

D6.5 Soil health and ecological impact effects

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from fisheries wastes**

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Document Summary

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Abstract

Deliverable 6.5 (D6.5) reports the results on the impact of bio-based fertilizers (BBF) on soil health during the field trial conducted in Estonia and Spain in the framework of WP5. In the field trial three common fertilizer products were used for both trials: NPK solution with amino acids (FER3), fish sludge pellet (FSP), protein fraction (CAT1), plus a local fertilizer was added at each site; Bokashi pellets (BP) in the case of Estonia and FER5' microalgae hydrolysate in the case of Spain. In Estonia the effects of different fertilization treatment on soil physico-chemical properties, bioindicators (dehydrogenase activity (DHA); basal soil respiration (RCO₂), microbial biomass carbon (MBC) and soil fauna (springtails and mites) were studied while in Spain enzyme activities were studied: alanine aminopeptidase, leucine aminopeptidase, β -glucosidase, chitinase, arylsulfatase, dehydrogenase, basal soil respiration and biomass carbon. These studies were carried out at two sampling times: one week after fertilization (T2), and after harvest (T5) and at two soil depths (0-10 cm and 10-30 cm). In both cases no significant differences in soil pH, organic matter (OM) contents, phosphorous (P) and potassium (K) were observed after short-term application of BBFs in the field. For both sites short-term applications of the tested biobased fertilizers (BBFs) derived from fishery waste and by-products had minimal influence on the soil's physical and chemical properties but significantly boosted microbial indicators such as microbial biomass carbon, dehydrogenase activity, highlighting an increased microbial activity. Organic fertilizer incorporation into the topsoil reflected significant treatment differences at 0-10 cm depth, with BP and CAT1 (for Estonia) and FSP in the case of Spain showing the highest values of DHA and MBC, followed by other BBFs. In contrast, the mineral fertilizer (MF) and unfertilized treatments (CON) exhibited the lowest values at both sampling times. Clear trends although no significant differences were found for soil basal respiration and soil biota, while the abundance of springtails and mites decreased after the fertilization. In addition, clear trends although no significant differences in DHA, RCO₂ of MBC found at the 10-30 cm depth, irrespective of treatment, or sampling time. Overall, organic fertilizers demonstrated a positive impact on biochemistry, particularly in the topsoil, highlighting their potential to enhance soil microbial activity and soil health.



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Glossary

AMGA: Annotated Model Grant Agreement

BBF: Biobased Fertilizer

BP: Bokashi Pellet

CAT1: protein fraction

CON: control, unfertilized treatment

D: Deliverable

DHA: Dehydrogenase Activity

FER3: the NPK solution with amino acids

FER5': microalgae hydrolysate

FSP: Fish Sludge Pellet

MBC: Microbial Biomass Carbon

METK: Centre of Estonian Rural Research and Knowledge

MF: Mineral Fertilizer

OM: Organic Matter

RCO2: Soil Basal Respiration

TOC: Total Organic Carbon

UGENT: Ghent University

WP: Work Package



1 Introduction

Waste and/or by-products from fishery, fish and seafood processing and aquaculture exhibit high nutrient content and thus can support plant growth when applied as fertilizing products. Assessing their role in replacing mineral fertilizers should be supported by assessing their impact on soil health.

This document presents the results of Task 6.6 "Soil health and ecological impact effects on soil biochemical processes, study of changes in soil biomass, - taxonomy, and functionality". Soil chemical properties and bio-indicators were characterized in the Estonian and Spanish field trials under WP5. Four fertilizing products were used in each field trial: the NPK solution with amino acids (FER3) from Cantabrian Sea, fish sludge pellet (FSP) from North Sea and protein fraction (CAT1) from Atlantic Sea besides a local biobased fertilizer was included at each site Bokashi pellet (BP) from Baltic Sea in the case of Estonia and Microalgae biostimulant (FER5') in Spain. Selection of used fertilizing products was based on the results of the quality and safety assessment of the fertilizing products in WP6 and pot trials in WP5. Simultaneously soil health was assessed as a set of soil bio-indicators as soil enzyme activities (alanine aminopeptidase, leucine aminopeptidase, β -glucosidase, chitinase and arylsulfatase and dehydrogenase) were determined trying to evaluate the effects of the different BBFs on the soil quality at metabolism level.

2 Materials and methods

2.1 Estonia

2.1.1 Field description and climate

The field trial was carried out in Jõgeva, Estonia (58° 45' 47.22"N, 26° 24' 14.40"E, altitude 67 m above sea level). The field has been cultivated organically since 2010. Red clover was plowed in the soil in 2022 as green manure. The soil is characterized as Endogleyic Luvisol (FAO, 2014) with silty loam texture with 16.6% sand (2-0.060 mm), 70.0% silt (0.060-0.002 mm) and 13.4% clay (< 0.002 mm). At the beginning of the trial (10.05.2023, 15 days before fertilization), the two upper layers (0-10 cm and 10-30 cm) were sampled and characterized as given in Table 1.



Table 1 Physical-chemical characteristics (mean \pm SD, n=4) of the soil (depth of 0-10 cm and 10-30 cm) at the beginning of field trial in Jõgeva, Estonia.

Characteristics	0-10 cm	10-30 cm
pH-H ₂ O	6.14 \pm 0.06	6.12 \pm 0.07
pH-KCl	5.21 \pm 0.11	5.24 \pm 0.07
Organic matter/%	5.09 \pm 0.25	5.09 \pm 0.20
Total N/g kg ⁻¹ DW	1.39 \pm 0.02	1.32 \pm 0.04
Total P/g kg ⁻¹ DW	0.78 \pm 0.10	0.82 \pm 0.05
Plant available P/g kg ⁻¹ DW	0.33 \pm 0.01	0.32 \pm 0.03
K/g kg ⁻¹ DW	1.19 \pm 0.03	1.33 \pm 0.05
Mg/g kg ⁻¹ DW	1.82 \pm 0.09	1.80 \pm 0.04
Ca/g kg ⁻¹ DW	2.68 \pm 0.20	2.79 \pm 0.08
Na/g kg ⁻¹ DW	0.06 \pm 0.02	0.07 \pm 0.01
S/g kg ⁻¹ DW	0.20 \pm 0.03	0.21 \pm 0.00

Estonia belongs to the mixed-forest subregion of the Atlantic continental region of the temperate zone. Historical weather data was obtained from a nearby weather station operated by the Estonian Environmental Agency (2024). The long-term average air temperature from 1991 to 2020 is 5.8 °C, with absolute maximum air temperature is 34.6 °C and absolute minimum air temperature is -36.7 °C. The long-term annual accumulated sum of precipitation is 672 mm.

The on-site meteorological weather station at Jõgeva provided accurate weather data during the field trial (Figure 1). The average temperature during the first half of June was 15.3°C, but from June 14 to the end of the month, daily highs rose to 27–29°C. In July, the average temperature was 16.3°C, with daily highs ranging between 19 and 25°C. The 30-year average temperatures for May, June, July, and August were 10.5 °C, 15.0 °C, 17.5 °C and 16.0 °C, respectively. Only 5.4 mm of precipitation were seen between June 1 to June 20. Hence, the field trial watered on June 20 and June 30 (in total, 10 L m⁻²). The first rainfall occurred at the end of June and by July, with a cumulative rainfall amounted to 104.4 mm. The long-term average precipitation of these months were 45 mm, 85 mm, 65 mm, and 90 mm, with an accumulated total of 285 mm.



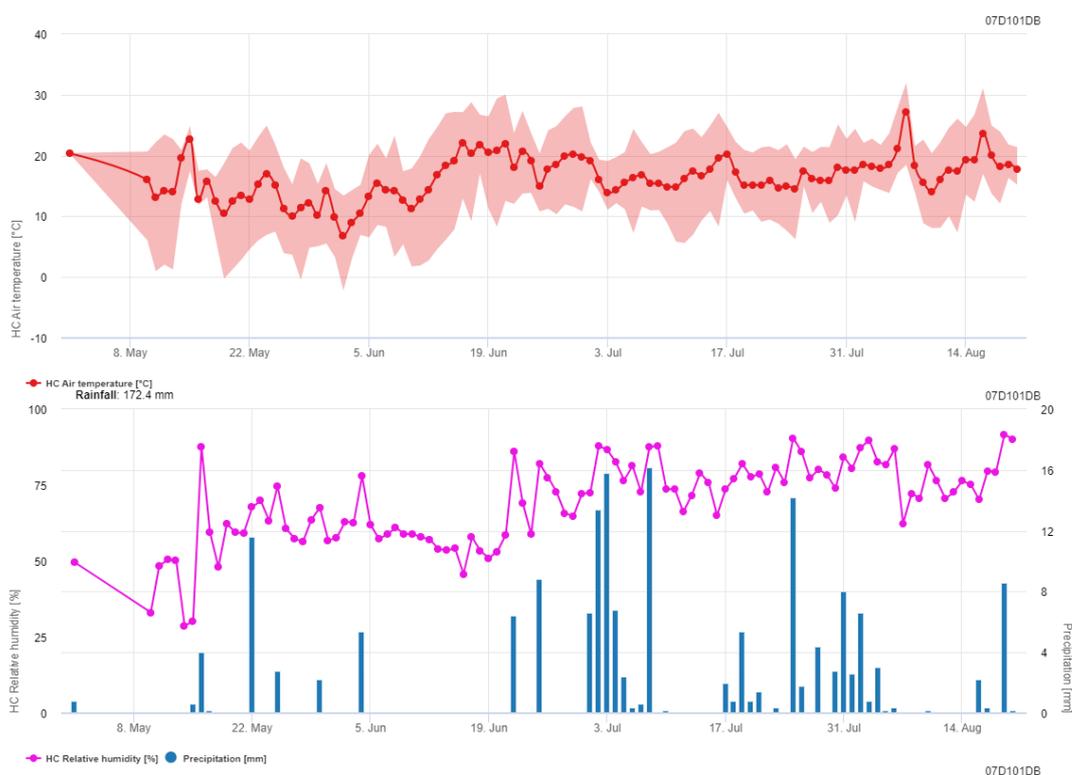


Figure 1 Temperature and precipitation during the field experiment.

2.1.2 Field trials

The field was ploughed in autumn 2022 and harrowed to a depth of 10 cm in spring 2023. The plot sizes were 1.4×8 m (11.2 m²), with a spacing of 2.8 m between plots. The field trial with broccoli (*Brassica oleracea* L. convar. *botrytis* var. *italica* `Cezar`) was conducted between June 5 (transplanting of seedlings) and August 4 (harvesting), 2023 (Figure 2). The field trials involved six treatments with three replicates as randomized complete block design: (1) control (unfertilized, CON), (2) mineral fertilizer (MF), (3) solid biobased fertilizer (bokashi pellet, BP), (4) solid biobased fertilizer (fish sludge pellet, FSP), (5) solid biobased fertilizer (protein fraction, CAT1), and (6) liquid biobased fertilizer (NPK solution with amino acids, FER3). Fertilizers were applied on the same day before planting. The amount of BBFs (treatments 2-6) to be applied was calculated by the N content corresponding to a fertilization rate of 120 N kg ha⁻¹. The N in the mineral fertilizers was in the form of ammonium (NH₄⁺-N). The nutrient application rate of the treatments is given in Table 2.





Figure 2 Field trial with broccoli in Jõgeva, Estonia.

Table 2 Nutrient application rates of biobased and mineral fertilizers in Estonian field trial.

Fertilizing products	Control, unfertilized (CON)	Mineral fertilizer (MF)	Bokashi pellet (BP)	Fish sludge pellet (FSP)	Protein fraction (CAT1)	NPK solution with amino acid (FER3)
Dosage, kg ha ⁻¹	-	775	4563	1939	1575	2260
N/kg ha ⁻¹	-	120	120	120	120	120
P/kg ha ⁻¹	-	22.0	47.7	53.2	48.6	36.0
K/kg ha ⁻¹	-	124.5	87.1	28.3	9.7	50.6
S/kg ha ⁻¹	-	35.0	11.4	16.9	8.0	16.5
Mg/kg ha ⁻¹	-	-	28.3	10.4	2.1	1.1

2.1.3 Soil samples

Soil samples for DHA, RCO₂, MBC and chemical properties was collected from the top layer (0-10cm) and subsurface layer (10-30cm) of each replicated block. In details, five sub-samples were taken from each plot with a hand auger (Ø 2 cm), separated according to depth (0-10 cm and 10-30 cm). Soils were sampled two weeks before the fertilization (T1) (10.05.2023), one week after fertilization (T2) (14.06.2023) and five days after harvesting (T5) (10.08.2023).

Soil samples for the determination of springtails and mites were collected using a soil corer (Ø 5 cm) at a depth of 0-20 cm, four soil samples were taken from each sample plot, a total of 12 soil samples were collected per treatment.



2.1.4 Analytical methods

Microbial activities

Dehydrogenase activity (DHA) was measured in the laboratory of Centre of Estonian Rural Research and Knowledge, Estonia, in accordance with Tabatabai (1982). Samples were sieved (\varnothing 2 mm) and stored at 4 °C until they were analyzed. Soil samples (5 g) were incubated at 30 °C for 24 h in the presence of an alternative electron acceptor (triphenyl-tetrazolium chloride). The red-tinted product, triphenyl formazan (TPF), was extracted with acetone and measured in a spectrophotometer at 546 nm (BioPhotometer plus).

Basal respiration (RCO₂) and microbial biomass carbon (MBC) were measured in the laboratories of Ghent University, Belgium. Basal respiration was assessed by measuring the CO₂ release rate from fresh soil through titration in a static system, following the ISO 16072:2011 standard. The soil was incubated in a closed vessel for 24 hours, during which the released CO₂ was absorbed into a 0.05M sodium hydroxide (NaOH) solution. The amount of CO₂ evolved was quantified by titrating the unreacted NaOH with 0.1M HCl to pH 8.3 in the presence of BaCl₂, as described by De Neve et al. (2003).

Microbial biomass carbon (MBC) was quantified using the chloroform fumigation-extraction method with a 0.5 M K₂SO₄ solution at a soil-to-solution ratio of 1:2 (w/v). Total organic carbon (TOC) in the extracts was analysed using a TOC analyser (TOC-VCPN, Shimadzu Corporation, Kyoto, Japan). The MBC was calculated from the difference in TOC between fumigated and non-fumigated soil samples, applying the conversion factor of 0.45 (Joergensen and Mueller, 1996; Vance et al., 1987).

Springtails (*Collembola*) and mites (*Acari*)

Springtails (*Collembola*) and mites (*Acari*) were determined in the laboratory of Estonian University of Life Sciences, Estonia. Samples were extracted using Tullgren funnels, following the method described by Coleman et al. (2017). Soil samples were placed on a metal sieve and exposed to light, which facilitated the collection of springtails and mites into sample bottles containing ethanol. The soil samples remained under light for 48 hours. All specimens were identified to the species level using identification keys provided by Fjellberg (1980, 1998) and Hopkin (2007).

Soil physical-chemical parameters

Macro- and micronutrients, as well as metals, were detected following microwave digestion of the samples using nitric acid (Ultra wave 1 and 2, Milestone Srl, Italy). The resulting extracts were analysed using Inductively Coupled Plasma Optical Emission (ICP-



OES; Thermo Fisher iCAP Q Scientific, USA). Plant available phosphorus (P) was extracted with an ammonium lactate-acetic acid buffer and quantified via ICP-OES (MEN 5793:2008). Dry matter (DM) content was calculated by drying samples in an oven at 105 °C until a constant weight was achieved, following ISO 18134-2:2017. Organic matter (OM) content was measured by placing the samples in a calcination furnace at 550 °C for 4 hours, in accordance with ISO 18122:2015. The pH in water (pH-H₂O) was determined using a pH-meter (Orion520A, USA) after extracting the samples with water (ratio 1:5 w/v) and allowing them to stand for 16 hours (ISO 2917:1999). The pH-KCl was measured using the extracts prepared with 1M KCl, with a soil-to-solution ratio of 1:5 (w/v), after 30 minutes equilibration period. Electrical conductivity (EC) was measured using a conductivity meter (WTW Tetra Con 96, Xylem Analytics, Weilheim in Oberbayern, Bavaria, Germany) from water extracts prepared at a 1:5 w/v ratio after 1 hour for shaking and filtration (EN 13038, 2011). All analyses were performed in triplicates.

2.1.5 Statistical analysis

The DHA, RCO₂, and MBC data were analysed using ANOVA using the Tukey-Kramer Honest Significant Difference (HSD). Statistical analyses were conducted using RStudio v1.3.1093 running R software v4.1.2 (R Core Team, 2020). Figures were generated using the ggplot2 package (Ginestet, 2011). Normality of data was checked with the Shapiro-Wilk normality test using the Shapiro. Test function and homogeneity of variance was tested with Levene's test using the Levene Test function.

The average values of the groups of springtails (*Collembola*) and mites (*Acari*) and their arithmetic mean errors (\pm SE) were calculated. The data were processed using Excel, CANOCO 4.52, and STATISTICA 8.0 programs, employing non-parametric Spearman correlation analysis methods.

2.2 Spain

2.2.1 Field description and climate

Two field trials were established in Zamudio (Bizkaia, the Basque Country, Spain) at NEIKER facilities (43° 17' 24''N, 20° 52' 15'' W) during two consecutive years in the same field: September 2023 – March 2024 and March 2024 – July 2024 under irrigated conditions. We will refer to the first field experiment as 2023 and to the second as 2024.



The soil at the field trial site was classified as Aquic Dystric Eutrudepts (SSS, 1999). The soil of the plot where field trials were placed was rotavated on 21/08/2023. To control the adventitious weeds, a weed control geotextile was installed on 22/08/2023.

The climate of the area is warm maritime according to the water regime of Papadakis' (1966) classification (Papadakis, 1966). The meteorological conditions during the growing seasons were described in Figure 3 (2023) and Figure 4 (2024). The climatic data employed in this study were collected from the weather station "Derio" (located 2 km away from the field trials), which is the property of the Basque meteorology agency (Euskamet, 2024; <http://www.euskalmet.euskadi.eus/>).

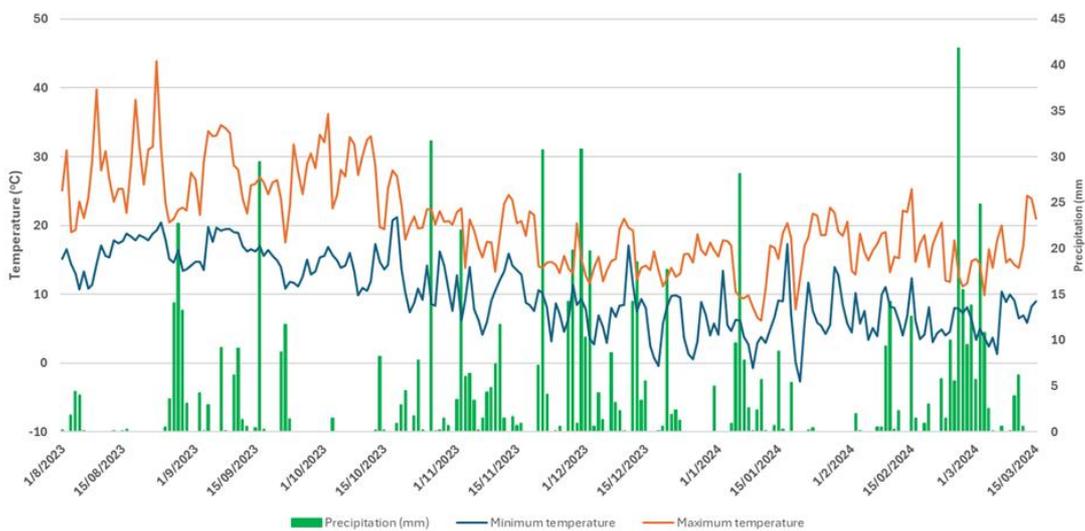


Figure 3 Precipitation (mm), air maximum and minimum temperatures (°C) during the first field experiment: broccoli – spring onion (2023).

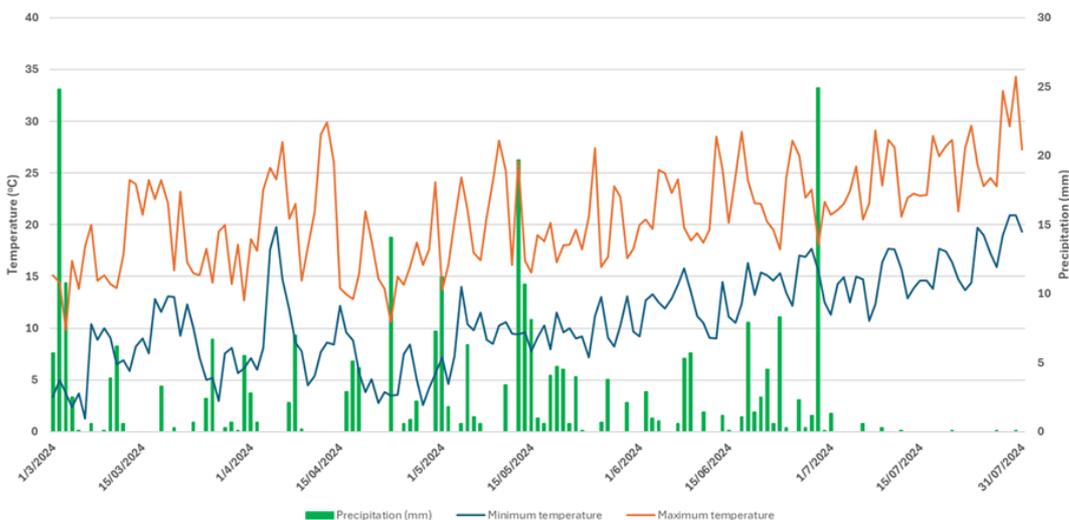


Figure 4 Precipitation (mm), air maximum and minimum temperatures (°C) during the second field experiment: broccoli – lettuce (2024).



Broccoli seedlings

Broccoli seeds (*Brassica oleracea* var. *italica* cv. *parthenon*) were sown on 07/08/2023 and on 19/02/2024 in trays 42x42x62 mm with volume 0,053 liter per hole. Broccoli seedlings were manually transplanted on the field on 13/09/2023 and on 19/03/2024 (BBCH 14, Feller et al., 1995) at 0.45 x 0.45 cm inter- and intra-row spacing. With the aim of measuring the residual N after the broccoli harvest, in 2023 spring onion seedlings (*Allium fistulosum*), and in 2024 lettuce seedlings (*Lactuca sativa*) were grown.

2.2.2 Fertilizer treatments

In 2023, four types of initial fertilization were applied as basal dressing (protein fraction from CATAR (CAT1), fish sludge pellet from GRONN (FSP), NPK solution with amino acids from FERTINAGRO (FER3), and microalgae hydrolysate used as a plant biostimulant from NEIKER (FER5')) (Table 3). CAT1, FSP and FER3 biofertilizers were equalized to 120 kg N ha⁻¹ rate. The biofertilizers were applied just before the transplanting of the broccoli seedlings (BBCH 14, Feller et al., 1995) on 13/09/2023. FER3 was diluted three times in water for application. The application of FER5' is described two paragraphs below.

In 2024, five types of initial fertilization were applied as basal dressing (CAT1, FSP, FER3, FER5'). CAT1 and FSP were matched at a dose of 120 kg N ha⁻¹ while FER3 and FER5' were applied as biostimulants reducing the N fertilization to 70% of the recommended rate, i.e. 84 kg/ha together with an application of 1.5l/ha of the corresponding biostimulant (FER3 and FER5') following the advice of FERTINAGRO (as plant biostimulant). In 2024, the biofertilizers were applied one day before the transplanting of the broccoli seedlings (BBCH 14, Feller et al., 1995) on 18/03/2024.

As recommended, applications of the hydrolysate from NEIKER (FER5') in 2023 and 2024 and the NPK solution with amino acids from FERTINAGRO (FER3) in 2024, were made when the nutrient requirement of the crop was highest (Ronga et al., 2019). Both were root applied on the broccoli crop three times at a rate of 1.5 L ha⁻¹. The first application was made when the broccoli seedlings were transplanted (BBCH 14, Feller et al., 1995) immediately 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)) and 84 kg N ha⁻¹ in FER3 (70% of the N provided in the other treatments of the field trial (120 kg N ha⁻¹)). The second application of FER5' and FER3 treatments was made when the broccoli head started to form (BBCH 41, Feller et al., 1995) on 30/10/2023 and on



22/04/2024. Finally, the third application was made when the broccoli head reached the 50% of the expected harvestable size (BBCH45, Feller et al., 1995) on 15/11/2023 and on 21/05/2024.

In 2023 and 2024, a control plot without N fertilization (CON) and a mineral control plot (MF; NAC 27%; 120 kg N ha⁻¹) were also established. All treatments were supplemented as basal dressing with 40 kg P ha⁻¹ and 150 kg K ha⁻¹, applied as Ca(H₂PO₄)₂ and K₂SO₄, respectively to avoid that any deficiency could affect the crop masking the effects of the BBFs.

Table 3 Nutrient content of biobased fertilizers in Spanish field trials.

	UNIT	FER3		FER5		FSP		CAT1	
		Average	SD	Average	SD	Average	SD	Average	SD
pH		4.82	0.02	4.28	0.04	6.15	0.01	6.05	0.02
Dry matter content	%	34.46	0.16	46.36	0.28	94.4	0.23	98.06	0.24
Moisture content	%	65.54	0.16	53.64	0.28	5.6	0.23	1.94	0.24
Ash content	%	21.22	0.17	6.82	0.06	17.5	0.39	1.618	0.74
Organic matter content	%	78.78	0.17	93.18	0.06	82.5	0.39	8.382	0.74
EC	mS/cm	18.71	0.03	17.48	0.23	7.15	0.47	437	0.36
TC	% FW	14.16	0.15	18.59	0.19	39.3	0.61	46.59	0.29
IC	% FW	0.002	0.000	0.002	0.001	0.169	0.005	0.047	0.002
TOC	% FW	14.16		18.58		39.13		46.54	
TN	% FW	4.34	0.24	3.9	0.08	6.19	0.07	762	0.72
NH + -N 4	g/kg FW	2.56	0.01	8.81	0.37	0.62	0.06	034	002
NO - -N 3	g/kg FW	<0.002		<0.002		<0.002		<0.002	
Soluble P	g/kg FW	13.72	0.03	11.76	0.12	13.88	0.13	2.474	092
P	g/kg DW	46.23	3.22	28.75	0.75	29.04	4.77	31.5	0.73
K	g/kg DW	64.96	5.48	4.06	0.24	15.49	2.16	6.28	0.12
Ca	g/kg DW	2.18	0.36	1.00	0.07	52.12	7.53	53.66	1.32
Mg	g/kg DW	1.35	0.22	0.51	0.04	5.68	0.96	1.34	0.03
Na	g/kg DW	7.67	0.84	0.96	0.04	16.72	1.6	7.22	0.63
S	g/kg DW	21.11	1.51	65.76	1.53	9.19	2.00	5.23	0.09
Fe	mg/kg DW	118.5	19.51	20.47	1.73	1144.83	200.32	004	0.00
Cu	mg/kg DW	93.76	15.52	<LOD		<LOD		< 3.82	
Co	mg/kg DW	<LOD		<LOD		<LOD		< 2.54	
Mn	mg/kg DW	13.61	2.29	30.42	2.17	115.02	21.67	< 0.27	
Zn	mg/kg DW	577.61	95.11	10.42	0.84	400.61	71.01	6.762	3.34
Al	mg/kg DW	125.53	4.93	106.54	10.81	773.09	109.65	011	0.02
Cd	mg/kg DW	1.16	0.13	<LOD		<LOD		< 0.85	
Cr	mg/kg DW	2.82	0.46	0.82	0.09	<LOD		202	0.88
Pb	mg/kg DW	<LOD		<LOD		<LOD		< 2.71	
Ni	mg/kg DW	4.22	0.39	0.76	0.29	<LOD		< 1.06	
As	mg/kg DW	3.00	0.13	0.25	0.05	1.04	0.18		
Hg	mg/kg DW	0.26	0.01	0.01	0.00	0.04	0.00		
Iodine	mg/kg DW	1.11	0.04	0.31	0.03	4.45	0.36	374	0.17
Free amino acids	%	9.8		0.8					
Pathogen									
Salmonella spp.	Detected/25 g	n. d.		n. d.		n. d.			
Faecal coliforms	UFC/g	<10		<10		<10			
E. coli	UFC/g	<10		<10		<10			

2.2.3 Broccoli, spring onion and lettuce harvest

Broccolis were collected by hand at optimal maturity at phenological stage BBCH59 (Feller et al., 1995). In the first growing season, 2023, FSP, MF, FER5' and CAT1 were harvested on 29/11/2023 whereas FER3 and CON treatments were harvested on 11/12/2023. In the second growing season, 2024, broccolis were collected all together on 28/05/2024.

2.2.4 Soil microbial parameters

For the determination of soil microbial parameters soil composite samples (four cores randomly collected) were collected at 0-10c m and 10-30 cm. During the first growing season, 2023, samples were collected one week after the fertilizer application (T2;



25/09/2023) and at harvest (T5), when broccoli flower petals were visible, but flowers were still closed on 29/11/2023 in FSP, MF, FER5' and CAT1 on 11/12/2023 in FER3 and CON.

During the second growing season, 2024, samples were collected one week after the fertilizer application (T2; 04/04/2024) and at harvest (T5; 28/05/2024) when broccoli flower petals were visible (but flowers were still closed) just before transplanting the lettuce seedlings.

After collection, the soil was stored at 4°C for a maximum of one month until analysis. Soil was sieved to less than 2 mm. Soil enzyme activities (alanine aminopeptidase, leucine aminopeptidase, β -glucosidase, chitinase and arylsulfatase) were determined following ISO/TS 22939. Dehydrogenase enzyme activity was determined following ISO-FDIS 23753-2. Potentially mineralisable N, an indicator of biologically active soil N, was measured as described Powers (1980). Respiration (RCO₂) was measured following ISO 16072 Norm (2002). Microbial biomass carbon (MBC) was determined according to Vance et al. (1987).

2.2.5 Statistical Analysis

All data were analysed using one-way ANOVA, followed by Duncan's test (De Mendiburu, 2009) with a significance level of $\alpha = 0.05$. The normality of residuals was tested using the Shapiro-Wilk test. Analyses were conducted with R (R Core Team, 2013).

3 Results and discussion

3.1 Estonia

3.1.1 Dehydrogenase activity (DHA)

Dehydrogenase activity (DHA) reflects the ability of microorganisms to catalyse redox reactions (Gu et al., 2009) and, as such, serves as an indicator of soil microbial activity and functionality. The average soil DHA results before fertilization, after fertilization and harvest are presented in Figure 5. Overall, the lowest DHA levels were observed before fertilization (ranging from 3.58 to 4.19 $\mu\text{g TPF g}^{-1} \text{h}^{-1}$), while the highest values were recorded one week after fertilization at a depth of 0-10 cm (ranging from 4.01 to 10.33 $\mu\text{g TPF g}^{-1} \text{h}^{-1}$). No significant differences were observed at a depth of 10-30 cm, regardless of the sampling time or treatments. Since the fertilizers were incorporated into the top layers of the soil, significant differences between treatments were only detected at a depth of 0-10 cm. More precisely, significantly higher soil DHA was observed at both



sampling time (T2, T5) in the 0–10 cm soil layer in BP treatment followed by CAT1 (Figure 5). On the contrary, the lowest value was recorded in the mineral fertilizer treatment (MF), followed by unfertilized treatment (CON), both after fertilization and after harvesting. There was a trend where treatments with organic fertilizers showed higher values of DHA in a depth of 0-10 cm compared to deeper depth 10-30 cm. In conclusion, organic fertilizers (BP, FSP, CAT1, FER3) showed a positive impact on DHA.

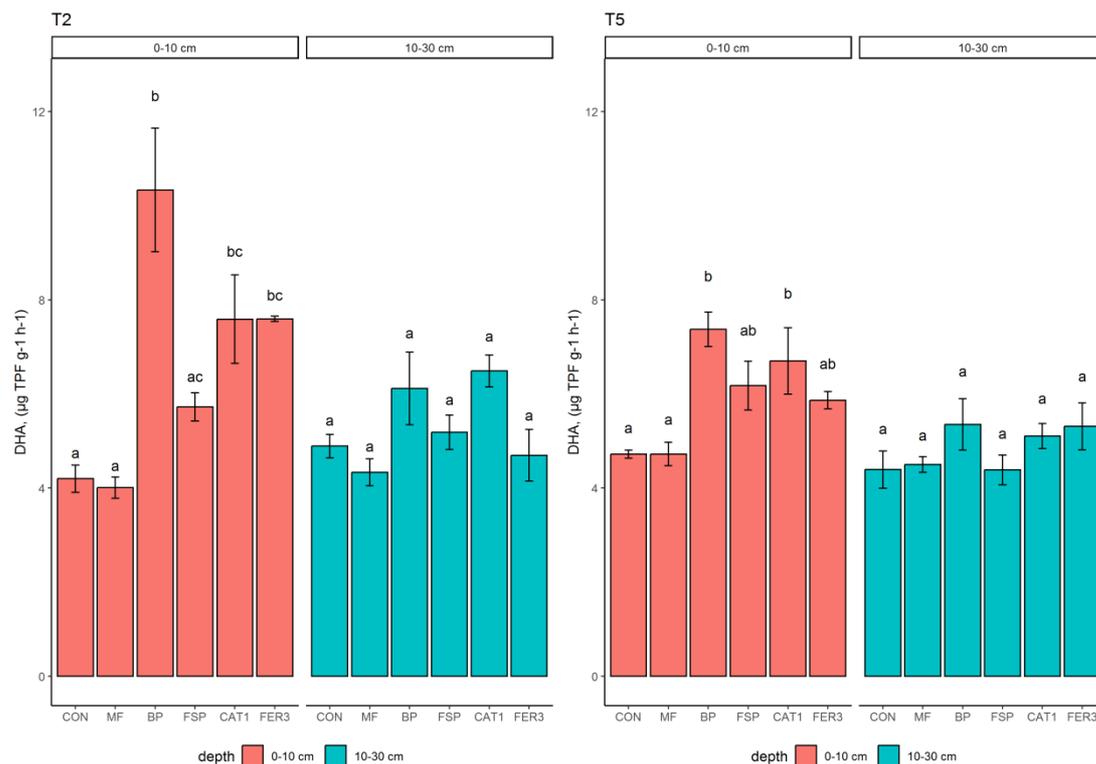


Figure 5 Soil dehydrogenase (DHA) in the 0–10 and 10–30 cm layers one week after fertilization (T2) and after harvest (T5). Unfertilized (CON), mineral fertilizers (MF) and biobased fertilizers; bokashi pellet (BP), fish sludge pellet (FSP), protein fraction (CAT1) and NPK solution with amino acids (FER3). Different letters indicate significant differences between treatments in the 0-10 cm layer and the 10–30 cm layer (Tukey's test, $p < 0.05$); error bars represent standard errors of the mean ($n = 3$).

3.1.2 Basal respiration and microbial biomass carbon

Basal respiration (RCO₂) was expressed in mg CO₂ released in per gram of soil per hour. Basal respiration is an indication of the oxidative capacity of soil microorganisms, and is therefore influenced both by the energy sources in the soil and the number of microorganisms (Bastida et al., 2008). The average RCO₂ results are presented in Figure 6. Overall, the RCO₂ values ranged from 0.001 to 0.009 mg CO₂ g⁻¹ h⁻¹ from 0-10 cm and 10-30 cm deep of soil. Contrary to the DHA trend, FSP treatment had the highest RCO₂ values from 10-30 cm deep soil after fertilization (T2) and after harvesting (T5). However,



no significant differences were observed between treatments at T2 and T5 in both soil layers. It demonstrated that fertilization did not significantly contribute to soil respiration.

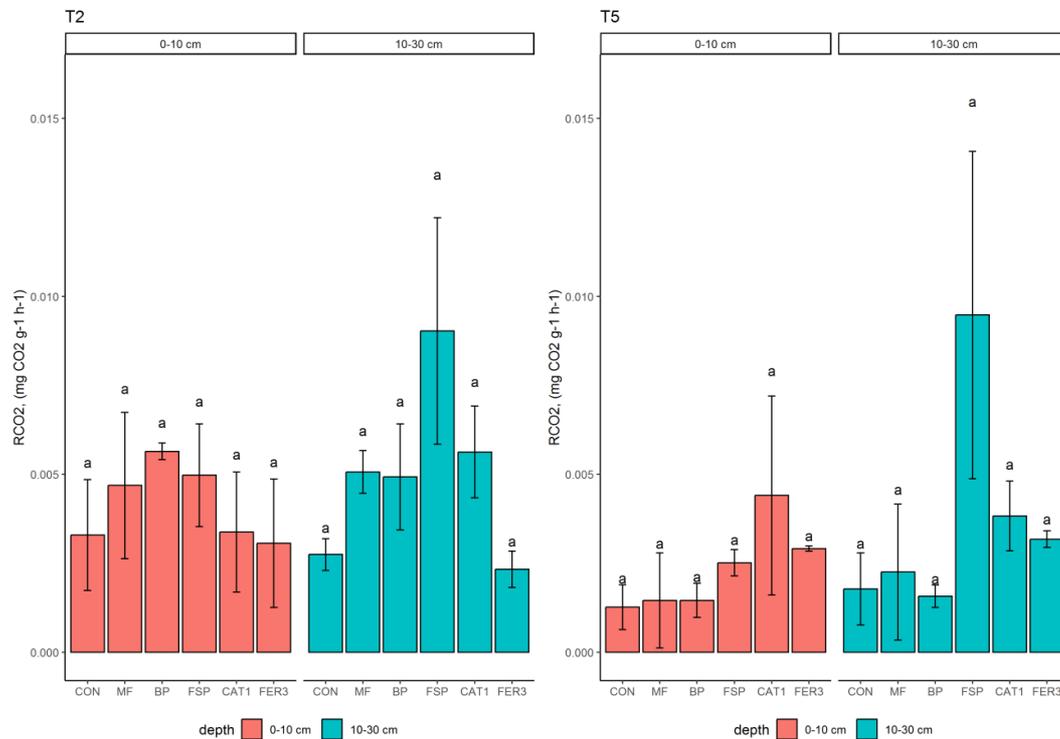


Figure 6. Soil basal respiration (RCO₂) in the 0–10 and 10–30 cm layers one week after fertilization (T2) and after harvest (T5). Unfertilized (CON), mineral fertilizers (MF) and biobased fertilizers; bokashi pellet (BP), fish sludge pellet (FSP), protein fraction (CAT1) and NPK solution with amino acids (FER3). No significant differences were observed between treatments (Tukey's test, $p < 0.05$); error bars represent standard errors of the mean ($n = 3$).

The MBC is primarily affected by soil organic carbon (SOC), soil pH and soil management, and is generally used as an early indicator to detect changes mainly induced by abiotic stress, where higher values indicate less stress (Amahnui et al., 2023; Anderson and Domsch, 1989; Kallenbach and Grandy, 2011; Yu et al., 2020). The average MBC results are presented in Figure 7. By T2 at a depth of 0-10 cm, the MBC values were in line with the trend of the DHA results (Figure 5). The lowest values were from treatments CON and MF, while the treatments amended with BP, CAT1 and FER3 had higher MBC values and had positive impact to the shallow 0-10 cm deep layer of soil. The treatment CAT1 demonstrated the highest MBC levels at $340 \mu\text{g C g}^{-1}$ soil DM ($p = 0.0022$), followed by the treatment BP at $207 \mu\text{g C g}^{-1}$ soil DM by T2. Conversely, the treatment MF exhibited the lowest MBC levels at $60 \mu\text{g C g}^{-1}$ soil DM by T2. Most probably, the available C and nutrients introduced by fertilization caused the differences in MBC values in the 0-10 cm soil layer. However, after the harvesting of the broccoli (T5), the treatments had no significant



differences in MBC, probably because crop residues provided an abundant carbon source and nutrient to the microbes. In summary, the treatments with BBFs positively affected MBC values and are highly consistent with the DHA results (Figure 5).

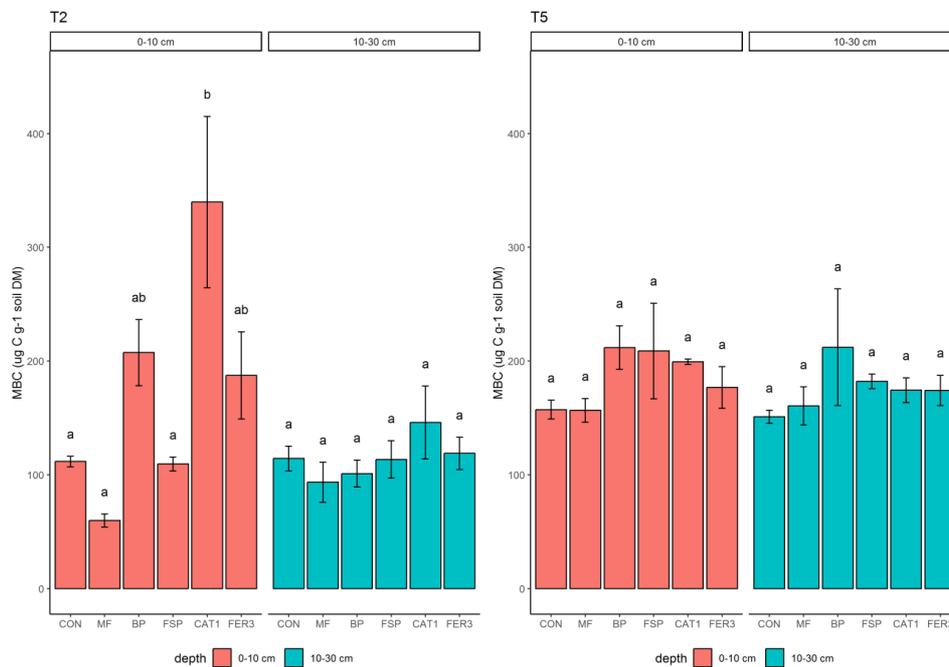


Figure 7. Soil microbial biomass carbon (MBC) in the 0–10 and 10–30 cm layers one week after fertilization (T2) and after harvest (T5). Unfertilized (CON), mineral fertilizers (MF) and biobased fertilizers; bokashi pellet (BP), fish sludge pellet (FSP), protein fraction (CAT1) and NPK solution with amino acids (FER3). Different letters indicate significant differences between treatments in the 0–10 cm layer (Tukey's test, $p < 0.05$); error bars represent standard errors of the mean ($n = 3$).

3.1.3 Springtails (*Collembola*) and mites (*Acari*)

Microarthropods, such as mites and springtails, play an important role in the decomposition of organic matter and produce fecal matter which serve as a substrate for soil microbes (Betancur-Corredor et al., 2023). Table 4 presents the average abundance of springtails and mites at a depth of 0–20 cm. No statistically significant differences in the abundance of springtails and mites were found between treatments, according to the Tukey-Kramer test. The highest springtails abundance was observed at the beginning of experiment, two weeks before fertilization (T1). After fertilization (T2) and after harvest (T5), the abundance of springtails remained relatively low and similar across treatments. These fluctuations in abundances were likely due to weather conditions, particularly the low precipitation levels observed in early June (see Figure 1).

A total of 28 springtail species were recorded throughout the experiment, with the species count provided in Table 4. The highest number of species (19) was found in the



treatment CON after fertilization, while the lowest species count (10) was observed in CAT1, also after fertilization. The dominant species included *Protaphorura armata*, *Folsomia spinosa* and *Parisotoma notabilis*. The highest diversity index (1.96) was recorded in the CON plot after the fertilization (T2), while the lowest index (1.35) was observed in FSP plot, also after fertilization (T2).

After fertilization, the abundance of springtails remained relatively similar and low across all treatments. After the harvest, springtail abundance was slightly higher in the BP treatment (55.6 ± 10.7 individuals). In the case of mites, lower abundance values were observed in FSP, CAT1 and FER3 treatments after fertilization, ranging from 4.4 to 5.3 individuals. The highest mite abundance was found in the unfertilized treatment CON (16.0 ± 4.4 individual $m^{-2} \times 1000$). In autumn, after harvest, the average abundance of mites stabilized, with the highest values recorded in the treatment FER3 (22.7 ± 4.9 individuals $m^{-2} \times 1000$). Overall, a decrease in the abundance of springtails and mites was observed after the fertilization, and followed by a slight increase after the harvesting, which highlights the need for a long-term monitoring of the soil fauna.

Table 4 Abundance of springtails and mites in soil 0-20 cm layer at three sampling time (T1- before fertilization, T2- one week after fertilization and T5- after harvest) under different fertilizer treatments (mean \pm SE, n=12). S- species number: individual m^{-2} ($\times 1000$); H-Shannon-Wiener diversity index.

Organisms	Treatment	T1	S	H	T2	S	H	T5	S	H
Springtails	CON	74.2 \pm 22.8	14	1.668	20.5 \pm 4.9	19	1.956	22.1 \pm 4.5	14	1.778
	MF	108.1 \pm 42.2	16	1.547	22.7 \pm 5.0	14	1.858	22.3 \pm 3.8	15	1.698
	BP	74.9 \pm 25.2	18	1.665	24.5 \pm 5.5	14	1.660	55.6 \pm 10.7	18	1.846
	FSP	36.7 \pm 7.7	17	1.595	23.9 \pm 3.9	14	1.343	36.9 \pm 5.6	13	1.920
	CAT1	59.2 \pm 12.1	15	1.609	31.1 \pm 12.9	10	1.489	40.8 \pm 8.5	14	1.712
	FER3	60.5 \pm 13.9	18	1.666	21.6 \pm 3.9	14	1.705	24.3 \pm 3.1	12	1.536
Mites	CON	14.4 \pm 3.5	-	-	16.0 \pm 4.5	-	-	12.1 \pm 1.5	-	-
	MF	25.1 \pm 5.4	-	-	11.6 \pm 1.5	-	-	19.1 \pm 2.5	-	-
	BP	32.4 \pm 5.1	-	-	10.3 \pm 1.9	-	-	21.1 \pm 1.8	-	-
	FSP	24.1 \pm 2.5	-	-	5.3 \pm 1.6	-	-	16.0 \pm 2.6	-	-
	CAT1	21.5 \pm 5.2	-	-	5.6 \pm 1.3	-	-	16.1 \pm 2.6	-	-
	FER3	11.4 \pm 1.8	-	-	4.4 \pm 0.7	-	-	22.7 \pm 4.9	-	-

Unfertilized (CON), mineral fertilizers (MF) and biobased fertilizers; bokashi pellet (BP), fish sludge pellet (FSP), protein fraction (CAT1) and NPK solution with amino acids (FER3).

3.1.4 Soil chemical-physical properties

The application of organic-rich BBFs led to increased soil OM content; however, no significant differences were observed between the treatments at either T2 or T5 (Table 5). This may be attributed to the addition of N, which decreased the C/N ratio of the soil, meeting the requirement of microbes, and thereby increasing the decomposition of the



added OM. Soil pH values varied only slightly in our study, with the BP treatment showing a higher pH. This increase was most probably attributed to both the higher pH of the BP product itself and the larger quantity used during fertilization. No significant differences in total P were found in the 0-10 cm soil layer, but significant differences in plant-available P were observed between treatments in this same depth by T2. The MF treatment had significantly higher K content than the unfertilized CON in the 0-10 cm soil layer by T2. However, no significant differences in plant-available P and K were detected between treatments after harvesting. The Ca contents showed no significant differences throughout the cultivation period, while Na contents varied slightly, ranging 0.05-0.07 g kg⁻¹ DW. The MF treatment had comparatively higher levels of Ca and S in the shallow 0-10 cm soil layer. Similarly, all treatments did not display significant differences in these nutrients after harvesting.



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Table 5 Soil physical-chemical characteristics in soil 0-10 and 10-30 cm layers at two sampling times (T2- two weeks after fertilization and T5- after harvest) under different fertilizer treatments (mean \pm SD, n=3).

Sampling time	Treatment	OM/%		pH-H ₂ O		Total P/g kg ⁻¹ DW		Plant available P/g kg ⁻¹		K/g kg ⁻¹ DW	
		0-10cm	10-30cm	0-10cm	10-30cm	0-10cm	10-30cm	0-10cm	10-30cm	0-10cm	10-30cm
T2	CON	5.34 \pm 0.09	5.49 \pm 0.04	6.24 \pm 0.20	6.25 \pm 0.17	0.81 \pm 0.02	0.81 \pm 0.05b	0.31 \pm 0.02b	0.32 \pm 0.03	1.32 \pm 0.02b	1.35 \pm 0.11
	MF	5.35 \pm 0.26	5.39 \pm 0.05	6.06 \pm 0.18	6.16 \pm 0.28	0.86 \pm 0.04	0.84 \pm 0.04ab	0.35 \pm 0.02ab	0.33 \pm 0.03	1.68 \pm 0.15a	1.38 \pm 0.14
	BP	5.42 \pm 0.25	5.28 \pm 0.14	6.49 \pm 0.07	6.43 \pm 0.45	0.84 \pm 0.00	0.83 \pm 0.01ab	0.32 \pm 0.01b	0.32 \pm 0.03	1.29 \pm 0.10b	1.38 \pm 0.09
	FSP	5.49 \pm 0.19	5.33 \pm 0.11	6.28 \pm 0.01	6.38 \pm 0.05	0.90 \pm 0.13	0.84 \pm 0.02ab	0.32 \pm 0.01b	0.32 \pm 0.01	1.37 \pm 0.21ab	1.36 \pm 0.04
	CAT1	5.69 \pm 0.19	5.50 \pm 0.06	6.32 \pm 0.27	6.38 \pm 0.16	0.89 \pm 0.10	0.90 \pm 0.01a	0.35 \pm 0.02ab	0.33 \pm 0.02	1.30 \pm 0.10b	1.37 \pm 0.06
	FER3	5.42 \pm 0.12	5.47 \pm 0.32	6.38 \pm 0.43	6.22 \pm 0.16	0.85 \pm 0.03	0.87 \pm 0.01ab	0.37 \pm 0.01a	0.35 \pm 0.01	1.43 \pm 0.07ab	1.4 \pm 0.030
T5	CON	5.70 \pm 0.16	5.70 \pm 0.05	6.18 \pm 0.16ab	6.20 \pm 0.07	0.80 \pm 0.02b	0.78 \pm 0.03	0.31 \pm 0.02	0.31 \pm 0.02	1.39 \pm 0.10	1.52 \pm 0.03
	MF	5.75 \pm 0.14	5.74 \pm 0.16	6.30 \pm 0.20ab	6.37 \pm 0.20	0.86 \pm 0.02ab	0.80 \pm 0.05	0.32 \pm 0.03	0.31 \pm 0.04	1.49 \pm 0.06	1.43 \pm 0.07
	BP	5.97 \pm 0.15	5.84 \pm 0.28	6.57 \pm 0.15a	6.26 \pm 0.11	0.84 \pm 0.05ab	0.82 \pm 0.02	0.31 \pm 0.02	0.30 \pm 0.02	1.22 \pm 0.12	1.35 \pm 0.17
	FSP	5.68 \pm 0.15	5.57 \pm 0.12	6.12 \pm 0.12b	6.18 \pm 0.08	0.87 \pm 0.02ab	0.84 \pm 0.01	0.35 \pm 0.03	0.32 \pm 0.00	1.31 \pm 0.18	1.50 \pm 0.13
	CAT1	5.74 \pm 0.11	5.61 \pm 0.15	6.08 \pm 0.15b	6.22 \pm 0.13	0.93 \pm 0.08a	0.84 \pm 0.01	0.34 \pm 0.05	0.31 \pm 0.01	1.33 \pm 0.03	1.47 \pm 0.07
	FER3	5.60 \pm 0.13	5.37 \pm 0.16	6.19 \pm 0.11ab	6.18 \pm 0.06	0.87 \pm 0.03ab	0.87 \pm 0.07	0.32 \pm 0.01	0.32 \pm 0.02	1.38 \pm 0.03	1.40 \pm 0.15

Unfertilized (CON), mineral fertilizers (MF) and biobased fertilizers: bokashi pellet (BP), fish sludge pellet (FSP), protein fraction (CAT1) and NPK solution with amino acids (FER3); OM, organic matter. Different letters indicate significant differences between treatments at $p < 0.05$ with Tukey-Kramer (HSD) test.



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Table 50 Soil physical-chemical characteristics in soil 0-10 and 10-30 cm layers at two sampling times (T2- two weeks after fertilization and T5- after harvest) under different fertilizer treatments (mean \pm SD, n=3). (Continued)

Sampling time	Treatment	pH-KCl		Mg/g kg ⁻¹ DW		Ca/g kg ⁻¹ DW		Na/g kg ⁻¹ DW		S/g kg ⁻¹ DW	
		0-10cm	10-30cm	0-10cm	10-30cm	0-10cm	10-30cm	0-10cm	10-30cm	0-10cm	10-30cm
T2	CON	5.26 \pm 0.06	5.35 \pm 0.12	1.84 \pm 0.02b	1.82 \pm 0.07	2.86 \pm 0.10	2.80 \pm 0.21	0.07 \pm 0.00ab	0.06 \pm 0.02	0.21 \pm 0.00b	0.20 \pm 0.01b
	MF	5.37 \pm 0.26	5.36 \pm 0.34	2.37 \pm 0.22a	1.88 \pm 0.02	3.44 \pm 0.19	2.93 \pm 0.21	0.07 \pm 0.01a	0.06 \pm 0.00	0.25 \pm 0.01a	0.22 \pm 0.01ab
	BP	5.66 \pm 0.11	5.39 \pm 0.21	1.92 \pm 0.11b	1.95 \pm 0.10	3.18 \pm 0.07	3.04 \pm 0.15	0.05 \pm 0.01b	0.05 \pm 0.01	0.21 \pm 0.01b	0.21 \pm 0.01b
	FSP	5.35 \pm 0.08	5.37 \pm 0.09	1.98 \pm 0.17b	1.97 \pm 0.12	3.27 \pm 0.56	3.02 \pm 0.15	0.05 \pm 0.01b	0.04 \pm 0.00	0.22 \pm 0.01b	0.21 \pm 0.00b
	CAT1	5.51 \pm 0.37	5.48 \pm 0.22	1.92 \pm 0.05b	1.99 \pm 0.08	3.21 \pm 0.29	3.20 \pm 0.14	0.05 \pm 0.01b	0.06 \pm 0.00	0.22 \pm 0.02b	0.23 \pm 0.01a
	FER3	5.66 \pm 0.37	5.35 \pm 0.07	2.02 \pm 0.08ab	1.99 \pm 0.08	3.19 \pm 0.04	3.06 \pm 0.09	0.05 \pm 0.01b	0.05 \pm 0.01	0.21 \pm 0.00b	0.22 \pm 0.01ab
T5	CON	5.33 \pm 0.15	5.35 \pm 0.05	2.03 \pm 0.05	2.07 \pm 0.08	3.23 \pm 0.17	3.12 \pm 0.20	0.07 \pm 0.00	0.07 \pm 0.01	0.21 \pm 0.01	0.20 \pm 0.01
	MF	5.22 \pm 0.16	5.47 \pm 0.27	2.06 \pm 0.06	2.03 \pm 0.11	3.15 \pm 0.08	3.05 \pm 0.20	0.06 \pm 0.00	0.06 \pm 0.01	0.22 \pm 0.01	0.20 \pm 0.01
	BP	5.78 \pm 0.17	5.40 \pm 0.12	2.03 \pm 0.26	1.96 \pm 0.10	3.31 \pm 0.23	2.93 \pm 0.03	0.06 \pm 0.00	0.06 \pm 0.00	0.21 \pm 0.00	0.21 \pm 0.01
	FSP	5.32 \pm 0.10	5.40 \pm 0.10	2.10 \pm 0.04	2.08 \pm 0.13	3.32 \pm 0.13	3.14 \pm 0.10	0.07 \pm 0.01	0.07 \pm 0.01	0.22 \pm 0.00	0.21 \pm 0.00
	CAT1	5.28 \pm 0.18	5.45 \pm 0.20	2.04 \pm 0.02	2.05 \pm 0.05	3.31 \pm 0.37	3.16 \pm 0.21	0.07 \pm 0.01	0.06 \pm 0.01	0.22 \pm 0.01	0.21 \pm 0.01
	FER3	5.26 \pm 0.08	5.34 \pm 0.10	2.27 \pm 0.28	2.02 \pm 0.09	3.52 \pm 0.57	3.13 \pm 0.17	0.07 \pm 0.01	0.06 \pm 0.01	0.22 \pm 0.01	0.22 \pm 0.01

Unfertilized (CON), mineral fertilizers (MF) and biobased fertilizers; bokashi pellet (BP), fish sludge pellet (FSP), protein fraction (CAT1) and NPK solution with amino acids (FER3); OM, organic matter. Different letters indicate significant differences between treatments at $p < 0.05$ with Tukey-Kramer (HSD) test.



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3.2 Spain

In the Spanish case, different enzymatic activities were analyzed to determine the effect on soil main processes.

3.2.1 Alanine aminopeptidase

This enzyme plays a key role in the nitrogen (N) cycle. It hydrolyzes peptides into amino acids, which is a critical step in the depolymerization and mineralization of organic nitrogen. Its activity serves as an indicator of nitrogen cycling processes and can be influenced by fertilizer application, environmental conditions, and other factors. The following Figure 8 shows the alanine aminopeptidase activity across different treatments at two time points: immediately after treatment application and at crop harvest.

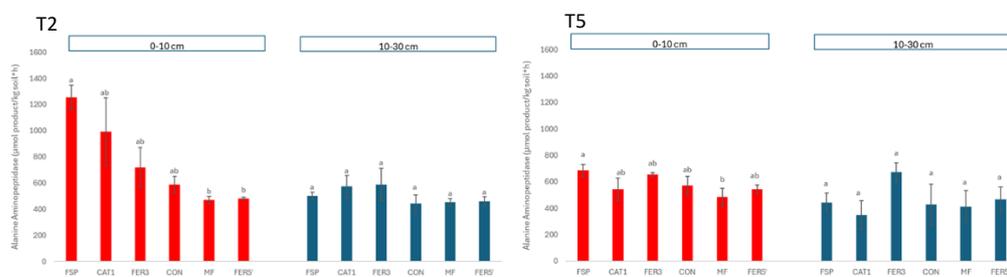


Figure 8 Alanine aminopeptidase enzymatic activity in soil 0-10 cm and 10-30 cm. Sampling time: T2-one week after fertilization; T5-after harvesting. Treatments: fish sludge pellet (FSP); protein fraction (CAT1); NPK solution with amino acids (FER3); unfertilized (CON); mineral fertilizer (MF) and biostimulant from microalgae (FER5').

Regarding alanine aminopeptidase activity (Figures 8), the soil fertilized with FSP showed the highest enzymatic activity in the 0–10 cm soil layer for both T2 and T5. The lowest activity was observed in the mineral fertilizer (MF) treatment in T5, and in both the MF and FER5' treatments in T2. At the 10–30 cm depth, no significant differences were found among treatments.

In all cases, enzyme activity decreased over time, and there were no statistical differences between the treatments at T5. Since enzymatic activity is closely related to the availability of suitable substrates, the decline may be due to substrate depletion. Nonetheless, the application of organic fertilizers (particularly those from FSP and CAT1) appears to enhance soil biological activity related to organic matter processes. Higher



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enzymatic activity was likely observed in treatments fertilized with BBFs that had a higher organic matter content and were subjected to less aggressive processing during production.

3.2.2 Leucine aminopeptidase

This enzyme is associated with nitrogen metabolism in soil, and it showed a response to fertilizer treatments similar to that of alanine aminopeptidase, as shown in Table 7 and Figure 9. This similarity is expected, as both enzymes are involved in similar nitrogen transformation processes. It catalyzes the hydrolysis of leucine and other hydrophobic amino acids from the N-terminus of peptides and proteins helping soil microbes access organic nitrogen by breaking down proteins into usable amino acids, especially leucine. This is crucial in nitrogen-limited environments (Štursová and Baldrian, 2010). Thus, its activity reflects microbial demand for nitrogen and can be used to assess soil biological functioning.

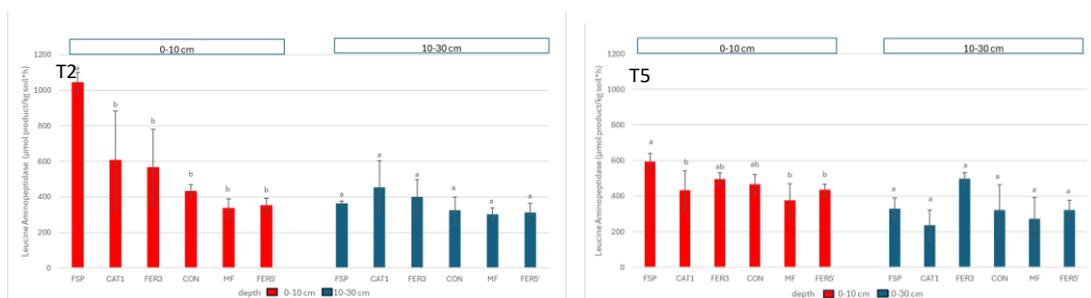


Figure 9 Leucine aminopeptidase enzymatic activity in soil 0-10 cm and 10-30 cm. Sampling time: T2-one week after fertilization; T5-after harvesting. Treatments: fish sludge pellet (FSP); protein fraction (CAT1); NPK solution with amino acids (FER3); unfertilized (CON); mineral fertilizer (MF) and biostimulant from microalgae (FER5').

More relevant results are observed for FSP, as it is the only one showing significant differences as it presents higher OM. This suggests that these results are closely linked to the applied organic matter with BBFs (Table 3).

3.2.3 Beta-glucosidase

Beta-glucosidase (beta-Glu) activity has been found to be sensitive to soil management and has been proposed as a soil quality indicator because it provides an early indication of changes in organic matter status and its turnover. This enzyme provides info related to



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organic matter quality. In the SEA2LAND case, as shown in the figures 103 Beta-glucosidase activity did not show significant differences between treatments, even among those that included organic matter inputs.

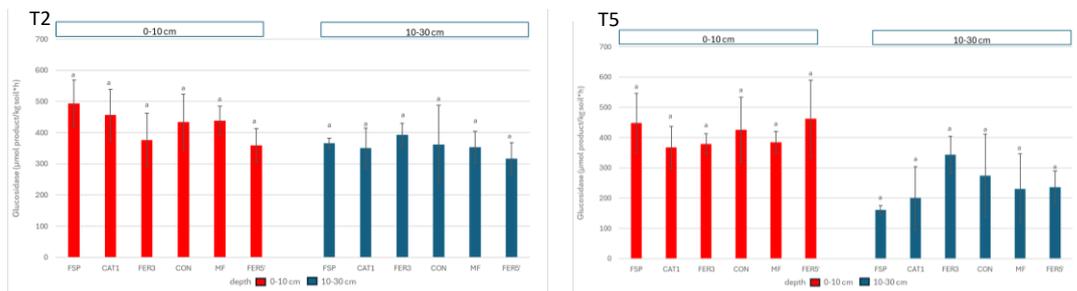


Figure 10 Beta-glucosidase enzymatic activity in soil 0-10 cm. Sampling time: T2-one week after fertilization; T5-after harvesting. Treatments: fish sludge pellet (FSP); protein fraction (CAT1); NPK solution with amino acids (FER3); unfertilized (CON); mineral fertilizer (MF) and biostimulant from microalgae (FER5').

3.2.4 Chitinase

Chitinase activity in soil is vital for nutrient cycling, particularly in facilitating nitrogen release from chitin-rich organic matter. It also plays a key role in strengthening plant defense mechanisms against fungal pathogens. This enzyme, produced by various microorganisms and plant roots, breaks down chitin—an essential component of fungal cell walls and insect exoskeletons—releasing nitrogenous compounds that plants can efficiently absorb and utilize for growth. The treatment has a clear effect on chitinase activity, as shown in the figure 11, with all treatments exhibiting higher activity than the control. Notably, FER3 demonstrates significantly elevated activity at both depths.

In the case of chitinase it can be observed a significant difference between treatments being higher for FER3 and FER5', specifically in the first sampling.

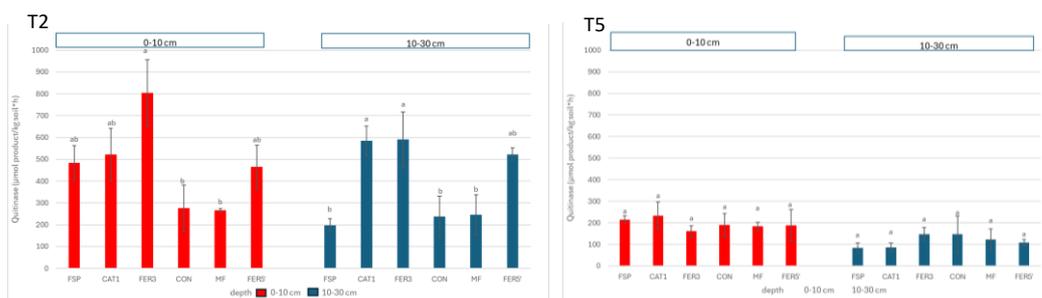


Figure 11 Chitinase enzymatic activity in soil 0-10 cm and 10-30 cm on M5 sampling. FSP, biobased fertilizer from Gronn; CAT1, biobased fertilizer from Catar; FER3, biobased fertilizer from Fertinagro; CON, negative control; MF, mineral fertilizer; FER5', microalgae-based biostimulant.

T5



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This effect diminishes over time. The high chitinase activity observed in the FER3 and FER5' treatments is likely due to the raw materials used in the BBF production, which may contain chitin or chitinase. These components could originate from arthropods included in the trout feed used to produce the hydrolysates for these fertilizers. The chitinase activity may therefore result from the hydrolysis of chitin present in these materials.

Notably, even a very small application—just 1.5 l/ha—produced a clear effect. It is important to emphasize that FER3 and FER5' were applied at biostimulant levels, i.e., less than 1 ml per plant. This suggests that any observed effects should be attributed to the biostimulant action rather than to nutrient supplementation.

3.2.5 Arylsulfatase

During soil organic S mineralization, arylsulfatase (ARS) catalyzes the hydrolysis of organic sulfate esters to sulfate-S. Because of its key role in soil organic S mineralization, ARS activity may reflect the turnover and cycling of S in soil. No significant effects have been recorded for this enzyme as no remarkable differences in soil enzymatic activities (Figure 12).

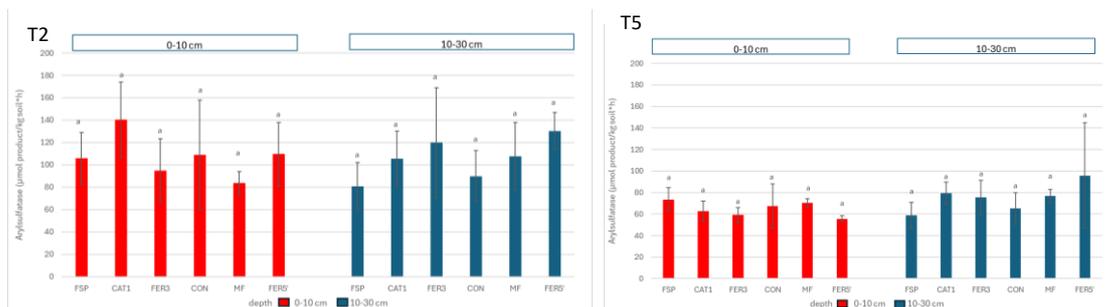


Figure 12. Arylsulfatase enzymatic activity in soil 0-10 cm and 10-30 cm. Sampling time: T2-one week after fertilization; T5-after harvesting. Treatments: fish sludge pellet (FSP); protein fraction (CAT1); NPK solution with amino acids (FER3); unfertilized (CON); mineral fertilizer (MF) and biostimulant from microalgae (FER5').

3.2.6 Dehydrogenase

Dehydrogenases play a significant role in the biological oxidation of soil organic matter (OM) by transferring hydrogen from organic substrates to inorganic acceptors (Zhang et al., 2010). Its importance for soil organic matter decomposition and nutrients release is undoubted. In figure 13 it is shown the effect of different fertilizers on the activity of dehydrogenase. The only significant difference was a higher activity in the FSP treatment compared to the others. The amount of organic matter added could play a role leading



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to this effect. but if we consider that the dehydrogenase increases its activity with time (at least, in short term) for all the studied BBFs (from 0 to 10 cm depth we can conclude that is not the added Organic matter the sole reason for this increment

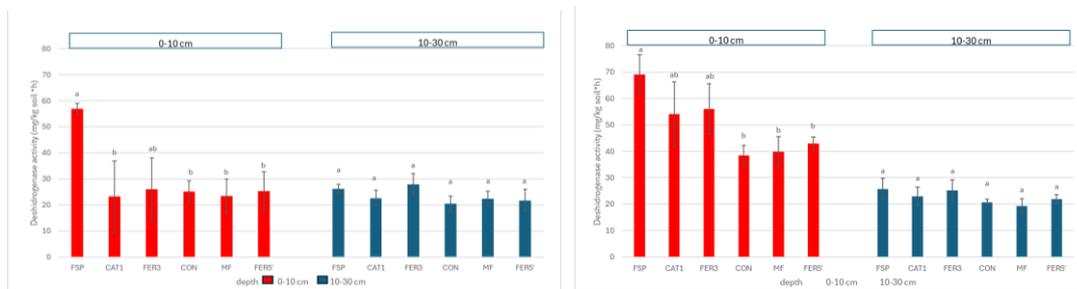


Figure 13 Dehydrogenase enzymatic activity in soil 0-10 cm and 10-30 cm. Sampling time: T2-one week after fertilization; T5-after harvesting. Treatments: fish sludge pellet (FSP); protein fraction (CAT1); NPK solution with amino acids (FER3); unfertilized (CON); mineral fertilizer (MF) and biostimulant from microalgae (FER5')

This increment is more notable for FER3 and CAT1 that duplicate their activity from T2 to T5 while FSP is more stable, that means a slower effect of these fertilizers on soil microbiota that could be due to a nutrient disequilibria (FSP was supplemented with NPK) or a more recalcitrant OM. More studies are needed.

3.2.7 Basal respiration and microbial biomass carbon

Basal respiration reflects the metabolic activity of soil microorganisms under natural conditions, offering insight into the health and vitality of the microbial community high basal respiration means active decomposition of organic matter, which releases nutrients like nitrogen and phosphorus for plant uptake and contributes to carbon cycling. This parameter is closely related to microbial biomass (MB) which defines the functional component of the soil microbiota, primarily responsible for the decomposition of organic matter and nutrient recycling. Moreover, it is considered a sensitive ecological indicator of environmental changes(Witter, 1996). High values in these parameters indicate a good soil health

As it is shown in figures 14 and 15 both parameters (microbial biomass carbon and basal respiration) are consistent with the enzyme activities. Basal respiration shows higher values for FSP and lower values for the control without fertilizer application (CON), due to the higher microbiological activity as discussed above when the results related to the enzyme activities were shown. In contrast to Estonia, in Spain it is only the FSP treatment



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that shows significant results as it happened for most of the enzymatic activities except chitinase.

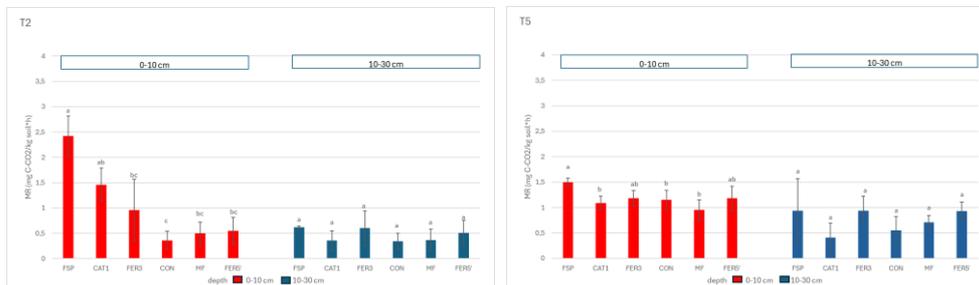


Figure 14 Basal respiration in soil 0-10 cm and 10-30 cm. Sampling time: T2-one week after fertilization; T5-after harvesting. Treatments: fish sludge pellet (FSP); protein fraction (CAT1); NPK solution with amino acids (FER3); unfertilized (CON); mineral fertilizer (MF) and biostimulant from microalgae (FER5').

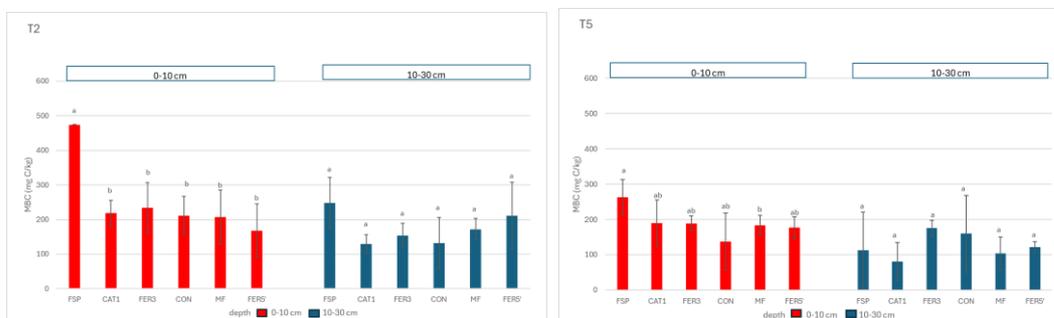


Figure 15 Microbial biomass carbon in soil 0-10 cm and 10-30 cm. Sampling time: T2-one week after fertilization; T5-after harvesting. Treatments: fish sludge pellet (FSP); protein fraction (CAT1); NPK solution with amino acids (FER3); unfertilized (CON); mineral fertilizer (MF) and biostimulant from microalgae (FER5').

4 Conclusions

For both sites (Estonia and Spain) short-term applications of the tested BBFs derived from fishery waste and by-products had minimal influence on the soil's physical and chemical properties but significantly boosted microbial indicators such as microbial biomass carbon, enzymes activity, highlighting an increased microbial activity. These findings suggest that such BBFs can promote soil microbial processes and potentially enhance soil fertility. Thus, it can be stated that soil health and microbiological activity improve, at least temporarily, with the addition of BBFs, especially in the case of enzymes related to organic matter decomposition and nitrogen metabolism. This effect decreases over time probably due to the resilience of the soil which tends to recover the original structure of the microbiome and that the target substrate of the enzymes is depleted so that their activity decreases.



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However, in Estonia, a decrease in soil fauna such as springtails and mites was observed after fertilisation, most likely due to weather conditions, soil disturbance or nutrient imbalances. This happened even though the organic fertilisers derived from fisheries improved soil fertility in the short term compared to the non-fertilised control. This was due to the increased soil activity which enhances the release of nutrients present not only in the fertilisers but also in the soil. However, long-term studies are necessary to fully assess their effects on soil health. Continued use of these fertilisers could probably lead to more stable changes and improved soil health in the medium term, but further studies are needed.

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