SEA2LAND

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D5.1 Agronomic behaviour and environmental effects of developed fertilizers

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Abstract

Deliverable 5.1 (D5.1) presents the agronomic performance of biobased fertilizers (BBFs) and shell residues in pot and field trials. First, 16 out of 26 BBFs characterized in WP6 were tested for nitrogen availability and 9 for phosphorous and potassium availability in pot trials with spring wheat and ryegrass, respectively (Chapter 2). The BBFs were selected based on nutrient content and the amount available for trials. The nitrogen and phosphorous use efficiency (NUE and PUE) of the BBFs ranged from close to the values of mineral fertilizers to very low. The potassium use efficiency (KUE) was moderate compared to mineral fertilizers.

Second, the three best performing BBFs were chosen for field trials and tested in broccoli cultivation in five countries. Each country included a local BBF of their own choice, to a total of 8 BBFs. The residual fertilizing effect was tested by cultivating either wheat, spring onions or lettuce following the broccoli, according to the crop rotation plan of each country.

The performance of the BBFs varied between the countries but were in most cases giving higher yield and nitrogen uptake than the non-fertilized control, but at large lower than for plots fertilized with mineral fertilizer. For most of the BBFs, the difference to the mineral fertilizer was however not statistically significant ($p > 0.05$). In a few cases, the yield/biomass and nitrogen uptake were also marginally higher (mainly not statistically different) in BBF treatments than in the mineral fertilizer control.

Based on the broccoli trial, there seems to be a correlation between the presence of higher levels of soil mineral nitrogen early in the season and yield. This correlation was however not valid for all countries and could not explain all the variation in yield (R^2 between 0.4 and 0.61).

Moreover, the liming effect of shell residues was tested in lab and field trials. In the lab, both finely and coarser ground shells gave a higher pH raise after 30 days than commercial liming agents, with the finer ground shells giving the highest pH raise. In the field, the finely ground shells gave a higher pH raise after two years in one of the sites, than the non-limed control. In the second field, no differences were observed and it was deemed necessary to have longer trials to determine differences at field scale. Especially since the commercial liming agents act slowly.

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Glossary

ANOVA: Analysis of variance

AMGA: Annotated Model Grant Agreement

BBCH: Scale to identify the phenological stages of plants

BBF: Bio-based fertilizer

BRIOAA: Belgian Research Institute of Organic Agriculture and Agroecology

CAPA: Chambre d'agriculture Pyrénées Atlantiques

CON0: Negative control

D: Deliverable

DM: Dry matter

FER3: Plant biostimulant. NPK solution with amino acids (FERTINAGRO)

FER5: Microalgae-based plant biostimulant with humic acids (NEIKER-FERTINAGRO, used in pots trial.

FER5': Plant biostimulant. Hydrolysate derived from microalgae biomass (NEIKER), used in field trial.

FSP: Organic fertilizer. Pelleted fish sludge (GRÖNN)

FiBL: The Research Institutes of Organic Agriculture

ICP-OES: Inductively Coupled Plasma Optical Emission

KUE: Potassium use efficiency

METK: Centre of Estonia Rural Research and Knowledge

MF: Mineral fertilizer

MFE: Mineral fertilizer equivalents

NEIKER: Basque Institute for Agricultural Research and Development)

NIBIO: Norwegian Institute for Bioeconomy Research

NIR: Near-Infrared Spectroscopy

NH_4^+: Ammonium

NO_3^- : Nitrate

NPK: Nitrogen Phosphorous Potassium

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NUE: Nitrogen use efficiency

OA2: Organic fertilizer.

PUE: Phosphorous use efficiency

SMN: Soil mineral nitrogen

UGent: University of Gent

ULB: Université Libre de Bruxelles.

UNIVPM: Universita Politecnica Delle Mache

WP: Work package

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1 Introduction

This deliverable presents findings on the agronomic performance and nitrogen leaching risks associated with the application of selected biobased fertilizers (BBFs) in both pot and field trials. It also evaluates the effectiveness of a shell mix as a sustainable liming agent. These investigations aim to support more circular, climate-resilient, and resource-efficient fertilization strategies across European agriculture when using fertilizing products derived from fishery waste and byproducts.

The reuse of organic residual streams in agriculture offers multiple benefits. Recycling nutrients from waste streams reduces reliance on synthetic mineral fertilizers, whose extraction and production processes are resource-intensive and environmentally damaging. Moreover, many European soils are experiencing a steady decline in soil organic matter (SOM), a key component of soil fertility, structure, and biological function. The application of organic fertilizers can help restore SOM levels, thereby improving soil health, nutrient cycling, and long-term agricultural productivity.

However, fertilizers must provide adequate nutrients to support crop growth without exceeding crop demand, as nutrient surpluses pose significant environmental risks, particularly in the form of nitrate leaching. Nutrients must also be available in plant-accessible forms at the right time to ensure optimal uptake. To assess both agronomic efficacy and environmental impact, this study monitored plant-available nitrogen, including mineral nitrogen (ammonium and nitrate), during the crop growing season. Nitrogen use efficiency and crop yield were measured under field conditions, and nitrate residues were monitored across multiple soil depths and time points to evaluate leaching risk due to rainfall and irrigation events.

Following an initial screening of BBFs for nutrient composition (see Deliverable D6.1), several promising ones were selected for pot trials assessing nitrogen, phosphorus, and potassium availability. The top-performing BBFs, based on both nutrient content and plant availability, were then tested in field trials conducted across five countries, offering diverse climatic and soil conditions.

In addition to nutrient-rich BBFs, Sea2Land also explored mussel shell-derived by-products for their liming potential. These calcium-rich residues can raise soil pH and reduce dependence on conventional liming agents, which are typically mined and energy-intensive to produce. By harnessing sea-based residual streams, this work contributes to more sustainable soil management and a circular bioeconomy approach in agriculture.

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2 Screening of BBFs for nitrogen, phosphorus, and potassium use efficiency

2.1 Materials and methods

The primary objective of the pot experiment under WP5.1 was to assess the uptake of nitrogen (N), phosphorus (P), and potassium (K) from newly developed bio-based fertilizers (BBFs) derived from fish and aquaculture by-products as created in WP3 and WP4. Based on the results of the pot tests, 3 promising BBFs were selected for subsequent field trials. Chemical analyses of these BBFs were conducted by UGENT prior to the pot tests (Table 1).

Table 1. Macronutrients and mineral forms of N and P in the bio-based fertilizers expressed as mean ± standard deviation by mass (fresh weight (FW)) (source: D6.1, UGENT).

Task	Area	Lead	Code	Form	TN/%	NH_4^+-N/ (g/kg)	NO_3^--N/ (g/kg)	P/(g/kg)	Plant available P/(g/kg)	K/(g/kg)
3.1	Baltic Sea	NUTRI	FS	l	0.12 ± 0.00	0.27 ± 0.01	0.007 ± 0.002	0.70 ± 0.02	0.43 ± 0.00	1.32 ± 0.01
			BP	s	2.63 ± 0.09	0.38 ± 0.02	<0.002	10.45 ± 3.80	5.50 ± 0.19	19.09 ± 1.68
			VER	s	0.89 ± 0.06	0.11 ± 0.00	<0.002	2.65 ± 0.05	0.86 ± 0.03	9.68 ± 0.53
3.2	Cantabrian Sea	FERTINAGRO	FER1	s	7.14 ± 0.15	12.00 ± 0.29	<0.002	1.02 ± 0.01	1.05 ± 0.02*	3.08 ± 0.08
			FER2	l	4.34 ± 0.24	2.23 ± 0.02	<0.002	3.71 ± 0.08	3.55 ± 0.09	4.38 ± 0.04
			FER3	l	5.31 ± 0.10	2.56 ± 0.01	<0.002	15.93 ± 1.11	13.72 ± 0.03	22.38 ± 1.89
			FER4	l	3.90 ± 0.08	4.74 ± 0.12	<0.002	2.93 ± 0.05	2.59 ± 0.05	3.29 ± 0.04
			FER5	l	11.13 ± 0.19	8.81 ± 0.37	<0.002	13.33 ± 0.35	11.76 ± 0.12	1.88 ± 0.11
3.3	Adriatic Sea	UNIVPM	UNI1	l	4.82 ± 0.17	10.95 ± 0.09	<0.002	3.25 ± 0.09	3.22 ± 0.03	6.08 ± 0.11
			UNI2	s	3.72 ± 0.08	4.79 ± 0.16	<0.002	7.09 ± 0.96	5.92 ± 0.31	10.93 ± 1.42
			UNI3	l	4.62 ± 0.03	10.48 ± 0.09	<0.002	3.17 ± 0.04	3.25 ± 0.10*	6.06 ± 0.06
			UNI4	s	0.18 ± 0.03	0.01 ± 0.00	<0.002	0.15 ± 0.02	0.031 ± 0.003	0.09 ± 0.01
4.1	North Sea	NIBIO	FSP	s	6.19 ± 0.07	0.62 ± 0.06	<0.002	27.42 ± 4.51	13.88 ± 0.13	14.62 ± 2.04
			FMP	s	9.77 ± 0.22	0.38 ± 0.05	0.007 ± 0.002	20.23 ± 1.70	14.34 ± 0.24	56.01 ± 0.74
4.2	Atlantic Sea	CATAR	CAT1	s	7.62 ± 0.72	0.34 ± 0.02	<0.002	30.89 ± 0.71	24.74 ± 0.92	6.15 ± 0.11
			CAT2	l	1.48 ± 0.03	0.28 ± 0.01	<0.002	0.76 ± 0.03	0.71 ± 0.01	2.04 ± 0.11
			CAT3	s	8.14 ± 0.17	0.07 ± 0.00	0.013 ± 0.002	24.69 ± 10.54	n.m**	4.44 ± 0.10
			CAT4	l	1.12 ± 0.00	0.21 ± 0.00	0.005 ± 0.002	0.77 ± 0.02	n.m	2.42 ± 0.05
4.3	Mediterranean Sea	UVIC	NRC1	l	0.098 ± 0.04	0.58 ± 0.01	<0.002	0.02 ± 0.00	0.0068 ± 0.00	0.19 ± 0.00
			OA1	s	1.89 ± 0.08	2.77 ± 0.12	<0.002	125.07 ± 13.20	18.81 ± 0.08	2.37 ± 0.09
4.4	Freshwater	UVIC	NRC2	l	0.009 ± 0.00	0.07 ± 0.00	<0.002	0.01 ± 0.00	0.0015 ± 0.0002	0.04 ± 0.00
			OA2	s	3.46 ± 0.11	7.09 ± 0.15	<0.002	14.99 ± 1.31	3.46 ± 0.31	9.35 ± 0.30
Chile	Chilean Sea	INIA	Pep	s	10.77 ± 0.07	3.42 ± 0.08	<0.002	14.89 ± 0.05	13.01 ± 0.31	14.33 ± 0.15
			SBF	s	7.80 ± 0.13	1.76 ± 0.06	<0.002	76.92 ± 1.40	38.72 ± 1.35	4.26 ± 0.12
			DFS	s	3.59 ± 0.00	0.60 ± 0.01	<0.002	58.21 ± 0.80	21.71 ± 1.09	1.29 ± 0.48
			Com	s	1.49 ± 0.34	0.71 ± 0.09	0.37 ± 0.05	6.78 ± 0.54	2.24 ± 0.17	4.66 ± 0.16

*The P of the products can be 100% mobile in the soil.

**n.m: not measured (due to later production of the products)

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BBFs with extremely low N, P, or K content, as well as those not yet available by the start of the pot trial, were excluded from the experiment. As a result, N uptake was assessed for 16 BBFs, P uptake for 9, and K uptake for 6 BBFs (Table 2).

Table 2. Tested bio-based fertilizers (BBF)

Task	Company	Country	Code	N availability (METK)	P availability (FiBL)	K availability (FiBL)
3.1 Baltic Sea	NUTRI	Estonia	**FS**	Low	Low	Low
			BP	x	X	x
			VER	x	X	x
3.2 Cantabrian Sea	FERTINAGRO	Spain	**FER1**	x	Low	Low
			FER2	x	Low	Low
			FER3	x	X	x
			FER4	x	Low	Low
			FER5	x	Low	Low
3.3 Adriatic Sea	UNIVPM	Italy	**UNI1**	x	Low	Low
			UNI2	x	x	x
			UNI3	x	Low	Low
			UNI4	Low	Low	Low
4.1 North Sea	GRONN	Norway	**FSP**	x	x	x
			FMP	x	x	x
4.2 Atlantic Sea	CATAR	France	**CAT1**	x	x	Low
			CAT2	x	Low	Low
			CAT3	Low	Low	Low
			CAT4	Low	Low	Low
4.3 Mediterranean	UVIC	Spain	**NRC1**	Low	Low	Low
			OA1	x	x	Low
4.4 Freshwater			**NRC2**	Low	Low	Low
			OA2	x	x	Low
Chilean Sea	INIA	Chile	**Pep**	NA	NA	NA
			SBF	NA	NA	NA
			DFS	NA	NA	NA
			Com	NA	NA	NA

x – tested in pot experiments

Pot trials to assess nitrogen (N) uptake were conducted at METK, while phosphorus (P) and potassium (K) uptake trials were carried out at FiBL under controlled conditions. Spring wheat (*Triticum aestivum* L., Var. Hiie) was used as the test crop for N uptake, with an experimental duration of 8 weeks in a greenhouse (Figure 1). The aboveground biomass of spring wheat was harvested by cutting and analyzed for nitrogen content using the Kjeldahl method. The experiment followed a randomized design with four replicates per treatment. To evaluate P and K uptake, experiments with ryegrass (*Lolium multiflorum*, Var. Gemini) were established at FiBL (Figure 2). These trials were conducted in a randomized block design, with each treatment replicated four times, each replicate

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consisting of three pots. Ryegrass grew for 14 weeks in a climate chamber, and P and K concentrations were determined from three cuttings (at 6, 10, and 14 weeks).

Figure 1. Spring wheat plants two weeks after experiment set up (top image) and before harvest (week 8, bottom image)

Figure 2. Ryegrass experiment directly after experimental set up (left picture) and grown for 10 days under controlled conditions in the climate chamber.

Shoot P concentration was measured using the molybdate blue method (Murphy and Riley, 1958) on a Segmented Flow Analyzer (Skalar Analytical B.V., San++ Automated Wet Chemistry Analyzer, Breda, Netherlands), and shoot K concentration was analyzed using an ICP-OES (PerkinElmer, model ICP-OES Optima 5300 DV) at the Core Facility Hohenheim, Stuttgart, Germany, following incineration and acid extraction of plant powder.

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The amount of BBF applied was based on the N, P, or K content of each BBF. For the N uptake test, BBFs were applied at a rate of 300 mg N per pot. For P uptake, the application rate was 50 mg P kg^{-1} substrate, and for K, it was 300 mg K kg^{-1} substrate. Each experiment included an unfertilized control: an N-free control (N0) for the N set, a P-free control (P0) for the P set, and a K-free control (K0) for the K set. Additionally, a fully fertilized control was established using readily available N, P, and K sources—namely, calcium nitrate tetrahydrate ($Ca(NO_3)_2$ x $4H_2O$), Triple Superphosphate (TSP, 20% P, Landor, Muttenz, Switzerland), and potassium sulfate (K_2SO_4).

Calculations

The effectiveness of BBFs was assessed by calculating the mineral fertilizer equivalent (MFE) and nutrient use efficiencies for N, P and K (NUE, PUE and KUE). MFE is a measure of the proportion of total N, P, and K from BBFs that is equally available to plants compared to the readily available N, P, and K applied in a mineral fertilizer. Nutrient use efficiency is expressed as the percentage of N, P, or K utilized by plants from the fertilizer. The MFE was calculated according to equation 1, and the nutrient use efficiency according to equation 2:

$$MFE\ (\%) = \frac{X_1 - X_0}{X_{MF} - X_0} * 100\ \% \qquad (1)$$

$$NUE, PUE\ and\ KUE\ (\%) = \frac{X_1 - X_0}{X_{applied}} * 100\ \% \qquad (2)$$

Where:
X_1 = N, P, or K content in the aboveground biomass fertilized with BBF,
X_0 = N, P, or K content in the aboveground biomass of unfertilized plants,
X_{MF} = N, P, or K content in the aboveground biomass fertilized with mineral fertilizer
$X_{applied}$ = total amount of N, P, or K applied (300, 50, and 300 mg kg^{-1} substrate, respectively)

Statistical Analysis

All data were analyzed using one-way ANOVA, followed by Tukey's Honestly Significant Difference (HSD) test with a significance level of α = 0.05. The normality of residuals was tested using the Shapiro-Wilk test. Analyses were conducted with JMP software, version 11 (SAS Institute Inc., North Carolina, USA).

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2.2 Results

Table 3 displays the calculated NUE, PUE, KUE, and MFE of the tested BBFs. BBF treatments significantly affected all evaluated parameters (Table 3). The pot trial results indicated significantly higher N-MFE% and NUE% in the FSP, CAT2, OA1, FER3, FER4, and FER5 treatments ($p < 0.05$) compared to other BBFs (Table 3). In these treatments, NUE% ranged between 36.4% and 43.9%, while N-MFE% ranged from 53.5% to 85.1%.

For P uptake, none of the BBFs achieved values comparable to Triple Superphosphate (Table 3). The highest PUE values were observed with BBFs FMP and CAT1, and a similar trend was seen for P-MFE, which ranged from 27% to 84%.

Regarding K uptake, none of the BBFs matched the K uptake level seen with potassium sulphate (Table 3). The highest KUE and K-MFE values were observed with BBFs FMP and FER3. The highest K-MFE was recorded for BBF FMP (68%), while the lowest K-MFE was found in BBF FSP (21%).

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Table 3. Nitrogen (N) use efficiency (NUE%), and mineral fertilizer equivalent (N-MFE%) of spring wheat under greenhouse conditions. Cumulative (sum of cut 1-3) P and K use efficiency (PUE and KUE) and cumulative (sum of cut 1-3) mineral fertilizer equivalent (P-MFE and K-MFE) of ryegrass under growth. BBFs selected for field trials are marked with a green background. Different letters following means (n = 4) and standard deviation (SD) indicate significant differences. Data were analyzed using one-way ANOVA followed by Tukey's honest significant difference test with a significance level of a = 0.05.

Treatment code	NUE%		N-MFE %		Cumulative PUE%		Cumulative P-MFE%		Cumulative KUE%		Cumulative K-MFE%	
	Mean±SD		Mean±SD		Mean±SD		Mean±SD		Mean±SD		Mean±SD	
Fert. control	63.4±11.4	a	100.0±15.0	a	53.6±4.7	a	100.0±8.8	a	81.0±7.3	a	100.0±9.0	a
BP	3.9±1.6	fg	5.7±2.3	fg	32.2±1.6	d	60.1±2.9	d	40.5±9.9	bc	50.1±12.3	bc
VER	1.3±0.8	g	1.9±1.1	g	15.5±0.5	e	29.0±1.0	e	29.4±8.1	cd	36.3±10.0	cd
FER1	35.6±1.1	c	60.7±1.8	cd								
FER2	33.4±0.8	cd	56.8±1.3	cd								
FER3	43.0±2.5	bc	73.3±4.2	bc	32.2±2.6	d	60.2±4.9	d	49.1±6.5	b	60.6±8.1	b
FER4	41.6±6.8	bc	70.8±11.6	bcd								
FER5	50.0±1.8	b	85.1±3.1	ab								
UNI1	10.6±4.0	efg	15.5±5.8	efg								
UNI2	5.7±1.9	fg	8.3±2.8	fg	35.3±2.0	cd	65.9±3.7	cd	43.2±7.8	bc	53.3±9.6	bc
UNI3	10.6±2.6	efg	15.5±3.8	efg								
FSP	43.9±1.9	bc	64.5±2.8	cd	21.0±2.1	e	39.1±3.8	e	16.7±6.2	d	20.7±7.6	d
FMP	15.3±2.3	ef	22.4±3.3	ef	43.0±5.4	bc	80.3±10.1	bc	55.0±7.1	b	67.9±8.8	b
CAT1	22.1±3.2	de	32.5±4.6	e	44.8±5.6	ab	83.6±10.4	ab				
CAT2	36.4±1.9	c	53.5±2.7	d								
OA1	42.1±3.8	bc	61.8±5.6	cd	14.3±3.9	e	26.7±7.2	e				
OA2	6.8±1.3	fg	10.0±2.0	fg	18.8±3.4	e	35.0±7.2	e				
Prob > F	<.0001		<.0001		<.0001		<.0001		<.0001		<.0001	

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2.3 Discussion and Conclusion

The results indicated that nitrogen uptake from BBFs was relatively low compared to mineral fertilizer treatments. Of the 16 BBFs tested, three demonstrated high N-MFE% values (70.8–85.1%), five showed moderate levels (53.5–64.5%), four were low (15.5–32.5%), and four were below 10%. The highest nitrogen availability (N-MFE% above 60%) was observed in BBFs FSP (fish sludge pellet), OA1 (organic amendment), and the FER1 (amino acids, organic matter, and humic extract), FER3 (NPK solution with amino acids), FER4 (foliar fertilizer with amino acids, humic extract, organic matter), and FER5 (fertilizer with humic acids).

For phosphorus availability, the BBFs FMP (pelleted fertilizer from fish sludge) and CAT1 (protein fraction derived from fishery by-products, including head, bone, and viscera) showed high efficacy, achieving 80–85% availability relative to Triple Superphosphate. These BBFs present promising alternatives to commercially available P fertilizers due to their high P content and potential to provide an effective nutrient source.

In terms of potassium, the BBFs achieved only moderate availability, with values between 50–68% compared to potassium sulphate. The highest potassium uptake was observed from the BBF FMP, likely due to its high organic matter content (71%). The organic matter content may also contribute to improved soil structure and fertility, making FMP a promising candidate as a substitute for mineral K fertilizers, particularly where enhanced organic C input to soil is desirable.

The selection of BBFs for field trials was based on both the pot test results (METK and FiBL) and the ability to produce the required amount of BBFs for field trials. Therefore, according to nitrogen uptake as well as the availability of the required amount, the following BBFs were tested in field trials: FER3, FSP, and CAT1. In addition, each partner could test one more BBF. Thus, in Estonia, BP, in Norway FMP, in Spain, FER5' and OA2, and in Belgium, UNI1, were also tested in the field trials.

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3 Field trials

The field trials were conducted in five countries over two years (2023 and 2024, Table 4). In 2023, broccoli was cultivated and fertilized with the BBFs selected from the pot trials (Table 5). In Spain, the experiment was repeated in 2024. Besides the BBFs, all countries included a negative (unfertilized) and a positive (mineral or commercially available organic fertilizer) control, as well one local BBF of own choice (two in the case of Spain in 2024). Unless stated otherwise in the text, all fertilized treatments received 120 kg total nitrogen per hectare.

Following broccoli cultivation, a residual crop was grown on the same fields with no further fertilization (Table 4): winter wheat in Belgium, Estonia and Norway, and onion and lettuce in Spain. Broccoli was cultivated twice in France and Spain. In France, the 2023 trial did not give any results due to poor weather and the trial was repeated in 2024. Due to poor weather also this season the residual crop could not be cultivated in France. In Spain the trial was repeated in 2024 on the same fields as used in 2023.

Table 4. Overview of field trials including cultivation period and crop variety. Green: Broccoli, yellow: winter wheat (residual crop), light green: other residual crops

<table>
<tr><th>Year</th><th colspan="9">2023</th><th colspan="10">2024</th></tr>
<tr><th>Month</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>11</th><th>12</th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th></tr>
<tr><td>Belgium</td><td></td><td colspan="4">Parthenon</td><td colspan="3"></td><td colspan="9">Mix Var: Christoph and Allessio</td><td colspan="2"></td></tr>
<tr><td>Estonia</td><td></td><td colspan="4">Cezar</td><td colspan="11">Peranaise</td><td colspan="3"></td></tr>
<tr><td>France</td><td colspan="2"></td><td colspan="4">Parthenon</td><td colspan="9"></td><td colspan="4">Parthenon</td></tr>
<tr><td>Norway</td><td></td><td colspan="4">Parthenon</td><td colspan="11">Kuban</td><td colspan="3"></td></tr>
<tr><td>Spain</td><td colspan="5"></td><td colspan="4">Parthenon</td><td></td><td colspan="2">Onion</td><td colspan="2">Parthenon</td><td colspan="2">Lettuce</td><td colspan="3"></td></tr>
</table>

Table 5. Fertilizers used in the five field trials.

<table>
<tr><th>Country</th><th>Common BBFs</th><th>Local BBF</th><th>Positive control</th></tr>
<tr><td>Belgium</td><td rowspan="5">FER3
FSP
CAT1</td><td>UNI1</td><td>Chicken manure (CM)</td></tr>
<tr><td>Estonia</td><td>BP</td><td>Mineral fertilizer (MF)</td></tr>
<tr><td>France</td><td>/</td><td>Sheep, cow & poultry manure (SCPM)</td></tr>
<tr><td>Norway</td><td>FMP</td><td>K8 from GRONN</td></tr>
<tr><td>Spain</td><td>FER5' and OA2 (only 2024)</td><td>Mineral fertilizer (MF)</td></tr>
</table>

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3.1 Soil and plant analysis, calculations and statistical analysis

Analysis of soil and plant materials were conducted collectively for all trials at the various institutions involved in WP5.

Soil standard parameters

The field soils were sampled prior to fertilization in 2023. Samples from 0-10, 10-30, and 30-60 cm depth were taken. For Spanish and Belgian fields, the soils were also sampled from 60-90cm, due to local regulations with respect to Nitrate Vulnerable Zones in the case of Belgium and research interest in the case of Spain. For each country, one sample was taken in the center, and four samples were taken 1 m towards every corner of the field. The five samples were mixed, and a sub-sample of 1 kg was sent for analysis. Soil carbon and nitrogen (ISO 10694), texture (NS-ISO 11277) and pH (H_2O) were analyzed at NIBIO, Norway.

Soil bulk density

For bulk density, samples were taken with cylinders in the depths 0-10 cm and 10-20 cm. Both fresh weight and dry weight (105 °C for 24 h) were recorded. The bulk density was calculated as $P = M V^{-1}$, where P is the bulk density, M is the weight of the dry soil and V is the volume of the cylinder. The bulk density was determined locally.

Mineral nitrogen

Samples for soil mineral nitrogen (SMN) were collected from each replicated block of each treatment including the unfertilized control, at the same depths as for determination of soil standard parameters. Soil was sampled two weeks before the fertilization (T1) to provide a baseline and then one week after fertilization (T2) to monitor the initial status of the fertilized soil. Additionally, soil samples were collected based on the phenological plant growth stages of the broccoli (Meier, 1997), T3 at BBCH 25 - visible formation of the 3rd side shoot, and T4 at BBCH 51 - the branches of inflorescence began to elongate, and the broccoli is ready for harvest. After the harvest, a fifth sample (T5) was taken to investigate the residual available N in the soil. The two last samples were collected after planting/sowing (T6) and harvest (T7) of the residual crop. Fresh soil samples were delivered in cool boxes soon after sampling and stored in the freezer at -20 °C until analysis at UGent.

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To prevent N loss, the thawed soil samples were used for total N test by the Kjeldahl digestion method, ammonium N (NH_4^+-N) and nitrate N (NO_3^--N), being extracted by 1M potassium chloride solution and analyzed on a continuous auto-flow analyzer (Chemlab System 4, Skalar, the Netherlands, Saju et al., 2022). The SMN levels were calculated as the sum content of NH_4^+-N and NO_3^--N of different layers of each treatment.

Plant analysis

The C and N concentration was determined using dry combustion with LECO equipment (CN828, LECO Corporation, Michigan, USA) at the facilities of NEIKER. Broccoli yield (kg ha^{-1}) was estimated from plot data at each location. Both fresh weight (FW) and dry weight (DW) were estimated for both stalks/leaves and heads. Commercial (i.e., salable) yield was determined as heads with a diameter of minimum 8 cm. Based on the nitrogen concentration of the plants, N uptake (kg N ha^{-1}) was determined per treatment.

Statistical analysis

First, data was analyzed at the level of the individual field trials (Chapters 3.2-3.6). Differences between fertilizer treatments were investigated using one-way ANOVA followed by either Tukey Honest Significant Difference test (Belgium, Estonia and France) or Duncan's test (Spain). The Norwegian data was analyzed using a two-way ANOVA mixed model in MiniTab followed by Tukey Honest Significant Difference test. Normality of residuals were assessed by Shapiro-Wilks test or by visual inspection of QQ-plots. Analyses were conducted either with R (R Core team, 2013) or with JMP version 11 (SAS institute Inc., North Carolina, USA). In cases where the residuals were not normally distributed, Kruskal-Wallis was used instead of ANOVA followed by the Dunn Test (applied for "Commercial yield" in France).

Second, all results were analyzed collectively (Chapter 3.7). Two-way ANOVA followed by Tukey HSD was used for analysis of the variables commercial yield, total aboveground biomass, nitrogen uptake and nitrogen use efficiency, as a function of treatment and country. The data were normalized by dividing by the mean of each variable for each country, prior to analysis. For analysis of yield as a function of soil mineral nitrogen content one week after fertilization, linear regression was used. All tests were performed in R (R Core team, 2013) at $\alpha = 0.05$ and assumptions were tested by inspection of Q-Q-plots.

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3.2 Belgium

3.2.1 Methods

The Belgian field experiment was conducted in Upigny, Belgium (GPS coordinates: 50.57163N, 4.87223E) at the research station of BRIOAA. The soil at the trial site is a clayey loam, considered as one of the most fertile in Belgium (SP Wallonie).

Prior to the trial, the field was ploughed to a depth of 25 cm on May 2, 2023 then prepared for broccoli planting. The experiment followed a randomized block design with four independent blocks, each receiving the six treatments once. Individual plots measured 16 m² (2 m × 8 m), with two rows of broccoli plants spaced by 75 cm per plot. The trial began on May 4, 2023, with harvesting on August 22, 2023.

One month old broccoli seedlings sourced from a local nursery (approximately at the four-leaf stage) were transplanted on May 4, 2023. Fertilizers were applied on May 22, 2023. The liquid BBFs FER3 and UNI1 were diluted in 5 liters of water and applied using a watering can, while the solid fertilizers (CAT1, FSP, and CM) were manually broadcast at the target rate. After application, the BBFs were lightly incorporated into the soil using a tool adapted to the plot size (market gardening hoe). The control treatment (CON0) received no fertilizer.

To ensure optimal broccoli growth, several agronomic measures were implemented:

- Pest protection: A protective net was placed over the plots during the first month,
- Weed control: Regular manual weeding.
- Irrigation: Supplemental watering was provided only once when rainfall was insufficient (equivalent to an application of 2.5 liters m^{-2}).
- Slug control: An organic anti-slug product was applied in July 2023.
- Predator deterrence: Natural methods was used to protect plants from birds (bird scarecrow) and rabbits (fences).

After harvest, the soil was left undisturbed for several weeks before being ploughed to a depth of 25 cm on January 30, 2024, in preparation for winter wheat sowing (variety Christoph + Allessio) at a density of 200 seeds m^{-2}. No additional fertilizers were applied to the wheat, and biomass assessment was conducted on August 7, 2024, by measuring the dry weight of plants within 1 m² quadrats in each plot (Table 6). Unfortunately, it was not

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possible to get results on the grain yield, nor on 1000 kernel weight. The entire experiment adhered strictly to organic farming regulations.

3.2.2 Results and discussion

Early field observations (15 days post fertilization) indicated that the liquid BBFs (UNI1 and FER3) had a light burning effect on young broccoli leaves. For this reason, plants treated with these BBF had a growth delay for one month. However, this growth delay had been completely recovered by the time of harvest since all the treatments gave the same total aboveground biomass (in tons per hectare, DW, see Table 6). Commercial yield of broccoli plants was not influenced by the treatments neither, but the high variability probably explains the lack of statistical significance between treatments. Indeed, we can observe that some treatments led to higher commercial yield such as fertilization with UNI1. The least performing treatment for commercial yield and total aboveground biomass (as expressed in both fresh and dry weight) was obtained with the FSP treatment. All the measured broccoli plants had similar nitrogen content (no difference between treatments). No residual effects were observed on the following wheat crop since all plots produced the same biomass.

Table 6. The fresh and dry commercial yield and total aboveground biomass of broccoli under different fertilizer treatments, N concentrations in broccoli plants (Ntot, %) and N use efficiency (NUE%) (mean ± SD, n=4). Different letters indicate significant differences between treatments at p<0.05 with Tukey-Kramer (HSD) test. ns: not significant.

	Commercial heads yield		Total aboveground biomass		Ntot	NUE	Winter wheat biomass
	$t\ ha^{-1}$ (FW)	$t\ ha^{-1}$ (DW)	$t\ ha^{-1}$ (FW)	$t\ ha^{-1}$ (DW)	%	%	$g\ DW\ m^{-2}$
CM	5.1±1.3	0.74±0.2	20.9±2.4bc	3.0±0.4	2.5±0.2ab	-0.3±7.5b	512±139
CON0	5.5±2.9	0.70±0.4	20.9±3.9c	2.7±0.5	2.8±0b	NA	416±111
CAT1	6.5±4.2	0.80±0.5	28.5±4.8a	3.5±0.6	2.9±0.2a	22.6±19.2a	476±67
FER3	6.4±1.6	0.80±0.2	28.7±3.2a	3.6±0.3	3.0±0.3a	28.4±10a	458±155
FSP	4.6±1.6	0.59±0.2	24.2±3.5ab	3.1±0.6	2.6±0.2ab	4.6±8ab	466±208
UNI1	9.7±2.0	1.15±0.3	29.5±1.2abc	3.5±0.3	3.0±0.2ab	25.6±3.3ab	4412±84
F value	2.11	2.09	5.64	2.62	3.88	5.69	0.23
Pr(>F)	Ns	Ns	0.0027	ns	0.0146	0.0054	ns

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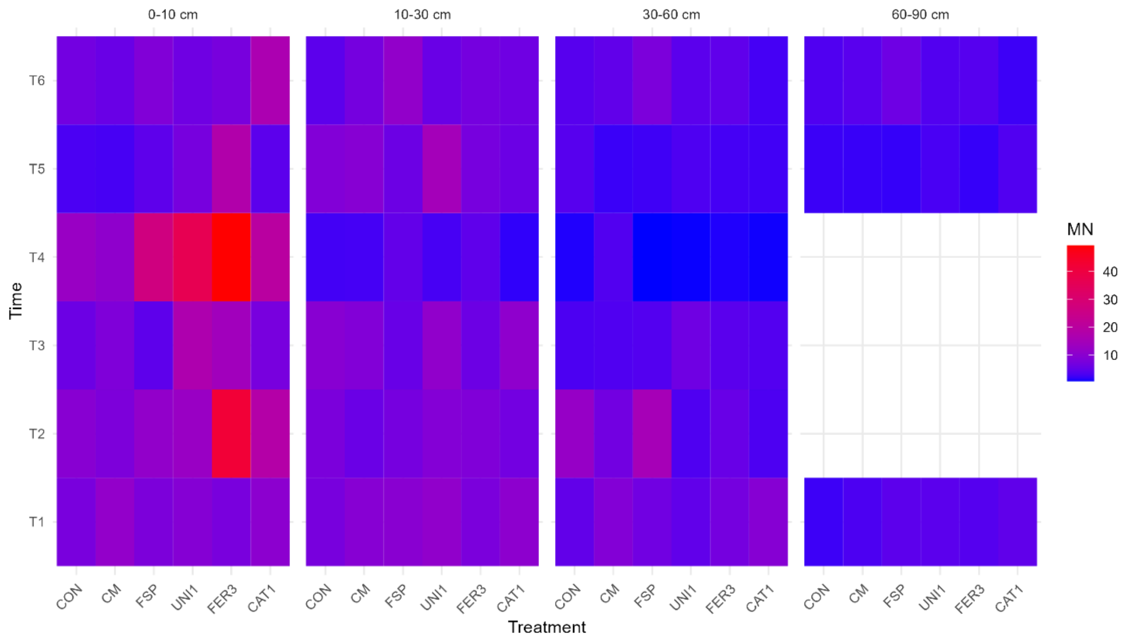

Figure 3. Heatmap of the soil mineral N concentration (mg kg^{-1}) in the Belgian field trial. CON: control, unfertilized treatment; CM: reference fertilizer; FSP: fish sludge pellet; UNI1: Amino acids and peptides; FER3: NPK solution with amino acids; CAT1: protein fraction.

The heat map (Figure 3) shows the mean concentration of mineral N (mg kg^{-1} DW) in the field from different treatments across even sampling time points. The soil sampled from 60-90 cm were collected to monitor the risk of nitrate leaching due to local regulations. Soil from T1 was analyzed to determine the mineral N concentration of the field soil before fertilization, while samples collected from the 60-90 cm deep layer after harvesting (T5 and T6) were used to investigate the nitrate concentration. Due to the prolonged and intense rainy season during the field trial in Belgium and the high content of clay in the soil, soil sampling was challenging. The study field also has a long history of organic farming, which could contribute to the continuous release of mineral N from organic residues in the soil. Furthermore, the range of mean SMN was narrow across the treatments and monitoring periods, reaching up to 49 mg kg^{-1} in the 0-10 cm soil layer at T4. This was also proved by the low NUE of applied fertilizers (Table 6). Additionally, rainfall and the moist condition of soil also influenced the N leaching and diffusion. So the heat map does not show a clear trend of decreasing mineral N concentrations with increasing soil depth, as typically observed in other fields.

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The soil before fertilization (T1) contained approximately 7-11 mg kg^{-1} of mineral N in the 0-10 cm and 10-30 cm layers, 5-9 mg kg^{-1} in the 30-60 cm layer, and around 2-5 mg kg^{-1} in the 60-90 cm soil layer. By T2, the mineral N concentration of CON0 was around 10 mg kg^{-1} in the top layer and around 8 mg kg^{-1} in the 10-30 cm layer and 12 mg kg^{-1} in the 30-60 cm layer. The positive control with chicken manure (CM) exhibited lower mineral N concentration compared to other fertilized treatments (11-19 mg kg^{-1}) in the top layer.

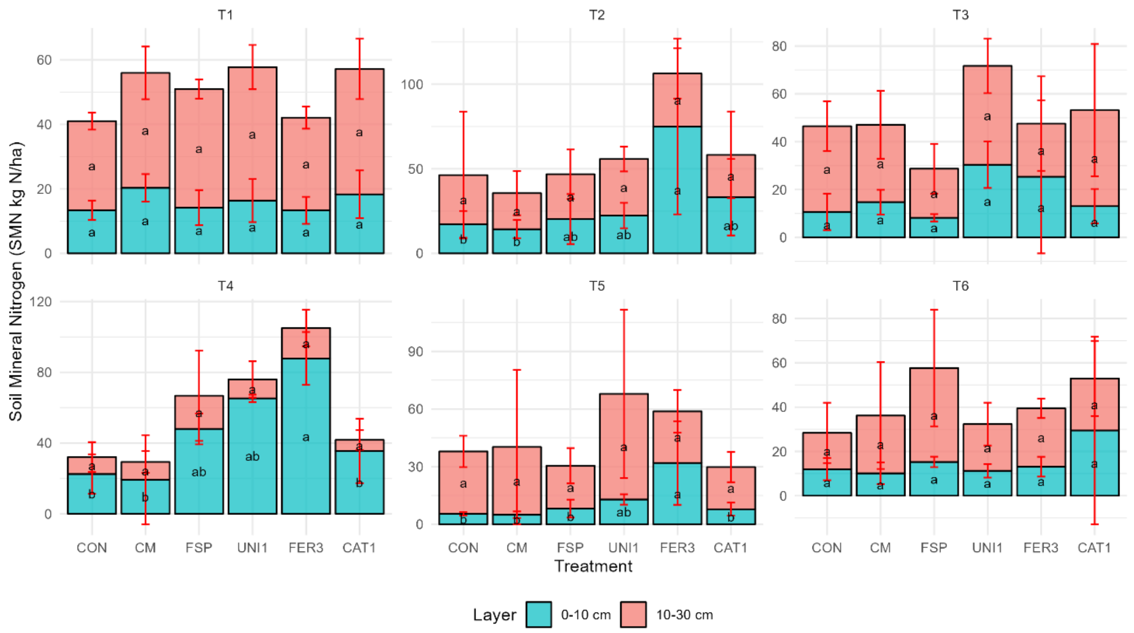

Figure 4. Soil mineral N levels (SMN kg ha^{-1}) by time and depth in the Belgian field trial. Letters refer to the statistical difference (one-way ANOVA, Tukey HSD, $p < 0.05$) of SMN between treatments at 0-10 cm and 10-30 cm layer, respectively. CON: control, unfertilized treatment; CM: reference fertilizer; FSP: fish sludge pellet; UNI1: Amino acids and peptides; FER3: NPK solution with amino acids; CAT1: protein fraction. Different letters imply significant differences between the treatments, within each soil layer and sampling time.

By T3, the mineral N of the top layer decreased, while some increases were observed in the deeper layers. The highest mineral N concentration was detected in samples from the UNI1 treatment (17 mg kg^{-1}) in the top layer. By T4, mineral N concentration increased in all treatments, mostly composed of nitrate N in the topsoil, most likely due to the mineralization of organic residues and fast nitrification under moist conditions. The mineral N concentration increased to 13 mg kg^{-1} in the CON0 treatment, while maintaining 20-37 mg kg^{-1} for treatment CAT1, REF, FSP, and UNI1 in the top layer. By T5, the mineral N concentrations decreased, with the highest concentrations of 18 mg kg^{-1} in the FER3 treatment in the top layer and 15 mg kg^{-1} in the UNI1 treatment in the subsurface layer.

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By T6, except the CAT1 treatment, no obvious differences of mineral N were observed compared to the soil before fertilization.

The SMN levels of the treatments show the potentially plant available N pool of the soil during study period (Figure 4). The initial SMN content was 41-58 kg ha^{-1} in the 0-30 cm layer. By T2, the SMN levels of the CON0 treatment were around 17 kg ha^{-1} in 0-10 cm soil layer, while the REF treatment only maintained 14 kg ha^{-1} in the top layer. Comparatively, the FER3 treatment had significantly higher SMN levels in the 0-10 cm layer (75 kg ha^{-1}). The other treatments applied with BBFs showed no significant differences in SMN levels. By T3, treatment UNI1 (30 kg ha^{-1}) exhibited higher SMN levels in the top layer, but no significant differences were observed between treatments. By T4, the SMN level of the FER3 treatment reached 88 kg ha^{-1} in the top layer, while the SMN levels of other fertilized treatments ranged from 36 to 65 kg ha^{-1}. However, no significant differences were shown between the treatments. The SMN levels at T4 were slightly higher than at T2, which indicated a slow N release in the field condition. It was also proved by the low NUE of the fertilizers and no significant difference between treatments considering dry biomass yield. Because of the shortage of the available N in the soil, no significant differences were observed between fertilized and control treatments. After harvesting, the FER3 treatment (32 kg ha^{-1}) again maintained significantly higher SMN level in the topsoil, followed by the CAT1 treatment, 13 kg ha^{-1}. By T6, no significant differences were observed from both layers.

3.3 Estonia

3.3.1 Methods

The field experiment was carried out in Jõgeva, Estonia (GPS coordinates: 58.76312N, 26.40399E 4, altitude 67 m above sea level). Crop rotation of the field was as follows: 2017/2018 winter wheat (*Triticum aestivum* L.), 2019 different vegetables, 2020 potato (*Solanum tuberosum* L.), 2021 barley (*Hordeum vulgare* L.), 2021/2022 red clover (*Trifolium pretense* L.). Red clover was cut and ploughed as green manure under the soil in 2022. The field was ploughed in autumn 2022 and harrowed at 10 cm in spring 2023. The plot sizes were 1.4 × 8 m (11.2 m^2). The trial plots followed a randomized complete block design. The field trial with broccoli took place between June and August 2023. The trial involved six treatments (Table 5), each with three replicates.

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Fertilizer was provided prior to broccoli planting and incorporated shallowly into the soil. In the case of the liquid biobased fertilizer FER3, it was diluted five times and watered to the trial plots with a watering can. In the positive control, an NPK fertilizer containing ammonium (NH_4^+), phosphorus pentoxide (P_2O_5) and potassium oxide (K_2O) was used. The level of fertilization was 120 kg N (as for the BBFs), 22 kg P, 125 kg K and 35 kg S ha^{-1}.
The broccoli seeds were sown in planting cassettes on May 2, 2023, and cultivated in a greenhouse until they were transplanted to the field. In each plot, 49 transplants at the 4-leaf stage were planted at a spacing of 0.45×0.45 m. The plots were managed by hand weeding for weed management and insect nets were used during the broccoli cultivation. After harvesting, the field trial was ploughed at a depth 20 cm and harrowed at 10 cm. To evaluate the BBFs residual effect, winter wheat was sowed on September 15th to evaluate the BBFs residual effect. The sowing rate was 230 kg ha^{-1} (500 seeds m^2). No chemical plant protection was applied during the cultivation of broccoli or winter wheat. The winter wheat was not fertilized.

Harvesting broccoli was done when the flower buds became compact and firm (60 days after transplanting). The middle 4 m of the middle row in each plot (9 plants per plot) were harvested on August 4, 2023, for further analysis (see chapter 3.1). Winter wheat was harvested on July 30, 2024, using a 1-meter-wide combine harvester, Wintersteiger. The harvested grain yield's dry matter content, 1000-grain weight and crude protein (NIR analysis method) was determined.

3.3.2 Results and discussion

The results of assessing the direct effect of BBFs on broccoli yield are presented in Table 7. No significant differences in the fresh or dry yield of marketable broccoli heads were observed between the treatments, with the head yield fresh weight ranging from 8.0 to 14.7 t ha^{-1}. Biomass dry yield was significantly dependent on the treatment (p = 0.0025) and the highest biomass was observed in the mineral fertilizer (MF) treatment (9.4 t ha^{-1}), followed by CAT1 and FER3 (8.5 and 8.3 t ha^{-1}, respectively). Significantly lower biomass was observed in the unfertilized control (CON0) and BP treatments (6.5 and 6.7 t ha^{-1}, respectively). This indicated that in the BP treatment did not release the N required for broccoli growth rapidly enough. The BBFs CAT1 and FER3 performed not significantly different from the mineral fertilizer in supplying plant available N and leading to considerable crop yields.

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The total N content of the harvested broccoli varied significantly among the treatments (*p = 0.0038*). The MF treatment exhibited the highest total N content (3.34%) within the crops, followed by FER3 (3.30%) and CAT1 (3.09%). Conversely, FSP, BP and CON0 treatments showed relatively low total N content (2.65, 2.45, and 2.35%, respectively). The crop N uptake and NUE% followed the same pattern, with especially BP having a low N uptake due to the low N availability in the soil during the growth period of broccoli.

Table 7. The fresh and dry commercial yield and total aboveground biomass of broccoli under different fertilizer treatments, N concentrations in broccoli plants and N use efficiency (NUE%) (mean ± SD, n=3). Different letters indicate significant differences between treatments at p<0.05 with Tukey-Kramer (HSD) test. ns: not significant.

	Commercial heads yield		Total aboveground biomass		NUE	Ntot	N
	$t\ ha^{-1}$ (FW)	$t\ ha^{-1}$ (DW)	$t\ ha^{-1}$ (FM)	$t\ ha^{-1}$ (DM)	%	%	$Kg\ ha^{-1}$
MF	14.1±6.2	1.42±0.5	79.4±8.2 a	9.4±1.4 a	135±48a	3.34±0.18a	315±57a
CON0	8.9±0.3	0.86±0.05	46.8±3.8 d	6.4±0.3 c		2.35±0.31b	152±26d
CAT1	11.9±0.4	1.31±0.2	65.8±2.7abc	8.3±0.4abc	85±14abc	3.09±0.34ab	254±17abc
FER3	14.7±2.3	1.36 ±0.2	68.2±4.6 ab	8.5±0.3 ab	108±14ab	3.30±0.24a	281±17ab
FSP	9.8±3.5	1.05±0.4	56.7±7.3 bcd	7.6±0.6 abc	42±30bc	2.65±0.39 ab	202±36bcd
BP	8.0±3.0	0.75±0.3	50.9±4.9 cd	6.7±0.6 bc	11±22c	2.45±0.27b	165±27cd
F	*2.11*	*2.48*	14.21	*7.16*	*9.31*	*6.50*	*11.81*
Pr(<F)	*ns*	*Ns*	<0.001	*0.0025*	*0.0021*	*0.001*	*0.0003*

The results of assessing the residual effect of bio-based fertilizers on winter wheat yield, 1000-kernel weight and crude protein content are presented in Table 8. No significant differences were observed between the treatments, but a slight tendency towards higher grain yield in treatments with BBFs was observed. The lowest yield was found in the treatment where the broccoli was fertilized with MF the previous year.

Table 8 The biobased fertilizers residual effect on winter wheat grain yield, 1000-kernel weight and crude protein content (mean ± SD, n=3). Different letters indicate significant differences between treatments at $p < 0.05$ with Tukey-Kramer (HSD) test. ns- not significant

Treatment	Yield (t DW ha^{-1})	1000- kernel weight (g)	Crude protein (%)
MF	5.28±0.4	45.9±0.4	10.5±1.13
CON0	5.48±0.5	47.2±0.4	10.8±0.89
CAT1	5.90±0.6	47.5±0.2	11.1±0.55
FER3	5.86±0.0	47.6±0.5	11.2±0.38
FSP	5.84±0.6	46.4±0.8	11.4±0.74
BP	5.61±0.3	46.4±2.0	10.7±0.80
Pr(>F)	*ns*	*ns*	*ns*

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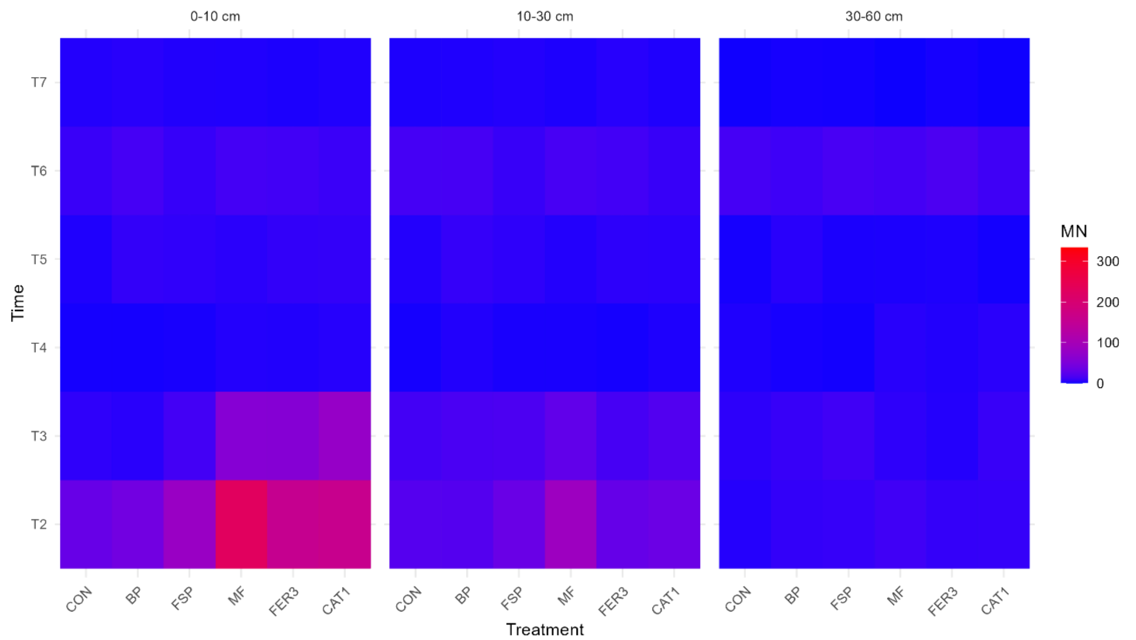

Figure 5. Heatmap of the soil mineral N concentration (mg kg^{-1}) in the Estonian field trial. CON: control, unfertilized treatment; MF: reference fertilizer; BP: Bokashi pellet; FSP: fish sludge pellet; FER3: NPK solution with amino acids; CAT1: protein fraction.

The heat map shows the mean concentration of mineral N (mg kg^{-1}) in the field from different treatments across several sampling time points (Figure 5). The heat map shows that N concentrations decreased with time and depth for most treatments during broccoli growth. The soil before fertilization (T1) contained 15, 16 and 14 mg kg^{-1} mineral N from the 0-10 cm deep layer to 36-60 cm layer, respectively. By T2, the mineral N concentration of field soil ranged from 37 to 64 mg kg^{-1} for three treatments with light colors, and from 171 to 239 mg kg^{-1} for the other three treatments. The MF treatment had the highest mean N concentration in the 0-10 cm layer, followed by FER3, CAT1 and FSP treatments, while the BP treatment and CON0 treatment resulted in lower mineral N concentration. The mineral N concentration of the subsurface layer had the same trends as that of the surface layer. By T3, the treatments MF, FER3 and CAT1 still had comparatively higher mineral N concentration in the soil at the 0-10 cm deep layer, while MF treatment contained more mineral N in the subsurface layer (10-30 cm). Although the unconsumed N within the soil released mineral N after the harvest of the crop and planting of winter crop (T6), the mineral N concentrations were 7-14 mg kg^{-1} at the surface layer, 8-13 mg kg^{-1} at the 10-30 cm deep layer, and 10-12 mg kg^{-1} at the 30-60 cm deep layer across the treatments, which were lower than those of the soil before fertilization. By T7, the mineral N of the soil further decreased down to 5-7 mg kg^{-1} at the

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surface layer. While the mineral N concentrations in the deeper layers ranged 7-15 mg kg^{-1} across the treatments.

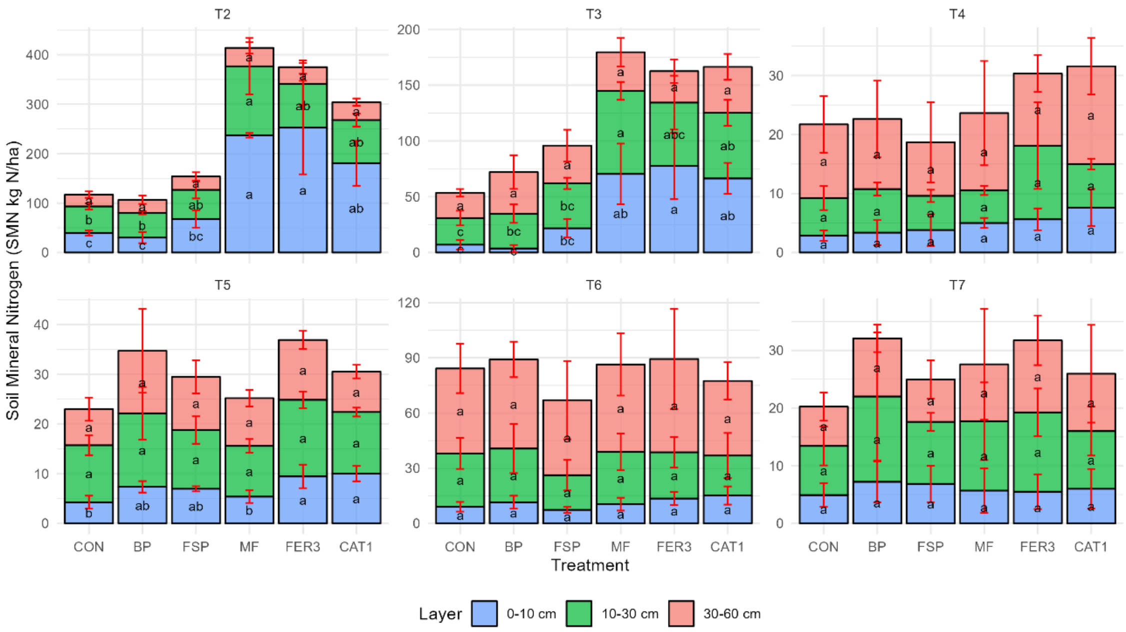

Figure 6. Soil mineral N levels (SMN kg ha^{-1}) of field in Estonia. Letters refer to the statistical difference (one-way ANOVA, Tukey HSD, $p < 0.05$) of SMN between treatments at 0-10 cm and 10-30 cm layer, respectively. CON: control, unfertilized treatment; MF: reference fertilizer; BP: Bokashi pellet; FSP: fish sludge pellet; FER3: NPK solution with amino acids; CAT1: protein fraction. Different letters imply significant differences between the treatments, within each soil layer and sampling time.

The SMN levels of the treatments referred to the potential plant available N pool of the soil. The SMN content before the fertilization (T1) was 18 ± 2 kg ha^{-1} 35 ± 4 kg ha^{-1} and 49 ± 12 kg ha^{-1} from 0-10 cm to 30-60 cm depth of soil, respectively (Figure 6). By T2, the SMN levels of the CON0 treatment increased from 53 ± 19 kg ha^{-1} to 93 ± 11 kg ha^{-1} in 0-30 cm soil layer due to the mineralization of soi organic matter. The BBF products FER3 and CAT1 performed no significant differences in supplying mineral N compared to the mineral fertilizer, reflected by high SMN levels in the 0-10 cm layer of the amended soil at T2. Simultaneously, the solid BP with high C/N ratio (12.3:1) led to the lowest SMN level (30 ± 11 kg ha^{-1}) in the top layer, which was lower than the unfertilized CON0 (40 ± 5 kg ha^{-1}) due to N immobilization. Although SMN level of the CAT1 treatment (181 ± 45 kg ha^{-1}) was much higher than that of the treatment FSP (68 ± 17 kg ha^{-1}) in the 0-10 cm layer by T2, no significant differences in yield were shown between these treatments. From T2 to T3, the SMN levels greatly decreased to 31, 35, 62 kg ha^{-1} for treatments CON0, BP and FSP, and to 125, 134, 145 kg ha^{-1} for the treatments CAT1, FER3 and MF in the 0-60 cm depth

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soil. From T3 to T4, the SMN levels of all treatments largely decreased to 9, 11, 10 kg ha^{-1} for the treatments CON0, BP and FSP, and to 15, 18, 11 kg ha^{-1} for the treatments CAT1, FER3 and MF in the 0-60 cm depth soil. By T4, there were no more significant differences in the SMN levels between treatments. From T4 to T5, SMN levels of all treatments slightly increased to 4-10 kg ha^{-1} in the 0-10 cm layer, and around 10-15 kg ha^{-1} in the 10-30 cm layer because of the crop residues. In the 30-60 cm layer SMN levels showed no significant differences between treatments at each sampling time. The SMN levels of the 30-60 cm layer of all treatments showed no significant differences across all sampling times.

Conclusions

In an Estonian field trial, the liquid fertilizer FER3 and loose solid fertilizer CAT1 resulted in crop yields and nitrogen use efficiency (NUE%) comparable to those of mineral fertilizer (MF) in broccoli cultivation. This suggests that FER3 and CAT1 could serve as viable alternatives to synthetic mineral nitrogen fertilizers. Additionally, the bio-based fertilizer FSP showed promise as a partial substitute for synthetic fertilizers.

3.4 France

3.4.1 Methods

The first field trial, led in 2023, couldn't be harvested. High rainfall and hot temperatures encouraged phenological problems as well as pests and diseases. A second trial was conducted in 2024 in Assat, in south-west of France, close to Pyrenees (GPS coordinates: 43.25399N, -0.3011370E). The soil of the site is classified as Brunisol.

The previous crop was parsley. The soil was very compacted and was therefore ploughed some days before planting. One day before plantation, a vibrashank was used. Broccoli seedlings (cultivated for six weeks) from a local nursery were transplanted on July 23rd, 2024. The trial plots followed a randomized complete block design with triplicates. The plot size was 1.8 x 10 m ($18m^2$). There were 2 rows of broccolis per plot, spaced by 65 cm. The distance between plants in the row was 40 cm.

The soil was fertilized just before plantation. FER3 was diluted three times in water and applied with a hand sprayer. The other BBFs were applied by hand. The soil was manually harrowed to a few centimetres' depth after application. All the plots were harvested 90 days after plantation, when the phenological stage BBCH59 was reached or close to.

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The middle 4m of the two rows in each plot were harvested. All the plants have been collected in this area for the fresh biomass measures but only five (the most representatives of the plot) have been dried. After the harvest, high rainfall till the end of the year prevented sowing of the residual crop.

3.4.2 Results and discussion

The direct effect of the BBFs on broccoli yield is presented in Table 9. There was no significant effect of the BBFs on the plant biomass or the yield. Nevertheless, some tendencies can be seen. Overall, the three BBFs gave at least as good results as the commercial organic fertilizer (SCPM) and better than the unfertilized treatment. FER3 obtained the highest yield and plant biomass, and less variability was observed for this treatment.

However, all the yield observed was very low, even for FER3. Weather could explain these results. Hot temperatures at the plantation penalized the vegetative recovery of the plants and caused a high plants mortality. On average, 34 % of plants were missing (between 5 % and 50 % mortality by plot). Moreover, in 2024, there were high temperature variations and high rainfall in this area. Bad development of cabbage was observed in this region this year.

Table 9 The effect of biobased fertilizers on commercial yield, total aboveground biomass, N concentrations in broccoli plants and N use efficiency (NUE%) (mean ± SD, n=3). Different letters indicate significant differences between treatments at $p < 0.05$ with Tukey test.

	Commercial heads yield		Total aboveground biomass	NUE	Ntot	N
	t ha^{-1} (FM)	t ha^{-1} (DM)	t ha^{-1} (DM)	%	%	Kg ha^{-1}
SCPM	1.30±1.1 ab	0.13±0.10 ab	0.98±0.20 bc	9.2±2.0b	3.17±0.40	30.8±2.4b
CON0	0.14±0.13 b	0.015±0.01 b	0.61±0.15 c		3.27±0.09	19.7±4.7b
CAT1	0.96±0.70 ab	0.10±0.07 ab	0.98±0.14 abc	8.6±3.0b	3.07±0.13	30.0±3.6b
FER3	2.10±0.33 a	0.20±0.04 a	1.51±0.06 a	23.6±1.5a	3.19±0.21	48.0±1.8a
FSP	1.23±0.82 ab	0.12±0.09 ab	1.28±0.36 ab	21.0±7.3a	3.58±0.34	44.9±8.7a

In this field trial, the fertilization of the broccoli was done once, based on the total nitrogen in the BBFs. Other mineral elements may have been limiting the growth. The N content of the broccolis was similar regardless of the treatment (Table 9), with slightly higher for FSP than the other treatments (not significant). The head had the highest N concentration with an average of 5 % against 2.9 % for the rest of biomass (data not

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shown).

Regarding the N in the aboveground biomass (kg ha^{-1}), FSP and FER3 were significantly higher than the three other treatments. For FER3, it is due to the amount of biomass produced, as the N concentration was not particularly high. For FSP, the amount of biomass produced is slightly below that of FER3 but the N concentration in the plant is a little higher than the other treatments. These elements explain the similar results of FSP and FER3.

The same similarities can be seen for the NUE with significantly higher NUE for FSP and FER3 than CAT1 and SCPM. The nitrogen present in the fertilizer was better utilized by the plant for FER3 and FSP.

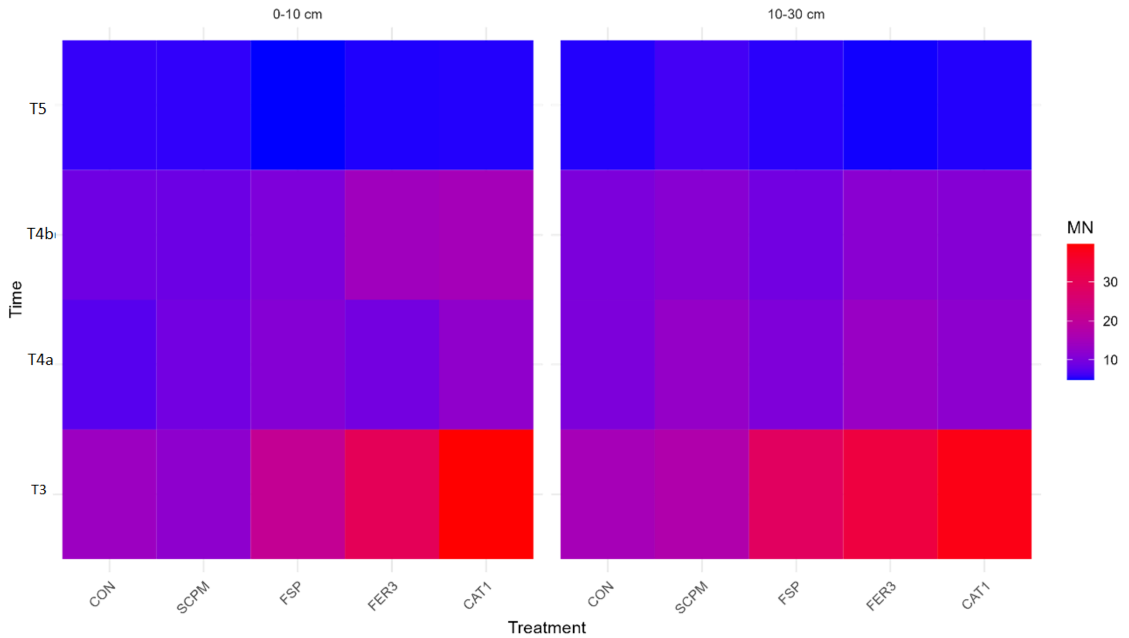

Figure 7. Heatmap of the soil mineral N concentration (mg kg^{-1}) of the field in France; The sampling point for this field — **T3:** one month after plantation (stage BBCH 25), **T4a:** BBCH stage 45 (50% of the expected size), **T4b:** at harvest, **T5:** after harvest (January). CON: control, unfertilized treatment; SCPM: reference fertilizer; FSP: fish sludge pellet; FER3: NPK solution with amino acids; CAT1: protein fraction.

The heatmap shows the mean concentration of mineral N (mg kg^{-1}) in the field from different treatments across several sampling time points (Figure 7) — T3: one month after plantation (stage BBCH 25), T4a: BBCH stage 45 (50% of the expected size), T4b: at

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harvest, T5: after harvest (January). The soil before fertilization (T1) contained 22 mg kg^{-1} mineral N in the 0-10 cm deep layer and 49 mg kg^{-1} mineral N in the 10-30 cm layer. By T3, the mineral N concentration ranged from 12 to 40 mg kg^{-1} among the treatments from the 0-10 cm deep layer and 15-38 mg kg^{-1} mineral N from the 10-30 cm layer, in which CAT1 treatment contained higher mineral N, followed by FER3 and FSP treatments. The N concentrations decreased with time and depth for most treatments during broccoli growth. The mineral N concentration of the subsurface layer had the same trends as that of the surface layer. By T5, mineral N concentration decreased to around 5-6 mg kg^{-1} at the surface and subsurface layers across the treatments.

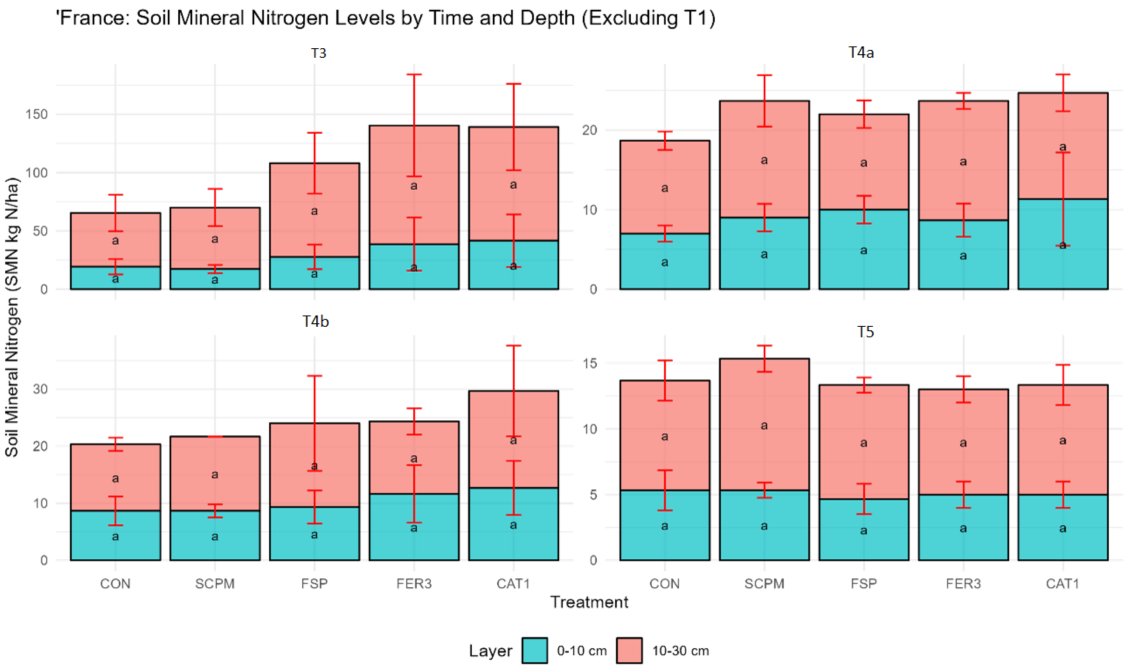

Figure 8. Soil mineral N levels (SMN kg ha^{-1}) of field in France; **T3:** one month after plantation (stage BBCH 25), **T4a:** BBCH stage 45 (50% of the expected size), **T4b:** at harvest, **T5:** after harvest (January). Letters refer to the statistical difference (one-way ANOVA, Tukey HSD, $p < 0.05$) of SMN between treatments at 0-10 cm and 10-30 cm layer, respectively. CON: control, unfertilized treatment; SCPM: reference fertilizer; FSP: fish sludge pellet; FER3: NPK solution with amino acids; CAT1: protein fraction. Different letters imply significant differences between the treatments, within each soil layer and sampling time.

The SMN levels of the treatments indicates the potential plant available N pool of the field soil (Figure 8). The SMN content before the fertilization (T1) was 22 kg ha^{-1} and 112 kg ha^{-1} in the 0-10 and 30-60 cm soil layers, respectively. One month after plantation (T3), the SMN levels of the treatments fertilized with BBFs increased to 28 kg ha^{-1}-42 kg ha^{-1} in

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the 0-10 cm soil layer and 80-102 kg ha^{-1} in the 10-30 cm layer. The SMN level of the CON0 treatment was only 65 kg ha^{-1} in the 0-30 cm layer, while the commercial organic fertilizer SCPM supplied around 70 kg ha^{-1} SMN. The mineralization of the SCPM may have lasted longer than the for the other BBFs. CAT1 and FER3 had values twice as high as CON and SCPM. The values of FSP are between the two. From T3 to one month before harvest (BBCH 45, T4a), the SMN levels greatly decreased to 19-25 kg ha^{-1} for all treatments in the 0-30 cm layer. Plant growth can explain this result but also the precipitation that may have caused leaching. From T4a to the harvest (T4b), the SMN levels of all treatments slightly varied to 20-30 kg ha^{-1} for the treatments in the 0-30 cm depth soil. At the beginning of the year 2025 (T5) SMN levels of all treatments decreased to 13-15 kg ha^{-1} in the 0-30 cm layer. At that time, the soil was bare, and the autumn precipitation continued to cause nitrogen leaching. However, no statistical significance was shown between treatments in SMN levels during the monitoring period.

FER3, which had the highest nitrogen rate one month after planting, obtained the highest yield (in trend). CAT1, which had a nitrogen content similar to FER3, had a lower yield than SCPM. This can be related to the NUE of CAT1 which is much lower than the other two BBFs. Other factors than nitrogen play a role in the composition of yield (recall that the yield was very low in the French trial).

3.5 Norway

3.5.1 Methods

The field trial was located at the NIBIO research station, Apelsvoll (GPS coordinates: 60.70047N, 10.86957E) on a moraine clay soil. The trial was laid out in a randomized block design with three replicates. Seeds of broccoli, variety 'Parthenon', were sown indoor in the greenhouse Mai 3rd and planted in field Mai 31st. When reaching the four-leaf stage, the plants were transplanted in field. Planting density was 45 cm between plants and between rows and planted in three rows. All fertilizers were given in field the day prior to planting. Fertilizers were spread by hand; liquid fertilizer was diluted five times. Following application, the fertilizers were shallowly incorporated into the soil by hand raking. To avoid insect pests, the field was covered with insect net. Weed management was done by hand-weeding twice. The field was irrigated twice applying an average of 5.5 mm at each irrigation. Broccoli was harvested on August 15th, 2023, when plants were mature

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and had reached BBCH 55-59. Each subplot was 8 meters long and 1.3 meters wide. To avoid any edge effect between treatments, only the middle four meters, all three rows of the plot was harvested. Soil tillage operation included ploughing in autumn 2022 and rotary tillage in spring 2023. The residual crop, winter wheat, variety Kuban, were sown September 9th, 2023, at a sowing rate of 352 kg ha^{-1}. The crop was harvested on August 19th, 2024.

3.5.2 Results and discussion

The yield in fresh weight of commercial yield did not vary between treatments (Table 10), only if compared with the negative control (CON0). The variation in dry yield of commercial heads was small. The dry yield in total biomass was significantly highest in FER3, CAT1 and FMP. The overall dry weight of FER3 was highest and of the fertilized treatments K8 lowest. NUE was lowest in K8 and FSP, as was also the N uptake in the plants. The N uptake will reflect the yield, and both were highest in FER3, CAT1 and FMP. FMP is the local fertilizer used in Norway consisting of a mix of fish sludge and bonemeal. As we used an organic fertilizer used by organic farmers as control we don't have any data on the fertilizer effect compared to mineral fertilizers. From the data here we find that FER3, CAT1 and FMP were the best performing fertilizers though FSP did not vary significantly from these in commercial yield.

Table 10. Yield, NUE and N in aboveground biomass. K8 is positive control and CON0 negative control.

	Commercial yield		**Biomass**	**NUE**	**N uptake**
	t FW ha^{-1}	**t DW ha^{-1}**	**t DW ha^{-1}**	**%**	**kg ha^{-1}**
K8	5.6±3.3 ab	0.53±0.3 bc	2.48± 1.3c	12.4±6.1 ab	38.3±33.6 b
CON0	4.1±1.4 b	0.44±0.1 c	3.49±0.7 bc		42.6±5.2b
CAT1	10.0±4.6 ab	0.97±0.4 abc	5.26± 1.4ab	33.0±8.4ab	82.2±10.1ab
FER3	13.2± 3.2a	1.28±0.3 a	6.22±0.3a	31.6±17.2ab	80.5±20.6ab
FSP	7.4±2.6 ab	0.75±0.2abc	4.34±0.9abc	11.4±12.6 b	56.2± 15.1ab
FMP	12.5±1.8 a	1.21± 0.1ab	5.46±0.5 ab	38.9±8.5a	89.2±10.1a

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There were no significant differences in yield or crude protein in the residual crop (Table 11). This indicated that the nitrogen had either been washed out or there was little available nitrogen left for the residual crop.

Table 11. Residual effect measured in winter wheat.

	Yield (kg DW ha^{-1})	Crude protein (%)
K8	2703±369	11.7±0.6
CON 0	2750±799	10.9±1.2
CAT1	2942±694	11.7±0.9
FER3	2456±350	11.9±0.1
FSP	2652±429	11.8±0.9
FMP	2808±246	11.8±0.3

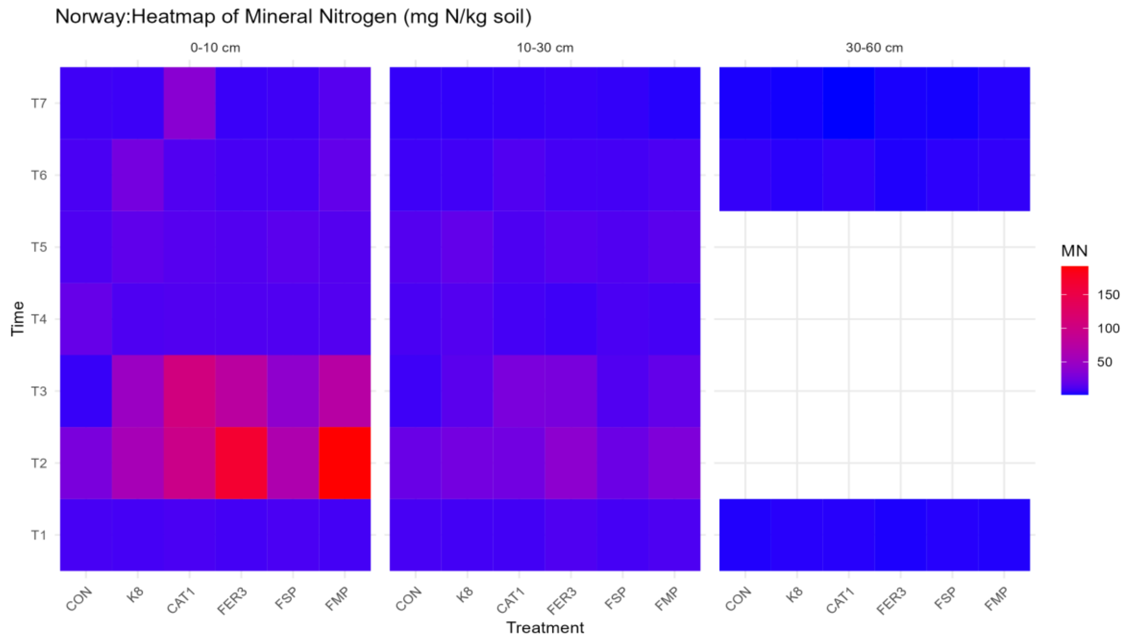

Figure 9. Heatmap of the soil mineral N concentration (mg kg^{-1}) of the field in Norway. CON: control, unfertilized treatment; K8: reference fertilizer; CAT1: protein fraction; FER3: NPK solution with amino acids; FSP: fish sludge pellet; FMP: fish mix pellet.

The heat map (Figure 9) shows the mean concentration of mineral N (mg kg^{-1} DW) in the field from different treatments across several sampling time points. The heat map shows that N concentrations decreased with time and depth for most treatments during broccoli growth, while slight increases were shown after broccoli harvesting. The original soil contained 9-10 mg kg^{-1} mineral N in the 0-10 cm deep layer, 8-12 mg kg^{-1} mineral N in the 10-30, and around 3 mg kg^{-1} mineral N in the 30-60 cm layer. By T2, the mineral N concentration of the FER3 treatment had increased to 169 mg kg^{-1}, CAT1 treatment to129 mg kg^{-1}, FMP treatment to 192 mg kg^{-1} and FSP treatment to 65 mg kg^{-1} in the 0-10 cm

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deep soil layer. The K8 treatment had the mean N concentration reaching 59 mg kg^{-1} in the 0-10 cm layer. The 10-30 cm deep soil layer had 21-39 mg kg^{-1} mineral N concentration across the treatments.

By T3, fertilized treatments still had higher mineral N concentration in the soil at the topsoil layer, so did the 10-30 cm soil layer. The treatment fertilized with solid CAT1 supplied the highest mineral N (106 mg kg^{-1}), followed by FER3 treatment (78 mg kg^{-1}), then FMP treatment (74 mg kg^{-1}) in the 0-10 cm soil layer. The K8 treatment contained 49 mg kg^{-1} mineral N in the topsoil layer, while the unfertilized CON0 treatment only had around 6 mg kg^{-1} of mineral N in the top layer and less than 8 mg kg^{-1} of mineral N in the second soil layer. At the 10-30 cm deep soil layer, CAT1 and FER3 treatments contained around 28 mg kg^{-1} of mineral N, and FSP and K8 treatments had around 15 mg kg^{-1} of mineral N. By T4, all layers of mineral N concentration decreased due to the N supplied to broccoli growth. After harvesting, the mineral N concentrated were increased due to the mineralization of the unconsumed N in the soil. By T6, the K8 treatment still had around 14 mg kg^{-1} of mineral N at the 0-10 cm soil layer. The mineral N CAT1 treatment increased to 37 mg kg^{-1} by T7. The 30-60 cm soil layer showed small variations.

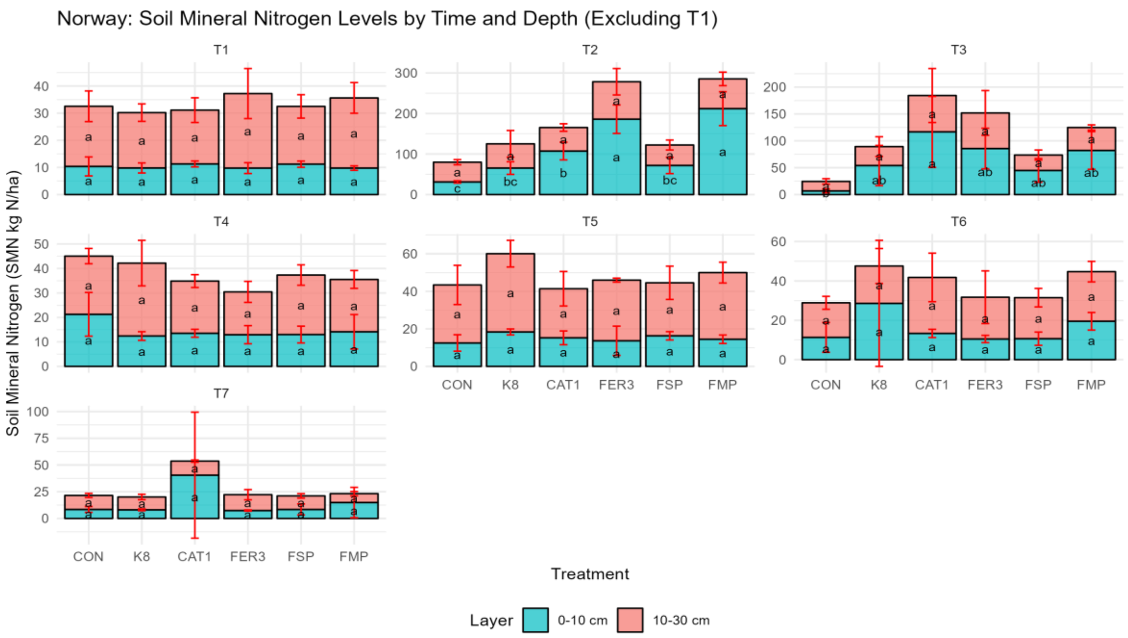

Figure 10. Soil mineral N levels (SMN kg ha^{-1}) of field in Norway. Letters refer to the statistical difference (one-way ANOVA, Tukey HSD, $p < 0.05$) of SMN between treatments at 0-10 cm and 10-30 cm layer, respectively. CON: control, unfertilized treatment; K8: reference fertilizer; CAT1: protein fraction; FER3: NPK solution with amino acids; FSP: fish sludge pellet; FMP: fish mix pellet. Different letters imply significant differences between the treatments, within each soil layer and sampling time.

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The SMN levels of the treatments represent the potential plant available N pool of the soil. The SMN content before the fertilization (T1) was 10-11 kg ha^{-1} from 0-10 cm and 20-27 kg ha^{-1} from 30-60 cm depth of soil (Figure 10). By T2, the SMN levels of the treatments fertilized with CAT1, FER3, FSP and FMP increased to 72-212 kg ha^{-1} in 0-10 cm soil layer, and 65 kg ha^{-1} from the treatment K8 and 31 kg ha^{-1} from in the unfertilized CON0. No significant differences of SMN (49-92 kg ha^{-1}) were observed in the 10-30 cm soil layer. By T3, SMN levels of the fertilized treatments CAT1, FER3, FSP, FMP, and K8 contained 117, 86 ,82, 45, 54 kg ha^{-1} in the top layer, respectively. The unfertilized CON0 treatment only had 24 kg ha^{-1} in the 0-30 cm soil layer. During the monitoring period, the SMN levels of the subsurface layers showed no significant differences. By T4, SMN levels of the fertilized treatments greatly decreased. From this sampling point, no significant differences of SMN levels were observed between treatments. The increase in SMN levels of the top layer of the FER treatment at T6 and CAT1 treatment at T7 might be due to the N mineralization of the N-containing residues.

The nutrient requirements for transplants like here broccoli is high already at planting as these have a well-developed root mass and ready to increase growth. Yield therefore likely reflects the time for increase in SMN as we find a tendency to a higher yield in the FER3 and FMP where the SMN were highest at T2 and a positive correlation between SMN at T2 and yield was demonstrated (see part 3.7). Release of N was seemingly slower for CAT1 but high at T3 and yield in the CAT1 treatment were the third highest. Fertilizer release of nutrients is an important factor defining crop yield. If we had a crop established from seeds the slower releasing fertilizers could have been better suited as the early available nutrients could have been washed out before the root system was well developed.

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3.6 Spain

3.6.1 Methods

Two field trials were established in Zamudio (Bizkaia, the Basque Country, Spain) at NEIKER facilities (43.2897N, 2.87417W) during two consecutive years under irrigated conditions in the same field: September 2023 – March 2024 and March 2024 – July 2024, hereafter referred to as 2023 and 2024 trials, respectively. The soil was classified as Aquic Dystric Eutrudepts (*SSS, 1999*). Six- and four-weeks old broccoli seedlings at phenological stage BBCH 14 were transplanted to the fields on September 13, 2023, and March 19, 2024, respectively. Four weeks prior to transplantation in 2023, the soil was rotavated and a weed control net was installed. Spring onions and lettuce were cultivated as residual crops following the two broccoli trials, respectively.

In 2023, four BBFs were tested in the trial: FER3, CAT1, FSP and FER5'. The three first are described in D6.1, while a short description of FER5' follows here: FER5' is similar to FER5 and was produced using microalgae biomass as a basis. The biomass was hydrolyzed to obtain a higher yield in free amino acids in the final product (basic characterization of FER5' is shown in deliverable 3.7). FER5' was aimed at performing as a biostimulant.

FER3, CAT1, and FSP were applied as fertilizer as basal dressing right before the transplanting of the broccoli seedlings. Those BBFs were applied at 120 kg N ha^{-1} rate and they were integrated in the soil as they were buried after their application. FER3 was diluted three times in water prior to application. The application of FER5' was done as root application divided into 3 applications at 1.5 L ha^{-1} in total, after the basal application of mineral fertilizer (NAC 27 %): 108 kg N ha-1 in FER5'(90% of the N provided in the other treatments of the field trial (120 kg N ha-1)

In 2024, an additional BBF, OA2, was included in the trial, also as fertilizer. At the moment in which the agronomic trial was started, this product was available, and it was considered worth assessing its agronomic performance in real conditions as a source of nitrogen. As in 2023, the BBFs were integrated into the soil after application.

Since several plants fertilized with FER3 were burnt in 2023, following the producers advice (FERTINAGRO), the BBF was rather applied as a biostimulant in 2024, following the same application indications as for FER5', 1.5 L ha^{-1} in three applications, after the basal

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application of mineral fertilizer (NAC 27%): 84 kg N ha-1 in FER3 (70% of the N provides in the other treatments of the field trial (120 kg N ha-1). FER5' was applied as a biostimulant as in 2023.

As recommended, applications of FER5' both years and FER3 in 2024, were made when the nutrient requirement of the crop was highest (Ronga et al., 2019). The detailed application procedure of FER3 and FER5' as biostimulant was made as follows: The first application was made at transplantation, immediately after the basal application of mineral fertilizer (calcium ammonium nitrate, NAC 27 %): 108 kg N ha-1 in FER5' and 84 kg N ha-1 in FER3 (in 2024), which provided 90 and 70 % of the N provided in the other treatments, respectively. Next, these liquid BBFs were applied when the broccoli head started to form (BBCH41) and when the broccoli head reached 50 % of the expected harvestable size (BBCH45). The positive control (MF) was fertilized in both years with 120 kg N (NAC 27 %), 40 kg P ha^{-1} ($Ca(H_2PO_4)_2$) and 150 kg K (K_2SO_4) per hectare.

Broccolis were harvested by hand at optimal maturity (BBCH 59), in which first flower's petals visible; flowers still closed (Meier, 1997, Feller et al., 1995). In 2023, FER3 and CON0 were harvested on 11th of December, while the other plots were harvested on 29th of November (89 and 77 days after transplantation, respectively). In 2024, broccolis were collected all together 70 days after transplantation (28th of May). Spring onions and lettuces were collected on 13th of March and 8th of July 2024, after 42 and 34 days, respectively.

The middle 4 m of the centre row of each plot was marked as harvest area. Diseases and damages and the number of plants were registered. Broccolis were collected by hand at optimal maturity at phenological stage BBCH59.

3.6.2 Results and discussion

Trial 1 – 2023

During the first field trial, the highest yield of broccoli heads was achieved in FER3 (0.70 t DW ha^{-1}) followed by the mineral fertilizer control (MF, 0.56 t DW ha^{-1}) and by CAT1, FSP, FER5' and CON0 (ranging from 0.36 to 0.45 t DW ha^{-1} , Table 12). In the case of commercial yield (heads Ø>8 cm, FW), the highest values were obtained in MF (5.0 t ha^{-1}), followed by the BBF treatments ranging between 2.8 and 4.0 t ha^{-1}. The lowest yields

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were detected in CON0 (1.5 t ha^{-1}). The same pattern was observed on dry weigh basis (Table 12). In the case of total aboveground biomass (FW), the only difference detected was between CON0 with the lowest values (17.4 t ha^{-1}) and the rest of the treatments (ranging from 28.0 t ha^{-1} to 33.6 t ha^{-1}). Regarding DW values, CAT1 and FSP (3.2 – 3.4 t ha^{-1}) achieved higher values than the CON0 (2.1 t ha^{-1}).

The highest NUE (%) values were found in the treatments with BBFs, especially in CAT1 (67.6 %), followed by FSP and FER3 (46.5 – 51.6 %). The lowest NUE (%) values were calculated for MF and FER5' (36.6 - 37.1 %). At the beginning of the trial, after the application of the BBFs, there was heavy rainfall, most probably causing the N applied in nitrate form in the MF and FER5' treatments to be partly washed away. This would justify that both treatments had the lowest NUE (%) values. Regarding the plant N uptake, all BBFs achieved higher values (ranging from 113 to 139 kg N ha^{-1}) than CON0 (69 kg N ha^{-1}).

The highest total aboveground biomass (DW) values were also found in FSP and CAT1. The heavy rains seemed to have little effect on the N uptake in the BBFs treatments, probably because most of the N was in organic form when they were applied to soils. Although CAT1 and FSP treatments achieved the highest aboveground biomass values (DW), this was not reflected in the yield of the heads. This could be since the crop was receiving N at the time when it was forming vegetative parts but stopped receiving it when it was forming heads. The mineral nitrogen values in soil shown in Figures 9 and 10 support this explanation. The high mineral nitrogen values found in the beginning of the trial (T1 and T2) in treatments FSP and CAT1 are not available by the time the crop requires nitrogen to start forming the head (T3 and T4). Conversely, FER3 treatment seemed to provide nitrogen throughout the whole period due to a continuous mineralization of nitrogen occurring concurrently with its uptake by the crop. Therefore, this was translated into a higher head yield with treatment FER3 in comparison to FSP or CAT1. Moreover, mineral nitrogen remained after the harvest in the FSP treatment and thus, a better adjustment of the application timing should be done. In summary, the treatments with the highest values in all heads (DW) and heads commercial yield (DW) were FER3 and MF, probably because they had more N available during head formation than the rest of the treatments.

While FER3 and CAT1 produced similar broccoli biomass as the MF, the residual effect of these two BBFs gave significantly higher spring onion biomass than the MF (Table 13). FER3 and CAT1 also achieved the highest nitrogen uptake. The lowest N uptake values were detected in MF and CON0. Those results showed that the residual fertilizer value of the bio-based fertilizers lasted over time, especially in FER3, CAT1 and FSP. The application of MF to the broccoli crop had no residual effect on the onion crop, as equal values were obtained between the MF and CON0.

Table 12. The effect of biobased fertilizers in 2023 on broccoli all heads yield (DW; kg ha^{-1}), commercial yield (FW and DW; kg ha^{-1}), total aboveground biomass (FW and DW; kg ha^{-1}), nitrogen use efficiency (NUE, %) and plant N uptake (kg N ha^{-1}).

Treatment	All heads yield	Commercial yield (Heads Ø>8 cm)		Total aboveground biomass		NUE	Plant N uptake
	t ha^{-1} (DW)	t ha^{-1} (FW)	t ha^{-1} (DW)	t ha^{-1} (FW)	t ha^{-1} (DW)	%	kg N ha^{-1}
MF	0.56±0.17 ab	5.0±2.1 a	0.50±0.22 a	30.6±6.8 a	3.07±0.6 ab	36.6±1.4 b	119±23 a
CON0	0.36±0.16 b	1.5±1.4 c	0.17±0.17 b	17.4±4.5 b	2.10±0.4 b		69±19 b
CAT1	0.44±0.05 b	3.1±0.6 ab	0.31±0.05 b	33.6±2.4 a	3.19±1.4 a	67.6±2.1 a	139±6.8 a
FER3	0.70± 0.20 a	4.0±1.4 ab	0.50±0.04 a	28±4.2 a	2.86±0.3 ab	51.6±20ab	130±11 a
FSP	0.45± 0.07 b	2.9±0.6 ab	0.28±0.07 b	29±4.6 a	3.43±0.6 a	46±5.5 ab	124±17 a
FER5'	0.43±0.13 b	2.8±1.4 ab	0.28±0.15 b	28.3±6.5 a	2.74±0.7 ab	37.3±14 b	113±30 a

Different letters indicate significant differences between treatments at $p < 0.05$ with Duncan test.

Table 13. The residual effect of biobased fertilizers in 2023 on spring onion total aboveground biomass (kg ha^{-1}) and plant N uptake (kg N ha $^{-1}$). Different letters indicate significant differences between treatments at $p < 0.05$ with Duncan test.

Treatment	Total aboveground biomass (kg DW ha^{-1})	Plant N uptake (kg ha^{-1})
MF	3.0±0.2 b	0.07±0.02 c
CON0	2.9±0.2 b	0.07±0.01 c
CAT1	3.7±0.5 a	0.09±0.03 b
FER3	3.8±0.8 a	0.12±0.03 a
FSP	3.2±0.1 ab	0.08±0.01 bc
FER5´	2.8±0.2 b	0.07±0.02 bc

During the first trial, several extreme weather events occurred making the field trial set-up difficult. The already mentioned heavy rainfall after fertilizer application probably caused part of the nitrate to be washed away. In addition, the site was flooded a large part of the time. Soil saturation and low winter temperatures did likely not allow the organic N present in the bio-based fertilizers to mineralize. However, with the arrival of milder temperatures in February and March and the oxygenation of the soil, the organic N in the fertilizers began to mineralize. This hypothesis is supported by the mineral nitrogen values in T6 shown in Figures 9 and 10. This residual nitrogen in the soil allowed the subsequent onion N uptake. Although a large proportion of broccoli plants survived the

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extreme rains during 2023, they were affected. Therefore, it was considered necessary to repeat the field trial.

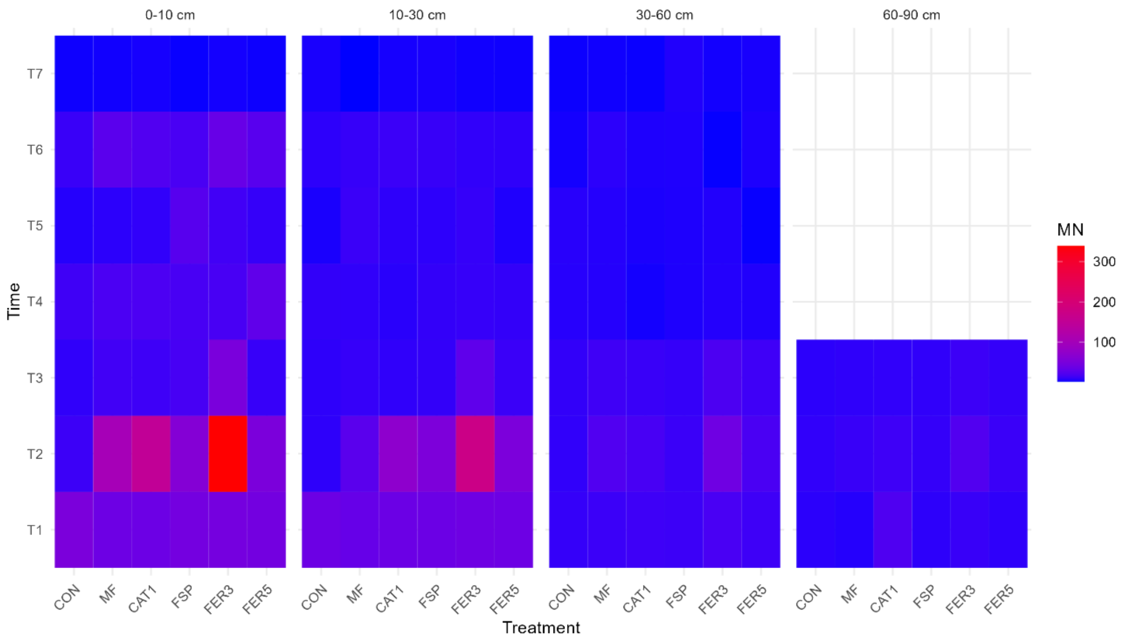

Figure 11. Heatmap of the soil mineral N concentration (mg kg^{-1}) of the field in Spain (Year 1: 2023). CON: control, unfertilized treatment; MF: reference fertilizer; CAT1: protein fraction; FSP: fish sludge pellet; FER3: NPK solution with amino acids; FER5: Fertilizer with humic acids.

The heat map (Figure 11) shows the mean concentration of mineral N (mg kg^{-1}) on the basis of dry mass in the field from different treatments across several sampling time points. Due to the weather conditions in the field area, soil sampling from T4-T7 was not possible at the 60-90 cm depth, showing as blanks in the heatmap. The heat map illustrates a clear decreasing trend in mineral N concentrations with increasing soil depth. The soil before fertilization contained 38-51 mg kg^{-1} mineral N in the 0-10 cm deep layer. The mineral N concentration was slightly higher in the 10-30 cm layer, showing values between 34 and 40 mg kg^{-1}. Further down, in the 30-60 cm layer, mineral N concentrations were down to 10-17 mg kg^{-1}. The lowest concentrations were observed in the deepest layer (60-90 cm), where mineral N ranged from 6 to 20 mg kg^{-1}.

By T2, the mineral N concentration of the unfertilized CON0 treatment decreased to 12 mg kg^{-1} in the top layer and around 8-9 mg kg^{-1} in the deeper soil layers. The concentration of the fertilized treatments increased notably, with the FER3 treatment reaching 339 mg kg^{-1} in the topsoil layer. This was followed by the CAT1 treatment at 151 mg kg^{-1}, the MF treatment at 103 mg kg^{-1}, the FSP treatment at 59 mg kg^{-1}, and the FER5'

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treatment at 51 mg kg⁻¹. The FER3 treatment also contained the highest mineral N concentrations in deeper soil layers. It contained approximately 174 mg kg⁻¹ mineral N in the subsurface layer, while the second highest CAT1 treatment had around 71 mg kg⁻¹.

Figure 12. Soil mineral N levels (SMN kg ha⁻¹) in Spain, 2023. Letters refer to the statistical difference (one-way ANOVA, Tukey HSD, $p < 0.05$) of SMN between treatments at 0-10 cm and 10-30 cm layer, respectively. CON: control, unfertilized treatment; MF: reference fertilizer; CAT1: protein fraction; FSP: fish sludge pellet; FER3: NPK solution with amino acids; FER5: Fertilizer with humic acids. Different letters imply significant differences between the treatments, within each soil layer and sampling time.

Along with the growth of the broccoli, the soil mineral N concentration decreased to 50 mg kg⁻¹ in the treatment FER3 in 0-10 cm soil layer by T3, while the other fertilized

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treatments had concentrations ranging from 10 to 17 mg kg^{-1}. In the topsoil layer, the mineral N ranged from 13 to 30 mg kg^{-1} at T4 and the range was between 6 and 25 mg kg^{-1} at T5. After the harvesting, slight increases of mineral N were observed at T6, followed by a subsequent decline in the top layer at T7. After T3, no remarkable variations were observed within the 10-30 cm and 30-60 cm soil layers.

The SMN levels of the treatments represents the potential plant available N pool of the soil. The initial SMN content (T1) were 44-59 kg ha^{-1} in the 0-10 cm soil layer, and 86-100 kg ha^{-1} in 10-30 cm depth of soil (Figure 12). By T2, the SMN levels of the unfertilized CON0 treatment was around 35 kg ha^{-1} in 0-30 cm soil layer. The fertilized treatments increased to 118 kg ha^{-1} in the top layer of the mineral fertilizer treatment MF, 59 kg ha^{-1} for treatment FER5', 69 kg ha^{-1} for treatment FSP, 392 kg ha^{-1} for treatment FER3, and 174 kg ha^{-1} for treatment CAT1. The treatment FER3 had significantly higher SMN level in the subsurface layer than the CON0 treatment. The treatment with the liquid BBF FER3 exhibited a high N release rate, though there were large deviations among replicates. These variations were primarily influenced by the rainfall, which resulted in the top two soil layers having a high moisture content exceeding 28% at T3. By T3, the FER3 treatment still showed significantly higher SMN levels in both layers than other treatments. By T4, FER5' treatment had higher SMN level in the 0-30 cm soil layer (59 kg ha^{-1}) than other treatments, but no significant differences were observed between treatments. The SMN levels of the 0-30 cm soil layers ranged from 24 to 48 kg ha^{-1} for the fertilized treatments by T5, and from 47 to 62 kg ha^{-1} by T6. By T7, the SMN levels of the 0-30 cm soil layers decreased to 7-13 kg ha^{-1} in the treatments.

Trial 2 – 2024

During the second field trial, both biomass (fresh weight and dry weight), NUE and N uptake (kg ha^{-1}) followed the order FER3, MF, FER5', FSP, CAT1, OA2, CON0 (Table 14). However, the order was slightly different for the total head and commercial heads yield, where MF gave the highest yield. The CON0 and OA2 treatments did not obtain any commercial heads .

Table 14. The effect of BBFs on broccoli cultivation in 2024. Different letters indicate significant differences between treatments at $p < 0.05$ with Duncan test.

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	All heads yield	Commercial yield		Total aboveground biomass		NUE	Plant N uptake
	t DW ha^{-1}	t FW ha^{-1}	t DW ha^{-1}	t ha^{-1} (FW)	t ha^{-1} (DW)	%	kg N ha^{-1}
MF	0.38±0.10 a	1.9±1.1 a	0.23±0.13 a	37.3±8.0 a	5.3±1.2 ab	51.2±26.5ab	102±22 ab
CON0	0.05±0.02 d	0 c	0 c	9.6±1.3 c	1.8±0.2 d		27±3 e
CAT1	0.18±0.06 bc	0.2±0.4 bc	0.03±0.04 bc	24.2±1.1 b	3.5±0.1 c	29.5±16.2ab	73±14.5 cd
FER3	0.38± 0.09 a	1.9±1.0 a	0.22±0.14 a	40.6±8.0 a	5.6±1.3 a	71.3±60.4 a	118±20 a
FSP	0.28± 0.06 ab	0.5±0.1 bc	0.06±0.004 bc	25.0±3.7 b	4.1±0.7 bc	32.1±9.4ab	65±11 d
FER5'	0.30±0.09 a	1.3±0.1 ab	0.15±0.07 ab	34.1±6.1 a	4.5±0.8 ab	47.4±3.0 ab	91±23 bc
OA2	0.14±0.02 cd	0 c	0 c	17.6±1.9 bc	3.0±0.2 cd	20±15.4 b	48±7 de

The results of assessing the residual effect of bio-based fertilizers on lettuce in 2024 are presented in Table 15. FER3, FSP, CAT1 and FER5' (378 – 361 kg ha^{-1}) achieved the highest total aboveground biomass values, followed by MF and CON0 (324 – 201 kg ha^{-1}). The lowest biomass values were detected in OA2 (143 kg ha^{-1}). Regarding the N uptake values, FER3, FSP and FER5' (13.6 – 12.5 kg N ha^{-1}) achieved the highest values, followed by CAT1 and MF (11.9 – 11.0 kg N ha^{-1}). The lowest values were achieved in CON0 and OA2 (6.5 – 4.3 kg N ha^{-1}).

Table 15. The residual effect of biobased fertilizers in 2024 on lettuce total aboveground biomass (kg ha^{-1}) and plant N uptake (kg N ha^{-1}). Different letters indicate significant differences between treatments at $p < 0.05$ with Duncan test.

Treatment	Total aboveground biomass (kg DM ha^{-1})	Plant N uptake (kg ha^{-1})
MF	324±91 ab	10.3±3.1 ab
CON0	201±19 bc	6.5±0.5 bc
CAT1	365±109 a	11.9±4.2 ab
FER3	378±37 a	12.8±1.6 a
FSP	436±40 a	13.6±0.9 a
FER5'	361 ±119 a	12.5±5.3 a
OA2	143±22 c	4.3±0.7 c

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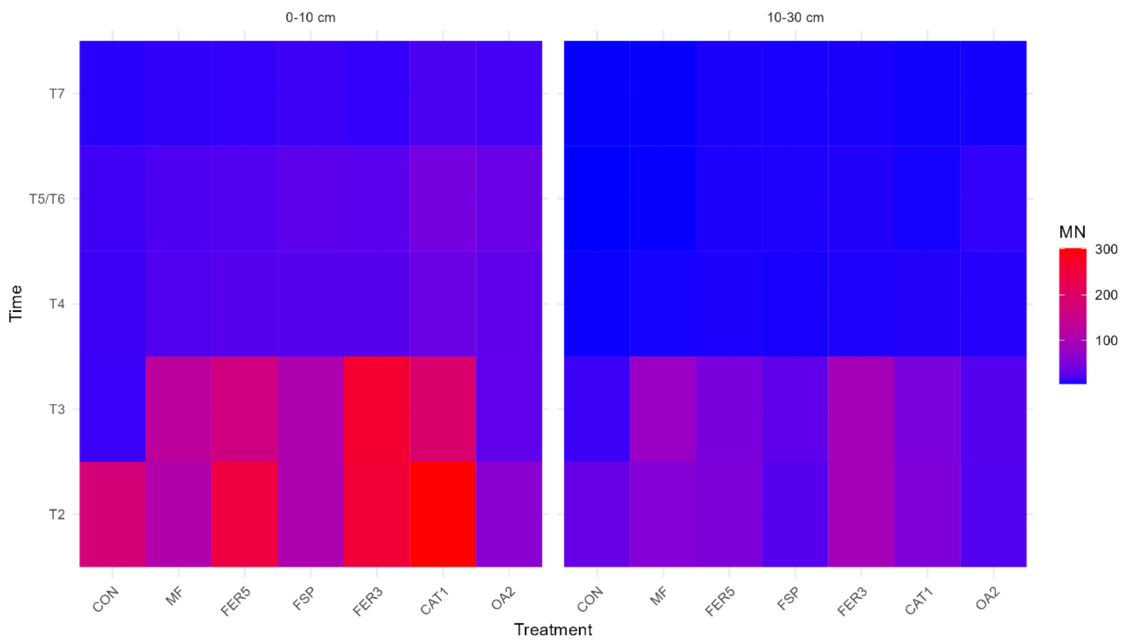

Figure 13. Heatmap of the soil mineral N concentration (mg kg^{-1}) of the field in Spain (Year 2: 2024). CON: control, unfertilized treatment; MF: reference fertilizer; CAT1: protein fraction; FSP: fish sludge pellet; FER3: NPK solution with amino acids; FER5: Fertilizer with humic acids, OA2: organic amendment.

The heat map (Figure 13) shows the mean concentration of mineral N (mg kg^{-1} dry weight) in the field from different treatments across several sampling time points from year 2024. For year 2024, only two layers of soil were sampled for mineral N monitoring. The mineral N of soil at T7 in 2023 corresponds to the initial concentration for year 2024 (T1), ranging from 2 to 3 mg kg^{-1} mineral N in the 0-10 cm soil layer, and 2-4 mg kg^{-1} mineral N in the 10-30 cm soil layer. The soil samples from T5 and T6 were combined into a single sample for analysis.

By T2, the mineral N concentration of the unfertilized CON0 treatment was around 176 mg kg^{-1} in the top layer and around 35 mg kg^{-1} in the deeper soil layers. The concentration of the fertilized treatments increased notably, with the FER3 treatment reaching 256 mg kg^{-1} mineral N, CAT1 treatment 302 mg kg^{-1} mineral N and FER5' treatment 247 mg kg^{-1} mineral N in the topsoil layer. This was followed by the MF treatment at 111 mg kg^{-1}, and the FSP treatment at 106 mg kg^{-1}. The OA2 treatment contained approximately 64 mg kg^{-1} mineral N in topsoil layers. The SMN concentration decreased with increasing depth of soil. The treatment fertilized with the liquid BBF FER3 contained higher mineral N in the subsurface layer (94 mg kg^{-1}), followed by MF, FER5' and CAT1 treatments, ranging from 52 to 57 mg kg^{-1} mineral N.

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During the growth of broccoli, the soil mineral N concentration varied. By T3, the fertilized treatments FSP, MF and FER3 had slightly higher mean mineral N concentrations, while lower concentrations were observed in other treatments. The OA2 treatment's mineral N concentration decreased to 31 mg kg^{-1} mineral N, while the CON treatment decreased down to 15 mg kg^{-1}. The mineral N concentrations of the subsurface soil exhibited minor variations. The FER3 treatment had the highest mineral N concentration at 93 mg kg^{-1}, followed by the OA2 treatment with 24 mg kg^{-1}.

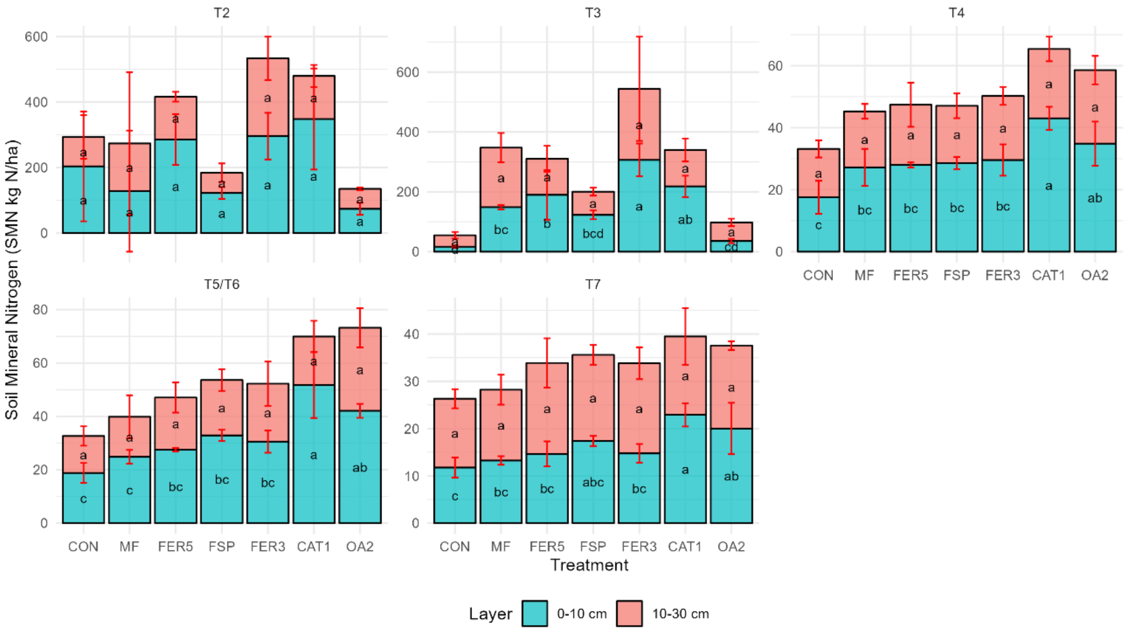

Figure 14. Soil mineral N levels (SMN kg ha^{-1}) of the field in Spain (Year 2: 2024). Letters refer to the statistical difference (one-way ANOVA, Tukey HSD, $p < 0.05$) of SMN between treatments at 0-10 cm and 10-30 cm layer, respectively. CON: control, unfertilized treatment; MF: reference fertilizer; CAT1: protein fraction; FSP: fish sludge pellet; FER3: NPK solution with amino acids; FER5: Fertilizer with humic acids, OA2: organic amendment. Different letters imply significant differences between the treatments, within each soil layer and sampling time.

The other fertilized treatments maintained mineral N levels ranging from 31 to 78 mg kg^{-1}. By T4, the mineral N concentrations largely decreased due to the N uptake by plants, but the fertilized treatments kept supplying more mineral N in the top layer, most probably due to the mineralization of the N-contained organic matters and unconsumed fertilizers. The subsurface layers' mineral N concentrations decreased to 6-9 mg kg^{-1}. The treatment CAT1 contained higher mineral N within the top layer soil by T4 and T5/T6. By T7, the soil mineral N concentrations further dropped, ranging from 10 to 20 mg kg^{-1} in the top layer and 6-8 mg kg^{-1} in the subsurface layer.

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The SMN levels of the treatments represents the potential plant available N pool of the soil in 2024. The initial SMN content was 7-13 kg ha^{-1} in the 0-30 cm deep layer (Figure 14). By T2, the SMN levels of the unfertilized CON0 treatment was 293 kg ha^{-1} in 0-30 cm soil layer, while the OA2 treatment maintained at 135 kg ha^{-1}. The SMN levels of the fertilized treatments increased to 128 kg ha^{-1} in the top layer of the mineral fertilizer treatment MF, 286 kg ha^{-1} for treatment FER5', 122 kg ha^{-1} for treatment FSP, 296 kg ha^{-1} for treatment FER3, and 348 kg ha^{-1} for treatment CAT1. The FER3 treatment also had the highest SMN level (238 kg ha^{-1}) in the subsurface layer. However, no significant differences were observed between treatments at this sampling time. By T3, the SMN level of the unfertilized CON0 treatment and the OA2 treatment decreased to 55 kg ha^{-1} and to 98 kg ha^{-1}, respectively. Other fertilized treatments exhibited high SMN levels in the soil, where treatment FER3 had significantly higher SMN level at 307 kg ha^{-1} in the top layer, followed by CAT1 treatment (218 kg ha^{-1}). Other fertilized treatments showed no significant difference in SMN levels compared to the MF treatment. By T4, the SMN levels in the 0-30 cm layer dropped to a range between 33-65 kg ha^{-1}. This range changed to 32-73 kg ha^{-1} by T5 and further decreased 26-40 kg ha^{-1} in subsequent measurements. Throughout the period from T4 to T7, the CAT1 and OA2 treatments exhibited significantly higher SMN levels compared to the CON0 treatment in the 0-10 cm deep layer, likely due to the mineralization of fertilizer residues in the topsoil. However, during the monitoring period, no significant differences were observed between treatments in the subsurface layer.

Conclusions

In a northern Spanish field trial, in terms of broccoli all head yield in milder weather conditions, FER3, FER5'and FSP BBFs were comparable to MF. In the case of commercial head yield, FER3, MF, and FER5' were comparable. In this case, the highest NUE was obtained with FER3. When weather conditions were unfavorable (long rainy periods, low temperatures and saturated soil), FER3 was the BBF that performed best for broccoli with all head yield and commercial head yield DW.

The residual N remaining in the soil after growing a broccoli crop was not the same after a period of favorable conditions (aerated soil and mild temperatures) as when weather conditions were not favorable (flooded soil and cold temperatures). When conditions were favorable, all BBFs except OA2 obtained higher lettuce biomass values than CON0.

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FER3, FSP, and FER5' were the treatments with the highest N uptake values in lettuce. When meteorological conditions were unfavorable, the onion crop obtained the highest biomass values in CAT1, FER3, and FSP, above MF. The highest N uptake values were achieved in the FER3 treatment.

3.7 Synthesis fertilizer effect and environmental risk of BBFs

Fertilizer effect

Of the 16 developed BBFs, three were deemed the best suited fertilizers based on their chemical characteristics and their performance in pot trials: CAT1, FER3, and FSP. Key results from this characterization are reproduced in Table 16.

Table 16. Chemical composition of the BBFs and the NUE, PUE and KUE obtained in the pot trials (data retrieved from Tables 1 and 3).

	Total N	NH_4^+-N	NUE	P	P-avail	PUE	K	KUE
	%	g kg^{-1}	%	g kg^{-1}	g kg^{-1}	%	g kg^{-1}	%
CAT1	7.6	0.34	22	30.9	24.7	45	6.16	NA
FER3	5.3	2.6	43	15.9	13.7	32	22.4	49
FSP	6.2	0.6	44	27.4	13.9	21	14.6	17

The performance of these BBFs and the negative control (CON0) in the field trials is shown in Figure 15. Due to differences in growth conditions, harvest protocols, determination of biomass and yield, fertilizer application etc., direct statistical comparison of the results between countries was not feasible. The use of the high-yielding broccoli variety 'Cezar' in Estonia also limits the comparability with the other trials where 'Parthenon' was used. Therefore, the results were normalized by dividing the individual observations by the average for each country across the four mentioned treatments (3 BBFs + CON0). In that way, the mean of each country across treatments is 1 and the relative variation between treatments within each country is kept. For Spain 2024, FER3 was let out of the analysis, as the BBF was supplemented with mineral fertilizer that year.

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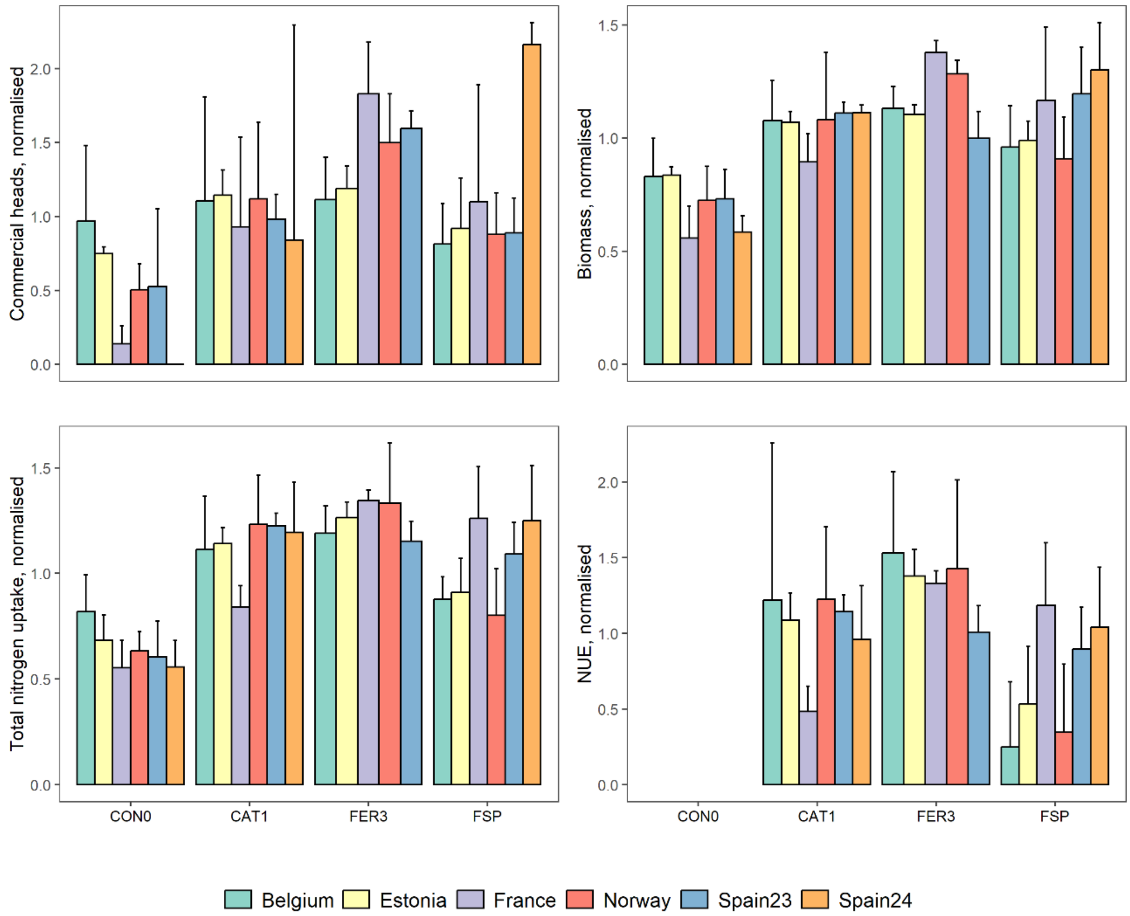

Figure 15. Yield of commercial heads, biomass, total plant nitrogen uptake and nitrogen use efficiency for all countries. The values have been normalized so that for each parameter and country, the mean is 1 across the here shown treatments.

Differences between treatment and country were tested by two-way ANOVA. For all variables, there were significant differences between the treatments, but not between countries (which is as expected as all countries have mean 1). However, the BBFs did not perform in a uniform way across countries, as was reflected by a significant interaction between treatment and country for all variables except NUE. The interaction hindered further analysis by post-hoc tests. From Figure 15 it can, however, graphically be seen a tendency towards better performance of the BBFs compared to the negative control. This was also confirmed by the individual ANOVA/post-hoc analysis performed for each country. A better performance of the BBFs than the unfertilized control is as expected as the BBFs add nutrients and organic matter to the soil.

Looking at the variations between the different BBFs, there is a slight tendency towards higher commercial yield, total biomass and nitrogen uptake following application of FER3

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than application of FSP. For the NUE, this pattern was confirmed statistically (Tukey HSD, α = 0.05). Both BBFs had a similar NUE and P-availability as determined by the pot trials / chemical analysis (Table 16). However, FER3 had a higher concentration of ammonia and higher PUE and KUE. Also, throughout the growth season, the soil fertilized with these BBFs in general had a higher soil mineral nitrogen concentration.

In line with this, an incubation trial assessing nitrogen mineralization from the BBFs, showed that the nitrogen in FER3 mineralized faster than the nitrogen in the other BBFs (Deliverable 6.3). Following FER3, CAT1 had the highest mineral N levels at most sampling times during the incubation period and had the same levels at FER3 at the last sampling day (day 120). Of these three, FSP had the lowest mineral N levels throughout the incubation period.

As written earlier, transplants like broccoli with a well-developed root system at transplanting need high amounts of nitrogen early in the season. The availability of SMN from the beginning of the season can then have large impact on the yield. This was examined statistically, using linear regression with broccoli yield (Total biomass, kg DW ha^{-1}) as the response and SMN at T2 for the uppermost two layers (0-30 cm) as explanatory variable. For Norway, Estonia and Spain 2023 there was a significant relationship between the two ($p < 0.05$), which explained 41, 63 and 40 % of the variation in yield, respectively (Figure 16). This relationship indicates that early SMN levels can be used as indicators of potential crop yield. Therefore, it is important to monitor SMN levels of the field to ensure sufficient mineral N availability in the growth season. Furthermore, it highlights that biobased fertilizers exhibiting rapid N release and high mineralization rates hold significant potential as viable alternatives to synthetic fertilizers. As mentioned earlier, BBFs with a slower release of nitrogen might be better suited for crops which are sown directly in the field.

However, as can be seen from the lacking relationship for the trial in Belgium and, as well as the part of the variation in yield which remains unexplained by early SMN, other factors such as plant availability of phosphorus and potassium content are also important.

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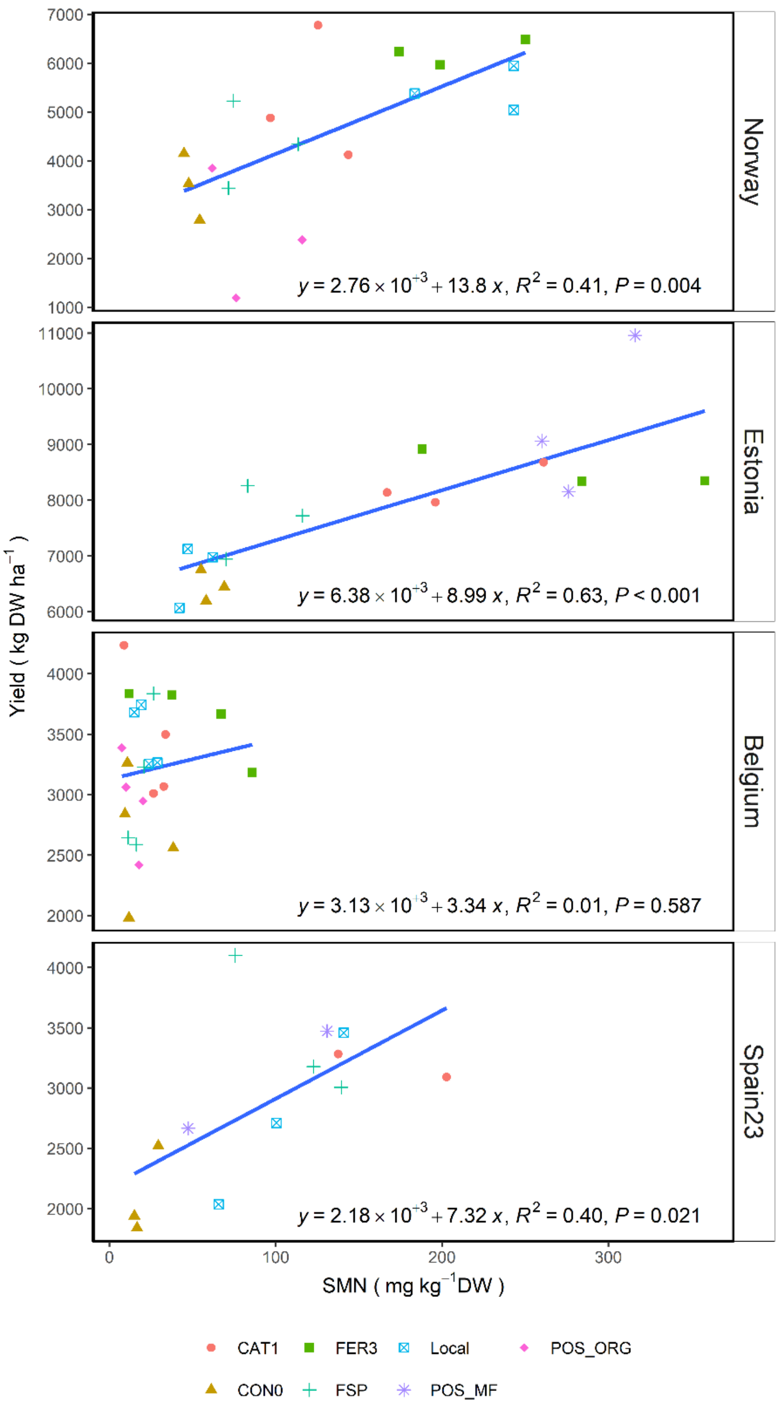

Figure 16. Broccoli yield (kg DW ha^{-1}) and soil mineral nitrogen (SMN) at T2 (mg kg^{-1} soil DW), i.e., one week after fertilization. The blue lines show the linear regression line, for which the formula, R2 and p-value is shown on top for each country. FER3 in Spain 2023 was excluded as the plants were burned from the high amounts of nitrogen. Soil samples for France at T2 was unavailable.

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To compare with mineral fertilizer (MF), the results from the two countries using MF as positive control are displayed in Table 17. The MF gave higher biomass, nitrogen uptake and NUE in Estonia and in Spain 2024. In Spain 2023 however the BBFs performed similar to the MF or better (NUE). It is difficult to predict in advance how both the BBFs and the MF will perform. In Spain 2023, CAT1 performed better than the MF, in 2024 it performed worse.

Table 17. Summary of results from Estonia and Spain, for the mineral fertilizer and the BBFs CAT1, FER3 and FSP. The numbers refer to the means, not individual replicates.

	Estonia	Spain 2023	Spain 2024
Total biomass			
Mineral fertilizer	9.4	3.1	5.3
BBFs	7.6 - 8.3	2.9 – 3.4	3.5 – 4.1
Kg N ha^{-1}			
Mineral fertilizer	315	119	102
BBFs	202 - 254	124 - 139	65 – 73
NUE			
Mineral fertilizer	135	37	51
BBFs	42 - 108	46 - 68	30 – 32

Residual fertilizer effect

BBFs have the possible benefit of long-term mineralization of nitrogen. While the MF in most cases gave higher broccoli yield and nitrogen uptake, the residual effect of MF was much poorer than for the BBFs in the Spanish field trials. In Estonia, there was a tendency towards higher yields in plots previously fertilized with BBFs compared to the MF, but the effect was not significant. Considering that part of the nitrogen in the BBFs is part of organic compounds which will mineralize with time, this residual fertilizing effect is as expected.

Risk of leaching

When mineral nitrogen is left in the soil after the growth season, there is a risk of leaching and hence pollution of aquatic bodies. Except Spain 2024, there was no statistical differences in the SMN levels in the upper 30 cm of the soil at T7. The average SMN levels varied from approximately 7 – 13 kg ha^{-1} in Spain 2023 to about 30 – 60 kg ha^{-1} in Belgium. For Spain 2024 however, the soil fertilized with CAT1 contained significantly

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higher levels of SMN at T7, than several of the other treatments. The variation in mean between the treatments in Spain 2024 were still rather small, ranging from around 28 to 40 kg ha^{-1}, which was lower than in Belgium and around the same as the levels in Norway. The treatment with CAT1 gave the highest SMN levels at T7 in Norway also, although not significantly higher.

Based on these measurements the risk of nitrogen leaching after use of BBFs seems limited compared to after use of mineral fertilizer or no fertilization.

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4 Shell residues as liming agents

Liming adjusts the soil pH to the optimal range for crop growth and is essential for reducing environmentally harmful runoff from agriculture. The optimal pH (H_2O) for grass and most other crops is 6.2. The aim of this study was to test the use of a liming agent produced from shell residue to raise the soil pH to an optimal level. Shells can provide a liming effect in field and a shell mix was compared to commercial liming agents both in a laboratory trial and in field trials.

4.1 Materials and methods

The tested shell mix contained residues of clam, mussel and murex shells ground to 1 mm, and was provided by UNIVPM. The shell mix contained 47.8 % CaO and 0.14 % MgO.

First, the liming efficiency of the shell mix was assessed during a 30-days incubation trial. Shells ground to below and above 1 mm were included (<1 mm and > 1 mm, respectively), along with the two commercial liming agents which were to be used in the field trials (named after the site they were used at, Sandnessjøen and Trofors). The properties of the commercial liming agents are given in Table 18.

Table 18. Properties of the commercial liming agents (positive control).

Site	Lime value 1/5 years	Mg (%)	CaO-equivalents (%)
Sandnessjøen	42/51	0.20	30
Trofors	39/48	0.45	56

The incubation trial was performed according to the UNI EN 14984: 2006 method. Briefly, samples were mixed with soil (pH 5.64 ±0.02; organic carbon 1.25 ± 0.74 %) in triplicate per liming agent. Each pot was composed as follows: 500 g of soil and 0.5 g of corrective, the mixtures created were then brought to a moisture corresponding to 70% of the full water holding capacity of the soil. Subsequently, after 1 day, 15 days and 30 days from the beginning of the incubation, two samples of soil corresponding to 10 mL were taken for each pot; 50 mL of water were added to each soil sample, and after 16 h the pH of the settling suspension was measured according to the ISO 10390: 2005 standard method. The results obtained were expressed by calculating the so-called index of efficiency E_1.

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$E_1=(pH_p-pH_0)/(pH_r-pH_0)$

where:

pH_p is the value achieved by the product tested

pH_0 is the value for the control (no addition)

pH_r is the value achieved by the reference material (CaO and $CaCO_3$ in this case)

$E_1<1$ or $E_1>1$ means that the product tested has a corrective power lower or higher than CaO or $CaCO_3$, respectively.

In field, the liming effect of the shell mix were tested in parallell at two sites in Norway in 2023 – 2025: Trofors and Sandnessjøen. Both are in the region of Helgeland, in the middle part of Norway. Trofors has inland climate, while Sandnessjøen has coastal climate. Therefore, Trofors has shorter growing season. Three treatments were included at each site: The finely ground shell mix (< 1 mm), a commercial liming agent (Sandnessjøen or Trofors), and a non-limed control.

The plot sizes were 7 * 1.5 meters, and there were three plots per treatment (i.e., 9 plots per site). Each of the limed plots received 4.2 kg liming agent. The plots were established during the summer of 2023. At Trofors, the plots were sown with a mixture of 80 % timothy (*Phleum pratense*) and 20 % meadow fescue (*Festuca pratensis*) on June 14th. At Sandnessjøen the plots were sown with a mix of 70 % timothy, 20 % meadow fescue and 10 % red clover (*Trifolium pratense*) on May 23rd. A NPK fertilizer of 22-3-10 were applied at 6.0 kg ha^{-1} at all plots.

Grass was sampled during the second season, at two cuttings. The yield of the cuttings was recorded, and the soil pH was measured after each cutting. For each plot, 10 soil sub-samples were pooled. The pH was measured in H_2O by a commercial laboratory. The soil pH was also measured prior to sowing, in one sample which was the result of pooling two sub-samples per site.

4.2 Results and discussion

Corrective power of liming agents during the pot trial

As shown in Table 19, the best performing sample was the shell mix ground to < 1 mm, exhibiting an E_1 higher than both commercial liming agents, and almost equal to $CaCO_3$.

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For this reason, the shell mix ground < 1 mm was chosen for the field trials. The performance of the two commercial liming agents is consistent with the properties provided by the LA companies shown in Table 18, the efficiency of the Trofors LA being higher than that of the LA used in Sandnessjøen.

Table 19. Index of liming efficiency E_I of shell mix and commercial liming agents (LA)

	E_I $CaCO_3$	E_I CaO
Sandnessjøen LA	0.245 ± 0.054	0.165 ± 0.051
Trofors LA	0.431± 0.063	0.291± 0.043
Shells < 1mm	0.822 ± 0.036	0.554 ± 0.023
Shells >1mm	0.478 ± 0.035	0.322 ± 0.024

Corrective power of the liming agents in the field.

At Sandnessjøen, the pH remained stable throughout the experiment, with a slightly higher (but not statistically different) pH in the two limed treatments (Table 20).

At Trofors, the pH remained stable till the 1st cutting, after which there was an increase in the pH in all treatments, including the non-limed control. The reason for the rise also in the negative control is difficult to explain and seems to be related to the field as a whole and not to the applied treatments. It could be due to a contamination of the field. In 2025 however, the pH had risen further in both the positive control and in the shell treatment and the plots limed with shell mix now had a significantly higher pH than in the non-limed control. The pH was also higher than in the plots with the commercial liming agent, although not statistically significant (Table 19). These results are in line with the results of the incubation study, and highlight the need of long-term studies for liming agents in field.

Table 20. pH prior to chalking (start), and after the 1st and 2nd cutting (2024) and spring 2025 at Trofors. Different letters imply significant difference between treatments.

Site	Treatment	Start	1st cutting	2nd cutting	2025
Sandnessjøen	Negative		5.7±0.2	5.7±0.2	5.7±0.1
	Positive	5.7	5.9±0.2	5.9±0.2	6.0±0.1
	Shell		5.8±0.2	5.9±0.3	5.8±0.2
Trofors	Negative		5.7±0.2	6.2±0.1	6.2±0.2 b
	Positive	5.8	5.9±0.2	6.1±0.1	6.3±0.1 ab
	Shell		5.9±0.3	6.2±0.2	6.5±0.1 a

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Both commercial liming agents were coarsely ground and act at a slow pace over several years. The shell residue mix were ground to a finer powder compared to the commercial products but can nevertheless be expected to act slowly as well. It has been common for a long time in Western and Norther Norway to use shell sand in agriculture. The shell sand contains native shells mixed with sand from the beach and is coarsely ground. The shell sand is mainly used as a soil improver but will increase the soil pH with time. The differences between the Norwegian shell sand and the here tested shell mix include both composition (shells from different species), grinding size, and the shell sand contain sand while the here tested shell mix does not. For future experiments the long-term effect of the here tested shell mix could be compared with the already used Norwegian shell sand.

Both cuttings the second season were harvested, and the yield were registered (Figure 17). Samples were sent for analysis of mineral elements and protein at a commercial lab (results not shown here). The yield differed between the sites, but not between the treatments (two-way ANOVA performed for both cuttings, a = 0.05).

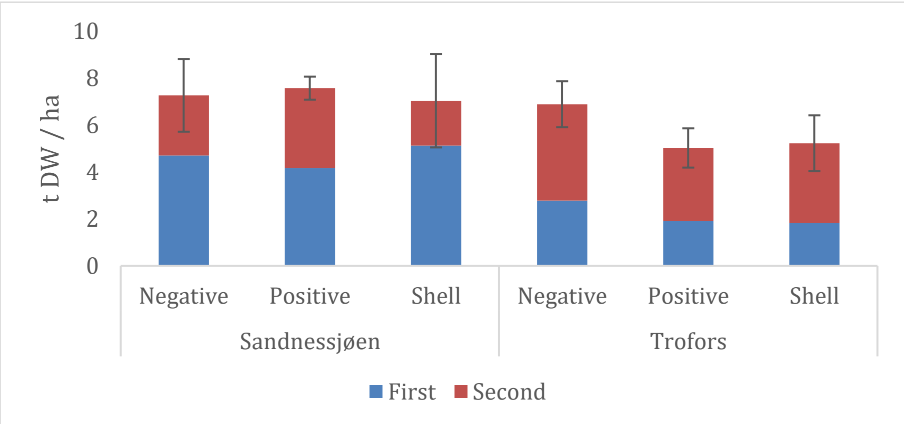

Figure 17. Grass yield at first and second cutting.

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5 Conclusion and recommendations

The here tested BBFs gave in many cases higher broccoli yields than commercial organic fertilizers. The yield was comparable to, or close to, the yield obtained using mineral fertilizer. To make BBFs attractive substitutes for mineral fertilizers it is however important to close the remaining yield gap, although relatively small.

Although being a correlation and not a causal relationship, it seems that the levels of soil mineral nitrogen early in the season is important for the yield at the end of the season. One way forward when further optimizing BBFs could be to combine them with mineral nitrogen, either as mineral fertilizer or as one of the recycled alternatives which are in the pipeline (e.g. urea from urine).

Further, the phosphorus availability in the BBFs will in many cases need optimization. Although this was not further investigated in the field trials, the results of the pot trials showed large variations in the phosphorus use efficiency. The same applies to potassium.

Marine residues also showed promising results as liming agent, with short-term liming efficiency being higher for the here tested shells, than for Norwegian commercial liming agents. When investigating liming agents it is however important to keep in mind that many agents are meant to act slowly over several years and hence are not raising the pH very rapidly in short-term studies. The historical use of shell sand in Norway also supports the inclusion of shells as liming agents.

Based on the here presented work, the group would recommend:

- To characterize each BBF thoroughly during development, to gain detailed knowledge on e.g., nitrogen mineralization and phosphorus plant availability.
- Ensure that the BBF can supply the plants with enough mineral nitrogen early in the season.
- Continued work on phosphorus availability in BBFs.

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