THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402
THIS OUTPUT REFLECTS THE VIEWS ONLY OF THE AUTHOR(S), AND THE EUROPEAN UNION CANNOT BE HELD RESPONSIBLE FOR ANY USE WHICH MAY BE MADE OF THE INFORMATION CONTAINED THEREIN

D6.3 Report on nitrogen mineralisation pattern and associated risks

Table of contents

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

Document Summary

Deliverable Title: D6.3 Report on nitrogen mineralisation pattern and associated risks

Version: 1.0

Deliverable Lead: Ghent University (UGENT)

Related Work package: WP6 - Quality and safety assessment of final products

Author(s): Jingsi Zhang, Çağrı Akyol, Erik Meers (UGENT)

Contributor(s): -

Reviewer(s): Miriam Pinto (NEIKER), Astrid Solvåg Nesse (NIBIO)

DIssemination level:

☒ PU, Public

☐ PP, Restricted to other programme participants (including the Commission Services)

☐ RE, Restricted to a group specified by the consortium (including the Commission Services)

☐ CO, Confidential, only for members of the consortium (including the Commission Services)

APPROVED BY: NEIKER, INI

Grant Agreement Number: 101000402

Programme: Horizon 2020 H2020-RUR-2020-1. Topic: CE-RUR-08-2018-2019-2020 - Closing nutrient cycles

Start date of Project: 01-01-2021

Duration: 54 months

Project coordinator: NEIKER-INSTITUTO VASCO DE INVESTIGACION Y DESARROLLO AGRARIO SA

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402
THIS OUTPUT REFLECTS THE VIEWS ONLY OF THE AUTHOR(S), AND THE EUROPEAN UNION CANNOT BE HELD RESPONSIBLE FOR ANY USE WHICH MAY BE MADE OF THE INFORMATION CONTAINED THEREIN

Abstract

The deliverable 6.3 (D6.3) reports nitrogen (N) mineralization patterns and associated risks (e.g., greenhouse gas (GHG) and ammonia (NH_3) emissions) of potential organic fertilizers produced from WP3 and WP4, characterized and selected based on D6.1. Eight potential organic fertilizers include Bokashi pellet (BP), NPK solution with amino acids (FER3), Hydrolysates (UNI1) and Chitin-rich fertilizer (UNI3), Protein fraction (CAT1), Fish sludge pelleted fertilizer (FSP) and Fish mix pelleted fertilizer (FMP), and Organic amendment (OA1).

N mineralization patterns of the fertilizers were determined through a 120-day soil incubation experiment. Ammonium N (NH_4^+-N) content dropped fast to the negligible level with obvious enhanced nitrate N (NO_3^--N) in all treatments due to the fast mineralization of amended organic N and quick nitrification. The treatments applied with OA1 and BP had the lowest net N release rates ($N_{rel,net}$) since only 27.5% and 19.0% N were released by day 120, respectively, because of their recalcitrant nature. While the $N_{rel,net}$ of other organic fertilizers ranged from 49.5 to 70.2%, mainly attributed to their organic N mineralization. FER3 and CAT1 showed higher net N mineralization ($N_{min,net}$), being 65.4% and 65.9%, respectively, followed by FSP and FMP with around 50% and then slurry-form fertilizers UNI1 (34.9%) and UNI3 (28.7%). Based on the soil incubation experiment, six BBFs with high N supply potential can (partially) substitute the synthetic N-fertilizers and is worth to further evaluate them in field conditions.

The gaseous emission test which were conducted on the same BBFs, showed varying emission patterns. The application of these fertilizers is unlikely to cause NH_3 leakage, because the fertilized treatment had a narrower range of NH_3 leakage, between 0.24 and 0.76 kg N ha^{-1}, while the highest NH_3 leakage was recorded in the unfertilized control (1.10 kg N ha^{-1}) during the incubation.

Regarding GHG emissions, the highest gas emission fluxes were observed at the initial stage, and came to the same levels as the unfertilized control after 7 days of incubation. The liquid FER3 fertilizer — with elevated mineralization rate — had significantly higher cumulative N_2O emission compared to other treatments. Its N and C emissions (N_2O, CO_2 and CH_4) resulted in 4.8% N and 30% C lost by emission after its application in the soil. It was followed by the slurry UNI1 with 1.8% N emitted as N_2O. The BP fertilizer with a C/N ratio of 12.3 led to significantly higher cumulative CO_2 and CH_4 emissions, accounting for 17% of its C content.

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

Global warming potential (GWP) of these fertilizers (only due to soil application) were mainly attributed to N_2O emission. The liquid fertilizer FER3 reached the greatest GWP of 13 CO_2 eq kg^{-1} N, while other organic fertilizers had lower GWP, ranging from 0.96 to 5.3 kg CO_2 eq kg^{-1} N. Although their GWP values were similar to other organic amendments, they were still lower than some commonly used synthetic fertilizers.

In summary, current findings are specific to the application of these BBFs under controlled conditions. To comprehensively understand their fertilizer efficacy and GWP, field-based trials and life-cycle analysis are essential to optimize their sustainable application.

Disclaimer:

Glossary

BBF: Biobased fertiliser

CH_4: methane

CO_2: Carbon dioxide

FW: Fresh weight

GHG: Greenhouse gas emission

GWP: Global warming potential

N_2O: Nitrous oxide

NH_3: Ammonia

NH_4^+-N: Ammonium-Nitrogen

$N_{min,net}$: Net Nitrogen Mineralisation

NO_3^--N: Nitrate-Nitrogen

$N_{rel,net}$: Net Nitrogen Release

PVC: Polyvinyl chloride

TC: Total Carbon

TN: Total Nitrogen

WFPS: Water Filled Pore Space

WP: Work Package

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

1 Introduction

The increasing global demand for sustainable agricultural practices has intensified interest in biobased fertilizers (BBFs) as alternatives to conventional mineral fertilizers. Derived from fishery waste and by-products, BBFs of Sea2Land offer the potential to recycle nutrients within agroecosystems, reduce dependence on synthetic inputs, and enhance soil health. However, to fully realize their environmental and agronomic benefits, it is crucial to understand the complex dynamics of nitrogen (N) release and mineralization from these fertilizing products, as well as their associated emissions of greenhouse gases (GHGs) and ammonia (NH_3).

N mineralization — the microbial conversion of organic N into plant-available inorganic forms such as ammonium and nitrate — determines the timing and efficiency of nutrient availability to crops. This process is influenced by a range of factors including the chemical composition of the fertilizer, soil properties, and climatic conditions. A mismatch between N release and crop demand can lead to nutrient losses, reduced yield potential, and increased environmental risks.

Simultaneously, BBFs can contribute to emissions of GHGs such as nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4), as well as NH_3 volatilization. These emissions have implications for climate change, air quality, and ecosystem health. Therefore, assessing the emission profiles of different BBFs is essential for identifying best practices and optimizing their use to minimize environmental impacts while maintaining agronomic performance.

This report explores the N release and mineralization as well as GHG and NH_3 emission characteristics of the pre-selected BBFs under controlled conditions. By examining these processes in tandem, the report aims to provide preliminary information on the development of more sustainable fertilizer strategies and support the transition toward circular, climate-smart agriculture.

2 Materials and methods

2.1 Biobased fertilisers' characteristics

Eight organic fertilizers were selected out of 26 fertilizing products obtained from the pilot installations in WP3 and WP4 (**Figure 1**). The characterization and selection of the fertilizing products are reported in D6.1. Feedstock materials, nutrient applied recovery

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402
THIS OUTPUT REFLECTS THE VIEWS ONLY OF THE AUTHOR(S), AND THE EUROPEAN UNION CANNOT BE HELD RESPONSIBLE FOR ANY USE WHICH MAY BE MADE OF THE INFORMATION CONTAINED THEREIN

technologies and final fertilizing products are summarized in **Table 1**. Characteristics of the selected eight BBFs are given in **Table 2**.

Figure 1 Eight selected biobased fertilizers (BBFs) for soil incubation and greenhouse gas emissions experiments.

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

Table 1 Fishery-waste derived fertilising products for lab-scale soil incubation experiments.

Region	Country	Input	Technology	Fertilizing products	Form	Code
Baltic sea	Estonia	Fish waste, food processing by-product, tree leaves and common reed litter, ash from oil shale industry	Bokashi fermentation, pelletization	Bokashi pellet	solid	BP
Cantabrian sea	Spain	Fish viscera from fish processing industry	Separation technologies, autolysis	NPK solution with amino acids	liquid	FER3
Adriatic sea	Italy	Organic residues from shellfish processing by-products, fish processing waste	Enzymatic hydrolysis	Hydrolysates	liquid	UNI1
		Organic residues from shellfish processing by-products, fish processing waste	Enzymatic hydrolysis, chemical chitin extraction	Chitin-rich fertiliser	liquid	UNI3
Atlantic sea	France	Fish processing waste (head, bone, viscera)	Enzymatic hydrolysis, Thermo-Mechano-Chemical (TMC) fractionation by twin-screw extrusion, drying	Protein fraction	solid	CAT1
North sea	Norway	Dried fish sludge	Drying, pelletization	Fish sludge pelleted fertiliser	solid	FSP
		26 % dried fish sludge, 38% blood meal, 20 % bone meal, 8 % sodium sulphate approved for organic agriculture and 8 % poly-sulphate	Drying, pelletization	Fish mix pelleted fertiliser	solid	FMP
Mediterranean sea	Spain	Marine fish sludge from aquaculture industry	Filtration, flocculation, biodrying	Organic amendment	solid	OA1

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

Table 2 Characteristics of biobased fertilizers.

Fertilizing product	Unit	Bokashi pellet	NPK solution with amino acid	Hydrolysate	Chitin-rich N fertilizer	Protein fraction	Fish sludge pellet	Fish mix pellet	Organic amendment
Code		BP	FER3	UNI1	UNI3	CAT1	FSP	FMP	OA1
pH-H_2O	-	7.21 ± 0.04	4.82 ± 0.02	5.78 ± 0.01	5.80 ± 0.01	6.05 ± 0.02	6.15 ± 0.01	6.17 ± 0.03	7.98 ± 0.02
EC	mS cm^{-1}	5.87 ± 0.22	18.7 ± 0.0	21.2 ± 0.2	20.9 ± 0.3	4.37 ± 0.36	8.32 ± 0.60	22.2 ± 0.14	7.06 ± 0.15
Moisture	%	9.35 ± 0.17	65.5 ± 0.2	57.9 ± 1.7	57.7 ± 0.17	1.94 ± 0.24	5.60 ± 0.23	5.49 ± 0.11	54.0 ± 0.24
DM	%	90.7 ± 0.2	34.5 ± 0.2	42.1 ± 1.7	42.3 ± 0.2	98.1 ± 0.2	94.4 ± 0.2	94.5 ± 0.1	46.0 ± 0.2
OM	%DM	61.9 ± 1.3	78.8 ± 0.2	82.5 ± 0.2	82.9 ± 0.1	83.8 ± 0.7	82.5 ± 0.4	71.1 ± 0.7	42.3 ± 0.3
Ash	%DM	38.1 ± 1.3	21.2 ± 0.2	17.5 ± 0.2	17.1 ± 0.1	16.2 ± 0.7	17.5 ± 0.4	28.9 ± 0.7	57.7 ± 0.3
TN	%	2.63 ± 0.09	5.31 ± 0.10	4.82 ± 0.17	4.62 ± 0.03	7.62 ± 0.72	6.19 ± 0.07	9.77 ± 0.22	1.89 ± 0.08
NH_4^+-N	g kg^{-1}	0.38 ± 0.02	2.56 ± 0.01	11.0 ± 0.09	10.5 ± 0.09	0.34 ± 0.02	0.62 ± 0.06	0.38 ± 0.05	2.77 ± 0.12
NO_3^--N	g kg^{-1}	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.007 ± 0.002	<0.002
TC	%	32.2 ± 0.4	14.2 ± 0.2	28.4 ± 1.2	27.9 ± 0.6	46.6 ± 0.3	39.3 ± 0.6	36.5 ± 0.8	10.8 ± 0.4
TOC/TC	-	0.98	1.00	1.00	1.00	1.00	1.00	1.00	0.96
C/N	-	12.3	2.67	5.88	6.04	6.11	6.35	3.73	5.70

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402
THIS OUTPUT REFLECTS THE VIEWS ONLY OF THE AUTHOR(S), AND THE EUROPEAN UNION CANNOT BE HELD RESPONSIBLE FOR ANY USE WHICH MAY BE MADE OF THE INFORMATION CONTAINED THEREIN

2.2 Soil collection and characterization

The soil for the incubation experiments was collected from the top layer (0 - 30 cm) of a field located in Upigny, Belgium. The soil type is characterized as silty loam, with 5.5% sand, 75.3% silt, and 19.2% clay. The soil was air-dried for 6 weeks until constant mass, sieved by 2 mm mesh, and stored in a cool room for further use. Dry matter content (DM) of the air-dried soil was calculated after drying sub-samples in the oven at 105°C until constant weight (ISO 18134-2:2017). Organic matter (OM) was determined by combusting the samples in the muffle furnace at 550°C for 4 hours (ISO 18122:2015). The soil organic carbon (SOC) was calculated by dividing the regional conversion factor 1.911 from OM (Sleutel et al., 2007). The pH values were determined by a pH-meter (Orion Star A211, Thermo Fisher Scientific, USA) after water extraction (ratio 1:5 w/v) and 16 hours standing (ISO 2917:1999). Electrical conductivity (EC) was determined by a conductivity meter (WTW Tetra Con 96, Xylem Analytics, Germany) after water extraction (ratio 1:5 w/w), 1 hour shaking and filtration (EN 13038, 2011). Total nitrogen (TN) was determined by Kjeldahl digestion (FOSS Kjeltec™ 8000, Denmark). For the determination of ammonium nitrogen ($NH4^+$-N) and nitrate nitrogen (NO_3^--N), the fertilising products were extracted by 1 mol/l potassium chloride (KCl) solution at a sample to KCl solution ratio of 1:5 (w/v), and then analysed on auto-flow analyser (Chemlab System 4, Skalar, the Netherlands). The characteristics of the soil is given in **Table 3**.

Table 3 Soil characteristics performed on air-dried soil.

Soil type	pH-H_2O	EC (µS cm^{-1})	OM (%)	OC (%)	TN (g kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})
Silty loam	6.8	96	4.9	2.6	1.1	u.d.	15

u.d.: under detection limit.

2.3 Experimental setups

2.3.1 Soil incubation setup

N release and mineralization dynamics of the selected BBFs were investigated through soil incubation experiments using 18-cm-deep poly vinyl chloride (PVC) tubes without plants under controlled conditions (see **Figure 2**). One week before soil incubation, sieved soil was watered to a moisture content of 35% water filled pore space (WFPS), covered and placed in a cool room at 20 ± 2 °C in the dark to activate soil microorganisms. A total of 10 treatments were prepared and incubated, including eight organic fertilizer products (**Table 1**), one unfertilized control (CON), and one treatment with mineral fertilizer calcium ammonium nitrate (CAN) as a positive control. The pre-incubated soil (240 g) was homogenized with the fertilizing products at a rate of 170 kg N ha^{-1} based on the TN contents of the fertilizers. Then the well-mixed soil was placed in

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

PVC tubes with a diameter of 4.6 cm and a height of 18 cm. The soil was compacted to a height of 10 cm, reaching a bulk density of 1.3 g cm^{-3}. The moisture was adjusted to 50% WFPS and maintained during the incubation. All tubes were covered with pin-holed parafilm to prevent moisture loss. The incubation lasted 120 days, during which the incubated soil tubes were destructively sampled every 20 days to determine mineral N contents, ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N), by Auto-Flow Analyser (Chemlab System 4, Skalar, the Netherlands) after 1M KCl extraction. Each treatment had three replicates and a total of 180 PVC tubes were prepared.

Figure 2 Set-up procedures of the soil incubation experiment. From left to right showed the addition of fertilizers to soil, mixing of the soil and fertilizers, transferring the soil to the PVC tubes, compaction to the aimed bulk density, adjustment of water content, tubes covered with the pin-holed gas permeable parafilm.

2.3.2 Microcosm setup

2.3.2.1 Experimental setup for greenhouse gas emissions

A separate incubation experiment was carried out in a temperature-controlled room at 20 ± 2°C for the measurement of GHG emissions. The pre-incubated soil (292 g) was thoroughly mixed with fertilizers at a rate of 170 N kg ha^{-1}, then transferred into a PVC tube (diameter = 6.9 cm, height = 7.5 cm). The soil was carefully compacted it a volume of 200 mL, reaching to an equivalent bulk density of 1.3 g cm^{-3}. The moisture content in each tube was increased to 70% WFPS to promote N_2O emission and maintained throughout the experiment. The PVC tubes were covered with pin-holed parafilm to prevent water evaporation before and after gas collection. The PE bottles (1L) were equipped with lids fitted with a rubber septum. A total of 10 treatments was tested and each had triplicates. The microcosm setup and gas sampling step were shown in **Figure 3**.

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402
THIS OUTPUT REFLECTS THE VIEWS ONLY OF THE AUTHOR(S), AND THE EUROPEAN UNION CANNOT BE HELD RESPONSIBLE FOR ANY USE WHICH MAY BE MADE OF THE INFORMATION CONTAINED THEREIN

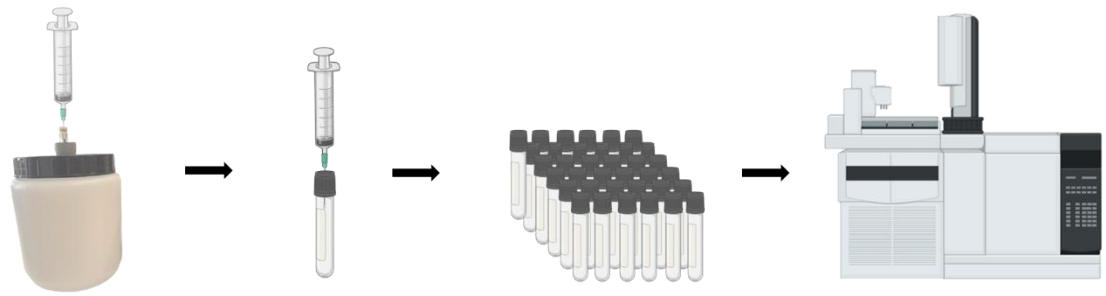


Figure 3 Illustration of gas sampling from the microcosm set-up for GC analysis. The PE bottle with a rubber septum and PVC tube with soil was placed in the PE bottle during the incubation, pre-vacuumed vials were prepared for gas collection.

2.3.2.2 Experimental setup for NH_3 leakage

The NH_3 leakage was measured based on the study of Egene et al. (2022). Each microcosm consists of a 1L Duran bottle adapted with a GL45-thread Smart Cap. The smart cap has two 2 mm threaded openings that can either be closed with blind plugs or fitted with valves that enable gas sampling. The experiment was carried out at a mean room temperature of 20 °C (temperature range: 19 – 21 °C). The setup included the same eight BBFs, one blank (unfertilized control soil), and one synthetic fertilizer CAN as a positive control.

The soil was pre-incubated for one week at 35% water-filled pore space (WFPS) to activate the soil microorganisms. 780 g of pre-incubated soil was thoroughly mixed with a fresh biobased or mineral fertilizer in a steel bowl and then transferred into the microcosm. The fertilizer amended soil was carefully packed to attain an equivalent bulk density of 1.4 kg m^{-3}. All fertilizers are applied at a rate of 170 kg N ha^{-1}. The moisture content in each bottle was brought to 70% WFPS and maintained throughout the experiment. The Duran bottles were covered with pin-holed parafilm to allow aerobic respiration and limit water evaporation. The microcosms were laid out in a randomized block design with three replicates for each treatment.

2.4 Sampling and analysis

2.4.1 Soil incubation

The soil incubated for N dynamics destructively sampled every 20 days. Soil samples were removed from the intact tubes and mixed thoroughly. Approximate 50 g of subsamples were stored in the freezer at -20°C before analysis. The net N release ($N_{rel,net}$) from the added products and the net N mineralization ($N_{min,net}$) of the added organic N fraction were determined by measuring the soil mineral N contents, NH_4^+-N and NO_3^--N.

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402
THIS OUTPUT REFLECTS THE VIEWS ONLY OF THE AUTHOR(S), AND THE EUROPEAN UNION CANNOT BE HELD RESPONSIBLE FOR ANY USE WHICH MAY BE MADE OF THE INFORMATION CONTAINED THEREIN

The soil extracts were prepared by mixing the soil and 1M potassium chloride solution (ratio 1:5 *w/v*) for 1h shaking, then analysed on a continuous auto-flow analyser (Chemlab System 4, Skalar, the Netherlands).

The potential N values of the added fertilizers were evaluated by net N release ($N_{rel,net}$), which is the fraction of released N from added fertilizing products subtracting that measured in the unfertilized control at any specified time. The mineral N (MN) content, the sum of of NH_4^+-N and NO_3^--N, was measured in the incubated soil. $N_{rel,net}$ was calculated by the formula (De Neve and Hofman, 1996), as follows:

$$N_{rel,net}(t,\%) = \frac{[MN - N_{\ treatment}]_t - [MN - N_{\ control}]_t}{N_{applied}} \times 100\% \qquad \text{Eq. 1}$$

At t_0, the $N_{rel,net}$ (%) equals to the MN ratio of total N in the products. Net N mineralization, $N_{min,net}$ (%), is the N mineralized from the organic fraction of the fertilizing products during the incubation (expressed as percentage of total N in the product), and is calculated by subtracting the amount of MN already present in the products at t_0 (De Neve and Hofman, 1996), as follows:

$$N_{min,net}(t,\%) = N_{rel,net}(t,\%) - N_{rel,net}(t_0,\%) \qquad \text{Eq. 2}$$

A positive $N_{min,net}$ value indicates net mineralization, whereas a negative $N_{min,net}$ value indicates net N immobilization.

2.4.2 Greenhouse gas emission

Flux measurements of soil N_2O, CO_2 and CH_4 emissions of all treatments were conducted for day 1, 3, 4, 7, 10, 14, and 17 during the incubation. The PVC tubes with prepared soil samples were put in the 1L PE bottles with lids fitted with a rubber septum for gas sampling. After sealing the bottle, 20 mL air was immediately injected by a plastic syringe, then 20 mL gas sample was taken and transferred into the 10 mL prepared vacuumed vials (Labco, Lampeter, UK) for analysis. Before taking gas samples, the syringe was flushed 2-3 times with the chamber air. The soil samples were incubated at 20°C and the gas were sampled again after 2 and 4 h. Concentrations of N_2O, CO_2 and CH_4 were measured by gas chromatography (7890 Agilent) outfitted with an ECD and FID and mechanizer and three valves (Agilent Technologies, USA). The emissions of N_2O, CO_2 and CH_4 were calculated from linear regression lines fitted to the observed increase of the gas concentrations in the headspace per flux measurement (Hu et al., 2024). The production rates (PR) of N_2O, CO_2 and CH_4, in µg N_2O-N kg^{-1} soil day^{-1}, or mg CO_2-C kg^{-1} soil day^{-1}, or µg CH_4-C kg^{-1} soil day^{-1}, were calculated using a modified ideal gas law as follows:

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

$$PR = \frac{P \times slope \times M \times 24 \times V}{R \times T \times mass}$$

P is the pressure in the chamber head space (atm); M is the elemental molar mass (e.g. 12 g mol^{-1} for CO_2-C and CH_4-C, 28 g mol^{-1}for N_2O-N; R is the ideal gas constant (0.08206 L atm mol^{-1} K^{-1}); T is the average atmospheric temperature (293 K); V is the volume of the chamber headspace (0.742 L), *mass* is the mass of the dry soil sample (0.260 kg) in each experimental setup. Cumulative gas emissions were calculated by multiplying the average gas production rates of two consecutive measurements by time intervals between the measurements. The net cumulative gas emissions were calculated by subtracting the cumulative gas emissions from the unfertilized control.

After the last batch of gas sampling, the soil was removed from the intact PVC tubes, well-mixed and collected in separated plastic bags, then frozen at 20°C for analyses. The net N release ($N_{rel,net}$) and the net N mineralization ($N_{min,net}$) were determined through mineral N measurement.

2.4.3 Ammonia emission

Over an incubation period of 20 days, ammonia (NH_3) leakage was monitored using the Gasera One Multi-gas analyser (Turku, Finland). At each sampling day, Duran bottles were connected to the analyser (**Figure 4**) for 8 minutes, during which NH_3 concentrations (ppm) were determined every two minutes for each replicate. Measurements were performed on day 0, 1, 2, 4, 6, 9, 12, 14, 16, 20. Measurements on day 0 were taken right after fertilizer incorporation.

Figure 4 NH_3 concentration monitoring via Gasera One Multi-gas analyzer.

The NH_3 leakage (kg N ha^{-1}) were calculated based on the ideal gas law as follows:

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402
THIS OUTPUT REFLECTS THE VIEWS ONLY OF THE AUTHOR(S), AND THE EUROPEAN UNION CANNOT BE HELD RESPONSIBLE FOR ANY USE WHICH MAY BE MADE OF THE INFORMATION CONTAINED THEREIN

$$NH_3\ leakage = \frac{P \times V \times M}{R \times T \times A}$$

P is the pressure in the chamber head space (0.839 atm); M is the elemental molar mass,14 g $mol^{-1}$for NH_3-N; R is the ideal gas constant (0.08206 L atm mol^{-1} K^{-1}); T is the average atmospheric temperature (293 K); V is the volume of the chamber headspace (0.623 L), *A* is the soil surface area in the Duran bottle (0.00694 m^2).

2.4.4 Global warming potential calculation

The global warming potential (GWP) of N_2O and CH_4 emissions was calculated and expressed as CO_2 equivalents per kg of N applied, using the latest GWP values of 273 for N_2O and 27 for CH_4 over a 100-year time horizon (IPCC, 2021). The GWP calculation in this section was based on the net cumulative GHG emissions from fertilized treatments, determined by subtracting the emissions from the unfertilized control to eliminate the influence caused by soil emission. From the life cycle assessment perspective, CO_2 emissions originating from the biogenic C within the biobased fertilizers were not considered in their GWP calculations (Egene et al., 2022).

3 Results and discussion

3.1 Nitrogen dynamics of the biobased fertilizers

3.1.1 Mineral Nitrogen evolution in the soil

At the beginning of the experiment, soils applied with UNI1, UNI3 and OA1 had higher ammonium N (NH_4^+-N) contents (**Figure 5**) due to their higher initial NH_4^+-N contents in the corresponding BBF products. During the initial stage of the incubation, organic N amended by the fertilization of BBFs quickly mineralized to NH_4^+-N and then nitrified to nitrate N (NO_3^--N). By day 20, the FMP treatment had the highest NH_4^+-N content (2.8 mg kg^{-1}) among all treatments. During the experimental period, NO_3^--N contents in BP and OA1 treatments increased to 22.2 and 30.1 mg kg^{-1}, respectively. The other six treatments fertilized with the BBFs contained higher NO_3^--N content, ranging from 43.1 to 61.6 mg kg^{-1}, while the synthetic CAN treatment exhibited the highest NO_3^--N content as 77.7 mg kg^{-1} and the unfertilized CON treatment as 25.7 mg kg^{-1}. After day 20, the mineral N contents of each treatment gradually increased. By the end of incubation, the mineral N content of synthetic CAN treatment reached to 92.4 mg kg^{-1}, followed by FER3 treatment 81.7 mg kg^{-1}, and the other fertilized ones between 69.5-79.4 mg kg^{-1}. The BP and OA1 treatments ended with 51.5 and 56.5 mg kg^{-1} after 120 days' incubation. The BP and OA treatments had the lowest mineral N contents along the incubation compared to other fertilized treatments.

The nitrification of NH_4^+-N of the fertilizing products added in the soil was fast and complete, proving by the negligible levels of the NH_4^+-N and the obvious enhanced NO_3^--N contents observed in the treatments throughout the incubation experiment.

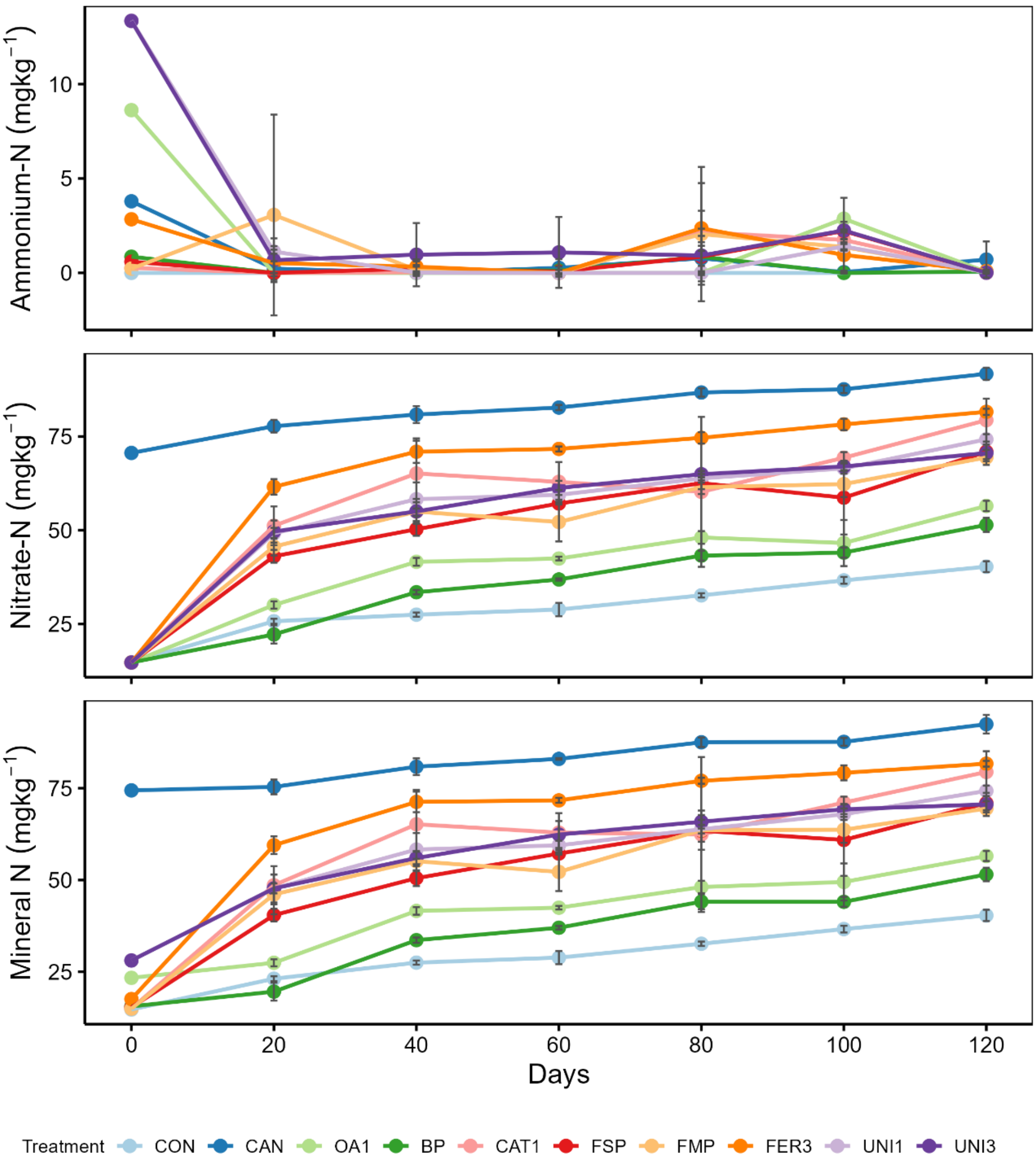


Figure 5 Evolution of mineral N (mg kg-1) in unfertilized control (CON) and fertilized soil with BBF products, manure and synthetic CAN fertilizer during the 120-day incubation experiment. Standard deviation is indicated by the error bar. CON: unfertilized control; CAN: calcium ammonium nitrate; BP: bokashi pellet; FER3: NPK solution with amnio acid; UNI1: hydrolysate; UNI3: chitin-rich fertilizer; FSP: fish sludge pellet; FMP: fish mix pellet; CAT1: protein fraction; OA1: organic amendment.

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

3.1.2 Nitrogen release and mineralization

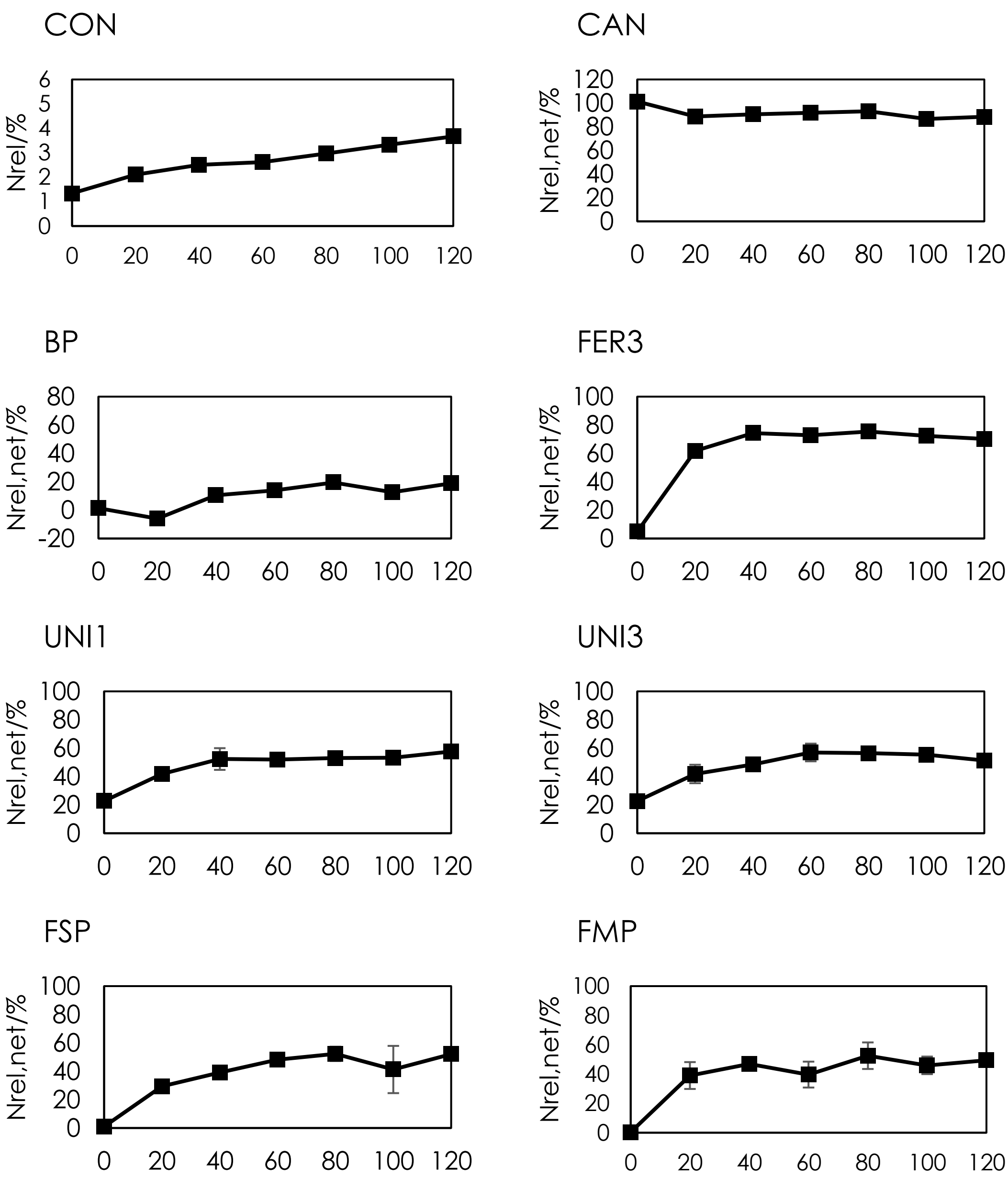
The unfertilized CON treatment resulted in 3.7 ± 0.1 % of native soil TN release at the end of the soil incubation experiment. The net N release of fertilized treatments is expressed as percentage of total N applied after removing the influence caused by the release of the soil native N (**Figure 6**). The released N from the CAN treatment peaked at day 80 reaching up to 93.2 ± 2.2 %, while only 88.5 ± 4.31% of released N left till day 120, indicating N immobilization or loss. The liquid fertilizer FER3 had higher $N_{rel,net}$ than other BBFs over time during the incubation period, reaching 75.4 ± 1.4% at day 80 and showing a decrease to 70.2 ± 1.3% at the end. The solid CAT1 released 66.4 ± 9.6% of applied N in the soil at day 120. UNI1 and UNI3 treatments released around 57.6 ± 2.4% and 51.4 ± 3.7% of applied N, respectively. The solid BP and OA1 treatments had lowest $N_{rel,net}$, 19.0 ± 3.1% and 27.5 ± 2.3% after 120 days' incubation, which attributed to their recalcitrant C introduced by lignin. The fish sludge derived fertilizers FSP and FMP released 52.0 ± 3.3% and 49.5 ± 3.4% of applied N at the end of the incubation experiment, respectively.

Overall, the N release rate of BBFs increased rapidly during the first 20 days' incubation, continued to rise more gradually until day 40, and then slowed down, becoming more stable thereafter, except for BP and OA1. It should be noted that all N in the CAN treatment was entirely mineral N, while other organic BBFs were composed mostly of organic N, ranging between 77.3-99.6% of the total N (**Table 2**). These results indicate that the organic mineralization rate is the limiting factor of N release from the BBFs when applied to soil.

The $N_{min,net}$ of the fertilized treatments of the sampling days is expressed as the percentage of the mineralized organic N fraction of the BBFs (Eq.2). Treatments applied with BP and OA1 exhibited negative $N_{min,net}$ values at day 20, indicating net N immobilization (**Figure 7**), and showed overall lower organic N mineralization rate along the incubation experiment compared to other BBFs. Including the recalcitrant C content, the product BP has the highest C/N ratio but low initial mineral N (**Table 2**), both of which contribute to N immobilization and reduced nitrogen release and mineralization during incubation. Till day 120, only 17.5 ± 3.1% of organic N within BP and 12.9 ± 2.3% of that in OA1 were mineralized as mineral N. Other BBFs showed rapid enhanced mineralization rate of organic C similar to their N release patterns. The viscous slurry products UNI1 and UNI3 displayed much lower N mineralization rates, resulting in $N_{min,net}$ as 34.9 ± 2.4% and 28.7 ± 3.7% by the end of the incubation due to their recalcitrant organic matter components (i.e., lipid, chitin), while their comparatively high N release rates can be attributed to their high initial mineral N contents (**Table 2**). The liquid FER3 outstood from other biobased fertilizers in terms of organic N mineralization, showing 70.6 ± 1.4% of

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

organic N mineralized at day 80 and slightly decreased to 65.4 ± 1.3% at the end, because of its low C/N ratio and labile N content (i.e., amino acids). The solid CAT1 product performed as the second highest organic N mineralization rate along the incubation, reaching 65.9 ± 9.6% at day 120, but displayed bigger variations in the replicates compared to other liquid fertilizers. Fish sludge derived products FSP and FMP performed similarly, although FMP has lower C/N ratio. By the end of the incubation experiment, the $N_{min,net}$ of FSP achieved 50.9 ± 3.3% and $N_{min,net}$ of FMP was 49.1 ± 3.4%.



THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

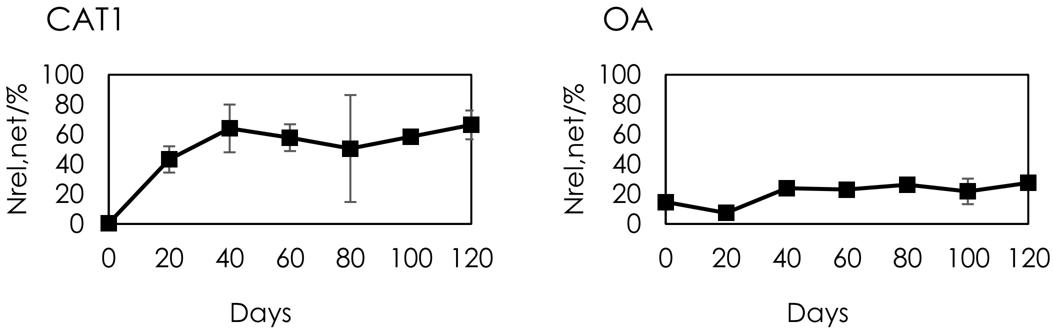


Figure 6 Net N release ($N_{rel,net}$, %) relative to the N input of added fertilisers in a 120-day soil incubation experiment. The N_{rel} (%) unfertilized control treatment is the proportion released from the native N in the soil over the incubation period. The value plotted at Day 0 refers to the percentage of mineral N from the fertilizers before their application in the soil. Standard deviation is indicated by the error bar (n=3). CON: unfertilized control; CAN: calcium ammonium nitrate; BP: bokashi pellet; FER3: NPK solution with amnio acid; UNI1: hydrolysate; UNI3: chitin-rich fertilizer; FSP: fish sludge pellet; FMP: fish mix pellet; CAT1: protein fraction; OA1: organic amendment.

Due to high organic N contents of the selected BBFs, mineral N release mainly resulted from the mineralization of organic matter applied to soil. BBFs with higher amount of labile organic N showed greater $N_{min,net}$; for liquid BBFs: FER3>UNI1 and UNI3, for solid BBFs: CAT1 > FSP and FMP. However, liquid BBFs was not necessarily associated with higher $N_{min,net}$. According to the soil incubation experiment, the hydrolysate FER3 had the highest N supply potential, followed by the CAT1 derived from purely fish processing waste. The pellet-form fertilizers FSP and FMP performed higher $N_{min,net}$ than slurry-form UNI1 and UNI3 fertilizers – which were derived from fish processing waste by enzymatic hydrolysis. Another fish sludge derived product OA1 exhibited much lower $N_{min,net}$ because of the plant tissue addition. BP produced from fermentation of fishery waste, food waste, recalcitrant C source plants and other waste showed the lowest $N_{min,net}$, both fertilizers (OA1 and BP) indicating its limited potential in supplying N as fertilizing products.

Overall, the order of N supply potential among the eight selected BBFs was : FER3 > CAT1 > FSP, FMP > UNI1, UNI3 > OA1 and BP. In a summary, except OA and BP, the other six organic fertilizers can be considered as potential (partial) substitutes for further evaluation in field conditions.

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

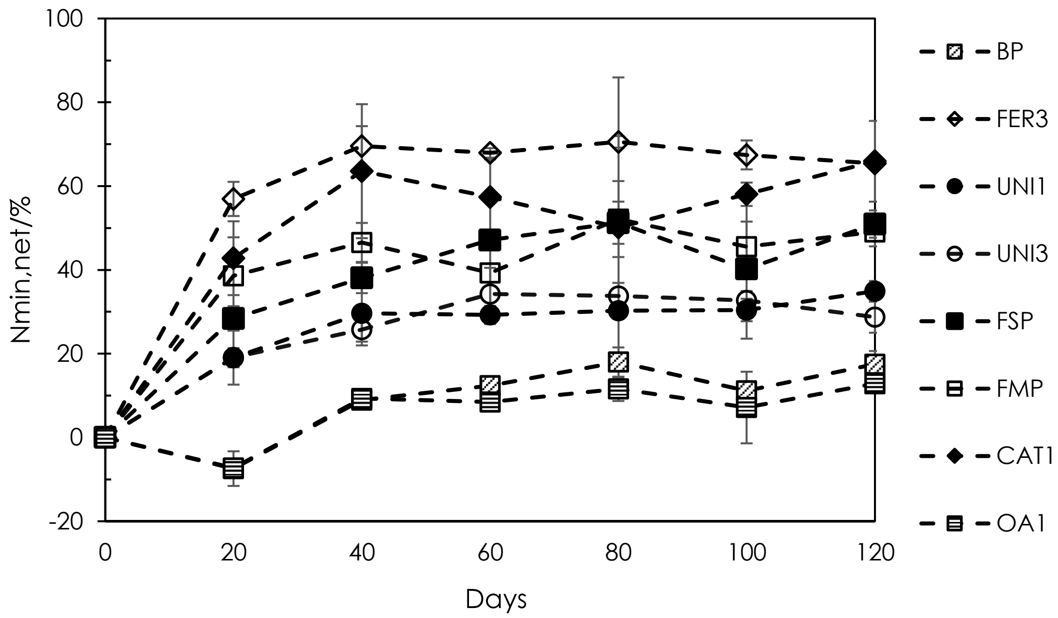


Figure 7 Net N mineralization ($N_{min,net}$, %) relative to the mineralized organic N of eight organic fertilisers in a 120-day soil incubation experiment. Standard deviation is indicated by the error bar (n=3). CON: unfertilized control; CAN: calcium ammonium nitrate; BP: bokashi pellet; FER3: NPK solution with amnio acid; UNI1: hydrolysate; UNI3: chitin-rich fertilizer; FSP: fish sludge pellet; FMP: fish mix pellet; CAT1: protein fraction; OA1: organic amendment.

3.2 Gaseous emissions

3.2.1 Greenhouse gas emission

3.2.1.1 N_2O emissions

Each treatment exhibited different N_2O emission dynamics, likely due to the varied characteristics of the applied BBFs. Considering their characteristics and N release and mineralization patterns determined through the soil incubation, eight BBFs were categorized into three subgroups for an easier comparison and discussion: 1) liquid BBFs (FER3, UNI1 and UNI3); 2) solid/pellet BBFs derived from fish processing waste and fish sludge (FSP and FMP); and 3) other solid BBFs produced from complex waste stream, including plant tissues (OA1 and CAT1).

The unfertilized control (CON) exhibited the fastest N_2O-N emission (20 ± 4 μg kg^{-1} day^{-1}) on the first day after adjusting the soil water content to 70% WFPS. The positive control treatment, fertilized with synthetic CAN solution, showed a similar N emission pattern as CON. Its N_2O flux was slightly higher due to the N introduction, peaking at 26 ± 11 μg N kg^{-1} day^{-1} on day 2. The highest N_2O fluxes of the liquid BBFs were observed on the first day of the experiment, reaching 5962 ± 984 μg N kg^{-1} day^{-1} from treatment FER3, 2220 ± 595 μg N kg^{-1} day^{-1} from treatment UNI1, and 1222 ± 919 μg N kg^{-1} day^{-1} from treatment

UNI3. Their fluxes quickly diminished to 94 ± 37, 65 ± 20, 36 ± 31 µg N kg^{-1} day^{-1} at day 3, respectively.

Treatments applied with solid BBFs - CAT1, FSP and FMP - showed increasing N_2O fluxes at the beginning of the experiment. FMP containing lower organic matter content displayed lower N_2O emission, compared to CAT1 and FSP. CAT1 derived from purely fish processing waste contained higher TOC (see **Table 2**). The highest N_2O flux from the FMP treatment was measured as 87 ± 11 µg N kg^{-1} day^{-1} on day 4. FSP has similar characteristics to FMP but contains higher OC content and MN/TN ratio, exhibiting the highest emission flux (258 ± 39 µg N kg^{-1}) day^{-1} on day 3.

The N_2O fluxes further decreased in the following days. N_2O-N flux of FER3 treatment dropped to around 10 µg kg^{-1} day^{-1}, while treatments UNI1 and UNI3 emitted approximately 10 µg N kg^{-1} day^{-1} by day 7.

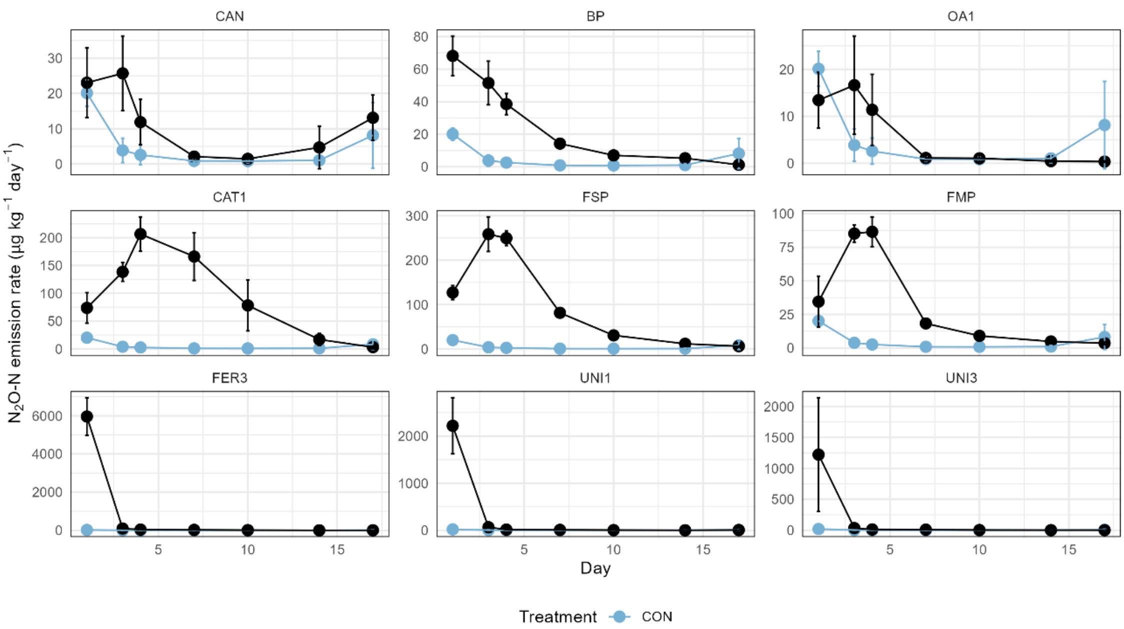


Figure 8 Comparison of mean fluxes of N_2O between fertilized treatments and the unfertilized control (CON). The error bars indicate the standard deviation of the measurements. CAN: calcium ammonium nitrate; BP: bokashi pellet; FER3: NPK solution with amnio acid; UNI1: hydrolysate; UNI3: chitin-rich fertilizer; FSP: fish sludge pellet; FMP: fish mix pellet; CAT1: protein fraction; OA1: organic amendment.

For the CAT1 treatment, the peak N_2O-N flux was recorded at 207 ± 31 µg kg^{-1} day^{-1} on day 4. The BP treatment exhibited the highest flux (68 ± 12 µg N kg^{-1} day^{-1}) on the first day, which gradually declined to approximately 1 µg N kg^{-1} day^{-1} by the end. The OA, which comprised of more than 50% mineral content and the lowest N and OC contents, emitted the least N_2O throughout the experimental period compared to the other

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

fertilizers, with a peak flux of 17 ± 10 μg N kg^{-1} day^{-1} on day 2. Overall, the N_2O emission rates of all fertilized treatments came down to the same levels with the unfertilized CON treatment by day 14.

The treatment applied with synthetic CAN showed no significant difference in cumulative N_2O-N emissions compared to the CON, because of no organic matter addition. Treatments fertilized with BP and OA1 were characterized by low N release and mineralization rates (**Figure 6**, **Figure 7**) as well as low N_2O fluxes (**Figure 8**). They resulted in no significant cumulative N_2O-N emissions compared to the CON treatment, approximately 0.07 and 0.31 mg N kg^{-1} throughout the experiment, respectively. The solid FMP application leaded to no significant difference in N_2O-N emissions, resulting in 0.44 ± 0.01 31 mg N kg^{-1} after 17 days' experiment. Compared to FMP treatment, treatments CAT1 and FSP exhibited higher N_2O-N initial fluxes and emitted around 1.53 and 1.42 mg N kg^{-1}, respectively, which were significantly higher than the CON treatment. The liquid FER3 with the extremely high initial N_2O flux showed significantly higher N_2O emission compared to other treatments, reaching 6.31 ± 0.86 mg N kg^{-1} during the test period, followed by UNI1 (2.41 ± 0.55 mg N kg^{-1}) and UNI3 (1.36 ± 0.95 mg N kg^{-1}) treatments.

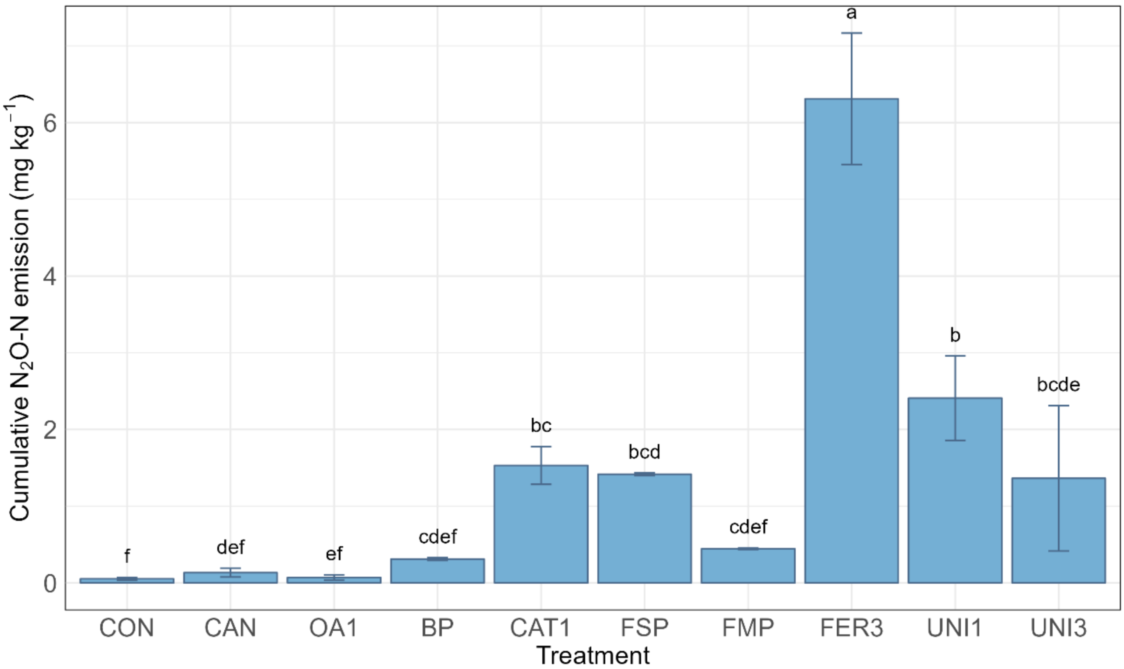


Figure 9 Cumulative emissions of N_2O from the unfertilized control (CON) and fertilized treatments. The error bars indicate the standard deviation of the measurements. CAN: calcium ammonium nitrate; BP: bokashi pellet; FER3: NPK solution with amnio acid; UNI1: hydrolysate; UNI3: chitin-rich fertilizer; FSP: fish sludge pellet; FMP: fish mix pellet; CAT1: protein fraction; OA1: organic amendment.

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402
THIS OUTPUT REFLECTS THE VIEWS ONLY OF THE AUTHOR(S), AND THE EUROPEAN UNION CANNOT BE HELD RESPONSIBLE FOR ANY USE WHICH MAY BE MADE OF THE INFORMATION CONTAINED THEREIN

3.2.1.2 CO_2 emissions

The CAN treatment exhibited a CO_2 emission pattern similar to the unfertilized CON, as no additional C was introduced after fertilization (**Figure 10**). The OA1 treatment showed almost identical CO_2 emissions pattern with the CAN and CON treatments, which can be attributed to its low C content (**Table 2**) and recalcitrant nature of the C component. This aligned with its mineralization pattern of the organic matter observed during the soil incubation (**Figure 7**). The cumulative CO_2-C emissions for treatments CON, CAN and OA1 were around 0.10, 0.09 and 0.09 g kg^{-1},respectively, which were significantly lower than other fertilized treatments. The solid BP, composed of around 32.3% total C and high C/N ratio around 12.3, exhibited high CO_2-C emission in the relative treatment. Initially, it showed an increase in CO_2-C flux from 58 to 69 mg C kg^{-1} day^{-1}, which later decreased and came down to levels observed in the unfertilized CON treatment. The BP treatment resulted in the highest cumulative CO_2-C emission among treatments, reaching 0.43 g CO_2-C kg^{-1}.

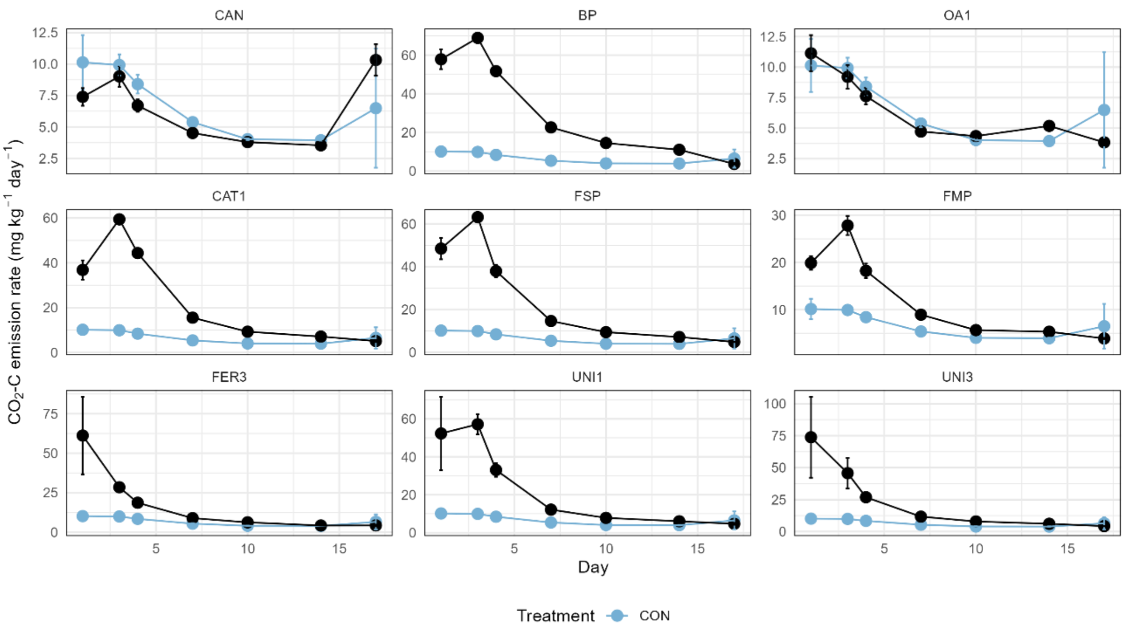


Figure 10 Comparison of mean fluxes of CO_2 between fertilized treatments and the unfertilized control (CON). The error bars indicate the standard deviation of the measurements. CAN: calcium ammonium nitrate; BP: bokashi pellet; FER3: NPK solution with amnio acid; UNI1: hydrolysate; UNI3: chitin-rich fertilizer; FSP: fish sludge pellet; FMP: fish mix pellet; CAT1: protein fraction; OA1: organic amendment.

Treatments fertilized with CAT1, FSP and FMP demonstrated similar CO_2-C emission patterns to the BP treatment, with peak emission mean fluxes (59, 63 and 28 mg kg^{-1} day^{-1}) on day 3, followed by a gradual decline toward the levels of the unfertilized CON treatment. Cumulative CO_2-C emission for treatments CAT1 and FSP were around 0.33 g

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

CO_2-C kg^{-1}, while the FMP treatment cumulatively emitted much lower (0.17 g C kg^{-1}) during the measurement period. The liquid BBFs FER3, UNI1 and UNI3, contained comparatively higher organic C, ranging from 35.6 to 46.6%. These treatments showed high initial CO_2-C emission flux and an overall decreasing trend in the following days. The highest CO_2 flux for FER3 and UNI3 treatments were observed on day 1, at approximately 61 and 74 mg C kg^{-1} day^{-1}, respectively, while the UNI1 treatment peaked on day 2 at 57 as mg C kg^{-1} day^{-1}. By day 7, their CO_2-C fluxes decreased to around 10 mg C kg^{-1} day^{-1}. However, due to the lower OC content, the FER3 treatment had significantly lower cumulative CO_2-C emission (0.21 g C kg^{-1}), compared to treatments UNI1 (0.30 g C kg^{-1}) and UNI3 (0.29 g C kg^{-1}).

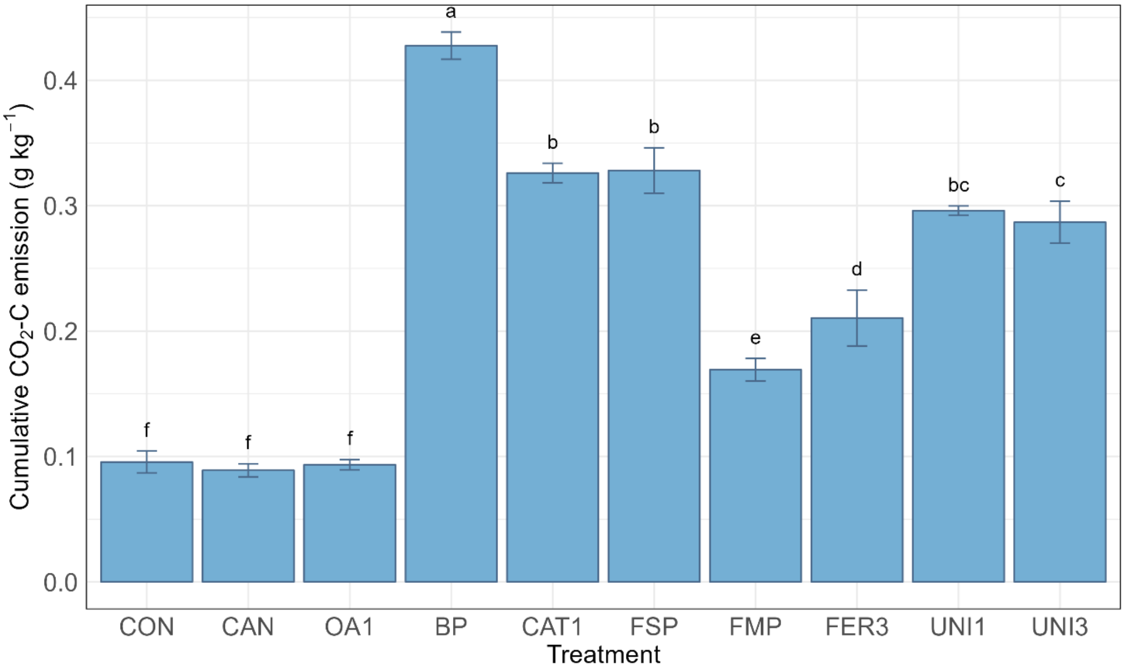


Figure 11 Cumulative emissions of N_2O from the unfertilized control (CON) and fertilized treatments. The error bars indicate the standard deviation of the measurements. CAN: calcium ammonium nitrate; BP: bokashi pellet; FER3: NPK solution with amnio acid; UNI1: hydrolysate; UNI3: chitin-rich fertilizer; FSP: fish sludge pellet; FMP: fish mix pellet; CAT1: protein fraction; OA1: organic amendment.

3.2.1.3 CH_4 emissions

The CH_4-C fluxes of the treatments aligned closely with their CO_2-C emission patterns, as illustrated in **Figure 12**. Among all treatments, the BP treatment had the highest cumulative CH_4-C emission, reaching 1.98 mg C kg^{-1}, which was significantly higher than that of other treatments (**Figure 13**). It was followed by FSP (1.53 mg C kg^{-1}), CAT1 (1.49 mg C kg^{-1}), UNI1 and UNI3 (1.38 and 1.39 mg C kg^{-1}). FMP and FER3 treatments exhibited lower cumulative CH_4-C emissions, at 0.76 and 0.97 mg C kg^{-1}, respectively. OA1 (0.44 mg

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402
THIS OUTPUT REFLECTS THE VIEWS ONLY OF THE AUTHOR(S), AND THE EUROPEAN UNION CANNOT BE HELD RESPONSIBLE FOR ANY USE WHICH MAY BE MADE OF THE INFORMATION CONTAINED THEREIN

C kg-1) and CAN (0.38 mg C kg-1) demonstrated no significant differences in cumulative CH_4-C emissions compared to the unfertilized CON treatment (0.42 mg C kg-1).

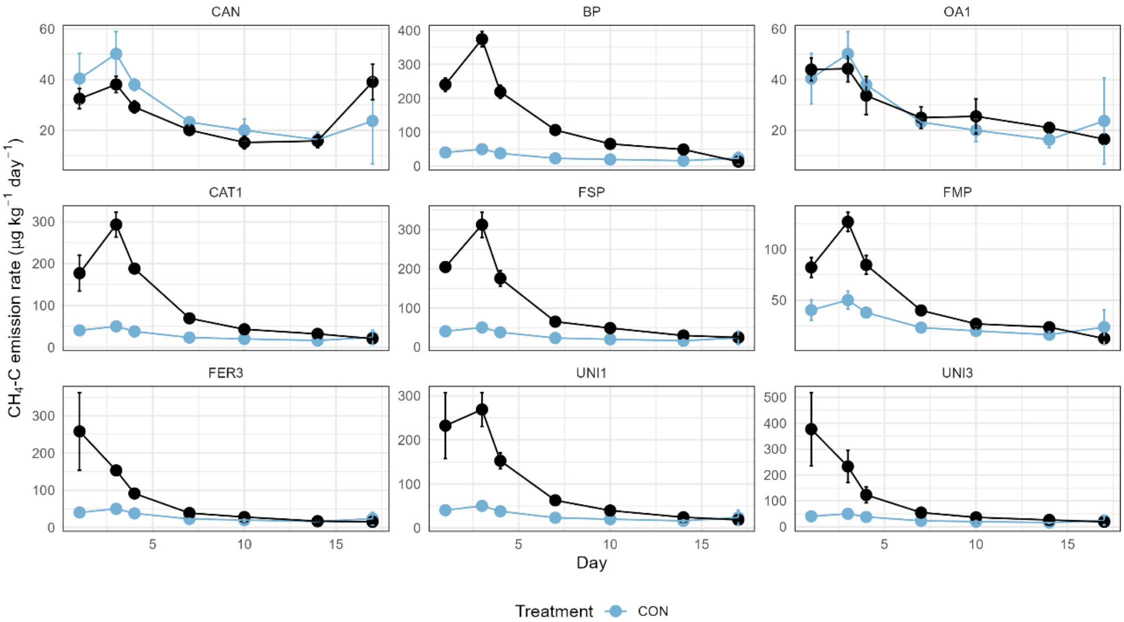


Figure 12 Comparison of mean fluxes of CO_2 between fertilized treatments and the unfertilized control (CON). The error bars indicate the standard deviation of the measurements.

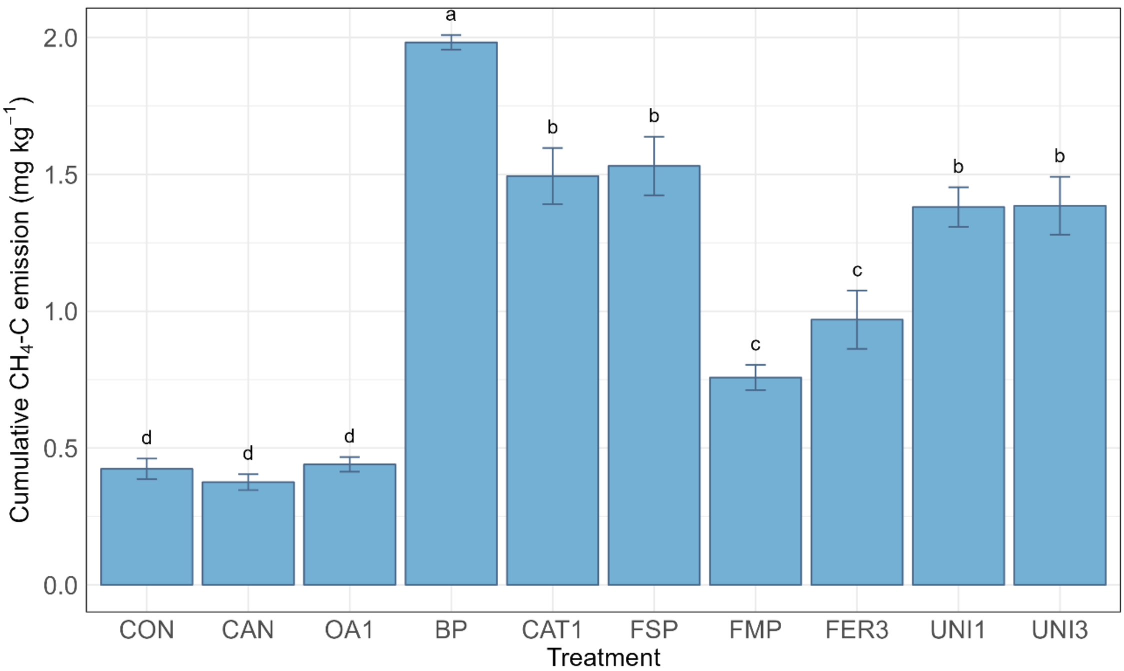


Figure 13 Cumulative emissions of CH_4-C from the unfertilized control (CON) and fertilized treatments. The error bars indicate the standard deviation of the measurements. CAN: calcium ammonium nitrate; BP: bokashi pellet; FER3: NPK solution with amnio acid; UNI1: hydrolysate; UNI3: chitin-rich fertilizer; FSP: fish sludge pellet; FMP: fish mix pellet; CAT1: protein fraction; OA1: organic amendment.

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

3.2.2 Carbon and nitrogen mineralization

For N_2O-N emissions, the FER3 treatment showed the highest emissions, with 18.4% of the applied N released into the air as N_2O (**Table 4**). Additionally, due to its high organic matter mineralization rate, 31% of the applied C was emitted in the form of CO_2 and CH_4. In contrast, treatments fertilized with the liquid fertilizers UNI1 and UNI3 exhibited lower N_2O-N emissions, with 6.9% and 3.9% of the applied N emitted, respectively. The N emission difference was likely due to the recalcitrant N source, chitin, present in UNI3. However, their C emissions were similar, because of their similar C and C/N ratio. The FMP treatment emitted significantly lower amounts of C (15%) and N (1.2%) compared to FSP and CAT1. N immobilization was observed in the OA1 treatment during soil incubation, yet it displayed similar organic matter mineralization dynamics in the gaseous emission experiment. Only 0.1% of the applied N from OA1 was emitted as N_2O, while the applied C was immobilized by soil microbes, resulting in a net C immobilization of -0.26%. Likewise, the BP treatment resulted only 0.8% of applied N emitted as N_2O. It showed the highest cumulative C emission, equals to 17% of applied C was released to the air. For the positive control using synthetic CAN, only 0.2% of the applied N was lost as N_2O, since no additional C was applied.

Table 4 Soil NH_4^+-N and NO_3^--N and net mineral N release ($N_{rel,net}$) in the soil on day 17, and the net N and C loss by emission ($N_{emitted,net}$ and $C_{emitted,net}$).

Treatment	NH_4^+-N	NO_3^--N	$N_{rel,net}$	$N_{emitted,net}$	$C_{emitted,net}$
	mg kg^{-1} FW		% $N_{applied}$	% $N_{applied}$	% $C_{applied}$
CON	0	50	-	-	-
CAN	0	98	47	0.06	-
OA1	0	65	15	0.02	-0.82
BP	0	34	-16	0.20	17
CAT1	0.7	80	30	1.1	28
FSP	0	61	11	1.0	26
FMP	0	70	20	0.3	14
FER3	0	110	62	4.8	30
UNI1	0.5	81	31	1.8	24
UNI3	0	87	36	1.0	23

Note: FW: fresh weight, indicating the soil NH_4^+-N and NO_3^--N concentrations at soil moisture content 70% WFPS on day 17; $C_{applied}$: calculated based on TC fertilizers' contents, CON: unfertilized control; CAN: calcium ammonium nitrate; BP: bokashi pellet; FER3: NPK solution with amnio acid; UNI1: hydrolysate; UNI3: chitin-rich fertilizer; FSP: fish sludge pellet; FMP: fish mix pellet; CAT1: protein fraction; OA1: organic amendment.

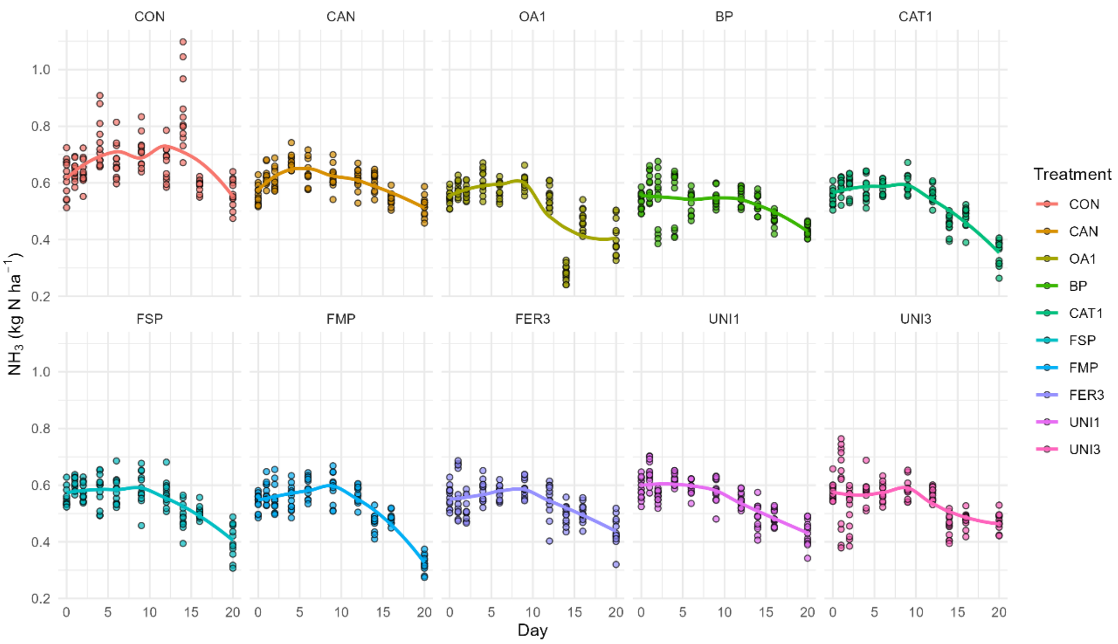
The mineral N of soil sampled right after the 17 days' gas collection were detected mainly composed of NO_3^--N (**Table 4**), which suggested that the NH_4^+-N within was quickly nitrified to NO_3^--N or immobilized by microbes. For example, BP treatment showed negative $N_{rel,net}$, referring to N immobilization by microbial activities, This is supported by

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

the field study in Estonia, where fertilization with BP significantly increased soil dehydrogenase (DHA) activity and microbial biomass carbon, with DHA showing the great increase compared to other treatments (see D6.5). FER3 treatment exhibited the highest mineralization of organic matters, resulted in the most net N release and emission, as well as net C emission. It was followed by two slurry form fertilizers UNI1 and UNI3. These two products led to no significant differences in C and N mineralization, release and emission in the soil and air. Regarding solid organic fertilizers, CAT1 - derived from purely fish processing waste - performed faster in organic matter mineralization and nitrification than FSP and FMP, resulting in 30% N released in the soil.

3.2.3 Ammonia emission

Ammonia (NH_3) emission from soils amended with and without fertilizers was monitored for 20 days. As shown in **Figure 14**, NH_3 leakage exhibited small variations and displayed an overall decreasing trend across all treatments. NH_3 leakage from the unfertilized control (CON) ranged from 0.47 to 1.10 kg N ha^{-1} during the incubation. In contrast, CAN treatment had a smaller range from 0.46 to 0.74 kg N ha^{-1}. Among the organic fertilizers, their NH_3 leakage had similar ranges, with the highest leakage as 0.76 kg N ha^{-1} from UNI3 treatment on day 1 and lowest 0.24 kg N ha^{-1}, from OA1 treatment on day 14. The fertilized treatments recorded similar NH_3 emission patterns compared to the unfertilized CON treatment, indicating that the fertilization practices did not contribute to any additional NH_3 emissions. Thus, fertilization with the selected fertilizers have no direct impact on N loss by NH_3 leakage. Accordingly, NH_3 leakage was not included in the later work on its global warming impact.



THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

Figure 14 Soil NH_3 leakage (kg N ha^{-1}) in microcosm experiment. CAN: calcium ammonium nitrate; BP: bokashi pellet; FER3: NPK solution with amnio acid; UNI1: hydrolysate; UNI3: chitin-rich fertilizer; FSP: fish sludge pellet; FMP: fish mix pellet; CAT1: protein fraction; OA1: organic amendment.

3.2.4 Global warming potential

The GWP of CO_2 emissions from the fertilized soils ranged from 0.53 to 2.5 kg CO_2 eq kg^{-1} N, while CAN and OA1 exhibited negative GWP due to their lower CO_2 emissions compared to the unfertilized CON. Figure 15 illustrates the GWP of the fertilized soils due to N_2O and CH_4 emissions. Although GWP of CO_2 emissions was included in the figure, they are not regarded as contributors to the overall GWP of fertilizer application in the subsequent discussion.

N_2O emission played a key role in GWP calculation, ranging from 0.04 to 13 kg CO_2 eq kg^{-1} N, while the GWP of CH_4 emissions were very low, showing the negligible GWP (0.32 kg CO_2 eq kg^{-1} N) from BP treatment. FER3 treatment, exhibiting the highest cumulative N_2O emission (**Figure 9**), resulted in the greatest GWP (13 kg CO_2 eq kg^{-1} N). OA1 — with lowest gas emissions — showed a GWP of only 0.05 kg CO_2 eq kg^{-1} N from N_2O and CH_4 emission. GWP values of other treatments varied between 0.96 and 5.3 kg CO_2 eq kg^{-1} N. Previous studies found a wide range of GWP due to the application of organic fertilizing products. Walling and Vaneeckhaute (2020) summarized that the GWP of compost was 0.06 to 5.6 kg CO_2 eq kg^{-1} N, 0.6 to 16 kg CO_2 eq kg^{-1} N for animal manure, and 0.15-18 kg CO_2 eq kg^{-1} N for digestate. Synthetic fertilizers had an even wider range, from 0.1 to 40 kg CO_2 eq kg^{-1} N.

In this study, fertilizer products were examined under optimal moisture conditions for microbial nitrification to promote N_2O emission. These organic fertilizers derived from fishery waste showed comparable GWP of their application to other organic-rich fertilizing products, whereas synthetic fertilizer CAN application did not cause gaseous C emissions and very limited N_2O emission, resulting in only 0.17 kg CO_2 eq kg^{-1} N. Other fertilized treatments with higher cumulative GHG emissions exhibited greater GWP than the CAN treatment, except for OA1.

It should be noted that the GWP here is limited to the GWP associated to the application only, it does not take into account the GWP of the production process and logistics. Synthetic mineral fertilisers have overall high GWP based on fossil resource usage and long-distance logistics required for reaching the field, whereas BBFs tend to be locally produced and used. In that sense, further systematic research under varying conditions is required to better understand the emission dynamics associated with the application of these BBFs.

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

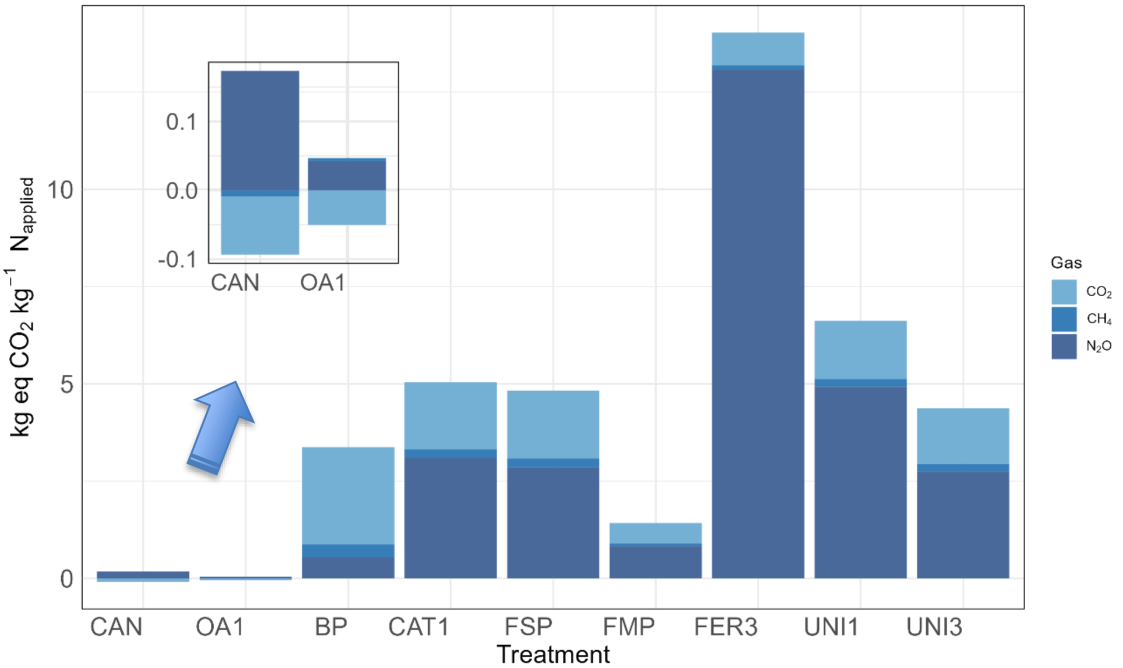


Figure 15 Global warming potential of synthetic and organic fertilizers, based on the 17-day microcosm experiment. CAN: calcium ammonium nitrate; BP: bokashi pellet; FER3: NPK solution with amnio acid; UNI1: hydrolysate; UNI3: chitin-rich fertilizer; FSP: fish sludge pellet; FMP: fish mix pellet; CAT1: protein fraction; OA1: organic amendment.

4 Conclusion

Six organic fertilizers — FER3, CAT1, FSP, FMP, UNI1 and UNI3 — showed considerable N supply capacity, with the potential to (partially) substitute synthetic N-fertilizers and warrant further application and evaluation under field conditions. The application of these fertilizers is unlikely to cause NH_3 emissions under the experimental condition. However, regarding GHG emissions, the global warming impact is predominantly driven by N_2O emission. While these organic fertilizers exhibited similar GHG emission patterns with other organic soil amendments, careful consideration of their global warming potential remains crucial, especially for the liquid fertilizer FER3.

While biobased fertilizers are generally expected to emit CO_2 rather than CH_4 in well-structured, aerobic soils with good aeration and moisture, CH_4 emissions under real-field dynamics needs to be critically evaluated under real-field dynamics how such emissions could be mitigated in future applications.

Concerning N_2O emissions, current findings are specific to this experimental setup and may not be universally representative. Prior studies showed that CAN often induces higher N_2O emissions than BBFs, suggesting that the observed N_2O response may not be

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402
THIS OUTPUT REFLECTS THE VIEWS ONLY OF THE AUTHOR(S), AND THE EUROPEAN UNION CANNOT BE HELD RESPONSIBLE FOR ANY USE WHICH MAY BE MADE OF THE INFORMATION CONTAINED THEREIN

universally representative. Further investigations are necessary to determine whether these BBFs systematically pose a higher N_2O emission risk under different environmental or soil conditions.

Additionally, the assessment of global warming potential in this study is limited to the soil application under controlled conditions without plants and does not account for the GWP associated with production and logistics. Synthetic fertilizers have a high embodied GWP due to fossil resource consumption and transportation, whereas BBFs are typically locally produced and used, potentially offering a lower overall carbon footprint. A comprehensive life-cycle analysis is recommended to fully compare their environmental impacts.

In summary, while these organic fertilizers show high potential for N supply and environmental compatibility under controlled conditions, their effects on GHG emission—particularly N_2O and CH_4 emissions—require further field-based research to ensure sustainable application strategies and minimize their impact on global warming.

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

Reference

De Neve, S., Hofman, G., 1996. Modelling N mineralization of vegetable crop residues during laboratory incubations. Soil Biology and Biochemistry 28(10-11), 1451-1457.

Egene, C.E., Regelink, I., Sigurnjak, I., Adani, F., Tack, F.M.G., Meers, E., 2022. Greenhouse gas emissions from a sandy loam soil amended with digestate-derived biobased fertilisers – A microcosm study. Applied Soil Ecology 178, 104577. https://doi.org/10.1016/j.apsoil.2022.104577.

EN13038:2011, Soil improvers and growing media. Determination of electrical conductivity.

Hu, J., et al., 2024. Increased N2O emissions by the soil nematode community cannot be fully explained by enhanced mineral N availability. Soil Biology and Biochemistry 191, 109314. https://doi.org/10.1016/j.soilbio.2024.109314.

IPCC, 2021. The Earth's Energy Budget, Climate Feedbacks and Climate Sensitivity, in: Intergovernmental Panel on Climate, C. (Ed.) Climate Change 2021 – The Physical Science Basis: Working Group I Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, pp. 923-1054. https://doi.org/10.1017/9781009157896.009.

ISO2917:1999, Measurement of pH — Reference method. https://www.iso.org/standard/24785.html.

ISO18122:2015, Determination of ash content. https://www.iso.org/standard/61515.html.

ISO18134-2:2017, Solid biofuels — Determination of moisture content — Oven dry method — Part 2: Total moisture — Simplified method. 2.

Sleutel, S., De Neve, S., Singier, B., Hofman, G., 2007. Quantification of organic carbon in soils: a comparison of methodologies and assessment of the carbon content of organic matter. Communications in soil science and plant analysis 38(19-20), 2647-2657.

Walling, E., Vaneeckhaute, C., 2020. Greenhouse gas emissions from inorganic and organic fertilizer production and use: A review of emission factors and their variability. Journal of Environmental Management 276, 111211. https://doi.org/10.1016/j.jenvman.2020.111211.

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRAN AGREEMENT NO 101000402

PROJECT PARTNERS