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ENVIRONMENTAL IMPACTS IN THE USE OF DIGESTATE AS SUBSTITUTE OF CHEMICAL FERTILIZERS

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Preface

The following research work is about the assessment of environmental impacts in the application of digestate for crop fertilization, at field scale level, and was supervised by Professor Fulvia Tambone in the frame of Horizon 2020 Nutri2Cycle project. The following PhD thesis is a collection of four research papers, of which one published, two submitted and a fourth under submission. Chapter one is an introduction and overview of the context of research, followed by four research papers (chapters 2-4), and followed by a discussion of the conclusions of this work (chapter 5). Titles of papers, authors and editors are listed below.

- Chapter 2: USE OF HIGHLY STABILIZED DIGESTATE TO REPLACE UREA IN OPEN FIELD AGRICULTURE: EFFECTS ON SOIL, ENVIRONMENT, AND MAIZE PRODUCTION (*submitted*)

  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 3: MEASURING AMMONIA AND ODOURS EMISSIONS DURING FULL FIELD DIGESTATE USE IN AGRICULTURE (*published*)

  Massimo Zilio, Ambrogio Pigoli, Bruno Rizzi, Gabriele Geromel, Erik Meers, Oscar Schoumans, Andrea Giordano, Fabrizio Adani
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- Chapter 4: IMPACT OF DIGESTATE FERTILIZATION ON NITROGEN CYCLE IN SOIL PROFILE (*under submission*)

  Bruno Rizzi, Massimo Zilio, Andrea Squartini, Piergiorgio Stevanato, Gabriele Geromel, Erik Meers, Oscar Schoumans, Andrea Giordano, Fabrizio Adani

- Chapter 5: ENVIRONMENTAL PERFORMANCE IN THE PRODUCTION AND USE OF RECOVERED FERTILIZERS FROM ORGANIC WASTES TREATED BY ANAEROBIC DIGESTION VS. SYNTHETIC MINERAL FERTILIZERS (*submitted*)

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

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1. Introduction

World population is constantly increasing and estimated to reach around 9.5 billion by 2050 (FAO 2017). In order to properly satisfy this demographic growth, it will be necessary to intensify food production at a global level, and therefore increase the environmental pressure on those resources linked to agricultural activities, such as soil, nutrients and water, which are at the same time fundamental for life on the planet.

In fact, the need to meet an increased food demand, has led to intensive use of chemical fertilizers and pesticides, monoculture cropping, and, in general, the adoption of an inappropriate agricultural management, with subsequent alteration of the natural nitrogen cycle.

1.1. Nitrogen cycle

Nitrogen is one of the most important nutrients and is essential for the life of many living beings. It can be found in the atmosphere, soil and water and is a crucial element for the production of plants in agriculture (LeBauer and Treseder 2008).

Plants take nitrogen they need mainly as nitrate (NO_3^-), in minor quantity as ammonia salts (NH_4^+), for example forest species that live in acid soils and poor in nitrates, and as nitrites (NO_2^-). The nitrogen reserves in the soil consist mainly of the organic substance present, which contains about 5% of it. Every year a fraction equal to 1-2% is mineralized, releasing the inorganic nitrogen necessary for plants. Furthermore, in the soil, there are small percentages of ammonia and nitric compounds in the mineral fraction. The amount of nitrates in a soil in fact depends on its nitrifying power, so on the activity of bacteria that transform ammonia nitrogen (NH_4^+) into nitric nitrogen

(NO_3^-). Mineral and organic nitrogen represents only 1/5 of the total nitrogen, the other 4/5 are represented by the molecular form (N_2) contained in the atmosphere, being the largest source on our planet, from which it is introduced into the ecosystems through atmospheric, chemical and biological fixation, the latter by far more important from a quantitative, ecological and agronomic point of view than the others.

Non-biological nitrogen fixation represents a small part in the cycle of the element itself. In fact, it represents that part of nitrogen that is transformed directly into nitrate in the atmosphere by the action of lightning, UV.rays, and cosmic radiation. The process also includes small quantities of ammonium (NH_4^+), which are produced by volcanic phenomena, forest fires and fumes from industrial plants.

Biological fixation of gaseous atmospheric nitrogen is by far more important from a quantitative, ecological and agronomic point of view than other fixation processes. The process occurs thanks to the nitrogenase enzyme, owned by nitrogen fixing bacteria, free or symbiotic, such as *Clostridium*, *Beijerinckia*, many cyanophytes, *Rhizobium*, just to name a few. Anyway, nitrogen-fixing microorganisms are very rare, as most of microorganisms use other forms of nitrogen, such as ammonium and nitrate ions.

In fact, once nitrogen reaches the ground, it is continuously recycled by microorganisms which, for their metabolism, draw energy from the chemical bonds of organic and inorganic molecules through ammonization (*Pseudomonas*) and nitrification (*Nitrosomonas* and *Nitrobacter*) processes; these make nitrogen available to the plant in the two most congenial forms for assimilation, ammonium ion (NH_4^+) and nitrate (NO_3^-).

The absorption in favor of one or the other form depends on the plant species and the environmental conditions, especially the pH: the absorption of NH_4^+ ions is favored in neutral conditions, depressed, on the other hand, in an acidic environment. The opposite occurs in the case of NO_3^- ions, which are more absorbed at lower pH values.

Ammonization is mainly regulated by various factors such as: temperature (from 15 ° to 60 ° C), humidity (60% of the total capacity of the soil), pH (neutral), presence of limestone and air, composition of the organic matter and the presence of microorganisms dedicated to the process (*Bacillus mycoides, Bacillus subtilis, Bacillus proteus, Bacillus vulgatus,* etc.). Subsequently, the oxidative process takes place involving ammonia present in the soil which leads to the formation of nitric acid, which takes place in two phases.

The first step of reaction is the oxydation of ammonium ion (NH_4^+) into nitrite (NO_2^-). This conversion is due to the activity of of soil ammonia-oxidising bacteria (AOB), such as *Nitrosospira, Nitrosomonas, Nitrococcus*, and ammonia-oxydising archaea (AOA), even doe the latter are not as important in agricultural soils (Hong J Di et al. 2010; Ferguson, Richardson, and Van Spanning 2007; Prosser 2007). In the second phase, the genus *Nitrobacter* provide for the final oxidation of the nitrite (NO_2^-) to nitrate (NO_3^-). Since this conversion is very rapid, concentrations of nitrite in soils are generally very low. Clearly, this process is influenced by significant factors such as: oxygen content (as an oxidative process, is scarce in anoxic soils), temperature (range between 4° and 40° C, with an optimum at 25° C), pH (neutral or slightly alkaline), humidity (60% of the total capacity of the soil) and the right amount of organic substance (as the excess or defect is an obstacle to nitrification).

Finally, the last process which close nitrogen cycle is that of denitrification. In fact, it represents the process through which nitrates (NO_3^-) are reduced through simpler intermediate combinations to elemental nitrogen (N_2), this process occurs in the presence of denitrifying bacteria (*Bacillus denitrificans, Vibrio denitrificans* and *Micrococcus denitrificans*), in presence of certain conditions, such as: presence of nitrates in significant quantities, presence of easily degradable organic substance, absence of free oxygen (it is more frequent in water-saturated soils), alkaline pH (7.9 - 8.2) and temperature included between 25° - 32° C.

In recent years, numerous studies have been carried out on the density and structure of soil microbial populations involved in the nitrogen cycle. These works are mainly based on a genomic approach, and aim to quantify the number of gene copies for key genes of the nitrification and denitrification processes in soils (C. Francis et al. 2005; Rotthauwe, Witzel, and Liesack 1997; C Rösch, Mergel, and Bothe 2002; Henry et al. 2004; Leininger et al. 2006).

The structure and size of soil microbial populations are generally influenced by environmental factors, such as pH, soil structure, moisture and nitrogen availability, even doe the complexity of interrelationships between microorganisms and soil chemical parameters has yet to be unveiled (Banning et al. 2015; Fierer 2017; Hermansson and Lindgren 2001; Wang et al. 2018; Huang et al. 2013; Mendum, Sockett, and Hirsch 1999; Hristova et al. 2004). Numerous studies on soil microorganisms observed that in most cases these do not carry out just a single type of nitrogen transformation, but for example the same ones that are able to fix nitrogen can also denitrify it (Kuypers, Marchant, and Kartal 2018).

Despite the existence of other genetic determinants, five key genes of the nitrogen cycle can generally be highlighted in soils, namely amoA, present in archaea and bacteria, nirK, nosZ, nifH. All bacteria and archaea capable of oxidizing ammonia have the AMO (ammonia monooxygenase) enzyme. Most of these bacteria belong to the *Betaproteobacteria* and *Gammaproteobacteria* class; among the archaea the most widespread are those belonging to the phylum *Thaumarchaeota*, present in sandy-clay soils. This reaction consists in the oxidation of ammonia (NH_3) to hydroxylamine (NH_2OH).

Up to date, two families of genes capable of carrying out nitrite reductase activities are known, nirK and nirS, converting nitrite (NO_2^-) into nitrogen monoxide (NO); bacteria hosting nirK mainly dominate nitrite reducers of the nirS type in most soils and rhizospheres (Ollivier et al. 2011). The nitrite reductase activity in soils can so be estimated by determining the abundance of gene copies of nosZ; this, being closely related to the presence of nirS, allows to deduce indirect information on the abundance of the first by analyzing the second (Graf, Jones, and Hallin 2014). Both enzymes are found in the periplasm and is possible the presence of both genes in the same microorganism. Regarding the nosZ gene, quantification studies of gene copies of soil microorganisms limit the detection to the terminal denitrification gene nosZ. Indeed, Domeignoz-Horta (Domeignoz-Horta et al. 2015), reported that: nosZ I community was consistently more abundant than the nosZ II community and no significant difference between the two groups could be attributed to different agricultural management practices, either in relation to crops or fertilization regimes. The same authors add that the lack of detectable variations between these subgroups is consistent with the fact that such differences have only been reported in long-term agronomic studies conducted over 50 years. This gene is capable of reducing nitrous oxide (N_2O) to harmless nitrogen (N_2), which returns to the atmosphere. Several bacteria, such as *Proteobacteria* and *Bacteroidetes*, and archea, such as *Crenarchaeotae Halobacteria*, possess this gene.

The nifH gene is generally used as a marker to identify microorganisms capable of fixing nitrogen in the soil (Kuypers, Marchant, and Kartal 2018).

The microbial activity of the soil is therefore of fundamental importance in determining the availability of the element for plants, given that, as already mentioned, both gaseous N_2 and organic compounds, like proteins or chitin, cannot be directly assimilated by plants (Emadodin et al. 2020). As a consequence, nitrogen fertilization has always been one of the most important limiting factors in agriculture, as necessary to gain high crop yields and an appropriate plant quality. In such context, agriculture, fossil fuels combustion of and other human activities have substantially altered the global cycle of N, generally increasing both the availability and mobility of N over large regions of the Earth. Crop production, and in particular the intensive use of mineral fertilizers, can be considered by far the single largest cause of human alteration of the global N cycle (Smil 1999).

1.2. Environmental impact of fertilizers

Since the invention and application of Haber-Bosch process at the beginning of XX century, huge quantities of mineral fertilizers have become available. This exponential increase of N fertilization, allowed, together with a strong land use intensification, and wide-ranging land reclamations, the doubling of world food production in the past four decades (Emadodin et al. 2020).

Production of nitrogen fertilizers has globally increased from 12 TgN in 1960 to 104 TgN in 2010 with an expected increase of 2% per year in the near future (Fowler et al. 2013; FAO 2017). It has been estimated that the amount of nitrogen used in agriculture from 1960 to 2010 is almost tenfold, and the amount of N_2 fixed by NH_4^+ from industrial processes for agriculture contributes about 45% of the nitrogen fixed each year on Earth (Canfield, Glazer, and Falkowski 2010; Fowler et al. 2013).

The overuse of nitrogen-based fertilizers leads to various environmental problems, which can all be traced back to a substantial alteration of the natural nitrogen cycle.

Agriculture is one of the main responsible in this process of altered nitrogen mobilization, as mineral fertilization to croplands contribute for half of the total global nitrogen input, which has been about 150 TgN for year 2000 (Schlesinger 2009). Several studies have shown that human alterations of the nitrogen cycle have doubled the input rate in the earth's nitrogen cycle, and these rates are still increasing (Green et al. 2004); this is accompanied by a global increase in the concentrations of N_2O, which is a powerful greenhouse gas, and an increase in the concentrations of other nitrogen oxides, which govern the formation of photochemical smog over vast regions of the Earth. In regions with intensive agriculture, the high amount on nitrogen inputs shows their impact on the environment, in terms of loss of biodiversity, dominance of certain weed species, and soil acidification (Ollivier et al. 2011).

Has been estimated that only 55% of the global applied N is adsorbed and used by crops, while the rest has to be considered in surplus. The remaining quantity indeed is lost, and leave the soil in different forms, in particular by leaching to groundwater (16%), therefore contributing to eutrophication (Stoate et al. 2009), surface and groundwater pollution by nitrate (Spalding and Exner 1993; Rahmati et al. 2015), by emissions of GHG and ozone- depleting gases, in particular N_2O (14%) (Davidson et al. 2000; Dobbie, McTaggart, and Smith 1999; Reinsch et al. 2018), and by soil erosion (15%) (Liu et al. 2010), with a relevant impact on human health and climate change.

1.3. Nitrogen leaching

Among the various mineral forms of nitrogen in the soil, mainly represented by nitrites (NO_2^-), nitrates (NO_3^-) and ammonium ion (NH_4^+), nitrate ion is the most mobile and susceptible to leaching; losses can reach 99% of the nitrates present, while for the ammonia form losses due to leaching are much lower than 1% (ISPRA 2015). This is also due to the fact that both nitrate and most soils are negatively charged, so this form of nitrogen is not retained by soils (H. J. Di and Cameron 2002).

Nitrate is produced in soil through the biological reaction of nitrification, which consists in a two step reaction of oxidation of ammonium carried out by two different genera of autotrophic soil microorganisms (Cameron, Di, and Moir 2013), as described in the previous chapter.

Conversion rate of ammonium into nitrate depends, as well as on ammonia concentrations in soil, also from pH (with an optimum of 4.5 - 7.5), soil temperature (with an optimum of 25 – 30° C) (Haynes and Sherlock 1986), and water content. The latter is very important, as nitrification has a maximum rate with soil at field-capacity water content, while rate are slower with higher and lower moisture content (Cameron, Di, and Moir 2013).

Soil texture and structure greatly affects the potential for nitrogen leaching: clay soil are generally less affected to this phenomena, due their greater potential for denitrification activity, and to the slower movement of draining water (Pratt, Lund, and Warneke 1980). Drainage actually can be considered the key factor, as an efficient field drainage, shortening the distance between topsoil and groundwater, and increasing the aeration of soil, with a consequent reduction of denitrification, determine an increase in the quantity of nitrate that can be lost by leaching (Scholefield et al. 1993).

The effect of land use systems on nitrate leaching are difficult to evaluate, considering the great amount of differences in soil, climate, fertiliser use and experimental conditions. In general, however, the following order of susceptibility to nitrate leaching has been identified: forests < cut grassland < grazed grassland < arable cropping < ploughing of pasture < horticultural crops (H. J. Di and Cameron 2002). Finally, an increased risk of nitrate leaching can occur from unfertilised bare soils, due to the absence of plat uptake (Low and Armitage 1970).

Following these premises, is not surprising that most of nitrate leaching losses occurs during late-autumn, winter and early-spring months, so in conditions of low N uptake by plants and net water drainage in soil profile (Wild and Cameron 1980). Fertilization residues remaining in soil after harvest can potentially be leached in autumn, together with nitrate released by mineralisation of soil organic nitrogen. This effect can even be more relevant in case of a previous dry summer, which results in a low N uptake by crops; in such situation autumn rainfall can potentially leach all nitrate accumulated in soil (Scholefield et al. 1993; Goulding 2000; Stout et al. 2000). Is fundamental to take into account that potential for nitrate leaching is always high when a rainfall event occurs shortly after the application of a nitrogen fertilizer.

The problem of nitrate leaching is not just the loss of a nutrient that could be available for crop growth, but also a matter of environmental pollution and a risk for human health (Cameron, Di, and Moir 2013).

Indeed, nitrates can cause environmental impacts on rivers, lakes, coastal and underground waters as they promote eutrophication, causing an uncontrolled proliferation of autotrophic species, and therefore determining the decrease in the quality of these environments (Val H. Smith and Schindler 2009).

Nitrate can cause serious health problems such as cancer and heart disease, in case of concentrations above 8.6 mg l^{-1} (Espejo-Herrera et al. 2016).

It has been estimated that 50% of the European population live in areas where nitrate concentrations in water exceed 5.6 mg NO_3^--N l^{-1} and about 20% live in areas where nitrate concentrations exceed the recommended level of 11.3 mg NO_3^--N l^{-1}, with variations between countries (Grizzetti et al. 2011). This is one of the reasons why in 1991, European Union issued the Nitrates Directive (91/676 / EEC), which indicated to member states the actions to be taken to identify areas vulnerable to nitrates of agricultural origin on their territory. Member states are also required to implement a series of measures to directly or indirectly reduce sources of nitrate pollution of waters; one of the most important measures concerns the definition of periods in which the application to the soil of certain types of fertilizers is prohibited and the definition of the maximum amount of fertilizer that can be spread on the ground (170 kg ha^{-1} of nitrogen. / year for Nitrate Vulnerable Zones).

1.4. Nitrogen emissions

Agriculture contributes to the release into the atmosphere of enormous quantities of greenhouse gases (GHGs), so gases able to retain the infrared radiation reflected from the earth's surface, atmosphere and clouds, causing the phenomenon known as the greenhouse effect. Main greenhouse gases in the earth's atmosphere are carbon dioxide (CO_2), nitrogen dioxide (N_2O), and methane (CH_4) (Snyder et al. 2009). Methane and nitrous oxide, although less common in the atmosphere, have a more powerful global warming potential than carbon dioxide; has been estimated that one unit of methane and nitrous oxide correspond respectively to 23 and 296 units of carbon dioxide (Houghton et al. 2001). The contribution of agricultural soils to CO_2, N_2O and CH_4 emissions generally depends on soil conditions: while aerobic conditions tend to increase CO_2, emissions, anaerobic conditions let to an increase in the production of CH_4 and N_2O, the latter through processes of nitrification and denitrification (Muñoz et al. 2010).

Carbon dioxide emissions from crops derives from biological processes of respiration, from soil, roots, and vegetation. However, net CO_2 emission of agricultural origin is relatively small; due to the fact that soils and crops are also able to be also a significant sink for carbon dioxide (Vita et al. 2017).

Agricultural soils in aerobic conditions are generally regarded as sinks for atmospheric methane, due to presence of methanotrophic microorganisms that use O_2 and CH_4 for their metabolism; on the other side, soils poor in oxygen content, typically flooded rice fields, are more subsceptible emitting methane (Oertel et al. 2016; Dutaur and Verchot 2007).

Agricultural soils have been addressed as the main source of global N_2O emissions (4.2 Tg N year^{-1}, approx. 62% of total emissions) (Thomson et al. 2012). Production of nitrous oxide derives from the activity of soil microorganisms, performing reactions of biological nitrification and denitrification, with the key driving factor being the concentration of oxygen, in turn depending on soil moisture content and structure. Other factors are temperature, concentrations of (NH_4^+) and (NO_3^-) (Snyder et al. 2009). As already mentioned, nitrification is the reaction of oxidation of NH_4^+ to NO_3^-; when this reaction occurs under oxygen-limited conditions, nitrifying microorganisms can use the intermediate reaction product (NO_2^-) as a final electron acceptor, determining a minor production of nitrous oxide and nitric oxide (NO) (IFA 2001). On the other side, denitrification occurs mainly in anaerobic soils, consisting in conversion of NO_3^- to dinitrogen gas (N_2); small amounts of N_2O can be produced and emitted when this reaction is not complete, with sporadic emissions occurring before, during, and after the growing season.

In addition to the three main greenhouse gases above mentioned, also emissions of ammonia NH_3 play a fundamental role; levels of ammonia emissions of the agro-zootechnical sector are estimated to constitute more than 90% of the total global emissions of this pollutant. Ammonia actively contributes to eutrophication of water, acid rain and the formation of particulate matter (PM 2.5) (Renard, Calidonna, and Henley 2004) which is related to onset of lung cancer (Raaschou-Nielsen et al. 2013). The contribution that ammonia makes to the formation of atmospheric particulate matter is linked to the level of urbanization and agricultural activity, to the orographic conformation of the area, in valleys and plains characterized by poor recirculation where the air stagnates, the level of ammonia grows exponentially),and weather conditions (Clarisse et al. 2009a).

1.5. Circular economy and nutrient recycling

As a consequence of the increased competition in the exploitation of natural resources, there's an unsustainable use of the resources themselves, which leads to a general environmental degradation, and an increase in competition for the remaining available resources. This depletion of natural resources is by the other side accompanied by an increase of waste disposal; in fact, large amounts of nutrients, such as Nitrogen (N), Phosphorus (P) and Potassium (K) are lost through incerination of waste, accumulation in soils and through harmful nutrient emissions to water and air.

In order to recover valuable resources from biological wastes and sludges, there's the need to shift from a linear economy model, based on the exploitation of natural resources and where every product is inexorably destined to become waste, to a circular economy model, based on the reuse, repair, refurbishing, and recycling of the existing materials and products, and on the general concept that wastes become a resource (Jurgilevich et al. 2016). In such context, biological wastes can be considered as a source of energy, organic matter and nutrients. Applying the concept of circular economy to agriculture means closing nutrient cycles as far as possible, using the recovered nitrogen, phosphorus and potassium as a direct substitute of fertilizers, or as a resource for the production of mineral fertilizers. The shift to a circular economy model aims to reduce environmental impact of the production of chemical fertilizers, to reduce depletion of non-renewable natural resources, such as phospates, and to reduce nutrient losses to environment.

1.6. Anaerobic digestion

Anaerobic digestion (AD) consists of a process of degradation of the organic matter contained in the biomass, mainly livestock waste, crop residues, wastewater and waste from the agro-food sector, a process that occurs thanks to the interaction of different microbial groups in conditions of absence of oxygen.

The biodegradative process consists of a first phase of hydrolysis of the complex substrates accompanied by acidification, with the formation of volatile fatty acids, ketones and alcohols. This is followed by a subsequent acetogenic phase, in which, starting from the fatty acids, there is the formation of acetic acid, formic acid, carbon dioxide and molecular hydrogen. Finally, a last phase in which, starting from the products of the previous phase, methanization is observed, i.e. the formation of methane starting from acetic acid or through the reduction of carbon dioxide using hydrogen as a co-substrate.

This degradation process leads to the development of biogas, consisting mainly of methane gas (60-70%), carbon dioxide (25-30%), nitrogen (2-5%), as well as minimal percentages of hydrogen sulphide and hydrogen. This gas mixture, whose CH_4 content varies according to the type of digested organic substance and the process conditions, has a high calorific value (5000-6000 kcal Nm^{-3}) and can be used for the production of electricity.

What remains at the end of the anaerobic digestion process is the digestate, a semi-solid product, biologically stable, with fertilizing properties similar to an organic fertilizer, due to the content of nitrogen, phosphorus, potassium and the amending capacity.

Digestate can be subjected to treatments aimed at simplifying subsequent management operations, or useful for reducing the nitrogen content, where necessary. For example, the first category includes the solid / liquid separation, which leads to the reduction of water content, and to the obtaining of a solid fraction with a high percentage of partially stabilized organic substance, and a liquid fraction with good nitrogen levels in ammonia form, exploitable in fertigation. Among the treatments aimed at reducing the nitrogen load of the digestate there’s the stripping of ammonia, consisting in the passage of the ammonia phase from the liquid phase, in which it is dissolved, to the gaseous phase by means of a steam flow. By combining the stripping of ammonia with the capture of the latter in an acid solution, such as sulfuric acid, ammonium sulfate $(NH_4)_2SO_4$ is obtained, which can be used as a fertilizer (Tambone et al. 2017).

1.7. Digestate: characteristics and agronomic use

Digestate is the result of a complex bio transformation, which lead to obtaining a product which has different characteristics with respect to the starting organic substrate.

Total carbon content of the digestates varies between 28 and 47% of the dry matter, while the organic carbon content varies between 30 and 80% of the dry matter (Tambone et al. 2010; Fouda et al. 2013). During the anaerobic digestion process the pH tends to increase, reaching average values of 8.3, due to the degradation of more than 90% of volatile fatty acids (VFAs) (Coutteau and Sorgeloos 1992).

Digestates generally show a lower content in polysaccharides, lipids and proteins, but are enriched in thermostable compounds, substances with high degree of aromaticity (such as aromatic lignin of about 30-60%) and long-chain aliphatic components, compared to residues not treated by digestion anaerobic. This is due to the fact that the organisms involved in the digestion process are not able to degrade this type of materials under anoxic conditions (Molinuevo-Salces et al. 2013).

Since during the process the more easily decomposable carbon compounds are subjected to decomposition, in the digestates obtained there is an increase in the biological compounds that are recalcitrant compared to the raw material used (Cuetos et al. 2009; Marcato et al. 2009; Gómez et al. 2011; Pognani et al. 2009). The organic substance of the digestate is more stabilized and has a lower decomposition rate after field application (Möller 2015).

During the digestion process, molecules containing organic nitrogen, such as proteins, are mineralized to produce biogas: the amino group is released in solution in the form of ammonia nitrogen, which is directly available for uptake by plants. The digestates therefore contain both organic and inorganic nitrogen, whose percentage distribution depends on the extent of the biochemical reactions, which in turn depend on the type of nitrogenous compound contained in the starting raw material, on the efficiency of the process and on any treatments which the digestate is subjected to. Digestates with an high NH_4-N content, are generally more efficient as nitrogen based fertilizers.

For example, by introducing only zootechnical effluents into the digester, the nitrogen introduced is already prevalent in ammonia form (up to 70-75% of the total nitrogen in pig sewage), therefore a product is obtained in which more than 80% of nitrogen becomes available as NH_4^+. Conversely, for digestates produced from organic waste, the NH_4^+ share often does not exceed 44–47% (Céline Vaneeckhaute et al. 2016).

A similar effect occurs on the phosphorus content of the digestates, which does not change compared to the content of the starting product, but through the anaerobic digestion process the organic phosphorus content is mineralized and becomes available to the plants. The total content of K, Ca, Mg and heavy metals is also not altered during anaerobic digestion (Céline Vaneeckhaute et al. 2016).

The anaerobic digestion process leads to a substantial reduction in the presence of pathogens, in particular there is a reduction of up to 5 logarithmic units of fecal coliforms found in the materials entering the plant. The effectiveness of this matrix sanitation process depends on factors such as temperature and residence time in the digester, as well as on the production of endogenous ammonia and competition with other microorganisms present (Pognani et al. 2009).

Given the characteristics of the organic carbon component of the digestate, this can be considered a soil improver, that is a material which, when brought to the soil, improves or maintains its physical, chemical or biological properties. Field studies have shown that soils treated with digestate increase their saturated hydraulic conductivity and water retention capacity (Nkoa 2014; Garg et al. 2005).

Several studies have shown that 90% of the nitrogen component of the digestate is rapidly available to plants, a value very similar to that of urea (Tambone and Adani 2017). Furthermore, compared to zootechnical fertilizers that have not undergone anaerobic digestion, the organic component of the digestate is much more stable, thus avoiding the immobilization of excess nitrogen which may in the future be leached or evaporate as a greenhouse gas.

Anaerobic digestion of livestock manure does not affect the N mineral content of the soil in autumn, so at the beginning of the main leaching period, therefore there is no difference in the risk of nitrate leaching from digestates compared to undigested animal manure. This means that anaerobic digestion by itself does not directly influence the risk of nitrate leaching after field application. It has also been proven that the use of crop residues treated with anaerobic digestion increases the availability of nitrogen in the soil, the yield of crops, and the efficiency of the use of N compared to the simple green manuring of these residues in the soil (Möller 2015).

The reduced C/N ratio of the digestate compared to untreated sludges, determines a reduction of the organic nitrogen content in the soil after the first growing season, consequently reducing the effect of residual nitrogen in the long term and the long-term risk of leaching of nitrates (Tambone et al. 2010).

The application of digestate in the field determines a reduction in odor emissions compared to the application of untreated livestock waste (Orzi et al. 2015). However, there is a high risk of ammonia volatilization following distribution due to the higher concentration values of ammonia nitrogen and pH; to reduce this risk, pre-sowing distribution with immediate burial, or distribution by injection into the soil is essential (Céline Vaneeckhaute et al. 2016).

1.8. Nutri2Cycle EU H2020 project

The H2020 Nutri2Cycle project proposes various solutions to reduce the use of non-renewable resources and close the nutrient cycle (N, P and C) of various European agricultural systems. The goal is to maintain a high level of productivity and economic efficiency by disconnecting from the use of mineral fertilizers, with a view to recovering nutrients and a circular economy. To achieve this goal, the project uses an approach based on the identification of efficient agricultural systems in closing nutrient cycles, which are evaluated on the basis of objective indicators and parameters; subsequently, pilot plant scale models are implemented, which will serve as a basis for effective upscaling. One fundamental point of the project is the spread of the results at regional, national and European level throughout a comprehensive network of regional operational groups, National Task Forces and European stakeholders, and the providing of scientific support on effective regulatory frameworks, in order to reduce emissions and increase self-reliance of Europe for food, energy and nutrients in the next century (“Nutri2Cycle” n.d.).

1.9. Research context and objectives

Digestate has potentially the characteristics of a low environmental impact fertilizer, which allows the recovery of nutrients in a circular economy perspective. However, the effects of an agronomic use of the digestate depend on its characteristics, which on turn depend on the composition of the starting substrates and on the process conditions, as well as on the methods of application.

The aim of this work was to evaluate the impacts resulting from the use of digestate as a fertilizer, in various environmental compartments, namely soil, water and atmosphere, and on the cycle of nutrients, in particular on the nitrogen cycle. The agronomic and environmental performances of the digestate were evaluated with respect to those of a synthetic fertilizer, through open field measurements and monitoring, chemical-physical analysis of the soil and digestate characteristics, and molecular biology studies, in order to obtain a complete and exhaustive view.

2. Use of highly stabilized digestate to replace urea in open field agriculture: effects on soil, environment, and maize production

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

2.1. Introduction

During the second half of the twentieth century, in particular since the late 1960s, agriculture throughout the world has undergone radical improvements, which overall have been defined as the "green revolution". The direct consequence of these improvements in the succeeding decades was a dramatic growth in agricultural yields which increased by up to 125% between 1966 and 2000 (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 ore 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. According to the same projections, the peak of phosphorus extraction, i.e., the point after which the annual extractable amount will no longer be able to increase, is expected for 2030, and the depletion of global reserves is likely before the end of the XXI century (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, which 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 (Molina et al. 2016; Padilla, Gallardo, and Manzano-Agugliaro 2018).

Using organic wastes as fertilizers in agriculture to replace synthetic fertilizers would therefore represent a solution to these problems, reducing the dispersion of nutrients in the environment, and it would also constitute an interesting model of circular economy (van Dijk, Lesschen, and Oenema 2016). However, untreated organic wastes do not represent acceptable fertilizers (Westerman and Bicudo 2005) because of their origin: in fact they can contain pathogens, heavy metals, or organic pollutants such as antibiotics or drug residues that would accumulate in agricultural soils, endangering the safety of food production and consumption (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. Lastly, they are often rich in water, which makes their management difficult and expensive both from an economic and environmental point of view, because of the CO_2 emissions associated with the transport of large volumes (Westerman and Bicudo 2005). To transform these biomasses into products which can be utilised in agriculture, technological/biotechnological treatments are therefore necessary (I. 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 to emissions of ammonia and greenhouse gases (N_2O) into the atmosphere (Cameron, Di, and Moir 2013; J. A. 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 light on the safety of digestates for agricultural use, as well as on the best process methods and accurate selection of infeed, there are still only very limited data available on the impact that the use of these biomasses have on full scale agriculture in the field (Koszel and Lorencowicz 2015; Pigoli et al. 2021; Massimo Zilio et al. 2021; Verdi et al. 2019).

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

2.2. Materials and Methods

2.2.1. Agronomic full field trials

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

Digestate was distributed at pre-sowing by injection into the soil at a depth of 15 cm by using a tank car joined to a rigid multi-anchor-subsoiler coupled with a Retrofit Variable-Rate Control (VRT control). Digestate was dosed adopting an N efficiency of 0.5, as suggested by the Regional Plan for Water Protection from Nitrate from Agriculture (Regione Lombardia 2020). 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 on the soil surface following a routine procedure (N_{tot} dosed: 185 kgN Ha^{-1} as urea and 100 kgN Ha^{-1} as ammonium sulphate; P_{tot} dosed: 39.3 kgP Ha^{-1} as 0/46/0 complex; K_{tot} dosed: 69.4 kg K Ha^{-1} as KCl). Fertilization date, fertilizers used, doses applied, and spreading methodology are summarized in Table 2.1.

Table 2.1. 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	Ammonium sulphate	100	100	Fertigation
	Synthetic fertilizer	23/04/2018	Pre-sowing	Urea	185	185	Spread in surface
		22/06/2018	Top-dressing	Ammonium sulphate	100	100	Fertigation
2019	Recovered fertilizer	16/04/2019	Pre-sowing	Digestate	370	229	Injection 15 cm
		1/08/2019	Top-dressing	Ammonium sulphate	100	100	Fertigation
	Synthetic fertilizer	16/04/2019	Pre-sowing	Urea	185	185	Spread in surface
		31/07/2019	Top-dressing	Ammonium sulphate	100	100	Fertigation
2020	Recovered fertilizer	28/05/2020	Pre-sowing	Digestate	370	200	Injection 15 cm
		31/07/2020	Top-dressing	Ammonium sulphate	90	90	Fertigation
	Synthetic fertilizer	28/05/2020	Pre-sowing	Urea	185	185	Spread in surface
		31/07/2020	Top-dressing	Ammonium sulphate	90	90	Fertigation

2.2.2. Fertilizer sampling and analysis

The digestates used in this work were sampled immediately before they were injected in the field. The analyses took place in the hours immediately following sampling and data represent the average of three years. pH was determined in aqueous solution using a 1:2.5 sample/water ratio. Total solids (TS) and total organic carbon (TOC) determinations were carried out following standard procedures of the American Public Health Association (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 method reported in Schievano et al. (2008).

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).

The main characteristics of digestate and ammonia sulphate used in this work are shown in Table 2.2. and 2.3. respectively.

Table 2.2. Main characteristics of the digestate dosed in pre-sown fertilization in this work (Mean ± SD).

Parameter	**Unit**	**Value[a]**
pH	pH unit	8.5 ± 0.1
Dry Matter 105°C	g kg^{-1} ww[b]	104 ± 3.9
Total Organic Carbon (TOC)	g kg^{-1} dw[b]	304 ± 34
Total Nitrogen (TKN)	g kg^{-1} dw	75 ± 5
$N\text{-}NH_4$ (TAN)	g kg^{-1} dw	43.3 ± 2.7
TAN/TKN	%	57.8
OD_{20}[c]	mg O_2 g^{-1} dw	22.6 ± 6.1
BMP[d]	L_{biogas} kg^{-1} dw	89 ± 17

Total P	g kg^{-1} dw	32.3 ± 1.7
Total K	g kg^{-1} dw	5.73 ± 0.5
Na	mg kg^{-1} dw	3209 ± 548
Mg	mg kg^{-1} dw	7895 ± 1136
Ca	mg kg^{-1} dw	50871 ± 6266
Al	mg kg^{-1} dw	21872 ± 2988
Cr	mg kg^{-1} dw	99.8 ± 25
Mn	mg kg^{-1} dw	451 ± 28
Fe	mg kg^{-1} dw	21563 ± 4398
Co	mg kg^{-1} dw	7.14 ± 1.3
Ni	mg kg^{-1} dw	62 ± 9.9
Cu	mg kg^{-1} dw	426 ± 68
Zn	mg kg^{-1} dw	984 ± 80
As	mg kg^{-1} dw	11.7 ± 2.6
Se	mg kg^{-1} dw	3.98 ± 0.6
Mo	mg kg^{-1} dw	11.7 ± 2.6
Cd	mg kg^{-1} dw	2.13 ± 0.5
Pb	mg kg^{-1} dw	66.1 ± 11
Hg	mg kg^{-1} dw	0.1 ± 0.3

PCDD/F+PCB-DL	ng TEQ kg^{-1} dw	10.6 ± 2.9[e]
Hydrocarbon C10-C40	mg kg^{-1} dw	284 ± 251[e]
AOX	mg kg^{-1} dw	< 0.6
DEHP	mg kg^{-1} dw	5.7 ± 5.3[e]
PAH	mg kg^{-1} dw	0.5 ± 0.5[e]
PCB	mg kg^{-1} dw	< 0.1
Salmonella	MPN g^{-1} dw	Absent
Faecal coliform	MPN g^{-1} dw	< 1,000

[a] Mean ± SD: *n*=11, except for Ca, Mn, Mg, Fe, Mo, Al, Co, Na: *n* = 9, and BMP: *n* = 10.

[b] ww and dw: wet weight and dry weight, respectively.

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

[d] BMP: biochemical methane production.

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

Table 2.3. Main characteristics (mean ± SD; n=17) of ammonium sulphate - $(NH_4)_2SO_4$ - used for topdressing fertilization in this work (all concentrations are expressed on wet basis).

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

[a] Sigurnjak et al., (2019), ammonium sulphate produced by air scrubbing

[b] Ivona Sigurnjak et al. (2016), air scrubber water from digestate treatment

[c] Vaneeckhaute et al. (2013), air scrubber water from digestate treatment

[d] Ledda, et al. (2013), ammonium sulphate produced by scrubbing with sulfuric acid

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

2.2.3. 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 2.2. 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) 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.

Potential nitrate leaching was assessed by the detection of nitrate presence at 1 m soil depth ($N\text{-}NO_3$) in soils. Sampling consisted in the withdrawal of soil cylinders up to a depth of one meter.

For each of the experimental plots three soil cylinders were sampled randomly. Each soil cylinder was divided into 4 sub-samples, each of 25 cm, corresponding to 0-25, 25-50, 50-75 and 75-100 cm layers in soil profile. In total eight sampling campaigns were carried out during in the period 2019-2020. The collected soil was brought immediately (the same day) to the lab and analysed immediately. In particular, the nitrate concentration was determined by Kjeldahl distillation, using Devarda's alloy (Faithfull 2002).

Table 2.4. Main chemical parameters of soil before the pre-sown fertilization on March 2018 and after the end of the third crop season on January 2021.

Parameter	**Unit**	**March 2018**	**January 2021**		
			Unfertilized	**Synthetic fertilizer**	**Recovered fertilizer**
Sand	%		47 ± 2.8[a]	49 ± 3.7	46 ± 4.4
Silt	%		41 ± 0.2	39 ± 1.5	43 ± 1.4
Clay	%		12 ± 2	12 ± 1.1	12 ± 2.6
pH	pH unit	7 ± 0.7(a)[b]	7.14 ± 0.2 (a)	7.06 ± 0.1 (a)	7.05 ± 0.2 (a)
CEC	C (mol kg^{-1})	24.2 ± 2.1 (ab)	23.8 ± 0.4 (a)	26.8 ± 0.8 (b)	22.3 ± 0.9 (a)
Total organic carbon (TOC)	g kg^{-1} dw[c]	10.3 ± 0.6 (a)	11.9 ± 0.2 (ab)	11.3 ± 0.4 (a)	12.3 ± 0.4 (b)
Total nitrogen	g kg^{-1} dw	1.27 ± 0.1 (a)	1.3 ± 0 (a)	1.41 ± 0 (b)	1.42 ± 0.9 (b)
Ratio C:N		8.13 ± 0.9 (ab)	9.22 ± 0 (b)	8.01 ± 0.1 (a)	8.65 ± 0.4 (ab)
P_{tot}	mg kg^{-1} dw	575 ± 11 (a)	521 ± 26 (a)	581 ± 32 (a)	550 ± 15 (a)
$P_{available}$	mg kg^{-1} dw	43.6 ± 2.6 (a)	46.4 ± 0 (a)	60.1 ± 16 (a)	58.9 ± 16 (a)
As	mg kg^{-1} dw	19.9 ± 1.1 (a)	22.9 ± 2.8 (a)	19.6 ± 0.5 (a)	21.1 ± 2.3 (a)
Cd	mg kg^{-1} dw	<0.5	<0.5	<0.5	<0.5
Hg	mg kg^{-1} dw	<0.5	<0.5	<0.5	<0.5
Cr	mg kg^{-1} dw	39.2 ± 2.3 (a)	42.6 ± 2 (a)	40 ± 4.1 (a)	40.2 ± 1.6 (a)
Ni	mg kg^{-1} dw	23.3 ± 2.3 (a)	25.7 ± 1.7 (a)	25.9 ± 3.7 (a)	26 ± 1.6 (a)
Pb	mg kg^{-1} dw	32.8 ± 0.1 (a)	34.2 ± 4.2 (a)	33.4 ± 2.2 (a)	33.6 ± 4.5 (a)
Cu	mg kg^{-1} dw	19.1 ± 1.3 (a)	22.2 ± 3.3 (a)	21.4 ± 3.5 (a)	24.4 ± 3.1 (a)

Zn	mg kg^{-1} dw	69.8 ± 0.5 (a)	71.4 ± 3 (a)	71.4 ± 1.3 (a)	70.8 ± 1.8 (a)
PCDD/PCDF + PCB DL	ng WHO-TEQ kg^{-1} dw	-	4.09 ± 0.1 (b)	4.3 ± 0.2 (b)	4.16 ± 0.1 (b)
Hydrocarbon C10-C40	mg kg^{-1} dw	< 30	< 30	< 30	< 30
Toluene	mg kg^{-1} dw	< 0.2	< 0.1	< 0.1	< 0.1
Phenols NPE + NP2EO + NP1EO	mg kg^{-1} dw	< 7.5	< 7.5	< 7.5	< 7.5
∑AOX	mg kg^{-1} dw	< 0.6	< 0.6	< 0.6	< 0.6
PCB	mg kg^{-1} dw	< 0.005	< 0.005	< 0.005	< 0.005
DEHP	mg kg^{-1} dw	0.24	< 0.1	< 0.1	< 0.1
Ciproflaxacin	mg kg^{-1} dw		<0.01	<0.01	<0.01
Sulfamethoxazole	mg kg^{-1} dw		<0.01	<0.01	<0.01
Fenofibrat	mg kg^{-1} dw		<0.01	<0.01	<0.01
Gemfibrozil	mg kg^{-1} dw		<0.01	<0.01	<0.01
Carbamazepina	mg kg^{-1} dw		<0.01	<0.01	<0.01
Metoprolol	mg kg^{-1} dw		<0.01	<0.01	<0.01
Diclofenac	mg kg^{-1} dw		<0.01	<0.01	<0.01
Ethinylestradiol	mg kg^{-1} dw		<0.01	<0.01	<0.01
Estradiol	mg kg^{-1} dw		<0.01	<0.01	<0.01

[a] mean ± SD; n=3

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

[c] dw: dry weight

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 (Environmental Protection Agency (EPA) 2007) and EPA 8321B 2007 methods (EPA. 2007).

2.2.4. Ammonia emission measurement

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

The experiments were repeated for three consecutive years on the same experimental plots. In particular, the soil used showed a neutral pH (7 ± 0.4), it was rich in silt (44% ± 2.1) and it was relatively poor in clay (10% ± 0.5). The amounts of ammonia nitrogen dosed at pre-sowing were kept almost unchanged for all the three years tested, i.e. 200 - 229 and 185 kg N Ha^{-1} for RF and SF, respectively (Table 2.1). 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, Thunderbeach Scientific, CA). The obtained dispersion coefficient (D; s m^{-1}) was used to determine the flow (S; ng NH^3 m^{-2} s^{-1}) emitted from the fertilized surface, on the basis of the concentrations measured in each plot (C; µg m^{-3}) and environmental (C_{bgd}; µg m^{-3}), according to the following equation:

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

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

2.2.5. Greenhouse gas (GHG) emissions measurement

GHG fluxes (N_2O, CH_4 and CO_2) were measured from 28/05/2020 to 17/03/2021 using the closed static chambers method (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 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.

2.2.6. Maize yield quantification and element content analysis

The annual grain yields for each of the experimental plots were assessed by manual harvesting of the grain. The data obtained from each plot were then aggregated in order to obtain final grain production (Mg Ha^{-1}) for each treatment, i.e. RF, SF and control.

Inorganic pollutant contents in grain (i.e., As, Cd, Hg, Cr, Ni, Pb, Cu and Zn) were assessed by inductively coupled plasma mass spectrometry (Varian, Fort Collins, USA), preceded by acid digestion (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. The elemental analyser used for the analysis was: Rapid max N exceed (model), produced by Elementar, Lomazzo (Italy).

2.2.7. Fertilizer use efficiency

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

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

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

2.2.8. Statistical analysis

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

2.3. Results and discussion

2.3.1. The effect of recovered fertilizers on soil

The use of recovered fertilizers for three consecutive years had no impact on soil properties apart from that on TOC content, which was positively and significantly affected by RF use (One-way ANOVA; $p<0.05$). The TOC content increased after three years from 10.3 ± 0.6 g kg^{-1} dw (March 2018) to 12.3 ± 0.4 g kg^{-1} dw (March 2021) (Table 2.4). Both the unfertilized and synthetic fertilized plots did not show any statistical differences with respect to the starting soil for the TOC contents (Table 2.4). The increase in TOC in soil fertilized with RF was most likely due to the contribution of digestate that was rich in organic carbon (TOC of 304 ± 34 g kg^{-1} dw) which was recalcitrant to biodegradation, as suggested by its high biological stability, measured by potential biogas production. In fact, the registered BMP of 89 ± 17 L_{biogas} kg^{-1} dw (see Table 2.2) 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 (One-way ANOVA; $p<0.05$) in March 2021, respectively. On the other hand, soil of the unfertilized plots did not show any variation in its N tot content, i.e., 1.3 ± 0 g kg^{-1} dw in January 2021.

2.3.2. Agronomic performance of recovered fertilizers and product safety

The amounts of maize grain produced (the average of 2018, 2019 and 2020 crop seasons) using digestate and derived ammonium sulphate as fertilizer (18.1 ± 2.9 Mg dried grain Ha^{-1}) (see Table 3.3) was similar and not statistically different from that produced with synthetic fertilizers (urea) (17.4 ± 1.2 Mg dried grain Ha^{-1}). This indicated that recovered fertilizers are capable of substituting for synthetic fertilizers.

Furthermore, the content of microelements and inorganic pollutants in the produced grains was quantified (Table 2.5). For all the elements analysed (except zinc), the concentrations in the grains produced using RF or SF as fertilizer were found to be equivalent.

However, RF fertilized plants produced grains containing more Zn than plants grown with synthetic fertilizers, i.e., 32.1 ± 1.9 vs 25 ± 2 mg kg^{-1} dw for RF and SF respectively.

Table 2.5. 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).

However, 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 naturally poor in it (Cakmak and Kutman 2018).

2.3.3. Environmental safety

2.3.3.1. Potential ammonia emissions and nitrate leaching

Ammonia (NH_3) emissions were measured directly at full scale during plot trials, as described in the M&M section. On average, the plots fertilized with RF emitted an amount of ammonia (25.6 ± 9.4 kg N Ha^{-1} i.e., 11.6 ± 4% TAN) that was not statistically different (One-way ANOVA, $p<0.05$, n=3, Tukey post-test) from that measured for plots fertilized with SF (24.8 ± 8.3 kg N Ha^{-1}, i.e., 13.4 ± 4.5% TAN). These data have previously been discussed in a paper published in this journal (Zilio et al., 2021) (see Table 3.3).

The risk of N leaching from the soil was assessed by analysing the concentration of NH_4^+ (Figure 2.1.) and NO_3^- (Figure 2.2.) taking soil samples during crop season in topical moments (i.e., before and after pre-sown fertilization, before and after topdressing fertilization and after harvest) both at the surface and at a depth of one meter, for all experimental soil plots during two consecutive agronomic seasons (2019 and 2020).

Figure 2.1. Concentrations of ammonia nitrogen (N-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.

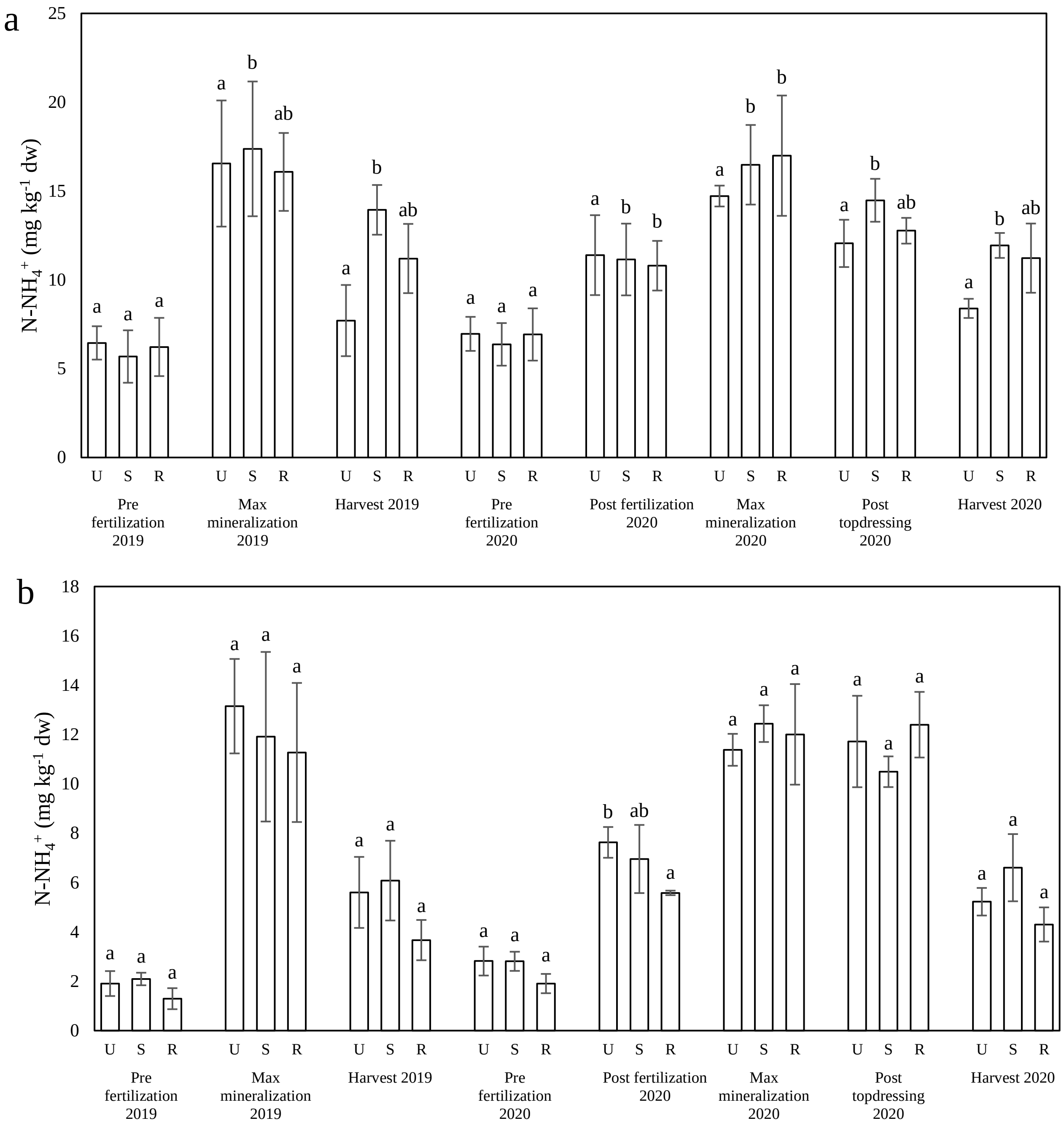
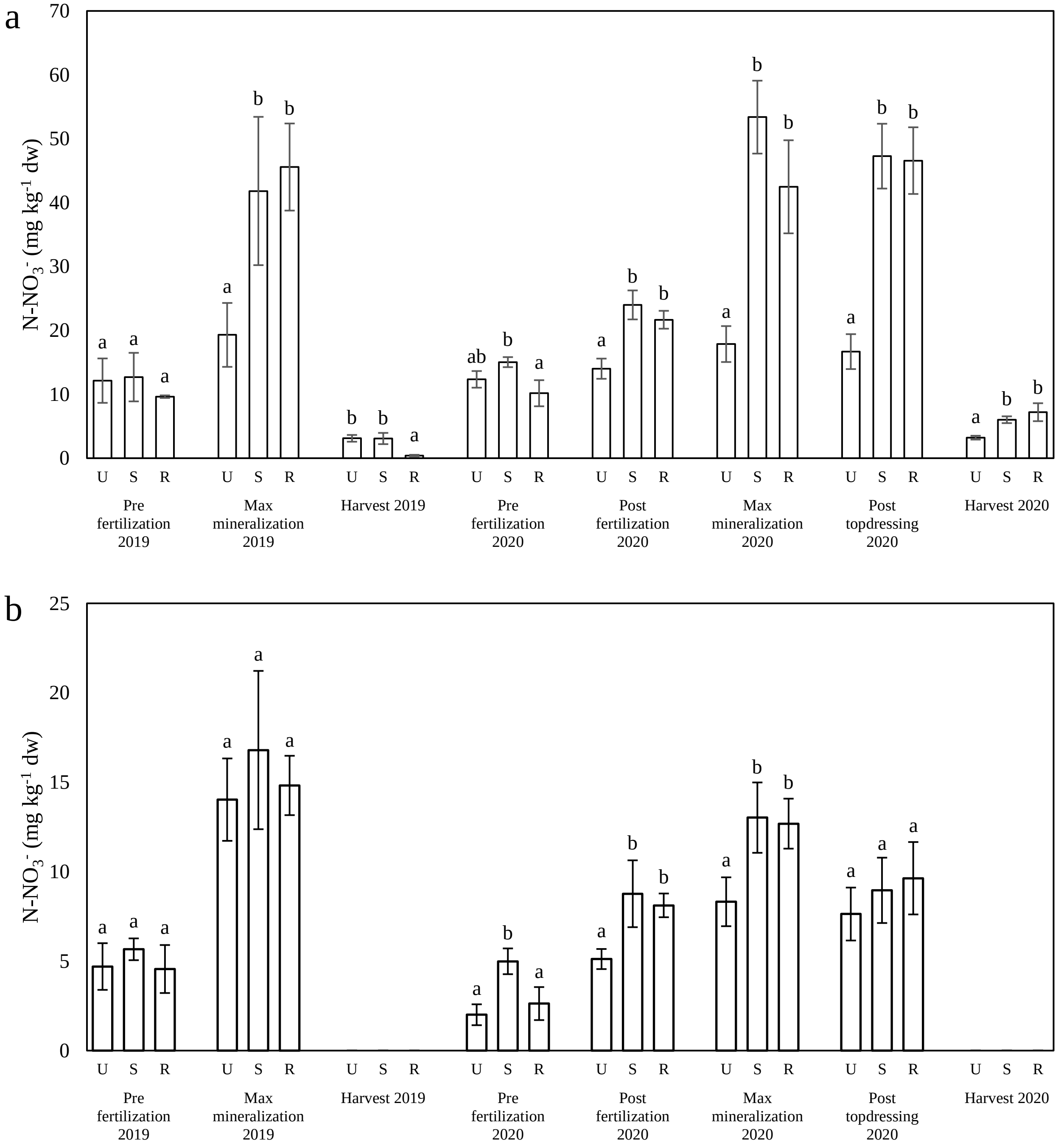


Figure 2.2. Concentrations of nitric nitrogen (N-NO$_3^-$) in experimental soils (a: surface; b: 1 meter depth) during the crop seasons 2019 and 2020 (mean ± SD; n=3). U: untreated, S: synthetic, R: recovered. Letters are referred to One-way ANOVA ($p<0.05$; Tukey post-test) comparing the three treatments within each sampling time.



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

The data also show that, in both monitored years, the concentrations of NO_3^- in soil fertilized with RF were never higher than those found in soil plots fertilized with SF (One-way ANOVA, $p<0.05$, $n=3$, Tukey post-test), and that in one case (pre-fertilization 2020) NO_3^- concentration was lower. The comparison between the NO_3^- concentrations measured in fertilized and unfertilized soil plots, showed, also, that the N doses used in this work did not cause leaching risks higher than those which occur in unfertilized soil. Average NO_3^- concentrations along the two monitored years were of 5.22 ± 4.65, 7.18 ± 5.89 and 6.56 ± 5.49 mg kg^{-1} dw for unfertilized, SF and RF, respectively: these values are similar to each other and in line with those found in the literature for undisturbed soils (9.6 mg kg^{-1}) (Ryden, Ball, and Garwood 1984). These figures are particularly interesting if it is considered that the unfertilized soil did not receive any N fertilizers throughout the three years, contrarily to the fertilized soil plots which every year received 470 and 285 kg Ha^{-1} of N, respectively, for RF and SF plots. All this suggests that dosing a correct amount of mineral N fertilizers (i.e., matching crop requirements) and using stable organic-N, did not lead to any nitrate leaching risk.

These results agree with those previously found in experiments on soils fertilized with either mineral or organic N fertilizers performed in the same geographical area (Lombardy, Po Valley) (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).

2.3.3.2. 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 2.6). The amounts of CO_2 emitted were 6,216 ± 1,160 kg C Ha^{-1} and 6,144 ± 1,491 kg C Ha^{-1} for RF and SF plots, respectively, suggesting that the addition to soil of organic matter by digestate did not lead to any C emission increase. These results confirmed that digestate organic matter was quite stable and did not mineralize, becoming part of the soil organic matter. This was more evident when unfertilized soil, that did not receive any fertilization for three years (CO_2 emission of 5,698 ± 935 kg C Ha^{-1}), was compared to RF that, on the contrary, was dosed yearly (CO_2 emission 6,216 ± 1,160 kg C Ha^{-1}).

Table 2.6. 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).

Methane did not contribute greatly to C emissions and in any case, again, there were no statistically significant differences between different plot trials i.e., 0.066 ± 0.06 kg C Ha^{-1}, 0.053 ± 0.04 kg C Ha^{-1} and 0.036 ± 0.03 kg C Ha^{-1}, for Unfertilized, SF and RF treatments, respectively.

Nitrous oxide (N_2O) emitted was of 1.71 ± 1.1, 10.3 ± 6.8 and 7.59 ± 3.2 kgN Ha^{-1} for Unfertilized, SF and RF, respectively. Plots fertilized with SF emitted more N_2O that those fertilized with RF, although no statistical differences were found.

As expected, the unfertilized soil, that did not receive any N fertilization during the three years of experimentation, emitted much less N_2O than fertilized soil plots, confirming the contribution of N fertilization to N_2O emission from soils (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 measured experimentally.

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 becomes important in understanding the fate of N in the soil (potential NO_3^- leaching and potential N_2O production).

2.3.3.3. 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 2.4). For all of the pollutants analysed, no significant increase was observed in the soils of all the experimental plots.

These data confirmed previous reports in the literature for similar work, namely that after the use of digestate in agriculture, no significant accumulations of heavy metals are found in the soil

(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 2.4, 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 2.4), in that case the increase in the concentration of carbon in the soil was detected. This shows that such variations can be measured, and confirms that most of the heavy metals brought to the soil dosed with digestate did not accumulate in the soil.

Regarding the concentrations of persistent organic pollutants (POPs) in the experimental soils, in no case was any increase found in their concentration after three years of experimentation, for all the plots studied, including unfertilized plots (Table 2.4). 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 2.4). 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).

2.3.4. 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 2.7), and therefore similar to that of 50% suggested by Lombardy Region and adopted in this work. These figures were obtained taking into consideration total N dosed, independently of N forms (mineral vs. organic). Nevertheless, nitrogen dosed with the digestate was represented for 57.8% TKN by NH_4-N that was readily available for plants as well as N from SF, and by 42.2% TKN by organic N that was quite stable (no mineralization occurred) because of the high biological stability of digestate. The stability of organic N was confirmed, such as discussed earlier, by measuring CO_2 and CH_4 evolution from soils treated with RF that showed similar figures to those of both plots fertilized with SF and unfertilized, and by measuring both NH_4^+ and NO_3^- soil contents at different topical moments, that were similar for all soils studied, independently of the fertilizers used. As a consequence of the results obtained, it can be considered that the organic N of digestate, substantially, did not contribute to mineral soil N, since it became part of the soil organic matter, and that only the ammonia form should be considered for FUE calculation. Doing so, the re-calculated RF FUE was of 85.3 ± 10%, comparable to that calculated for SF (FUE of 93.6 ± 4.4%). Consequently, the N fertilizer replacement value (NFRV) obtained for RF used to replace SF, when referred only to the mineral N form, was of 83.7%. Obviously, this value assumes validity only if the digestate characterization is performed to attest the high biological stability of the organic matter which it contains.

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

Table 2.7. 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

2.4. 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.

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.

3. 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.*

3.1. 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 (Herrero 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 (I. 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) (J. a 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. 2009b).

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 (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 (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).

3.2. Material and Methods

3.2.1. 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 3.1). 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 3.1.

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 3.1. 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.

3.2.2. 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 3.2. 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 3.2. 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

3.2.3. 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 Table 2.4. 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.

3.2.4. 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 3.1). 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 Table 2.4. 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 3.1).

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-NH_3 (kg ha^{-1}) and the calculated amount of ammonia nitrogen (N-NH_4; kg ha^{-1}) spread onto the soil with fertilizations.

3.2.5. 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).

3.2.6. 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.

3.2.7. 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.

3.3. Results and discussion

3.3.1. Maize yield

At the end of each of the crop seasons, the grain yield from soils fertilized with digestate and urea was evaluated (Table 3.3). 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).

3.3.2. Ammonia emission

The ammonia emission measurements were done using passive ALPHA samplers and processing the data with dispersion models (see Ammonia emissions measurements). 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 3.3. 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 3.1. Observing each of the three graphs alone (Figure 3.1), 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, 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 3.1). 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 3.3). 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 3.3). 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 3.1. 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.

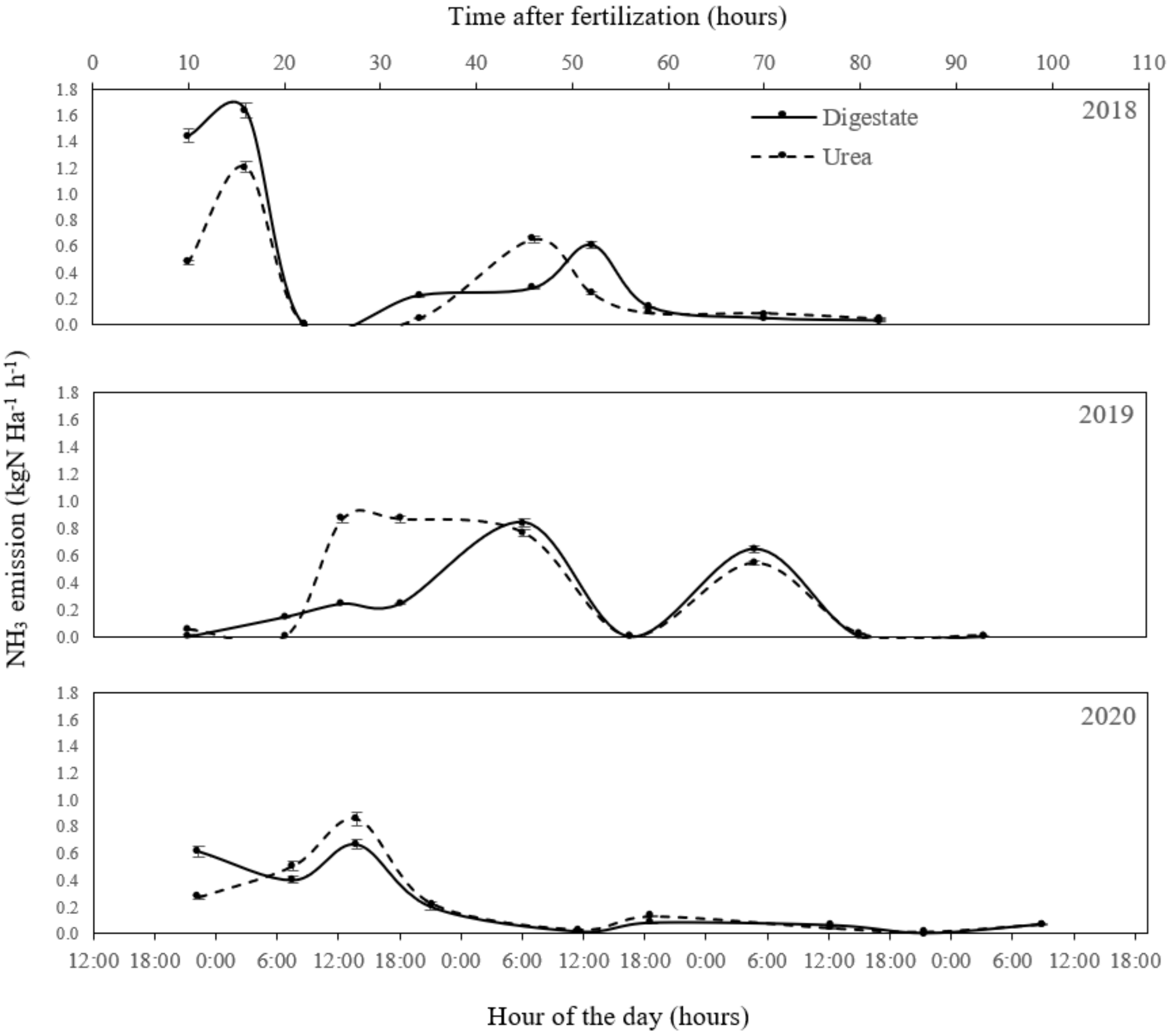
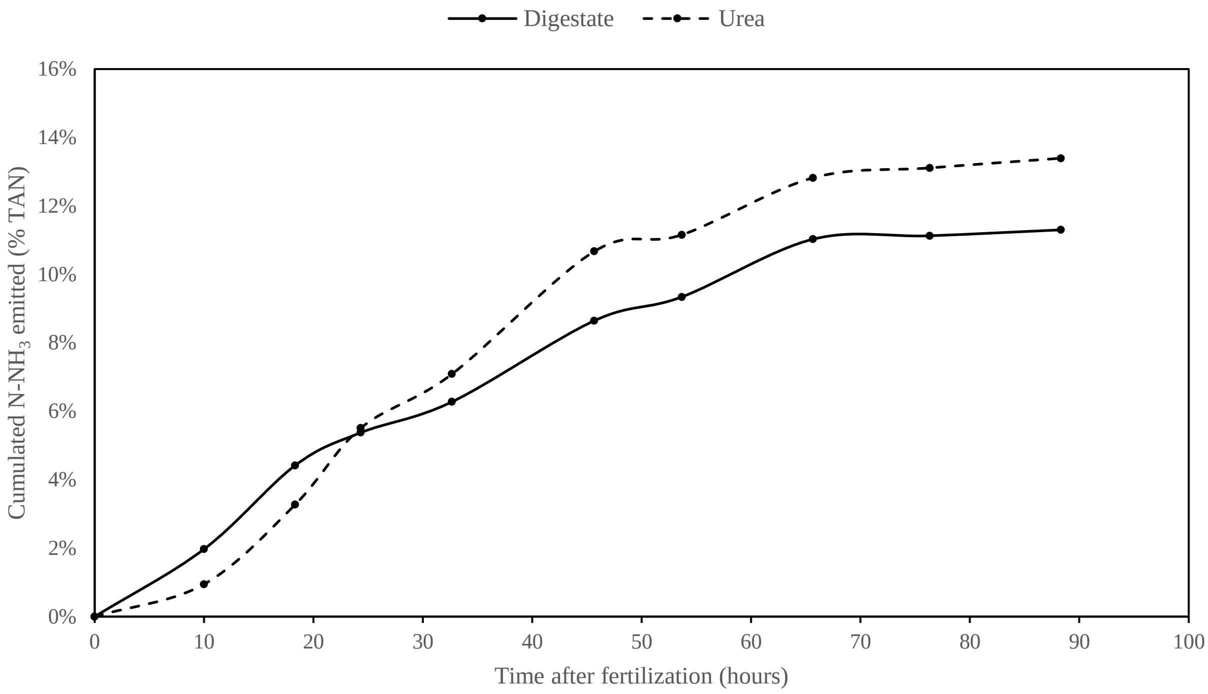


Figure 3.2. Cumulated ammonia loss (% TAN) in the hours following the spreading. The data reported refer to the average of the three years of experimentation on maize fields (2018, 2019 and 2020).



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 3.2). 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 3.2).

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 3.4) both injected and spread on the surface, and urea (Table 3.5) 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 3.4. 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	Ryegrass	Spring	86	67	12	14	17.9	Wind tunnels	(Rubæek et al. 1996)

	5 cm	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 3.3) was very similar to the data reported by Riva and colleagues (10.8%, Table 3.4) (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 3.5. 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 3.4) 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 3.5) 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 3.5).

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 3.5), 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.

3.3.3. 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).

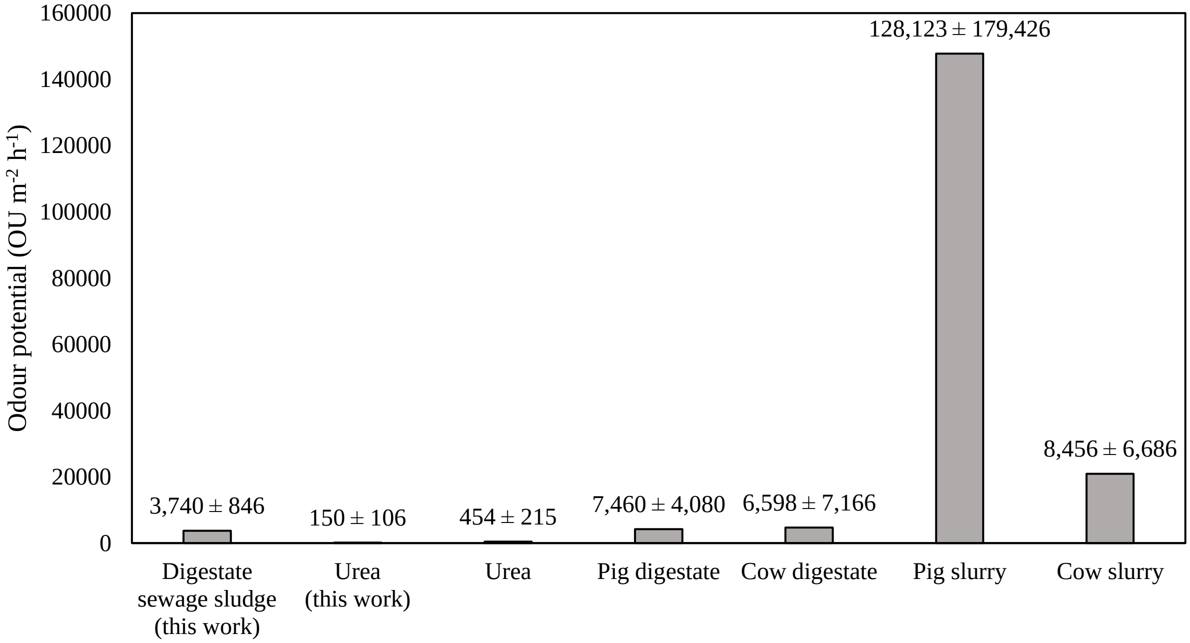
However, odors emission detections suffer for high variability that is an intrinsic characteristic of these measurements (Hudson et al. 2007), making it difficult to carry out statistically robust comparisons. The variability is due to both the large number of factors affecting odour emission, especially 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 3.3) 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 (Orzi et al. 2018; Orzi et al. 2015). From Figure 3.3 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 3.3. 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).

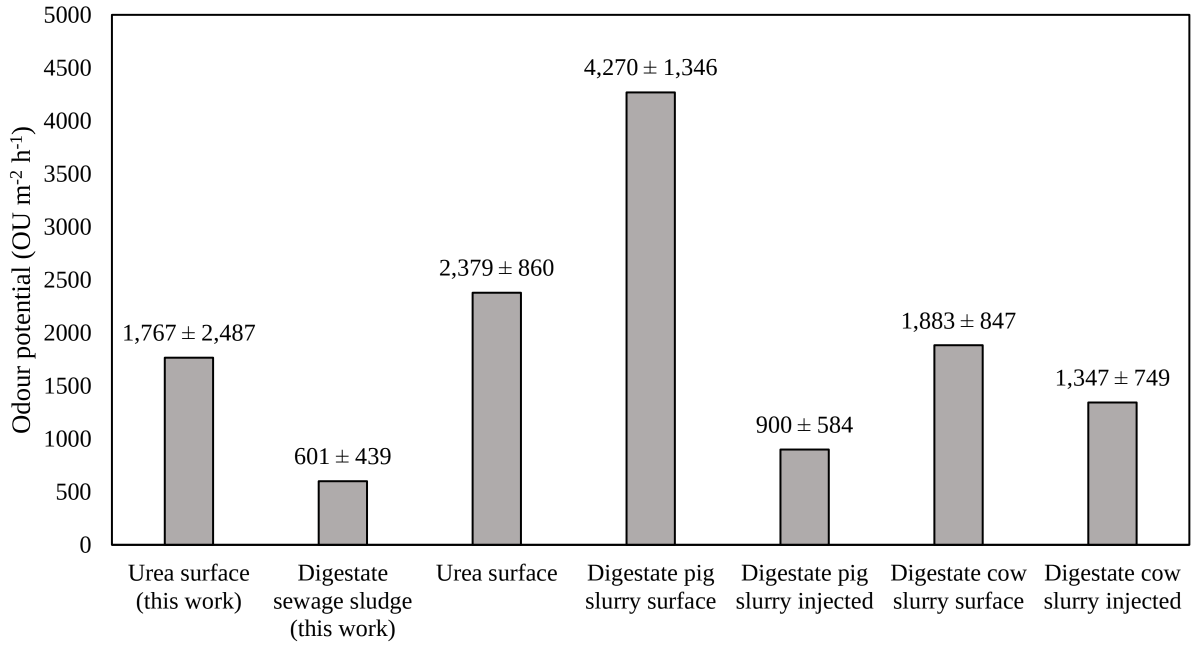
The urea, as expected, showed the lowest potential odour emission value, i.e. 150 ± 106 OU m^{-2} h^{-1}, not so far with previous data 454 ± 215 OU m^{-2} h^{-1} (V. Orzi et al. 2018).

In open field experiments (2018, 2019 and 2020) odour emissions from the experimental plots were measured each year immediately after fertilization (Table 3.3). 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 3.3). 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; 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 3.4, 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 (Orzi et al. 2018), i.e. 900 ± 584 OU m^{-2} h^{-1} and 1347 ± 749 OU m^{-2} h^{-1}, respectively).

Figure 3.4. 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.

3.4. 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.

4. Impact of digestate fertilization on nitrogen cycle in soil profile

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Digestate has proven to be a valuable nitrogen fertilizer and soil amendment; however, in order to optimize the use of this resource in agriculture, it is necessary to minimize as much as possible the environmental impacts deriving from the application in the field, especially in terms of nitrogen losses in the various environmental compartments, in particular groundwater and the atmosphere. It is therefore necessary to deepen the knowledge of the nitrogen cycle in the soil, with particular reference to the microorganisms, on which the transformations of this element depend. The presence of different forms of nitrogen, in particular ammonium and nitrates, in the soil profile of a corn crop was monitored during the year for three years. At the same time, emissions of nitrous oxide and ammonia were measured. Microbial communities correlated with nitrogen cycle were investigated through qPCR. Results showed that application of a highly stabilized digestate had

effects similar to those obtained with chemical fertilizaation, avoiding risks of nitrogen losses typical of unstabilized organic fertilizers.

4.1. Introduction

Modern intensive agriculture relies on the use of high amounts of mineral fertilizers, in particular nitrogen (N), which is an essential nutrient to plants. In last decades, the use of synthetic N fertilizers has led to an unprecedented increase in agricultural production, especially in developing countries, which reached 125% between 1966 and 2000 (Foley et al. 2011).

However, the production and use of N fertilizers has a strong ecological impact. In fact, their production through the Haber-Bosh process, requires large amounts of energy and therefore the emission of carbon dioxide (CO_2). It is estimated that annually, the production of N fertilizers requires about 2-3% of the energy consumed globally, and is responsible for 1.2% of the CO_2 emitted (Gaidajis and Kakanis 2020; C. Smith, Hill, and Torrente-Murciano 2020). Moreover the production and use of these fertilizers is responsible also for conspicuous emission of other greenhouse gases (GHG), in particular nitrous oxide (N_2O) (Hasler et al. 2015).

A solution to limit energy consumption and greenhouse gases emissions is the use of organic wastes and animal slurries. In fact, these products contain significant amounts of nutrients, which could be potentially recovered and used for the production of fertilizers, in order to substitute synthetic products, in a view of circular economy. (Tambone et al. 2017)

In fact, animal manures and other organic by-products have been traditionally applied to land as fertilizers for growing crops, and so improving physical and chemical properties of soil. However, the direct use of these by-products as fertilizers in agriculture can lead to various problems. In particular, the strong variability in the nutrient content of these biomasses can lead to an unbalanced release of nutrients in the soil. This, in addition to significantly decreasing the fertilization efficiency, can also cause environmental problems due to the loss of nutrients, in particular phosphorus (P) and nitrogen (N) which in soluble forms can be leached up to reach the water bodies. In addition, gaseous forms of nitrogen, such as ammonia (NH_3) and nitrous oxide (N_2O), if released into the atmosphere are responsible for pollution and an increase in the greenhouse effect (Tambone et al. 2017) (Westerman and Bicudo 2005).

Such issues can be overcome with a process of stabilization of organic wastes and slurries.

Several technologies, both chemical, physical and biological have been developed in order to stabilize manure and slurries; among these technologies anaerobic digestion (AD) has gained increased attention in the last decades as an effective technology to convert untreated organic wastes into useful products, with the added benefit of producing renewable energy (I. Sigurnjak et al. 2019). AD is an anaerobic, microbial-driven process, which convert organic matter (OM) into biogas and digestate. Biogas produced is composed mainly of methane (~60%) and carbon dioxide (~40%), and can be used to produce heat and electricity. (Sheets et al. 2015)

In addition, the AD process leaves as a by-product a nutrient-rich organic sludge, called digestate, which has proved to be a valuable fertilizer and soil amendment. Several studies have shown that 90% of the nitrogen component of the digestate is rapidly available to plants, a value very similar to that of urea (Tambone and Adani 2017). Digestates also contains organically bound nitrogen, which is not ready available for plants but contributes to the formation of nitrogen pool in soil. On the other side, digestate can be defined as a soil improver due to the presence of a highly stable organic fraction.

Despite this, some doubts remain about the risks of the use of digestate in agriculture, in particular with regard to nitrogen losses in the form of nitrates, and ammonia. In fact, digestates can have a higher potential of nitrogen losses, due to their higher pH and NH_3/NH_4 contents (Nkoa 2014). Further research is so necessary to assess the environmental risks associated with agronomic use of digestate.

Objective of this study was to evaluate environmental impacts of the application of a stabilized digestate from organic wastes as fertilizer. The experiment has been carried on for three years (2018 – 2019 – 2020), in full scale field trials, comparing digestate application with conventional mineral nitrogen fertilization (urea) on maize crop; data obtained were compared with available literature. The dynamics of nitrogen mineralization were monitored during experiment, in particular to evaluate potential nutrient losses to atmosphere in form of NH_3 and N_2O, and leaching to groundwater as nitrate (NO_3^-).

4.2. Material and Methods

4.2.1. Experiment site and setup

The experiments were carried out over three consecutive agronomic seasons (2018 - 2019 - 2020), with triplicate experimental plots and using a randomized scheme on maize crop. The experimental field was located in the Po valley (Northern Italy). All the experiments compared the use of two different fertilizers in addition to an unfertilized control. Fertilization with slurry-like digestate from sewage sludge was compared with fertilization with solid granular urea; for both treatments, ammonium sulphate was used for topdressing fertilization.

Distribution and dosing of digestate occurred in pre-sown, assuming a nitrogen efficiency of 0.5, in compliance with Italian legislation, and in order to meet agronomic requirements for maize (Regione Lombardia 2020). Adopting this procedure, the amount of efficient nitrogen is the same as chemical fertilizer. A tank car joined to a rigid multi-anchor-subsoiler coupled with a Retrofit Variable-Rate Control (VRT control) was used for this operation. Distribution of granular urea occurred in pre sown, through distribution on soil surface, as is common practice in Northern Italy. Quantities of nitrogen dosed and chronological list of agronomic operations is reported in Table 2.1.

4.2.2. Fertilizer sampling and analysis

The digestate used in this work was sampled immediately before the spreading, directly from the tank car, then analysed in the immediately following hours.

The main characteristics of digestate used in this experiment are shown in Table 2.2. Determination of pH was performed in aqueous solution using a 1:2.5 sample/water ratio. Total Organic Carbon (TOC) and nitrogen content, in terms of Total Kjeldahl Nitrogen (TKN) and Total Ammonia Nitrogen (TAN) were determined in compliance with standard procedures of the American Public Health Association and analytical methods for wastewater sludges (APHA 1992). (IRSA CNR 1994). Inductively coupled plasma mass spectrometry (Varian, Fort. Collins, USA), preceded by acid digestion (EPA 1998) was used for the determination of phosphorus content. All the analyses were carried out in triplicate.

4.2.3. Soil sampling and analysis

Soil was sampled several times during the three agronomic seasons under study, on dates reported in Table 4.1. The samplings were carried out by coring (three for each plot), taking samples at increasing depth, one every 25 cm up to a depth of one meter (0-25 cm, 25-50 cm, 50-75 cm and 75-100 cm). The samples were then immediately transported to the laboratory and stored both at 4° C, for chemical analysis and at -20° C, for DNA extraction. All samples were analyzed within a short time from the sampling date.

For chemical analysis, samples were air dried, sieved to 2 mm and then ground to 0.5 mm. 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, ammonia nitrogen, and nitrate nitrogen were determined by Kjeldahl method (Faithfull 2002). All the analyses were carried out in triplicate.

Table 4.1. Chronological list of agronomic operations and soil samplings carried out during the experimentation.

Date	Sampling	Agronomic operation
23/04/2018	Pre sown 2018	Pre sown spreading
03/04/2019	Pre sown 2019	
16/04/2019		Pre sown spreading
28/06/2019	Pre topdressing 2019	
30/07/2019		Topdressing fertilization
01/08/2019	Post topdressing 2019	
23/09/2019		Harvest
24/09/2019	Harvest 2019	
16/05/2020	Pre sown 2020	
28/05/2020		Pre sown spreading
18/06/2020	Post sown 2020	
14/07/2020	Pre topdressing 2020	
31/07/2020		Topdressing fertilization
07/08/2020	Post topdressing 2020	
28/10/2020		Harvest
05/11/2020	Harvest 2020	
12/01/2021	Three years after experiment start	

4.2.4. Nitrous oxide emissions measurement

Determination of fluxes of nitrous oxide (N_2O) was performed from 28/05/2020 (pre-sowing) to 17/03/2021, through the use of non-steady-state chambers, as reported in literature (Bertora et al. 2018; Gregorich et al. 2005).

Chambers were supported by anchors inserted in soil down to 20 cm depth, in order to ensure the isolation of soil column; chambers were set up in triplicate for each of the treatments (Digestate fertilization, chemical fertilization and untreated control (Tang, Cape, and Sutton 2001; Peyron et al. 2016).

Air flows were sampled at predefined times during the monitoring period; collected air samples were analyzed in laboratory for their concentrations in N_2O by gas chromatography, as reported by (Piccini et al. 2017).

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 air volume and soil surface isolated from the chamber, corresponding to the height of the chamber (m), and t is the closing time of the chamber.

The dC/dt ration was calculated by linear regression between concentrations and sampling times, if the increase in gas concentrations in the chamber was linear; HM model was applied in case of non-linear accumulation trend (Peyron et al. 2016). Cumulative emissions were estimated through linear interpolation between sampling days.

4.2.5. Ammonia emissions measurement

Emissions of ammonia (NH_3) were monitored by the use of passive ALPHA samplers (Adapted Low-cost Passive High Absorption), as described by (Tang, Cape, and Sutton 2001; Riva et al. 2016; Zilio et al. 2021). For each parcel, three ALPHA samplers were set up, plus an additional sampling point placed at a distance of about 1000 meters away from the fertilized fields and other possible point sources of NH_3, in order to obtain background environmental concentration values. Each sampling point located in the parcel was replaced a minimum of 2 times a day near sunrise and sunset, in order to monitor the variation in atmospheric turbulence. During the day of the spreading and the following day, the replacement frequency took place at the entrance in field of fertilization vehicles.

Atmospheric turbulence parameters were monitored through the use of an 10 Hz ultrasonic anemometer. WindTrax dispersion model (Tunderbeach scientific, CA) was used to byprocess NH_3 concentration information in order to determine the relative flow, taking into account the atmospheric turbulence parameters obtained with the anemometer, thus obtaining a dispersion coefficient (D; s m^{-1}). The flux emitted by the fertilized surface (S; ng NH_3 m^{-2} s^{-1}) was determined on the basis of the sampled ammonia concentrations (C; µg m^{-3}) and the ambient background ammonia concentration (C_{bgd}; µg m^{-3}), according to the following equation:

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

The Emission Factor, representing the ratio between the volatilized NH_3, expressed in kg N-NH_3 ha^{-1}, and ammonia fraction of the fertilizer used, was calculated using the following equation:

$$EF\% = fc \times N^{-1}$$

Where EF% is ammonia emission factor, fc the cumulative flux of ammonia (g m^{-2}), and N the nitrogen input with fertilization (kg m^{-2}).

4.2.6. Quantification of soil microorganisms by qPCR

The consistency of microbial populations linked with transformation of nitrogen in soil was assessed targeting five key genes (*amoA archea*, *amoA eubacteria*, *nifH*, *nirK*, *nosZ*) by qPCR. In brief, *amoA* gene in both archea and eubacteria are responsible for ammonia oxidation, the rate-limiting step of nitrification, *nifH* gene, encodes the iron protein of nitrogenase, the enzyme that catalyzes N_2 fixation, *nirK* gene encodes for nitrite reductase activity, converting nitrite into nitrous oxide, while the denitrifier populations were assessed by sequence analysis of *nosZ*, the gene for the terminal enzyme in denitrification, N_2O reductase.

Determination occurred on same soils sampled concurrently for chemical characterization, for seasons 2019 and 2020, following the method already used in Zilio et al., 2020.

For total DNA extraction, the PowerSoil® (Qiagen) kit was used, following producer's protocol. The total DNA extracted was then quantified using NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific). Real time PCR reactions were performed on QuantStudio 12K Flex Real-Time PCR System (ThermoFisher Scientific), using SyberGreen technology, in a final volume of 10µl. The sequences of the primers used are reported in Table 4.2. As templates for the standard curves, amplicons for each of the target genes were cloned into purified plasmids (pGem-T; Promega Corp.) and inserted into *E. coli* JM101 by electroporation; standard calibration curve was built using five points in triplicate, equal to fifteen reactions. The number of copies per ng of DNA and the corresponding amounts to be used for each of the quantitative PCR calibration curves, were calculated upon the size of the vector (3,015 bp), the size of each insert, and measuring the plasmid DNA concentration.

Calibration curve calculated for each gene and the output of the qPCR reaction were subsequently used for the determination of the number of gene copies per gram of soil in each sample; in order to ensure a robust statistical basis, each sample was analyzed in triplicate.

Table 4.2. Primers used in the quantification of nitrogen related microorganisms in soil. For each primer are shown name, nucleotide sequence, target gene and reference. N = undefined nucleotide.

Primer Name	**Sequence 5’ -> 3’**	**Gene Name**	**GenBank ID**	**References**
Arch-amoAF	NTAATGGTCTGGCTTAGACG	Archaeal *amoA*	MH318568.1	(C. A. Francis et al. 2005)
Arch-amoAR	GCGGCCATCCATCTGTATGT	Archaeal *amoA*	MH318568.1	(C. A. Francis et al. 2005)
amoA1F	GGGGTTTCTACTGGTGGT	Bacterial *amoA*	MG913925.1	(Rotthauwe, Witzel, and Liesack 1997)
amoA2R	CCCCTCNGNAAAGCCTTCTTC	Bacterial *amoA*	MG913925.1	(Rotthauwe, Witzel, and Liesack 1997)
nosZF	CGNTGTTCNTCGACAGCCAG	*nosZ*	CP000490.1	(Christopher Rösch, Mergel, and Bothe 2002)
nosZR	CATGTGCAGNGCNTGGCAGAA	*nosZ*	CP000490.1	(Christopher Rösch, Mergel, and Bothe 2002)
nirK876F	ATNGGCGGNCANGGCGA	*nirK*	KJ147768.1	(Henry et al. 2004)
nirK1040	NGCCTCGATCAGNTTNTGGTT	*nirK*	KJ147768.1	(Henry et al. 2004)
nifHF	AAAGGNGGNATCGGNAANTCCACCAC	*nifH*	KC445685.1	(Christopher Rösch, Mergel, and Bothe 2002)
nifHR	TTGTTNGCNGCNTACATNGCCATCAT	*nifH*	KC445685.1	(Christopher Rösch, Mergel, and Bothe 2002)

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. Plots and graphs were obtained through the use of Microsoft EXCEL 2016.

4.3. Results and discussion

4.3.1. Determination of organic carbon and pH in soil profile

Total Organic Carbon (TOC) and pH were determined for all samples; results are reported in Table 4.4., and Table 4.3., for TOC and pH respectively.

The pH value remains mostly homogeneous, in conditions of sub acidity, both by comparing the different treatments and over time, even if values of digestate and urea are slightly lower than in untreated soils. It is possible to observe a slightly increasing trend with increasing soil depth, approaching neutrality, for both treatments and control, probably due to the lesser influence of fertilization and atmospheric agents in the deeper layers than in the superficial ones.

The organic carbon concentrations detected in the soils did not show differences both between treatments and in the different sampling times. In all sampling times, a negative trend is observed along the profile, with a higher concentration of carbon in the surface layer compared to that of the deeper layers. This result was obtained similar for all three treatments, and is most likely due to microbial activity and the presence of crop residues.

Table 4.3. Soil pH (in water) (mean ± SD, n=3) in the experimental plots. The sampling dates are reported in Table 3. The analyses were carried out at four levels of depth: 0-25, 25-50, 50-75 and 75-100 cm. Letters are referred to One-way ANOVA analysis (Tukey post-test, $p<0.05$), which compares values of the three treatments at the same depth. The triplets of symbols used are: a – b – c; x – y – z; α – β – γ; χ – ψ – ω.

		pH (in water)								
Treatment	**Depth (cm)**	**Pre sown 2019**	**Pre topdressing 2019**	**Post topdressing 2019**	**Harvest 2019**	**Pre sown 2020**	**Post sown 2020**	**Pre topdressing 2020**	**Post topdressing 2020**	**Harvest 2020**
Unfertilized	0-25	6.66 ± 1.19 (a)	6.04 ± 0.52 (a)	6.44 ± 0.17 (a)	6.27 ± 0.61 (ab)	7.03 ± 0.73 (a)	6.57 ± 0.69 (a)	6.06 ± 0.31 (a)	6.16 ± 0.12 (a)	6.09 ± 0.32 (a)
	25-50	6.96 ± 0.77 (y)	6.26 ± 0.48 (y)	6.64 ± 0.28 (x)	5.82 ± 0.11 (x)	7.14 ± 0.70 (x)	6.74 ± 0.62 (x)	6.37 ± 0.10 (x)	6.40 ± 0.20 (x)	6.16 ± 0.34 (x)
	50-75	7.31 ± 0.44 (α)	6.64 ± 0.62 (α)	6.76 ± 0.36 (α)	6.78 ± 0.30 (α)	7.54 ± 0.49 (β)	7.25 ± 0.71 (α)	6.81 ± 0.25 (αβ)	6.64 ± 0.34 (α)	6.83 ± 0.63 (αβ)
	75-100	7.73 ± 0.10 (χ)	6.50 ± 0.93 (χ)	5.99 ± 0.78 (χ)	6.47 ± 0.16 (χ)	7.54 ± 0.47 (χ)	7.29 ± 0.84 (χ)	6.19 ± 1.03 (χ)	6.40 ± 0.54 (χ)	7.14 ± 0.97 (χ)
Urea	0-25	6.75 ± 0.86 (ab)	5.75 ± 0.30 (a)	6.39 ± 0.51 (a)	5.67 ± 0.46 (a)	6.87 ± 0.77 (a)	6.24 ± 0.55 (a)	5.57 ± 0.58 (a)	5.62 ± 0.73 (a)	5.80 ± 0.43 (a)
	25-50	6.77 ± 0.72 (x)	5.84 ± 0.17 (x)	6.52 ± 0.19 (x)	6.27 ± 0.37 (x)	7.30 ± 0.52 (x)	6.39 ± 0.65 (x)	6.42 ± 0.47 (x)	6.33 ± 0.33 (x)	6.11 ± 0.61 (x)

	50-75	7.24 ± 0.51 (α)	6.35 ± 0.18 (α)	6.84 ± 0.22 (α)	6.64 ± 0.19 (α)	7.74 ± 0.32 (β)	7.30 ± 0.27 (α)	7.10 ± 0.23 (β)	7.03 ± 0.16 (α)	7.00 ± 0.51 (β)
	75-100	6.63 ± 1.30 (χψ)	6.13 ± 0.22 (χ)	7.16 ± 0.42 (χ)	6.62 ± 0.18 (χ)	8.01 ± 0.22 (χψ)	7.57 ± 0.46 (χ)	7.14 ± 0.73 (χ)	7.16 ± 0.75 (χ)	7.12 ± 0.65 (χ)
Digestate	0-25	6.33 ± 0.86 (b)	5.82 ± 0.37 (a)	5.80 ± 0.60 (a)	5.88 ± 0.11 (b)	6.65 ± 0.68 (a)	6.17 ± 0.40 (a)	5.67 ± 0.10 (a)	5.61 ± 0.24 (a)	5.69 ± 0.57 (a)
	25-50	6.84 ± 0.52 (x)	5.75 ± 0.23 (x)	6.49 ± 0.26 (x)	6.13 ± 0.18 (x)	6.92 ± 0.94 (x)	6.39 ± 0.44 (x)	6.28 ± 0.20 (x)	6.14 ± 0.30 (x)	6.16 ± 0.24 (x)
	50-75	7.09 ± 0.44 (α)	6.43 ± 0.68 (β)	6.92 ± 0.31 (α)	6.53 ± 0.12 (α)	7.34 ± 0.44 (α)	7.14 ± 0.39 (α)	7.01 ± 0.64 (α)	6.85 ± 0.20 (α)	7.12 ± 0.43 (α)
	75-100	7.00 ± 0.63 (ψ)	6.35 ± 0.61 (χ)	6.94 ± 0.28 (χ)	7.03 ± 0.28 (χ)	7.66 ± 0.34 (ψ)	7.40 ± 0.66 (χ)	6.83 ± 0.38 (χ)	6.81 ± 0.38 (χ)	7.58 ± 0.42 (χ)

d.w.: dry weight

Table 4.4. Soil total carbon (TOC) in mg kg-1 d.w. (mean ± SD, n=3) in the experimental plots. The sampling dates are reported in Table 3. The analyses were carried out at four levels of depth: 0-25, 25-50, 50-75 and 75-100 cm. Letters are referred to One-way ANOVA analysis (Tukey post-test, p<0.05), which compares values of the three treatments at the same depth. The triplets of symbols used are: a – b – c; x – y – z; α – β – γ; χ – ψ – ω.

		TOC (mg kg^{-1} d.w.)								
Treatment	**Depth (cm)**	**Pre sown 2019**	**Pre topdressing 2019**	**Post topdressing 2019**	**Harvest 2019**	**Pre sown 2020**	**Post sown 2020**	**Pre topdressing 2020**	**Post topdressing 2020**	**Harvest 2020**
Unfertilized	0-25	10287.15 ± 618.74 (a)	8256.43 ± 2190.91 (a)	10706.48 ± 1692.79 (a)	12199.35 ± 2141.24 (a)	11397.95 ± 700.41 (a)	12123.70 ± 884.13 (a)	10324.39 ± 867.03 (a)	10048.61 ± 690.64 (a)	11722.41 ± 1176.35 (a)
	25-50	3416.47 ± 555.75 (x)	2919.06 ± 575.07 (x)	4191.34 ± 1045.55 (x)	10086.62 ± 764.90 (x)	8463.04 ± 260.94 (x)	7354.33 ± 1151.69 (x)	8758.91 ± 102.16 (x)	6989.33 ± 1665.22 (xy)	6033.67 ± 1956.58 (x)
	50-75	2071.90 ± 623.28 (β)	323.39 ± 221.47 (α)	2905.59 ± 1153.06 (β)	3919.63 ± 868.14 (α)	1969.89 ± 201.96 (α)	2271.57 ± 453.01 (α)	1570.96 ± 419.46 (β)	856.85 ± 26.71 (α)	1821.37 ± 296.55 (α)
	75-100	1217.82 ± 191.17 (χ)	50.25 ± 0.92 (χ)	1170.68 ± 101.72 (ω)	2811.03 ± 782.41 (χ)	881.60 ± 196.42 (χ)	1793.76 ± 514.25 (χ)	1562.18 ± 99.27 (χ)	0.00 ± 0.00 (ψ)	823.83 ± 313.11 (χ)
Urea	0-25	11013.25 ± 542.47 (a)	10619.71 ± 1448.49 (a)	9572.92 ± 432.36 (a)	12962.82 ± 1237.96 (a)	11373.80 ± 378.89 (a)	10285.63 ± 1527.54 (a)	11181.56 ± 1442.15 (a)	10266.03 ± 625.95 (a)	10360.70 ± 1262.07 (a)

	25-50	6633.61 ± 1192.61 (y)	6140.40 ± 1703.82 (xy)	5482.95 ± 1727.00 (x)	11149.69 ± 1732.81 (x)	6387.21 ± 310.24 (x)	7573.56 ± 1381.47 (x)	4775.85 ± 1222.46 (y)	5340.77 ± 293.75 (y)	5941.51 ± 1814.28 (x)
	50-75	1656.14 ± 545.45 (αβ)	4535.39 ± 1020.40 (β)	767.42 ± 79.57 (α)	2926.47 ± 428.46 (α)	2380.51 ± 268.80 (α)	1972.18 ± 533.66 (α)	2509.23 ± 422.70 (αβ)	972.37 ± 676.42 (α)	1555.56 ± 231.64 (α)
	75-100	1166.21 ± 121.85 (χ)	2832.90 ± 529.31 (ω)	343.94 ± 17.56 (ψ)	2450.32 ± 299.79 (χ)	1841.15 ± 270.72 (χ)	1887.78 ± 242.70 (χ)	1496.89 ± 424.71 (χ)	582.58 ± 245.90 (χ)	905.15 ± 125.93 (χ)
Digestate	0-25	9841.01 ± 755.13 (a)	9446.02 ± 956.69 (a)	10043.00 ± 1509.94 (a)	11782.25 ± 1919.26 (a)	13893.06 ± 928.66 (a)	11537.15 ± 1077.78 (a)	11897.92 ± 1485.77 (a)	10232.79 ± 538.55 (a)	10302.83 ± 1299.05 (a)
	25-50	5233.61 ± 221.67 (xy)	6564.94 ± 1494.83 (y)	4788.98 ± 1157.66 (x)	7613.47 ± 1700.27 (x)	7033.70 ± 718.79 (x)	7953.13 ± 1690.65 (x)	5665.19 ± 1534.43 (y)	8416.29 ± 780.45 (x)	4910.54 ± 1146.76 (x)
	50-75	860.77 ± 16.47 (α)	2119.60 ± 1198.76 (α)	1020.56 ± 337.49 (α)	2484.48 ± 572.18 (α)	4719.62 ± 258.19 (α)	1809.50 ± 566.73 (α)	4950.75 ± 23.92 (α)	3174.53 ± 1805.48 (α)	3229.53 ± 1279.85 (α)
	75-100	1413.09 ± 183.26 (χ)	1489.73 ± 319.17 (ψ)	0.00 ± 0.00 (χ)	2498.87 ± 81.88 (χ)	2191.29 ± 131.00 (χ)	1275.05 ± 370.03 (χ)	1414.59 ± 683.13 (χ)	2040.92 ± 1406.14 (χ)	1493.59 ± 283.28 (χ)

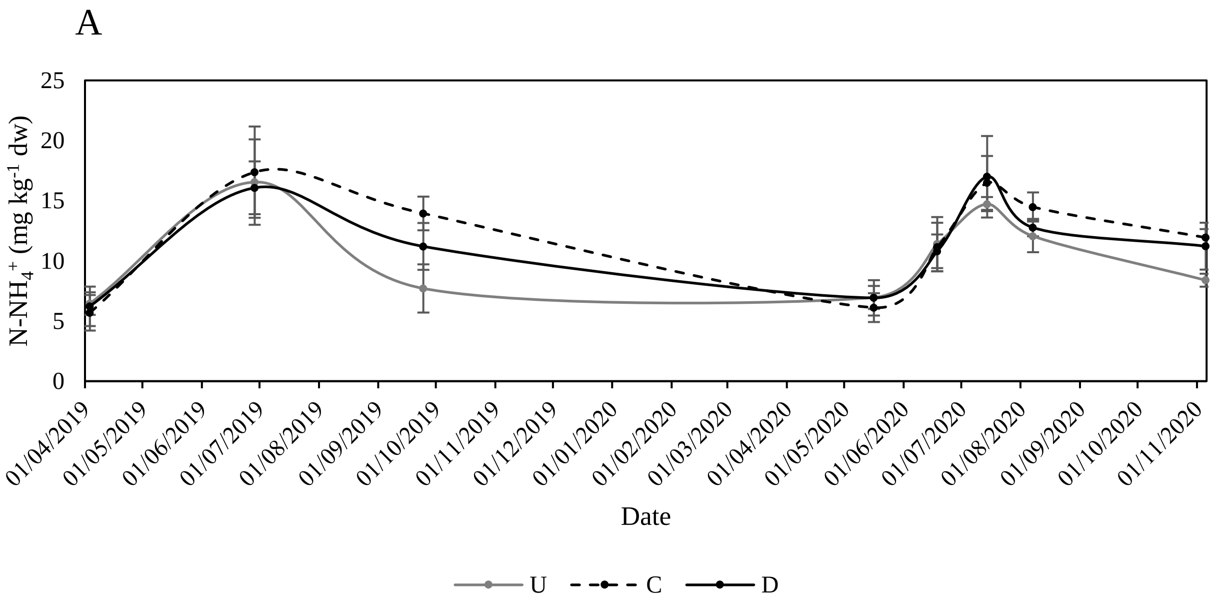
d.w.: dry weight

4.3.2. Nitrogen speciation in soil profile

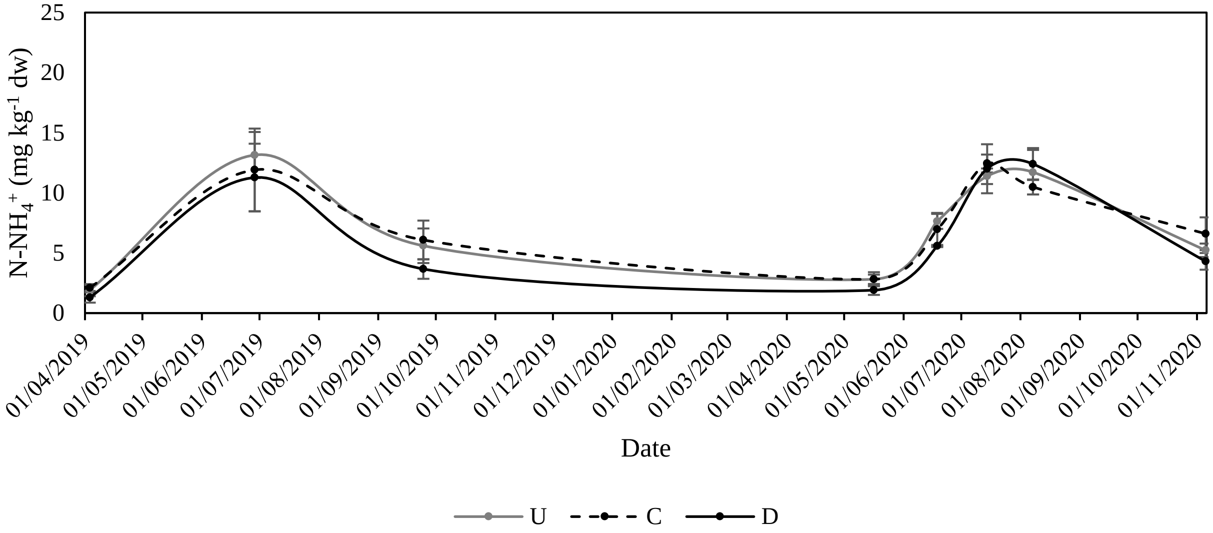
Complete results of determination of ammonia nitrogen (NH_4^+), nitrate (NO_3^-) and Total Kjeldahl Nitrogen (TKN), are reported in Table 4.5, Table 4.6,and Table 4.7 respectively.

Figure 4.1 reports trend of ammonia concentration during experiment, for layer 0-25, and layer 75-100.

Figure 4.1. Concentration of ammonium nitrogen ($N\text{-}NH_4^+$) in experimental soils (a: surface; b: 1 meter depth) during the 2019 - 2020 crop seasons (n = 3). Error bars show Standard deviation. U: untreated, C: chemical fertilizer, D: digestate.



B



Results show that ammonia nitrogen concentrations generally decrease from the surface soil to the deeper layers; this general trend can be found both for treatments with urea, for treatments with digestate and for control plots. This type of gradient is not surprising, as the presence of ammonia nitrogen in the soil is closely linked to plant activity and the presence of external inputs, such as fertilization; moreover, as a positively charged molecule, has a low mobility in soil (Cameron, Di, and Moir 2013). In addition, ammonia form of nitrogen in soil ($N\text{-}NH_4^+$) is rapidly converted to nitric form ($N\text{-}NO_3^-$), which instead tends to leach towards deeper layers, or volatilizes in gaseous form (NH_3), thereby leaving the soil.

In layer (0-25), concentrations of NH_4 show a very similar trend between treatments, with the same concentrations, around 6 mg kg^{-1} dw, at the beginning of season, a peak around first days of July for all treatments, and again a decrease at the end of the season. The only statistically significant difference between treatments is at the end of the season (post topdressing and harvest), for both

2019 and 2020; in particular, at the moment of post topdressing sampling, values of digestate fertilization are higher compared to values of chemical fertilization. Anyway, concentrations at beginning of season 2020 are again not statistically different.

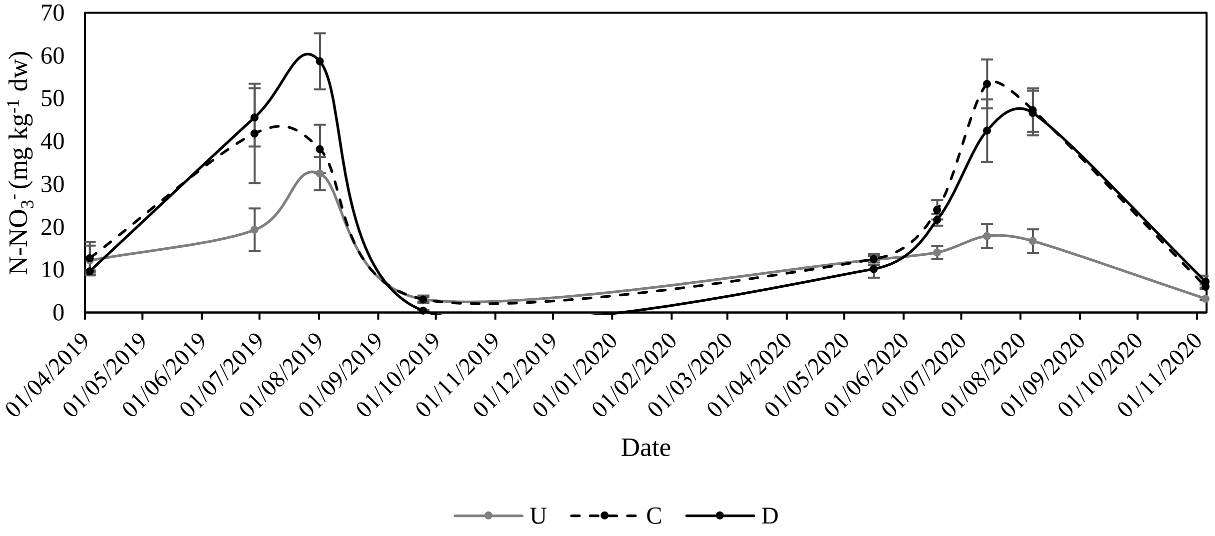
Same trend can be spotted for layer (75-100), where the concentrations are lower, and follow a similar trend to surface concentrations.

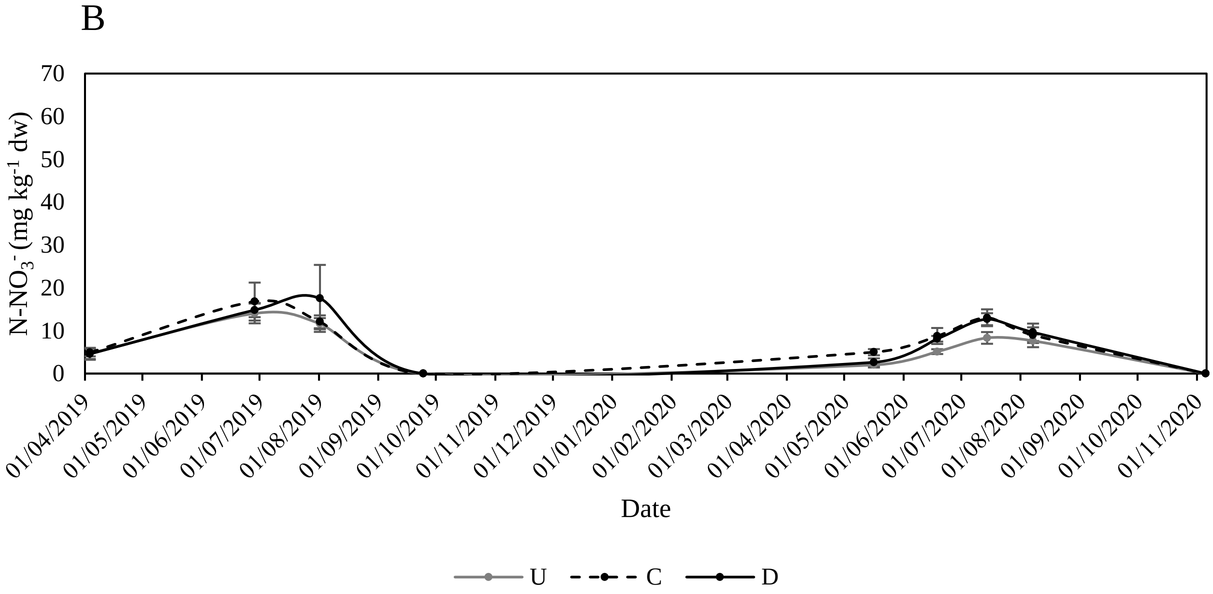
The fact that ammonia concentrations are same for chemical and digestate treatment during all season, can indicate that ammonia nitrogen inputs due to fertilizations for both treatments are equally available for plants uptake.

Trend of nitrate (NO_3^-) concentrations for soil layers 0-25 and 75-100 is reported in Figure 4.2.

Figure 4.2. Concentration of nitrate nitrogen ($N\text{-}NO_3^-$) in experimental soils (a: surface; b: 1 meter depth) during the 2019 - 2020 crop seasons (n = 3). Error bars show Standard deviation. U: untreated, C: chemical fertilizer, D: digestate.

A





In opposition to ammonia nitrogen, nitrate is highly mobile in soil due to his presence in aqueous solution; and as well as ammonia concentrations, nitrate concentrations in soil profile are generally higher in topsoil, and lower at 100 cm of depth. Results show that NO_3^- concentrations along soil profiles have similar trends between treatments, with a decrease from top soil to deep layers. As for ammonium, there is a evident seasonal trend, with concentrations in soil surface that reach a peak in summer; even if this trend can be seen also for untreated parcels, and therefore can be a result of climate and season, values for fertilized parcels are significantly higher, highlighting a relevant contribution of fertilizer to nitrate concentration in soil. This is even more evident considering that values at the beginning of the season are not statistically different between treatments. Despite high concentrations on surface, concentrations at 100 cm of depth are relatively low, never reaching above 20 mg kg^{-1} dw; as for topsoil values, concentrations increase during the season, reaching a peak in post topdressing sampling time, followed by a decrease proceeding to the end of the season.

Table 4.5. Soil ammonia nitrogen ($N\text{-}NH_4^+$) in mg kg^{-1} d.w. (mean ± SD, n=3) in the experimental plots. The sampling dates are reported in Table 3. The analyses were carried out at four levels of depth: 0-25, 25-50, 50-75 and 75-100 cm. Letters are referred to One-way ANOVA analysis (Tukey post-test, p<0.05), which compares values of the three treatments at the same depth. The triplets of symbols used are: a – b – c; x – y – z; α – β – γ; χ – ψ – ω.

		$N\text{-}NH_4^+$ (mg kg^{-1} d.w.)								
Treatment	**Depth (cm)**	**Pre sown 2019**	**Pre topdressing 2019**	**Post topdressing 2019**	**Harvest 2019**	**Pre sown 2020**	**Post sown 2020**	**Pre topdressing 2020**	**Post topdressing 2020**	**Harvest 2020**
Unfertilized	0-25	6.45 ± 0.94 (a)	16.55 ± 3.55 (a)	12.99 ± 0.68 (a)	7.71 ± 2.00 (a)	6.96 ± 0.96 (a)	14.72 ± 0.59 (a)	12.05 ± 1.33 (a)	8.39 ± 0.54 (b)	8.39 ± 0.54 (b)
	25-50	3.30 ± 0.73 (x)	15.36 ± 2.41 (x)	11.06 ± 1.35 (x)	7.47 ± 3.51 (x)	4.07 ± 0.77 (x)	12.98 ± 0.92 (x)	12.48 ± 0.83 (x)	6.58 ± 0.97 (y)	6.58 ± 0.97 (y)
	50-75	2.67 ± 0.97 (α)	13.83 ± 1.70 (α)	10.38 ± 1.36 (α)	6.79 ± 3.73 (α)	3.36 ± 0.84 (α)	11.87 ± 1.07 (α)	10.44 ± 0.94 (α)	7.06 ± 1.50 (α)	7.06 ± 1.50 (α)
	75-100	1.91 ± 0.51 (χ)	13.15 ± 1.91 (χ)	9.50 ± 1.05 (χ)	7.01 ± 2.34 (χ)	2.82 ± 0.58 (χ)	11.38 ± 0.65 (χ)	11.72 ± 1.85 (χ)	5.22 ± 0.56 (χ)	5.22 ± 0.56 (χ)
Urea	0-25	5.68 ± 1.48 (a)	17.37 ± 3.79 (a)	12.91 ± 1.18 (a)	13.94 ± 1.40 (b)	6.37 ± 1.20 (a)	16.48 ± 2.24 (a)	14.48 ± 1.21 (a)	11.94 ± 0.70 (a)	11.94 ± 0.70 (a)

	25-50	3.88 ± 0.47 (x)	15.04 ± 3.41 (x)	11.63 ± 1.16 (x)	5.88 ± 1.12 (x)	4.76 ± 0.48 (x)	14.94 ± 2.05 (x)	11.18 ± 1.91 (x)	11.27 ± 1.47 (x)	11.27 ± 1.47 (x)
	50-75	2.68 ± 0.77 (α)	12.15 ± 3.13 (α)	10.82 ± 1.49 (α)	5.98 ± 2.47 (α)	3.37 ± 0.86 (α)	13.80 ± 1.74 (α)	12.23 ± 0.44 (α)	8.38 ± 0.33 (α)	8.38 ± 0.33 (α)
	75-100	2.09 ± 0.25 (χ)	11.91 ± 3.44 (χ)	10.05 ± 0.90 (χ)	6.48 ± 1.01 (χ)	2.81 ± 0.39 (χ)	12.44 ± 0.74 (χ)	10.49 ± 0.62 (χ)	6.60 ± 1.36 (χ)	6.60 ± 1.36 (χ)
Digestate	0-25	6.22 ± 1.64 (a)	16.08 ± 2.19 (a)	23.11 ± 7.33 (b)	11.20 ± 1.95 (ab)	6.93 ± 1.47 (a)	16.99 ± 3.39 (a)	12.77 ± 0.72 (a)	11.22 ± 1.95 (ab)	11.22 ± 1.95 (ab)
	25-50	3.54 ± 0.57 (x)	14.43 ± 2.52 (x)	14.29 ± 3.31 (x)	9.25 ± 2.86 (x)	4.30 ± 0.64 (x)	14.06 ± 2.16 (x)	12.69 ± 0.30 (x)	10.52 ± 2.16 (xy)	10.52 ± 2.16 (xy)
	50-75	2.60 ± 0.73 (α)	13.40 ± 3.31 (α)	11.83 ± 2.09 (α)	8.23 ± 5.01 (α)	3.48 ± 0.99 (α)	12.07 ± 1.89 (α)	10.60 ± 1.15 (α)	6.37 ± 1.30 (α)	6.37 ± 1.30 (α)
	75-100	1.29 ± 0.43 (χ)	11.27 ± 2.82 (χ)	11.24 ± 2.29 (χ)	7.74 ± 1.79 (χ)	1.91 ± 0.39 (χ)	12.01 ± 2.04 (χ)	12.40 ± 1.33 (χ)	4.30 ± 0.69 (χ)	4.30 ± 0.69 (χ)

d.w.: dry weight

Table 4.6. Soil nitrate nitrogen (N-NO_3^-) in mg kg^{-1} d.w (mean ± SD, n=3) in the experimental plots. The sampling dates are reported in Table 3. The analyses were carried out at four levels of depth: 0-25, 25-50, 50-75 and 75-100 cm. Letters are referred to One-way ANOVA analysis (Tukey post-test, $p<0.05$), which compares values of the three treatments at the same depth. The triplets of symbols used are: a – b – c; x – y – z; α – β – γ; χ – ψ – ω.

		N-NO_3^- (mg kg^{-1} d.w.)								
Treatment	**Depth (cm)**	**Pre sown 2019**	**Pre topdressing 2019**	**Post topdressing 2019**	**Harvest 2019**	**Pre sown 2020**	**Post sown 2020**	**Pre topdressing 2020**	**Post topdressing 2020**	**Harvest 2020**
Unfertilized	0-25	12.14 ± 3.48 (a)	19.30 ± 5.00 (a)	32.45 ± 3.88 (a)	3.11 ± 0.53 (a)	12.34 ± 1.30 (ab)	14.01 ± 1.58 (b)	17.87 ± 2.81 (b)	16.68 ± 2.74 (b)	3.22 ± 0.30 (b)
	25-50	7.93 ± 0.74 (x)	17.53 ± 3.28 (x)	20.23 ± 5.11 (x)	1.45 ± 0.38 (x)	8.74 ± 0.56 (x)	11.53 ± 1.19 (y)	12.84 ± 0.65 (x)	13.60 ± 1.14 (x)	2.30 ± 0.44 (y)
	50-75	5.53 ± 1.15 (α)	16.25 ± 2.21 (α)	13.61 ± 1.57 (α)	0.00 ± 0.00	5.66 ± 0.66 (α)	7.83 ± 1.70 (α)	9.90 ± 1.64 (α)	7.61 ± 0.62 (α)	0.00 ± 0.00
	75-100	4.70 ± 1.30 (χ)	14.03 ± 2.30 (χ)	11.64 ± 1.30 (χ)	0.00 ± 0.00	2.00 ± 0.58 (χ)	5.12 ± 0.56 (Ψ)	8.32 ± 1.37 (ψ)	7.63 ± 1.48 (χ)	0.00 ± 0.00
Urea	0-25	12.69 ± 3.80 (a)	41.82 ± 11.60 (a)	38.17 ± 5.67 (a)	3.08 ± 0.88 (a)	15.04 ± 0.79 (a)	23.99 ± 2.27 (a)	53.38 ± 5.70 (a)	47.28 ± 5.07 (a)	6.02 ± 0.53 (a)

	25-50	8.41 ± 2.25 (x)	24.29 ± 5.57 (x)	17.68 ± 1.55 (x)	2.22 ± 0.49 (x)	8.71 ± 1.24 (x)	16.62 ± 2.13 (x)	16.08 ± 2.13 (x)	15.68 ± 1.77 (x)	4.03 ± 0.11 (x)
	50-75	5.67 ± 0.45 (α)	19.53 ± 5.72 (α)	14.50 ± 0.76 (α)	1.07 ± 0.29 (β)	5.52 ± 0.67 (α)	9.73 ± 1.03 (α)	12.88 ± 0.90 (α)	10.09 ± 2.60 (α)	1.65 ± 1.00 (α)
	75-100	4.92 ± 0.91 (χ)	16.80 ± 4.42 (χ)	12.08 ± 1.50 (χ)	0.00 ± 0.00	4.99 ± 0.72 (ψ)	8.77 ± 1.87 (χ)	13.02 ± 1.97 (χ)	8.96 ± 1.83 (χ)	0.00 ± 0.00
Digestate	0-25	9.63 ± 0.20 (a)	45.57 ± 6.81 (b)	58.66 ± 6.56 (b)	0.41 ± 0.12 (b)	10.17 ± 2.04 (b)	21.67 ± 1.40 (a)	42.48 ± 7.28 (a)	46.58 ± 5.22 (a)	7.20 ± 1.40 (a)
	25-50	7.39 ± 1.76 (x)	22.20 ± 6.18 (x)	24.08 ± 7.47 (x)	0.29 ± 0.07 (β)	9.85 ± 1.73 (x)	15.85 ± 1.08 (x)	15.62 ± 0.81 (x)	14.29 ± 0.62 (x)	2.80 ± 0.12 (y)
	50-75	6.12 ± 1.36 (α)	17.17 ± 3.61 (α)	19.36 ± 7.93 (α)	0.00 ± 0.00	5.37 ± 1.25 (α)	10.34 ± 1.81 (α)	13.12 ± 1.66 (α)	11.67 ± 1.75 (α)	1.62 ± 0.78 (α)
	75-100	4.56 ± 1.34 (χ)	14.82 ± 1.66 (χ)	17.54 ± 7.81 (χ)	0.00 ± 0.00	2.63 ± 0.92 (χ)	8.12 ± 0.66 (χΨ)	12.69 ± 1.40 (χ)	9.63 ± 2.02 (χ)	0.00 ± 0.00

d.w.: dry weight

Table 4.7. Soil total nitrogen (Ntot) in mg kg-1 d.w. (mean ± SD, n=3) in the experimental plots. The sampling dates are reported in Table 3. The analyses were carried out at four levels of depth: 0-25, 25-50, 50-75 and 75-100 cm. Letters are referred to One-way ANOVA analysis (Tukey post-test, p<0.05), which compares values of the three treatments at the same depth. The triplets of symbols used are: a – b – c; x – y – z; α – β – γ; χ – ψ – ω.

		Ntot (mg kg^{-1} d.w.)								
Treatment	**Depth (cm)**	**Pre sown 2019**	**Pre topdressing 2019**	**Post topdressing 2019**	**Harvest 2019**	**Pre sown 2020**	**Post sown 2020**	**Pre topdressing 2020**	**Post topdressing 2020**	**Harvest 2020**
Unfertilized	0-25	1388.16 ± 214.19 (a)	1285.98 ± 30.39 (a)	1269.78 ± 23.89 (a)	1345.05 ± 51.84 (a)	1291.57 ± 132.85 (a)	1151.55 ± 71.99 (a)	1215.68 ± 80.64 (a)	1295.94 ± 78.03 (a)	1295.94 ± 78.03 (a)
	25-50	1039.54 ± 120.19 (x)	703.89 ± 35.73 (x)	724.87 ± 157.87 (x)	1145.83 ± 86.68 (x)	868.32 ± 85.60 (x)	705.95 ± 253.88 (x)	896.37 ± 667.07 (x)	911.72 ± 15.20 (y)	911.72 ± 15.20 (y)
	50-75	588.58 ± 65.82 (β)	182.04 ± 20.98 (α)	229.86 ± 15.64 (β)	319.77 ± 122.63 (α)	253.17 ± 7.11 (α)	206.98 ± 22.10 (α)	288.87 ± 68.31 (α)	290.32 ± 34.73 (α)	290.32 ± 34.73 (α)
	75-100	103.32 ± 26.23 (χ)	119.64 ± 42.59 (ψ)	157.16 ± 20.67 (χ)	305.05 ± 77.04 (ψ)	208.08 ± 80.43 (χ)	162.31 ± 37.12 (χ)	168.07 ± 37.28 (χ)	195.11 ± 53.66 (χ)	195.11 ± 53.66 (χ)
Urea	0-25	1107.19 ± 99.47 (a)	1306.18 ± 66.61 (a)	1237.38 ± 84.52 (a)	1306.48 ± 66.58 (a)	1204.86 ± 144.90 (a)	1341.18 ± 106.37 (a)	1233.31 ± 173.09 (a)	1274.10 ± 173.89 (a)	1274.10 ± 173.89 (a)

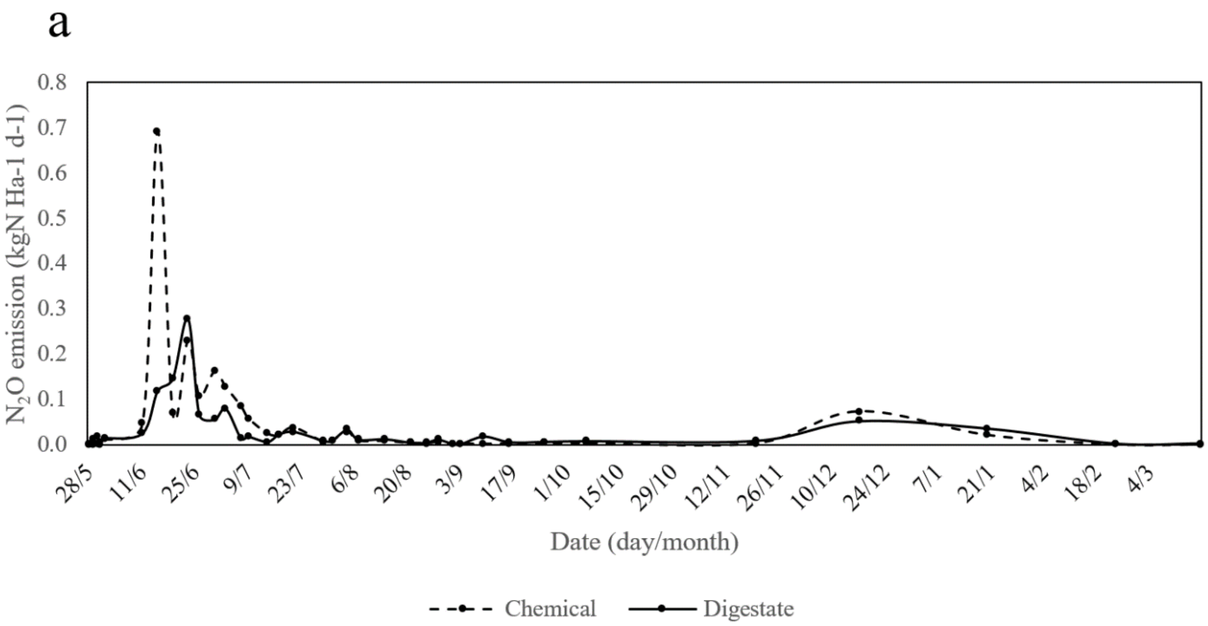
	25-50	1013.85 ± 182.36 (x)	860.28 ± 165.09 (x)	503.99 ± 56.54 (x)	1049.88 ± 135.60 (x)	998.23 ± 202.90 (x)	447.63 ± 241.56 (x)	627.17 ± 243.74 (x)	1010.26 ± 149.30 (xy)	1010.26 ± 149.30 (xy)
	50-75	242.34 ± 127.64 (α)	346.89 ± 93.77 (α)	250.55 ± 42.70 (αβ)	259.53 ± 12.88 (α)	318.24 ± 74.40 (α)	320.19 ± 87.42 (α)	245.21 ± 50.91 (α)	314.23 ± 74.58 (α)	314.23 ± 74.58 (α)
	75-100	58.10 ± 21.73 (χ)	323.43 ± 33.57 (χ)	187.70 ± 71.31 (χ)	170.20 ± 4.03 (χ)	251.65 ± 32.45 (χ)	143.81 ± 93.93 (χ)	206.47 ± 97.12 (χ)	333.05 ± 104.42 (χ)	333.05 ± 104.42 (χ)
Digestate	0-25	1159.43 ± 65.83 (a)	1367.03 ± 89.65 (a)	1350.21 ± 79.63 (a)	1238.00 ± 81.45 (a)	1380.22 ± 164.11 (a)	1556.43 ± 289.82 (a)	1329.52 ± 68.66 (a)	1369.95 ± 78.64 (a)	1369.95 ± 78.64 (a)
	25-50	1071.32 ± 100.16 (x)	1020.00 ± 141.27 (x)	511.88 ± 108.97 (x)	616.99 ± 20.23 (y)	956.43 ± 310.69 (x)	551.67 ± 478.17 (x)	1138.88 ± 50.68 (x)	1223.52 ± 73.99 (x)	1223.52 ± 73.99 (x)
	50-75	188.29 ± 59.15 (α)	367.29 ± 124.95 (α)	401.35 ± 90.30 (α)	250.66 ± 38.33 (α)	416.31 ± 99.56 (α)	418.04 ± 218.47 (α)	327.32 ± 221.83 (α)	330.39 ± 40.84 (α)	330.39 ± 40.84 (α)
	75-100	175.15 ± 82.65 (χ)	99.51 ± 40.19 (ψ)	373.63 ± 87.79 (ψ)	217.63 ± 29.78 (χψ)	279.30 ± 99.13 (χ)	402.02 ± 329.18 (χ)	286.59 ± 147.59 (χ)	306.28 ± 24.92 (χ)	306.28 ± 24.92 (χ)

d.w.: dry weight

4.3.3. Nitrous oxide emissions trend

Emissions of nitrous oxide (N_2O) from field were evaluated over the agronomic season of year 2020; measurement of N_2O fluxes was performed on a daily basis from 28/05/2021 to 17/03/2021. Results of monitoring are shown in Figure 4.3.

Figure 4.3. Daily nitrous oxide emissions (a) from the experimental plots from 28/05/2020 (pre-sowing) to 17/03/2021. Pearson correlation (b) between the N_2O emissions detected for each sampling point, the temperature recorded in the corresponding days and the cumulative rainfall recorded in the 72 hours before each measurement. *: $p < 0.05$; n= 36.



b

	Rainfall (cumulated of 72h before N_2O measurements)	Temperature (during N_2O measurements)
N_2O emission Chemical fertilizer	0.225	0.118
N_2O emission Digestate	**0.418***	0.092

Nitrous oxide emissions are generally low, both for digestate and urea, generally remaining below 0.1 kgN Ha^{-1} d^{-1}.

Emissions remain very low for the first 20 days after spreading, following which there is a significant peak of N_2O emissions for the treatment with chemical fertilizer. The concentrations of gas emitted rapidly pass from 0.0114 kg N Ha^{-1} d^{-1} of 01/06/2020 to 0.6918 kg N Ha^{-1} d^{-1} of 15/06/2020, and then decrease just as rapidly. Emissions therefore have a second peak, albeit to a lesser extent, reaching a value of approximately 0.3 kg N Ha^{-1} d^{-1}; in this second case, this emission peak is associated with high concentrations also as regards the emissions from the plots treated with digestate. A third peak can be spotted shortly after, where the quantity emitted are even lower, and emissions form digestate are half of emissions from chemical fertilizer (0.0794 and 0.1619 kg N Ha^{-1} d^{-1} respectively). The period of low emissions shortly after the spreading can be explained with the low availability of nitrogen in nitrate form. Instead, the period of high emissions has instead to be imputed to microbial activity of nitrification, so conversion of nitrite to gaseous nitrous oxide. Emissions then remain almost zero for both treatments and control.

Is interesting to notice that nitrous oxide emissions do not increase after the nitrogen input of topdressing fertilization; this can be imputed to the presence of crop, which uptake reduces the amount of nitrogen available for microbial nitrification.

At the same time is interesting to notice the presence of an increase of emissions around the half of December, when the activity of soil microorganisms should be very low due to the decrease of temperature. Unfortunately data of chemical soil parameters and of microbial activity are unavailable; other studies have investigated winter emissions of nitrous oxide, concluding that these have to be imputed to microbial activity, even with frozen soil (Röver, Heinemeyer, and Kaiser 1998).

Pearson correlation between N_2O emissions, temperature recorded in the corresponding days and the cumulative rainfall recorded in the 72 hours before each measurement, showed a low correlation only for N_2O emission from digestate and rainfall (0.418).

4.3.4. Ammonia cumulated emissions

Results of monitoring of ammonia emissions are extensively described in Chapter 3.

In general, cumulated emissions were not statistically different between treatments with digestate and treatments with chemical fertilizer, respectively 25.6 ± 9.4 kg N Ha^{-1} and 24.8 ± 8.3 kg N Ha^{-1}.

4.3.5. Distribution of N functional genes in soil profile

The presence of genes related to the metabolization and transformation of nitrogen in soil was evaluated for all samples (n = 108). In particular, the number of gene copies for the amoA archea, amoA eubacteria, nifH, nirK, nosZ genes, key genes for the biological reactions of nitrogen transformation in the soil, was quantified.

The complete results of the determination are reported in Table 4.8.

As expected, the greatest microbial activity was concentrated in the agricultural horizon, or in the samples of layers 0-25 and 25-50, where the number of gene copies found is greater than an order of magnitude compared to the deeper layers. Partial exception to this general trend was represented by the number of gene copies of nifH, which tends to be in the order of 10^7-10^8 even in the deeper layers.

The results show that there are essentially no significant differences between the number of gene copies between treatments for all investigated microbial genes, so the addition of digestate has no influence on the balance of the microbial communities of the soil, at least in terms of metabolism. Further investigations could instead highlight a diversification in terms of species.

Table 4.8. Number of gene copies per gram of soil in the experimental plots (mean ± SD, n=3) of four key genes in soil nitrogen cycle. amoA: ammonia mono oxigenase from archaea and eubacteria. nifH: nitrogenase iron protein from eubacteria. nirK: nitrite reductase from eubacteria. nosZ: nitrous oxide reductase from eubacteria. The sampling dates are reported in Table 3. The analyses were carried out at four levels of depth: 0-25, 25-50, 50-75 and 75-100 cm. Letters are referred to One-way ANOVA analysis (Tukey post-test, $p<0.05$). The ANOVA was applied within each sample time separately. Each one compared the gene copies number (for each gene) between the three treatments at the same depth. The triplets of symbols used are: a – b – c; x – y – z; α – β – γ; χ – ψ – ω.

Gene	**Treatment**	**Depth (cm)**	**Gene copies mg soil^{-1}**								
			Pre sown 2019	**Pre topdressing 2019**	**Post topdressing 2019**	**Harvest 2019**	**Pre sown 2020**	**Post sown 2020**	**Pre topdressing 2020**	**Post topdressing 2020**	**Harvest 2020**
amoA (nitrifying archaea)	Unfertilized	0-25	3.51E+07 ± 2.31E+07 (a)	4.69E+07 ± 1.32E+07 (a)	8,26E+07 ± 4,55E+07 (a)	1.16E+08 ± 2.76E+07 (a)	2,12E+08 ± 1,12E+08 (a)	1,10E+08 ± 1,55E+07 (b)	4,66E+08 ± 3,54E+08 (a)	1,78E+09 ± 4,49E+08 (b)	1,78E+09 ± 4,49E+08 (b)
		25-50	4.41E+07 ± 1.32E+07 (y)	4.48E+06 ± 1.62E+06 (x)	7,58E+07 ± 4,06E+07 (x)	1.46E+08 ± 3.60E+07 (y)	1,70E+08 ± 6,72E+07 (x)	1,18E+08 ± 2,17E+07 (xy)	3,04E+08 ± 1,54E+08 (x)	1,24E+08 ± 7,41E+06 (x)	1,24E+08 ± 7,41E+06 (x)
		50-75	1.13E+06 ± 6.04E+05 (α)	0.00E+00 ± 0.00E+00	2,91E+06 ± 1,85E+06 (α)	2.80E+06 ± 1.65E+06 (α)	4,09E+06 ± 3,89E+06 (α)	5,49E+06 ± 1,09E+06 (α)	3,10E+07 ± 2,78E+07 (α)	1,66E+06 ± 0,00E+00 (α)	1,66E+06 ± 0,00E+00 (α)
		75-100	5.59E+06 ± 4.21E+06 (ψ)	0.00E+00 ± 0.00E+00	9,48E+05 ± 0,00E+00 (ψ)	9.06E+06 ± 2.22E+06	4,22E+06 ± 1,06E+06 (χ)	3,38E+05 ± 0,00E+00 (ω)	8,39E+05 ± 5,81E+05 (ψ)	7,75E+05 ± 0,00E+00 (ψ)	7,75E+05 ± 0,00E+00 (ψ)

	Urea	0-25	7.55E+07 ± 5.17E+07 (a)	1.79E+07 ± 6.95E+06 (a)	1,90E+08 ± 1,17E+08 (a)	1.09E+08 ± 2.91E+07 (a)	7,10E+07 ± 1,75E+07 (a)	3,81E+08 ± 1,48E+08 (a)	1,87E+08 ± 8,77E+07 (a)	2,70E+08 ± 2,01E+08 (a)	2,70E+08 ± 2,01E+08 (a)
		25-50	4.11E+07 ± 2.40E+07 (y)	1.39E+07 ± 5.83E+06 (x)	1,05E+08 ± 6,51E+07 (x)	1.51E+07 ± 3.69E+06 (x)	1,13E+08 ± 1,29E+07 (x)	1,32E+07 ± 1,38E+05 (x)	2,21E+08 ± 1,77E+08 (x)	1,49E+08 ± 2,55E+07 (x)	1,49E+08 ± 2,55E+07 (x)
		50-75	1.20E+07 ± 3.68E+06 (β)	0.00E+00 ± 0.00E+00	2,50E+06 ± 5,50E+04 (α)	0.00E+00 ± 0.00E+00	7,69E+06 ± 1,99E+06 (α)	1,52E+07 ± 1,28E+07 (α)	1,36E+07 ± 9,76E+06 (α)	3,05E+07 ± 3,00E+07 (α)	3,05E+07 ± 3,00E+07 (α)
		75-100	2.89E+06 ± 1.13E+06 (χψ)	0.00E+00 ± 0.00E+00	3,52E+07 ± 0,00E+00 (χ)	0.00E+00 ± 0.00E+00	4,22E+06 ± 1,41E+06 (χ)	6,24E+06 ± 0,00E+00 (χ)	3,58E+06 ± 1,10E+06 (χ)	4,48E+06 ± 0,00E+00 (χ)	4,48E+06 ± 0,00E+00 (χ)
	Digestate	0-25	2.65E+08 ± 6.49E+07 (b)	1.77E+08 ± 8.35E+07 (b)	1,21E+08 ± 5,27E+07 (a)	9.08E+07 ± 2.75E+07 (a)	6,62E+07 ± 1,39E+07 (a)	2,48E+08 ± 1,10E+07 (ab)	1,88E+09 ± 5,84E+08 (b)	1,44E+08 ± 3,70E+07 (a)	1,44E+08 ± 3,70E+07 (a)
		25-50	3.85E+06 ± 1.21E+06 (x)	2.10E+08 ± 7.86E+07 (y)	2,73E+07 ± 1,27E+07 (x)	8.83E+06 ± 2.31E+05 (x)	1,50E+08 ± 6,70E+06 (x)	3,88E+08 ± 1,89E+08 (y)	8,07E+08 ± 7,94E+07 (x)	2,12E+08 ± 6,93E+07 (x)	2,12E+08 ± 6,93E+07 (x)
		50-75	4.43E+06 ± 1.62E+06 (α)	0.00E+00 ± 0.00E+00	3,86E+07 ± 3,39E+07 (α)	6.88E+06 ± 5.04E+06 (α)	8,13E+06 ± 5,14E+06 (α)	2,14E+07 ± 5,99E+06 (α)	2,42E+06 ± 7,57E+05 (α)	5,78E+06 ± 5,36E+06 (α)	5,78E+06 ± 5,36E+06 (α)
		75-100	5.40E+05 ± 1.73E+05 (χ)	0.00E+00 ± 0.00E+00	1,87E+07 ± 1,63E+07 (χψ)	0.00E+00 ± 0.00E+00	3,93E+06 ± 2,82E+06 (χ)	2,31E+06 ± 7,81E+05 (ψ)	1,86E+06 ± 6,64E+05 (χψ)	1,71E+06 ± 1,65E+06 (ψ)	1,71E+06 ± 1,65E+06 (ψ)
amoA (nitrifying eubacteria)	Unfertilized	0-25	9.64E+06 ± 6.50E+06 (b)	2.68E+06 ± 1.09E+06 (ab)	2,41E+06 ± 8,78E+05 (a)	1.40E+06 ± 3.67E+05 (a)	6,98E+07 ± 2,60E+07 (a)	5,24E+07 ± 3,74E+07 (a)	2,82E+07 ± 9,98E+06 (b)	1,81E+07 ± 2,51E+06 (a)	1,81E+07 ± 2,51E+06 (a)
		25-50	8.31E+05 ± 3.08E+05 (y)	2.76E+05 ± 1.43E+05 (x)	1,10E+06 ± 5,03E+05 (x)	1.12E+06 ± 2.77E+05 (y)	3,05E+07 ± 9,32E+06 (y)	2,24E+07 ± 1,41E+07 (x)	4,52E+07 ± 1,44E+07 (x)	6,08E+06 ± 4,30E+06 (x)	6,08E+06 ± 4,30E+06 (x)
		50-75	1.28E+05 ± 6.63E+04 (α)	0.00E+00 ± 0.00E+00	2,56E+05 ± 0,00E+00 (α)	7.87E+05 ± 7.99E+04	0,00E+00 ± 0,00E+00	3,11E+07 ± 0,00E+00 (β)	0,00E+00 ± 0,00E+00	0,00E+00 ± 0,00E+00	0,00E+00 ± 0,00E+00
		75-100	6.31E+04 ± 7.64E+03 (χ)	0.00E+00 ± 0.00E+00	0,00E+00 ± 0,00E+00	0.00E+00 ± 0.00E+00	0,00E+00 ± 0,00E+00	0,00E+00 ± 0,00E+00	0,00E+00 ± 0,00E+00	0,00E+00 ± 0,00E+00	0,00E+00 ± 0,00E+00

	Urea	0-25	9.17E+06 ± 2.05E+06 (b)	1.45E+06 ± 6.65E+05 (ab)	5,01E+06 ± 3,77E+06 (a)	9.86E+06 ± 4.32E+06 (b)	5,92E+07 ± 7,71E+06 (a)	7,80E+07 ± 1,85E+06 (a)	2,99E+08 ± 5,47E+07 (a)	2,35E+07 ± 4,01E+06 (a)	2,35E+07 ± 4,01E+06 (a)
		25-50	2.13E+05 ± 1.47E+05 (x)	6.85E+05 ± 3.31E+05 (x)	6,06E+05 ± 3,86E+05 (x)	4.40E+05 ± 1.57E+05 (x)	5,98E+06 ± 3,09E+06 (x)	1,67E+08 ± 1,51E+08 (x)	1,20E+07 ± 1,04E+07 (x)	1,36E+07 ± 1,22E+07 (x)	1,36E+07 ± 1,22E+07 (x)
		50-75	1.17E+05 ± 5.74E+04 (α)	0.00E+00 ± 0.00E+00	2,98E+05 ± 0,00E+00 (α)	0.00E+00 ± 0.00E+00	1,56E+07 ± 0,00E+00 (α)	3,82E+06 ± 0,00E+00 (α)	0,00E+00 ± 0,00E+00	1,91E+06 ± 8,47E+05 (α)	1,91E+06 ± 8,47E+05 (α)
		75-100	2.58E+05 ± 1.61E+05 (χ)	0.00E+00 ± 0.00E+00	7,77E+05 ± 0,00E+00 (χ)	0.00E+00 ± 0.00E+00	0,00E+00 ± 0,00E+00	3,05E+06 ± 0,00E+00 (χ)	0,00E+00 ± 0,00E+00	0,00E+00 ± 0,00E+00	0,00E+00 ± 0,00E+00
	Digestate	0-25	2.70E+06 ± 6.12E+05 (a)	3.82E+06 ± 4.54E+05 (b)	3,02E+06 ± 1,25E+06 (a)	4.63E+06 ± 1.52E+06 (a)	7,63E+07 ± 5,26E+07 (a)	1,09E+08 ± 4,38E+07 (a)	2,30E+08 ± 5,62E+05 (a)	4,18E+07 ± 2,25E+07 (a)	4,18E+07 ± 2,25E+07 (a)
		25-50	1.50E+05 ± 4.93E+04 (x)	1.31E+06 ± 3.05E+05 (y)	6,11E+05 ± 2,64E+05 (x)	0.00E+00 ± 0.00E+00	1,81E+07 ± 6,99E+06 (xy)	5,84E+07 ± 5,19E+07 (x)	7,78E+07 ± 7,45E+07 (x)	4,40E+05 ± 2,63E+05 (x)	4,40E+05 ± 2,63E+05 (x)
		50-75	0.00E+00 ± 0.00E+00	0.00E+00 ± 0.00E+00	4,17E+05 ± 1,70E+05 (α)	0.00E+00 ± 0.00E+00	6,37E+06 ± 0,00E+00 (β)	1,62E+07 ± 1,49E+07 (αβ)	0,00E+00 ± 0,00E+00	7,12E+05 ± 0,00E+00 (α)	7,12E+05 ± 0,00E+00 (α)
		75-100	0.00E+00 ± 0.00E+00	0.00E+00 ± 0.00E+00	7,87E+05 ± 0,00E+00 (χ)	0.00E+00 ± 0.00E+00	0,00E+00 ± 0,00E+00	2,97E+06 ± 1,23E+06 (χ)	0,00E+00 ± 0,00E+00	0,00E+00 ± 0,00E+00	0,00E+00 ± 0,00E+00
nifH (N fixers eubacteria)	Unfertilized	0-25	8.47E+08 ± 3.27E+08 (a)	8.14E+08 ± 1.71E+08 (b)	8,68E+08 ± 3,05E+08 (a)	5.68E+08 ± 1.28E+08 (b)	2,60E+08 ± 9,83E+07 (a)	2,42E+08 ± 1,06E+08 (a)	3,61E+09 ± 2,04E+09 (b)	3,19E+08 ± 7,83E+07 (a)	3,19E+08 ± 7,83E+07 (a)
		25-50	1.00E+09 ± 1.39E+08 (y)	2.57E+08 ± 6.83E+07 (x)	1,36E+09 ± 2,02E+08 (x)	7.46E+08 ± 3.96E+08 (xy)	3,34E+08 ± 1,46E+08 (x)	4,06E+08 ± 5,38E+07 (y)	2,60E+09 ± 1,54E+09 (x)	6,58E+08 ± 1,68E+08 (x)	6,58E+08 ± 1,68E+08 (x)
		50-75	8.42E+07 ± 5.82E+07 (α)	1.23E+07 ± 5.19E+06 (α)	3,91E+08 ± 1,02E+08 (β)	1.39E+08 ± 7.59E+07 (α)	3,90E+07 ± 4,91E+07 (α)	1,58E+08 ± 9,90E+07 (α)	4,16E+09 ± 1,12E+09 (β)	4,16E+09 ± 1,12E+09 (β)	4,16E+09 ± 1,12E+09 (β)

		75-100	3.78E+08 ± 2.04E+08 (ψ)	1.80E+07 ± 4.87E+06 (χ)	2,61E+08 ± 1,12E+08 (ψ)	3.72E+07 ± 8.20E+06 (χ)	5,93E+07 ± 3,98E+07 (χ)	2,74E+07 ± 1,76E+07 (χ)	1,06E+08 ± 5,34E+07 (χ)	1,02E+07 ± 7,55E+06 (χ)	1,02E+07 ± 7,55E+06 (χ)
	Urea	0-25	5.91E+08 ± 2.55E+08 (a)	2.79E+08 ± 8.98E+07 (a)	8,13E+08 ± 3,02E+08 (a)	6.36E+08 ± 1.75E+08 (b)	9,34E+08 ± 6,59E+08 (a)	1,99E+08 ± 1,12E+08 (a)	2,14E+08 ± 1,19E+08 (a)	3,00E+08 ± 2,49E+08 (a)	3,00E+08 ± 2,49E+08 (a)
		25-50	7.43E+08 ± 5.24E+08 (y)	9.73E+08 ± 4.63E+08 (y)	1,24E+09 ± 1,92E+08 (x)	1.22E+09 ± 1.98E+08 (y)	5,71E+08 ± 3,73E+08 (x)	1,84E+08 ± 1,31E+07 (x)	1,26E+09 ± 1,00E+08 (x)	7,06E+08 ± 1,06E+08 (x)	7,06E+08 ± 1,06E+08 (x)
		50-75	1.56E+08 ± 9.25E+07 (α)	1.00E+08 ± 2.66E+07 (β)	6,88E+07 ± 6,68E+07 (α)	3.08E+08 ± 5.93E+07 (β)	2,37E+07 ± 1,29E+07 (α)	8,25E+07 ± 7,41E+07 (α)	9,54E+07 ± 7,79E+07 (α)	9,54E+07 ± 7,79E+07 (α)	9,54E+07 ± 7,79E+07 (α)
		75-100	2.78E+08 ± 1.70E+08 (χψ)	0.00E+00 ± 0.00E+00	1,56E+07 ± 8,16E+06 (χ)	3.57E+07 ± 2.04E+07 (χ)	4,57E+07 ± 2,96E+07 (χ)	2,05E+07 ± 0,00E+00 (χ)	8,85E+07 ± 9,60E+07 (χ)	7,17E+06 ± 5,12E+06 (χ)	7,17E+06 ± 5,12E+06 (χ)
	Digestate	0-25	7.51E+08 ± 2.72E+08 (a)	1.24E+09 ± 2.33E+08 (c)	6,97E+08 ± 3,09E+08 (a)	2.63E+08 ± 7.56E+07 (a)	6,53E+08 ± 4,35E+08 (a)	1,99E+08 ± 5,70E+07 (a)	4,63E+08 ± 2,72E+08 (a)	4,16E+08 ± 2,71E+08 (a)	4,16E+08 ± 2,71E+08 (a)
		25-50	1.08E+08 ± 6.83E+07 (x)	1.39E+09 ± 3.76E+08 (y)	1,46E+09 ± 1,42E+08 (x)	3.16E+08 ± 9.10E+07 (x)	3,96E+08 ± 4,45E+07 (x)	4,83E+08 ± 2,25E+07 (y)	3,21E+08 ± 2,34E+08 (x)	2,21E+07 ± 9,26E+06 (y)	2,21E+07 ± 9,26E+06 (y)
		50-75	7.40E+07 ± 3.10E+07 (α)	1.35E+08 ± 3.54E+07 (β)	1,50E+08 ± 1,07E+08 (αψ)	3.14E+07 ± 1.07E+07 (α)	2,01E+08 ± 3,17E+07 (β)	2,13E+08 ± 8,67E+07 (α)	4,65E+08 ± 4,37E+08 (α)	4,65E+08 ± 4,37E+08 (α)	4,65E+08 ± 4,37E+08 (α)
		75-100	8.99E+07 ± 3.85E+07 (χ)	2.76E+08 ± 5.60E+07 (ψ)	5,01E+07 ± 2,87E+07 (χ)	1.30E+08 ± 3.95E+07 (ψ)	4,23E+07 ± 6,03E+07 (χ)	2,46E+07 ± 1,18E+07 (χ)	1,68E+08 ± 1,45E+08 (χ)	1,14E+07 ± 6,33E+06 (χ)	1,14E+07 ± 6,33E+06 (χ)
nirK (denitrifying eubacteria)	Unfertilized	0-25	1.54E+06 ± 5.87E+05 (a)	4.73E+08 ± 7.58E+07 (a)	6,88E+08 ± 1,43E+08 (b)	2.34E+08 ± 4.33E+07 (a)	4,72E+09 ± 3,14E+09 (a)	1,65E+09 ± 5,16E+08 (a)	4,50E+09 ± 1,56E+09 (a)	2,63E+09 ± 1,66E+09 (a)	2,63E+09 ± 1,66E+09 (a)
		25-50	7.37E+05 ± 4.17E+05 (y)	2.87E+07 ± 6.09E+06 (x)	4,54E+08 ± 3,07E+08 (x)	1.07E+08 ± 2.91E+07 (y)	2,18E+09 ± 1,43E+09 (x)	2,31E+09 ± 9,44E+08 (β)	1,13E+09 ± 1,65E+09 (x)	3,26E+09 ± 1,72E+09 (x)	3,26E+09 ± 1,72E+09 (x)
		50-75	3.45E+04 ± 2.92E+04 (α)	3.42E+06 ± 1.80E+06 (α)	6,42E+06 ± 5,30E+06 (α)	1.56E+07 ± 5.83E+06 (β)	1,34E+07 ± 6,18E+06 (α)	3,11E+07 ± 1,15E+07 (α)	1,06E+08 ± 8,33E+07 (α)	2,32E+06 ± 1,07E+05 (α)	2,32E+06 ± 1,07E+05 (α)

		75-100	3.20E+04 ± 1.72E+04 (ψ)	3.87E+06 ± 2.91E+05 (ψ)	3,05E+06 ± 1,65E+06 (χ)	5.73E+06 ± 3.68E+06 (χ)	2,23E+06 ± 5,05E+05 (χ)	1,37E+06 ± 2,13E+05 (χ)	3,11E+06 ± 1,06E+06 (χ)	9,41E+05 ± 8,57E+05 (χ)	9,41E+05 ± 8,57E+05 (χ)
	Urea	0-25	7.76E+05 ± 4.92E+04 (b)	2.61E+08 ± 3.13E+07 (a)	6,77E+08 ± 7,21E+07 (ab)	4.39E+08 ± 1.01E+08 (b)	9,69E+09 ± 1,06E+09 (a)	1,90E+09 ± 4,94E+08 (a)	3,71E+09 ± 5,91E+08 (a)	2,21E+09 ± 1,51E+09 (a)	2,21E+09 ± 1,51E+09 (a)
		25-50	1.81E+05 ± 1.28E+05 (x)	4.57E+08 ± 2.02E+08 (y)	4,42E+08 ± 1,37E+08 (x)	5.39E+07 ± 1.59E+07 (x)	6,64E+08 ± 2,88E+08 (x)	2,26E+08 ± 1,60E+08 (x)	9,46E+08 ± 7,31E+08 (x)	1,51E+10 ± 9,90E+09 (x)	1,51E+10 ± 9,90E+09 (x)
		50-75	3.00E+04 ± 1.74E+04 (α)	5.13E+06 ± 5.44E+05 (α)	2,76E+06 ± 2,30E+06 (α)	1.64E+06 ± 6.67E+05 (α)	6,25E+07 ± 5,07E+07 (α)	3,05E+07 ± 2,97E+07 (α)	2,25E+07 ± 1,08E+07 (α)	6,29E+07 ± 6,03E+07 (α)	6,29E+07 ± 6,03E+07 (α)
		75-100	2.79E+04 ± 7.64E+03 (χψ)	1.19E+06 ± 5.34E+05 (χ)	5,02E+07 ± 4,33E+07 (χ)	1.06E+06 ± 1.59E+05 (χ)	1,36E+07 ± 1,20E+07 (χ)	1,37E+07 ± 1,30E+07 (χ)	1,02E+07 ± 7,78E+06 (χ)	1,82E+06 ± 3,41E+05 (χ)	1,82E+06 ± 3,41E+05 (χ)
	Digestate	0-25	1.33E+06 ± 3.92E+05 (ab)	1.18E+09 ± 3.03E+08 (b)	3,34E+08 ± 1,71E+08 (a)	3.56E+08 ± 1.22E+08 (b)	4,15E+09 ± 1,93E+09 (a)	3,08E+09 ± 2,12E+09 (a)	4,65E+09 ± 2,38E+09 (a)	3,17E+09 ± 1,53E+09 (a)	3,17E+09 ± 1,53E+09 (a)
		25-50	1.06E+04 ± 5.13E+03 (x)	3.61E+08 ± 6.18E+07 (y)	7,31E+07 ± 2,47E+07 (x)	8.73E+07 ± 1.35E+07 (xy)	2,45E+09 ± 1,00E+09 (x)	5,27E+08 ± 3,26E+08 (y)	4,20E+09 ± 3,94E+08 (y)	4,19E+06 ± 4,41E+05 (x)	4,19E+06 ± 4,41E+05 (x)
		50-75	2.90E+04 ± 2.00E+04 (α)	3.41E+06 ± 1.27E+06 (α)	4,45E+06 ± 1,79E+06 (α)	2.04E+06 ± 7.78E+05 (α)	2,89E+07 ± 9,00E+06 (α)	1,93E+08 ± 1,02E+08 (β)	3,61E+06 ± 3,49E+05 (α)	3,74E+06 ± 1,74E+06 (α)	3,74E+06 ± 1,74E+06 (α)
		75-100	1.36E+04 ± 4.54E+03 (χ)	1.07E+06 ± 2.55E+05 (χ)	1,95E+06 ± 7,01E+05 (χ)	1.49E+06 ± 2.06E+05 (χ)	1,28E+07 ± 1,08E+07 (χ)	7,12E+07 ± 3,32E+07 (ψ)	1,30E+07 ± 8,50E+06 (χ)	2,70E+06 ± 1,94E+06 (χ)	2,70E+06 ± 1,94E+06 (χ)
nosZ (denitrifying eubacteria)	Unfertilized	0-25	2.08E+07 ± 1.01E+07 (a)	6.61E+07 ± 2.80E+07 (a)	4,23E+07 ± 1,03E+07 (a)	2.34E+07 ± 7.99E+06 (a)	2,33E+08 ± 4,09E+07 (a)	2,63E+08 ± 1,29E+08 (a)	4,68E+08 ± 3,40E+08 (a)	1,51E+08 ± 1,31E+08 (a)	1,51E+08 ± 1,31E+08 (a)
		25-50	2.31E+07 ± 1.31E+07 (y)	8.74E+06 ± 3.09E+06 (x)	2,75E+07 ± 1,27E+07 (x)	2.21E+07 ± 1.45E+07 (y)	5,00E+07 ± 8,00E+04 (y)	1,77E+08 ± 1,36E+08 (x)	2,01E+08 ± 1,63E+08 (x)	1,02E+08 ± 8,77E+07 (x)	1,02E+08 ± 8,77E+07 (x)

	50-75	6.43E+05 ± 4.27E+05 (α)	2.15E+06 ± 1.32E+06 (α)	2,28E+06 ± 1,04E+06 (β)	5.24E+06 ± 2.31E+06 (β)	1,95E+06 ± 1,09E+06 (α)	9,12E+06 ± 5,51E+06 (α)	4,30E+07 ± 1,91E+07 (α)	6,41E+05 ± 3,34E+05 (α)	6,41E+05 ± 3,34E+05 (α)
	75-100	7.21E+05 ± 4.77E+05 (χ)	0.00E+00 ± 0.00E+00	1,62E+05 ± 1,41E+05 (χ)	6.93E+05 ± 1.75E+05 (ψ)	4,32E+06 ± 2,64E+06 (χ)	3,28E+05 ± 1,99E+05 (χ)	5,67E+05 ± 2,80E+05 (χ)	6,87E+04 ± 1,67E+04 (χ)	6,87E+04 ± 1,67E+04 (χ)
Urea	0-25	8.30E+07 ± 8.73E+06 (b)	6.71E+07 ± 2.24E+07 (a)	4,12E+07 ± 8,91E+05 (a)	5.90E+07 ± 1.43E+07 (b)	1,25E+09 ± 4,34E+08 (b)	4,00E+08 ± 9,62E+07 (a)	7,27E+08 ± 4,17E+08 (a)	1,90E+08 ± 3,13E+07 (a)	1,90E+08 ± 3,13E+07 (a)
	25-50	1.75E+06 ± 9.09E+05 (x)	2.89E+07 ± 1.55E+07 (y)	3,53E+07 ± 7,25E+06 (x)	1.39E+07 ± 4.46E+06 (xy)	1,65E+08 ± 4,94E+07 (xy)	4,68E+08 ± 4,22E+08 (x)	4,43E+07 ± 3,16E+07 (x)	3,49E+08 ± 3,21E+08 (x)	3,49E+08 ± 3,21E+08 (x)
	50-75	1.05E+06 ± 6.56E+05 (α)	2.58E+06 ± 2.45E+05 (α)	7,46E+05 ± 8,35E+04 (αβ)	3.13E+06 ± 8.77E+05 (αβ)	6,88E+06 ± 4,05E+06 (α)	2,16E+07 ± 1,91E+07 (α)	1,27E+06 ± 8,63E+05 (α)	7,42E+06 ± 6,72E+06 (α)	7,42E+06 ± 6,72E+06 (α)
	75-100	1.63E+05 ± 2.82E+04 (χ)	6.73E+04 ± 2.35E+04 (χ)	2,70E+04 ± 0,00E+00 (χ)	1.57E+05 ± 8.27E+04 (χ)	1,28E+07 ± 1,73E+07 (χ)	1,17E+07 ± 0,00E+00 (ψ)	5,02E+06 ± 3,24E+05 (ψ)	4,92E+06 ± 3,45E+06 (χ)	4,92E+06 ± 3,45E+06 (χ)
Digestate	0-25	1.09E+07 ± 1.71E+06 (a)	7.80E+07 ± 1.55E+07 (a)	4,51E+07 ± 1,12E+07 (a)	4.26E+07 ± 1.30E+07 (ab)	4,19E+08 ± 1,13E+08 (a)	6,56E+08 ± 3,40E+08 (a)	5,33E+08 ± 2,98E+08 (a)	7,45E+07 ± 1,17E+07 (a)	7,45E+07 ± 1,17E+07 (a)
	25-50	1.12E+06 ± 5.51E+05 (x)	2.17E+07 ± 5.21E+06 (xy)	5,26E+06 ± 1,75E+06 (y)	2.60E+06 ± 1.07E+06 (x)	2,43E+08 ± 1,07E+08 (x)	4,16E+07 ± 1,95E+07 (x)	1,07E+08 ± 1,03E+08 (x)	1,58E+06 ± 1,86E+04 (x)	1,58E+06 ± 1,86E+04 (x)
	50-75	1.61E+05 ± 1.99E+04 (α)	2.04E+06 ± 4.06E+05 (α)	4,01E+05 ± 2,70E+05 (α)	6.14E+05 ± 2.22E+05 (α)	2,65E+07 ± 2,98E+07 (α)	4,56E+06 ± 2,97E+06 (α)	4,90E+07 ± 4,65E+07 (α)	4,66E+05 ± 1,65E+05 (α)	4,66E+05 ± 1,65E+05 (α)
	75-100	2.24E+05 ± 1.26E+05 (χ)	1.49E+05 ± 3.11E+04 (ψ)	3,21E+04 ± 0,00E+00 (χ)	8.80E+04 ± 1.45E+04 (χ)	3,67E+05 ± 2,93E+04 (χ)	4,02E+06 ± 2,64E+06 (χ)	1,97E+06 ± 1,56E+06 (χ)	9,56E+05 ± 4,86E+05 (χ)	9,56E+05 ± 4,86E+05 (χ)

4.4. Conclusions

Although considerable amounts of ammonium were added with urea and digestate (185 kg N ha^{-1} urea and 229 kg N ha^{-1} digestate), no significant differences in the concentrations of this element were obtained between the different treatments. Likewise, the quantification of nitrate concentration did not show a significant difference between the two treatments; in particular with regard to the concentrations in the deeper soil layers. In carrying out this study, the concentrations of nitrates dissolved in the water were not measured, for example by means of lysimeters, but only the concentrations present in the circulating solution of the sampled soil. An absolute quantification of concentrations is therefore not possible, and it is certainly an aspect to be investigated with further studies and monitoring. Also with regard to nitrous oxide and ammonia emissions, the behavior of the plots fertilized with digestate was similar to that of the plots fertilized with urea.

Finally, observing the bacterial quantification data, it was possible to state that the addition of digestate has no influence on the balance of soil microbial communities, at least in terms of metabolism. Further investigations could instead highlight a diversification in terms of species.

Digestate is therefore not different from a synthetic fertilizer as regards the nitrogen content brought to soil, and does not involve greater risks than synthetic fertilizers as regards the alterations of the nitrogen cycle and the losses of this element in the different environmental compartments.

5. 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.

5.1. 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; I. 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 (Shen 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; 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.

5.2. Materials and Methods

5.2.1. 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 ammonia sulphate).

5.2.2. System description

5.2.2.1. 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 5.1a.).

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 5.1a.) 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.

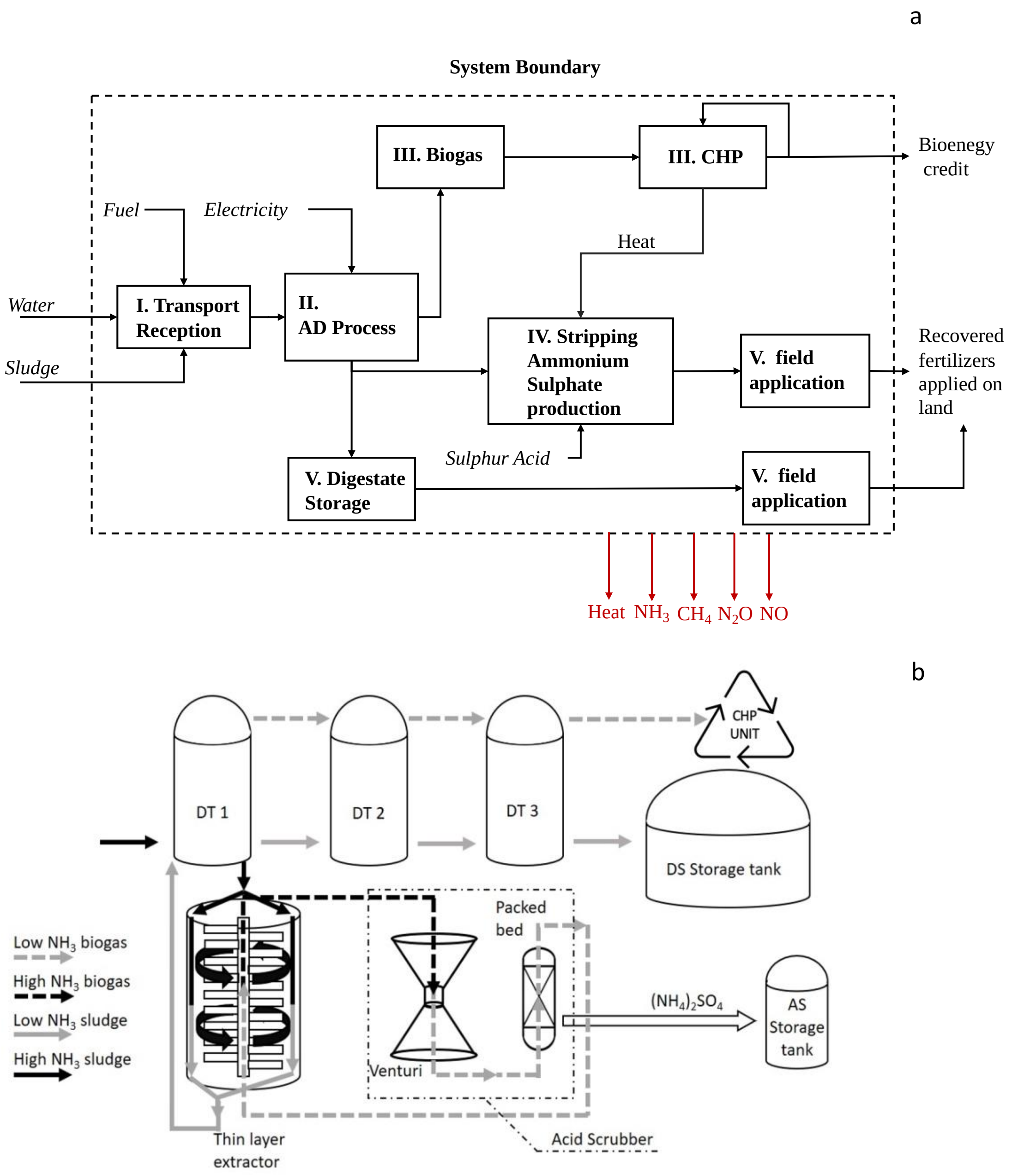


Figure 5.1. AD plant and N-stripping unit layouts (a); system boundaries and main processes for the Recovered Fertilizers (RF) (b).

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).

5.2.2.2. Recovered fertilizers produced

Recovered fertilizers (renewable fertilizers) characteristics can be found in Pigoli et al. (2021). The previous characterization made also included organic contaminants and target emerging organic contaminants (see Table 2.2.).

5.2.3. 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 Table 2.1. and summarised in Table 5.1.

5.2.3.1. 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 (Tang, Cape, and Sutton 2001; Riva et al. 2016). 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.

5.2.4. System boundaries and data inventory

5.2.4.1. System boundaries

The system boundary starts from the organic waste collection and transport, encompasses the production of digestate/bio-fertilizer and ammonia sulphate, the correlated processes for producing biogas which is transformed into electric energy and thermal energy and finally the use of the digestate in the field. The system boundary was represented by the dashed line in Figure 5.1b. 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 5.1., 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 5.1.). 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 (Xu et al. 2013; 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 Table 2.2.).

5.2.4.2. 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 5.1.).

5.2.4.3. 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 5.1. 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$_2$O)	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$_2$O)	kg ha^{-1}	9[a]	Detected on-site by the authors (Chapter 4)
Indirect dinitrogen monoxide (N-N$_2$O)	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 1 ha 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 1 ha 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.

5.2.5. 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).

5.3. 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 5.2. 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 5.2.). Analysis and contributions of the processes to the categories are discussed below.

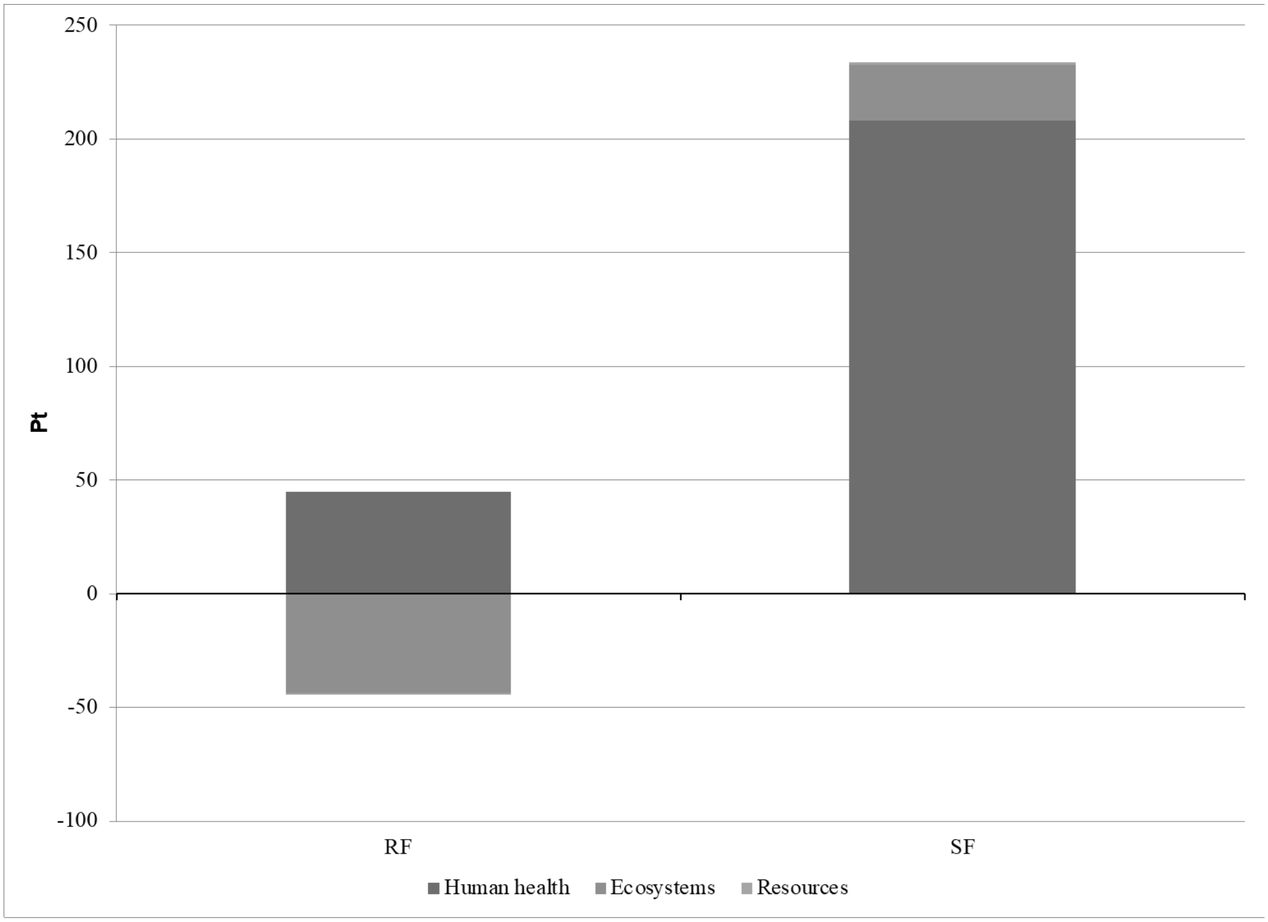


Figure 5.2. Comparative environmental results for Scenarios RS and SF. Impacts assessment calculated according to ReCiPe 2016 endpoint (H) V 1.03 method.

5.3.1. 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 kg$CO_{2eq.}$, 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 5.2.). 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 5.2. 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: 1 ha 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} (Table 2.2.) 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) (Table 2.2.), 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) 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 5.2.). 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 5.3a.). 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.

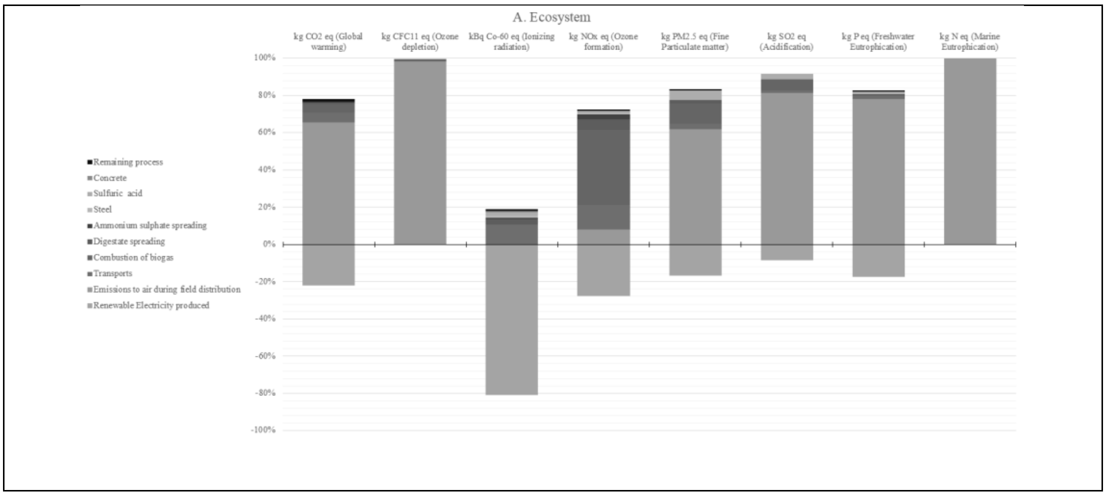
Impact due to *Fine particulate matter formation* was almost identical for the two scenarios (Table 5.2.). This result was because this impact was generated mostly by the ammonia emissions during field fertilization, which was similar for the two Scenarios investigated (Chapter 3). Particulate matter 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.

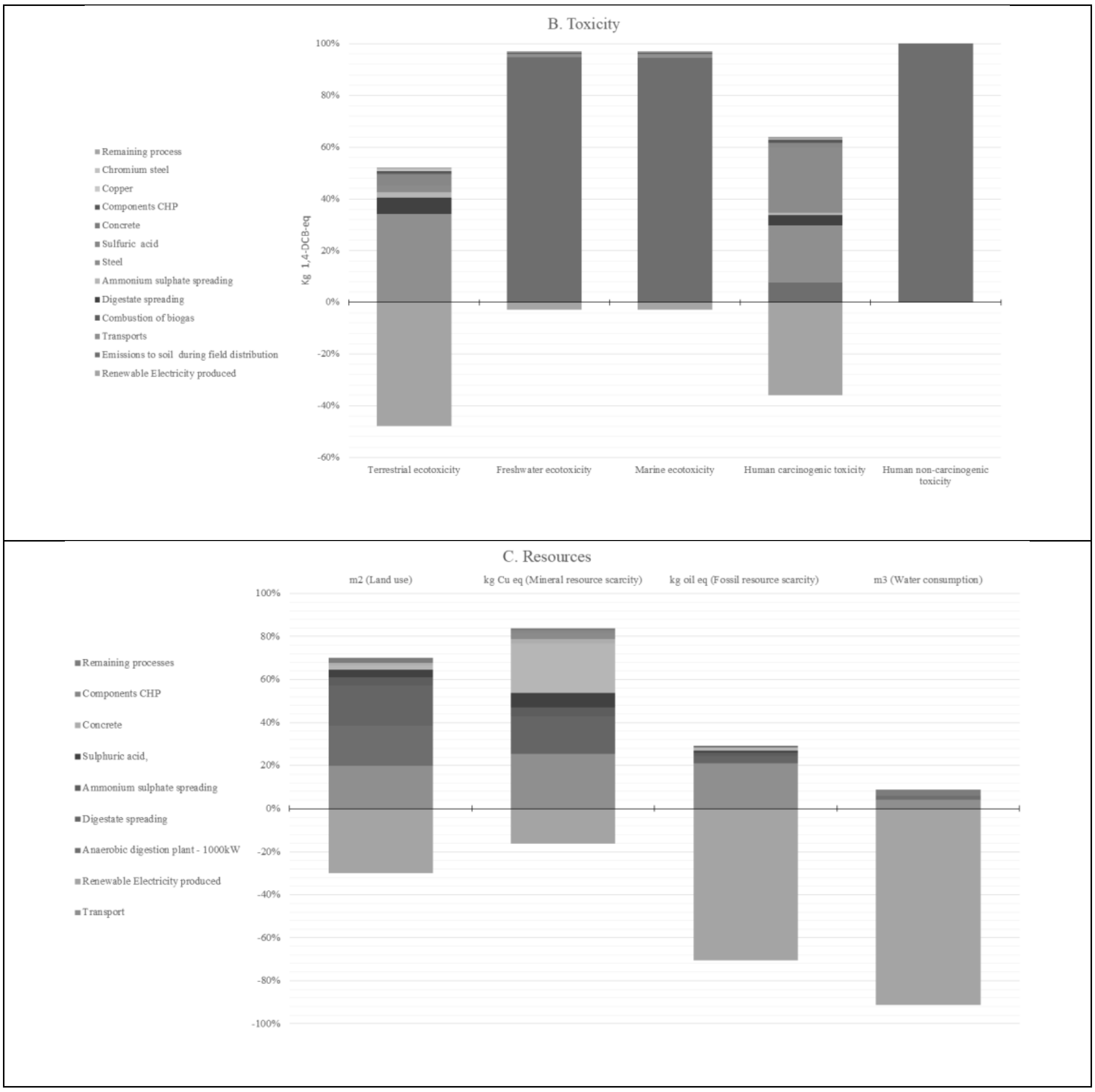
Terrestrial acidification which is related to nutrients supplied, i.e. deposition of ammonia, nitrogen oxides and sulphur dioxide in acidifying forms, displayed similar values for the Scenario RF and Scenario SF (Table 5.2.). Scenario RF had a slightly higher impact due to fertilizers distribution because 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 (Table 2.2.). 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.

Figure 5.3. Process contribution to impact categories of Scenario RF, focusing on the ecosystem (a), toxicity (b) and resources (c). Impacts assessment were calculated according to ReCiPe 2016 midpoint (H) V 1.03 method





5.3.2. Midpoint results of impact categories related to human health protection

The inclusion of toxicity categories (USEtox) (Table 5.2.) 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 5.2.); in particular, for Scenario RF, the impact was due above all to the transport of sludge to the AD plant (Figure 5.3b.), 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 5.3b.).

5.3.3. Midpoint results of impact categories related to Resources scarcity protection

The use of both renewable energy (biogas) and recovered material (sewage sludge) to produce fertilizers (digestate and ammonia sulphate) led, also, to high efficiency in terms of *Land use*, *Mineral resource use*, *Fossil resources*, reducing, until negative, these impacts (Table 5.2.).

5.3.4. 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 5.2.). 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).

5.3.5. 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 5.3.), 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 5.3a. and 5.3b.). 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 5.2. and Figure 5.2.), confirmed this virtuous approach. Nevertheless, already stated, LCA can help in optimize processes, further reducing impact.

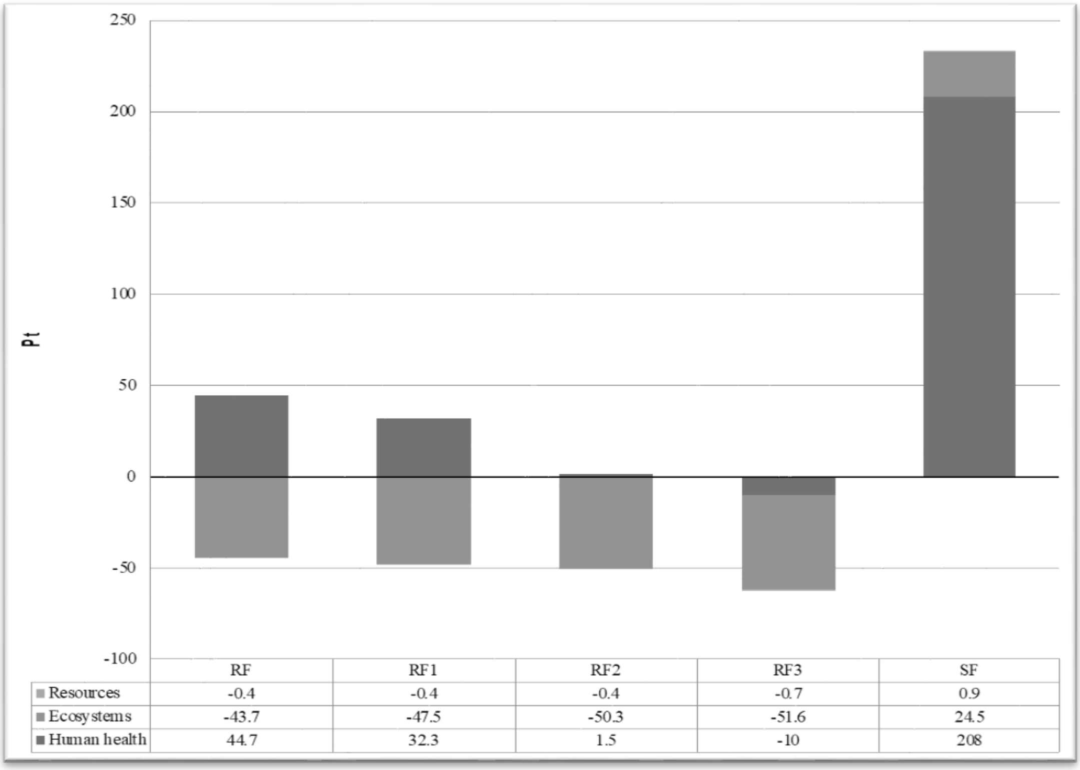
Nitrogen dioxide emissions have been reported to be greatly reduced by using nitrification inhibitors (NI) (Menéndez et al. 2012; Herr et al. 2020). From the literature, it was calculated, on average, that the use of NI allowed a reduction of 44% in total N_2O emissions (Qiao et al. 2015), further reducing total Scenario RF impacts (Scenario RF_1), with reference to Ecosystem and Human Health impacts (Figure 5.4.), 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 5.4. (Scenario RF_2).

Another important activity that plays an important role in determining impact is transport. Transport affected a lot the *Terrestrial Ecotoxicity* (Figure 5.3b.) and, although much less severely, many other sub-categories within *Ecosystem* and *Resources* categories (Figure 5.3a. and 5.3c.), 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 5.1.) 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 5.4.).

Figure 5.4. 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.



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

5.4. 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.

6. General conclusions

The need to meet an increased food demand due to world population growth, has led to intensive use of chemical fertilizers and pesticides, monoculture cropping, and, in general, the adoption of an inappropriate agricultural management. The exponential increase of nitrogen fertilization in the past four decades, allowed the doubling of world food production, but also has lead to various environmental problems. Indeed, has been estimated that only half of nitrogen supplied to crops is actually absorbed by plants, while the remaining part leaves the soil in different forms, such as nitrates in groundwater, ammonia and nitrous oxide emissions in atmosphere, all of which contributes to environmental pollution, with a relevant impact on human health and climate change. A transition towards a more sustainable model of agriculture is necessary, shifting from a model of linear economy to a circular economy view, where nutrients are recovered from organic wastes, valorised, and reused as crop fertilizers.

In such sense, Anaerobic Digestion has proven to be a mature technology, capable of valorizing organic wastes, recovering the nutritional elements contained in them, leading to the production of highly efficient nitrogen fertilizers with soil improver properties.

The simultaneous production of biogas, which can be converted into electricity, determines further advantages from an economic and, above all, environmental point of view.

Application of digestate as crop fertilizer showed no negative impacts on soil quality, nor on the accumulation of organic and inorganic pollutants. Instead, content of organic carbon in soil seems to increase after repeated use of digestate, confirming its characteristics of soil amendment.

Nitrogen applied via organic fertilization with digestate is efficiently used by crops, as demonstrated by the yields of maize grain, which were equivalent to those obtained using a similar dose of urea N from a synthetic fertilizer, with very similar fertilizer use efficiencies (FUE).

Digestate fertilization seems to have not a higher environmental impact in terms of GHG and ammonia emissions, compared to conventional chemical fertilization. This can be mainly imputed to the use of soil injection application technique, which reduces ammonia emission, down to levels comparable to those typical of surface fertilization with urea. In addition, for what concerns the emission of odour, it has been observed that with digestate injection, emission was reduced to a level that was no longer distinguishable from that of non-fertilized soil. Concentrations of nitrate in deep soil layers seems to be equal between digestate fertilization and chemical fertilization, indicating that there are no differences for what concerns losses of nitrogen by leaching. The addition of digestate seems to have no influence on the balance of soil microbial communities, at least in terms of metabolism.

The value of digestate as a substitute for synthetic fertilizers is also confirmed by the LCA analysis, which indicates that producing and using renewable fertilizers led to a strong environmental impact reduction, compared to producing and using synthetic fertilizers. LCA approach also confirmed the importance of assuring high biological stability of digestate, and using it at the right time and according to crop requirements, as well as the distribution by injection, in order to avoid losing all the advantages because of impacts derived from their incorrect use.

In conclusion, anaerobic digestion plus liquid digestate injection is confirmed as a good practice to provide a suitable fertilizer, replacing the synthetic fertilizer in an environmentally sustainable way. Nevertheless, is of fundamental importance the correct management of digestate production parameters, in order to obtain a product with a high stability. In fact, all 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), therefore constituting a valid bio-based fertilizer, that can replace mineral N fertilizers, without loss of performance or environmental risks.

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