FERTIMANURE

Deliverable

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Grant Agreement No. 862849

D4.5. Final - Report on agronomic performance of the obtained BBFs and TMFs in laboratory setting

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Preface

This study was carried out and published as a part of the European demonstration project FERTIMANURE funded by the H2020 programme (project number 862849). The FERTIMANURE project focuses on the implementation of nutrient recovery and reuse technologies at 5 pilot installations with aim to produce bio-based fertilisers (BBFs) from animal manure and tailor-made fertilisers (TMFs) as blends of BBFs and (synthetic) mineral fertilisers for crop specific applications.

One of the tasks within the FERTIMANURE project is to assess BBFs and TMFs produced in the context of FERTIMANURE for their ability to substitute current mineral fertilisers that are produced based on finite fossil-based resources and on high energy consumption. The mentioned assessments take part on laboratory scale and in a full field scale. Deliverable D4.5 'Final - Report on agronomic performance of the obtained BBFs and TMFs in laboratory setting' gives insight into final results of the BBF and TMF testing in laboratory settings, whereas the full field scale results are reported in D4.6 'Final - Report on agronomic and environmental performance in field trial experiences'. The D4.5 more specifically reports on nitrogen (N) and carbon (C) dynamics of tested BBFs via incubation tests, phosphorus (P) plant availability of BBFs by plant growth assay, the effect of biologically activated BBFs, and lastly effect of the produced biostimulant.

We would like to acknowledge the researchers and staff of RITTMO Agroenvironnement (France), Fraunhofer-Institut für Umwelt, Sicherheits und Energietechnik (Germany), Ghent University (Belgium), BETA – University of Vic (Spain) and University of Milano (Italy) for their work and contribution.

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Summary

Deliverable 4.5 ‘‘Final - Report on agronomic performance of the obtained BBFs and TMFs in laboratory setting” is a part of FERTIMANURE work package (WP) 4. The WP4 aims to assess bio-based fertilisers (BBFs; produced in WP2) and tailor-made fertilisers (TMFs; produced in WP3) for their ability to substitute conventional synthetic mineral fertilisers whose production is based on finite fossil-based resources. The results from year 1 were previously reported in D4.1, and D4.5 aims to summarise the final results (of 3-year work) per each sub-task under Task 4.1 ‘Assessment of novel fertilisers under controlled conditions’. The Task 4.1 is divided over the following 4 sub-tasks:

Sub-task 4.1.1. Assessment of N release patterns via soil incubation assay

Sub-task 4.1.2. Assessment of P plant availability via dedicated plant growth assay

Sub-task 4.1.3. Assessment of biological activated bio-based fertilisers

Sub-task 4.1.4. Assessment of biostimulants

The information compiled in this report is divided into 7 chapters. The introduction is given in **Chapter 1**.

Chapter 2 focuses on the assessment of nitrogen (N) release and mineralisation patterns via soil incubation assay. The rate of mineral N (NO_3-N + NH_4-N) that is released over the course of a growing season, determines whether BBF can be deemed as a worthy substitute for mineral N fertilisers which themselves are by definition 100% in the form of mineral N. This assessment was done for ammonium nitrate (BE-AN), ammonium sulphate (BE-AS), ammonium water (BE-AW), nutrient rich concentrate (ES-NC), liquid K fertiliser (FR-LK) and bio-dried solid fraction (ES-DSC). Results have shown that for ammonium salts (BE-AS, BE-AN and BE-AW) N release is 100%, because these BBFs contain N in 100% mineral form. Additionally, application of ammonium salts can even stimulate additional release of mineral N from soil organic matter which is known as priming effect. For ES-NC, depending on the type of membrane systems used (reverse osmosis - RO, microfiltration - MFR or combination of both – MFRO) and freeze concentration, the following N release was determined: ES-NC-RO = 92 % N release, ES-NC-MFR = 85 % N release and ES-NC-MFRO = 52% N release. The FR-LK had N release of 53%, and the lowest N release was measured in ES-DSC amounting to 21%.

Although not initially mentioned in Description of the action (DOA), carbon (C) release from BBFs that are rich in C and therefore might have a potential to contribute to C sequestration has also been assessed and is reported in **Chapter 3**. The only C rich BBFs were biochar (FR-BC and DE-BC) and ES-DSC. All three BBFs are quite stable, with having less than 3% of applied OC being mineralised as CO_2.

Chapter 4 concerns an assessment of P plant availability via a P incubation and dedicated plant growth assay with rye-grass. The P potential was assessed for ES-DSC, ES-NC, P ashes (ES-PA), ammonium phosphate (DE-AP) and biochar (FR-BC and DE-BC). In P incubations the following P release was noted: ES-DSC = 76% P release, ES-PA = 54% P release, ES-NC-MFRO = 53% P release and ES-NC-MFR = 48% P release, as compared to 100% P release of triple superphosphate. In pot trial with ryegrass, on the other hand, the same BBFs exhibited higher yield and P fertiliser replacement value (PFRV) than TSP. When it comes to DE-AP, it was assessed only in pot trial and ammonium phosphate on perlite resulted in lower crop yield and PFRV of 45% as the distribution of mono ammonium phosphate on the perlite material may be uneven, less P was available to the plants than intended by the fertilisation plan. Therefore, an isolation of the mono ammonium phosphate from perlite in its pure form was done and this BBF resulted in similar performance as TSP, with a PRFV value of 106%. For biochars, depending on the type of production process applied and used feedstock, different PFRVs were noted: 38% - 48% PFRV for FR-BC, and 100% PFRV for DE-BC.

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Chapter 5 involves the testing of biological activated BBFs by adding the microbial consortia obtained in WP3 to selected BBFs and testing the activated BBFs in tomato, radish and lettuce pot trial to check the effect of microbial consortia on crop yield and quality. In tomato cultivation, the increased efficacy of microbial consortia in terms of total productivity respect to the non-activated treatments was observed. In radish an effect of BBF treatments was not noted, whether activated or not, probably due to the short cultivation cycle. In lettuce the application of BBFs exerted a positive effect on quantitative (yield) parameters, particularly the treatment with biochar (FR-BC) and the activated biochar (FR-BC_A).

Chapter 6 deals with the assessment of biostimulants in terms of efficiency as plant growth promoters for nutrient uptake and tolerance against abiotic stress (hydric, saline and temperature stress). In tomato cultivation during hydric stress, AA-biostimulant increased crop yield by 54% and 16%, respectively, as compared to the control fertilisation and commercial biostimulant; whereas in saline stress no effect of AA-biostimulant was seen as the salt stress was probably not high enough to produce stress conditions. In spinach under hydric stress the general effect of the AA-biostimulant was not observed, but rather depended on the stress level applied. Under saline stress, AA-biostimulant increased the crop yield on average by c. 25%. In lettuce, AA-biostimulant effect observed only under hydric stress, and not under saline stress. For swiss chard under temperature stress there was no effect on the yield, however, AA-biostimulant application increased chlorophyll and reduced proline levels (i.e. reduced the stress overall).

Finally, **Chapter 7** discusses the overall conclusion and recommendations on the performance of all the tested BBFs from FERTIMANURE pilots.

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Content

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List of Tables

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List of Figures

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List of Abbreviations

AN	Ammonium nitrate
ANR	Apparent nitrogen recovery
APR	Apparent phosphorus recovery
AS	Ammonium sulphate
AW	Ammonia water
BBF	Bio-based fertiliser
CAN	Calcium ammonium nitrate
DM	Dry matter
EC	Electrical conductivity
FM	Fresh matter
FPR	Fertilizing Products Regulation
GHG	Greenhouse gas emission
LF	Liquid fraction
MAP	Mono ammonium phosphate
MF	Microfiltration
NFRV	Nitrogen fertilizer replacement value
NRR	Nutrient recovery and reuse
OC	Organic carbon
OM	Organic matter
PFRV	Phosphorus fertilizer replacement value
PS	Pig slurry
RO	Reverse osmosis
SF	Solid fraction
SOM	Soil organic matter
TMF	Tailor-made fertiliser
TOC	Total organic carbon
TSP	Triple superphosphate
WFPS	Water filled pore space
WHC	Water holding capacity

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1. Introduction

In keeping with the idea of a circular economy, nutrient recovery from biomass streams like animal manure has accelerated, leading to the development of bio-based fertilisers (BBFs). The European Commission has implemented the EU Fertilising Products Regulation (FPR, 2019/1009), which took effect on July 16, 2022, to ease the transition. The regulation's main objective is to promote the manufacture of fertilisers from renewable raw resources that fall under certain categories. The use of organic and organo-mineral fertilisers is receiving a lot of attention. However, the regulations on the use of animal manure derived BBFs are still not fully clear.

The recent work of European Commission's Joint Research Centre proposes harmonised standards for nitrogen (N) fertiliser obtained from manure that may be applied above the N application standard for manure as a replacement for (synthetically produced) mineral N fertilisers (Huygens et al., 2020). The implementation of these proposed harmonised standards could permit the use of N fertilisers, either partially or entirely derived from processed manure, in areas subject to the 170 kg total N/ha/yr limit set by the Nitrates Directive (91/676/EEC). This implementation, however, is not yet initiated. Therefore, the potential of animal manure BBFs remains to be not fully explored.

FERTIMANURE project aims to stimulate further processing of animal manure and assess agronomic and environmental performance of recovered BBFs as compared to their conventional counterparts (i.e. synthetic mineral fertilisers). Specifically FERTIMANURE will develop, integrate, test and validate innovative Nutrient Management Strategies to efficiently recover mineral nutrients and other products with agronomic value from manure, to finally obtain reliable and safe fertilisers that can compete on the European Union (EU) fertilisers market. This will be achieved by:

(i) implementing **5 on-farm experimental innovative and integrated nutrient recovery pilots** in the most relevant European countries in terms of livestock production (Spain, France, Germany, Belgium, the Netherlands), and

(ii) addressing the **nutrient management through 3 different strategies** adapted to mixed and specialised farming systems:
   a. (Strategy #1) On-farm production and use of BBF,
   b. (Strategy #2) On-farm BBF production and Centralised TMF production and
   c. (Strategy #3) On-farm TMF production and use.

One of the project tasks is to assess BBFs from animal manure and TMFs containing manure-derived nutrients for their ability to substitute current synthetic mineral fertilisers whose production is based on finite fossil-based resources. Deliverable D4.5 *Final - 'Report on agronomic performance of the obtained BBFs and TMFs in laboratory setting'* reports on overall results of work done in laboratory settings within the 3 year period 2021-2023, whereas the full field scale results from the same period (2021-2023) are reported in D4.6 *'Final - Report on agronomic and environmental performance in field trial experiences'*.

The main aim of D4.5 is to analyse the agronomical impact of BBFs in terms of N, C and P dynamics, biological activated BBFs and biostimulants performance (Table 1). It is hypothesized that the application of recovered products could (a) increase fertiliser efficiency and decrease nutrient losses as compared to the use of raw manure/digestate, and (b) increase C storage in agricultural soils as compared to the use of synthetic fertiliser.

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Table 1. List of BBFs tested by consortium partners under the FERTIMANURE project in laboratory setting (Task 4.1). '-' indicates that respective BBF is not considered for laboratory assessment or the mentioned sub-task assessment is not relevant for the respective BBF.

Pilot	BBF product description	Codes	N incubation	C incubation	P availability	Biological activated BBFs	Biostimulant
NL	Ammonium sulphate solution	NL-AS	-	-	-	-	-
	Liquid K-fertiliser	NL-LK	-	-	-	-	-
	Soil conditioner	NL-SC	-	-	-	Done	-
	Wet organic P-rich fertiliser	NL-WP	-	-	-	-	-
	90% dried organic P-rich fertiliser	NL-DP	-	-	-	-	-
ES	Nutrient-rich concentrate	ES-NC	Done	Done	Done	-	-
	Bio-dried solid fraction	ES-DSC	Done	Done	Done	Done	
	Phosphorous (ashes)	ES-PA	-	-	Done	-	-
	Ammonium salts	ES-AS	Done	-	-	-	-
	AA-based biostimulants	ES-AA	-	-	-	-	Done
DE	Biochar	BE-BC	Done	Done	Done	-	-
	Ammonium phosphate on perlite	DE-AP	Done	-	Done	-	-
BE	Ammonium nitrate	BE-AN	Done	-	-	-	-
	Ammonium sulphate	BE-AS	Done	-	-	-	-
	Ammonium water	BE-AW	Done	-	-	-	-
FR	Biochar	FR-BC	-	Done	Done	Done	-
	Ammonium sulphate	FR-AS	Done	-	-	-	-
	Liquid K-fertiliser	FR-LK	Done	-	-	-	-

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2. Assessment of nitrogen release patterns via soil incubation assay

N use efficiency and the ability of BBF/TMF to substitute mineral fertilisers is fully dependent on the speciation of applied N following application to the soil. The rate of mineral species (i.e. nitrate + ammonium) that is released over the course of a growing season, determines whether BBF/TMF can be deemed as a worthy substitute to mineral fertilisers which themselves are by definition 100% in the form of mineral N species. Within the FERTIMANURE project, N release dynamics were assessed and compared with mineral fertilisers in soil incubation assays under controlled conditions and in absence of plants. This was achieved by collecting and homogenising soil of different soil texture classes in different treatments containing the BBF/TMF, mineral fertiliser, raw animal manure and unfertilised blank treatment. Soils amended or not with BBF/TMF were incubated at fixed temperature and moisture and those samples were scarified at fixed times to estimate mineral N content. These results serve to determine N release and N mineralisation potential of applied BBF/TMF throughout the time by correcting it for the effect of the soil stock (i.e. mineralisation from soil organic matter (SOM)) and allow to compare N release from the evaluated BBF as compared to the release performance of mineral fertilisers.

N incubation experiments were performed by the following partners: UGhent (Belgium), Fraunhofer (Germany), RITTMO (France) and UVIC-UCC (Spain). In this chapter the following BBFs were assessed for their N dynamics:

- **RITTMO**: ammonium sulphate (FR-AS) and liquid K-fertiliser (FR-LK)
- **Fraunhofer**: mono-ammonium phosphate on perlite (DE-AP1) and biochar (DE-BC)
- **UGent**: ammonium nitrate (BE-AN), ammonium sulphate (BE-AS) and ammonium water (BE-AW)
- **UVIC-UCC:** bio-dried solid fraction (ES-DSC), ammonium sulphate (ES-AS) and nutrient rich concentrate (ES-NC) from the treatment of liquid fraction via combination of different membrane systems and freeze concentration

Each partner followed their own local/national protocol (detailed description can be found in D4.4 *'Homogenised procedures to assess agronomic performance in pot tests and field trials'*). However, in order to harmonise reporting, the results of incubation trials are expressed using net N-release and N-mineralisation rate. The net N-release ($N_{rel,net}$, Equation (1)) defines the net N release as the difference between the mineral N available in the fertilised soil and the mineral N available in the control (Ncontrol) treatment, as follows (De Neve and Hofman, 2000):

$$N_{rel,net}(\%) = \frac{([NO_3^- - N,treatment] - [NO_3^- - N,control]) + ([NH_4^+ - N,treatment] - [NH_4^+ - N,control])}{N_{total\ applied}} \times 100 \quad \textit{(Eq. 1)}$$

At t = 0, the $N_{rel,net}$ (%) equals the product $N_{mineral}/N_{total}$ ratio x 100. $N_{min,net}$ (%) is the N mineralised from the organic fraction of the product (expressed as a percentage of total N in the product), and is calculated by subtracting the amount of mineral N already present in the products at t = 0, as follows (Sigurnjak et al., 2017):

$$N_{min,net}(t; \%total\ N) = N_{rel,net}(t) - N_{rel,net}(t = 0) \quad \textit{(Eq. 2)}$$

In the equation above, a positive value denotes net mineralisation, and a negative value denotes immobilisation.

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2.1. Ammonium sulphate (FR-AS), Liquid K-fertiliser (FR-LK) from French pilot and Biochar (DE-BC) from German pilot (RITTMO)

For more information on this study, please contact the authors from RITTMO Agroenvironnement: Lionel Ruidavets (lionel.ruidavets@rittmo.com) or Fiona Ehrhardt (fiona.ehrhardt@rittmo.com).

2.1.1 Introduction

The objective of this experiment was to evaluate the N release contained in BBFs generated from the French pilot (i.e. ammonium sulphate (FR-AS) and liquid K-fertiliser (FR-LK)) and biochar obtained from German pilot (DE-BC). The N release is determined by the incubation of different soil/fertiliser mixtures under controlled conditions, which are then assessed in time for their mineral N concentration. In these laboratory conditions, 91-day monitoring is equivalent to a 1-year full scale field trial.

2.1.2 Methodology

Laboratory incubations were conducted in aluminium cups filled with 25 g of dry soil sieved to 4 mm. Each tested BBF (Table 2) was mixed into the soil at an equivalent dose of 170 kg total N/ha. After that, soil humidity was regulated at 50% of soil water holding capacity (WHC). Afterwards soil mixtures were incubated at 28°C +/- 1°C; WHC 50%, over 91 days during which N mineral forms concentration into the soils were measured at 0, 7, 14, 28, 49, 70 and 91 days. The experiment was done on two different soils: one agricultural soil corresponding to the criteria of the French standard FD U 44-163 : "**standard soil**" (N content of 0.10 % DM, OM content 1.6 %) and one agricultural soil from fields used during maize French fields trials (Brittany region) in FERTIMANURE project (D4.6): "**agricultural soil**" (content of 0.33 % DM, OM content 5.49 %). The following treatments were tested in 3 replicates:

(1) Soil only (negative control)
(2) Soil + FR-AS
(3) Soil + pig slurry (feedstock for FR-AS and FR-LK)
(4) Soil + FR-LK (liquid fraction of pig slurry after NH_3 extraction by N stripping)
(5) Soil + compost (organic reference)
(6) Soil + CAN (calcium ammonium nitrate: mineral reference).
(7) Soil + DE-BC (biochar produced on German pilot)

Table 2. Products characteristics on (g/kg) on fresh weight basis.

Parameters	FR-AS	Compost	Pig slurry	FR-LK	CAN	DE-BC
Dry matter	207	382	13.3	14.3	9980	750.0
Total carbon	0.0	194.5	2.7	1.2	ND	243.6
Total N	43.8	19.1	1.8	0.5	160	17.8
NH_4-N	43.8	2.3	1.4	<0.2	10	<0.2
NO_3-N	0.0	<0.003	<0.2	<0.2	150	ND
Total P	0.0	22.7	0.11	0.10	0.0	ND
Total potassium	0.0	9.84	2.57	2.3	0.0	ND
Total sulphur	163.5	ND	0.12	0.15	0.0	11.8

ND: not determined; FR-AS: ammonium sulphate; FR-LK: liquid K fertiliser; CAN: calcium ammonium nitrate, DE-BC: biochar from cattle manure)

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At each measuring point, mineral N was determined in soil by using 1M KCl extraction. The NH_4-N and NO_3-N measurements were performed by an external laboratory (Laboratoire Départemental d'Analyses et de Recherches de Laon, France) by NF EN ISO 11732 (method by flow analysis and spectrometric detection) and NF EN ISO 13395 (method by flow analysis and spectrometric detection) respectively. Statistical tests (ANOVA) were performed with STATGRAPHICS V15-2-06. A Shapiro-Wilk test was performed to validate the normality of the residuals, and a Levene test was performed for validation of the homogeneity of variances. The means comparison test (student t-test) is carried out for each parameter and for which the ANOVA shows a significant effect at 5% threshold. If the means cannot be compared, a non-parametric Kruskal-Wallis test allows the medians to be compared.

2.1.3 Results and discussion

(i) N release in the standard soil

The average net N release was 123.9 ± 8.6% for FR-AS, 129.6 ± 21.6% for CAN, 59.9 ± 24.1% for pig slurry, 52.8 ± 20.6% for FR-LK, and 20.6 ± 2.9% for compost at the end of day 91 (Figure 1). Products with high mineral N content present similar evolution profile of net N release. For FR-AS and CAN (100% mineral N) N net release showed value near 100% or > 100% compared to initial N mineral applied by day 91. The observed net N mineralisation > 100% could be result of a positive effect on microbial activity present in the soil which may have increased the mineralisation of organic N present in the soils.

In the case of compost treatment, net N release was higher than the initial addition of mineral N. This confirm that a part of organic N was mineralised during the incubation. This was also observed with FR-LK, with an average increase of 12.4% for N mineral content. In the case of pig slurry, a slight positive net mineralisation is also observed during incubations. However, the net N release was slowly released throughout the incubation period and reached the initial value of mineral N applied. This slight increase during incubation may also result from organic N mineralisation.

For DE-BC, even if the N concentration is 1.78%, it is not in mineral form. During incubation, an observation of slight increase in the N content in the soil: + 9.1% +/- 4.6% at 49 days, finally dropping to 0 at the end of the test. DE-BC did not provide any form of mineral N and the N contained in this BBF is not mineralized.

(ii) N release in agricultural soil

The average net N release was 109.6 ± 8.6% for FR-AS, 129.6 ± 21.6% for CAN, 59.90± 36.6% for pig slurry, and 25.1 ± 3.4% for compost at the end of day 91 (Figure 2). Impact of FR-AS was similar to that measured in the standard soil with a positive effect on SOM mineralisation. Results for compost treatment was nearly the same as the results measured with standard soil.

Concerning CAN and pig slurry, dynamic of N net release was different with values measured during incubation that were below the initial mineral N input value to finally reached 100% on the 91st day. One of the main differences between the two soils was the OM content with respectively 1.6 % for standard soil and 5.49 % for agricultural soil. So, in the agricultural soil, this temporary immobilisation of mineral N could be explained by the blocking of part of the N in the microorganisms which could have developed more intensely due to the higher concentration of C.

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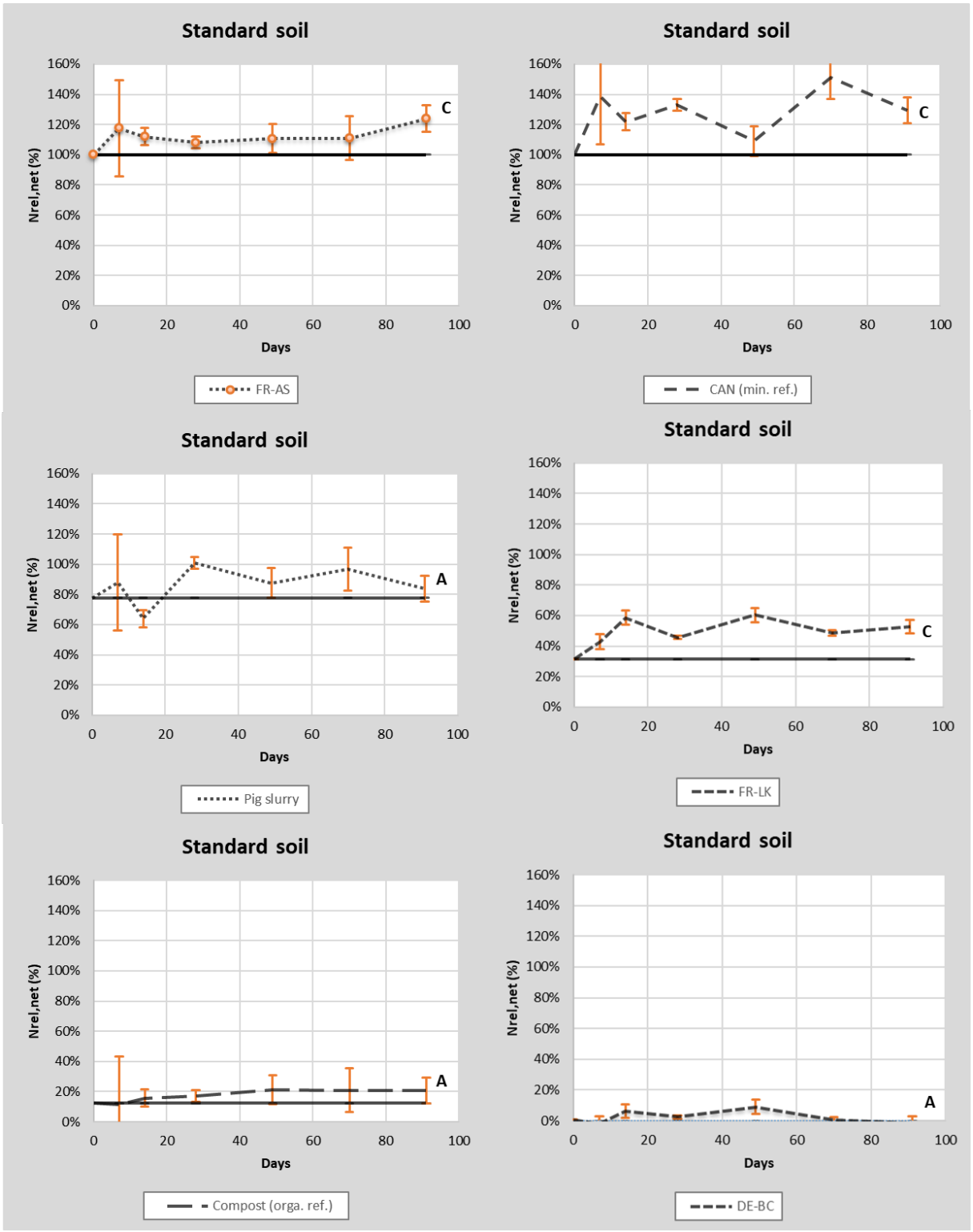

Figure 1. Standard soil N release (Nrel,net; %) relative to the N input of added products in 91-day incubation experiment on standard soil. Value plotted at t = 0 indicates the percentage of mineral N in applied product and is presented with straight line throughout 91 days of incubation time. Error bars indicate standard deviations (n = 3). Letters indicate the statistical difference between treatments (1 letter = 1 group). Values observed above the line indicate net N mineralisation, while values below the line indicate net N immobilisation.

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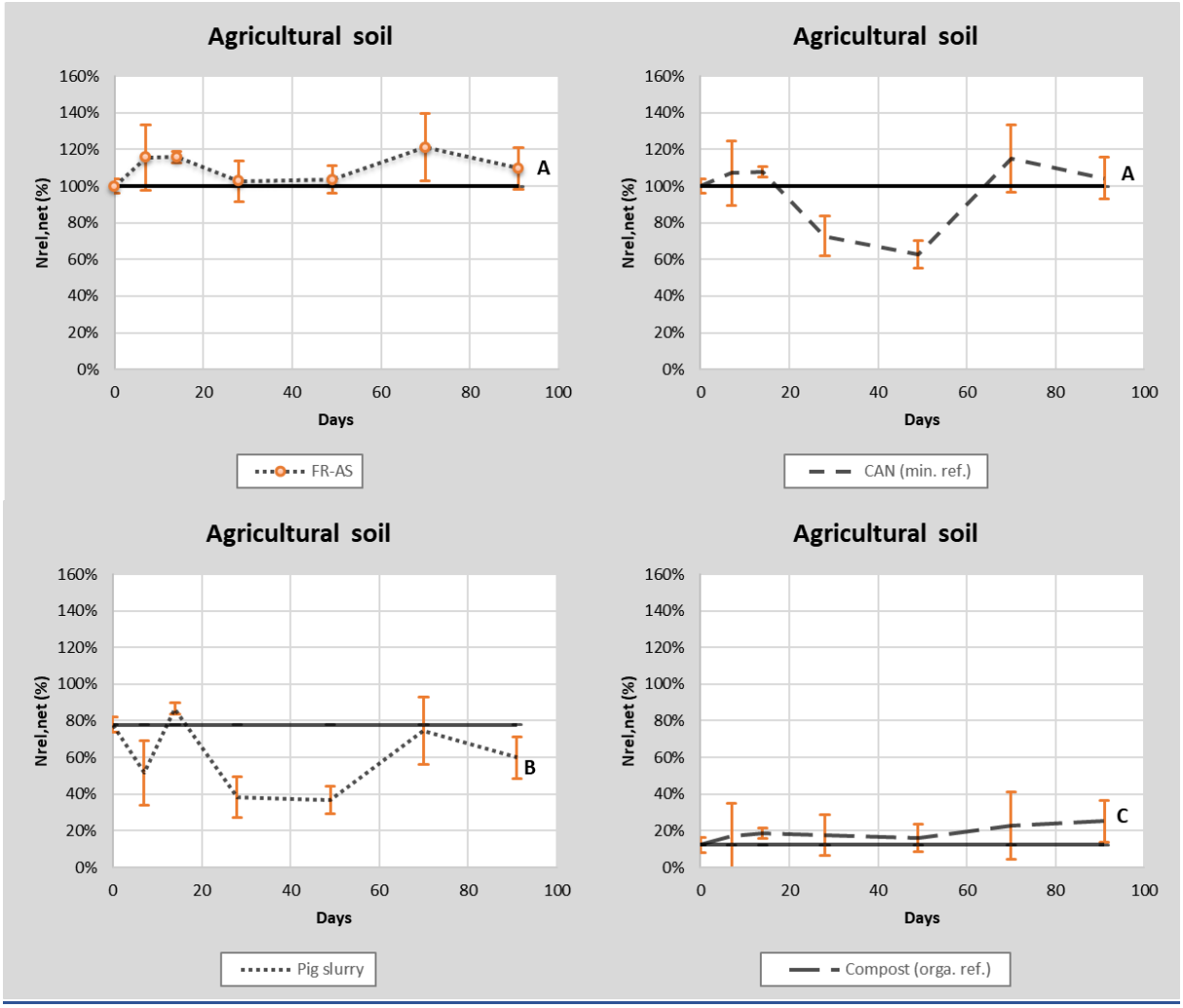

Figure 2. Agricultural soil N release (Nrel,net; %) relative to the N input of added products in 91-day incubation experiment on agricultural soil. Value plotted at t = 0 indicates the percentage of mineral N in applied product (t=0) and is presented with straight line throughout 91 days of incubation time. Error bars indicate standard deviations (n = 3). Letters indicate the statistical difference between modalities (1 letter = 1 group). Values observed above the line indicate net N mineralisation, while values below the line indicate net N immobilisation.

2.1.4 Conclusion and recommendation

This experiment compared the dynamics of the N released by the BBFs in comparison with controls of synthetic mineral N (CAN), and the raw manure from which they are derived. FR-AS acted like a synthetic mineral N fertiliser as results demonstrated that 100% of applied total N remained in mineral form during incubation. FR-AS supplies the soil with mineral N and it seems that its addition to the soil induced a positive effect with a stimulation of soil N mineralisation. In case of FR-LK, the mineral N supplied remained to be available throughout the course of incubation. DE-BC did not provide any form of mineral N and the N contained in this BBF is not mineralized.

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2.2. Mono-ammonium phosphate (DE-AP) and biochar (DE-BC) from German pilot (Fraunhofer)

For more information on this study, please contact the author from Fraunhofer Institute: Sophie Schönfeld (Sophie.schoenfeld@umsicht.fraunhofer.de).

2.2.1 Introduction

To verify the ability of BBFs from German pilot, i.e., biochar (DE-BC) and mono-ammonium phosphate on perlite (DE-AP1), to replace conventional synthetic fertilisers, the availability of N in the soil after fertilisation has to be examined. The determining factor is the presence of nutrients available to plants (NO_3-N and NH_4-N). Therefore, N release dynamics after fertilisation were assessed in soil incubation tests.

2.2.2 Methodology

(i) Soil properties

For the soil incubation a soil consisting of 14% sand, 58% silt and 28% clay was used. Following the German classification (Wittmann, 1977) this soil is referred to as silty loam and according to FAO-classification (FAO, 2014) it is a silty clay loam. Primary parameters of the soil were pH 5.2, Corg 2.80%, C/N 9, humus content 4.8% and 16% water content. P_2O_5 and K_2O were determined by using calcium acetate lactate (CAL) method and amounted to respectively 1.5 and 7.5 mg/100 g soil. The very low status of plant available P and K is necessary to judge any effects of the fertilisers in the experiments. The relatively low pH is helpful for the application of fertilisers - some of which have a considerable liming effect (such as biochar: DE-BC has a pH of 12.3) - to prevent an increase of pH above useful values for nutrient availability and plant growth.

(ii) Soil incubation tests on pH

To ensure acceptable pH-values of the soil for the experiments, it was tested in incubation tests how the fertiliser application will change the soil pH. For this, 200 g of dry soil (< 2 mm) were put into a plastic bag, and 25 mL of fully demineralised water was added. The DE-BC was ground with a Retsch cutting mill to a particle size of 0.25 mm, and subsequently sieved to a fineness of 180 µm using a sieve tower. The sieved product was added to the soil in increments of 1.3 g, 2.6 g, 3.9 g, 5.2 g, 7.8 g, 10.4 and 13 g, respectively. As a control treatment, a similar incubation series was carried out with lime (1.3 g, 2.6 g, 3.9 g, 5.2 g and 7.8 g). The bags were sealed and incubated at 22°C for 10 days. Each bag was kneaded twice a day for 2 min. After 10 days the bags were opened and the pH value was determined following the protocol of the Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (method A 5.1.1; VDLUFA, 2016), where 16 g of soil was extracted with 40 mL of a calcium chloride solution (c = 0.0125 mol/L). The pH values were determined with a SevenExcellence pH meter S400 from Metler Toledo using a pH sensor electrode InLab Expert.

(iii) Soil incubation tests N availability

Soil incubation tests for the examination of the N availability were performed in plastic cups with 200 g of soil, which were sealed with cling film to prevent the drying of the samples. In Table 3, the amount of fertiliser and additional balancing nutrients used within the experiments are listed. Table 11 (section 4.2.2) gives an overview of important fertilising parameters of the respective BBFs. After 0, 1, 3, 7, 14, 21, 35, 49 and 70 days the mineral N of each sample was measured. For each fertiliser and each measuring date, 6 replicates were

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carried out. For the overall evaluation, the respective mean values of the data were used. For the measurement of the mineral N, 75 mg of soil was extracted with 300 mL of a c = 0.0125 mol/L $CaCl_2$ solution and filtered afterwards. The content of NH_4-N and NO_3-N was determined spectroscopically by an external laboratory.

Table 3. Amount of fertiliser and additional balancing nutrients in mg used for each 200 g sample of soil.

	Fertiliser	Lime	NH_4NO_3	K_2SO_4	$CaSO_4$	KH_2PO_4	$Na_2HPO_4 \cdot 12\ H_2O$	KCl	$MgCl_2 \cdot 6\ H_2O$
1	-	-	-	-	-	-	-	-	-
2	-	-	143	76	-	108	-	160	82
3	-	600	143	76	-	108	-	160	82
4	300	-	129	-	70	-	181	-	43
5	300	400	129	-	70	-	181	-	43
6	63	-	128	76	-	-	-	219	82
7	63	600	128	76	-	-	-	219	82

1) Control 2) Full Fertilisation 3) Full Fertilisation + Lime 4) DE-BC 5) DE-BC + Lime 6) DE-AP1 7) DE-AP1 + Lime

Regarding the statistics, in our soil incubation trials, we repeated each sample four times to ensure our results were reliable. We used the median as a way to find the middle value of our data. This helped us get a good average, especially since our soil samples were quite different from each other.

2.2.3 Results and discussion

(i) Soil incubation tests on pH

The pH of soil plays a critical role in determining its suitability for plant growth and nutrient availability. In this study, incubation tests were conducted to investigate the effects of adding biochar (DE-BC) or lime on soil pH. Biochar, with a high pH of 12.3, was found to gradually increase soil pH upon application, though not as significantly as lime (Figure 3). Lime, in contrast, led to a more pronounced pH increase, following a typical saturation curve due to carbonate buffering. These findings suggest that both biochar and lime can be used to adjust soil pH, with lime having a stronger and more predictable effect. Proper management of soil pH is essential for optimizing nutrient availability and promoting healthy plant growth. Soil incubation tests with lime addition showed significant fluctuations in NH_4-N and NO_3-N concentrations over time, attributed to microbial activity and nitrification processes (Figure 4-7). Lime application seemed to shift the composition of inorganic N towards NO_3-N, likely due to increased microbial activity favored by less acidic conditions. These findings underscore the importance of pH in influencing nutrient dynamics and microbial processes in soil ecosystems.

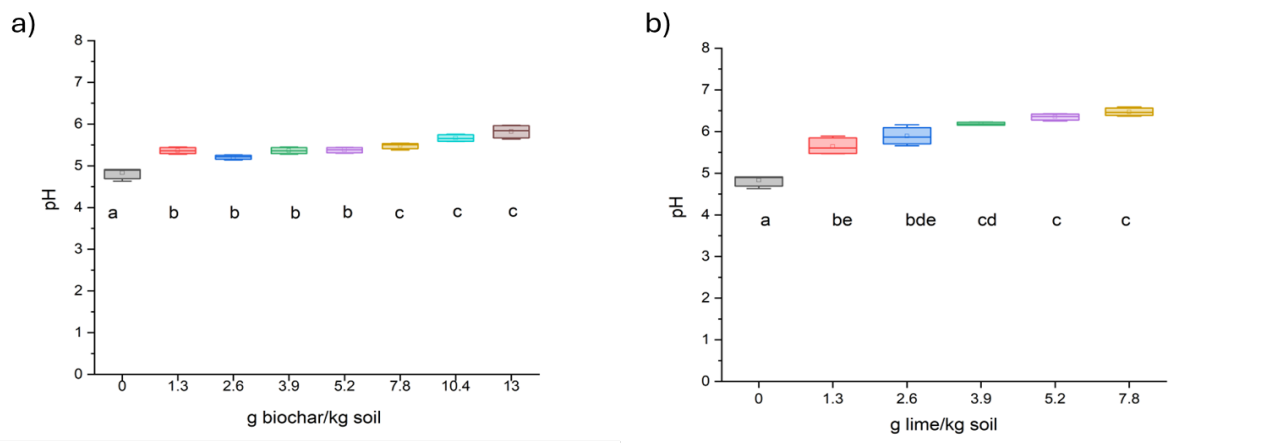

Figure 3. Change of pH upon soil incubation with biochar (DE-BC) over a period of 10 days depending on the quantity of char added (a) and change of pH upon soil incubation with lime over a period of 10 days depending

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on the quantity of lime added (b). Different letters indicate significant differences between the individual test members (Tukey test, $p < 0.05$).

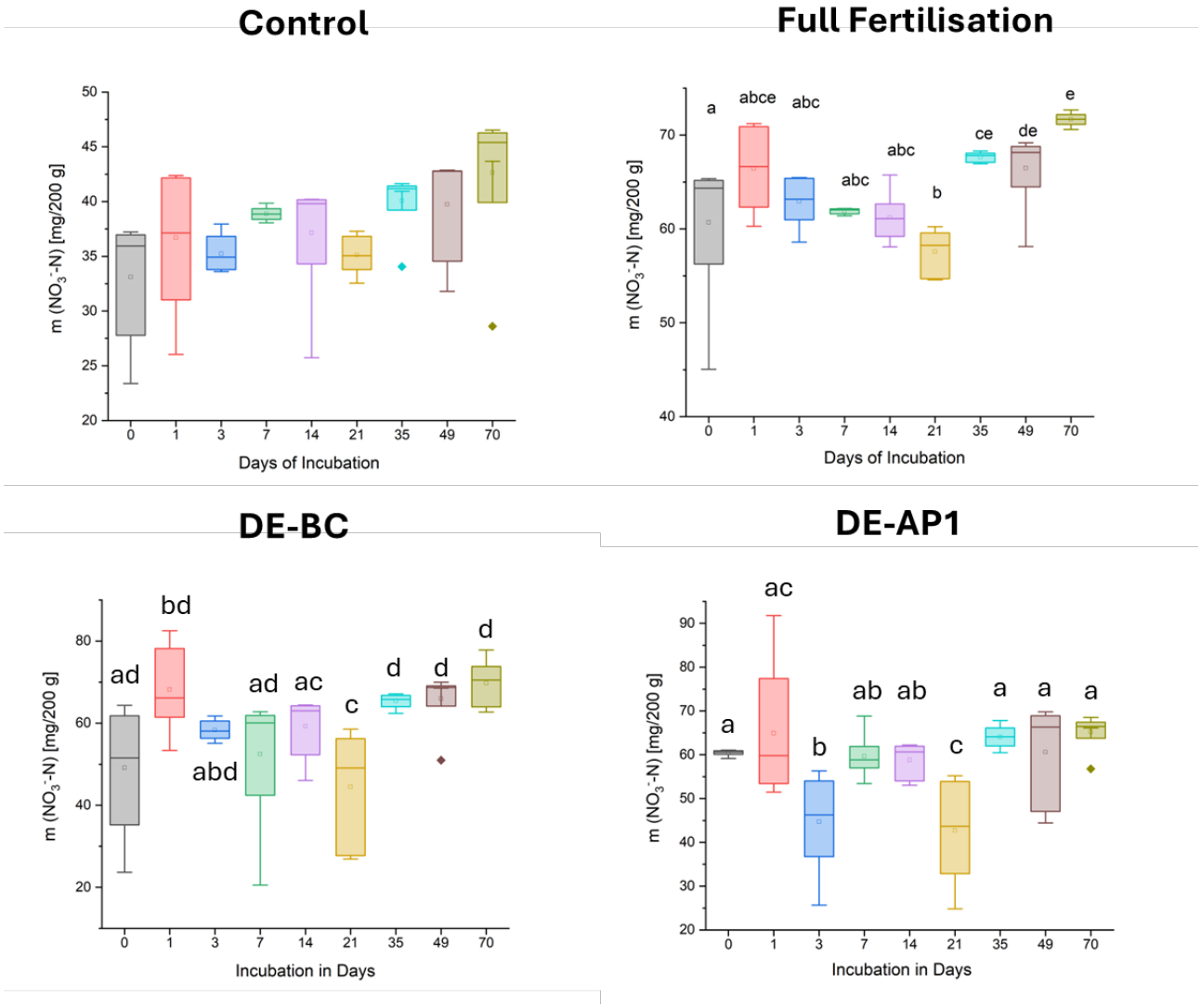

Figure 4. Amount of NO_3-N per 200 g of soil after incubation with the different fertiliser combinations without lime for 70 days. Different letters indicate significant differences between the individual test members (Tukey test, $p < 0.05$).

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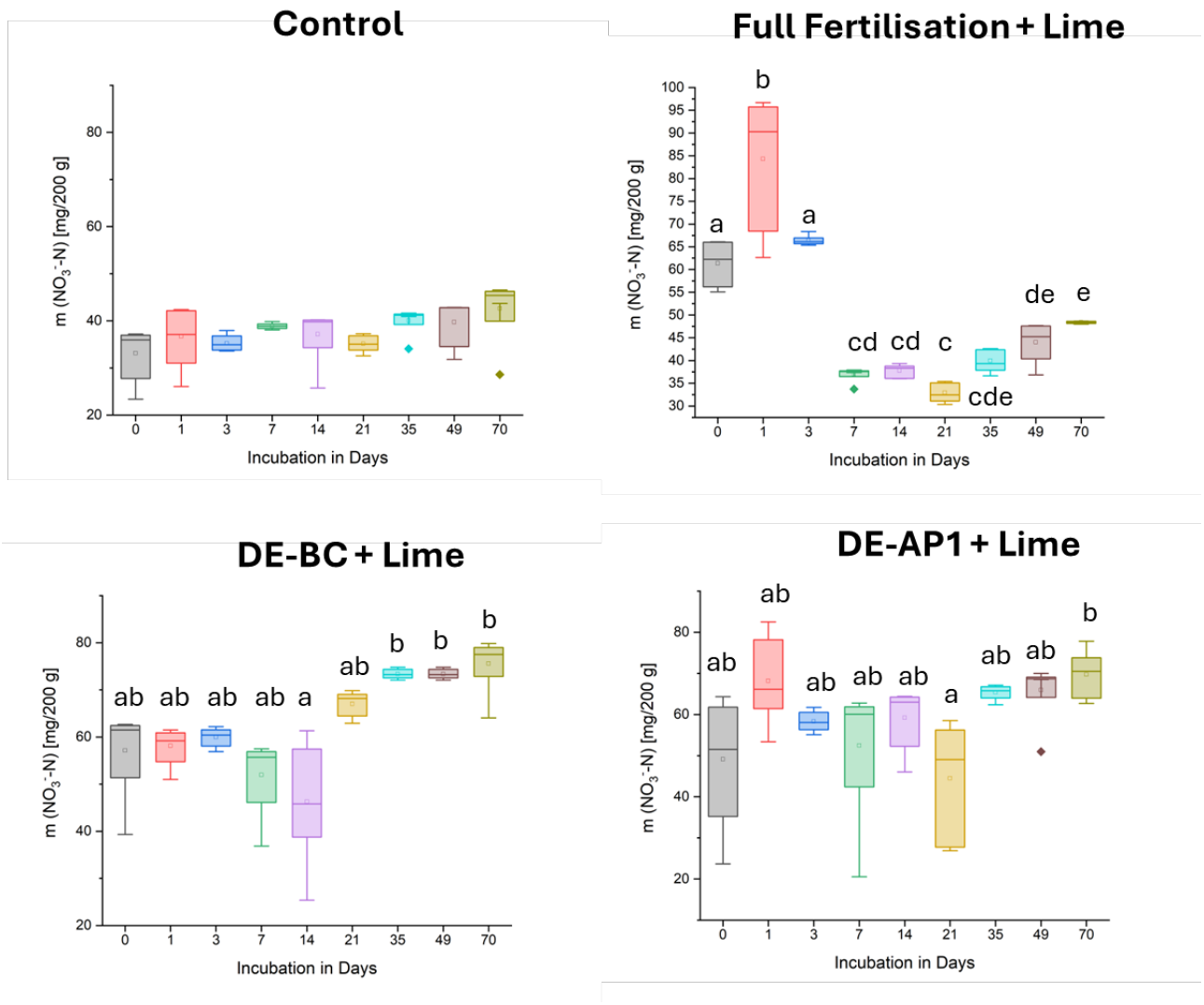

Figure 5. Amount of NO_3-N per 200 g of soil after incubation with the different fertiliser combinations with lime for 70 days. Different letters indicate significant differences between the individual test members (Tukey test, $p < 0.05$).

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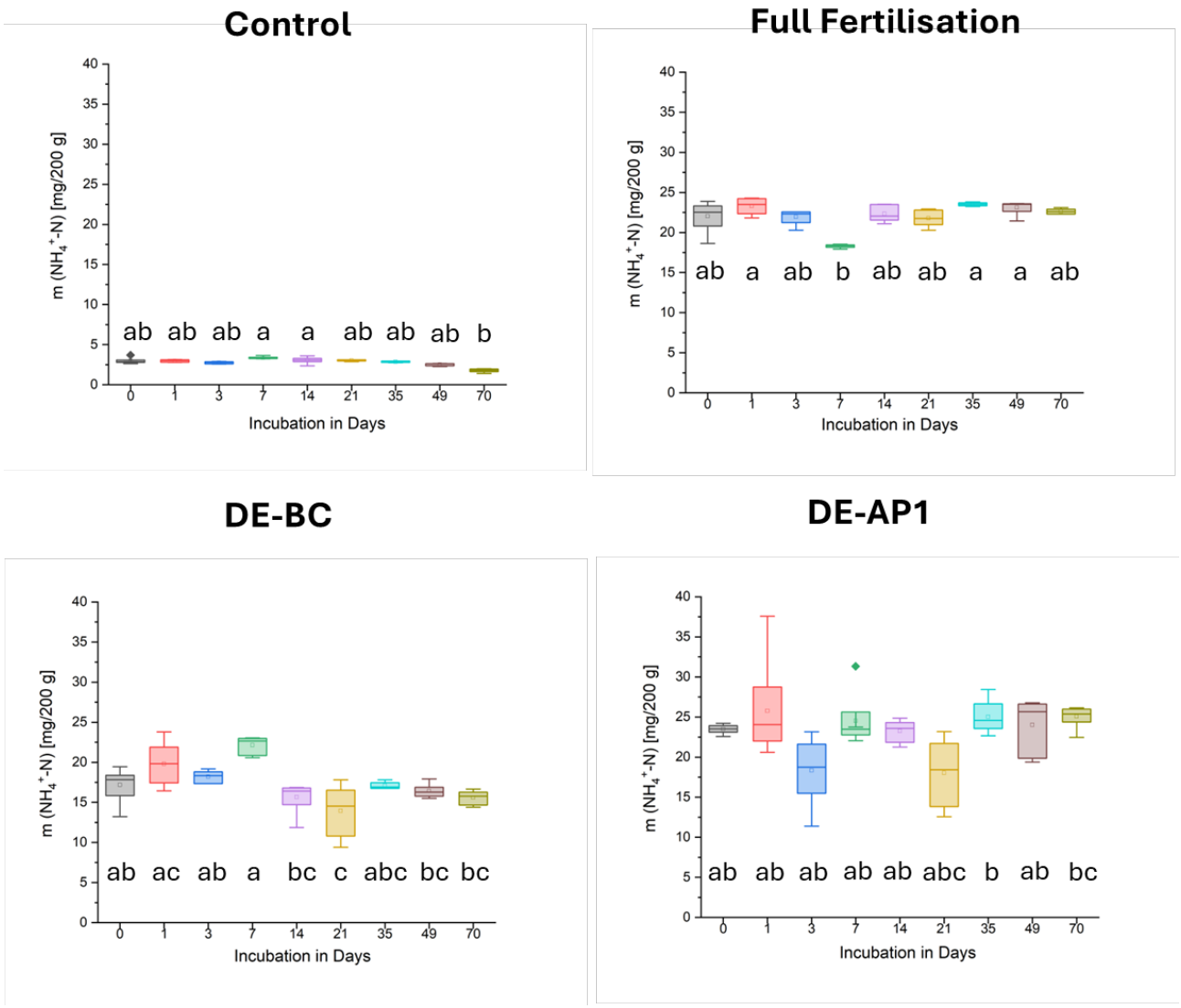

Figure 6. Amount of NH_4-N per 200 g of soil after incubation with the different fertiliser combinations for 70 days. Different letters indicate significant differences between the individual test members (Tukey test, $p < 0.05$).

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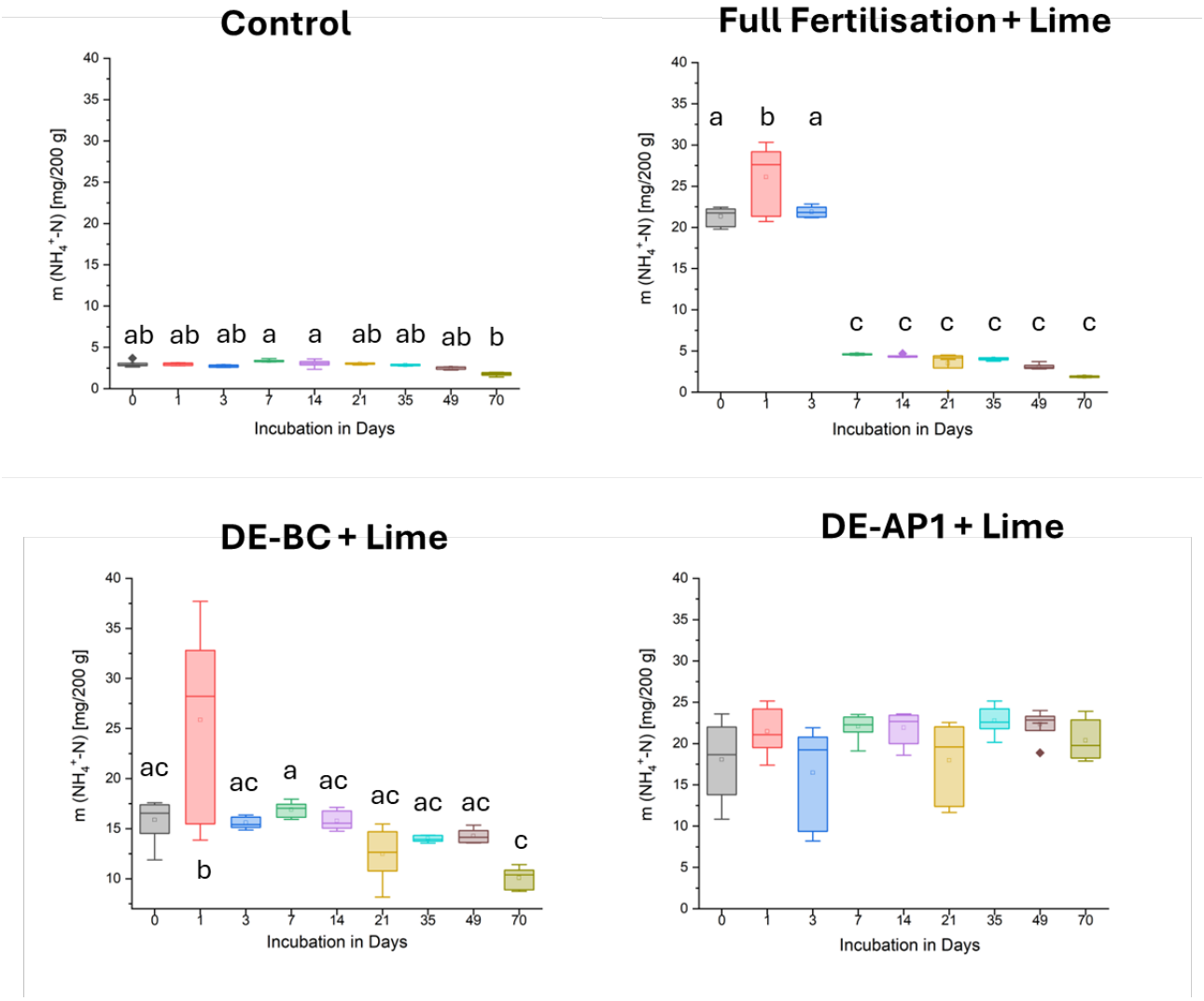

Figure 7. Amount of NH_4-N per 200 g of soil after incubation with the different fertiliser combinations with lime for 70 days. Different letters indicate significant differences between the individual test members (Tukey test, $p < 0.05$).

(ii) Soil incubation tests N availability

The treatment with full fertilisation shows a constant release rate of approx. 73 ± 9 % over the entire period (Figure 8). The DE-BC shows similar behaviour during the first 7 days of the incubation. Afterwards, a clearly lower availability can be observed, which only seems to recover from incubation day 35 onwards but is slightly below the performance of the full fertilisation until the end of the experiment. The DE-AP1 fertiliser shows a constantly high release rate (>60%), similar to the full fertilisation, with the exception of two test days with a significantly lower release rate. This does not necessarily have to be due to the nature of the fertiliser but can also be attributed to experimental reasons. The fact that the release rate is below 100% even with full fertilisation may be due to the adsorption of NH_4-N and NO_3-N towards negatively and positively charged soil particles. Another explanation might be the distribution of the mineral fertiliser within the analysed soil.

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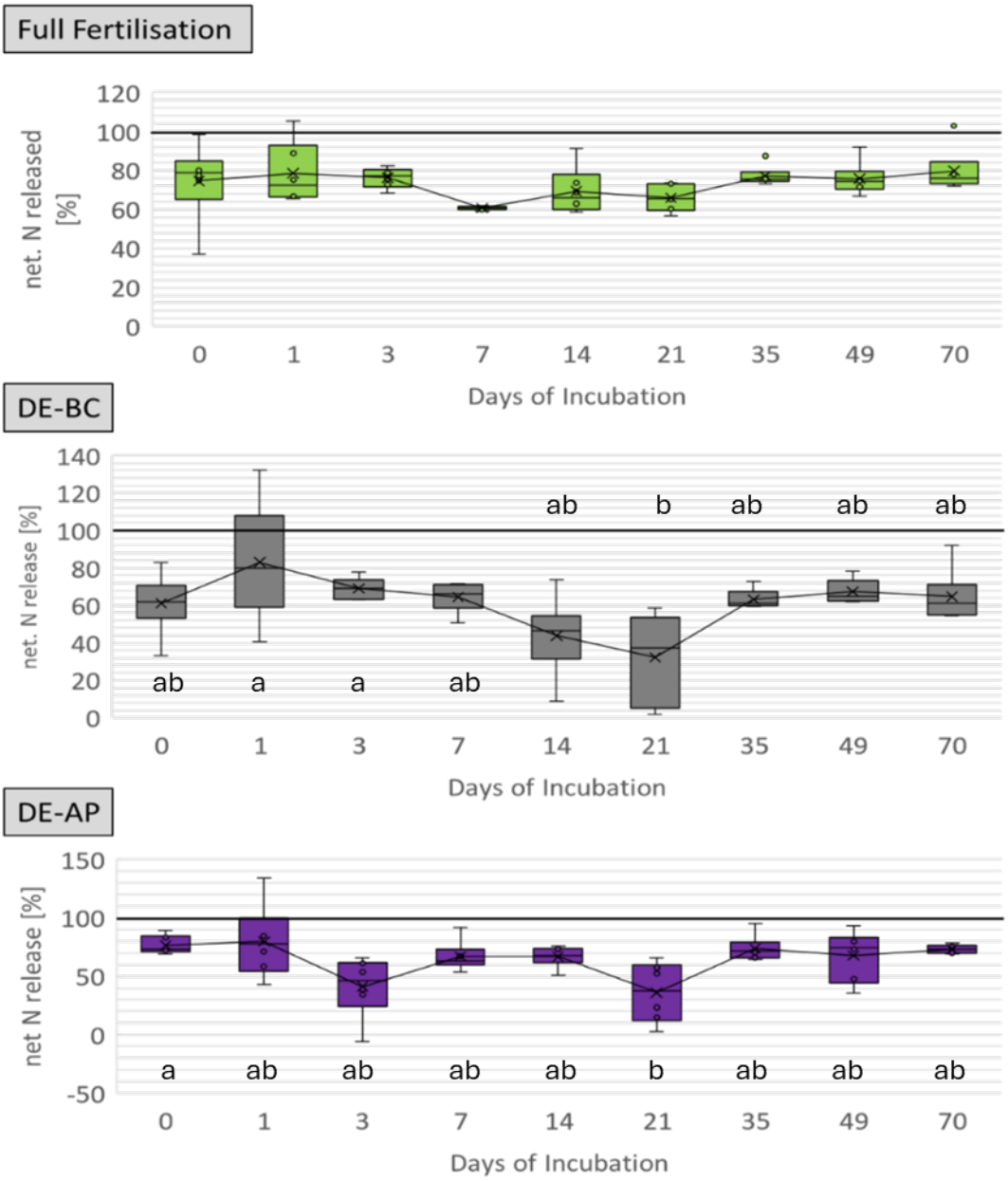

Figure 8. Box plot diagram of the net N release (in % of total N applied) with standard deviations for applied fertilisers over a time span of 70 days.

2.2.4 Conclusion and recommendation

Soil incubation trials with DE-BC and lime are associated with an increase in pH of 0.23 to 0.99 depending on the amount of product added (1 to 13 g biochar (DE-BC) and 1 to 8 g lime). Thanks to buffering effects of the soil, an amendment of the thoroughly alkaline substances is not accompanied by a linear increase of the pH value, which ensures a sufficient amendment in the context of soil fertilisation.

The examined fertilisers show small differences in the availability of mineral N at day 70. While the DE-AP1 is barely different from a purely synthetic fertiliser in terms of mineral N availability (65 ± 15% for MAP vs 73 ±

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9% for Full Fertilisation), with DE-BC fertilisation slightly less mineral N is available (61 ± 13%). This is probably due to the sorption of NH_4-N to biochar through high cation exchange capacity of the biochar. When amended with lime the availability of N is slightly shifted to NO_3-N most likely because nitrification is supported by moderate acid conditions. This was obvious in all treatments. In case of the full fertilisation after only 3 days no additional effect from the fertilisation can be observed. Finally, there is only one explanation possible: there was a technical error which lead for NH_4-N and NO_3-N not to be added during the experiment trial and the data obtained are not representative of a full fertilisation supported by lime.

2.3. Bio-dried solid fraction (ES-DSC), Ammonium sulphate (ES-AS) and Nutrient rich concentrate (ES-NC) from Spanish pilot (UVIC-UCC)

For more information on this study, please contact the authors from UVIC-UCC: Berta Singla Just (berta.singla@uvic.cat) and Laura Diaz-Guerra (laura.diaz.guerra@uvic.cat).

This section will be published as "Singla Just, B., Castaño, O., Guerra-Gorostegui, N., Vilaplana, R., Meers, E., Robles Aguilar, A., Díaz-Guerra, L., Llenas, L. Nitrogen release and kinetics assessment in Bio Based Fertilizers derived from pig manure through a soil incubation study. Under Preparation"

2.3.1 Introduction

This study aimed to estimate the N release using a long-term aerobic incubation from the BBFs obtained from the Spanish pilot. This was done by comparing the dynamics of N-mineralisation in soil amended using BBFs with those in soil amended by conventional mineral fertiliser (CAN) and pig slurry (PS) from the Spanish pilot. The BBFs studied are biodried solid fraction (ES-DSC), ammonium sulphate (ES-AS), and a nutrient-rich concentrate (ES-NC) produced by subjecting the liquid fraction to a combination of various membrane systems and freeze concentration. First NC derives from microfiltration and reverse osmosis retentates treated via freeze concentration (ES-NC-MFRO), second NC is generated from retentate of microfiltration treated by freeze concentration (ES-NC-MFR), and the third, from retentate of reverse osmosis in which membrane contactor was skipped and treated by freeze concentration (ES-NC-RO).

2.3.2 Methodology

(i) Soil properties

The soil used for the incubation experiment was collected from the surface layer (0–30 cm) of a forest soil from the region of Osona (Catalonia, Spain). This soil was poor in nutrients which allowed the detection of different properties of the fertilising products. The main soil parameters were: pH-KCl (1M) = 6.01 ± 0.06; EC (mS ·cm^{-1}) = 0.11 ± 0.00; WHC (%) = 30.36 ± 0.87; bulk density (kg· m^{-3}) = 1348 ± 17; water content (%) = 2.64 ± 0.01; organic matter (%) = 2.97 ± 3.20; total P (mg P · kg soil^{-1}) = 62 ± 3; P soluble in water (mg P · kg soil^{-1}) = 1.65 ± 0.05; P available-CAL = 5.49 ± 0.27; total C (%) = 0.05 ± 0.03; total N (%) = 0.97 ± 0.02.

(ii) BBFs sampling and characterization

The pilot plant was installed at the "Cal Ros" a pig farm located in the municipality of Muntanyola (Barcelona, Spain), under the management of "Cooperativa Plana de Vic". This farm specializes in breeding both sown and fattening pigs. The pilot had the capacity to process up to 3m^3 of pig slurry daily and incorporated two different treatment trains either to treat solid or liquid streams from pig slurry. All BBFs were sourced directly

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from the pilot plant with airtight polyethylene sampling bottles. They were kept at 4°C before their characterization which is shown in the Table 4.

Table 4. Chemical composition of pig slurry (PS), calcium ammonium nitrate (CAN) and the BBFs (ES-DSC, ES-AS, ES-NC-MFRO, ES-NC-MFR and ES-NC-RO) used in the N incubations (mean value ± standard deviation, *n*=3).

Parameter	Unit	PS	CAN	ES-DSC	ES-AS	ES-NC-MFRO	ES-NC-MFR	ES-NC-RO
pH	-	6.39± 0.01	ND	7.19±0.04	ND	7.33±0.01	7.42±0.03	7.64±0.00
EC	$mS \cdot cm^{-1}$	25.49±0.14	ND	*6.5±0.14	ND	22.30±0.46	27.40±0.30	17.20±0.20
Total C	%	3.44±0.22	1.79±0.02	26.74±0.27	0.19±0.00	1.99±0.00	1.89±0.01	0.76±0.01
Total N	%	0.45±0.00	16.25±0.01	2.28±0.02	1.11±0.03	0.38±0.00	0.47±0.01	0.19±0.01
C/N ratio	-	7.64	0.11	11.72	0.17	5.23	4.02	4.00
NH_4-N	$g \cdot kg^{-1}$	3.07±0.01	ND	1.80±0.03	12.28±0.26	2.75±0.03	3.71±0.07	1.59±0.04
NH_4-N/tot N	%	70.33	ND	8.24	101.71	72.10	85	83.73
DM	%	5.39 ±0.60	ND	66.57±0.28	20.51±2.66	3.31±0.02	3.07±0.00	1.01±0.00

** The unit is $\mu s \cdot cm^{-1}$ ND: data not available ; ES-DSC: bio-dried solid fraction; ES-AS: ammonium sulphate; ES-NC-MFRO: nutrient rich concentrate from microfiltration and reverse osmosis retentates treated via freeze concentration; ES-NC-MFR: nutrient rich concentrate from - retentate of microfiltration treated by freeze concentration; ES-NC-RO: nutrient rich concentrate from retentate of reverse osmosis in which membrane contactor was skipped and treated by freeze concentration.*

(iii) Incubations

To investigate the N mineralization rate of BBFs, an incubation experiment was set up for 160 days. Before the main incubation, a soil pre-incubation in dark a temperature of 22°C was conducted for one week with the purpose of stimulating microbial activity. Afterwards, 147 g of pre-incubated soil was placed in a 100 mL container and mixed with each BBF at a rate of 170 kg N/ha/yr. In addition, a negative unfertilised control was included, along with two positives references with calcium ammonium nitrate (CAN) and pig slurry (PS) sourced from the corresponding Spanish pilot. Then, using distilled water, the WHC of soil-BBF mixtures was adjusted to 50%. The containers were closed with a layer of parafilm with pin holes, which minimizes the moisture losses during the pre-incubation and ensures gas exchange. To capture the N dynamics, ten samplings were done on days 5, 10, 20, 40, 60, 80, 100, 120, 140, and 160 with destructive samples. In all treatments, four replicates were used on each sampling day, to offer an adequate resolution of the dynamics of N mineralisation during the experiment. Every sampling day, mineral N (NO_3-N + NH_4-N) in the mixtures and the controls were analysed. Then, for each incubation day, the total N release was calculated as described in the start of Chapter 2.

(iv) Statistical analyses

The obtained results were analysed statistically through a one-way ANOVA using R Studio software, and subsequently, a Tukey's test for honestly significant differences (HSD) was applied at a significance level of $p \leq 0.05$. This allowed the comparison of effects of the tested fertilisers among them and against the reference fertilisation treatments.

2.3.3 Results and discussion

At the end of the incubation period, the average N release (mean ± standard deviation, expressed in %) in each treatment was 112 ± 27 for ES-AS, 108 ± 19 for CAN, 92 ± 25 for ES-NC-RO, 85 ± 35 for ES-NC-MFR, 62 ± 18 for PS, 52 ± 20 for ES-NC-MFRO and 21 ± 11 for ES-DSC (Figure 9). However, only ES-AS and ES-NC-RO showed the same release pattern as CAN, as 100% of applied N was retained in mineral form during incubation. This similarity with CAN could be related to the high NH_4-N/total N ratio found in these BBFs.

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However, in the case of PS, ES-NC-MFR and ES-NC-MFRO, positive mineralisation was also observed due to the mineralisation of organic N present in the product. Despite this, the N release throughout the incubation period was slow as compared to the reference mineral. The C/N ratio from ES-DSC was balanced, suggesting a rapid mineralisation, but the results showed that only 20% of the N applied was released to the soil. Considering an agronomical perspective, this BBF does not present adequate performance for N fertilization.

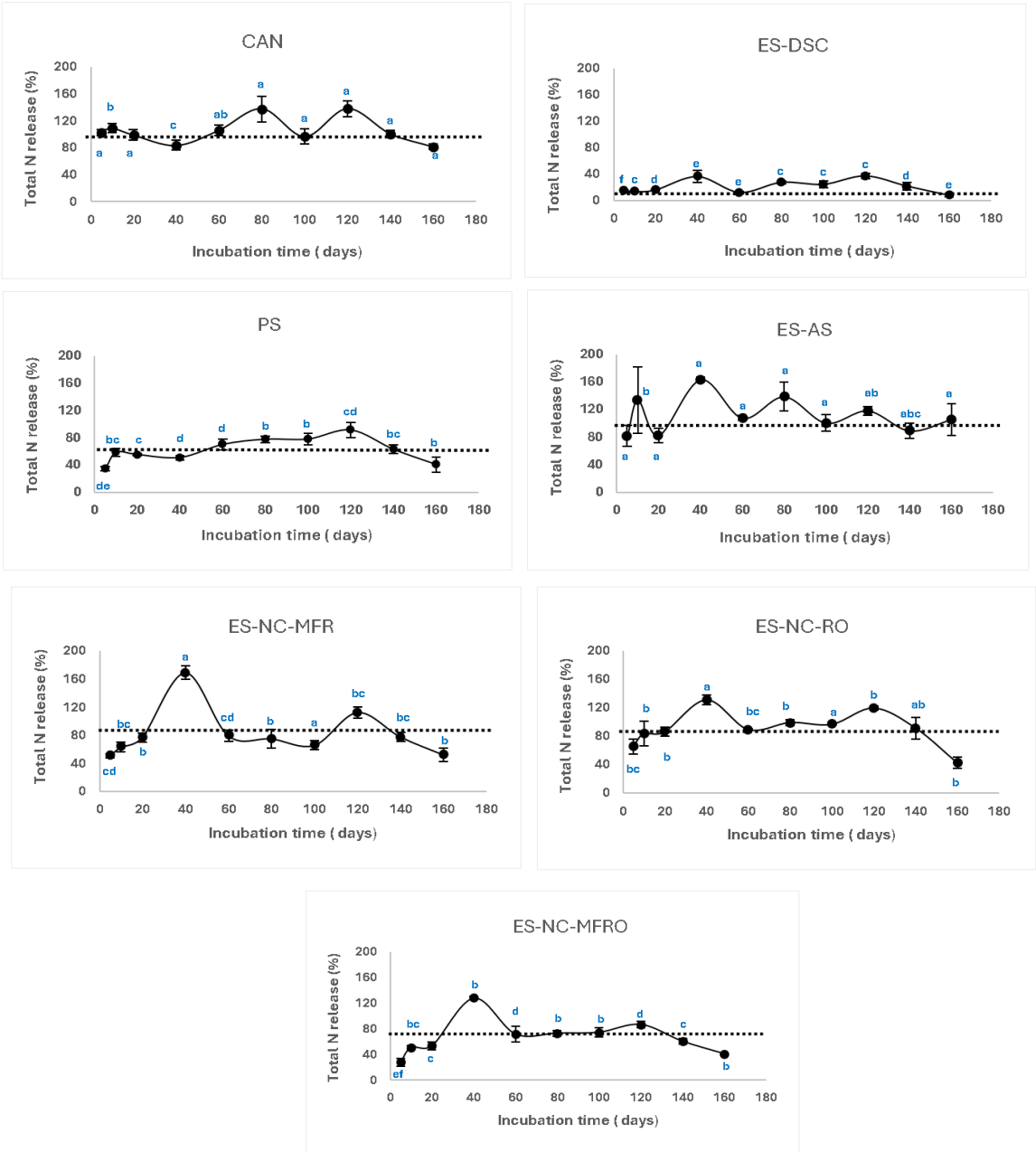

Figure 9. Dynamics of N mineralisation from BBFs produced under Spanish pilot plant. Different lower case letters (a–f) denote statistically significant differences in means (Tukey's Test for $p < 0.05$) among the products for each sample time (t = 5, 10, 20, 40, 60, 80, 100, 120, 140, and 160 days). The dot lines in each graph represent the initial percentage of inorganic N present in each treatment.

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2.3.4 Conclusion and recommendation

Compared to synthetic mineral N fertilisers, ES-AS and ES-NC-RO exhibited comparable pattern release, as the findings showed that 100% of the applied N stayed in a mineral state during incubation. However, ES-NC-MFRO and ES-NC-MFR, while not exhibiting the same release pattern as mineral fertilisers, displayed a high average release rate, surpassing 50%. In contrast, ES-DSC did not perform as effectively as expected in terms of N release, showing an average release rate of only 20% of the total N applied.

2.4. Ammonium sulphate (BE-AS), Ammonium nitrate (BE-AN) and Ammonium water (BE-AW) from Belgian pilot (UGent)

For more information on this study, please contact the authors from Ghent University: Vaibhav Shrivastava (vaibhav.shrivastava@ugent.be) and Ivona Sigurnjak (Ivona.sigurnjak@ugent.be).

This section will be published as "Shrivastava, V., Saju, A., Sigurnjak, I., Edayilam, N., Van De Sande, T., & Meers, E. (2024). Evaluating Agronomic and Environmental Performance of Bio-Based vs. Synthetic Fertilisers: Compilation of 4-year field trials. Under Preparation"

2.4.1 Introduction

The purpose of this study was to estimate the N release by comparing the dynamics of N-mineralisation in soil amended with BBFs to those in soil amended with conventional mineral fertiliser (CAN) and pig slurry (PS). This was done by studying the N decomposition kinetics of the Belgian BBFs ammonium sulphate (BE-AS), ammonium nitrate (BE-AN), and ammonia water (BE-AW) through incubation studies and relating them to some of their physiochemical/agrochemical features (e.g. C/N ratio, mineral N, pH).

2.4.2 Methodology

(i) BBFs sampling and characterisation

The BE-AS was obtained from a pig farm equipped with an air scrubbing unit located in Houthulst, Belgium: it is not a part of Belgian pilot. BE-AN and pig slurry were collected from a pig farm in Gistel, Belgium: a Belgian pilot in the first two years (2020-2021) of FERTIMANURE project. Here, centrifugation was used to separate the digestate into solid fraction (SF) and liquid fraction (LF), and the LF was then subjected to NH_3 stripping and scrubbing to extract N in the form of AN. The BE-AW was obtained from a Belgian AD facility that uses the centrifugation-based initial separation of digestate: it is not a part of the Belgian pilot. The LF of the digestate is sent to evaporator from where the NH_3 condensate was obtained. The next stage involves the passing of the obtained evaporation condensate through an NH_3 stripper from where the BE-AW is obtained as a final product. Airtight polyethylene sampling bottles of 1 L each were used to collect all of the BBFs for product characterisation (Table 5). According to the producer's directions, BE-AN and BE-AS were kept in the refrigerator at 4°C, while BE-AW was kept in the lab's fume hood where the temperature was typically between 15-20°C.

(ii) Incubations

To investigate the rate of mineralisation of BBFs, an incubation experiment was set up. The sandy soil used in the experiment was collected from a farm in Wingene, Belgium, which was also the part of Belgian field trials (D4.6). The soil had 0.97 ± 0.21 % TC, pH-KCl = 5.86 ± 0.31 and EC (µS/cm) = 59.62 ± 8.57. For nutrient

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content, the following was measured in g/kg dry matter basis: TN = 0.77 ± 0.16 ; TP = 1.08 ± 0.07 ; TK = 1.12 ± 0.04 ; TS = 0.23 ± 0.11. The soil was sieved through a 2 mm screen and air dried for 5 weeks before the incubations. The pre-incubation of soil took place at 35% water-filled pore space (WFPS) for a week. After that, 271 g of the pre-incubated soil was combined with the BE-AS, BE-AN, BE-AW, PS, and calcium ammonium nitrate (CAN; 16%), which served as the experiment's reference. Then, using distilled water, the mixture's WFPS was raised to 50%. For 120 days, 144 tubes in total were incubated. Following the Nitrates Directive, fertilisers were added at a rate equivalent to 170 kg of total N ha^{-1} yr^{-1}. At every 20-day interval, destructive sampling was carried out, in which 10 g of soil in each tube was combined with 50 mL of 1M KCl, the mixture was agitated end-over-end for 30 min, and then it was filtered. To determine NH_4-N and NO_3-N, the filtrate was analysed colourimetrically by a continuous flow auto-analyser (Chemlab System 4, Skalar, the Netherlands).

Table 5. Chemical composition of tested bio-based fertilisers, pig slurry (PS) and calcium ammonium nitrate (CAN) used in the N incubations.

Parameters	Unit	BE-AS	BE-AN	BE-AW	PS	CAN
Dry matter	%	21	24	N/A	9.60	100
C tot	%	0.07	0.03	1.03	3.92	N/A
N tot	%	4.28	8.75	13	0.74	16
NH_4-N	%	4.28	4.25	13	0.40	1
NO_3-N	%	0.01	4.50	0.01	0.01	15
S tot	%	6.62	ND	ND	0.84	ND
pH	-	5.24	5.17	11.2	7.33	ND
EC	mS	199	303	312	42	ND

ND: not determined; BE-AS: ammonium sulphate; BE-AN: ammonium nitrate; BE-AW: ammonium water; PS: pig slurry; CAN: calcium ammonium nitrate.

(iii) Statistics

The results were analysed using one-way ANOVA and Tukey's honestly significant difference (HSD). The effects of tested fertilisers were compared with the treatments and also against the used reference treatments. Using the SPSS 22.0 software for Windows, all tests were run at a probability (p) level of 0.05.

2.4.3 Results and discussion

Throughout the incubation, the average net N release was 140 ± 8% for BE-AS, 122 ± 22% for BE-AN, 122 ± 29% for BE-AW, 120 ± 17% for PS, and 113 ± 7% for CAN at the end of day 120 (Figure 10). Because of the high NH_4-N/total N ratio of BBFs, the net N release of BE-AS, BE-AN, and BE-AW was generally comparable with CAN and showed nearly 100% of N-released by day 40. The observed net N mineralisation is thought to be the result of the positive priming effect of BE-AS on OM present in the soil, resulting in an N-release value >100%, as all ammonium salt solutions are 100% in mineral N form. In the case of PS, positive net mineralisation is observed due to the mineralisation of organic N present in the product. However, the net N was slowly released throughout the incubation period and reached a 100% value by the end of day 100. This is due to the C/N ratio > 7, resulting in slow release over the period.

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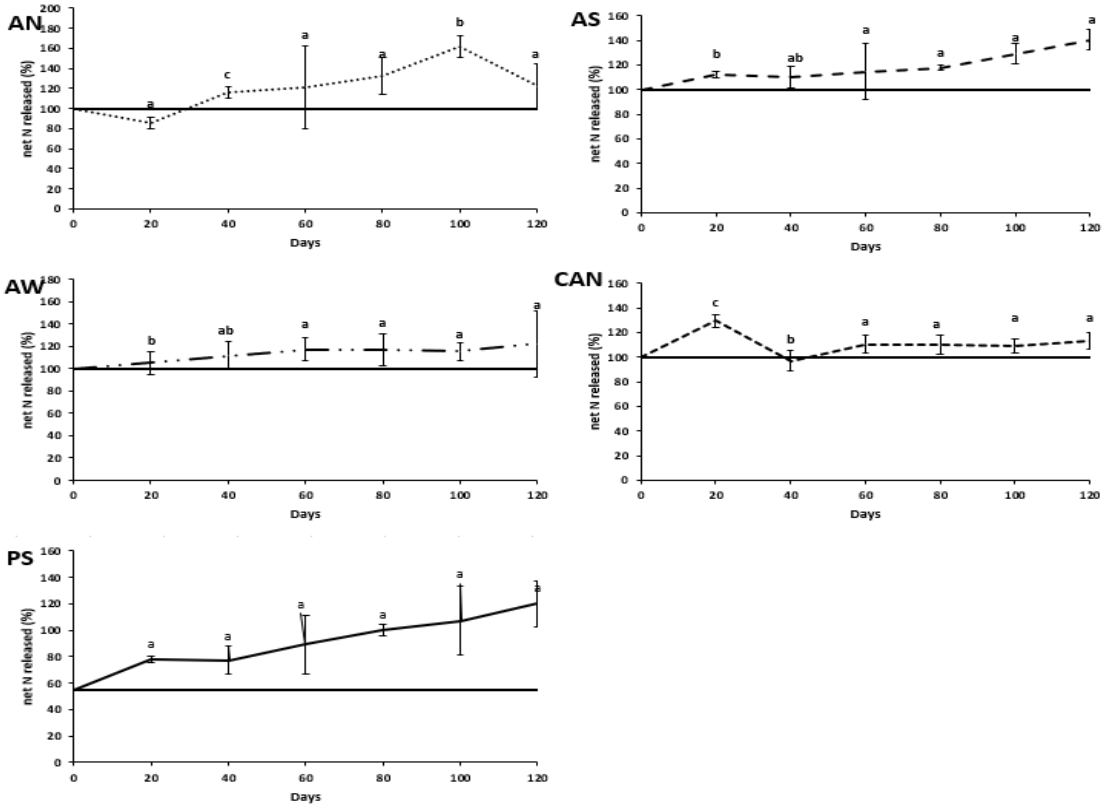

Figure 10. Net N release (in % of total N applied) for applied fertilisers over a time span of 120 days. Legend: AS-ammonium sulphate = BE-AS, AN-ammonium nitrate = BE-AS, AW-ammonia water = BE-AW, CAN-calcium ammonium nitrate and PS- pig slurry. Lower case letters (a–d) denote statistically significant differences in means (Tukey's Test for $p < 0.05$) among the products for each sample time (t = 20, 40, 60, 80, 100, and 120). The straight lines in each graph represent the initial percentage of inorganic N present in treatments.

2.4.4 Conclusion and recommendation

In terms of N, the BBFs as well as CAN have shown 100% of net N released by day 40. This was due to the high NH_4-N/total N ratio of BBFs, making them readily available for N-release. For PS, the release dynamics were rather slow due to the presence of organic N in the product. In overall, the study shows the positive potential for BBFs to replace synthetic mineral fertilisers.

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3. Assessment of Carbon release patterns via soil incubation assay

Biochars and soil amendments produced at FERTIMANURE on-farm pilots do not contain only nutrients (i.e. N, P, K, etc), but also valuable organic matter (OM) which helps to ameliorate soil physicochemical and biological properties. When added to soil, microorganisms use OM as a source of energy, thereby emitting CO2 via respiration. The fraction of OM that is less degradable remains and eventually contributes to soil organic matter (SOM). The important constituent pool of SOM is soil organic carbon (SOC). SOC has been recognized by European policy as an instrument to reduce CO2 emission through soil C sequestration (Lugato et al., 2014). This is also reflected in an European initiative to increase the SOC stock in the soil with 4 promille (so called '4 promille initiative', https://www.4p1000.org/).

Although C incubations are not stated in the DOA of FERTIMANURE project, the FERTIMANURE partners acknowledge the importance of C and have decided to conduct C incubation experiments. These experiments allow to assess the dynamics of C-mineralisation in soil amended with C–rich BBFs (e.g. biochars and soil amendments), compared with conventional organic amendment (e.g. commercial compost, in this case the FITA compost was used), in order to quantify the mineralisable C and hence determine stable OC that would remain in the soil after 1 year from the application moment. Partners that performed the experiments on BBFs/TMFs are UVIC-UCC (Spain: bio-dried solid fraction (ES-DSC) and nutrient rich concentrate (ES-NC)) and RITTMO (France: biochar (FR-BC)). Each partner followed their own local/national protocol. German pilot plant also produces a biochar (DE-BC) and this BBF was sent by Fraunhofer to RITTMO to assess the DE-BC along with FR-BC. This chapter includes the report of final results from RITTMO and UVIC-UCC.

Across this chapter, the accumulated content of mineralized C, which evolved as CO_2 from each BBFs, was calculated as the difference between the cumulative CO_2 -C from the unamended soil and the BBFs-treated soil, and was calculated using the following equation:

$$C_{\min} = \frac{C_{min,treatment} - C_{min control}}{TOC\ applied} \times 100 \qquad \textit{(Eq. 3)}$$

3.1. Biochar from French (FR-BC) and German (DE-BC) pilots (RITTMO)

For more information on this study, please contact the authors from RITTMO Agroenvironnement: Lionel Ruidavets (lionel.ruidavets@rittmo.com) or Fiona Ehrhardt (fiona.ehrhardt@rittmo.com).

3.1.1 Introduction

The objective was to determine the organic carbon (OC) dynamics of biochars when mixed with soil in order to evaluate their potential to add stable C that would remain in soil for a long time. The biochars C mineralisation capacity was examined under controlled conditions during the period of 91 days.

3.1.2 Methodology

The experiment was conducted based on French standard FD U 44-163 standard («Organic amendments and growing media - Characterisation of organic matter by the potential mineralisation of carbon and nitrogen").

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For this, a soil/BBF mixtures (25g of soil and biochar added at 2 g of organic C/kg dry soil) was placed in hermetically sealed jars in the presence of NaOH. Carbon dioxide (CO_2), produced during the mineralisation of the OC present in the mixture, is trapped during the test by NaOH (0.5 M). The CO_2emission was measured by acid base dosage measuring the remaining NaOH content in jars. The trial took place inside a heat chamber regulated at 28°C +/- 1°C and for 91 days during which 7 measuring points were taken (initial point and after 7, 14, 28, 49, 70 and 91 days). The product characterisation of the tested fertilisers is reported in Table 6. The following treatments were tested in 3 replicates:

(1) Soil only (negative control)
(2) Soil + FR-BC (obtained from poultry manure pyrolysis)
(3) Soil + Poultry manure (source of FR-BC)
(4) Soil + DE-BC (obtained from cattle manure pyrolysis)
(5) Soil + Compost (organic reference).

Table 6. Products characteristics of tested fertilisers on fresh matter basis.

Parameters	Unit	FR-BC	Compost	Poultry manure	DE-BC
Dry matter	g/kg	977	382	862	ND
Total carbon	g/kg	ND	194.5	ND	243.6
Organic carbon	g/kg	544.0	129.0	351.0	ND
Total N	g/kg	20.7	19.1	33.1	ND
NH_4-N	g/kg	<0.05	2.3	2.97	ND
NO_3-N	g/kg	ND	<0.003	ND	ND
Total P	g/kg	22.1	22.7	11.7	ND
Total potassium	g/kg	73.2	9.84	25.2	ND
Total sulphur	g/kg	ND	ND	ND	ND

ND: not determined; FR-BC: French biochar; DE-BC: German biochar

For FR-BC, the experiment was done on two different soils: one agricultural soil corresponding to the criteria of the French standard FD U 44-163 : "**standard soil,**" and one agricultural soil from fields used during maize French fields trials (Brittany region) in FERTIMANURE project : "**agricultural soil.**" (please see section 2.1.2 for more info on the soil). For DE-BC, this test was done only using the **standard soil.**

Statistical tests (ANOVA) were performed with STATGRAPHICS V15-2-06. A Shapiro-Wilk test was performed to validate the normality of the residuals, and a Levene test was performed for validation of the homogeneity of variances. The means comparison test (student t-test) is carried out for each parameter and for which the ANOVA shows a significant effect at 5% threshold. If the means cannot be compared, a non-parametric Kruskal-Wallis test allows the medians to be compared.

3.1.3 Results and discussion

(i) Carbon mineralisation with FR-BC

On standard soil, the mineralisation results made it possible to separate 3 significantly different groups (ANOVA, $p<0.05$; Figure 11): i) **Poultry manure** with a high C mineralisation rate. For this manure, 63.2 ± 4.6% of added organic C was mineralised on the 91st day. This manure contained 341 g/kg fresh matter (FM) of organic C which was easily mineralised by microorganisms; ii) **Compost** treatment showed a mineralisation on the 91st day of 36.8 ± 3.4% of its initial C. Thus only 1/3 of its initial C was mineralised which suggests that the compost contained a part of C more recalcitrant to biodegradation and therefore more stable which could participate to enrich the soil C pool. These results are conform to composting process during which the easiest accessible part of the OM is already degraded. Microorganisms need more time or specific metabolisms to degrade the remaining OC; iii) No real C mineralisation was measured with **FR-BC** addition to soil. C mineralisation was measured at 2.6 ± 2.1%. On agricultural soil, the results classified products into 2 different

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groups (Figure 11): i) **Poultry manure and compost** with a C mineralisation of 59.6 ± 7.9% and 53.3% ± 3.0% respectively; ii) **FR-BC** presented a mineralisation rate of 0.1 ± 2.3%.

In general, results were similar between the two soils for poultry manure and FR-BC. For compost treatment, C mineralisation was clearly higher on agricultural soil compared to standard soil. Regarding soils characteristics (section 2.1.2), agricultural soil presented potentially more active microbiological population. It was also biologically more active than the standard soil due to its exploitation as an agricultural field year over year (contrary to the standard soil, which has been stored sifted and dried for over a year).

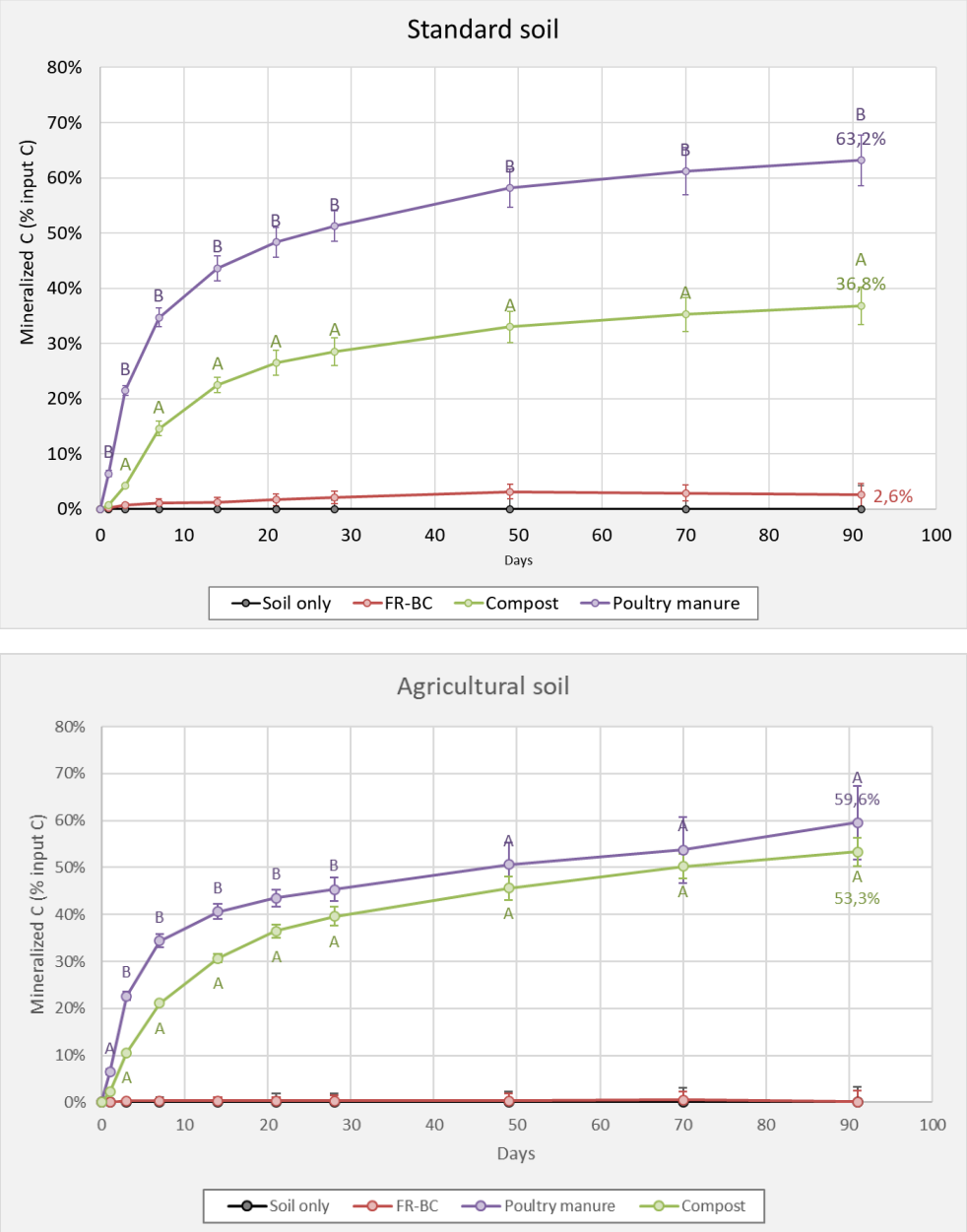

Figure 11. Part of organic carbon mineralised during the trial with FR-BC on two different soils. Letters indicate the significantly different modalities (1 letter = 1 group, no letter = control).

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(ii) Carbon mineralisation with DE-BC

The tests carried out with DE-BC show similar results as those obtained with FR-BC on standard soil. Compost treatment presented a C mineralisation of 38.8 ± 2.4% and C mineralisation of DE-BC was 2.0 ± 3.2% (Figure 12). In general, results obtained with these trials are in agreement with other results shown in scientific publications. The evaluation of the C biochar stability is carried out mainly on incubation studies in the laboratory, often in the short term. The rate of mineralisation determined during 222 days of incubation by Naisse et al. (2015) represents a loss of 1.4% of the initial C, whereas after 87 days of incubation a mineralisation of 0.14 to 0.18% observed by Luo et al., (2011), and after 60 days a mineralisation of 1.84 to 2.10% for Kuzyakov et al. (2009). The differences in the rate of biochar mineralisation between the different studies are both to be attributed to the types of soil used, the incubation conditions (temperature, humidity, etc.), and the quality of the biochars used.

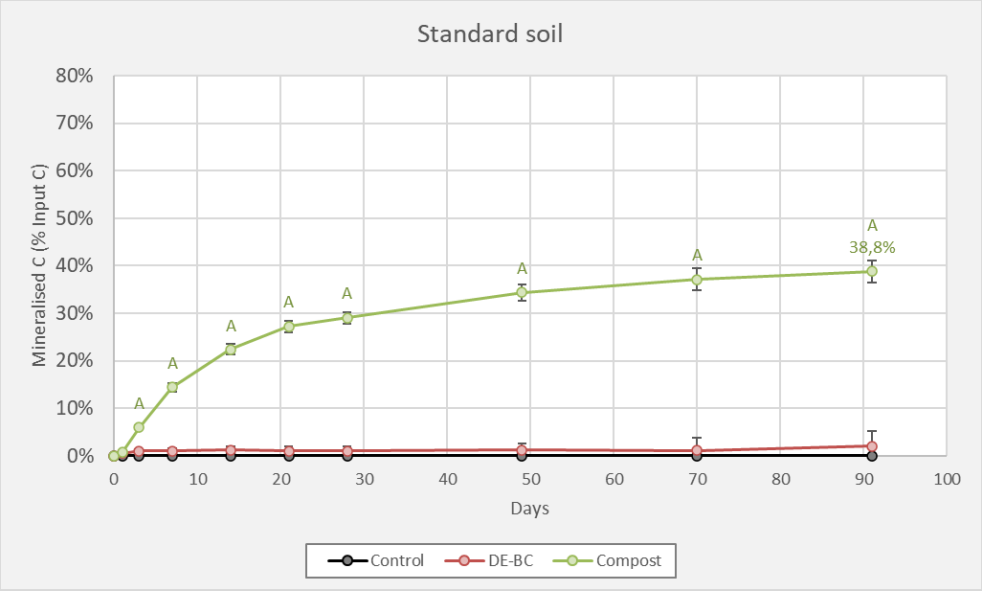

Figure 12. Part of organic carbon mineralised (% input OC) during the trial with DE-BC. Letters indicate the significantly different modalities (1 letter = 1 group, no letter = control).

3.1.4 Conclusion and recommendation

Biochars tested during these trials are products containing C recalcitrant to degradation, contrary to the compost or the poultry manure which was used as raw material in pyrolysis process. Depending on soil, 36.8 to 53.3% of the compost OC was mineralised, and 59.6% of the poultry manure organic OC was mineralised during the trials. FR-BC and DE-BC carbon mineralisation did not exceed an average value of 2.6%. This implied that the OC of biochars was not easily mineralised. Soil enrichment with biochars would make it possible to sequester more stable carbon in the soil, in accordance with the objective of 4 per 1000 initiative announced by the French Minister of Agriculture Stéphane Le Foll during 2015 Paris Climate Change Conference (COP 21, November 30 to December 12, 2015 at Le Bourget in France).

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3.2. Bio-dried solid fraction (ES-DSC) and Nutrient rich concentrate (ES-NC) from Spanish pilot (UVIC-UCC)

For more information on this study, please contact the authors from UVIC-UCC: Berta Singla Just (berta.singla@uvic.cat), Laura Diaz-Guerra (laura.diaz.guerra@uvic.cat) and Ana Robles Aguilar (ana.robles@uvic.cat).

3.2.1 Introduction

This study aims to quantify C mineralization (i.e. the amount of CO_2 released) through soil incubations using the BBFs obtained from the Spanish pilot. The objective was achieved comparing the C mineralisation in soils amended with BBFs against soils amended with commercial compost (FITA) and pig slurry (PS) from the Spanish pilot. The BBFs studied were the biodried solid fraction (ES-DSC), as well as one of the nutrient-rich concentrate from the retentate of microfiltration treated by freeze concentration (ES-NC-MFR).

3.2.2 Methodology

To investigate the rate of C mineralisation of BBFs, an aerobic soil experiment was performed for 85 days. The soil used for the incubation experiment was the same as the soil used for N incubation in section 2.3.2. A one-week soil pre-incubation at 22°C in dark conditions was conducted prior to the incubation to stimulate the microbial activity. After this, 147 g of soil was placed into a 100 mL container and mixed with ES-DSC and ES-NC-MFR at a rate of 9000 kg OC/ha, following literature recommendations aimed at improved soil health (Egene et al., 2021). The two BBFs, ES-DSC and ES-NC-MFR, were sourced directly from the Spanish pilot plant (section 2.3.2) and their product characterization is reported in Table 7.

Table 7. Chemical composition of pig slurry (PS), commercial compost FITA, and the two BBFs (ES-DSC and ES-NC-MFR) used in the C incubations (mean value ± standard deviation, n=3).

Parameter	Unit	PS	FITA	ES-DSC	ES-NC-MFR
pH	-	6.39±0.01	n/a	7.19±0.04	7.42±0.03
EC	$mS \cdot cm^{-1}$	25.49±0.14	n/a	*6.5±0.14	27.40±0.30
Total C	%	3.44±0.22	19±0.2	26.74±0.27	1.89±0.01
Total TOC	%	3.1±0.35	9.23±0.20	23.9±0.10	1.96±0.23
Total N	%	0.45±0.00	1.91±0.03	2.27±0.02	0.38±0.00
C/N ratio	-	7.64	4.83	11.7	4.02
DM	%	5.39 ±2.47	38±1.20	66.57±0.50	5.04±0.05

ES-DSC: bio-dried solid fraction; ES-NC-MFR: nutrient rich concentrate from - retentate of microfiltration treated by freeze concentration.
** The unit is $\mu s \cdot cm^{-1}$.*

A negative unfertilized control and two positive references, commercial compost FITA and pig slurry (PS) from the Spanish pilot were also included. These mixtures were then adjusted to 70% WHC using distilled water. To capture the C dynamics, a total of 12 destructive samplings were done on days 1, 2, 3, 5, 9, 14, 21, 36, 45, 59, 75, and 85 of the experiment. For every day, four replicates were included to ensure a detailed data collection of C mineralisation dynamics throughout the experiment. CO_2 release was measured using the NaOH trap method (Pell et al., 2006). Cumulative amounts of mineralized C were expressed as the percentage of TOC applied and will be hereafter referred as percentage of C mineralized (%C min). The obtained results were analysed statistically through a one-way ANOVA using R Studio software, and subsequently, a Tukey's test for honestly significant differences (HSD) was applied at a significance level of $p \leq 0.05$ only in the last of the incubation. This allowed the comparison of effects of the tested fertilisers among them and against the reference fertilisation treatments

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3.2.3 Results and discussion

The C mineralisation rates are shown in Figure 13. As expected, PS demonstrated the highest C mineralisation compared to BBFs. None of the treatments showed any mineralisation after day 14. In contrast to PS, the ES-DSC had the lowest mineralisation rates during the incubation, indicating that a significant amount of C may remain in the soil. Therefore, the results suggest that it is a sustainable practice to improve soil quality, despite the limited mineralisation. Additionally, the Tukey test indicated no statistical differences between ES-NC-MFR and compost FITA since they both exhibited a similar pattern in C mineralisation.

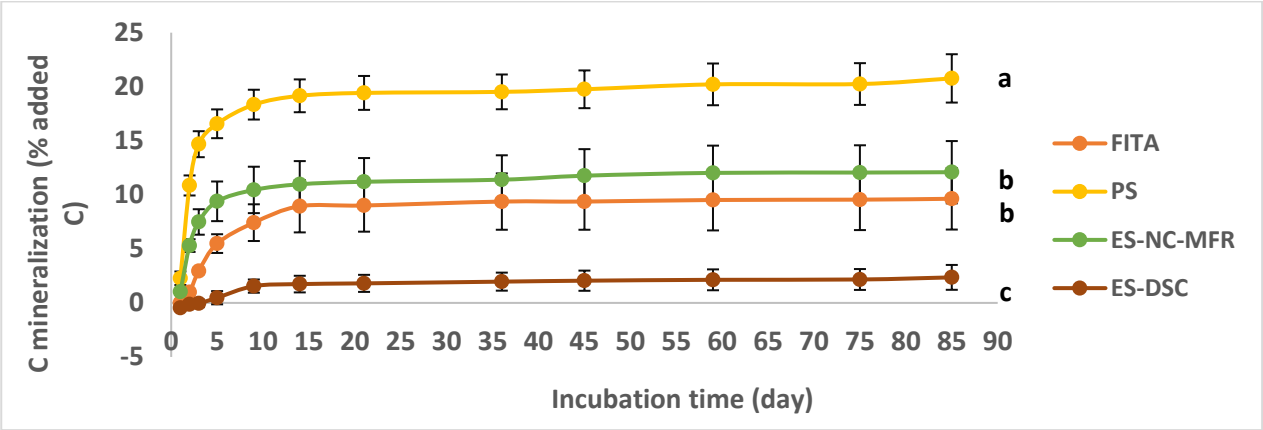

Figure 13. Cumulative C release from BBFs produced in the Spanish pilot plant. Different lower case letters (a–d) denote statistically significant differences in means (Tukey's Test for $p \leq 0.05$) among the products at day 85.

3.2.4 Conclusion and recommendation

The ES-DSC may be a promising choice for sustainable soil management due to the lack of C mineralisation observed during the soil incubation (less than 3%). On the other hand, both ES-NC-MFR (12%) and PS (20%) showed significantly higher C mineralization rates, making them potential nutrient sources for plants. Furthermore, ES-NC-MFR can replace compost in terms of C dynamics within the soil, since both had similar C mineralization rates. This finding represents new opportunities for diversifying organic amendments in agriculture and improving the overall sustainability of soil management practices.

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4. Assessment of Phosphorus plant availability via dedicated plant growth assay

For estimating efficiency of novel BBF for P fertilisation, analyses of total P content and soluble P in various extracting solution are not sufficient, especially for organic amendments in which OM contained in products may interact with soils and impact P bioavailability. Therefore, two approaches were followed to estimate potential efficiency of BBF for P fertilisation:

i. **Incubation** of soils with low level of P amended or not with BBF under controlled temperature and moisture. After fixed time, samples were sacrificed to estimate concentrations of P extractable through adequate extracting solutions.
ii. **Pot trials** with plants (rye-grass) grown on soils with low level of P amended or not with BBF. After 3 fixed times, rye-grass was harvested and analysed to estimate evolution of apparent P recovery (APR).

For both approaches, 3 doses were tested for each BBF as doses of P may influence P bioavailability. In addition, triple super phosphate (TSP) was used as positive reference. Moreover, FERTIMANURE partners have decided to use also raw manure from the respective pilot plant as an additional reference in P incubations and pot trials. FERTIMANURE partners that have conduct P experiments are:

- **RITTMO** (France): biochar (FR-BC)
- **Fraunhofer** (Germany): biochar (DE-BC) and mono-ammonium phosphate on perlite (DE-AP)
- **UVIC-UCC** (Spain): bio-dried solid fraction (ES-DSC), phosphorus ashes (ES-PA) and nutrient rich concentrate (ES-NC) and extraction of phosphoric acid (ES-EPA) from phosphorous-rich ashes (ES-PA)

To compare the P bioavailability of each product, calculation of APR (Apparent P recovery) was done and corresponded to the part of P absorbed by plants minus P uptake in unfertilised control treatment in relation to the amount of P that was to the soil:

$$APR\ (\%) = \frac{[P]_{uptake\ treatment}\ (mg\ P) - [P]uptake\ control(soil)\ (mg\ P)}{[P]_{total\ applied}\ (mg\ P)}\ x\ 100 \qquad \textit{(Eq. 4)}$$

The APR is required to calculate P fertiliser replacement value (PFRV) which indicates the replacement potential of a certain BBF as compared to the conventional synthetic mineral fertiliser:

$$PFRV_{BBF} = APR_{BBF} / APR_{Min} \qquad \textit{(Eq. 5)}$$

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4.1. Biochar (FR-BC) from French pilot (RITTMO)

For more information on this study, please contact the authors from RITTMO Agroenvironnement: Lionel Ruidavets (lionel.ruidavets@rittmo.com) or Fiona Ehrhardt (fiona.ehrhardt@rittmo.com).

4.1.1 Introduction

The goal of this study was to assess the bioavailability of P in biochars derived from poultry manure pyrolysis (FR-BC1) and solid digestate pyrolysis (FR-BC2) under pot conditions in cultivation of Italian rye grass. Two pot experiments were conducted to assess the P uptake by plants and to calculate the apparent P recovery (APR) and the P fertiliser replacement value (PFRV) of BBF in comparison with mineral reference triple super phosphate (TSP).

4.1.2 Methodology

This trial was based on the micro-culture technic described by Chaminade (1960, 1964), updated by Lemaire (1977), and summarised by Lombaert (1992). The assay is similar to the method developed by INRA "*Conduite d'une culture de ray-grass en conditions contrôlées, avec traçage isotopique (32P) du phosphore du sol*".

The tests consisted of mixing biochars and control products (Table 8) at different doses of P (30, 60 and 100kg P_2O_5 per hectare) with soil (2 L pots). Products were added to the concerned pots and the soil and product mixtures were homogenised. Preliminary fertilisation was provided to be sure that only P may be limiting plant growth. Then pots were sown with Italian rye grass seeds (seeding density of 2 g per pot), and placed in cultivation greenhouses and watered in a way that the humidity was optimal (70% of soil water retention capacity). A nutritive solution of KNO_3 regularly provides other essential nutrients (N, K) so that they are not limiting. At the start of the test, other microelements (Mg, S, Na, B, Mn, Mo, Fe, Cu, Zn) were added for the same purpose. The ryegrass was harvested 3 times during the first trial, 4 times during the 14 weeks second trial in order to deplete the substrate and evaluate the amount of biomass produced; the biomass was later analysed to quantify the amount of P taken up by plant and calculate the APR from biochars. All data undergone a statistical analysis consisting of an analysis of variance (SNK 95% threshold) with verification of the validity hypotheses of the ANOVA.

Table 8. Products characteristics (g/kg on fresh weight).

Parameters	FR-BC1	FR-BC2	Poultry manure	Compost	TSP
Dry matter	977	957	862	382	1000
Total carbon	ND	526	ND	194.5	0.0
Organic carbon	544.0	306	351.0	129.0	0.0
Total N	20.7	17	33.1	19.1	0.0
NH_4-N	<0.05	<0.05	2.97	11.3	0.0
NO_3-N	ND	<0.05	ND	<0.003	0.0
Total P	22.1	15,7	11.7	22.7	218
Total potassium	73.2	53.12	25.2	9.84	0.0

ND: not determined; FR-BC1: French biochar derived from poultry manure pyrolysis; FR-BC2: French biochar derived from solid digestate pyrolysis; TSP: triple superphosphate.

During the first experiment the following treatments were tested in 5 replicates at incremental rates (30, 60 and 100kg P_2O_5 per hectare), and 3 cuts were done : i) soil (control); ii) Soil + FR-BC1; iii) Soil + Poultry manure (source of FR-BC1); iv) Soil + Compost (organic reference); v) Soil + TSP (mineral reference). The experiment was done on two different soils: one agricultural soil corresponding to the criteria of the French standard FD U

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44-163 : “standard soil”, and one agricultural soil from fields used during maize French fields trials (Brittany region) in FERTIMANURE project : “agricultural soil” (section 2.1.2).

For the second experiment, only agricultural soil was used and FR-BC1 and FRBC2 were compared to TSP and tested at two doses (Table 9) corresponding to :

- a “fertiliser” dose equivalent to 200 kgP_2O_5/ha (FR-BC1_200U; FR-BC2_200U and TSP-200U)
- an “amendment/soil improver” dose where biochars were applied at 8 t/ha (FR-BC1_8T/ha; TSP equivalent to BC1_8T/ha; FR-BC2_8T/ha and TSP equivalent to FR-BC2_8T/ha.

Table 9. Treatments carried out in second experiment.

Treatments for the second experiment	Unfertilized control	FR-BC1_200U	FR-BC2_200U	TSP-200 U	FR-BC1_8 T/ha	TSP equivalent to -BC1_8T/ha	FR-BC2_8 T/ha	TSP equivalent to FR-BC2_8T/ha
P_2O_5 intake (kg P2O5/ha)	0	**200**	**200**	**200**	**405,6**	**405,6**	**288**	**288**

4.1.3 Results and discussion

(i) Experiment 1

Treatments had no influence on crop dry biomass that was cultivated on standard soil (Figure 14). In agricultural soil, all tested products had a substantial influence, excluding poultry manure at 30 kg P_2O_5/ha and mineral reference at 30 and 60 kg P_2O_5/ha.

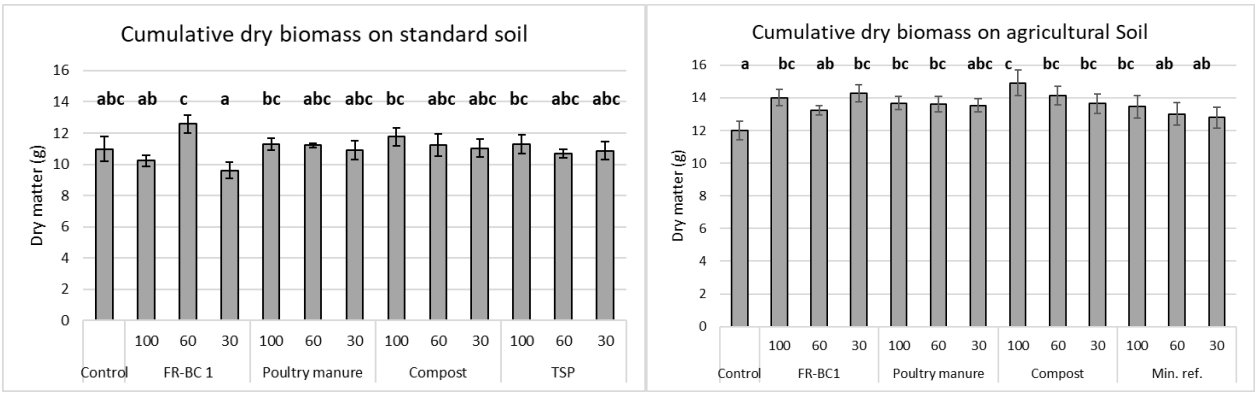

Figure 14. Experiment 1 - Cumulative harvested dry biomass (g per pot) for each treatment. Above the histogram, letters indicate significant different means (one way ANOVA, $p<0.05$) between products for the sum of the three cuts.

When it comes to the APR, results for compost and mineral reference must be considered with attention: due to an initial laboratory analysis error, P content in TSP (mineral reference) and compost (organic reference) were underestimated. Initial P intake in these treatments was too high compared to biochar and poultry manure. No calculation of PFRV was done. In standard soil, a global poor assimilation of P was measured. For FR-BC, only 60 kg P_2O_5/ha dose presented a positive APR with 20.76 ± 3.9%. The other APR doses did not present any difference with unamended soil. For poultry manure, APR was 8.8 ± 3.0%; 16.8 ± 2.4% and 15.5 ± 3.5% for 30, 60 and 100 kg P_2O_5/Ha respectively. For compost, results were equal regardless of dose with APR of 5.6 ± 4.6%; 5.9 ± 5.2% and 5.4 ± 5.0% for 30, 60 and 100 kg P_2O_5/Ha respectively. For TSP, APR were also quite low with APR of 14.1 ± 3.3%; 12.1 ± 3.8% and 12.5 ± 4.9% for 30, 60 and 100 kg P_2O_5/Ha respectively. These results matched with observations on pH and dry biomass production: soil pH was too high and could have limited P bioavailability for plants. Lack of bioavailable P induced a global reduction of

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biomass production similar in each treatment. In agricultural soil, bioavailable P was higher than those measured in standard soil. For FR-BC, APR was 48.9 ± 2.1%; 28.6 ± 2.2% and 22.9 ± 3.5% for 30, 60 and 100 kg P_2O_5/ Ha respectively. By taking into account the doses 30 and 60 kg P_2O_5/Ha. There was a tendency for the APR to decrease with increasing applied dose. For poultry manure, APR was 57.5 ± 2.8%; 39.9 ± 4.1% and 32.4± 3.3% for 30, 60 and 100 kg P_2O_5/ Ha respectively. There is also a tendency for the APR to decrease with increasing applied dose.

(ii) Experiment 2

Biochars in this experiment were used at higher doses than in experiment 1. Statistical analysis revealed that treatment had a significant effect on dry biomass cumulated on the 4 cuts (Figure 15). More specifically, the FR-BC1 increased rye-grass biomass significantly (compared to the unfertilised control) at both treatment rates. The increase is considerably superior to the mineral reference (TSP) at the fertiliser dose, but inferior to the reference at the amendment dose. The FR-BC2 did not substantially enhance cumulated biomass, regardless of fertiliser or amendment dosage. We measured differences between soils, suggesting that the products may have influenced soil characteristics such as pH, conductivity, and structure. Initially, there was a contrast between the standard (alkaline soi) and the agricultural (acidic soil) soil utilized in this study. Soil acidity can diminish the availability of essential nutrients like phosphate and sulphate (Marsh et al., 1987; Haynes, 1984; Mora et al., 1999) as well as minor elements such as copper, molybdenum, and selenium (Cavallaro and McBride, 1980; Jeffery and Uren, 1983). In addition, it can worsen the release of harmful materials such as aluminium (Bolan, 2008). Usually, soil pH is increased by using organic residues like biochar (Chintala et al, 2013), compost (Walker et al, 2004) or poultry manure (Dikinya, 2010). On the contrary use of TSP as mineral fertiliser may reduce the soil pH (Peryea, 1990). That could explain why there was no visible effect on standard soil (alkaline soil pH masks the effect of amendment), but an effect with an acid soil like the agricultural soil. To confirm this hypothesis, soil pH was measured after the third harvest. Results on soil final pH showed a difference between the two soils, but no differences were measured between treatments of the same soil. The pH was between 8.6 and 8.7 for standard soil and between 5.4 to 5.7 for agricultural soil. The analysis on soil conductivity found a difference between standard soil (72-83 µS/cm) and agricultural soil (10-18 µS/cm).

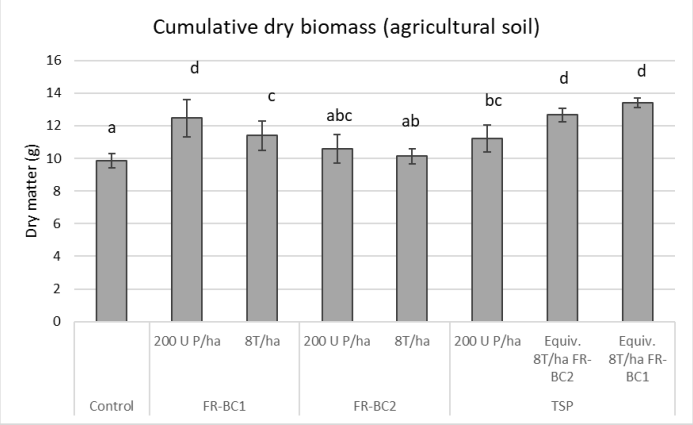

Figure 15. Cumulative harvested dry biomass (g per pot) for each treatment in experiment 2. Above the histogram, letters indicate significant different means (one way ANOVA, $p<0.05$) between products for the sum of the 4 cuts.

When it comes to the APR, it was low across all treatments, with a highest APR of 14% ± 3% in TSP at 200 kg P_2O_5 (Table 10). The finding might be explained by a phosphate supply that exceeds ryegrass needs. This confirms the tendency for the APR to decrease with increasing applied dose, observed in experiment 1. Nevertheless, the APR of the two biochars incorporated at amendment dose are comparable, even if the P

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supply dose differs; the same applies for the APR of the TSP at two doses. When applied at lower doses (fertiliser dose 200 units of P_2O_5) the APR of the two biochars is significantly higher (compared to the amendment doses), but the APR of the TSP applied under the same conditions is significantly higher.

Table 10. APR and PFRV values obtained in experiment 2.

			Agricultural soil	
	Treatments	**Applied dose (Kg P_2O_5/Ha)**	**APR(%)**	**PFRV %**
Experiment 2	**FR-BC1**	200	7% ± 1%	47.6%
		8T/ha (405.6 Kg P_2O_5)	2% ± 1%	17.5%
	FR-BC2	200	5% ± 2%	37.9%
		8T/ ha (288 Kg P_2O_5)	3% ± 1%	24.8%
	TSP	200	14% ± 3%	
		Equiv. 8T/ha FR-BC2 (405.6 Kg P_2O_5)	11% ± 0%	
		Equiv. 8T/ha FR-BC1 (288 Kg P_2O_5)	9% ± 2%	

4.1.4 Conclusion and recommendation

FR-BC provides a significant amount of bioavailable P. For an applied dose of 200 kg P_2O_5/Ha, PFRV value was 48 % of TSP can be used in partial substitution of conventional mineral P fertilisation. With biochar as P fertiliser, plant biomass production is similar to production obtained with TSP. However, it is very important to take into account the properties of the soils: in alkaline soil, P fertilisation is not efficient, and the use of biochar seems to not be a correct practice. Biochar presented interesting APR values when it is used at low intake doses (minus 100 kg P_2O_5). This could be in accordance to realistic biochar addition to soil with inputs at 4 T/Ha.

4.2. Biochar (DE-BC) and Mono-ammonium phosphate (DE-AP) from German pilot (Fraunhofer)

For more information on this study, please contact the author from Fraunhofer Institute: Sophie Schönfeld (Sophie.schoenfeld@umsicht.fraunhofer.de).

4.2.1 Introduction

The agronomic performance of biochar (DE-BC) from the German pilot plant and the ammonium phosphate fertiliser (DE-AP1 and DE-AP2) was assessed for their P. Three pot trials have been performed with two crops: ryegrass and maize. Within the scope of the project two different modifications of DE-AP were produced. DE-AP1 refers to mono ammonium phosphate on perlite and was used for the first two pot trials with rye grass and maize and DE-AP2 refers to pure mono ammonium phosphate isolated from the process and was used within the third pot trial with rye grass. The trials aimed to determine the availability of the P contained in the two BBFs to plants. However, there is a difference in methodology within the three trials. In the first two trials with ryegrass and maize the P provided was only partially derived from the BBFs. The last trial with rye grass addressed this fact, and a changed fertilising protocol where all P originated from the BBFs was used. Lime as an additional amendment was rejected in the third experiment since the first two trials indicated that it does not improve the nutrient availability or growth of the plants.

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4.2.2 Methodology

Pot trial experiments with ryegrass and maize were performed using Mitscherlich pots containing 6 kg (first rye grass experiment) or 5.5 kg (maize trials and second ryegrass experiment) of soil. The randomised complete block design was used to place the treatments in four replicates. In the specific treatment group, missing nutrients were supplemented in a water-soluble form. The significance of soil pH was assessed by an additional treatment group with the addition of lime (target pH 5.7-6.0; not done in third experiment). The plants were regularly assessed and watered to maintain 70% water capacity. Based on the requirements of the ryegrass and maize for the duration of the experiment, a target fertilisation was defined in mg per pot: i) pot trial 1 ryegrass: 1500 N, 420 S, 730 P, 3960 K and 300 Mg; ii) pot trial 2 maize: 1200 N, 420 S, 730 P, 3960 K and 300 Mg; iii) pot trial 3 rye grass: 1200 N, 420 S, 730 P, 6000 K and 500 Mg. A reduction of the target value of N in rye grass was chosen because within the first trial there was no sign of a limitation of N even during the last harvesting cycles. The target values for potassium and magnesium were adjusted in pot trial 3 because of the higher amounts of DE-BC used. The BBF DE-BC has significant amounts of K and Mg. In order to keep the values of these nutrients equal within all fertilising groups, the target fertilisation for these two nutrients was increased. The product characterization of the BBFs from German pilot is given in Table 11, whereas the application amount of nutrients for each pot experiment is given in Tables 12 - 14.

Table 11. Nutrition parameters of biochar (DE-BC), MAP1 on perlite (DE-AP1) and MAP2 (DE-AP2).

Fertiliser	Parameter	Value [% solid matter]
Biochar (DE-BC)	Total nitrogen (N)	1.6
	Ammonium nitrogen (NH_4^+)	<0.2
	Phosphate (P_2O_5) water soluble	0.1
	Phosphate (P_2O_5) neutral ammonium citrate soluble	2.04
	Phosphate (P_2O_5) soluble in mineral acid	2.88
	Potassium oxide (K_2O)	8.8 (43.8)*
	Magnesium total (MgO)	1.6
	Calcium total (CaO)	3.29
MAP 1 (DE-AP1) on perlite	Total nitrogen	11.8
	Ammonium nitrogen (NH_4^+)	11.8
	Phosphate (P_2O_5) water soluble	32.4
	Phosphate (P_2O_5) neutral ammonium citrate soluble	38.4
	Phosphate (P_2O_5) soluble in mineral acid	38.7
	Potassium oxide (K_2O)	0.26
	Magnesium total (MgO)	0.05
	Calcium total (CaO)	0.13
MAP 2 (DE-AP2)	Total nitrogen (N)	12.2
	Ammonium nitrogen (NH_4^+)	12.2
	Phosphate (P_2O_5) water soluble	24.3
	Phosphate (P_2O_5) neutral ammonium citrate soluble	26.8
	Phosphate (P_2O_5) soluble in mineral acid	26.8
	Potassium oxide (K_2O)	0
	Magnesium total (MgO)	0
	Calcium total (CaO)	0

Table 12. Amount of fertiliser applied in treatments (TRT) during **pot trial 1 (rye grass)** and additional balancing nutrients in g used for each 6 kg sample.

TRT	Fertiliser	Lime	NH_4NO_3	K_2SO_4	$CaSO_4$	KH_2PO_4	$Na_2PO_4 \cdot 12H_2O$	KCl	$MgCl_2 \cdot 6\ H_2O$
1	0	0	0	0	0	0	0	0	0
2	-	0	4.29	2.28	0	3.25	0	4.8	2.47
3	-	18	4.29	2.28	0	3.25	0	4.8	2.47
4	9	0	3.88	0	2.10	0	5.44	0.03	1.29
5	9	12	3.88	0	2.10	0	5.44	0.03	1.29

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6	1.88	0	3.84	2.28	0	0	0	6.57	2.47
7	1.88	18	3.84	2.28	0	0	0	6.57	2.47

1) Control 2) Full Fertilisation 3) Full Fertilisation + Lime 4) Biochar (DE-BC) 5) Biochar (DE-BC) + Lime 6) MAP (DE-AP) 7) MAP (DE-AP) + Lime

Table 13. Amount of fertiliser applied in treatments (TRT) during **pot trial 2 (maize)** and additional balancing nutrients in g used for each 5.5 kg sample.

TRT	Fertiliser	Lime	NH_4NO_3	K_2SO_4	$CaSO_4$	KH_2PO_4	$Na_2PO_4 \cdot 12H_2O$	KCl	$MgCl_2 \cdot 6\ H_2O$
1	0	0	0	0	0	0	0	0	0
2	-	0	3.14	2.09	0	3.25	0	4.4	2.27
3	-	16,5	3.14	2.09	0	3.25	0	4.4	2.27
4	8,25	0	2.92	0	1.96	0	4.78	5.62	1.18
5	8,25	11	2.92	0	1.96	0	4.78	5.62	1.18
6	1.88	0	3.84	2.28	0	0	0	6.57	2.47
7	1.88	18	3.84	2.28	0	0	0	6.57	2.47

1) Control 2) Full Fertilisation 3) Full Fertilisation + Lime 4) Biochar (DE-BC) 5) Biochar (DE-BC) + Lime 6) MAP1 (DE-AP1) 7) MAP1 (DE-AP1) + Lime

Table 14. Amount of fertiliser applied in treatments (TRT) during **pot trial 3 (rye grass)** and additional balancing nutrients in g used for each 5.5 kg sample.

TRT	Fertiliser	NH_4NO_3	K_2SO_4	$CaSO_4$	KH_2PO_4	KCl	$MgCl_2 \cdot 6\ H_2O$
1	0	0	0	0.55	0	0	0
2	0	3.14	2.09	0.55	3.25	7.99	3.78
3	45.52	1.90	1.23	0.55	0	3.52	0
4	2.53	2.25	2.09	0.55	0	9.60	3.78
5	3.07	3.14	2.09	0.55	0	9.60	3.78

1) Control 2) Full Fertilisation 3) Biochar (DE-BC) 4) MAP2 (DE-AP2) 5) Triple superphosphate (TSP).

The experiment was arranged in a completely randomised design with four replications. Single factor analysis of variance (ANOVA) with a pairwise t test was used to examine the effects of fertiliser treatment.

4.2.3 Results and discussion

(i) Pot trial 1 (Rye grass)

The first harvest of biomass took place after 21 days. Five additional harvests were carried out after 33, 47, 57, 71 and 85 days. Figure 16 gives an overview of the total of 6 harvests and each fertiliser's overall performance over the whole growth period. Depending on the treatment and the harvest date, the biomass production of ryegrass varied considerably. The principle is that nutrient availability, i.e., N, P, K, and pH may cause differences in growth. Each harvest will remove nutrients and thereby change the supply for the regrowth.

In the first harvest, the best yield was achieved with the use of biochar (DE-BC) as a fertiliser, closely followed by biochar (DE-BC) with lime. Biochar performed exceptionally well in the early growth cycles but showed a decrease in biomass production towards the end of the experiment. Biochar appeared to provide optimal nutrient supply to young plants, promoting faster growth. Toward the end of the assessment period, the fully fertilised treatment produced the highest biomass, followed by the MAP (DE-AP1) fertiliser. The addition of lime did not seem to significantly benefit treatments.

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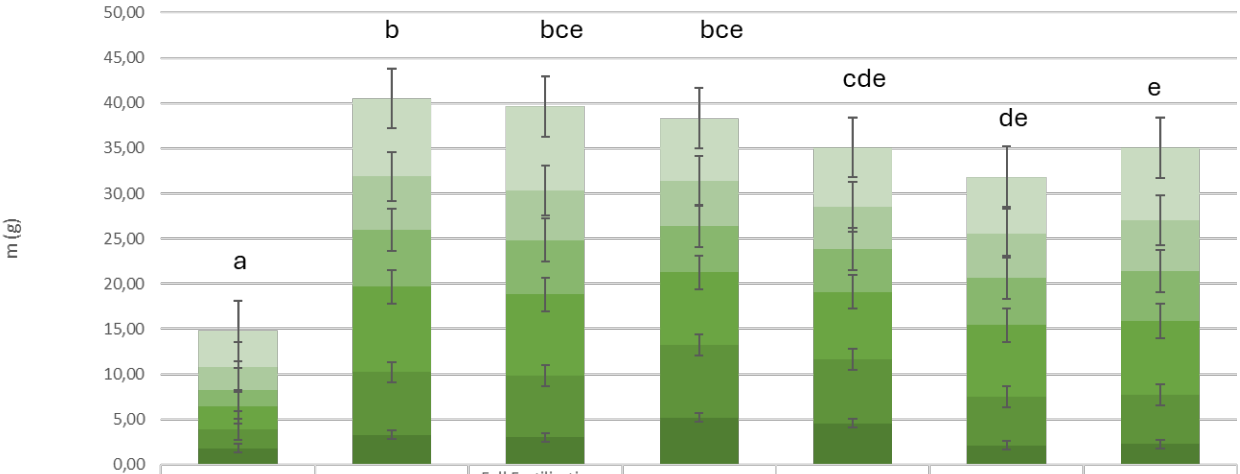
Tables 15 gives an overview of the amount of P that was supplied and the overall sum of the six harvests of the P content that was assimilated by the plants. Based on this data the APR and PFRV were determined. The APR values for the biochar treatment (DE-BC) are slightly higher (14.8%) compared to full fertilisation (13.9%). Lime does not enhance P uptake; instead, it slightly decreases APR values in lime-supplemented treatments. DE-BC biochar, with a PFRV of 106%, is a promising alternative to synthetic P fertilisers. On the other hand, the DE-AP biobased fertiliser has half the APR size, and lime improves P availability. However, the fertilising effects of DE-AP are suboptimal (PFRV of 45-56%).



	Control	Full Fertilisation	Full Fertilisation + Lime	DE-BC	DE-BC + Lime	DE-AP1	DE-AP1 + Lime
Harvest 6 (g/pot)	3,97	8,64	9,32	6,88	6,59	6,22	8,04
Harvest 5 (g/pot)	2,53	5,88	5,42	5,02	4,65	4,89	5,62
Harvest 4 (g/pot)	1,88	6,30	6,04	5,13	4,75	5,28	5,52
Harvest 3 (g/pot)	2,49	9,46	9,00	8,02	7,47	7,92	8,13
Harvest 2 (g/pot)	2,13	6,93	6,77	8,04	7,04	5,37	5,46
Harvest 1 (g/pot)	1,81	3,30	3,05	5,20	4,59	2,14	2,29

Figure 16. Harvestable amount of ryegrass (g/pot) during the six harvests done within the **pot trial 1.**

Table 15. Overview of the amount of phosphorus (P) added to each treatment, the amount of P which was assimilated into the plants over six harvest cycles with the respective apparent P recovery (APR) value and the P fertiliser replacement value (PFRV).

Treatment	Amount P Fertilised [mg/pot]	Sum P Uptake [mg/pot]	APR [%]	PFRV [%]
Control	0	20	-	-
Full Fertilisation	739	123	13.9	-
Full Fertilisation + Lime	739	112	12.5	-
DE-BC	729	128	14.8	106.3
DE-BC + Lime	729	106	11.8	94.8
DE-AP	722	65	6.2	44.7
DE-AP +Lime	722	70	6.9	55.6

(ii) Pot trial 2 (Maize)

Dry biomass results (Figure 17) show that highest yield was observed in full fertilization and biochar treatment. Lime widens data spread and tends to negatively affect the yield. DE-AP1 treatment results in significantly lower biomass, noticeable in plant growth assessment, with almost no advantage over the unfertilized control for maize. This might be due to an even distribution of fertiliser on perlite which led to reduced nutrient uptake.

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Full fertilisation and DE-BC treatment provide sufficient P supply, while the addition of lime scatters results and worsens P uptake in DE-BC.

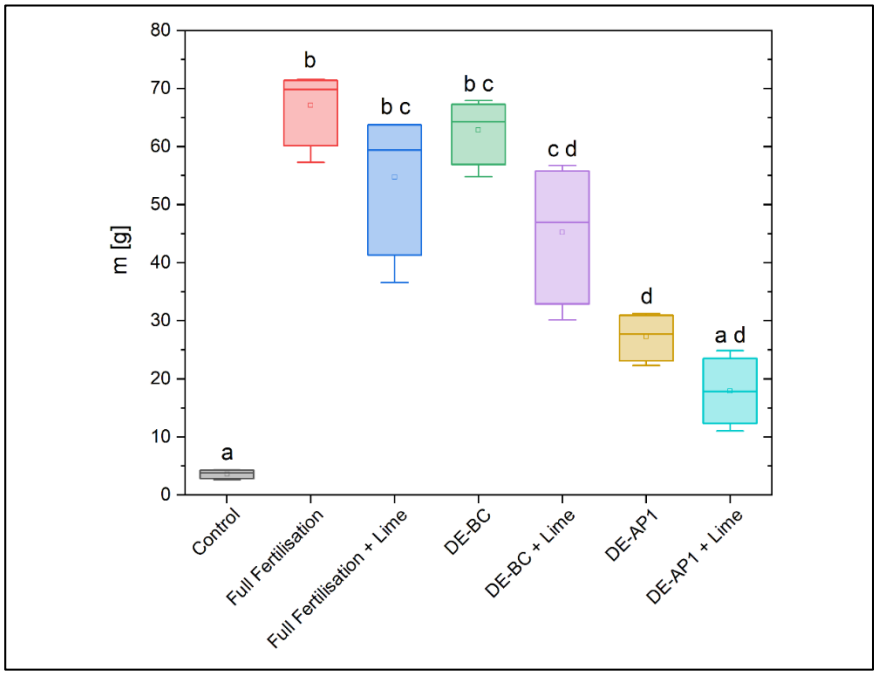

Figure 17. Dry matter yield (g per pot). Different letters indicate significant differences between the individual test members (Tukey test, $p < 0.05$).

The DE-BC treatment shows a higher APR of 12.6% compared to other treatments, suggesting better utilization of P (Table 16). However, when lime is added to the DE-BC treatment, the APR decreases to 7.6%, indicating a potential reduction in P uptake efficiency. Similarly, the PFRV values indicate the effectiveness of P fertilizer application relative to the control treatment. Treatments with higher PFRV percentages demonstrate greater P uptake efficiency compared to the control. For example, the DE-BC treatment exhibits a PFRV of 112.8%, indicating that it effectively replaces the P from the control treatment and promotes enhanced P utilization by plants. However, the addition of lime to the DE-BC treatment reduces the PFRV to 68.1%, suggesting a decrease in P fertilizer replacement efficiency. However, the addition of lime to certain treatments appears to have reduced P uptake efficiency, as evidenced by lower APR and PFRV values. This could be attributed to factors such as altered soil pH affecting P availability or interactions between lime and P in the soil. Overall, the observed results suggest that treatments like DE-BC can enhance P uptake and utilization efficiency, potentially contributing to improved agricultural productivity. Conversely, the addition of lime may have varying effects on P utilization efficiency, underscoring the importance of considering soil amendments' interactions in agricultural practices.

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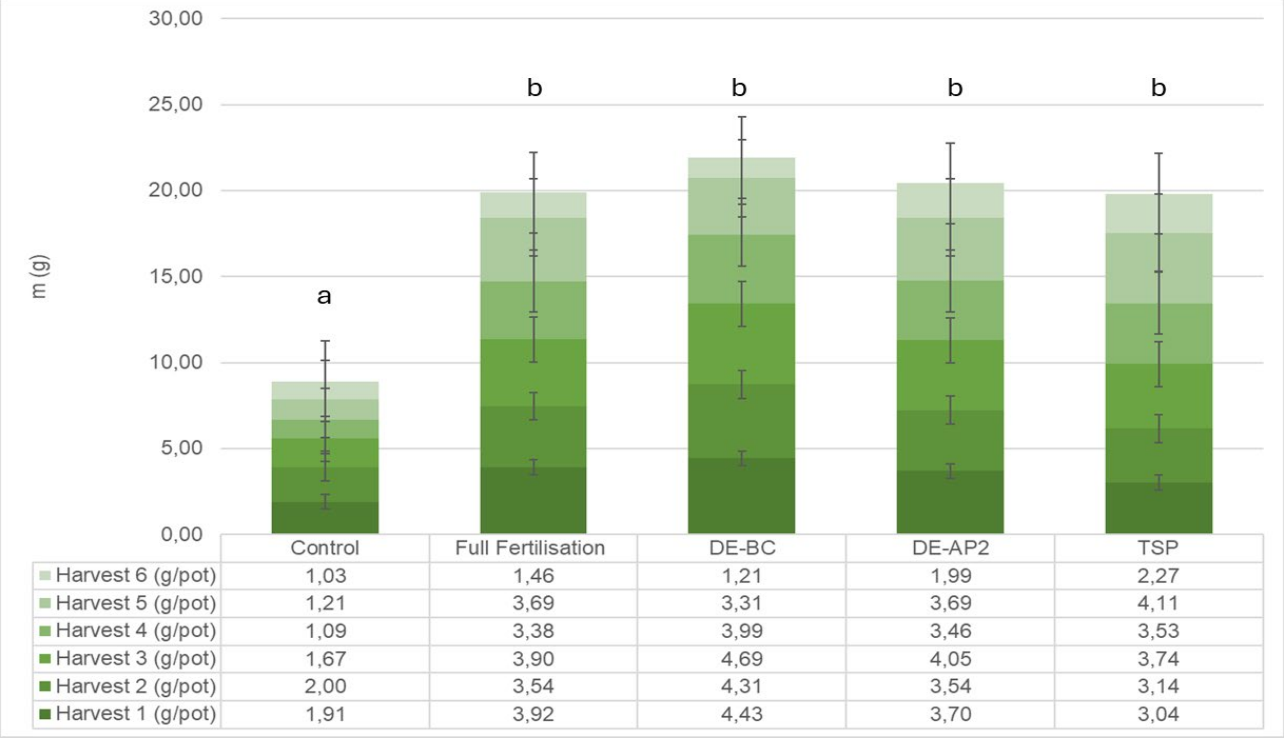
Table 16. Overview of the amount of phosphorus (P) added to each treatment, the amount of P which was assimilated into the plants after 7 weeks growth with the respective apparent P recovery (APR) value and the P fertiliser replacement value (PFRV).

Treatment	Amount P Fertilised [mg/pot]	Sum P Uptake [mg/pot]	APR [%]	PFRV [%]
Control	0	3.58	-	-
Full Fertilisation	730	85.18	11.2	-
Full Fertilisation + Lime	730	78.04	10.2	91.3
DE-BC	730	95.61	12.6	112.8
DE-BC + Lime	730	59.15	7.6	68.1
DE-AP1	730	32.02	3.9	34.9
DE-AP1 + Lime	730	20.22	2.3	20.4

(iii) Pot trial 3 (Rye grass)

In comparison to the first rye grass trial in this third trial all P applied comes from the BBFs DE-BC and DE-AP2. As stated, before DE-AP2 was isolated from the optimized process of the German pilot plant and corresponds to pure mono ammonium phosphate. The first harvest of biomass took place after 18 days. Five additional harvests were carried out after 26, 35, 43, 55 and 68 days. In Figure 18, an overview of the harvestable amount. Similar to the first pot trial, the first harvests of DE-BC fertilised plants had the highest biomass production within the first four harvests. The last two harvest showed a decrease in the harvestable amount in all plants.



	Control	Full Fertilisation	DE-BC	DE-AP2	TSP
Harvest 6 (g/pot)	1,03	1,46	1,21	1,99	2,27
Harvest 5 (g/pot)	1,21	3,69	3,31	3,69	4,11
Harvest 4 (g/pot)	1,09	3,38	3,99	3,46	3,53
Harvest 3 (g/pot)	1,67	3,90	4,69	4,05	3,74
Harvest 2 (g/pot)	2,00	3,54	4,31	3,54	3,14
Harvest 1 (g/pot)	1,91	3,92	4,43	3,70	3,04

Figure 18. Harvestable amount of ryegrass (g/pot) during the six harvests done within the **pot trial 3**.

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Within this trial the optimized product DE-AP2 was used. As can be seen from Table 17 the P uptake from this product is as sufficient as mineral fertilisation. This supports our claim that we indeed did recover pure mono ammonium phosphate with our MAP reactor. Additionally, DE-BC shows a quite high PFRV indicating better P availability than conventional mineral fertilisation. These findings show that the P in DE-BC biochar is fully plant available and can be used as additional fertiliser. This enables the product not only to be a soil conditioner but instead a soil conditioner with fertilising effect.

Table 17. Overview of the amount of phosphorus (P) added to each treatment, the amount of P which was assimilated into the plants after 6 harvests and 68 days of growth with the respective apparent P recovery (APR) value and the P fertiliser replacement value (PFRV).

Treatment	Amount P Fertilised [mg/pot]	P Uptake [mg/pot]	APR [%]	PFRV [%]
Control	0	14.18	N/A	N/A
Full Fertilisation	730	60.44	6.3	N/A
DE-BC	730	65.45	7.0	110.8
DE-AP2	730	62.94	6.7	105.4
TSP	730	60.82	6.4	100.8

N/A: not applicable

4.2.4 Conclusion and recommendation

In our study, the performance of DE-BC across all three experiments stood out prominently. Notably, in the third experiment, DE-BC showcased a performance akin to that of TSP, highlighting its efficacy as a P fertilizer. The consistent performance of DE-BC underscores its potential as a reliable option for promoting P uptake and enhancing crop growth across different growth stages. Moreover, the comparison between DE-AP1 and DE-AP2 revealed distinct differences in their P availability. DE-AP2 demonstrated significantly improved P availability compared to DE-AP1, indicating its potential for more effective P supplementation. Both DE-AP1 and DE-AP2, however, exhibited comparable performance to TSP, suggesting their viability as alternatives to traditional P fertilizers. These findings emphasize the importance of selecting appropriate P fertilizers tailored to specific agricultural needs. DE-BC emerges as a promising option, offering consistent performance comparable to conventional fertilizers like TSP. Meanwhile, the superior performance of DE-AP2 in P availability highlights its potential for optimizing nutrient uptake and enhancing crop productivity. Overall, our study underscores the significance of P fertilization in promoting plant growth and crop yield. By evaluating the performance of DE-BC and DE-AP1/DE-AP2 against traditional fertilizers like TSP, we provide valuable insights into optimizing P management strategies for sustainable agriculture practices. Further research and field trials can provide additional validation and refinement of these findings, contributing to the advancement of P fertilization practices in agricultural systems.

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4.3. Bio-dried solid fraction (ES-DSC), Phosphorus ashes (ES-PA) and Nutrient rich concentrate (ES-NC) from Spanish pilot (UVIC-UCC)

For more information on this study, please contact the authors from UVIC-UCC: Berta Singla Just (berta.singla@uvic.cat), Laura Diaz-Guerra (laura.diaz.guerra@uvic.cat) and Ana Robles Aguilar (ana.robles@uvic.cat).

This section will be published as "Singla Just, B., Castaño, O., Guerra-Gorostegui, N., Vilaplana, R., Meers, E., Robles Aguilar , A., Díaz-Guerra, L., Llenas, L. Assessing P bioavailability in Bio Based Fertilizers derived from animal manure through incubation study and pot trial. Under Preparation"

Some of the results in this section are included in the following publication: "Singla Just, B., Binder, P., Guerra-Gorostegui, N., Díaz-Guerra, L., Vilaplana, R., Frison, N., Meers, E., Llenas, L., Robles Aguilar, A., Phosphorus Release Dynamics from Ashes during a Soil Incubation Study: Effect of Feedstock Characteristics and Combustion Conditions. Agronomy. 2024, 14(5), 935. https://doi.org/10.3390/agronomy14050935"

4.3.1 Introduction

The main objective of this study was to assess the mineralisation kinetics and the agronomic performance of the novel BBFs obtained from the Spanish pilot. To achieve this objective, two distinct methodologies were employed: incubations and pot trials.

Soil incubations with a low level of P amended under controlled temperature and moisture, following the same approach as N incubations (section 2.3.2). This was done by comparing the dynamics of P mineralisation in soil amended using BBFs with those in soil amended by conventional mineral fertiliser (TSP) and pig slurry (PS) from the Spanish pilot. Hence, the BBFs studied were: bio-dried solid fraction (ES-DSC) and P ashes (ES-PA) from the solid fraction, and two combinations of nutrient rich concentrate (i.e., ES-NC- MFRO and ES-NC-MFR) from the liquid fraction.

Pot trials were conducted using ryegrass as the test crop to assess the effectiveness and P bioavailability of the BBFs from the Spanish pilot that contained certain P amounts. Therefore, this study involved a comparison of P mineralisation dynamics in soils amended with BBFs against those amended with conventional mineral fertiliser (TSP) and pig slurry (PS). The treatments included the biodried solid fraction (ES-DSC) and the extraction of phosphoric acid (ES-EPA) from P-rich ashes (ES-PA). The process of obtaining this acid involved pelletizing and incinerating the ES-DSC in a biomass boiler, resulting in P-rich ashes. Following this, 1.2 M sulfuric acid was added to produce phosphoric acid. The reverse osmosis retentates treated via freeze concentration (ES-NC-MFRO) and retentate of microfiltration treated by freeze concentration (ES-NC-MFR) were also assessed.

4.3.2 Methodology

The soil used for the incubation and pot experiment was the same as the one that was used for N (section 2.3.2). The tested BBFs were collected from the Spanish pilot plant as described in section 2.3.2, and their characterization is shown in the Table 18.

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Table 18. Chemical composition of pig slurry (PS), mineral fertiliser triple super phosphate (TSP) and BBFs used in the P incubations and pot trial (mean value ± standard deviation, *n*=3)

Parameter	Unit	PS	TSP	ES-DSC	ES-PA	ES-EPA	ES-NC-MFRO	ES-NC-MFR
pH	-	6.39±0.01	ND	7.19	ND	2.61±0.2	7.33±0.01	7.42±0.03
EC	$mS \cdot cm^{-1}$	25.49±0.14	ND	*6.5±0.14	ND	ND	22.3±0.46	27.40±0.30
C total	%	3.44±3.44	ND	26.74±0.27	ND	ND	1.99±0.00	1.89±0.01
N total	%	0.45±0.00	ND	2.27±0.02	ND	ND	0.38±0.00	0.47±0.01
C/N ratio	-	7.64	ND	11.77	ND	ND	5.23	4.02
P total	$mg\ P \cdot kg^{-1}$	810±10.68	21.8	5445.53±49.82	72.9±0.15	11.37±0.5**	572.94±19.64	1136.70±44.34
DM	%	5.39±0.05	ND	66.57±0.5	ND	ND	3.31±0.14	5.04±0.2

** The unit is in $\mu S \cdot cm^{-1}$; **The unit is mg/L. ND: not determined; ES-DSC: bio-dried solid fraction; ES-PA: phosphorous ashes; ES-EPA: phosphoric acid from phosphorus ashes; ES-NC-MFRO: nutrient rich concentrate from microfiltration and reverse osmosis retentates treated via freeze concentration; ES-NC-MFR: nutrient rich concentrate from - retentate of microfiltration treated by freeze concentration.*

(i) Incubations

P incubation in the absence of plant was done in a similar approach to N incubations (as is described in section 2.3.2), but in this case the application rate was 48 kg $TP \cdot ha^{-1}$. Before the main incubation, a soil pre-incubation in the dark at 22 °C was conducted for one week to stimulate microbial activity. After this, 147 g soil was placed in a 100 mL container and mixed with ES-DSC, ES-PA, ES-NC-MFRO, and NC-MFR at the rate of 48 kg $TP \cdot ha^{-1}$. In addition, a negative control and two positives references such as triple super phosphate (TSP) and pig slurry (PS) from the Spanish pilot were included. Then, using distilled water, the mixtures were raised and adjusted to 50% WHC. The containers were closed with a layer of parafilm with pin holes to minimize the moisture losses, ensuring gas exchange during the pre-incubation. To capture the P dynamics, 10 destructive samplings were done on days 5, 10, 20, 40, 60, 80, 100, 120, 140, and 160. In all treatments, four replicates were collected in each sampling to offer an adequate data set of the dynamics of P mineralisation during the experiment. The available P was analysed using the calcium acetate lactate (CAL) method on each sampling day.

(ii) Pot trial

In this case, one litter-capacity pot of 10 cm in height was used for the pot trial. Following the same approach as the incubations, a soil pre-incubation in the dark at 22 °C was done in order to stimulate microbial activity. After this, 1.2 kg of pre-incubated soil was added to each pot and mixed with the BBFs. In addition, a negative and two positive controls (TSP and pig slurry from the Spanish pilot) were added. The test was done at 3 application levels: 30%, 60% and 100% of the recommended P dosage (48 kg TP/ha), considering that the estimated production will be 5 tonnes per hectare and the amount of P available in the soil is low. Ryegrass seeds were sown at 1 cm below the surface of each pot at 3.5 $g \cdot m^{-2}$ (Teagasc, 2014). Pots were kept in an indoor growth chamber at room temperature with controlled light conditions (2000 LUX, daytime = 12 hours, night-time = 12 hours). In addition, potassium nitrate was added as a Holland solution to balance mainly N levels along the treatments. The experiment followed a randomized block design, with each watering up to 50% WHC and four replicates per treatment. In addition, three cuttings of the foliar ryegrass biomass were carried out (once a month) throughout the pot trial. Afterwards the plant material was dried at 60°C for at least 48 h until it reached a constant dry weight. The plant material was examined for its total P content in the laboratory.

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4.3.3 Results and discussion

(i) Incubations

At the end of the incubation period, the average P release (mean ± standard deviation, expressed in %) in each treatment was: 125±56 for TSP, 76±15 for ES-DSC, 71±9 for PS, 54±20 for ES-PA, 53±7 for ES-NC-MFRO, and 48±7 for ES-NC-MFR (Figure 19). The results indicated that the mineral reference (TSP) achieved the highest P release due to the high content of inorganic P. Notably, none of the tested BBFs exhibited a release P pattern similar to that of TSP. However, ES-DSC displayed the second-highest P release pattern, particularly at the early stage of the incubation period (on day 10). However, all BBFs that were tested showed a P release pattern of more than 50%, except ES-PA, which released only an average of 54%.

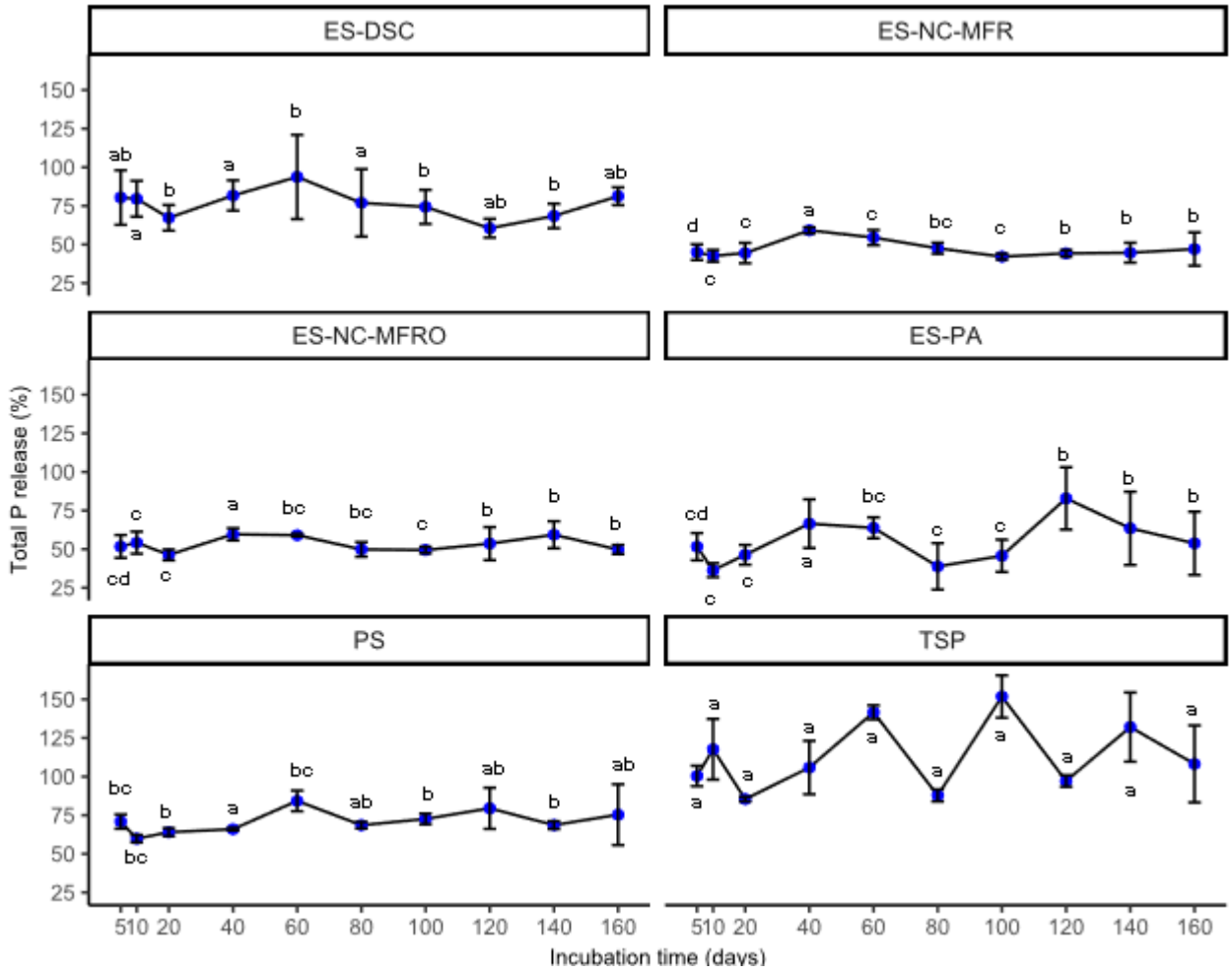

Figure 19. P release (in % of total P applied) for applied fertilisers over a time of 160 days (mean value ± standard deviation, n=4). Lower case letters (a–d) denote statistically significant differences in means (Tukey's Test for p ≤ 0.05) among the products for each sample time (t = 5, 10, 20, 40, 60, 80, 100, 120, 140, and 160 days).

(ii) Pot trial

Figure 20 illustrates the cumulative dry yield obtained from each treatment across the three cuts. There was a notable increase in dry yield, particularly evident during the final cut (at the end of the experiment), indicating the effectiveness of slow-release fertilizers. In contrast, the negative control demonstrated inferior agronomic performance compared to the other treatments (average: 0.77 g DM·pot^{-1}). Also, most treatments bested the

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mineral fertilizer TSP at dosages of 60% and 100%. However, no significant statistical differences were found at the 30% dosage level. Additionally, among the BBFs, ES-NC-MFR exhibited a higher yield (on average) compared to the other treatments.

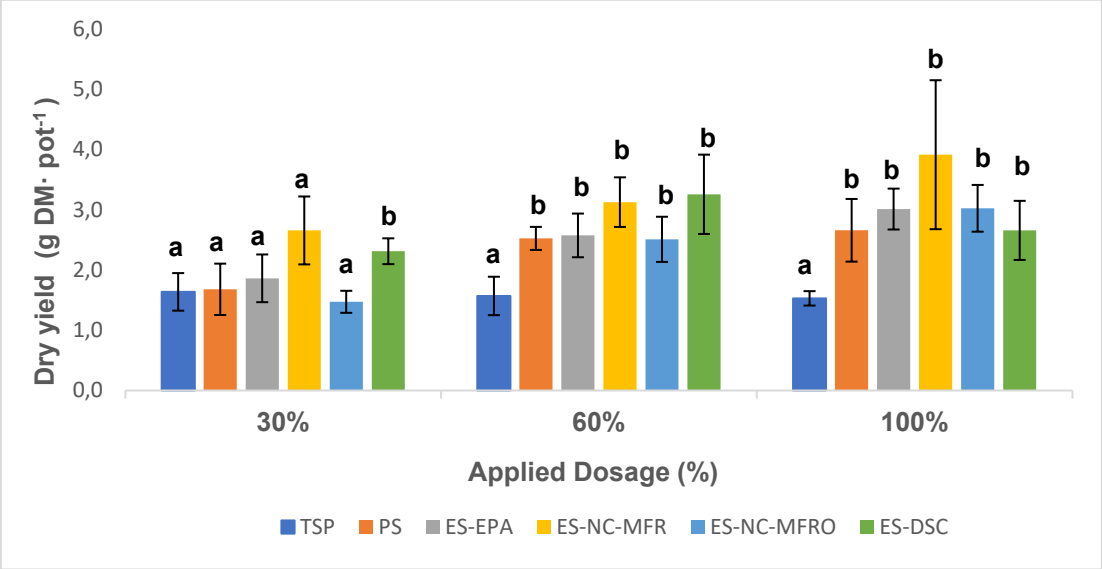

Figure 20. Cumulative dry yield of ryegrass (g/pot) for each treatment (mean value ± standard deviation, n=4). Above the histogram, lowercase letters indicate significant different means (one-way ANOVA, $p \leq 0.05$) between products for the sum of the 3 cuts.

The Table 19 displays the effectiveness of BBFs compared to conventional fertilizers (TSP) and pig slurry (PS) in terms of APR and PFRV. In general, the treatments including ES-DSC, ES-EPA, ES-NC-MFRO, and ES-NC-MFR, exhibited different APR and PFRV values that varied across different application rates. This indicates differences in P availability and uptake by the ryegrass plants. For instance, ES-DSC and ES-NC-MFR treatments showed relatively higher APR percentages, suggesting their potential effectiveness in supplying P to the plants. On the other hand, ES-PA had lower values but achieved comparable results to the mineral fertilizer TSP. Moreover, in terms of PFRV, treatments with ES-NC-MFR and ES-DSC showed the highest values, indicating their superior performance compared to TSP.

Contrary to expectations, the BBFs performed better than TSP, and this could be explained for several reasons. Firstly, the inorganic P fixed in soils as a part of SOM is less stable than P minerals fixed in soils, which could quickly become available P for the plants (Jindo et al., 2023). Several studies such as Ohno & Crannell (1996) and Chase et al. (2018) have also shown that adding organic matter, such as the BBFs tested (ES-NC-MFR, ES-NC-MFRO, and ES-DSC), can prevent soil P fixation and enhance the amount of phytoavailable P. On the other hand, considering that only P and N were balanced in terms of nutrients, the high amount of N and K could have increased the N to P or K to P ratio, also enhancing the P release of some BBFs (Bogdan et al. 2021) Additionally, Nardi (2017) found that organic molecules with hormone-like structures or other compounds that stimulate plant growth (mainly root growth) can enhance P uptake. This also contributes to explain the results obtained, since TSP does not contain these molecules while BBFs do.

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Table 19. Overview of the amount of P uptake which was assimilated into the plants after 90 days of growth with the respective apparent P recovery (APR) value and the P fertiliser replacement value (PRFV).

Treatment	Average of Sum P Uptake [mg/pot]	APR (%)	PFRV (%)
TSP, 30%	2.8	32.6	
TSP, 60%	2.9	16.6	
TSP, 100%	2.5	8.3	
PS, 30%	2.2	23.6	72
PS, 60%	4.0	24.7	149
PS, 100%	5.7	21.6	261
ES-PA, 30%	2.4	26.7	82
ES-PA, 60%	3.9	23.9	145
ES-PA100%	5.0	18.7	225
ES-DSC, 30%	2.8	32.3	99
ES-DSC, 60%	6.1	38.9	235
ES-DSC, 100%	6.2	23.7	286
ES-NC-MFR, 30%	4.4	55.0	169
ES-NC-MFR, 60%	9.0	59.0	356
ES-NC-MFR, 100%	12.8	51.3	619
ES-NC-MFRO, 30%	2.1	22.6	69
ES-NC-MFRO, 60%	4.1	25.3	153
ES-NC-MFRO 100%	5.6	21.4	258

4.3.4 Conclusion and recommendation

In conclusion, the analysis revealed significant variations in the effectiveness of the BBFs studied. The ES-DSC, ES-NC-MFRO, ES-NC-MFR, and ES-PA, exhibit different effectiveness in supplying P to ryegrass plants compared to conventional fertilisers (TSP) and pig slurry (PS). While treatments like ES-DSC and ES-NC-MFR demonstrate relatively higher P recovery rates, ES-PA achieves comparable results to TSP. Notably, treatments with ES-NC-MFR and ES-DSC show superior performance in terms of PFRV, indicating their potential as effective alternatives to conventional fertilisers in sustainable agricultural practices. Further research and optimization of application strategies are recommended to maximize the effectiveness of BBFs in nutrient management and crop productivity while minimizing environmental impacts.

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5. Assessment of biological activated biobased fertilisers (UMIL+AGRI)

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*This section has been redrafted according to Clagnan, E., Cucina, M., De Nisi, P. et al. Effects of the application of microbiologically activated bio-based fertilizers derived from manures on tomato plants and their rhizospheric communities. Sci Rep **13**, 22478 (2023). https://doi.org/10.1038/s41598-023-50166-5*

5.1 Introduction

The use of biobased fertilisers (BBF) is a promising strategy to optimise the exploitation of resources and generate products that can be effective in agriculture. However, the processes used for their production are generally detrimental for the soil beneficial microorganisms. Soil fertility is characterised by three different dimensions: physical, chemical, (micro) biological. While the supply of solid BBFs to soil can have a positive effect on the first two dimensions, microbiological diversity in BBFs is reduced by the production system, making it necessary to enrich BBFs with microorganisms that are useful to plants and soils. Soil microorganisms play a key role in maintaining the productive balance of plants and the health of soil. They have numerous functions, either on a nutritional level: by increasing phosphorous use efficiency, as in the case of mycorrhizae; or by fixing N in the soil, as in the case of rhizobia. In addition, some microorganisms can penetrate inside the plant by acting as endophytes. Endophytes generate a series of metabolic changes within the plant itself, making it more responsive to environmental stresses, some of these microorganisms are even good degrader of organic matter, and it helps to slowly release nutrients to plants when they need for it. Finally, there are a series of microorganisms that inhabit the rhizosphere, the part of the soil closest to the plant roots, that produce a series of metabolites capable of stimulating plant growth, which is why they are called Plant Growth Promoting Rhizobacteria (PGPR). Therefore, it is of paramount importance to enrich BBFs with microbial consortia to enable them to fulfil all three fertility dimensions.

5.2 Methodology

(i) Microbial consortia development

Specific microbial consortia were developed by Agrifutur according to the characteristics of the different BBFs. The consortia were designed with microorganisms that complement each other and cover the major functional groups required for the proper development of plants in the soil: PGPR, Soil Organic Matter (SOM) degrader, N-fixer, P-use efficiency. The consortia developed are presented in Table 20.

(ii) Biobased fertilisers characterization

Three biobased fertilisers (BBF) were selected from WP2 and characterised (Table 21): biodried solid fraction, coming from a biodrying process developed at ES pilot plant, solid fraction of digestate coming from the NL pilot plant and biochar coming from FR pilot plant. The three BBF showed different characteristics and properties and they were coupled with microorganisms taking into consideration these characteristics (Table 21).

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Table 20. Composition of microbial consortia.

Microbial consortium	Biobased Fertiliser	Microorganism	Functional group
Microbial consortium 1	Biodried solid fraction (ES)	*Rhizobium pisi*	PGPR[a]
		Sinorhizobium meliloti	PGPR
		Lactobacillus plantarum	PGPR
		Trichoderma harzianum	SOM[b]-degrader
Microbial consortium 2	Solid fraction of digestate (NL)	*Azospirillum brasilense*	N-fixer
		Trichoderma viride	SOM-degrader
		Rhizophagus irregularis	P-use efficiency
		Lactobacillus plantarum	PGPR
Microbial consortium 3	Biochar (FR)	*Trichoderma viride*	SOM-degrader
		Rhizophagus irregularis	P-use efficiency

[a]*Plant growth promoting*
[b]*Soluble organic matter*

Table 21. Biobased fertilisers characterization

Parameter	Unit	Biodried solid fraction	Solid fraction of digestate	Biochar
Country	-	Spain	The Netherlands	France
Code	-	ES	NL	FR
Total solids	%	52.8[a] ± 0.7	26.2 ± 0.4	90.3 ± 1.1
pH	pH unit	7 ± 0.1	7.4 ± 0.1	9.1 ± 0.1
Total OC	%	17 ± 1	37 ± 2	54 ± 3
Total N	%	2.8 ± 0.1	2.1 ± 0.1	2.7 ± 0.1
Total P	%	0.6 ± 0	1.1 ± 0.2	2.2 ± 0.1
Total K	%	1.2 ± 0.1	1.7 ± 0.1	7.3 ± 0.2
DRI	mmol O_2 h^{-1} $kgVS^{-1}$	42.2 ± 35	n.d.[b]	n.d.[b]

[a]*Mean Value ± SD (n =3)*
[b]*Not determined*
Data are expressed on dry weight basis except for total solids and pH

(iii) Pot experiments

Three pot experiments were carried out using three different crop species.The first pot experiment was carried out between March and July 2022 using tomato plants (*Solanum lycopersicon* cv. Minuet). The second and third experiments were carried out between April and May 2023 using radish (*Raphanus sativus* L. cv. Pablo) and lettuce (*Lactuca sativa* cv. Canasta) respectively. ES-BBF and NL-BBF were applied as N organic fertilisers, i.e. they were used substituting completely mineral fertilisers, while FR-BBF was applied as organic amendment. Each treatment was replicated in four pots. Each pot was filled with 2 kg of soil for tomato, 1 kg of soil for radish and lettuce; pots were watered to obtain 60 % of WHC (water content was maintained throughout the experiment by weighing pots and adding lost water) and non-activated or activated fertilisers were applied following experimental plan reported in Table 22. Plants were then transplanted and grown in greenhouse ensuring the optimal photoperiod (16 h light and 8 h dark per day). Basing on the results obtained for tomato, an additional treatment was included in the radish and lettuce experiments: NL activated with Trichoderma (NL T). Experiment lasted for 110 days for tomato, 14 days for radish and 35 days for lettuce. The characteristics of the soil used are reported in Clagnan et al., 2023.

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Table 22. Biobased fertilisers (BBFs) application doses and experimental design for tomato, radish and lettuce plants

Tomato					
Treatment	**Description**	**BBF amount (g)**	**N fertilization (g Urea)**	**K fertilization (g K_2SO_4)**	**Biological activation**
NT	Non-fertilized soil	-	-	-	-
C	Chemical Fertilization	-	0.13	0.22	-
ES	BBF1	2.1	-	0.15	-
ES A	Activated BBF1	2.1	-	0.15	X
NL	BBF2	2.8	-	0.11	-
NL A	Activated BBF2	2.8	-	0.11	X
FR	BBF3	16	0.13	0.22	-
FR A	Activated BBF3	16	0.13	0.22	X
Radish					
Treatment	**Description**	**Amount of BBF (g)**	**N fertilization (g Urea)**	**K fertilization (gK_2SO_4)**	**Biological activation**
NT	Non-fertilized soil	-	-	-	-
C	Chemical Fertilization	-	0.022	-	-
ES	BBF1	0.372	-	-	-
ES A	Activated BBF1	0.372	-	-	X
NL	BBF2	0.496	-	-	-
NL A	Activated BBF2	0.496	-	-	X
NL T	Trichoderma act. BBF2	0.496		-	X
FR	BBF3	8	0.022	-	-
FR A	Activated BBF3	8	0.022	-	X
Lettuce					
Treatment	**Description**	**Amount of BBF (g)**	**N fertilization (g Urea)**	**K fertilization (gK_2SO_4)**	**Biological activation**
NT	Non-fertilized soil	-	-	-	-
C	Chemical Fertilization	-	0.097	0.104	-
ES	BBF1	1.607	-	0.061	-
ES A	Activated BBF1	1.607	-	0.061	X
NL	BBF2	2.143	-	0.023	-
NL A	Activated BBF2	2.143	-	0.023	X
NL T	Trichoderma act. BBF2	2.143	-	0.023	X
FR	BBF3	8	0.097	0.104	-
FR A	Activated BBF3	8	0.097	0.104	X

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(iv) Crop production evaluation and qualitative characterisation of products

Tomatoes were collected through all crop season, at full ripeness assessed using a colorimeter (Chroma Meter CR-410, Konica-Minolta, Milan, Italy). Colour parameters were set as: a (redness): 25 ± 2; L (darkness): 40 ± 2 (Bakir et al., 2020). Once harvested, tomatoes were weighed and then characterised for the following parameters: total sugar, titrable acidity, sugar: acid ratio, lycopene, carotenoids, taste index, and protein. At the end of the trial plants were collected and both fresh and dry weight were registered.

Radish roots and leaves were harvested 14 days after transplanting. The following parameters were measured on radish roots immediately after harvest: root diameter, fresh weight, dry weight, macro and microelements, including Na, Mg, K, Ca, P, Mn, Fe, Cu, Zn, Cr, Co, Ni, As, Se, Mo, Cd, Pb, and the total glucosinolate concentration. Fresh weight and dry weight were determined also on radish leaves.

Lettuce was harvested 35 days after transplanting. The fresh and dry weight of harvested panicles was determined. Macro and microelement concentrations in panicles, including Na, Mg, K, Ca, P, Mn, Fe, Cu, Zn, Cr, Co, Ni, As, Se, Mo, Cd, Pb were determined. Twenty-five days after transplanting and immediately before harvesting, a portable leafclip meter DUALEX Scientific (Force A, Centre Universitaire Paris-Sud Batiment 503, rue du belvédère, 91400 Orsay France) was used to obtain indexes related to leaf chlorophyll content (Chl), flavonols (Flv,), anthocyanins (Anth) and a Nitrogen Balance Index (NBI, ratio Chl/Flv) in intermediate leaves (Cerovic et al., 2012).

Statistical analysis was performed by one-way ANOVA and Tukey's post hoc test ($p<0.05$), and, when needed, by Student's *t* test between each treatment and the control ($p<0.05$) using SPSS software.

(v) Metagenomic analysis - 16S and ITS rRNA sequencing

Samples for molecular biology analyses were collected at the beginning (Time 0; n=1), middle (Time 1; n=1) and end (Time 2; n=3, pooled sample) of the experimental period for tomato while for radish and lettuce only at Time 0 and Time 2. All analyses were carried out as per Clagnan et al., 2023.

5.3 Results and discussion

(i) Tomato pot trial

In terms of both fresh and dry plant weight, both activated and non-activated treatments for ES (biodried material) and NL (solid fraction digestate) showed similar values to the untreated control, while lower values than the chemical fertilization were observed (Table 23). Biochar (FR) showed highest plant weight for both treatments. Unfertilized control showed the lowest weight in both fresh and dry tomatoes while the highest production was achieved with activated solid digestate (NL). In general, all activated BBF showed higher tomato weight than the non-activated treatments, however mostly lower than chemical fertilization. It was interesting to note that BBF used as a mineral fertiliser substitute produced less than chemical fertiliser. Still, when inoculated with microbial consortia, they produced similar (ES) or more (NL), indicating that microorganisms could probably overcome the low nutrient availability typical of organic fertilisers. Differences between biodried (ES) and solid digestate (NL), depended probably by the low biological stability of biodried materials exercising phytotoxicity. Biochar (FR) showed a different pattern as production obtained with the sole biochar was similar to that of chemical fertilisers. In this case, because biochar was used as organic amendment with the addition of mineral fertilisers, it could be that the presence of biochar made up a more efficient nutrient availability. More interesting was to observe that by adding microbial consortia, total tomatoes production increased a lot with the application of biochar. In conclusion, the data obtained seemed to indicate that microbial consortia play a role in enhancing total productivity of tomatoes (Table 23).

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Table 23. Productive yields of tomato plants (plant biomass and tomato fruits) in the pot experiment

Parameter	Unit	NT[a]	C[b]	Bodried-ES[c]		Solid digestate NL[d]		Biochar-FR[e]	
	-	Unfertilised control	Chemical fertilisation	BBF[f]	Biologically activated BBF	BBF	Biologically activated BBF	BBF	Biologically activated BBF
Fresh plant weight	g per pot	20.2[g] ± 3.3a	30.4 ± 2.8b	18.4 ± 2.8a	21.4 ± 2.2a	20 ± 1.2a	23.3 ± 3a	49 ± 2.7c	49.9 ± 4.4c
Dried plant weight	g per pot	9.1 ± 1.5a	13.1 ± 1.2b	8.1 ± 0.6a	10.3 ± 1a	8.6 ± 0.5a	9.8 ± 1.3a	24.5 ± 1.4c	25 ± 2.2c
Fresh tomato weight	g per pot	52.6 ± 7.6a	107.9 ± 3.8c	88.2 ± 8.4b	101.6 ± 3.9c	89 ± 5.5b	120.5 ± 2.4d	110.5 ± 6.9b	127 ± 4.8d
Dried tomato weight	g per pot	5.8 ± 1.1a	12.1 ± 0.9c	9.4 ± 0.9b	11.7 ± 0.7c	7.9 ± 1.4b	13.1 ± 0.5d	11.8 ± 0.3b	14.1 ± 2.1d

[a]Unfertilised control, [b]Chemical fertilisation, [c]Spanish bio dried solid fraction, [d]Dutch solid fraction of digestate, [e]French biochar, [f]Biobased fertiliser, [g]Mean value ± SD (n = 3). Means followed by the same letters are not statistically different according to the Tukey test (P ≤ 0.05)

Total solids, tritable acidity and pH were similar for all tomatoes under all treatments. Total soluble sugars were always higher for all treatments than the unfertilized control. Biodried material (ES) and solid digestate (NL) showed lower total soluble sugars than the chemical fertilization treatment while biochar (FR) showed highest or similar values to chemical fertilization. Sugar-acid ratio showed the highest value again with chemical fertilization while the lowest in the unfertilized control and with non-activated NL and FR and no difference was present between non-activated and activated ES. Taste index was again the lowest in the unfertilized control while the highest with chemical fertilization and both FR treatments. Protein content was the lowest in the unfertilized control while the highest for the activated treatment in conjunction with ES and FR while NL showed similar values in both treatments. Both carotenoids and lycopene concentrations were the lowest in unfertilized control, NL showed similar values for both treatments while ES and FR showed higher values in the activated respect to the non-activated treatments. In conclusion, tomatoes characterization indicated that fertilization enhanced tomatoes quality but that there were no differences between fertilisers (chemical vs. organic) as well as between activated and non-activated organic fertilisers.

From the results obtained (i.e. presence of the bacteria only at Time 0 or retrieved after Time 0 without a specific pattern) it seemed that these bacteria might be not effective or effective at the early stage. After Time 0, their survival might be hindered by the soil and rhizospheric communities or reduced below detectable limits. Another reason for their low retrieval might be that the fertilization technique, that is mimicking an on field spreading, might not be as suitable for these bacteria as it might be for their fungal “partners”. Commonly known techniques that enhance their efficiency might be seed coating or seedling’s roots dipping, however new techniques need to be developed to use microbial activation on-field especially in combination with BBFs application (Ptaszek et al., 2023). When compared to bacterial inoculants, fungal inoculants showed a higher permanence in soil and were retrieved also after Time 0. *Rhizophagus* was present at low abundance and sporadically which might hint to a “casual presence” probably not correlated to the fertilization. Similarly, *Trichoderma* was also retrieved in all samples leading to the conclusion that Trichoderma is already a component of the soil community under study. However, when “spiked” its presence was higher across time in the ABBFs, leading to that conclusion that *Trichoderma* is playing the leading role out of all microorganisms and driving the biomass and nutritional differences. The fertilization technique seems to be better suited to fungi showing less problematic than for their bacterial counterparts. Complete results for tomato can be found in Clagnan et al. (2023).

(ii) Radish pot experiment

Table 24 shows the productive yields of both fresh and dry weight of radish shoot (aerial plant portion) and root (the edible part). Both fresh and dry weight of radish shoots treated with non-activated and activated ES

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(biodried material) and NL (digestate solid fraction) were similar to the untreated control (NT). Plants treated with Trichoderma-activated digestate solid fraction (NL T) and those treated with both non-activated biochar (FR) showed fresh and dry weights higher than control (NT) according to Student's t test ($p < 0.05$). Considering radish roots, the edible and commercially valuable part of radish, both fresh and dry weights were similar for NT and C. Roots treated with both non-activated and activated ES (biodried material) showed fresh and dry weight similar (even lower) to NT and C. NL-treated roots, both activated and non-activated, gave yields similar to NT, as well as those treated with Trichoderma-activated NL. On the contrary, activated-FR plants gave root yields higher than the control (NT).The higher productivity of plants treated with activated-FR could be attributed to an availability of NPK present in biochar that is higher than expected and further increased by the activation with the microbial consortium. This effect is more evident in root than in shoot weight. The low yields shown by ES-treated plants, particularly by roots, could probably be attributed to the low biological stability of biodried materials, leading to phytotoxicity, as noted for tomato.

Table 24. Productive yields of radish (plant and root biomass) in the pot experiment.

Radish shoot	**FW**	**DW**	**DW**
	(g per pot)	**(g per pot)**	**(%)**
NT	6.44 ± 0.65	0.56 ± 0.06	8.75 ± 0.38
C	7.05 ± 0.34	0.64 ± 0.02	9.11 ± 0.13
ES	6.62 ± 1.49	0.58 ± 0.09	8.90 ± 0.77
ES A	6.35 ± 1.50	0.55 ± 0.16	8.60 ± 0.60
NL	5.97 ± 2.00	0.54 ± 0.19	9.08 ± 0.68
NL A	6.77 ± 1.18	0.57 ± 0.10	8.43 ± 0.64
NL T	7.85 ± 0.89*	0.77 ± 0.10*	9.78 ± 0.27
FR	8.67 ± 1.29*	0.75 ± 0.11*	8.70 ± 0.60
FR A	7.73 ± 1.11	0.68 ± 0.06	9.10 ± 1.40
Radish root	**FW**	**DW**	**DW**
	(g per pot)	**(g per pot)**	**(%)**
NT	14.96 ± 1.39	0.8 ± 0.16	5.35 ± 0.62
C	13.18 ± 0.83	0.71 ± 0.07	5.4 ± 0.23
ES	11.78 ± 1.78	0.69 ± 0.16	5.84 ± 0.47
ES A	12.69 ± 2.26	0.74 ± 0.14	5.81 ± 0.64
NL	15.04 ± 1.85	0.79 ± 0.05	5.3 ± 0.78
NL A	13.45 ± 0.27	0.76 ± 0.11	5.62 ± 0.8
NL T	13.56 ± 4.40	0.89 ± 0.22	6.66 ± 0.87
FR	16.34 ± 3.96	0.94 ± 0.06	5.9 ± 1.04
FR A	18.74 ± 2.29*	1.01 ± 0.04*	5.43 ± 0.59

Asterisks (*) indicate significant differences in a treatment respect to the control (NT) according to Student's t test ($p < 0.05$).

The diameter of radish roots was measured at harvest (data not shown). A high variability was found in this parameter within each treatment. Nor the samples treated with chemical fertiliser (C) neither those treated with BBFs (weather activated or not) exhibited any difference respect to the control (NT) ones (data not shown). The glucosinolate content (on a fresh weight basis, data not shown) did not change in chemically fertilized roots (C) respect to the non-treated ones, nor in the sample treated with BBFs. Taken together these data indicate that the fertilization with BBFs and activated BBFs does not induce significant changes in the organoleptic of radish roots, nor in the root horizontal diameter. The different fertilizing treatments did not induce significant changes in K, Na, P and Fe composition of radish roots respect to the control (Table 25). Only slight differences were found for Mg and Ca concentrations, not implying any nutrient unbalance. No

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differences were found in the microelement composition of differently fertilized radish roots (data not shown). The heavy metal concentration is always lower than 1 ppm except for Cr radish grown with the biochar (3.6±1.2 ppm for FR and 3.2±1.1 ppm for FR A). Arsenic is in the concentration interval 0.1-0.2 ppm, Co 0.04-0.15 ppm, Cd 0.07-0.14 ppm and Pb 0.1-0.2 ppm (data not shown).

Table 25. Macro- and meso- element composition of radish roots in tested treatments (TRT).

TRT	K (mg g^{-1})	Na (mg g^{-1})	Mg (mg g^{-1})	Ca (mg g^{-1})	P (mg g^{-1})	Fe (mg g^{-1})
NT	40.42 ± 2.78	12.08 ± 2.68	1.87 ± 0.07^{ab}	3.33 ± 0.10^{ab}	4.04 ± 0.19	0.13 ± 0.05
C	47.32 ± 2.04	10.79 ± 2.01	2.85 ± 0.25^{ab}	5.48 ± 0.73^{ab}	3.50 ± 0.10	0.16 ± 0.10
ES	40.72 ± 6.84	9.49 ± 1.25	3.01 ± 0.77^{ab}	5.51 ± 0.55^{ab}	2.96 ± 0.12	0.11 ± 0.03
ES A	31.43 ± 0.02	11.64 ± 4.28	1.56 ± 0.23^{b}	2.63 ± 0.44^{b}	2.81 ± 0.03	0.08 ± 0.03
NL	44.03 ± 4.41	10.41 ± 0.10	3.39 ± 0.96^{a}	5.79 ± 1.60^{a}	3.78 ± 0.32	0.15 ± 0.03
NL A	36.30 ± 4.03	8.99 ± 2.56	1.82 ± 0.09^{ab}	3.78 ± 0.40^{ab}	2.96 ± 0.07	0.12 ± 0.02
NL T	46.95 ± 10.62	14.04 ± 6.57	2.46 ± 0.43^{ab}	4.63 ± 1.25^{ab}	3.44 ± 0.54	0.14 ± 0.05
FR	49.99 ± 4.02	6.62 ± 3.49	2.03 ± 0.43^{ab}	3.35 ± 0.85^{ab}	4.49 ± 0.97	0.11 ± 0.02
FR A	40.15 ± 6.62	11.99 ± 2.57	2.35 ± 0.24^{ab}	4.14 ± 0.35^{ab}	4.53 ± 1.49	0.11 ± 0.02

Different letters indicate significant differences among the treatments according to one-way ANOVA and post hoc Tukey's test ($p < 0.05$).

In terms of bacterial presence, *Rhizobium* and *Ensifer* were present across most samples with an increase in abundance at T2 while *Azospirillum* and *Lactiplantibacillus* showed a decrease in abundance from T0 to T2 in the inoculated sample. *Azospirillum* and *Lactiplantibacillus* reinforced the results obtained in tomato while *Rhizobium* and *Ensifer* showed a possible better adaptation to this crop. The presence of all strains after 15 days might hint to the need of multiple reinoculation on crops with a longer growth/productive phase. In terms of fungal presence, *Rhizophagus* was only retrieved in the initial consortia possibly showing a need for higher amounts within the inoculum. Similarly to tomato, *Trichoderma* was present across all samples with a significant higher abundance in the activated BBFs again hinting at its leading role and better adaptability out of all microorganisms.

(iii) Lettuce pot experiment

The fresh and dry weight of the edible part of the lettuce plants, i.e. the panicle, treated with the different BBFs is reported in Table 26. The fresh weight of chemically fertilized (C) lettuce panicles was higher than the not treated ones, while the dry weights were similar. When fertilized with biodried material (ES), whether activated or not, no differences were found in panicle fresh and dry weight respect to NT ones. The treatment with non-activated NL (digestate solid fraction) induced the same fresh and dry weight as in the NT plants, while the NL activation with both the consortium (NL A) and the sole Trichoderma (NL T) induced an increase in fresh weight respect to the non-activated NL, even if not significantly different from the control (NT). The highest fresh and dry weight were found in panicles treated with the biochar (FR), especially the activated one (FR A).

Table 26. Productive yields of lettuce plants (panicle biomass) in tested treatments (TRT).

TRT	FW (g)	DW (g)	DW (%)
NT	60.22 ± 7.82^{de}	23.33 ± 1.38^{bc}	38.20 ± 4.27^{ab}
C	79.54 ± 6.43^{bc}	24.79 ± 0.47^{abc}	30.73 ± 2.59^{bcd}
ES	56.17 ± 0.9^{de}	22.32 ± 0.26^{c}	39.84 ± 0.39^{a}
ES A	59.85 ± 2.51^{de}	22.34 ± 0.28^{c}	37.63 ± 1.69^{ab}
NL	51.05 ± 7.34^{e}	22.30 ± 0.78^{c}	40.78 ± 2.17^{a}

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NL A	66.05 ± 3.48^{cd}	23.07 ± 0.72^{bc}	35.51 ± 1.22^{abc}
NL T	69.39 ± 4.39^{cd}	23.96 ± 0.62^{abc}	35.83 ± 1.30^{abc}
FR	89.22 ± 1^{b}	25.72 ± 0.04^{ab}	28.90 ± 0.48^{cd}
FR A	111.63 ± 2.33^{a}	26.68 ± 1.53^{a}	26.34 ± 2.59^{d}

Different letters indicate significant differences among the treatments according to one-way ANOVA and post hoc Tukey's test ($p < 0.05$).

Table 27 shows the Chlorophyll, Flavonoid, Anthocyanin and N Balance indexes in lettuce panicles 15 days after transplanting. The chemical fertilization induced a more pronounced increase in Chl than in Flv. Panicles treated with ES and NL, both non-activated and activated, exhibited a Chl even lower than in the not treated control (NT). FR and FR A showed a Chl similar to the control. This result could be interpreted as an indication that the treatment with BBFs, weather activated or not, induce an acceleration of the vegetative cycle, leading to earlier senescence symptoms. The chemically fertilized sample (C) exhibits the highest Flv index, while all the BBF-treated samples have Flv values lower than the control (NT). The treatment with biochar activated with Trichoderma induced the highest NBI value. At harvest time (data not shown) the Chl index is higher than the control in the panicles treated with the BBFs, reaching in some samples the value of the chemically fertilized ones.

Table 27. Chlorophyll, Flavonoid, Anthocyanin and Nitrogen Balance indexes in lettuce panicles 15 days after transplanting.

TRT	Chl	NBI*	Flv	Anth
NT	21.16 ± 1.48^{bc}	34.11 ± 2.98	0.62 ± 0.06^{ab}	0.27 ± 0.01^{ab}
C	26.42 ± 1.27^{a}	35.56 ± 13.44	0.80 ± 0.22^{a}	0.26 ± 0.02^{ab}
ES	15.78 ± 0.95^{e}	36.72 ± 5.04	0.44 ± 0.07^{b}	0.27 ± 0.04^{ab}
ES A	16.66 ± 1.56^{de}	34.44 ± 4.19	0.49 ± 0.05^{b}	0.30 ± 0.02^{a}
NL	17.18 ± 1.46^{de}	39.53 ± 6.83	0.44 ± 0.14^{b}	0.29 ± 0.01^{a}
NL A	18.52 ± 1.92^{cde}	39.56 ± 9.53	0.50 ± 0.15^{b}	0.27 ± 0.01^{ab}
NL T	18.74 ± 1.23^{cd}	41.67 ± 7.90	0.45 ± 0.15^{b}	0.28 ± 0.01^{ab}
FR	21.96 ± 0.66^{b}	44.16 ± 5.09	0.50 ± 0.05^{b}	0.25 ± 0.01^{b}
FR A	20.54 ± 0.83^{bc}	38.05 ± 11.2	0.53 ± 0.19^{ab}	0.28 ± 0.03^{ab}

**Chl=chlorophyll index; * *NBI= Nitrogen balance index (Chl/Flv); ***Flv=flavonoid index; ****Anth=anthocyanin index. Different letters indicate significant differences among the treatments according to one-way ANOVA and post hoc Tukey's test ($p < 0.05$).*

The different fertilizing treatments (Table 28) did not induce significant changes in Ca composition of lettuce panicles respect to the control. The treatment with non-activated and activated biochar (FR and FR A) induced an increase in K ad also in Mg concentration. The P content was higher than the control and similar to or even higher than the chemically fertilized panicle in all the BBF-treated plants, whether activated or not, indicating that the BBF fertilization positively affects P nutrition. Fe was significantly higher only in FR A plants, suggesting an effect of biochar activation on Fa availability.

No differences were found in the microelement composition of differently fertilized lettuce plants (data not shown). The heavy metal concentration in lettuce panicles is always lower than 1 ppm except for Cr in lettuce grown with chemical fertiliser (C: 1.27±0.39 ppm) and with the activated biodried solid fraction (ES A: 1.46±1.98 ppm) (data not shown).

In terms of bacterial presence, *Azopirillum*, *Rhizobium* and *Ensifer* were present across multiple samples with a generally lower abundance at T2 while *Lactiplantibacillus* was not retrieved at T2 hinting to similar results to tomato with lower adaptation to this crop and need of multiple reinoculation.

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Table 28. Macro- and meso- element composition of lettuce panicles

TRT	K	Na	Mg	Ca	P	Fe
	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})
NT	22.70 ± 0.53^{bc}	2.65 ± 0.01^{c}	3.31 ± 0.02^{abc}	8.25 ± 0.20	1.50 ± 0.08^{b}	0.08 ± 0.01^{b}
C	30.73 ± 2.45^{c}	2.35 ± 0.03^{c}	3.07 ± 0.18^{ab}	9.73 ± 0.09	2.16 ± 0.30^{ab}	0.23 ± 0.06^{b}
ES	34.80 ± 0.27^{b}	2.12 ± 0.06^{c}	2.97 ± 0.08^{abc}	10.00 ± 0.62	2.55 ± 0.09^{a}	0.10 ± 0.01^{b}
ES A	33.05 ± 2.61^{b}	4.32 ± 0.24^{a}	3.06 ± 0.32^{abc}	9.61 ± 0.87	2.49 ± 0.10^{a}	0.63 ± 0.79^{b}
NL	34.55 ± 0.08^{b}	2.15 ± 0.08^{c}	3.14 ± 0.26^{abc}	10.98 ± 0.05	2.38 ± 0.20^{ab}	0.21 ± 0.05^{b}
NL A	29.52 ± 3.29^{bc}	3.22 ± 0.17^{abc}	3.74 ± 0.40^{a}	10.56 ± 1.86	2.51 ± 0.19^{a}	0.12 ± 0.00^{b}
NL T	31.13 ± 4.30^{bc}	4.12 ± 0.73^{a}	3.04 ± 0.06^{abc}	9.42 ± 0.85	2.24 ± 0.51^{ab}	0.11 ± 0.03^{b}
FR	48.85 ± 3.17^{a}	2.75 ± 0.08^{bc}	2.44 ± 0.14^{c}	8.94 ± 0.74	3.01 ± 0.22^{a}	0.15 ± 0.06^{b}
FR A	49.66 ± 0.14^{a}	3.84 ± 0.26^{ab}	2.50 ± 0.13^{bc}	9.15 ± 0.19	2.81 ± 0.14^{a}	0.42 ± 0.02^{a}

Different letters indicate significant differences among the treatments according to one-way ANOVA and post hoc Tukey's test ($p < 0.05$).

5.4 Conclusion and recommendation

The results obtained in the pot experiment with tomato indicate the efficacy of microbial consortia in terms of total productivity respect to the treatment with the BBFs alone. For what concerns the fruit quality, improvements in the qualitative parameters were detected in the BBF-treated fruits respect to the untreated and chemically fertilized ones. Anyway, no improvements were found following the activation of BBFs with the microbial consortia.

The results obtained with radish did not show any effect of BBF treatments, whether activated or not, nor quantitative (yield) neither for qualitative parameters, probably due to the short cultivation cycle.

The results obtained with lettuce indicate that the application of BBFs exerted a positive effect on quantitative (yield) parameters, particularly the treatment with biochar (FR) and the activated biochar (FR A). All the treatments seem to accelerate the vegetative phase, leading to earlier senescence respect to the control (NT) and the chemically fertilised (C) plants.

When compared to bacterial inoculants, fungal inoculants showed a higher permanence in soil and were retrieved also after Time 0. In particular, Trichoderma presence was higher across time in the soil treated with activated BBFs, leading to the conclusion that Trichoderma is playing the leading role out of all microorganisms and driving the biomass and nutritional differences.

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6. Assessment of biostimulants (UVIC-UCC, UGent)

For more information on this study, please contact the authors from UVIC-UCC: Omar Castaño-Sanchez (omar.castano@uvic.cat) and Laura Diaz-Guerra (laura.diaz.guerra@uvic.cat).

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Parts of this section will be published as Castaño-Sánchez, O., Díaz-Guerra, L., Singla-Just, B., Vilaplana, R., Llenas. L. Harnessing Algae-Protein Hydrolysate as a Sustainable Biostimulant for Enhancing Crop Resilience and Growth. In preparation

6.1 Introduction

The aim of this study was to evaluate the agronomic performance of the biostimulant product (ES-AA), developed by the Spanish pilot. This product was produced in 2 batches that differed in the concentration of amino acids. ES-AA from 1st batch (ES-AA1) contained 0.11% of amino acids, whereas ES-AA from 2nd batch (ES-AA2) contained 1.31% of amino acids (Table 31). The effect of ES-AA1 was tested in Swiss chard cultivation to resist temperature stress and varying fertiliser dosages. Apart from this, ES-AA1 was applied in tomato to study the effects of the biostimulant on plant growth and biochemistry, including different application methods and environmental stresses. In the third experiment, ES-AA2 was applied in lettuce and spinach grown with different levels of hydric and saline stress to test the capacity of the biostimulant for improving plant resistance and production under stress conditions. The objective is to understand the effectiveness and the impact of the biostimulant in agricultural applications, thus contributing to the overall objectives of the FERTIMANURE project.

6.2 Methodology

(i) Biostimulant produced

Table 29 shows the free amino acids content of both biostimulants (ES-AA 1 and ES-AA 2) that were used throughout all the agronomic trials compared to the commercial biostimulant (Isabión®). ES-AA 1 and ES-AA 2 had a lower free amino acid concentration in comparison with the commercial reference. To be able to compare the biostimulants, the application dose was adjusted to apply the same amount of free amino acids in all treatments.

(ii) Pot experiment with Swiss chard

Here the aim was to investigate the biostimulant effects (ES-AA1) on responses of Swiss chard (*Beta vulgaris*) to temperature stress and varying fertiliser dosages. The experimental design is a factorial experiment with two main factors: temperature stress levels and fertiliser dosages. The temperature stress levels were incrementally increased up to 38ºC, while three fertiliser dosages (70%, 100%, and 130% of the 120 kg total N crop requirement = 100%; Table 30) were applied. Swiss chard plant was selected and prepared for the experiment. Soil testing and preparation ensured optimal conditions for growth. The planting process followed standard agricultural practices, including seed selection and germination for 15 days, followed by planting into the pots. Two types of fertilisers were used in this study: TMF blend (ammonium sulphate (NL-AS) + nutrient-

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rich concentrate (ES-NC)) and calcium ammonium nitrate (CAN). TMF contained 6.31% of total N and CAN 17% of total N.

Table 29. Free amino acids content in the biostimulant products from FERTIMANURE (ES-AA 1 and ES-AA 2) and the commercial biostimulant Isabión®. The ES-AA 1 biostimulant was applied in the Swiss chard and tomato pot experiment, while ES-AA 2 was applied to the lettuce and spinach experiment.

Amino acids	ES-AA 1 (%)	ES-AA 2 (%)	Isabión® (%)
ASP	0.0042	0.052	0.35
GLU	0.0053	0.115	0.27
SER	0.0094	0.053	0.13
HYS	0.0138	0.014	0.10
GLY	0.0155	0.038	3.80
THR	0.0042	0.075	0.08
ARG	0.0240	0.039	0.12
ALA	0.0008	0.166	1.87
TYR	0.0034	0.079	0.33
CYS-CYS	0.0034	0.021	-
VAL	0.0035	0.101	0.09
MET	0.0017	0.036	0.08
PHE	0.0030	0.095	0.16
ILE	0.0014	0.072	0.07
LEU	0.0047	0.166	0.20
LYS	0.0019	0.098	0.35
PRO	0.0019	0.035	-
HYP	0.0097	0.021	1.45
TRP	0.0011	0.016	-
GLN	0.0006	0.005	-
ASN	0.0004	0.018	-
TOTAL	**0.1140**	**1.314**	**10.3**

Table 30. Design of experiment with different treatments and parameters.

Normal Conditions	Treatments + Biostimulants (B)	Treatments + Stress (S)	Treatments + Stress (S) + Biostimulants (B)
Unfertilized Control	Unfertilized Control + B	Unfertilized Control + S	Unfertilized Control + B + S
TMF 70%	TMF 70% + B	TMF 70% + S	TMF 70% + B + S
TMF 100%	TMF 100% + B	TMF 100% + S	TMF 100% + B + S
TMF 130%	TMF 130% + B	TMF 130% + S	TMF 130% + B + S
CAN 70%	CAN 70% + B	CAN 70% + S	CAN 70% + B + S
CAN 100%	CAN 100% + B	CAN 100% + S	CAN 100% + B + S
CAN 130%	CAN 130% + B	CAN 130% + S	CAN 130% + B + S

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Temperature stress was applied starting from the 2nd week of July 2022. The stress was incrementally intensified as follows: 1st hour: 28ºC, 2nd hour: 32ºC, 3rd hour: 38ºC, 4th hour: Open-air cooling. The closed oven increment method was used with ventilation at 100% and the valve at 100%. The total stress time was 4 hours per day for a period of 15 days, subject to weather conditions. Fertilisers were applied both foliar and through root application. The biostimulant was applied during the vegetative growth stages, estimated to be after 30 days of plantation. This was done once, with an application amount of 2 L/ha. Data on various parameters were collected, including plant growth (biomass), nutrient uptake, and stress-related responses (Chlorophyll, MDA and proline). All mentioned parameters were analysed at the end of the experiment after harvest of Swiss chard. The detailed information is given in Shrivastava et al. (2024).

(iii) Pot experiment with tomato

The potential agronomic performance of biostimulants produced in the Spanish pilot plant was assessed on tomato crops grown in a growth chamber with controlled conditions of temperature (24°C-26°C), humidity (50%-65%) and 12h/12h light period. Tomato seeds were sown in 1L capacity pots with 1.2 kg of a <5 mm sieved sandy-loam soil. Plants were fully fertilised on N-criteria and P-criteria of the crop nutrient demand (Tomato: 180 N, 75 P_2O_5, 315 K_2O) with commercial ammonium sulphate $(NH4)_2SO_4$ and calcium superphosphate, respectively. Fully fertilised plants without biostimulant application acted as a negative control (C). Thus, three different treatments were applied: plants with foliar spray biostimulant application (BL), plants with soil biostimulant application (BS) and a comparison with a commercial biostimulant application (Isabión® Syngenta) (BC). These treatments were combined with different plant conditions: hydric stress, saline stress, or optimal conditions. By measuring the daily evapotranspiration (ETP) of the soil, 100% ETP water was calculated and daily added in the optimal conditions (OC) treatments while 60% ETP water was used to create the hydric stress (HS) conditions. Saline stress (SS) was applied by daily irrigating 100% ETP water to the plants, including a 135 mM NaCl solution, until getting the desired EC of the soil (day 90). For each treatment, five replicates were maintained. An overview of the different treatments is provided in Table 31.

Table 31. Overview of the treatments of the biostimulant assay in tomato.

Optimal conditions (OC)	**Hydric stress (HS)**	**Saline stress (SS)**
Fully fertilised plants (OC-C)	Fully fertilised plants (HS-C)	Fully fertilised plants (SS-C)
Fully fertilised plants + ES-AA1 soil application (OC-BS)	Fully fertilised plants + ES-AA1 soil application (HS-BS)	Fully fertilised plants + ES-AA1 soil application (SS-BS)
Fully fertilised plants + ES-AA1 foliar application (OC-BL)	Fully fertilised plants + ES-AA1 foliar application (HS-BL)	Fully fertilised plants + ES-AA1 foliar application (SS-BL)
Fully fertilised plants + Isabión® soil application (OC-BC)	Fully fertilised plants + Isabión® soil application (HS-BC)	Fully fertilised plants + Isabión® soil application (SS-BC)

The biostimulant product was applied 5 times during the crop cycle, matching the different phenological stages of the plants (days 22, 39, 56, 71 and 106). To be able to compare the ES-AA1 biostimulant with a commercial biostimulant, the free aminoacids that were applied with a commercial dosage (2L/ha) using the commercial biostimulant (Isabión® Syngenta) were equated to the free aminoacyls applied with the FERTIMANURE biostimulant, which meant applying ES-AA1 with a much higher dosage (175L/ha). Following the recommended dose, since ES-AA1 had a lower free amino acid concentration, the volume of biostimulant solution needed per application was higher (87,5 µL) than with the commercial biostimulant (1.0 µL). Despite this, not significant differences were observed in the watering regimes between the control plants and those receiving biostimulant treatments. Changes in plant growth and biochemistry in response to the different biostimulants and/or the kinds of biostimulant applications, in combination with the stresses, were assessed in terms of fresh weight, NPK content and other biochemical parameters related to plant stress such as proline,

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MDA, and chlorophyll content. Soil parameters consisted of the determination of total N, NO_3^-, NH_4^+, total P, soluble P, exchangeable K, pH, and EC.

(iv) Pot experiment with lettuce and spinach

A comparative study to evaluate the effects of biostimulant (ES-AA2, previously described in Table 29) on spinach and lettuce was done under different levels of water and saline stress. For each treatment, four replicates were maintained. Table 32 provide an overview of the different treatments used in this study. Furthermore, the crops were assessed in a controlled growth chamber, maintaining a temperature range of 24-26°C, humidity levels of 50-60%, and a 12-hour light period. Changes in plant growth and biochemistry were assessed in terms of fresh weight, NPK content and biochemical parameters associated to plant stress such as proline, MDA, chlorophyll and carotenoids, and leaf relative water content (RWC). Spinach nutrient requirement was 120 N, 42 P_2O_5, 200 K_2O, and for lettuce 90 N, 40 P_2O_5, 180 K_2O.

Table 32. Overview of the treatments applied in the biostimulant assay with spinach and lettuce.

Hydric stress for both crops		Saline stress for spinach		Saline stress for lettuce
Control	-1.18 kPa soil matric potential	Control	Tap water	Tap water
H1	-120 kPa soil matric potential	S1	7.5 ds/m (1.5% NaCl)	1.5 ds/m (0.3% NaCl)
H2	-219 kPa soil matric potential	S2	9 ds/m (1.8% NaCl)	2.5 ds/m (1.5% NaCl)
H3	-376 kPa soil matric potential	S3	12 ds/m (2.4% NaCl)	5 ds/m (1% NaCl)
H4	-645 kPa soil matric potential	S4	15 ds/m (3% NaCl)	7.5 ds/m (1.5% NaCl)

6.3 Results and discussion

(i) Pot experiment with Swiss chard

In a pot experiment with Swiss chard, varying doses of CAN and TMF were tested under different conditions for thermal stress and biostimulants application. Overall, there were no statistically significant differences in yield for CAN under normal conditions and biostimulants application conditions, nor for TMF with biostimulants conditions and stress conditions (Figure 21). However, significant differences were observed between the 70%, 100%, and 130% N dosages for TMF and CAN in other conditions, with higher N uptake at the 130% dosage leading to increased yield. CAN outperformed TMF at the 100% and 130% N dosages due to the presence of plant-available N in CAN compared to the organic N in TMF. The higher C/N ratio in TMF potentially led to N immobilisation in the soil, reducing its accessibility for plant uptake and subsequently lowering yield.

The application of biostimulants positively influenced plant growth and yield, particularly with increasing TMF dosages, possibly by mineralizing organic N and enhancing nutrient availability and uptake. Various mechanisms, including the formation of metal-amino acids/peptides and stimulation of nutrient-solubilising bacteria/fungi, were proposed to contribute to improved nutrient availability and uptake.

Temperature stress did not significantly affect yield but caused physical signs of stress, such as leaf folding or rolling. This response can be interpreted as an adaptive mechanism to mitigate transpiration loss. Additionally, biostimulant application led to higher chlorophyll concentrations across various treatments and dosages, although the direct effect on yield from this increased chlorophyll content was not evident. Biostimulant application also increased proline levels, suggesting improved stress tolerance. MDA levels indicated greater membrane damage in the case of CAN under stress conditions, but biostimulants helped plants withstand and recover from temperature stresses. An additional field based testing of biostimulants is recommended to fully understand the implications of biostimulant-induced changes in chlorophyll content and proline levels on different plant morphology and stress responses.

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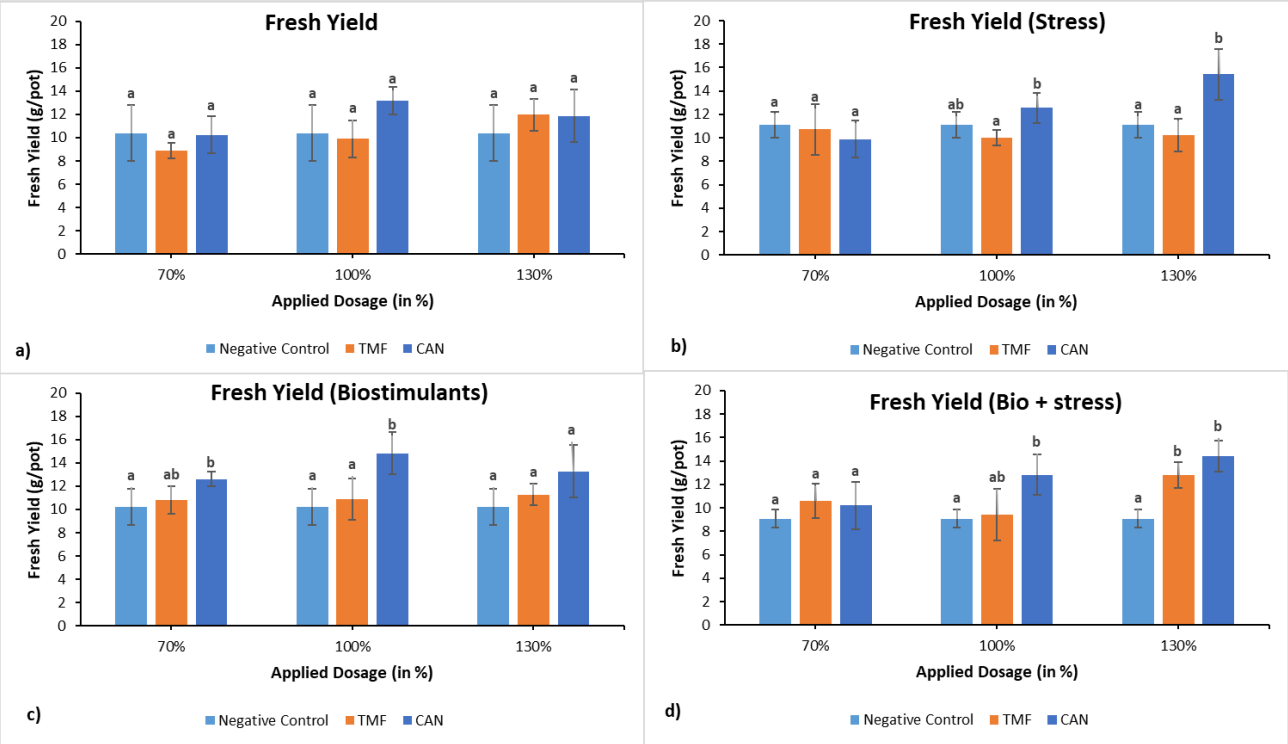

Figure 21. Fresh yield for Swiss chard a) under normal conditions when no stress or biostimulants were applied, b) under condition when only stress application was done, c) under condition when only biostimulants application was done, d) under condition when both stress and biostimulants application was done. The lowercase letters "a, b, c, d" indicate the statistically significant differences (Tukey HSD $P < 0.05$) for different treatments at a particular N rate.

(ii) Pot trials with tomato

As main results, under optimal conditions, the plant biomass increased significantly with the ES-AA1, specifically with foliar application, reaching a similar growth to those with the commercial biostimulant (Figure 22A). The same was observed in plants under hydric stress. In contrast, regarding saline stress, no differences were observed with any biostimulant application and the control. In fact, fresh yield from the saline treatments was similar to those under control conditions. This could mean that the saline treatment was not high enough to produce stress conditions in the tomato plants.

Regarding the biochemical parameters, it was expected to find a correlation between the plant biomass and the different metabolites analysed. Proline is an amino acid that is overproduced as a response to certain stress conditions, specially to osmotic stress processes (Hayat et al. 2012). Its determination is, therefore, very useful for assessing the physiological status and, more generally, for understanding plant stress tolerance. However, despite the biostimulant-induced increment in plant biomass, proline content was similar in all treatments under optimal conditions (Figure 22B). In the hydric stress, the biostimulant treatments had significantly lower proline content than the control treatment, probably related to stress mitigation in those plants receiving the biostimulant application. Thus, proline content in HS-BL and HS-BC treatments was lower than HS-BS and HS-C, which is in accordance with the biomass results. Regarding the saline treatments, despite not finding differences in growth, plants receiving the ES-AA1, specifically with foliar application, showed a lower proline content than control ones.

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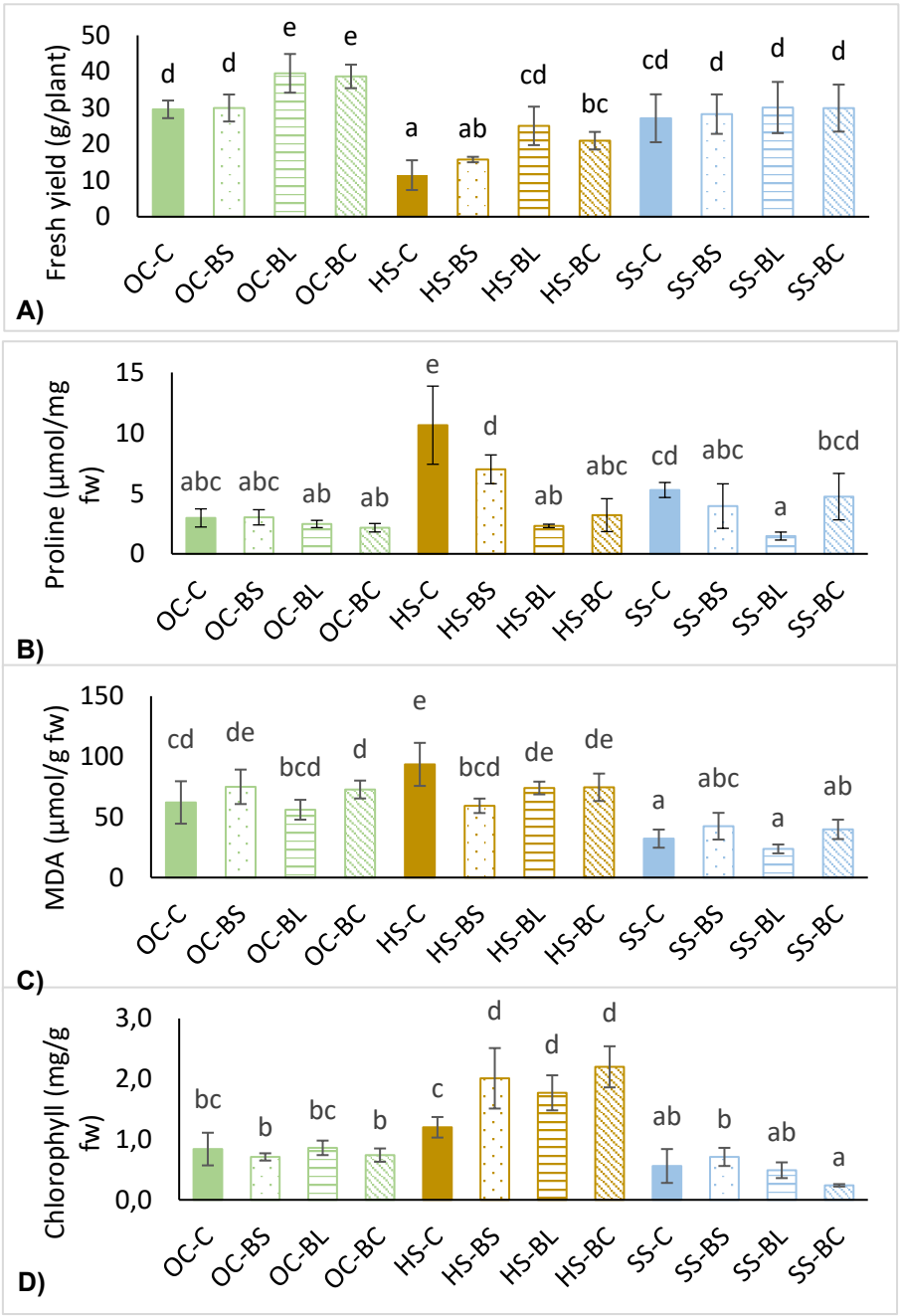

Figure 22. Fresh yield (A), proline content (B), MDA content (C) and chlorophyll content (D) of tomato plants under different conditions and biostimulant applications. OC: Optimal conditions. HS: Hydric stress. SS: Saline stress. C: Control. BS: Biostimulant-Soil. BL: Biostimulant-Leaf. BC: Biostimulant-Commercial. Each bar represents the mean values ± SD. Different letters indicate significant differences among treatments, according to Duncan's test ($p \leq 0.05$).

The main molecular manifestation of increased oxidative stress is the peroxidation of membrane lipids. Malondialdehyde (MDA) is one of the three breakdown products of polyunsaturated fatty acids in membranes because of this peroxidation process (Davey et al., 2005). Consequently, MDA is considered a stress indicator in various conditions and pathologies. Regarding this biochemical parameter, there were no differences between the treatments in optimal conditions and under saline stress (Figure 22C). However, with hydric

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stress, plants had lower MDA concentrations when they received a biostimulant treatment, especially the ES-AA1 applied in the soil. Again, this result could indicate a stress mitigating effect in tomato plants produced by the ES-AA1.

Chlorophyll determination is useful to quantify the photosynthetic capacity of the plants. Scientific literature has widely reported that chlorophyll content decreases under stress conditions (Solarte et al. 2010). Again, there were no differences between the treatments in optimal conditions and under saline stress (Figure 22D). In contrast, under hydric stress, the different biostimulant treatments increased the chlorophyll content compared to control, although no differences were detected between the ES-AA1 and the commercial biostimulant.

Concerning the nutrient content, the control plants had higher N and K in all conditions (data not shown) than those under the biostimulant treatments. There were no differences in terms of P content in hydric and saline stress between treatments but in optimal conditions, P content was higher in plants receiving the biostimulant (BS and BC treatments). Among the treatments in each stress conditions plants in some biostimulant treatments, especially with foliar application, showed higher biomass but lower nutrient concentration than the control, which might indicate a similar nutrient uptake and greater resource investment in growth by the plants receiving the biostimulant. It could be hypothesized that the biostimulant might be improving nutrient uptake by the plant in some cases, also affecting the nutrient mobilisation and allocation inside the plant and enhancing nutrient efficiency, then resulting in better plant development with a similar amount of nutrients.

(iii) Pot trials with lettuce and spinach

The main results of this experiment are presented in Figure 23 (spinach) and Figure 24 (lettuce). In spinach plants, a general effect of the biostimulant was not observed but depended on the stress level applied. Under drought conditions, we found slight increases in plant growth due to the biostimulant in some levels of this stress, specifically in H1 and H3 treatments (Figure 23A). Similarly, the saline treatment significantly modulated the effect of the biostimulant. Thus, while control plants slightly reduced their growth as salt stress increased, those treated with the biostimulant showed a similar fresh yield as control regardless of the saline stress level (Figure 23B). The biostimulant increased growth rate in spinach plants and, this effect was emphasized by the stress intensity, finding the largest differences in plants subjected to the highest level of saline stress (S4).

Concerning the lettuce plants, the biostimulant effect of the ES-AA2 was mainly observed under hydric stress (Figure 24A) since the biostimulant-induced increase in plant biomass was significant in plants grown with water scarcity. In contrast, this effect was not detected under saline stress. In both cases (Figure 24A-B), the control plants were significantly larger than those grown under stress. So, for the lettuce, there was a significant reduction in the crop yield despite the application of the biostimulant in all stress levels, but this yield loss was mitigated with the biostimulant application.

The results obtained regarding the nutrient content of spinach show that the effect of water and salt stress had no effect on the NPK content, although salt stress did affect the Na content, with a higher concentration of Na correlating with increased severity of stress. Additionally, there was an effect of the biostimulant on the P and Na content. In the case of P, biostimulant application increased its concentration in treatments S3 and S4, while it reduced the concentration of Na in treatments S1, S3, and S4. For lettuce, both water and salt stress reduced the concentration of P, and salt stress reduced the concentration of K while increasing the concentration of Na. No effect of the biostimulant was observed on the N content. There was also no mitigation of Na content, which could explain the lack of differences in crop yield after biostimulant application. However, it was observed that in water stress treatments (H2 and H3), the biostimulant increased the P content in plants and also mitigated the reduction in K concentration in salt stress treatments. Other authors as Shehata et al. (2011) also found that the application of amino acids could have positive effects on P and K contents and fresh weight.

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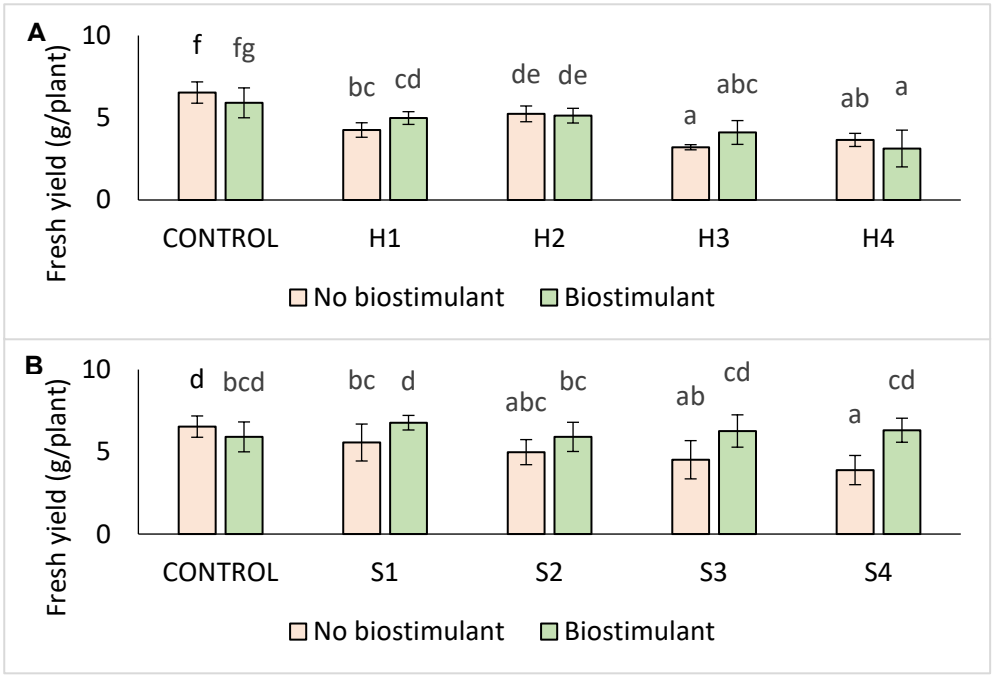

Figure 23. Fresh yield of spinach plants under hydric (H; A) and saline (S; B) stress in combination with the biostimulant application. Each bar represents the mean values ± SD. Bars with different letters indicate significant differences, according to Duncan's test ($p \leq 0.05$).

Regarding the biochemical parameters, no clear biostimulant effects were observed on the leaf content of chlorophyll, carotenoids, MDA or RWC. Regarding proline content, some treatments that showed differences in crop yield with the application of ES-AA2 in spinach (S2 and S3) and in lettuce (H3 and H4) also showed differences in the proline content, being significantly lower than plants without the biostimulant application at the same stress level. The literature contains a wealth of studies focusing particularly on the accumulation of proline due to its role as an osmolyte in response to osmotic stress (Hayat et al., 2012). This behavior is observed in various types of stress and plants, especially under conditions of salt stress (Kissoudis et al., 2016) and water stress (Claussen, 2005). A reduction in these elevated levels of proline can be associated with a reduction in the stress experienced by the plant.

To sum up, even though a biostimulant product can act as an enhancer of nutrient uptake and plant growth, our findings suggest that it could also influence the activation of other metabolic pathways related to stress resistance. Given the biomass results, our biostimulant might have acted as a promoter of protective mechanisms that have allowed the stressed plants to maintain its vegetative growth, especially in spinach plants under saline stress. Identifying the mechanisms triggered by biostimulants can be challenging and is currently the subject of recent research (Paul et al. 2019).

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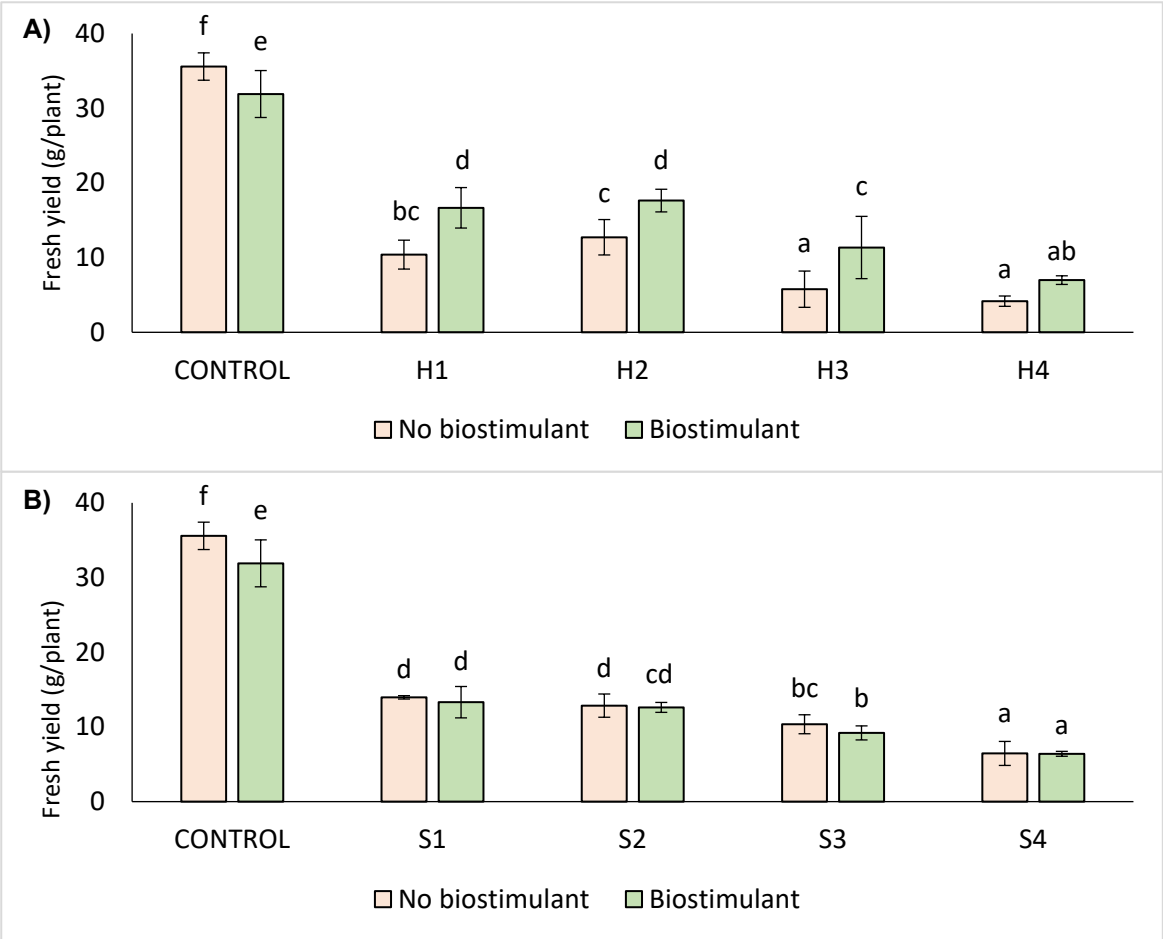

Figure 24. Fresh yield of lettuce plants under hydric (H; A) and saline (S; B) stress in combination with the biostimulant application. Each bar represents the mean values ± SD. Bars with different letters indicate significant differences, according to Duncan's test ($p \leq 0.05$).

6.4 Conclusion and recommendation

In Swiss chard study clear patterns emerged at 70%, 100%, and 130% dosages of TMF and CAN. Enhanced N uptake at 130% significantly improved yield, highlighting N optimization's importance. Biostimulant application consistently benefited Swiss chard, manifesting in increased chlorophyll and proline levels. Temperature stress had a limited impact on yield but induced notable physiological stress responses, with CAN-treated plants showing higher MDA levels.

In the tomato test, the biostimulant had more intense and remarkable effects when it was applied directly to the leaves than in the soil. The ES-AA1 improved the biomass in tomato plants, especially under optimal conditions and hydric stress, reaching similar crop yield to those grown with the commercial biostimulant.

In the spinach and lettuce test, the ES-AA2 biostimulant was able to effectively increase crop yield and this effect was dependent on the species-related plant responses to stress condition, being observed mainly in spinach under saline stress and in lettuce subjected to hydric stress. Thus, this experiment also confirmed that the biostimulant can be helpful for increasing plant resistance against a range of different hydric and saline stresses.

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7. General conclusions and recommendations

7.1. Ammonium salts (UGent, RITTMO, UVIC-UCC)

Ammonium salts tested in a laboratory setting are ammonium sulphate (FR-AS, BE-AS, ES-AS), ammonium nitrate (BE-AN) and ammonium water (BE-AW). All BBFs are 100% in mineral N form, and as such where tested only for their N dynamics via N incubation experiment. The results of RITTMO, UVIC-UCC and UGent have shown that all BBFs acted like a synthetic mineral N fertiliser (same CAN used in all experiments) in terms of N release: 100% of applied N remained in mineral form during incubation test. From these tests it can be concluded that ammonium salts supply the soil with mineral N and it seems that their addition to the soil can also induce a positive effect with a stimulation of N mineralisation from SOM, i.e. priming effect (N release > 100%). Therefore, in regard to the obtained results, ammonium salts (FR-AS, BE-AS, ES-AS and BE-AN) can be recommended for use as substitutes for synthetic N fertilisers. However, for ammonium water (BE-AW) it is a known fact that this BBF has an alkaline pH which can lead to NH_3 emissions upon to its application. For more information on BE-AW, please see *D4.6 "Final – Report on agronomic and environmental performance in field trial experience"* where results on its performance, in lettuce cultivation and NH_3 and greenhouse gas emissions, are reported.

7.2. Liquid K-fertiliser (RITTMO)

Liquid K-fertiliser (FR-LK) is produced by French pilot after stripping and scrubbing NH_3 from liquid fraction of manure. The results of RITTMO have shown that mineral N supplied remained to be available throughout the course of the N incubation and organic N was mineralized. In total, at the end of the incubation experiment 53% of total N applied from FR-LK was found in the mineral form. In general, FR-LK should be considered as a raw manure with a lower NH_4-N content. This could facilitate the field application of this BBF because a large part of the N initially present in ammoniacal form has been extracted (which simplifies its use in the field in connection with the Nitrates Directive), and on the other hand, this will make it possible to take advantage of the contribution of K associated to this BBF. It would be of interest in future (outside of FERTIMANURE project) to carry out tests on FR-LK by studying the bioavailability of the K in this BBF. In *D4.6 "Final – Report on agronomic and environmental performance in field trial experience"* results from 1 year field trial in sugar beet cultivation are reported, however, due to dry weather conditions it was not possible to draw conclusions on FR-LK potential to be used as a K-fertiliser.

7.3. Biochar (RITTMO, Fraunhofer)

Biochars (FR-BC and DE-BC) were tested for their potential to ameliorate soil physicochemical and biological properties by providing a stable OC, and as a potential source of P.

The work on C stability of the tested biochars has shown that both BBFs are quite stable, with having less than 3% of applied OC being mineralised as CO_2. The soil OC is recognised by European policy as an instrument to reduce CO_2 emission through soil C sequestration. Therefore, the use of biochars can help with achieving a goal of an European initiative to increase the SOC stock in the soil with 4 promille (so called '4 promille initiative', https://www.4p1000.org/). However, it should be noted that the duration of the project and the tests carried out do not make it possible to precisely study the amending properties of the biochar in the long term.

The work on P plant availability from biochars, has shown that in pot cultivation of rye grass the use of biochars can result in a similar plant biomass production as with synthetic P fertilisation. The PFRV values, however, varied depending on the applied P dose (in the experiment by RITTMO):

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- In the case of FR-BC1, for an applied dose of 200 kg P_2O_5/ha and 8 T/Ha (405 kg P_2O_5/ha) PFRV values were respectively 47.6% and 17.5 %.
- In the case of FR-BC2, for an applied dose of 200 kg P_2O_5/ha and 8 T/Ha (288 kg P_2O_5/ha) PFRV values were respectively 37.9% and 24.8%.

In general, the PFRV values for FR-BC1 and FR-BC2 were quite low. This indicates that FR-BC cannot replace TSP in full, but partial replacement (c. 50% of TSP) might be possible. On the other hand, DE-BC that was obtained with TCR technology (applied in the study by Fraunhofer) had PFRV of 110% (at applied dose of 260 kg P_2O_5/ha), meaning it performed even slightly better than TSP. The TCR technology (vs. pyrolysis used in studies by RITTMO) and used additives in the TCR process allowed production of the DE-BC with P in plant available form, resulting in higher PFRV for DE-BC as compared to FR-BC. Additionally, DE-BC seems to promote plant growth in the early stages of growth and can give advantage for fast growing crops. Therefore, soil application of DE-BC before sowing is recommended.

7.4. Mono ammonium phosphate (Fraunhofer)

The mono ammonium phosphate on perlite (DE-AP1) fertiliser could not compete with full mineral fertilisation in terms of P supply. Both, biomass yields and the supply of P to the plants were reduced compared to the plants from the full mineral fertilisation treatment. The PFRV of DE-AS1 was only 45%. As the distribution of mono ammonium phosphate on the perlite material may be uneven, less P was available to the plants than intended by the fertilisation plan. This is held accountable for the lower yields received with this treatment. Therefore, an isolation of the mono ammonium phosphate from perlite in its pure form was done by Fraunhofer.

After optimising the recovery of ammonia from the TCR gas stream DE-AP2 in its pure form could be isolated. The DE-AP2 is chemically identical with mono ammonium phosphate ($NH_4H_2PO_4$) and thus can be used like a conventional fertilizer. P uptake within the rye grass plants was as good as TSP treatment, with a PRFV value of 106%. Therefore, the isolation of the mono ammonium phosphate from perlite in its pure form (DE-AP2) demonstrated significantly improved P availability compared to DE-AP1, indicating its potential for more effective P supplementation.

7.5. Biologically activated BBFs (UMIL, AGRI)

The work of UMIL + AGRI on biological activation of biodried soild fraction (ES-DSC), soil amendment (NL-SC) and biochar (FR-BC) has shown that there is efficacy of microbial consortia in terms of total productivity (tomatoes). Biological activation increased dried tomato yield by 24%, 66% and 19% for ES-DSC, NL-SC and FR-BC, respectively (27%, 14% and 2% for dried plant weight, respectively). For what concerns the fruit quality, improvements in the qualitative parameters were detected in the BBF-treated fruits respect to the untreated and chemically fertilized ones. Anyway, no improvements were found following the activation of BBFs with the microbial consortia.

The results obtained with radish did not show any effect of BBF treatments, whether activated or not, nor quantitative (yield) neither for qualitative parameters, probably due to the short cultivation cycle. The results obtained with lettuce indicate that the application of BBFs exerted a positive effect on quantitative (yield) parameters, particularly the treatment with biochar (FR) and the activated biochar (FR A). All the treatments seem to accelerate the vegetative phase, leading to earlier senescence respect to the control (NT) and the chemically fertilised (C) plants.

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7.6. Biostimulant (UVIC-UCC, UGent)

The biostimulant had more intense and remarkable effects when it was applied directly to the leaves than in the soil. The ES-AA1 improved the biomass in tomato plants, especially under optimal conditions and hydric stress, reaching similar crop yield to those grown with the commercial biostimulant. This experiment allowed us to better understand the effect of the ES-AA1 biostimulant on plant metabolism and development as well as on plant resistance against stress conditions, also testing different application methods. The ES-AA2 biostimulant was able to effectively increase crop yield and this effect was dependent on the species-related plant responses to stress condition, being observed mainly in spinach under saline stress and in lettuce subjected to hydric stress. Thus, this experiment also confirmed that the biostimulant can be helpful for increasing plant resistance against a range of different hydric and saline stresses.

In case of study with Swiss chard as a test crop, although most conditions lacked statistical significance, increased N uptake at the TMF 130% N dosage notably enhanced yield, emphasising N optimisation's importance. Biostimulants consistently improved chlorophyll concentration and proline content, likely due to better N uptake and stress mitigation. Temperature stress had minimal impact on yield, showing Swiss chard's adaptability, though physiological stress responses were evident across TMF treatments. Higher MDA levels in CAN treatments under stress indicated increased membrane damage.

The effectiveness of the biostimulant in increasing biomass and stress tolerance has been confirmed, with the crops treated with this product showing 20-50% higher yield depending on the plant species and stress level. It is highly recommended to apply it according to the method described in the previous experiments, which consist of 3-5 foliar applications following the phenological stages of the crop. The biostimulant solution should adjust its concentration to the amino acid content of the product, always aiming to apply the same amount of free amino acids as with a commercial product

7.7. Bio-dried solid fraction (UVIC-UCC)

Biodried solid fraction (ES-DSC) derived from the solid fraction was tested for its potential to ameliorate soil physicochemical and biological properties by providing a stable OC, and as a potential source of P and N. The results of the C incubations indicate that the product is stable, with the lowest mineralization rates (< 3% of applied OC) observed during the incubation process: comparable even to the ones of biochar. This suggests that a significant amount of C may remain in the soil, making it a sustainable practice to enhance soil quality. Moreover, the P incubations showed a high amount of P release (76%) and superior performance in terms of PFRV (99% at 30% P dose, 235% at 60% P dose and 286% at 100% P dose), making ES-DSC an effective alternative to conventional P fertilizers in sustainable agricultural practices. In addition, ES-DSC outperforms TSP in terms of dry yield at all doses, with 44%, 106%, and 80% higher dry yields at the 30%, 60%, and 100% doses, respectively. However, this product might not be suitable for N fertilization due to its low N release value (21%).

7.8. Phosphorous-rich ashes (UVIC-UCC)

These ashes were produced to create phosphoric acid using the wet extraction method. Tests were conducted to see if they could be used as a substitute for mineral P fertilizer (i.e. TSP). The results showed that, while they were not as effective as TSP, they did had an average P release of 54%.Therefore, ES-PA could partially replace mineral P fertilizers. However, additional pot trials are required to confirm the observed pattern during the P incubation.

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7.9. Extraction of phosphoric acid (UVIC-UCC)

The phosphoric acid obtained from the ES-PA was extracted using sulphuric acid 1.2 M to obtain all the organic P in the form of inorganic P. The results of the pot trial showed that ES-EPA performs even better compared to the TSP. For instance, the PFRVs were 82% at 30% P dose, 145% at 60% P dose, and 225% at 100% P dose. The dry yield increase with ES-EPA was 19% at 30% P dose, 63% at 60% P dose, and 100% at a 100% P dose.

7.10. Nutrient-rich concentrate (UVIC-UCC)

After separating the solid and liquid fractions, a nutrient-rich concentrate (ES-NC) was produced by subjecting the liquid fraction to a combination of various membrane systems and freeze concentration. The ES-NC comes in three combinations: ES-NC-MFRO, ES-NC-MFR, and ES-NC-RO. These BBFs were obtained from retentates treated by microfiltration and reverse osmosis and had undergone freeze concentration. Specifically, ES-NC-MFR was obtained from the retentate of microfiltration that had undergone freeze concentration, while ES-NC-RO was obtained from the retentate of reverse osmosis that had undergone freeze concentration, but without the membrane contactor.

The three BBFs were tested for its N potential and the results showed that ES-NC-RO had a similar pattern of N release as the CAN, since the average N release was 92%. This means that it could serve as a good N alternative in terms of N fertilization. The N release for ES-NC-MFRO and ES-NC-MFR was above 50%. In fact, ES-NC-MFR, despite not achieving the same results as CAN, had a release pattern very similar to that of CAN, at 85% N release.

Additionally, ES-NC-MFR was tested with the aim of potentially improving soil physicochemical and biological properties by providing stable OC. However, most of the OM in the product is released as carbon dioxide, so it is not suitable for C sequestration. Nevertheless, it could still be a feasible option to increase dissolved OM in soils and microbial activity. Finally, ES-NC-MFR and ES-NC-MFRO were tested in P incubations and pot trials. Regarding the P incubations, both BBFs did not achieve the same level of P release as TSP. In this case, they released on average 53% and 48% of the total P applied in ES-NC-MFR and ES-NC-MFRO treatment, respectively. In contrast, in the pot trial, the PFRV percentages were 169% at a 30% P dose, 356% at a 60% P dose, and 619% at a 100% P dose in ES-NC-MFR, and 69% at a 30% P dose, 153% at a 60% P dose, and 258% at a 100% P dose in ES-NC-MFRO. In addition, regarding the dry yield, the BBFs also had a better performance compared to TSP. For this reason, these high percentages emphasize that both BBFs have the potential for providing a more effective P supplementation.

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References

1. Bakir, S., Capanoglu, E., Hall, R. D. & de Vos, R. C. H. (2020) Variation in secondary metabolites in a unique set of tomato accessions collected in Turkey. Food Chemistry 317, 126406. https://doi.org/10.1016/j.foodchem.2020.126406
2. Bogdan, A.; Robles-Aguilar, A.A.; Liang, Q.; Pap, S.; Michels, E.; Meers, E. (2022) Substrate-Driven Phosphorus Bioavailability Dynamics of Novel Inorganic and Organic Fertilizing Products Recovered from Municipal Wastewater—Tests with Ryegrass. *Agronomy* 2022, *12*, 292
3. Bolan N.S, Rowarth J, de la Luz Mora M, D. Curtin A and D. (2008) Biological transformation and bioavailability of nutrient elements in acid soils as affected by liming, chapter 17 in Developments in Soil Science, volume 32, Ravendra Naidu (Editor)
4. Cavallaro N. and McBride M. B. (1980). Activities of Cu2+ and Cd2+ in soil solutions as affected by pH. Soil Science Society of America Journal 44, 729-732.
5. Cerovic Z. G., Masdoumier G., Ben Ghozlen N., Latouche G. (2012). A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. Physiologia Plantarum 146: 251–260.
6. Chaminade R. (1960) Expérimentations en petits vases de végétation – Types d'essais pour tester l'efficacité des engrais humiques. Annales Agronomiques, 121-133
7. Chaminade R. (1964) Diagnostic des carences minérales du sol par l'expérimentation en petits vases de végétation. Science du Sol, 157-168
8. Chase, A.J., Erich, M.S., Ohno, T., 2018. Bioavailability of phosphorus on iron (oxy) hydroxide not affected by soil amendment-derived organic matter. Agric.Environ.Lett. 3, 170042. https://doi.org/10.2134/ael2017.12.0042. Chen, C., Dynes, J.J., Wang, J., Sparks, D.L., 2014. Propert
9. Chintala Rajesh, Mollinedo Javier, Schumacher Thomas E., Malo Douglas D. & James L. Julson (2014) Effect of biochar on chemical properties of acidic soil, Archives of Agronomy and Soil Science, 60:3, 393-404, DOI: 10.1080/03650340.2013.789870
10. Clagnan, E., Cucina, M., De Nisi, P. et al. (2023) Effects of the application of microbiologically activated bio-based fertilizers derived from manures on tomato plants and their rhizospheric communities. Sci Rep 13, 22478 (2023). https://doi.org/10.1038/s41598-023-50166-5
11. Claussen, W. (2005) 'Proline as a measure of stress in tomato plants', Plant Science, 168(1), pp. 241–248.
12. Davey, M. W., Stals, E., Panis, B., Keulemans, J., Swennen, R. L. (2005) 'High-throughput determination of malondialdehyde in plant tissues', Analytical Biochemistry, 347(2), pp. 201–207.
13. De Neve S. and Hofman G. (2000). Influence of soil compaction on C and N mineralization from soil. Biology and Fertility of Soils 30, 544-549.
14. Dikinya Oagile and Mufwanzala Namasiku (2010) Chicken manure-enhanced soil fertility and productivity: Effects of application rates. Journal of Soil Science and Environmental Management Vol. 1(3), pp. 46-54

This project has received funding from
the EU Horizon 2020 Research and Innovation Programme
under grant agreement No. 862849

15. Egene, C. E., Sigurnjak, I., Regelink, I. C., Schoumans, O. F., Adani, F., Michels, E., … Meers, E. (2021). Solid fraction of separated digestate as soil improver : implications for soil fertility and carbon sequestration. Journal of Soils and Sediments, 21(2), 678–688. https://doi.org/10.1007/s11368-020-02792-z
16. FAO (2014). *World reference base for soil resources 2014: International soil classification system for naming soils and creating legends for soil maps*. *World soil resources reports: Vol. 106*. Rome: FAO. Retrieved from http://www.fao.org/3/i3794en/I3794en.pdf
17. French Standard FD U44-163 - Amendements organiques et supports de culture -Caractérisation de la matière organique par la minéralisation potentielle du carbone et de l'azote. « Organic amendments and growing media - Characterisation of organic matter by the potential mineralisation of carbon and N"
18. Hayat, S., Hayat, Q., Alvemeni, M. N., Wani, A. S., Pichtel, J., Ahmad, A. (2012) 'Role of proline under changing environments: A review', Plant Signaling and Behavior, 7(11), pp. 1–11.
19. Haynes, R.J., (1984). Lime and phosphate in the soil-plant systems. Adv. Agron. 37, 249–467.
20. Huygens, D., Orveillon, G., Lugato, E., Tavazzi, S., Comero, S., Jones, A., Gawlik, B. and Saveyn, H., 2020. Technical proposals for the safe use of processed manure above the threshold established for Nitrate Vulnerable Zones by the Nitrates Directive (91/676/EEC), EUR 30363 EN, Publications Office of the European Union, Luxembourg, 2020, ISBN 978-92-76-21540-0, doi:10.2760/984729, JRC121636.
21. Jeffery, J.J.; Uren, U.C. 1983). Copper and zinc species in the soil solution and the effects of soil pH. Australian Journal of Soil Science, v.21, p.479-488, 1983.
22. Jindo, K., Audette, Y., Olivares, F.L., Canellas, L.P., Smith, D.S., Voroney, R.P., 2023. Biotic and abiotic effects of soil organic matter on the phytoavailable phosphorus in soils: a review. Chem. Biol. Technol. Agric. 10, 29. https://doi.org/10.1186/s40538- 023-00401-y.
23. Kissoudis, C., Sumarti, S., Van De Wiel, C., Visser, R. G., Van Der Linden, C. G., Bai, Y. (2016) 'Responses to combined abiotic and biotic stress in tomato are governed by stress intensity and resistance mechanism', Journal of Experimental Botany, 67(17), pp. 5119–5132.
24. Kuzyakov Y., Subbotina I., Chen H., Boqomolova I., Xu, X. (2009). Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labelling. Soil Biology & Biochemistry Vol.41, p210–219.
25. Lemaire F. (1977) Nouvelles observations sur l'appréciation de la fertilité des sols cultivés au moyen de l'expérimentation en petits vases de végétation. Annales Agronomiques, n° 28, 425-444
26. Lombaert V. (1992) Microcultures Méthode Chaminade, Dossiers Agronomiques d'Aspach-le-Bas, n° 5, 35-51
27. Lugato E, Bampa F, Panagos P, Montanarella L, Jones A. (2014) Potential carbon sequestration of European arable soils estimated by modelling a comprehensive set of management practices. Glob Change Biol 20:3557–3567. https://doi.org/10.1111/gcb.12551

This project has received funding from
the EU Horizon 2020 Research and Innovation Programme
under grant agreement No. 862849

28. Luo Y., Durenkamp M., De Nobili M., Lin Q., Brookes P.C. (2011) Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. Soil Biology & Biochemistry Vol.43, p2304–2314
29. Ma Y. L., Matsunaka T. (2013). Biochar derived from dairy cattle carcasses as an alternative source of P and amendment for soil acidity. Soil Science and Plant Nutrition Vol. 59, p628–641.
30. Marsh, K.B., Tillman, R.W., Syers, J.K., (1987). Charge relationships of sulphate sorption by soils. Soil Sci. Soc. Am. J. 51, 318–323.
31. Mora, M.L., Garcia, J., Santander, J., Demanet, R., (1999). Rol de los fertilisantes Nados y fosfatados en los procesos de acidificacion de los suelos. Frontera Agricola 5(1&2), 59–81.
32. Naisse C., Girardin C., Lefevre R., Pozzi A., Maas R., Stark A., Rumpel C. (2015). Effect of physical weathering on the carbon sequestration potential of biochars and hydrochars in soil. Global Change Biology Bioenergy Vol.7,p488-496.Ma Y. L., Matsunaka T. (2013). Biochar derived from dairy cattle carcasses as an alternative source of P and amendment for soil acidity. Soil Science and Plant Nutrition Vol. 59, p628–641.
33. Nardi, S., Pizzeghello, D., Ertani, A (2017). Hormone-like activity of the soil organic matter. Applied Soil Ecology. http://dx.doi.org/10.1016/j.apsoil.2017.04.
34. NF EN ISO 11732: 2005 (revised 2019). Water quality - Determination of ammonium nitrogen - Method by flow analysis (CFA and FIA) and spectrometric detection.
35. NF EN ISO 13395:1996 (revised 2017). Water quality — Determination of nitrite nitrogen and nitrate nitrogen and the sum of both by flow analysis (CFA and FIA) and spectrometric detection
36. Ohno, T., Crannell, B.S., 1996. Green and animal manure-derived organic matter effects on phosphorus sorption. J. Environ. Qual. 25, 1137–1143.
37. Pell, M., Stenstrom, J. & Granhall, U. (2006). Soil respiration. *In* J. Bloem, D.W. Hopkins & A. Benedetti, eds. *Microbiological methods for assessing soil quality*, p. King's Lynn, CABI Publishing.
38. Peryea F. J. (1990). Phosphate-FertilisFertiliser-Induced Salt Toxicity of Newly Planted Apple Trees. Soil Science Society of America, Volume54, Issue6, Pages 1778-1783
39. Ptaszek, M., Canfora L., Pugliese M., Pinzari F., Gilardi G., Trzciński P., Malusà E. (2023) Microbial-based products to control soil-borne pathogens: Methods to improve efficacy and to assess impacts on microbiome. Microorganisms 11(1), 224. https://doi.org/10.3390/microorganisms11010224
40. Shehata, S.M.; Abou El-Yazied, A. (2011). Effect of Foliar Spraying with Amino Acids and Seaweed Extract on Growth Chemical Constitutes, Yield and its Quality of Celeriac Plant Effect of Bio-stimulants on Yield and Quality of Head Lettuce Grown Under Two Sources of Nitrogen View project. Eur. J. Sci. Res, 58, 257–265.
41. Shrivastava, V., Edayilam, N., Singla Just, B., Castaño-Sanchez, O., Díaz-Guerra, L., & Meers, E. (2024) Evaluation of agronomic efficiency and stress resistance on Swiss chard via use of biostimulants. Scientia Horticulturae, 330, 113053. https://doi.org/10.1016/j.scienta.2024.113053

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42. Sigurnjak, I., De Waele, J., Michels, E., Tack, F.M.G., Meers, E., De Neve, S. (2017). Nitrogen release and mineralization potential of derivatives from nutrient recovery processes as substitutes for fossil fuel based nitrogen fertilizers. Soil Use and Management 33, 437-446
43. Singla Just, B., Binder, P., Guerra-Gorostegui, N., Díaz-Guerra, L., Vilaplana, R., Frison, N., Meers, E., Llenas, L., Robles Aguilar, A. (2024) Phosphorus Release Dynamics from Ashes during a Soil Incubation Study: Effect of Feedstock Characteristics and Combustion Conditions. Agronomy. 2024, 14(5), 935. https://doi.org/10.3390/agronomy14050935"
44. Solarte, M., Moreno, L., Melgarejo, L. M. (2010) 'Fotosíntesis y pigmentos vegetales.', Experimentos en Fisiología vegetal, pp. 77–87.
45. Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (2016). *Die Untersuchung von Böden* (4. Auflage). *Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik: Bd. 1,2*. Darmstadt: VDLUFA-Verlag.
46. Walker David J, Clemente Rafael, Pilar Bernal, M. (2004). Contrasting effects of manure and compost on soil pH, heavy metal availability and growth of Chenopodium album L. in a soil contaminated by pyritic mine waste, Chemosphere, Volume 57, Issue 3,Pages 215-224.
47. Wittmann, O. (1997). Soil Classification of the Federal Republic of Germany. *Mitteilung Der Deutschen Bodenkundlichen Gesellschaft*, *1997*(84), 253–275.

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FERTIMANURE

FERTIMANURE

INNOVATIVE NUTRIENT RECOVERY FROM SECONDARY SOURCES-PRODUCTION OF HIGH-ADDED VALUE FERTILISERS FROM ANIMAL MANURE

PROJECT COORDINATOR

Fundació Universitària Balmes (Spain)

CONSORTIUM

Ghent University (Belgium)
Wageningen Environmental Research (The Netherlands)
University of Milan (Italy)
Leitat (Spain)
GreenWin (Belgium)
European Landowners Organisation (Belgium)
IPS Konzalting (Croatia)
Fraunhofer (Germany)
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PROJECT WEBSITE:

https://www.fertimanure.eu

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Brief project summary

The mission of the FERTIMANURE project is to provide innovative solutions (technology, end-products, and business models) that solve real issues, ie the manure challenge, and help farmers with the challenges that they are currently facing. FERTIMANURE will develop, integrate, test and validate innovative nutrient management strategies so as to efficiently recover and reuse nutrients and other products with agronomic value from manure, to ultimately obtain reliable and safe fertilisers that can compete in the EU fertiliser market.

The FERTIMANURE project will cover both technological and nutrient management approaches. The technological side will be addressed with the implementation of 5 innovative & integrated on-farm experimental pilots for nutrient recovery in the most relevant European countries in terms of livestock production (Spain, France, Germany, Belgium, The Netherlands), whereas nutrient management will be addressed through 3 different strategies adapted to mixed and specialised farming systems:

Strategy #1 with on-farm production and use of bio-based fertilisers (BBF)(1) , **Strategy #2** with on-farm BBF production and centralised tailor-made fertilisers (TMF)(2) production, and **Strategy #3** with on-farm TMF production and use.

Definition of Bio-based fertilisers (BBFs): *Bio-based fertilisers (BBFs) are fertilising products or a component to be used in the production of (Tailor-Made) Fertilisers that are derived* ***from biomass-related resources.***

The BBFs of FERTIMANURE are "obtained through a ***physical, thermal/thermo-chemical, chemical, and/or biological processes for the treatment*** *of manure or digestate that result into a change in composition due to a change in concentration of nutrients and their ratios compared to the input material(s) in order to get better marketable products providing farmers with nutrients of sufficient quality".*

However, just separation of manure in a solid and liquid fraction (as first processing step) is excluded. These products are not conceived as a BBF, although they are valuable sources to supply nutrients on agricultural land.

LIST OF BBFs Produced in FERTIMANURE

Number	BBF-code	BBF product description
1	NL-AS	Ammonium sulphate solution
2	NL-LK	Liquid K-fertiliser
3	NL-SC	Soil conditioner
4	NL-WP	Wet organic P-rich fertiliser
5	NL-DP	*90% dried organic P rich fertiliser (calc)*
6	ES-NC	Nutrient-rich concentrate
7	ES-DSC	Bio-dried solid fraction
8	ES-PA	Phosphorous (ashes)
9	ES-AM	Ammonium salts
10	ES-AA	AA-based biostimulants
11	DE-AS	Ammonium sulphate solution (liquid)
12	DE-BC	Biochar (solid)
13	DE-AP	Ammonium phosphate on perlite (solid)
14	BE-AN	Ammonium nitrate
15	BE-AS	Ammonium sulphate
16	BE-AW	Ammonium water
17	FR-BC	Biochar
18	FR-AS	Ammonium sulphate
19	FR-LK	Liquid K-fertiliser

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Definition of Tailor-Made Fertilisers (TMFs): *A tailor-made fertiliser (TMF) is a customized fertiliser that meets with the nutrient requirements of a specific crop by taking into account the soil type, soil fertility status, and growing conditions and fertilisation practises.*

The TMFs obtained in FERTIMANURE are produced from BBFs (produced from manure or digestate and/or other recovered fertilising products that are available) and/or mineral fertilisers (MF) (and/or biostimulants).

Fully crop specific TMFs can be defined and centrally produced assuming e.g. a sufficient nutrient status of a soil type and no additional fertilisation practice.

However, on farm level the soil-crop requirements will be different due to another nutrient status of the soil and the fact that often manure/digestate will be applied on the fields which has to be taken into account as nutrient supplier. Consequently, the composition of the TMF (combination of BBF and MF) that will be used by the farmer can differ from the one produced in a centralised way.

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