UNIVERSITÀ DEGLI STUDI DI MILANO

Ph.D. in Environmental Science – XXIV cycle

FROM SEWAGE SLUDGE TO RENEWABLE FERTILIZER: EFFICACY AND ENVIRONMENTAL RISKS IN OPEN FIELD EXPERIMENT.

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Preface

This work is part of the Horizon 2020 project Nutri2Cycle, under the supervision of Professor Fulvia Tambone. This thesis is divided into several chapters, the abstract and an introduction are followed by four chapters which are a collection of research papers. Papers in chapter three and four are already published, while the ones in chapter five and six are submitted to the journal. The titles of the papers, with their authors and editors are listed below.

- Chapter 3: THERMOPHILIC ANAEROBIC DIGESTION AS SUITABLE BIOPROCESS PRODUCING ORGANIC AND CHEMICAL RENEWABLE FERTILIZERS: A FULL-SCALE APPROACH. *Ambrogio Pigoli, Massimo Zilio, Fulvia Tambone, Stefania Mazzini, Micol Schepis, Erik Meers, Oscar Schoumans, Andrea Giordano, Fabrizio Adani* Waste Management 124 (2021) 356–367
- Chapter 4: MEASURING AMMONIA AND ODOURS EMISSIONS DURING FULL FIELD DIGESTATE USE IN AGRICULTURE.
  Massimo Zilio, Ambrogio Pigoli, Bruno Rizzi, Gabriele Geromel, Erik Meers, Oscar Schoumans, Andrea Giordano, Fabrizio Adani
  Science of the Total Environment 782 (2021) 146882 Contents

- Chapter 5: USE OF HIGHLY STABILIZED DIGESTATE TO REPLACE UREA IN OPEN FIELD AGRICULTURE: EFFECTS ON SOIL, ENVIRONMENT, AND MAIZE PRODUCTION.

  Massimo Zilio, Ambrogio Pigoli, Bruno Rizzi, Axel Herrera, Fulvia Tambone, Gabriele Geromel, Erik Meers, Oscar Schoumans, Andrea Giordano, Fabrizio Adani

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- Chapter 6: ENVIRONMENTAL PERFORMANCE IN THE PRODUCTION AND USE OF RECOVERED FERTILIZERS FROM ORGANIC WASTES TREATED BY ANAEROBIC DIGESTION VS. SYNTHETIC MINERAL FERTILIZERS

  Axel Herrera, Giuliana D'Imporzano, Massimo Zilio, Ambrogio Pigoli, Bruno Rizzi, Erik Meers, Oscar Schouman, Micol Schepis, Federica Barone, Andrea Giordano, Fabrizio Adani.

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1 ABSTRACT

The increasing of human population worldwide set, among others, two challenges: food provision, which lead to fertilizers production and waste disposal. Sewage sludge, that can be harmful if not-correctly managed, if properly treated with anaerobic digestion can be used as worthy fertilizer/soil conditioner, while producing energy through biogas combustion. This thesis focusses on a full-scale anaerobic digestion plant treating mainly sewage sludge at thermophilic condition (55°C) for relatively long time (HRT about 50 days) leading to the production of a highly stabilized digestate. Furthermore, the plant has a stripping system which recover ammonia from the substrate, producing ammonium sulphate that can be used as N fertilizer.

The recovered fertilizers produced (digestate and ammonium sulphate) were characterized and monitored along three years. Moreover, an experimental maize field was set up for three crop seasons to study the use in agriculture of the recovered fertilizers produced (digestate in pre-sown and ammonium sulphate in topdressing), in comparison with parallel use of synthetic fertilizers (urea in pre-sown and ammonium sulphate in topdressing). The digestate was injected in soil at a depth of 15 cm, urea was spread on soil surface, and ammonium sulphate given as fertigation.

During the pre-sown fertilizations were measured ammonia, and odour emissions. The emission of greenhouse gases from the experimental fields was also determined for the ten months following the spreading in pre-sowing. Furthermore. Along the three years yield were quantified and grain quality was tested. At the beginning and at the end of the period were run complete soil analyses. Finally, an LCA study was conducted comparing the environmental impacts of the recovered fertilizers studied and the traditional synthetic urea fertilization.

Digestate characterization showed a high N (47% in ammonia form) and P concentration, while a lower K content. All organic and inorganic pollutants (emerging pollutants among the others) were far below the legal limit for its use in agriculture and microbiologic analyses showed an almost complete sanitation of the substrate. Ammonium sulphate (7% N) was free from pollutant, showing a high purity level. Ammonia emissions in open field were similar for digestate and urea, and odour were slightly lower for digestate than urea, being ammonia coming from urea hydrolysis responsible for odour productions. The emission of greenhouse gases throughout the crop season was equivalent between fields fertilized with recovered fertilizers and synthetic fertilizers. Furthermore, soil analyses showed no significant differences in organic or inorganic pollutants. Digestate ensured an ammonia availability for plant equal to urea fertilization and agronomic yield were similar for digestate and urea treatment, while significantly higher than untreated plot, as expected. Finally, in the LCA study, digestate showed a better environmental performance than urea, due the impact of synthetic urea production and considering the renewable energy production of biogas. In conclusion the high stabilized digestate and ammonium sulphate can effectively and safely substitute the traditional chemical fertilization on crop, bringing the advantage of being renewable and helping waste management.

2 INTRODUCTION

This work focuses on the exploitation of the Anaerobic Digestion (AD) to process Sewage Sludge (SS) before its use in agriculture as fertilizer and soil conditioner. Our aim is to demonstrate that this pathway can efficiently and safely dispose SS while recovering energy (as biogas) and producing a fertilizer with performance at least the same than traditional chemical fertilizers.

World population will grow for 30 years at least, and can reach 12 billion in year 2100 according to other scenarios (Abel et al. 2016). For this reason and for the increase of pro-capita income the food demand almost tripled in the last fifty years and is going to increase further. Moreover, the share of animal based calories escalates accordingly with the increase of income, so to aggravate the need of agricultural production in the time being (Sabola 2017; Gouel and Guimbard 2019).

On the other hand, to an increase of population corresponds also an increase in the production of wastes and SS only in Europe are produced 10,130,000 MG y^{-1} of sludge (expressed as dry solids) (European Commission 2009).

Hereafter are collected some definitions of SS found in literature:

- As an output of wastewater treatment plant, [SS] is a critical biologically active mixture of water, organic matter (derived from human wastes, food wastes, etc.), dead and alive microorganisms (including pathogens), and inorganic and organic toxic contaminants (e.g., metallic trace elements, PAHs). (Kacprzak et al. 2017)
- Domestic wastewater treatment uses both physical removal and the biological transformation of particles, pathogens, organic compounds and nutrients to dramatically improve the quality of effluent water. The solids removed during

a primary sedi- mentation step and the settled microorganisms produced during biological transformation processes after secondary clarification are collectively called “SS”, which is the major byproduct of wastewater treatment.(Peccia and Westerhoff 2015)

- SS can be defined as the solid or semi-solid residue left over after the treatment of wastewater. In literature it can be defined as by-product, yet it shall be treated as a waste in the process of wastewater treatment. SS may be used as a source of energy (AD, thermal treatment), treated and used on land as a fertilizer and soil conditioner, or may even be used as a source to extract valuable compounds (phosphorous recovery). (Fijalkowski et al. 2017)
- SS, the most important byproduct of biological wastewater treatment, is considered an important source of secondary pollution in aquatic environments, linked to health problems and even deaths in humans. (Feng, Luo, and Chen 2015)
- [SS] can simply be defined as the residual semi-solid material which is inevitably left over from municipal or industrial wastewater or sewage treatment processes. (Raheem et al. 2018)

2.1 Laws and regulation about use of SS in agriculture

Since the European directive 86/278 (known as sludge directive) SS use in agriculture has increased over time, and further law and regulations has been promulgated. In the scheme below are reported the most important European, Italian and regional laws concerning the agricultural uses of SS.

Directive 86/278, called “sludge directive” (COUNCIL DIRECTIVE 1986)	It encourages the correct use of SS in agriculture avoid dangerous effects on humans, animals and environment. It prohibits the use of raw SS unless injected or incorporate in soil and set limits for pollutants.
D.Lgs. n99/92 (Italian Parliament 1992)	This law decree transposes the EU directive 86/278 into Italian national law. For the use of sludges in agriculture three points are underlined: I. SS cannot be used raw, but it must undergo treatment. II. SS must have a positive effect as fertilizer and/or soil conditioner. III. SS does not contain substance which are toxic and harmful and/or persistent, and/or bio-accumulative in such concentration that can be harmful for

	cultures, animals, humans and environmental.
Working document on sludge. 3rd draft. ENV.E.3/LM (European Commission 2000)	Proposed then withdrawn limits for organic pollutants in SS used in agriculture, few countries adopted similar values (Mininni et al. 2015)
Lombardy Region.D.G.R n.715944/2003	Specify the definitions of products that can be used in agriculture.
Directive 2008/98/EC (European Parliament 2008)	This European directive introduced the “End-of-Waste Criteria” which specify that a waste after recovery or recycling loses its status of “waste” and gain a new status of “product” or “secondary raw material”. Four conditions must be satisfied because this may happen: I. The substance or object is commonly used for specific purposes. II. A market or demand exists for such substance or object. III. The substance or object fulfils the technical requirements for specific purposes and meets the existing legislation and standards applicable to product.

	IV. The use of the substance or object will not lead to overall adverse environmental or human health impacts.
(IPTS 2014)	This Final report of IPTS includes compost and digestate in the “End-of-Waste” criteria, and it adds SS as input material in the production of these two substrates. This document (Annex 2 to 18) reports different definitions, limitations, protocols and standards for digestate and compost all over EU. In the Annexes 19 and 20 it proposes some end of waste criteria for these products.
D.lgs n 109/18 (Italian Parliament 2018)	This law decree introduces new limitation, specially about hydrocarbon C10-C40, PCDD/F, PCB-DL, and few elements, i.e. Se, Be, As, total Cr, Cr VI.
D.G.R 6665/2019 (Lombardy Region 2019)	This regional decree set new standards and limits for SS “suitable for agriculture” and for “high quality SS”, the latter having more severe limits.

2.2 Harmful effect that can follow the use of raw SS in agriculture

The use of SS in agriculture as fertilizer and/or soil conditioner can be very positive on both economic and environmental sides. Though, when used in agriculture it can also lead to health hazard due to the high presence of biological contaminants as pathogen bacteria, fungi, viruses and enteric parasites, and to the possible presence of chemical pollutants like heavy metals and organic xenobiotic. Unpleasant odour emission is also an issue that must be considered.

2.2.1 Biological hazard.

Back to the seventies a paper (Matthews 1979) considered the suitable ways to dispose SS and agricultural utilization was considered the second more common (after marine disposal) and underline the importance of the biological risk. It identifies the three most dangerous group of organisms in SS as Salmonellae, intestinal parasites (mainly *Taenia saginata)* and potato cyst nematode. A later study (Barbier et al. 1990) focused on parasites (family of *Taeniidae*, *Ascaris* spp., *Toxocara* spp., and family *Trichuridae*) and the risk derived from these organism when SS is spread in field for agricultural purposes. The conclusions were that while it seemed not dangerous in arable land, thanks to the fast (within 24 hours) incorporation in soil, the practice could be harmful on grazing or pasture land. An interesting study (Ross et al. 1992) focused on consequences of the spreading of SS on land for the marine environment, starting from the increase of concentration of pathogens and parasites in coastal sea, near where sludge was used in field. A very vast paper (Straub, Pepper, and Gerba 1993) shows a longlist of pathogens commonly found in SS that is reported in this work in Table 1. Moreover, it describes the common way of pathogens exposure for human being when sludge is spread in field. For agricultural personnel direct contact is the most frequent way, also contact with clothing and equipment can transport pathogens to other people. Spreading and burying SS can produce aerosol containing pathogens, and with the presence of wind, can bring contamination far from spreading site and then can be transmitted by inhalation or contact with surfaces. The last two ways of exposure are mediated by water, that can be both superficial (run-off to superficial water bodies) or deep (leaching to groundwater).

Note: it wasn't possible to find more recent papers describing pathogens consortia in SS, most of the late paper deal with sanitation of SS, the topic will be treated in the following section.

Table 1 Virus, bacteria, parasites and fungi pathogenic to humans that can be present in SS. Source: (Straub, Pepper, and Gerba 1993) modified.

Human viruses shed in feces that may be present in SS		
Virus Group	N. of Serotypes	Illness Caused
Adenovirus	41	Pharyngitis, conjunctivitis, respiratory illness, vomiting, diarrhea.
Astrovirus	5	Vomiting, diarrhea
Calicivirus	2	Vomiting, diarrhea
Coronavirus	1	Vomiting, diarrhea
Enterovirus		
Poliovirus	3	Paralysis, meningitis, fever
Coxsackie A	24	Herpangina, respiratory illness, meningitis, fever

Coxsackie B	6	Myocarditis, congenital heart anomalies, rash, fever, meningitis, respiratory illness, pleurodynia
Echovirus	34	Meningitis, encephalitis, respiratory disease, rash, diarrhea, fever
Enterovirus 68 – 72	4	Meningitis, encephalitis, respiratory illness, acute haemorrhagic conjunctivitis, fever
Hepatitis A virus	1	Hepatitis
Hepatitis E virus	1	Hepatitis
Norwalk virus	1	Epidemic vomiting and diarrhea
Reovirus	3	Not clearly established
Rotavirus	4	Vomiting, diarrhea
“Small round viruses”	3	Vomiting, diarrhea

Bacteria, parasites and fungi pathogenic to humans that may be present in SS

Group	Pathogen	Disease caused
Bacteria	*Salmonella* (2000 types)	Typhoid, paratyphoid, salmonellosis
	Shighella (4 spp.)	Bacillary dysentery
	Enteropathogenic *E.coli*	Gastroenteritis
	Yersinia enterocolitica	Gastroenteritis
	Campylobacter jejuni	Gastroenteritis
	Vibrio cholerae	Cholera

	Leptospira	Weil's disease
Protozoa	Entamoeba histolytica	Amebic dysentery, liver abscess, colonic ulceration
	Giardia lamblia	Diarrhea, malabsorption
	Balantidium coli	Mild diarrhea, colonic ulceration
	Cryptosporidium	Diarrhea
Helminths	Ascaris lumbricoides (roundworm)	Ascariasis
	Ancyclostoma duodenale (hookworm)	Anemia
	Necator americanus (hookworm)	Anemia
	Taenia saginata (tapeworm)	Taeniasis (tapeworm from uncooked beef and pork)
	Trichuris (whipworm)	Abdominal pain, diarrhea
	Toxocara (roundworm)	Fever, abdominal pain
	Strongyloides (threadworm)	Abdominal pain, nausea, diarrhea
Fungi	Aspergillus fumigatus	Respiratory otomycosis
	Candida albicans	Candidiasis
	Cryptococcus neoformans	Subacute chronic meningitis
	Epidermophyton spp. and Trichophyton spp.	Ringworm and athlete's foot
	Trchosporon spp.	Infection of hair follicles
	Phialophora spp.	Deep tissue infection

2.2.2 Chemical hazard

Another well-known issue in the use of SS in field, indeed it contain 70-90% of Cd, Cr, Cu, Pb and Zn present in wastewater (Lake, Kirk, and Lester 1984) these metals can be found in different forms in SS, like soluble, precipitated, coprecipitated in metal oxides, adsorbates and associated with biological residues (Lester, Sterritt, and Kirk 1983). Starting from the late seventies some studies were conducted on pollutants accumulation in soil, for example (Chang et al. 1984) analysed a cropland soil treated with SS for a period of six years; the study concluded that heavy metals accumulated almost entirely in the shallow 15 cm of soil, but luckily crop absorption and removal was very low (less than 1%). Heavy metals availability for plant uptake on the long run is a most interesting issue, Antoniadis et al. (2007) studied how metal competition for sorption sites in soil can make some of them more available, also during organic matter decomposition, how it happens after spreading SS on cropland. Results showed that Cd can become more available over time, when soil organic matter decreases, and metal competition increases.

Because these pollutants are non-volatile elements their amount in SS depends on the sources of wastewater, we can highlight few main sources of the latter:

1. Tap water
2. Human faeces and urine
3. Water from atmospheric events (through hydraulic derivation)
4. Industrial discharge
5. Washing effluents (often car washing)
6. Surfaces cleaning effluents

Tap water contamination comes mostly from piping system, although the water itself has very low concentration, due to the huge amount of water discharged and the accumulation of metals in SS the final concentration can result quite high.

Some interesting studies estimate the origin of different metals some results are summarized in table 2. An interesting case concerns Pb, leaded gas for vehicles fuel used to be one of the most important founts of Pb pollution, especially in water from atmospheric deposition. Since leaded gas is illegal in Europe from the late nineties, the all-over concentration is decreasing, and migrating to different sources.

Table 2: Comparison between two studies about the main sources of heavy metals in wastewater.

Metal	Main source	
	(Koch and Rotard 2001)	(Sörme and Lagerkvist 2002)
Cd	Uncertain, probably industrial discharge (59-79%)	Uncertain, probably washing effluents.
Cr	Uncertain, probably industrial discharge (90-91%)	Uncertain, probably industrial or washing effluents.
Cu	Tap water (60-77%)	Tap water and water from atmospheric event (trough roof gutters)
Pb	Water from atmospheric event	Uncertain, probably industrial or washing effluents.
Zn	Drinking water (30-39%) and a largely from industrial discharge.	Tap water, food and washing effluent.

Another study, (Rule et al. 2006), focused on time distribution of pollutants, other than space distribution. It concluded that highest concentrations are found in the morning more than in the afternoon/evening, on Mondays more than in other days, and in the first hour of rain more than after longer time raining. This phenomenon is due to a "first flush" effect, according to which there is an accumulation of metals when water is stagnant, and the concentration becomes lower and lower when water flows constantly.

Chemical hazard can come also from organic compounds, these are often petroleum hydrocarbons that can be both cyclic or chain molecules, heteroatomic compounds or high molecular weight polycondensation compounds (resins and asphalts) (Gennadiev et al. 2015). Usually these substances, although toxic, are likely to show very low concentrations in SS and even lower in soil. Moreover, some organic compounds are reactive in soil, so to rapidly decrease their concentration (O'Connor 1996) (Petersen et al. 2003). According to (P. Zhang and Chen 2017) most soil pollution by these substances comes from traffic exhaust and oil spill, so that SS is not of the main causes.

On the other hand, emerging contaminants can be an issue in SS. These compounds are mostly represented by the so called PPCPs (which stands for Pharmaceutical and Personal Care Products), also by illegal drugs, metabolites of PPCPs, and other similar anthropogenic chemicals.

Pharmaceuticals are the wider share of PPCPs found in wastewater and consequently in SS, the most common are analgesic, psychoactive drugs, beta-blockers. lipid regulators, steroid hormones, and most of all antibiotics (Daughton & Ternes, 1999; Fatta-Kassinos et al., 2011). The latter are one of the most effective drugs for both humans and livestock, their spreading in the environment is particularly worth of care due to the possibility to enhance and maintain antibiotic resistance genes in bacteria and in soil environment.

As mentioned, not only pharmaceutical drugs, but also illicit drugs can be found in SS and soil. The paper by Jones-Lepp et al. (2004) was one of the first to report the presence of methylenedioxymethamphetamine (MDMA) and methamphetamine in waste water treatment plant. Later on, another study (Noguera-Oviedo and Aga 2016), focused more on cocaine and its metabolite (benzoylecgonine) in surface and deep waters. An interesting branch of this topic can abandon the environmental field to look in the sociological one; indeed according to Pal et al. (2013) the most frequent residues in wastewater treatment plant are benzoylecgonine, ecgonine methyl ester, MDMA, methamphetamine, amphetamine and morphine.

At the last point of this list of emerging pollutants there is personal care products, of which the most of concern is UV filters, deriving from sunscreen products, that can have an estrogenic-similar effect on animals (Jurado et al. 2014).

This topic is studied for no more than 20 years, this is due the very low concentration at which these substances can have effects on environment and human health, so that an increase of technology was necessary for their detection and quantification (Kolpin, Furlong, and Meyer 2002).

Our focus in this work is on SS, but is interesting to notice that although most of PPCPs are found in SS, still some can go in superficial water discharge (Benotti et al. 2009).

Emerging pollutants are often transformed in other chemical species in natural environment or in wastewater treatment. These processes reduce the concentration of emerging pollutants but some of the products can have an higher toxic effect than the original compounds (Escher and Fenner 2011; Celiz, Tso, and Aga 2009).

2.2.3 Odour emissions

Is the last issue we will treat here about the use of SS in agriculture, as said before during the application of SS pathogens can be spread with the aerosol and gaseous emissions can occur (Wing et al. 2014; Zhu et al. 2016), though the odour itself is an important concern, especially for people living in rural areas. Odour composition is very complex, though is possible to roughly highlight two groups of compounds, the volatile inorganic compounds (VICs) and the volatile organic compounds (VOCs). VICs are mostly represented by ammonia and hydrogen sulphide, while VOCs refer mainly to sulphur organic compounds, alcohols, terpenes, carbonyls, aromatics, volatile fatty acids, alkanes, alkenes, ketones and esters (Fang et al. 2015; He et al. 2012).

2.2.4 Ammonia emissions

As said in the previous paragraph, during digestate spreading a significative amount of ammonia undergoes volatilization to air. This amount has been estimated of about 15% (Sommer and Hutchings 2001; Matsunaka et al. 2006; Terhoeven-Urselmans et al. 2009; Möller and Stinner 2009). It is of maximum importance to reduce these emissions, in fact ammonia molecules in the air reacts with different pollutant to form $PM_{2.5}$, which is particulate matter smaller than 2.5 μm and is known to cause health danger, especially lung cancer. (Stokstad 2014). Furthermore, gaseous ammonia in the atmosphere reacts with SO_3, forming sulfuric acid, causing therefore acid rains, which are well-known to be dangerous for the environment (Bandyopadhyay, Kumar, and Biswas 2017). Problem of lesser concern are water eutrophication and the effective N loss during fertilization, meaning a reduction of the fertilizing power of digestate. In the last years more and more attention focused on the application technics in order to reduce ammonia emissions, in particular trailing shoes reduce ammonia emissions compared with plate spreader, and injection reduces more than trailing shoes (Nicholson et al. 2018).

2.2.5 Nitrous Oxide emissions

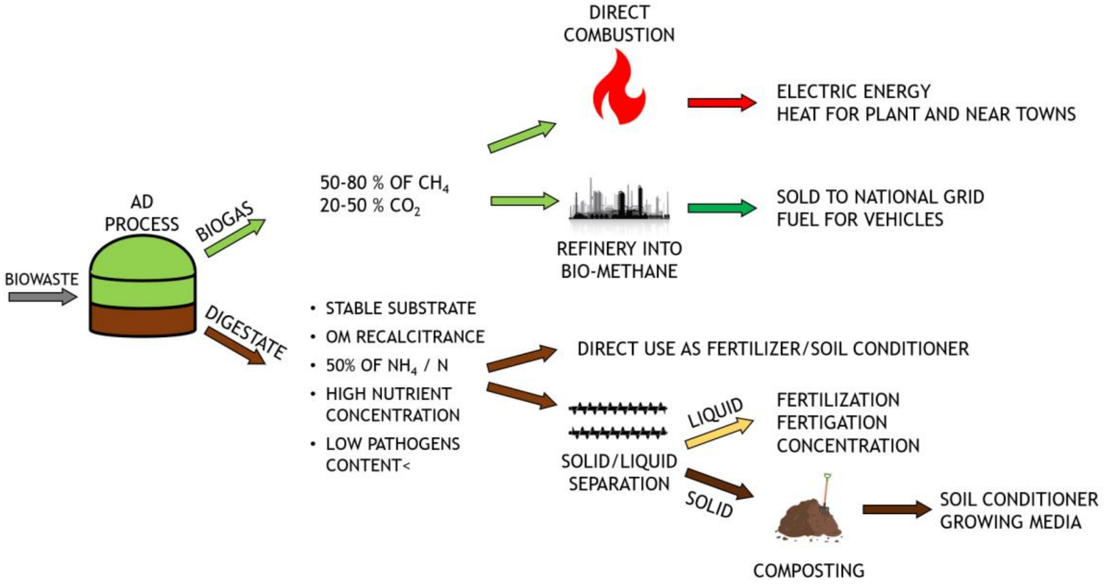
Nitrous Oxide (N_2O) is the greenhouse gas of major concern related with digestate spreading, its global warming potential is 310 times higher compared to carbon dioxide (P. Börjesson and Berglund 2007). Moreover, this gas has an ozone depletion effect in the stratosphere, increasing the importance of reducing its emissions. While, as said before, ammonia emissions are higher for digestate than for undigested feedstock, for nitrous oxides appears to be the opposite (Collins et al. 2011). For example, (P. Börjesson and Berglund 2007) reported for manure applying in field, 40g N_2O / ton $_{applied}$ and 25g N_2O / ton $_{applied}$ for respectively undigested and digested. Finally, as for ammonia, agricultural practices can contribute to cut down nitrous oxide emissions.

2.3 AD to treat SS and its positive effect for the use in agriculture.

AD is a biologic process, through hydrolysis, acidogenesis, acetogenesis and methanogenesis, of stabilization of an organic substrate, in one or more controlled reactors, in absence of oxygen, of the substrate biodegradable fraction. The main effect of the process is to stabilize the substrate while producing valuable biogas (Figure 1), which usually contains 50-80% of methane and the remaining part of carbon dioxide (Ahring 2003). Usually, biogas is used immediately by the plant to produce electric energy, sold to the national grid, and heat, exploited by the plant itself or transferred to the nearest towns. In the last years is putting more and more attention to the upgrade to biomethane (Adnan et al. 2019), a gas composed mainly by methane (>95%) and with no trace of H_2S, an acid that can seriously damage piping lines and cylinders whew biomethane is transported and/or conserved.

Since the seventies increasingly attention focused on the AD process to treat bio-wastes, especially animal manure and/or household wastes (Tani et al. 2006).

Figure 1. AD process, its two by products and their main uses



The increase in the development of this technology is not only due the interest in waste disposal, but also due the concerns on energy prices and shortage of those years, other than increasing attention to climate changes. Nowadays, we may reassume the main input bio-waste in the following groups:

- Treatment of wastes from agro-food and fermentation industries.

- Treatment of organic fraction of municipal solid waste
- Treatment of municipal SS
- Treatment of energy crops
- Treatment of livestock effluent
- Treatment of livestock effluent co-digested with energy crops
- Treatment of organic fraction of municipal solid wastes co-digested with livestock effluents.

Although the production of biogas is a positive consequence of the AD progress, in the last years research focused more on the stabilization, sanitation, increase of fertilizing and soil conditioning properties of the substrate.

2.3.1 Amending properties of digestate

A soil improver, amendment or conditioner is a substance which given to soil increase the physical, chemical, or biological quality of the latter, with different effects than apporting nutrients (e.g., liming for pH correction or apporting organic matter).

Due the great variance of substances that can be used as infeed, the recalcitrance of the final product can greatly vary (Provenzano et al. 2011). Nevertheless, AD process increases the stability and the recalcitrance of the infeed, independently by the substrate. Tambone et al. in (2009) and in (2013) studied different substrates during AD process using the CPMAS 13C NMR spectroscopy and highlighted a relative increasing of recalcitrance molecules (e.g. lignin, non-hydrolysable lipids, aromatics and phenols) thanks to the degradation of the more labile carbohydrates-like compounds. On this topic (Teglia, Tremier, and Martel 2011) had similar results.

2.3.2 Fertilizing properties of digestate

Similarly to what said before, also for nutrient the main factor that influence the digestate characterization is the composition of the infeed, especially for P and K, which are non-volatile macro-nutrient, the final content depends on the concentration of the input. This notwithstanding, during AD process a portion of organic matter is consumed, this lead to a concentration of nutrients in the substrate (Möller and Müller 2012; Möller, Schulz, and Müller 2010). The high content in nutrient (table 3 and 4), especially nitrogen in ammonia form, phosphorus and potassium allow an efficient use of digestate as fertilizer, at least similar to chemical fertilization (Ahmad and Jabeen 2009; Chantigny et al. 2008).

Moreover, organic nitrogen is partially mineralized so to increase the amount of ammonium, this is a positive effects thanks to the high availability of ammonium to plants (Riva et al. 2016) (Tambone and Adani 2017).

Table 3. Typical values for total N content, NH_4^+-N share on total N, and C : N ratio as well as a characterization of the biodegradability of theorganic material and mineral-fertilizer equivalents (MFE%) for several organic fertilizers. Modified by (Gutser et al. 2005).

Fertilizer	**Total N**	**Dry matter**	**NH_4-N : N**	**C:N**	**Short term MFE**	**OM biodegradability**
	Kg Mg^{-1}	%	%	ratio	%	
Legume coarse meal	40-60	95	0-5	10-13	35-45	High
Horn/feather/leather meal	130	95	0-5	3-4	50-70	High
Brewery/distillery residues	3	6	0-5	8-10	30-35	High
Meat/blood/bone meal	75-120	95	5-10	3-5	60-80	Very high
Green manure	10-35	100	0-10 (N-NO_3^-)	10-30	10-40	Low-medium
Biocompost	6	60	0-15	13-20	0-20	Low
Solid manure	6	25	5-20	12-15	10-20	Low
SS (high DM)	4-5	25	5-20	6-8	15-30	Medium
Dried poultry excrements	30	55	5-30 (uric acid)	5	60-70	High
SS low (DM)	1-2	5	30-40	3-5	45-55	Medium
Slurry (cattle)	4	7.5	40-60	8	35-45	Low
Digestate vegetal biomass	2-3	8	35-60	5-8	40-60	Low
Digestate co-digestion	3-15	5	45-70	2-5	50-70	Low
Slurry (poultry)	10	15	60-80	4	70-85	Medium
Urine	4	2	80-90	1-2	90-100	-

Table 4. Characteristics of liquid digestates from different origin. Modified by (Makadi, Tomòcsik, and Orosz 2012).

Ingestate	Digestion process	Total N	NH4-N	Total P	Total K	Source of data
Swine manure	Mesophilic	2.93 (g L-1)	2.23 (g L-1)	0.93 (g L-1)	1.37 (g L-1)	(Loria et al. 2007)
Liquid cattle manure	Mesophilic	4.27 (% DM)	52.9 (‰ N tot)	0.66 (%DM)	4.71 (% DM)	(Moller et al. 2008)
Co-digestion (Energy crops, cow manure slurry and agro-industrial wastes)	Thermophilic	105 (g kg-1 TS)	2.499 (g L-1)	10.92 (g kg-1 TS)	-	(Pognani et al. 2009)
Co-digestion (Energy crops, cow manure slurry, agro-industrial wastes and OFMSW)	Thermophilic	110 (g kg-1 TS)	2.427 (g L-1)	11.79 (g kg-1 TS)	-	(Pognani et al. 2009)
Co-digestion (Cow manure, plant residues and offal)	Mesophilic and Thermophilic	0.2013 (%m/m, fresh matter)	0.157 (%m/m, fresh matter)	274.5 (mg kg-1 fresh matter)	736.45 (mg kg-1 fresh matter)	(Makadi et al. 2008)
Co-digestion (Clover/grass or pea straw or cereal straw or silage maize and clover/grass silage -mean-)	Mesophilic	0.253 (%m/m, fresh matter)	0.176 (%m/m, fresh matter)	0.62 (% DM)	18.5	(Stinner, Möller, and Leithold 2008)

2.3.3 Sanitation of digestate

The AD process has a positive effect on SS greatly reducing pathogens and parasites in the infeed. According to Ottoson et al. (2008) and Sahlström, (2003) few features of the process can influence these effects, the most relevant are:

- Temperature (Thermophilic – 55°C- more than Mesophilic -35°C-)
- pH (more sanitation at higher pH levels)
- Ammonia concentration (the higher the more sanitation effect)
- Hydraulic retention time (more effective in longer time)

A study by Scaglia et al., (2014) studied lab scale sanitation of SS with and without a small addition of ammonia and at different temperatures. The results showed how at all temperature the sanitation was effective, though the addiction of ammonia didn't comport any noticeable advantage. Later on, another study (Valentina Orzi et al. 2015), studied sanitation in ten full scale plants, working mainly with livestock effluent, at mesophilic conditions, and concluded showing optimal reduction of pathogens, confirming what said before about pH and ammonia relation with sanitation capacity of the process.

3 THERMOPHILIC ANAEROBIC DIGESTION AS SUITABLE BIOPROCESS PRODUCING ORGANIC AND CHEMICAL RENEWABLE FERTILIZERS: A FULL-SCALE APPROACH.

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This work reports a full-scale study in which organic wastes were transformed by high-solid thermophilic anaerobic digestion (HSAD), into N fertilizers and organic fertilizers, i.e. digestate. The produced fertilizers were characterized over 42 months and their properties were discussed in comparisons with literature data. HSAD coupled with N stripping technology led to ammonium sulphate production having high N concentration (74 ± 2 g kg^{-1} *wet weight), neutral pH (6.8 ± 1.3) and low traces of other elements. Digestate showed both higher carbon (C) content (314 ± 30 g* kg^{-1} *on dry matter (DM) and biological stability than green composts, indicating good amendment properties. Digestate was also interesting for its N (77 ± 3.7 g* kg^{-1} *dry matter – DM) content, half of it in the ammonia form, and P content (28 ± 4.1 g* kg^{-1} *DM) that was 43% readily available as soluble P-orthophosphate. K content was low (6.5 ± 1.3 g* kg^{-1} *DM), indicating poor fertilizing ability of digestate for this element. All organic pollutants investigated were much lower than the limits required for agricultural use and levels of some of them were lower than the content revealed for other organic matrices such as agricultural and energy crop digestates and compost. Emerging pollutants (i.e., pharmaceuticals) were tested as markers and they were found to be below the detection limit (<0.01 mg* kg^{-1} *DM) indicating very low content. The results obtained showed that HSAD coupled with N stripping allowed transforming sewage sludge into fertilizers and soil improvers exploitable in agriculture.*

3.1 Introduction

With the increase of the global population, the production of organic wastes is likewise increasing. Sewage sludges (SS), in particular, which are the semi-solid residual materials left over after municipal and industrial wastewater treatment processes (Raheem et al. 2018), have been produced at the rate of 181 and 1,850 Gg dry matter (DM) $year^{-1}$, i.e. 21 ± 4 kg DM $person^{-1}$ $year^{-1}$ in Europe's top 13 producing countries for the period 2010 -2017 (Di Capua et al. 2020), i.e. 8 million m^3 $year^{-1}$

(https://ec.europa.eu/environment/waste/sludge/), posing environmental and economic problems for their management.

Population expansion not only causes an increase of waste production but also an escalation of food demand and consequently of fertilizer requirements. It has been estimated that the world demand for fertilizer will increase by 7.9% in 5 years (from 2017 to 2022) according to Food and Agriculture Organization (FAO) (FAO 2019). Producing fertilizers requires energy and/or fossil-origin raw material. The nitrogen production industry as a whole uses more than 1% of the world's total energy and emits more than 300 million Mg of CO_2 per year (Cherkasov, Ibhadon, and Fitzpatrick 2015). Most of the phosphate rock mines sites are concentrated in a few countries (Morocco, China, USA, Jordan and South Africa) which can cause supply issues especially for Europe. Moreover, the quality of these sources is decreasing, causing a higher content of pollutants (e.g. for P-based fertilizers) (Günther, Grunert, and Müller 2018). P and N are also dangerous pollutants in both deep and fresh waters and especially in the latter, in which they can cause eutrophication and consequent death zones (Conley et al. 2009) if misused.

Soil contains the largest pool of active C, about 1,500 – 2,000 Pg C (considering one-meter-depth) (Janzen 2004). This fraction is important because it directly affects soil fertility, but also the world C stocking capacity (Ayuke et al. 2011; Lal 2009; P. Smith et al. 2018). Furthermore, C content in soil is decreasing because of both land use changes and intensive agriculture depleting soil C. Therefore, there is a need to recover nutrients but also organic matter from organic matrices contributing to soil C balance.

Although there are different pathways for sewage sludge disposal, i.e. landfill, incineration, land reclamation, composting, pyrolysis, gasification and co-incineration, the use of sewage sludge in agriculture represents the most common practice, taking almost 40% of sewage sludge in EU (European Commission 2009). This is because sewage sludge contains both organic matter (OM) and nutrients, it can act as both an organic amendment and as fertilizer (Di Capua et al. 2020). Sewage sludge's fertilizer properties can be enhanced by SS pre-treatment such as anaerobic digestion (AD), which also brings other benefits (Di Capua et al. 2020). The AD process produces a substrate, called digestate, that has different characteristics from the feedstock of the digester. The AD, by promoting organic matter (OM) degradation of easily decomposable organic components, leads to relative high biological stability of the digestate, increasing its amendment properties (Tambone et al. 2010) and nullifying phytoxicity (i.e. high maturity); moreover, by promoting OM mineralization it increases nutrient availability for plants (Tambone et al. 2010), reducing, also, potential odour production (Valentina Orzi et al. 2015). The OM stabilization, at a right combination of temperature, microbial competition and ammonia production, reduces or annuls pathogen contents above all under thermophilic conditions (Scaglia et al. 2014; Qi et al. 2018; Valentina Orzi et al. 2015) guaranteeing hygienic products and avoiding pathogens ending up in the soil (Bonetta et al. 2014). Therefore, digestate can be proposed as both organic amendment and fertilizer, as it can provide both OM to replace soil OM losses (Tambone et al. 2010) and nutrients, replacing mineral synthetic fertilizers (Riva et al. 2016). However, in many cases there are dangers that nutrients can overload crop requirements or digestate may not prove to be directly suitable for fertilizer use (e.g. nutrient imbalance in the product compared to crop requirements, limits imposed by law for a specific component - e.g. Nitrate directive in EU - and topdressing fertilization). Therefore, nutrient recovery from digestate may be necessary to produce new biobased fertilizers, which can be easily used and/or transported. AD has been reported to be a useful biotechnology for facilitating the subsequent nutrient recovery to produce fertilizers, because of nutrient speciation (e.g. organic N to mineral N(Ledda et al. 2013; Amekan 2020)). One of the most studied and exploited digestate post-treatments for recovering a nutrient is ammonia stripping (Ivona Sigurnjak et al. 2019). Ammonium is often the most represented mineral N form in digestate and at high pH and/or high temperatures, ammonia can volatilize from digestate and extracted by exploiting a gas (air, biogas or N_2). The ammonia rich gas can be scrubbed with an acid substance (most often sulphuric acid or nitric acid), recovering N and producing an N-salt solution having high N content (N of 6-8 % weight/weight). This technique can also enhance biogas production, avoiding ammonia inhibition during the AD process (Zhang and Jahng, 2010; Abouelenien et al., 2010; Walker et al., 2011; Bousek et al., 2016).

This work aims to describe amendment and fertilizer properties of bio-fertilizers obtained from a full-scale thermophilic high-solid thermophilic anaerobic digestion (THSAD plant) designed to produce fertilizers, i.e. digestate and ammonium sulphate, starting from organic wastes represented mainly by sewage sludge. The fertilizers obtained were analysed throughout 2017-2020, comparing analytical data with those of other bio-fertilizers (agricultural digestates and composts) and with the legal limits in force for agricultural use.

Potentially, both inorganic and organic pollutants can affect the safe use of digestate in agriculture, especially because the AD process degrading the OM can cause an increase in the relative concentration of pollutants (Al Seadi et al. 2013; Kupper et al. 2014). On the other hand, literature has reported the ability of AD to degrade organic pollutants (Verlicchi and Zambello 2015). As consequence of that, in addition to agronomic properties, inorganic and organic pollutants have been detected, introducing, also, some emerging organic pollutants as markers.

This paper represents a rare case in which a detailed description of chemical and biological properties of biofertilizer obtained at full scale plant monitored for long time (years) have been done, becoming data collected very useful to give a detailed description of the biofertilizer properties. No many data exist with reference to ammonium sulfate charateristics and their variability with time. Again there is no so detailed description of digestate that included the use of both common and less common analytical approaches (e.g. biological stability, ^{13}C-and ^{31}P- NMR) helping in tracing amendment and fertilizers properties. In addition becasue biofertilizers were produced treating organic wastes, environmental issues have been considered making a great analytical effort in the investigation of common pollutants, i.e. heavy metals, but also organic pollutants, including emerging pollutant, and pathogens. All these allowed giving a full picture of biofertilizers produced by anaerobis digestion at full scale to be used in agricultrue in substitution of conventional fertilizers.

3.2 Materials and methods

3.2.1 Plant description

The THSAD plant, located in Lombardy Region (northern Italy), transform different types of wastes, mainly sewage sludge, into a useful soil improver (i.e. digestate) and N-based mineral fertilizer (i.e. ammonium sulphate), i.e. renewable fertilizers, and the electrical and thermal energy needs of the plant.

The data reported in the present paper refer to a period covering 42 months (2017, 2018, 2019 and the first 6 months of 2020).

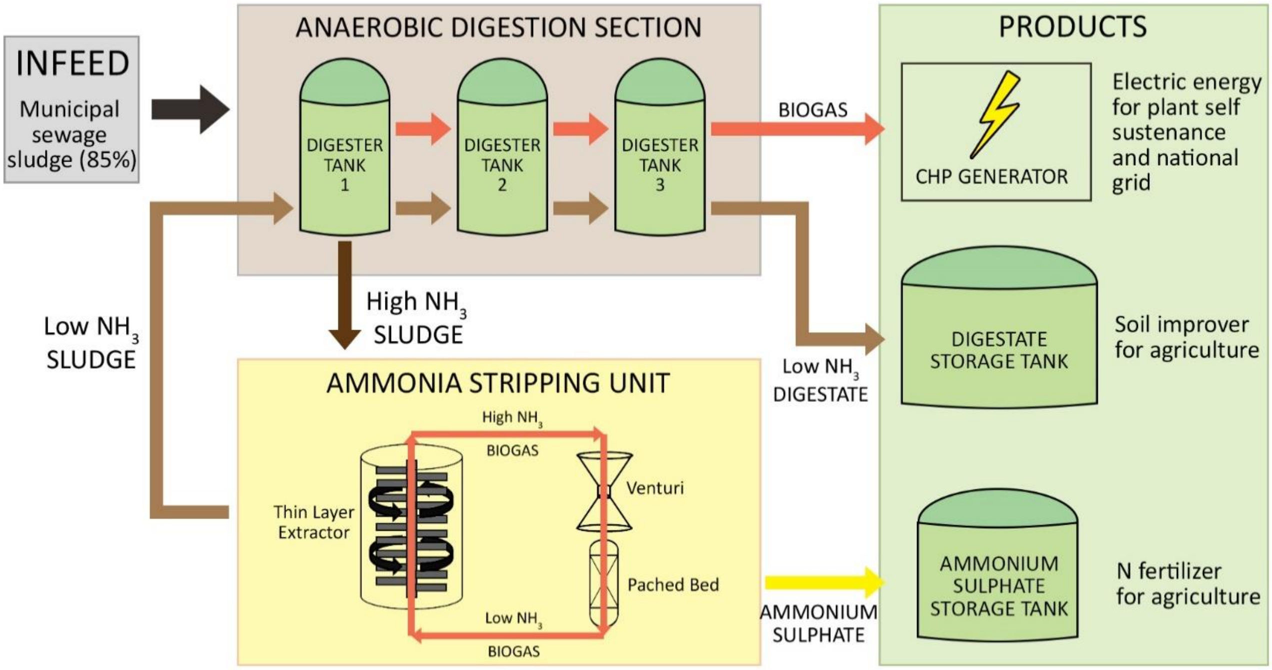
3.2.1.1 Anaerobic Digestion section.

High-solid anaerobic digestion (Di Capua et al. 2020) took place in three reactors in series of 4,500 m^3 each, made of carbon steel (Giordano et al. 2019) and it was performed under thermophilic conditions (55 °C) using hot water and steam as thermal energy carriers. The biogas produced by the plant is fed to a combined heat and power (CHP) unit in order to recover heat and electrical energy, primarily consumed on-site (about 30%), while the excess is sent to the national grid. During the cold season, an auxiliary boiler fed with methane (starting from month 38 of the observation periods with biogas) can be used if necessary. Reactor tanks have no mechanical mobile part inside, and the feedstock/digestate mixing is guaranteed by continuous circulation through external pumps. Biogas was collected in the headspace of each reactor equipped with a double-membrane gasometer dome maintained at a constant pressure which covers the tanks (Figure 1).

Feedstock was represented by 85% of municipal sewage sludge, 5% by sewage sludge from the agro-food industry and the remaining fraction by the liquid fraction of food waste from separate collection. The feedstock received was first gathered in basins and then transferred into a mixing unit where it was heated (by direct steam injection, to reach 55°C) and homogenized with digestate coming from the third digester and water to obtain a solid content of about 140 g kg^{-1} wet weight (ww) before being fed to the first digester. About 0.3 Mg of water was added per Mg of feed (these data represent both water vapour injected directly into digestate to assure thermophilic conditions and water used to lower DM content). The weekly organic loading rate (OLR) during the whole period was in the range 0.7 – 3 kg VS m^{-3} d^{-1} with a mean value of 2.0 ± 0.5 kg VS m^{-3} d^{-1}. This means an average Hydraulic Retention time (HRT) of about 50 days. The weekly specific methane production was of about 200 ± 29 CH_4 $kg^{-1}VS_{in}$.

During the 42 months of observation, the AD plant treated about 240,000 Mg of waste producing about 320,000 Mg of digestate and about 13,500 Mg of biogas.

Figure 1. Scheme of the digestion plant and ammonia stripping system. On the left is represented the infeed of the plant. On the right are shown the three main outputs: energy, digested sludge and ammonium sulphate.



3.2.1.2 Ammonia stripping unit.

The ammonia stripping unit is used to control the total ammonia nitrogen (TAN) level in the digestate to avoid AD inhibition due to TAN accumulation and toxic circumstances for the micro-organisms (Capson-Tojo et al. 2020). The system withdraws sludge from the digester tank to a thin film evaporator (TFE), where ammonia is stripped from digestate by biogas collected from the headspace of the three digesters. Then, biogas is fed to an absorption unit constituted by a Venturi scrubber followed by an upstream packed bed column where ammonia reacts with sulphuric acid producing an ammonium sulphate solution (Costamagna et al. 2020). The resulting low ammonia digestate is pumped back to the first reactor.

The stripping and adsorption unit was modified in 2020 by increasing the liquid/gas, using air as stripping gas, and finally using three Venturi scrubbers in the absorption section.

During the first 38 months (30 months of effective work), the stripping unit removed about 6 % of the input N waste, corresponding about to 12% of TAN. In the following 4 months, by tuning the stripping and ammonia trap units, about 12% of the input N-waste was removed, i.e. 22-27% of TAN. During the 42 months of observation, about 2,180 Mg of ammonium sulphate solution (7.2 ± 0.2 % N-NH_4) was produced.

3.2.2 Renewable fertilizers characterization

3.2.2.1 Chemical Analyses Method.

Ingestate, digestate and ammonium sulphate, if not better specified in tables, were sampled during a period of 42 months (from January 2017 to June 2020) and characterized from a chemical point of view according, also, (digestate) to Lombardy Region regulation (Regione Lombardia 2019) for agricultural use of these products.

The following parameters were determined: pH (EPA 9045D) (EPA, 2004), dry matter at 105°C (DM 105°C), dry matter at 600°C (DM 600°C) and Total Organic Carbon (TOC) (APHA 1998), total nitrogen (TKN) (EN 13652) (EN 2001). Ammonia-N (NH_4-N) was determined by ISO 5664 method (ISO 1984).

In addition, nutrients (P, K, Ca, Mg, Fe, Mo, Mn), heavy metals contents (Cd, Cr tot, Ni, Pb, Cu, Zn), and micropollutants/element (Hg, As, Al, Co, Se, Na) were determined by inductively coupled plasma mass spectrometry (ICP-MS, Varian Inc., Fort Collins, CO, USA) according to DIN EN ISO 11885 (ISO, 2009) (Ca, Mn, Mg, Fe, Mo, Al, Co, Na), and according to UNI-EN 16174 (UNI-EN, 2012) for all the other elements.

Organic micropollutants were detected as follows: C10-C40 hydrocarbons by UNI EN 14039 (UNI, 2005) method, halogenated organic compounds (AOX) by Gas Chromatography (GC) approach (UNI EN ISO 22155:20161) (UNI, 2016) and EPA 8270E 20181 (EPA, 2014) + EPA 3550C 2007) (EPA, 2007a). PCDD/Fs were measured using UNI 11199 (UNI, 2007) method, PCBs through UNI EN 16167 (UNI, 2012) and UNI EN 16167 (UNI, 2019), and DEHP through EPA 3550C (EPA, 2007a) + EPA 8270E (EPA, 2018) methods.

Emerging organic pollutants (pharmaceuticals), i.e. Ciproflaxacin, Sulfamethoxazole, Fenofibrat, Gemfibrozil, Carbamazepine, Metoprolol, Diclofenac, Ethinylestradiol and Estradiol were detected by HPLC-MS following EPA 3550C (EPA, 2007a) and EPA 8321B 2007 methods (EPA, 2007b).

Faecal coliform and *Salmonella* were determined as reported in CNR IRSA 3 (CNR IRSA, 1983) (Fecal coliform) and ISTISAN 14/18 (ISTISAN, 2018) + APAT CNR IRSA 7080 (*Salmonella*) (APAT, 2003).

3.2.2.2 Biological stability determination

Biological stability of the digestates was evaluated by measuring the oxygen uptake by microorganisms to degrade organic matter and by measuring the residual Biochemical Methane Potential (BMP).

In particular, the Substrate Oxygen Uptake Rate test (SOUR test) was used (V. Orzi et al. 2010). In brief, 1 g of wet matter sample was placed in a flask with 500 ml of deionized water, and added of 12 mL of phosphate buffer solution (KH_2PO_4 0.062 mol L^{-1}, K_2HPO_4 0.125 mol L^{-1}, $Na_2HPO_4 \cdot 7H_2O$ 0.125 mol L^{-1}), and 5 mL of nutritive solution ($CaCl_2$ 0.25 mol L^{-1}, $FeCl_3$ 0.9 mmol L^{-1}, and $MgSO_4$ 0.09 mol L^{-1}), prepared according to the standard BOD test procedures (V. Orzi et al. 2010). The oxygen uptake rate was reported as the result of the oxygen demand occurring in a 20-h tests (OD_{20}, mg O_2 g DM^{-1}). All SOUR-OD_{20} tests were performed in triplicate.

Biological stability was determined, also, by a long-term degradation test (60 d) using the anaerobic potential biogas production test (BMP test), performed according to Schievano et al. (2008). In brief, in a 100 mL serum bottle, 0.62 g of dried sample (Ø < 1 mm) was added to 37.5 mL of inoculum and 22 mL of deionized water. The batch tests were carried out with 60 mL samples (about 35 g kg^{-1} TS) and 40 mL of headspace. The fresh feedstock and inoculum percentages of TS were respectively 35% and 65%. Control blanks were prepared using 60 mL of inoculum. All batches were sealed with Teflon hermetic caps, flushed with an N2 atmosphere, and incubated at 37 ± 1°C, until no further biogas production was detected (normally around 60 d).

3.2.2.3 Cross-Polarization Magic Angle Spinning Carbon-13 Nuclear Magnetic Resonance.

Three samples of digestate were taken directly from the plant at three different times over the years 2018, 2019 and 2020. Before analyses, the samples were dried at 105°C and milled (diameter ≤0.2 mm).

The solid-state Cross-Polarization Magic Angle Spinning Carbon-13 Nuclear Magnetic Resonance (CPMAS ^{13}C NMR) spectra of the digestate samples were acquired at 10 kHz on a Bruker AMX 600 spectrometer (Bruker BioSpin GmbH, Rheinstetten) using a 4-mm CP-MAS probe. The pulse repetition rate was set at 0.5 s, the contact time at 1 ms, and the number of scans was 3200. The chemical shift scale of CPMAS^{13}C NMR spectra were referred to tetramethylsilane (δ = 0 ppm). Spectra were elaborated using TOPSPIN 1.3 software (Bruker BioSpin GmbH, Rheinstetten, Germany).

3.2.2.4 P fractionation and its characterization by ^{31}P-NMR.

Sequential extraction of phosphorus

The different phosphorus (P) species characterizing the samples of digestate, collected at three different times during the year, were carried out by the sequential extraction suggested by Turner and Leytem (2004). This procedure was able to assess the different P fractions by using $NaHCO_3$, NaOH-EDTA, and HCl solvents. In this way it was possible to extract P considered readily soluble ($NaHCO_3$) and also, poorly soluble P fractions, such as

P associated with amorphous iron/aluminium hydroxides and organic matter, and Ca^{2+}, Mg^{2+} and NH_4^+ - phosphates, which were extracted by NaOH-EDTA and HCl.

In brief, the first extraction was performed by using a 0.5 mol L^{-1} $NaHCO_3$ solution (1:60 dry biomass/solution ratio) for 4h. After centrifugation at 10,000 rpm for 30 min and then filtration through a 0.45 μm cellulose-nitrate membrane, the residual fraction was extracted for 16 h with 1 mol L^{-1} HCl solution (1:60 dry biomass/solution ratio) or with a 0.5 mol L^{-1} NaOH plus 50 mmol L^{-1} EDTA (1:20 dry biomass/solution ratio).

An aliquot of each supernatant was frozen at -80°C and lyophilized for NMR spectroscopy characterization. Another aliquot was mineralized and subsequently the P content was detected by ICP-MS analysis.

^{31}P Nuclear Magnetic Resonance Spectroscopy

^{31}P Nuclear Magnetic Resonance Spectroscopy was performed by using 300 mg of lyophilized sample dissolved in 3 mL of a solution made by 1 mol L^{-1} NaOH and 0.1 mol L^{-1} EDTA (H_2O/D_2O 9:1) (pH > 13). The samples were vortexed and centrifuged at 5,000 rpm for 4 minutes at 20 °C. The supernatant was placed in a 10 mm NMR tube and the NMR spectra recorded on a Bruker AV600 spectrometer operating at a frequency of 600.13 MHz and 242.94 MHz for ^{1}H and ^{31}P nuclei, respectively. The ^{31}P spectra were acquired with a 10 mm BB probe at 20 °C and were referenced to external 85% H_3PO_4. P_{90°=15 μs, D1=2 s, SW=7763, 975 Hz, NS ranging from 4,000 to 27,000 depending on the samples; chemical shifts (δ) were measured in ppm. The NMR spectra were transformed with a line broadening (LB=-2 Hz and GB= 0.02) by TOPSPIN software and the baseline was corrected using a polynomial function. All NMR signals were integrated and the sum of the integrals was assigned the value of the total phosphorus concentration (mg kg^{-1}) obtained by the ICP-MS analysis. The complete assignment of phosphorus species was performed following the literature relative to the composition of the phosphorus species in soil, manure and slurry (Turner and Leytem 2004).

3.3 Results and discussion

3.3.1 Renewable fertilizers characterization

3.3.1.1 Ammonium sulphate solution characterization

A full characterization of the ammonium sulphate solution was made on samples collected during the trial period; every sample was tested in triplicate. This characterization was very important because little information is available in the literature on the complete chemical characteristics of ammonium sulphate solutions produced by ammonia stripping from digestate or effluents. Ammonium sulphate solution (Table 1) appeared as a yellowish/red liquid, with a density of about 1,200 g L^{-1}. Ammonium sulphate solution (ASS) recovered had a NH_4-N average concentration of 7.2 ± 0.1%, electric conductivity of 119 ± 27 mS cm^{-1} (1:2.5 v/v digestate:water at 25°C); the dry matter (DM) content was of 35.5 ± 0.37% and so, higher than that reported in other works, which however reported data with a strong variability. pH of the solution was almost neutral, i.e. 6.8 ± 1.3, and so higher than values found in the literature (average of 3.7 ± 2.2). pH close to neutrality represent an advantage for agricultural purposes, as it allows an easier use as fertilizer, reducing corrosion of machinery and risk of damage for crop plants when it is used as a topdressing; in addition, soil acidification is reduced. As expected, almost all nitrogen (Total N), which had a concentration of 74 ± 2 g kg^{-1} ww was found in ammonia form (i.e. 97%); this value was higher than those found in literature that was, as average, of Total N of 41 ± 32 g kg^{-1} ww (Table 1).

Organic carbon was found only in traces i.e. TOC of <0.1 g kg^{-1}, according to data reported in the literature (Table 1). Other macronutrients, namely P and K, as expected, had no a significant concentration in ammonium sulphate solution, i.e. 11.7 ± 4.7 mg kg^{-1} and 14.2 ± 7.9 mg kg^{-1}, respectively, showing the high purity of the solution produced. Higher concentrations of the elements were found for Ca (68 ± 18 mg kg^{-1} ww), Mg (10 ± 4 mg kg^{-1} ww) and Na (22.4 ± 7.7 mg kg^{-1} ww) with all the other elements being lower than 10 mg kg^{-}

^{1} ww. Micronutrients and inorganic micropollutants were found only in traces. Currently there are no other data in the literature on the content of micronutrients or pollutants in ammonium sulphate obtained from biofertilizers from anaerobic digestion, so it was not possible to make any comparisons.

Table 1. Main characteristics (mean ± SD; n=17) of ammonium sulphate - $(NH_4)_2SO_4$ - produced by the plant (all concentrations are expressed on wet basis) - data referred to the period 01/01/2017- 30/06/2020.

	Unit	Value	Literature Comparison
pH	pH	6.8 ± 1.3	3.7 ± 2.2 [a, b, c]
EC	mS cm^{-1}	119 ± 27 (1:2.5 v/v 25 °C)	192 ± 61 [a, b, c] (dilution not reported)
Dry Matter 105°C	% of ww	35.5 ± 0.4	20 ± 10 [a, b, c, d]
Total Organic Carbon	g kg^{-1} ww	< 0.1	0.4 [a]
Total N	g kg^{-1} ww	74 ± 2	41 ± 32 [a, b, c, d]
N-NH$_4$	g kg^{-1} ww	71.7 ± 1.9	41 ± 32 [a, b, c, d]
P tot	mg kg^{-1} ww	11.7 ± 4.7	16 ± 28 [a, b, d]
K tot	mg kg^{-1} ww	14.2 ± 7.9	59 ± 93 [a, b, d]
S tot	g kg^{-1} ww	88 ± 2	39 ± 12 [a, b, c]
Ca tot	mg kg^{-1} ww	68 ± 18	
Mg tot	mg kg^{-1} ww	10 ± 4	
Fe tot	mg kg^{-1} ww	< 11	
Mo tot	mg kg^{-1} ww	0.1 ± 0.1	
Cu tot	mg kg^{-1} ww	< 6	0.3[b]
Zn tot	mg kg^{-1} ww	2.5 ± 2.4[e]	2.9[b]
Mn tot	mg kg^{-1} ww	0.7 ± 0.5[e]	
Al tot	mg kg^{-1} ww	3.6 ± 5.5	
Co tot	mg kg^{-1} ww	0.01 ± 0	
Se tot	mg kg^{-1} ww	0.04 ± 0	

Na tot	mg kg^{-1} ww	22.4 ± 7.7
Cr tot	mg kg^{-1} ww	< 1
Pb tot	mg kg^{-1} ww	< 1
As tot	mg kg^{-1} ww	< 1
Cd tot	mg kg^{-1} ww	< 0.25
Ni tot	mg kg^{-1} ww	< 1
Hg tot	mg kg^{-1} ww	< 0.25
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.

3.3.2 Digestate characterization vs. amendment and fertilizers properties.

Digestate produced represented a fertilizer that contained organic matter (amendment properties) and nutrients (fertilizers properties). Therefore, the next sections are devoted to discussing digestate data in its amendment and fertilizing properties in comparison with other organic matrices, i.e. agriculture order to establish both co-digested (agricultural digestate) and energy crops digestates (Tambone et. al., 2017; Scaglia et al., 2018 and organic amendments, i.e. green composts (Scaglia et al. 2018) (Table 2).

Table 2. 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.

Parameter	**Unit**	**Infeed[a] (this work)**	**Digestate[b] (this work)**	**Lombardy Law N. 6665/2019 - Legal limits[c]**	**Agricultural digestate[d]**	**Energy Crop digestate[e]**	**Green Compost 1[e]**	**Green Compost 2[e]**
pH		7.3 ± 2.5	8.5 ± 0.3	5.5 < pH < 11		8.4 ± 0.1	8.8 ± 0.2	8.9 ± 0.3
Dry Matter 105°C	g kg^{-1} ww[f]	191 ± 45	103 ± 3.7		61.1 ± 12.5			
Dry Matter 600°C	g kg^{-1} ww	60 ± 48	40.4 ± 2.5					
Total Organic Carbon	g kg^{-1} DM[f]	351 ± 78	314 ± 30	> 200		432 ± 14	292 ± 30	210 ± 10
TKN	g kg^{-1} DM	54 ± 20	77 ± 3.7	> 15	80.6 ± 13.3	70.3 ± 0.8	15.2 ± 0.18	15.2 ± 0.6
$N\text{-}NH_4$	g kg^{-1} DM	n.d.[g]	35.9 ± 2.4		48.9 ± 26.7	44.1 ± 3.8	0.12 ± 0.01	0.5 ± 0.0
$N\text{-}NH_4$/TKN	%	n.d.	46.6		60.66	62.27	0.7	3.2
OD_{20}[g]	mg O_2 g^{-1} DM	n.d.	22.6 ± 6.1			66.8 ± 1	15.6 ± 0.3	10.3 ±1.1
BMP[h]	L_{biogas} kg^{-1} DM	n.d.	57 ± 23			229 ± 31	144 ± 3.8	201 ± 20
P	g kg^{-1} DM	19±11	28 ± 4.1	> 4	15 ± 5	13.8 ± 5	3 ± 0	20.75 ± 0.12

K	g kg^{-1} DM	n.d.	6.5 ± 1.3			14.8 ± 3	9.2 ± 0.1	12.01 ± 0.09
Ca	g kg^{-1} DM	n.d	43 ± 7			9.2 ± 0.1	31.47 ± 0.17	30.9 ± 0.2
Mg	g kg^{-1} DM	n.d	5.2 ± 0.6			2.8 ± 4	8.57 ± 165	7.5 ± 17.1
Fe	g kg^{-1} DM	n.d	26.2 ± 6.4			3.4 ± 0.0	10.6 ± 0.1	13.2 ± 0.1
Mo	mg kg^{-1} DM	n.d	10 ± 1					
Cu	mg kg^{-1} DM	277 ± 142	408 ± 60	≤ 1,000	71.1 ± 30.6	83.3 ± 1.1	53.5 ± 1.6	53.5 ± 0.86
Zn	mg kg^{-1} DM	673 ± 413	1,020 ± 120	≤ 2,500	353 ± 204	393 ± 4.4	151 ± 3	159 ± 0.1
Mn	mg kg^{-1} DM	n.d	444 ± 35					
Al	g kg^{-1} DM	n.d	25.8 ± 4.5					
Co	mg kg^{-1} DM	n.d	6.6 ± 2.3					
Se	mg kg^{-1} DM	4[l]	3.7 ± 2.1	≤ 10				
Na	g kg^{-1} DM	n.d	1.9 ± 0.4			11.41 ± 0.034	0.807 ± 0.017	0.571 ± 0.001
Cr	mg kg^{-1} DM	54.2 ± 55.6	95 ± 22	< 200	8.56 ± 1.93	17.24 ± 0.4	88.8 ± 0.9	37.78 ± 0.8
Pb	mg kg^{-1} DM	45 ± 44	64 ± 11	≤ 750	1.97 ± 0.91	2.99 ± 0.04	24 ± 0.2	51.2 ± 0.08
Ni	mg kg^{-1} DM	36.8 ± 36.2	61 ± 13	≤ 300	10.3 ± 3.36	9.55 ± 0.47	41.8 ± 1	26.11 ± 2.2
As	mg kg^{-1} DM	6.3 ± 4.7	9.0 ± 2.2	< 20		1.05 ± 0.02	0.51 ±0.06	0.57 ± 0.15

Cd	mg kg^{-1} DM	0.6 ± 1.1[i]	1 ± 0.5[i]	≤ 20	0.39 ± 0.17	0.37 ± 0.05	0.17 ± 0.03	0.34 ± 0.01
Hg	mg kg^{-1} DM	0.3 ± 0.7[i]	0.1 ± 0.3[i]	≤ 10		0.24 ± 0.1	0.75 ± 0.02	0.22 ± 0.01
PAH	mg kg^{-1} DM	0.2 ± 0.5[i]	0.5 ± 0.5[i]	∑< 6		1.08	0.04	< 0.83
PCB	mg kg^{-1} DM	0.04 ± 0.51[i]	< 0.1	∑< 0.8		0.12	0.008	0.03
PCDD/F+PCB-DL	ng TEQ kg^{-1} DM	2.3 ± 4.4[i]	10.6 ± 2.9[i]	∑≤ 25		0.87	1.02	1.01
DEHP	mg kg^{-1} DM	2.8 ± 7.0[i]	5.7 ± 5.3[i]	< 100		< 1.54	< 0.14	0.15
Hydrocarbon C10-C40	mg kg^{-1} ww mg kg^{-1} DM	807 ± 1,093[i]	284 ± 251[i] (2,757)	≤ 1,000				
AOX	mg kg^{-1} DM	0.4 ± 3.3[i]	< 0.6	∑ < 500		< 0.46	2.75	0.04
Ciproflaxacin	mg kg^{-1} DM		< 0.01[m]					
Sulfamethoxazole	mg kg^{-1} DM		< 0.01[m]					
Fenofibrat	mg kg^{-1} DM		< 0.01[m]					
Gemfibrozil	mg kg^{-1} DM		< 0.01[m]					
Carbamazepine	mg kg^{-1} DM		< 0.01[m]					
Metoprolol	mg kg^{-1} DM		< 0.01[m]					
Diclofenac	mg kg^{-1} DM		< 0.01[m]					
Ethinylestradiol	mg kg^{-1} DM		< 0.01[m]					

Estradiol	mg kg^{-1} DM		< 0.01[m]					
Salmonella	MPN g^{-1} DM	47[n]	Absent	< 100		Absent	Absent	Absent
Faecal coliform	MPN g^{-1} DM	110,000[l]	< 1,000	< 10,000		Absent	Absent	Absent

[a]Calculated as average of input material: *n* = 1,021; PHA: n = 973; PCB: *n* = 1,000; PCDD/F+PCB-DL: n = 370; DEHP: *n* = 704; Hydrocarbon C10-C40: *n* = 297; AOX: *n* = 717

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

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

[d]Tambone, et al. (2017) average of n=13 mix of livestock effluent and energy crops.

[e]Scaglia et al., (2018).

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

[g]n.d.: not determined.

[g]OD$_{20}$: Oxygen Demand after 20h

[h]BMP: potential biogas production.

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

[l]Data from this work.

[m]Analysis performed on 2020; *n*=4.

[n]Mean calculated considering 80th percentile because other data were below detection limits.

3.3.3 Organic matter content vs. amendment properties.

It is widely recognized that conservation of soil organic carbon (SOC) is the result of the combination of three different processes: biochemical, physical and chemical organic matter (OM)-protection (Castellano et al. 2015). Biochemical protection is related to the molecular and chemical recalcitrance of organic substrates to prevent degradation (Papa et al. 2014). Rapid degradation contributes to faster OM turnover (months, years); non-protected OM assumes agronomic relevance, i.e. amendment properties. On the other hand, physical and chemical protection are related to the occlusion of OM by soil microaggregates' surface interaction with soil minerals (Marschner, Winkler, and Jödemann 2005; Bachmann et al. 2008). These latter mechanisms of organic C protection are reported to contribute to long term OM turnover (centuries, millennia), assuming less agronomic importance and its amount is limited in soil and finite (Chung et al. 2010; Castellano et al. 2015).

As discussed above, the ability of an organic matrix to contribute to soil fertility acting as an organic amendment depends on the amount of organic matter (TOC) (quantitative aspect) and its degradability, i.e. biological stability (qualitative aspects).

TOC content of the digestate from sewage sludge produced in this work (314 ± 30 g kg^{-1} DM) (Table 2), was lower than that reported in the literature for digestates obtained from energy crops (432 ± 14 g kg^{-1} DM) (Table 2), but higher than those reported for green compost (292 ± 30 and 210 ± 10 g kg^{-1} DM). These figures suggest good amendment properties for digestate. Amendment properties also depend on TOC biological stability, i.e. TOC degradability (biochemical OM protection). Therefore, digestate studied was characterized also for biological stability and the results obtained (Table 2) were compared with those for agricultural digestates and above all for green composts, since the latter are recognized as very good organic amendments (Adugna 2016). Specific Oxygen Uptake Rate (OD_{20}) and residual biogas potential (BMP) are two interesting techniques to estimate OM stability of biomasses, as previously recognized (Schievano, D'Imporzano, and Adani 2009).

OD_{20} values for digestate studied, i.e. 22.6 ± 6.1 mg O_2 g^{-1} DM, was lower than values reported for energy crop digestate, i.e. 66.8 ± 1 mg O_2 g^{-1} DM, and only slightly higher than those of composts (15.6 ± 0.3 and 10.3 ±1.1 mg O_2 g^{-1} DM) (Table 2), suggesting good biological stability of TOC. BMP gave different figures as data registered for digestate, i.e. 57 ± 23 L_{biogas} kg^{-1} DM was lower than those for energy crop digestate, i.e. 229 ± 31 L_{biogas} kg^{-1} DM, and for green compost, i.e. 144 ± 3.8 and 201 ± 20 L_{biogas} kg^{-1} DM. Differences obtained were due to dissimilarity in methods used with particular reference to length of time of the measurements. OD_{20} was performed for a short time (20h) so that above all only easily degradable OM contributed to the whole oxygen consumption. Because digestate contained volatile fatty acids (VFAs) coming from anaerobic digestion, short time measurement gave an underestimation of the biological stability (higher oxygen consumption to degrade VFAs). On the other hand, BMP took place for a longer time, i.e. 60 d, so that contribution to biogas (anaerobic biodegradation of organic matter) was due to not only due to easily degradable organic matter but also to intermediate types of degradable organic matter such as lignocellulosic material.

Therefore, based on both TOC content and biological stability data it can be concluded that thermophilic digestate is a valuable product to contribute to the maintenance of organic matter content of the soil (Scaglia et al. 2018; Tambone et al. 2010).

In order to complete the description of the amendment properties of digestate, CPMAS ^{13}C NMR was used to better describe organic carbon composition of the digestate (Table 3). Results obtained indicated a constancy in C composition of digestate during the period considered.

The region (Figure S1) between 47 and 115 ppm dominates the ^{13}C NMR spectra of all the samples, with an average of 54.8 ± 10 % of the total integrated area that is typical of polysaccharides and protein. The strong chemical shift at 73 ppm was attributable to O-alkyl-C of cellulose/hemicellulose-like material and the pronounced signal at 105 ppm was related to anomeric-C compounds in C2 and C6 of carbohydrates (Kögel-Knabner 2002;

Fernandez-Bayo et al. 2018). A shoulder centered at 56 ppm represents the metoxhy-C indicating the presence of lignin and lignin-derived molecules, which was confirmed by the presence of aromatic C phenol or phenyl ether C (115-160 ppm), i.e. 7.63 ± 2.5 %. These compounds (hemicellulose, cellulose and lignin) are typically clustered forming a 3D complex characterized by medium-low degradability (Papa et. al, 2013): they are partially preserved and concentrated in relative terms during the anaerobic digestion process (Tambone et al. 2009; Fernandez-Bayo et al. 2018).

Chemical shifts in the region 0-47 ppm constituted the second largest region characterizing digestates spectra (29.2 ± 6.7 %). According to previous work, this region was represented by hydrocarbons (Tambone et al., 2010, 2013, 2009; Baldock et al., 1992). Jardé et al. (2005), studying the lipid fraction in sewage sludge reported that hydrocarbons were mainly constituted by vegetal hydrocarbons derived from the presence of vegetable fragments, oil and human feces containing both microbial and food hydrocarbons. Payet et al. (1999), confirmed the presence of natural hydrocarbons in sewage sludge due to the presence of plant hydrocarbons preserved in the human feces, in agreement with data from Réveillé et al. (2003). The intense signal at 174 ppm in the carbonyl region (160–210 ppm), suggested the contribution of carbonyl groups of peptide bonds of amino acid moieties and carbonyl C of hydrocarbons (Martinez-Balmori et al. 2014).

Comparing the spectroscopic characteristics of these digestates (Table 3) with those of digestates of different origins, the similarity with those originating from animal manure was evident because of the presence of fecal material and vegetable matter (Table 3). On the other hand, digestates derived from the organic fraction of municipal solid waste (OFMSW), were characterized by higher aliphatic carbon, probably due to the high presence in the infeed mixture of vegetable waste and oil/fat (from food) contributing to this fraction (cutin, suberin). More interesting was the fact that ^{13}C-NMR spectra of digestate resembled that of composts (Table 3), apart from the higher presence of aromatic-C and lower presence of alkyl fractions due to biomass origin (lignocellulosic material vs. fecal origin material).

Therefore, taking into consideration total C content, biological stability data and ^{13}C-NMR, it should be noted that digestate was very little different from compost and indeed it contained more C and the C was more recalcitrant to biological degradation. These results indicate that anaerobic digestion as well as composting degrade the easily degradable organic matter, concentrating most recalcitrant matter, so that digestate can be considered an alternative for giving good organic amendment properties, such as previously suggested by Tambone et al. (2015) that reported that well performed AD was able to achieve high biological stability and that the subsequent composting did not add any notable advantage (Tambone et al. 2015).

Table 3. Spectroscopic (CPMAS 13-C NMR) characteristics of digestate and comparison with other biomasses (literature data).

SAMPLES	**C-type (ppm)**			
	Total aliphatic C (0-47 ppm)	**O-CH_3 or N-alkyl O-alkyl C di-O-alkyl C (47-115 ppm)**	**Aromatic C phenol or phenyl ether C (115-160 ppm)**	**Carboxyl C keto C (160-210 ppm)**
Digestate (n=3)[a]	29.2 ± 6.7ab	54.8 ± 10b	7.63 ± 2.5a	8.37 ± 2.9a
Farm digestate (n=8)[d, e]	31.1 ± 4.5a	49.9 ± 9.1b	8.85 ± 1.9a	10.13 ± 6.1a
OFMSW digestate (n=12)[c,d]	44 ± 2.1b	36.3 ± 2a	8.13 ± 0.6a	11.61 ± 1.1a
Compost[d]	21.6±3.6a	55.4±1.8b	13.9±1.6b	8.9±0.5a

[a] Digestates of this work.

[b] Values of the same column followed by different letters are statistically different ($P < 0.001$, Gabriel test).

[c] Tambone et al. (2009).

[d] Tambone et al. (2010).

[e] Tambone et al. (2013).

3.3.4 Digestate Nutrient contents

3.3.4.1 N, P and K

Total N concentration for digestate was of 77 ± 3.7 g kg^{-1} DM, in line with those for agricultural and energy crop digestates, i.e. 80.6 ± 13.3 g kg^{-1} DM and 70.3 ± 0.8 g kg^{-1} DM, respectively (Table 2). Ammonia concentration in the digestate was of 46.6 % of Total N, lower than that found for agricultural and energy crop digestates (Table 2), but much higher, as expected, than those of composts. Lower ammonium content than that of other digestates was related to the fact that it was partially removed by the N stripping unit, as described earlier. On the other hand, because of the high dry matter content, 103 ± 3.7 g kg^{-1} ww, the N-NH_4^+ content of digestate was of 7.9 g kg^{-1} ww and so, much higher than that measured for agricultural digestates, i.e. 2.98 g kg^{-1} ww. About 53.4% of Total N was under organic forms that were recalcitrant to mineralization, as suggested by the high biological stability as discussed before, which was much higher than that of green compost. In this way, organic-N contributed to maintain the soil-N pool.

The P content of the digestate from sewage sludge, i.e. 28 ± 4 g kg^{-1} DM, was higher than that reported for other digestates i.e. 15 ± 5 and 13.8 ± 5 g kg^{-1} DM for agricultural digestate and energy crops, respectively (Table 2). During anaerobic digestion organic P forms becomes modified, depending on the biomass origin, AD condition and chemical and physical properties of ingestate vs. digestate (Mazzini et al. 2020). Therefore, to understand chemical speciation of P in the digestate under study, P fractionation of digestate samples was carried out to assess its fertilizer properties. Data obtained showed good P recoveries, equal to 86 ± 2% of the total P detected for all digestates (Table 4). Average P fractions contents (mg kg^{-1} DM) were, as the average of three replicates, as in the following: P-$NaHCO_3$ of 11,590 ± 1,100, P-HCl of 7,932 ± 913 and P-NaOH-EDTA of 6,891 ± 1,179. This means that, on average, more than the 43% of the total P in digestate can be considered readily soluble, i.e. P-$NaHCO_3$ (Table 4): these data are in line with previous findings (Mazzini et al. 2020).

^{31}P NMR performed on digestate samples allowed us to get more information about different P fractions. In this regard, the NMR spectra of digestate (Table 4) were able to identify the main peaks due to inorganic P-forms, i.e. orthophosphate (6.1 ppm) and pyrophosphate (-4.4 ppm) (Figure S2). Another signal at -0.2 ppm was assigned to P of DNA. Small peaks in the monoester region (4.85 - 4.64 ppm and 5.23 ppm) attributed to α and β – glycerophosphate and phosphatidic acid, respectively, indicated the presence of P organic compounds. Other signals in the phosphate monoesters region (4.95 - 4.10 ppm) were due to inositol-phosphate and mononucleotides originating from the hydrolysis of RNA. Each P-species detected was quantified and the results are reported in Table 4. Data clearly showed that P extracted by $NaHCO_3$ was 100 % inorganic-P made up by orthophosphate P, which is considered soluble and readily available for plants. Inorganic forms also dominated the HCl and NaOH-EDTA fractions, and they were made, above all, by orthophosphate P (Mg/Ca phosphate) (Table 4). The HCl fraction showed, also, low concentrations of phosphate monoester and pyrophosphate P. On the other hand, NaOH-EDTA extracts showed the presence of low amounts of organic P such as DNA and phospholipids.

All these figures were very similar to the average data calculated for agricultural digestate previously studied (Table 4) (Mazzini et al. 2020).

The concentration of K in the digestate produced (6.5 ± 1.3 g kg^{-1} DM) was lower than that reported in the literature both for digestates from energy crops (14.8 ± 3 g kg^{-1} DM) and for green compost (9.16 ± 0.13 and 12.01 ± 0.09 g kg^{-1} DM). This makes the digestate from sewage sludge slightly poorer in K than other bio-fertilizers.

Table 4. Phosphorous fractionation and speciation detected by ^{31}P NMR for digestate studied in this work in comparison with literature.

Digestate	Extractant	P-total[a]	P distribution[b]	Orthophosphate	Phosphate monoesters	Pyrophosphate	DNA	Phospholipid	Pi[c]	Po[c]
		mg kg^{-1}	%Ptot			mg kg^{-1}			(% P for fraction)	
Digestate of this work	P-NaHCO3	11,590 ±1,100	44 ± 3	11,590 ± 1,100	n.d.d	n.d.	n.d.	n.d.	100	0
	P-HCl	7,932 ±913	30 ± 2	7,749 ± 899	131±28	52±6	n.d.	n.d.	98.3±0.4	1.65±0,37
	P-NaOH + EDTA	6,891 ±1,179	26 ± 5	6,514 ± 1,106	262±54	61±15	29±6	24±5	95.4±0.4	4.57±0.37
Agricultural digestate[e]	P-NaHCO3	5,139 ± 1,398	44 ± 14	5,097 ± 1,423	21.3 ± 52.2	20.7 ± 25.1	n.d.	n.d.	98.3±2c	1.67±1c
	P-HCl	3,769 ± 1,461	31 ± 7	3,188 ± 1,539	572 ± 489	8.53 ± 11.1	n.d.	n.d.	100 ± 1	1 ± 1
	P-NaOH + EDTA	3,094 ± 1,400	25 ± 7	2,411 ± 1,583	567 ± 631	32.4 ± 26.0	56.1 ± 27.4	27.8 ± 28.2	86 ± 9	14 ± 9

[a] Quote extracted by the three different solvents.

[b] Quote extracted by the three different solvents reported as % of the total P.

[c] Inorganic (Pi) and organic (Po) phosphorous in each extract calculated on the base of ^{31}P NMR data.

[d] n.d. no detectable

[e] Mazzini et al. (2020), average different agricultural digestates (n=6).

3.3.4.2 Other meso- and micronutrient nutrients, and inorganic micropollutants

Digestate characterization showed that it contained, also, other meso and microelements useful for plant nutrition (e.g. Ca, Mg, Fe etc.) the presence of which was in line with the other organic matrices indicated for comparison (Table 2). AD processes, because of organic matter degradation, concentrated micro-pollutants contained in the feed matrices (Boix et al. 2016; Bloem et al. 2017) so that detecting and discussing them can be useful in view of the agricultural use. From Table 2, it can be seen that digestate from sewage sludge had a content of pollutants, as expected, higher than those contained in agricultural digestate and compost; on the other hand, they were much lower than the legal limits for the use of this kind of digestate in agriculture (Lombardy Region Law N. 6665/2019). It should be reported, also, that digestate are dosed in order to fulfil nutrient requirements of crops and that doses are about 1/10 of the compost amount used as organic amendment.

3.3.4.3 Organic micro-pollutants

Persistent Organic Pollutants (POPs) are those organic compounds that can show dangerous effects on people and/or on the environment, and are not easily degraded in soil, water or sediment. These compounds are, also, mostly non-degradable during AD process. For this reason, their content in digestate depends on the quality of the infeed (Al Seadi et al. 2013). Therefore, an AD plant whose products will go to fields must carefully select infeed with very low concentrations of POPs, in order, also, to fulfil the very strict limits required for agricultural use (Table 2). Any non-compliance with rules suggests the need to take a different path such as incineration or pyrolysis.

In this study, several organic pollutants were tested (Table 2). All organic pollutants were much lower than the limits imposed for agricultural use and some were lower than the contents revealed for other organic matrices such as agricultural and energy crop digestate and compost (e.g. PHA, PCB, AOX). Again, it is important to remember that amounts of digestate fertilizer commonly applied is about 8-10 times lower than that of compost used as organic amendment. Unfortunately, no data about hydrocarbons (C10-C40) were available for other biomasses. From Table 2, it can be seen that hydrocarbons reduced a lot after anaerobic digestion indicating a biological origin of hydrocarbons for sewage sludge, as well reported in the literature (Payet et al., 1999). An investigation performed by the Environmental Protection Agency of Veneto Region (Italy) on the presence of hydrocarbons (C>12) (ARPAV 2013) in 17 agricultural digestates, reported hydrocarbons concentration in the range of 720-4,600 mg kg^{-1} DM and even of 31,800 mg kg^{-1} DM for slaughterhouse derived digestate (high fat content). These values can be compared with data for digestate from this work (Table 2), referring to DM, i.e. 2,757 mg kg^{-1} DM.

Less studied until recently is the presence of emerging pollutants (pharmaceuticals) in digestate. Konradi and Vogel (2013), suggested, taking into consideration parameters related to pollutants such as residence time in the soil, solubility and eco-toxicity, to detect 9 emerging pollutants to be used as markers: antibiotics (Ciproflaxacin and Sulfamethoxazole), lipid regulators (Fenofibrate and Gemfibrozil), psychiatric drugs (Carbamazepine), beta-blockers (Metoprolol), analgesic (Diclorofenac) and hormones (Ethinylestradiol and Estradiol). Results obtained (Table 2) showed a concentration for all compounds below the detection limit (<0.01 mg kg^{-1} DM) indicating low concentration (Konradi and Vogel, 2013). These values were in line with those reported in the literature for the same classes of compounds in digestates from sewage sludge, i.e. 0.001 – 1 mg kg^{-1} DM for antibiotics, 0.0001 – 1 mg kg^{-1} DM for psychiatric drugs, 0.004 – 1 mg kg^{-1} DM for analgesics and 0.001 – 10 mg kg^{-1} DM for hormones (Verlicchi and Zambello 2015). Low contents can be ascribed, also, to the AD process since it is reported as the most effective type of treatment in reducing the concentration of these compounds in sewage sludge (Verlicchi and Zambello 2015). Panseri et al., (2013), studying the ability of AD in reducing antibiotics content, reported an 80% of reduction.

Unfortunately, a comparison with similar organic pollutants in other fertilizers is not possible because of the lack of systematic studies on this subject. Furthermore, most of the classes of emerging pollutants analysed in this work were exclusively for human use, and therefore not easily traceable in animal waste or agricultural biomasses, with the exception of antibiotics, that are furthermore considered very toxic for soil organisms ($EC_{50} > 1$ mg kg^{-1}) (Konradi and Vogel 2013). Literature reported concentrations of veterinary antibiotics in a range of 0.005 – 7.5 mg kg^{-1} DM in pig slurry (Gros et al. 2019), 2.0 – 22.8 mg kg^{-1} DM in pig manure (Van den Meersche et al. 2016), and 0.02 – 8.0 mg kg^{-1} DM in cattle feces (Berendsen et al. 2015), all of these data reported being much higher than those observed for digestate analysed in this paper (<0.01 mg kg^{-1} DM (Table 2).

3.3.4.4 Pathogen content

The pathogen contents (Salmonella and Faecal coliform) was detected in both infeed and digestate (Table 2). Salmonella was present in the feed, i.e. 47 MPN g^{-1} DM, but after anaerobic digestion it was absent. AD showed a strong effect also on Faecal coliform that dropped from 110,000 MPN g^{-1} DM (feed) to less than 1,000 MPN g^{-1} DM in the digestate, being this data much lower than Regional legal limits (<10,000 MPN g^{-1} DM) (Regione Lombardia 2019). Anaerobic digestion has been reported reducing pathogen content thanks to the joined action of alkaline pH, high ammonia concentration and biological process, i.e. microbial competition for organic substrate (Orzi et al, 2015). pH and ammonia actions are magnified under thermophilic condition leading to excellent digestate sanitation such as previously reported (Mendez et al., 2002; Nordin and Nyberg, 2009). Scaglia et al., (2014) studying sewage sludge sanitation by AD, indicated that temperature above 55 °C and ammonia concentration higher than 0.4 g kg^{-1} ww resulted in optimum digestate sanitation. Since process studied in this work showed alkaline pH, i.e. 8.5 ± 0.3 and high ammonia concentration, i.e. 3.7 ± 0.2 g kg^{-1} ww, the reduction of the pathogen contents observe was expected.

3.4 Conclusions

Anaerobic digestion is an interesting biotechnology able to produce renewable energy (biogas) but also renewable fertilizers. This work aimed to describe the fertilizer properties of fertilizers produced at full scale starting from a mix of organic wastes (sewage sludge, organic fraction of municipal solid waste and minor food industry wastes). Results indicated that ammonia stripping allowed the production of N mineral fertilizers that can be useful for topdressing purposes. On the other hand, digestate can act as both an organic amendment because of high organic carbon content and high biological stability, i.e. high recalcitrance of biomolecules contained, and as fertilizers because of high nutrient contents (except for K). Inorganic and organic pollutants were much lower than the limits imposed by rules for agricultural use and sometimes lower that those detected for other biomasses. Emerging pollutants were present only at very low levels, emphasizing the role of HSAD in reducing their content. Unfortunately, a systemic comparison with other organic matrices used in agriculture (e.g. agricultural digestate and compost) was not possible because of the lack of literature data regarding emerging pollutants and suggesting further investigation in this direction.

In conclusion, this paper was able describing renewable fertilizers obtained from anaerobic digestion plant and discussing fertilizers properties. A limit of this paper consist in the fact that fertilizers properties have been indirectly studied and no directly tested at full field. In this way, agronomical trials are ongoing within the same European project and three years of experimental data will be presented in next scientific papers.

Credits

AP and MZ contributed equally to this work

FA: designed the project, elaborated data, interpreted the data and wrote the paper

MZ: collected, elaborated, interpreted the data and wrote the paper

AP: collected, elaborated, interpreted the data and wrote the paper

FT: collected the data, 13CNMR analyses and wrote the paper

SM: 31P-NMR analyses

MS: Full scale plant collection and management

AD: Full scale plant collection and management

EM and OS: Scientific contribution and manuscript correction.

4 MEASURING AMMONIA AND ODOURS EMISSIONS DURING FULL FIELD DIGESTATE USE IN AGRICULTURE.

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The use of digestate in agriculture represents an opportunity for reducing the use of synthetic fertilizers while promoting nutrient and organic matter recycling, i.e. contributing to a circular economy. However, some environmental impacts could result from digestate use, with particular reference to N emissions, which can contribute to particulate matter formation in the atmosphere. So, correct digestate spreading methods need to be tested to reduce ammonia emission and, possibly, also to avoid annoyance to the inhabitants. In this work a digestate from organic wastes was used as a fertilizer by its injection at 15 cm, in comparison with a synthetic one (urea) for three consecutive years in open fields, measuring ammonia and odours emission. On average, the ammonia emission from digestate was of 25.6 ± 9.4 kg N ha^{-1} *(11.6% ± 4 of Total Ammonia Nitrogen - TAN - dosed), while urea emitted 24.8 ± 8.3 kg N ha*$^{-1}$ *(13.4% ± 4.5 of TAN dosed). The injected digestate also emitted less odour than urea (601 ± 531 and 1,767 ± 2,221 OU m*$^{-2}$ *h*$^{-1}$*, respectively), being ammonia coming from urea hydrolysis responsible for odour productions.*

The different N fertilizers did not lead to differences in crop yields, i.e. 18.5 ± 2.9 Mg grain ha^{-1} *and 17.4 ± 1.2 Mg grain ha*$^{-1}$ *for digestate and urea respectively.*

4.1 Introduction

Climate change is pushing the world into shifting production processes towards more sustainable models, lowering environmental impacts and reducing greenhouse gas emissions (Frantzeskaki et al. 2019; European Commission 2019). One of the main challenges is how to manage the transition towards circular economy models based on the recovery of wastes, that become raw material for the subsequent production cycle (Pieroni, McAloone, and Pigosso 2019; Lüdeke-Freund, Gold, and Bocken 2019).

Nutrient recovery from organic wastes represents an interesting circular economy model able to upgrade waste into fertilizers to be used in substitution for synthetic ones (Toop et al. 2017). Indeed, N and P dispersion in the environment causes many problems and these two elements have been reported to be over “planetary boundaries” (Johan Rockström et al. 2009a; W. Steffen et al. 2015). Moreover, fertilizer production requires large amounts of energy consumption or the exploitation of non-renewable mineral deposits that strongly impact on environmental and climate change (Springmann et al. 2018).

However, untreated organic wastes do not represent acceptable fertilizers (Westerman and Bicudo 2005). Technology/biotechnology is needed to transform them into useful products (Ivona Sigurnjak et al. 2019). In the last decades, anaerobic digestion has been proposed as a valid biotechnology for producing bioenergy but, also, to produce bio fertilizers, i.e. the digestate, to be used in agriculture as a substitute for synthetic fertilizers (Tambone et al. 2019; Verdi et al. 2019; Riva et al. 2016).

Furthermore, the possibility of using digestate in agriculture has raised many doubts regarding its possible environmental impacts. The high amounts of nitrogen in the mineral form (ammonia-N), which is useful for plant nutrition, can cause environmental problems due to both nitrates (NO_3^-) leaching and N emission to the atmosphere (N_2O and NH_3) (Delgado 2002; Cameron, Di, and Moir 2013). Although problems connected with nitrate leaching have received much attention in the past (Padilla, Gallardo, and Manzano-Agugliaro 2018), less is known regarding N emissions.

The anthropogenic emission of ammonia causes a series of impacts on both climate, ecosystems and health. In fact, once in the upper atmosphere, ammonia combines with other molecules generating a wide range of nitrogen compounds which fall to the soil causing acidification and eutrophication of ecosystems (Hautier et al. 2014; Clark and Tilman 2008). Furthermore, ammonia in the atmosphere contributes to the formation of secondary particulate matters

(Erisman and Schaap 2004) influencing the planet climate because they act as condenser nuclei for atmospheric water forming clouds (Bianchi et al. 2016). In addition particulate matters affect human health causing acute or chronic respiratory diseases (Losacco and Perillo 2018; Fennelly 2020; Comunian et al. 2020; Setti et al. 2020). In previous work (Riva et al. 2016) it was reported for the Lombardy Region (North Italy) that about 96% of ammonia polluting the air was due to agricultural activity (livestock), with these data being confirmed by the international literature (Clarisse et al. 2009).

In recent decades, many studies have tried to clarify the ammonia emissions from both mineral and animal fertilizers (Sommer and Hutchings 2001; Sommer and Olesen 2000), but not many data are available for digestate. Getting real data is sometimes very difficult because working at full field scale is costly and complicated. Therefore, the data proposed have often been obtained at lab or pilot scale (Finzi et al. 2019) and so, rather distant from the reality. In addition, studying only at lab scale makes it impossible to test innovative technologies such as digestate injection into the soil, coupled with precision farming, to reduce ammonia emissions (Morken and Sakshaug 1998; Nicholson et al. 2018).

Ammonia has been reported having a very low odour threshold causing inhabitants annoyance during fertilization. Therefore, reducing ammonia emission means, also, reducing odour emission. In addition, organic fertilizers contain organic matter that can produce many volatile organic odorous molecules as the result of microbial bioprocess such as fermentation and anaerobic respiration (Orzi et al., 2015). Thus, spreading organic fertilizers such as digestate in the field introduces another problem in addition to ammonia emission, i.e. odour impact, which is interesting to study.

In previous work it was reported that anaerobic digestion, because it degrades the easily degradable organic matter, strongly reduced the potential odour emission but, because it mineralize organic-N to ammonia, odours potentially can increase if ammonia emission are not controlled (Valentina Orzi et al. 2015). The digestate injection into the soil has been reported reducing odour emission at values even below that measured for mineral fertilizers spreaded onto the surface, such as urea (V. Orzi et al. 2018), taking into consideration that odours from urea is the result of ammonia coming from its hydrolysis.

Digestate is incresingly indicated as a useful N-fertilzers able to replace mineral fertilizers (e.g. urea) for crop production (Riva et al., 2016). Therefore its use should be promoted, but taking into account correct and safe management.

The objective of this study was to provide data on ammonia and odour emissions resulting from the use of digestate from organic wastes (mainly sewage sludge) in the open field in a full-scale production context, by adopting a low emission strategy, i.e. digestate injection, to reduce both ammonia and odour emissions.

The study was performed by comparing digestate with conventional N-fertilization (urea) and discussing the results obtained with the literature data. The experiments were repeated for three consecutive years (2018, 2019 and 2020). They were carried out on a maize crop located in the Po valley (northern Italy), one of the most intensely cultivated areas in Europe, and consequently with serious problems about ammonia in the atmosphere (ISPRA 2019).

4.2 Material and Methods

4.2.1 Spreading and experimental setup

All the experiments were carried out to compare emissions and agronomic performance of two different fertilizers (slurry-like digestate and solid granular urea, plus an unfertilized control) dosed following standard agricultural procedure used in the Po Valley. The experimental fields were located in the Po valley, northern Italy, and the experiments were carried out on a maize field, with experimental plots in triplicate and using a randomized scheme.

Digestate was spread by injection in 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 in order to satisfied N maize requirements, adopting an N efficiency of 0.5, such as suggested by the Regional Plan for Water Protection from Nitrate from Agriculture (Regione Lombardia 2020). Doing so efficient N dosed for digestate was equal to that coming from urea (Table 1). Urea was spread as the solid form on the soil surface following a routine procedure typical of Po Valley. Fertilization date, fertilizers used and doses applied, and spreading methodology are reported in Table 1.

The ammonia and odours measurements took place at pre-sowing fertilization in three consecutive years, 2018-2019-2020, adopting the same agronomic and emission measurement technique, and fertilizer doses.

Table 1. Main information regarding fertilization plan adopted: fertilization date, fertilizers used, and dose applied.

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

Urea	28/05/2020	Pre-sowing	Urea	185	185	185 [b]	Spread in surface
	31/07/2020	Top-dressing	Ammonia sulphate	90	90	90	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 (Regione Lombardia 2020).

[b] Ureic ammonia considered as 100% ammonia.

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.

The main characteristics of the digestate used in this work are shown in Table 2. Digestate 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 (APHA 1992). Total nitrogen (TKN) and ammonia nitrogen (TAN) were determined according to the analytical method for wastewater sludges (IRSA CNR 1994). Total P content was assessed by inductively coupled plasma mass spectrometry (Varian, Fort. Collins, USA), preceded by acid digestion (EPA 1998) of the samples. All the analyses were carried out in triplicate.

Table 2. Main characteristics of the digestates used in this work (mean ± SD, n=3).

Parameter	Unit	2018	2019	2020
pH	pH unit	8.6 ± 0.3	8.4 ± 0.3	8.5 ± 0.4
Total solids (TS)	%	10.3 ± 0.48	10.5 ± 0.5	10.5 ± 0.2
Total Organic Carbon (TOC)	% dw[a]	29.2 ± 4.13	31.2 ± 4.2	30.9 ±0.2
Total Nitrogen (TKN)	% dw	7.7 ± 0.3	7.5 ± 0.5	7.3 ± 0.8
N-NH_4 (TAN)	% dw	4.6 ± 0.4	4.5 ± 0.3	3.9 ± 0.1
TAN/TKN	%	60	60	53

[a] dw = dry weight

4.2.3 Soil sampling and analysis

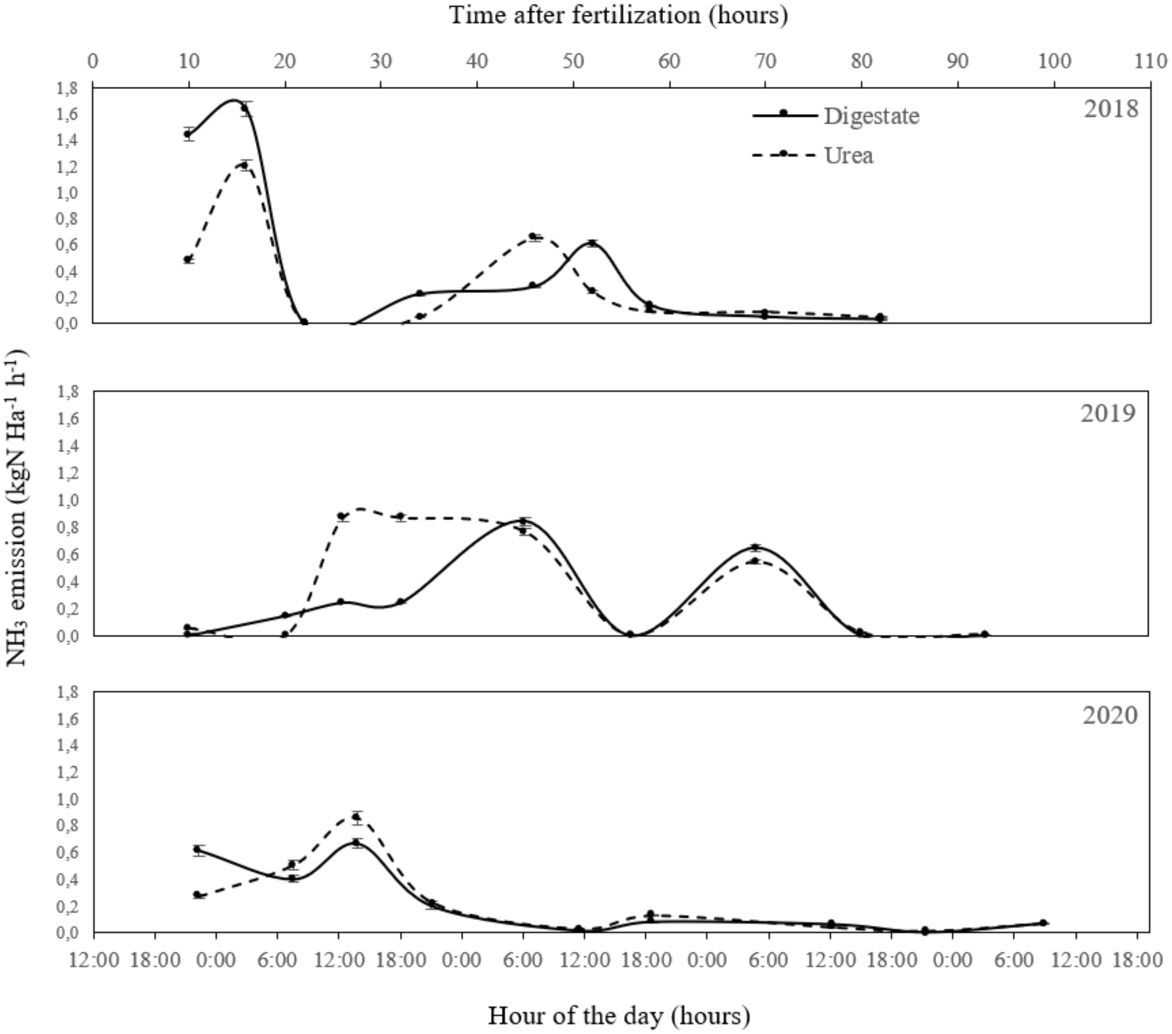
The soils studied in this work were sampled just before the spreading by taking three random samples (made by 3 sub-samples)/plot of soil (20 cm); this procedure was repeated each year and no statistical differences occurred. Samples were air dried, sieved to 2 mm and then ground to 0.5 mm. The main characteristics of soils are reported in Chapter 5 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 (Gee and Bauder 1986). Cation Exchange Capacity (CEC) was determined by saturating the samples with $BaCl_2$ (Rhoades 1982). Total organic carbon (TOC) was determined using the Walkley and Black method (Olsen, Sommers, and Page 1982), total nitrogen by the Kjeldahl method (Faithfull 2002). All the analyses were carried out in triplicate.

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 (Figure 1). 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, which main soil chemical characteristics are reported in Chapter 5 Table 2. 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 digestate and urea, respectively (Table 1).

Figure 2. Ammonia emissions (kg N ha^{-1} h^{-1}, *n* = 3) measured in the hours following injection/spreading of fertilizers on maize crop in the years 2018, 2019 and 2020. X axis on the top of the figure shows the time after fertilization (hours), while X-axis on the bottom of the figure shows the daytime. Error bars show the SD.



The concentration of NH_3 was monitored by the exposure of ALPHA passive samplers (Tang, Cape, and Sutton 2001; Riva et al. 2016). 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, Tunderbeach 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:

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

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

4.2.5 Potential odour emission and field odour emission measurement

Potential odour emissions were measured on gas samples collected in the laboratory following the protocol reported by Riva and colleagues (Riva et al. 2016). The sampling was carried out by spreading the sample homogeneously on a surface that was then covered with a steel chamber having a sampling area of 0.127 m^2. A continuous flow of air was continuously flushed inside the chamber for 5 minutes (rate 0.38 m^3 h^{-1}). Output gas from the chamber was collected in Nalophan sampling bags, which were then analysed through dynamic olfactometry (CEN 2003) within 24 hours from sampling. Analyses were performed in three replicates.

The same method was used for full field sampling. The chamber was placed above the newly fertilized soil, taking care to eliminate any air leaks from the edges. All measurements were made once per plot.

The results of the Dynamic Olfactometry were expressed as odour concentration value (OU m^{-3}). The specific odour emission rate SOER (OUE m^{-2} h^{-1}) was calculated by using the following equation:

$$SOER = 1000 \times (C \times Q/S)$$

in which C is the odour concentration (OU m^{-3}), Q is the incoming air rate to the flux chamber (0.38 m^3 h^{-1}) and S the surface covered by the chamber (0.127 m^2).

4.2.6 Maize yield quantification and N content in grain

The annual 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 a value (in Mg ha^{-1}) for each treatment, i.e. digestate, urea and control.

The quantification of the N content in the harvested maize grain was performed through combustion method (Dumas method) (Saint-Denis and Goupy, 2004). Before analysis, the grain samples (20 g of dry matter) were prepared by grounding them using a ball mill. N was detected by using an elemental analyser (Rapid max N exceed model, Elementar, Lomazzo, Italy). Each analysis was performed in triplicate.

4.2.7 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 Maize yield

At the end of each of the crop seasons, the grain yield from soils fertilized with digestate and urea was evaluated (Table 3). In agreement with data from previous work (Walsh et al. 2012; Verdi et al. 2019; Riva et al. 2016), the production, as a three-year average, for the plots fertilized with digestate (18.1 ± 2.9 Mg ha^{-1}) was very similar to that obtained from the plots fertilized with urea (17.4 ± 1.2 Mg ha^{-1}) (one-way ANOVA analysis, $p = 0.72$, $n = 3$). Low standard deviation indicated that the yields were very similar throughout the three years. The use of digestate determined, as an average value over the three years, an N content in the grain of 12 ± 0.9 gN kg^{-1} DM, higher than that of the control (9.26 ± 0.6 gN kg^{-1} DM) and treatment with urea (11.3 ± 0.7 gN kg^{-1} DM) (one-way ANOVA, $n = 6$, $p<0.01$, Tukey post-test).

4.3.2 Ammonia emission

The ammonia emission measurements were done using passive ALPHA samplers and processing the data with dispersion models (see Section 2.4). This approach has advantages and disadvantages: passive samplers fully exposed to the atmosphere do not allow maintaining controlled micro-environmental conditions, unlike other methods such as wind tunnels (Misselbrook et al. 2005). Therefore, measurement made at different time can be affected by environmental parameters, introducing variability. On the other hand, passive ALPHA sampler, taking into consideration the environmental conditions occurred during the measurements, allows realistic measurements of emission that occurred at that particular time and condition (Misselbrook et al. 2005).

Table 3. Ammonia emissions, maize productions yield and N content in grain for the three years of experiments. Ammonia and odour emission are reported as mean ± SD (*n*=3). Yield are reported as dry grain yield produced per hectare (mean ± SD; *n*=3). N content in grain are reported as grams of N per kilograms of dry grain material (mean ± st. dev)

Campaign	Fertilizer	Total cumulated ammonia emission (kg N ha^{-1})	Loss of NH_3 (%Ntot)	Loss of NH_3 (%TAN)	Odour emission (OU m^{-2} h^{-1})	Grain yield DM (Mg ha^{-1})	N content in grain (gN kg^{-1})
2018	Unfertilized	Undetectable[a]	-	-	277 ± 7a	6.5 ± 0.8a	9.08 ± 0.2a
	Digestate	34.2	9.25	14.9	262 ± 52a	16.8 ± 1.4b	11.4 ± 0.8b
	Urea	25	13.5	13.5	259 ± 31a	17.4 ± 2.1b	11.3 ± 0.5b
2019	Unfertilized	Undetectable	-	-	367 ± 22a	11.6 ± 1.2a	9.12 ± 0.3a
	Digestate	26.9	7.44	12	444 ± 122a	16.1 ± 1.4b	12.8 ± 0.2c
	Urea	33	17.8	17.8	404 ± 54a	16.2 ± 1.6b	11.3 ± 0.9b
2020	Unfertilized	Undetectable	-	-	1,257 ± 311a	13.3 ± 1.8a	9.56 ± 1a
	Digestate	15.6	4.33	7.8	1,097 ± 730a	21.4 ± 3.1c	11.7 ± 0.7b
	Urea	16.4	8.85	8.9	4,638 ± 1,097b	18.6 ± 2.1b	11.4 ± 0.9b
Mean	Unfertilized	Undetectable	-	-	633 ± 494a	10.4 ± 3.5a	9.26 ± 0.6a
	Digestate	25.6 ± 9.4a[b]	7.01 ± 2.5a	11.6 ± 4a	601 ± 531a	18.1 ± 2.9b	12 ± 0.9c
	Urea	24.8 ± 8.3a	13.4 ± 4.5b	13.4 ± 4.5a	1,767 ± 2,221a	17.4 ± 1.2b	11.3 ± 0.7b

[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).

The fluxes of NH_3 released from the soil after spreading (years 2018, 2019 and 2020) are shown in Figure 1. Observing each of the three graphs alone (Figure 1), it can be clearly seen that there is a strong overlap between the emission curves of NH_3 from soils fertilized with digestate (solid lines) and urea (dashed lines). In each of the three years, the soils fertilized with urea and digestate were therefore found to have emitted similar amounts of ammonia over time, thus responding in a similar way to the main environmental factors that may have influenced this process (Bouwmeester, Vlek, and Stumpe 1985; Sommer and Hutchings 2001; Cameron, Di, and Moir 2013). Among these factors, the most important in this specific case were the climatic conditions in the days preceding and following the spreading (Tables S2 and S3), given that the chemical characteristics of the soil remained unchanged. However, it was not possible to obtain a coherent model that correlated emission flows with climatic conditions by using multivariate statistical analysis (Partial Least Squares Analysis, PLS), probably due to the high variability of data acquired between years and complexity of the factors involved in the open field.

Comparing instead the emission flows year by year (2018, 2019 and 2020), they appeared very variable (Figure 1). In fact, the graphic corresponding to the experiment of 2018 showed a strong emission peak between the 10th and 20th hour after fertilization, corresponding to the night, followed by a higher modest peak close to the 50th hour after spreading, corresponding instead to the morning hours. The ammonia losses at the end of the experiment were of 32.2 kg N ha^{-1} and 25 kg N ha^{-1} for digestate and urea, respectively, corresponding to a loss of 14.9% and 13.5% of the TAN dosed, and were very similar between digestate and urea (Table 3). The graph reporting data on 2019 showed a completely different pattern: in this case, in fact, in the first 20 hours after fertilization, the emission flows appeared very low, then increased starting from the 20th h after fertilization with urea and later for fertilization with digestate. Emission peaks were reached after 45 hours, at 6:00 in the morning. A second peak of similar intensity was then recorded at the 70th h after the spreading, again at 6:00 in the morning. In this case, the loss of ammonia was 26.9 kg N ha^{-1} and 33 kg N ha^{-1} for digestate and urea (12% and 17.8% loss of TAN dosed), respectively (Table 3). 2019 was the year in which urea lost more ammonia (%TAN) than in other years of experimentation. This was probably due to climatic conditions; in fact, the soil received several showers of rain in the days before the spreading and the low temperatures combined with the high atmospheric humidity probably contributed to maintaining high soil moisture. It is well known that these conditions tend to increase the loss of ammonia from urea, especially if dosed on the surface, because of moisture enhanced urea hydrolysis (Cameron, Di, and Moir 2013).

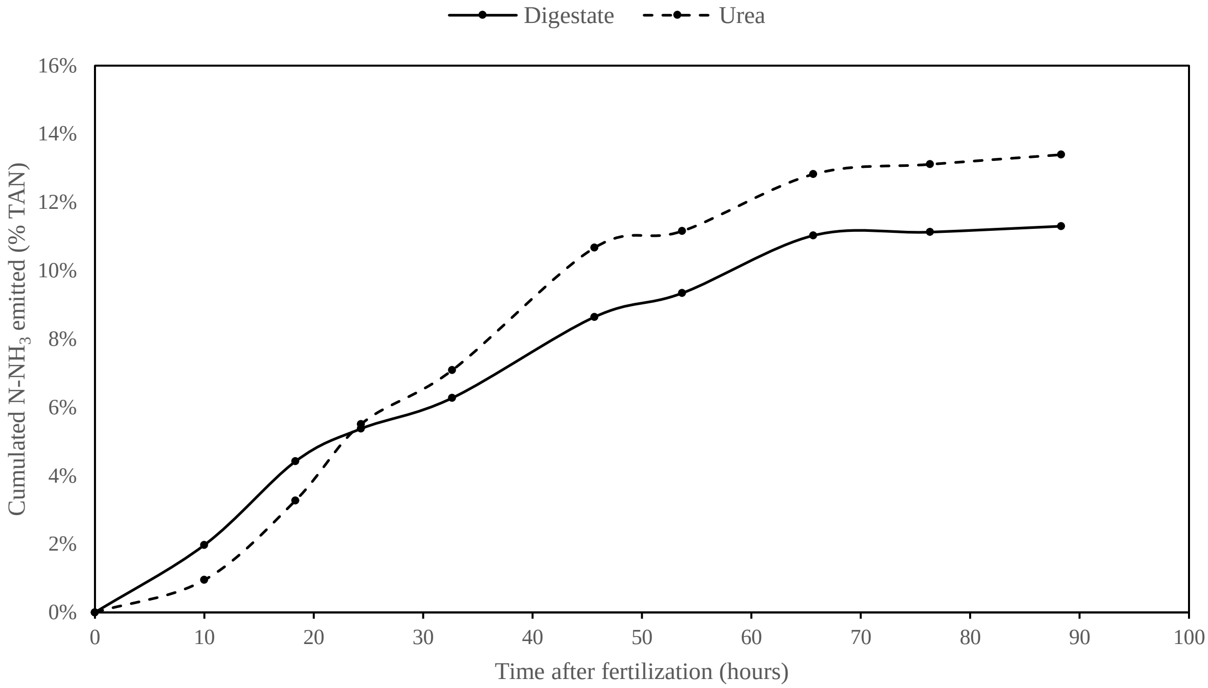
Finally, for the year 2020 high emission levels were already observed during the first measurement, i.e. 10 hours after fertilization, reaching a peak at the 25th h, at noon. After this single peak, the ammonia emission was reduced to very modest values and was close to zero for the rest of the experiment.

At the end of the experiment, the total ammonia emitted was of 15.6 kg N ha^{-1} and of 16.4 kg N ha^{-1} for digestate and urea respectively, corresponding to a loss of 7.8% and 8.9% of the TAN dosed. These were the lowest values measured over the three years of experiments, for both digestate and urea. Such low emissions were probably caused by the particularly dry environmental conditions, especially in the days before and immediately following the spreading. On the third day after spreading, rains were recorded (2.6 mm) which, however, were not enough to have a significant effect on ammonia emission.

Ultimately, observing all the data from the three years together it was not possible to identify a similar pattern for ammonia emissions, since they showed such a strong variability between the three years due probably to environmental condition. In particular, from the above discussion and taking into consideration environmental parameters, both highest solar radiation and wind speed for the year 2020 led to dry condition (lowest air moisture) reducing ammonia emission, according, also, to what reported in the literature (Cameron et. al, 2013).

Therefore, data collected on 2018, 2019 and 2020 in this work, represented real emissions occurred under those particular environmental conditions. However, considering that three measurements were made in three different years characterized by diverse conditions, the average emission values obtained can be assumed as a good approximation of real ammonia emission occurred during fertilizers injection/spreading.

Figure 2. Cumulated ammonia loss (% TAN) in the hours following the spreading. The data reported refer to the average of the three years of experimentation on maize fields (2018, 2019 and 2020).



At the end of the trials, the average ammonia losses were similar and not statistically different (One-way ANOVA p = 0.92, *n* = 6), i.e. 11.6 ± 4 % TAN and 13.4 ± 4.5% TAN, respectively for digestate and urea (mean of the years 2018, 2019 and 2020) (Figure 2). Furthermore, according to Sommer and Olesen (2000), on average, about 48% of the total ammonia emitted during such experiments was likely to be emitted in the first 24 hours after fertilization. For all the three years, the ammonia emissions recorded were stable and close to zero after 80 h from the spreading (Figure 2).

To include the data reported in this work in a broader context, a comparative study was carried out with data from the literature, deriving from similar studies carried out using digestate (Table 4) both injected and spread on the surface, and urea (Table 5) distributed onto the surface. Unfortunately, not many data were reported from digestate used on maize so that the comparisons made include other crops. In addition, the use of different methods to measure ammonia emission make this comparison more difficult and this must be taken into consideration discussing the result afterwards.

Table 4. Ammonia emission measured in this work in comparison with literature data reporting experiment performed at full field.

Digestate origin	Spreading technique	Crop	Season	Ntot dosed (kg N ha^{-1})	NH_4^+ dosed (kg N ha^{-1})	NH_3 cumulated emission (kg N ha^{-1})	Loss of NH_3 (%Ntot)*	Loss of NH_3 (%TAN)^a	Measurement method	Reference
Sewage sludge	Injection 15 cm	Maize	Spring	370	229	34.2	9.24	14.9	ALPHA passive samplers	This work
		Maize	Spring	370	229	26.9	7.27	11.7		This work
		Maize	Spring	370	200	15.6	4.22	7.82		This work
Cattle slurry + energy crops	Injection 15 cm	Maize	Spring	130	65.7	7.1	5.46	10.8	ALPHA passive samplers	(Riva et al. 2016)
Food waste	Injection 10 cm	Grass	Spring	142	100	17	12	17	Wind tunnels	(Nicholson et al. 2018)
		Grass	Spring	106	75.3	17	16	22.6		(Nicholson et al. 2018)
		Grass	Autumn	117	79.6	12	10.3	15.1		(Nicholson et al. 2018)
		Grass	Autumn	151	122	43	28.5	35.2		(Nicholson et al. 2018)
Cattle + pig slurry	Injection 5 cm	Ryegrass	Spring	86	67	12	14	17.9	Wind tunnels	(Rubæek et al. 1996)
		Ryegrass	Spring	106	80	9	8.49	11.3		(Rubæek et al. 1996)
Pig slurry	Surface spreading	Timothy	Spring	700	485	200	28.6	41.2	Wind tunnels	(Chantigny et al. 2004)
Liquid Pig slurry		Timothy	Spring	140	-	17.7	12.6	-		(Chantigny et al. 2007)

Cattle + pig slurry	Ryegrass	Spring	110	70	35	31.8	50		(Rubæek et al. 1996)
	Ryegrass	Spring	106	78	20	18.9	25.6		(Rubæek et al. 1996)

The loss of ammonia (% TAN dosed) reported in this work for digestate (11.6% ± 4 on average, Table 3) was very similar to the data reported by Riva and colleagues (10.8%, Table 4) (Riva et al. 2016), which were carried out in the same climatic zone (Lombardy, Italy), with the same distribution technique (injection at 15 cm) and crop (maize) and adopting the same measurement method, i.e. passive sampler. However, it is interesting to note that Riva et al. (2016) dosed an amount of ammonia N (65.7 kg N ha^{-1}) equal to about one third to the amount used in this work (200 - 229 kg N ha^{-1}), from which it seems that the amount of N dosed was probably less relevant than other variables (i.e. climate and spreading techniques) in determining ammonia loss.

Table 5. Literature summary of ammonia emissions measured by fertilizing with urea spread on the surface in open fields (data from Rochette *et al.*, 2013).

N dosed (kgN ha^{-1})	N-NH_3 cumulated emission (kgN ha^{-1})*	Loss of N-NH_3/Ntot (%)	Reference
185	25	13.5	This work
185	33	17.8	This work
185	16.4	8.85	This work
89	12.5	14	(Musa 1968)
178	28.5	16	(Musa 1968)
255	51	20	(Musa 1968)
75	33	44	(Cai et al. 2002)
200	96	48	(Cai et al. 2002)
135	20.3	15	(Fan et al. 2005)
225	42.8	19	(Fan et al. 2005)

30	3	10	(Black, Sherlock, and Smith 1987)
100	17	17	(Black, Sherlock, and Smith 1987)
300	99	33	(Black, Sherlock, and Smith 1987)
80	11.2	14	(Rojas et al. 2012)
160	28.8	18	(Rojas et al. 2012)
56	26.3	47	(Ellington 1986)
112	73.9	66	(Ellington 1986)

*: values calculated (not reported in original paper)

Comparison made with other literature data (Table 4) was more difficult because all data were obtained using a different methodology, i.e. wind tunnel. Anyway, by using digestate distributed by injection on grass (Nicholson et al., 2018) and on ryegrass (Rubæek et al., 1996) emission measured were not so far from those measured in this work, i.e. N loss of 22.5± 9.1% TAN and N loss of 14.6 ± 4.7% TAN (average data), respectively. These values were lower than N loss obtained by distributing the digestate on the surface, which was, as average, of 38.9 ± 12% TAN (Chantigny et al. 2004; Rubæek et al. 1996). These data underlined the importance to inject digestate reducing N emission.

Ammonia emission due to urea use in this work have been compared with data in the literature (Table 5) that, like the spreading modality used in this work, all considered surface spreading. In our work the ammonia loss (% TAN dosed) registered was of 13.4 ± 4.5% TAN and so lower than the average calculated from the literature, i.e. 24.8% ± 16.6 (n = 17). However, since different methods have been used, the comparison made is only indicative. However, reports revealed a very wide range of data, from 10% TAN to 66% TAN (Musa 1968; Cai et al. 2002; Black, Sherlock, and Smith 1987; Fan et al. 2005; Rojas et al. 2012; Ellington 1986) (Table 5).

These differences may be due to multiple factors related to both climatic conditions and soil characteristics (Harrison and Webb 2001). Unfortunately, from such a heterogeneous group of studies, it was not possible to reconstruct a complete picture. However, as regards the data reported in this work, it is possible to hypothesize that the low percentage of ammonia lost by urea, compared to the average of the other works (Table 5), may be attributable to the low rainfall at our site during the period of the observations, since moisture is one of the main drivers for ammonia emission from urea (Cameron, Di, and Moir 2013).

Taking into consideration results obtained and the literature data, some suggestions can be given to reduce the ammonia emission using N fertilizers. First of all, spreading or distributing fertilizers onto the surface causes large ammonia emission so that it becomes essential to inject liquid fertilizers (i.e. digestate) and bury solid fertilizers (i.e. urea) (Sommer and Hutchings 2001), above all in presence of humid soil. Humidity has been reported playing an important role in ammonia emission from urea because it promotes its hydrolysis releasing ammonium (Cameron, Di, and Moir 2013). On the other hand, abundant rainfall or irrigation immediately after spreading have the effect to reduce ammonia emission for both urea and digestate, thanks to water that drains the dissolved ammonium in deep soil removing it from the soil-atmosphere interface (Sanz-Cobena et al. 2011).

Contrary to what one might think the amount of nitrogen dosed does not seem to have an impact on the percentage of ammonia lost.

4.3.3 Odour emission

The odour emission measurements reported in this work were carried out in both lab scale (potential odour emission) and open field. Measuring potential odour (lab scale) is very useful because it allows to measure the odour emitted by different fertilizers and so their potentiality in emitting odours when then they are used in full field. In addition, this measurement allows measuring odours from fertilizers excluding all environmental variables that in the open field can heavily influence the result (Riva et al. 2016; V. Orzi et al. 2018). The successive comparison between potential odour emission (lab scale measurement) and odour emitted in the open field for the same fertilizer, allows estimating the impact of environmental variables on the values measured in open field, including soil injection and soil incorporation (Orzi et al., 2018).

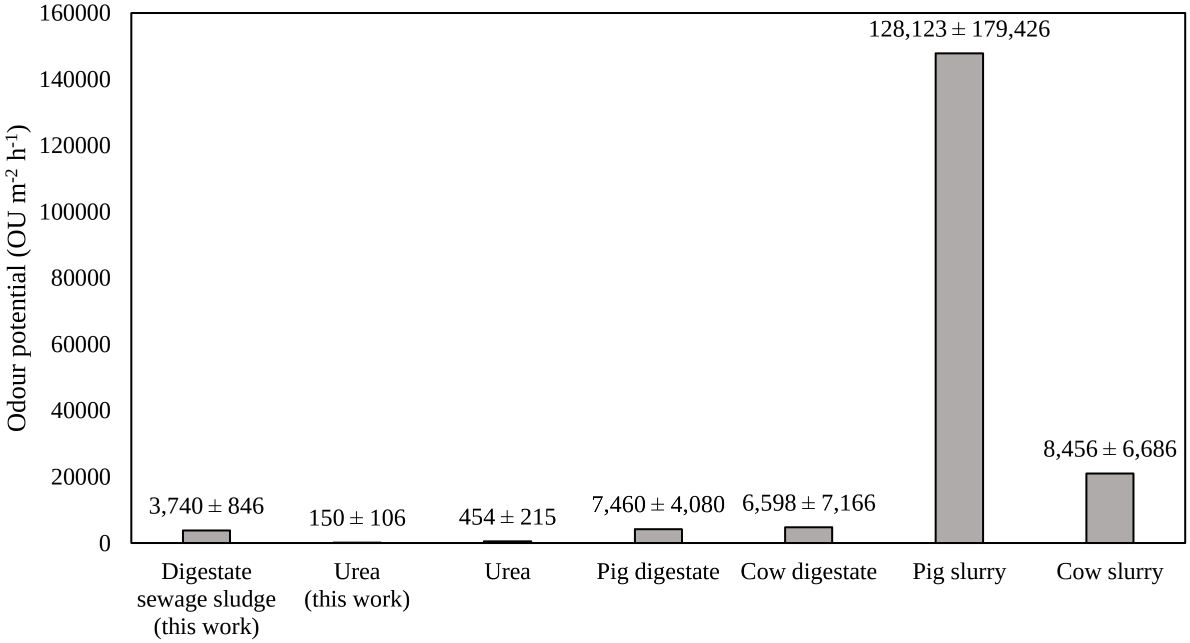
However, odors emission detections suffer for high variability that is an intrinsic characteristic of these measurements (Hudson et al. 2007), making it difficult to carry out statistically robust comparisons. The variability is due to both the large number of factors affecting odour emission, especially from biomass (M. Zilio et al. 2020), and technical difficulties in performing measurements (Hudson et al. 2007). In addition, the dynamic olfactometry method, despite it being the reference method for this type of measurement, suffers from low reproducibility of data due to human error (Van Harreveld et al., 1999; Hove et al., 2017). Keeping in mind these limitations, data obtained in this work are below discussed.

Digestate used in this work showed a potential odour emission measured at lab scale of 3,740 ± 846 OU m^{-2} h^{-1} (Figure 3) in line with data reported, on average, for agricultural digestate ($OU_{dig.}$ = 4,454 ± 5,217 OU m^{-2} h^{-1}; n = 25) (Orzi et al., 2015; Orzi et al., 2018).

Literature reported that anaerobic digestion, because it degrades the easily degradable organic matter and concentrates the more recalcitrant compounds (Orzi et al., 2015; Orzi *et al.*, 2018; Zilio *et al.*, 2020) reduces potential odour production (Orzi et al., 2015). Therefore, it was interesting, for the purposes of the discussion, to compare the odour emission values from the same substrates before and after anaerobic digestion. Unfortunately, in this work it was not possible to test feed sewage sludge, because it was represented by a mix of different substrates (more than 60) that varied during the year. However, the liquid digestate used, because it was stocked in a 50,000 m^3 tank before agricultural use, allowed taking representative samples to be measured.

Therefore, data obtained for the digestate used in this work were compared with those coming from previous studies for both digestates and non-digested material (V. Orzi et al. 2018; Valentina Orzi et al. 2015). From Figure 3 the digestate for this work showed a lower potential odour emission than those reported for pig and cow digestates (7,460 ± 4,080 and 6,598 ± 7,166 OU m^{-2} h^{-1} respectively), although the high standard deviation did not allow statistical differences to be established. On the other hand, observing the potential odour emission from the same undigested biomasses, very high values were registered for pig slurry (128.123 ± 179.426 OU m^{-2} h^{-1}), unlike cow slurry that showed a potential odour emission (8.456 ± 6.686 OU m^{-2} h^{-1}) not so far from that of cow digestate.

Figure 3. Potential odour emissions measured in laboratory for the digestate used in this work in comparison with other organic matrices (data from Orzi *et al.*, 2015, 2018) (mean ± SD).



The difference between pig and cow slurry can be ascribed to the fact that the second one was made by lignocellulosic residual material partially anaerobically digested (by a polygastric mammal), which underlined the importance, in addition to anaerobic digestion, of the organic substrate's origin (Scaglia et al. 2018). In this way, because sewage sludge represents a partially digested organic material coming from a wastewater treatment plant, low potential odour emission can be ascribed to both the material origin and to the subsequent anaerobic digestion. This fact was confirmed by biological stability degree of digestate measured by both aerobic (OD_{20}) and anaerobic (BMP) tests (Scaglia et al., 2018), i.e. OD_{20} of 22.7 ± 6.1 mg O_2 g^{-1} dw and BMP of 57 ± 23 L_{biogas} kg^{-1} dw that were in line (OD_{20}) or lower (BMP) than those measured for two green composts, i.e. 15.06 ± 0.3 mg O_2 g^{-1} dw and 10.3 ± 1.1 mg O_2 g^{-1} dw, and 144 ± 3.8 L_{biogas} kg^{-1} dw and 201 ± 20 L_{biogas} kg^{-1} dw, respectively (Scaglia et al., 2018).

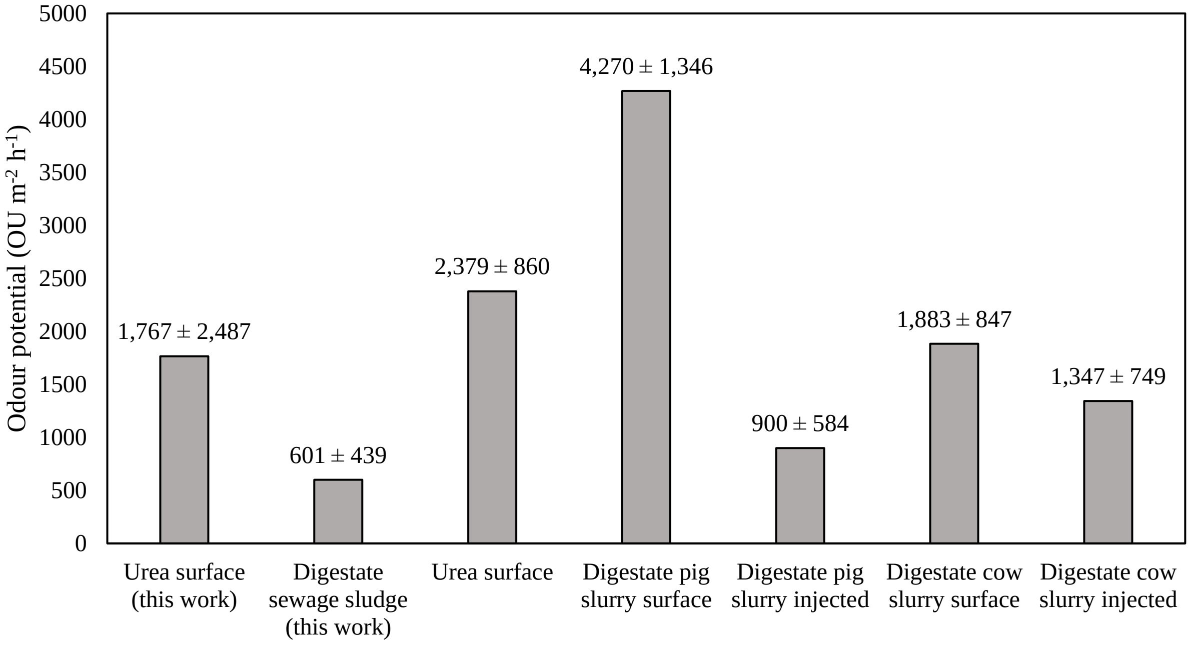
The urea, as expected, showed the lowest potential odour emission value, i.e. 150 ± 106 OU m^{-2} h^{-1}, not so far with previous data 454 ± 215 OU m^{-2} h^{-1} (V. Orzi et al. 2018).

In open field experiments (2018, 2019 and 2020) odour emissions from the experimental plots were measured each year immediately after fertilization (Table 3). The results showed that, considering the three years average, the odour emitted by the plots fertilized with digestate was very low, i.e. 601 ± 531 OU m^{-2} h^{-1}, and similar to that emitted by non-fertilized plots (633 ± 494 OU m^{-2} h^{-1}). The plots fertilized with urea, on the other hand, showed a higher average odour emission (1,767 ± 2,221 OU m^{-2} h^{-1}) than the digestate-fertilized plots, but were not statistically different, probably due to the high variability that is typical of odour measurements.

Therefore, odour emission measured for digestate studied in this work in the open field was much lower than the potential odour measured at lab scale (Figure 3). This difference was most likely due to the injection of digestate into the soil which was able to reduce the odour emission, as previously described (Riva et al. 2016; V. Orzi et al. 2018). On the other hand, urea odour emission measured in the full field, was, as an average of the three years tested, of 1,767 ± 2,221 OU m^{-2} h^{-1} much higher than the potential measured i.e. 150 ± 106 OU m^{-2} h^{-1}. Probably in this case soil and air moisture, promoting a fast urea hydrolysis, stimulated ammonia emission. As known, ammonia has a low olfactory threshold (odour threshold between 0.0266 and 39.6 mg m^{-3}) (Rice and Netzer 1982), thus its rapid release may have produced an increase in odour emission.

Observing the data reported in Figure 4, the digestate used in this work by injection showed odour emission that was not so different from data reported for injected pig and cow digestates, measured previously adopting the same methodologies (V. Orzi et al. 2018), i.e. 900 ± 584 OU m^{-2} h^{-1} and 1347 ± 749 OU m^{-2} h^{-1}, respectively).

Figure 4. Odour emissions measured in full field for different fertilizers (data from Orzi *et al.*, 2018) compared with those measured for digestate and urea used in this work (mean ± SD).



These data confirmed the validity of the injection method to limit odour emission, confirmed by the comparison of data for injected pig digestate with spread pig digestate, i.e. 900 ± 584 OU m^{-2} h^{-1} vs 4,280 ± 1,346 OU m^{-2} h^{-1}, respectively (Orzi et al., 2016). On the other hand, no substantial differences can be observed between injected cow digestate and surface spread cow digestate, i.e. 1,347 ± 749 OU m^{-2} h^{-1} vs 1,883 ± 847 OU m^{-2} h^{-1}, respectively (Orzi et al., 2018), indicating that the most important factors involved in odour reduction during agronomic use of digestate are the spreading technique (injection vs. surface spread), the treatment (digestate vs. non-digestate) and the biomass origin.

4.4 Conclusions

This work showed that the use of digestate from sewage sludge as a fertilizer in agriculture can replace urea without increasing ammonia emission. The injection of digestate into the soil has been confirmed as a good technique for reducing ammonia emission, allowing it to reach levels comparable to those typical of surface fertilization with urea. Ammonia emission can be further reduced by improving the injection system: preliminary data indicated that the use of a flexible anchor reduced emissions with respect to the use of rigid ones.

Concerning the emission of odour, it has been observed that digestate from sewage sludge emits less odour than digestates from livestock manure, and if injected into the soil its emission was reduced to a level that was no longer distinguishable from that of non-fertilized soil.

The digestate dosed allowed producing maize at the same rate as the urea confirmed the good fertilizing properties of both dressings.

In conclusion, anaerobic digestion plus liquid digestate injection were confirmed as good practice to provide a suitable fertilizer, replacing the synthetic fertilizer in an environmentally sustainable way, i.e. with low ammonia and odours emissions.

Author Contributions

FA: designed the project, elaborated and interpreted the data and wrote the paper

MZ: collected, elaborated and interpreted the data and wrote the paper

AG and GG: managed the experimental field and the agronomic operations

AP and BR: collected the data

EM and OS: Scientific contribution and manuscript correction.

5 Using highly stabilized digestate and digestate-derived ammonium sulphate to replace synthetic fertilizers: the effects on soil, environment, and crop production.

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Recovered fertilizers (a highly stabilized digestate and ammonium sulphate) obtained from anaerobic digestion of sewage sludge, were used on plot trials with a maize crop, in a comparison with synthetic fertilizers. After three consecutive cropping seasons, the soils fertilized with the recovered fertilizers (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 utilising 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.

5.1 Introduction

During the second half of the twentieth century, in particular since the late 1960s, agriculture throughout the world underwent 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 (Khush 2001). 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 (Foley et al. 2011).

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 (Pingali 2012). 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 (FAOSTAT, 2021) 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 (Fowler et al. 2013; Galloway et al. 2014). 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 (C. Smith, Hill, and Torrente-Murciano 2020).

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 (Ridder et al. 2012). Current reserves of phosphate minerals are estimated at 67,000 TgP 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 (Geissler, Mew, and Steiner 2019) 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. The peak of phosphorus extraction, i.e., the point after which the annual extractable amount will no longer be able to increase is difficult to predict, and there is no univocal consensus in the literature. However, in accordance with numerous works it is expected for 2030, and the depletion of global reserves is likely before the end of the XXI century (Cordell, Drangert, and White 2009; Schoumans et al. 2015). 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: this in turn affects the cost of food (Cordell and White 2011). 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 (W. Steffen et al. 2015; Toop et al. 2017; Johan Rockström et al. 2009a). 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 (Bennett, Carpenter, and Caraco 2001; V. H. Smith, Tilman, and Nekola 1999). 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 (Espejo-Herrera et al. 2016; Padilla, Gallardo, and Manzano-Agugliaro 2018).

Using organic wastes as fertilizers (recovered 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 a circular economy (van Dijk, Lesschen, and Oenema 2016). However, untreated organic wastes do not represent acceptable fertilizers (Westerman and Bicudo 2005) because of their origin and therefore intrinsic risks for potential contamination: 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 (Gros et al. 2019; Van den Meersche et al. 2016; Berendsen et al. 2015; Albihn 2002; Zwolak et al. 2019). Furthermore, their nutrient content and plant availability are difficult to control, so that they are not often able to replace synthetic mineral fertilizers. Finally, 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 (Westerman and Bicudo 2005). To transform these biomasses into products which can be utilised in agriculture, technological/biotechnological treatments are therefore necessary (Ivona Sigurnjak et al. 2019). 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 (Massimo Zilio et al. 2021; Verdi et al. 2019; Pigoli et al. 2021; Tambone et al. 2019).

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 lead to emissions of ammonia and greenhouse gases (N_2O) into the atmosphere (Cameron, Di, and Moir 2013; Delgado 2002).

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 (Govasmark et al. 2011). Although progressively more studies in recent years are shedding additional light on the safety of digestates for agricultural use, as well as on the best process methods and accurate selection of feedstock, there are still only very limited data available on the impacts that the use of these biomasses have on full scale agriculture in the field (Koszel and Lorencowicz 2015; Pigoli et al. 2021; Massimo Zilio et al. 2021; Verdi et al. 2019).

There are very few full scale works which report on the ability of digestate and digestate-derived fertilizers to replace synthetic fertilizers (Riva et al. 2016; Verdi et al. 2019) and none which has considered both agronomic, soil chemical and environmental aspects together. All these points are very important to establish the suitability of recovered fertilizers in promoting circular economy approaches in crop fertilization, i.e. nutrient and organic matter recycling. Therefore, the power of this work consists in testing recovered fertilizers produced by full scale plants (Pigoli et al. 2021) vs. synthetic mineral fertilizers by using a holistic approach which considers agronomic (crop production and N efficiency), soil chemistry (soil chemical-physical characteristics) and environmental performance. In this latter case, GHG (N_2O, CO_2 and CH_4 gases) and ammonia emission, nitrate leaching, and inorganic and organic pollutants have been considered.

In addition, the use of well stabilized digestate allowed focusing on the importance of the biological stability of organic matter composing the biofertilizers in contributing to both soil organic carbon turnover (amendment properties) and in limiting N-derived impacts such as nitrate leaching and nitrous oxide emission.

5.2 Materials and Methods

5.2.1 Agronomic full field trials

Fertilization date, fertilizers used, doses applied, and spreading methodology for the different treatments are summarized in Table 1. Agronomic trials tested, at plot scale, the fertilizer properties of recovered fertilizers (digestate from organic wastes combined with digestate-derived ammonium sulphate) vs. synthetic fertilizers; an unfertilized treatment was included as control. Fertilizers were tested on plots of 350 m^2 (width 5.9 m, length 60 m) 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. The arrangement of the plots is shown in Figure S1.

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 (Regione Lombardia 2020). Efficiency suggested by regional rules takes into consideration the content of readily available N (ammonium) that roughly coincides with digestate ammonium content used in this work, since the organic fraction is not considered to be readily available for plant nutrition. Nitrogen fertilization was completed by using ammonium sulphate produced starting from digestate (Pigoli et al., 2021) 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 by using routine procedures. Urea was used at pre-sowing and commercial ammonium sulphate as a top dressing (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). Digestate-derived ammonium sulphate and commercial ammonium sulphate have been considered equivalent. Regarding urea, it was dosed all in a single spread in order to standardize N fertilization with that of plots treated with digestate. Finally, the unfertilized plots (U) did not receive any type of fertilization for the entire duration of the experiment.

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

5.2.2 Fertilizer sampling and analysis

The digestate used in this work derives from anaerobic digestion of sewage sludge, while ammonium sulphate used in RF treatment was obtained by ammonia stripping during the same anaerobic digestion process. The details of the process to produce fertilizers and their characteristics have been widely described and discussed in previous work (Pigoli et al., 2021; Zilio et al., 2021); fertilizer data are reported in Chapter 3 Tables 1 and 2. The digestate in particular showed a high biological stability (BMP: 89 ± 17 L_{biogas} kg^{-1} dw) compared to other agricultural digestates (Pigoli et al. 2021). The N (75 ± 5 g kg^{-1} dw, TAN/TKN: 57.8%) and P (32.3 ± 1.7 g kg^{-1} dw) total contents, were significantly higher than those reported in literature for agricultural digestates (H. Xu et al. 2020; Yun et al. 2021; Wang et al. 2021), while the total K content (5.73 ± 0.5 g kg^{-1} dw) was slightly below (H. Xu et al. 2020; Yun et al. 2021; Wang et al. 2021). Ammonium sulphate showed a high total N content (74 ± 2 g kg^{-1} on wet weight, TAN/TKN: 97%) and low concentration of heavy metals.

The digestates used in this work were sampled immediately before they were injected in the field and the analyses took place in the hours immediately following sampling. 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 (APHA 1992). Total nitrogen (TKN) and ammonia nitrogen (TAN) were determined according to the analytical method for wastewater sludges (IRSA CNR 1994). Heavy metals, total P and K content was assessed by inductively coupled plasma mass spectrometry (Varian, Fort Collins, USA), preceded by acid digestion (EPA 1998) of the samples. All the analyses were carried out in triplicate. Biochemical methane production (BMP) was determined following the biological method reported in Schievano et al. (2008), according also to the European regulations for fertilizers (EU 2019). Nevertheless, physical methods such as thermal analysis can also be proposed (Li et al. 2021; Xing et al. 2021).

Organic micropollutants were detected as follows: C10-C40 hydrocarbons by UNI EN 14039 (UNI, 2005) method, halogenated organic compounds (AOX) by Gas Chromatography (GC) approach (UNI EN ISO 22155:20161) (UNI, 2016) and EPA 8270E 20181 (EPA, 2014) + EPA 3550C 2007) (EPA, 2007a). PCDD/Fs were measured using UNI 11199 (UNI, 2007) method, PCBs through UNI EN 16167 (UNI, 2012) and UNI EN 16167 (UNI, 2019), and DEHP through EPA 3550C (EPA, 2007a) + EPA 8270E (EPA, 2018) methods.

Emerging organic pollutants (pharmaceuticals), i.e. Ciproflaxacin, Sulfamethoxazole, Fenofibrat, Gemfibrozil, Carbamazepine, Metoprolol, Diclofenac, Ethinylestradiol and Estradiol were detected by HPLC-MS following EPA 3550C (EPA, 2007a) and EPA 8321B 2007 methods (EPA, 2007b).

Faecal coliforms and *Salmonella* were determined as reported in CNR IRSA 3 (CNR IRSA, 1983) (Faecal coliforms) and ISTISAN 14/18 (ISTISAN, 2018) + APAT CNR IRSA 7080 (*Salmonella*) (APAT, 2003).

5.2.3 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 (Gee and Bauder 1986). Cation Exchange Capacity (CEC) was determined by saturating the samples with $BaCl_2$ (Rhoades 1982), texture by the pipette method (Gee and Bauder 1986), total organic carbon by the Walkley and Black method (Olsen, Sommers, and Page 1982) and total nitrogen by the Kjeldahl method (Faithfull 2002). 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 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, i.e., three samplings in 2019 (before pre-sowing fertilization, at the moment of maximum mineralization of N added to the soil and at harvest). In 2020, in order to consider, also, nitrate leaching between pre-sowing fertilization and the maximum mineralization and after topdressing fertilization, two further samplings were added for a total of five. Details on sampling dates and agronomic operations are reported in Table S3. 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 (Faithfull 2002).

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 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 (EPA 2007a) and EPA 8321B 2007 methods (EPA 2007b).

The amounts of carbon and organic pollutants added to the soil by digestate fertilization were calculated using the following formula:

$$\frac{C_{dig} \times Dig\ dosed}{(S \times d \times b) \times C_{soil}}$$

Where:

C_{dig} is the concentration of the element in the digestate dosed (Table S1), Dig dosed is the amount of digestate dosed per hectare (48,544 kg ha^{-1}), S is the soil surface (10,000 m^{2}), d is the reference soil depth (0.15 m), b is the soil bulk density (1,200 kg m^{-3}), and C_{soil} is the concentration of the element in the soil before receiving fertilization (Table 2).

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
ΣAOX	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

5.2.4 Ammonia and greenhouse gas (GHG) emission measurement

The ammonia emission data reported in this work, together with the methods used for the measurements, were previously published in Zilio et al. (2021) (see Supporting information).

GHG fluxes (N_2O, CH_4 and CO_2) were measured from 28/05/2020 to 17/03/2021 (a detailed calendar of the sampling dates is reported in Table S4) using the closed static chambers method (Bertora et al. 2018). 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 (Piccini et al. 2017).

The original Hutchinson and Mosier (HM) three-points calculation was used when non-linear accumulation was detected; otherwise, Linear Regression has been used (Maris et al. 2021; Bertora et al. 2018). In order to preserve linear conditions, big volume chambers and short deployment times (0-10-20 mins) were used.

Measured environmental temperature has been exploited to correct gas concentrations for each sampling date. Since insulated metal chambers were used, increase in temperature inside the chamber over the deployment time was negligible (Bertora et al. 2018). 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 (Peyron et al. 2016). Finally, the cumulative emissions were obtained by estimating the flows in the non-sampling days, by means of linear interpolation.

5.2.5 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 (EPA 1998) of the samples. All the analyses were carried out in triplicate.

N grain content was assessed by the combustion method (Dumas method) (Saint-Denis and Goupy 2004). 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 by using an elemental analyser (Rapid max N exceed, Elementar, Lomazzo, Italy).

5.2.6 Fertilizer use efficiency

The N fertilizer use efficiency (FUE) for Synthetic and Recovered fertilizers, and N fertilizer replacement value (NFRV) for Recovered fertilizers only were calculated according to Sigurnjak et al. (2017). The two parameters were calculated following the formula:

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

$$NFRV(\%) = \left[\frac{\dfrac{(N\,uptake\,recovered\,fertilizers - N\,uptake\,unfertilized)}{total\,N\,applied\,recovered\,fertilizers}}{\dfrac{(N\,uptake\,synthetic\,fertilizers - N\,uptake\,unfertilized)}{total\,N\,applied\,synthetic\,fertilizers}} \right] \times 100$$

5.2.7 Statistical analysis

The comparisons between treatments were carried out through One-way ANOVA analysis, with Tukey post-test. Unless otherwise specified, the significance limit value p was set at 0.05 for all the analyses carried out. The statistical analyses were carried out using IBM SPSS® 23 software. The plots were obtained through the use of Microsoft EXCEL 2016.

5.3 Results and discussion

5.3.1 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. 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). In fact, during the three years of experimentation, the plots fertilized with RF received a total of 4,427 kg C ha^{-1} dw, equal to 24% of the organic carbon already present in the surface 15 cm of soil at the beginning of the experiment. Furthermore, the organic carbon contained in the digestate was particularly 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) (Pigoli et al. 2021). 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 (Greenberg et al. 2019; Alburquerque, de la Fuente, and Bernal 2012), 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 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.

Given that the concentrations of reactive nitrogen (N-NH_4^+ and N-NO_3^-) remained similar between all the experimental soils (see section 3.3.1), the observed increase can be attributed to the organic N component to which contributed both high-stabilized organic matter added with RF and plant root and bacterial biomass developed in response to the high availability of nitrogen (SF) (Geisseler and Scow 2014; Singh 2018).

5.3.2 Agronomic performance of recovered fertilizers and product safety

The amounts of maize grain produced (the average of 2018, 2019 and 2020 crop seasons) using recovered fertilizer (18.1 ± 2.9 Mg dried grain ha^{-1}) (Chapter 4 Table 3)were similar and not statistically different from that produced with synthetic fertilizers (17.4 ± 1.2 Mg dried grain ha^{-1}). This indicated that recovered fertilizers are capable of substituting for synthetic fertilizers.

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 detected did not show significant differences between the grains produced using SF and RF as fertilizers.

However, RF fertilized plants produced grains containing more Zn than plants grown with SF, i.e., 32.1 ± 1.9 vs 25 ± 2 mg kg^{-1} dw for RF and SF respectively. This was probably due to the high intake of Zn supplied to the soil with the digestate, as discussed in paragraph 2.2.2, which increased its availability for plants. These values were in line with those reported in the literature for both maize grain and other cereals (i.e., rice and wheat) (Ullah, Ali, and Farooqi 2010; Ertl and Goessler 2018). Furthermore, zinc is an essential element, and among cereals, maize is usually poor in it (Cakmak and Kutman 2018).

Table 3. Element content in maize grain produced in 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).

5.3.3 Environmental safety

5.3.3.1 Potential ammonia emissions and nitrate leaching

Ammonia (NH_3) emissions were measured directly during plot trials, as described in the M & M section. These data have been already discussed in a previous work (Massimo Zilio et al. 2021) and they are here reported to complete the data set provided by the experimentation made. Therefore, ammonia emission data are briefly discussed, referring any details to the previous work done.

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 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 (Zilio et al., 2021) (Chapter 4 Table 3).

Regarding the risk of N leaching from the soils, obtained results 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 (Figure 1).

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, and that in one case (pre-fertilization 2020) NO_3^- concentration was lower (Figure 2). Indeed, average NO_3^- concentrations at 1-meter depth 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}). These figures are particularly interesting if it is considered that the unfertilized soil did not receive any N fertilizers throughout the three years, unlike the fertilized soil plots which every year received 470 and 285 kg ha^{-1} of N, respectively, for RF and SF plots. Although eight samplings during two crop seasons cannot be considered a continuous monitoring, able to exclude leaching due for example to abundant and rapid water flows, data obtained for the eight samplings (Figure 2) seem to suggest similar behaviour for SF and RF.

Soil texture can greatly influence the tendency of a soil to leach nitrate (Gaines and Gaines 1994). The experimental soil used in this experimentation was classified as a loamy soil (Table S7), although the soil layer between 50 and 75 cm from the surface showed high percentages of clay (36.6 %) that can slowly nitrate leaching. These results agree with those previously found in experiments on soils with different texture characteristics fertilized with either mineral or organic N fertilizers performed in the same geographical area (Lombardy, Po Valley) (Massimo Zilio et al. 2020). 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 (Zilio et al., 2020).

Figure 1. Concentrations of ammonia nitrogen ($N\text{-}NH_4^+$) 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.

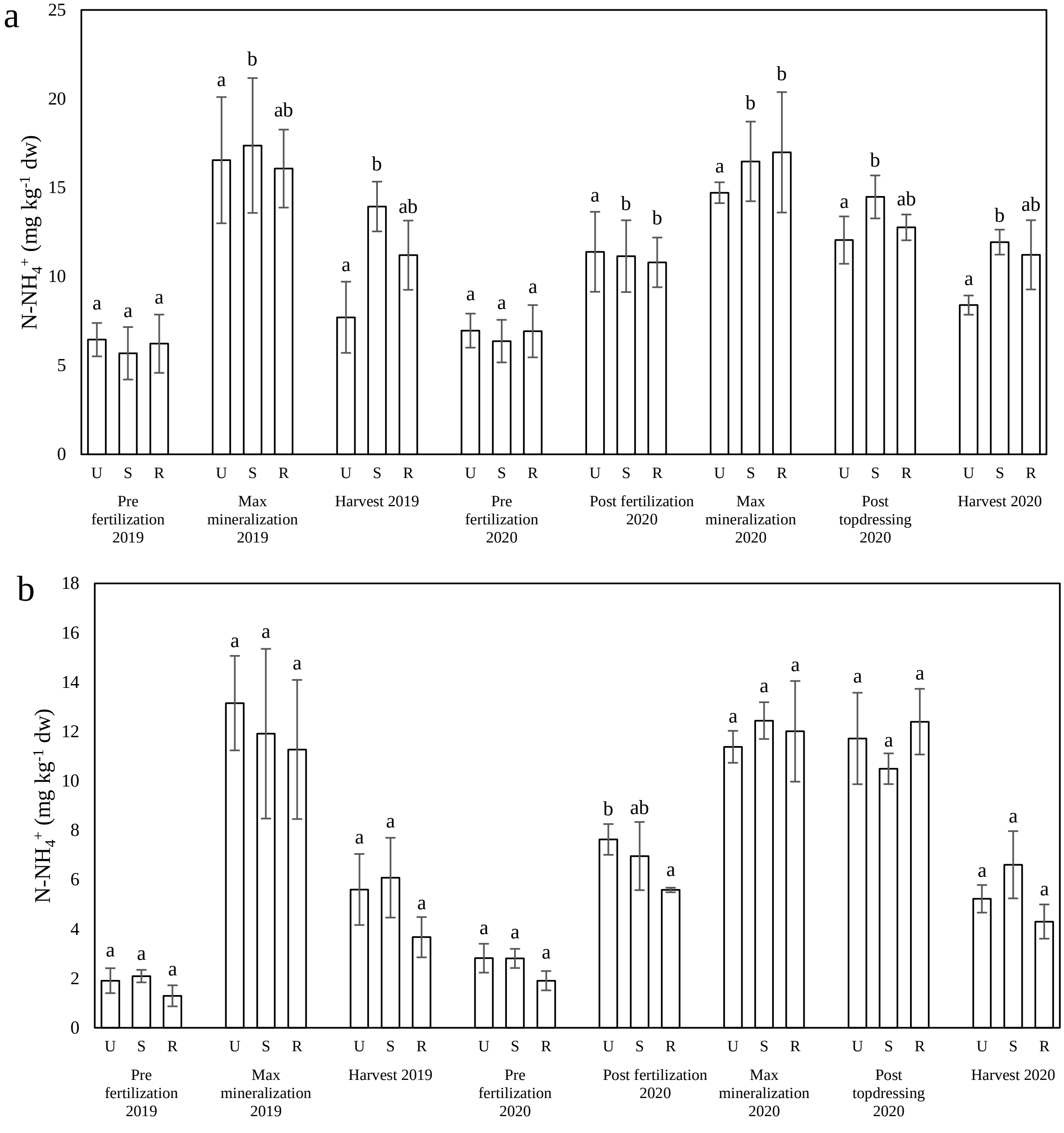
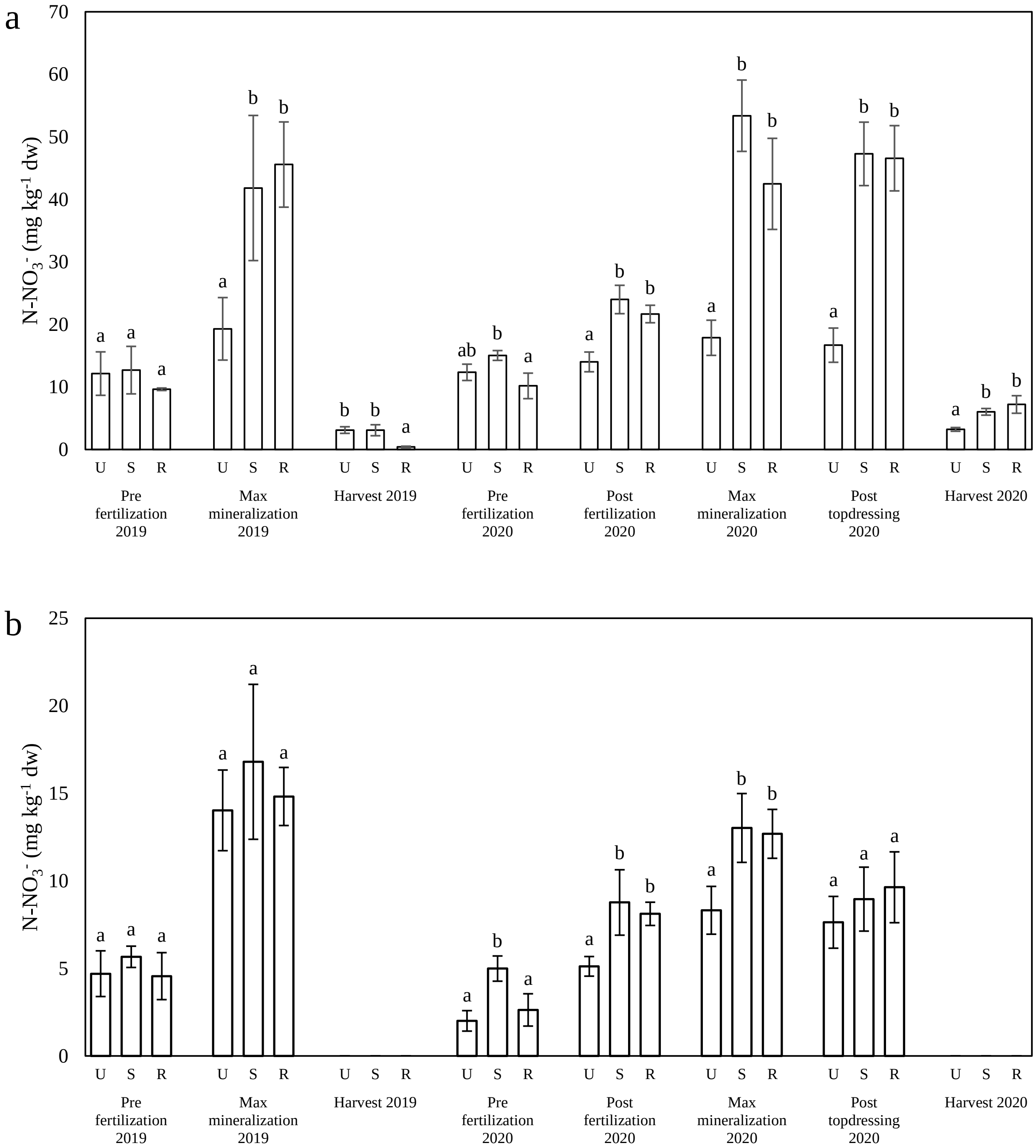


Figure 2. Concentrations of nitric nitrogen (N-NO_3^-) in experimental soils (a: surface; b: 1 meter depth) during the crop seasons 2019 and 2020 (mean ± SD; n=3). U: untreated, S: synthetic, R: recovered. Letters are referred to One-way ANOVA ($p<0.05$; Tukey post-test) comparing the three treatments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



5.3.3.2 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 cumulative amounts of CO_2 emitted in the 10 months of monitoring 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 treatments 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. The plots fertilized with RF emitted an amount of N_2O not statistically different from those fertilized with SF.

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 (Davidson 2009). 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 validated by the experimental measurements.

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 (Montemayor et al. 2019; Verdi et al. 2019). 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 is an important factor in understanding the fate of N in the soil (potential NO_3^- leaching and potential N_2O production).

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).

5.3.3.3 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 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 (Dragicevic, Sogn, and Eich-Greatorex 2018; Barłóg, Hlisnikovský, and Kunzová 2020).

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 after three years of application.

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 (Fabietti et al. 2010; Tran et al. 2015; Manz et al. 2001). 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 (Konradi and Vogel 2013). 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) (Pigoli et al. 2021).

5.3.4 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), because it is impossible to separate ammonium from organic forms. 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. Taking into consideration data above reported it could be reasonably assumed for the ammonium fraction of RF a FUE very close to that of SF, and that organic-N was not available.

The stability of organic N was confirmed, 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 ammonium 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

5.4 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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Author Contributions

FA: designed the project, elaborated and interpreted the data and wrote the paper.

MZ: designed the project, collected data, elaborated and interpreted the data and wrote the paper.

FT: managed the soil analysis, collected the data.

AP: managed field samplings and fertilizers/plants analysis.

BR: managed field samplings and soil analysis.

AH: managed field samplings, help in collecting the data.

AG: designed the project, managed the experimental field.

GG: managed the experimental field and the agronomic operations.

EM and OS: Scientific contribution and manuscript correction.

6 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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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 aims to demonstrate that the use of RF (Scenario RF) reduced total impact related to crop fertilization compared to SF (Scenario SF).

Scenario RF showed better environmental performances than the system encompassing the production and use of urea and synthetic fertilizers (Scenario SF). In particular, 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.

6.1 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.(J Rockström, Gaffney, and Thunberg 2021) 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.(Will Steffen et al. 2015) 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.(Johan Rockström et al. 2009b)

The regular production and use of mineral fertilizers in agriculture carries a long term negative footprint in the environment(Welch 2002) 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,(Christensen et al. 2014) 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),(Galloway et al. 2003; FAOSTAT 2020) generating big environmental problems. In addition, the production of P and K fertilizers relies upon non-renewable and extracted resources that are becoming depleted(Daneshgar et al. 2018) and are concentrated (e.g. P) in only a few countries.(Desmidt et al. 2015) 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.(Stahel 2019)

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,(Pigoli et al. 2021) producing fertilizers to replace synthetic ones.(McDonough 2002) 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.(Yasar et al. 2017; Mazzini et al. 2020; Tambone et al. 2010) 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.(Pepè Sciarria et al. 2019; Ivona Sigurnjak et al. 2019; Ledda et al. 2013; Pigoli et al. 2021)

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),(Sutton et al. 2018) 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,(Anastasiou et al. 2015) which are attributed to the high carbon dioxide emissions which result from the intensification of agricultural practices.(Lal 2009)

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.(X. Zhang et al. 2015; Galloway et al. 2003) 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.(Riva et al. 2016; Massimo Zilio et al. 2021) 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%).(Webb et al.

2010; Basosi et al. 2014) Therefore, the increase of NUE and environmental outcomes of recovered fertilizers represent challenges for modern agriculture.(Webb et al. 2013)

Recently, a scientific paper described,(Pigoli et al. 2021) 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.

6.2 Materials and Methods

6.2.1 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”(Klöpffer 2012) as it analysed a large full-scale anaerobic digestion plant used to transform organic wastes into bio-fertilizers (production phase),(Pigoli et al. 2021) and the subsequent full field application of the recovered bio-fertilizers (digestate and ammonium sulphate).

6.2.2 System description

6.2.2.1 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).(Pigoli et al. 2021) 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 parameters 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.(Pigoli et al. 2021) 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.(Di Capua et al. 2021; Pigoli et al. 2021) 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.

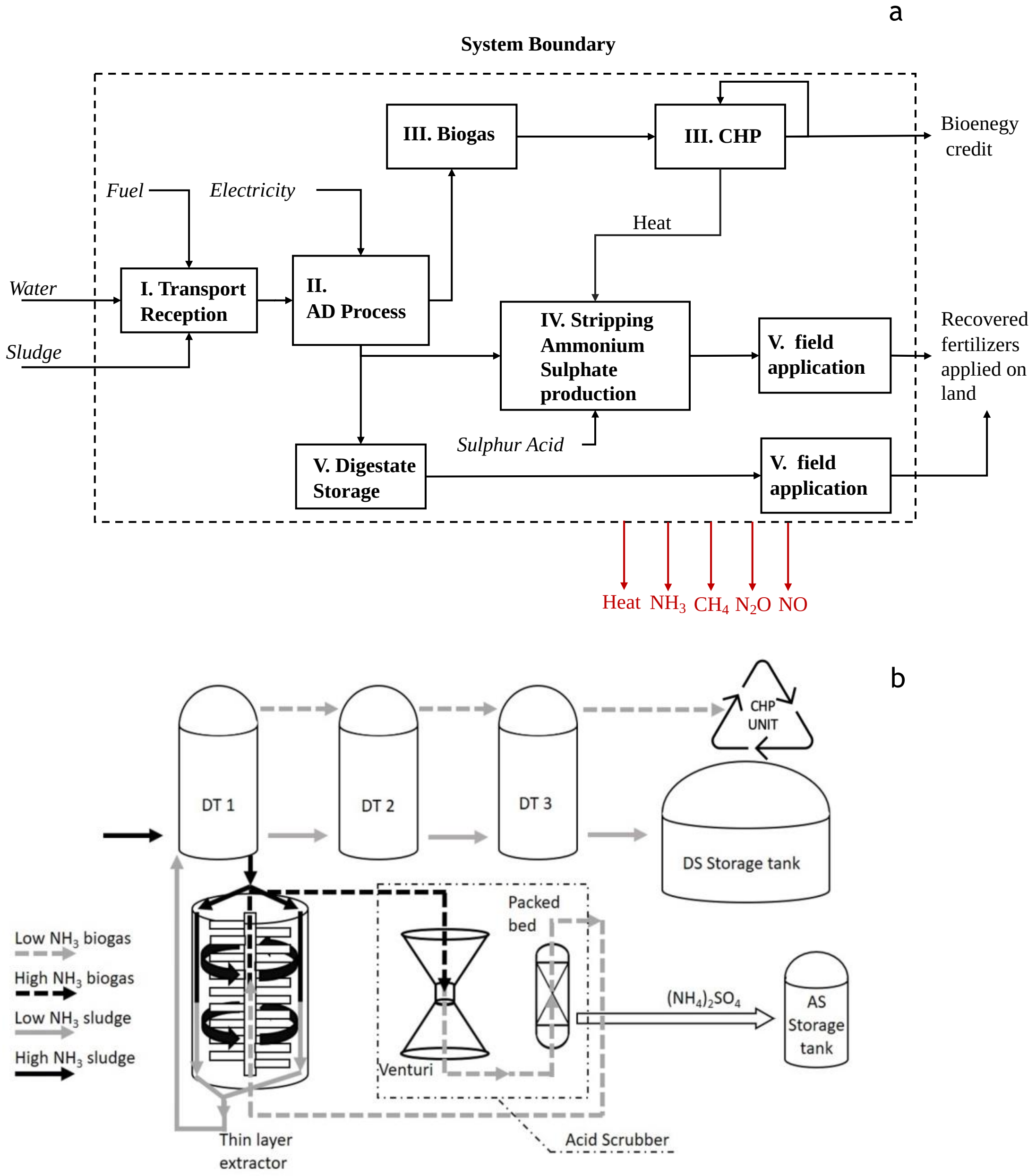

Figure 1. AD plant and N-stripping unit layouts (a); system boundaries and main processes for the Recovered Fertilizers (RF) (b).

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).

6.2.2.2 Recovered fertilizers produced

Recovered fertilizers (renewable fertilizers) characteristics can be found in Pigoli et al. (2021).(Pigoli et al. 2021) The previous characterization made also included organic contaminants and target emerging organic contaminants (Chapter 3 Table 2).

6.2.3 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.(Regione Lombardia 2020) 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 Chapter 5 Table 1 and summarised in Table 1.

6.2.3.1 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.(Bertora et al. 2018) 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.(Piccini et al. 2017) The cumulative emissions were obtained by estimating the flows in the non-sampling days, by linear interpolation.(Peyron et al. 2016)

The concentration of NH_3 was monitored by the exposure of ALPHA passive samplers.(Riva et al. 2016; Tang, Cape, and Sutton 2001) 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.

6.2.4 System boundaries and data inventory

6.2.4.1 System boundaries

The system boundary starts from the organic waste collection and transport, encompasses the production of digestate/bio-fertilizer and ammonium 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).(Weidema et al. 2013)

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). Indirect dinitrogen monoxide and NOx were estimated according to IPCC (2006).(IPCC (Intergovernmental Panel on Climate Change) 2006) Nitrate leaching was calculated according to IPCC (2006)(IPCC (Intergovernmental Panel on Climate Change) 2006) 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. Phosphorus in soil, leaching and run off was modelled according to Ecoinvent report 15.(Nemecek and Kägi 2007) Heavy metals supplied were included in the model according to the characterization data of digestate, plant uptake and accumulation rate in the soil system.(Y. Xu et al. 2013; G. Börjesson, Kirchmann, and Kätterer 2014) The input of organic pollutants was considered for PCDD/F, DEHP, PAH contained in digestate, as a proper numerical quantification was workable (see Chapter 3 Table 2).

6.2.4.2 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).

6.2.4.3 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-NH_4)	kg ha^{-1}	25.2	Detected on-site by the authors (Chapter 4)
Direct dinitrogen monoxide (N-N_2O)	kg ha^{-1}	9[a]	Detected on-site by the authors (Chapter 4)
Indirect dinitrogen monoxide (N-N_2O)	kg ha^{-1}	0.8	IPCC 2006
Nitrate leaching (N-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-NH_4)	kg ha^{-1}	25.2	Detected on-site by the authors (Chapter 4)
Direct dinitrogen monoxide (N-N_2O)	kg ha^{-1}	9[a]	Detected on-site by the authors (Chapter 4)
Indirect dinitrogen monoxide (N-N_2O)	kg ha^{-1}	0.8	IPCC 2006
Nitrate leaching (N-NO_3)	kg ha^{-1}	83[b]	IPCC 2006
NOx (N-NOx)	kg ha^{-1}	0.3	IPCC 2006
Carbon dioxide (C-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 Chapter 4).

[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 chapter 4).

[c] RF: Recovered Fertilizer scenario, and SF: Synthetic Fertilizer scenario.

6.2.5 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(Goedkoop et al. 2008) 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),(Huijbregts et al. 2017) 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.(Hauschild and Huijbregts 2015)

6.3 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.

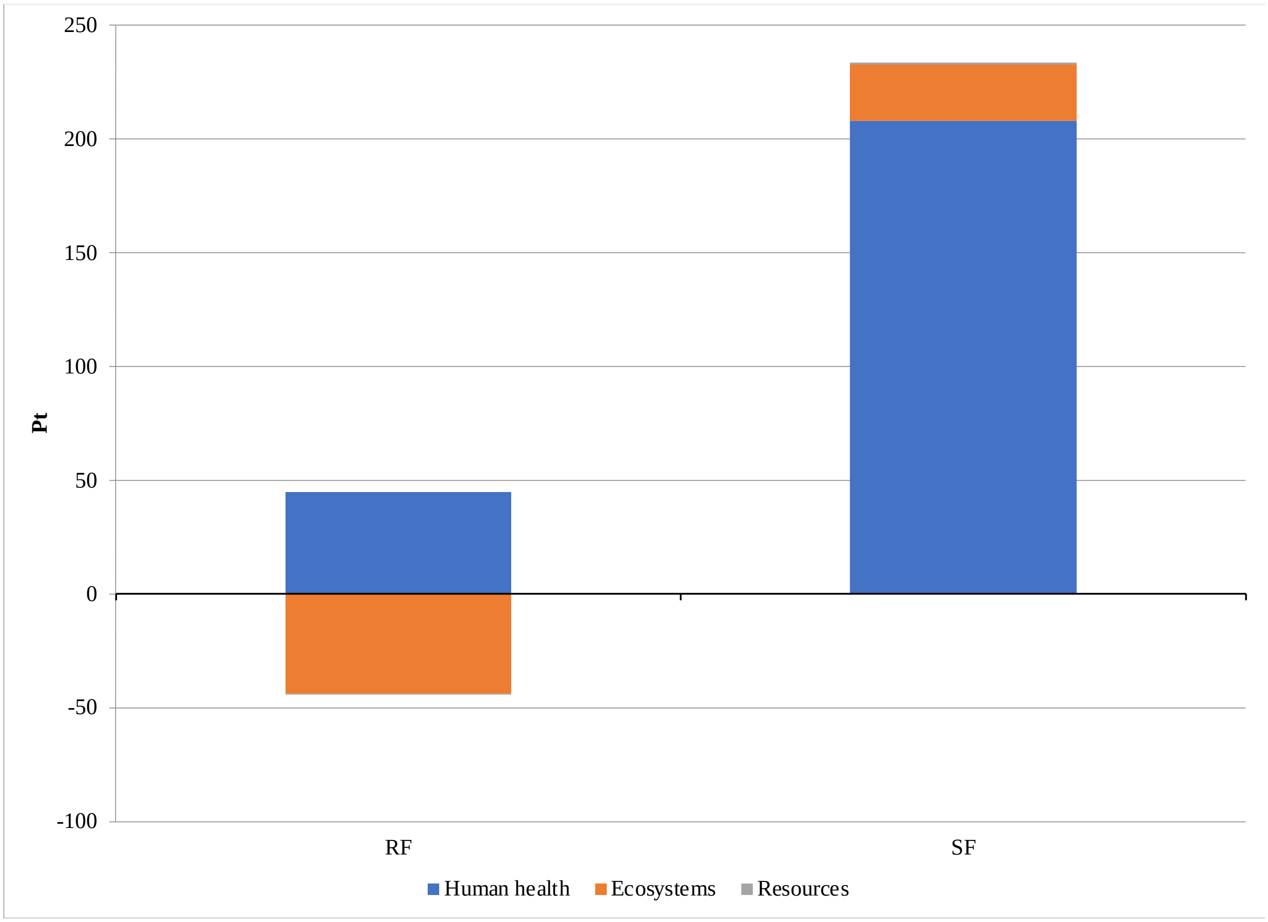

Figure 2. Comparative environmental results for Scenarios RS and SF. Impacts assessment calculated according to ReCiPe 2016 endpoint (H) V 1.03 method.

6.3.1 Midpoint results of impact categories related to Ecosystem quality

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,(Paolini et al. 2018) 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 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 370 kg ha^{-1} (Chapter 5 Table 1) than in Scenario SF, i.e. 185 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 185 kg ha^{-1} and 185 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) (Chapter 3 Table 2), that was even lower (i.e. with higher biological stability) than those reported for well-matured composts,(Scaglia et al. 2018) 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)(Tambone and Adani 2017) 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 (Chapter 4) 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.(Montemayor et al. 2019)

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,(Brentrup et al. 2000; IPCC (Intergovernmental Panel on Climate Change) 2006) 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 (Chapter 4) thanks to the digestate injection that resulted in a strong mitigation in ammonia emissions in comparison with superficial spreading,(Massimo Zilio et al. 2021) as confirmed also by the literature.(Riva et al. 2016) 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,(Emmerling, Krein, and Junk 2020) 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 (Chapter 4).

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(Huijbregts et al. 2017) 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 (Chapter 4). 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.(Björnsson et al. 2013; Montemayor et al. 2019) 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.(Willén et al. 2017; Bacenetti, Lovarelli, and Fiala 2016)

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 (Chapter 5 Table 1). N:P ratio imbalance is well known and documented for animal slurries and digestates,(Macura et al. 2019) 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.(Peccia and Westerhoff 2015)

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 S4 supporting information).


A. Ecosystem
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)
100%
80%
60%
40%
20%
0%
-20%
-40%
-60%
-80%
-100%
Remaining process
Concrete
Sulfuric acid
Steel
Ammonium sulphate spreading
Digestate spreading
Combustion of biogas
Transports
Emissions to air during field distribution
Renewable Electricity produced
B. Toxicity
100%
80%
60%
40%
20%
0%
-20%
-40%
-60%
Kg 1,4-DCB-eq
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
Terrestrial ecotoxicity
Freshwater ecotoxicity
Marine ecotoxicity
Human carcinogenic toxicity
Human non-carcinogenic toxicity
C. Resources
m2 (Land use)
kg Cu eq (Mineral resource scarcity)
kg oil eq (Fossil resource scarcity)
m3 (Water consumption)
100%
80%
60%
40%
20%
0%
-20%
-40%
-60%
-80%
-100%
Remaining processes
Components CHP
Concrete
Sulphuric acid,
Ammonium sulphate spreading
Digestate spreading
Anaerobic digestion plant - 1000kW
Renewable Electricity produced
Transport

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

6.3.2 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.*(Bacenetti, Lovarelli, and Fiala 2016; Montemayor et al. 2019)

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.(Provolo et al. 2018; Leclerc and Laurent 2017)

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).

6.3.3 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 ammonium sulphate) led, also, to high efficiency in terms of *Land use, Mineral resource use, Fossil resources,* reducing, until negative, these impacts (Table 2).

6.3.4 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.(Niero et al. 2014; Bacenetti et al. 2016; Piippo, Lauronen, and Postila 2018; Styles et al. 2018; Yoshida et al. 2018)

6.3.5 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.(Lam, Zlatanović, and van der Hoek 2020)

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 are the more important factors 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).(Menéndez et al. 2012; Herr et al. 2020) From the literature, it was calculated, on average, that the use of NI allowed a reduction of 44% in total N_2O emissions,(Qiao et al. 2015) 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.

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).

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.(Scarlat, Dallemand, and Fahl 2018) In the Lombardy region alone, about 580 AD plants are operating producing biogas and now, are starting to produce biomethane.(Benato and Macor 2019; GSE (Gestore dei Servizi Energetici) 2021) Recently a particular interest has been devoted to liquid biomethane (Bio-LNG) as a substitute for fossil fuels in truck transportation,(EBA et al. 2020) 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 assuming the biogas production from organic wastes (OFMSW and sludge), the purification and compression, and the transport by 30 ton trucks and average consumption of fuel equal to 0.34 kg LNG per kilometre travelled.(Smajla et al. 2019)

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).

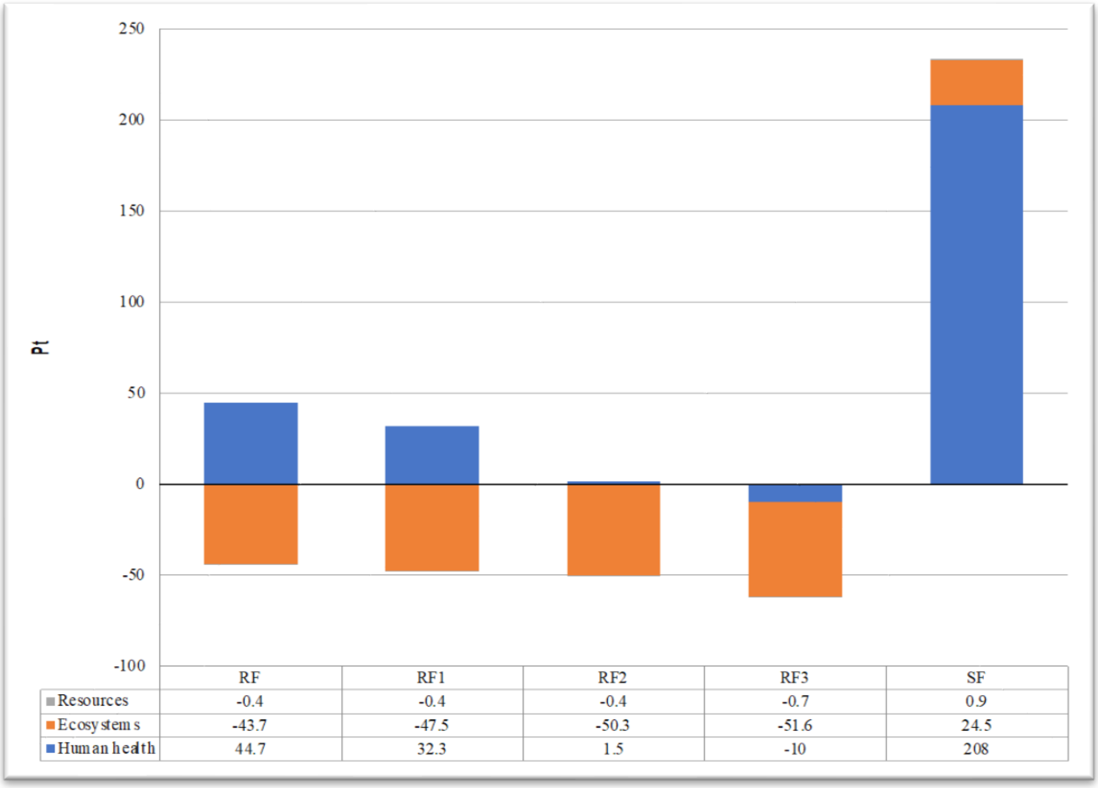

Figure 4. Comparative environmental results for Scenario RF, Scenario RF_1 (RF + nitro inhibitor), Scenario RF_2 (RF + nitro inhibitor + anchor), Scenario RF_3 (RF + nitro inhibitor + anchor + biomethane for transportation) and Scenario SF. Impacts assessment calculated according to ReCiPe 2016 endpoint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

6.4 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 (Pigoli et al. 2021) 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.

Author Contributions

AH: Methodology, formal analysis, investigation, writing-original draft preparation, writing-review editing.

GD: Contribution: Conceptualization, methodology, formal analysis, investigation, data curation, writing-original draft preparation, writing-review editing, supervision.

MZ: Methodology, soil sampling, analyses and emission detections

AP: Plant data collection and soil analyses

BR: Soil analysis, emission detection and nitrate leaching detection

EM: Scientific consultant and final draft revision

OS: Scientific consultant and final draft revision

MS: Plant data acquisition and draft revision

FB: Plant data acquisition and draft revision

AG: AD plant management, agronomical trials management and final draft revision

FA: Conceptualization, methodology, formal analysis, investigation, data curation, writing-original draft preparation, writing-review editing, supervision, project administration, funding acquisition, resources.

7 GENERAL CONCLUSIONS

Sewage sludge is a waste which need to be disposed, though, on the other hand, is reach of compounds that can be useful exploited in agriculture. AD process is well-known to valorise organic substrates, through stabilization of the organic fraction, sanitation and production of biogas. This notwithstanding, the scientific community has some concerns about its use in agriculture, due to possible soil pollution, ammonia emission, nutrients leaching and so on.

This work, through a full-scale approach and open field experimental trials, demonstrated that highly stabilized digestate is suitable as fertilizer/soil conditioner in order to substitute synthetic fertilizers. Furthermore, digestate can also be considered an organic amendment thanks to the high carbon content and biologic stability. On the other hand, safety is assured by the low content of both inorganic and organic pollutants, beside a substantial sanitation of the input sewage sludge. Moreover, if correctly managed, the use of digestate does not increase ammonia emissions and can even reduce odours. The overall performance was positive, showing same yields as traditional fertilizers, besides the production of renewable energy and the sewage sludge safe disposal which leads to a lower environmental impact compared to traditional fertilization.

These results showed once more that AD is a key passage in the disposal of sewage sludge and other organic wastes, greatly increasing the efficacy and efficiency of the process, in a circular economy perspective.

8 BIBLIOGRAPHY

Abel, Guy J., Bilal Barakat, Samir Kc, and Wolfgang Lutz. 2016. “Meeting the Sustainable Development Goals Leads to Lower World Population Growth.” *Proceedings of the National Academy of Sciences of the United States of America* 113 (50): 14294–99. https://doi.org/10.1073/pnas.1611386113.

Abouelenien, Fatma, Wataru Fujiwara, Yuzaburo Namba, Maria Kosseva, Naomichi Nishio, and Yutaka Nakashimada. 2010. “Improved Methane Fermentation of Chicken Manure via Ammonia Removal by Biogas Recycle.” *Bioresource Technology* 101 (16): 6368–73. https://doi.org/10.1016/j.biortech.2010.03.071.

Adnan, Amir Izzuddin, Mei Yin Ong, Saifuddin Nomanbhay, Kit Wayne Chew, and Pau Loke Show. 2019. “Technologies for Biogas Upgrading to Biomethane: A Review.” *Bioengineering* 6 (4): 1–23. https://doi.org/10.3390/bioengineering6040092.

Adugna, Getinet. 2016. “A Review on Impact of Compost on Soil Properties, Water Use and Crop Productivity.” *Academic Research Journal of Agricultural Science and Research* 4 (3): 93–104. https://doi.org/10.14662/ARJASR2016.010.

Ahmad, Rafiq, and Nusrat Jabeen. 2009. “Demonstration of Growth Improvement in Sunflower (Helianthus Annuus L.) by the Use of Organic Fertilizers under Saline Conditions.” *Pakistan Journal of Botany* 41 (3): 1373–84.

Ahring, Birgitte Kiœr. 2003. *Biomethanation II*. Berlin: Spinger.

Albihn, Ann. 2002. “Recycling Biowaste - Human and Animal Health Problems.” *Acta Veterinaria Scandinavica* 43 (SUPPL. 1): 69–75. https://doi.org/10.1186/1751-0147-43-S1-S69.

Alburquerque, José Antonio, Carlos de la Fuente, and María Pilar Bernal. 2012. “Chemical Properties of Anaerobic Digestates Affecting C and N Dynamics in Amended Soils.”

Agriculture, Ecosystems and Environment 160: 15–22. https://doi.org/10.1016/j.agee.2011.03.007.

Amekan, Yumechris. 2020. “The Influence of Microbial Community Dynamics on Anaerobic Digestion Efficiency and Stability: A Review.” *International Journal of Renewable Energy Development* 9 (1): 85–95. https://doi.org/10.14710/ijred.9.1.85-95.

Anastasiou, Dimos, Miquel Aran, Jérôme Balesdent, Gottlieb Basch, Borbala Biró, Isabel de Maria Mourão, Edoardo Costantini, et al. 2015. “Soil Organic Matter in Mediterranean Regions.” Brussels, Belgium.

Antoniadis, Vasileios, Christos D. Tsadilas, and Daniel J. Ashworth. 2007. “Monometal and Competitive Adsorption of Heavy Metals by Sewage Sludge-Amended Soil.” *Chemosphere* 68 (3): 489–94. https://doi.org/10.1016/j.chemosphere.2006.12.062.

APAT - IRSA/CNR. 2003. “APAT CNR IRSA 7080 - Metodi Analitici per Le Acque.”

APHA. 1992. “Standard Methods for the Examination of Water and Wastewater.” In *Standard Methods for the Examination of Water and Wastewater*, 18 th. Washington, DC, USA: American Public Health Association.

———. 1998. “Standard Methods for Examination of Water and Wastewater.” In , edited by American Public Health Association, 20th ed. Washington, DC.

ARPAV. 2013. “Caratteristiche Del Digestato Da Impianti Di Digestione Anaerobica Campagna Di Monitoraggio Maggio-Settembre 2013.”

ASTM International. 2016. “ASTM D7485 - 16 - Standard Test Method for Determination of Nonylphenol, p-Tert-Octylphenol, Nonylphenol Monoethoxylate and Nonylphenol Diethoxylate in Environmental Waters by Liquid Chromatography/Tandem Mass Spectrometry.”

Ayuke, F. O., L. Brussaard, B. Vanlauwe, J. Six, D. K. Lelei, C. N. Kibunja, and M. M. Pulleman. 2011. “Soil Fertility Management: Impacts on Soil Macrofauna, Soil Aggregation and Soil Organic Matter Allocation.” *Applied Soil Ecology* 48 (1): 53–62. https://doi.org/10.1016/j.apsoil.2011.02.001.

Bacenetti, Jacopo, Daniela Lovarelli, and Marco Fiala. 2016. “Mechanisation of Organic Fertiliser Spreading, Choice of Fertiliser and Crop Residue Management as Solutions for Maize Environmental Impact Mitigation.” *European Journal of Agronomy* 79 (September): 107–18. https://doi.org/10.1016/J.EJA.2016.05.015.

Bacenetti, Jacopo, Cesare Sala, Alessandra Fusi, and Marco Fiala. 2016. “Agricultural Anaerobic Digestion Plants: What LCA Studies Pointed out and What Can Be Done to Make Them More Environmentally Sustainable.” *Applied Energy* 179: 669–86. https://doi.org/10.1016/j.apenergy.2016.07.029.

Bachmann, Jörg, Georg Guggenberger, Thomas Baumgartl, Ruth H. Ellerbrock, Emilia Urbanek, Marc O. Goebel, Klaus Kaiser, Rainer Horn, and Walter R. Fischer. 2008. “Physical Carbon-Sequestration Mechanisms under Special Consideration of Soil Wettability.” *Journal of Plant Nutrition and Soil Science* 171 (1): 14–26. https://doi.org/10.1002/jpln.200700054.

Baldock, J. A., J. M. Oades, A. G. Waters, X. Peng, A. M. Vassallo, and M. A. Wilson. 1992. “Aspects of the Chemical Structure of Soil Organic Materials as Revealed by Solid-State13C NMR Spectroscopy.” *Biogeochemistry* 16 (1): 1–42. https://doi.org/10.1007/BF02402261.

Bandyopadhyay, Biman, Pradeep Kumar, and Partha Biswas. 2017. “Ammonia Catalyzed Formation of Sulfuric Acid in Troposphere: The Curious Case of a Base Promoting Acid Rain.” *Journal of Physical Chemistry A* 121 (16): 3101–8. https://doi.org/10.1021/acs.jpca.7b01172.

Barbier, D., D. Perrine, C. Duhamel, R. Doublet, and P. Georges. 1990. “Parasitic Hazard with Sewage Sludge Applied to Land.” *Applied and Environmental Microbiology* 56 (5): 1420–22. https://doi.org/10.1128/aem.56.5.1420-1422.1990.

Barłóg, Przemysław, Lukáš Hlisnikovský, and Eva Kunzová. 2020. “Concentration of Trace Metals in Winter Wheat and Spring Barley as a Result of Digestate, Cattle Slurry, and Mineral Fertilizer Application.” *Environmental Science and Pollution Research* 27 (5): 4769–85. https://doi.org/10.1007/s11356-019-07304-2.

Basosi, R., D. Spinelli, A. Fierro, and S. Jez. 2014. “MIneral Nitrogen Fertilizers: Environmental Impact of Production and Use.” In *Fertilizers Components, Uses in Agriculture and Environmental Impacts*, edited by Fernando López-Valdez and Fabían Fernández-Luqueño, First, 316. New York, NY, USA: Nova Science Publisher, Inc.

Benato, Alberto, and Alarico Macor. 2019. “Italian Biogas Plants: Trend, Subsidies, Cost, Biogas Composition and Engine Emissions.” *Energies* 12 (6): 1–31. https://doi.org/10.3390/en12060979.

Bennett, Elena M., Stephen R. Carpenter, and Nina F. Caraco. 2001. “Human Impact on Erodable Phosphorus and Eutrophication: A Global Perspective.” *BioScience* 51 (3): 227–34. https://doi.org/10.1641/0006-3568(2001)051[0227:HIOEPA]2.0.CO;2.

Benotti, Mark J., Rebecca A. Trenholm, Brett J. Vanderford, Janie C. Holady, Benjamin D. Stanford, and Shane A. Snyder. 2009. “Pharmaceuticals and Endocrine Disrupting Compounds in U.S. Drinking Water.” *Environmental Science and Technology* 43 (3): 597–603. https://doi.org/10.1021/es801845a.

Berendsen, Bjorn J.A., Robin S. Wegh, Joost Memelink, Tina Zuidema, and Linda A.M. Stolker. 2015. “The Analysis of Animal Faeces as a Tool to Monitor Antibiotic Usage.” *Talanta* 132: 258–68. https://doi.org/10.1016/j.talanta.2014.09.022.

Bertora, Chiara, Matteo Peyron, Simone Pelissetti, Carlo Grignani, and Dario Sacco. 2018. “Assessment of Methane and Nitrous Oxide Fluxes from Paddy Field by Means of Static Closed Chambers Maintaining Plants within Headspace.” *Journal of Visualized Experiments*, no. 139: 1–7. https://doi.org/10.3791/56754.

Bianchi, F., J. Tröstl, H. Junninen, C. Frege, S. Henne, C. R. Hoyle, U. Molteni, et al. 2016. “New Particle Formation in the Free Troposphere: A Question of Chemistry and Timing.” *Science* 352 (6289): 1109–12. https://doi.org/10.1126/science.aad5456.

Björnsson, Lovisa, Mikael Lantz, Pål Börjesson, Thomas Prade, Sven-Erik Svensson, and Håkan Eriksson. 2013. “Impact of Biogas Crop Production on Greenhouse Gas Emissions, Soil Organic Matter and Food Crop Production–A Case Study on Farm Level.” The Swedish Knowledge Centre for Renewable Transportation Fuels.

Black, A. S., R. R. Sherlock, and N. P. Smith. 1987. “Effect of Timing of Simulated Rainfall on Ammonia Volatilization from Urea, Applied to Soil of Varyingmoisture Content.” *Journal of Soil Science* 38 (4): 679–87. https://doi.org/10.1111/j.1365-2389.1987.tb02165.x.

Bloem, E., A. Albihn, J. Elving, L. Hermann, L. Lehmann, M. Sarvi, T. Schaaf, J. Schick, E. Turtola, and K. Ylivainio. 2017. “Contamination of Organic Nutrient Sources with Potentially Toxic Elements, Antibiotics and Pathogen Microorganisms in Relation to P Fertilizer Potential and Treatment Options for the Production of Sustainable Fertilizers: A Review.” *Science of the Total Environment* 607–608: 225–42. https://doi.org/10.1016/j.scitotenv.2017.06.274.

Boix, C., M. Ibáñez, D. Fabregat-Safont, E. Morales, L. Pastor, J. V. Sancho, J. E. Sánchez-Ramírez, and F. Hernández. 2016. “Behaviour of Emerging Contaminants in Sewage Sludge after Anaerobic Digestion.” *Chemosphere* 163: 296–304.

https://doi.org/10.1016/j.chemosphere.2016.07.098.

Bonetta, Silvia, Sara Bonetta, Elisa Ferretti, Giorgio Fezia, Giorgio Gilli, and Elisabetta Carraro. 2014. "Agricultural Reuse of the Digestate from Anaerobic Co-Digestion of Organic Waste: Microbiological Contamination, Metal Hazards and Fertilizing Performance." *Water, Air, and Soil Pollution* 225 (8): 1–11. https://doi.org/10.1007/s11270-014-2046-2.

Börjesson, Gunnar, Holger Kirchmann, and Thomas Kätterer. 2014. "Four Swedish Long-Term Field Experiments with Sewage Sludge Reveal a Limited Effect on Soil Microbes and on Metal Uptake by Crops." *Journal of Soils and Sediments* 14 (1): 164–77. https://doi.org/10.1007/s11368-013-0800-5.

Börjesson, Pål, and Maria Berglund. 2007. "Environmental Systems Analysis of Biogas Systems-Part II: The Environmental Impact of Replacing Various Reference Systems." *Biomass and Bioenergy* 31 (5): 326–44. https://doi.org/10.1016/j.biombioe.2007.01.004.

Bousek, J., D. Scroccaro, Jan Sima, Norbert Weissenbacher, and W. Fuchs. 2016. "Influence of the Gas Composition on the Efficiency of Ammonia Stripping of Biogas Digestate." *Bioresource Technology* 203: 259–66. https://doi.org/10.1016/j.biortech.2015.12.046.

Bouwmeester, R. J. B., P. L. G. Vlek, and J. M. Stumpe. 1985. "Effect of Environmental Factors on Ammonia Volatilization from a Urea-Fertilized Soil." *Soil Science Society of America Journal* 49 (2): 376–81. https://doi.org/10.2136/sssaj1985.03615995004900020021x.

Brentrup, Frank, Jiirgen Kusters, Joachim Lammel, and Hermann Kuhlmann. 2000. "Methods to Estimate On-Field Nitrogen Emissions from Crop Production as an Input to LCA Studies in the Agricultural Sector." *International Journal of Life Cycle*

Assessment 5 (6): 349–57. https://doi.org/10.1007/bf02978670.

Cai, G., D. Chen, R. E. White, X. H. Fan, A. Pacholski, Z. L. Zhu, and H. Ding. 2002. “Gaseous Nitrogen Losses from Urea Applied to Maize on a Calcareous Fluvo-Aquic Soil in the North China Plain.” Edited by X.M. Yang. *Soil Research* 40 (5): 737. https://doi.org/10.1071/SR01011.

Cakmak, I., and U. B. Kutman. 2018. “Agronomic Biofortification of Cereals with Zinc: A Review.” *European Journal of Soil Science* 69 (1): 172–80. https://doi.org/10.1111/ejss.12437.

Cameron, K. C., H. J. Di, and J. L. Moir. 2013. “Nitrogen Losses from the Soil/Plant System: A Review.” *Annals of Applied Biology* 162 (2): 145–73. https://doi.org/10.1111/aab.12014.

Capson-Tojo, G., R. Moscoviz, S. Astals, Robles, and J. P. Steyer. 2020. “Unraveling the Literature Chaos around Free Ammonia Inhibition in Anaerobic Digestion.” *Renewable and Sustainable Energy Reviews* 117 (September 2019): 109487. https://doi.org/10.1016/j.rser.2019.109487.

Capua, Francesco Di, Fabrizio Adani, Francesco Pirozzi, Giovanni Esposito, and Andrea Giordano. 2021. “Air Side-Stream Ammonia Stripping in a Thin Film Evaporator Coupled to High-Solid Anaerobic Digestion of Sewage Sludge: Process Performance and Interactions.” *Journal of Environmental Management* 295 (May): 113075. https://doi.org/10.1016/j.jenvman.2021.113075.

Capua, Francesco Di, Danilo Spasiano, Andrea Giordano, Fabrizio Adani, Umberto Fratino, Francesco Pirozzi, and Giovanni Esposito. 2020. “High-Solid Anaerobic Digestion of Sewage Sludge: Challenges and Opportunities.” *Applied Energy* 278 (September): 115608. https://doi.org/10.1016/j.apenergy.2020.115608.

Castellano, Michael J., Kevin E. Mueller, Daniel C. Olk, John E. Sawyer, and Johan Six. 2015. “Integrating Plant Litter Quality, Soil Organic Matter Stabilization, and the Carbon Saturation Concept.” *Global Change Biology* 21 (9): 3200–3209. https://doi.org/10.1111/gcb.12982.

Celiz, Mary D., Jerry Tso, and Diana S. Aga. 2009. “Pharmaceutical Metabolites in the Environment: Analytical Challenges and Ecological Risks.” *Environmental Toxicology and Chemistry* 28 (12): 2473–84. https://doi.org/10.1897/09-173.1.

CEN. 2003. “Air Quality — Determination of Odour Concentration by Dynamic Olfactometry. Standard Method n. 13725.” In . Brussels, B.: European Committee for Standardization.

Chang, A. C., J. E. Warneke, A. L. Page, and L. J. Lund. 1984. “Accumulation of Heavy Metals in Sewage Sludge-Treated Soils.” *Journal of Environmental Quality* 13 (1): 87–91. https://doi.org/10.2134/jeq1984.00472425001300010016x.

Chantigny, Martin H., Denis A. Angers, Gilles Bélanger, Philippe Rochette, Nikita Eriksen-Hamel, Shabtai Bittman, Katherine Buckley, Daniel Massé, and Marc Olivier Gasser. 2008. “Yield and Nutrient Export of Grain Corn Fertilized with Raw and Treated Liquid Swine Manure.” *Agronomy Journal* 100 (5): 1303–9. https://doi.org/10.2134/agronj2007.0361.

Chantigny, Martin H., Denis A. Angers, Philippe Rochette, Gilles Bélanger, Daniel Massé, and Denis Côté. 2007. “Gaseous Nitrogen Emissions and Forage Nitrogen Uptake on Soils Fertilized with Raw and Treated Swine Manure.” *Journal of Environmental Quality* 36 (6): 1864–72. https://doi.org/10.2134/jeq2007.0083.

Chantigny, Martin H., Philippe Rochette, Denis A. Angers, Daniel Massé, and Denis Côté. 2004. “Ammonia Volatilization and Selected Soil Characteristics Following Application of Anaerobically Digested Pig Slurry.” *Soil Science Society of America*

Journal 68 (1): 306–12. https://doi.org/10.2136/sssaj2004.3060.

Cherkasov, N., A. O. Ibhadon, and P. Fitzpatrick. 2015. "A Review of the Existing and Alternative Methods for Greener Nitrogen Fixation." *Chemical Engineering and Processing: Process Intensification* 90: 24–33. https://doi.org/10.1016/j.cep.2015.02.004.

Christensen, Bjarne, Frank Brentrup, Laetitia Six, Christian Pallière, and Antoine Hoxha. 2014. "Assesing the Carbon Footprint of Fertilizers at Production and Full Life Cycle." In *Proceedings 751*, 751:20. London, UK: International Fertiliser Society.

Chung, Haegeun, Kathie J. Ngo, Alain Plante, and Johan Six. 2010. "Evidence for Carbon Saturation in a Highly Structured and Organic-Matter-Rich Soil." *Soil Science Society of America Journal* 74 (1): 130–38. https://doi.org/10.2136/sssaj2009.0097.

Clarisse, Lieven, Cathy Clerbaux, Frank Dentener, Daniel Hurtmans, and Pierre François Coheur. 2009. "Global Ammonia Distribution Derived from Infrared Satellite Observations." *Nature Geoscience* 2 (7): 479–83. https://doi.org/10.1038/ngeo551.

Clark, Christopher M., and David Tilman. 2008. "Loss of Plant Species after Chronic Low-Level Nitrogen Deposition to Prairie Grasslands." *Nature* 451 (7179): 712–15. https://doi.org/10.1038/nature06503.

CNR IRSA. 1983. "Quaderno n. 64 - METODI ANALITICI PER I FANGHI."

Collins, H. P., A. K. Alva, J. D. Streubel, S. F. Fransen, C. Frear, S. Chen, C. Kruger, and D. Granatstein. 2011. "Greenhouse Gas Emissions from an Irrigated Silt Loam Soil Amended with Anaerobically Digested Dairy Manure." *Soil Science Society of America Journal* 75 (6): 2206–16. https://doi.org/10.2136/sssaj2010.0360.

Comunian, Silvia, Dario Dongo, Chiara Milani, and Paola Palestini. 2020. "Air Pollution and Covid-19: The Role of Particulate Matter in the Spread and Increase of Covid-

19’s Morbidity and Mortality.” *International Journal of Environmental Research and Public Health* 17 (12): 1–22. https://doi.org/10.3390/ijerph17124487.

Conley, Daniel J., Hans W. Paerl, Robert W. Howarth, Donald F. Boesch, Sybil P. Seitzinger, Karl E. Havens, Christiane Lancelot, and Gene E. Likens. 2009. “Ecology - Controlling Eutrophication: Nitrogen and Phosphorus.” *Science* 323 (5917): 1014–15. https://doi.org/10.1126/science.1167755.

Cordell, Dana, Jan Olof Drangert, and Stuart White. 2009. “The Story of Phosphorus: Global Food Security and Food for Thought.” *Global Environmental Change* 19 (2): 292–305. https://doi.org/10.1016/j.gloenvcha.2008.10.009.

Cordell, Dana, and Stuart White. 2011. “Peak Phosphorus: Clarifying the Key Issues of a Vigorous Debate about Long-Term Phosphorus Security.” *Sustainability* 3 (10): 2027–49. https://doi.org/10.3390/su3102027.

Costamagna, P., M. Delucchi, G. Busca, and Andrea Giordano. 2020. “System for Ammonia Removal from Anaerobic Digestion and Associated Ammonium Sulfate Production: Simulation and Design Consideration,” no. xxxx: 1–15.

COUNCIL DIRECTIVE. 1986. “COUNCIL DIRECTIVE of 12 June 1986 on the Protection of the Environment, and in Particular of the Soil, When Sewage Sludge Is Used in Agriculture (86/278/EEC).”

Daneshgar, Saba, Arianna Callegari, Andrea G. Capodaglio, and David Vaccari. 2018. “The Potential Phosphorus Crisis: Resource Conservation and Possible Escape Technologies: A Review.” *Resources* 7 (2). https://doi.org/10.3390/resources7020037.

Daughton, Christian G., and Thomas A. Ternes. 1999. “Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change?” *Environmental Health*

Perspectives 107 (SUPPL. 6): 907–38. https://doi.org/10.1289/ehp.99107s6907.

Davidson, Eric A. 2009. “The Contribution of Manure and Fertilizer Nitrogen to Atmospheric Nitrous Oxide since 1860.” *Nature Geoscience* 2 (9): 659–62. https://doi.org/10.1038/ngeo608.

Delgado, J. A. 2002. “Quantifying the Loss Mechanisms of Nitrogen.” *Journal of Soil and Water Conservation* 57 (6): 389–98.

Desmidt, Evelyn, Karel Ghyselbrecht, Yang Zhang, Luc Pinoy, Bart Van Der Bruggen, Willy Verstraete, Korneel Rabaey, and Boudewijn Meesschaert. 2015. “Global Phosphorus Scarcity and Full-Scale P-Recovery Techniques: A Review.” *Critical Reviews in Environmental Science and Technology* 45 (4): 336–84. https://doi.org/10.1080/10643389.2013.866531.

Dijk, Kimo C. van, Jan Peter Lesschen, and Oene Oenema. 2016. “Phosphorus Flows and Balances of the European Union Member States.” *Science of the Total Environment* 542: 1078–93. https://doi.org/10.1016/j.scitotenv.2015.08.048.

Dragicevic, Ivan, Trine A. Sogn, and Susanne Eich-Greatorex. 2018. “Recycling of Biogas Digestates in Crop Production—Soil and Plant Trace Metal Content and Variability.” *Frontiers in Sustainable Food Systems* 2 (August): 1–14. https://doi.org/10.3389/fsufs.2018.00045.

EBA, GIE, NGVA, and SEA-LNG. 2020. “BioLNG in Transport : Making Climate Neutrality a Reality A Joint White Paper about BioLNG Production.” Brussels, Belgium.

Ellington, A. 1986. “Ammonia Volatilization Losses from Fertilizers Applied to Acid Soil in the Field.” *Fertilizer Research* 8 (3): 283–96.

Emmerling, Christoph, Andreas Krein, and Jürgen Junk. 2020. “Meta-Analysis of Strategies to Reduce NH3 Emissions from Slurries in European Agriculture and

Consequences for Greenhouse Gas Emissions.” *Agronomy* 10 (11): 14. https://doi.org/10.3390/agronomy10111633.

EN. 2005. “UNI EN 14039 - Characterization of Waste - Determination of Hydrocarbon Content in the Range of C10 to C40 by Gas Chromatography.”

———. 2009. “DIN EN ISO 11885 - Water Quality - Determination of Selected Elements by Inductively Coupled Plasma Optical Emission Spectrometry.”

———. 2012a. “UNI EN 16167 - Soil, Treated Biowaste and Sludge - Determination of Polychlorinated Biphenyls (PCB) by Gas Chromatography with Mass Selective Detection (GC-MS) and Gas Chromatography with Electron-Capture Detection (GC-ECD).”

———. 2012b. “UNI EN 16174 - Sludge, Treated Biowaste and Soil - Digestion of Aqua Regia Soluble Fractions of Elements.”

———. 2016. “UNI EN ISO 22155 - Soil Quality - Gas Chromatographic Determination of Volatile Aromatic and Halogenated Hydrocarbons and Selected Ethers - Static Headspace Method.”

———. 2018. “UNI EN 16167 - Soil, Treated Biowaste and Sludge - Determination of Dioxins and Furans and Dioxin-like Polychlorinated Biphenyls by Gas Chromatography with High Resolution Mass Selective Detection (HR GC-MS).”

EN, UNI. 2012c. “UNI EN 16167 - Soil, Treated Biowaste and Sludge - Determination of Polychlorinated Biphenyls (PCB) by Gas Chromatography with Mass Selective Detection (GC-MS) and Gas Chromatography with Electron-Capture Detection (GC-ECD).”

EPA. 1998. “Method EPA 3051 -Microwave Assisted Acid Digestion of Sediments, Sludges, Soils and Oils.” Washington, DC.

———. 2004. “EPA 9045D - Soil and Waste PH.”

———. 2007a. “METHOD 3550C - Ultrasonic Extraction.”

———. 2007b. “METHOD 8321B - Solvent-Extractable Nonvolatile Compounds by High-Performance Liquid Chromatography/Thermospray/Mass Spectrometry (Hplc/Ts/Ms) or Ultraviolet (Uv) Detection.”

———. 2014. “EPA 8270E - Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC-MS).”

Erisman, J. W., and M. Schaap. 2004. “The Need for Ammonia Abatement with Respect to Secondary PM Reductions in Europe.” *Environmental Pollution* 129 (1): 159–63. https://doi.org/10.1016/j.envpol.2003.08.042.

Ertl, Kathrin, and Walter Goessler. 2018. “Grains, Whole Flour, White Flour, and Some Final Goods: An Elemental Comparison.” *European Food Research and Technology* 244 (11): 2065–75. https://doi.org/10.1007/s00217-018-3117-1.

Escher, Beate I., and Kathrin Fenner. 2011. “Recent Advances in Environmental Risk Assessment of Transformation Products.” *Environmental Science and Technology* 45 (9): 3835–47. https://doi.org/10.1021/es1030799.

Espejo-Herrera, Nadia, Esther Gràcia-Lavedan, Elena Boldo, Nuria Aragonés, Beatriz Pérez-Gómez, Marina Pollán, Antonio J. Molina, et al. 2016. “Colorectal Cancer Risk and Nitrate Exposure through Drinking Water and Diet.” *International Journal of Cancer* 139 (2): 334–46. https://doi.org/10.1002/ijc.30083.

EU. 2019. “Regulation of the European Parliament and of the Council Laying down Rules on the Making Available on the Market of EU Fertilising Products and Amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and Repealing Regulation (EC) No 2003/2003.” *Official Journal of the European Union*.

European Commission. 2000. “European Commission (2000) Working Document on Sludge. 3rd Draft. ENV.E.3/LM, 27 April, 2000.”

———. 2009. “Environmental, Economic and Social Impacts of the Use of Sewage Sludge on Land.” *Consultation Report on Options and Impacts, Report by RPA. Milieu Ltd and WRc for the European Commission, DG Environment under Study Contract DG ENV.G.4/ ETU/2008/0076r.*, 1–20. https://ec.europa.eu/environment/archives/waste/sludge/pdf/part_i_report.pdf.

———. 2019. “The European Green Deal.” Brussels.

European Committee for Standardization. 2001. “EN 13652.” Brussels: Comité Europeén De Normalisation.

European Parliament. 2008. “Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on Waste and Repealing Certain Directives.”

Fabietti, Gabriele, Mattia Biasioli, Renzo Barberis, and Franco Ajmone-Marsan. 2010. “Soil Contamination by Organic and Inorganic Pollutants at the Regional Scale: The Case of Piedmont, Italy.” *Journal of Soils and Sediments* 10 (2): 290–300. https://doi.org/10.1007/s11368-009-0114-9.

Faithfull, Nigel T. 2002. *Methods in Agricultural Chemical Analysis*. Bristol: CABI publishing.

Fan, X. H., Y. S. Song, D. X. Lin, L. Z. Yang, and J. M. Zhou. 2005. “Ammonia Volatilization Losses from Urea Applied to Wheat on a Paddy Soil in Taihu Region, China.” *Pedosphere* 15 (1): 59–65.

Fang, Jingjing, Fan Lü, Hua Zhang, Liming Shao, and Pinjing He. 2015. “Odor Compounds Released from Different Zones of Two Adjacent Waste Treatment Facilities: Interactive Influence and Source Identification.” *Journal of the Air and Waste*

Management Association 65 (12): 1446–55. https://doi.org/10.1080/10962247.2015.1104401.

FAO. 2019. *World Fertilizer Trend and Outlook to 2022*. Rome.

FAOSTAT, Database (FAO). 2020. “Food and Agriculture Organization of the United Nations.” *The Contribution of Agriculture to Greenhouse Gas Emissions*. Rome, Italy.

Fatta-kassinos, Despo, Sureyya Meric, and Anastasia Nikolaou. 2011. “Pharmaceutical Residues in Environmental Waters and Wastewater : Current State of Knowledge and Future Research,” 251–75. https://doi.org/10.1007/s00216-010-4300-9.

Feng, Leiyu, Jingyang Luo, and Yinguang Chen. 2015. “Dilemma of Sewage Sludge Treatment and Disposal in China.” *Environmental Science and Technology* 49 (8): 4781–82. https://doi.org/10.1021/acs.est.5b01455.

Fennelly, Kevin P. 2020. “Particle Sizes of Infectious Aerosols: Implications for Infection Control.” *The Lancet Respiratory Medicine* 8 (9): 914–24. https://doi.org/10.1016/S2213-2600(20)30323-4.

Fernandez-Bayo, Jesus D., Ramin Yazdani, Christopher W. Simmons, and Jean S. VanderGheynst. 2018. “Comparison of Thermophilic Anaerobic and Aerobic Treatment Processes for Stabilization of Green and Food Wastes and Production of Soil Amendments.” *Waste Management* 77: 555–64. https://doi.org/10.1016/j.wasman.2018.05.006.

Fijalkowski, Krzysztof, Agnieszka Rorat, Anna Grobelak, and Malgorzata J. Kacprzak. 2017. “The Presence of Contaminations in Sewage Sludge – The Current Situation.” *Journal of Environmental Management* 203: 1126–36. https://doi.org/10.1016/j.jenvman.2017.05.068.

Finzi, Alberto, Elisabetta Riva, Alda Bicoku, Viviana Guido, Seit Shallari, and Giorgio Provolo. 2019. “Comparison of Techniques for Ammonia Emission Mitigation during Storage of Livestock Manure and Assessment of Their Effect in the Management Chain.” *Journal of Agricultural Engineering* 50 (1): 12–19. https://doi.org/10.4081/jae.2019.881.

Foley, Jonathan A., Navin Ramankutty, Kate A. Brauman, Emily S. Cassidy, James S. Gerber, Matt Johnston, Nathaniel D. Mueller, et al. 2011. “Solutions for a Cultivated Planet.” *Nature* 478 (7369): 337–42. https://doi.org/10.1038/nature10452.

Food and Agriculture Organization of the United Nations (FAOSTAT). 2021. “Http://Www.Fao.Org/Faostat/En/#data/RFN.” 2021.

Fowler, David, Mhairi Coyle, Ute Skiba, Mark A. Sutton, J. Neil Cape, Stefan Reis, Lucy J. Sheppard, et al. 2013. “The Global Nitrogen Cycle in the Twentyfirst Century.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 368 (1621): 20130164. https://doi.org/10.1098/rstb.2013.0164.

Frantzeskaki, Niki, Katharina Hölscher, Ian P. Holman, Simona Pedde, Jill Jaeger, Kasper Kok, and Paula A. Harrison. 2019. “Transition Pathways to Sustainability in Greater than 2 °C Climate Futures of Europe.” *Regional Environmental Change* 19 (3): 777–89. https://doi.org/10.1007/s10113-019-01475-x.

Gaines, T. P., and S. T. Gaines. 1994. “Soil Texture Effect on Nitrate Leaching in Soil Percolates.” *Communications in Soil Science and Plant Analysis* 25 (13–14): 2561–70. https://doi.org/10.1080/00103629409369207.

Galloway, James N., John D. Aber, Jan Willem Erisman, Sybil P. Seitzinger, Robert W. Howarth, Ellis B. Cowling, and B. Jack Cosby. 2003. “The Nitrogen Cascade.” *BioScience* 53 (4): 341–56. https://doi.org/10.1641/0006-3568(2003)053[0341:TNC]2.0.CO;2.

Galloway, James N., Wilfried Winiwarter, Adrian Leip, Allison M. Leach, Albert Bleeker, and Jan Willem Erisman. 2014. “Nitrogen Footprints: Past, Present and Future.” *Environmental Research Letters* 9 (11): 115003. https://doi.org/10.1088/1748-9326/9/11/115003.

Gee, G. W., and J. W. Bauder. 1986. “Particle-Size Analysis.” In *Methods of Soil Analysis, Part 2*, edited by A. L. Page, 2nd ed Agr, 383–411. Madison WI: ASA and SSSA.

Geisseler, Daniel, and Kate M. Scow. 2014. “Long-Term Effects of Mineral Fertilizers on Soil Microorganisms - A Review.” *Soil Biology and Biochemistry* 75: 54–63. https://doi.org/10.1016/j.soilbio.2014.03.023.

Geissler, Bernhard, Michael C. Mew, and Gerald Steiner. 2019. “Phosphate Supply Security for Importing Countries: Developments and the Current Situation.” *Science of the Total Environment* 677: 511–23. https://doi.org/10.1016/j.scitotenv.2019.04.356.

Gennadiev, A. N., Yu I. Pikovskii, A. S. Tsibart, and M. A. Smirnova. 2015. “Hydrocarbons in Soils: Origin, Composition, and Behavior (Review).” *Eurasian Soil Science* 48 (10): 1076–89. https://doi.org/10.1134/S1064229315100026.

Giordano, Andrea, Francesco Di Capua, Giovanni Esposito, and Francesco Pirozzi. 2019. “Long-Term Biogas Desulfurization under Different Microaerobic Conditions in Full-Scale Thermophilic Digesters Co-Digesting High-Solid Sewage Sludge.” *International Biodeterioration and Biodegradation* 142 (May): 131–36. https://doi.org/10.1016/j.ibiod.2019.05.017.

Goedkoop, Mark, Anne De Schryver, Michiel Oele, Sipke Durksz, and Douwe de Roest. 2008. “Introduction to LCA with SimaPro 7.” *PRé Consultants, The Netherlands*.

Gouel, Christophe, and Houssein Guimbard. 2019. “Nutrition Transition and the Structure of Global Food Demand." *American Journal of Agricultural Economics* 101 (2): 383–403. https://doi.org/10.1093/ajae/aay030.

Govasmark, Espen, Jessica Stäb, Børge Holen, Douwe Hoornstra, Tommy Nesbakk, and Mirja Salkinoja-Salonen. 2011. "Chemical and Microbiological Hazards Associated with Recycling of Anaerobic Digested Residue Intended for Agricultural Use." *Waste Management* 31 (12): 2577–83. https://doi.org/10.1016/j.wasman.2011.07.025.

Greenberg, Isabel, Michael Kaiser, Anna Gunina, Philipp Ledesma, Steven Polifka, Katja Wiedner, Carsten W. Mueller, Bruno Glaser, and Bernard Ludwig. 2019. "Substitution of Mineral Fertilizers with Biogas Digestate plus Biochar Increases Physically Stabilized Soil Carbon but Not Crop Biomass in a Field Trial." *Science of the Total Environment* 680: 181–89. https://doi.org/10.1016/j.scitotenv.2019.05.051.

Gros, Meritxell, Josep Mas-Pla, Mercè Boy-Roura, Irma Geli, Francesc Domingo, and Mira Petrović. 2019. "Veterinary Pharmaceuticals and Antibiotics in Manure and Slurry and Their Fate in Amended Agricultural Soils: Findings from an Experimental Field Site (Baix Empordà, NE Catalonia)." *Science of the Total Environment* 654: 1337–49. https://doi.org/10.1016/j.scitotenv.2018.11.061.

GSE (Gestore dei Servizi Energetici). 2021. "Atlaimpianti Di Produzione Di Energia Elettrica." 2021.

Günther, Susanne, Michael Grunert, and Susann Müller. 2018. "Overview of Recent Advances in Phosphorus Recovery for Fertilizer Production." *Engineering in Life Sciences* 18 (7): 434–39. https://doi.org/10.1002/elsc.201700171.

Gutser, R., Th Ebertseder, A. Weber, M. Schraml, and U. Schmidhalter. 2005. "Short-Term and Residual Availability of Nitrogen after Long-Term Application of Organic Fertilizers on Arable Land." *Journal of Plant Nutrition and Soil Science* 168 (4): 439–46. https://doi.org/10.1002/jpln.200520510.

Harreveld, A. P. Van, Paul Heeres, and Hendrik Harssema. 1999. “A Review of 20 Years of Standardization of Odor Concentration Measurement by Dynamic Olfactometry in Europe.” *Journal of the Air and Waste Management Association* 49 (6): 705–15. https://doi.org/10.1080/10473289.1999.11499900.

Harrison, Roland, and J. Webb. 2001. “A Review of the Effect of N Fertilizer Type on Gaseous Emissions.” *Advances in Agronomy* 73: 65–108. https://doi.org/10.1016/s0065-2113(01)73005-2.

Hauschild, Michael Z., and Mark A.J. Huijbregts, eds. 2015. *LCA Compedium - The Complete World of Life Cycle Assessment*. First. Springer Dordrecht Heidelberg New York London.

Hautier, Yann, Eric W. Seabloom, Elizabeth T. Borer, Peter B. Adler, W. Stanley Harpole, Helmut Hillebrand, Eric M. Lind, et al. 2014. “Eutrophication Weakens Stabilizing Effects of Diversity in Natural Grasslands.” *Nature* 508 (7497): 521–25. https://doi.org/10.1038/nature13014.

He, Pin-Jing, Jia-Fu Tang, Na Yang, Jing-Jing Fang, Xiao He, and Li-Ming Shao. 2012. “The Emission Patterns of Volatile Organic Compounds during Aerobic Biotreatment of Municipal Solid Waste Using Continuous and Intermittent Aeration The Emission Patterns of Volatile Organic Compounds during Aerobic Biotreatment of Municipal Solid Waste Us.” *Journal of the Air & Waste Management Association* 62:4: 461–70. https://doi.org/10.1080/10962247.2012.658954.

Herr, Christina, Thomas Mannheim, Torsten Müller, and Reiner Ruser. 2020. “Effect of Nitrification Inhibitors on N2O Emissions after Cattle Slurry Application.” *Agronomy* 10 (8): 1–23. https://doi.org/10.3390/agronomy10081174.

Hove, Nathalie C.Y., Peter Demeyer, Caroline Van der Heyden, Stephanie Van Weyenberg, and Herman Van Langenhove. 2017. “Improving the Repeatability of

Dynamic Olfactometry According to EN 13725: A Case Study for Pig Odour.” *Biosystems Engineering* 161: 70–79. https://doi.org/10.1016/j.biosystemseng.2017.06.004.

Hudson, N., K. Bell, E. McGahan, S. Lowe, G. Galvin, and K. Casey. 2007. “Odour Emissions from Anaerobic Piggery Ponds. 2: Improving Estimates of Emission Rate through Recognition of Spatial Variability.” *Bioresource Technology* 98 (10): 1888–97. https://doi.org/10.1016/j.biortech.2006.06.013.

Huijbregts, Mark A.J., Zoran J.N. Steinmann, Pieter M.F. Elshout, Gea Stam, Francesca Verones, Marisa Vieira, Michiel Zijp, Anne Hollander, and Rosalie van Zelm. 2017. “ReCiPe2016: A Harmonised Life Cycle Impact Assessment Method at Midpoint and Endpoint Level.” *International Journal of Life Cycle Assessment* 22 (2): 138–47. https://doi.org/10.1007/s11367-016-1246-y.

IPCC (Intergovernmental Panel on Climate Change). 2006. “Guidelines for National Greenhouse Gas Inventories: Agriculture, Forestry and Other Land Use.” *In: H. Dong, J. Mangino, T. A. McAllister, J. L. Hatfield, D. E. Johnson, … A. Romanovskaya (Working Group)*. Geneva, Switzerland.

IPTS. 2014. “IPTS (Institute for Prospective Technological Studies) End-of- Waste Criteria for Biodegradable Waste Subjected to Biological Treatment (Compost & Digestate): Technical Proposals - Final Report 2013, European Commission, JRC Scientific and Policy Reports.”

IRSA CNR. 1994. *Metodi Analitici per Le Acque*. Edited by Istituto Poligrafico e Zecca dello Stato. *Quaderni N. 100*. Rome, IT.

ISO. 1984. “ISO 5664 - Water Quality — Determination of Ammonium — Distillation and Titration Method.”

ISPRA. 2019. “Italian Emission Inventory 1990 - 2017.” *ISPRA, Rapporti 306/2019*.

Istituto Superiore di Sanità. 2018. “Rapporto ISTISAN 14/18.”

Italian Parliament. 1992. “D.Lgs. 27 Gennaio 1992, n. 99 (1). Attuazione Della Direttiva 86/278/CEE Concernente La Protezione Dell’ambiente, in Particolare Del Suolo, Nell’utilizzazione Dei Fanghi Di Depurazione in Agricoltura.”

———. 2018. “D.Lgs 28 Settembre 2018 n. 109, Coordinato Con La Legge Di Conversione 16 Novembre 2018 n.130. Recante:<<Disposizioni Urgenti per La Città Di Genova, La Sicurezza Della Rete Nazionale Delle Infrastrutture e Dei Trasporti, Gli Eventi Sismici Del 2016 e 201” 269.

Janzen, H. H. 2004. “Carbon Cycling in Earth Systems - A Soil Science Perspective.” *Agriculture, Ecosystems and Environment* 104 (3): 399–417. https://doi.org/10.1016/j.agee.2004.01.040.

Jardé, Emilie, Laurence Mansuy, and Pierre Faure. 2005. “Organic Markers in the Lipidic Fraction of Sewage Sludges.” *Water Research* 39 (7): 1215–32. https://doi.org/10.1016/j.watres.2004.12.024.

Jones-Lepp, T J, D A Alvarez, J D Petty, and J N Huckins. 2004. “E Nvironmental C Ontamination a n d T Polar Organic Chemical Integrative Sampling and Liquid Chromatography – Electrospray / Ion-Trap Mass Spectrometry for Assessing Selected Prescription and Illicit Drugs in Treated Sewage Effluents.” *Archives of Environmental Contamination and Toxicology* 439: 427–39. https://doi.org/10.1007/s00244-004-3146-6.

Jurado, Anna, Pablo Gago-Ferrero, Enric Vàzquez-Suñé, Jesus Carrera, Estanislao Pujades, M. Silvia Díaz-Cruz, and Damià Barceló. 2014. “Urban Groundwater Contamination by Residues of UV Filters.” *Journal of Hazardous Materials* 271

(April): 141–49. https://doi.org/10.1016/J.JHAZMAT.2014.01.036.

Kacprzak, Małgorzata, Ewa Neczaj, Krzysztof Fijałkowski, Anna Grobelak, Anna Grosser, Małgorzata Worwag, Agnieszka Rorat, Helge Brattebo, Åsgeir Almås, and Bal Ram Singh. 2017. “Sewage Sludge Disposal Strategies for Sustainable Development.” *Environmental Research* 156 (June 1986): 39–46. https://doi.org/10.1016/j.envres.2017.03.010.

Khush, Gurdev S. 2001. “Green Revolution: The Way Forward.” *Nature Reviews Genetics* 2 (10): 815–22. https://doi.org/10.1038/35093585.

Klöpffer, Walter. 2012. “The Critical Review of Life Cycle Assessment Studies According to ISO 14040 and 14044.” *International Journal of Life Cycle Assessment* 17 (9): 1087–93. https://doi.org/10.1007/s11367-012-0426-7.

Koch, M., and W. Rotard. 2001. “On the Contribution of Background Sources to the Heavy Metal Content of Municipal Sewage Sludge.” *Water Science and Technology* 43 (2): 67–74. https://doi.org/10.2166/wst.2001.0074.

Kögel-Knabner, Ingrid. 2002. “The Macromolecular Organic Composition of Plant and Microbial Residues as Inputs to Soil Organic Matter.” *Soil Biology and Biochemistry* 34 (2): 139–62. https://doi.org/10.1016/S0038-0717(01)00158-4.

Kolpin, Dana W, Edward T Furlong, and Michael T Meyer. 2002. “Pharmaceuticals , Hormones , and Other Organic Wastewater Contaminants in U . S . Streams , 1999 - 2000 : A National Reconnaissance” 36 (6): 1202–11.

Konradi, Sabine, and Ines Vogel. 2013. “Fate of Pharmaceuticals (PhCs) in Sewage Sludge and Proposal of Indicator Substances for Monitoring.” In *Pharmaceuticals in Soil, Sludge and Slurry*. Dessau.

Koszel, Milan, and Edmund Lorencowicz. 2015. “Agricultural Use of Biogas Digestate as a

Replacement Fertilizers.” *Agriculture and Agricultural Science Procedia* 7: 119–24. https://doi.org/10.1016/j.aaspro.2015.12.004.

Kupper, Thomas, Diane Bürge, Hans Jörg Bachmann, Sabine Güsewell, and Jochen Mayer. 2014. “Heavy Metals in Source-Separated Compost and Digestates.” *Waste Management* 34 (5): 867–74. https://doi.org/10.1016/j.wasman.2014.02.007.

Lake, D. L., P. W. W. Kirk, and J. N. Lester. 1984. “Fractionation, Characterization, and Speciation of Heavy Metals in Sewage Sludge and Sludge-Amended Soils: A Review.” *Journal of Environmental Quality* 13 (2): 175–83. https://doi.org/10.2134/jeq1984.00472425001300020001x.

Lal, R. 2009. “Challenges and Opportunities in Soil Organic Matter Research.” *European Journal of Soil Science* 60 (2): 158–69. https://doi.org/10.1111/j.1365-2389.2008.01114.x.

Lam, Ka Leung, Ljiljana Zlatanović, and Jan Peter van der Hoek. 2020. “Life Cycle Assessment of Nutrient Recycling from Wastewater: A Critical Review.” *Water Research* 173: 16. https://doi.org/10.1016/j.watres.2020.115519.

Leclerc, Alexandra, and Alexis Laurent. 2017. “Framework for Estimating Toxic Releases from the Application of Manure on Agricultural Soil: National Release Inventories for Heavy Metals in 2000–2014.” *Science of the Total Environment* 590–591: 452–60. https://doi.org/10.1016/j.scitotenv.2017.01.117.

Ledda, C., A. Schievano, S. Salati, and Fabrizio Adani. 2013. “Nitrogen and Water Recovery from Animal Slurries by a New Integrated Ultrafiltration, Reverse Osmosis and Cold Stripping Process: A Case Study.” *Water Research* 47 (16): 6157–66. https://doi.org/10.1016/j.watres.2013.07.037.

Lester, J. N., R. M. Sterritt, and P. W.W. Kirk. 1983. “Significance and Behaviour of Heavy

Metals in Waste Water Treatment Processes II. Sludge Treatment and Disposal." *Science of the Total Environment, The* 30 (C): 45–83. https://doi.org/10.1016/0048-9697(83)90003-7.

Li, Bingjie, Sining Yun, Tian Xing, Kaijun Wang, Teng Ke, and Jinhang An. 2021. "A Strategy for Understanding the Enhanced Anaerobic Co-Digestion via Dual-Heteroatom Doped Bio-Based Carbon and Its Functional Groups." *Chemical Engineering Journal* 425 (February): 130473. https://doi.org/10.1016/j.cej.2021.130473.

Lombardy Region. 2019. "Decr. Reg. Lombardia n. 6665 Del 14/05/2019 Ricognizione Dei Limiti Di Concentrazione Caraterrizzanti i Fanghi Di Depurazione Ideonei per l'utilizzo in Agricoltura, a Seguito Delle Nuove Disposizioni Normative Nazionali Di Cui Alla Legge 16/11/2018, n.130," 1–10.

Loria, Esteban R., John E. Sawyer, Daniel W. Barker, John P. Lundvall, and Jeffery C. Lorimor. 2007. "Use of Anaerobically Digested Swine Manure as a Nitrogen Source in Corn Production." *Agronomy Journal* 99 (4): 1119–29. https://doi.org/10.2134/agronj2006.0251.

Losacco, Caterina, and Antonella Perillo. 2018. "Particulate Matter Air Pollution and Respiratory Impact on Humans and Animals." *Environmental Science and Pollution Research* 25 (34): 33901–10. https://doi.org/10.1007/s11356-018-3344-9.

Lüdeke-Freund, Florian, Stefan Gold, and Nancy M.P. Bocken. 2019. "A Review and Typology of Circular Economy Business Model Patterns." *Journal of Industrial Ecology* 23 (1): 36–61. https://doi.org/10.1111/jiec.12763.

Macura, Biljana, Solveig L. Johannesdottir, Mikołaj Piniewski, Neal R. Haddaway, and Elisabeth Kvarnström. 2019. "Effectiveness of Ecotechnologies for Recovery of Nitrogen and Phosphorus from Anaerobic Digestate and Effectiveness of the

Recovery Products as Fertilisers: A Systematic Review Protocol.” *Environmental Evidence* 8 (1): 1–9. https://doi.org/10.1186/s13750-019-0173-3.

Makadi, Marianna, Attila Tomòcsik, Bettina Eich-Lobermann, and Katja Schiemenz. 2008. “NUTRIENT CYCLING BY USING RESIDUES OF BIOENERGY PRODUCTION - EFFECTS OF BIOGAS-DIGESTATE ON PLANT AND SOIL PARAMETERS.” In *Vol. 36, Supplement: Proceedings of the VII. Alps-Adria Scientific Workshop, 28 April-2 May 2008,* 1807–10. Stara Lesna, Slovakia. https://www.jstor.org/stable/90003077.

Makadi, Marianna, Attila Tomòcsik, and Viktòria Orosz. 2012. “Digestate: A New Nutrient Source - a Review.” *Biogas* 14: 295–312.

Manz, M., K. D. Wenzel, U. Dietze, and G. Schüürmann. 2001. “Persistent Organic Pollutants in Agricultural Soils of Central Germany.” *Science of the Total Environment* 277 (1–3): 187–98. https://doi.org/10.1016/S0048-9697(00)00877-9.

Maris, Stefania Codruta, Federico Capra, Federico Ardenti, Roberta Boselli, Davide Pochintesta, Gian Maria Beone, Vincenzo Tabaglio, and Andrea Fiorini. 2021. “The Interaction between Types of Cover Crop Residue and Digestate Application Methods Affects Ammonia Volatilization during Maize Cropping Season.” *Journal of Environmental Quality* 50 (2): 504–12. https://doi.org/10.1002/jeq2.20205.

Marschner, B., R. Winkler, and D. Jödemann. 2005. “Factors Controlling the Partitioning of Pyrene to Dissolved Organic Matter Extracted from Different Soils.” *European Journal of Soil Science* 56 (3): 299–306. https://doi.org/10.1111/j.1365-2389.2004.00678.x.

Martinez-Balmori, Dariellys, Riccardo Spaccini, Natália Oliveira Aguiar, Etelvino Henrique Novotny, Fábio Lopes Olivares, and Luciano Pasqualoto Canellas. 2014. “Molecular Characteristics of Humic Acids Isolated from Vermicomposts and Their Relationship to Bioactivity.” *Journal of Agricultural and Food Chemistry* 62 (47): 11412–19.

https://doi.org/10.1021/jf504629c.

Matsunaka, T., T. Sawamoto, H. Ishimura, K. Takakura, and A. Takekawa. 2006. “Efficient Use of Digested Cattle Slurry from Biogas Plant with Respect to Nitrogen Recycling in Grassland.” *International Congress Series* 1293: 242–52. https://doi.org/10.1016/j.ics.2006.03.016.

Matthews, P. J. 1979. “THE USE OF SEWAGE SLUDGE ON LAND.” *Water Science and Technology* 19 (8): 1–8. https://doi.org/10.2166/wst.1987.0038.

Mazzini, Stefania, Gigliola Borgonovo, Leonardo Scaglioni, Floriana Bedussi, Giuliana D’Imporzano, Fulvia Tambone, and Fabrizio Adani. 2020. “Phosphorus Speciation during Anaerobic Digestion and Subsequent Solid/Liquid Separation.” *Science of the Total Environment* 734: 139284. https://doi.org/10.1016/j.scitotenv.2020.139284.

Mc Lean, E. O. 1982. “Soil PH and Lime Requirement.” In *Methods of Soil Analysis. Part 2.*, edited by A. L. Page, 2nd ed. Ag, 199–224. Madison WI: ASA and SSSA.

McDonough, William. 2002. *Cradle to Cradle : Remaking the Way We Make Things*. 1st ed. New York : North Point Press, 2002.

Meersche, Tina Van den, Els Van Pamel, Christof Van Poucke, Lieve Herman, Marc Heyndrickx, Geertrui Rasschaert, and Els Daeseleire. 2016. “Development, Validation and Application of an Ultra High Performance Liquid Chromatographic-Tandem Mass Spectrometric Method for the Simultaneous Detection and Quantification of Five Different Classes of Veterinary Antibiotics in Swine Manure.” *Journal of Chromatography A* 1429: 248–57. https://doi.org/10.1016/j.chroma.2015.12.046.

Mendez, J M, B Jimenez, and C Maya. 2002. “Disinfection Kinetics of Pathogens in Physicochemical Sludge Treated with Ammonia,” no. 1: 67–74.

Menéndez, Sergio, Iskander Barrena, Igor Setien, Carmen González-Murua, and José María Estavillo. 2012. “Efficiency of Nitrification Inhibitor DMPP to Reduce Nitrous Oxide Emissions under Different Temperature and Moisture Conditions.” *Soil Biology and Biochemistry* 53: 82–89. https://doi.org/10.1016/j.soilbio.2012.04.026.

Mininni, G., A. R. Blanch, F. Lucena, and S. Berselli. 2015. “EU Policy on Sewage Sludge Utilization and Perspectives on New Approaches of Sludge Management.” *Environmental Science and Pollution Research* 22 (10): 7361–74. https://doi.org/10.1007/s11356-014-3132-0.

Ministero dell’ambiente. 2019. “DM 2019/46.”

Misselbrook, T. H., F. A. Nicholson, B. J. Chambers, and R. A. Johnson. 2005. “Measuring Ammonia Emissions from Land Applied Manure: An Intercomparison of Commonly Used Samplers and Techniques.” *Environmental Pollution* 135 (3 SPEC. ISS.): 389–97. https://doi.org/10.1016/j.envpol.2004.11.012.

Möller, Kurt, and Torsten Müller. 2012. “Effects of Anaerobic Digestion on Digestate Nutrient Availability and Crop Growth: A Review.” *Engineering in Life Sciences* 12 (3): 242–57. https://doi.org/10.1002/elsc.201100085.

Möller, Kurt, Rudolf Schulz, and Torsten Müller. 2010. “Substrate Inputs, Nutrient Flows and Nitrogen Loss of Two Centralized Biogas Plants in Southern Germany.” *Nutrient Cycling in Agroecosystems 2010 87:2* 87 (2): 307–25. https://doi.org/10.1007/S10705-009-9340-1.

Möller, Kurt, and Walter Stinner. 2009. “Effects of Different Manuring Systems with and without Biogas Digestion on Soil Mineral Nitrogen Content and on Gaseous Nitrogen Losses (Ammonia, Nitrous Oxides).” *European Journal of Agronomy* 30 (1): 1–16. https://doi.org/10.1016/j.eja.2008.06.003.

Moller, Kurt, Walter Stinner, Arno Deuker, and Gunter Leithold. 2008. “Effects of Different Manuring Systems Witch and without Biogas Digestion on Nitrogen Cycle and Crop Yield in Mixed Organic Dairy Farming Systems.” *Nutrient Cycling in Agroecosystems* 82: 209–32. https://doi.org/10.1007/s10705-008-9196-9.

Montemayor, Erica, August Bonmatí, Marta Torrellas, Francesc Camps, Carlos Ortiz, Francesc Domingo, Victor Riau, and Assumpció Antón. 2019. “Environmental Accounting of Closed-Loop Maize Production Scenarios: Manure as Fertilizer and Inclusion of Catch Crops.” *Resources, Conservation and Recycling* 146 (March): 395–404. https://doi.org/10.1016/j.resconrec.2019.03.013.

Morken, John, and Stein Sakshaug. 1998. “Direct Ground Injection of Livestock Waste Slurry to Avoid Ammonia Emission.” *Nutrient Cycling in Agroecosystems* 51 (1): 59–63. https://doi.org/10.1023/A:1009756927750.

Musa, M. M. 1968. “Nitrogen Fertilizer Transformations in the Sudan Geriza Soil.” *Plant Soil* 28: 413–21.

Nemecek, Thomas, and Thomas Kägi. 2007. “Life Cycle Inventories of Agricultural Production Systems.” *Ecoinvent*.

Nicholson, F. A., A. Bhogal, A. Rollett, M. Taylor, and J. R. Williams. 2018. “Precision Application Techniques Reduce Ammonia Emissions Following Food-Based Digestate Applications to Grassland.” *Nutrient Cycling in Agroecosystems* 110 (1): 151–59. https://doi.org/10.1007/s10705-017-9884-4.

Niero, Monia, Massimo Pizzol, Henrik Gundorph Bruun, and Marianne Thomsen. 2014. “Comparative Life Cycle Assessment of Wastewater Treatment in Denmark Including Sensitivity and Uncertainty Analysis.” *Journal of Cleaner Production* 68: 25–35. https://doi.org/10.1016/j.jclepro.2013.12.051.

Noguera-Oviedo, Katia, and Diana S. Aga. 2016. “Lessons Learned from More than Two Decades of Research on Emerging Contaminants in the Environment.” *Journal of Hazardous Materials* 316: 242–51. https://doi.org/10.1016/j.jhazmat.2016.04.058.

Nordin, Annika, and Karin Nyberg. 2009. “Inactivation of Ascaris Eggs in Source-Separated Urine and Feces by Ammonia at Ambient Temperatures ” 75 (3): 662–67. https://doi.org/10.1128/AEM.01250-08.

O’Connor, George A. 1996. “Organic Compounds in Sludge-Amended Soils and Their Potential for Uptake by Crop Plants.” *Science of the Total Environment* 185 (1–3): 71–81. https://doi.org/10.1016/0048-9697(95)05043-4.

Olsen, S. R., L. E. Sommers, and A. L. Page. 1982. *Methods of Soil Analysis*. Edited by A. L. Page. 2nd ed. Madison WI: Agron Monogr 9. ASA and ASSA.

Orzi, V., E. Cadena, G. Dimporzano, A. Artola, E. Davoli, M. Crivelli, and F. Adani. 2010. “Potential Odour Emission Measurement in Organic Fraction of Municipal Solid Waste during Anaerobic Digestion: Relationship with Process and Biological Stability Parameters.” *Bioresource Technology* 101 (19): 7330–37. https://doi.org/10.1016/j.biortech.2010.04.098.

Orzi, V., C. Riva, B. Scaglia, G. D’Imporzano, F. Tambone, and F. Adani. 2018. “Anaerobic Digestion Coupled with Digestate Injection Reduced Odour Emissions from Soil during Manure Distribution.” *Science of the Total Environment* 621: 168–76. https://doi.org/10.1016/j.scitotenv.2017.11.249.

Orzi, Valentina, Barbara Scaglia, Samuele Lonati, Carlo Riva, Gabriele Boccasile, Giovanni Loris Alborali, and Fabrizio Adani. 2015. “The Role of Biological Processes in Reducing Both Odor Impact and Pathogen Content during Mesophilic Anaerobic Digestion.” *Science of the Total Environment* 526: 116–26. https://doi.org/10.1016/j.scitotenv.2015.04.038.

Ottoson, J.R., A. Schurer, and B Vinneras. 2008. "In Situ Ammonia Production as Sanitation Agent during Anaerobic Digestion at Mesophilic Temperature." *Applied Microbiology*. https://doi.org/10.1111/j.1472-765X.2007.02317.x.

Padilla, Francisco M., Marisa Gallardo, and Francisco Manzano-Agugliaro. 2018. "Global Trends in Nitrate Leaching Research in the 1960–2017 Period." *Science of the Total Environment* 643 (2): 400–413. https://doi.org/10.1016/j.scitotenv.2018.06.215.

Pal, Raktim, Mallavarapu Megharaj, K Paul Kirkbride, and Ravi Naidu. 2013. "Science of the Total Environment Illicit Drugs and the Environment — A Review." *Science of the Total Environment* 463–464: 1079–92. https://doi.org/10.1016/j.scitotenv.2012.05.086.

Paolini, Valerio, Francesco Petracchini, Marco Segreto, Laura Tomassetti, Nour Naja, and Angelo Cecinato. 2018. "Environmental Impact of Biogas: A Short Review of Current Knowledge." *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering* 53 (10): 899–906. https://doi.org/10.1080/10934529.2018.1459076.

Papa, Gabriella, Barbara Scaglia, Andrea Schievano, and Fabrizio Adani. 2014. "Nanoscale Structure of Organic Matter Could Explain Litter Decomposition." *Biogeochemistry* 117 (2–3): 313–24. https://doi.org/10.1007/s10533-013-9863-z.

Payet, C., C. Bryselbout, J. L. Morel, and E. Lichtfouse. 1999. "Fossil Fuel Biomarkers in Sewage Sludges: Environmental Significance." *Naturwissenschaften* 86 (10): 484–88. https://doi.org/10.1007/s001140050659.

Peccia, Jordan, and Paul Westerhoff. 2015. "We Should Expect More out of Our Sewage Sludge." *Environmental Science and Technology* 49 (14): 8271–76. https://doi.org/10.1021/acs.est.5b01931.

Pepè Sciarria, Tommy, Giovanni Vacca, Fulvia Tambone, Luca Trombino, and Fabrizio Adani. 2019. *Nutrient Recovery and Energy Production from Digestate Using Microbial Electrochemical Technologies (METs)*. *Journal of Cleaner Production*. Vol. 208. Elsevier Ltd. https://doi.org/10.1016/j.jclepro.2018.10.152.

Petersen, S. O., K. Henriksen, G. K. Mortensen, P. H. Krogh, K. K. Brandt, J. Sørensen, T. Madsen, J. Petersen, and C. Grøn. 2003. "Recycling of Sewage Sludge and Household Compost to Arable Land: Fate and Effects of Organic Contaminants, and Impact on Soil Fertility." *Soil and Tillage Research* 72 (2): 139–52. https://doi.org/10.1016/S0167-1987(03)00084-9.

Peyron, Matteo, Chiara Bertora, Simone Pelissetti, Daniel Said-Pullicino, Luisella Celi, Eleonora Miniotti, Marco Romani, and Dario Sacco. 2016. "Greenhouse Gas Emissions as Affected by Different Water Management Practices in Temperate Rice Paddies." *Agriculture, Ecosystems and Environment* 232: 17–28. https://doi.org/10.1016/j.agee.2016.07.021.

Piccini, Irene, Fabrizio Arnieri, Enrico Caprio, Beatrice Nervo, Simone Pelissetti, Claudia Palestrini, Tomas Roslin, and Antonio Rolando. 2017. "Greenhouse Gas Emissions from Dung Pats Vary with Dung Beetle Species and with Assemblage Composition." *PLoS ONE* 12 (7): 1–15. https://doi.org/10.1371/journal.pone.0178077.

Pieroni, Marina P.P., Tim C. McAloone, and Daniela C.A. Pigosso. 2019. "Business Model Innovation for Circular Economy and Sustainability: A Review of Approaches." *Journal of Cleaner Production* 215: 198–216. https://doi.org/10.1016/j.jclepro.2019.01.036.

Pigoli, Ambrogio, Massimo Zilio, Fulvia Tambone, Stefania Mazzini, Micol Schepis, Erik Meers, Oscar Schoumans, Andrea Giordano, and Fabrizio Adani. 2021. "Thermophilic Anaerobic Digestion as Suitable Bioprocess Producing Organic and Chemical

Renewable Fertilizers: A Full-Scale Approach." *Waste Management* 124 (2021): 356–67. https://doi.org/10.1016/j.wasman.2021.02.028.

Piippo, Sari, Maria Lauronen, and Heini Postila. 2018. "Greenhouse Gas Emissions from Different Sewage Sludge Treatment Methods in North." *Journal of Cleaner Production* 177: 483–92. https://doi.org/10.1016/j.jclepro.2017.12.232.

Pingali, Prabhu L. 2012. "Green Revolution: Impacts, Limits, Andthe Path Ahead." *Proceedings of the National Academy of Sciences of the United States of America* 109 (31): 12302–8. https://doi.org/10.1073/pnas.0912953109.

Pognani, Michele, Giuliana D'Imporzano, Barbara Scaglia, and Fabrizio Adani. 2009. "Substituting Energy Crops with Organic Fraction of Municipal Solid Waste for Biogas Production at Farm Level: A Full-Scale Plant Study." *Process Biochemistry* 44 (8): 817–21. https://doi.org/10.1016/j.procbio.2009.03.014.

Provenzano, Maria Rosaria, Giuseppina Iannuzzi, Claudio Fabbri, and Nicola Senesi. 2011. "Qualitative Characterization and Differentiation of Digestates from Different Biowastes Using FTIR and Fluorescence Spectroscopies" 2011 (March): 83–89. https://doi.org/10.4236/jep.2011.21009.

Provolo, Giorgio, Giulia Manuli, Alberto Finzi, Giorgio Lucchini, Elisabetta Riva, and Gian Attilio Sacchi. 2018. "Effect of Pig and Cattle Slurry Application on Heavy Metal Composition of Maize Grown on Different Soils." *Sustainability (Switzerland)* 10 (8): 16. https://doi.org/10.3390/su10082684.

Qi, Guangdou, Zhifei Pan, Yasuha Sugawa, Fetra J. Andriamanohiarisoamanana, Takaki Yamashiro, Masahiro Iwasaki, Keiko Kawamoto, Ikko Ihara, and Kazutaka Umetsu. 2018. "Comparative Fertilizer Properties of Digestates from Mesophilic and Thermophilic Anaerobic Digestion of Dairy Manure: Focusing on Plant Growth Promoting Bacteria (PGPB) and Environmental Risk." *Journal of Material Cycles and*

Waste Management 20 (3): 1448–57. https://doi.org/10.1007/s10163-018-0708-7.

Qiao, Chunlian, Lingli Liu, Shuijin Hu, Jana E. Compton, Tara L. Greaver, and Quanlin Li. 2015. "How Inhibiting Nitrification Affects Nitrogen Cycle and Reduces Environmental Impacts of Anthropogenic Nitrogen Input." *Global Change Biology* 21 (3): 1249–57. https://doi.org/10.1111/gcb.12802.

Raheem, Abdul, Vineet Singh Sikarwar, Jun He, Wafa Dastyar, Dionysios D. Dionysiou, Wei Wang, and Ming Zhao. 2018. "Opportunities and Challenges in Sustainable Treatment and Resource Reuse of Sewage Sludge: A Review." *Chemical Engineering Journal* 337 (December 2017): 616–41. https://doi.org/10.1016/j.cej.2017.12.149.

Regione Lombardia. 2019. "DGR 6665/2019."

———. 2020. "Programma d'Azione Regionale per La Protezione Delle Acque Dall ' Inquinamento Provocato Dai Nitrati Provenienti Da Fonti Agricole Nelle Zone Vulnerabili Ai Sensi Della Direttiva Nitrati 91/676/CEE."

Rhoades, J. D. 1982. "Cation Exchange Capacity." In *Methods of Soil Analysis, Part 2*, edited by A. L. Page, Agron. Mon, 149–57. Madison WI: ASA and SSSA.

Rice, R.G., and A. Netzer. 1982. *Handbook of Ozone Technology and Application. Vol 1*. Ann Arbor. Stoneham, MA.: Butterworth Publishers.

Ridder, M De, S De Jong, J Polchar, and S Lingemann. 2012. *Risks and Opportunities in the Global Phosphate Rock Market: Robust Strategies in Times of Uncertainty. … Pdf (Accessed on 18 October 2013 …*.

Riva, C., Valentina Orzi, M. Carozzi, M. Acutis, G. Boccasile, S. Lonati, Fulvia Tambone, G. D'Imporzano, and Fabrizio Adani. 2016. "Short-Term Experiments in Using Digestate Products as Substitutes for Mineral (N) Fertilizer: Agronomic Performance, Odours, and Ammonia Emission Impacts." *Science of The Total Environment* 547:

206–14. https://doi.org/10.1016/j.scitotenv.2015.12.156.

Rochette, Philippe, Denis A. Angers, Martin H. Chantigny, Marc Olivier Gasser, J. Douglas MacDonald, David E. Pelster, and Normand Bertrand. 2013. “NH3 Volatilization, Soil NH4+concentration and Soil PH Following Subsurface Banding of Urea at Increasing Rates.” *Canadian Journal of Soil Science* 93 (2): 261–68. https://doi.org/10.4141/CJSS2012-095.

Rockström, J, O Gaffney, and G Thunberg. 2021. *Breaking Boundaries: The Science of Our Planet*. Dorling Kindersley Limited.

Rockström, Johan, Will Steffen, Kevin Noone, Åsa Persson, F. Stuart Chapin, Eric F. Lambin, Timothy M. Lenton, et al. 2009a. “A Safe Operating Environment for Humanity.” *Nature* 461|24 (September): 472–75. https://doi.org/10.5751/ES-03180-140232.

Rockström, Johan, Will Steffen, Kevin Noone, Åsa Persson, F Stuart Chapin, Eric F Lambin, Timothy M Lenton, et al. 2009b. “A Safe Operating Space for Humanity.” *Nature* 461 (7263): 472–75. https://doi.org/10.1038/461472a.

Rojas, Carlos Andrés Leguizamón, Cimélio Bayer, Sandra Mara Vieira Fontoura, Mirla Andrade Weber, and Fernando Vieiro. 2012. “Volatilização de Amônia Da Ureia Alterada Por Sistemas de Preparo de Solo e Plantas de Cobertura Invernais No Centro-Sul Do Paraná.” *Revista Brasileira de Ciencia Do Solo* 36 (1): 261–70. https://doi.org/10.1590/S0100-06832012000100027.

Ross, A.D., R.A. Lawrie, J.P. Keneally, and M.S. Whatmuff. 1992. “Risk Characterisation and Managment of Sewage Sludge on Agricultural Land - Implications Fot the Environment and Food-Chain.” *Australian Veterinary Journal* 69 (8): 177–81.

Rubæek, G. H., K. Henriksen, J. Petersen, B. Rasmussen, and S. G. Sommer. 1996.

"Effects of Application Technique and Anaerobic Digestion on Gaseous Nitrogen Loss from Animal Slurry Applied to Ryegrass (Lolium Perenne)." *Journal of Agricultural Science* 126 (4): 481–92. https://doi.org/10.1017/s0021859600075572.

Rule, K L, S D W Comber, D Ross, A Thornton, C K Makropoulos, and R Rautiu. 2006. "Diffuse Sources of Heavy Metals Entering an Urban Wastewater Catchment" 63: 64–72. https://doi.org/10.1016/j.chemosphere.2005.07.052.

Sabola, Taonga. 2017. "Power Reforms Progressing Well' | The Times Group," 1–27. https://doi.org/10.5281/zenodo.31008.

Sahlström, Leena. 2003. "A Review of Survival of Pathogenic Bacteria in Organic Waste Used in Biogas Plants." *Bioresource Technology* 87 (2): 161–66. https://doi.org/10.1016/S0960-8524(02)00168-2.

Saint-Denis, Thierry, and Jacques Goupy. 2004. "Optimization of a Nitrogen Analyser Based on the Dumas Method." *Analytica Chimica Acta* 515 (1): 191–98. https://doi.org/10.1016/j.aca.2003.10.090.

Sanz-Cobena, Alberto, Thomas Misselbrook, Vicci Camp, and Antonio Vallejo. 2011. "Effect of Water Addition and the Urease Inhibitor NBPT on the Abatement of Ammonia Emission from Surface Applied Urea." *Atmospheric Environment* 45 (8): 1517–24. https://doi.org/10.1016/j.atmosenv.2010.12.051.

Scaglia, Barbara, Giuliana D'Imporzano, Gilberto Garuti, Marco Negri, and Fabrizio Adani. 2014. "Sanitation Ability of Anaerobic Digestion Performed at Different Temperature on Sewage Sludge." *Science of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2013.07.114.

Scaglia, Barbara, Fulvia Tambone, Luca Corno, Valentina Orzi, Yuri Lazzarini, Gilberto Garuti, and Fabrizio Adani. 2018. "Potential Agronomic and Environmental

Properties of Thermophilic Anaerobically Digested Municipal Sewage Sludge Measured by an Unsupervised and a Supervised Chemometric Approach.” *Science of the Total Environment* 637–638: 791–802. https://doi.org/10.1016/j.scitotenv.2018.04.426.

Scarlat, Nicolae, Jean-François Dallemand, and Fernando Fahl. 2018. “Biogas: Developments and Perspectives in Europe.” *Renewable Energy* 129: 457–72. https://doi.org/https://doi.org/10.1016/j.renene.2018.03.006.

Schievano, Andrea, Giuliana D’Imporzano, and Fabrizio Adani. 2009. “Substituting Energy Crops with Organic Wastes and Agro-Industrial Residues for Biogas Production.” *Journal of Environmental Management* 90 (8): 2537–41. https://doi.org/10.1016/j.jenvman.2009.01.013.

Schievano, Andrea, Michele Pognani, Giuliana D’Imporzano, and Fabrizio Adani. 2008. “Predicting Anaerobic Biogasification Potential of Ingestates and Digestates of a Full-Scale Biogas Plant Using Chemical and Biological Parameters.” *Bioresource Technology* 99 (17): 8112–17. https://doi.org/10.1016/j.biortech.2008.03.030.

Schoumans, Oscar F., Fayçal Bouraoui, Christian Kabbe, Oene Oenema, and Kimo C. van Dijk. 2015. “Phosphorus Management in Europe in a Changing World.” *Ambio* 44 (2): 180–92. https://doi.org/10.1007/s13280-014-0613-9.

Seadi, Teodorita Al, Bernhard Drosg, Werner Fuchs, Dominik Rutz, and Rainer Janssen. 2013. *Biogas Digestate Quality and Utilization*. *The Biogas Handbook: Science, Production and Applications*. https://doi.org/10.1533/9780857097415.2.267.

Setti, Leonardo, Fabrizio Passarini, Gianluigi De Gennaro, Pierluigi Barbieri, Sabina Licen, Maria Grazia Perrone, Andrea Piazzalunga, et al. 2020. “Potential Role of Particulate Matter in the Spreading of COVID-19 in Northern Italy: First Observational Study Based on Initial Epidemic Diffusion.” *BMJ Open* 10 (9): 1–9.

https://doi.org/10.1136/bmjopen-2020-039338.

Sigurnjak, I., C. Vaneeckhaute, E. Michels, B. Ryckaert, G. Ghekiere, F. M.G. Tack, and E. Meers. 2017. “Fertilizer Performance of Liquid Fraction of Digestate as Synthetic Nitrogen Substitute in Silage Maize Cultivation for Three Consecutive Years.” *Science of the Total Environment* 599–600: 1885–94. https://doi.org/10.1016/j.scitotenv.2017.05.120.

Sigurnjak, Ivona, C. Brienza, E. Snauwaert, A. De Dobbelaere, J. De Mey, C. Vaneeckhaute, E. Michels, O. Schoumans, Fabrizio Adani, and E. Meers. 2019. “Production and Performance of Bio-Based Mineral Fertilizers from Agricultural Waste Using Ammonia (Stripping-)Scrubbing Technology.” *Waste Management* 89: 265–74. https://doi.org/10.1016/j.wasman.2019.03.043.

Sigurnjak, Ivona, Evi Michels, Sara Crappé, Saskia Buysens, Filip M.G. Tack, and Erik Meers. 2016. “Utilization of Derivatives from Nutrient Recovery Processes as Alternatives for Fossil-Based Mineral Fertilizers in Commercial Greenhouse Production of Lactuca Sativa L.” *Scientia Horticulturae* 198: 267–76. https://doi.org/10.1016/j.scienta.2015.11.038.

Singh, Bijay. 2018. “Are Nitrogen Fertilizers Deleterious to Soil Health?” *Agronomy* 8 (4). https://doi.org/10.3390/agronomy8040048.

Smajla, Ivan, Daria Karasalihović Sedlar, Branko Drljača, and Lucija Jukić. 2019. “Fuel Switch to LNG in Heavy Truck Traffic.” *Energies* 12 (3): 19. https://doi.org/10.3390/en12030515.

Smith, Collin, Alfred K. Hill, and Laura Torrente-Murciano. 2020. “Current and Future Role of Haber-Bosch Ammonia in a Carbon-Free Energy Landscape.” *Energy and Environmental Science* 13 (2): 331–44. https://doi.org/10.1039/c9ee02873k.

Smith, P., S. Lutfalla, W. J. Riley, M. S. Torn, M. W.I. Schmidt, and J. F. Soussana. 2018. “The Changing Faces of Soil Organic Matter Research.” *European Journal of Soil Science* 69 (1): 23–30. https://doi.org/10.1111/ejss.12500.

Smith, V.H., G.D. Tilman, and J.C. Nekola. 1999. “Eutrophication: Impacts of Excess Nutrient Inputs on Freshwater, Marine, and Terrestrial Ecosystems.” *Environmental Pollution* 100 (1–3): 179–96. https://doi.org/10.1016/S0269-7491(99)00091-3.

Sommer, S. G., and N. J. Hutchings. 2001. “Ammonia Emission from Field Applied Manure and Its Reduction - Invited Paper.” *European Journal of Agronomy* 15 (1): 1–15. https://doi.org/10.1016/S1161-0301(01)00112-5.

Sommer, S. G., and J. E. Olesen. 2000. “Modelling Ammonia Volatilization from Animal Slurry Applied with Trail Hoses to Cereals.” *Atmospheric Environment* 34 (15): 2361–72. https://doi.org/10.1016/S1352-2310(99)00442-2.

Sörme, L., and R. Lagerkvist. 2002. “Sources of Heavy Metals in Urban Wastewater in Stockholm.” *Science of the Total Environment* 298 (1–3): 131–45. https://doi.org/10.1016/S0048-9697(02)00197-3.

Springmann, Marco, Michael Clark, Daniel Mason-D’Croz, Keith Wiebe, Benjamin Leon Bodirsky, Luis Lassaletta, Wim de Vries, et al. 2018. “Options for Keeping the Food System within Environmental Limits.” *Nature* 562 (7728): 519–25. https://doi.org/10.1038/s41586-018-0594-0.

Stahel, Walter R. 2019. *The Circular Economy: A User’s Guide*. Edited by Ellen Foundation MacArthur. First. New York, NY, USA: Taylor & Francis.

Steffen, W., K. Richardson, J. Rockstrom, S. E. Cornell, I. Fetzer, E. M. Bennett, R. Biggs, et al. 2015. “Planetary Boundaries: Guiding Human Development on a Changing Planet.” *Science* 347 (6223): 1259855–1259855.

https://doi.org/10.1126/science.1259855.

Steffen, Will, Katherine Richardson, Johan Rockström, Sarah E. Cornell, Ingo Fetzer, Elena M. Bennett, Reinette Biggs, et al. 2015. “Planetary Boundaries: Guiding Human Development on a Changing Planet.” *Science* 347 (6223): 10. https://doi.org/10.1126/science.1259855.

Stinner, Walter, Kurt Möller, and Günter Leithold. 2008. “Effects of Biogas Digestion of Clover/Grass-Leys, Cover Crops and Crop Residues on Nitrogen Cycle and Crop Yield in Organic Stockless Farming Systems.” *European Journal of Agronomy* 29 (2–3): 125–34. https://doi.org/10.1016/j.eja.2008.04.006.

Stokstad, Erik. 2014. “Ammonia Pollution from Farming May Exact Hefty Health Costs.” *Science* 343 (6168): 238. https://doi.org/10.1126/science.343.6168.238.

Straub, T. M., I. L. Pepper, and C. P. Gerba. 1993. “Hazards from Pathogenic Microorganisms in Land-Disposed Sewage Sludge.” *Reviews of Environmental Contamination and Toxicology* 132: 55–91. https://doi.org/10.1007/978-1-4684-7065-9_3.

Styles, David, Paul Adams, Gunnar Thelin, Céline Vaneeckhaute, David Chadwick, and Paul J.A. Withers. 2018. “Life Cycle Assessment of Biofertilizer Production and Use Compared with Conventional Liquid Digestate Management.” *Environmental Science and Technology* 52 (13): 7468–76. https://doi.org/10.1021/acs.est.8b01619.

Sutton, M.A., A. Bleeker, C.M. Howard, M. Bekunda, B. Grizzetti, W. de Vries, H.J.M. van Grinsven, et al. 2018. *Our Nutrient World: The Challenge to Produce More Food and Energy with Less Pollution. Global Overview of Nutrient Management. Centre for Ecology and Hydrology, Edinburgh on Behalf of the Global Partnership on Nutrient Management and the International Nitrogen Initiative*. Vol. 47.

Tambone, Fulvia, and Fabrizio Adani. 2017. “Nitrogen Mineralization from Digestate in Comparison to Sewage Sludge, Compost and Urea in a Laboratory Incubated Soil Experiment.” *Zeitschrift Fur Pflanzenernahrung Und Bodenkunde* 180 (3): 355–65. https://doi.org/10.1002/jpln.201600241.

Tambone, Fulvia, Fabrizio Adani, Giovanni Gigliotti, Daniela Volpe, Claudio Fabbri, and Maria Rosaria Provenzano. 2013a. “Organic Matter Characterization during the Anaerobic Digestion of Different Biomasses by Means of CPMAS 13C NMR Spectroscopy.” *Biomass and Bioenergy* 48: 111–20. https://doi.org/10.1016/j.biombioe.2012.11.006.

———. 2013b. “Organic Matter Characterization during the Anaerobic Digestion of Different Biomasses by Means of CPMAS 13C NMR Spectroscopy.” *Biomass and Bioenergy* 48: 111–20. https://doi.org/10.1016/j.biombioe.2012.11.006.

Tambone, Fulvia, Pierluigi Genevini, Giuliana D’Imporzano, and Fabrizio Adani. 2009. “Assessing Amendment Properties of Digestate by Studying the Organic Matter Composition and the Degree of Biological Stability during the Anaerobic Digestion of the Organic Fraction of MSW.” *Bioresource Technology* 100 (12): 3140–42. https://doi.org/10.1016/j.biortech.2009.02.012.

Tambone, Fulvia, Valentina Orzi, Giuliana D’Imporzano, and Fabrizio Adani. 2017. “Solid and Liquid Fractionation of Digestate: Mass Balance, Chemical Characterization, and Agronomic and Environmental Value.” *Bioresource Technology* 243 (June): 1251–56. https://doi.org/10.1016/j.biortech.2017.07.130.

Tambone, Fulvia, Valentina Orzi, Massimo Zilio, and Fabrizio Adani. 2019. “Measuring the Organic Amendment Properties of the Liquid Fraction of Digestate.” *Waste Management* 88: 21–27. https://doi.org/10.1016/j.wasman.2019.03.024.

Tambone, Fulvia, Barbara Scaglia, Giuliana D’Imporzano, Andrea Schievano, Valentina

Orzi, Silvia Salati, and Fabrizio Adani. 2010. “Assessing Amendment and Fertilizing Properties of Digestates from Anaerobic Digestion through a Comparative Study with Digested Sludge and Compost.” *Chemosphere* 81 (5): 577–83. https://doi.org/10.1016/J.CHEMOSPHERE.2010.08.034.

Tambone, Fulvia, Laura Terruzzi, Barbara Scaglia, and Fabrizio Adani. 2015. “Composting of the Solid Fraction of Digestate Derived from Pig Slurry: Biological Processes and Compost Properties.” *Waste Management* 35: 55–61. https://doi.org/10.1016/j.wasman.2014.10.014.

Tang, Y.S., J.N. Cape, and M.A. Sutton. 2001. “Development and Types of Passive Samplers for Monitoring Atmospheric NO2 and NH3 Concentrations.” *The Scientific World JOURNAL* 1 (2): 513–29. https://doi.org/10.1100/tsw.2001.82.

Tani, M., N. Sakamoto, T. Kishimoto, and K. Umetsu. 2006. “Utilization of Anaerobically Digested Dairy Slurry Combined with Other Wastes Following Application to Agricultural Land.” *International Congress Series* 1293: 331–34. https://doi.org/10.1016/j.ics.2006.03.013.

Teglia, C., A. Tremier, and J. L. Martel. 2011. “Characterization of Solid Digestates : Part 2 , Assessment of the Quality and Suitability for Composting of Six Digested Products,” 113–26. https://doi.org/10.1007/s12649-010-9059-x.

Terhoeven-Urselmans, Thomas, Edwin Scheller, Markus Raubuch, Bernard Ludwig, and Rainer Georg Joergensen. 2009. “CO2 Evolution and N Mineralization after Biogas Slurry Application in the Field and Its Yield Effects on Spring Barley.” *Applied Soil Ecology* 42 (3): 297–302. https://doi.org/10.1016/j.apsoil.2009.05.012.

Toop, Trisha A., Shane Ward, Thomas Oldfield, Maria Hull, Marie E. Kirby, and Michael K. Theodorou. 2017. “AgroCycle - Developing a Circular Economy in Agriculture.” *Energy Procedia* 123: 76–80. https://doi.org/10.1016/j.egypro.2017.07.269.

Tran, Bich Chau, Marie Jeanne Teil, Martine Blanchard, Fabrice Alliot, and Marc Chevreuil. 2015. “Fate of Phthalates and BPA in Agricultural and Non-Agricultural Soils of the Paris Area (France).” *Environmental Science and Pollution Research* 22 (14): 11118–26. https://doi.org/10.1007/s11356-015-4178-3.

Turner, Benjamin L., and April B. Leytem. 2004. “Phosphorus Compounds in Sequential Extracts of Animal Manures: Chemical Speciation and a Novel Fractionation Procedure.” *Environmental Science and Technology* 38 (22): 6101–8. https://doi.org/10.1021/es0493042.

Ullah, Ikram, Muhammad Ali, and Arifa Farooqi. 2010. “Chemical and Nutritional Properties of Some Maize (Zea Mays L.) Varieties Grown in NWFP, Pakistan.” *Pakistan Journal of Nutrition* 9 (11): 1113–17. https://doi.org/10.3923/pjn.2010.1113.1117.

UNI. 2007. “UNI 11199 - Caratterizzazione Dei Rifiuti - Determinazione Di Policlorodibenzo-p- Diossine (PCDD) e Policlorodibenzofurani (PCDF) in Rifiuti Solidi.”

UNI EN. 2004. “ISO 16703:2004 - Soil Quality — Determination of Content of Hydrocarbon in the Range C10 to C40 by Gas Chromatography.”

———. 2007. “UNI 11199 - Caratterizzazione Dei Rifiuti - Determinazione Di Policlorodibenzo-p- Diossine (PCDD) e Policlorodibenzofurani (PCDF) in Rifiuti Solidi.”

———. 2016. “UNI EN ISO 22155 - Soil Quality - Gas Chromatographic Determination of Volatile Aromatic and Halogenated Hydrocarbons and Selected Ethers - Static Headspace Method.”

———. 2018. “UNI EN 16167 - Soil, Treated Biowaste and Sludge - Determination of Dioxins and Furans and Dioxin-like Polychlorinated Biphenyls by Gas Chromatography with High Resolution Mass Selective Detection (HR GC-MS).”

Vaneeckhaute, C., E. Meers, E. Michels, G. Ghekiere, F. Accoe, and Filip M.G. Tack. 2013. “Closing the Nutrient Cycle by Using Bio-Digestion Waste Derivatives as Synthetic Fertilizer Substitutes: A Field Experiment.” *Biomass and Bioenergy* 55: 175–89. https://doi.org/10.1016/j.biombioe.2013.01.032.

Verdi, L., P. J. Kuikman, S. Orlandini, M. Mancini, M. Napoli, and A. Dalla Marta. 2019. “Does the Use of Digestate to Replace Mineral Fertilizers Have Less Emissions of N 2 O and NH 3 ?” *Agricultural and Forest Meteorology* 269–270 (June 2017): 112–18. https://doi.org/10.1016/j.agrformet.2019.02.004.

Verlicchi, P., and E. Zambello. 2015. “Pharmaceuticals and Personal Care Products in Untreated and Treated Sewage Sludge: Occurrence and Environmental Risk in the Case of Application on Soil - A Critical Review.” *Science of the Total Environment* 538: 750–67. https://doi.org/10.1016/j.scitotenv.2015.08.108.

Walker, M., K. Iyer, S. Heaven, and C. J. Banks. 2011. “Ammonia Removal in Anaerobic Digestion by Biogas Stripping: An Evaluation of Process Alternatives Using a First Order Rate Model Based on Experimental Findings.” *Chemical Engineering Journal* 178: 138–45. https://doi.org/10.1016/j.cej.2011.10.027.

Walsh, John J., Davey L. Jones, Gareth Edwards-Jones, and A. Prysor Williams. 2012. “Replacing Inorganic Fertilizer with Anaerobic Digestate May Maintain Agricultural Productivity at Less Environmental Cost.” *Journal of Plant Nutrition and Soil Science* 175 (6): 840–45. https://doi.org/10.1002/jpln.201200214.

Wang, Kaijun, Sining Yun, Tian Xing, Bingjie Li, Yasir Abbas, and Xinming Liu. 2021. “Binary and Ternary Trace Elements to Enhance Anaerobic Digestion of Cattle Manure: Focusing on Kinetic Models for Biogas Production and Digestate Utilization.”

Bioresource Technology 323 (October 2020): 124571. https://doi.org/10.1016/j.biortech.2020.124571.

Webb, J., Brian Pain, Shabtai Bittman, and John Morgan. 2010. “The Impacts of Manure Application Methods on Emissions of Ammonia, Nitrous Oxide and on Crop Response-A Review.” *Agriculture, Ecosystems and Environment* 137 (1–2): 39–46. https://doi.org/10.1016/j.agee.2010.01.001.

Webb, J., Peter Sørensen, Gerard Velthof, Barbara Amon, Miriam Pinto, Lena Rodhe, Eva Salomon, Nicholas Hutchings, Piotr Burczyk, and Joanne Reid. 2013. “An Assessment of the Variation of Manure Nitrogen Efficiency throughout Europe and an Appraisal of Means to Increase Manure-N Efficiency.” In *Advances in Agronomy*, 119:371–442. Elsevier. https://doi.org/10.1016/B978-0-12-407247-3.00007-X.

Weidema, Bo Pedersen, C. Bauer, R. Hischier, C. Mutel, T. Nemecek, J. Reinhard, C. O. Vadenbo, and G. Wernet. 2013. “Overview and Methodology. Data Quality Guideline for the Ecoinvent Database Version 3.” Vol. 3. St. Gallen: The ecoinvent Centre.

Weissengruber, Lina, Kurt Möller, Markus Puschenreiter, and Jürgen K. Friedel. 2018. “Long-Term Soil Accumulation of Potentially Toxic Elements and Selected Organic Pollutants through Application of Recycled Phosphorus Fertilizers for Organic Farming Conditions.” *Nutrient Cycling in Agroecosystems* 110 (3): 427–49. https://doi.org/10.1007/s10705-018-9907-9.

Welch, Ross M. 2002. “The Impact of Mineral Nutrients in Food Crops on Global Human Health.” *Plant and Soil* 247 (1): 83–90. https://doi.org/10.1023/A:1021140122921.

Westerman, P. W., and J. R. Bicudo. 2005. “Management Considerations for Organic Waste Use in Agriculture.” *Bioresource Technology* 96 (2): 215–21. https://doi.org/10.1016/j.biortech.2004.05.011.

Willén, A., C. Junestedt, L. Rodhe, M. Pell, and H. Jönsson. 2017. "Sewage Sludge as Fertiliser - Environmental Assessment of Storage and Land Application Options." *Water Science and Technology* 75 (5): 1034–50. https://doi.org/10.2166/wst.2016.584.

Wing, Steve, Amy Lowman, Alex Keil, and Stephen W. Marshall. 2014. "Odors from Sewage Sludge and Livestock: Associations with Self-Reported Health." *Public Health Reports* 129 (6): 505–15. https://doi.org/10.1177/003335491412900609.

Xing, Tian, Sining Yun, Bingjie Li, Kaijun Wang, Jiageng Chen, Bo Jia, Teng Ke, and Jinhang An. 2021. "Coconut-Shell-Derived Bio-Based Carbon Enhanced Microbial Electrolysis Cells for Upgrading Anaerobic Co-Digestion of Cow Manure and Aloe Peel Waste." *Bioresource Technology* 338 (July): 125520. https://doi.org/10.1016/j.biortech.2021.125520.

Xu, Hongfei, Sining Yun, Chen Wang, Ziqi Wang, Feng Han, Bo Jia, Jiageng Chen, and Bingjie Li. 2020. "Improving Performance and Phosphorus Content of Anaerobic Co-Digestion of Dairy Manure with Aloe Peel Waste Using Vermiculite." *Bioresource Technology* 301 (December 2019): 122753. https://doi.org/10.1016/j.biortech.2020.122753.

Xu, Y., Wantai Yu, Q. Ma, and H. Zhou. 2013. "Accumulation of Copper and Zinc in Soil and Plant within Ten-Year Application of Different Pig Manure Rates." *Plant, Soil and Environment* 59 (11): 492–99. https://doi.org/10.17221/121/2013-pse.

Yasar, Abdullah, Rizwan Rasheed, Amtul Bari Tabinda, Aleena Tahir, and Friha Sarwar. 2017. "Life Cycle Assessment of a Medium Commercial Scale Biogas Plant and Nutritional Assessment of Effluent Slurry." *Renewable and Sustainable Energy Reviews* 67 (January): 364–71. https://doi.org/10.1016/J.RSER.2016.09.026.

Yoshida, Hiroko, Marieke ten Hoeve, Thomas H. Christensen, Sander Bruun, Lars S.

Jensen, and Charlotte Scheutz. 2018. “Life Cycle Assessment of Sewage Sludge Management Options Including Long-Term Impacts after Land Application.” *Journal of Cleaner Production* 174: 538–47. https://doi.org/10.1016/j.jclepro.2017.10.175.

Yun, Sining, Tian Xing, Feng Han, Jing Shi, Ziqi Wang, Qingyang Fan, and Hongfei Xu. 2021. “Enhanced Direct Interspecies Electron Transfer with Transition Metal Oxide Accelerants in Anaerobic Digestion.” *Bioresource Technology* 320 (PA): 124294. https://doi.org/10.1016/j.biortech.2020.124294.

Zhang, Lei, and Deokjin Jahng. 2010. “Enhanced Anaerobic Digestion of Piggery Wastewater by Ammonia Stripping: Effects of Alkali Types.” *Journal of Hazardous Materials* 182 (1–3): 536–43. https://doi.org/10.1016/j.jhazmat.2010.06.065.

Zhang, Pei, and Yinguang Chen. 2017. “Polycyclic Aromatic Hydrocarbons Contamination in Surface Soil of China: A Review.” *Science of the Total Environment* 605–606: 1011–20. https://doi.org/10.1016/j.scitotenv.2017.06.247.

Zhang, Xin, Eric A. Davidson, Denise L. Mauzerall, Timothy D. Searchinger, Patrice Dumas, and Ye Shen. 2015. “Managing Nitrogen for Sustainable Development.” *Nature* 528 (7580): 51–59. https://doi.org/10.1038/nature15743.

Zhu, Yan-Li, Guo-Di Zheng, Ding Gao, Tong-Bin Chen, Fang-Kun Wu, Ming-Jie Niu, & Ke-Hua Zou, and Ke-Hua Zou. 2016. “Odor Composition Analysis and Odor Indicator Selection during Sewage Sludge Composting Odor Composition Analysis and Odor Indicator Selection during Sewage Sludge Composting.” *Journal of Air 6 & Waste Management Association* 66:9: 930–40. https://doi.org/10.1080/10962247.2016.1188865.

Zilio, M., V. Orzi, M. E. Chiodini, C. Riva, M. Acutis, G. Boccasile, and F. Adani. 2020. “Evaluation of Ammonia and Odour Emissions from Animal Slurry and Digestate Storage in the Po Valley (Italy).” *Waste Management* 103: 296–304.

https://doi.org/10.1016/j.wasman.2019.12.038.

Zilio, Massimo, Silvia Motta, Fulvia Tambone, Barbara Scaglia, Gabriele Boccasile, Andrea Squartini, and Fabrizio Adani. 2020. “The Distribution of Functional N-Cycle Related Genes and Ammonia and Nitrate Nitrogen in Soil Profiles Fertilized with Mineral and Organic N Fertilizer.” *PLoS ONE* 15 (6): 1–19. https://doi.org/10.1371/journal.pone.0228364.

Zilio, Massimo, Ambrogio Pigoli, Bruno Rizzi, Gabriele Geromel, Erik Meers, Oscar Schoumans, Andrea Giordano, and Fabrizio Adani. 2021. “Measuring Ammonia and Odours Emissions during Full Fi Eld Digestate Use in Agriculture.” *Science of the Total Environment* 782: 146882. https://doi.org/10.1016/j.scitotenv.2021.146882.

Zwolak, Aneta, Magdalena Sarzyńska, Ewa Szpyrka, and Kinga Stawarczyk. 2019. “Sources of Soil Pollution by Heavy Metals and Their Accumulation in Vegetables: A Review.” *Water, Air, and Soil Pollution* 230 (7). https://doi.org/10.1007/s11270-019-4221-y.