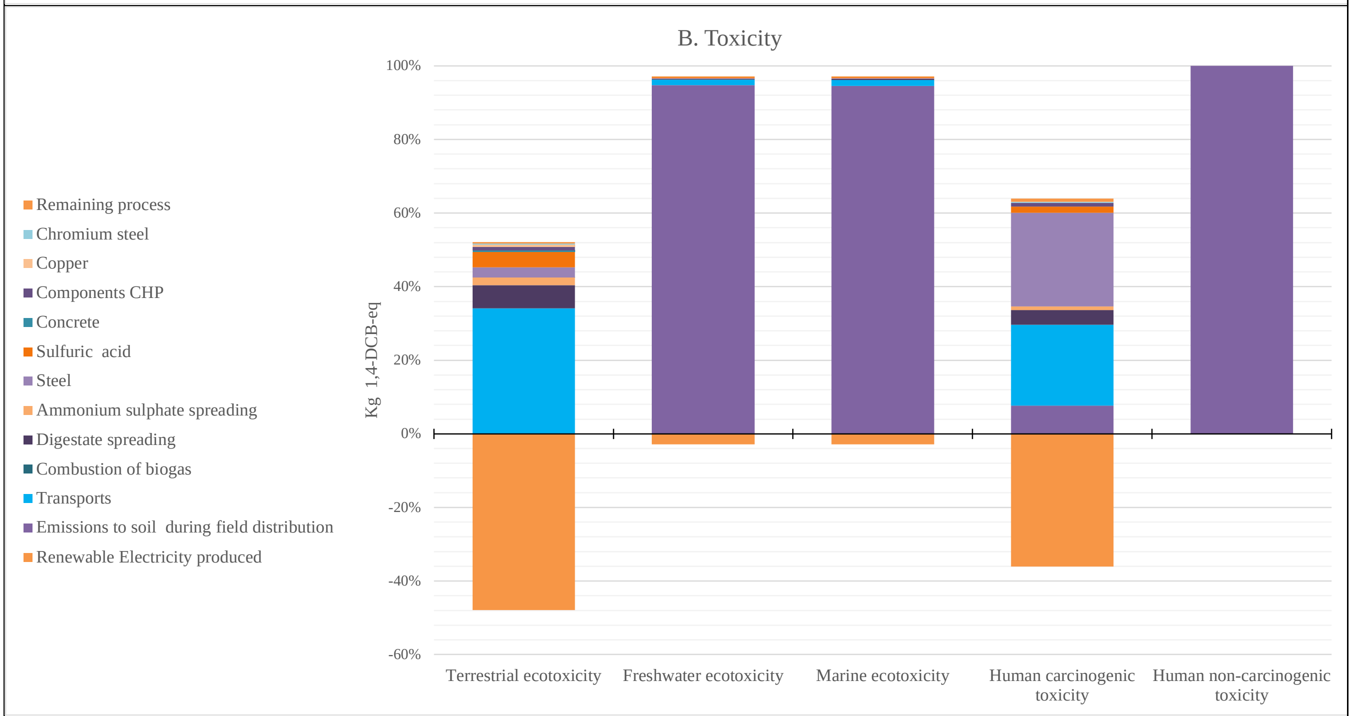
Impact due to *Fine particulate matter formation* was almost identical for the two scenarios (Table 2). This result was because this impact was generated mostly by the ammonia emissions during field fertilization, which was similar for the two Scenarios investigated (Table S4). Particulate matter due to biogas burning in the CHP unit (producing both heat and electricity), fuel combustion for sludge transport to the plant and digestate field distribution were balanced by credits due to renewable energy produced, determining only a slightly lower value than that calculated for the Scenario SF.

Terrestrial acidification, which is related to nutrients supplied, i.e. deposition of ammonia, nitrogen oxides and sulphur dioxide in acidifying forms, displayed similar values for the Scenario RF and Scenario SF (Table 2). Scenario RF had a slightly higher impact due to fertilizers distribution because of NOx emissions related to the greater use of machinery necessary for the distribution of digestate. Previous studies reported opposing results, i.e. an increase in potential acidification when N mineral fertilizer was replaced by digestate.[158,161] On the other hand, when the use of proper timing and distribution techniques were considered, previous LCA results were in line with those of this work.[162,163]

Freshwater and marine eutrophication deal with the increase of nutrients (namely P and N) leading to excessive primary productivity and finally biodiversity losses. Freshwater eutrophication (expressed as P equivalent) displayed a higher value for the Scenario RF than Scenario SF, because the total amount of P brought to the soil by digestate, was greater than the crop requirement and so higher than P dosed in the Scenario SF. Phosphorus overdose depended on the N:P ratio that determined an excess of P when dosing the correct amount of efficient N required by a crop (Table S3). N:P ratio imbalance is well known and documented for animal slurries and digestates,[25] and it is even more accentuated in the case of digestates produced by sewage sludge, in which the previous wastewater purification process mainly determines an accumulation of P, while the denitrification processes displace part of the nitrogen.[164]

For *marine eutrophication*, the impact measured for the two scenarios was equivalent, as the N leached assessed in full-field trials was recorded as equal for the two scenarios studied (see Table S4 supporting information).


A. Ecosystem
Remaining process
Concrete
Sulfuric acid
Steel
Ammonium sulphate spreading
Digestate spreading
Combustion of biogas
Transports
Emissions to air during field distribution
Renewable Electricity produced
100%
80%
60%
40%
20%
0%
-20%
-40%
-60%
-80%
-100%
kg CO2 eq (Global warming)
kg CFC11 eq (Ozone depletion)
kBq Co-60 eq (Ionizing radiation)
kg NOx eq (Ozone formation)
kg PM2.5 eq (Fine Particulate matter)
kg SO2 eq (Acidification)
kg P eq (Freshwater Eutrophication)
kg N eq (Marine Eutrophication)


B. Toxicity
Remaining process
Chromium steel
Copper
Components CHP
Concrete
Sulfuric acid
Steel
Ammonium sulphate spreading
Digestate spreading
Combustion of biogas
Transports
Emissions to soil during field distribution
Renewable Electricity produced
Kg 1,4-DCB-eq
100%
80%
60%
40%
20%
0%
-20%
-40%
-60%
Terrestrial ecotoxicity
Freshwater ecotoxicity
Marine ecotoxicity
Human carcinogenic toxicity
Human non-carcinogenic toxicity

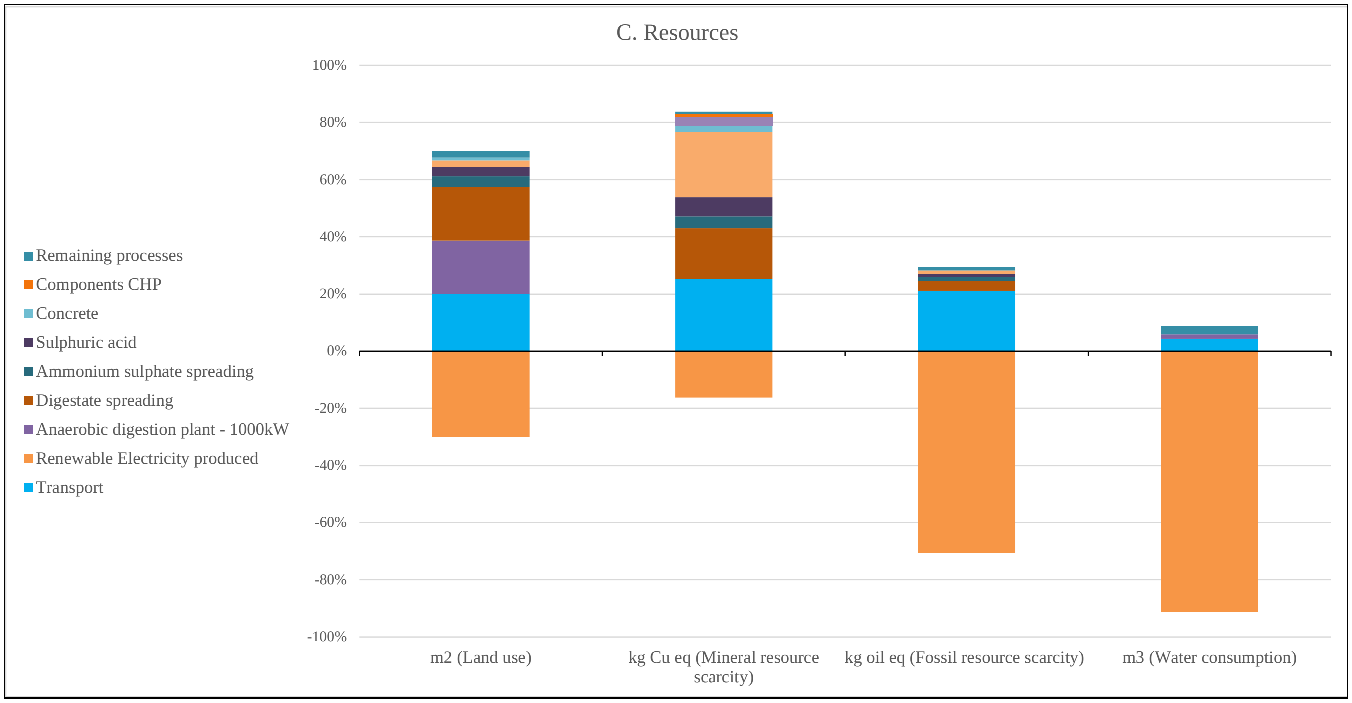


Figure 3. *Process contribution to impact categories of Scenario RF, focusing on the ecosystem (a), toxicity (b) and resources (c). Impacts assessment were calculated according to ReCiPe 2016 midpoint (H) V 1.03 method*

3.3.2 Midpoint results of impact categories related to human health protection

The inclusion of toxicity categories (USEtox) (Table 2) in the ReCiPe 2016 methodology, allowed us to better focus the impacts of the production and use of fertilizers when compared with previous work done that considered only the main agricultural-related indicators, such as *Global Warming Potential, eutrophication and acidification.*[158,163]

The use of fertilizers determined a higher impact for the Scenario RF than Scenario SF for the toxicity categories, i.e. *Freshwater and marine ecotoxicity* and *Human non-carcinogenic toxicity*, because of heavy metals (HM) (above all Zn) supplied to soil with digestate. This figure has been already been highlighted in literature for other organic fertilizers (pig slurries) because of their very high Zn and Cu contents.[165,166]

The terrestrial ecotoxicity impact was mainly generated during the fertilizer production (Table 2); in particular, for Scenario RF, the impact was due above all to the transport of sludge to the AD plant (Figure 3b), while for Scenario SF, it was the N fixation process (ammonia steam reforming) that determined the impact. Nevertheless, Scenario RF benefitted from the production of electricity, significantly reducing the impacts. Finally, the category *Human carcinogenic toxicity* also showed a better environmental outcome for Scenario RF than Scenario SF, thanks to the credits from the production of renewable energy (Figure 3b).

3.3.3 Midpoint results of impact categories related to Resources scarcity protection

The use of both renewable energy (biogas) and recovered material (sewage sludge) to produce fertilizers (digestate and ammonia sulphate) led, also, to high efficiency in terms of *Land use, Mineral resource use, Fossil resources*, reducing, until negative, these impacts (Table 2).

3.3.4 Single endpoint indicator

The single endpoint indicator provided by the ReCiPe method allows one to view the normalized and weighted impacts in a synthetic manner and is divided into the three areas of protection, i.e. ecosystem, toxicity and resources (Figure 2). The Scenario RF was significantly better than Scenario SF, and in particular the indicators showed for Scenario RF, not only an impact reduction but, also, the prevention of impact in the areas of protection of Resources and Human health, as previously reported.[167–171]

3.3.5 Further scenarios reducing environmental impacts in producing and using renewable fertilizers.

Life Cycle Assessment is a powerful tool for describing impacts due to fertilizer production and use, highlighting positive and negative effects for renewable fertilizers vs. synthetic mineral fertilizers in a real case study.

However, LCA is also a potent tool to design potential scenarios in terms of environmental impacts, from which to learn how to improve productive processes, and further reduce environmental impacts. This process can be done by observing in detail impacts categories and the contribution of each process activity to the category impact to find solutions by combining individual technologies.[172]

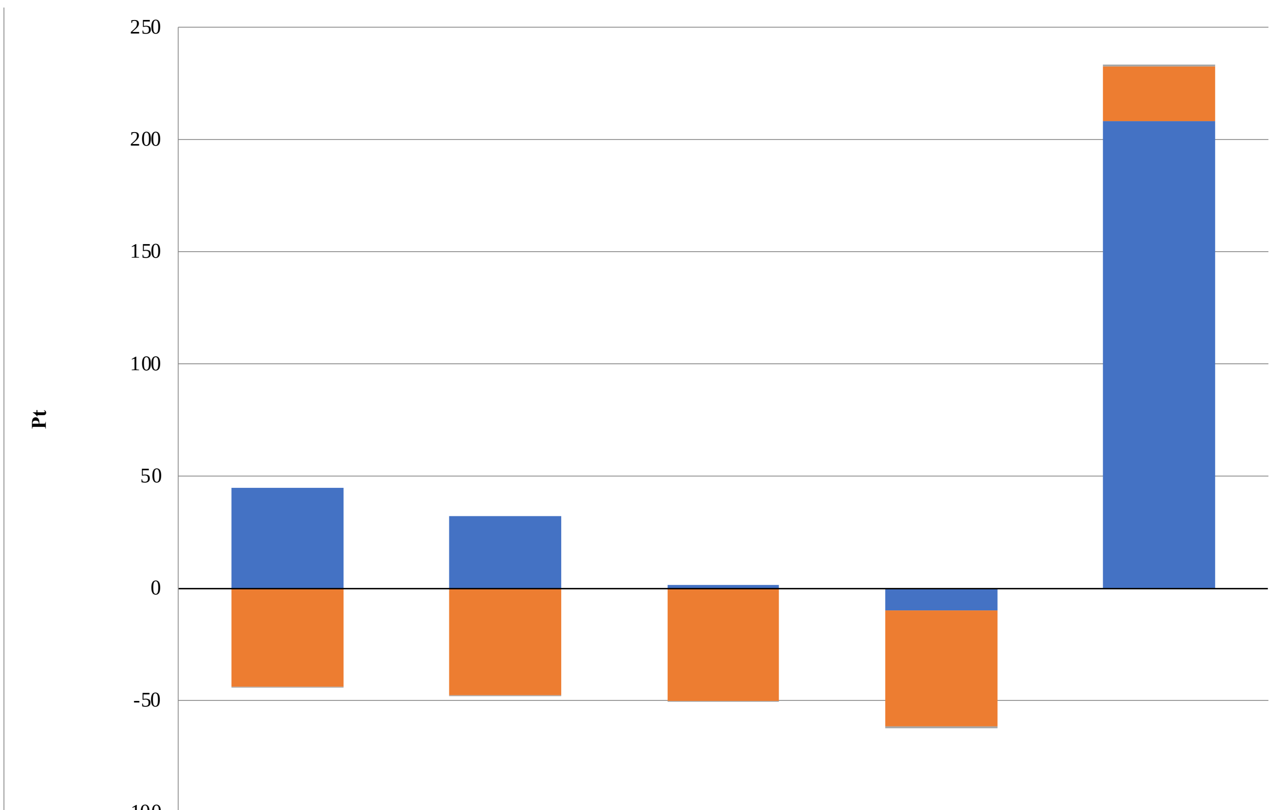
The results discussed above indicate that the recovery of sewage sludge producing renewable fertilizers by AD allowed environmental benefits when the renewable fertilizers produced were used correctly and by efficient timing in substituting for synthetic mineral fertilizers, suggesting that the application of the Circular Economy in agriculture in terms of fertilization resulted in a win-win approach which makes it more sustainable. However, as for all productive processes, impacts remain, and they cannot be nullified completely but only further reduced.

The detailed observation of every single impact, divided for impact categories and activities affecting each impact (Figure 3), allowed us to understand what the more important factors are in determining impacts. Emissions to air during field distribution of fertilizers (i.e. NH_3 and N_2O emission) seemed to affect greatly the Ecosystem and Human toxicity categories as they interacted with many impact subcategories (Figure 3a and 3b). Therefore, reducing air emissions allows the further reduction of ecosystem and human impacts because of renewable fertilizer production and use. Digestate and ammonium sulphate produced by the plant studied in this work were used correctly following the best practice, i.e. digestate and ammonia injection, while the digestate was characterized by high biological stability, avoiding N mineralization and nitrate leaching. The strong impact reduction obtained by substituting synthetic mineral fertilizers with renewable fertilizers (Table 2 and Figure 2), confirmed this virtuous approach. Nevertheless, already stated, LCA can help in optimize processes, further reducing impact.

Nitrogen dioxide emissions have been reported to be greatly reduced by using nitrification inhibitors (NI).[173,174] From the literature, it was calculated, on average, that the use of NI allowed a reduction of 44% in total N_2O emissions,[175] further reducing total Scenario RF impacts (Scenario RF_1), with reference to Ecosystem and Human Health impacts (Figure 4), if these data are implemented in the LCA. The modelling of this scenario considered just the addition of NI and the emissions of N_2O, for the other data the scenario remained the same as the original RF. On the other hand, total ammonia emitted during digestate distribution can be reduced by optimizing the injection system. Preliminary data coming from work performed at full scale at the AD plant studied in this work, indicated that by modifying the distribution equipment, i.e. Vervaet Terragator equipped with flexible anchors and a roller postposed to the anchors, allowed a reduction of ammonia emission of 44% (data not shown). The future integration of this practice will allow a further reduction of impacts, as shown in Figure 4 (Scenario RF_2). The new anchor system is applied to the digestate distribution system already in use, so the only change in the scenario modelling is the emission of ammonia.

Another important activity that plays an important role in determining impact is transport. Transport affected a lot the *Terrestrial Ecotoxicity* (Figure 3b) and, although much less severely, many other sub-categories within *Ecosystem* and *Resources* categories (Figure 3a and 3c), because of the fossil fuel used. Today, in the EU, anaerobic digestion represents a well consolidated bioprocess treating organic wastes and dedicated energy crops, producing biogas/biomethane.[176] In the Lombardy region alone, about 580 AD plants are operating producing biogas and now, are starting to produce biomethane.[177,178] Recently a particular interest has been devoted to liquid biomethane (Bio-LNG) as a substitute for fossil fuels in truck transportation,[179] and the first plants have started operating in Lombardy Region, very close to the AD plant studied in this work. A new scenario was modelled (RF3) assuming the biogas production from organic wastes (OFMSW and sludge), the purification and compression of biomethane, and the transport by 30 ton trucks and average consumption of fuel equal to 0.34 kg LNG per kilometre travelled.[180] Emissions from trucks were recalculated accordingly.

Assuming an ability to substitute all fossil fuels with Bio-LNG produced from the organic fraction of municipal solid waste (Table 1) for transportation, a further strong impact reduction was obtained, nullifying completely the environmental impacts due to production and use of recovered fertilizers (Scenario RF_3) (Figure 4).



	RF	RF1	RF2	RF3	SF
Resources	-0.4	-0.4	-0.4	-0.7	0.9
Ecosystems	-43.7	-47.5	-50.3	-51.6	24.5
Human health	44.7	32.3	1.5	-10	208

Figure 4. *Comparative environmental results for Scenario Recovered Fertilisers (RF), Scenario RF_1 (RF + nitro inhibitor), Scenario RF_2 (RF + nitro inhibitor + anchor), Scenario RF_3 (RF + nitro inhibitor + anchor + biomethane for transportation) and Scenario Synthetic Fertilisers (SF). Impacts assessment calculated according to ReCiPe 2016 endpoint (H) V 1.03 method.*

3.4 Conclusions

Nutrient recovery from organic waste represents a great opportunity to design a new approach in crop fertilization in the framework of the Circular Economy. Nevertheless, recycling nutrients is not enough, as recovered fertilizers should be able to substitute synthetic mineral fertilizers that contain high nutrient concentrations with high nutrient efficiency. A previous paper of ours[128] that RF could be effectively obtained thanks to AD and that these RFs were good candidates for replacing SF. In this paper, the LCA approach indicates that producing and using those RFs instead of producing and using SF, led to a strong environmental impact reduction. This result was due above all to the AD process that makes all this possible because of renewable energy production, and biological processes modifying the fertilizer properties of digestate. Nevertheless, a correct approach in using RF is mandatory, to avoid losing all the advantages of producing RF because of impacts derived from incorrect RF use. In this way, a well-performed AD process assuring high biological stability of digestate, limiting RF-N_2O emission and RF-NO_3^- leaching, and RF injection limiting NH_3 emissions, as well as using RF at the right time and according to crop requirements should be assured.

Acknowledgements

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3.5 Supporting information

Table S1. *Main characteristics of infeed (mean ± SD; n=42) and full characterization of digestate in comparison with legal limits for its use as fertilizer in agriculture, and with data from literature for digestate and composts (mean of three-years monitoring, from Pigoli et al., 2021)*

Parameter	**Unit**	**Digestate**[a]	**Lombardy Law N. 6665/2019 – Legal limits**[b]
pH		8.5 ± 0.3	5.5 < pH < 11
Dry Matter 105°C	g kg^{-1} ww[c]	103 ± 3.7	
Dry Matter 600°C	g kg^{-1} ww	40.4 ± 2.5	
Total Organic Carbon	g kg^{-1} DM[c]	314 ± 30	> 200
TKN	g kg^{-1} DM	77 ± 3.7	> 15
$N\text{-}NH_4$	g kg^{-1} DM	35.9 ± 2.4	
$N\text{-}NH_4$/TKN	%	46.6	
OD_{20}[d]	mg O_2 g^{-1} DM	22.6 ± 6.1	
BMP[e]	L_{biogas} kg^{-1} DM	57 ± 23	
P	g kg^{-1} DM	28 ± 4.1	> 4
K	g kg^{-1} DM	6.5 ± 1.3	
Ca	g kg^{-1} DM	43 ± 7	
Mg	g kg^{-1} DM	5.2 ± 0.6	
Fe	g kg^{-1} DM	26.2 ± 6.4	
Mo	mg kg^{-1} DM	10 ± 1	
Cu	mg kg^{-1} DM	408 ± 60	≤ 1,000
Zn	mg kg^{-1} DM	1,020 ± 120	≤ 2,500
Mn	mg kg^{-1} DM	444 ± 35	
Al	g kg^{-1} DM	25.8 ± 4.5	
Co	mg kg^{-1} DM	6.6 ± 2.3	
Se	mg kg^{-1} DM	3.7 ± 2.1	≤ 10
Na	g kg^{-1} DM	1.9 ± 0.4	
Cr	mg kg^{-1} DM	95 ± 22	< 200
Pb	mg kg^{-1} DM	64 ± 11	≤ 750
Ni	mg kg^{-1} DM	61 ± 13	≤ 300
As	mg kg^{-1} DM	9.0 ± 2.2	< 20
Cd	mg kg^{-1} DM	1 ± 0.5[f]	≤ 20
Hg	mg kg^{-1} DM	0.1 ± 0.3[f]	≤ 10

PAH	mg kg^{-1} DM	0.5 ± 0.5[f]	$\sum < 6$
PCB	mg kg^{-1} DM	< 0.1	$\sum < 0.8$
PCDD/F+PCB-DL	ng TEQ kg^{-1} DM	10.6 ± 2.9[f]	$\sum \leq 25$
DEHP	mg kg^{-1} DM	5.7 ± 5.3[f]	< 100
Hydrocarbon C10-C40	mg kg^{-1} ww mg kg^{-1} DM	284 ± 251[f] (2,757)	≤ 1,000
AOX	mg kg^{-1} DM	< 0.6	$\sum < 500$
Ciproflaxacin	mg kg^{-1} DM	< 0.01[g]	
Sulfamethoxazole	mg kg^{-1} DM	< 0.01	
Fenofibrat	mg kg^{-1} DM	< 0.01	
Gemfibrozil	mg kg^{-1} DM	< 0.01	
Carbamazepine	mg kg^{-1} DM	< 0.01	
Metoprolol	mg kg^{-1} DM	< 0.01	
Diclofenac	mg kg^{-1} DM	< 0.01	
Ethinylestradiol	mg kg^{-1} DM	< 0.01	
Estradiol	mg kg^{-1} DM	< 0.01	
Salmonella	MPN g^{-1} DM	Absent	< 100
Faecal coliform	MPN g^{-1} DM	< 1,000	< 10,000

[a]Mean ± SD: *n*=42, except for Ca, Mn, Mg, Fe, Mo, Al, Co, Na: *n* = 9, and BMP: *n* = 10.

[b]Legal limit referred to the digestate described in this work.

[c]ww and DM: wet weight and dry matter, respectively.

[d]OD_{20}: Oxygen Demand after 20h

[e]BMP: potential biogas production.

[f]Mean and SD calculated considering data below detection limits = 0.

[g]Analysis performed in 2020; *n*=4.

Table S2. *Main characteristics of ammonium sulphate - $(NH_4)_2SO_4$ - derived from digestate, used in field trials (mean three years ± SD, n=17).*

Parameter	**Unit**	**Value**
pH	pH	6.8 ± 1.3
EC	mS cm^{-1}	119 ± 27 (1:2.5 v/v 25 °C)
Dry Matter 105°C	% of ww	35.5 ± 0.4
Total Organic Carbon	g kg^{-1} ww	< 0.1
Total N	g kg^{-1} ww	74 ± 2
$N-NH_4$	g kg^{-1} ww	71.7 ± 1.9

Data related to agronomic use of fertilizers in the two systems, RF and SF came from fertilization trials performed in the seasons 2018-2020. Fertilizers were tested on plots of 350 m^2 cropped with maize in 6 replicates, using a randomized experimental scheme. Thesis included the use of digestate from organic wastes combined with digestate-derived mineral fertilizer (ammonium sulphate) (RF) vs. synthetic fertilizers (SF); an unfertilized treatment was included as control. Digestate was distributed at pre-sowing by injection into the soil at a depth of 15 cm. Table S3 resume the main information about the fertilization plans. The crop yield was statistically no different between the RF and SF theses in the 3 years.

Table S3. *Main information regarding fertilization plan adopted: fertilization date, fertilizers used, and dose applied (RF = Recovered Fertilizers and SF = Synthetic Fertiliser).*

Period	**Plots**	**Fertilization**	**Fertilizer**	**Ntot applied (kg N ha^{-1})**	**Efficient N applied[a] (kg N ha^{-1})**	**Type of spreading**
2018 - 2020	RF	Pre-sowing	Digestate	370	185	Injection 15 cm
		Top-dressing	Ammonia sulphate	100	100	Fertigation
	SF	Pre-sowing	Urea	185	185	Spread in surface
		Top-dressing	Ammonia sulphate	100[b]	100[b]	Fertigation

[a]Data calculated taking into consideration N efficiency for digestate of 0.5 and for urea of 1, according to Regional Plan for Water Protection from Nitrate from Agriculture [144].

[b]On 2020: 90 kgN ha^{-1}

Table S4. *Comparison between emissions (Ammonia, GHG and Nitrate leaching) and grain production measured from experimental soils fertilized with digestate and urea during the agronomic season (maize) (RF = Recovered Fertilizers and SF = Synthetic Fertiliser). The column "unfertilized" refers to the control plots set during the experimental design.*

Parameter	Unit	RF	SF	Unfertilized
NH_3[a]	kgN ha^{-1}	25.6 ± 9.4(a)[b]	24.8 ± 8.3(a)	Undetectable[c]
N_2O[d]	kgN ha^{-1}	7.59 ± 3.2(ab)	10.3 ± 6.8(b)	1.71 ± 1.1(a)
CO_2[d]	kgC ha^{-1}	6216 ± 1160(a)	6144 ± 1491(a)	5698 ± 935(a)
CH_4[d]	kgC ha^{-1}	0036 ± 0.03(a)	0.053 ± 0.04(a)	0.066 ± 0.06(a)
NO_3^- [e]	mgN kg^{-1}	6.45 ± 7.6(a)	7.24 ± 8.6(a)	6.23 ± 7.1(a)
Grain Yield	Mg ha^{-1} DM[f]	18.1 ± 2.9(b)	17.4 ± 1.2(b)	10.4 ± 3.5(a)

[a]Cumulative emissions measurements carried out up to 90 hours after spreading (n = 9). The measures were repeated for three consecutive years (2018-2019-2020). Total N dosed: 370 kgN ha^{-1} (Digestate), 185 kgN ha^{-1} (Urea) (from Zilio et al., 2021)

[b]Letters in brackets are referred to One-way ANOVA analysis carried out for each of the emission source reported in the table (Tukey post-test, $p < 0.05$; $n = 3$).

[c]Ammonia emission in unfertilized plots did not differ from background.

[d]Cumulative emissions measurements carried out from 28/05/2020 (spreading) to 17/03/2021 (293 days, n = 36). Total N dosed: 370 kgN ha^{-1} (Digestate), 185 kgN ha^{-1} (Urea)

[e]Average concentration of NO_3^- in the soil at 1-meter depth. The measurements were carried out in 3 moments of the season (before spreading in pre-sowing, 20 days after spreading and after harvesting). n for each measure = 3. Total N dosed: 370 kgN ha^{-1} (Digestate), 185 kgN ha^{-1} (Urea)

[f]DM = dry matter

Chapter IV

Using highly stabilized digestate and digestate-derived fertilizer to replace synthetic fertilizers in open field agriculture: the effects on soil, environment, and crop production.

Massimo Zilio, Ambrogio Pigoli, Bruno Rizzi, Axel Herrera, Fulvia Tambone, Gabriele Geromel, Erik Meers, Oscar Schoumans, Andrea Giordano, Fabrizio Adani
(Submitted Journal Science of the Total Environment).

4 Using highly stabilized digestate and digestate-derived fertilizer to replace synthetic fertilizers in open field agriculture: the effects on soil, environment, and crop production.

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ABSTRACT ART

Abstract

A highly stabilized digestate (biochemical methane production – BMP – of 89 ± 17 L_{biogas} kg^{-1} dw) and a digestate-derived fertilizer (ammonium sulphate) obtained from anaerobic digestion of sewage sludge, were used as fertilizer on an open field maize crop, in a comparison with synthetic fertilizers. After three consecutive crop seasons, the soils fertilized with the recovered fertilizers (digestate + ammonium sulphate) (RF), compared to those fertilized with synthetic fertilizers (SF), did not show significant differences either in their chemical characteristics or in the accumulation of inorganic and organic pollutants (POPs). The RF ensured an ammonia N availability in the soil equal to that of the soil fertilized with SF, during the whole period of the experiment. Furthermore, no risks of N leaching were detected, and the use of RF did not result in a greater emission of ammonia or greenhouse gases than the use of SF. The agronomic results obtained using RF were equivalent to those obtained with SF (fertilizer use efficiency of 85.3 ± 10 and 93.6 ± 4.4% for RF and SF respectively). The data show that pushing the anaerobic digestion up to obtain a very stable digestate can be a good strategy to produce a bio-based fertilizer with similar performance to that of a synthetic fertilizer, without environmental risks.

Keywords: Digestate; Environmental impacts; Fertilizer use efficiency; Soil quality; Sewage Sludge.

4.1 Introduction

During the second half of the twentieth century, in particular since the late 1960s, agriculture throughout the world has undergone radical improvements, which overall have been defined as the "green revolution". The direct consequence of these improvements in the succeeding decades was a dramatic growth in agricultural yields which increased by up to 125% between 1966 and 2000.[181] This new availability of calories supported economic development in many areas of the world, allowing populations to grow without increasing the cultivated areas, thus also safeguarding forests and natural lands.[182]

One of the main improvements introduced by the green revolution to agriculture was the use of large amounts of synthetic fertilizers to provide nutrients to crops.[183] From the late 1960s to the present day, the use of synthetic fertilizers in the world increased by 500%, and included an 800% increase in the use of nitrogen (N) fertilizers[124](but it is evident that this high usage is becoming progressively less sustainable. The amount of N fertilizers produced on a global scale rose from 12 TgN in 1960 to 104 TgN in 2010, with an expected increase of 2.3% per year in the near future. This amount now contributes to 45% of the total nitrogen fixed annually on the planet, effectively causing strong imbalances in the natural nitrogen cycle, with harmful consequences for terrestrial and aquatic ecosystems.[184,185] Almost all the N fertilizers are produced by fixing atmospheric N into ammonia, through a process devised in 1909 by Fritz Haber and Carl Bosch (the so-called Haber-Bosch process), which today is considered one of the most energy-consuming industrial processes on a global scale, responsible for 1.2% of the annual anthropogenic CO_2 emissions.[186]

Phosphorus-based fertilizers are no less problematic. Essentially, all the phosphorus (P) used to produce fertilizers derives from mineral deposits that are located in a few areas of the planet, and these are limited.[187] Current reserves of phosphate ore are estimated at 67,000 Tg P and about 75% of them are located in Morocco (West Africa). China and the US also have significant reserves, but these are considered strategic resources and are therefore not sold on the global market. Recent estimates of the extraction rate[188] quantify the annual amount of phosphate minerals extracted in the world at 255 million metric tons (MMT), and the projections foresee an increase of 50-100% by 2050. According to the same projections, the peak of phosphorus extraction, i.e., the point after which the annual extractable amount will no longer be able to increase, is expected for 2030, and the depletion of global reserves is likely before the end of the XXI century.[189,190] The limited reserves of phosphorus available, as well as the fact that it is considered a strategic resource because it is crucial for agriculture, exposes its price on the global market to strong and unpredictable fluctuations, which are also linked to geopolitical conditions, as already happened in 2008, which in turn affects the cost of food.[191] Many nations, including those comprising the European Union, which do not possess significant reserves of phosphate minerals within their borders, are particularly exposed to these risks.

On the other hand, these same nutrients (N and P) are generally present in large amounts in the wastewater and organic wastes from the food production industry, which includes agriculture and livestock. Paradoxically, these waste biomasses are a problem because their uncontrolled dispersion into the environment, together with the excessive use of synthetic fertilizers in agriculture, causes an excess of nutrients in soils and waters in many areas of the planet, with serious consequences for ecosystems and the balancing of biogeochemical cycles on a global scale.[192–194] The excess of phosphorus, in particular, causes eutrophication in aquatic ecosystems, resulting in the loss of entire ecosystems and of the fish resources dependent upon them.[195,196] Nitrogen, in the form of nitrate (NO_3^-), is leached within the soil until it reaches the groundwater, often destined for human consumption, leading to public health problems.[197,198]

Using organic wastes as fertilizers in agriculture to replace synthetic fertilizers would therefore represent a solution to these problems, reducing the dispersion of nutrients in the environment, and it would also constitute an interesting model of circular economy.[199] However, untreated organic wastes do not represent acceptable fertilizers[200] because of their origin: in fact they can contain pathogens, heavy metals, or organic pollutants such as antibiotics or drug residues that would accumulate in agricultural soils, endangering the safety of food production and consumption.[201–205] Furthermore, their nutrient content and plant availability are difficult to control, so that they are not often able to replace synthetic mineral fertilizers. Lastly, they are often rich in water, which makes their management difficult and expensive both from an economic and environmental point of view, because of the CO_2 emissions associated with the transport of large volumes.[200] To transform these biomasses into products which can be utilised in agriculture, technological/biotechnological treatments are therefore necessary.[133] Among these treatments, in recent decades the anaerobic digestion process has been proposed as a valid technology to valorise organic wastes of different types, producing biogas, and also as a source of biofertilizers such as digestate which can be used in agriculture as a substitute for synthetic fertilizers.[128,140,206,207]

However, the possibility of using digestate in agriculture to replace synthetic fertilizers is still debated, especially as regards the possible environmental impacts. The high concentration of nutrients contained in these biomasses, which is useful for plant nutrition, can cause leaching of N and P in the soil with consequent water pollution, and also to emissions of ammonia and greenhouse gases (N_2O) into the atmosphere.[208,209] Furthermore, originating from organic wastewater and organic wastes, digestate also has the same problems of contamination by heavy metals, pathogens and organic pollutants typical of these biomasses, which could therefore pollute the soil.[210] Although progressively more studies in recent years are shedding light on the safety of digestates for agricultural use, as well as on the best process methods and accurate selection of infeed, there are still only very limited data available on the impact that the use of these biomasses have on full scale agriculture in the field.[128,140,206,211]

The aim of this work was to analyse the effects of the use of a highly stabilized digestate and ammonium sulphate derived from digestate from sewage sludge as fertilizers on a full-scale field crop for three consecutive years. All observations were carried out by comparing the parallel use of the recovered fertilizers (digestate and digestate-derived ammonium sulphate) and synthetic mineral fertilizers. In particular, the effects of using these fertilizers on soil chemical-physical characteristics and crop yields were assessed as well as the environmental impacts, i.e. on NO_3^- leaching, NH_3, N_2O, CO_2 and CH_4 emission, and presence of inorganic and organic contaminants in soil and in the grain produced.

4.2 Material and Methods

4.2.1 Agronomic full field trials

Agronomic trials tested, at full field scale, the fertilizer properties of digestate from organic wastes combined with digestate-derived mineral fertilizer (ammonium sulphate) vs. synthetic fertilizers; an unfertilized treatment was included as control. Fertilizers were tested on plots of 350 m^2 cropped with maize (*Zea mays* L.; hybrid Pioneer P1547, FAO 600), in triplicate, using a randomized experimental scheme and following the standard agronomic procedures used in the Po Valley (northern Italy), where the experimental fields were located.

Digestate was distributed at pre-sowing by injection into the soil at a depth of 15 cm by using a tank car joined to a rigid multi-anchor-subsoiler coupled with a Retrofit Variable-Rate Control (VRT control). Digestate was dosed adopting an N efficiency of 0.5, as suggested by the Regional Plan for Water Protection from Nitrate from Agriculture.[144] Nitrogen fertilization was completed by using ammonium sulphate produced starting from digestate[128] in topdressing by fertigation (N_{tot} dosed: 370 kgN Ha^{-1} as digestate and 100 kgN Ha^{-1} as ammonium sulphate; P_{tot} dosed: 134 kgP Ha^{-1} as digestate; K_{tot} dosed: 24.1 kg K Ha^{-1} as digestate and 44.82 kg K Ha^{-1} as K_2O).

Synthetic fertilizers were spread on the soil surface following a routine procedure (N_{tot} dosed: 185 kgN Ha^{-1} as urea and 100 kgN Ha^{-1} as ammonium sulphate; P_{tot} dosed: 39.3 kgP Ha^{-1} as 0/46/0 complex; K_{tot} dosed: 69.4 kg K Ha^{-1} as KCl). Fertilization date, fertilizers used, doses applied, and spreading methodology are summarized in Table 1.

Table 1. *Fertilization plan adopted: fertilization date, fertilizers used, and dose applied.*

Year	Plots	Date	Fertilization	Fertilizer	Ntot applied (kg N Ha^{-1})	NH_4^+ applied (kg N Ha^{-1})	Type of spreading
2018	Recovered fertilizer	23/04/2018	Pre-sowing	Digestate	370	229	Injection 15 cm
		22/06/2018	Top-dressing	Ammonia sulphate	100	100	Fertigation
	Synthetic fertilizer	23/04/2018	Pre-sowing	Urea	185	185	Spread in surface
		22/06/2018	Top-dressing	Ammonia sulphate	100	100	Fertigation
2019	Recovered fertilizer	16/04/2019	Pre-sowing	Digestate	370	229	Injection 15 cm
		1/08/2019	Top-dressing	Ammonia sulphate	100	100	Fertigation
	Synthetic fertilizer	16/04/2019	Pre-sowing	Urea	185	185	Spread in surface
		1/08/2019	Top-dressing	Ammonia sulphate	100	100	Fertigation
2020	Recovered fertilizer	28/05/2020	Pre-sowing	Digestate	370	200	Injection 15 cm
		31/07/2020	Top-dressing	Ammonia sulphate	90	90	Fertigation
	Synthetic fertilizer	28/05/2020	Pre-sowing	Urea	185	185	Spread in surface
		31/07/2020	Top-dressing	Ammonia sulphate	90	90	Fertigation

4.2.2 Fertilizer sampling and analysis

The digestates used in this work were sampled immediately before they were injected in the field. The analyses took place in the hours immediately following sampling and data represent the average of three years. pH was determined in aqueous solution using a 1:2.5 sample/water ratio. Total solids (TS) and total organic carbon (TOC) determinations were carried out following standard procedures of the American

Public Health Association.[212] Total nitrogen (TKN) and ammonia nitrogen (TAN) were determined according to the analytical method for wastewater sludges.[213] Heavy metals, total P and K content was assessed by inductively coupled plasma mass spectrometry (Varian, Fort Collins, USA), preceded by acid digestion[214] of the samples. All the analyses were carried out in triplicate. Biochemical methane production (BMP) was determined following the method reported in Schievano et al. (2008).[215]

Organic micropollutants were detected as follows: C10-C40 hydrocarbons by UNI EN 14039[216] method, halogenated organic compounds (AOX) by Gas Chromatography (GC) approach (UNI in ISO 22155:20161 and EPA 8270E 20181 + EPA 3550C 2007).[217–220] PCDD/Fs were measured using UNI 11199[221]method, PCBs through UNI EN 16167 and UNI EN 16167,[222,223] and DEHP through EPA 3550C[217] + EPA 8270E methods.[218] Emerging organic pollutants (pharmaceuticals), i.e. Ciproflaxacin, Sulfamethoxazole, Fenofibrat, Gemfibrozil, Carbamazepine, Metoprolol, Diclofenac, Ethinylestradiol and Estradiol were detected by HPLC-MS following EPA 3550C[217] and EPA 8321B 2007 methods.[220] Faecal coliforms and *Salmonella* were determined as reported in CNR IRSA 3[224] (Faecal coliforms) and ISTISAN 14/18 + APAT CNR IRSA 7080 (*Salmonella*).[225,226] The main characteristics of the digestate and ammonia sulphate used in this work are shown in Table S1 and Table S2.

4.2.3 Soil sampling and analysis

The soils studied in this work were sampled just before the fertilization in March 2018 by taking three random samples (each one made by 3 sub-samples) at 0-20 cm. After three years, the soil was sampled again in March 2021, maintaining the same sampling procedure, taking three random samples/plot. Samples were air dried, sieved to 2 mm and then ground to 0.5 mm. The main characteristics of soils are reported in Table 2. Soil pH was determined in aqueous solution using a 1:2.5 sample/water ratio (McLean, 1982), and texture by the pipette method.[228] Cation Exchange Capacity (CEC) was determined by saturating the samples with $BaCl_2$,[229] total organic carbon (TOC) by the Walkley and Black method[230] and total nitrogen by the Kjeldahl method.[231] All the analyses were carried out in triplicate. Total P and K contents were determined using the same method used for fertilizers analysis (*see section 4.2.2*).

Potential nitrate leaching was assessed by the detection of nitrate presence at 1 m soil depth ($N\text{-}NO_3$) in soils. Sampling consisted in the withdrawal of soil cylinders up to a depth of one meter. For each of the experimental plots three soil cylinders were sampled randomly. Each soil cylinder was divided into 4 sub-samples, each of 25 cm, corresponding to 0-25, 25-50, 50-75 and 75-100 cm layers in soil profile. In total eight sampling campaigns were carried out during in the period 2019-2020. The collected soil was brought immediately (the same day) to the lab and analysed immediately. In particular, the nitrate concentration was determined by Kjeldahl distillation, using Devarda's alloy.[231]

Inorganic and organic pollutants were detected at the start and the end of the trial; in particular, heavy metals (HV) were determined by the method already reported for fertilizers (*see section 4.2.2*). The determination of the organic pollutants in the soils was carried out using the following methods: PCDD/PCDF + PCB DL: UNI EN 16167:2012, AOX: UNI EN ISO 22155:2016, Hydrocarbon C10-C40: ISO 16703:2004, Toluene: UNI EN ISO 22155:2016, Phenols: ASTM D7485-16, DEHP: EPA 3510C 1996 + EPA 8270E 2017. Emerging organic pollutants (pharmaceuticals), i.e. Ciproflaxacin, Sulfamethoxazole, Fenofibrat, Gemfibrozil, Carbamazepine, Metoprolol, Diclofenac, Ethinylestradiol and Estradiol were detected at the end of the trial by HPLC-MS following EPA 3550C[217] and EPA 8321B 2007 methods.[220] A complete list of samplings and agronomic operations carried out is reported in Table S3.

4.2.4 Ammonia emission measurement

For all the experiments, the ammonia emitted from the experimental plots was measured in the hours following the pre-sowing injection/spreading. All the digestate injections took place at the same hour (h. 11:00), and the first sampling was always carried out 10 hours later (21:00).

The experiments were repeated for three consecutive years on the same experimental plots. In particular, the soil used showed a neutral pH (7 ± 0.4), it was rich in silt (44% ± 2.1) and it was relatively poor in clay (10% ± 0.5). The amounts of ammonia nitrogen dosed at pre-sowing were kept almost unchanged for all the three years tested, i.e. 200 - 229 and 185 kg N Ha^{-1} for RF and SF, respectively (Table 1). The concentration of NH_3 was monitored by the exposure of ALPHA passive samplers.[148,234] For each plot, the ALPHA samplers were exhibited in sets of three. To obtain background environmental concentration values, an additional sampling point was placed at a distance of about 1,000 meters away from the fertilized fields and other possible point sources of NH_3. Each sampler located in the plot was replaced a minimum of twice a day near sunrise and sunset, to be able to monitor the variation of atmospheric turbulence which has a direct effect on the dispersion of pollutants. During the application day and the following day, the substitution was done when the vehicles entered the field, for fertilization and for incorporation. The study of atmospheric turbulence was carried out by using an ultrasonic anemometer (10 Hz) positioned in the plots near to the samplers.

By processing the NH_3 concentration information, an analysis of the dispersion of NH_3 in the atmosphere was performed through the application of the dispersion model (WindTrax, Thunderbeach Scientific, CA). The obtained dispersion coefficient (D; s m^{-1}) was used to determine the flow (S; ng NH^3 m^{-2} s^{-1}) emitted from the fertilized surface, on the basis of the concentrations measured in each plot (C; µg m^{-3}) and environmental (C_{bgd}; µg m^{-3}), according to the following equation:

$$S = (C - C_{bgd}) \times D^{-1}$$

The ammonia emission factor (EF%) was obtained from the ratio between the released N-NH_3 (kg ha^{-1}) and the calculated amount of ammonia nitrogen (N-NH_4; kg ha^{-1}) spread onto the soil with fertilizations.

4.2.5 Greenhouse gas (GHG) emissions measurement

GHG fluxes (N_2O, CH_4 and CO_2) were measured from 28/05/2020 to 17/03/2021 using the closed static chambers method.[145] Anchors were inserted into the soil (three for each plot) up to a depth of 20 cm, to isolate the soil column. The chambers were placed on the surface of the soil above the columns and closed with a lid. The air inside the chambers was sampled and analysed in the laboratory through gas chromatography.[146] The emissive flow of the gas from the soil was estimated using the following general equation:

$$F = H \times dC/dt$$

where *F* is the flow, *H* is the ratio between the air volume and the soil surface isolated from the chamber, corresponding to the height of the chamber (m), and *t* is the time the chamber remains closed. If the increase in GHG concentration inside the chamber was linear, the *dC*/*dt* ratio was obtained by linear regression between concentrations and sampling times. In case of non-linear accumulation, the HM model was applied.[147] Finally, the cumulative emissions were obtained by estimating the flows in the non-sampling days, by means of linear interpolation.

4.2.6 Maize yield quantification and element content analysis

The annual grain yields for each of the experimental plots were assessed by manual harvesting of the grain. The data obtained from each plot were then aggregated in order to obtain final grain production (Mg Ha^{-1}) for each treatment, i.e. RF, SF and control. Inorganic pollutant contents in grain (i.e., As, Cd, Hg, Cr, Ni, Pb, Cu and Zn) were assessed by inductively coupled plasma mass spectrometry (Varian, Fort Collins, USA), preceded by acid digestion[214] of the samples. All the analyses were carried out in triplicate. N grain content was assessed by the combustion method (Dumas method).[235] Before analysis, the grain samples (20 g dry weight per plot) were prepared by grinding them using a ball mill. Each analysis was made on two experimental replicates. The elemental analyser used for the analysis was: Rapid max N exceed (model), produced by Elementar, Lomazzo (Italy).

4.2.7 Fertilizer use efficiency

The N fertilizer use efficiency (FUE), and N fertilizer replacement value (NFRV) assessments for nitrogen carried out on soils with treated with both types of fertilizers were calculated according to Sigurnjak et al. (2017). The two parameters were calculated following the formula:

$$FUE(\%) = \frac{N\,uptake_{fert}}{N\,applied} \times 100$$

$$NFRV(\%) = \left[\frac{\frac{(N\,uptake\,biobased\,treatment - N\,uptake\,control)}{total\,N\,applied\,biobased\,treatment}}{\frac{(N\,uptake\,mineral\,treatment - N\,uptake\,control)}{total\,N\,applied\,mineral\,treatment}}\right] \times 100$$

4.2.8 Statistical analysis

The statistical analyses were carried out using IBM SPSS® 23 software. Unless otherwise specified, the significance limit value p was set at 0.05 for all the analyses carried out. The plots were obtained through the use of Microsoft EXCEL 2016.

4.3 Results and discussion

4.3.1 The effect of recovered fertilizers on soil

The use of recovered fertilizers for three consecutive years had no impact on soil properties apart from that on TOC content, which was positively and significantly affected by RF use (One-way ANOVA; $p<0.05$). The TOC content increased after three years from 10.3 ± 0.6 g kg^{-1} dw (March 2018) to 12.3 ± 0.4 g kg^{-1} dw (March 2021) (Table 2). Both the unfertilized and synthetic fertilized plots did not show any statistical differences with respect to the starting soil for the TOC contents (Table 2). The increase in TOC in soil fertilized with RF was most likely due to the contribution of digestate that was rich in organic carbon (TOC of 304 ± 34 g kg^{-1} dw) which was recalcitrant to biodegradation, as suggested by its high biological stability, measured by potential biogas production. In fact, the registered BMP of 89 ± 17 L_{biogas} kg^{-1} dw (Table S1) was much lower than values reported in the literature (on average) for both energy crop digestate (229 ± 31 L_{biogas} kg^{-1} dw) and composts (144 ± 3.8 - 201 ± 20 L_{biogas} kg^{-1} dw), and not far from previous data reported for a similar digestate (i.e., 57 ± 23 L_{biogas} kg^{-1} dw).[128] This confirms that the organic matter contained in the digestate used was very stable, preventing the rapid degradation of the carbon added to the soil, which accumulated over time,[237,238] as will be discussed later. The total soil nitrogen content (N tot) increased for both plots fertilized with SF and RF, which moved from a starting value of 1.27 ± 0.1 g kg^{-1} dw (March 2018) to 1.41 ± 0 and 1.42 ± 0.9 g kg^{-1} dw (One-way ANOVA; $p<0.05$) in March 2021, respectively. On the other hand, soil of the unfertilized plots did not show any variation in its N tot content, i.e., 1.3 ± 0 g kg^{-1} dw in January 2021.

4.3.2 Agronomic performance of recovered fertilizers and product safety

The amounts of maize grain produced (the average of 2018, 2019 and 2020 crop seasons) using digestate and derived ammonium sulphate as fertilizer (18.1 ± 2.9 Mg dried grain Ha^{-1}) (Table S4) was similar and not statistically different from that produced with synthetic fertilizers (urea) (17.4 ± 1.2 Mg dried grain Ha^{-1}). This indicated that recovered fertilizers are capable of substituting for synthetic fertilizers.

Table 2. *Main chemical parameters of soil before the pre-sown fertilization on March 2018 and after the end of the third crop season on January 2021.*

Parameter	Unit	March 2018	January 2021		
			Unfertilized	Synthetic fertilizer	Recovered fertilizer
Sand	%		47 ± 2.8[a]	49 ± 3.7	46 ± 4.4
Silt	%		41 ± 0.2	39 ± 1.5	43 ± 1.4
Clay	%		12 ± 2	12 ± 1.1	12 ± 2.6
pH	pH unit	7 ± 0.7(a)[b]	7.14 ± 0.2 (a)	7.06 ± 0.1 (a)	7.05 ± 0.2 (a)
CEC	C (mol kg^{-1})	24.2 ± 2.1 (ab)	23.8 ± 0.4 (a)	26.8 ± 0.8 (b)	22.3 ± 0.9 (a)
Total organic carbon (TOC)	g kg^{-1} dw[c]	10.3 ± 0.6 (a)	11.9 ± 0.2 (ab)	11.3 ± 0.4 (a)	12.3 ± 0.4 (b)
Total nitrogen	g kg^{-1} dw	1.27 ± 0.1 (a)	1.3 ± 0 (a)	1.41 ± 0 (b)	1.42 ± 0.9 (b)
Ratio C:N		8.13 ± 0.9 (ab)	9.22 ± 0 (b)	8.01 ± 0.1 (a)	8.65 ± 0.4 (ab)
P_{tot}	mg kg^{-1} dw	575 ± 11 (a)	521 ± 26 (a)	581 ± 32 (a)	550 ± 15 (a)
$P_{available}$	mg kg^{-1} dw	43.6 ± 2.6 (a)	46.4 ± 0 (a)	60.1 ± 16 (a)	58.9 ± 16 (a)
As	mg kg^{-1} dw	19.9 ± 1.1 (a)	22.9 ± 2.8 (a)	19.6 ± 0.5 (a)	21.1 ± 2.3 (a)
Cd	mg kg^{-1} dw	<0.5	<0.5	<0.5	<0.5
Hg	mg kg^{-1} dw	<0.5	<0.5	<0.5	<0.5
Cr	mg kg^{-1} dw	39.2 ± 2.3 (a)	42.6 ± 2 (a)	40 ± 4.1 (a)	40.2 ± 1.6 (a)
Ni	mg kg^{-1} dw	23.3 ± 2.3 (a)	25.7 ± 1.7 (a)	25.9 ± 3.7 (a)	26 ± 1.6 (a)
Pb	mg kg^{-1} dw	32.8 ± 0.1 (a)	34.2 ± 4.2 (a)	33.4 ± 2.2 (a)	33.6 ± 4.5 (a)
Cu	mg kg^{-1} dw	19.1 ± 1.3 (a)	22.2 ± 3.3 (a)	21.4 ± 3.5 (a)	24.4 ± 3.1 (a)
Zn	mg kg^{-1} dw	69.8 ± 0.5 (a)	71.4 ± 3 (a)	71.4 ± 1.3 (a)	70.8 ± 1.8 (a)
PCDD/PCDF + PCB DL	ng WHO-TEQ kg^{-1} dw	-	4.09 ± 0.1 (b)	4.3 ± 0.2 (b)	4.16 ± 0.1 (b)
Hydrocarbon C10-C40	mg kg^{-1} dw	< 30	< 30	< 30	< 30
Toluene	mg kg^{-1} dw	< 0.2	< 0.1	< 0.1	< 0.1
Phenols NPE + NP2EO + NP1EO	mg kg^{-1} dw	< 7.5	< 7.5	< 7.5	< 7.5
∑AOX	mg kg^{-1} dw	< 0.6	< 0.6	< 0.6	< 0.6
PCB	mg kg^{-1} dw	< 0.005	< 0.005	< 0.005	< 0.005
DEHP	mg kg^{-1} dw	0.24	< 0.1	< 0.1	< 0.1
Ciproflaxacin	mg kg^{-1} dw		<0.01	<0.01	<0.01
Sulfamethoxazole	mg kg^{-1} dw		<0.01	<0.01	<0.01
Fenofibrat	mg kg^{-1} dw		<0.01	<0.01	<0.01
Gemfibrozil	mg kg^{-1} dw		<0.01	<0.01	<0.01
Carbamazepina	mg kg^{-1} dw		<0.01	<0.01	<0.01
Metoprolol	mg kg^{-1} dw		<0.01	<0.01	<0.01
Diclofenac	mg kg^{-1} dw		<0.01	<0.01	<0.01
Ethinylestradiol	mg kg^{-1} dw		<0.01	<0.01	<0.01
Estradiol	mg kg^{-1} dw		<0.01	<0.01	<0.01

[a]mean ± SD; n=3

[b]Letters are referred to One-way ANOVA comparing values in each row (p<0.05; n=3; Tukey post-test).

[c]dw: dry weight

Furthermore, the content of microelements and inorganic pollutants in the produced grains was quantified (Table 3). For all the elements analysed (except zinc), the concentrations in the grains produced using RF or SF as fertilizer were found to be equivalent. However, RF fertilized plants produced grains containing more Zn than plants grown with synthetic fertilizers, i.e., 32.1 ± 1.9 vs 25 ± 2 mg kg^{-1} dw for RF and SF respectively. However, these values were in line with those reported in the literature for both maize grain and other cereals (i.e., rice and wheat).[239,240] Furthermore, zinc is an essential element, and among cereals, maize is naturally poor in it.[241]

4.3.3 Environmental safety

4.3.3.1 Potential ammonia emissions and nitrate leaching

Ammonia (NH_3) emissions were measured directly at full scale during plot trials, as described in the M & M section. On average, the plots fertilized with RF emitted an amount of ammonia (25.6 ± 9.4 kg N Ha^{-1} i.e., 11.6 ± 4% TAN) that was not statistically different (One-way ANOVA, $p<0.05$, n=3, Tukey post-test) from that measured for plots fertilized with SF (24.8 ± 8.3 kg N Ha^{-1}, i.e., 13.4 ± 4.5% TAN). These data have previously been discussed in a paper published in this journal (Table S5).[140]

The risk of N leaching from the soil was assessed by analysing the concentration of NH_4^+ (Figure 1) and NO_3^- (Figure 2) taking soil samples during crop season in topical moments (i.e., before and after pre-sown fertilization, before and after topdressing fertilization and after harvest) both at the surface and at a depth of one meter, for all experimental soil plots during two consecutive agronomic seasons (2019 and 2020).

Table 3. *Element content in maize grain produced on 2020.*

Element	Element content in maize grain (mg kg^{-1} dw[a])		
	Unfertilized	**Synthetic fertilizer**	**Recovered fertilizer**
N	9,565 ± 100[b] (a)[c]	11,421 ± 936 (b)	11,778 ± 780 (b)
P	2,771 ± 191 (a)	2,585 ± 239 (a)	2,743 ± 174 (a)
Na	473 ± 77.8 (a)	498 ± 48.2 (a)	516 ± 22.7 (a)
Mg	943 ± 48.8 (a)	919 ± 59.6 (a)	914 ± 66.4 (a)
Al	< 0.01	< 0.01	< 0.01
K	3,438 ± 330 (a)	3,167 ± 212 (a)	3,176 ± 346 (a)
Ca	1,104 ± 157 (a)	1,226 ± 205 (a)	1,178 ± 45.4 (a)
Cr	< 0.01	< 0.01	< 0.01
Mn	< 0.01	< 0.01	< 0.01
Fe	23.4 ± 1.33 (a)	26.6 ± 8.98 (a)	28.8 ± 8.34 (a)
Co	< 0.01	< 0.01	< 0.01
Ni	< 0.01	< 0.01	< 0.01
Cu	10.7 ± 7.02 (a)	8.50 ± 2.06 (a)	7.98 ± 1.69 (a)
Zn	26.2 ± 3.67 (a)	25.0 ± 1.98 (a)	32.1 ± 1.9 (b)
As	< 0.01	< 0.01	< 0.01
Se	< 0.01	< 0.01	< 0.01
Mo	< 0.01	< 0.01	< 0.01
Cd	< 0.01	< 0.01	< 0.01
Pb	< 0.01	< 0.01	< 0.01
Hg	< 0.01	< 0.01	< 0.01

[a]dw: dry weight
[b]mean ± SD; n=3.
[c]Letters are referred to One-way ANOVA analysis comparing values in each row (Tukey post-test, $p < 0.05$; n=3).

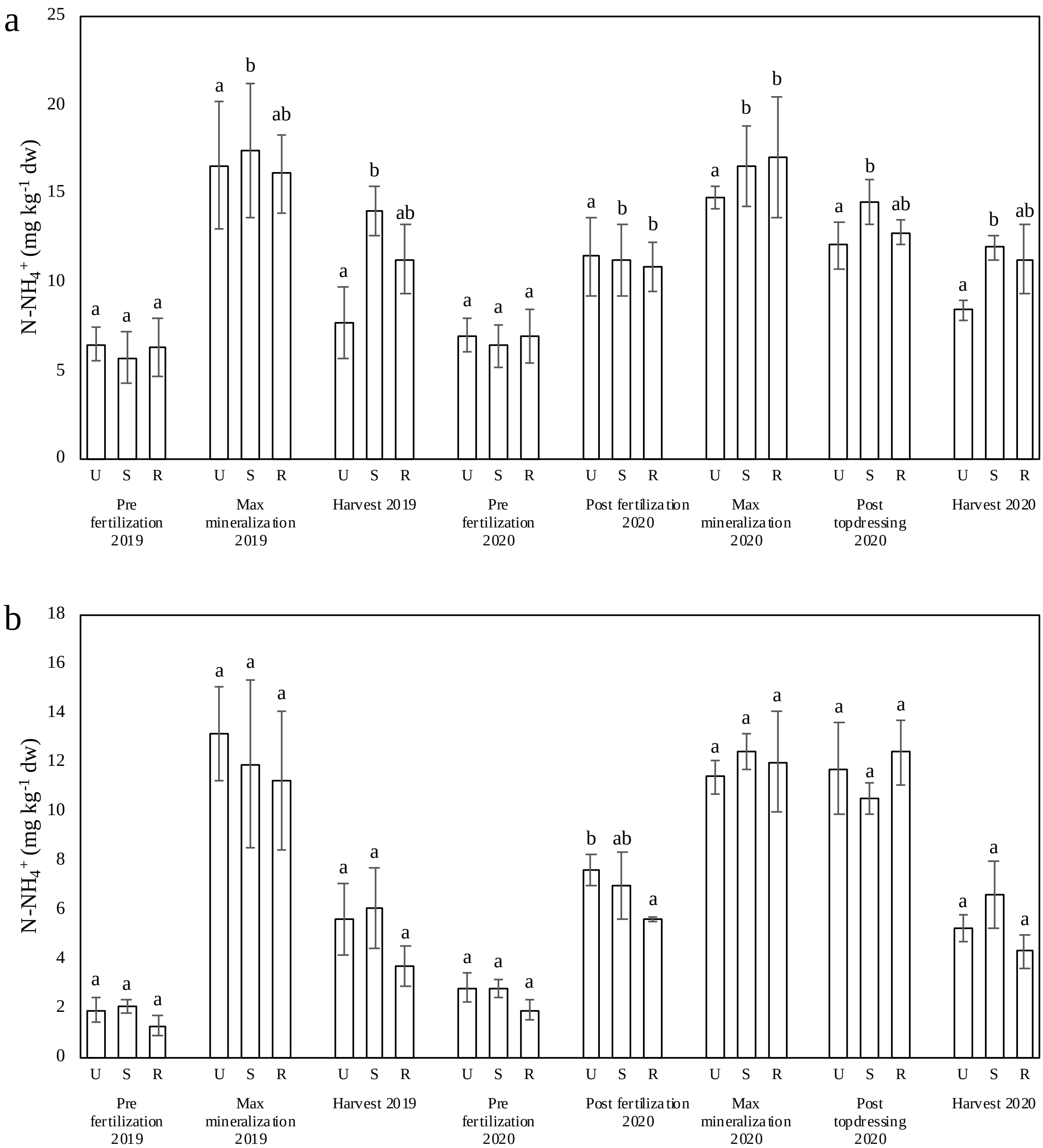

Figure 1. *Concentrations of ammonia nitrogen (N-NH4+) in experimental soils (a: surface; b: 1 meter depth) during the crop seasons 2019 and 2020 (mean ± SD; n=3). U: untreated, S: synthetic, R: recovered. Letters are referred to One-way ANOVA (p<0.05; Tukey post-test) comparing the three treatments within each sampling time.*

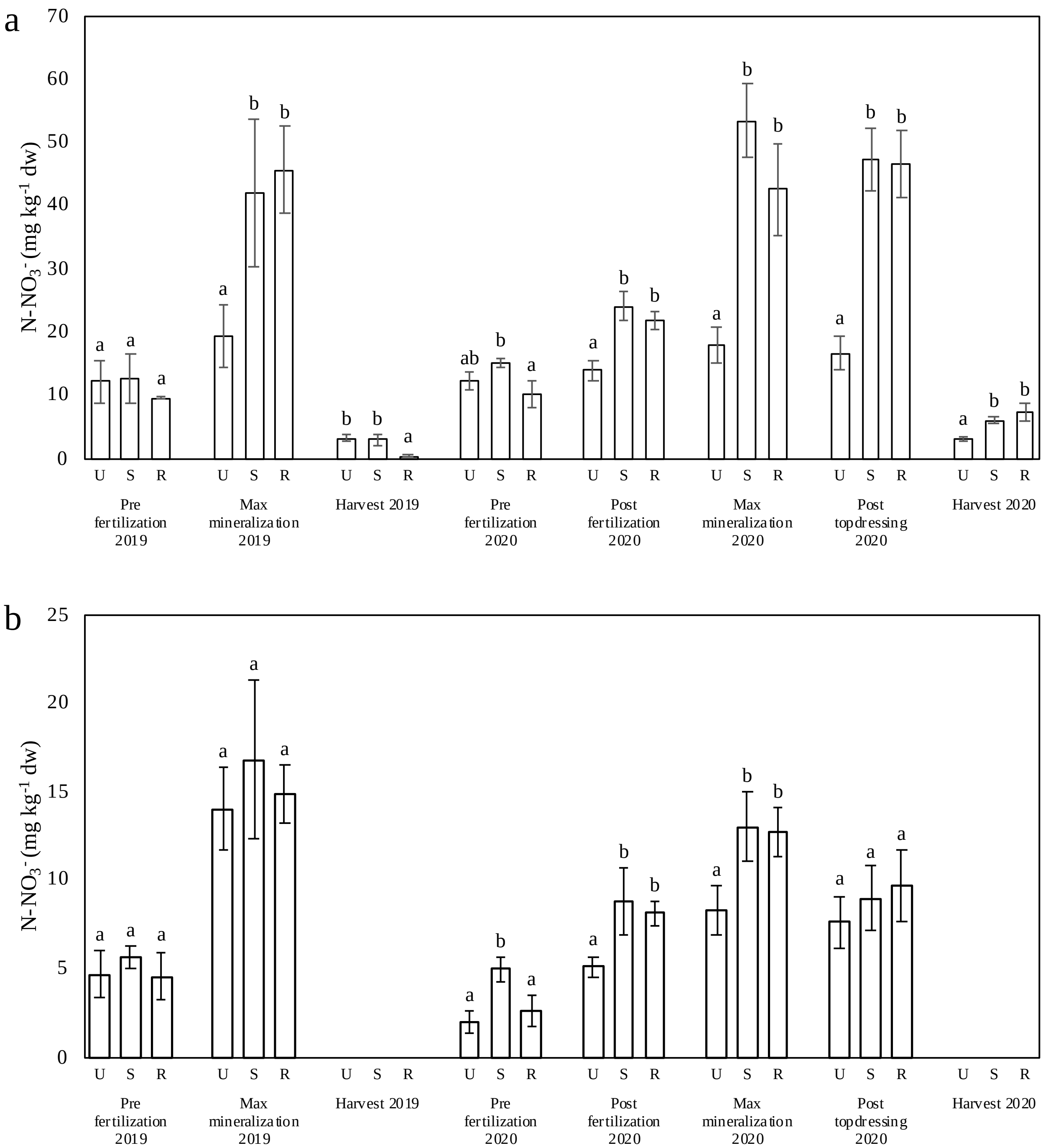

Figure 2. *Concentrations of nitric nitrogen (N-NO_3^-) in experimental soils (a: surface; b: 1 meter depth) during the crop seasons 2019 and 2020 (mean ± SD; n=3). U: untreated, S: synthetic, R: recovered. Letters are referred to One-way ANOVA (p<0.05; Tukey post-test) comparing the three treatments within each sampling time.*

Results obtained showed that during the two years of monitoring and for all sampling campaigns, the NH_4^+ concentrations in the experimental plots fertilized with RF were always comparable to those detected for plots fertilized with SF, both at the surface and at one-meter depth in the soil.

The data also show that, in both monitored years, the concentrations of NO_3^- in soil fertilized with RF were never higher than those found in soil plots fertilized with SF (One-way ANOVA, $p<0.05$, $n=3$, Tukey post-test), and that in one case (pre-fertilization 2020) NO_3^- concentration was lower. The comparison between the NO_3^- concentrations measured in fertilized and unfertilized soil plots, showed, also, that the N doses used in this work did not cause leaching risks higher than those which occur in unfertilized soil. Average NO_3^- concentrations along the two monitored years were of 5.22 ± 4.65, 7.18 ± 5.89 and 6.56 ± 5.49 mg kg^{-1} dw for unfertilized, SF and RF, respectively: these values are similar to each other and in line with those found in the literature for undisturbed soils (9.6 mg kg^{-1}).[242] These figures are particularly interesting if it is considered that the unfertilized soil did not receive any N fertilizers throughout the three years, contrarily to the fertilized soil plots which every year received 470 and 285 kg Ha^{-1} of N, respectively, for RF and SF plots. All this suggests that dosing a correct amount of mineral N fertilizers (i.e., matching crop requirements) and using stable organic-N, did not lead to any nitrate leaching risk. These results agree with those previously found in experiments on soils fertilized with either mineral or organic N fertilizers performed in the same geographical area (Lombardy, Po Valley).[243] In that occasion, the results obtained also indicated that soil microorganisms related to the N-cycle played a role in controlling nitrate leaching, i.e., nitrification-denitrification soil activity, so that N dosed up to 450 kg N ha^{-1} per year did not show any problem for nitrate leaching in a different full field scale study performed in the Po Valley.[243]

4.3.3.2 GHG emissions from soils

Greenhouse gas (GHG) emissions were measured in 2020 starting from pre-sowing fertilization and thereafter for the following 10 months (from 28/05/2020 to 17/03/2021), with periodic measurements (Table 4). The amounts of CO_2 emitted were 6,216 ± 1,160 kg C Ha^{-1} and 6,144 ± 1,491 kg C Ha^{-1} for RF and SF plots, respectively, suggesting that the addition to soil of organic matter by digestate did not lead to any C emission increase. These results confirmed that digestate organic matter was quite stable and did not mineralize, becoming part of the soil organic matter. This was more evident when unfertilized soil, that did not receive any fertilization for three years (CO_2 emission of 5,698 ± 935 kg C Ha^{-1}), was compared to RF that, on the contrary, was dosed yearly (CO_2 emission 6,216 ± 1,160 kg C Ha^{-1}). Methane did not contribute greatly to C emissions and in any case, again, there were no statistically significant differences between different plot trials i.e., 0.066 ± 0.06 kg C Ha^{-1}, 0.053 ± 0.04 kg C Ha^{-1} and 0.036 ± 0.03 kg C Ha^{-1}, for Unfertilized, SF and RF treatments, respectively. Nitrous oxide (N_2O) emitted was of 1.71 ± 1.1, 10.3 ± 6.8 and 7.59 ± 3.2 kgN Ha^{-1} for Unfertilized, SF and RF, respectively. Plots fertilized with SF emitted more N_2O that those fertilized with RF, although no statistical differences were found. As expected, the unfertilized soil, that did not receive any N fertilization during the three years of experimentation, emitted much less N_2O than fertilized soil plots, confirming the contribution of N fertilization to N_2O emission from soils.[244] These results showed that dosing a much higher amount of N with recovered fertilizers (in total 470 kg N Ha^{-1}) than with synthetic fertilizers (285 kg N Ha^{-1}) did not lead to N_2O emissions increasing. This can be ascribed, as already discussed for the potential nitrate leaching and CO_2 emissions, to the high biological stability of organic matter contained in the digestate, which limited N mineralization and nitrification. Therefore, taking into consideration that only the mineral N fraction was responsible for N_2O emission, i.e., 290 kg N Ha^{-1} for RF and 275 kg N Ha^{-1} for SF, an equal N_2O emission was expected, as was then measured experimentally.

Table 4. *Cumulated emissions of N_2O, CO_2 and CH_4 measured from the experimental plots during the crop season 2020 and the following months (from 28/05/2020 to 17/03/2021).*

Fertilizer	Total nitrogen dosed (kgN Ha^{-1})	Total N_2O emitted (kgN Ha^{-1})	Total CO_2 emitted (kgC Ha^{-1})	Total CH_4 emitted (kgC Ha^{-1})
Unfertilized	0	1.71 ± 1.1[a](a)[b]	5698 ± 935(a)	0.066 ± 0.06(a)
Synthetic fertilizer	285	10.3 ± 6.8(b)	6144 ± 1491(a)	0.053 ± 0.04(a)
Recovered fertilizer	461	7.59 ± 3.2(ab)	6216 ± 1160(a)	0.036 ± 0.03(a)

[a]mean ± SD, n = 6
[a]letters are referred to One-way ANOVA comparing values in each column ($p<0.05$; n=6; Tukey post-test).

These data may appear to contrast with some of those previously reported which indicated that there were higher N_2O emissions for recovered fertilizers than for synthetic fertilizers.[158,206] However, in these previous studies, the biological stability of the organic matter was not measured/reported. Therefore, the degradability of the organic fraction which leads to mineral N that is then responsible for N_2O production was not known. It therefore appears that the measurement of the biological stability of the organic substrate becomes important in understanding the fate of N in the soil (potential NO_3^- leaching and potential N_2O production).

4.3.3.3 Soil pollutants

The concentration of inorganic pollutants in the soil (i.e., As, Cd, Hg, Cr, Ni, Pb, Cu, Zn) was measured before the start of the experiment and after three years (Table 2). For all of the pollutants analysed, no significant increase was observed in the soils of all the experimental plots. These data confirmed previous reports in the literature for similar work, namely that after the use of digestate in agriculture, no significant accumulations of heavy metals are found in the soil.[245,246] In particular, as regards our study, the amount of heavy metals applied to the soil every year represented a minimal fraction compared to the content of the same metals already present in it (0.5% on average), with the exceptions only of Cu and Zn. In fact, every year, the quantity of Cu and Zn applied to the soil with the digestate corresponds respectively to 6% and 3.8% of what was already present in the 15 cm of surface soil. However, as reported in Table 2, after three years of experimentation the concentration of these two metals in the soil fertilized with recovered fertilizers was no higher than that measured at the beginning of the experiment, nor any higher than that of the unfertilized soil at the end of the experiment. One might think that three years of experimentation are not enough to measure an increase in the concentration of an element in the soil, even if it is dosed with a consistent quantity. However, in this work, fertilization with RF brought into the soil every year an amount of carbon equal to 8% of what was already present, and as previously observed (Table 2), in that case the increase in the concentration of carbon in the soil was detected. This shows that such variations can be measured and confirms that most of the heavy metals brought to the soil dosed with digestate did not accumulate in the soil.

Regarding the concentrations of persistent organic pollutants (POPs) in the experimental soils, in no case was any increase found in their concentration after three years of experimentation, for all the plots studied, including unfertilized plots (Table 2). Furthermore, all values complied with the legal limits established in Italy for agricultural soils (DM 2019/46, Ministero dell'ambiente), and the values were in line with data reported for European agricultural soils as regards PCB, dioxins and DEHP for which data are available in the literature.[248–250] Weissengruber and colleagues (2018) applying a forecasting model, reported that the risk of POPs accumulating in soils using digestate as fertilizer for several years (200) is negligible.

In addition to POPs, also the concentrations of emerging pollutants in soils (pharmaceuticals) were measured after three years of experimentation. These types of molecules can in fact be present in bio fertilizers, and therefore accumulate in the soil, with potentially toxic effects for ecosystems and public health.[252] However, for these types of compounds there are still no laws that set limits or identify a group of molecules to be monitored, so the choice was made based on what was suggested by Konradi and Vogel (2013), taking into consideration parameters such as residence time in the soil, solubility and ecotoxicity. The 9 compounds chosen were: antibiotics (Ciproflaxacin and Sulfamethoxazole), lipid regulators (Fenofibrate and Gemfibrozil), psychiatric drugs (Carbamazepine), beta-blockers (Metoprolol), analgesic (Diclorofenac) and hormones (Ethinylestradiol and Estradiol) (Table 2). The analyses showed that after the third year, the soil concentrations of all the pharmaceuticals were always below the instrumental detection limit (<0.01 mg kg^{-1} dw), for all the experimental plots, with no differences between soils fertilized with RF, SF or not fertilized. In a previous work it was already reported that the concentration of emerging organic pollutants in this type of digestate was very low, and always below detection limit and often lower than the values reported for other types of organic matrices routinely used as fertilizers (i.e., animal slurries and manures).[128]

4.3.3.4 Recovered fertilizers nitrogen efficiency

The N fertilizer use efficiency (FUE) measured for SF was of 93.6 ± 4.4% to be compared with that calculated for RF which was of 55.5 ± 6.6% (Table 5), and therefore similar to that of 50% suggested by Lombardy Region and adopted in this work. These figures were obtained taking into consideration total N dosed, independently of N forms (mineral vs. organic). Nevertheless, nitrogen dosed with the digestate was represented for 57.8% TKN by NH_4-N that was readily available for plants as well as N from SF, and by 42.2% TKN by organic N that was quite stable (no mineralization occurred) because of the high biological stability of digestate. The stability of organic N was confirmed, such as discussed earlier, by measuring CO_2 and CH_4 evolution from soils treated with RF that showed similar figures to those of both plots fertilized with SF and unfertilized, and by measuring both NH_4^+ and NO_3^- soil contents at different topical moments, that were similar for all soils studied, independently of the fertilizers used. As a consequence of the results obtained, it can be considered that the organic N of digestate, substantially, did not contribute to mineral soil N, since it became part of the soil organic matter, and that only the ammonia form should be considered for FUE calculation. Doing so, the re-calculated RF FUE was of 85.3 ± 10%, comparable to that calculated for SF (FUE of 93.6 ± 4.4%). Consequently, the N fertilizer replacement value (NFRV) obtained for RF used to replace SF, when referred only to the mineral N form, was of 83.7%. Obviously, this value assumes validity only if the digestate characterization is performed to attest the high biological stability of the organic matter which it contains.

It therefore appears that high FUE and NFRV for recovered fertilizers can be achieved by well performed anaerobic digestion which is able to transform as much as possible of the organic-N into ammonia, leaving a very stable organic fraction containing a low mineralizable organic-N that contributes to the stable soil N-pool. The separate mineral N fraction can then be assumed to have the same efficiency as that of a common synthetic fertilizer (e.g. urea) and the organic fraction to have an efficiency close to zero, contributing to the soil organic matter pool.

Table 5. *Fertilizer use efficiency (FUE) and N fertilizer replacement value (NFRV) for the maize crop fertilized with SF and RF in the year 2020. Letters are referred to One-way ANOVA (n=6, p<0.05, Tukey post-test).*

	Unfertilized	**Synthetic fertilizer**	**Recovered fertilizer**	
N uptake (kgN Ha^{-1} dw[a])	175 ± 19	267 ± 13	256 ± 31	
N tot applied (kgN Ha^{-1})	0	285	460 (Ntot)[b]	290 ($N\text{-}NH_4^+$)[c]
FUE (%)	-	93.6 ± 4.4 (b)	55.5 ± 6.6 (a)	85.3 ± 10 (b)
NFRV (%)	-	-	54.5	83.7

[a]dw: dry weight
[b]N applied considering the N tot contained in the digestate dosed
[c]N applied considering only the $N\text{-}NH_4^+$ contained in the digestate dosed

4.4 Conclusions

In conclusion, the use of highly stabilized digestate and digestate-derived ammonium sulphate as a fertilizer replacing synthetic fertilizers did not have negative impacts on soil quality, nor on the accumulation of inorganic and organic pollutants (POPs), but instead caused an increase in the portion of organic carbon in the soil, contributing to the improvement of its quality. All the data reported indicate that a very stable digestate can solve problems of uncontrolled mineralization typical of less stable biomasses used in agriculture (i.e., slurry or manure), without risks of N leaching, nor of gas emissions (ammonia or GHG). If the digestate is dosed by equating the amount of NH_4-N to a synthetic fertilizer, and the amount of organic N assimilated to that to a well stabilized soil improver, the grain yield produced is equivalent to those obtained using a similar dose of urea N (SF), with fertilizer use efficiencies (FUE) which are very similar. The stabilization of the digestate can therefore constitute a strategy to obtain a bio-based fertilizer that can replace mineral N fertilizers, without loss of performance or environmental risks.

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4.5 Supporting information

Table S1. *Main characteristics of infeed (mean ± SD) and full characterization of digestate in comparison with legal limits for its use as fertilizer in agriculture, and with data from literature for digestate and composts (mean of three-years monitoring, from Pigoli et al., 2021)*

Parameter	**Unit**	**Digestate**[a]	**Lombardy Law N. 6665/2019 – Legal limits**[b]
pH		8.5 ± 0.3	5.5 < pH < 11
Dry Matter 105°C	g kg^{-1} ww[c]	103 ± 3.7	
Dry Matter 600°C	g kg^{-1} ww	40.4 ± 2.5	
Total Organic Carbon	g kg^{-1} DM[c]	314 ± 30	> 200
TKN	g kg^{-1} DM	77 ± 3.7	> 15
$N\text{-}NH_4$	g kg^{-1} DM	35.9 ± 2.4	
$N\text{-}NH_4$/TKN	%	46.6	
OD_{20}[d]	mg O_2 g^{-1} DM	22.6 ± 6.1	
BMP[e]	L_{biogas} kg^{-1} DM	57 ± 23	
P	g kg^{-1} DM	28 ± 4.1	> 4
K	g kg^{-1} DM	6.5 ± 1.3	
Ca	g kg^{-1} DM	43 ± 7	
Mg	g kg^{-1} DM	5.2 ± 0.6	
Fe	g kg^{-1} DM	26.2 ± 6.4	
Mo	mg kg^{-1} DM	10 ± 1	
Cu	mg kg^{-1} DM	408 ± 60	≤ 1,000
Zn	mg kg^{-1} DM	1,020 ± 120	≤ 2,500
Mn	mg kg^{-1} DM	444 ± 35	
Al	g kg^{-1} DM	25.8 ± 4.5	
Co	mg kg^{-1} DM	6.6 ± 2.3	
Se	mg kg^{-1} DM	3.7 ± 2.1	≤ 10
Na	g kg^{-1} DM	1.9 ± 0.4	
Cr	mg kg^{-1} DM	95 ± 22	< 200
Pb	mg kg^{-1} DM	64 ± 11	≤ 750
Ni	mg kg^{-1} DM	61 ± 13	≤ 300
As	mg kg^{-1} DM	9.0 ± 2.2	< 20
Cd	mg kg^{-1} DM	1 ± 0.5[f]	≤ 20
Hg	mg kg^{-1} DM	0.1 ± 0.3[f]	≤ 10
PAH	mg kg^{-1} DM	0.5 ± 0.5[f]	∑< 6
PCB	mg kg^{-1} DM	< 0.1	∑< 0.8
PCDD/F+PCB-DL	ng TEQ kg^{-1} DM	10.6 ± 2.9[f]	∑≤ 25

DEHP	mg kg^{-1} DM	5.7 ± 5.3[f]	< 100
Hydrocarbon C10-C40	mg kg^{-1} ww mg kg^{-1} DM	284 ± 251[f] (2,757)	≤ 1,000
AOX	mg kg^{-1} DM	< 0.6	∑ < 500
Ciproflaxacin	mg kg^{-1} DM	< 0.01[g]	
Sulfamethoxazole	mg kg^{-1} DM	< 0.01	
Fenofibrat	mg kg^{-1} DM	< 0.01	
Gemfibrozil	mg kg^{-1} DM	< 0.01	
Carbamazepine	mg kg^{-1} DM	< 0.01	
Metoprolol	mg kg^{-1} DM	< 0.01	
Diclofenac	mg kg^{-1} DM	< 0.01	
Ethinylestradiol	mg kg^{-1} DM	< 0.01	
Estradiol	mg kg^{-1} DM	< 0.01	
Salmonella	MPN g^{-1} DM	Absent	< 100
Faecal coliform	MPN g^{-1} DM	< 1,000	< 10,000

[a]Mean ± SD: *n*=42, except for Ca, Mn, Mg, Fe, Mo, Al, Co, Na: *n* = 9, and BMP: *n* = 10.
[b]Legal limit referred to the digestate described in this work.
[c]ww and DM: wet weight and dry matter, respectively.
[d]OD_{20}: Oxygen Demand after 20h
[e]BMP: potential biogas production.
[f]Mean and SD calculated considering data below detection limits = 0.
[g]Analysis performed in 2020; *n*=4.

Table S2. *Main characteristics (mean ± SD; n=17) of ammonium sulphate - $(NH_4)_2SO_4$ - used for topdressing fertilization in this work (all concentrations are expressed on wet basis).*

Parameter	**Unit**	**Value**
pH	pH	6.8 ± 1.3
EC	mS cm^{-1}	119 ± 27 (1:2.5 v/v 25 °C)
Dry Matter 105°C	% of ww	35.5 ± 0.4
Total Organic Carbon (TOC)	g kg^{-1} ww	< 0.1
Total N (TKN)	g kg^{-1} ww	74 ± 2
$N\text{-}NH_4$ (TAN)	g kg^{-1} ww	71.7 ± 1.9
Total P	mg kg^{-1} ww	11.7 ± 4.7
Cd tot	mg kg^{-1} ww	< 0.25
Hg tot	mg kg^{-1} ww	< 0.25
Ni tot	mg kg^{-1} ww	< 1
Pb tot	mg kg^{-1} ww	< 1
Cu tot	mg kg^{-1} ww	< 6
Zn tot	mg kg^{-1} ww	2.5 ± 2.4[e]
Salmonella		Absent
E. Coli		Absent
Enterococcaceae		Absent

[a]Sigurnjak et al., (2019), ammonium sulphate produced by air scrubbing
[b]Ivona Sigurnjak et al. (2016), air scrubber water from digestate treatment
[c]Vaneeckhaute et al. (2013), air scrubber water from digestate treatment
[d]Ledda, et al. (2013), ammonium sulphate produced by scrubbing with sulfuric acid
[e]Mean and SD calculated considering data below detection limits = 0.

Table S3. *Chronological list of agronomic operations and soil samplings carried out during the experimentation.*

Date	Sampling	Agronomic operation
23/04/2018	Pre sown 2018	Pre sown spreading
3/04/2019	Pre sown 2019	
16/04/2019		Pre sown spreading
28/06/2019	Pre topdressing 2019	
1/08/2019		Topdressing fertilization
23/09/2019		Harvest
24/09/2019	Harvest 2019	
16/05/2020	Pre sown 2020	
28/05/2020		Pre sown spreading
18/06/2020	Post sown 2020	
14/07/2020	Pre topdressing 2020	
31/07/2020		Topdressing fertilization
7/08/2020	Post topdressing 2020	
28/10/2020		Harvest
5/11/2020	Harvest 2020	
12/01/2021	Three years after experiment start	

Table S4. *Average maize productions yield in grain for the three years of experiments (mean ± SD; n=9). Table modified from Zilio et al., 2021.*

Fertilizer	**Grain yield dw[a] (Mg Ha^{-1})**
Unfertilized	10.4 ± 3.5 (a)[b]
Synthetic fertilizer	17.4 ± 1.2 (b)
Recovered fertilizer	18.1 ± 2.9 (b)

[a]dw: dry weight
[b]Letters are referred to One-way ANOVA analysis (Tukey post-test, $p < 0.01$; n=9).

Table S5. *Average ammonia emissions (mean ± SD, n=9) for the three years of experiments (2018, 2019 and 2020). Table modified from Zilio et al., 2021.*

Fertilizer	**Total cumulated ammonia emission (kg N Ha^{-1})**	**Loss of NH_3 (%Ntot)**	**Loss of NH_3 (%TAN)**
Synthetic fertilizer	24.8 ± 8.3 a	13.4 ± 4.5 b	13.4 ± 4.5 a
Recovered fertilizer	25.6 ± 9.4 a[b]	7.01 ± 2.5 a	11.6 ± 4 a

[a]ammonia emission in unfertilized plots did not differ from background.
[b]Letters are referred to One-way ANOVA analysis carried out comparing for each year the odour emitted from the three treatments (Tukey post-test, $p < 0.01$; $n = 3$).

Chapter V

Conclusions.

5 Conclusions

This PhD thesis was aimed to assess diverse cycling approaches in the management of nutrients from full-scale technologies using different types of waste streams regarding their global environmental impact. In general, our results showed that the reuse of end-products is critical for positive environmental impacts due to avoided emissions from production, transport, and mineral sources. Within the improvement of soil condition (e.g. digestate) satisfying the aim of supporting a closed-loop integrated system.

When comparing directly recovered fertilizers with synthetic fertilizers, results indicated that lower impact on fossil resource depletion and energy consumption could be obtained from biobased sources. However, there is also an increase in eutrophication and acidification potential due to N and P leaching and NH_3 losses, respectively. Although our findings show, this contrasting impact can be reduced by assuring a stable bio compound and integrating the proper practices in its use (i.e. precision agriculture).

Global warming potential as a critical indicator could vary depending on the energy intensity of the processing, and on the other hand, from the possible co-production of energy from the process. As in our case, AD systems can induce lower or even negative impact (savings) because of both fossil energy substitution and GHGs reduction from the whole recovering processing, affecting positively as well other indicators, especially the ones belonging to the resources group.

LCA is a crucial environmental management tool, comprehensive and in constant upgrading and expansion. It can be exploited by performing more studies with a product perspective on recycled nutrient products and even optimizing individual recovery processes by combining recovery technologies. However, there are challenges to finding more common standards in its application (i.e. mixed results; depending on FU, boundaries, allocation, an emission method, and so on). This is also stressed by the rapid advance in information technologies and the perceived expansion to encompass other social and economic dimensions.

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