

Article

Assessment of the Carbon and Nitrogen Mineralisation of Digestates Elaborated from Distinct Feedstock Profiles

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Abstract: The carbon (C) and nitrogen (N) mineralisation rates of five digestates were studied and compared with pig slurry, compost, and a solid fraction of digestate in aerobic incubation experiments. The objective was to identify the most relevant drivers of C and N mineralisation based on the physicochemical properties of the products. Net organic nitrogen mineralisation of digestates ($N_{\min,net}$) was on average 30%, although the range was relatively wide, with digestate from pig manure (39%) reaching double the value of digestate from sewage sludge (21%). The total carbon to total nitrogen (TC:TN) ($r = -0.83, p < 0.05$) and ammonium nitrogen to total nitrogen ($NH_4^+ - N:TN$) ($r = 0.83, p < 0.05$) ratios of the products were strongly correlated with $N_{\min,net}$, adequately mirroring the expected fertilising potential of the products. The digestates had C sequestration values between 50 and 81% of applied total organic carbon (TOC), showcasing their potential to contribute to C build-up in agricultural soils. The carbon use efficiency of the amended soils was negatively correlated with dissolved organic carbon (DOC) ($r = -0.75, p < 0.05$) suggesting that catabolic activities were promoted proportionately to the DOC present in these products. Ratios of DOC:TOC ($r = -0.88, p < 0.01$) and TC:TN ($r = 0.92, p < 0.01$) were reliable predictors of the fraction of C that would remain one year after its incorporation and thus could be used as simple quality parameters to denote the C sequestration potential of digestates prior to their use in the field.

Keywords: digestate; carbon use efficiency; carbon sequestration; nitrogen mineralisation; nitrification; Nitrates Directive



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1. Introduction

The European Union (EU) has resolutely set its sights on transitioning away from the linear paradigm of “take, make, dispose” towards a circular economy model that fosters the reducing, reusing, and recycling of resources, as exemplified by the adoption of the Fertilising Products Regulation (FPR) ((EU) 2019/1009), which sets the stage for a unified European market of organic waste-derived fertilisers. At the heart of the European Green Deal lies the ambitious long-term goal to reach net-zero greenhouse gas (GHG) emissions by 2050, while the recent adoption of the cross-sectoral “Fit for 55” package by the European Commission (EC) aims to crank up the EU’s commitment to reduce emissions by 55% by 2030 (compared to 1990 levels). As a milestone towards this overarching goal, the Regulation on Land Use, Forestry and Agriculture proposes to increase carbon (C) removal by natural sinks to 310 MT CO₂ equivalent by 2030 and achieve climate neutrality in this combined sector by 2035. On a broad scale, this vision will involve the upscaling and incentivisation of C farming initiatives, understood as a series of practices intended to increase C removal and storage in the land sector. This roadmap should translate into concrete actions such as the enhancement and conservation of soil organic carbon (SOC) in arable land.

The policy framework portrayed above arguably makes anaerobic digestion (AD) an interesting prospect as it can simultaneously provide renewable energy from a variety of organic waste streams, produce fertilisers in the form of nutrient-rich digestate and reduce GHG emissions [1]. By the end of 2019, the number of active biogas plants was just shy of 19,000 and total biogas production had reached 16 billion m³ [2]. At the same time, biomethane is rapidly gaining traction for its potential to decarbonise both the transportation sector and the public gas grid [3,4]. It follows that with an increasing capacity to process organic waste streams owing to the European AD sector's steady growth, the opportunity arises to develop adequate management strategies for digestate [5]. Concomitantly, a new category of fertilisers, branded the "RENURE" products (recovered nitrogen from manure), which falls between unprocessed livestock manure and conventional chemical fertilisers [6], is under way in the EU. With this new hybrid class of fertilisers, among which digestate is featured, the current maximum nitrogen (N) application limit of 170 kg N ha⁻¹ y⁻¹ etched in the Nitrates Directive (ND) (91/676/EEC) would be lifted for certain animal manure-derived products in Nitrate Vulnerable Zones (NVZ).

It has been shown that certain nutrient recovery techniques [7] can improve the mineral N availability of the upgraded digestate and, consequently, its compliance with the RENURE criteria [6,8]. On the other hand, the use of raw digestate is still widespread as energy production takes precedence over nutrient recovery for AD plants [9]. In the same vein, the EC recently confirmed that at present, post-processed digestates, which include separation into a liquid and solid fraction, were not included in the FPR, de facto leaving the field open to unseparated (raw) biogas slurry as the sole ambassador for digestate products in the near future. In light of these developments, and with the RENURE derogation just around the corner, a better understanding of the fate of nutrients, N in particular, from digestate is all the more relevant. In this regard, several N-driven field studies have examined the potential of digestate as a chemical fertiliser substitute and its beneficial effects on crop growth [10–12]. Adverse effects include N losses from leaching due to a rapid nitrification of the initial ammonium nitrogen (NH₄⁺-N) content [13,14]. Other reported drawbacks from the application of digestate to soil include volatilisation of ammonia (NH₃), due to the high NH₄⁺-N content and elevated pH [15]. While some of the underlying mechanisms have not been completely elucidated, nitrous oxide (N₂O) emissions are also associated with the application of digestate [16], which have been reported to be affected by the moisture content of the soil [17], and the organic C [18] or NH₄⁺-N contents [19] of digestate.

Moreover, the variability of N concentrations between different digestate qualities and the ensuing mineralisation pattern of the remaining organic N (N_{org}) can further compound the unpredictability of N kinetics [20]. Additional parameters influencing mineralisation, such as composition of the organic materials, soil temperature and water content, drying and rewetting events and soil physicochemical properties, must also be taken into consideration [21]. As a result, laboratory scale microcosm incubations constitute an important step to narrow down some of the key parameters behind N release dynamics in soil (i.e., the amount of available inorganic N), which in turn can help delineate more efficient management systems for digestate. In this respect, a few studies have examined the fate of C and N from a limited number of digestates in incubated soil experiments [22–24].

As alluded to earlier, the importance of the C removal imprint on current and future EU policies relating to agricultural systems makes it all the more pertinent to examine digestate not only from a fertilising point of view (N dynamics) but also as a possible contributor to SOC build-up (C dynamics). To further build upon this topic, the present study selected digestates elaborated from five of the most representative feedstock streams in the European AD sector (pig manure, poultry manure, energy crops, sewage sludge, food waste) to better compare their C and N mineralisation kinetics in aerobic incubation experiments.

Conversely, as the centrepiece of SOC decomposition and stabilisation dynamics, the understanding of soil microbiological processes has made great strides over the last decade, to the point where the inclusion of microbial mechanics in global C models can improve the

accuracy of their predictions [25]. One such parameter, the microbial carbon use efficiency (CUE), quantifies the fraction of C that is taken up by microbial consortia and is effectively retained in the microbial biomass in lieu of being respired, whereby a higher CUE may be an indicator of increased C storage. While it is still unclear how these elements interact, it is widely recognised that temperature, water availability (abiotic factors), the microbial communities and the addition of exogenous C to soil (in this case from digestate) affect CUE [26,27]. As a novel factor that might bear significance in the frame of the bustling C farming strategies [28], and for which digestate-driven studies are still scarce to our knowledge [29], we also examined the CUE of the different digestate-amended treatments. To better earmark the fertilising and/or soil improving properties of the digestates, the incubated soil treatments included three benchmark products: a conventional organic fertiliser (pig slurry), a soil improver (compost) and the solid fraction of digestate (as a hybrid between the two former categories). The objective of the study was to identify the most relevant parameters driving C (sequestration/respiration) and N mineralisation based on the physicochemical properties of the digestates.

2. Materials and Methods

2.1. Physicochemical Characterization of Products

Samples of raw digestate were collected from five full-scale AD facilities across Europe. Four of the five digestates were of co-digested feedstocks, the fifth was of the mono-digestion of poultry manure. Table 1 provides an overview of the biogas plants and the feedstock composition of the products. Each digestate (D) was named after its predominant feedstock, as follows: biowaste (D_BW); sewage sludge (D_SS); corn silage (D_CS); pig manure (D_PM); chicken manure (D_CM). For comparison purposes, three benchmark products were included: (i) commercial compost (COM) as representative of a typical soil improver with expected organic amendment characteristics (COM_1 for the N incubations, COM_2 for the C incubations); (ii) undigested pig slurry (U_PS) as representative of a conventional fertiliser often used in farming systems; (iii) the solid fraction of digestate from chicken manure (SF_CM) as a hybrid between the two former categories, with an expected C and N mineralisation behaviour between that of the above-mentioned soil improver and the fertilisers.

All products were collected in polyethylene sampling bottles and stored at 4 °C until further use. Total solids were determined on fresh samples placed in an oven at 105 °C until constant mass was reached (48 h). Organic matter (OM) was determined on the oven-dried samples by loss on ignition in a muffle furnace after 4 h at 550 °C. The pH was determined using an Orion Star A211 (Thermo Fisher Scientific, Waltham, MA, USA) pH meter in a 1:5 ratio (*w/v*) of fresh sample to 1 M potassium chloride (KCl). The suspension was stirred and allowed to settle for 15' before the reading was taken. Electrical conductivity (EC) was measured on an Orion Star A212 conductivity meter in a 1:5 ratio (*w/v*) of fresh sample to deionised water. The soil suspension was placed in an orbital shaker for 60' and filtered (Whatman No. 43, Maidstone, UK) prior to the reading.

Total nitrogen (TN), total carbon (TC) and total organic carbon (TOC) were determined on a PRIMACS100 Analyzer series (Skalar Analytical B.V., Breda, Netherlands). Ammonium-N and nitrates (NO₃⁻-N) were analysed on 1 M KCl filtrates on a Skalar SA 1050 flow injection analyser. The KCl extractions were prepared in a 1:5 ratio (*w/v*) of fresh sample to 1 M KCl. They were placed in an orbital shaker for 30' and subsequently filtered (Whatman No. 43). Total phosphorus (P) was determined on 0.3 g of fresh product by nitric acid digestion (HNO₃ 65%) using a Vista-MPX inductively coupled plasma emission spectrometer (Varian Inc., Palo Alto, CA, USA).

Table 1. Description of the products used for C and N incubation experiments and the facilities from which they originate *.

| Product | | Facility | Yearly Ratio of Feedstock Composition (%) |
|---------|--------------------------|------------------------------|---|
| Acronym | Description | | |
| D_BW | Digestate | Am-Power (BE) | Biowaste (food): 69 Food industry sludge: 11 Animal manure: 7 Glycerine: 6 Other substrates: 4 Manure solid fraction: 3 |
| D_SS | Digestate | Acqua & Sole (IT) | Sewage sludge: 85 Biowaste (food): 9 Digestate from biowaste: 7 |
| D_CS | Digestate | BENAS-GNS (DE) | Corn silage: 44 Rye silage: 31 Chicken manure: 14 Grass: 5 Corn grain: 4 Other solids: 1 Millet: <1 |
| D_PM | Digestate | Groot Zevert Vergisting (NL) | Pig manure: 67 By-products from dairy and feed industry: 16 Dairy by-products: 11 Slaughterhouse manure: 9 Dairy cattle manure: 4 Glycerine: 4 |
| D_CM | Digestate | Anonymous (UK) | Chicken manure: 100 |
| COM_1 | Commercial compost | / | Source-separated waste from households and gardens |
| COM_2 | Commercial compost | / | Source-separated waste from gardens and parks |
| U_PS | Undigested pig slurry | Anonymous (BE) | Pig slurry: 100 |
| SF_CM | Solid fraction digestate | Rika Biofuels (UK) | Chicken manure: 100 |

* Soil and products were identical for both C and N mineralisation assays except for COM_1 (used of N incubations) and COM_2 (used for the C incubations).

Biochemical oxygen demand over 5 days (BOD₅) was determined according to the Macherey-Nagel (Macherey-Nagel GmbH & Co. KG, Düren, Germany) protocol reference 985 825 [30]. Dissolved organic carbon (DOC) was determined according to Macherey-Nagel protocol reference 985 093 [31]. BOD₅ and DOC were performed by a certified laboratory (Innolab, Oostkamp, Belgium).

2.2. Soil Characteristics

The methods (pH, EC, DM, OM, NH₄⁺-N and NO₃⁻-N, TC, TN, TOC) were identical to those described in Section 2.1 (Table 2). The soil for the incubation experiments was taken from the top layer (0–30 cm) of an unfertilised private patch in Wortegem-Petegem, East Flanders, Belgium (50°50'21.6323" N, 3°33'22.852" E). It is classified as a luvisol [32] with a loamy sand texture (75% sand; 14% clay; 11% silt). The soil was air dried and sieved through a 2-mm screen before use. Total P, potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) were determined via the Aqua Regia method, in a 1:3 ratio of HCl:HNO₃, on 1 g of soil using a Vista-MPX inductively coupled plasma emission spectrometer.

Table 2. Soil physicochemical properties ($n = 3$, mean value \pm standard deviation).

| Parameter | |
|--|------------------|
| pH KCl | 5.3 \pm 0.1 |
| EC (mS cm ⁻¹) | 0.2 \pm 0.0 |
| DW (g kg ⁻¹ FW) | 991 \pm 1.0 |
| OM (g kg ⁻¹) | 42 \pm 1.1 |
| TC (g kg ⁻¹) | 18 \pm 0.7 |
| TOC (g kg ⁻¹) | 18 \pm 0.1 |
| TN (g kg ⁻¹) | 1.63 \pm 0.21 |
| NH ₄ ⁺ -N (mg kg ⁻¹) | 11.87 \pm 0.48 |
| NO ₃ ⁻ -N (mg kg ⁻¹) | 28.35 \pm 0.48 |
| P (g kg ⁻¹) | 0.78 \pm 0.02 |
| K (g kg ⁻¹) | 2.15 \pm 0.08 |
| Ca (g kg ⁻¹) | 2.07 \pm 0.03 |
| Mg (g kg ⁻¹) | 2.00 \pm 0.04 |
| S (g kg ⁻¹) | 0.28 \pm 0.01 |

EC = electrical conductivity; DW = dry weight; FW = fresh weight; OM = organic matter; TC = total carbon; TOC = total organic carbon; TN = total nitrogen.

2.3. N Incubations

The C and N mineralisation rates of soil amended with five digestates (D_BW; D_SS; D_CS; D_PM; D_CM) and three reference products (COM; U_PS; SF_CM) were studied in consecutive soil microcosm incubation experiments. For assessing the N mineralisation dynamics, 162 polyvinyl chloride (PVC) tubes (9 treatments \times 6 sampling times \times 3 replicates), including the unfertilised control soil, were monitored over a four-month period (127 days) according to the protocol established by the Flemish Institute for Technological Research [33]. First, the air-dried soil was preincubated at 35% water-filled pore space (WFPS) for 7 days at 20.9 °C in order to stimulate and stabilise the initial microbial activity. Except for COM_1 and SF_CM (high DM content), all products were placed on a magnetic stirrer to ensure better sample homogeneity during transfer to the soil microcosms. Each tube received 260 g of preincubated soil (233 g dry soil), previously mixed to the products. The mixture was packed to a bulk density of 1.4 Mg m⁻³, corresponding to a soil volume of 166 cm³ inside the tube. The targeted application rate of the products in the tubes was 170 kg N ha⁻¹ based on the Nitrates Directive (91/676/EEC). Assuming a field bulk density of 1.4 Mg m⁻³ and a 30 cm depth, the known surface and height of the mixed material inside the tube (4.6 cm diameter, 10 cm height) allowed us to convert kg ha⁻¹ to g kg⁻¹ of product applied. In the end however, the chosen application rate of 170 kg N ha⁻¹ was not met because the initial TN characterisation values of the products, upon which the calculations were based, turned out to be inaccurate due to equipment failure. Once the samples had been reanalysed and the correct TN values had been established (the experiment was already ongoing), the treatments proved to have different application rates (Table 3).

The amount of water contained in each product was accounted for, and the total moisture level of the soil treatments brought to 50% WFPS. Each tube was covered with perforated parafilm for adequate gas exchange and was left in the dark at a constant temperature of 20.9 °C in a completely randomised design. The mass of each tube was registered, and the tubes regularly weighed to maintain a constant moisture level (50% WFPS) throughout the experiment. Destructive sampling was carried out every 20 days over a total of 127 days (due to COVID restrictions the last sampling date was on day 127 instead of 120). At each interval, 27 tubes (3 replicates of 9 treatments) were removed to determine NH₄⁺-N and NO₃⁻-N content. The sum of NH₄⁺-N and NO₃⁻-N was considered as the amount of mineral nitrogen (N_{min}).

Table 3. Application rates of total nitrogen for N incubations (mg TN 100 g⁻¹ DM soil) (kg ha⁻¹) for each treatment and of total organic carbon for C incubations (mg TOC 100 g⁻¹ DM soil) with their equivalent field application rates (kg ha⁻¹).

| Unit | | TN Product Application Rate | | | | | | | |
|--------------|--------------------------------------|------------------------------|------|------|------|------|-------|------|-------|
| | | D_BW | D_SS | D_CS | D_PM | D_CM | COM * | U_PS | SF_CM |
| N incubation | mg TN 100 g ⁻¹ DM soil | 9.8 | 7.0 | 7.9 | 10.1 | 10.0 | 7.3 | 11.4 | 6.0 |
| | kg TN ha ⁻¹ (equivalent) | 275 | 197 | 223 | 283 | 281 | 204 | 318 | 167 |
| C incubation | | TOC Product Application Rate | | | | | | | |
| | | D_BW | D_SS | D_CS | D_PM | D_CM | COM * | U_PS | SF_CM |
| C incubation | mg TOC 100 g ⁻¹ DM soil | 180 | 303 | 346 | 158 | 173 | 647 | 54 | 694 |
| | kg TOC ha ⁻¹ (equivalent) | 2519 | 4236 | 4843 | 2216 | 2418 | 9051 | 758 | 9721 |

Digestate from biowaste (D_BW); sewage sludge (D_SS); corn silage (D_CS); pig manure (D_PM); chicken manure (D_CM); compost (COM_1 for N incubations and COM_2 for C incubations); undigested pig slurry (U_PS); solid fraction of digestate from chicken manure (SF_CM). * Soil and products were identical for both experiments except for COM_1 (used of N incubations), and COM_2 (used for the C incubations). TN = total nitrogen; TC = total carbon, TOC = total organic carbon.

2.4. C Incubations

For soil incubations aimed at assessing the C mineralisation dynamics, the soil and treatments were identical to those of the N incubations, except for COM_2 being substituted for COM_1. The incubation experiment was based on the protocol from the Public Waste Agency of Flanders [34]. There was a total of 30 tubes, counting triplicates of 8 products and one control (unfertilised soil). For this experiment, the TOC loading rate of each treatment was maximised to trigger measurable microbial respiratory activity while attention was also given to the amount of N supplied by each treatment so that it would not constitute a limiting factor for microbial growth. As the DM range of the different products was quite heterogenous, a 25% WFPS soil level was opted for over a 7-day preincubation period at 20 °C. This made it possible to increase the amount of product being applied, by factoring in its water content, to reach 50% WFPS for the incubation phase.

Except for COM_1 and SF_CM (high DM content), all products were placed on a magnetic stirrer to ensure better sample homogeneity during addition to the soil microcosms. For digestates, the amount of product added per tube ranged between 23 and 24 g fresh matter (FM) (applied amounts are presented in Table 3). Each tube (7 cm height × 6.8 cm diameter) received 275 g of preincubated soil (256 g dry soil), which had previously been thoroughly mixed with the products, and was then packed to a bulk density of 1.4 Mg m⁻³. Once the WFPS level had been adjusted to 50%, the tubes were placed inside glass jars (1 L) with airtight lids. In each jar, the PVC tubes (182 cm³) were topped with a wire mesh, on top of which a vial (120 cm³) containing 20 mL of sodium hydroxide (NaOH) 0.5 M was placed. Upon placing each NaOH trap, the glass jars were immediately sealed shut and the time registered so as to ensure equal incubation times across all treatments. The jars were incubated at a constant temperature of 20 °C, in the dark, in a completely randomised design. The initial mass of each jar was registered and regularly weighed to maintain 50% WFPS over the course of the experiment. The treatments were incubated over 149 days during which time the NaOH 0.5 M vials were removed and back titrated on a 702 SM Titrino automatic potentiometric titrator (Metrohm, Herisau, Switzerland) with 0.5 M HCl to measure the amount of evolved CO₂, after having precipitated the carbonates with 2 mL of 0.5 M barium chloride (BaCl₂) [35]. In total, there were 16 sampling times (on days 1, 2, 3, 4, 6, 8, 11, 15, 18, 24, 35, 53, 74, 102, 134, 149). Upon removing the NaOH traps, the glass jars were left open for 4 h for full oxygen replenishment, after which fresh NaOH 0.5 M vials were placed back inside.

2.5. Soil Microbial Biomass

At the end of the C incubation experiment (day 149), the microbial biomass carbon (MBC) was determined on all treatments (control soil and fertilised soil treatments) via the

chloroform fumigation-extraction method [36]. A homogenised quantity of 30 g of each replicate (3×9 treatments) was weighed for both fumigated and unfumigated samples. The fumigated samples were then placed inside a vacuum desiccator, in a chloroform-saturated environment (CHCl_3) from which the air was evacuated. After a 24-h fumigation period in the dark, the samples were removed and extracted with 0.5 M potassium sulphate (K_2SO_4) in a 1:2 ratio (w/v) before being placed on an orbital shaker for 60'. The non-fumigated samples underwent the same K_2SO_4 extraction process. All samples were then filtered (Whatman No. 6) and stored at -18°C until analysis of TC contents on a Formacs^{HT-I} TOC/TN analyser (Skalar Analytical B.V., Breda, Netherlands).

2.6. Calculations

For each sampling time (t), results for N release (N_{rel}) were expressed as the difference between N_{min} (taken as the sum of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) measured in the treatment (fertilised soils) and N_{min} contained in the control (unfertilised soil) over the amount of TN added with each product, as follows:

$$N_{\text{rel}} (\% \text{TN}) = \frac{N_{\text{min,treatment}}(t) - N_{\text{min,control}}(t)}{\text{TN}_{\text{applied}}} \times 100 \quad (1)$$

Having subtracted the N_{min} of the control, net N mineralisation ($N_{\text{min,net}}$) was calculated as the difference between N_{min} content of the treatment on day 0 and N_{min} of all subsequent measurements, expressed as percentage of N_{org} . A positive $N_{\text{min,net}}$ value suggests net mineralisation, a negative N value suggests net N immobilisation [37] as follows:

$$N_{\text{min,net}} (\% N_{\text{org}}) = \frac{N_{\text{min}}(t = x) - N_{\text{min}}(t = 0)}{N_{\text{org}}} \times 100 \quad (2)$$

The mineralised C (C_{min}) was expressed as the difference between the amount of $\text{CO}_2\text{-C}$ evolved from the soil treatment and that of the control (unfertilised soil) over the amount of added TOC from each product:

$$C_{\text{min}} (\%) = \frac{\text{CO}_2 - C_{\text{treatment}}(t) - \text{CO}_2 - C_{\text{control}}(t)}{\text{TOC}_{\text{applied}}} \times 100 \quad (3)$$

A second-order kinetic model was fitted to the C_{min} results of each of the tested products, based on 16 sampling times taken over 149 days. This model assumed that the rate of mineralisation is proportional to the product of the C concentration of the substrate and the microorganisms derived from the substrate [38]:

$$C(t) = C_A - \frac{C_A}{1 + k_2 a (1 - a) C_A t} \quad (4)$$

where $C(t)$ is the cumulative amount of C mineralised at time t ; C_A the amount of mineralised C; k_2 the second-order mineralisation rate; a the amount of mineralised C substrate that becomes part of the microbial biomass itself and further influences the mineralisation process. Variables k_2 and a are expressed as a single variable $k_2 a (1 - a)$. The model was then used to extrapolate the experimental results and predict the amount of effective OC (EOC), defined as the amount of C remaining after 365 days.

The data from the experiment were corrected to reflect an average annual field temperature of 9.7°C in Belgium, using a temperature dependence model for C mineralisation [39]:

$$k(T) = k_{\text{opt}} \exp\left(-k\left(1 - \frac{T}{T_{\text{opt}}}\right)^2\right) \quad (5)$$

where $k(T)$ is the C mineralisation rate at temperature T ; k_{opt} the mineralisation rate at optimum temperature; $k(2.55)$ a rate parameter reflecting the temperature sensitivity of

the mineralisation rate k ; and T_{opt} the optimum temperature for C mineralisation. The k parameter was based on a previous estimate established in the same laboratory [39]. More specifically, k was empirically derived from measurements of cumulative crop residue C mineralisation at 5.5, 10, 16 and 25 °C in a Flemish loamy sand cropland soil, highly comparable to the soil used in the present study. Thus, the mineralisation rates at temperatures T and at 9.7 °C were calculated from this equation, from which a ratio $k(T):k(9.7)$ was derived. The extrapolated incubation time (365 days) at temperature T was then multiplied by this ratio to obtain an equivalent number of days at 9.7 °C.

The cumulative CO₂-C results, stemming from the initial TOC application rates (Table A1, Appendix A), expressed per 100 g soil, were normalised based on the amount of added TOC applied with each product:

$$\text{CO}_2 - \text{C} \left(\mu\text{g CO}_2 - \text{C mg}^{-1} \text{ TOC} \right) = \frac{\sum_{t=0}^{t=x} \text{CO}_2 - \text{C}}{\text{TOC}_{\text{applied}}} \quad (6)$$

The CUE, defined as the ratio of C gain to C uptake over a period of time, was assessed to gauge the efficiency with which microbial communities assimilated the C supplied by the treatments [40,41]:

$$\text{CUE} = \frac{\Delta\text{MBC}}{(\Delta\text{MBC} + \Sigma\text{CO}_2 - \text{C})} \times k_{EC}^{-1} \quad (7)$$

MBC is the microbial biomass carbon determined on the last day of the C incubation and CO₂-C the amount of evolved CO₂-C by day 149; ΔMBC and ΣCO₂-C taken together represent C uptake; k_{EC} is a correction factor which considers that about 45% of the killed biomass C is evolved as CO₂-C [42].

2.7. Statistical Analysis

All statistical analyses were performed on SPSS 27.0 software (IBM Corp, Armonk, NY, USA) for Windows. Differences in treatments were assessed using one-way ANOVA and Tukey's honestly significant difference (HSD) post-hoc test. The normal distribution of data was checked using the Shapiro–Wilk test. Homogeneity of variance was checked using Levene's test. Correlation coefficients between treatment effects and product characteristics were determined using Pearson's correlation.

3. Results

3.1. Product Characteristics

All digestates and U_PS had pH values in the alkaline range, between 8.3 and 8.7 (Table 4). DM content of the five digestates ranged from 8 to 11%, while values for COM_1 and COM_2 were comparatively much higher (53 and 35%).

As was foreseeable, the highest DM content was observed with SF_CM (81%), while the lowest was from U_PS (2.9%). TC of digestates varied somewhat, ranging from 256 (D_PM) to 369 g TC kg⁻¹ (D_CS). For U_PS, TC was in the lower range (277 g TC kg⁻¹ DM) while its TC:TN ratio was the lowest of all at 1.99 (as an order of magnitude, the second lowest was D_PM at 2.60). There was some degree of variability for OM from digestates (600–749 g OM kg⁻¹ DM), the lowest value being associated with SF_CM (398 g OM kg⁻¹ DM). TOC:TC was relatively widespread across products (0.77–0.99), the highest value coming from COM_1, the lowest from U_PS (Table 4). The lowest and highest TN values for digestates were, respectively, 71 (D_SS) and 99 (D_PM) g TN kg⁻¹. The TC:TN ratios of digestates ranged between 2.6 (D_PM) and 4.5 (D_CS), in agreement with previously reported values [43,44].

Table 4. Physicochemical properties of the products used for C and N incubations, expressed on dry matter basis unless specified ($n = 3$, mean value \pm standard deviation).

| Parameter | D_BW | D_SS | D_CS | D_PM | D_CM | COM_1 | COM_2 | U_PS | SF_CM |
|---|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|----------------|
| pH KCl | 8.7 \pm 0.0 | 8.5 \pm 0.0 | 8.5 \pm 0.1 | 8.5 \pm 0.0 | 8.4 \pm 0.0 | 8.0 \pm 0.7 | 5.1 \pm 0.1 | 8.3 \pm 0.0 | 8.3 \pm 0.0 |
| pH H ₂ O | 8.2 \pm 0.0 | 8.3 \pm 0.0 | 8.2 \pm 0.0 | 8.2 \pm 0.0 | 8.2 \pm 0.0 | / | 5.9 \pm 0.1 | 8.3 \pm 0.0 | / |
| EC (mS/cm) | 1.9 \pm 0.1 | 0.9 \pm 0.0 | 1.8 \pm 0.0 | 2.0 \pm 0.1 | 2.1 \pm 0.1 | 0.7 \pm 0.0 | 1.2 \pm 0.1 | 0.0 \pm 0.0 | 0.0 \pm 0.0 |
| DM (g kg ⁻¹ FM) | 85 \pm 5.9 | 110 \pm 1.6 | 107 \pm 5.6 | 78 \pm 7.5 | 80 \pm 0.9 | 531 \pm 7.1 | 349 \pm 9.0 | 29 \pm 0.3 | 812 \pm 0.3 |
| OM (g kg ⁻¹) | 601 \pm 2.2 | 644 \pm 2.8 | 749 \pm 3.8 | 732 \pm 3.4 | 748 \pm 2.4 | 298 \pm 0.8 | 839 \pm 9.9 | 569 \pm 3.0 | 398 \pm 1.4 |
| TC (g kg ⁻¹) | 259 \pm 6.8 | 307 \pm 6.1 | 369 \pm 11.1 | 256 \pm 5.3 | 272 \pm 1.4 | 153 \pm 1.7 | 467 \pm 0.0 | 277 \pm 2.7 | 194 \pm 0.2 |
| TOC (g kg ⁻¹) | 227 \pm 22.6 | 285 \pm 15.4 | 336 \pm 23.8 | 215 \pm 29.0 | 232 \pm 28.2 | 151 \pm 1.2 | 435 \pm 0.0 | 214 \pm 44.6 | 181 \pm 0.0 |
| DOC (g kg ⁻¹) | 101.1 | 60.7 | 122.4 | 106.4 | 97.2 | / | 4.9 | 63.0 | 11.1 |
| TN (g kg ⁻¹) | 85.2 \pm 0.2 | 71.3 \pm 0.4 | 82.8 \pm 1.4 | 98.7 \pm 2.2 | 96.0 \pm 0.4 | 14.3 \pm 0.4 | 17.3 \pm 0.0 | 139.0 \pm 0.0 | 34.6 \pm 0.4 |
| P (g kg ⁻¹) | 25.4 \pm 0.2 | 35.5 \pm 0.1 | 17.3 \pm 0.6 | 22.7 \pm 0.8 | 10.4 \pm 2.3 | 3.2 \pm 0.4 | 1.1 \pm 0.1 | 15.4 \pm 0.7 | 5.4 \pm 0.2 |
| NH ₄ ⁺ -N (g kg ⁻¹) | 55.2 \pm 5.2 | 38.0 \pm 1.1 | 44.1 \pm 2.2 | 63.0 \pm 1.0 | 67.5 \pm 7.8 | 0.0 \pm 0.0 | / | 100.5 \pm 2.7 | 13.1 \pm 0.0 |
| NO ₃ ⁻ -N (g kg ⁻¹) | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 0.7 \pm 0.0 | / | 0.0 \pm 0.0 | 0.0 \pm 0.0 |
| TC:TN | 3.04 | 4.31 | 4.46 | 2.60 | 2.83 | 10.68 | 26.97 | 1.99 | 5.60 |
| TOC:TC | 0.88 | 0.93 | 0.91 | 0.84 | 0.85 | 0.99 | 0.93 | 0.77 | 0.94 |
| TC:N _{org} | 8.64 | 9.22 | 9.54 | 7.18 | 9.53 | 11.23 | / | 7.19 | 9.0 |
| TOC:TN | 2.67 | 4.00 | 4.05 | 2.18 | 2.41 | 10.59 | 25.15 | 1.54 | 13.9 |
| NH ₄ ⁺ -N:TN | 0.65 | 0.53 | 0.53 | 0.64 | 0.70 | 0.00 | / | 0.72 | 0.38 |
| N _{org} :TN | 0.35 | 0.47 | 0.47 | 0.36 | 0.30 | 0.95 | / | 0.28 | 0.61 |
| DOC:TOC | 0.44 | 0.21 | 0.36 | 0.49 | 0.42 | / | 0.01 | 0.30 | 0.06 |

EC = electrical conductivity; DM = dry matter; FM = fresh matter; OM = organic matter; TC = total carbon; TOC = total organic carbon; DOC = dissolved organic carbon; TN = total nitrogen; NH₄⁺-N = ammonium nitrogen; NO₃⁻-N = nitrate nitrogen; N_{org} = organic nitrogen; P = phosphorus. Tested products were: digestate (D) from biowaste (D_BW); sewage sludge (D_SS); corn silage (D_CS); pig manure (D_PM); chicken manure (D_CM); compost (COM_1 for N incubations and COM_2 for C incubations); undigested pig slurry (U_PS); solid fraction digestate from chicken manure (SF_CM).

The N_{min} was predominantly in NH₄⁺-N form, as can be expected after AD [45], with NH₄⁺-N:TN ratios ranging from 0.53 to 0.70, also in line with previously reported values [37]. The U_PS product had the highest amount of NH₄⁺-N (101 g kg⁻¹ DM) and TN (139 g kg⁻¹ DM) and also the highest NH₄⁺-N:TN ratio (0.72), suggesting it contained the largest amount of plant-available N for immediate supply. The SF_CM values were comparatively lower, with 13 g NH₄⁺-N kg⁻¹ DM and 35 g TN kg⁻¹ DM, resulting in a much lower NH₄⁺-N:TN (0.38), while TOC:TN (13.9) was noticeably higher than for digestates and closer to the composts. The highest TC:TN and TOC:TN belonged to the two composts (COM_1 and COM_2), which also had the lowest amounts of total Kjeldahl N, indicating a better suitability as a soil improver than fertiliser [23]. COM_2 had the lowest DOC at 4.9 g DOC kg⁻¹, which agreed with the findings of Zmora-Nahum et al. [46], who reported 4 g DOC kg⁻¹ as indicative of stable compost. The C:N ratio of COM_2 (27) also pointed to a mature compost, within the optimal range [47]. The DOC:TOC ratio of digestates ranged from 0.20 to 0.42, indicating that in some cases, relatively substantial amounts of C were still present in water-soluble form. However, this variability is expected from organic-based fertilisers, as evidenced by the similar ratios reported in other studies ranging from as little as 0.03 to as much as 0.64 [22,48]. In this respect, the SF_CM was much closer to COM_2, with these products displaying DOC:TOC ratios of 0.06 and 0.01, respectively. The BOD value (data not shown) of COM_2 was the lowest at 9.7 g O₂ kg⁻¹, which would tend to indicate stable C compounds. The COM_1 contained small amounts of NO₃⁻-N (0.74 g NO₃⁻-N kg⁻¹ DM), whereas NO₃⁻-N went undetected in the other products. The N_{org}:TN ratio ranged from 0.30 (D_CM) to 0.47 (D_CS and D_SS). The digestates exhibited TC:N_{org} ratios ranging from 7.2 (D_PM) to 9.5 (D_CS). As for U_PS, N_{org}:TN was 0.31 and TC:N_{org} was the lowest of all at 6.2. N_{org}:TN from SF_CM (0.61) was roughly midway between U_PS and raw digestates.

3.2. C Mineralisation

The addition of all products to the soil induced an increased microbial activity as highlighted by the curves of respired CO₂-C (Figure 1).

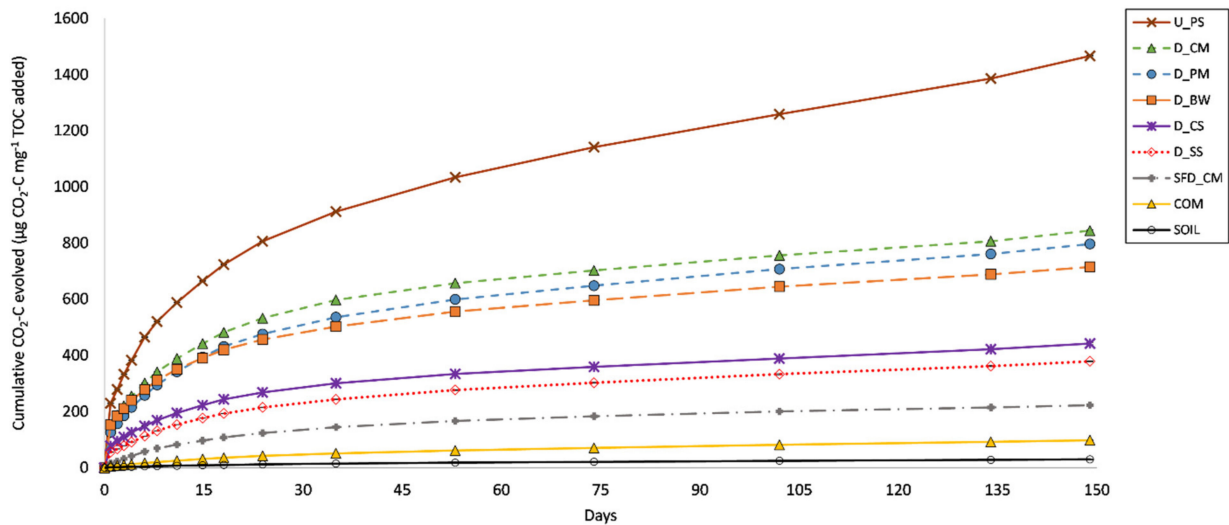


Figure 1. CO₂-C efflux ($\mu\text{g CO}_2\text{-C mg}^{-1}\text{ TOC}$) of unfertilised and fertilised soils over 149-day incubation experiment ($n = 3$, mean value \pm standard deviation, where absent, error bars fall within symbols). Tested products are: digestate (D) from biowaste (D_BW); sewage sludge (D_SS); corn silage (D_CS); pig manure (D_PM); chicken manure (D_CM); compost (COM_1); undigested pig slurry (U_PS); solid fraction digestate from chicken manure (SF_CM).

The C_{\min} pattern was significantly different for all treatments ($p < 0.05$) on day 149. In general, the microbial response was much higher from the added digestates and U_PS, than from SF_CM and COM_2 (Figure 2). The U_PS product triggered the highest respiratory response ($1466 \mu\text{g CO}_2\text{-C mg}^{-1}\text{ TOC}$), with almost double the value of the second highest treatment (Table A1, Appendix A), while the lowest response came from COM_2 ($98 \mu\text{g CO}_2\text{-C mg}^{-1}\text{ TOC}$). Cavalli et al. [49] had reported a similar trend in which microbial respiration from undigested slurry was markedly higher than for digestate, which was ascribed to the undigested slurry containing significantly higher amounts of volatile fatty acids. The C_{\min} (Equation (3)), expressed as a percentage of added TOC, followed almost the same order as evolved CO₂-C, except for D_CM (53%) finding itself in front of U_PS (46%), tailed by D_BW (41%), D_CS (29%), D_SS (20%), SF_CM (14%) and COM_2 (1%) (symbols in Figure 2).

The experimental data was fed into several models (parallel first- and zero-order, first-order with 2 pools, second-order), and the best fit was obtained with the second-order model. Indeed, the amount of mineralised C (C_A) determined by this model (Table 5) showed the highest coefficients of determination and followed a similar order as the experimental C_{\min} results (Table 6), with the slight difference that U_PS and D_PM switched places. The second-order model was used to extrapolate the amount of effective organic carbon (EOC) remaining after 365 days at the average annual field temperature in Belgium (9.7°C) as a proxy for the stable, potentially sequestrable, fraction of C supplied by each product. The EOC expressed as a percentage of added TOC contained in each product was as follows (Table 5): COM_2 (99%); SF_CM (86%); D_SS (81%); D_CS (74%); D_BW (61%); D_PM (57.3%); U_PS (56.9%); D_CM (50%). Almost identical EOC coefficients were reported for compost (93–95%) and solid fractions (70–75%) under similar experimental conditions [50,51].

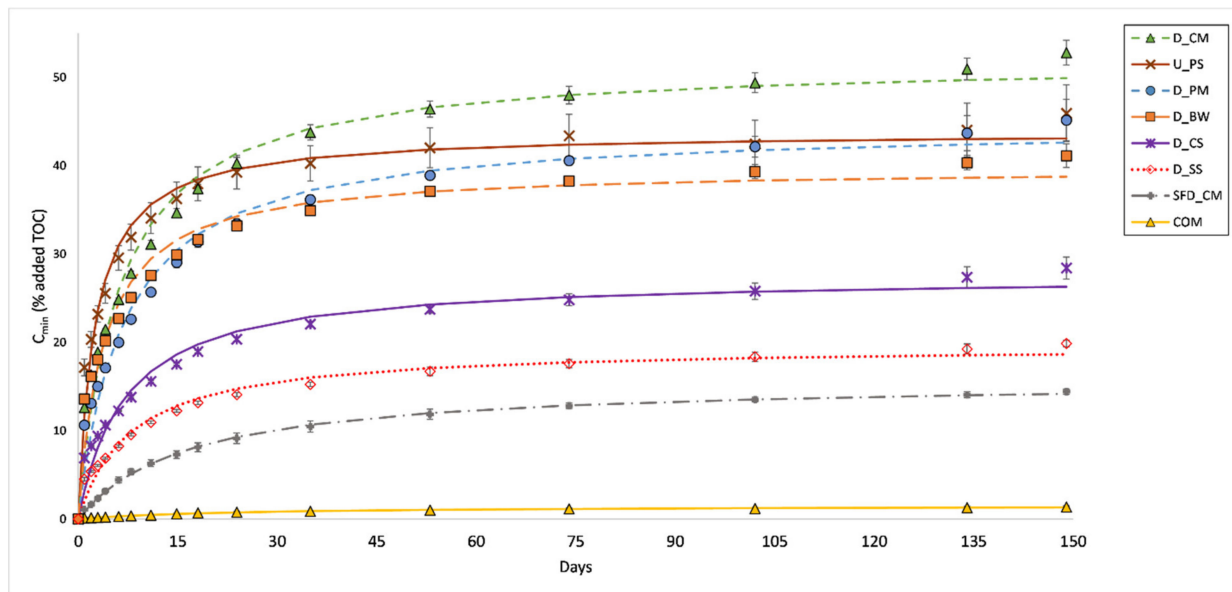


Figure 2. C mineralisation (% added TOC) of fertilised soils over 149-day incubation experiment ($n = 3$, mean value \pm standard deviation, where absent, error bars fall within symbols). Lines represent the curve-fitting result; symbols are experimental data. Tested products are: digestate (D) from biowaste (D_BW); sewage sludge (D_SS); corn silage (D_CS); pig manure (D_PM); chicken manure (D_CM); compost (COM_1); undigested pig slurry (U_PS); solid fraction digestate from chicken manure (SF_CM).

Table 5. Main parameters of the 2nd order kinetic model from the C incubation experiment and CUE results.

| Product | C_A (%) | $k_2a(1 - a)$ | R^2 | EOC (%) | CUE |
|---------|-----------|---------------|-------|---------|-----------------|
| D_BW | 39.7 | 0.0132 | 0.967 | 61.2 | 16.2 \pm 1.5 |
| D_SS | 19.6 | 0.0129 | 0.976 | 81.3 | 13.7 \pm 0.8 |
| D_CS | 27.5 | 0.0103 | 0.968 | 73.7 | 19.5 \pm 7.2 |
| D_PM | 44.6 | 0.0065 | 0.978 | 57.3 | 13.3 \pm 10.3 |
| D_CM | 52.0 | 0.0063 | 0.979 | 50.0 | 13.3 \pm 8.7 |
| COM_2 | 1.5 | 0.0533 | 0.995 | 98.7 | 40.9 \pm 10.7 |
| U_PS | 43.8 | 0.0181 | 0.978 | 56.9 | 2.3 \pm 3.4 |
| SF_CM | 15.8 | 0.0076 | 0.999 | 85.8 | 40.9 \pm 3.3 |

C_A = the amount of mineralised C; k_2 = the second-order mineralisation rate; a = the amount of mineralised C substrate that becomes part of the microbial biomass; R^2 = coefficient of determination of the 2nd order model; EOC = effective organic carbon (stable C after 365 days); CUE = carbon use efficiency of the fertilised soil treatments on day 149 of C incubation experiment.

Table 6. Main N_{rel} and C_{min} results from the incubation experiments ($n = 3$, mean value \pm standard deviation).

| Parameter | D_BW | D_SS | D_CS | D_PM | D_CM | COM * | U_PS | SF_CM |
|------------------|------------------------------|-----------------------------|------------------------------|------------------------------|------------------------------|----------------------------|-----------------------------|-----------------------------|
| N_{rel} (%TN) | 72.2 \pm 4.8 ^{cd} | 62.6 \pm 2.8 ^c | 68.6 \pm 8.4 ^{cd} | 77.9 \pm 2.3 ^{de} | 80.8 \pm 6.3 ^{de} | 8.7 \pm 1.7 ^a | 86.8 \pm 1.5 ^e | 47.7 \pm 4.3 ^b |
| C_{min} (%TOC) | 41.1 \pm 1.4 ^e | 19.9 \pm 0.4 ^c | 28.4 \pm 1.2 ^d | 45.1 \pm 2.4 ^{ef} | 52.8 \pm 1.4 ^g | 1.4 \pm 0.2 ^a | 45.9 \pm 3.2 ^f | 14.4 \pm 0.3 ^b |

N_{rel} = the amount (%) of added TN mineralised on the last day of the N incubation experiment (day 127); C_{min} = the amount of added TOC mineralised on the last day of the C incubation experiment (day 149). Treatments with the same letters are not statistically different according to Tukey’s HSD test (significance level of 0.05). * Soil and products were identical for both experiments except for COM_1 (used of N incubations), and COM_2 (used for the C incubations).

The EOC was significantly positively correlated to TC:TN ($r = 0.92$, $p < 0.01$) and negatively to the initial NH_4^+ -N:TN ratio ($r = -0.93$, $p < 0.01$) (Table 7). The highest

amounts of EOC were found in COM_2 and SF_CM, which also happened to have the lowest DOC values, respectively at 4.9 and 11.1 g DOC kg⁻¹ DM. For digestates, the highest EOC was found in the D_SS treatment (81%), while this product also had the lowest amount of DOC (60.7 g DOC kg⁻¹ DM).

Table 7. Overview of the most significant correlations observed between initial digestate compositional properties, C and N mineralisation kinetics and carbon use efficiency (CUE) ($n = 8$).

| | CUE | EOC | C _{min} | CO ₂ -C | N _{rel} | N _{min,net} | NH ₄ ⁺ -N:TN | TC:TN | DOC | DOC:TOC |
|------------------------------------|----------|----------|------------------|--------------------|------------------|----------------------|------------------------------------|---------|---------|---------|
| CUE | 1 | | | | | | | | | |
| EOC | 0.82 * | 1 | | | | | | | | |
| C _{min} | -0.82 * | -1.00 ** | 1 | | | | | | | |
| CO ₂ -C | -0.87 ** | -0.84 ** | 0.84 ** | 1 | | | | | | |
| N _{rel} | -0.90 ** | -0.93 ** | 0.93 ** | 0.91 ** | 1 | | | | | |
| N _{min,net} | -0.74 | -0.7 | 0.71 | 0.88 ** | 0.87 ** | 1 | | | | |
| NH ₄ ⁺ -N:TN | -0.82 * | -0.93 ** | 0.94 ** | 0.81 * | 0.99 ** | 0.83 * | 1 | | | |
| TC:TN | 0.77 * | 0.92 ** | -0.92 ** | -0.79 * | -0.99 ** | -0.83 * | -0.99 ** | 1 | | |
| DOC | -0.75 * | -0.75 * | 0.75 * | 0.56 | 0.53 | 0.36 | 0.76 * | -0.72 * | 1 | |
| DOC:TOC | -0.80 * | -0.88 ** | 0.89 ** | 0.68 * | 0.72 | 0.46 | 0.81 * | -0.78 * | 0.95 ** | 1 |

N_{rel} = the amount (%) of added TN mineralised on the last day of the N incubation experiment (day 127); N_{min,net} = the amount (%) of added N_{org} mineralised on the last day of the N incubation experiment (day 127); NH₄⁺-N = ammonium nitrogen (g kg⁻¹ DM); TN = total nitrogen (g kg⁻¹ DM); C_{min} = the amount of added TOC mineralised on the last day of the C incubation experiment (day 149); CO₂-C = the amount of evolved CO₂-C (μg mg⁻¹ TOC) from the fertilised soil treatments on the last day of the C incubation experiment (day 149); CUE = carbon use efficiency of the fertilised soil treatments on the last day of the C incubation experiment (day 149); EOC = the effective organic carbon that remains after 365 days (%TOC); DOC = the dissolved organic carbon (g kg⁻¹ DM). * Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

As a general pattern, the higher the observed CUE, the higher the EOC ($r = 0.82$, $p < 0.05$) of the product and, conversely, the higher the DOC of the product, the lower the CUE ($r = -0.75$, $p < 0.05$) (Table 7). In effect, suggesting that the easily accessible DOC induced a higher respiration and a lower microbial biosynthesis, as evidenced for instance by U_PS, which had the lowest CUE (2%), the highest CO₂-C respiratory activity and one of the lowest EOC values (Table 5). On the contrary, COM_2 and SF_CM had the highest CUE (both 41%) and also exhibited the lowest respired CO₂-C and the highest EOC potentials. In agreement with Albuquerque et al. [22], a significantly positive correlation was observed between BOD and DOC content ($r = 0.86$, $p = 0.01$; not shown). In particular, it was noted that COM_2 and the SF_CM had the lowest BOD values (data not shown), respectively 9.7 and 51.0 mg O₂ kg⁻¹ DM, and the lowest C_{min} rates. However for this study, BOD taken as an indicator of OM stability, seemingly did not yield significant relationships with C_{min} activity ($r = 0.57$; not shown), but when BOD was expressed over TOC (BOD:TOC), the positive relationship with C_{min} increased somewhat ($r = 0.67$; not shown), thus effects of BOD on C kinetics should not be ruled out.

3.3. N Mineralisation

On day 0, NH₄⁺-N content of the treatments was based on the added amounts of each product and their initial properties. From highest NH₄⁺-N content to lowest, the order was as follows: U_PS > D_CM > D_PM > D_BW > D_CS > D_SS > SF_CM > COM_1 = SOIL. Thus, all products supplied readily available amounts of N in the form of NH₄⁺-N on day 0. NH₄⁺-N concentrations dropped rapidly to trace amounts by day 40 (Figure 3), up until the end of the experiment (day 127), which suggests the absence of anaerobic conditions [39]. Ammonium-N losses during incubations, with similar moisture conditions, have been reported to be minimal [52] especially considering the acidic pH of our soil (5.3). Furthermore the loamy sand soil used in this experiment had low clay content (Section 2.2) with likely negligible NH₄⁺ fixation in the clay interlayer space [53].

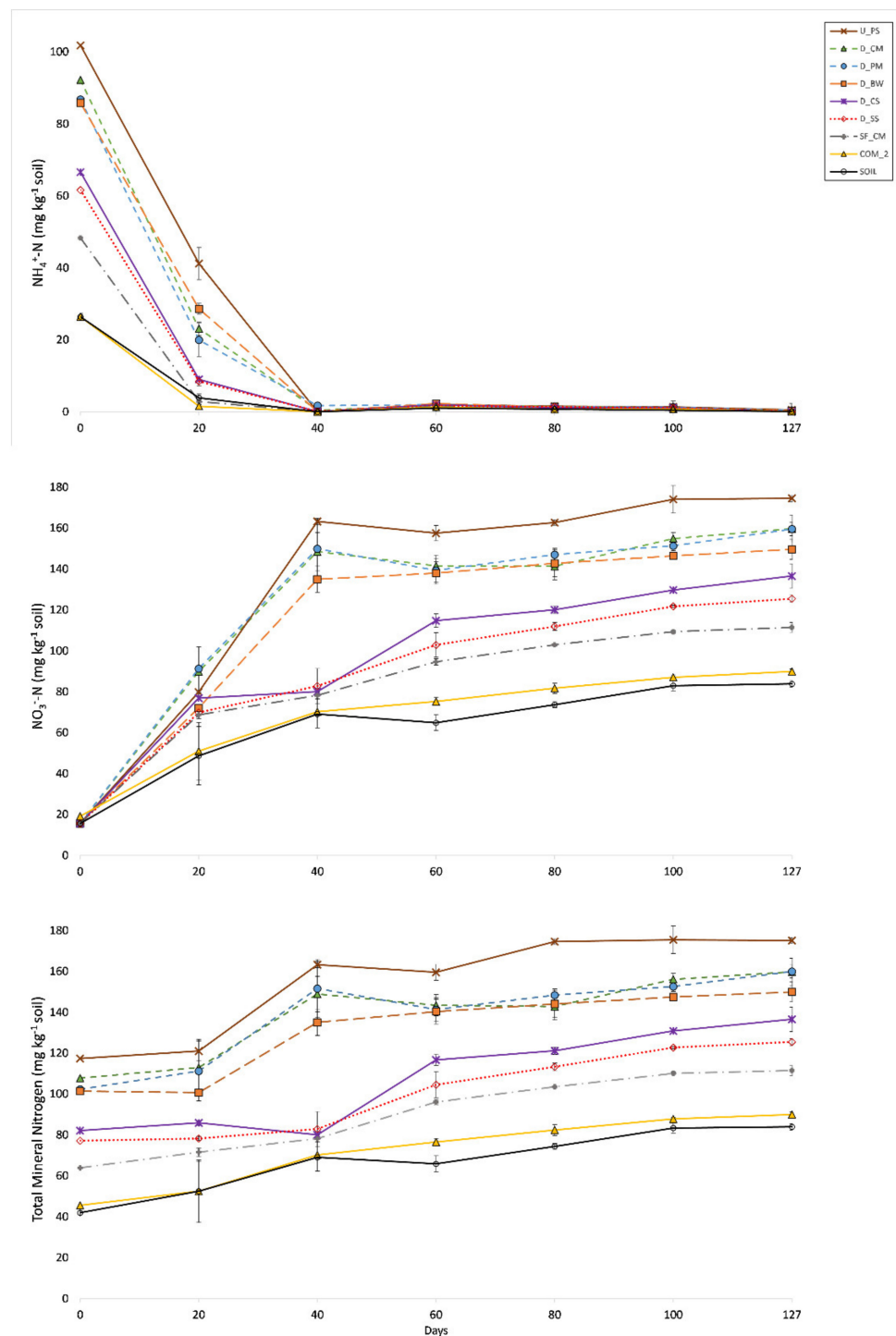


Figure 3. Evolution of NH₄⁺-N (top), NO₃⁻-N (middle); total mineral N (bottom) (mg kg⁻¹) in unfertilised soil and soil treated with digestate and reference products ($n = 3$, mean value \pm standard deviation, where absent, error bars fall within symbols) over 127-day incubation experiment. Tested products are: digestate (D) from biowaste (D_BW); sewage sludge (D_SS); corn silage (D_CS); pig manure (D_PM); chicken manure (D_CM); compost (COM_1); undigested pig slurry (U_PS); solid fraction digestate from chicken manure (SF_CM).

From day 0 to 20, NH₄⁺-N in the control soil dropped from 26.4 (\pm 0.1) to 3.84 (\pm 1.1) mg NH₄⁺-N kg⁻¹ soil and by day 40 onwards never exceeded 1 mg NH₄⁺-N kg⁻¹ soil. As for NO₃⁻-N, a steady increase in NO₃⁻-N could be observed from the initial

15.58 (± 2.1) NO_3^- -N kg^{-1} soil, with a slight drop from day 40 to day 60 (64.8 ± 3.8), which can be ascribed to rewetting events on days 40 and 80, which might have triggered a slightly higher mineralisation and nitrification activity. From day 60 onwards, a steady increase was observed, finally reaching $83.87 (\pm 1.7)$ mg NO_3^- -N kg^{-1} soil by the end of the experiment on day 127. The COM_1 closely followed the same pattern as the unfertilised soil (Figure 3) with only trace amounts of NH_4^+ -N detected on day 0. All the digestate treatments and U_PS, which had higher amounts of initially available NH_4^+ -N, reached values neighbouring the 0 mark by day 40. Concomitantly, a rapid increase in NO_3^- -N could be observed (Figure 3), which suggests that the most probable fate of NH_4^+ -N was nitrification [23,54,55]. On day 0, the amount of NO_3^- -N was similar for all treatments and was supplied mostly by the soil (15.6 mg NO_3^- -N kg^{-1} soil), as NO_3^- -N was not detected in the digestates and only a small quantity was already present in COM_1 (Table 4).

On the final day of the incubations, N_{rel} (Equation (1)) calculated as a percentage of total added N from each treatment after having subtracted the N_{min} contribution of the unfertilised soil control, was as follows (Table 6): 87% (U_PS); 81% (D_CM); 78% (D_PM); 72% (D_BW); 69% (D_CS); 63% (D_SS); 48% (SF_CM); 9% (COM_1). In other words, the raw digestates displayed fertilising values, in terms of plant-available mineral N, between 60 and 80% of the TN applied. Net N mineralisation ($N_{\text{min,net}}$) was calculated by subtracting the N_{min} content of the treatment on day 0 from the N_{min} at all subsequent measurements and expressed as percentage of organic N (% N_{org}) (Equation (2)). All treatments yielded positive $N_{\text{min,net}}$ results on day 127: 52% (U_PS); 39% (D_PM); 35% (D_CM); 33% (D_CS); 21% (D_BW); 20% (D_SS); 16% (SF_CM); 4% (COM_1).

These results generally agree with previously reported N mineralisation ranges for digestates [23,49,56]. On the final day, N_{rel} ($r = 0.99$, $p < 0.01$) and $N_{\text{min,net}}$ ($r = 0.83$, $p < 0.05$) were significantly positively correlated with the initial NH_4^+ -N:TN ratio of the products (Table 7), thus reconfirming the validity of this parameter to predict plant-available N from the products [20,57].

It is generally accepted that products within a 1 to 15 C:N ratio are more likely to trigger a rapid release of N, whereas C:N ratios > 35 usually favour net N immobilisation [58]. Given that the C:N ratios of digestates usually fall between 3 and 8.5 [59], they most often lead to a rapid net N mineralisation in the soil and concomitantly a sharp increase of plant-available N [60]. This study reaffirmed this observation, as it was found that the digestates with higher NH_4^+ -N:TN and lower TC:TN ratios (than COM_1 and SF_CM), exhibited higher N_{rel} and $N_{\text{min,net}}$ results. As illustrated by the three highest performing treatments in terms of $N_{\text{min,net}}$ (D_PM, U_PS and D_CM), which were characterised by a lower TC:TN range (2.0 to 2.8), while the NH_4^+ -N:TN ratio was on the higher end of the spectrum (0.6 to 0.7). This observation was supported by the significantly negative relationships found between, respectively, N_{rel} ($r = -0.99$, $p < 0.01$) and $N_{\text{min,net}}$ ($r = -0.83$, $p < 0.05$) with the initial TC:TN of the products, thus reasserting the importance of the C:N ratio of the input materials to predict N mineralisation outcome in soil, in agreement with other findings [61,62].

4. Discussion

4.1. C Mineralisation

A substrate's C:N ratio is generally recognised as a crucial parameter linked to both C and N dynamics resulting from the addition of OM to soil [63,64]. Here, C_{min} was strongly negatively correlated with TC:TN, and evolved CO_2 -C was positively correlated to C_A , taken as the easily mineralised pool of C. This was in general agreement with Riffaldi et al. [65], who reported that lower C:N ratios led to higher C mineralisation activity. A highly significant positive relationship between initial NH_4^+ -N content of the products and respired CO_2 -C on the last day of incubations, and a negative correlation between N_{org} :TN of products with both CO_2 -C and C_{min} points towards NH_4^+ -N having stimulated soil heterotrophic activity, as was already observed in previous studies [23,66]. It cannot be excluded that high NH_4^+ -N contents were just indicative of inherent high biological lability

of the added OM, and so via mutual covariation with indicators of C-lability, co-varied with C_{\min} . Indeed products with a high NH_4^+ -N content also had high DOC:TOC ratio and low TC: N_{org} ratio, while C_{\min} was positively correlated to the DOC:TOC of the products ($r = 0.89$, $p < 0.01$).

The DOC content is usually closely related to microbial activity, owing to the dissolved fraction being generally more biologically available to microorganisms [67]. Compared with COM_2 and the control soil, the addition of digestates and U_PS resulted in a marked increase in microbial activity as indicated by the higher respiration (Figure 1) and C_{\min} rates (Figure 2). These observations were also supported by the positive correlations found between the C_A parameter of the second-order kinetic model with the measured C_{\min} and respired CO_2 -C on day 149 of C incubations ($r = 0.99$, $p < 0.01$; $r = 0.82$, $p < 0.05$, respectively). As already pointed out (Section 3.2), a negative correlation between CUE and the amount of DOC initially present in the products ($r = -0.75$, $p < 0.05$) and their DOC:TOC ratio ($r = -0.80$, $p < 0.05$) was also noted. This would suggest that the higher amounts of DOC present induced higher microbial respiration, hence resulting in a lower microbial biosynthesis, and in turn, a lower CUE. This pattern was illustrated for instance by U_PS, which was behind the lowest CUE (2%) and the highest CO_2 -C efflux. While the initial DOC content of U_PS was not the highest, the DOC:TOC ratio proved a meaningful indicator of C dynamics overall as it showed significantly positive correlations with the CO_2 -C efflux and the C_{\min} rate ($r = 0.68$, $p < 0.05$ and $r = 0.89$, $p < 0.01$, respectively). In other words, as a general trend, the higher the DOC and DOC:TOC of the product, the higher the incumbent CO_2 -C efflux and C_{\min} rates of a given treatment, as exemplified for instance by COM_2 and SF_CM, both containing comparatively low amounts of DOC (5 and 11 g DOC kg^{-1} , respectively) and exhibiting the lowest C_{\min} and CO_2 -C activity, while higher DOC concentrations in digestates and U_PS led to a much higher microbial activity. Thus, DOC and DOC:TOC appeared to be important drivers of C dynamics in this study, in light of which it might be argued that the digestates (and U_PS) could have contained more easily degradable organic compounds, as evidenced by DOC content, than the aerobically stabilised COM_2, which in turn led to higher CO_2 -C and C_{\min} activities. This observation is in agreement with findings from Kirchmann & Bernal [68], who compared anaerobically-treated and composted materials, and pointed to the former containing less stable C than the latter, thus leading to higher respiration rates.

The CUE (Equation (7)) was intended to measure the TOC that is metabolised and allocated to microbial growth, thus serving as an indicator of the efficiency with which microbial communities could assimilate the C supplied by the treatments. In this regard, CUE results in this study seemed to support the aforementioned observations. Interestingly, a negative correlation ($r = -0.75$, $p < 0.05$) was also observed between the amount of DOC and EOC. This tends to corroborate the accuracy of the second-order kinetic model as this would signify that the higher the amount of DOC contained in the products, considered as the easily degradable fraction of C, the lower the expected EOC value. In one of the few studies to our knowledge that looked into the CUE of soils treated with digestate, Cattin et al. [29] reported a generally positive effect of the solid fraction of digestate on CUE. Our findings agree with this observation, though admittedly additional studies are probably still required to draw any further comparisons or trends.

4.2. C Sequestration Potential and Possible Implications for C Farming Strategies

The strong fit with the second-order kinetic model allowed us to predict the fate of C after 365 days, considered as the effective pool of organic C (EOC), with a high degree of confidence (Table 5). The results showed that, without reaching the levels of EOC contained in conventional soil improvers such as compost (99% for COM_2), the application of digestate could bring an added environmental benefit in terms of C build-up in agricultural soils. The extrapolation of the model showed that the digestates in this study could theoretically contribute between 205 (D_CM) and 553 (D_SS) kg EOC based on an application rate of 170 kg N ha^{-1} year $^{-1}$ in NVZ. Incidentally, among these products, three

of the digestates (D_BW; D_PM and D_CM) qualified under the $\text{TOC:TN} \leq 3$ RENURE criterion [6], meaning their EOC contribution could be higher and would depend on the specific N demands of the considered crop. Continuing with the exercise, SF_CM held a potential $2185 \text{ kg EOC ha}^{-1}$, thus markedly differentiating itself from the raw digestates and positioning itself closer to a soil improver, in this case COM_2, which provided an estimated $4245 \text{ kg EOC ha}^{-1}$.

The metabolic mechanisms that give rise to C release into the environment, or on the contrary to C stabilisation, are still largely misunderstood, while evolving views on the matter have been put forward to better explain soil microbial dynamics. One such conceptual framework, the 'microbial carbon pump', argues that most of the stable C, in this case represented by EOC, is derived from the labile pool of C, which is converted via *in vivo* turnover (anabolism) into microbial necromass, considered a precursor for stable C (the so-called entombing effect) [69–71]. In our study, DOC:TOC ratios of digestates ranged from 0.21 to 0.49, which hints at relatively high amounts of labile C still being present in some cases, whereas the EOC of digestates ranged from 50 to 81% (in some cases relatively high). It should thus not be excluded that part of the DOC contained in digestate, as a readily available source of C to microbes, was assimilated and transformed into more recalcitrant forms, via microbial necromass, as this theory proposes. On the other hand, other studies point towards the physical protection of organic C aggregates [72,73] and heterogeneous binding mechanisms [74] as the main drivers behind C storage dynamics, parameters which were not assessed in this study.

4.3. N Mineralisation

An initial drop in $N_{\text{min,net}}$ activity was observed, which could indicate N immobilisation, between days 0 and 20 for some products (D_PM; D_CM; D_BW) and between days 0 and 40 for others (SF_CM; D_CS; D_SS), followed by subsequent remineralisation (Figure 4). This could be explained by the fact that a strong respiratory activity was registered at the beginning of the incubation experiment. Thus, as was noted by Albuquerque et al. [22], N might have been immobilised initially into microbial biomass when the applied labile C from the products was in relative abundance. For the digestates in particular, a seemingly steeper immobilisation seemed to have occurred in the case of D_CS and D_SS. It may be explained by these products having higher TC:TN and $N_{\text{org}}:\text{TN}$ ratios, thus a lower amount of readily available $\text{NH}_4^+\text{-N}$. This observation is supported by the significant relationships observed between $N_{\text{min,net}}$ and, respectively, $N_{\text{org}}:\text{TN}$ ($r = -0.83$, $p < 0.05$) and TC:TN ($r = -0.83$, $p < 0.05$).

A possible lower N availability might have induced N-acquiring microorganisms to use the available labile C as primary energy source to break down the N embedded in the recalcitrant OM, in accordance with the microbial N mining hypothesis [63,75]. In support of this observation, D_CS and D_SS had the highest amounts of EOC of the studied digestates. Similarly, U_PS was the only product that apparently gave off a positive $N_{\text{min,net}}$ activity from the start (i.e., no immobilisation) and also had the lowest TC:TN, $N_{\text{org}}:\text{TN}$ and EOC, which would tend to lend further credibility to the aforementioned explanation. Finally, in the absence of a monitoring of pH, N_2O and NH_3 emissions during the incubation experiment, an initial volatilisation of $\text{NH}_4^+\text{-N}$ between days 0 and 40 cannot be completely ruled out either (in spite of all the precautions that were taken (Section 3.3)).

Digestate is generally reported as having a superior N-fertilising value compared to the undigested feedstock, mostly due to a higher $\text{NH}_4^+\text{-N}$ content, lower DM (better infiltration) and a lower C:N ratio [49,57,59,61]. Unexpectedly, U_PS, which was included to represent the fate of available N from undigested manure against that of digested feedstocks, outperformed the digestates in terms of N mineralisation (Figure 4). While this result may seem contradictory at first, a closer look at some of the agrochemical properties of U_PS (Table 4) confirmed the importance of the abovementioned parameters. Indeed, U_PS exhibited the highest $\text{NH}_4^+\text{-N}$ content of all products ($101 \text{ g NH}_4^+\text{-N kg}^{-1} \text{ DM}$), the lowest DM content ($29 \text{ g kg}^{-1} \text{ FM}$) and the lowest TC:TN and TOC:TN ratios (1.9 and

1.5, respectively). The explanation behind these comparatively exceptional values is that U_PS was collected from the surface of an unmixed slurry pit, where the sedimentation of solids over time led to a thinned out upper layer and a thick bottom layer. This would explain the low DM and the high $\text{NH}_4^+\text{-N}$ content, owing to the slurry containing a larger volume of ammonium-containing liquid fraction in the form of pig urine and less solids (C compounds). Thus, resulting overall in a lower TC:TN ratio. In this respect, U_PS was the exception that confirmed the rule, insofar that $\text{NH}_4^+\text{-N}:\text{TN}$ ($r = 0.83$, $p < 0.05$) and TC:TN ($r = -0.83$, $p < 0.05$) were found to be sound predictors of N mineralisation patterns and tended to corroborate the importance of such parameters to describe N_{min} availability. Nonetheless, it is usually more likely to find such characteristics in digested, rather than undigested, materials [59]. Makara et al. [76] found DM and BOD to be statistically correlated in undigested manures, where a higher DM content led to a higher BOD. In this respect also, the outstanding character of U_PS was reconfirmed as it was on the lower end of the spectrum for DM ($28.6 \text{ g kg}^{-1} \text{ FM}$) and BOD ($1.5 \text{ g O}_2 \text{ L}^{-1}$), when compared with values reported in the literature, which ranged anywhere from 1.0 [77] up to as much as $80 \text{ g O}_2 \text{ L}^{-1}$ [78].

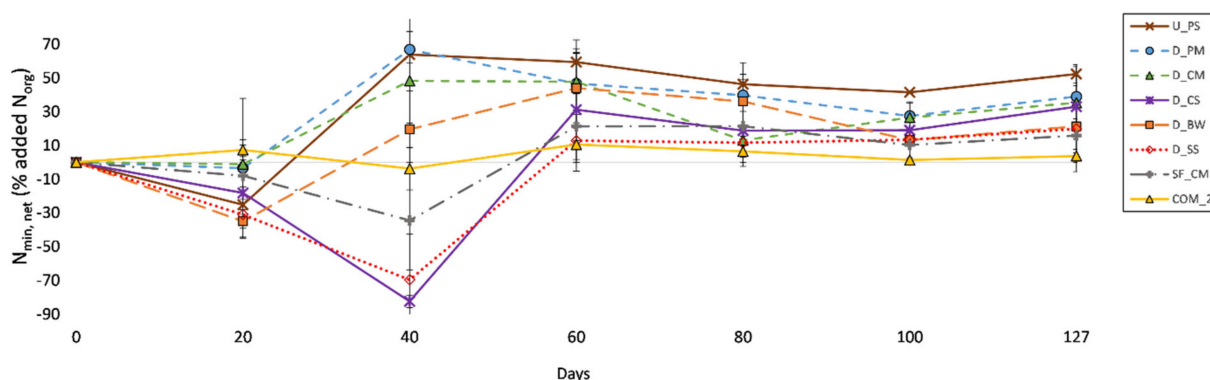


Figure 4. Net N mineralisation (%N_{org}) of soil treated with digestate and reference products (T, mean value \pm standard deviation, where absent, error bars fall within symbols) over 127-day incubation experiment. Tested products are: digestate (D) from biowaste (D_BW); sewage sludge (D_SS); corn silage (D_CS); pig manure (D_PM); chicken manure (D_CM); compost (COM_1); undigested pig slurry (U_PS); solid fraction digestate from chicken manure (SF_CM).

By and large, when setting the $N_{\text{min,net}}$ and N_{rel} performance of COM_2 (4 and 9%, respectively) against that of the digestates, there seemed to be little room for interpretation as regards digestate's first vocation as a fertiliser, with net $N_{\text{min,net}}$ rates ranging between 20 and 39% by day 127, and 63% and 81% for N_{rel} . These results tend to agree with previously reported trends [23,56] and point towards digestate being a suitable quick-release fertiliser. It is however also worth paying attention to the remaining N_{org} pool contained in the digestates, as inferred by their $N_{\text{min,net}}$ values, which suggest that anywhere between 60 to 80% of N_{org} was still present in the treated soils after 127 days (excluding any N priming effects from the native soil). In this respect, a study of the longer-term N dynamics of digestates in a plant–soil system in regard to the subsequent mineralisation kinetics of the remaining N_{org} would be warranted. This could provide valuable insight into the synchrony or asynchrony between plant N uptake and N mineralisation kinetics, thereby also assessing any risk of N leaching. In addition, in this study, an initial N immobilisation phase might have occurred for all digestate treatments, in light of which appropriate timing of N fertilisation to avoid any counterproductive effects on crop growth might also be taken into consideration.

5. Conclusions

The C and N mineralisation rates of five digestates from distinct feedstock profiles (i.e., pig manure, poultry manure, energy crops, sewage sludge, food waste) were compared to those of a conventional soil improver (compost), a conventional organic fertiliser (pig slurry) and the solid fraction of digestate (as a hybrid between the 2 former categories) in laboratory microcosm incubations. After 127 days, net N mineralisation (%N_{org}) of digestates ranged from 21 to 39% (63–81% N_{rel}), highlighting the fertilising affinity of these organo-mineral products, but also the wide range of N mineralisation results from the different products, as digestate from pig manure (39%) reached almost double the value of digestate from sewage sludge (21%). The TC:TN and NH₄⁺-N:TN ratios of the products proved to be good predictors of net N mineralisation and, as such, constituted sound indicators of their expected fertilising potential. The observed variability in N mineralisation makes it highly advisable at the very least to systemically proceed to a full physicochemical characterisation of digestate products before each use in the field.

The five tested digestates had EOC values ranging from 50 to 81% of applied TOC. Thus, without reaching the levels of >90% EOC found in composts, the application of digestate would have the potential to meaningfully contribute to C build-up in agricultural soils, in alignment with European C farming policies, and on top of its primary function as N fertiliser. Ratios of DOC:TOC and TC:TN proved good predictors of the fraction of added C that would remain undecomposed in the field one year after its incorporation and could likewise be used as a simple quality parameter denoting the C-sequestration potential of digestates or derived products prior to their use in the field.

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Appendix A

Table A1. Main results of evolved CO₂-C from C incubation experiment after 149 days (*n* = 3, mean value ± standard deviation).

| Parameter | D_BW | D_SS | D_CS | D_PM | D_CM | COM * | U_PS | SF_CM |
|--|---------|---------|---------|---------|---------|--------|----------|---------|
| CO ₂ -C (μg mg ⁻¹ TOC) | 714 ± 2 | 379 ± 1 | 442 ± 4 | 796 ± 4 | 843 ± 2 | 98 ± 1 | 1466 ± 2 | 223 ± 2 |

CO₂-C = the amount of evolved CO₂-C (μg mg⁻¹ TOC) from the fertilised soil treatments on the last day of the C incubation experiment (day 149); TOC = total organic carbon. * Soil and products were identical for both experiments except for COM_1 (used of N incubations), and COM_2 (used for the C incubations).

References

1. Chynoweth, D.P.; Owens, J.M.; Legrand, R. Renewable Methane from Anaerobic Digestion of Biomass. *Renew. Energy* **2001**, *22*, 1–8. [[CrossRef](#)]
2. EBA (European Biogas Association). *EBA Statistical Report 2020*; EBA: Brussels, Belgium, 2020.
3. Leprich, U.; Hoffmann, P.; Luxenburger, M. Certificates in Germany's Renewable Energy Market. In *Marketing Renewable Energy*; Springer: Cham, Switzerland, 2017. [[CrossRef](#)]
4. Horschig, T.; Adams, P.W.R.; Röder, M.; Thornley, P.; Thrän, D. Reasonable Potential for GHG Savings by Anaerobic Biomethane in Germany and UK Derived from Economic and Ecological Analyses. *Appl. Energy* **2016**, *184*, 840–852. [[CrossRef](#)]
5. Logan, M.; Visvanathan, C. Management Strategies for Anaerobic Digestate of Organic Fraction of Municipal Solid Waste: Current Status and Future Prospects. *Waste Manag. Res.* **2019**, *37* (Suppl. S1), 27–39. [[CrossRef](#)] [[PubMed](#)]
6. Huygens, D.; Orveillon, G.; Lugato, E.; Tavazzi, S. *Technical Proposals for the Safe Use of Processed Manure above the Threshold Established for Nitrate Vulnerable Zones by the Nitrates Directive (91/676/EEC)*; JRC: Ispra, Italy, 2020. [[CrossRef](#)]
7. Vaneekhaute, C.; Lebuf, V.; Michels, E.; Belia, E.; Vanrolleghem, P.A.; Tack, F.M.G.; Meers, E. Nutrient Recovery from Digestate: Systematic Technology Review and Product Classification. *Waste Biomass Valoriz.* **2017**, *8*, 21–40. [[CrossRef](#)]
8. Reuland, G.; Sigurnjak, I.; Dekker, H.; Michels, E.; Meers, E. The Potential of Digestate and the Liquid Fraction of Digestate as Chemical Fertiliser Substitutes under the RENURE Criteria. *Agronomy* **2021**, *11*, 1374. [[CrossRef](#)]
9. Romero-güiza, M.S.; Mata-alvarez, J.; María, J.; Rivera, C. Nutrient Recovery Technologies for Anaerobic Digestion Systems: An Overview. *Tecnologías de Recuperación de Nutrientes Para Los Sistemas de Digestión Anaeróbica: Revisión Tecnologías de Recuperação de Nutrientes Para Os Sistemas de Digestão Anaeróbica: R. Bucaramanga* **2015**, *29*, 7–26.
10. Sánchez-Rodríguez, A.R.; Carswell, A.M.; Shaw, R.; Hunt, J.; Saunders, K.; Cotton, J.; Chadwick, D.R.; Jones, D.L.; Misselbrook, T.H. Advanced Processing of Food Waste Based Digestate for Mitigating Nitrogen Losses in a Winter Wheat Crop. *Front. Sustain. Food Syst.* **2018**, *2*, 1–14. [[CrossRef](#)]
11. Insam, H.; Gómez-Brandón, M.; Ascher, J. Manure-Based Biogas Fermentation Residues - Friend or Foe of Soil Fertility? *Soil Biol. Biochem.* **2015**, *84*, 1–14. [[CrossRef](#)]
12. Jurgutis, L.; Šlepetienė, A.; Amalevičiūtė-Volungė, K.; Volungevičius, J.; Šlepetys, J. The Effect of Digestate Fertilisation on Grass Biogas Yield and Soil Properties in Field-Biomass-Biogas-Field Renewable Energy Production Approach in Lithuania. *Biomass Bioenergy* **2021**, *153*, 106211. [[CrossRef](#)]
13. Haraldsen, T.K.; Andersen, U.; Krogstad, T.; Sørheim, R. Liquid Digestate from Anaerobic Treatment of Source-Separated Household Waste as Fertilizer to Barley. *Waste Manag. Res.* **2011**, *29*, 1271–1276. [[CrossRef](#)]
14. Schwager, E.A.; VanderZaag, A.C.; Wagner-Riddle, C.; Crolla, A.; Kinsley, C.; Gregorich, E. Field Nitrogen Losses Induced by Application Timing of Digestate from Dairy Manure Biogas Production. *J. Environ. Qual.* **2016**, *45*, 1829–1837. [[CrossRef](#)] [[PubMed](#)]
15. Nicholson, F.; Bhogal, A.; Cardenas, L.; Chadwick, D.; Misselbrook, T.; Rollett, A.; Taylor, M.; Thorman, R.; Williams, J. Nitrogen Losses to the Environment Following Food-Based Digestate and Compost Applications to Agricultural Land. *Environ. Pollut.* **2017**, *228*, 504–516. [[CrossRef](#)] [[PubMed](#)]
16. Verdi, L.; Kuikman, P.J.; Orlandini, S.; Mancini, M.; Napoli, M.; Dalla Marta, A. Does the Use of Digestate to Replace Mineral Fertilizers Have Less Emissions of N₂O and NH₃? *Agric. For. Meteorol.* **2019**, *269–270*, 112–118. [[CrossRef](#)]
17. Pezzolla, D.; Bol, R.; Gigliotti, G.; Sawamoto, T.; López, A.L.; Cardenas, L.; Chadwick, D. Greenhouse Gas (GHG) Emissions from Soils Amended with Digestate Derived from Anaerobic Treatment of Food Waste. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 2422–2430. [[CrossRef](#)] [[PubMed](#)]
18. Johansen, A.; Carter, M.S.; Jensen, E.S.; Hauggard-Nielsen, H.; Ambus, P. Effects of Digestate from Anaerobically Digested Cattle Slurry and Plant Materials on Soil Microbial Community and Emission of CO₂ and N₂O. *Appl. Soil Ecol.* **2013**, *63*, 36–44. [[CrossRef](#)]
19. Dietrich, M.; Fongen, M.; Foereid, B. Greenhouse Gas Emissions from Digestate in Soil. *Int. J. Recycl. Org. Waste Agric.* **2020**, *9*, 1–19. [[CrossRef](#)]
20. Sharifi, M.; Baker, S.; Hojabri, L.; Hajiaghaei-kamrani, M. Short-Term Nitrogen Dynamics in a Soil Amended with Anaerobic Digestate. *Can. J. Soil Sci.* **2019**, *99*, 173–181. [[CrossRef](#)]
21. Cabrera, M.L.; Kissel, D.E.; Vigil, M.F. Nitrogen Mineralization from Organic Residues. *J. Environ. Qual.* **2005**, *34*, 75–79. [[CrossRef](#)]
22. Alburquerque, J.A.; de la Fuente, C.; Bernal, M.P. Chemical Properties of Anaerobic Digestates Affecting C and N Dynamics in Amended Soils. *Agric. Ecosyst. Environ.* **2012**, *160*, 15–22. [[CrossRef](#)]
23. Tambone, F.; Adani, F. Nitrogen Mineralization from Digestate in Comparison to Sewage Sludge, Compost and Urea in a Laboratory Incubated Soil Experiment. *Z. Pflanzenernahr. Bodenkd.* **2017**, *180*, 355–365. [[CrossRef](#)]
24. de la Fuente, C.; Alburquerque, J.A.; Clemente, R.; Bernal, M.P. Soil C and N Mineralisation and Agricultural Value of the Products of an Anaerobic Digestion System. *Biol. Fertil. Soils* **2013**, *49*, 313–322. [[CrossRef](#)]
25. Qiao, Y.; Wang, J.; Liang, G.; Du, Z.; Zhou, J.; Zhu, C.; Huang, K.; Zhou, X.; Luo, Y.; Yan, L.; et al. Global Variation of Soil Microbial Carbon-Use Efficiency in Relation to Growth Temperature and Substrate Supply. *Sci. Rep.* **2019**, *9*, 1–8. [[CrossRef](#)] [[PubMed](#)]
26. Domeignoz-Horta, L.A.; Pold, G.; Liu, X.J.A.; Frey, S.D.; Melillo, J.M.; DeAngelis, K.M. Microbial Diversity Drives Carbon Use Efficiency in a Model Soil. *Nat. Commun.* **2020**, *11*, 1–10. [[CrossRef](#)]

27. Simon, E.; Canarini, A.; Martin, V.; Séneca, J.; Böckle, T.; Reinthaler, D.; Pötsch, E.M.; Piepho, H.P.; Bahn, M.; Wanek, W.; et al. Microbial Growth and Carbon Use Efficiency Show Seasonal Responses in a Multifactorial Climate Change Experiment. *Commun. Biol.* **2020**, *3*, 1–10. [CrossRef]
28. Kallenbach, C.M.; Wallenstein, M.D.; Schipanski, M.E.; Stuart Grandy, A. Managing Agroecosystems for Soil Microbial Carbon Use Efficiency: Ecological Unknowns, Potential Outcomes, and a Path Forward. *Front. Microbiol.* **2019**, *10*, 1146. [CrossRef] [PubMed]
29. Cattin, M.; Semple, K.T.; Stutter, M.; Romano, G.; Lag-Brotons, A.J.; Parry, C.; Surridge, B.W. Changes in Microbial Utilization and Fate of Soil Carbon Following the Addition of Different Fractions of Anaerobic Digestate to Soils. *Eur. J. Soil Sci.* **2021**, *2020*, 1–16. [CrossRef]
30. Macherey-Nagel GmbH & Co. KG. REF 985 825, Test 8-25, 12.16, BOD5-TT. Available online: <https://vendart.com.au/app/uploads/2019/10/985825-INSTRUCTIONS.pdf> (accessed on 4 February 2022).
31. Macherey-Nagel GmbH & Co. KG. REF 985 093, Test 0-93 08.16, Total Organic Carbon. Available online: <https://vendart.com.au/app/uploads/2019/10/985093-INSTRUCTIONS.pdf>. (accessed on 4 February 2022).
32. IUSS Working Group WRB. World Reference Base for Soil Resources. In *2015 International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*; World Soil Resources Reports No. 106; FAO: Rome, Italy, 2015. Available online: <https://www.fao.org/3/i3794en/I3794en.pdf> (accessed on 4 February 2022).
33. VITO. Bodem-Bepaling van Snel Vrijkomende Organische Stikstof. Available online: https://esites.vito.be/sites/reflabos/2010/Onlinedocumenten/BAM_deel1_12.pdf (accessed on 20 December 2021).
34. OVAM. Oriënterend Onderzoek Naar de Invulling van de Begrippen Mineralenrijk-Mineralenarm, Humusrijk; D/2002/5024/06; OVAM: Mechelen, Belgium, 2002.
35. Anderson, J.P.E. Soil Respiration. In *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties*, 9.2.2, 2nd ed.; Page, A.L., Ed.; John Wiley & Sons: New York, NY, USA, 1982; Volume 9, pp. 831–871. [CrossRef]
36. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An Extraction Method for Measuring Soil Microbial Biomass C. *Soil Biol. Biochem.* **1987**, *19*, 1–5. [CrossRef]
37. Sigurnjak, I.; De Waele, J.; Michels, E.; Tack, F.M.; Meers, E.; De Neve, S. Nitrogen Release and Mineralization Potential of Derivatives from Nutrient Recovery Processes as Substitutes for Fossil Fuel-Based Nitrogen Fertilizers. *Soil Use Manag.* **2017**, *33*, 437–446. [CrossRef]
38. Sleutel, S.; De Neve, S.; Prat Roibás, M.R.; Hofman, G. The Influence of Model Type and Incubation Time on the Estimation of Stable Organic Carbon in Organic Materials. *Eur. J. Soil Sci.* **2005**, *56*, 505–514. [CrossRef]
39. De Neve, S.; Pannier, J.; Hofman, G. Temperature Effects on C- and N-Mineralization from Vegetable Crop Residues. *Plant Soil* **1996**, *181*, 25–30. [CrossRef]
40. Tiemann, L.K.; Billings, S.A. Changes in Variability of Soil Moisture Alter Microbial Community C and N Resource Use. *Soil Biol. Biochem.* **2011**, *43*, 1837–1847. [CrossRef]
41. Joergensen, R.G.; Wu, J.; Brookes, P.C. Measuring Soil Microbial Biomass Using an Automated Procedure. *Soil Biol. Biochem.* **2011**, *43*, 873–876. [CrossRef]
42. Jenkinson, D.S.; Brookes, P.C.; Powelson, D.S. Measuring Soil Microbial Biomass. *Soil Biol. Biochem.* **2004**, *36*, 5–7. [CrossRef]
43. Sawada, K.; Toyota, K. Effects of the Application of Digestates from Wet and Dry Anaerobic Fermentation to Japanese Paddy and Upland Soils on Short-Term Nitrification. *Microbes Environ.* **2015**, *30*, 37–43. [CrossRef] [PubMed]
44. Müller-Stöver, D.S.; Sun, G.; Kroff, P.; Thomsen, S.T.; Hauggaard-Nielsen, H. Anaerobic Co-Digestion of Perennials: Methane Potential and Digestate Nitrogen Fertilizer Value. *J. Plant Nutr. Soil Sci.* **2016**, *179*, 696–704. [CrossRef]
45. Möller, K. Effects of Anaerobic Digestion on Soil Carbon and Nitrogen Turnover, N Emissions, and Soil Biological Activity. A Review. *Agron. Sustain. Dev.* **2015**, *35*, 1021–1041. [CrossRef]
46. Zmora-Nahum, S.; Markovitch, O.; Tarchitzky, J.; Chen, Y. Dissolved Organic Carbon (DOC) as a Parameter of Compost Maturity. *Soil Biol. Biochem.* **2005**, *37*, 2109–2116. [CrossRef]
47. Akratos, C.S.; Tekerlekopoulou, A.G.; Vasiliadou, I.A.; Vayenas, D.V. *Cocomposting of Olive Mill Waste for the Production of Soil Amendments*; Elsevier Inc.: Philadelphia, PA, USA, 2017. [CrossRef]
48. Scaglia, B.; Pognani, M.; Adani, F. The Anaerobic Digestion Process Capability to Produce Biostimulant: The Case Study of the Dissolved Organic Matter (DOM) vs. Auxin-like Property. *Sci. Total Environ.* **2017**, *589*, 36–45. [CrossRef]
49. Cavalli, D.; Corti, M.; Baronchelli, D.; Bechini, L.; Marino Gallina, P. CO₂ Emissions and Mineral Nitrogen Dynamics Following Application to Soil of Undigested Liquid Cattle Manure and Digestates. *Geoderma* **2017**, *308*, 26–35. [CrossRef]
50. Egene, C.E.; Sigurnjak, I.; Regelink, I.C.; Schoumans, O.F.; Adani, F.; Michels, E.; Sleutel, S.; Tack, F.M.G.; Meers, E. Solid Fraction of Separated Digestate as Soil Improver: Implications for Soil Fertility and Carbon Sequestration. *J. Soils Sedim.* **2020**, *21*, 678–688. [CrossRef]
51. De Neve, S.; Sleutel, S.; Hofman, G. Carbon Mineralization from Composts and Food Industry Wastes Added to Soil. *Nutr. Cycl. Agroecosyst.* **2003**, *67*, 13–20. [CrossRef]
52. de la Fuente, C.; Clemente, R.; Martinez, J.; Pilar Bernal, M. Optimization of Pig Slurry Application to Heavy Metal Polluted Soils Monitoring Nitrification Processes. *Chemosphere* **2010**, *81*, 603–610. [CrossRef] [PubMed]
53. Nieder, R.; Benbi, D.K.; Scherer, H.W. Fixation and Defixation of Ammonium in Soils: A Review. *Biol. Fertil. Soils* **2011**, *47*, 1–14. [CrossRef]

54. Gómez-Brandón, M.; Juárez, M.F.D.; Zangerle, M.; Insam, H. Effects of Digestate on Soil Chemical and Microbiological Properties: A Comparative Study with Compost and Vermicompost. *J. Hazard. Mater.* **2016**, *302*, 267–274. [[CrossRef](#)]
55. Goberna, M.; Podmirseg, S.M.; Waldhuber, S.; Knapp, B.A.; García, C.; Insam, H. Pathogenic Bacteria and Mineral N in Soils Following the Land Spreading of Biogas Digestates and Fresh Manure. *Appl. Soil Ecol.* **2011**, *49*, 18–25. [[CrossRef](#)]
56. Sigurnjak, I.; Vaneckhaute, C.; Michels, E.; Ryckaert, B.; Ghekiere, G.; Tack, F.M.G.; Meers, E. Fertilizer Performance of Liquid Fraction of Digestate as Synthetic Nitrogen Substitute in Silage Maize Cultivation for Three Consecutive Years. *Sci. Total Environ.* **2017**, *599–600*, 1885–1894. [[CrossRef](#)]
57. Fouda, S.; Von Tucher, S.; Lichti, F.; Schmidhalter, U. Nitrogen Availability of Various Biogas Residues Applied to Ryegrass. *J. Plant Nutr. Soil Sci.* **2013**, *176*, 572–584. [[CrossRef](#)]
58. Brust, G.E. *Management Strategies for Organic Vegetable Fertility*; Elsevier Inc.: Philadelphia, PA, USA, 2019. [[CrossRef](#)]
59. Möller, K.; Müller, T. Effects of Anaerobic Digestion on Digestate Nutrient Availability and Crop Growth: A Review. *Eng. Life Sci.* **2012**, *12*, 242–257. [[CrossRef](#)]
60. Watson, C.A.; Atkinson, D.; Gosling, P.; Jackson, L.R.; Rayns, F.W. Managing Soil Fertility in Organic Farming Systems. *Soil Use Manag.* **2002**, *18*, 239–247. [[CrossRef](#)]
61. Gutser, R.; Ebertseder, T.; Weber, A.; Schraml, M.; Schmidhalter, U. Short-Term and Residual Availability of Nitrogen after Long-Term Application of Organic Fertilizers on Arable Land. *J. Plant Nutr. Soil Sci.* **2005**, *168*, 439–446. [[CrossRef](#)]
62. Barduca, L.; Wentzel, S.; Schmidt, R.; Malagoli, M.; Joergensen, R.G. Mineralisation of Distinct Biogas Digestate Qualities Directly after Application to Soil. *Biol. Fertil. Soils* **2021**, *57*, 235–243. [[CrossRef](#)]
63. Chen, R.; Senbayram, M.; Blagodatsky, S.; Myachina, O.; Dittert, K.; Lin, X.; Blagodatskaya, E.; Kuzyakov, Y. Soil C and N Availability Determine the Priming Effect: Microbial N Mining and Stoichiometric Decomposition Theories. *Glob. Chang. Biol.* **2014**, *20*, 2356–2367. [[CrossRef](#)] [[PubMed](#)]
64. Hicks, L.C.; Meir, P.; Nottingham, A.T.; Reay, D.S.; Stott, A.W.; Salinas, N.; Whitaker, J. Carbon and Nitrogen Inputs Differentially Affect Priming of Soil Organic Matter in Tropical Lowland and Montane Soils. *Soil Biol. Biochem.* **2019**, *129*, 212–222. [[CrossRef](#)]
65. Riffaldi, R.; Saviozzi, A.; Levi-Minzi, R. Carbon Mineralization Kinetics as Influenced by Soil Properties. *Biol. Fertil. Soils* **1996**, *22*, 293–298. [[CrossRef](#)]
66. Calderón, F.J.; McCarty, G.W.; Reeves, J.B. Analysis of Manure and Soil Nitrogen Mineralization during Incubation. *Biol. Fertil. Soils* **2005**, *41*, 328–336. [[CrossRef](#)]
67. Silveira, M.L.A. Dissolved Organic Carbon and Bioavailability of N and P as Indicators of Soil Quality. *Sci. Agric.* **2005**, *62*, 502–508. [[CrossRef](#)]
68. Kirchmann, H.; Bernal, M.P. Organic Waste Treatment and C Stabilization Efficiency. *Soil Biol. Biochem.* **1997**, *29*, 1747–1753. [[CrossRef](#)]
69. Zhu, X.; Jackson, R.D.; DeLucia, E.H.; Tiedje, J.M.; Liang, C. The Soil Microbial Carbon Pump: From Conceptual Insights to Empirical Assessments. *Glob. Chang. Biol.* **2020**, *26*, 6032–6039. [[CrossRef](#)]
70. Liang, C.; Zhu, X. The Soil Microbial Carbon Pump as a New Concept for Terrestrial Carbon Sequestration. *Sci. China Earth Sci.* **2021**, *64*, 545–558. [[CrossRef](#)]
71. Liang, C.; Schimel, J.P.; Jastrow, J.D. The Importance of Anabolism in Microbial Control over Soil Carbon Storage. *Nat. Publ. Gr.* **2017**, *2*, 1–6. [[CrossRef](#)]
72. Chevallier, T.; Blanchart, E.; Albrecht, A.; Feller, C. The Physical Protection of Soil Organic Carbon in Aggregates: A Mechanism of Carbon Storage in a Vertisol under Pasture and Market Gardening (Martinique, West Indies). *Agric. Ecosyst. Environ.* **2004**, *103*, 375–387. [[CrossRef](#)]
73. Luo, Z. Modelling the Dynamic Physical Protection of Soil Organic Carbon: Insights Modelling the Dynamic Physical Protection of Soil Organic Carbon: Insights into Carbon Predictions and Explanation of the Priming Effect. *Glob. Chang. Biol.* **2017**, *23*, 5273–5283. [[CrossRef](#)]
74. Solomon, D.; Lehmann, J.; Harden, J.; Wang, J.; Kinyangi, J.; Heymann, K.; Karunakaran, C.; Lu, Y.; Wirick, S.; Jacobsen, C. Micro- and Nano-Environments of Carbon Sequestration: Multi-Element STXM–NEXAFS Spectromicroscopy Assessment of Microbial Carbon and Mineral Associations. *Chem. Geol.* **2012**, *329*, 53–73. [[CrossRef](#)]
75. Moorhead, D.L.; Sinsabaugh, R.L. A Theoretical Model of Litter Decay and Microbial Interaction. *Ecol. Monogr.* **2006**, *76*, 151–174. [[CrossRef](#)]
76. Makara, A.; Kowalski, Z.; Saeid, A. Properties of the Filtrate from Treatment of Pig Manure by Filtration Method. *Open Chem.* **2017**, *15*, 19–27. [[CrossRef](#)]
77. Sommer, S.G.; Mathanpaal, G.; Dass, G.T. A Simple Biofilter for Treatment of Pig Slurry in Malaysia. *Environ. Technol.* **2005**, *26*, 303–312. [[CrossRef](#)] [[PubMed](#)]
78. Brookman, S.K.E. Estimation of Biochemical Oxygen Demand in Slurry and Effluents Using Ultra-Violet Spectrophotometry. *Water Res.* **1997**, *31*, 372–374. [[CrossRef](#)]