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Prediction of nitrogen mineralization from novel bio-based fertilizers using chemical extractions

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ABSTRACT

Bio-based fertilizers (BBFs) are an increasingly important source of nutrients in agriculture, promoted by the new EU fertilizer regulation aiming to enable a circular bioeconomy. Predicting the mineralization-dependent nutrient release of BBFs is critical for their appropriate use and to minimize environmental losses. We assessed mineralizable nitrogen (N) and carbon (C) of a representative selection of 32 BBFs and evaluated a set of chemical extraction methods to predict their N mineralization dynamics. In 84-day aerobic incubations, cumulative mineral N release varied between −13 and 100 % of amended N. Mineralized C ranged from 10 % to 117 % of amended C. Based on the dynamics of N and C mineralization, BBFs were classified into five significantly different groups. Among the tested chemical indicators of N mineralization from BBFs, cold and hot water presented the lowest extraction intensities, followed by hot potassium chloride and hot sulfuric acid extractions, while C:N ratio is based on total contents. Mineral N released almost immediately was best predicted by cold water extractable N, while hot sulfuric acid extractable N and C:N ratio predicted N released after the first two weeks and after 84 days, respectively. The combination of these three indicators was able to discriminate BBFs into four out of five mineralization classes. Such a cost-effective yet accurate estimation of N mineralization dynamics from BBFs can therefore be used as a basis to inform farmers on suitable timing and amount of BBF application, improving the synchrony between N release from BBFs and crop N demand.

1. Introduction

Bio-based fertilizers (BBFs) are becoming increasingly important, offering opportunities to close nutrient cycles, but their handling is more knowledge-intensive than for traditional fertilizers. BBFs include all fertilizers derived from animal or vegetal bio-waste and derivatives from bio-waste processing and waste water treatment. Recent efforts to support the use of BBFs culminated in the current European Fertilizer Regulation (Fertilizing Products Regulation (EU) 2019/1009), which entered into force in June 2022. In this regulation, BBFs are classified based on their component materials, state of matter, and organic material content (European Council,

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2019). However, their international commerce and environmentally friendly application have been constrained by the scarcity of scientific knowledge needed for practical guidelines and to support international guidelines and policies (European Council, 2016).

The prediction of nitrogen (N) release from BBFs is crucial for their efficient use, and to avoid environmental losses. The availability of N from BBFs is largely controlled by their N mineralization dynamics, which is best assessed by in-vitro biological mineralization assays (Calderón et al., 2005; Lashermes et al., 2010; Lazicki et al., 2020; Morvan et al., 2006; Parnaudeau et al., 2004; Thuriès et al., 2001). The resulting mineralizable N is defined as the sum of ammonium (NH₄-N) and nitrate (NO₃-N) extractable after aerobically incubating a soil-BBF mixture for a defined period of time.

Mineralizable N determined in aerobic incubations is widely accepted as it derives directly from biologically driven mineralization processes (Ros et al., 2011). However, such mineralization assays are time-consuming and thus costly, and they tend to overestimate mineralizable N due to ideal incubation conditions. With the continuous development of new BBFs, there is a need for holistic yet simple, fast and low-cost methods for predicting N mineralization dynamics. Basic parameters such as total N (TN), total carbon (TC) and C:N ratios are commonly determined, but the prediction of N mineralization could potentially be improved by applying chemical extraction methods that have been developed since the 1970s to predict mineralization of N from soil organic matter (SOM).

We selected four chemical N extraction methods developed for extracting different proportions of soil N and adapted them to BBFs. Mild methods extracting less than 20 % of SOM-N were selected for their potential to target highly labile organic N pools. EN 13652 defines a standard method using cold water (CW-N) to determine water-soluble nutrients from soil improvers and growing media. Hot water extractable N (HW-N) has been used to extract labile organic SOM-N (Curtin et al., 2006; Gregorich et al., 2003) and was originally proposed as an index for N availability in soils (Keeney and Bremner, 1966). Hot potassium chloride extractable N (hKCl-N) is one of the most widespread methods to assess highly labile organic N pools (Ros et al., 2011). Finally, sulfuric acid extractable N (H₂SO₄-N) has been proposed by Martínez and Galantini (2017) as a fast and low-cost method to assess moderately labile organic N pools of particulate organic matter (POM-N), which is considered a good predictor for mineralizable N (Curtin and Wen, 1999).

Table 1

Description, country of origin, component material category (CMC) and product function category (PFC) of bio-based fertilizers (BBFs) included in the present study. Component material numbers in parentheses only apply to a minor portion of materials contained in the BBF. The table was modified from Wester-Larsen et al. (2022) and additionally includes SWG and SLA20. For information on BBF properties, in addition to those presented in Fig. 1, see Wester-Larsen et al. (2022).

BBF	Origin	Description	CMC	PFC
BA5	AT	Organic fertilizer based on bioethanol residues amended with molasses, potato fruit water, maize steeping water and wheat gluten.	4, (6)	1 A-I
BA6	AT	Organic fertilizer based on bioethanol residues amended with molasses, potato fruit water and maize steeping water.	4, (6)	1 A-I
BIH	CH	Organic fertilizer based on horn meal.	10	1 A-I
BIL	FI	Organic fertilizer based on broiler manure and seaweed.	10	1 A-I
BIO	FI	Organo-mineral fertilizer containing meat and bone meal, apatite, vinasse, chicken manure and potassium sulphate.	10	1 B-I
BIP	DK	Liquid organic fertilizer based on by-products from sugar beets' and potatoes' processing.	6	1 A-II
BVC	DK	Organic fertilizer based on composted municipal organic food waste.	3	1 A-I
CGR	UK	Mineral fertilizer based on recovered nutrients from wastewater supernatants with a special coating for nutrient release optimization.	12	1 C-I-a
ECO	FI	Organic fertilizer based on meat and bone meal.	10	1 B-I
FEK	BE	Organic fertilizer fully based on chicken manure.	10	1 A-I
FEL	FI	Organic fertilizer based on chicken manure.	10	1 A-I
HDG	DK	Liquid organic fertilizer based on digested pig's slurry	5	1 A-II
ILF	IT	Liquid organic fertilizer based on gelatin produced by the enzymatic hydrolysis of shavings from treated hides and skins.	10	1 A-II
MAL	DE	Organo-mineral fertilizer based on malt sprouts, vinasse from sugar beets processing and minerals.	6	1 A-I
MB2	CH	Organic fertilizer based on meat and bone meal.	10	1 B-I
MO13	NL	Organic pelleted fertilizer based on feather meal and vegetal by-products.	10	1 A-I
NAD	DE	Organic fertilizer based on fermented manure and vegetal by-products.	4	1 B-I
OG1	DK	Organo-mineral fertilizer based on meat and bone meal.	10	1 B-I
OG2	DK	Organic fertilizer based on horn meal from pigs' bristles.	10	1 A-I
OOO	ES	Composted by-products from the processing of olives.	3	1 A-I
OPU	CH	Organic fertilizer based on chicken manure.	10	1 B-I
PAL	DE	Organo-mineral fertilizer based on vegetal and animal by-products amended with fermented biochar, clay and stone powder.	4	1 A-I
PCS	DK	Liquid mineral fertilizer based on wastewater treatment by-products.	12	1 C-I-a
PCW	DK	Liquid organic fertilizer based on cell water from potato protein production.	6	1 B-II
PRI	NL	Organo-mineral fertilizer integrated with animal and vegetal by-products.	3, 6	1 B-I
SDG	DK	Liquid organic fertilizer based on fermented agricultural and food industry by-products, amended with seaweed.	5, 6	1 A-II
SIF	NL	Organic fertilizer based on hair meal, feather meal, meat and bone meal and vegetable by-products from the food industry.	6, (10)	1 A-I
SLA20	CH	Liquid organic fertilizer based on digested slurry.	5	1 A-II
SWG	CH	Organic fertilizer based on sheep wool.	10	1 A-I
SYS	NL	Liquid mineral fertilizer based on scrubber water from a biogas facility.	6	1 C-II-b
TRS	FR	Organo-mineral fertilizer based on fish soluble, proteins and ash.	10	1 A-I
VEC	ES	Vermicompost from horse manure.	3	1 A-I

The effectiveness of chemical extractions as predictors for mineralizable N is often assessed by correlating extracted vs. mineralizable N. However, considering multiple predictors simultaneously enhances the predictive power of chemical proxies, better representing the complexity of N mineralization (Braos et al., 2016; Lashermes et al., 2010; Ros et al., 2011). This has been achieved with multi-variate clustering methods (Lashermes et al., 2010; Morvan et al., 2006; Parnaudeau et al., 2004). Lashermes et al. (2010) applied this approach to generate solid multi-predictor decision trees using stepwise chemical digestion (SCD) properties and Kjeldahl organic N. This allowed the characterization of BBFs based on multiple defining N features rather than a single indicator. However, existing decision trees are based on chemical indicators which are expensive and labor intensive. Therefore, the aim of this study was to identify a set of chemical indicators that can be cheaply quantified in a standard laboratory.

The objective of this study was to identify a set of chemical extractions that can predict N mineralization dynamics of 32 BBFs as assessed in an aerobic incubation assay.

2. Materials and methods

2.1. Bio-based fertilizers

Thirty-two commercial or prototyped N-rich BBFs from 10 European countries were selected to represent the range of products currently available or under development in Europe. This selection included fertilizing products from six component material categories (CMCs) and six product function categories (PFCs) according to the European Fertilizing Product Regulation (Table 1). Products were stored following manufacturers' recommendations to keep them unaltered, and the storage period did not exceed one year. Fresh samples such as digestates were stored at -20°C . In preparation for incubation and extraction experiments, subsamples of solid BBFs were homogenized and ground to less than 2 mm using a pestle and mortar.

Basic characterization of BBFs included dry matter content (DMC, 105°C , EN 13040), OM content (combustion for 1 h at 350°C + 5 h at 480°C), C:N ratio (Trumac or CHN628, Leco), nitrate-N and ammonium-N (1:60 (v:v) water extracts analyzed with Skalar San++ system).(Fig. 1)

2.2. Biological characterization of mineralizable N and C

Apparent net mineralizable N and CO_2 release induced by the BBFs were assessed using aerobic incubation assays carried out over

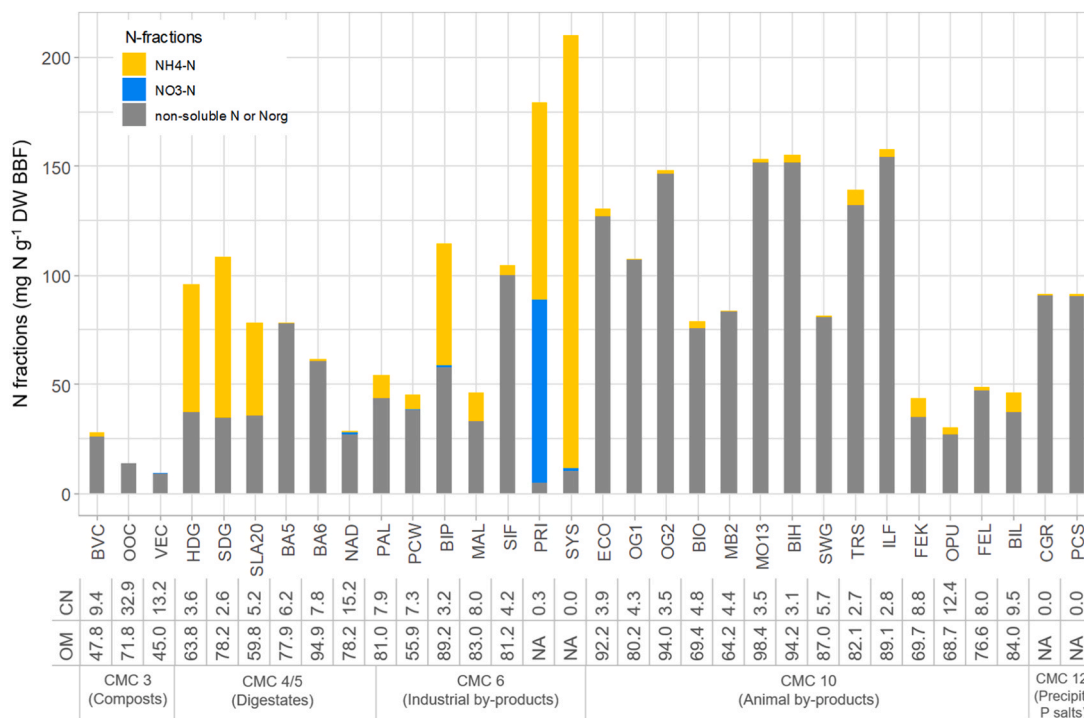


Fig. 1. Nitrogen (N) content of 32 bio-based fertilizers (BBFs) relative to their dry matter content (DW). Total N measured by dry combustion is further divided into cold-water soluble ammonium ($\text{NH}_4\text{-N}$), cold-water soluble nitrate ($\text{NO}_3\text{-N}$) and cold-water insoluble or organic N. Organic matter (OM) content expressed in percent of DW, CN ratios and component material categories (CMC) are noted in the table underneath the plot. NA. An accurate assessment of OM from PRI, SYS, CGR and PCS by calcination was not possible as indicated by NA (OM not available). Data partially redrawn from those presented in Wester-Larsen et al. (2022).

84 days in three replicates. C and N mineralization dynamics were assessed in separate samples, incubated simultaneously at 15°C and 80 % air humidity in a loamy topsoil (Loamy1, Table A1). Soil moisture was kept constant at 55 % of maximal water holding capacity of soil-BBF mixtures gravimetrically. In addition to the BBFs, unfertilized soil (UNF), ammonium sulfate (AMS) and potassium nitrate (PTN) were included as controls. N mineralization was assessed following the Swiss reference method (Agroscope, 1998a). In a nutshell, BBFs were surface applied to the equivalent of 120 g dry weight (DW) soil in a ratio of 800 mg N kg⁻¹ DW soil. N mineralization was quantified at six time points (day 0 (30 min after mixing BBFs with soil), 7, 14, 28, 56, 84) by extracting the BBF-soil mixture with 1 M KCl (ratio 1:4). Extracts were stored at -20°C until analysed photometrically (Smartchem 450 Discrete Analyser, AMS Alliance - Alliance Instruments, Switzerland). Nitrate (NO₃-N) content of the extracts was determined according to Keeney and Nelson (1983), while ammonium (NH₄-N) was determined using the modified indophenol blue reaction (Krom, 1980). Cumulative apparent N mineralization of the incubated soil-BBF mixture (N_{min}-N g¹ amended TN) was calculated as the sum of NO₃-N and NH₄-N in the sample minus the sum of NO₃-N and NH₄-N in the unfertilized control. To test the influence of soil type on N mineralization dynamics, an additional incubation experiment was conducted with 4 BBFs (BIO, ECO, PAL and OG2) and five additional soils with textures ranging from loamy sand to clay and organic C contents between 10.4 and 28.6 mg C g⁻¹ DW (Table A1, [supplementary material A1](#)).

In parallel, microbial respiration was measured based on a modified version of the Swiss reference method (Agroscope, 1998b). Essentially, BBFs were amended to the equivalent of 20 g DW soil in a ratio of 800 mg N kg⁻¹ DW soil and incubated in a closed system. Respired CO₂-C was trapped in 0.1 M NaOH and quantified at five time points (day 1, 7, 14, 56, 84) by the addition of 1 mL BaCl₂ followed by inverse titration with 0.1 M HCl on an automated titrator (complete Titrand modular setup, Metrohm AG, Herisau, Switzerland). Respired C from unfertilized soil was subtracted to obtain cumulative apparent C release from BBFs (mg CO₂-C g⁻¹ amended TC).

A detailed description of biological characterization methods is given in [supplementary material A1](#). Dynamics of N_{min}-N and respiration rates of UNF, mineral controls (AMS and PTN) and internal organic references are given in [supplementary material A2 and Figures A2, A3 and A4](#).

2.3. Chemical characterization of BBFs

Four chemical extraction methods originally developed for the extraction of N from soil were adapted to extract N from BBFs: 1:60 CW-N_{min} modified from EN 13652, hot water extractable N (HW-N, [Curtin et al., 2006](#)), hot potassium chloride extractable N (hKCl-N, [Chadwick et al., 2000](#)) and sulfuric acid extractable N (H₂SO₄-N, [Martínez and Galantini, 2017](#)). In pre-tests, different BBF-extractant ratios, incubation times and temperatures ([Table A2](#)) were tested on a subset of eight BBFs to select final protocols based on the requirement of extracting at least 5 % of TN. All final protocols followed the same extraction scheme with different settings ([Table A3](#)). In brief, a pre-defined amount of homogenized BBF was weighted into 50 mL centrifuge tubes and mixed with the extractant. Suspensions were incubated at a given temperature for a specific period in an oven (Binder GmbH, Tuttlingen, Germany). For 1:60 CW-N_{min}, HW-N and hKCl-N, a pre-extraction was performed to differentiate between N released during chemical extraction and easily soluble mineral N already present in the BBFs ([Table A3](#)). Pre-extracts were discarded and extracts were separated from the residue by centrifugation (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany, settings: 2600 x g, 15°C, 10 min) and stored at -20°C until further analysis. All extractions were run in duplicate (coefficients of variance mostly below 5 % and never exceeding 10 %). Extracted N was quantified by measuring Total Nitrogen bound (TNb) in the extracts (mg TNb g⁻¹ TN of BBFs) with a multi-N/C analyzer (Analytik Jena GmbH, Jena, Germany) calibrated with glycine.

In addition, we performed SCD but only for a subset of eleven solid and fully organic BBFs to quantify the soluble, cellulose-like and lignin-like fractions ([AFNOR, 2016](#)). A detailed description is given in [supplementary material A3](#).

2.4. Statistical analysis

All statistical analyses were performed using R, version 4.1.0 or 4.1.2 ([R Core Team, 2021](#)). BBFs were classified according to their N and C mineralization dynamic using a multivariate approach. In detail, a principal component analysis (PCA) was performed jointly on N_{min}-N and CO₂-C data, and the obtained principal components served as input data for the agglomerative clustering algorithm (euclidean distance, ward.D2 method) provided by the “eclust” function of the package “factoextra” ([Kassambara and Mundt, 2020](#)). This approach represents a simplification of the clustering technique used by [Parnaudeau et al. \(2004\)](#) and [Morvan et al. \(2006\)](#) and enables to reduce the effect of multicollinearity on the clustering result. The suitability of predictors for N mineralization estimation was evaluated based on linear correlations (Pearson) between predictors and N_{min}-N values performed with packages “PerformanceAnalytics” ([Peterson and Peter, 2020](#)) and “Hmisc” ([Harrell, 2021](#)). To avoid an underestimation of correlation coefficients caused by differences in moisture contents of BBFs ([Lazicki et al., 2020](#)), all values used for correlation analysis were expressed based on BBFs' DW. The suitability of selected N mineralization predictors to separate BBFs into distinct groups similar to the clustering based on C and N mineralization dynamics was evaluated based on significance levels obtained from analyses of variance (ANOVA) followed by Tukey tests with $\alpha < 0.05$. Visual assessments of the data with diagnostic plots were used to check for homogeneity of variance.

3. Results

3.1. Basic BBF properties

Overall, total N in the BBFs ranged from 9 to 210 mg TN g⁻¹ DW and total C from 3 to 528 mg TC g⁻¹ DW (Fig. 1). Composts had the lowest TN values and highest C:N ratios. Animal by-products other than manures presented the lowest C:N ratios. For digestates and the industrial by-products BIP, PRI and SYS, cold-water-soluble NH₄-N constituted over 50 % of TN. PRI was the only BBF containing substantial amounts of CW-soluble NO₃-N. Composts and chicken manures had lower OM contents than BBFs derived from animal by-products. OM contents of PRI, SYS, CGR and PCS were below detection limit. Dry matter content varied from 56 % to 97 % for solid BBFs and from 4 % to 56 % for liquid ones.

3.2. Biological characterization of mineralizable N

Apparent net mineralizable N was driven by increasing NH₄-N values during the first seven days of incubation (Figure A5), by increasing NO₃-N values and proportionally decreasing NH₄-N values between day 7 and day 28 (Figures A5 and A6), and by stagnating NO₃-N values for the remaining incubation time (Figure A6). Mineralizable N_{min}-N followed four main patterns: stagnation throughout incubation (e.g., VEC), stagnation followed by a decrease towards day 84 (e.g., SDG), increase or decrease following a log-type curve (e.g., SIF, OOC), increase following a linear curve (e.g., CGR).

Composts (CMC 3) showed very low or negative apparent net N mineralization, achieving N_{min}-N values between -134 mg N_{min}-N g⁻¹ amended TN (OOC) and 260 mg N_{min}-N g⁻¹ amended TN (VEC) on the last sampling day (Fig. 2). Fresh crop digestates (CMC 4) achieved higher final N_{min}-N values than composts (15.5–52.9 % of amended TN), either as a result of higher initial N_{min}-N values or higher net N mineralization rates (Fig. 2). In contrast, dried and solid crop digestates (CMC 5) showed very low net N mineralization rates, with N_{min}-N values tending to decrease towards the end of the incubation as a result of decreasing NH₄-N. Nonetheless, N_{min}-N values of these BBFs remained high throughout the incubation period because of initial NH₄-N values equivalent to an average of 50 % of amended TN. Similar to dried and solid crop digestates, TRS and BIP showed considerable negative N_{min}-N trends after day 28.

Industrial by-products (CMC 6) and precipitated phosphate salts and derivatives (CMC 12) produced very diverse apparent net N mineralization patterns. Especially for BBFs low in OM, cumulative N_{min}-N increased linearly or remained constant throughout the incubation period, reaching N_{min}-N values from 87 % to 105 % of amended TN after 84 days of incubation. This pattern was mainly NO₃-N driven, as NH₄-N values only increased by a maximum of 12.8 % in the first 2 weeks of incubation. For the remaining BBFs in these categories, the highest increases of N_{min}-N were recorded during the first seven days of incubation, achieving apparent net N_{min}-N values from 43 % to 55 % of amended TN on day 84. BBFs derived from animal by-products (CMC 10) presented similar N

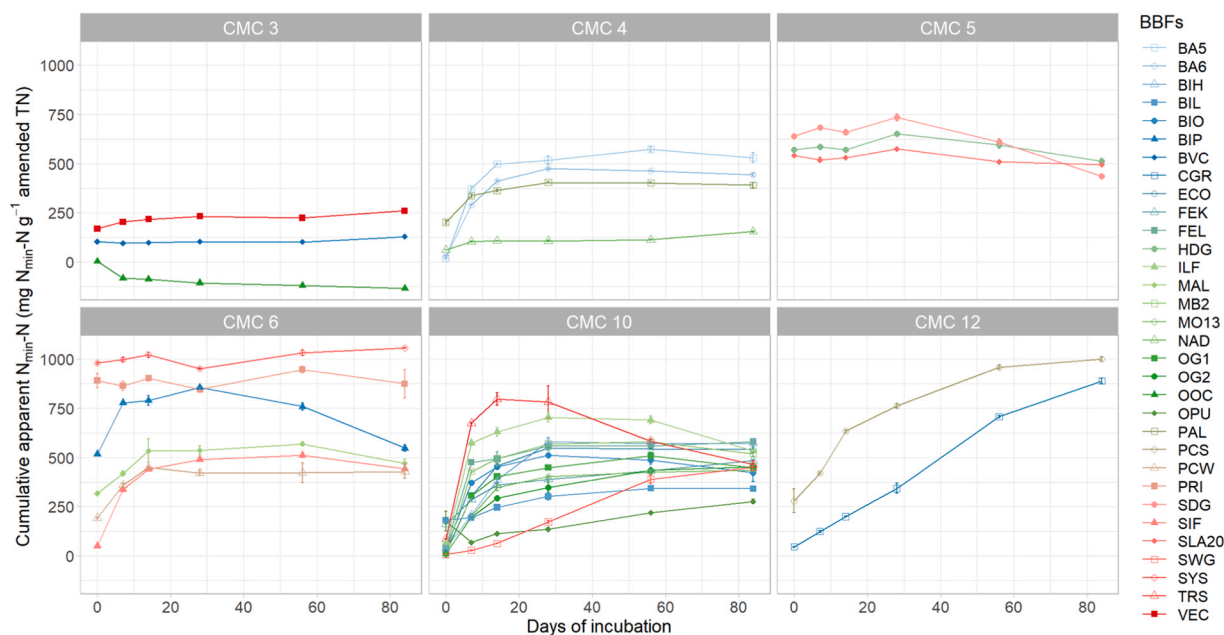


Fig. 2. Cumulative apparent mineralized nitrogen (N_{min}-N) values (means and standard errors) expressed relative to total amended nitrogen (TN) (800 mg N kg⁻¹ DW soil) and shown in sub-groups based on the component material category (CMC) of bio-based fertilizers (BBFs). Values of unfertilized soil were subtracted. The N_{min}-N value on day 0 is not derived from mineralization, but mainly reflects the intrinsic mineral N content of the BBFs. Assuming linear mineralization rates between time points (except for day 0), the slope of the lines connecting measured values represents N_{min}-N mineralization rate.

mineralization curves, with an accentuated N_{\min} -N increase in the first seven days of incubation and steady values between 34 % and 58 % of amended TN from day 28 onwards. Exceptions were SWG and OPU, which showed lower N_{\min} -N values until day 56 (SWG) or until the end of incubation (OPU).

In the additional experiment on the effect of soil type on N mineralization of four BBFs, only the sandy soil showed significantly lower mineralization rates than the other soils (Figure A7, Table A4).

3.3. Biological characterization of mineralizable C

Respiration rates representing apparent C mineralization from BBFs covered a wide range of patterns overall and even within each CMC group (Fig. 3). For most BBFs, the highest respiration rates were recorded within the first 24 h of incubation, with a maximum value of $13.2 \pm 0.3 \mu\text{g CO}_2\text{-C g}^{-1} \text{ DW soil h}^{-1}$ for BA6 (data not shown). BBFs inducing the highest respiration rates during the first seven days were either liquid and fully organic (BIP, PCW, ILF), or based on meat and bone meal (ECO, OG1) or on chicken manure (FEK, OPU, BIL). After the initial rapid mineralization phase, $\text{CO}_2\text{-C}$ values stabilized at a constant level until day 84 for all BBFs.

Over the entire incubation period, between 14 % and 112 % of amended TC was respired. For BBFs very low in TC (CGR, PCS, SYS), respiration rates were similar to those of UNF and thus not included in statistical analyses.

3.4. Classification of BBFs based on mineralizable N and C

BBFs were classified according to their N and C mineralization dynamic using a multivariate approach. This classification produced five strongly data-supported BBF clusters as indicated by bootstrapping p -values lower than or equal to 0.05 (Fig. 4, A10, A11).

Cluster 1 consisted of ten fully organic, solid BBFs and one organo-mineral, liquid BBF. Animal protein (meat and bone meal) and vegetal by-products from the food industry represented the main component materials (Table 2). Despite the mainly low initial N_{\min} -N levels ($<50 \text{ mg } N_{\min}\text{-N g}^{-1} \text{ amended TN}$), BBFs of cluster 1 showed high initial N mineralization dynamics, achieving the overall highest N_{\min} -N levels of tested BBFs. Cluster 2 encompassed seven fully organic or organo-mineral, solid BBFs based mainly on animal protein (hairs or feather meal) or chicken manure. N_{\min} -N values from cluster 2 showed similar dynamics to cluster 1 but were about 30 % lower than those of cluster 1. Cluster 3 contained three liquid or easily soluble BBFs based on animal proteins and vegetal by-products from the food industry. These BBFs were characterized by high initial N_{\min} -N values (logarithmic-type N_{\min} curves) and negative N mineralization rates from day 28 onwards. Cluster 4 exclusively included solid, composted BBFs characterized by the overall lowest N_{\min} -N levels on day 84. N mineralization dynamics in this cluster presented almost linear curves dominated by very low or even negative N mineralization rates within the first seven days of incubation. Cluster 5 comprised only digestates. These liquid BBFs were characterized by the overall highest initial N_{\min} -N values and constant negative N mineralization rates from day 28 onwards.

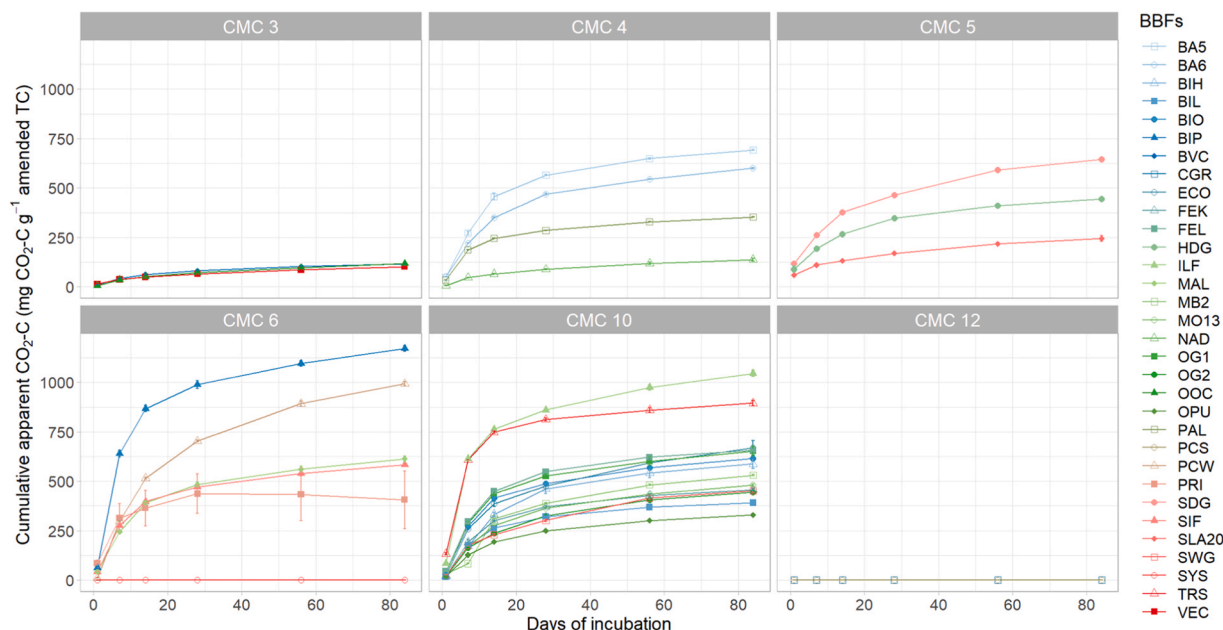


Fig. 3. Cumulative apparent respired carbon ($\text{CO}_2\text{-C}$) values (means and standard errors) expressed relative to the amount of amended total carbon (TC) and shown in sub-groups based on the component material category (CMC) of bio-based fertilizers (BBFs). Values of the unfertilized soil were subtracted and values of CGR, PCS and SYS set to zero. Assuming linear respiration rates between time points, the slope of the lines connecting measured values represent C mineralization rates.

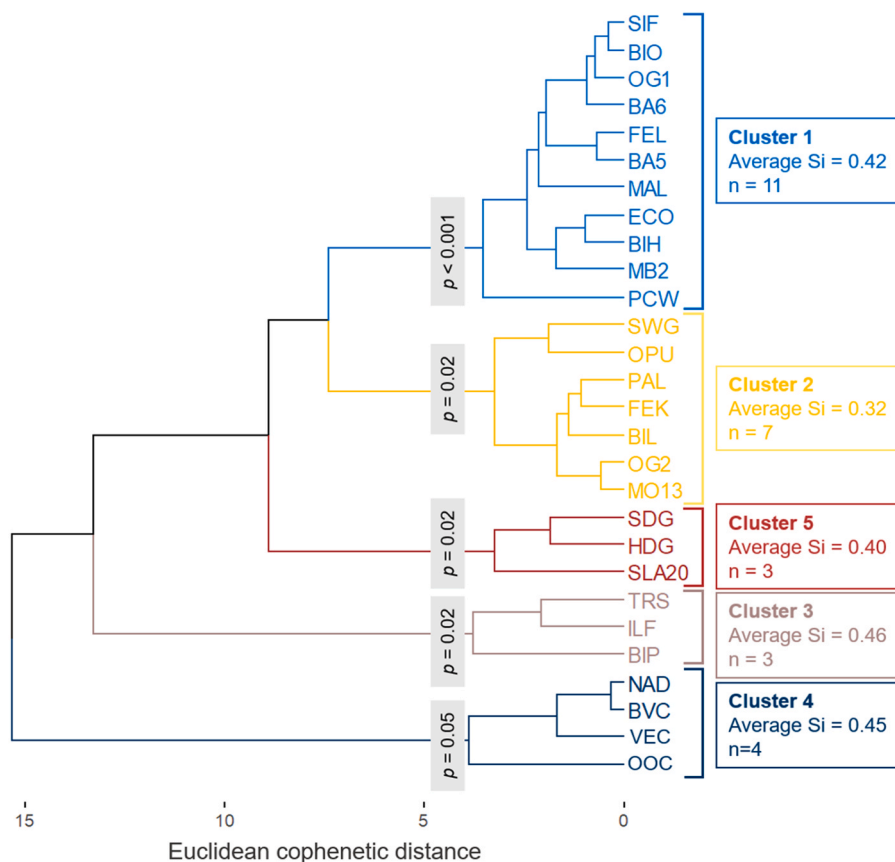


Fig. 4. Classification of bio-based fertilizers (BBFs) based on N_{\min} -N and CO_2 -C data. Clusters result from aggregative hierarchical clustering applied to principal components of N_{\min} -N and CO_2 -C data. Cluster information includes cluster number, average silhouette width (Average Si), number of included BBFs (n) and bootstrapping-generated p-values. Note that clusters are not ordered numerically ascending.

Table 2

Characterization of bio-based fertilizers (BBF) clusters based on nitrogen (N) and carbon (C) mineralization data (Fig. 4) with respect to component material categories (CMCs), product function categories (PFCs), average initial N_{\min} -N (N_{\min} - N_{d0}), average cumulative apparent N_{\min} -N at the end of incubation (N_{\min} - N_{d84}), average apparent mineralizable N represented by N_{\min} -N differences between days 0 and 84 of incubation (ΔN_{\min}) and average cumulative apparent CO_2 -C at the end of incubation (CO_2 - C_{d84}). Values indicate the cluster mean \pm standard deviation. UNF values were subtracted.

Cluster	CMCs	PFCs	Mean N_{\min} - N_{d0} mg N_{\min} -N g ⁻¹ amended TN	Mean N_{\min} - N_{d84}	Mean ΔN_{\min}	Mean CO_2 - C_{d84} mg CO_2 -C g ⁻¹ amended TC
1	(4), 6, 10	1A-I, 1B-I, 1B-II	71 \pm 28	491 \pm 17	419 \pm 36	654 \pm 35
2	(3), 4, 10	1A-I, 1B-I	107 \pm 32	404 \pm 26	297 \pm 51	415 \pm 20
3	6, 10	1A-I, 1A-II	223 \pm 120	516 \pm 21	292 \pm 109	1038 \pm 65
4	3	1A-I, 1B-I	85 \pm 30	103 \pm 73	18 \pm 47	118 \pm 7
5	5, (6)	1A-II	583 \pm 24	480 \pm 19	-102 \pm 41	445 \pm 95

3.5. Chemical characterization

Across all BBFs, 1:60 CW- N_{\min} yielded the lowest extractable N values (Table 3) reflecting the mineral N contents of BBFs (Fig. 1) and was highest for liquid BBFs low in OM. Also, HW-N yielded low extractable N values (0–25 % of TN), followed by hKCl-N (0–47 % of TN) and H_2SO_4 -N (9–100 % of TN). Liquid and easily soluble BBFs (TRS) achieved the lowest HW-N (< 90 mg TNb g⁻¹ TN BBF) and hKCl-N (< 200 mg TNb g⁻¹ TN BBF) values coupled with highest H_2SO_4 -N levels (> 790 mg TNb g⁻¹ TN BBF), whereas solid BBFs yielded intermediate extractable N values.

The prediction power of chemical extractions in relation to mineralizable N at different stages of incubation was evaluated based on Pearson correlations (Table A5). At the start of the incubation, 1:60 CW- N_{\min} showed the strongest correlation with N_{\min} -N ($R^2 = 0.97$),

Table 3

Extractable nitrogen (N) fractions of bio-based fertilizers (BBFs) obtained using the following chemical extraction methods: hot-water extractable N (HW-N, mg TNb g⁻¹ TN BBF), hot-KCl extractable N (hKCl-N, mg TNb g⁻¹ TN BBF), sulfuric acid extractable N (H₂SO₄-N, mg TNb g⁻¹ TN BBF) and cold water extractable mineral nitrogen (1:60 CW-N_{min}, mg N_{min}-N g⁻¹ TN BBF). Data were collected at the Research Institute of Organic Agriculture (FiBL) and the Natural Resources Institute of Finland (LUKE).

BBF	HW-N	hKCl-N	H ₂ SO ₄ -N	1:60 CW-N _{min}
BA5	32.1	100.6	403.7	12.6
BA6	96.9	260.8	435.7	14.4
BIH	159.2	200.5	257.1	21.0
BIL	132.2	355.7	513.9	199.5
BIO	200.3	393.5	502.1	35.2
BIP	10.6	24.8	1137.9	495.4
BVC	147.3	174.1	254.7	82.8
CGR	23.7	290.4	346.9	7.2
ECO	48.8	114.5	332.4	26.7
FEK	303.3	466.1	438.4	203.0
FEL	156.3	174.2	280.0	33.3
HDG	81.8	6.0	941.9	613.6
ILF	7.0	22.8	921.3	21.5
MAL	26.6	366.2	706.4	279.2
MB2	248.3	401.0	558.3	5.0
MO13	59.6	161.3	308.7	9.9
NAD	112.4	226.1	203.1	40.2
OG1	95.6	249.2	485.3	6.9
OG2	48.8	97.8	242.2	10.1
OOC	87.3	105.7	95.1	0.0
OPU	218.3	375.8	355.4	111.5
PAL	93.3	221.5	410.6	196.3
PCS	18.6	196.0	502.6	10.7
PCW	40.6	129.0	1040.6	156.9
PRI	105.1	229.2	871.9	973.5
SDG	77.9	177.0	891.7	682.9
SIF	139.1	270.7	391.5	46.4
SLA20	59.2	103.5	790.7	558.1
SWG	64.2	102.1	174.5	2.6
SYS	0.0	0.3	963.8	951.7
TRS	6.4	18.1	989.8	50.1
VEC	148.3	328.9	428.6	33.9

while H₂SO₄-N was strongly correlated with N_{min}-N at day 7 ($R^2 = 0.72$) and 14 ($R^2 = 0.63$) of the incubation. At the late stage of incubation, C:N ratio showed the strongest correlation with N_{min}-N on day 56 ($R^2 = 0.68$) and day 84 ($R^2 = 0.73$). In contrast, HW-N and hKCl-N did not correlate strongly with N_{min}-N values at any incubation stage.

3.6. Chemical indicators as cluster predictors

Based on the results of Pearson correlations, 1:60 CW-N_{min}, H₂SO₄-N and C:N ratio were selected to be evaluated as explanatory variables for BBF classes. In the multivariate space defined by principal components from PCA with cumulative N_{min}-N and CO₂-C values over the entire mineralization period, 1:60 CW-N_{min} notably distinguished BBFs in cluster 5 from the rest (Fig. 5). The trends of H₂SO₄-N and C:N ratio were almost opposite to each other and discriminated between BBFs of clusters 1, 2, 3 and 4. Tukey tests were conducted to assess differences between BBF clusters for the selected predictors (Table A7). Cluster 5 had significantly higher 1:60 CW-N_{min} values compared to all other clusters ($p < 0.001$), cluster 3 and 5 had significantly higher H₂SO₄-N values compared to clusters 1, 2 and 4 ($p < 0.05$), and cluster 4 showed significantly higher C:N ratios compared to the other clusters ($p < 0.01$). Combining all selected predictors, cluster 4 could be discriminated based on its high C:N ratios, cluster 3 based on its high H₂SO₄-N values, and cluster 5 by its high 1:60 CW-N_{min} levels combined with high H₂SO₄-N values. Only clusters 1 and 2 could not be significantly distinguished from each other with any of the selected predictors, despite a measurable difference in value distribution of 1:60 CW-N_{min}, H₂SO₄-N and C:N ratio.

4. Discussion

4.1. Biological characterization

In 84-day aerobic incubations, cumulative mineral N release ranged from -13–106 % amended N (Fig. 2). A similar range was found by Levavasseur et al. (2022) in their database containing N mineralization data for 663 exogenous organic materials (EOMs) and in previous studies (Calderón et al., 2005; Cordovil et al., 2005; Morvan et al., 2006).

Composts (CMC 3) showed low respiration rates (0–20 %), in some cases combined with net N immobilization (e.g., OOC). This N

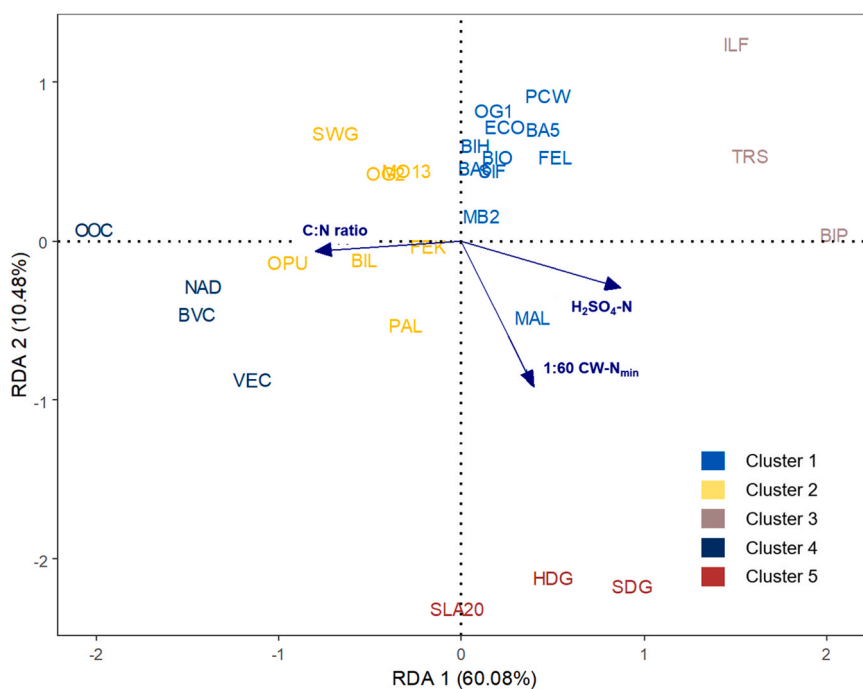


Fig. 5. Redundancy analysis (RDA) based on principal components of mineral nitrogen (N_{\min} -N) and respired carbon (CO_2 -C) data (dependent variables) displaying selected chemical predictors as explanatory variables. Selected predictors are cold water extractable mineral N (1:60 CW- N_{\min}), sulfuric acid extractable N (H_2SO_4 -N) and carbon to nitrogen ratio (C:N ratio). Bio-based fertilizers (BBFs) names are colored according to the cluster they are assigned to by aggregative hierarchical clustering (Fig. 4).

limitation likely results from high initial C:N ratios of composted materials rather than from the composting process itself (Amlinger et al., 2003; Lazicki et al., 2020; Sradnick and Feller, 2020). Similarly to Pansu and Thuriès (2003), no remineralization of immobilized N was observed, confirming that BBFs with high C:N ratios need to be regarded as mineral N sinks rather than sources during short- or medium-term incubation periods.

Non-fresh crop digestates (CMC 5), mainly including digested slurries, presented considerably higher N_{\min} -N values than similar materials in the study by Levavasseur et al. (2022), where up to 50 % of N was mineralized after a 1-year incubation under field conditions. However, the initially high mineral N contents and the rapidly decreasing NH_4 -N values coupled with low respiration rates indicate the predominance of NH_4 -N removing processes (nitrification, volatilization, immobilization) over ammonification. Indeed, potential volatilization has been shown to account for losses of up to 15 % of added N for digestates in laboratory incubation experiments (Wester-Larsen et al., 2022).

Precipitated phosphate salts and derivatives (CMC 12) did not show pronounced logarithmic first-order-kinetic-type mineralization curves, achieving cumulative N_{\min} -N values higher than 80 % of amended N after 84 days of incubation despite very low respiration rates throughout the incubation period. These results resemble N-release patterns of controlled release fertilizers (Du et al., 2006). Given the low OM content of CGR and PCS, N_{\min} release from these BBFs is primarily driven by the solubilization of the coating of the granules and not by mineralization processes. Similar N release patterns were observed for SYS and PRI. For these reasons, CGR, PSC, PRI and SYS should be considered similar to mineral or slow-release fertilizers and were thus excluded from the evaluation as in the present study.

Animal by-products (CMC 10) and fresh crop digestates (CMC 4) presented a first-order kinetic-type N mineralization in accordance with previously reported results (Lazicki et al., 2020; Levavasseur et al., 2022). Based on the collected cumulative N_{\min} -N, no evidence for N immobilization or volatilization was found. However, Wester-Larsen et al. (2022) observed cumulative potential NH_3 volatilization above 10 % of added TN for all animal BBFs after 44 days of incubation when applied on the soil surface. When incorporated into the soil, volatilization decreased. This highlights the importance of assessing NH_3 volatilization potential in a given system to achieve a comprehensive estimation of mineralizable N from BBFs.

It should also be noted that gross N mineralization cannot be measured with the setup presented here. However, as losses also occur under field conditions, gross N mineralization is less relevant for estimating plant N availability of BBFs.

4.2. Chemical characterization

Concerning CMCs, C:N ratios and original mineral N values, BBFs in the present study showed similar ranges as reported by Lazicki et al. (2020) and Levavasseur et al. (2022). In contrast to other incubation studies (Levavasseur et al., 2022; Parnaudeau et al., 2004),

precipitated phosphate salts and derivatives in the present study (CGR, PCS) were very low in OM and TC content, resulting in C:N ratios close to zero.

To the best of our knowledge, no published studies assessed HW-N or H₂SO₄-N of EOMs as potential predictors for their mineralizable N values. hKCl-N extractions have been applied to EOMs before (Chadwick et al., 2000), with similar results as observed in the present study. HW-N and hKCl-N included a pre-extraction in order to separate readily available N from highly labile N. Thus, for liquid or highly soluble BBFs (e.g., BIP, TRS) the pre-extraction step removed up to 99 % of TN from the BBFs, resulting in very low HW-N and hKCl-N. On the other hand, H₂SO₄-N (no pre-extraction) values of these BBFs were close to 100 % of the BBF's TN.

For HW-N, hKCl-N and H₂SO₄-N, standard errors were under 5 % and never exceeded 10 %, attesting that the protocols used can be considered reliable. However, sample homogenization in preparation for extractions is a critical step, especially for suspended BBFs such as digestates or BBFs based on mixed animal and vegetal by-products (e.g., MO13), which showed the highest standard errors despite careful sub-sampling.

Extraction intensity and leaching mechanisms are generally critical features that can influence the prediction power of chemical N extraction methods. Of particular relevance is the thermal hydrolysis of heat-labile organic N compounds to NH₄-N, which can be expected to occur during extraction procedures at elevated temperatures (Curtin et al., 2006; Gregorich et al., 2003). This implies that mineral-to-organic N ratios measurable in the extracts are not fully representative of the original ratios of untreated samples. To compensate for this effect, for the screening intentions of the present study, extractable N was quantified as TNb rather than by N_{min} or Kjeldahl N assessment methods. However, this choice implied the lack of a further subdivision of extracted N into mineral and organic species, which could give a deeper insight into the nature of extracted N and lead to different correlation patterns with mineralizable N. Nonetheless, even with TNb quantification NH₄-N remained a critical N species to assess because especially at high concentrations losses could not be avoided, as was clearly visible from the H₂SO₄-N value of the fully mineral and liquid BBF "SYS" (Table 3).

The proposed classification of extraction procedures for SOM-N of Ros et al. (2011) based on TN percentages discriminating between mild, medium and strong methods extracting < 5 %, < 35 % and > 35 % of TN, could also be applied to BBF-adapted methods, as a similar range of extraction intensities was observed in our study. Expanding on various extraction intensities in SOM-N mineralization studies, some authors noted stronger correlations between mineralizable N and mild chemical indicators. They argued that such extractions are less likely to extract recalcitrant organic N pools (Braos et al., 2016; Curtin et al., 2006). However, neither Ros et al. (2011) nor this study confirmed this observation.

In fact, extraction intensity influenced N_{min}-N prediction power, but methods with low extraction intensities (HW-N and hKCl-N) correlated only weakly with mineralizable N, supporting the findings of Chadwick et al. (2000). In contrast, H₂SO₄-N and SCD-derived variables showed a considerably higher correlation strength with N_{min}-N values (Table A5, A6) as reported in former studies (Jensen et al., 2005; Lashermes et al., 2009; Morvan et al., 2006). The prediction power of C:N ratios and TN for mineralizable N has been widely studied showing highest correlations to N_{min}-N at late stages of incubation (Jensen et al., 2005; Lashermes et al., 2009; Morvan et al., 2006). Similarly to Lazicki et al. (2020), C:N ratios appeared to be better indicators of N_{min}-N than TN, as C:N ratios are more decisive for the affinity of EOMs to mineralization.

The strong correlation between 1:60 CW-N_{min} and N_{min}-N levels at the start of the incubation can be explained by considering the methodological aspects of these N quantifications. In fact, both variables were obtained following similar protocols, which are designed to target readily available N pools exclusively. The correlation between N_{min}-N and DMC can be explained by the fact that the total N of liquid BBFs often has a higher proportion of inorganic N. Also, physical degradation properties (e.g., affinity to solubilization or crumbling) of BBFs could serve as explanatory variables of C and N mineralization dynamics in the first week of incubation, but they were not considered in the present study.

4.3. Evaluation of chemical predictors

In the present study, the suitability of a number of chemical BBF characterization methods was evaluated with respect to i) the estimation of cumulative apparent N_{min}-N levels in different incubation phases and ii) the discrimination of significantly different C and N mineralization patterns. Based on correlation coefficients, coefficients of determination and levels of significance, three predictors were considered suitable for estimating N_{min}-N at given incubation stages. Threshold values of the correlation coefficient $r > 0.8$, $R^2 > 0.6$ and $p < 0.001$ were applied as evaluation criteria for the selection of suitable chemical indicators. In contrast, for fertilizer recommendations based on mineralizable SOM-N it has been suggested that only characterization methods associated with $R^2 > 0.83$ can be reliably used to extrapolate mineralizable SOM-N levels (Malley et al., 2004). However, even the best predictors for mineralizable SOM-N did not achieve $R^2 > 74$ % (Ros et al., 2011), suggesting that $R^2 \geq 0.6$ can be considered indicative of reasonably high prediction power for the estimation of N_{min}-N from BBFs.

In this regard, it is important to notice how the reliability of predictors changes in relation to different phases of N mineralization. The C:N ratio, for example, could be considered a reliable predictor of cumulative N_{min}-N at the end of the incubation, as reported by several authors (Chadwick et al., 2000; Lazicki et al., 2020; Morvan et al., 2006), but has been reported as only a poor proxy for N_{min}-N levels in the first stages of incubation (Jensen et al., 2005). Thus, the C:N ratio is not a suitable candidate for a holistic single-predictor-based estimation of BBFs' mineralization dynamics.

Ros et al. (2011) suggested that the combination of multiple predictors could lead to higher confidence levels of N_{min}-N prediction through a more comprehensive characterization of the mineralized materials. Furthermore, considering the incubation stage-dependent correlation patterns, a multi-predictor approach comprising a combination of reliable proxies for different stages of N mineralization could further improve prediction power. A number of EOM classifications build on this principle, using multivariate approaches to generate and evaluate chemical predictor-based EOM clusters (Bareha et al., 2018; Lashermes et al., 2010; Morvan et al.,

2006; Parnaudeau et al., 2004).

Following this approach, C:N ratios, $\text{H}_2\text{SO}_4\text{-N}$ and 1:60 CW- N_{\min} were selected as chemical predictors for the reconstruction of the $\text{N}_{\min}\text{-N}$ and $\text{CO}_2\text{-C}$ -based BBF clustering (Fig. 5). Based on the analyses of variance, C:N ratios could significantly discriminate BBF classes characterized by low N mineralization or immobilization (cluster 4) from those with medium to high cumulative $\text{N}_{\min}\text{-N}$ values, with similar thresholds to those observed by other authors for plant materials (Trinsoutrot et al., 2000), wastewater sludges (Parnaudeau et al., 2004) and EOMs (Lashermes et al., 2010; Pansu et al., 2003). Values of 1:60 CW- N_{\min} discriminated BBFs contained in cluster 5 mainly as a result of their high initial $\text{N}_{\min}\text{-N}$ but did not remarkably contribute to subdividing BBFs based on their N and C mineralization patterns in the mid to late stages of incubation. This finding is related to the rapidly decreasing influence of initial mineral N concentrations on N mineralization within the first seven days of incubation. Amongst selected chemical indicators, $\text{H}_2\text{SO}_4\text{-N}$ was the predictor significantly discriminating the highest number of BBF clusters due to its high prediction power for mid-incubation $\text{N}_{\min}\text{-N}$ values. Similarly, SDC was able to predict mid-incubation $\text{N}_{\min}\text{-N}$ values and could help to further discriminate between clusters 1 and 2. As SDC is also a rather costly and time-consuming method, near infrared spectroscopy (NIRS), which was found to correlate well with SDC fractions (Henriksen et al., 2007; Peltre et al., 2009), may be a suitable indicator to be tested.

Using a set of standardized and soil-independent predictors for mineralizable N is highly desirable to facilitate the application BBFs and to avoid time-consuming soil specific aerobic incubation assays. Especially when considering that N mineralization depends not only on soil texture, as shown in the present study (Figure A7, Table A4), but also on SOC content and environmental parameters such as soil moisture and temperature (Elrys et al., 2021). Nevertheless, field validation of chemical predictors with selected BBFs from each BBF cluster is needed to refine the effective mineralization dynamics of the BBFs under specific pedo-climatic conditions also considering potential interactions between N and C fractions of BBFs which might influence mineralization of organic matter.

4.4. Classification of BBFs

Classification of BBFs based on principal components of $\text{N}_{\min}\text{-N}$ and $\text{CO}_2\text{-C}$ data generated five strongly data-supported clusters. This number is similar to other studies on EOMs where multivariate clustering approaches generated five (Parnaudeau et al., 2004, 17 EOMs) to six (Lashermes et al., 2010, 273 EOMs; Morvan et al., 2006, 47 EOMs) clusters. Similarly to these publications, BBF clusters generally discriminated between i) very high and fast N mineralization associated with high C mineralization (cluster 3), ii) high N mineralization associated with medium-high C mineralization (cluster 1), iii) medium N mineralization associated with medium-low C mineralization (cluster 2), iv) low or negative N mineralization associated with medium-low C mineralization (cluster 4), and v) low or negative N mineralization associated with medium C mineralization (cluster 5).

In terms of CMCs (i.e., raw materials and their processing), only clusters 4 and 5 could be assigned uniquely to one category, whereas clusters 1, 2 and 3 all included a combination of fully organic or organo-mineral BBFs based on animal, vegetal or mixed raw materials. Thus, even within the same CMC, BBFs differ with respect to their C and N mineralization patterns. In this respect, it should be mentioned that the CMCs are primarily intended to simplify trading and are less suitable for application recommendations. In particular, BBFs from CMC 10 formed three sub-groups: animal proteins (e.g., meat and bone meal) represented in cluster 1, animal proteins high in beta-keratin content (e.g., feather meal, animal hairs) and (chicken) manures represented in cluster 2, easily soluble animal proteins (e.g., fish soluble) represented in cluster 3. Wester-Larsen et al. (2022) proposed a more detailed CMC grouping (especially CMC10), which could potentially better fit clusters in our study, which groups BBFs according to a combination of feedstock and processing technology. Similarly to the CMC distribution amongst clusters of the present study, also Parnaudeau et al. (2004) and Morvan et al. (2006) discriminated municipal wastewater sludges and composted materials, respectively, into four significantly different N mineralization classes. Thus, it appears unlikely that CMC is a suitable predictor for C and N mineralization dynamics of BBFs. In contrast, the proposed multi-predictor approach can provide practical information on N mineralization dynamics needed by end-users for efficient field application of BBFs.

5. Conclusions

The 32 investigated BBFs displayed diverse mineralizable N and C patterns, forming five distinct clusters that did not align with the European Fertilizing Products Regulation's CMC classification. Tested chemical BBF characterization methods covered a spectrum of extraction intensities. Their prediction power for mineralizable N levels of BBFs varied depending on the considered stage of N mineralization. The correlations between mineralizable N and cold water extractable mineral N, sulfuric acid extractable N and C:N ratio were satisfactory for the estimation of mineralizable N from BBFs at different mineralization stages. The combination of the three chemical indicators was able to classify BBFs in four out of the five groups previously established based on their N and C mineralization patterns. SCD-fractions and near-infrared spectroscopy could potentially serve as additional indicators to address this uncertainty.

This study shows that a multiple-predictor approach based on chemical indicators targeting differently labile organic N pools of BBFs can be a realistic alternative to biological characterization methods, provided that a sufficient number of suitable predictors, e.g. three as in this study, is available. We recommend these indicators for the development of a novel BBF classification system to facilitate the agronomical application of BBFs and ensure a link between N release dynamics of BBFs and their context-specific application (pedoclimate, crop, growth stage).

CRedit authorship contribution statement

L. Agostini: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Formal

analysis, Data curation, Conceptualization. **Bunemann E.K. Bunemann:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **C. Jakobsen:** Resources, Methodology, Formal analysis, Conceptualization. **T. Salo:** Resources, Methodology, Formal analysis, Data curation. **L. Wester-Larsen:** Writing – review & editing, Validation, Resources, Methodology, Formal analysis, Data curation. **S. Symanczik:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are deposited at Zenodo repository 10.5281/zenodo.12746383

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.eti.2024.103781](https://doi.org/10.1016/j.eti.2024.103781).

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