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

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## Abstract

Recovered fertilizers (a highly stabilized digestate and ammonium sulphate) obtained from anaerobic digestion of sewage sludge, were used on plot trials with a maize crop, in a comparison with synthetic fertilizers. After three consecutive cropping seasons, the soils fertilized with the recovered fertilizers (RF), compared to those fertilized with synthetic fertilizers (SF), did not show significant differences either in their chemical characteristics or in the accumulation of inorganic and organic pollutants (POPs). The RF ensured an ammonia N availability in the soil equal to that of the soil fertilized with SF, during the whole period of the experiment. Furthermore, no risks of N leaching were detected, and the use of RF did not result in a greater emission of ammonia or greenhouse gases than the use of SF. The agronomic results obtained using RF were equivalent to those obtained with SF (fertilizer use efficiency of 85.3 ± 10 and 93.6 ± 4.4% for RF and SF respectively). The data show that utilising a very stable digestate can be a good strategy to produce a bio-based fertilizer with similar performance to that of a synthetic fertilizer, without environmental risks.

**Keywords:**

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

## 1. Introduction

During the second half of the twentieth century, in particular since the late 1960s, agriculture throughout the world underwent radical improvements, which overall have been defined as the "green revolution". The direct consequence of these improvements in the succeeding decades was a dramatic growth in agricultural yields which increased by up to 125% between 1966 and 2000 (Khush, 2001). This new availability of calories supported economic development in many areas of the world, allowing populations to grow without increasing the cultivated areas, thus also safeguarding forests and natural lands (Foley et al., 2011).

One of the main improvements introduced by the green revolution to agriculture was the use of large amounts of synthetic fertilizers to provide nutrients to crops (Pingali, 2012). From the late 1960s to the present day, the use of synthetic fertilizers in the world increased by 500%, and included an 800% increase in the use of nitrogen (N) fertilizers (FAOSTAT, 2021) but it is evident that this high usage is becoming progressively less sustainable. The amount of N fertilizers produced on a global scale rose from 12 TgN in 1960 to 104 TgN in 2010, with an expected increase of 2.3% per year in the near future. This amount now contributes to 45% of the total nitrogen fixed annually on the planet, effectively causing strong imbalances in the natural nitrogen cycle, with harmful consequences for terrestrial and aquatic ecosystems (Fowler et al., 2013; Galloway et al., 2014). Almost all the N fertilizers are produced by fixing atmospheric N into ammonia, through a process devised in 1909 by Fritz Haber and Carl Bosch (the so-called Haber-Bosch process), which today is considered one of the most energy-consuming industrial processes on a global scale, responsible for 1.2% of the annual anthropogenic $CO_2$ emissions (Smith et al., 2020).

Phosphorus-based fertilizers are no less problematic. Essentially, all the phosphorus (P) used to produce fertilizers derives from mineral deposits that are located in a few areas of the planet, and these are limited (Ridder et al., 2012). Current reserves of phosphate minerals are estimated at 67,000 TgP and about 75% of them are located in Morocco (West Africa). China and the US also have significant reserves, but these are considered strategic resources and are therefore not sold on the global market. Recent estimates of the extraction rate (Geissler et al., 2019) quantify the annual amount of phosphate minerals extracted in the world at 255 million metric tons (MMT), and the projections foresee an increase of 50-100% by 2050. The peak of phosphorus extraction, i.e., the point after which the annual extractable amount will no longer be able to increase is difficult to predict, and there is no univocal consensus in the literature. However, in accordance with numerous works it is expected for 2030, and the depletion of global reserves is likely before the end of the XXI century (Cordell et al., 2009; Schoumans et al., 2015). The limited reserves of phosphorus available, as well as the fact that it is considered a strategic resource because it is crucial for agriculture, exposes its price on the global market to strong and unpredictable fluctuations, which are also linked to geopolitical conditions, as already happened in 2008: this in turn affects the cost of food (Cordell and White, 2011). Many nations, including those comprising the European Union, which do not possess significant reserves of phosphate minerals within their borders, are particularly exposed to these risks.

On the other hand, these same nutrients (N and P) are generally present in large amounts in the wastewater and organic wastes from the food production industry, which includes agriculture and livestock. Paradoxically, these waste biomasses are a problem because their uncontrolled dispersion into the environment, together with the excessive use of synthetic fertilizers in agriculture, causes an excess of nutrients in soils and waters in many areas of the planet, with serious consequences for ecosystems and the balancing of biogeochemical cycles on a global scale (Rockström et al., 2009; Steffen et al., 2015; Toop et al., 2017). 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 et al., 2001; Smith et al., 1999). Nitrogen, in the form of nitrate ($NO_3^-$), is leached within the soil until it reaches the groundwater, often destined for human consumption, leading to public health problems (Espejo-Herrera et al., 2016; Padilla et al., 2018).

Using organic wastes as fertilizers (recovered fertilizers) in agriculture to replace synthetic fertilizers would therefore represent a solution to these problems, reducing the dispersion of nutrients in the environment, and it would also constitute an interesting model of a circular economy (van Dijk et al., 2016). However, untreated organic wastes do not represent acceptable fertilizers (Westerman and Bicudo, 2005) because of their origin and therefore intrinsic risks for potential contamination: in fact they can contain pathogens, heavy metals, or organic pollutants such as antibiotics or drug residues that would accumulate in agricultural soils, endangering the safety of food production and consumption (Albihn, 2002; Berendsen et al., 2015; Gros et al., 2019; Van den Meersche et al., 2016; Zwolak et al., 2019). Furthermore, their nutrient content and plant availability are difficult to control, so that they are not often able to replace synthetic mineral fertilizers. Finally, they are often rich in water, which makes their management difficult and expensive both from an economic and environmental point of view, because of the $CO_2$ emissions associated with the transport of large volumes (Westerman and Bicudo, 2005). To transform these biomasses into products which can be utilised in agriculture, technological/biotechnological treatments are therefore necessary (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 (Pigoli et al., 2021; Tambone et al., 2019; Verdi et al., 2019; Zilio et al., 2021).

However, the possibility of using digestate in agriculture to replace synthetic fertilizers is still debated, especially as regards the possible environmental impacts. The high concentration of nutrients contained in these biomasses, which is useful for plant nutrition, can cause leaching of N and P in the soil with consequent water pollution, and also lead to emissions of ammonia and greenhouse gases ($N_2O$) into the atmosphere (Cameron et al., 2013; Delgado, 2002). Furthermore, originating from organic wastewater and organic wastes, digestate also has the same problems of contamination by heavy metals, pathogens and organic pollutants typical of these biomasses, which could therefore pollute the soil (Govasmark et al., 2011). Although progressively more studies in recent years are shedding additional light on the safety of digestates for agricultural use, as well as on the best process methods and accurate selection of feedstock, there are still only very limited data available on the impacts that the use of these biomasses have on full scale agriculture in the field (Koszel and Lorencowicz, 2015; Pigoli et al., 2021; Verdi et al., 2019; Zilio et al., 2021).

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

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

## 2. Material and Methods

### *2.1 Agronomic full field trials*

Fertilization date, fertilizers used, doses applied, and spreading methodology for the different treatments are summarized in Table 1. Agronomic trials tested, at plot scale, the fertilizer properties of recovered fertilizers (digestate from organic wastes combined with digestate-derived ammonium sulphate) vs. synthetic fertilizers; an unfertilized treatment was included as control. Fertilizers were tested on plots of 350 $m^2$ (width 5.9 m, length 60 m) cropped with maize (*Zea mays* L.; hybrid Pioneer P1547, FAO 600), in triplicate, using a randomized experimental scheme and following the standard agronomic procedures used in the Po Valley (northern Italy), where the experimental fields were located. The arrangement of the plots is shown in Figure S1.

Digestate was distributed at pre-sowing by injection into the soil at a depth of 15 cm by using a tank car joined to a rigid multi-anchor-subsoiler coupled with a Retrofit Variable-Rate Control (VRT control). Digestate was dosed adopting an N efficiency of 0.5, as suggested by the Regional Plan for Water Protection from Nitrate from Agriculture (Regione Lombardia, 2020). Efficiency suggested by regional rules takes into consideration the content of readily available N (ammonium) that roughly coincides with digestate ammonium content used in this work, since the organic fraction is not considered to be readily available for plant nutrition. Nitrogen fertilization was completed by using ammonium sulphate produced starting from digestate (Pigoli et al., 2021) in topdressing by fertigation ($N_{tot}$ dosed: 370 kgN $ha^{-1}$ as digestate and 100 kgN $ha^{-1}$ as ammonium sulphate; $P_{tot}$ dosed: 134 kgP $ha^{-1}$ as digestate; $K_{tot}$ dosed: 24.1 kg K $ha^{-1}$ as digestate and 44.82 kg K $ha^{-1}$ as $K_2O$).

Synthetic fertilizers were spread by using routine procedures. Urea was used at pre-sowing and commercial ammonium sulphate as a top dressing ($N_{tot}$ dosed: 185 kgN $ha^{-1}$ as urea and 100 kgN $ha^{-1}$ as ammonium sulphate; $P_{tot}$ dosed: 39.3 kgP $ha^{-1}$ as 0/46/0 complex; $K_{tot}$ dosed: 69.4 kg K $ha^{-1}$ as KCl). Digestate-derived ammonium sulphate and commercial ammonium sulphate have been considered equivalent. Regarding urea, it was dosed all in a single spread in order to standardize N fertilization with that of plots treated with digestate. Finally, the unfertilized plots (U) did not receive any type of fertilization for the entire duration of the experiment.

### *2.2 Fertilizer sampling and analysis*

The digestate used in this work derives from anaerobic digestion of sewage sludge, while ammonium sulphate used in RF treatment was obtained by ammonia stripping during the same anaerobic digestion process. The details of the process to produce fertilizers and their characteristics have been widely described and discussed in previous work (Pigoli et al., 2021; Zilio et al., 2021); fertilizer data are reported in Tables S1 and S2. The digestate in particular showed a high biological stability (BMP: 89 ± 17 $L_{biogas}$ $kg^{-1}$ dw) compared to other agricultural digestates (Pigoli et al., 2021). The N (75 ± 5 g $kg^{-1}$ dw, TAN/TKN: 57.8%) and P (32.3 ± 1.7 g $kg^{-1}$ dw) total contents, were significantly higher than those reported in literature for agricultural digestates (Wang et al., 2021; Xu et al., 2020; Yun et al., 2021), while the total K content (5.73 ± 0.5 g $kg^{-1}$ dw) was slightly below (Wang et al., 2021; Xu et al., 2020; Yun et al., 2021). Ammonium sulphate showed a high total N content (74 ± 2 g $kg^{-1}$ on wet weight, TAN/TKN: 97%) and low concentration of heavy metals.

The digestates used in this work were sampled immediately before they were injected in the field and the analyses took place in the hours immediately following sampling. pH was determined in aqueous solution using a 1:2.5 sample/water ratio. Total solids (TS) and total organic carbon (TOC) determinations were carried out following standard procedures of the American Public Health Association (APHA, 1992). Total nitrogen (TKN) and ammonia nitrogen (TAN) were determined according to the analytical method for wastewater sludges (IRSA CNR, 1994). Heavy metals, total P and K content was assessed by inductively coupled plasma mass spectrometry (Varian, Fort Collins, USA), preceded by acid digestion (EPA, 1998) of the samples. All the analyses were carried out in triplicate. Biochemical methane production (BMP) was determined following the biological method reported in Schievano et al. (2008), according also to the European regulations for fertilizers (EU, 2019). Nevertheless, physical methods such as thermal analysis can also be proposed (Li et al., 2021; Xing et al., 2021).

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

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

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

### *2.3 Soil sampling and analysis*

The soils studied in this work were sampled just before the fertilization in March 2018 by taking three random samples (each one made by 3 sub-samples) at 0-20 cm. After three years, the soil was sampled again in March 2021, maintaining the same sampling procedure, taking three random samples/plot. Samples were air dried, sieved to 2 mm and then ground to 0.5 mm. The main characteristics of soils are reported in Table 2. Soil pH was determined in aqueous solution using a 1:2.5 sample/water ratio (McLean, 1982), and texture by the pipette method (Gee and Bauder, 1986). Cation Exchange Capacity (CEC) was determined by saturating the samples with $BaCl_2$ (Rhoades, 1982), texture by the pipette method (Gee and Bauder, 1986), total organic carbon by the Walkley and Black method (Olsen et al., 1982) and total nitrogen by the Kjeldahl method (Faithfull, 2002).

All the analyses were carried out in triplicate. Total P and K contents were determined using the same method used for fertilizers analysis (see section 2.2.).

Potential nitrate leaching was assessed by the detection of nitrate presence at 1 m soil depth ($N-NO_3$) in soils. Sampling consisted in the withdrawal of soil cylinders up to a depth of one meter. For each of the experimental plots three soil cylinders were sampled randomly. Each soil cylinder was divided into 4 sub-samples, each of 25 cm, corresponding to 0-25, 25-50, 50-75 and 75-100 cm layers in soil profile. In total eight sampling campaigns were carried out during in the period 2019-2020, i.e., three samplings in 2019 (before pre-sowing fertilization, at the moment of maximum mineralization of N added to the soil and at harvest). In 2020, in order to consider, also, nitrate leaching between pre-sowing fertilization and the maximum mineralization and after topdressing fertilization, two further samplings were added for a total of five. Details on sampling dates and agronomic operations are reported in Table S3. The collected soil was brought immediately (the same day) to the lab and analysed immediately. In particular, the nitrate concentration was determined by Kjeldahl distillation, using Devarda's alloy (Faithfull, 2002).

Inorganic and organic pollutants were detected at the start and the end of the trial; in particular, heavy metals (HV) were determined by the method already reported for fertilizers (see section 2.2). The determination of the organic pollutants in the soils was carried out using the following methods: PCDD/PCDF + PCB DL: UNI EN 16167:2012, AOX: UNI EN ISO 22155:2016, Hydrocarbon C10-C40: ISO 16703:2004, Toluene: UNI EN ISO 22155:2016, Phenols: ASTM D7485-16, DEHP: EPA 3510C 1996 + EPA 8270E 2017.

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

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

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

Where:

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

A complete list of samplings and agronomic operations carried out is reported in Table S3.

### *2.4 Ammonia and Greenhouse gas (GHG) emissions measurement*

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

GHG fluxes ($N_2O$, $CH_4$ and $CO_2$) were measured from 28/05/2020 to 17/03/2021 (a detailed calendar of the sampling dates is reported in Table S4) using the closed static chambers method (Bertora et al., 2018). Anchors were inserted into the soil (three for each plot) up to a depth of 20 cm, to isolate the soil column. The chambers were placed on the surface of the soil above the columns and closed with a lid. The air inside the chambers was sampled and analysed in the laboratory through gas chromatography (Piccini et al., 2017).

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

Measured environmental temperature has been exploited to correct gas concentrations for each sampling date. Since insulated metal chambers were used, increase in temperature inside the chamber over the deployment time was negligible (Bertora et al., 2018). The emissive flow of the gas from the soil was estimated using the following general equation:

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

where $F$ is the flow, $H$ is the ratio between the air volume and the soil surface isolated from the chamber, corresponding to the height of the chamber (m), and $t$ is the time the chamber remains closed.

If the increase in GHG concentration inside the chamber was linear, the $dC/dt$ ratio was obtained by linear regression between concentrations and sampling times. In case of non-linear accumulation, the HM model was applied (Peyron et al., 2016). Finally, the cumulative emissions were obtained by estimating the flows in the non-sampling days, by means of linear interpolation.

### *2.5 Maize yield quantification and element content analysis*

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

Inorganic pollutant contents in grain (i.e., As, Cd, Hg, Cr, Ni, Pb, Cu and Zn) were assessed by inductively coupled plasma mass spectrometry (Varian, Fort Collins, USA), preceded by acid digestion (EPA, 1998) of the samples. All the analyses were carried out in triplicate.

N grain content was assessed by the combustion method (Dumas method) (Saint-Denis and Goupy, 2004). Before analysis, the grain samples (20 g dry weight per plot) were prepared by grinding them using a ball mill. Each analysis was made on two experimental replicates by using an elemental analyser (Rapid max N exceed, Elementar, Lomazzo, Italy).

### *2.6 Fertilizer use efficiency*

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

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

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

### 2.7 Statistical analysis

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

## 3. Results and discussion

### 3.1 The effect of recovered fertilizers on soil

The recovered fertilizers used in this work (digestate from sewage sludge and ammonium sulphate) were described in the section "Material and Methods" (paragraph 2.2). Furthermore, the main chemical characteristics and the presence of contaminants are shown in tables S1 and S2.

The use of recovered fertilizers for three consecutive years had no impact on soil properties apart from that on TOC content, which was positively and significantly affected by RF use. The TOC content increased after three years from 10.3 ± 0.6 g $kg^{-1}$ dw (March 2018) to 12.3 ± 0.4 g $kg^{-1}$ dw (March 2021) (Table 2). Both the unfertilized and synthetic fertilized plots did not show any statistical differences with respect to the starting soil for the TOC contents (Table 2). The increase in TOC in soil fertilized with RF was most likely due to the contribution of digestate that was rich in organic carbon (TOC of 304 ± 34 g $kg^{-1}$ dw). In fact, during the three years of experimentation, the plots fertilized with RF received a total of 4,427 kg C $ha^{-1}$ dw, equal to 24% of the organic carbon already present in the surface 15 cm of soil at the beginning of the experiment. Furthermore, the organic carbon contained in the digestate was particularly recalcitrant to biodegradation, as suggested by its high biological stability, measured by potential biogas production. In fact, the registered BMP of 89 ± 17 $L_{biogas}$ $kg^{-1}$ dw (Table S1) was much lower than values reported in the literature (on average) for both energy crop digestate (229 ± 31 $L_{biogas}$ $kg^{-1}$ dw) and composts (144 ± 3.8 - 201 ± 20 $L_{biogas}$ $kg^{-1}$ dw), and not far from previous data reported for a similar digestate (i.e., 57 ± 23 $L_{biogas}$ $kg^{-1}$ dw) (Pigoli et al., 2021). This confirms that the organic matter contained in the digestate used was very stable, preventing the rapid degradation of the carbon added to the soil, which accumulated over time (Alburquerque et al., 2012; Greenberg et al., 2019), as will be discussed later.

The total soil nitrogen content (N tot) increased for both plots fertilized with SF and RF, which moved from a starting value of 1.27 ± 0.1 g $kg^{-1}$ dw (March 2018) to 1.41 ± 0 and 1.42 ± 0.9 g $kg^{-1}$ dw in March 2021, respectively. On the other hand, soil of the unfertilized plots did not show any variation in its N tot content, i.e., 1.3 ± 0 g $kg^{-1}$ dw in January 2021.

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

### *3.2 Agronomic performance of recovered fertilizers and product safety*

The amounts of maize grain produced (the average of 2018, 2019 and 2020 crop seasons) using recovered fertilizer (18.1 ± 2.9 Mg dried grain $ha^{-1}$) (Table S5) were similar and not statistically different from that produced with synthetic fertilizers (17.4 ± 1.2 Mg dried grain $ha^{-1}$). This indicated that recovered fertilizers are capable of substituting for synthetic fertilizers.

Furthermore, the content of microelements and inorganic pollutants in the produced grains was quantified (Table 3). For all the elements analysed (except zinc), the concentrations detected did not show significant differences between the grains produced using SF and RF as fertilizers.

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

## *3.3 Environmental safety*

### *3.3.1 Potential ammonia emissions and nitrate leaching*

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

On average, the plots fertilized with RF emitted an amount of ammonia (25.6 ± 9.4 kg N ha$^{-1}$ i.e., 11.6 ± 4% TAN) that was not statistically different from that measured for plots fertilized with SF (24.8 ± 8.3 kg N ha$^{-1}$, i.e., 13.4 ± 4.5% TAN). These data have previously been discussed in a paper published in this journal (Zilio et al., 2021) (Table S6).

Regarding the risk of N leaching from the soils, obtained results showed that during the two years of monitoring and for all sampling campaigns, the $NH_4^+$ concentrations in the experimental plots fertilized with RF were always comparable to those detected for plots fertilized with SF (Figure 1).

The data also show that, in both monitored years, the concentrations of $NO_3^-$ in soil fertilized with RF were never higher than those found in soil plots fertilized with SF, and that in one case (pre-fertilization 2020) $NO_3^-$ concentration was lower (Figure 2). Indeed, average $NO_3^-$ concentrations at 1-meter depth along the two monitored years were of 5.22 ± 4.65, 7.18 ± 5.89 and 6.56 ± 5.49 mg $kg^{-1}$ dw for unfertilized, SF and RF, respectively: these values are similar to each other and in line with those found in the literature for undisturbed soils (9.6 mg $kg^{-1}$). These figures are particularly interesting if it is considered that the unfertilized soil did not receive any N fertilizers throughout the three years, unlike the fertilized soil plots which every year received 470 and 285 kg $Ha^{-1}$ of N, respectively, for RF and SF plots. Although eight samplings during two crop seasons cannot be considered a continuous monitoring, able to exclude leaching due for example to abundant and rapid water flows, data obtained for the eight samplings (Figure 2) seem to suggest similar behaviour for SF and RF.

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

### *3.3.2 GHG emissions from soils*

Greenhouse gas (GHG) emissions were measured in 2020 starting from pre-sowing fertilization and thereafter for the following 10 months (from 28/05/2020 to 17/03/2021), with periodic measurements (Table 4). The cumulative amounts of $CO_2$ emitted in the 10 months of monitoring were 6,216 ± 1,160 kg C $ha^{-1}$ and 6,144 ± 1,491 kg C $ha^{-1}$ for RF and SF plots, respectively, suggesting that the addition to soil of organic matter by digestate did not lead to any C emission increase. These results confirmed that digestate organic matter was quite stable and did not mineralize, becoming part of the soil organic matter. This was more evident when unfertilized soil, that did not receive any fertilization for three years ($CO_2$ emission of 5,698 ± 935 kg C $ha^{-1}$), was compared to RF that, on the contrary, was dosed yearly ($CO_2$ emission 6,216 ± 1,160 kg C $ha^{-1}$).

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

Nitrous oxide ($N_2O$) emitted was of 1.71 ± 1.1, 10.3 ± 6.8 and 7.59 ± 3.2 kgN $ha^{-1}$ for Unfertilized, SF and RF, respectively. The plots fertilized with RF emitted an amount of $N_2O$ not statistically different from those fertilized with SF.

As expected, the unfertilized soil, that did not receive any N fertilization during the three years of experimentation, emitted much less $N_2O$ than fertilized soil plots, confirming the contribution of N fertilization to $N_2O$ emission from soils (Davidson, 2009). These results showed that dosing a much higher amount of N with recovered fertilizers (in total 470 kg N $ha^{-1}$) than with synthetic fertilizers (285 kg N $ha^{-1}$) did not lead to $N_2O$ emissions increasing. This can be ascribed, as already discussed for the potential nitrate leaching and $CO_2$ emissions, to the high biological stability of organic matter contained in the digestate, which limited N mineralization and nitrification. Therefore, taking into consideration that only the mineral N fraction was responsible for $N_2O$ emission, i.e., 290 kg N $ha^{-1}$ for RF and 275 kg N $ha^{-1}$ for SF, an equal $N_2O$ emission was expected, as was then validated by the experimental measurements.

These data may appear to contrast with some of those previously reported which indicated that there were higher $N_2O$ emissions for recovered fertilizers than for synthetic fertilizers (Montemayor et al., 2019; Verdi et al., 2019). However, in these previous studies, the biological stability of the organic matter was not measured/reported. Therefore, the degradability of the organic fraction which leads to mineral N that is then responsible for $N_2O$ production was not known. It therefore appears that the measurement of the biological stability of the organic substrate is an important factor in understanding the fate of N in the soil (potential $NO_3^-$ leaching and potential $N_2O$ production).

### *3.3.3 Soil pollutants*

The concentration of inorganic pollutants in the soil (i.e., As, Cd, Hg, Cr, Ni, Pb, Cu, Zn) was measured before the start of the experiment and after three years (Table 2). For all of the pollutants analysed, no significant increase was observed in the soils of all the experimental plots.

These data confirmed previous reports in the literature for similar work, namely that after the use of digestate in agriculture, no significant accumulations of heavy metals are found in the soil (Barłóg et al., 2020; Dragicevic et al., 2018).

In particular, as regards our study, the amount of heavy metals applied to the soil every year represented a minimal fraction compared to the content of the same metals already present in it (0.5% on average), with the exceptions only of Cu and Zn. In fact, every year, the quantity of Cu and Zn applied to the soil with the digestate corresponds respectively to 6% and 3.8% of what was already present in the 15 cm of surface soil. However, as reported in Table 2, after three years of experimentation the concentration of these two metals in the soil fertilized with recovered fertilizers was no higher than that measured at the beginning of the experiment, nor any higher than that of the unfertilized soil at the end of the experiment. One might think that three years of experimentation are not enough to measure an increase in the concentration of an element in the soil, even if it is dosed with a consistent quantity. However, in this work, fertilization with RF brought into the soil every year an amount of carbon equal to 8% of what was already present, and as previously observed (Table 2), in that case the increase in the concentration of carbon in the soil was detected. This shows that such variations can be measured, and confirms that most of the heavy metals brought to the soil dosed with digestate did not accumulate in the soil after three years of application.

Regarding the concentrations of persistent organic pollutants (POPs) in the experimental soils, in no case was any increase found in their concentration after three years of experimentation, for all the plots studied, including unfertilized plots (Table 2).  Furthermore, all values complied with the legal limits established in Italy for agricultural soils (DM 2019/46, Ministero dell'ambiente), and the values were in line with data reported for European agricultural soils as regards PCB, dioxins and DEHP for which data are available in the literature (Fabietti et al., 2010; Manz et al., 2001; Tran et al., 2015). Weissengruber and colleagues (2018) applying a forecasting model, reported that the risk of POPs accumulating in soils using digestate as fertilizer for several years (200) is negligible.

In addition to POPs, also the concentrations of emerging pollutants in soils (pharmaceuticals) were measured after three years of experimentation. These types of molecules can in fact be present in bio fertilizers, and therefore accumulate in the soil, with potentially toxic effects for ecosystems and public health (Konradi and Vogel, 2013). However, for these types of compounds there are still no laws that set limits or identify a group of molecules to be monitored, so the choice was made based on what was suggested by Konradi and Vogel (2013), taking into consideration parameters such as residence time in the soil, solubility and ecotoxicity. The 9 compounds chosen were: antibiotics (Ciproflaxacin and Sulfamethoxazole), lipid regulators (Fenofibrate and Gemfibrozil), psychiatric drugs (Carbamazepine), beta-blockers (Metoprolol), analgesic (Diclorofenac) and hormones (Ethinylestradiol and Estradiol) (Table 2). The analyses showed that after the third year, the soil concentrations of all the pharmaceuticals were always below the instrumental detection limit (<0.01 mg $kg^{-1}$ dw), for all the experimental plots, with no differences between soils fertilized with RF, SF or not fertilized. In a previous work it was already reported that the concentration of emerging organic pollutants in this type of digestate was very low, and always below detection limit and often lower than the values reported for other types of organic matrices routinely used as fertilizers (i.e., animal slurries and manures) (Pigoli et al., 2021).

### 3.4 *Recovered fertilizers nitrogen efficiency*

The N fertilizer use efficiency (FUE) measured for SF was of 93.6 ± 4.4% to be compared with that calculated for RF which was of 55.5 ± 6.6% (Table 5), and therefore similar to that of 50% suggested by Lombardy Region and adopted in this work. These figures were obtained taking into consideration total N dosed, independently of N forms (mineral vs. organic), because it is impossible to separate ammonium from organic forms. Nevertheless, nitrogen dosed with the digestate was represented for 57.8% TKN by $NH_4$-N that was readily available for plants as well as N from SF, and by 42.2% TKN by organic N that was quite stable (no mineralization occurred) because of the high biological stability of digestate. Taking into consideration data above reported it could be reasonably assumed for the ammonium fraction of RF a FUE very close to that of SF, and that organic-N was not available.

The stability of organic N was confirmed, as discussed earlier, by measuring $CO_2$ and $CH_4$ evolution from soils treated with RF that showed similar figures to those of both plots fertilized with SF and unfertilized, and by measuring both $NH_4^+$ and $NO_3^-$ soil contents at different topical moments, that were similar for all soils studied, independently of the fertilizers used. As a consequence of the results obtained, it can be considered that the organic N of digestate, substantially, did not contribute to mineral soil N, since it became part of the soil organic matter, and that only the ammonium form should be considered for FUE calculation. Doing so, the re-calculated RF FUE was of 85.3 ± 10%, comparable to that calculated for SF (FUE of 93.6 ± 4.4%). Consequently, the N fertilizer replacement value (NFRV) obtained for RF used to replace SF, when referred only to the mineral N form, was of 83.7%. Obviously, this value assumes validity only if the digestate characterization is performed to attest the high biological stability of the organic matter which it contains.

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

## 4. Conclusions

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

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## Notes

## 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: soil analyses.

AP: soil samplings and fertilizers/plants analyses.

BR: soil samplings and soil C and N analyses and data elaboration.

AH: soil samplings and data elaboration.

AG: designed the project and managed the experimental field.

GG: managed the experimental field and agronomic operations.

EM and OS: Scientific contribution and manuscript correction.

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

| Year | Plots | Date | Fertilization | Fertilizer | Fertilizer dosed (Mg ha$^{-1}$ ww$^{a}$) | 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 | 48.5 | 370 | 229 | Injection 15 cm |
| | | 22/06/2018 | Top-dressing | Ammonium sulphate [b] | 1.35 | 100 | 100 | Fertigation |
| | Synthetic fertilizer | 23/04/2018 | Pre-sowing | Urea | 0.4 | 185 | 185 | Spread in surface |
| | | 22/06/2018 | Top-dressing | Ammonium sulphate [c] | 0.46 | 100 | 100 | Spread in surface |
| **2019** | Recovered fertilizer | 16/04/2019 | Pre-sowing | Digestate | 48.5 | 370 | 229 | Injection 15 cm |
| | | 1/08/2019 | Top-dressing | Ammonium sulphate | 1.35 | 100 | 100 | Fertigation |
| | Synthetic fertilizer | 16/04/2019 | Pre-sowing | Urea | 0.4 | 185 | 185 | Spread in surface |
| | | 1/08/2019 | Top-dressing | Ammonium sulphate | 0.46 | 100 | 100 | Spread in surface |
| **2020** | Recovered fertilizer | 28/05/2020 | Pre-sowing | Digestate | 48.5 | 370 | 200 | Injection 15 cm |
| | | 31/07/2020 | Top-dressing | Ammonium sulphate | 1.26 | 90 | 90 | Fertigation |
| | Synthetic Fertilizer | 28/05/2020 | Pre-sowing | Urea | 0.4 | 185 | 185 | Spread in surface |
| | | 31/07/2020 | Top-dressing | Ammonium sulphate | 0.46 | 90 | 90 | Spread in surface |

[a] ww: wet weight

[b] Digestate-derived ammonium sulphate used in recovered fertilizer treatment has an N title of 7% ww/ww

[c] Ammonium sulphate used in synthetic fertilizer treatment has an N title of 21% ww/ww.

Table 2. Main chemical parameters (mean ± SD; n=3) 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 | 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)[a] | 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[b] | 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] Letters in parenthesis are referred to One-way ANOVA comparing values in each row ($p<0.05$; n=3; Tukey post-test).

[b] dw: dry weight

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

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

[a] dw: dry weight

[b] mean ± SD; n=3.

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

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

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

[a] mean ± SD, n = 6

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

Table 5. Fertilizer use efficiency (FUE) and N fertilizer replacement value (NFRV) for the maize crop fertilized with SF and RF in the year 2020.

| | **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)[d] | 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

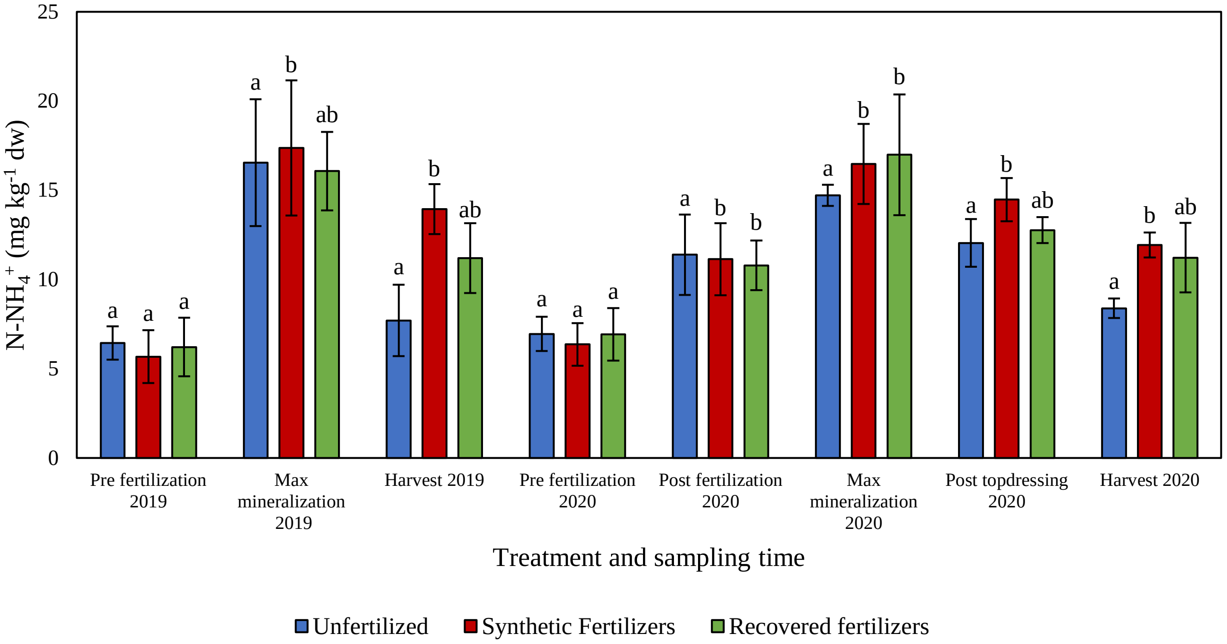
[c] N applied considering only the $N\text{-}NH_4^+$ contained in the digestate dosed

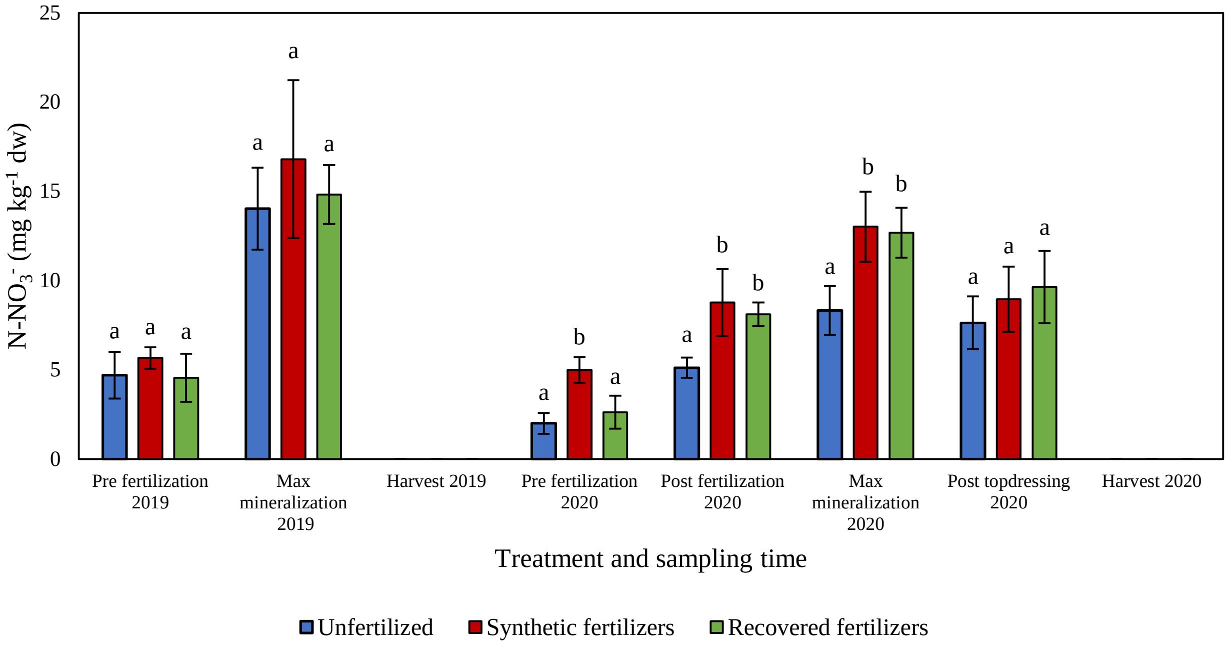
[d] Letters are referred to One-way ANOVA (n=6, $p<0.05$, Tukey post-test).

Caption Figures

**Figure 1.** Concentrations of ammonia nitrogen (N-$NH_4^+$) in experimental soils (soil layer between surface and 25 cm depth) during the crop seasons 2019 and 2020 (mean ± SD; n=3). Letters are referred to One-way ANOVA ($p<0.05$; Tukey post-test) comparing the three treatments within each sampling time.

**Figure 2.** Concentrations of nitric nitrogen (N-$NO_3^-$) in experimental soils (soil layer between 75 and 100 cm depth from surface) during the crop seasons 2019 and 2020 (mean ± SD; n=3). Letters are referred to One-way ANOVA ($p<0.05$; Tukey post-test) comparing the three treatments within each sampling time.


N-NH$_4^+$ (mg kg$^{-1}$ dw)
25
20
15
10
5
0
a
a
a
a
b
ab
a
b
ab
a
a
a
a
b
b
a
b
b
a
b
ab
a
b
ab
Pre fertilization 2019
Max mineralization 2019
Harvest 2019
Pre fertilization 2020
Post fertilization 2020
Max mineralization 2020
Post topdressing 2020
Harvest 2020
Treatment and sampling time
Unfertilized
Synthetic Fertilizers
Recovered fertilizers


25
20
15
10
5
0
N-NO3- (mg kg-1 dw)
a
a a a
a a
b
a b a
b
a b
b
b
a
a a
a a
Pre fertilization 2019
Max mineralization 2019
Harvest 2019
Pre fertilization 2020
Post fertilization 2020
Max mineralization 2020
Post topdressing 2020
Harvest 2020
Treatment and sampling time
Unfertilized
Synthetic fertilizers
Recovered fertilizers