

## Dynamics of carbon mineralization and *in situ* nutrient turnover in soil amended with anaerobic digestate

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### ABSTRACT

Anaerobic digestate (AD) is an increasingly prominent component of circular agriculture, yet its rapid biogeochemical effects require nuanced evaluation across laboratory and field settings. This study integrated a fully controlled 28-day soil incubation with an *in situ* longitudinal field tracking trial (15 m<sup>3</sup>·ha<sup>-1</sup> AD applied prior to winter wheat cultivation) to assess organic carbon mineralization kinetics and seasonal nutrient dynamics. In the incubation experiment, AD addition triggered an immediate acceleration of carbon mineralization, doubling the mineralization rate constant ( $k = 0.41 \text{ day}^{-1}$  vs.  $0.19 \text{ day}^{-1}$  in the unamended control) and significantly reducing the biological carbon half-life from 3.7 to 1.7 days. In the field, the immediate post-application phase (Day 14, Term II) was marked by a transient flush of available N (+25%) and S (+67%), confirming rapid early-stage nutrient mobilization. Across the 295-day seasonal cycle (culminating after crop harvest in Term IV), the soil exhibited significant increases in organic matter, available P, and exchangeable K, alongside a pH shift from 7.0 to 7.5 and a reduction in bioavailable Fe and Mn. Because the field component employed a longitudinal baseline-tracking design, late-season nutrient and carbon increments represent the combined legacy of AD conditioning, ambient seasonal dynamics, and crop rhizosphere turnover. These findings demonstrate that AD serves as an active organic-mineral conditioner, optimizing early nutrient supply while supporting the progressive restructuring of the soil sorption complex over the cropping season.

**Keywords:** CO<sub>2</sub> emission kinetics, organic waste, soil fertility, nutrient availability.

### INTRODUCTION

Organic waste is a highly heterogeneous group of materials that can be broadly categorised as biodegradable waste (e.g. wood, paper, agricultural residues and municipal sewage sludge) and biowaste (e.g. food waste, kitchen waste and garden waste) (Jakubus and Sychalski 2022). The management of organic waste generally follows two primary pathways: agricultural ‘waste-to-matter’ recovery (e.g. use as fertilisers or soil amendments for degraded land, or anaerobic digestion and co-composting), and non-agricultural ‘waste-to-energy’ recovery (e.g. incineration, pyrolysis, and gasification) (Jakubus 2024). Meanwhile, modern efforts increasingly focus on efficiently recycling bio- and agro-wastes as

renewable energy sources within the bioeconomy framework, where anaerobic digestion serves as a crucial intersection for these recovery pathways. The fermentation process yields valuable biogas and a byproduct known as anaerobic digestate (AD). The modern agroecological paradigm no longer views digestate application merely as a waste disposal method (Anacleto et al. 2025); instead, anaerobic digestion has become a subject of intense research as a multifunctional soil conditioner capable of long-term soil restructuring, carbon sequestration, and restoring soil fertility (Zapata-Morales and Moreno-Andrade 2025).

AD represents the partly degraded organic material remaining after fermentation process (Dietrich et al. 2020). As highlighted by Lee et al. (2021) digestate is a low-cost and environmentally

friendly alternative to conventional organic fertilizers, offering a more balanced nutrient profile than pure livestock waste slurries. Furthermore, AD is considered safer for the environment as it is typically free from pathogens often found in raw manure (Kovačić et al. 2022). AD is rich in nitrogen (primarily in the form of  $\text{N-NH}_4$ ), phosphorus, and potassium, and usually exhibits an alkaline reaction (Zhang et al. 2018). These properties underscore the importance of AD in balancing C, N, P as well as pH values which is particularly relevant to sustainable environmental management (Garcia-Lopez et al. 2023). However, in a modern agroecological context, AD is no longer treated solely as a simple substitute for mineral fertilizers (NPK). Instead, it is studied as a multifunctional conditioner capable of long-term soil matrix restructuring and carbon sequestration (Nyang'au et al. 2022).

Despite its widespread use, the rapid biogeochemical phenomena initiated immediately after the application of this highly labile organic matter remain a subject of intense scientific debate. Van Midden et al. (2023) and Möller (2015) have demonstrated varying effects of AD on soil properties, particularly emphasizing positive changes in physical properties and the enhancement of microbial activity. While existing literature extensively covers N and P dynamics following AD application, the biogeochemical cycling of S remains largely marginalized. The innovative contribution of this study lies in explicitly coupling the short-term release of S from the digestate. Unlike previous agronomic research (Holatko et al. 2023, Panakhyd et al. 2026), which primarily focuses on simple mineral S fertilizers or evaluates total soil S pools in isolation, this study assesses AD as a complex organic-mineral matrix. Furthermore, the impact of AD on short- and long-term soil biogeochemical dynamics, including the so-called “priming effect” and the processes of secondary nutrient stabilization within the sorption complex, is less frequently analyzed. This gap is particularly evident regarding S transformations.

The priming effect refers to the short-term, robust change in the turnover rate of native soil organic matter (SOM) triggered by the addition of exogenous labile organic substrates (Bernard et al. 2022). In agricultural soil systems, this phenomenon is primarily driven by the sudden influx of easily available carbon, which rapidly stimulates microbial biomass and extracellular enzyme activity. Consequently, this microbial

activation temporarily accelerates the mineralization of more recalcitrant carbon pools, leading to a synchronized, rapid release of plant-available nutrients into the soil solution. Evidence of the priming effect following AD application has been noted by Barlóg et al. (2020) and Abubaker et al. (2012). Möller (2015) remarked that while AD application offers many positive effects, they may be short-lived compared to the impact of other undigested feedstocks. Recent studies (Ferraz-Almeida 2022, Olayemi et al. 2022) indicate that the kinetics of this process are highly non-linear; following an initial phase of rapid nutrient release, the soil environment undergoes transformations toward the formation of more stable chemical bonds.

The research objectives were:

- To determine the kinetic parameters of organic carbon mineralization and potential soil respiration following AD application under controlled laboratory conditions;
- To trace the *in situ* temporal trajectories of soil physicochemical properties and nutrient availability across the cropping season using exploratory multivariate techniques (PCA, HCA).

The following research hypotheses were formulated:

- H1: Anaerobic digestate (AD) application triggers an immediate acceleration of carbon mineralization kinetics under controlled conditions, evidenced by a substantially higher mineralization rate constant ( $k$ ) and a reduced biological half-life of labile carbon ( $t_{1/2}$ ) compared to unamended soil.
- H2: In the field, AD application induces an immediate transient mobilization of available nutrients (specifically N and S), followed by a progressive shift in soil chemical equilibrium that enhances base saturation and promotes the seasonal stabilization of nutrients within the soil sorption complex across the crop vegetative cycle.

## MATERIALS AND METHODS

### Field experiment

The experiment was established at the experimental station of the Poznań University of Life Sciences in Wielkopolska Province, Poland

(52°31'19"N, 16°39'40"E). The soil, classified as *Haplic Phaeozems* (WRB, 2022) was a sandy loam. Baseline physicochemical and chemical soil characteristics prior to digestate application are summarized as Term I in Tables 1, 2, and 3 in the Results section.

The field experiment followed a randomized block design with four replicates, with an individual plot area of 15 m<sup>2</sup>. The AD was sourced from an agricultural biogas plant operating at the same experimental station and had a pH of 8.02. Its chemical composition (g·kg<sup>-1</sup> of d.m.) was: TOC 426, TN 55.6, AN 12.4, P 6.2, K 20.8, Mg 3.5, Ca 38.7, Na 13.8, and Fe 32.4. Micronutrient amounts (mg·kg<sup>-1</sup> of d.m.) were: Mn 132.6, Cu 54.2, and Zn 182.3. AD was applied in the autumn at a rate of 15 m<sup>3</sup>·ha<sup>-1</sup> and thoroughly mixed into the soil. This rate provided approximately 40–50 kg N·ha<sup>-1</sup> (depending on dry matter content) and adhering to the European Nitrates Directive limits (1991). Soil samples were collected from the arable layer (0–25 cm depth) at four independent time points using a soil auger:

- Term I– soil, prior to AD application, in autumn (Day 0)
- Term II – soil after AD application, in autumn (Day 14)
- Term III– soil at the onset of the growing season, in spring (Day 195)
- Term IV – final soil sampling term, in summer (Day 295).

A multi-point composite sampling strategy ensured spatial representativeness at each of term. Approximately 15 - 20 sub-samples were collected from the arable layer (0–25 cm) following a diagonal transect and immediately homogenized. The resulting bulk soil was reduced via the standard quartering method to obtain a representative 1.5 kg laboratory sample for subsequent physicochemical and incubation assays.

### Methodological framing of field tracking

Rather than establishing adjacent unfertilized fallow plots—which inevitably alter weed dynamics, soil water regimes, and microclimatic conditions differently than a fully cultivated crop, this study adopted an *in situ* longitudinal monitoring design. Here, the pre-application soil status (Term I) served as the authentic baseline reference ( $t_0$ ). This temporal tracking approach evaluates the progressive trajectory of soil biogeochemical

shifts across the vegetative cycle under the combined influence of AD conditioning, agronomic crop growth, and seasonal turnover. Focusing on temporal dynamics enabled the precise tracking of early nutrient mobilization and subsequent nutrient stabilization, comparing each post-application term directly against the authentic baseline environmental status. The soil samples were air-dried and then sieved through a 2 mm mesh (Van-Reeuwijk 1992).

### Incubation experiment

The field research was expanded to include an assessment of potential CO<sub>2</sub> emissions from the AD-fertilized soil. For this purpose, an incubation experiment was conducted using soil from the experimental field (*Haplic Phaeozems*). To ensure comparability, soil was sampled from the arable layer (0–25 cm depth) prior to the field AD application (baseline). Two treatments were established in sealed 1 L plastic containers: CS – control soil (1 kg) and S+AD – soil (1 kg) with AD applied at a rate of 15 m<sup>3</sup>·ha<sup>-1</sup>, was conducted.

Incubation was performed in triplicate over a 28 – day period at a constant temperature (22 °C) and 60% water holding capacity (WHC). This temperature, standard for soil microbiological assays, ensured optimal microbial activity. This laboratory approach aimed to determine the absolute intrinsic degradability of the digestate and the maximum potential carbon mineralization pool ( $C_0$ ), ensuring the reproducibility of the kinetic parameters rather than mimicking fluctuating field conditions.

CO<sub>2</sub> emissions were measured by placing a beaker containing 20 mL of a 0.5 mol·L<sup>-1</sup> NaOH solution inside each container. Measurements were performed on days 3, 7, 10, 14, 21, and 28. To ensure a non-destructive, longitudinal monitoring NaOH-containing beakers were replaced at each interval while incubation containers remained sealed. Residual NaOH solution was titrated with 0.5 mol·L<sup>-1</sup> HCl in the presence of BaCl<sub>2</sub> to a phenolphthalein endpoint (Li et al. 2018). The soil respiration rate was calculated as the difference in the amount of HCl consumed. Given the 1 kg of soil mass, the absolute CO<sub>2</sub> measured (in mg) directly corresponds to emissions per kilogram of soil (mg · kg<sup>-1</sup>). The daily soil respiration rate (mg · kg<sup>-1</sup> · d<sup>-1</sup>) for each interval was calculated by dividing the CO<sub>2</sub> amount by the number of days in the specific period. Although empty

containers (without soil) were not used to account for background CO<sub>2</sub>, the use of identical vessels for all treatments ensures that any background absorption constitutes a constant systematic bias. Consequently, the relative differences between treatments, net priming effect calculations, and first-order kinetic curves remain mathematically robust and unaffected by this limitation.

## Analytical methods

The following determinations were made in the soil samples: pH – potentiometric method using a pH meter with a glass electrode in 1 mol·L<sup>-1</sup> KCl; Total nitrogen (TN), total organic carbon (TOC), and total sulfur (TS) – using a Vario Max CNS apparatus; available (mineral) nitrogen (AN) was determined according to the procedure by Keeney and Nelson (1982) with steam distillation in 1 mol·L<sup>-1</sup> KCl extracts; available sulfur (AS) was extracted with 2% CH<sub>3</sub>COOH according to the procedure by Bardsley and Lancaster (1960). Sulfur concentration was determined using a nephelometric method based on barium chloride precipitation and spectrophotometric measurement at a wavelength of 495 nm;

Available phosphorus (AP) was determined using the Olsen method (1954); exchangeable cations (Exch: Ca, Mg, K, and Na) were determined by the Pallmann method (Van-Reeuwijk 1992).

Total cation exchange capacity (CEC) was deliberately omitted in this study. Given the neutral to slightly alkaline soil reaction (pH 7.0–7.5), exchangeable acidity (H<sup>+</sup>, Al<sup>3+</sup>) is virtually negligible. Under these conditions, the sum of the measured exchangeable base cations (Ca, Mg, K, and Na) accurately reflects the effective cation exchange capacity (eCEC). Furthermore, tracking the dynamic fluctuations of individual basic cations was far more relevant to the study's primary objective, assessing plant stoichiometric responses and specific nutrient availability, than reporting a single, aggregated CEC index.

The bioavailable forms of microelements (AZn, ACu, AMn, AFe) were determined using the DTPA test (ISO 2001); Total amounts of macro- and microelements (TCa, TK, TMg, TNa, TCu, TFe, TMn, TZn) were determined according to Ostrowska et al. (1991). Following dry mineralization, the resulting ash was dissolved in 6 mol·L<sup>-1</sup> HCl and concentrations were determined by atomic absorption spectrophotometry (AAS) using a Varian SpectraAA 220FS

apparatus. The total phosphorus (TP) was determined spectrophotometrically after coloring with ammonium metavanadate. The same procedure (Ostrowska et al. 1991) was applied to determine the amounts of macro- and microelements in AD.

## Statistical analysis

For the field parameters, a one-way analysis of variance (ANOVA) was performed to test the effect of sampling term (evaluating temporal progression from Term I through Term IV). Upon rejection of the null hypothesis, post-hoc mean comparisons were conducted using Tukey's honestly significant difference (HSD) test at  $\alpha = 0.05$ . Homogeneous groups are designated by common letters in the tables. For the incubation experiment, differences in cumulative CO<sub>2</sub> emissions and kinetic parameters ( $C_0$ ,  $k$ ,  $t_{1/2}$ ) between the unamended control (CS) and AD-amended soil (S+AD) were evaluated using Student's t-test at  $p < 0.05$ . Multivariate patterns across experimental terms were analyzed using principal component analysis (PCA) and hierarchical cluster analysis (HCA, Ward's method with squared Euclidean distance). Data analysis was carried out using STATOBL software running on Windows 11. Multivariate statistical analyses, including principal component analysis (PCA) and hierarchical cluster analysis (HCA), as well as data visualization, were performed using Python 3.10 software (Python Software Foundation, Wilmington, DE, USA).

Kinetics and stoichiometric modeling decomposition dynamic of the organic matter introduced with the AD were modeled using a first-order kinetics equation. Cumulative CO<sub>2</sub> emissions were fitted to an asymptotic curve via the non-linear least squares regression method (Levenberg-Marquardt algorithm) via the equation:

$$C_t = C_0 \cdot (1 - e^{-kt}) \quad (1)$$

where:  $C_t$  – cumulative amount of carbon emitted after time  $t$  [mg·kg<sup>-1</sup>],  $C_0$  – potential maximum pool of mineralizable carbon [mg·kg<sup>-1</sup>],  $k$  – mineralization rate constant [day<sup>-1</sup>],  $t$  – incubation time [days].

Based on the calculated constant  $k$  for each treatment, the carbon half-life ( $t_{1/2}$ ) was determined, defining the time required to mineralize 50% of the easily available carbon pool, using the relationship:

$$t_{1/2} = \frac{\ln 2}{k} \quad (2)$$

## RESULTS

### Changes in soil physicochemical properties

Quantitative changes in the analyzed soil parameters are presented in Tables 1–3, supporting the assumptions formulated for H2. AD application positively influenced soil properties by increasing the content of most components. Between Term I and Term IV, TN amounts exhibited a substantial increase of 53%; however, this difference was not statistically significant at  $p < 0.05$ . In the soil from the fourth sampling term (Term IV), a 30–38% higher amounts of OM, TOC, AP, and TCa were recorded compared to the baseline (Tables 1 and 2). Notably, AN and AS contents increased rapidly and significantly by 25% and 67%, respectively, immediately following AD application (Term II), before subsequently decreasing. Regarding exchangeable cations, levels recorded after the wheat harvest (Term IV) were twofold higher for K, and 10% and 15% higher for Mg and Ca, respectively (Table 3). Conversely, total and available amounts

of Fe and Mn decreased over the experimental period. Consequently, soil prior to AD application (Term I) exhibited 1.5-fold higher content of available Fe and Mn, as well as 32% and 43% higher total amounts of Fe and Mn, respectively (Table 3).

### Spatiotemporal transformation of the soil environment

Figure 1 supplements the tabular data with a PCA biplot, illustrating the spatiotemporal transformation of soil physicochemical properties following AD application (from Term I to Term IV). The data reveal significant soil shifts, transitioning from a phase characterized by the rapid release of labile components (left side of the biplot) to a phase marked by the formation of stable nutrient associations within the soil matrix (right side of the biplot).

The total amounts of most components were vectorially associated with the early sampling stages (Terms I and II). By the final sampling stage (Term IV), strong correlations were observed

**Table 1.** Quantitative changes in the analyzed soil properties depending on the sampling term

| Term | pH   | C:N | OM                         | TOC   | TN    | AN     | TP    | AP    | TS    | AS     |
|------|------|-----|----------------------------|-------|-------|--------|-------|-------|-------|--------|
|      |      |     | g·kg <sup>-1</sup> of d.m. |       |       |        |       |       |       |        |
| I    | 7.0b | 14  | 36.8b                      | 21.4b | 1.53a | 0.16ab | 0.72a | 0.34b | 0.24a | 0.06c  |
| II   | 7.2b | 10  | 35.6b                      | 20.6b | 2.0a  | 0.2a   | 0.65a | 0.34b | 0.20a | 0.10a  |
| III  | 7.2b | 12  | 39.2b                      | 22.7b | 1.92a | 0.16ab | 0.68a | 0.33b | 0.21a | 0.06bc |
| IV   | 7.5a | 12  | 49.8a                      | 29.0a | 2.33a | 0.14b  | 0.68a | 0.47a | 0.19a | 0.08ab |

**Table 2.** Total macronutrient content and exchangeable cations (g·kg<sup>-1</sup> of d.m.) in soil samples depending on the sampling term

| Term | Total amounts |      |       |      | Exchangeable cations |        |       |       |
|------|---------------|------|-------|------|----------------------|--------|-------|-------|
|      | Ca            | Mg   | K     | Na   | Ca                   | Mg     | K     | Na    |
| I    | 21.4b         | 2.8a | 25.7a | 3.0a | 5.85ab               | 0.96ab | 0.26b | 0.17a |
| II   | 16.4c         | 2.7b | 24.9a | 2.9a | 5.30b                | 0.90b  | 0.32b | 0.19a |
| III  | 17.5c         | 2.8a | 24.4a | 3.2a | 5.51ab               | 0.93b  | 0.33b | 0.18a |
| IV   | 27.5a         | 2.8a | 22.0a | 3.1a | 6.72a                | 1.06a  | 0.55a | 0.19a |

**Table 3.** Total and available amounts of microelements (mg·kg<sup>-1</sup> of d.m.) in soil samples depending on the sampling term

| Term | Fe     |           | Cu     |           | Zn     |           | Mn      |           |
|------|--------|-----------|--------|-----------|--------|-----------|---------|-----------|
|      | Total  | Available | Total  | Available | Total  | Available | Total   | Available |
| I    | 7.24a  | 1.63a     | 8.39a  | 1.31a     | 29.47a | 3.90a     | 164.0a  | 7.38a     |
| II   | 7.01a  | 1.33b     | 7.17ab | 1.28a     | 31.98a | 4.80a     | 141.4ab | 7.81a     |
| III  | 6.36ab | 1.32b     | 7.39ab | 1.31a     | 31.96a | 3.90a     | 163.1a  | 8.00a     |
| IV   | 5.48b  | 0.61c     | 8.43a  | 1.39a     | 29.24a | 4.80      | 114.6b  | 4.6b      |

between increasing organic matter (OM) and pH levels and total calcium (TCa), its exchangeable forms, as well as the amounts of exchangeable Mg, K, and Na cations. The dendrogram of sampling terms (Figure 2, left panel) reveals a significant divergence in soil properties between Terms I and II and Term IV. The linkage distance for the soil from Term IV is more than twice as large as that for the other terms, providing evidence of a complete restructuring of the soil's sorptive and biological properties under the influence of the applied digestate. Hierarchical Cluster Analysis using Ward's method (Figure 2, right panel) identified precise functional clusters comprising pH, OM, TOC, exchangeable K, and AP. Additionally, a clear separation was noted for parameters such as TFe, AFe, TMn, AMn, TCu, ACu, TZn, and AZn from the buffering parameters (pH, OM). Furthermore, a structural association was demonstrated between total calcium (TCa), exchangeable Ca and Mg cations, and the total phosphorus (TP) pool (Figure 2).

### Effect of AD on soil respiration kinetics

AD application induced rapid C-mineralization kinetics (Table 4). The fertilization significantly stimulated microbial activity, as evidenced by the dynamics of CO<sub>2</sub> emission (Figure 3).

Cumulative CO<sub>2</sub> emission over the 28-day of incubation period reached 67.8 mg·kg<sup>-1</sup> in the control soil, increasing to 100.7 mg·kg<sup>-1</sup> in the AD-treated soil. The most intense CO<sub>2</sub> emission occurred within the first three days, indicating a substantial pool of labile carbon within the digestate that is highly susceptible to transformation.

To evaluate the microbiological priming effect, the actual measurement data were fitted to a first-order kinetics model. Non-linear analysis (Table 4) showed that AD increased the potential pool of easily mineralizable carbon (C<sub>0</sub>) by nearly 44%. Simultaneously, the kinetic constant doubled ( $k = 0.41 \cdot \text{day}^{-1}$  compared to  $k = 0.19 \cdot \text{day}^{-1}$  for the control), which significantly shortened the carbon biological half-life. The carbon half-life decreased from 3.7 days in the control to 1.7 days in the fertilized treatment. This rapid kinetics confirm that the tested AD acts as a highly reactive microbial starter, capable of the instantaneous release of nutrients immediately upon application to the soil environment.

### DISCUSSION

The significance of AD as an alternative source of TOC, TN, AN, AP, AS and TCa is consistent with previous literature reports (Kovačič

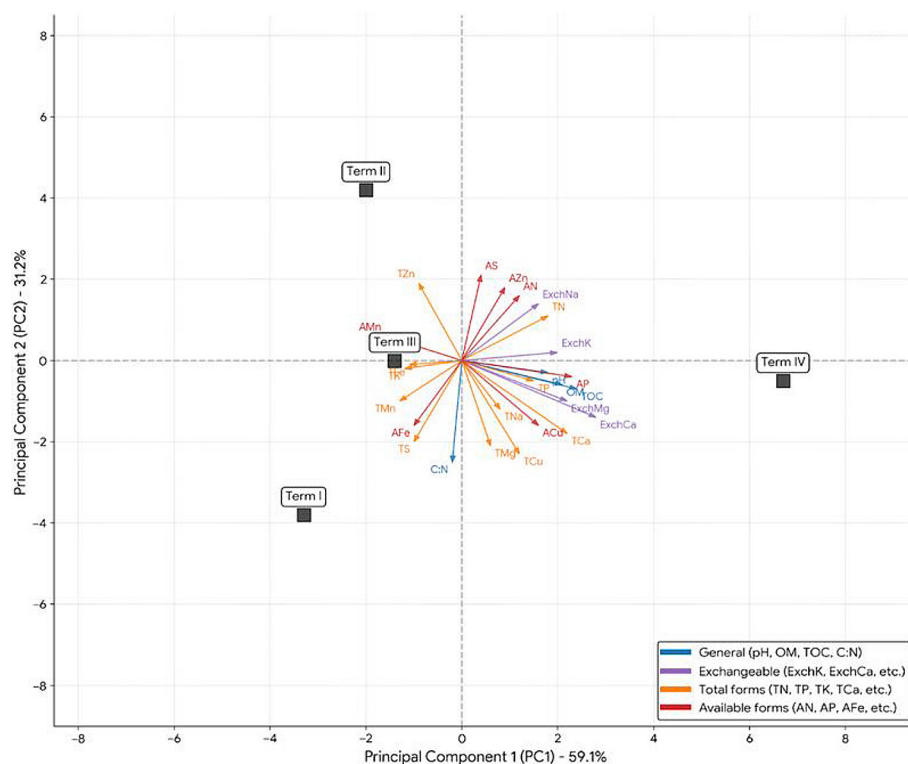


Figure 1. Principal component analysis (PCA) biplot

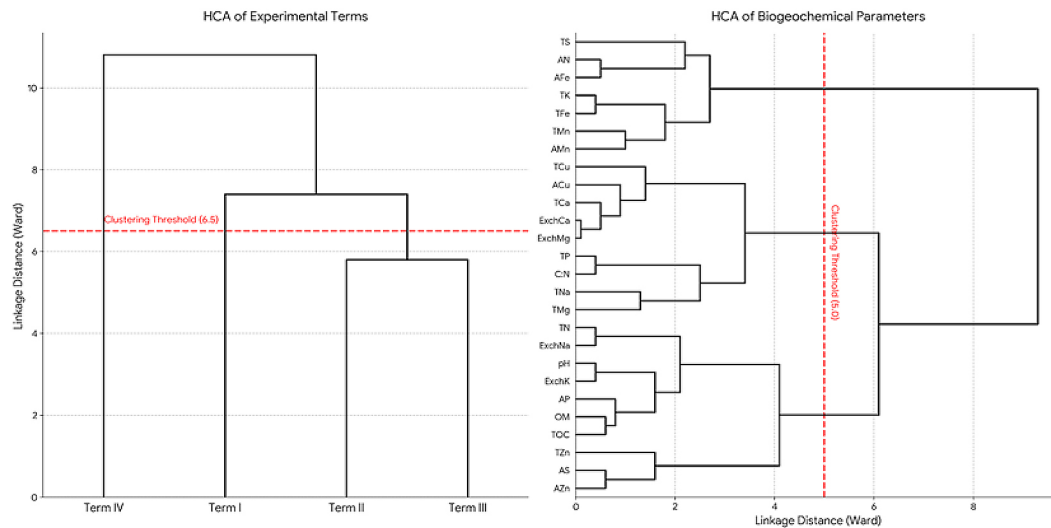


**Figure 2.** Hierarchical cluster analysis (HCA) dendrograms

**Table 4.** Parameters of the first-order kinetics model

| Treatment | C <sub>0</sub> (mg) | k (day <sup>-1</sup> ) | t <sub>1/2</sub> (days) |
|-----------|---------------------|------------------------|-------------------------|
| CS        | 64.1                | 0.19                   | 3.7                     |
| S+AD      | 92.4                | 0.41                   | 1.7                     |

et al. 2022, Garcia –Lopez et al. 2023). However, the specific quality of the organic matter introduced with the digestate is a key driver of soil dynamic. Unlike traditional organic fertilizers such as compost or manure, AD constitutes a unique matrix. On the one hand, it contains a pool of highly labile carbon (residual volatile fatty acids) and ammonium nitrogen ( $\text{N-NH}_4^+$ ), and on the

other, recalcitrant fractions (lignocellulose) that survived methanogenesis (Lee et al. 2021). A different pattern of changes was determined for the poorly studied macronutrient as S. The study by Yekta et al. (2014) shows that the S in the digestate was dominated by insoluble iron sulfides followed by reduced organic S and zero-valent S. Inorganic sulfate i.e., available form for plants was found at low concentrations. Even though these are small amounts, they are quickly activated, temporarily increasing the pool of available nutrients, as in these studies. However, over time, this amount is reduced as the nutrients become immobilized. This is consistent with reports of Fontain et al. (2020) or Weimers et al. (2022).

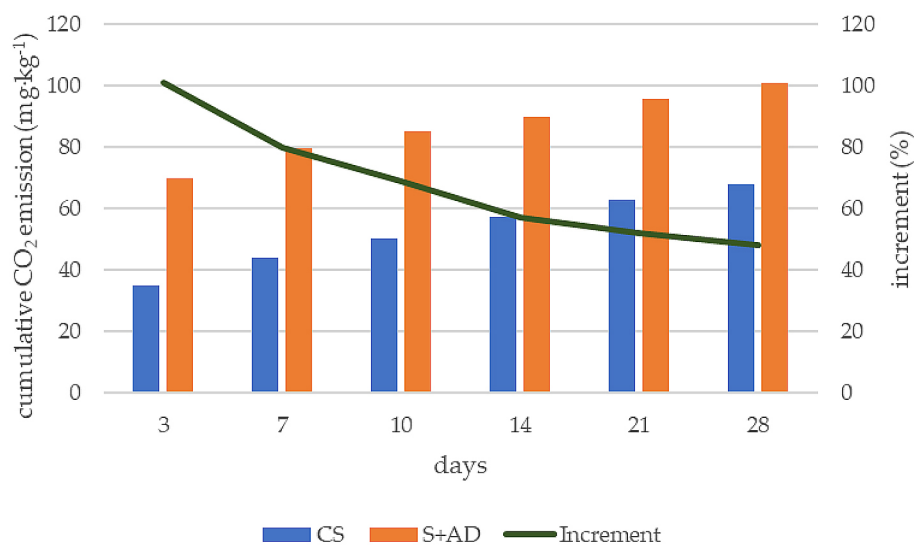


**Figure 3.** Cumulative CO<sub>2</sub> emission kinetics during the 28-day soil incubation period. The green line (increment, right axis) represents the relative percentage increase in CO<sub>2</sub> emission from the AD-treated soil compared to the unfertilized control, illustrating the dynamics of the microbial priming effect

This study confirms a priming effect, a phenomenon also emphasized by Barlóg et al. (2020) and Abubaker et al. (2012), associated with the stimulation of OM decomposition under the influence of applied AD. AD with a high organic matter degradability exerts a stronger effect on short-term soil microbial activity than AD with low degradability (Lin et al. 2022). These results reveal a shortening of the biological carbon half-life ( $t_{1/2} = 1.7$  days), a clear manifestation of a positive priming effect. This aligns with incubation studies by Häfner et al. (2022), who observed rapid C mineralization from AD, with peak  $\text{CO}_2$  emission occurring after two days of soil incubation.

HCA models demonstrated that AD application synchronized the rapid release of available forms of nitrogen and sulfur. This is highly desirable, as it ensures a synchronized availability of macronutrients within the soil solution, enhancing the soil's capacity to support biogeochemical cycles during the active growing season.

When interpreting the marked increases in OM, TOC, and exchangeable cations recorded at Term IV (Tables 1–3), it is essential to consider the integrated nature of the field agroecosystem. The applied rate of  $15 \text{ m}^3 \cdot \text{ha}^{-1}$  provided an initial exogenous carbon and nutrient input that stimulated early microbial activity and transient nutrient flushes (as reflected by Term II data and incubation kinetics). However, the substantial accumulation of TOC and OM observed after 295 days (Term IV) exceeds the direct stoichiometric carbon mass added via digestate alone. This late-season enrichment reflects the combined legacy of AD as an active biochemical starter and the subsequent biological inputs from the winter wheat crop, including fine root turnover, rhizodeposition, and crop residue incorporation under ambient seasonal conditions. Hence, AD functions not solely as a direct biogen donor, but as an active soil conditioner that improves soil physical and chemical conditions, thereby facilitating greater stabilization and retention of organic matter and nutrients within the arable layer over the cropping year.

The significance of the OM, TOC, as well as Ca, Mg, and P introduced with the AD suggests the simultaneous release of these elements from the fertilizer's organic matrix during the environmental stabilization process. The association of total phosphorus with Ca and Mg in the HCA model suggests that a portion of the phosphorus may be associated with calcium and magnesium, potentially forming insoluble phosphates. While

direct speciation analysis is required for confirmation, this association is consistent with the observed pH-dependent stabilization. The slow degradation of organic Ca and Mg complexes also induced a successive increase in pH and an improvement in the soil's sorption capacity. This phenomenon directly translated into the dynamics of K and AP. Specially, P availability in this system stems from AD mineralization and protection against retrogradation via stable pH while the environmental conditions created by AD promote K ions sorption, protecting them from possible leaching beyond the soil profile. These findings are corroborated by literature reports (Gerke 2022) indicating the fundamental role of processed organic matter in building the cation exchange capacity of arable soils.

The HCA model identified a clear structural association between buffering parameters (pH, OM, TOC) and exchangeable cations, indicating that AD application enhances the soil's buffering capacity. Conversely microelements (TFe, TMn, TCu, and TZn) were grouped separately from these buffering parameters. Combined with the observed pH increase (Term IV), this suggests a potential improvement in the soil's sorptive capacity and the formation of stable organo-mineral complexes. While direct measurements of CEC and metal speciation were beyond this study's scope, these findings, coupled with the observed shifts in SOM and pH, align with the hypothesis that AD facilitates metal chelation and precipitation into insoluble forms. These processes likely contribute to the stabilization of Fe and Mn, as evidenced by their reduced availability in Term IV. The susceptibility of Fe and Mn to these changing conditions is well-documented, as these elements typically show decreased mobility in neutral to slightly alkaline environments (Walna et al. 2010). This creates a distinct agronomic trade-off because the AD-induced alkalization, which is beneficial for phosphorus availability and soil physical structure, may simultaneously necessitate more precise management of micronutrient availability. From a practical perspective, AD application should be accompanied by periodic soil micronutrient monitoring. In intensive wheat cultivation, if soil tests indicate a sustained decline in available Fe or Mn following AD application, producers should consider supplemental measures, such as foliar applications or adjusting AD dosages. Recognizing this trade-off is essential for transitioning from simple waste disposal

to a nuanced, site-specific circular economy management strategy, ensuring that the structural benefits of AD do not inadvertently compromise soil micronutrient bioavailability.

### Study limitations

Several methodological considerations should be contextualized. First, the field trial was conducted at a single experimental location over one growing season, reflecting the specific pedoclimatic conditions of Central Europe. Second, the field study implemented a longitudinal baseline-tracking design (monitoring temporal shifts relative to Term I baseline levels) rather than maintaining parallel, unfertilized zero-control plots across the entire vegetative season. While this approach maintains crop continuity and avoids microclimatic artefacts associated with uncropped fallow plots, it inherently tracks the combined trajectory of AD conditioning, crop growth, and seasonal turnover. Crucially, the direct, unconfounded kinetics of carbon mineralization from the digestate were independently verified under strictly controlled conditions in the 28-day incubation experiment, which included an unamended control (CS). Furthermore, the rapid pulse of available N and S recorded at Term II (Day 14), prior to substantial crop nutrient uptake or winter dormancy, provides clear *in situ* confirmation of digestate-induced nutrient mobilization. Future multi-year trials incorporating parallel mineral fertilizer benchmarks will further refine the partitioning between digestate inputs, root-derived carbon, and seasonal turnover.

### CONCLUSIONS

This study demonstrates that anaerobic digestate functions as a high-reactivity soil conditioner rather than a passive, slow-release fertilizer. Controlled incubation assays confirmed accelerated carbon mineralization dynamics, evidenced by a doubling of the mineralization rate constant and a shortened carbon half-life of 1.7 days (vs. 3.7 days in unamended soil). *In situ* field tracking demonstrated that this dynamic translated into an immediate, synchronized release of plant-available nitrogen and sulfur within 14 days of application. Over the 295-day cropping cycle, digestate application supported an enhanced soil sorption complex, characterized by higher base cation levels, elevated pH, and phosphorus stabilization,

accompanied by a decline in bioavailable iron and manganese. By distinguishing immediate digestate mobilization from the broader seasonal legacy of crop cultivation, these findings underline the necessity of pairing AD application with routine micronutrient monitoring. In the context of the circular bioeconomy, utilizing AD as an active soil conditioner represents an agronomically viable strategy for sustainable soil fertility management.

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