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Research article

The degree of hydrolysis of meat and bone meals fertilizers drives carbon mineralisation and the availability of nitrogen and phosphorus in soil

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ABSTRACT

Meat and bone meals (MBMs) are animal by-products that can be recycled in agriculture as organic fertilizers following appropriate sanitization treatments. However, nutrients bioavailability depends on soil mineralisation processes and often does not match crops' nutrient demand. Alkaline thermobaric hydrolysis (ATH) is a scalable physico-chemical process commonly applied to animal biomasses to enhance nutrient availability, yet its effects on the fertilising potential of MBMs remains poorly understood.

In this study, two ATH protocols were applied to an MBM to obtain products with different degrees of hydrolysis (DH). The resulting fertilizers were characterized in term of DH and subsequently incubated into the soil to assess organic carbon (C) mineralisation and nitrogen (N) and phosphorous (P) availability.

Increasing ATH intensity resulted in higher DH values, ranging from 7 to 8% in non-hydrolysed MBM to 13-15% and 40-60% in low- and high-hydrolysed MBMs, respectively. Organic C mineralisation was not significantly affected by ATH treatment. In contrast, soil N availability increased with increasing DH, with potentially mineralizable N rising from 37% in the non-hydrolysed MBM to 48-52% in the hydrolysed products. Soil available P, however, was not enhanced by the hydrolytic process.

These findings demonstrate that alkaline thermobaric hydrolysis can modulate nutrient release from MBMs, primarily increasing N availability, while having limited effects on organic C mineralisation and P availability. Overall, the results suggest that the degree of hydrolysis may influence nutrient release dynamics and should be considered when developing MBM-based fertilizers with targeted agronomic properties.

1. Introduction

Recycling organic waste as fertilizers in agriculture represents an important and common practice for improving soil fertility (Luo et al., 2018), and it is completely in line with the principles of the circular economy. Organic and organic-based fertilizers (hereinafter referred to as organic fertilizers throughout the text) can alleviate threats to soil fertility, such as desertification and erosion, by improving the physical, chemical and biological properties of soils (Bebber and Richards, 2022; Diacono et al., 2019; Wang et al., 2019). Organic fertilizers can reduce the use of chemical fertilizers, the production of which has a significant environmental impact (Galloway et al., 2008). Moreover, recycling waste biomass as organic fertilizers helps address issues related to the end-use of these materials, which are usually landfilled or incinerated, resulting in significant economic costs and potential environmental risks (Sharma et al., 2019).

One important source of organic waste for fertilizer production is animal by-products (ABPs), such as meat meal, fish meal, feather meal, leather meal, horn meal and hoof meal (Bhunia et al., 2021). Limited data are available regarding the global amount of ABPs suitable for organic fertilizer production, but it is estimated to represent approximately one-third of the live weight of animals, equivalent to around 150 million tonnes per year (Limeneh et al., 2022).

Meat meal, bone meal and meat and bone meal (MBM) are ABPs from slaughterhouses and are produced from animal processing offal. MBMs were previously used as a source of proteins and minerals in animal feed (Hendriks et al., 2002), but their use as feed is now strictly regulated (in the EU by Regulation (EC) No. 1069/2009), following cases in which transmissible spongiform encephalopathy (TSE) was linked to feeding ruminants with MBMs contaminated with the causative agent of the

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disease. Under Regulation (EC) No. 1069/2009, ABPs are classified into three categories: materials suspected of being infected with TSE (Category 1), materials presenting a risk of infection other than TSE (Category 2), and materials not showing any signs of disease transmissible to humans or other animals (Category 3). According to Regulation (EC) No. 1069/2009, Category 1 ABPs must be used as fuel or disposed of as waste through incineration or co-incineration. Category 2 and 3 ABPs must undergo sterilisation (>133°C, >3 bar, >20 min for Category 2; and >70°C, >1 h for Category 3) before being used as feed or fertilizers. The strict restrictions on the use of MBM as feed have increased the interest of organic fertilizer producers in these materials, owing to their content of organic carbon (C), nitrogen (N), phosphorus (P) and calcium (Ca) (Jatana et al., 2020; Jeng et al., 2004; Nogalska and Załuszniewska, 2021).

In addition, the use of ABP-derived materials as fertilising products in the European Union is currently regulated within the framework of Regulation (EU) 2019/1009, which establishes requirements for CE-marked EU fertilising products and interfaces with the animal by-products legislation. Therefore, the valorisation of MBMs as fertilizers requires not only compliance with sanitary requirements but also conformity with product quality, safety, contaminant and labelling standards. Within this regulatory context, technologies capable of modulating nutrient release while preserving product quality are of particular interest.

Meat meal, bone meal and MBM have been used as organic fertilizers for many crops (Jeng et al., 2004, 2006; Kivelä et al., 2015; Nogalska and Załuszniewska, 2021), and studies have examined organic C mineralisation (Cayuela et al., 2008; Mondini et al., 2008), organic N mineralisation (Chaves et al., 2014; Rapisarda et al., 2022) and P availability (Bøen and Haraldsen, 2013; Jeng and Vagstad, 2009; Nogalska and Zalewska, 2013) under different environmental conditions and across different soil types. As MBMs are rich in organic matter (60–80% dry matter), their nutrients (e.g. N and P) become available only after mineralisation, which is mainly regulated by soil characteristics and the microbial community (Creamer et al., 2015; Jatana et al., 2020; Jeng et al., 2006; Ylivainio et al., 2008a). Nitrogen in MBMs is mainly present in organic forms (3–12% dry matter), such as polypeptides, with a C/N ratio ranging from 3.2 to 4.8 (Jeng et al., 2004). MBMs also contain a substantial amount of P (2–10% dry matter), occurring as apatite P in the bone fraction and in organic forms within the meat fraction (Bøen and Haraldsen, 2013). When used as organic fertilizers, MBMs have shown high N-use efficiency in crops (60–80% of the applied N), with rapid N release following soil application (Jatana et al., 2020; Jeng et al., 2006; Mondini et al., 2008). In contrast, P becomes available to crops more slowly, with effects lasting for several years depending on soil characteristics (e.g. pH) (Bøen and Haraldsen, 2013; Jeng et al., 2006). The effects of MBMs on soil fertility, and on N and P availability, have been extensively investigated under a wide range of environmental conditions and soil types (Cayuela et al., 2008; Chaves et al., 2014; Kivelä et al., 2015; Liu et al., 2019; Mondini et al., 2008; Nogalska and Załuszniewska, 2021; Spångberg et al., 2011; Tenuta and Lazarovits, 2004). However, considerably less attention has been paid to how processing treatments applied to MBMs before soil application influence their subsequent nutrient release dynamics. In particular, despite the growing interest in alkaline hydrolysis as a strategy to modify the physicochemical properties of protein-rich biomasses, little information is available on how different degrees of hydrolysis of the same MBM feedstock affect soil C mineralisation, N mineralisation and P availability. Consequently, the extent to which hydrolysis can be used to modulate the agronomic behaviour of MBM-based fertilisers remains poorly understood.

Partial alkaline hydrolysis of protein biomasses, such as MBMs, is currently used to solubilise proteins and peptides into water-soluble forms (Friedman et al., 1984; Garcia et al., 2011; Matinong et al., 2022; Piazza and Garcia, 2010). The application of alkaline hydrolysis to MBMs is of interest not only for increasing their safety (Kalambura et al., 2008) but also for modulating nutrient release to better match crop requirements. For example, increasing protein solubility could increase the rate of mineralisation of organic C and N, as well as increase P availability. Alkaline hydrolysis is commonly carried out using sodium (Na) or calcium hydroxide solutions; however, these alkaline solutions are not fully compatible with the production of high-quality fertilizers (Gousterova et al., 2008; Kang et al., 2019), because they increase pH and salinity, induce the precipitation of Ca phosphates (through both increasing pH and supplying Ca) and increase the risk of N losses through ammonia (NH_3) volatilisation after field application (Wester-Larsen et al., 2022). Furthermore, the relatively high temperatures (e.g. 60–85°C) and long hydrolysis times (e.g. 2–48 h) commonly used to solubilise collagen from ABPs may substantially alter their fertilising characteristics (Matinong et al., 2022; Salim et al., 2024). For example, they may cause losses of C and N (as carbon dioxide, CO_2 and NH_3) or reduce the agronomic quality of polypeptides by excessively decreasing peptide size or causing complete racemisation of amino acids (Bellagamba et al., 2015; Liu et al., 2025). The use of a basic salt such as sodium carbonate, combined with thermobaric alkaline hydrolysis involving high temperatures under pressure for short time intervals, may represent a logical solution to preserve the agronomic quality of polypeptides while simultaneously modulating nutrient release into the soil.

The general objective of this work was therefore to investigate how different degrees of hydrolysis influence nutrient release dynamics from MBM-based fertilisers. To this end, MBMs with different DH were prepared by means of alkaline thermobaric hydrolysis in the presence of sodium carbonate. These materials were characterised to identify parameters capable of estimating their degree of hydrolysis and to document the physicochemical modifications induced by the treatment. Subsequently, they were applied to a model calcareous soil, and soil C mineralisation, N mineralisation and P availability were evaluated. The hypotheses tested were as follows: (1) increasing the intensity of alkaline thermobaric hydrolysis applied to MBMs increases the degree of hydrolysis of the material; and (2) differences in the degree of hydrolysis are associated with differences in nutrient release dynamics following soil application, particularly with respect to C and N mineralisation.

2. Materials and methods

2.1. Meat and bone meal samples

2.1.1. Thermobaric alkaline hydrolysis (TAH)

A sample of MBM was purchased on the market and processed to obtain hydrolysed MBMs (HMBMs) using a pilot-scale reactor. The MBM sample was mixed with 3 wt% sodium carbonate (pH 11.5 ± 0.1), a relatively inexpensive alkaline agent, immediately before thermobaric hydrolysis. The industrial production process of HMBM was simulated using a specially designed hermetically sealed, high-pressure resistant steel container with a capacity of approximately 1 L. Once filled to approximately one-third of its volume (~0.7 kg) with MBM and a small amount of water (~5–10 g) to allow steam generation, the container was placed inside an industrial reactor for thermobaric hydrolysis and autoclaved under saturated steam under the conditions reported in Table 1.

The different hydrolytic treatments were selected to obtain two organic fertilisers with increasing DH (low, HMBM-L and high, HMBM-H), while avoiding conditions severe enough to induce complete

Table 1
Description of hydrolysis processes applied on the meat and bone meal (MBM).

Sample	Description
MBM	Meat and bone meal, as received
HMBM-L	MBM hydrolysed in 3% Na_2CO_3 at 5 bar for 20 min (158 ± 3°C)
HMBM-H	MBM hydrolysed in 3% Na_2CO_3 at 7 bar for 20 min (170 ± 3°C)

hydrolysis of the MBM sample. Each hydrolysis treatment was repeated three times, and the resulting hydrolysates were combined and homogenised to obtain the final samples used for analysis. Following treatment, the samples were dried at 40 ± 2°C in a ventilated oven until constant weight, then milled and sieved to 0.5 mm. The samples were stored at room temperature in closed bottles protected from light.

2.1.2. Physico-chemical characterisation

All samples were analysed for their main physicochemical characteristics. The pH was determined in a 0.01 M $CaCl_2$ extract (1.5:25, w/v) using a combined electrode (5209, Crison Instruments SA, Alella, Spain) connected to a pH metre (Compact titrator, Crison Instruments SA, Alella, Spain). Salinity was measured in a water extract (1:5, w/v) using a conductivity cell (CDC 749, Radiometer Analytical, Loveland, CO, USA) connected to a conductivity metre (CDM 210, Radiometer Analytical, Loveland, CO, USA). Total solids were determined by drying to constant weight at 105°C using a static oven (FD 23, Binder GmbH, Tuttlingen, Germany). Ash content was determined by loss on ignition at 550°C for 8 h (FM 3, FALC, Italy). Total organic C (TOC) was determined according to the wet oxidation method using potassium bichromate (Springer and Klee, 1954). Total Kjeldahl nitrogen (TKN) was determined using an automatic Kjeldahl instrument (KjelFlex K360, Büchi Labortechnik AG, Flawil, Switzerland) after acid digestion with concentrated sulphuric acid (96%) and selenium–potassium persulfate as a catalyst. The inorganic forms of N, ammonium (NH_4^+) and nitrate (NO_3^-), were extracted with 1 M potassium chloride (1:50, w/v) for 2 h at room temperature and subsequently analysed using the Kjeldahl automatic instrument after the addition of 1 g magnesium oxide for NH_4^+–N determination and reduction with 0.5 g Devarda alloy for NO_3^-–N determination, respectively. Total N (TN) was considered equal to TKN because the NO_3^-–N concentrations were below the detection limit of the method for all MBM samples. Total organic N (TON) was calculated as the difference between TN and NH_4^+–N. Total P (TP) and metals were determined by inductively coupled plasma optical emission spectroscopy (Spectro Arcos ICP–OES Analyser, Spectro Analytical Instrument GmbH, Kleve, Germany) after microwave-assisted aqua regia digestion (Ethos Start D, Milestone, Sorisole, Italy). Heavy metal concentrations were subsequently compared with the limit values established for PFC 1(A) Organic Fertilisers under Regulation (EU) 2019/1009. Phosphorus soluble in neutral ammonium citrate (pH 7.0) and water was determined according to methods 3.1.4 and 3.1.6 of the Official methods for the Analysis of Fertilisers of the European Union (European Parliament and Council, 2003).

2.1.3. Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopic analysis

ATR–FTIR spectroscopy was used to identify structural modifications induced by alkaline thermobaric hydrolysis and to provide complementary information on changes in the organic fractions of MBM products. ATR–FTIR analysis was performed using an infrared spectrophotometer (Tensor 27, Bruker Corporation, Billerica, USA). A small aliquot of each sample was deposited onto the diamond ATR device (Quest Single Reflection ATR Accessory, Specac Ltd., Orpington, England) connected to the infrared spectrophotometer. Spectra were recorded over the range 4000–400 cm^{-1}, with a spectral resolution of 4 cm^{-1} and 64 scans, and were processed using OPUS 6.5 software (Bruker, Karlsruhe, Germany).

2.1.4. Differential thermal (DTA) and thermogravimetric (TGA) analyses

DTA and TGA were used to characterise changes in the thermal stability and decomposition behaviour of MBM products following hydrolysis. DTA and TGA were carried out using a TG–DTA92 instrument (Setaram, Caluire-et-Cuire, France). Approximately 10 mg of sample was weighed into an alumina crucible and heated from 30 to 700°C at a heating rate of 10°C min^{-1} under a dynamic air atmosphere with a flow rate of 8 L h^{-1}. An empty crucible was used as the reference material. The DTA profile represents the temperature difference (ΔT) between the sample and the reference as a function of time. DTA data, reported as heat flow (mW mg^{-1} of sample), provide qualitative information on exothermic and endothermic transformations. TGA data were reported as thermogravimetric (TG) profiles, representing the percentage weight loss experienced by the sample, and differential thermogravimetric profiles, obtained from the first derivative of the TG profile and representing the rate of weight loss (percentage per unit time).

2.1.5. Degree of hydrolysis determination

The DH of MBMs was determined using the water-extractable (WE) fraction, obtained following the procedure adopted by Cayuela et al. (2009). Briefly, 10 g (dry weight) of products was added to 100 mL of deionised water and extracted using an end-over-end shaker (120 rpm) for 2 h at room temperature (20 ± 2°C). The mixtures were centrifuged (6.000 *g* for 30 min at 4 ± 1°C) and filtered through a 0.4 μm hydrophilic polycarbonate membrane (Isopore, Millipore, USA) under vacuum. The filtrate was analysed for water-extractable organic C (WE_{OC}) and water-extractable total N (WE_{TN}) using a TOC-VCSN analyser (Shimadzu Corporation, Kyoto, Japan), and for water-extractable ninhydrin-reactive N (WE_{NRN}) after reaction with a reagent solution supplied by Sigma-Aldrich (N7285). This reagent consists of a solution of ninhydrin and hydrindantin in dimethyl sulphoxide and lithium acetate buffer (pH 5.2) (Moore, 1968). Briefly, 0.2 mL of diluted water extract and 0.2 mL of ninhydrin reagent solution were added to a 2.0 mL microtube. The solution was rapidly mixed by inversion, and the samples were placed in a boiling water bath for exactly 10 min. The samples were then cooled using an ice-water bath to room temperature, after which 1.0 mL of ethyl alcohol (95%) was added. Absorbance was measured at 570 nm using a spectrophotometer (Jasco 7700, Jasco Corporation, Japan). The WE_{NRN} concentration was determined by comparing the absorbance at 570 nm with a standard calibration curve prepared using an L-alanine solution (0.05% in glacial acetic acid).

The DH of MBMs was estimated from the ratios between WE_{OC} and TOC, and between WE_{TN} and TN, according to the following equations:

$$DH_C(\%) = \frac{WE_{OC}}{TOC} \cdot 100$$

$$DH_N(\%) = \frac{WE_{TN}}{TN} \cdot 100$$

where DH_C and DH_N represent the degree of hydrolysis of C and N, respectively.

The DH based on WE_{NRN} was estimated from the ratio between WE_{NRN} and WE_{TN} according to the following equation:

$$DH_{NRN}(\%) = \frac{WE_{NRN}}{WE_{TN}} \cdot 100.$$

2.1.6. Hot-water-extractable nitrogen (HWEN) analysis

Hot-water-extractable N in MBMs was determined according to Rapisarda et al. (2022). Briefly, 5 g of fertiliser was mixed with 100 mL of deionised water and placed in a water bath for 16 h at 80 ± 2°C under end-over-end agitation. The mixture was then centrifuged (30,000 *g*, 15 min, 20°C) and filtered through Whatman No. 42 filter paper. The filtered solution was analysed for TN as described in Section 2.1.2. HWEN is expressed as a percentage of TN.

2.2. Soil incubation experiment

The incubation experiment was carried out using a *Tipic Xeropsamment* sandy soil (main characteristics: clay 11%, silt 14%, sand 75%, pH 8.1, cation exchange capacity 18 cmol (+) kg^{-1}, total $CaCO_3$ 62%, active $CaCO_3$ 18%, TOC 1.27%, TN 0.082%, C/N ratio 15.5) collected from the top 0.3 m of an agricultural field near Rimini (Northern Italy, 44°01′27.57″ N, 12°36′45.89″ E; mean annual temperature 14.2 ± 0.1°C;

mean annual precipitation 626 ± 38 mm). This soil was selected because it is representative of agricultural areas where MBM-based fertilisers are commonly used. The experimental design was intended to investigate the effects of different degrees of hydrolysis under controlled conditions in a relevant agricultural soil, rather than to evaluate the response of a wide range of soil types. The soil was adjusted to 50% of its water-holding capacity and pre-incubated for 10 days in an incubator set at 23 ± 1°C. MBMs were added to the soil at a rate of 100 mg N kg^{-1} dry soil. This application rate was selected in accordance with commonly adopted laboratory incubation procedures for evaluating N mineralisation from organic amendments, including ISO 14238:2012, and was intended to provide sufficient analytical sensitivity for comparing nutrient release dynamics among treatments under controlled conditions. Because the conversion of mg N kg^{-1} soil to field-equivalent application rates depends on assumptions regarding soil bulk density and incorporation depth, no direct agronomic application rate was inferred from the experimental dosage (Table S2). The treated soils were divided into two parallel sets of incubations: the first dedicated to organic C mineralisation, and the second to organic N mineralisation and P availability.

2.2.1. Carbon mineralisation in soil

Soil CO_2–C evolution fluxes were determined using static incubation vessels (23 ± 1°C) containing the equivalent of 10 g dry soil treated with MBMs and 10 mL of 0.05 M NaOH. The NaOH was titrated with 0.025 M HCl to a pH endpoint of 8.3 after the addition of 2 mL of 0.5 M $BaCl_2$ (Anderson, 1982). The cumulative amount of CO_2–C evolved was calculated based on the measured CO_2–C evolution rate. The experiment was conducted for 21 days, until the respiration rate of treated soils declined to that of the untreated control soil. The data were fitted over incubation time (t) using the logistic model proposed by Cayuela et al. (2008):

$$CO_2 - C(t) = \frac{C_{max}}{1 + \left(\frac{t}{t_{½}}\right)^{k_C}}$$

where C_{max} is the maximum cumulative respiration level (mg CO_2–C kg^{-1} dry soil), k_C is the respiration rate constant ($days^{-1}$), and $t_{½}$ is the half-time (days), representing the time required to reach half of C_{max}.

2.2.2. Nitrogen mineralisation and available P in soil

After periods of 0, 7, 14, 21, 28, 35, 49, 63, 84 and 105 days following MBM application, soil from four replicate microcosms (~200 g dry weight basis) was analysed for proteolytic activity, inorganic N (NH_4^+–N and NO_3^-–N + NO_2^-–N) and available P.

The activity of protease hydrolysing casein was determined using 1% casein as the substrate, incubated for 2 h at 50 ± 1°C in 0.05 M Tris-HCl buffer (pH 9.0), and the released tyrosine was quantified using the Folin–Ciocalteu colorimetric method (Ladd and Butler, 1972). Soil proteolytic activity was expressed as mg tyrosine h^{-1} kg^{-1} dry soil.

Inorganic N (N_i) was extracted with 1 M KCl (1:10, w/v) for 2 h at room temperature, and concentrations of NH_4^+–N and NO_2^-–N + NO_3^-–N were determined by automated flow analysis (AA3, Bran-Lubbe, Germany). The data were used to calculate mineralised organic N (N_m) using the equation proposed by Agehara and Warncke (2005):

$$N_m = [(N_i)_{t=n} - (N_i)_{t=0}]_{FERT} - [(N_i)_{t=n}]_{CK}$$

where t is the sampling time, *FERT* is the fertilised soil, and *CK* is the control soil.

The N_m data were fitted using a first-order kinetic model proposed by Griffin and Honeycutt (2000) and Stanford and Smith (1972):

$$N_m(t) = N_0 \cdot (1 - e^{-k_N \cdot t})$$

This model estimates the potentially mineralizable N (N_0) and the first-order rate constant (k_N) over the incubation period. N_m denotes the amount of mineralised N (sum of inorganic N forms) at a given time (t).

Available P was determined using the Olsen colorimetric method (Olsen et al., 1954). The data were used to calculate net available P (P_n) using the following equation:

$$P_n = [(P_{available})_{t=n}]_{FERT} - [(P_{available})_{t=n}]_{CK}$$

where t is the sampling time, *FERT* is the fertilised soil, and *CK* is the control soil.

The P_n data were fitted using an exponential asymptotic model (Archontoulis and Miguez, 2015):

$$P_m(t) = P_{asym} + (P_0 - P_{asym}) \cdot e^{-k_P \cdot t}.$$

This model estimates the amount of net available P (Olsen-P) at the beginning of incubation (P_0), the asymptotic value (P_{asym}) and the exponential rate constant (k_P). Moreover, P_m denotes the amount of net available P at a specific time (t).

2.3. Statistical analysis and data management

All chemical characterisations were carried out in duplicate independent analyses. Data in Tables 2 and 3 are reported as means, and relative standard deviations were lower than 5% for each parameter. All data were analysed using the R environment for statistical computing (R Core Team, 2022). For the soil incubation experiment, means of all parameters at each sampling time were compared for significant differences using Tukey's HSD test (α = 0.05). Parameters of the nonlinear models (logistic, first-order and exponential asymptotic) were estimated using nonlinear regression with the Marquardt–Levenberg algorithm and an iterative procedure to minimise the residual sum of squares (Benbi and Richter, 2002). The selected models were chosen based on their widespread application and biological relevance for describing C mineralisation, N mineralisation and nutrient release processes in soil incubation studies. Model performance was evaluated through residual analysis, parameter significance, 95% confidence intervals, and goodness-of-fit statistics. When alternative model formulations were tested, model selection was supported using information criteria (AIC and BIC) together with biological interpretability of the fitted parameters. Goodness of fit of the final models was additionally assessed using pseudo-R^2 (P-R^2), calculated as P-R^2 = 1 − (RSS/TSS), where RSS is the residual sum of squares and TSS is the total sum of squares (Schabenberger and Pierce, 2001). The half-time (t½) of the first-order

Table 2
Main chemical characteristics of meat and bone meal (MBM) and hydrolysed MBM (HMBM), data reported on fresh (fw) or dry weight (dw).

Parameters	Unit	MBM	HMBM-L	HMBM-H
Moisture	% fw	3.55	3.92	4.23
Ashes	% dw	34.3	35.0	36.8
Volatile solids	% dw	65.7	65.0	63.2
pH ($CaCl_2$)		6.6	7.7	8.4
Electrical Conductivity (EC)	dS m^{-1}	3.45	3.67	4.51
Total Organic C (TOC)	% dw	40.4	39.5	39.1
Total N (TN)	% dw	7.71	7.63	7.57
Ammonium-N	% dw	0.16	0.16	0.22
Nitrate-N	% dw	<0.01	<0.01	<0.01
Total Organic N (TON)	% dw	7.56	7.47	7.35
C/N ratio		5.2	5.1	5.2
Total P	% dw	5.88	5.79	5.75
Ammonium Citrate-soluble P	% dw	1.81	1.97	2.75
Water-soluble P	% dw	0.186	0.156	0.155
C/P ratio		6.9	6.8	6.8
N/P ratio		1.3	1.3	1.3
Total S	% dw	0.277	0.288	0.311
Total K	% dw	0.490	0.558	0.570
Total Ca	% dw	8.79	8.56	8.45
Total Mg	% dw	0.196	0.188	0.182
Total Na	% dw	1.06	1.45	2.12

Table 3
Water extractable organic C (WE_{OC}), water extractable total N (WE_{TN}), water extractable ninhydrin reactive N (WE_{NRN}), degree of hydrolysis of organic C (DH_C), total nitrogen (DH_N), water extractable total N (WE_{NRN}) and hot-water extractable N (HWEN) of MBMs samples.

Parameters	Unit	MBM	HMBM-L	HMBM-H
WE_{OC}	g C kg^{-1}	28.5	57.6	151
WE_{TN}	g N kg^{-1}	5.9	9.6	44.5
WE_{NRN}	g N kg^{-1}	0.222	0.272	0.567
DH_C	%	7.3	14.6	40.2
DH_N	%	7.9	12.9	61.4
DH_{NRN}	%	3.76	2.83	1.27
HWEN	% TN	27.0	40.3	70.9

model was calculated as ln(2)/kN. Model parameters were considered significantly different ($\alpha = 0.05$) when their 95% confidence intervals did not overlap. All equations were fitted using all data points, although only mean values are shown in the figures.

3. Results

3.1. Chemical characterisation of meat and bone meal and HMBMs

The results of the main chemical characterisation of MBM and HMBM are reported in Table 2. Alkaline hydrolysis tended to increase ash content, ranging from 34.3% to 36.8% on a dry weight (dw) basis from MBM to HMBM-H. Conversely, volatile solids decreased from 65.7% (MBM) to 63.2% dw (HMBM-H). pH also increased with alkaline hydrolysis, being slightly acidic in the untreated MBM (pH 6.6), slightly alkaline in HMBM-L (pH 7.7) and moderately alkaline in HMBM-H (pH 8.4). Electrical conductivity (EC) ranged from 3.45 to 4.51 dS m^{-1}, increasing with hydrolysis intensity. TOC ranged from 39.1 to 40.4% dw and decreased slightly with increasing hydrolysis intensity. TN also decreased slightly, ranging from 7.57 to 7.71% dw. Inorganic N represented less than 5% of TN, with only NH_4^+–N detectable. Consequently, TON ranged from 7.35 to 7.56% dw, accounting for more than 95% of TN. The C/N ratio was similar across all MBM products, ranging from 5.1 to 5.2. TP, similar to TOC and TN, slightly decreased with hydrolysis, ranging from 5.75 to 5.88% dw. Soluble P fractions showed different trends: water-soluble P ranged from 0.155 to 0.186% dw and decreased with hydrolysis, whereas ammonium citrate–soluble P ranged from 1.81 to 2.75% dw and increased with hydrolysis. Total S and total K increased with hydrolysis, ranging from 0.277 to 0.311% dw and 0.49 to 0.57% dw, respectively. Total Ca and total Mg slightly decreased with hydrolysis, ranging from 8.45 to 8.79% dw and 0.182 to 0.196% dw, respectively. Total Na increased markedly with hydrolysis, ranging from 1.06 to 2.12% dw. The concentrations of potentially toxic elements (PTEs) are reported in Table S1. All measured PTE concentrations were substantially below the limit values established for PFC 1(A) Organic Fertilisers under Regulation (EU) 2019/1009, indicating that the tested materials complied with the current European requirements regarding heavy metal content.

The ATR–FTIR spectra in the range 4000–400 cm^{-1} are shown in Fig. 1. The spectral regions between 3500 and 2800 cm^{-1} and 1750–900 cm^{-1} appeared to be the most affected by hydrolysis. The main band assignments of ATR–FTIR profiles are reported in Table S3 and were interpreted considering product chemical composition and origin (Keshavarzi et al., 2020; Mehta et al., 2020; Wu et al., 2022). The ATR–FTIR profiles of the products were similar; however, HMBMs showed overall more intense signals than MBMs. The band at 3281 cm^{-1}, attributed to O–H stretching of carboxylic, alcoholic or phenolic groups, increased with DH. Similarly, the bands at 2292 and 2853 cm^{-1}, attributed to C–H stretching of aliphatic structures, were more intense in HMBMs than in MBM. The same trend was observed for the band at 1739 cm^{-1}, attributed to C=O stretching in carboxylic or ester groups, which showed higher intensity in HMBMs than in MBM. In the spectral region from 1700 to 900 cm^{-1}, band intensities increased with DH, following the order HMBM-H > HMBM-L > MBM. Overall, the ATR–FTIR results indicate progressive modifications of the proteinaceous and oxygen-containing organic fractions with increasing hydrolysis intensity, supporting the differentiation of MBMs according to their degree of hydrolysis.

Fig. 2 shows the TG and DTA profiles of MBM and HMBMs. TG profiles showed an initial weight loss (4–5%) below 200°C, associated with a small exothermic peak in the DTA profile, commonly attributed to the loss of residual water. Three main exothermic regions were observed: the first with a maximum at 364–377°C, the second at 459–492°C and the third at 519–555°C. Above 600°C, no further weight loss or heat flow was observed. The three products showed different patterns in DTA peak intensities. For MBM, the three peaks had similar heat flows (1.0–1.2 mW/mg), with the highest at 474°C. In HMBM-L, the first peak (371°C) was the lowest (0.8 mW/mg), while the other two were similar (1.4–1.5 mW/mg). In HMBM-H, the second peak (459°C) was the lowest (0.8 mW/mg), while the other two were similar (1.5–1.6 mW/mg). The TG profiles showed a similar pattern with minor differences. MBM showed a weight loss of 29.9% corresponding to the first exothermic peak, followed by losses of −11.3% and −7.4% for the second and third peaks, respectively. HMBM-L showed a similar trend with slightly higher losses across all stages. Finally, HMBM-H showed increased weight losses associated with the first and third peaks (−34.5% and −12.8%, respectively) and a decrease for the second peak (−8.6%). Overall, the TG-DTA profiles confirmed that ATH altered the thermal decomposition behaviour of MBM, with the magnitude of the changes increasing with the degree of hydrolysis.

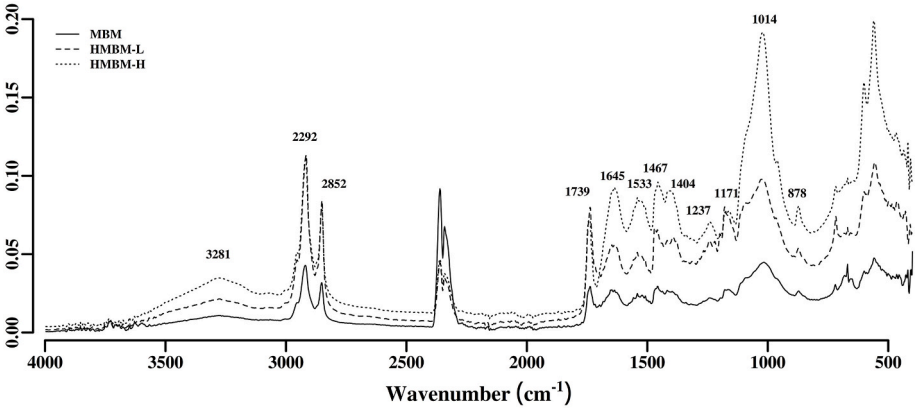

Fig. 1. Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectra of MBM and hydrolysed MBMs.

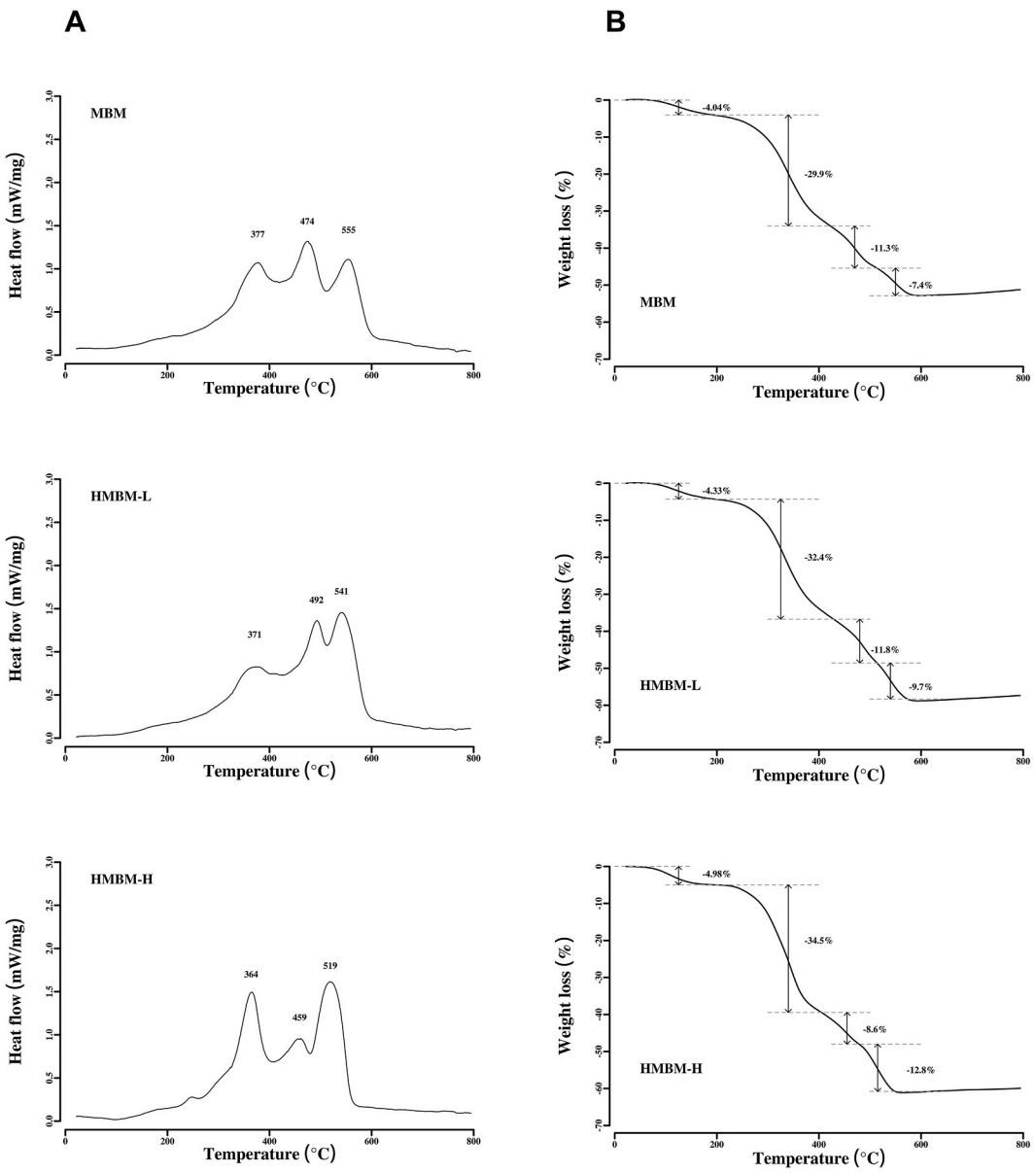

Fig. 2. Differential Thermal Analysis (A) and Thermogravimetric (B) profiles of MBM and hydrolysed MBMs.

3.2. Degree of hydrolysis and hot-water-extractable N

The results of DH and HWEN analyses for MBM and HMBM samples are reported in Table 3. The concentrations of water-extractable organic C (WE_{OC}), TN (WE_{TN}) and ninhydrin-reactive N (WE_{NRN}) increased with alkaline hydrolysis: WE_{OC} increased from 28.5 to 151 g C kg^{-1}, WE_{TN} from 5.9 to 44.5 g N kg^{-1} and WE_{NRN} from 0.222 to 0.567 g N kg^{-1}. Accordingly, the DH of C (DH_C) and N (DH_N) showed similar trends, increasing markedly from MBM to HMBM-H: DH_C increased from 7.3 to 40.2%, and DH_N from 7.9 to 61.4%. In contrast, the DH of NRN (DH_{NRN}) decreased with hydrolysis, from 3.76% to 1.27%. The calculated HWEN was 27.0%, 40.3% and 70.9% of TN for MBM, HMBM-L and HMBM-H, respectively (Table 3).

3.3. Soil incubation experiment

3.3.1. Carbon mineralisation in soil

The results of soil respiration rate (SRR) (mg C–CO_2 h^{-1} kg^{-1}) and cumulative CO_2 released (mg C–CO_2 kg^{-1}) from soil amended with MBM and HMBMs are reported in Fig. 3(A and B, respectively). Soils treated with MBMs showed a sharp increase in respiration rate at the beginning of incubation (maximum at day 2), followed by a gradual decrease, reaching values comparable to the unamended soil (0.25–0.30 mg C–CO_2 h^{-1} kg^{-1}) after approximately three weeks. However, significant differences in SRR between treatments were observed only after one day of incubation ($p < 0.001$). The highest SRR was recorded for HMBM-H (3.23 mg C–CO_2 h^{-1} kg^{-1}), followed by HMBM-L (2.41 mg C–CO_2 h^{-1} kg^{-1}), MBM (2.06 mg C-CO_2 h^{-1} kg^{-1}) and bare soil (1.12 mg C–CO_2 h^{-1} kg^{-1}). No significant differences in SRR or cumulative CO_2 release were observed among the three fertiliser treatments during the remainder of the incubation, except in comparison with the bare soil, with the exception of the last two sampling times for SRR. The logistic model applied to soil C mineralisation data fitted the extra CO_2 released from soils treated with MBMs well (p-$R^2 > 0.99$; Fig. 3 C). No significant differences in the estimated kinetic parameters were observed among treatments. The model showed no significant differences among the three products, with maximum C mineralised (C_{max}) ranging from 41.7% to 47.6% of TOC added, and a half-time of C mineralisation ($t_{½}$) ranging from 2.69 to 3.0 days (Table 4).

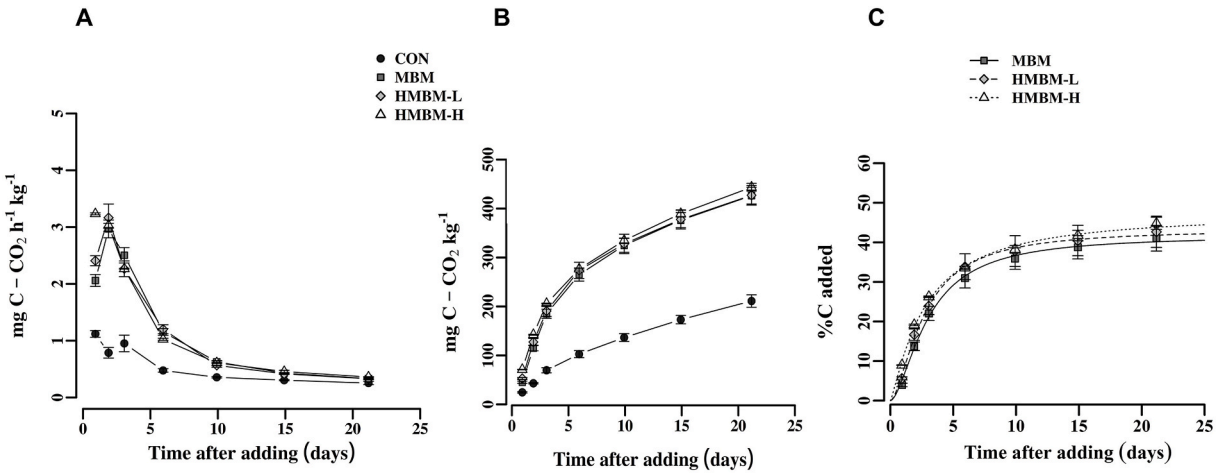

Fig. 3. Respiration rate during the incubation experiment of meat and bone meal in soil A); cumulative carbon dioxide released from soil treated with meat and bone meals B); logistic model fitting of extra CO_2-C released from soil during meat and bone meals incubation in soil C). Data reported as mean, error bars are one standard error of the mean.

Table 4
Parameters estimated according to the logistic model for the total organic C (TOC) mineralisation of different products into the soil (data reported as means ± one standard error of the means, C_{max} is the quantity of maximum mineralizable organic C; k_C is the rate constant; $t_{½}$ is the half-time, RSS is the residual sum-of-squares, P-R^2 is the pseudo-R square of the model; different letters indicate that the 95% confidence intervals between means did not overlap).

Products	C_{max} % TOC added	k_C	$t_{½}$ days	RSS	P-R^2
MBM	41.7 ± 1.2 a	−1.653 ± 0.145 a	3.00 ± 0.18 a	4.094	0.996
HMBM-L	43.3 ± 1.0 a	−1.601 ± 0.120 a	2.69 ± 0.14 a	3.145	0.997
HMBM-H	47.6 ± 2.0 a	−1.203 ± 0.122 a	2.76 ± 0.27 a	4.665	0.995

3.3.2. Nitrogen mineralisation in soil

The results of soil proteolytic activity (mg Tyr h^{-1} kg^{-1}) during the incubation experiment are reported in Fig. 4. Amendment of soil with MBM and HMBMs significantly increased proteolytic activity ($p < 0.001$), reaching maximum values ranging from 455 to 490 mg Tyr h^{-1} kg^{-1} after one week of incubation, compared with the bare soil (200 mg Tyr h^{-1} kg^{-1}). Thereafter, activity decreased in soils amended with MBMs, although in different ways. For MBM and HMBM-L, activity decreased more rapidly and stabilised at approximately 280–330 mg Tyr h^{-1} kg^{-1} from day 14 to day 28. In contrast, in HMBM-H, proteolytic activity decreased more slowly and remained stable at around 360 mg Tyr h^{-1} kg^{-1} up to the fourth sampling time (day 28). Subsequently, no significant differences in proteolytic activity were observed among soils amended with MBMs, and by the sixth sampling time (day 63) values converged to those of the bare soil (160–200 mg Tyr h^{-1} kg^{-1}).

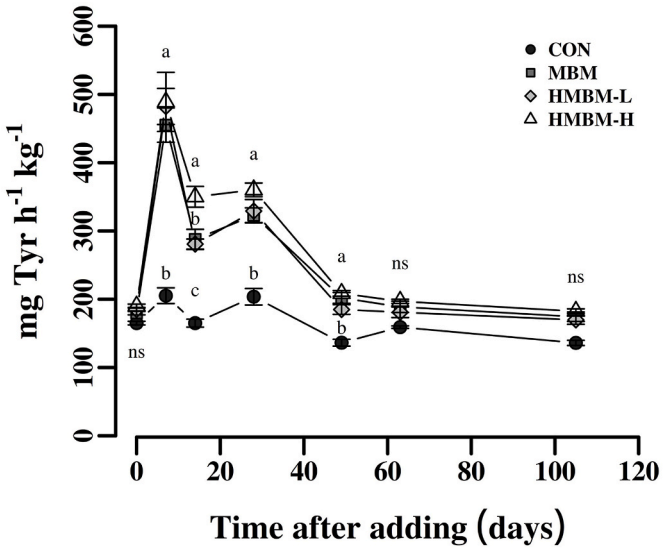

Fig. 4. Proteolytic activity in soil treated with meat and bone meals (data reported as mean, error bars are one standard error of the mean, different letters indicate significant differences at the HSD Tukey's test within each sampling time, ns = not significant).

Biological mineralisation of organic N in soil produces NH_4^+–N initially, followed by NO_3^-–N via NO_2^-–N (Manzoni and Porporato, 2009; Schimel and Bennett, 2004). Kinetics of NO_3^-N and NH_4^+–N release in soils amended with MBM and HMBMs are reported in Fig. 5(A and B, respectively). NH_4^+–N concentrations in soil were always <10 mg N kg^{-1} and did not show significant differences ($p = 0.87$) among treatments at any sampling time. In contrast, NO_3^-–N concentrations, excluding the initial sampling time (day 0), showed significant differences ($p < 0.001$) among treatments. Bare soil showed the lowest NO_3^-–N levels, ranging from 56 mg N kg^{-1} at the beginning to 86 mg N kg^{-1} at the end of incubation. In soils amended with MBMs, NO_3^-–N followed a typical first-order kinetic pattern (Benbi and Richter, 2002), with a rapid increase at the beginning of incubation and reaching maximum values (131–143 mg N kg^{-1}) at the end of the experiment. HMBMs showed significantly higher nitrate-N release than MBMs from the third (day 14) to the seventh (day 49) sampling time.

The first-order kinetic model fitted the net N mineralisation data well (p-R^2 = 0.88–0.97; Fig. 5 C). The model indicated significant differences ($p < 0.05$) in potentially mineralizable organic N (N_0), with the lowest values observed for MBM (36.6% of N added), followed by HMBM-L (47.5%) and HMBM-H (52.1%) (Table 5). The first-order rate constant (k_N) and the half-time of N mineralisation did not show significant differences among treatments (Table 5).

3.3.3. Available P in soil

The kinetics of available P (Olsen-P) in soil following fertiliser application are reported in Fig. 6 A. At the beginning of incubation, Olsen-P showed significant differences ($p < 0.05$) only between the bare soil (47.1 mg P kg^{-1}) and MBM (54.5 mg P kg^{-1}). The initial Olsen-P concentration of the control soil was already high (approximately

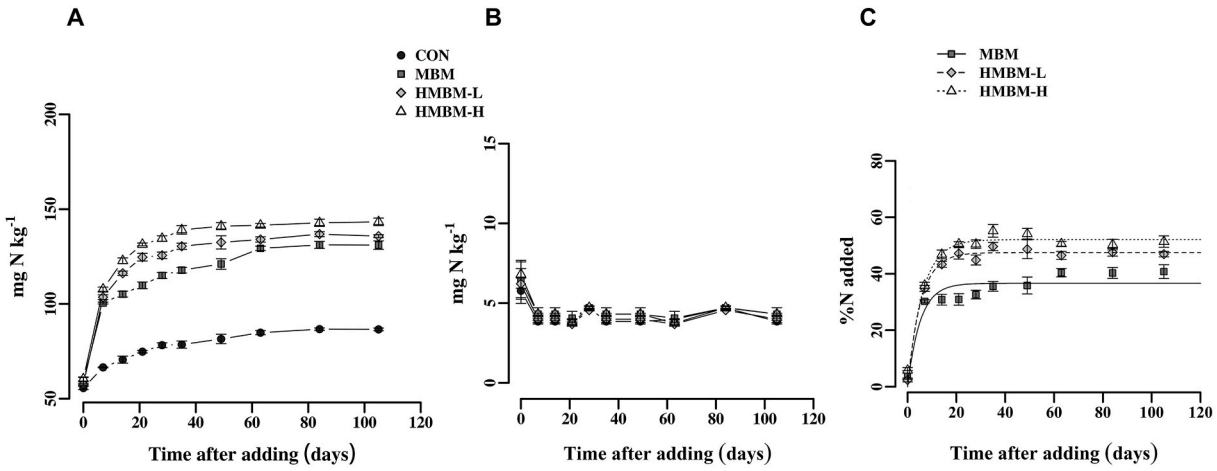

Fig. 5. Nitrate-N A) and ammonium-N B) released in soil treated with meat and bone meals; first-order model fitting of net N mineralisation C). Data reported as mean, error bars are one standard error of the mean.

Table 5
Parameters estimated according to the 1st order model for the organic N mineralisation of different products into the soil (data reported as means ± one standard error from the means, N_0 is the quantity of potentially mineralizable organic N; k_N is the rate constant; $t_{½}$ is the half-time, RSS is the residual sum-of-squares, P-R^2 is the pseudo-R square of the model; different letters indicate that the 95% confidence intervals between means did not overlap).

Products	N_0 % TN added	k_N days^{-1}	$t_{½}$ days	RSS	P-R^2
MBM	36.6 ± 1.5 a	0.194 ± 0.059 a	3.57 ± 0.83 a	124.7	0.878
HMBM-L	47.5 ± 0.6 b	0.185 ± 0.017 a	3.75 ± 0.32 a	19.92	0.989
HMBM-H	52.1 ± 1.1 c	0.166 ± 0.022 a	4.18 ± 0.49 a	55.66	0.972

47 mg P kg^{-1}), indicating a well-supplied soil with respect to available phosphorus. Consequently, the capacity of the system to exhibit further increases in Olsen-P following fertiliser application was likely limited. Thereafter, Olsen-P increased slightly by day 20, reaching values between 59 and 64 mg P kg^{-1}, but without significant differences among treatments. Finally, it decreased to 55–60 mg P kg^{-1} by the end of the incubation period. Fig. 6 B shows the fit of net Olsen-P in soil following application of MBM and HMBMs. Net Olsen-P in soil amended with MBMs did not differ significantly among fertiliser treatments but was higher than in the untreated control at the beginning of incubation (5–10% of P added). It then followed a declining exponential trend after two weeks, and by the end of the incubation, no significant differences were observed relative to the control (i.e. zero net effect, equivalent to bare soil). The results of the net available P fitting are reported in Table 6. Fig. 6 B shows the net available P fitting to the asymptotic exponential decay model.The model provided a satisfactory fit only for soil treated with HMBM-L (P-R^2 = 0.978), whereas the limited temporal variation observed in the other treatments reduced the ability of the model to capture P dynamics. The estimated amount of net available P at the beginning of incubation (P_0) did not differ significantly among treatments and ranged from 3.51% to 9.65% of P added. The asymptotic parameter (P_{asym}) was significantly higher in soil treated with MBM (4.16% of P added) compared with soils treated with HMBMs (−2.87% and −0.825% of P added). The limited treatment effects observed for Olsen-P should therefore be interpreted in the context of the high initial P availability and the strongly calcareous nature of the soil, both of which may have buffered differences in P release among MBMs with different degrees of hydrolysis.

4. Discussion

4.1. Meat and bone meal characterisation

According to the producer, the MBMs used in this study belong to

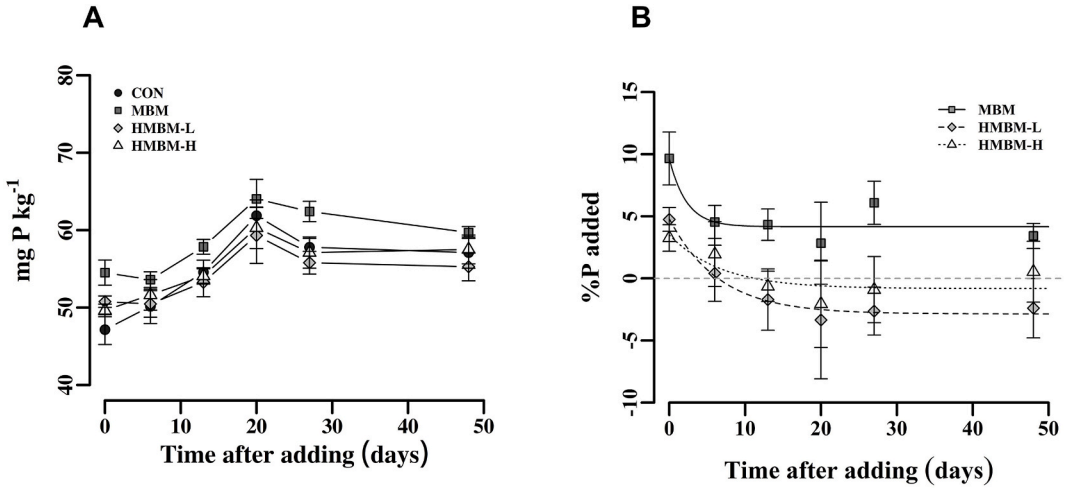

Fig. 6. Available P A) and asymptotic descendent model fitting of net available P in soil treated with meat and bone meals B). Data reported as mean, error bars are one standard error of the mean.

Table 6
Parameters estimated according to the exponential asymptotic model for the available P of different products into the soil (data reported as means ± one standard error from the means, P_0 is the amount of net available P released at the begin of incubation (day 0); P_{asym} is the asymptotic amount of net available P; k_P is the exponential rate constant; RSS is the residual sum-of-squares; P-R^2 is the pseudo-R square of the model; different letters indicate that the 95% confidence intervals between means did not overlap).

Products	P_0 %P added	P_{asym} %P added	k_P days^{-1}	RSS	P-R^2
MBM	9.65 ± 1.42 a	4.16 ± 0.73 a	0.442 ± 0.688 a	6.031	0.803
HMBM-L	4.81 ± 0.57 a	−2.87 ± 0.40 b	0.153 ± 0.035 a	0.9887	0.978
HMBM-H	3.51 ± 1.31 a	−0.825 ± 0.932 b	0.148 ± 0.135 a	5.224	0.730

Category 3 under EU Regulation 1069/2009 and are therefore 'not affected by any sign of disease transmissible to humans or other animals'. For this reason, after appropriate sterilisation, they can be used for fertiliser production. In addition to the sanitary requirements established by Regulation (EC) No. 1069/2009, the marketing of MBM-derived fertilising products within the European Union is subject to the requirements of Regulation (EU) 2019/1009 concerning product quality, contaminant content, safety and labelling. Therefore, the agronomic valorisation of MBMs requires compliance with both animal by-product and fertiliser legislation. MBMs are generally characterised by a combination of high N, P and Ca contents and low K concentration (Bøen and Haraldsen, 2013; Chaves et al., 2014; Garcia et al., 2006; Nogalska and Załuszniewska, 2021), as observed in the samples used in this study (Table 2). The applied hydrolysis processes only slightly affected this composition, with a small reduction in nutrient concentrations due to the addition of sodium carbonate (Na_2CO_3) at the beginning of the process. Indeed, ash content tended to increase in HMBMs, and the high ash content of MBMs is mainly attributable to the presence of bone particles (Garcia and Phillips, 2009). Both pH and EC increased with the degree of hydrolysis as a result of the addition of the alkaline salt (Na_2CO_3) and the hydrolytic process, although without exceeding reasonable agronomic thresholds (i.e. pH < 9 and EC < 5 dS m^{-1}). This is a well-known drawback of alkaline hydrolysis; moreover, alternative hydrolytic processes, such as acid and/or enzymatic hydrolysis, also have disadvantages, including the need for acid/base addition for pH adjustment and the use of more expensive hydrolytic agents (e.g. proteases) than Na_2CO_3 (Garcia et al., 2011; Zhang et al., 2017). Alkaline hydrolysis had only a mild effect on the organic matter content of MBM; TOC, N concentrations and the C/N ratio of HMBMs showed only slight reductions compared with the original MBM, indicating that minimal mineralisation of organic matter occurred during the hydrolysis process. In contrast, soluble P fractions were affected by alkaline hydrolysis, with a marked increase in the neutral ammonium citrate–soluble fraction and a slight decrease in the water-soluble fraction. Neutral ammonium citrate–soluble P is an indicator of fertiliser quality (Duboc et al., 2022; Kratz et al., 2019); thus, alkaline hydrolysis improved the plant-available P fraction. To our knowledge, no studies have addressed the effect of alkaline hydrolysis on P availability in MBMs; however, higher-temperature processes (e.g. pyrolysis), as well as alkali and metal concentrations (e.g. Ca, Mg), are well known to affect P solubility in similar materials (Ahmed et al., 2021; Chen et al., 2007; Nochefranca et al., 2020; Sarrion et al., 2023; Stark et al., 2006; Zwetsloot et al., 2015). Zwetsloot et al. (2015) reported that high-temperature pyrolysis (>750°C) of slaughterhouse waste increased total P and the formic acid-soluble fraction, but reduced the water-soluble and organic P fraction, resulting in higher plant-available P. Hydrolysis may have degraded the organic P fraction, increasing its solubility in neutral ammonium citrate, despite the substantial presence of Ca, which is likely present in an insoluble form. The small variations in total S, K, Ca and Mg concentrations, together with the marked increase in total Na after alkaline hydrolysis, can be attributed to the decrease in volatile solids and the increase in ash content due to organic matter mineralisation and the addition of Na_2CO_3. The concentrations of potentially toxic elements measured in the tested materials (Table S1) remained substantially below the limit values established by Regulation (EU) 2019/1009 for PFC 1(A) for organic fertilisers, indicating no critical constraints related to heavy metal content for the investigated products.

The main effect of the alkaline hydrolysis process was on the characteristics of the MBM organic fraction. Alkaline hydrolysis, by breaking chemical bonds such as peptide bonds of proteins and peptides (Dąbrowska et al., 2022; Garcia et al., 2011), increased the solubility of the organic fraction (i.e. WE_{OC}, WE_{TN}, WHN) and the DH of MBMs (i.e. DH_C, DH_N). The hydrolysis of proteins and polypeptides, which constitute the major part of the MBM organic fraction (Garcia et al., 2006), and other organic compounds, is confirmed by ATR–FTIR spectra (Fig. 1) and DTA–TG profiles (Fig. 2). The increased intensity of signals in the ATR–FTIR spectra at 1645, 1533, 1467, 1404 and 1237 nm in HMBMs, which can be assigned to amide groups and CH_2 deformation of proteins (Mehta et al., 2020), is consistent with modifications of proteinaceous structures induced by alkaline hydrolysis. Similarly, the DTA–TG profiles (Fig. 2) show an increased weight loss in the first endothermic region in HMBMs, corresponding to the more labile organic fraction (Conesa et al., 2003), confirming degradation of the MBM organic fraction following alkaline hydrolysis.

4.2. Degree of hydrolysis of meat and bone meals

The DH of a polymer (e.g. protein or polypeptide) is commonly defined as the percentage of cleaved peptide bonds relative to the total number of peptide bonds (Nielsen et al., 2001). Some proposed DH indices (DH_C and DH_N) are based on the ratio between the WE and total fractions of C and N, assuming that the former represents an estimate of cleaved bonds and the latter represents total bonds. In practical terms, an increase in the WE fraction corresponds to an increase in DH. The strong alkaline hydrolysis conditions applied to the HMBM-H product resulted in higher DH_C and DH_N values than in HMBM-L and MBM. As expected, alkaline hydrolysis increased the WE C and N fractions in proportion to the intensity of the applied process. Therefore, it is possible to modulate the characteristics of the final product by adjusting the intensity of the hydrolytic process.

The DH_{NRN} parameter highlights the importance of the amino acid N fraction within the WE N pool, as a specific DH index of the WE fraction. In this case, NRN represents the cleaved peptide bonds, whereas WE_{TN} represents the total N fraction. DH_{NRN} decreases as the intensity of alkaline hydrolysis increases, due to the lower yield of WE_{NRN} relative to WE_{TN}. This likely indicates that cleavage of peptide bonds releasing ninhydrin-reactive N was less efficient than the solubilisation of other N-containing compounds (i.e. proteins and polypeptides). HWN is a chemical index of N availability in soil from organic fertilisers, based on the assumption that the hot-WE N fraction represents plant-available N. The results for HWN are broadly consistent with those reported for organic fertilisers derived from tannery and slaughterhouse by-products (Rapisarda et al., 2022), which ranged between 11% and 54%. Alkaline hydrolysis increased this index from 27% (MBM) to 70.9% (HMBM-H), improving the hot-water solubility of N-containing compounds.

We propose DH_C, DH_N, DH_{NRN} and HWN as indicators of the release of soluble compounds as influenced by hydrolytic processes. To validate this, a soil incubation experiment was conducted to evaluate the release

of soluble forms of C and N.

4.3. Soil incubation experiment

4.3.1. Carbon mineralisation in soil

The immediate increase in CO_2 respiration rate (maximum after 1–2 days) indicates that MBMs contain a significant amount of easily bioavailable compounds that can be used as substrates for microbial respiration (Reischke et al., 2015). These results are consistent with previous studies on MBM mineralisation in soil (Cayuela et al., 2008; Mondini et al., 2008), where this effect was attributed to the presence of amino acids and polypeptides derived from partial hydrolysis of proteins in MBMs. Mondini et al. (2008) reported that, after 14 days of incubation at 15–20°C, the evolved extra CO_2–C represented 10–16% of the C added. In our experiment, a substantially higher emission of extra CO_2–C was observed (42–48% of C added), likely due to the intensity of the preparative thermal treatment applied to MBMs. In any case, these results are in agreement with previous studies using similar products (Chaker et al., 2023; Mondini et al., 2017; Thuriès et al., 2001). It should also be highlighted that CO_2 release from carbonate degradation cannot be ruled out (Sun et al., 2023), nor can a potential priming effect (Zheng et al., 2024).

Surprisingly, alkaline hydrolysis of MBM does not appear to induce a marked increase in extra CO_2–C release from soil (Table 4). This result seems to disagree with WE_{OC}, an indicator of readily available C for soil microorganisms, which increased substantially in HMBM (3–15% of C), and does not correspond to the extra CO_2–C released from soil after MBM application (from 42 to 48% of C). Evidently, only a fraction of WE_{OC} in MBM is available for soil respiration, and alkaline hydrolysis has only a limited effect on this fraction. The lack of a proportional increase in soil respiration despite the higher WE_{OC} content of hydrolysed MBMs suggests that the quality and microbial accessibility of the released organic compounds may be as important as their quantity. One possible, but unverified, explanation is that alkaline hydrolysis may alter the biochemical composition of the soluble organic fraction, potentially generating compounds that are less readily metabolised by soil microorganisms. For example, the formation of D-amino acid isomers during alkaline treatment has been reported in the literature (Hopkins and Ferguson, 1994; O'Dowd et al., 1997); however, this mechanism was not investigated in the present study and therefore remains speculative.

4.3.2. Nitrogen mineralisation in soil

The organic N mineralisation in soil is a widely studied process (Benbi and Richter, 2002; Manzoni and Porporato, 2009; Schimel and Bennett, 2004). Organic N is mineralised to NH_4^+ and subsequently oxidised to NO_3^- through enzymatic processes mediated by soil heterotrophic microorganisms. Therefore, soil organic N mineralisation is regulated by soil microbial activity, pH and SOC content (Gao et al., 2023). Considering soil microbial activity, soil proteolytic activity is enhanced by the application of organic biomasses such as compost, sewage sludge, biochar and ABPs (Vranova, 2013). Cayuela et al. (2009) observed an increase of up to 1500 mg Tyr h^{-1} kg^{-1} in proteolytic activity after adding 5–20 mgg^{-1} of MBM and 9 days of incubation in an agricultural soil. Jatana et al. (2020) and Mondini et al. (2008) reported proteolytic activities of 300–500 mg Tyr h^{-1} kg^{-1} after 2–5 days of incubation following the addition of 200–400 kg N ha^{-1}. Our results are consistent with these studies, with an increase of up to 500 mg Tyr h^{-1} kg^{-1} after the addition of approximately 1.3 mg g^{-1} and one week of incubation. A high DH of MBMs appears to induce a rapid increase in proteolytic activity (Fig. 4). There are no published data on the effect of MBM DH on proteolytic activity; however, protease synthesis is induced by organic N supply (Geisseler and Horwath, 2008) and is repressed by high concentrations of amino acids (Shivanand and Jayaraman, 2009). The enhanced proteolytic activity following soil addition with HMBMs compared with MBM indicates that thermal treatments induce partial hydrolysis of proteins, releasing peptides and amino acids that increase WE_{ON} and/or the substrates available for soil proteases (Mondini et al., 2008). This increase in proteolytic activity is also correlated with CO_2 release and soil microbial activity, indicating that MBM DH is an effective indicator of soil microbiome functionality in response to these products.

As reported by other authors (Jatana et al., 2020; Mondini et al., 2008), organic N added with MBMs (mainly proteins, peptides and amino acids) is rapidly mineralised in soil. Organic N is readily transformed to NH_4^+–N and subsequently to NO_3^-N; however, under the experimental conditions, no significant accumulation of NH_4^+–N was observed in soil, except at the beginning of the experiment, as also reported by Agehara and Warncke (2005), Azeez and Van Averbeke (2010) and Rapisarda et al. (2022). This is due to the experimental conditions and the low C/N ratio of MBMs, both of which favour rapid NH_4^+–N oxidation (Geisseler and Horwath, 2008). In contrast to NH_4^+–N, NO_3^-–N accumulation in soil proceeded rapidly, but after three weeks, the overall nitrification rate was significantly reduced, indicating progressive exhaustion of the available substrate (Jatana et al., 2020; Mondini et al., 2008), in agreement with the reduction in soil proteolytic activity. Chaves et al. (2014), in 12 incubation experiments using three different soils and five MBMs with different characteristics, observed net N mineralisation between 38.1% and 48.4% of total N added. Similarly, Mondini et al. (2008) reported net N mineralisation between 31.6% and 51.5% in incubation experiments with two soils and two MBMs (defatted and non-defatted). Our results fall within a similar range (36.6–52.1%), but net N mineralisation increased with MBM DH. These results suggest that the hydrolytic process, by increasing the fraction of total and ninhydrin-reactive N extractable in water, also increases net N mineralisation in soil, and that MBM N mineralisation can be modulated through thermal hydrolysis processes. These findings support the hypothesis that the degree of hydrolysis can be used as a practical parameter to modulate N release from MBM-based fertilisers.

4.3.3. Available P in soil

The availability of P from organic NP fertilisers is a challenging issue to manage (Kratz et al., 2019). In these fertilisers, P is commonly present in both organic and inorganic forms, which become available to plants as phosphate through different pathways and with different kinetics (Brod et al., 2015; Duboc et al., 2017). Organic P must be mineralised to phosphate before becoming plant-available, and this process depends on microbial activity. In contrast, inorganic P is subject to immobilisation and precipitation processes, which are mainly controlled by soil pH, temperature and moisture. The P made available by fertilisers does not remain in an available form indefinitely; instead, it is typically subject to precipitation, immobilisation, adsorption, leaching and runoff processes that progressively reduce plant availability (Gasser et al., 2023). MBM-based fertilisers are an effective P source, but the immediately available P in soil is generally low, typically less than 20% of total P (Ylivainio et al., 2008b), or up to 50% during the first year or growing season (Jeng et al., 2006). An experimental study (Jeng and Vagstad, 2009) on P leaching in soil treated with MBM (42–126 kg/ha total P) showed very low P losses (0.16–0.58 kg/ha) after 78 days of incubation. Our results confirm this behaviour, as the Olsen-P fraction remained below 10% of the added TP following MBM application. It should also be noted that the soil used in this study was characterised by a high carbonate content (62% total $CaCO_3$) and an already elevated initial Olsen-P concentration (>45 mg P kg^{-1}). Such values are generally considered indicative of a well-supplied soil with respect to plant-available phosphorus. Therefore, the potential for further increases in Olsen-P following fertiliser application was likely limited, and phosphorus dynamics were strongly influenced by the buffering capacity of the calcareous soil. Thermobaric alkaline hydrolysis of MBM resulted in a reduction of soil Olsen-P (Fig. 6). This result is somewhat unexpected, as the intensity of hydrolytic treatment significantly increased ammonium citrate–extractable P, which is commonly used as

an indicator of P availability. One possible explanation is microbial immobilisation of phosphorus stimulated by the greater availability of organic C and N in hydrolysed MBMs. However, this mechanism was not directly measured and therefore should be regarded as a hypothesis requiring further investigation. The resulting increase in organic C and N may stimulate microbial activity and, consequently, increase microbial P demand, leading to P immobilisation within the microbial biomass (Dai et al., 2020). This could explain the rapid decline in available P in soils incubated with HMBMs. However, although hydrolysis increases the fraction of potentially available P, incubation experiments show an opposite trend in soil. Under the conditions of the present experiment, chemical indicators of P availability did not fully predict the observed Olsen-P dynamics in soil, highlighting the importance of considering soil-specific factors such as carbonate content, pH and background P status.

5. Conclusions

Recycling biomass as fertiliser represents an important strategy for improving resource efficiency and advancing circular-economy approaches in agriculture. However, the effective valorisation of organic residues requires a detailed understanding of how processing technologies influence nutrient availability and fertiliser performance.

The results of this study demonstrate that alkaline thermobaric hydrolysis can effectively modify the physicochemical properties of meat and bone meal (MBM), increasing its degree of hydrolysis and the proportion of soluble organic compounds. The degree of hydrolysis was closely associated with changes in nitrogen availability, as hydrolysed MBMs exhibited significantly higher potentially mineralizable N than the untreated material. In contrast, organic C mineralisation was only marginally affected by hydrolysis despite the substantial increase in soluble organic C fractions, suggesting that factors other than the quantity of soluble C may regulate microbial utilisation of hydrolysis-derived compounds.

The effects of hydrolysis on phosphorus availability were less clear. Under the conditions of the present study, conducted in a calcareous soil characterised by high pH, high carbonate content and elevated background Olsen-P concentrations, hydrolysis did not increase soil available P. These results indicate that soil properties may play a dominant role in controlling P dynamics and that the effects of hydrolysis on P availability should not be generalized beyond the investigated conditions.

In addition, the combination of ATR–FTIR spectroscopy, TG-DTA analysis and hydrolysis indices proved useful for characterising the physicochemical modifications induced by alkaline thermobaric hydrolysis. The systematic changes observed in thermal decomposition patterns, spectroscopic signatures and soluble C and N fractions allowed the differentiation of MBMs according to their degree of hydrolysis. Although the direct prediction of nutrient release dynamics from these parameters requires further investigation, the results suggest that they may represent valuable tools for process monitoring, quality control and the future development of indicators linking product characteristics to agronomic performance.

Overall, the findings suggest that the degree of hydrolysis may represent a useful parameter for modulating nutrient release from MBM-based fertilisers, particularly with respect to nitrogen availability. Further research is required to evaluate the performance of hydrolysed MBMs across contrasting soil types, to better understand phosphorus dynamics, and to assess the potential of hydrolysis-based processing strategies for developing fertilisers tailored to crop nutrient requirements.

Informed consent statement

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CRediT authorship contribution statement

Andrea Ciurli: Data curation, Writing – review & editing. **Luigi Sciubba:** Formal analysis, Investigation, Methodology. **Giampaolo Di Biase:** Data curation, Formal analysis, Methodology. **Martina Mazzon:** Writing – review & editing. **Claudio Ciavatta:** Conceptualization, Resources, Supervision, Writing – review & editing. **Luciano Cavani:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2026.130354.

Data availability

The datasets generated and analysed during the current study are publicly available in the AMSacta institutional research repository, accessible at (https://doi.org/10.6092/unibo/amsacta/8714)

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