

# Deliverable

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## D4.6. Final – Report on agronomic and environmental performance in field trial experiences

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Author(s) and Co-author(s)	Ivona Sigurnjak, Vaibhav Shrivastava, Nimisha Edayilam, Omar Castano Sánchez, Laura Díaz Guerra, Carlos Ortiz, Ignasi Salaet, Herminia De La Varga, Begoña Arrufat, José Antonio Rodríguez-Sánchez, Mariana Moreira, Clément Munier, Pierre-Baptiste Blanchant, Anna Adriani, Nicolas Thevenin, Lionel Ruidavets, René Rietra, Erik Meers
Contributor(s)	UGent, UVIC-UCC, Fertinagro, CRAB, CRAGE, CA80, WENR, RITTMO, DARP

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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 'Report on agronomic performance of the obtained BBFs and TMFs in laboratory setting' gives insight into final results of the of testing in laboratory settings, whereas the full field scale results from 2021 - 2023 are reported in D4.6 'Report on agronomic and environmental performance in field trial experiences'. This report concerns D4.6 which summaries results from 25 field trials and 8 pot trials that took place in the period 2021 - 2023 in four partner countries (Spain, France, Belgium and the Netherlands). In these field and pot experiments three TMFs and seven BBFs were tested by cultivating 8 different crop types. The aim of the experiments is to draw conclusions on i) agronomic performance of BBFs and TMFs in respect to crop yield and fertiliser replacement value, and ii) environmental performance in regards to nitrate leaching and ammonia and greenhouse gas emissions.

We would like to acknowledge the researchers and staff of Fertinagro Biotech (Spain), RITTMO Agroenvironnement (France), Ghent University (Belgium), BETA Technological Centre – University of Vic (Spain), Departament d'Accio Climatica, Alimentacio i Agenda Rural (Spain), Wageningen Environmental Research (the Netherlands), Assemblée Permanente des Chambres d'Agriculture (France), for their work and contribution.

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## Summary

Deliverable 4.6 ‘*Final - Report on agronomic and environmental performance in field trial experiences*’ 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 and on high energy consumption. The D4.6 reports on results and final conclusion under the following two tasks of WP4:

### **Task 4.2. Field validation of agronomic performance in quadruplicate-randomised block design**

*Sub-task 4.2.1. Spain*

*Sub-task 4.2.2. France*

*Sub-task 4.2.3. Belgium*

*Sub-task 4.2.4. The Netherlands*

### **Task 4.3. Field assessment of environmental performance: nutrient losses**

Deliverable 4.6 aims to summarise the final outcome for each task and sub-task, thus it is divided into 7 chapters. **Chapter 1** provides an introduction to the deliverable. In **Chapter 2** the final results of testing three tailor-made fertilisers (TMFs) in Spanish field trials for winter wheat and potatoes, as well as pot cultivation of spinach and lettuce, are presented. The TMFs were developed through collaboration between UVIC-UCC and Fertinagro, with the former using a Spanish/Dutch pilot and the latter employing patented technology for pig slurry. **Chapter 3** explores experiments conducted using ammonium sulphate BBF by French partners (CRAB, CRAGE, and CA80 under APCA), WENR and UGent. This BBF was tested in 19 field trials and 4 pot trials. This chapter also investigates the N performance of ammonium nitrate and ammonium water from the Belgian pilot, with a pot experiment testing ammonium water (BE-AW) on lettuce and a field trial testing ammonium nitrate (BE-AN) on potatoes and maize. **Chapter 4** focuses on testing of biochar-based BBFs from the French partners in three field trials and one pot trial involving crops such as potatoes, sugar beet, ryegrass, and sauerkraut. **Chapter 5** features the evaluation of liquid potassium-based BBFs by the French partner CRAGE in a field trial with sugar beet as the target crop. In **Chapter 6**, the deliverable reports on the environmental monitoring campaigns (Task 4.3) that investigated ammonia and gaseous emissions resulting from the on-field and pot application of the various BBFs. Finally, **Chapter 7** engages in an overall discussion and offers recommendations regarding the performance of all the tested BBFs from the FERTIMANURE pilots.

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## List of Abbreviations

<b>AA</b>	Amino acids
<b>AKR</b>	Apparent potassium recovery
<b>ANR</b>	Apparent nitrogen recovery
<b>APR</b>	Apparent phosphorus recovery
<b>BBF</b>	Bio-based fertiliser
<b>CAN</b>	Calcium ammonium nitrate
<b>CEC</b>	Cation exchange capacity
<b>DM</b>	Dry matter
<b>EC</b>	Electrical conductivity
<b>FM</b>	Fresh matter
<b>GHG</b>	Greenhouse gas emissions
<b>KFRV</b>	Potassium fertiliser replacement value
<b>NFRV</b>	Nitrogen fertiliser replacement value
<b>OC</b>	Organic carbon
<b>OM</b>	Organic matter
<b>PFRV</b>	Phosphorus fertiliser replacement value
<b>PS</b>	Pig slurry
<b>SOM</b>	Soil organic matter
<b>TMF</b>	Tailor-made fertiliser
<b>TSP</b>	Triple superphosphate
<b>TOC</b>	Total organic carbon
<b>UAA</b>	Utilised agricultural area



## 1. Introduction

Keeping with the idea of a circular economy, nutrient recovery from biomass streams like animal manure has accelerated in recent years, leading to the development of bio-based fertilisers (BBFs). The European Commission has implemented the EU Fertilising Product Regulation (FPR, 2019/1009), which took effect on July 16, 2022, to ease the transition from conventional fertilisers to BBFs. 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 much 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 is not yet 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 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 some of 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 and TMFs for their ability to substitute current synthetic mineral fertilisers whose production is based on finite fossil-based resources. Assessments are conducted at laboratory, pot and field scale. Deliverable D4.5 '*Final - Report on agronomic performance of the obtained BBFs and TMFs in laboratory setting*' reports on results in laboratory settings, whereas the full field scale results from 2021 - 2023 are reported in D4.6 '*Final - Report on agronomic and environmental performance in field trial experiences*'.

The main aim of D4.6 is to assess agronomic and environmental performance of BBFs and TMFs (Table 1) in terms of crop yield, nutrient recovery efficiency, nitrate leaching and ammonia and greenhouse gas (GHG) emissions, in a full field scale (when not feasible then on pot scale) conditions. For this purpose, in the period 2021 - 2023 FERTIMANURE consortium conducted in total 26 field trials (maize trial by Fertinagro failed and is not reported) and 8 pot experiments across Spain, France, Belgium and the Netherlands. Field trials took place in several regions of Europe which are soil/climate defined to build solid results and access the variability of novel fertiliser efficiency on studied crops.

The short-term crop response to N was assessed by N fertiliser replacement value (NFRV). The NFRV has been defined by several different academic papers and there has been much discussion about what the definitions should be (Schils et al., 2020). These papers were compiled and reviewed by Schils et al. (2020), and in this article they define the NFRV as the N fertiliser replacement value, which specifies the amount of standard mineral N needed from a novel fertiliser to give a similar N uptake response as by a conventional





fertiliser. The NFRV is calculated from the ratio of Apparent N Recovery (ANR) of the test fertiliser to the reference fertiliser. The ANR is the amount of N from the applied fertiliser taken up by the crop after subtracting the amount of N taken up by the control treatment (no N fertilisation) (Schils et al., 2020). ANR and NFRV are calculated with the following equations:

$$ANR_{fertilizer} = \left( \frac{crop\ N\ uptake_{fertilizer} - crop\ N\ uptake_{control}}{total\ N\ applied_{fertilizer}} \right) \quad (Eq. 1)$$

$$NFRV = \frac{ANR_{BBF}}{ANR_{CAN}} \quad (Eq. 2)$$

Similar approach can be used for assessing P. However, in this case ANR and NFRV are named as APR and PFRV.

$$APR_{fertilizer} = \left( \frac{crop\ P\ uptake_{fertilizer} - crop\ P\ uptake_{control}}{total\ P\ applied_{fertilizer}} \right) \quad (Eq. 3)$$

$$PFRV = \frac{APR_{BBF}}{APR_{CAN}} \quad (Eq. 4)$$

Understanding the agronomical effects of using TMFs and BBFs under various circumstances is crucial. 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 (assessed in D4.5).



**Table 1.** Overview of conducted field trials and pot experiments in period 2021-2023. Classification of the field trial (i.e. scientific or demo): Scientific field trials comply with the proposed basic protocol in D4.4. Demo field trials do not comply with the proposed basic protocol.

	#	PP in charge	Growing season 2021		Growing season 2022		Growing season 2023		Type of trial
			Crop 2021	Tested product	Crop 2021	Tested product	Crop 2021	Tested product	
<b>ES</b>	Field 1	UVIC + DARP	Winter Wheat	TMF <sup>1</sup>	Winter Wheat	TMF <sup>1</sup>	-	-	Scientific
	Field 2	Fertinagro	Potatoes	TMF <sup>2</sup>	Potatoes	TMF <sup>2</sup>	Potatoes	TMF <sup>2</sup>	Demo
	Pot 1	UVIC	Spinach	TMF <sup>3</sup>	-	-	-	-	Scientific
	Pot 2	UVIC	Lettuce	TMF <sup>3</sup>	-	-	-	-	Scientific
<b>FR</b>	Field 3	CRAB	Silage maize	FR-AS	Silage maize	FR-AS	-	-	Scientific
	Field 4	CRAB	Spinach	FR-AS	Spinach	FR-AS	Winter wheat	FR-AS	Scientific
	Pot 3	CRAB/RITTMO	Rye-grass	FR-BC	Rye-grass	FR-BC	-	-	Scientific
	Field 5	CA80	Potatoes	FR-BC	-	-	-	-	Scientific
	Field 6	CA80	Potatoes	FR-AS	Potatoes	FR-AS, "FR-BC + FR-AS "	Potatoes	FR-AS	Scientific
	Field 7	CRAGE	Sauerkraut cabbage	FR-AS	Cabbage	FR-AS, "FR-BC + FR-AS "	Cabbage	FR-AS	Scientific
	Field 8	CRAGE	Sugar beet	FR-AS, FR-LK	Sugar beet	FR-AS, FR-BC, FR-LK	-	-	Scientific
<b>BE</b>	Pot 4	UGent	Lettuce	BE-AW	-	-	-	-	Scientific
	Field 9	UGent	Maize	BE-AN, BE-AS	Maize	BE-AN, BE-AS	-	-	Scientific
<b>NL</b>	Pot 5	WENR	Maize	NL-AS	-	-	-	-	Scientific
	Pot 6	WENR	Maize	NL-AS	-	-	-	-	Scientific
	Pot 7	WENR	Grass	NL-AS	-	-	-	-	Scientific
	Pot 8	WENR	Grass	NL-AS	-	-	-	-	Scientific
	Field 10	WENR	Maize	NL-AS	-	-	-	-	Demo
	Field 11	WENR	Maize	NL-AS	-	-	-	-	Demo
	Field 12	WENR	Grass	NL-AS	-	-	-	-	Demo
	Field 13	WENR	Grass	NL-AS	-	-	-	-	Demo

ES: Spain; FR: France, BE: Belgium; NL: the Netherlands; PP: project partner; TMF: tailor-made fertiliser; AA: amino-acid; AS: ammonium sulphate; BC: biochar; LK: liquid potassium fertiliser; AW: ammonium water; AN: ammonium nitrate. <sup>1</sup> UVIC-UCC TMF concerns pre-sowing application of the biodried soil fraction (ES-DSC) plus a top-dress application of the ammonium sulphate solution (ES-AS) and the biostimulant (ES-AA) product. <sup>2</sup> Fertinagro's TMF is on-farm combination of pig slurry, synthetic mineral fertilisers, bio-stimulants, humic acids and additives. <sup>3</sup> UVIC-UCC TMF concerns the biodried soil fraction (ES-DSC) plus ammonium sulphate solution (ES-AS).

## 2. Tailor-made fertilisers (TMFs)

### 2.1. Winter wheat field cultivation (UVIC-UCC, Spain)

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

This study will be published as Castaño-Sánchez, O. et al. Testing the agricultural adequacy of a TMF in a 2-year field experiment with winter wheat cultivation. Under preparation.

#### 2.1.1 Introduction

The Spanish field trial at UVIC-UCC was designed to test the agricultural adequacy of a TMF made from Spanish BBFs. The TMF concerns pre-sowing application of the bio-dried solid fraction (ES-DSC) plus a top-dress application of the ammonium sulphate solution (ES-AS) and the biostimulant (ES-AA) product. Due to delays and technical issues at Spanish pilot prior to TMF application for winter wheat cropping season 2021, the ammonium sulphate from Dutch pilot (NL-AS) was used to formulate the Spanish TMF. The general objective was to assess the TMF against the synthetic fertiliser reference and the direct application of pig slurry in terms of agronomic value, environmental impact, and potential improvement in soil quality. The assessment took place for two cropping seasons 2021/2022 and 2022/2023.

#### 2.1.2 Methodology

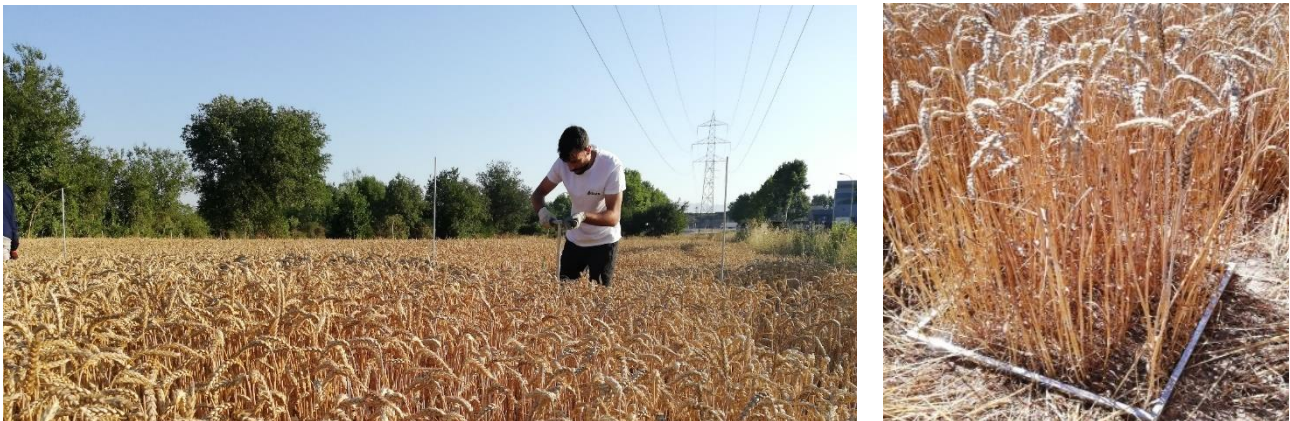
##### (i) Experimental design

The experimental field is located in Vic (41°56'44.9"N 2°16'40.4"E, Catalonia, Spain), a zone with a humid sub-Mediterranean climate. The study area was divided into 40 plots of 8 m<sup>2</sup>, with 4 m of distance between plots. The experiment followed a randomised complete two blocks design (Block A and Block B). Before starting the field experiment, ryegrass was cultivated the previous year to partially extract and reduce the content of soil nutrients, resulting in a soil more suitable for an agronomic assay.

The TMF was formulated according to the nutritional requirements of winter wheat crop, taking into account the maximum legislative application of total N (170 kg/ha/y), the soil characteristics, and the BBFs properties. The BBFs selected for the TMF formulation were: bio-dried solid fraction (ES-DSC) of pig slurry from the Spanish pilot plant (applied on pre-sowing), ammonium sulphate (NL-AS) from the Dutch pilot plant, and biostimulant (ES-AA) coming from the Spanish pilot plant (applied on top-dress). ES-DSC covered half of the desired N application, while NL-AS covered the other half as a top dress. The TMF was compared against commercial ammonium sulphate used for top-dress fertilisation (referred as "Mineral" treatment) and with the direct use of pig slurry as fertiliser ("Raw Manure" treatment). Additionally, plots with no fertilisation were used as a negative control ("Control" treatment). Considering each plot as a replicate, every treatment consisted of 4 replicates (2 replicates in Block A and 2 in Block B), to ensure randomness in the experiment. All fertilisation plans were applied at 3 different doses (50%, 75% and 100%) of the allowed maximum N application (170 kg N/ha). These allowed us to compare the different fertilisers at doses used by farmers in Catalonia and to investigate whether a smaller dose could maintain the crop yield and soil quality, and therefore, could be feasible to apply. In addition, to test the effect of the biostimulant ES-AA, this product was applied to half of each plot, in combination with the corresponding treatment. Thus, each half plot receiving this biostimulant was compared with the other half of the plot. The first year of the field experiment was finished in July 2022 and the second one in July 2023, although the final soil sampling and characterisation was done in October 2023. The data set was statistically analysed by IBM® SPSS 28 (IBM SPSS statistics, Corporation, Chicago, USA). One-way ANOVA was employed with Tukey's test to evaluate the effects of the treatments on most of the parameters analysed, except for crop yield, for which Duncan's test was performed.

(ii) Soil parameters

Soil samplings were performed in the no-biostimulant half of each plot for all treatments, except for the TMF plots that were sampled in the biostimulant-applied half. This soil sampling design aims to compare the application of the three BBFs (including the biostimulant ES-AA) considering this mixture as a complete TMF, with the other treatments that are common in agricultural practices (raw manure direct application and conventional fertilisation, both non-including the biostimulant). Soil nitrates were measured periodically from 0 to 90 cm depth throughout the two years of the experiment (Figure 1). In addition, a complete soil characterisation (including  $\text{NO}_3^-$ , Olsen P, total P, exchange K, total K, total N,  $\text{NH}_4^+$ , TOC, total Zn, total Cu, pH and EC) from 0 to 30 cm depth were done at the end of the second year of the experiment (October 2023) and compared with the initial values (October 2021).



**Figure 1.** Soil sampling and crop harvest on July 2022 (1<sup>st</sup> crop year).

(iii) Plant parameters

Plant sampling and analyses were done at the end of the first year of the experiment (July 2022), and at the end of the second year (July 2023). Plant growth responses to the different treatments was assessed in terms of dry weight, NPK content, Zn and Cu content with the same sampling scheme as the soil sampling. The grain yield was also assessed via Near Infrared (NIR) Grain Analyser for both the non-biostimulant treatments and the biostimulant ones. The analysed parameters by the NIR Grain Analyzer were total protein, lipids, ash, acid-detergent fiber, neutral-detergent fiber content, total fiber, total P, phytic P and starch contents. For the first year, these parameters were statistically analysed with a sample size of  $n=4$ . However, in the second year, only one analysis ( $n=1$ ) per treatment could be conducted in the laboratory due to a poor crop yield and grain size associated to the anomalous rainfall regime that occurred in 2023 in Catalonia region. This resulted in an insufficient sample size for proper statistical analysis in the second year of the experiment. Therefore, the samples analysed in the first year correspond to the grain from each plot separately, while the sample analysed in the second year is a composite sample from the 4 plots that define the treatment.

### 2.1.3 Results and discussion

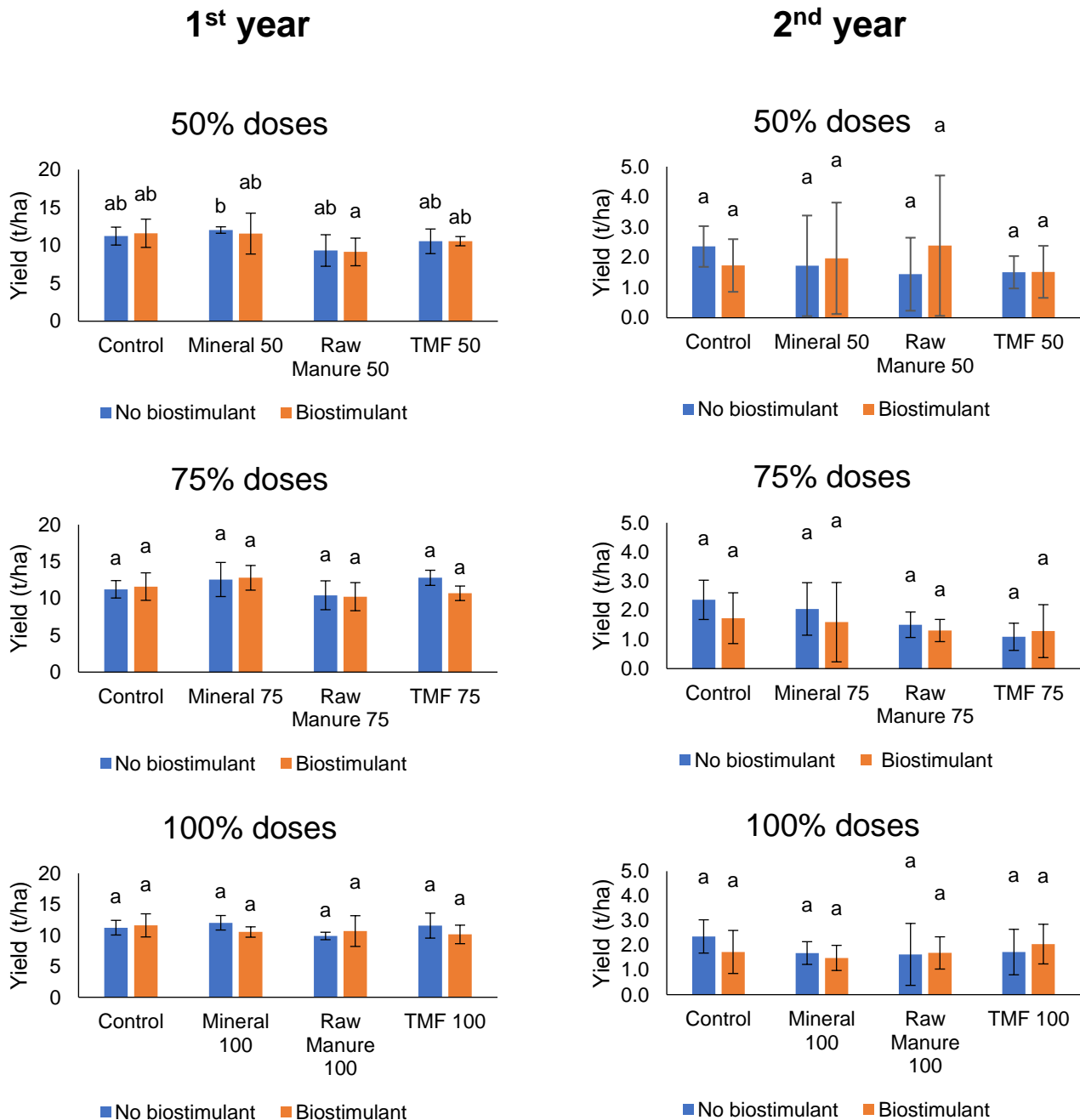
(i) Initial soil characterisation and crop nutrient requirements

Wheat (whole plant) average production in Catalonia is 5.1 t/ha (dry weight) and average nutrient uptake amounts to 148 kg N/ha, 71 kg  $\text{P}_2\text{O}_5$ /ha and 122 kg  $\text{K}_2\text{O}$ /ha according to the Catalonian Agriculture Department (DACC, 2019). Based on the soil analysis (data not shown), it was determined that the amount of  $\text{P}_2\text{O}_5$  and  $\text{K}_2\text{O}$  was high enough for a good crop development. For all soil parameters analysed, there were no significant differences between the treatments.

(ii) Crop yield

The results of crop yield for the first year showed significant differences only in plots receiving the lowest fertilisation dose (Figure 2). Specifically, the crop yield in “Mineral 50” treatment was significantly higher than

the “Raw Manure 50 + biostimulant” treatment. In general, the mineral fertilisation always had higher mean values of crop yield, although the differences were not significant. In contrast, “Raw Manure” treatments generally showed lower crop yield. In addition, there were no significant differences in terms of crop yield (t/ha) between the biostimulant and non-biostimulant treatments. In general, the harvest in July 2022 was profitable (12 t per ha on average) since almost all treatments doubled the average production of the area. These results could indicate that initial soil fertility was enough to allow the proper growth and development of winter wheat plants.



**Figure 2.** Yield (t/ha) of wheat from first and second crop year at different doses (50%, 75% and 100% of maximum allowed N application). Each column represents the mean values  $\pm$  SD. Columns with different letters represent significant differences between treatments according to Duncan's test ( $p \leq 0.05$ ).

In the second year, the crop yield was much lower than in the first year, being 2-2.5 t per ha per treatment on average. This represents a reduction of 80%-85% compared to the previous year crop yield. These results can be explained by the exceptional weather conditions in 2023 when there was a very prolonged period of extreme drought in Spain and particularly in Catalonia region. Normally, the rain at the beginning of the year led to appropriate crop development, but in 2023 the rain came very late, favoring the weed growth and proliferation. Because of these weeds, high standard deviation was observed in some treatments linked to the plots that were completely overrun by weeds, having wheat hardly developed. Unfortunately, regarding the different treatments and the biostimulant application, we cannot draw relevant conclusions from the results of the crop yield obtained in the second year.

(iii) ANR and NFRV

Regarding the ANR and NFRV, the N uptake by the plants in “Control” treatment was similar to that found in the “TMF” treatment in all doses, which is in accordance with the lack of significant differences observed in crop yield. So, contrary to expectations, the additional N fertilisation with the TMF did not increase N uptake by the wheat plants during the first and second year of the field trial. This could be also related to the high soil N content obtained in “Control” plots at the beginning of the experiment (October 2021), although the differences in soil total N were not significant either in October 2021 or in July 2022.

**Table 2.** Apparent N recovery (ANR) and N fertiliser replacement value (NFRV) of the different treatments in wheat crop for the two years of experiment.

Treatments	2022		2023	
	ANR	NFRV	ANR	NFRV
TMF 50	-0.04 ± 0.27 a	-0.12 ± 0.89 a	-0.01 ± 0.12 a	-0.04 ± 0.41 a
MINERAL 50	0.30 ± 0.26 a		-0.02 ± 0.13 a	
TMF 75	-0.06 ± 0.19 a	-0.19 ± 0.61 a	-0.03 ± 0.06 a	-0.10 ± 0.21 a
MINERAL 75	0.42 ± 0.35 a		-0.00 ± 0.08 a	
TMF 100	0.03 ± 0.12 a	0.08 ± 0.41 a	-0.02 ± 0.04 a	-0.08 ± 0.13 a
MINERAL 100	0.37 ± 0.22 a		0.01 ± 0.06 a	

(iv) Grain quality parameters

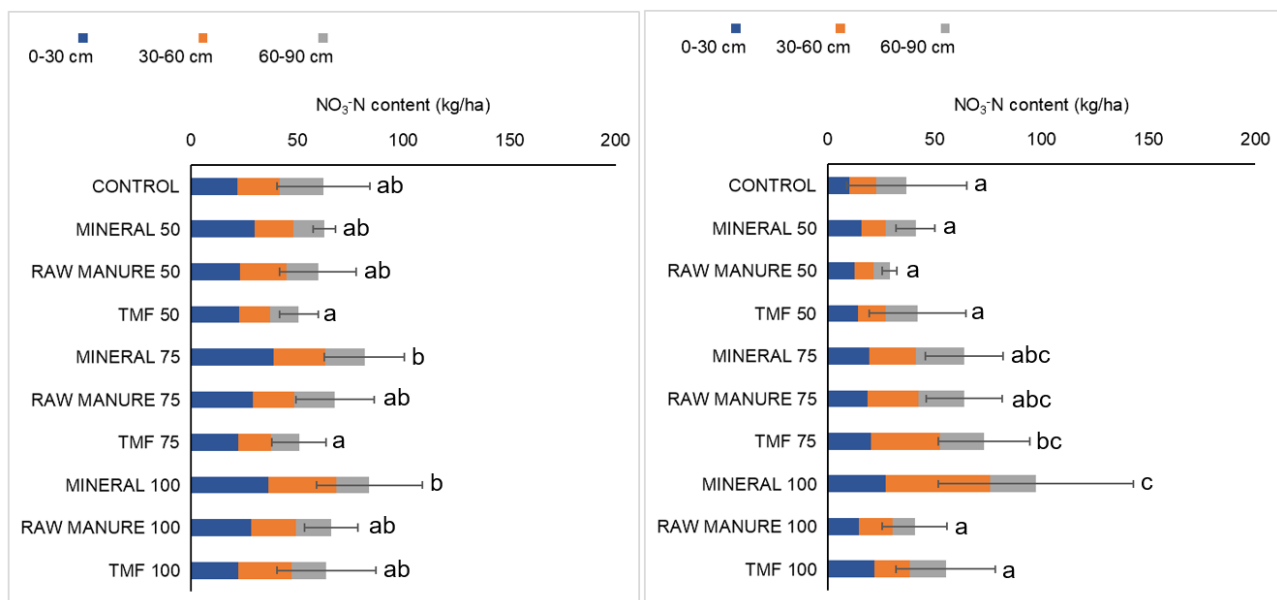
Regarding the protein content, the differences found were due to the type of fertiliser applied, and not to the biostimulant application. Thus, no differences were found between the grain from plants receiving the biostimulant and those grown without this product. In contrast, the grain produced by winter wheat plants fertilised with “Mineral 75” and “Mineral 100” treatments had the highest protein content, being significantly different to those subjected to the control and TMF treatments at the same dose. Despite this, no differences were detected in terms of total lipids, ash, ADF (acidic detergent fiber), NDF (neutral detergent fiber), total fiber, total P, phytic P and starch contents.

Comparing grain from first and second year, we found that their characteristics were very different. While there were no differences in terms of total lipids and fiber content, grain from second year had higher values of total protein, ash, ADF, NDF, total P, phytic P and lower starch content. The differences in characteristics between both grains may be attributed to the extreme drought conditions of the second year, potentially resulting in incomplete grain filling.



(v) N residue in the soil

Results obtained indicate that TMF formulation is a safer choice in comparison to the commercial ammonium sulphate application in terms of  $\text{NO}_3\text{-N}$  residue. At the beginning of the experiment (October 2021), there were no differences in the  $\text{NO}_3\text{-N}$  content between the treatments in any of the studied depths (0-30, 30-60, 60-90 cm). At the end of the first crop cycle (July 2022),  $\text{NO}_3\text{-N}$  residue at 0-90 cm depth of “Mineral 75” and “Mineral 100” was slightly higher, but not significantly different from control plots. Even though, at 0-30 and 0-90 cm depth, Mineral 75 was significantly higher than TMF 75 plots. At the end of the second crop cycle (July 2023), the “Mineral 100” treatment plots had a significantly higher amount of  $\text{NO}_3\text{-N}$  in the 0-90cm profile in comparison with other treatments at the same dose, and with control plots, as shown in Figure 3. Also, at the end of the second crop cycle, “Mineral 100”  $\text{NO}_3\text{-N}$  residue was significantly higher at 0-30 and 30-60 cm depth than control plots and significantly higher at 30-60 cm depth than “TMF 100” plots. If the increment in nitrate content in deeper profiles continues, this would represent a significant impact on nitrate leaching produced by the mineral fertilisation, which could potentially increase nitrate pollution also in groundwater.



**Figure 3.**  $\text{NO}_3\text{-N}$  content (kg/ha) in the soil profile after harvest sorted by depths in July 2022 (A) and July 2023 (B). Each column represents mean values for 0-30 cm depth (blue), 30-60 cm depth (orange) and 60-90 cm depth (grey). Error bars represent standard deviation from the total value for 0-90 cm depth. Columns with different letters represent significant differences between treatments according to Duncan's test ( $p \leq 0.05$ ).

#### 2.1.4 Conclusion and recommendation

The combination of ES-DSC and NL-AS as a TMF along with the biostimulant (ES-AA), the application of pig slurry and the fertilisation with commercial ammonium sulphate were not able to increase crop yield in the two harvests of the experiment in comparison to control plots. Also, the application of biostimulant was not effective neither increasing crop yield or grain quality parameters (data not shown). Regarding nutrient content, highest doses of commercial ammonium sulphate (Mineral treatments) increased N content first year of the trial, but also increased  $\text{NO}_3\text{-N}$  residue both years after harvest. In July 2022 the amount of  $\text{NO}_3\text{-N}$  residue in “Mineral 100” plots was 32% higher than “TMF 100” plots, and in July 2023 this difference increased to be 76% higher than in “TMF 100” plots”. At the end of the experiment (October 2023) this increase was as high as a 93%. This increment sustained over time can lead to nitrate pollution by leaching. So, it is recommended to use the TMF formulation to prevent these environmental harmful effects. If necessary to use a mineral fertiliser, its application should be done at low doses and with a very strict control of the crop and the soil.

## 2.2. Spinach and lettuce pot cultivation (UVIC-UCC, Spain)

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

### 2.2.1 Introduction

Spinach and lettuce are two crops widely cultivated and consumed globally, playing a key role in nutrition and the economy of many regions worldwide. As an effective strategy for the fertilisation of both crops, the introduction of new BBFs can result in a significant reduction in the use of mineral fertiliser, improving the environmental impact of the agriculture. To assess the agronomic value of TMF formulations, the following experiment compared a fertilisation with a TMF versus commercial ammonium sulphate on crop yield and plant nutrition of spinach (*Spinacia oleracea* var. Butterfly) and lettuce (*Lactuca sativa* var. Batavia), which were grown in pots under controlled environmental conditions.

### 2.2.2 Methodology

The effects of the TMF were tested in plants of spinach and lettuce grown in pots in mesocosms, under optimal conditions of temperature (24-26°C), humidity (45-65%) and photoperiod (12h/12h) (Figure 4). The soil used in this experiment was the same as in the experimental field (see section 2.1.2). The TMF consisted of a 4:1 mixture of two BBFs from the Spanish pilot plant, ammonium sulphate (ES-AA) and nutrient-rich concentrate (ES-NC), while the mineral fertilisation was done with commercial ammonium sulphate (21% N). ES-AS and ES-NC composition is shown in Table 3. Both fertilisation plans were applied in increasing doses, including 50%, 75% and 100% of the plant N requirements, and they were compared between them and with a negative control treatment. Consequently, this experimental set-up resulted in 7 different treatments per crop: control, TMF at 3 dosages, and Mineral at 3 dosages. Each treatment consisted of 6 replicates, having 42 pots in total per each crop. The determination of soil elements was performed at the beginning and the end of the experiment, and it consisted of total and available P, exchangeable K, total N, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, total organic C, Zn, Cu, pH and EC. Plant parameters were determined after harvesting the plants and consisted of fresh yield and analysis of the main nutrients for the plant (N and P) and other macro- and micronutrients (Ca, Mg, Fe, Zn, Cu and Na). The data set was statistically analysed performing one-way ANOVA ( $p \leq 0.05$ ) and Tukey's test honestly significant difference (HSD) using IBM® SPSS 28 (IBM SPSS statistics, Corporation, Chicago, USA). Regarding lettuce plants, soil data could not be statistically analysed due to only having one replicate per parameter.

**Table 3.** Characterisation (Mean  $\pm$  SD) of the BBFs: ammonium sulphate (ES-AS) and nutrient-rich concentrate (ES-NC).

Parameters	ES-AS	ES-NC
pH	7.00 $\pm$ 0.42	7.68 $\pm$ 0.49
EC (mS/cm)	-	23.00 $\pm$ 0.99
Organic matter (g/kg)	-	22.44 $\pm$ 0.47
Total N (g/kg)	13.85 $\pm$ 2.25	3.64 $\pm$ 0.12
Ammonium-N (g/kg)	13.85 $\pm$ 2.25	2.74 $\pm$ 0.14
Organic-N	-	0.93 $\pm$ 0.27
P (g/kg)	-	0.49 $\pm$ 0.01
K (g/kg)	12.91 $\pm$ 14.96	1.76 $\pm$ 0.00
S (g/kg)	30.09 $\pm$ 11.90	0.36 $\pm$ 0.00
Ca (g/kg)	5.00 $\pm$ 0.00	1.12 $\pm$ 0.23
Mg (g/kg)	0.28 $\pm$ 0.09	0.37 $\pm$ 0.12
Na (g/kg)	7.88 $\pm$ 0.41	1.13 $\pm$ 0.55



Cu (mg/kg)	0.05 ± 0.00	232.36 ± 51.42
Zn (mg/kg)	12.83 ± 22.14	992.36 ± 78.28



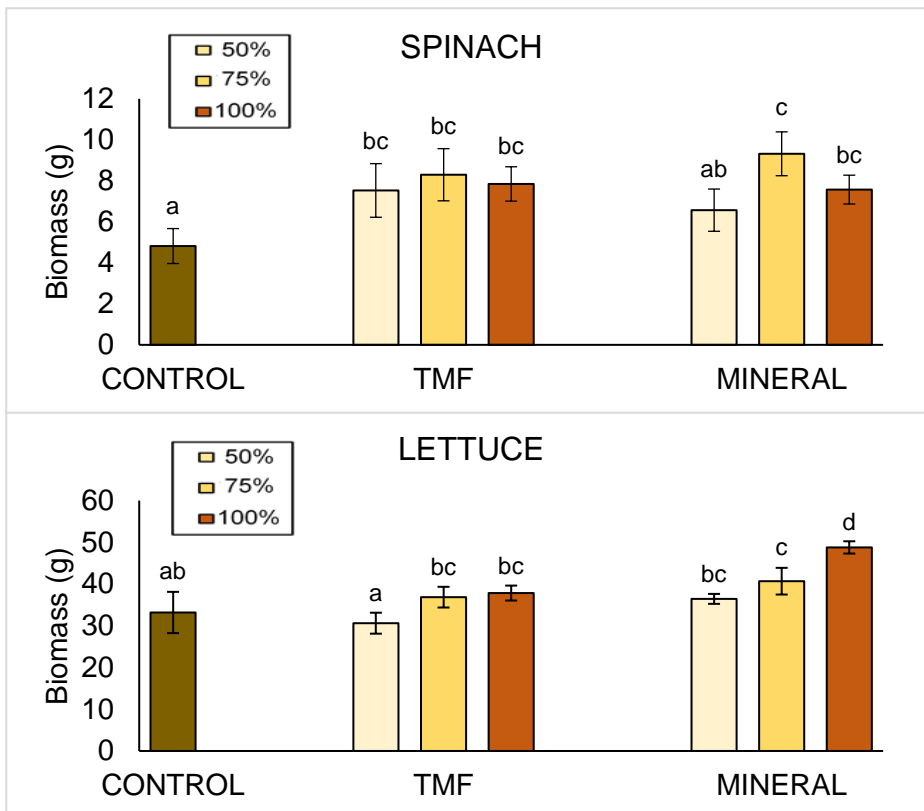
**Figure 4.** Lettuce plants at day 67.

### 2.2.3 Results and discussion

#### (i) Crop yield

Results displayed in Figure 5 shows that, in spinach, both fertilisation plans had a similar performance at all doses. The TMF increased the crop yield in spinach plants, reaching similar values to those obtained with the Mineral treatment. Thus, all doses had significant differences with the control except for Mineral 50.

In contrast, for lettuce plants, TMF treatments were not able to increase crop yield at any dose, while the mineral treatment (Mineral 75 and Mineral 100) improved crop yield in comparison with control, also performing better than the same dose of the TMF treatment. This result could be related to the high salinity of the ES-NC product. In fact, lettuce is known to be a salinity sensitive crop, so this could have limited the effects of TMF fertilisation on this crop. It is also important consider that the N supplied to the soil from the commercial ammonium sulphate is more available for the plants than those from the TMF mixture. Both factors might explain the low crop yield observed in the lettuces subjected to the TMF treatments. Despite these results, other studies have observed that the use of organo-mineral fertilisers can help reach optimum crop yield, nutrient uptake and quality in lettuce crops (Olaniyi, 2008), so more experiments with our TMF are probably needed to achieve more refined results.



**Figure 5.** Fresh weight (g) of spinach and lettuce plants grown under the TMF and mineral treatments at 3 different doses (50%, 75%, 100%). Bars represent mean values  $\pm$  SD. Different letters represent significant differences among treatments, according to Tukey test ( $p \leq 0.05$ ).

(ii) Nutrient content in plants

The main nutrient content in the TMF and Mineral treatments for both crops is shown in Table 4. In the case of the spinach, N content was significantly higher in plants grown with the Mineral treatments. Differences in P content were more subtle, finding only the TMF 50 different from the control. Regarding lettuce, similar results were found. The plant content of N was higher in Mineral treatments, while P content differences were smaller, obtaining the lowest value in Mineral 75 plants. In agreement with our results, several studies have verified the efficacy of ammonium sulphate as a N source for spinach, increasing crop yield (Gülser, 2005; Krężel and Kołota, 2010) and N content (Gülser, 2005). Additionally, in 2020, Machado et al. also demonstrated the effectiveness of fertilisation with ammonium sulphate when applied alongside with organic compost. In our case, the increase in crop yield and N content was only observed in plants with the commercial ammonium sulphate application (Mineral treatments), while those receiving the TMF treatments remained with no- or slight differences in comparison with the control.

Regarding the rest of macro- and micronutrients, highest doses of Mineral treatment also performed better in both crops. In the case of Ca, Mg, Fe, Zn, Cu and Na contents, plants with Mineral 100 treatment had the highest value, being significantly different to control. The rise in plant concentrations of N, Ca, and Mg could be attributed to the presence of nitrate in the rhizosphere, since the nutrient uptake can be stimulated when plants absorb nitrate through their roots (Jones, 2016). Thus, the increased availability of nitrate in Mineral treatments might have been responsible for the elevated levels of calcium and magnesium obtained in these plants. For the lettuce, results yield similar conclusions, but in this crop, Mineral 75 was the dosage that

performed better. In contrast, the values of macro- and micronutrients obtained in plants with the TMF treatments always remain equal or significantly lower than control ones.

**Table 4.** Main nutrients (% of dry matter) from the different treatments in spinach and lettuce (mean value  $\pm$  SD). Different letters represent significant differences among treatments, according to Tukey test ( $p \leq 0.05$ ).

Treatments	SPINACH		LETTUCE	
	N	P	N	P
CONTROL	3.43 $\pm$ 0.12 cd	0.45 $\pm$ 0.01 bc	1.36 $\pm$ 0.05 bc	0.35 $\pm$ 0.01 a
TMF 50	3.33 $\pm$ 0.13 de	0.58 $\pm$ 0.04 a	1.29 $\pm$ 0.01 c	0.33 $\pm$ 0.00 b
MINERAL 50	3.85 $\pm$ 0.05 b	0.49 $\pm$ 0.02 b	1.32 $\pm$ 0.05 bc	0.32 $\pm$ 0.00 b
TMF 75	3.13 $\pm$ 0.01 e	0.48 $\pm$ 0.02 b	1.41 $\pm$ 0.06 b	0.34 $\pm$ 0.00 ab
MINERAL 75	3.62 $\pm$ 0.08 bc	0.44 $\pm$ 0.05 bc	1.27 $\pm$ 0.01 c	0.30 $\pm$ 0.01 c
TMF 100	3.61 $\pm$ 0.05 bc	0.39 $\pm$ 0.01 c	1.40 $\pm$ 0.02 b	0.32 $\pm$ 0.01 b
MINERAL 100	4.51 $\pm$ 0.11 a	0.44 $\pm$ 0.01 bc	1.63 $\pm$ 0.03 a	0.32 $\pm$ 0.01 b

**Table 5.** Apparent N recovery (ANR) and N fertiliser replacement value (NFRV) of the different treatments in spinach and lettuce (mean value  $\pm$  SD). Different letters represent significant differences among treatments, according to Tukey test ( $p \leq 0.05$ ).

Treatments	SPINACH		LETTUCE	
	ANR	NFRV	ANR	NFRV
TMF 50	14.30 $\pm$ 7.29 b	0.98 $\pm$ 0.50	-20.81 $\pm$ 11.49 a	-2.13 $\pm$ 1.18
MINERAL 50	14.65 $\pm$ 6.63 b		9.76 $\pm$ 5.57 bc	
TMF 75	6.10 $\pm$ 2.57 a	0.55 $\pm$ 0.23	6.38 $\pm$ 3.26 b	1.04 $\pm$ 0.53
MINERAL 75	11.10 $\pm$ 2.51 ab		6.11 $\pm$ 3.80 b	
TMF 100	4.71 $\pm$ 1.21 a	0.67 $\pm$ 0.17	4.21 $\pm$ 1.35 b	0.23 $\pm$ 0.07
MINERAL 100	7.03 $\pm$ 1.27 ab		18.34 $\pm$ 1.28 c	

(iii) Nutrient content in soil

For spinach, results from the soil analysis showed no differences in terms of organic matter, total N, total P and total K between the treatments and the initial soil status (data not shown). Olsen P was significantly higher in initial soil but it showed no differences between treatments. Exchangeable K was higher in TMF treatments than in Mineral treatments at doses 75% and 100%, and non-different than the initial soil. This is probably due to the K input by the ES-NC. Lastly, N-NO<sub>3</sub><sup>-</sup> content after harvest was higher in Mineral treatments than TMF ones at all doses, being also higher in Mineral 100 than those at the beginning of the experiment. These results are consistent with those obtained in the field experiment with winter wheat crop (see section 2.1.3), where Mineral treatments also resulted in higher N-NO<sub>3</sub><sup>-</sup> residue in the soil.

(iv) ANR and NFRV

As shown in Table 5, NFRV for the TMF was 98%, 55% and 67% at doses 50%, 75% and 100% for spinach, respectively. These results demonstrated a high ability of the TMF to supply N to crops in comparison with the commercial fertiliser, specially at low doses. In the case of lettuce, values were 0%, 104% and 23%, respectively, at the same mentioned doses. NFRV values at TMF doses of 50% and 100% in lettuce were very low due to non-significant differences in terms of crop yield in comparison with the control plants. Ammonium sulphate seems to be a very reliable source of N for lettuce in terms of crop productivity, as El-Bassyouni (2016) concluded after comparing this mineral N fertiliser with a natural organic N source (organic manure). In this study, it was also concluded that the application of mineral-N sources resulted in higher N content in the plants, which is in accordance with the increment in N content detected in our spinaches and lettuces grown with the Mineral treatments.

### 2.2.4 Conclusion and recommendation

The combination of ES-AS and ES-NC as a TMF formulation was effective for spinach plants, increasing crop yield across all doses and having a NFRV, in comparison with the mineral fertilisation, of 98%, 55% and 67% at doses 50%, 75% and 100%, respectively. Also, results obtained from the N-NO<sub>3</sub><sup>-</sup> residue analysis showed that TMF application can reduce leaching risk in the soil, being an environmentally safer choice than commercial ammonium sulphate. Nevertheless, mineral fertilisation was able to increase N and micro-element content in both crops. Regarding lettuce crop, mineral fertilisation improved crop yield and nutrient plant content, while the fertilisation with TMF did not result in improvements in its cultivation. This is likely due to the high salinity of ES-NC, so the application of this TMF is not recommended for salinity-sensitive crops.

## 2.3. Potato field cultivation (Fertinagro, Spain, demo trial)

*For more information on this study, please contact the authors from Fertinagro: Ignasi Salaet ([ignasi.salaet@tervalis.com](mailto:ignasi.salaet@tervalis.com)), Herminia De La Varga ([herminia.delavarga@tervalis.com](mailto:herminia.delavarga@tervalis.com)), Begoña Arrufat ([bego.arrufat@tervalis.com](mailto:bego.arrufat@tervalis.com)) and José Antonio Rodríguez-Sánchez ([ja.rodriquez@tervalis.com](mailto:ja.rodriquez@tervalis.com)).*

### 2.3.1 Introduction

To date, in Spain, pig slurry is mainly used to fertilise extensive crops such as wheat and barley. It has a negative value for the farmer, because the value of pig slurry as a fertiliser is much lower than the costs of its disposal. Nevertheless, uses of the pig slurry are being studied and one option is to apply it on cash crops, since the economic margins of the system will increase. However, before applying a slurry in cash crops, a detailed study of the bottlenecks and opportunities for using it is necessary.

Fattening pig slurry is a poor fertiliser since it contains small percentages of NPK, as well as insoluble micronutrients. This means that pig slurry has to be applied to the field in large quantities to meet the needs of a particular crop, resulting in problems coming from the presence of antibiotics and substances containing heavy metals, which are potentially toxic to humans and the environment. The strategy is therefore to mitigate acute exposure as much as possible. For example, to mitigate the toxicity of a heavy metal, an adequate amount of high molecular weight humic substances can be incorporated into the slurry.

One of the roles of Fertinagro within the FERTIMANURE project is the implementation of its patented process (WO2019/106210) called Method For The Treatment Of Organic Animal Waste And Use Of The Thus Treated Product As A Fertiliser. This patented process can help to solve the shortcomings that pig slurry has by transforming it into an on-farm TMF, enriched with nutrients and biostimulants, reducing the toxic effects of heavy metals and antibiotics. The process of formulating and producing the TMF is reported in D3.6 '*Processes and technologies specification and set up to produce on-farm TMFs from animal manure*'. In this section of D4.6, results of TMF assessment in potato cultivation are reported. Potato was chosen as a test crop due to the regional situation and Spanish governmental efforts to make potatoes a high-value crop (capable to generate 9,000€/ha) in the region. In addition, this crop consumes many nutritional units, which makes it suitable for the experiment.

To use the pig slurry as fertiliser, one of the options is to enrich the slurry with stabilised N (urea with urease inhibitor) that allows the application of the TMF with all N before sowing, avoiding the addition of it as cover fertiliser. Next, Fertinagro has proven in its patent that the application of high molecular weight humic substances at a rate of 10 g/g of metal can reduce by 40% to 50% the number of water-soluble metals in the TMF. To mitigate the toxicity of heavy metal, an adequate amount of high molecular weight humic substances has been incorporated into the TMF. Finally, biostimulants can be added to improve the TMF.

Along this document, the experiments carried out with potato crops, in 2021, 2022 and 2023, are going to be explained. To evaluate the TMF produced, the experiments have been carried out in the same area, but in different plots. Moreover, different times and methods of application were tested.

### 2.3.2 Methodology

For the production of the TMF from pig slurry, Fertinagro has implemented the process described in the patent WO2019/106210. One big advantage of this patent is that the properties of the TMF can be adjusted in real-time according to the fertilisation program and the stage of the crop development. The TMF was prepared by mixing the pig slurry with urea, ammonium sulphate, potassium sulphate, diammonium phosphate, humic acids and amino acids in different proportions to complete the deficiencies presented by the slurry at each moment, to cover the requirements of the potato.

Three trials were conducted in three years (2021, 2022 and 2023) to test the application of TMF at different times (basal or top dressing) and with different application methods (direct application from the slurry distributor or through the irrigation water). The trials were carried out in the same area but in different plots each year. In all plots, the same tasks were carried out, except for the basal dressing and cover dressing. Before sowing, 8 metric tonnes per hectare of sheep manure were applied as fertiliser. Likewise, the control plots of each year were fertilised with 1800 kg/ha of mineral fertiliser (NPK) as basal dressing (Table 6). The basal dressing application on the TMF plots was as follows for each year (Table 6):

- In 2021, a single application of TMF was made at a rate of 20 t/ha. This application was made before sowing. The TMF was made directly at the slurry distributor and was applied to the plot just before sowing.
- In 2022, the same mineral fertiliser was used as basal dressing as in the control plot, but with a 33% reduction, only 1200 kg/ha.
- In 2023, no basal dressing was made on the TMF plot.

**Table 6.** Fertilisation applied in basal and top dressing.

Year	Basal Dressing				Top Dressing			
	Control		TMF		Control		TMF	
	Fertiliser	Kg/ha	Fertiliser	Kg/ha	Fertiliser	Kg/ha	Fertiliser	Kg/ha
2021	(NPK) 6.8.18	1800	TMF	20000	(NPK) 30.0.0	200	-	-
2022	(NPK) 8.5.15	1800	(NPK) 8.5.15	1200	(NPK) 26.46.0	375	TMF	17000
2023	-	-	-	-	(NPK) 30.0.0	300	TMF	14000

As for the top dressing of the control plots, a mineral fertiliser (NPK) was applied in different amounts each year depending on the characteristics of the soil and the fertiliser itself (Table 6). As for the TMF plots, the applications of top dressing fertiliser were as follows:

- In 2021, no cover dressing fertiliser was applied since a sufficient amount of TMF had been applied in the basal dressing to cover the requirements of the potato over the entire crop.
- In 2022, 17 t/ha of TMF were applied. This TMF was made, without prior transformation, in the slurry distributor and applied slowly in the irrigation water.
- In 2023, 14 t/ha of TMF were applied. For the preparation of this TMF, the slurry was processed (milled and filtered) to reduce its particle size and thus achieve a better distribution of the TMF throughout the plot. The application was made by applying the TMF slowly in the irrigation water.

The three trials the amount of NPK units provided to the crop was close to the requirements of this crop for an expected production of 35 t/ha (Table 7).

**Table 7.** Amount of NPK (kg/ha) required to produce 35 t of potato/ha.

Year	2021			2022			2023		
Treatment	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
Requirements	220	40	310	220	40	310	220	40	310
Control	202	92	290	226	90	270	180	90	270
TMF	225	95	280	211	126	339	194	93	263

### 2.3.3 Results and discussion

#### (i) Use of pig slurry for TMF fertiliser in potato

In the first trial, to test if pig slurry could be used for TMF fertiliser in potato crops, no differences were found in the quantity (Table 8) of potatoes produced between both plots, with mineral fertiliser or pig slurry-based TMF.

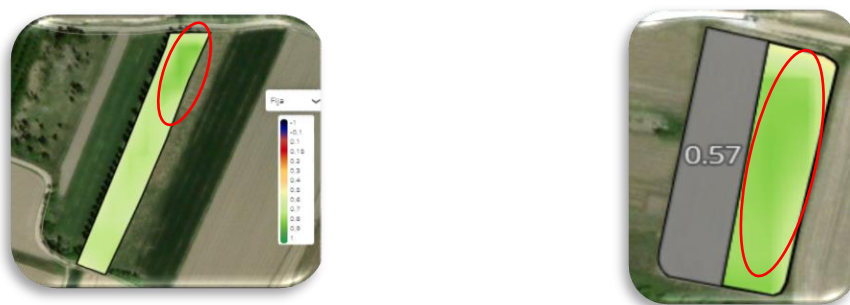
**Table 8.** Production of potatoes (t/ha).

Year	Control	TMF	% Reduction
2021	38.2	37.1	2.9%
2022	35.9	34.5	3.9%
2023	45.3	43.3	4.4%

The harvested potatoes also showed no physical or organoleptic differences between the two treatments, achieving the same quality. Thus, we can confirm that it is feasible to apply a pig slurry-based TMF on potato crops. Applying this TMF as a basal dressing greatly reduces fertiliser costs with respect to mineral fertiliser.

#### (ii) Application of the TMF through irrigation

After the above confirmation, in the second trial, the application of TMF as a top dressing fertiliser was evaluated. Since TMF cannot be applied as top dressing with the slurry distributor, the test of its application was directly done in irrigation water. In this case, flood irrigation. There were no significant differences observed in the production (Table 8) or quality of the potatoes obtained in this trial. However, analysing the chlorophyll index of the crop one month after the application of TMF, it was observed (Figure 6) that the areas close to the application point were greener than those farther away. This could be because, as the unprocessed slurry was used for the preparation of the TMF, the solid particles of the slurry were too large and the water could not carry them to the end of the plot.



**Figure 6.** Chlorophyll index one month after the application of TMF. Trials 2022 and 2023.

To try to avoid this poor distribution of the TMF along the plot, a third trial (2023) was carried out. In order to improve the distribution of TMF, and in addition to be able to use this product in fertigation, the slurry was transformed (milled and filtered) to reduce its particle size. In the same time, this process results in a more stable TMF, with a longer shelf life.

(iii) Reduction of fertiliser when incorporating the TMF in the program

In the 2023 trial, testing included the option of omitting basal dressing on the plot where the TMF was applied. In this case, a small reduction in the amount of potatoes harvested was observed (Table 8), but this is compensated by the savings achieved by not applying the basal dressing.

2.3.4 Conclusion and recommendation

With the obtained results, after three-year tests, it could be concluded that: (i) TMF made from pig slurry can be used in fertilisation of potatoes; (ii) the treatment of the TMF has been enhanced to facilitate its application through irrigation; (iii) transforming the slurry (to mild and filtering it) before preparing the TMF is crucial for its **fertigation** application; (iv) the obtained yield and quality of the crop are equivalent to those harvested with conventional fertilisers. In addition, by using this TMF pig slurry fertiliser, application of a basal dressing could be avoided, achieving equal incomes but reducing the fertilising units applied to the field.



### 3. Ammonium salts (ammonium sulphate, ammonium nitrate and ammonium water)

#### 3.1. Potato - maize field cultivation (UGent, Belgium): BE-AN and BE-AS

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

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##### 3.1.1 Introduction

To validate the agricultural and environmental performance of ammonium sulphate (BE-AS) and/or ammonium nitrate (BE-AN) produced by the Belgian pilot plant, Ghent University, in collaboration with Inagro vzw, conducted a full-scale field trial. The primary objective of this field trial was to assess the short-term N supply to the crops from BE-AS and BE-AN. To evaluate the NFRV of these biobased products in comparison to synthetic mineral N fertilisers, the field trial was carried out on potatoes in 2021 and maize in 2022.

##### 3.1.2 Methodology

The field trial took place at an experimental farm located in Wingene, Belgium. The primary objectives were to determine crop yield, assess N fertiliser value and risk for nitrate leaching. Therefore, soil samples were collected both before fertilisation and after harvest at depths of 0-90 cm to evaluate mineral N levels, whereas crop yield was determined at harvest time. The N application rates were set at three levels: 40%, 70%, and 100% of the recommended N rate, taking into account the specific demands of maize (150 kg N/ha, 45 kg P/ha, 100 kg K/ha) or potatoes (140 kg N/ha, 118 kg P/ha, K: 323 kg K/ha) and the existing soil N status. To assess the NFRV of each biobased treatment, a comparison was made by calculating the ANR of each treatment in relation to synthetic mineral N fertiliser (NPK mineral). Each treatment was replicated four times, except for the control and PK treatments, which had eight replicates, resulting in a total of 64 plots. The application of BBFs (Table 9) was carried out using a specialised machine equipped with both a vacuum pump and injector coulters designed for the precise application of organic liquid fertilisers with high viscosity. Additionally, a hose pump with a tube system was utilised for the application of pure and liquid fertilisers (Figure 7). This dual-method approach was employed to minimize the release of ammonia into the atmosphere, reducing ammonia volatilisation during the fertilisation process. The results were analysed using one-way ANOVA ( $p < 0.05$ ) and Tukey’s honestly significant difference (HSD). Data were interpolated using the software IBM SPSS 26.

**Table 9.** Chemical composition of BBFs used in Belgian field trials.

Product	TN (%)	TC (%)	pH	EC (mS/cm)
Ammonium Nitrate (BE-AN)	7.58±0.19	0.027±0.00	5.17	303.3
Ammonium Sulphate (BE-AS)	4.18±0.03	0.068±0.01	5.24	198.8



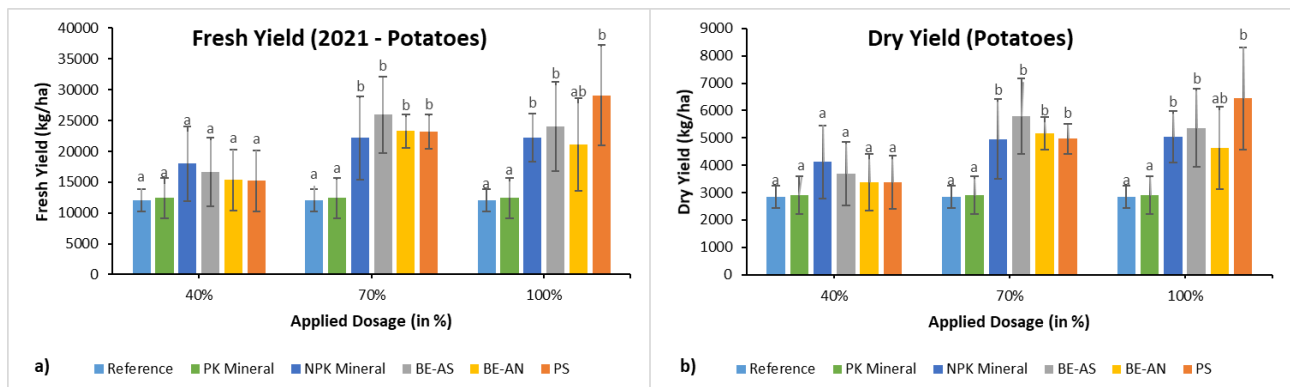


**Figure 7.** Product application and incorporation at Wingene, BE as a part of 2021 Belgian field trials for potatoes.

### 3.1.3 Results and discussion

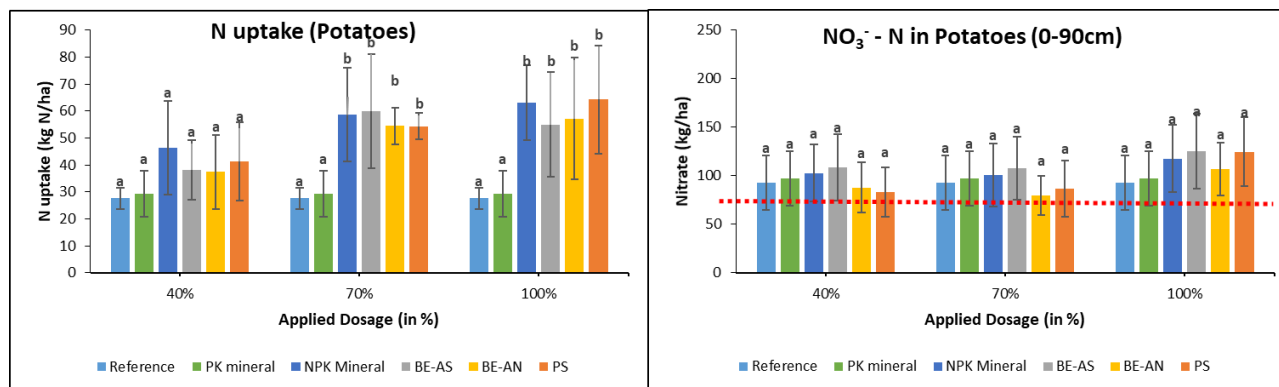
#### (i) 2021 Field trial with potatoes

The trial experienced high variability due to heavy rain, making it challenging to see the difference between the treatments. BE-AN and BE-AS, when compared to pig slurry 100% dosage, performed noticeably inferiorly, primarily due to nitrate leaching in the initial stages and organic N in pig slurry later benefiting crop growth. Unfertilised control and PK mineral underperformed BE-AS. At 40% of recommended dosage, crop yield was significantly lower than at 70% or 100%, likely due to a N-rich soil equalising nutrient uptake. BE-AN showed a lower yield on average but no significant differences as compared to BE-AS and NPK mineral (Figure 8).



**Figure 8.** Mean fresh yields and dry yield (tubers harvested in kg/ha) for tested treatments at different applied dosages (percentage of maximum applied dosage of 140 kg total N/ha) in 2021 Belgian field trials. Reference (unfertilised control) and (N)PK minerals are used as standards and are similar for all dosages. Standard deviation is represented by error bars (n=8 for reference and PK mineral, n=4 for all treatments). If significant differences exist, the lowercase letters "a, b, c, d" indicate the statistically significant differences (Tukey HSD  $P < 0.05$ ) for different treatments at a particular N dose.

Belgium sets a limit of 85 kg/ha for nitrate residues in the 0-90 cm soil layer during the winter period (1<sup>st</sup> – 15<sup>th</sup> November). Nitrate residues did not significantly increase leaching risk for BBFs compared to NPK mineral, but overall residues were high (i.e. higher than the imposed legal limit). High nitrate residue values for all treatments indicate significant leaching before crop uptake. This is common in potatoes due to their limited N absorption capacity. Rainfall inversely affected nitrate residue in the soil (Figure 9).



**Figure 9.** Mean N uptake by plants and nitrate-N (NO<sub>3</sub>-N) (in kg/ha) in the soil profile (0-90 cm) (percentage of maximum applied dosage of 140 kg N total /ha). Reference (unfertilised control) and (N)PK minerals are used as standards and are similar for all dosages. Standard deviation is represented by error bars (n=8 for reference and PK mineral, n=4 for all other treatments). If significant differences exist, the lowercase letters "a, b, c, d" indicate the statistically significant differences (Tukey HSD P < 0.05) for different treatments at a particular N dose.

The replaceability potential of BE-AS was highlighted by a high NFRV value at 70%. PS at 100% showed a relatively high NFRV, with pronounced differences between doses. Notably, BE-AS at 70% exhibited an NFRV exceeding 100, indicating its potential as an effective replacement for conventional NPK mineral. Overall, the 1<sup>st</sup> year of field trials suggests that BBFs, particularly BE-AS, has a potential to replace conventional mineral fertilisers effectively (Table 10).

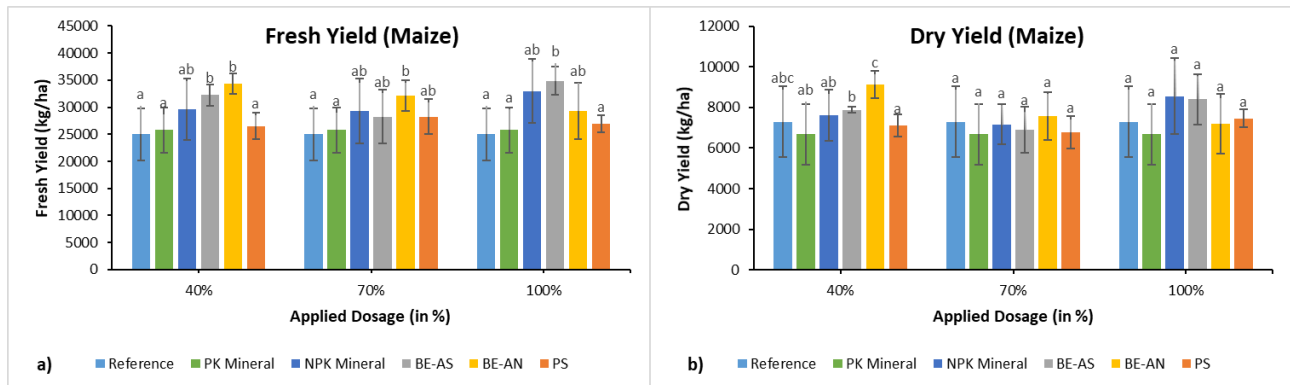
**Table 10** NFRV and ANR average and standard deviation values for BBFs in comparison to synthetic N fertiliser at different treatment dosages for 2021 Belgian field trials (potatoes). No significant difference.

Product	ANR	NFRV (%)
NPK Mineral 40	0.3 ± 0.3	
NPK Mineral 70	0.3 ± 0.2	
NPK Mineral 100	0.2 ± 0.1	
Pig slurry 40	0.2 ± 0.3	81 ± 99
Pig slurry 70	0.3 ± 0.1	99 ± 19
Pig slurry 100	0.3 ± 0.2	120 ± 69
Ammonium nitrate (BE-AN) 40	0.1 ± 0.2	47 ± 80
Ammonium nitrate (BE-AN) 70	0.3 ± 0.1	85 ± 22
Ammonium nitrate (BE-AN) 100	0.2 ± 0.2	82 ± 66
Ammonium sulphate (BE-AS) 40	0.2 ± 0.2	51 ± 64
Ammonium sulphate (BE-AS) 70	0.3 ± 0.2	104 ± 71
Ammonium sulphate (BE-AS) 100	0.2 ± 0.1	76 ± 57

(ii) 2022 Field trial with maize

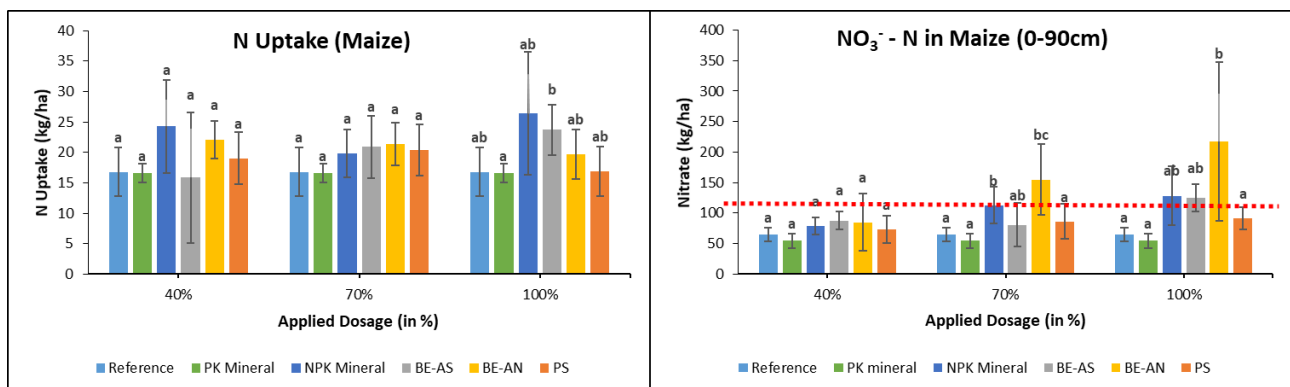
In 2022, field trial in Belgium was scheduled for maize from June to October. However, unforeseen challenges arose, including exceptionally high temperatures in July, reaching around 40°C in the fields, followed by drought conditions in August. To mitigate potential yield losses due to drought, an emergency harvest was initiated. Consequently, no significant differences were observed among treatments or dosages (Figure 10).

On average, both BE-AN and BE-AS outperformed NPK mineral, with BE-AN at 70% dosage and BE-AS at 100% dosage showing better performance, although the differences were not statistically significant ( $p > 0.05$ ). The resilient performance and comparative effectiveness of the Belgian BBFs, particularly in challenging climatic conditions, provide valuable insights into their potential as sustainable alternatives in modern agricultural practices.



**Figure 10.** Mean fresh yields and dry yield (maize harvested in kg/ha) for tested treatments at different applied dosages (percentage of maximum applied dosage of 150 kg total N/ha) in 2022 Belgian field trials. Reference (unfertilised control) and (N)PK minerals are used as standards and are similar for all dosages. Standard deviation is represented by error bars ( $n=8$  for reference and PK mineral,  $n=4$  for all treatments). If significant differences exist, the lowercase letters "a, b, c, d" indicate the statistically significant differences (Tukey HSD  $P < 0.05$ ) for different treatments at a particular N dose.

Continuing the pattern observed in yield, N uptake similarly displayed no significant differences among treatments or dosages (Figure 11). However, high standard deviations were evident, particularly in the case of BE-AS at 40% and NPK mineral at 100%. This variance may be attributed to an emergency harvest, where the supplied N to the maize was not fully utilised for plant growth. Furthermore, the accelerated N uptake, especially in maize, occurs during later reproductive stages (after 80–100 days), underscoring the impact of early harvest.



**Figure 11.** Mean N uptake by plants and nitrate-N ( $\text{NO}_3\text{-N}$ ) (in kg/ha) in the soil profile (0-90 cm) (percentage of maximum applied dosage of 150 kg N total/ha). Reference (unfertilised control) and (N)PK minerals are used as standards and are similar for all dosages in 2022. Standard deviation is represented by error bars ( $n=8$  for reference and PK mineral,  $n=4$  for all other treatments). If significant differences exist, the lowercase letters "a, b, c, d" indicate the statistically significant differences (Tukey HSD  $P < 0.05$ ) for different treatments at a particular N dose.

The NFRV exhibited promising potential for BE-AS and BE-AN in comparison to NPK mineral (Table 11). Nevertheless, no significant differences were noted in this case either. Despite BE-AS and BE-AN displaying

NFRV values of >100% at a 70% N dosage, the high standard deviations pose challenges in interpreting the results. In 2022, the Flemish government redefined the soil nitrate residue limit in the winter period based on the location of the field to 120 kg/ha. However, similar to the findings in 2021, nitrate residues did not significantly elevate leaching risk for BBFs compared to NPK mineral, although overall residues remained high in all cases (Figure 11). This is primarily due to the effect of emergency harvest and lower uptake, which resulted in high residues across all treatments.

**Table 11.** ANR and NFRV average and standard deviation values for BBFs in comparison to synthetic N fertiliser at different treatment dosages for 2022 Belgian field trials (Maize). No significant difference.

Product	ANR	NFRV (%)
NPK Mineral 40	0.13 ± 0.13	-
NPK Mineral 70	0.03 ± 0.04	-
NPK Mineral 100	0.07 ± 0.07	-
Pig slurry 40	0.05 ± 0.08	37 ± 63
Pig slurry 70	0.04 ± 0.05	135 ± 148
Pig slurry 100	0.00 ± 0.03	4 ± 48
Ammonium nitrate (BE-AN) 40	0.07 ± 0.04	54 ± 30
Ammonium nitrate (BE-AN) 70	0.04 ± 0.03	113 ± 82
Ammonium nitrate (BE-AN) 100	0.02 ± 0.02	24 ± 31
Ammonium sulphate (BE-AS) 40	-0.01 ± 0.17	-8 ± 135
Ammonium sulphate (BE-AS) 70	0.04 ± 0.05	129 ± 155
Ammonium sulphate (BE-AS) 100	0.05 ± 0.03	71 ± 40

### 3.1.4 Conclusion and recommendation

Based on the findings from the two-year FERTIMANURE field trials conducted in Belgium, the BBFs under examination demonstrated no significant differences in terms of fresh and dry biomass yields when compared to the NPK mineral. Similar N uptake levels were observed for both BE-AS and BE-AN in comparison to the CAN treatment. These results indicate a potential for the tested BE-AS and BE-AN as viable replacements for synthetic mineral N fertilisers. However, it is worth noting that while there were no significant differences in post-harvest soil mineral N residues between the BBFs and the NPK mineral, the presence of elevated soil nitrate levels could signal an increased risk of N leaching for all tested treatments. This highlights the importance of enhanced fertiliser management practices in the upcoming seasons to mitigate potential environmental impacts. Furthermore, it is essential to consider that varying climatic conditions played a pivotal role in both years of the study, with heavy rainfall in 2021 and drought conditions in 2022. These climatic variations had an impact on overall biomass production over time, emphasising the need for adaptable agricultural strategies to account for changing weather patterns.

## 3.2. Lettuce pot cultivation (UGhent, Belgium): BE-AW

This study was published as “Shrivastava, V., Sigurnjak, I., Edayilam, N., & Meers, E. (2023). Ammonia water as a biobased fertiliser: Evaluating agronomic and environmental performance for *Lactuca sativa* compared to synthetic fertilisers. *Biocatalysis and Agricultural Biotechnology*, 102907.”

### 3.2.1 Introduction

To assess the effectiveness of ammonia water (BE-AW) as a BBF as well as a potential replacement to synthetic mineral fertiliser, Ghent University tested BE-AW in a lettuce pot experiment. To mitigate the issues regarding volatile nature of BE-AW (due to high pH) and social concerns regarding its smell, the BE-AW was tested at two different pH levels: i) ammonia water at initial pH (BE-AW<sub>phini</sub>) and ii) ammonia water at pH 5 (BE-AW<sub>ph5</sub>). The performance at both pHs were tested against synthetic mineral fertilisers and pig slurry. This section includes details on the biomass performance, N uptake and NFRV at both tested pH of BE-AW.

### 3.2.2 Methodology

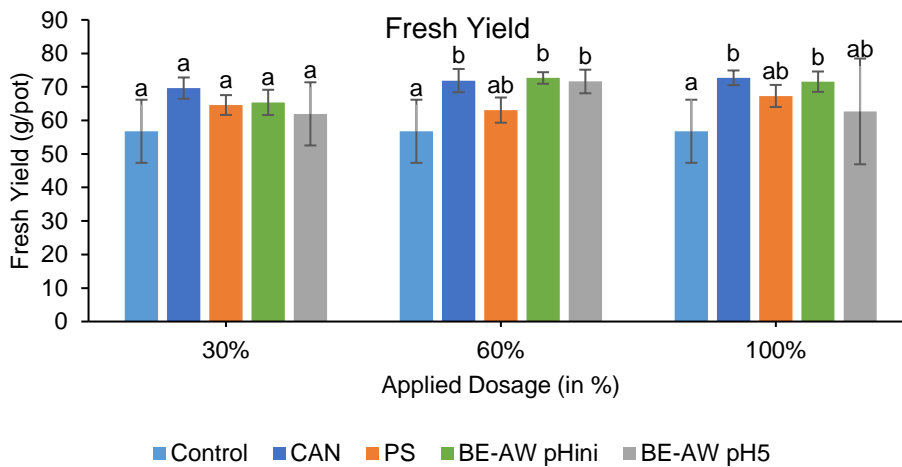
The test crop, lettuce (variety: *Lactuca sativa* L., cv. Cosmopolia; crop cycle: 1.5 - 2 months), has nutritional requirements of 200 kg N ha<sup>-1</sup>, 125 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, and 240 kg K<sub>2</sub>O ha<sup>-1</sup>. The BE-AW was added at two different pH levels (the baseline pH of 11.2 and pH 5 created by adding H<sub>2</sub>SO<sub>4</sub>) at three different dosages (30%, 60%, and 100%), and the results were compared. The BE-AW had the following properties: 13% TN, 1.03% TC, 130 g NH<sub>4</sub>-N/kg, 0.10 g NO<sub>3</sub>-N/kg, pH 11.2 and EC (mS/cm) of 312.8. Lettuce seedlings from Inagro vzw were used for the experiment. To conduct the pot trials, the soil was taken from a farm in Wingene, Belgium, with the following properties: 0.77 g TN/kg, 0.97% TC, 1.08 g TP/kg, 1.12 g TK/kg, pH (KCl) of 5.86 and EC (μS/cm) of 60. The soil-sand mixture weighed 1.65 kg in each pot, which measured 18 cm in height and 12.6 cm in top diameter. One week before setting up the experiment, a pre-incubation was done at 35% WFPS to ensure the activation of microorganisms present in the soil. The experimental setup included quadruplicate pots with treatments of BE-AW (two pH), one synthetic reference (CAN), raw pig slurry (PS), and an unfertilised control. The application rates were estimated based on the nutrients needed for the growth of lettuce. Synthetic fertilisation was carried out using potassium sulphate (PAT; 30% K<sub>2</sub>O, 10% MgO, and 42.5% SO<sub>3</sub>) and triple super phosphate (TSP; 46% P<sub>2</sub>O<sub>5</sub>) to support crop growth and ensure an equivalent application of P and K in all treatments. The seedlings were moved into each pot after fertilisation (Figure 12), and 80 - 100 mL of demineralised water was added to provide each pot with a 60% water-holding capacity. Three times a week, or as needed, watering was done, and the location of pots was alternated twice a week. All other details of the experimental design are reported in Shrivastava et al. (2023).



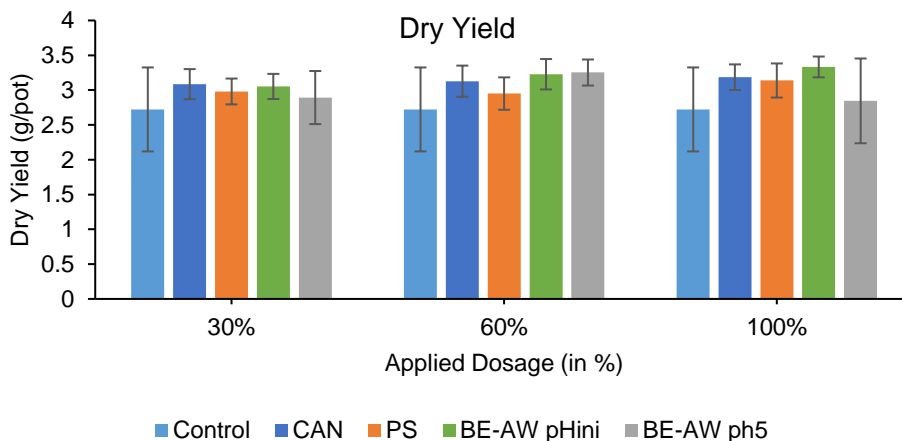
**Figure 12.** Experimental setup for the lettuce pot trials testing BE-AW at three N incremental doses.

### 3.2.3 Results and discussion

When compared to CAN 60%, lettuce fertilised with BE-AW<sub>phini</sub> 60% and BE-AW<sub>ph5</sub> 60% showed similar plant FW (71.8 g, 72.6 g, and 71.61 g, respectively), however, BE-AW<sub>ph5</sub> 100% treatment resulted on average in lower FW (62.7 g) than the other treatments (70.1 g). It was observed that BE-AW<sub>phini</sub> 100% (71.6 g) provided an average better yield in plant FW when compared to PS 100% (67.3 g) at a 100% dose (Figure 13). In terms of DM yield, it was shown that BE-AW<sub>ph5</sub> 100% plants produced nearly equal DM (2.8 g, respectively) to the unfertilised control (2.7 g). At either dose, there were no significant differences found between BE-AW<sub>phini</sub> and CAN (Figure 14). As anticipated, compared to the unfertilised plants, all fertilised treatments (apart from BE-AW<sub>ph5</sub> 100%) displayed a significantly greater crop total N. There were no discernible differences between applied dosages. Regarding P and K concentrations, no observable variations between the treatments were found.



**Figure 13.** Mean fresh yield (lettuce harvested in g/pot) for tested treatments at different applied dosages. Control is used as a standard and is similar for all dosages. The standard deviation is represented by error bars (n=4 for all treatments). If significant differences exist, the lowercase letters "a, b, c, d" indicate the statistically significant differences (Tukey HSD P < 0.05) for different treatments at a particular N dose.

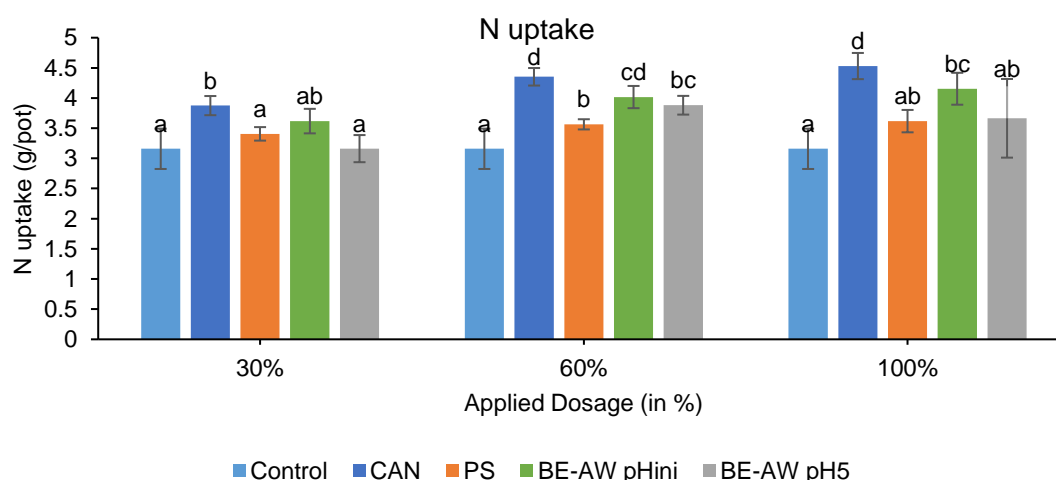


**Figure 14.** Mean dry yield (lettuce harvested in g/pot) for tested treatments at different applied dosages. Control is used as a standard and is similar for all dosages. The standard deviation is represented by error bars (n=4 for all treatments). No significant difference.



Since all of the total N in these products is in the form that plants can use, their performance at all three doses is comparable to that of CAN. Additionally, BE-AW<sub>pH5</sub> and BE-AW<sub>pHini</sub> were the treatments in this study that showed significant changes in FW based on dosage applied, with plants receiving a 60% N dose having a much greater FW than those receiving a 30% or 100% N dose. For BE-AW<sub>pHini</sub>, this result could be explained by the fact that the plants with 60% dosage already received enough N required in comparison to doses at 30% and 100%. For BE-AW<sub>pH5</sub>, the 100% dose showed significantly lower values as compared to the 60% dose. This might be due to the interference of the lower pH of BE-AW with soil properties at higher dosages.

The ANR and NFRV are used to assess BBFs performance (Table 12). NFRV, which measures the substitutability of a BBF versus a synthetic fertiliser, is the amount of synthetic fertiliser saved when employing a bio-based alternative while achieving the same crop output. NFRV was below 100% for all three dosages for BE-AW at both pHs. Although the volatility of BE-AW raises some questions about N losses, the very small amount of product applied along with the quick incorporation of the product into the soil, the soil's insulating property, and the controlled moisture conditions of the experiment caused negligible NH<sub>3</sub> volatilisation, ensuring ANR results that were comparable to the treatment with synthetic fertiliser. It is important to keep in mind that the data have substantial standard deviations, particularly with BE-AW<sub>pH5</sub> at 100% dosage. Because of the non-homogeneous N uptake (Figure 15) and yield displayed by the replicates of BE-AW<sub>pH5</sub> at 100%, the NFRV results within the treatment were variable. Further information regarding the impact of the tested products on plant growth came from the analysis of soil characteristics following harvest. As was previously mentioned, the alkaline/acidic pH of BE-AW could cause it to volatilize after being applied to the medium. N volatility can be reduced by using proper fertiliser management practices, although caution must be used when using concentrated volatile N products on field size. Although the BE-AW naturally have high EC values, their higher N content required that lesser amounts of each product be added to each pot. Therefore, no discernible effects on crop growth were found.



**Figure 15.** Mean N-uptake (in g/pot) after harvest for tested treatments. Control is used as standard and is similar for all dosages (n=4 for all treatments). If significant differences exist, the lowercase letters "a, b, c, d" indicate the statistically significant differences (Tukey HSD P < 0.05) for different treatments at a particular N dose.

**Table 12.** ANR and NFRV values for BBFs in comparison to synthetic N fertiliser at different treatment dosages.

Product	ANR	NFRV (%)
CAN 30%	1.1 ± 0.2c	-
CAN 60%	0.9 ± 0.1c	-
CAN 100%	0.6 ± 0.1b	-
PM 30%	0.4 ± 0.2ab	34 ± 15ab
PM 60%	0.3 ± 0.1a	33 ± 7a



PM 100%	0.2 ± 0.1a	33 ± 13a
AW pHini 30%	0.7 ± 0.3bc	64 ± 28b
AW pHini 60%	0.7 ± 0.1b	71 ± 15b
AW pHini 100%	0.5 ± 0.1ab	72 ± 19a
AW pH5 30%	0.0 ± 0.3a	0.39 ± 31a
AW pH5 60%	0.6 ± 0.1b	60 ± 13b
AW pH5 100%	0.2 ± 0.3a	36 ± 47a

CAN: calcium ammonium nitrate; PM: pig manure; AW: ammonium water. If significant differences exist, the lowercase letters "a, b, c, d" indicate the statistically significant differences (Tukey HSD  $P < 0.05$ ) for different treatments at a particular N dose.

### 3.2.4 Conclusion and recommendation

Overall, the current study provided useful information on BBFs like BE-AW while also posing new research topics regarding pH-based plant optimisation. The comprehensive field validation of the BE-AW under various environmental circumstances, with different types of soil and crops, is prioritised to collect data on their agronomic characteristics and environmental effects. In comparison to the synthetic N fertiliser (CAN), the effect of BBFs on lettuce growth showed improved fresh yield and nutrient concentration in the case of BE-AW fertilisation at both pHs for 30% and 60% dosages. However, BE-AW<sub>pH5</sub> performed poorly when dosed at 100% compared to its synthetic counterpart. The NFRV results showed that BE-AW<sub>pHini</sub> has a similar or higher potential for N replacement compared to synthetic CAN.

## 3.3. Grass and maize pot and field cultivation (WENR, the Netherlands, demo trial): NL-AS

This study was published as Rietra, René, Kimo van Dijk, and Oscar Schoumans (2024). "Environmental Effects of Using Ammonium Sulfate from Animal Manure Scrubbing Technology as Fertilizer" *Applied Sciences* 14, no. 12: 4998. <https://doi.org/10.3390/app14124998>

### 3.3.1 Introduction

The Arjan Prinsen Farm (APF), a dairy farm in the Netherlands, is home to a pilot plant which produces RENURE products from dairy manure. One of these products is ammonium sulphate (NL-AS), which is produced by first digesting the dairy slurry, separating it into the liquid fraction, and applying stripper/scrubber technology to the liquid fraction. In the stripper, part of the ammonium in the solution is transferred as ammonia to the air. In the scrubber, the ammonia is washed from the air into a sulphuric acid solution, resulting in the production of ammonium sulphate (NL-AS). The release of ammonia from the digestate is stimulated by heating the digestate or by increasing the pH.

Three main trials were performed to test the effectiveness of NL-AS as a N fertiliser: i) a pot experiment of maize and grass testing both yield and environmental emissions, ii) a field demonstration trial of maize and grass focussed on yield (2021), and iii) a field demonstration trial of grass with tests of both yield and environmental emissions. Each of the trials compared NL-AS to a combination of calcium ammonium nitrate (CAN) plus additional S in the form of gypsum, which was applied so that the total S dosage was equal to the NL-AS treatments: 328-478 kg S ha<sup>-1</sup> depending on soil and crop. The pot experiment was designed to test the NFRV. All trials were carried out on both clay and sandy soils. The method of application was injection to approximately 5 cm soil depth, while the application method of CAN was broadcast spreading, as it is a granular fertiliser. Emissions of ammonia, N<sub>2</sub>O, and CH<sub>4</sub> were also tested during the pot trial of 2021, and N<sub>2</sub>O emissions were tested during the field trial of 2022. The methods and results of this part are detailed in section **Error! Reference source not found.** Environmental monitoring campaigns.



### 3.3.2 Methodology

#### (i) Pot experiment, 2021

The pot experiment was set up to determine the NFRV of NL-AS (6%N, 7%S) for maize (*Zea mays* L.) and grass (*Lolium perenne* L.) on two soil types (sand and clay, both from Wageningen, the Netherlands; 51 58 N, 5 40 E). Furthermore, 5 rates of fertiliser were used based on recommendations determined by soil analysis made by the laboratory Eurofins, which offers fertilisation advice services to farmers. Eurofins recommended a N fertilisation rate of 120 kg N ha<sup>-1</sup> for grass and 165 kg N ha<sup>-1</sup> for maize (Figure 16). Five fertilisation rates were used in the pot experiment: 0%, 50%, 75%, 100%, and 125% of Eurofins' advice. CAN (26%N) was tested at all rates of fertiliser, and NL-AS was tested at 50% and 75% to focus on the fertiliser levels at which a fertiliser response is expected. Gypsum (24%S) was added to the CAN treatments to make the S dosage equal to that of NL-AS treatments. The trial was performed in triplicate, which resulted in 84 pots. Both fertilisers were applied via low-emission methods respective to their type: CAN pellets via broadcast application and NL-AS via injection. Soils were maintained at 60% water holding capacity. The grass was cut at 5 cm above the soil surface 23, 63 and 113 days after the fertilisation. Maize was cut at 3 cm above the soil 41 days after fertilisation. The fresh weight of maize and each grass cut was determined, and dry matter was determined after drying for 48 h at 70 °C. After the cuts, the N content of dry matter was determined, and soil mineral N was determined after removing quartz sand and plant roots. A more complete description of methodology can be found in *Rietra et al., 2024*.



**Figure 16.** Three grass pots on sandy soil from pot experiment shortly before harvest. Left: control no nitrogen fertilizer. Middle: CAN-75 treatment. Right: BBF-75 treatment.

#### (ii) Field trials 2021 and 2022

Three field trials were performed: maize 2021, grass 2021, and grass 2022. Each were prepared in spring of the corresponding year on clay and sand fields in Wageningen, the Netherlands (51 58 N, 5 40 E). Fertilisation advice was determined by Eurofins Agro and mineral N was measured prior to the experiment. Each field had 6 plots: 3 blocks and 2 randomised fertiliser treatments per block. The fertiliser treatments were NL-AS (6%N, 7%S) and S containing CAN- (Triferto, the Netherlands; Novasul 23%N, 7%S), and gypsum (Triferto, the Netherlands; gypsum, 24%S) were added to the CAN+S treatment to make the total S dosage equal to the NL-AS treatment. The NL-AS used in 2022 was slightly more concentrated, with 7%N and 8%S. In the grass 2022 experiment, one additional control plot was added to each field. Each plot was 10 m x 9 m. Weed control was applied. No irrigation was used as there was sufficient precipitation during the experiment. The soil used for maize was tilled in spring and was seeded with guidance by GPS. The grasslands were existing grasslands used by farmers with swards dominated by *Lolium perenne* L. Maize was harvested in September of 2021. The fresh yield was determined per two rows of maize, and a random sample was taken for dry matter content and chemical analysis. After cutting the maize, all plots were sampled in November 2021 for soil mineral N. Soil organic matter was also determined to calculate the soil density. In 2021, the first grass cut was in early June, and there was no second cut due to a mistake in the second fertilisation. In 2022, there were two cuts (May and June), after which the experiment was stopped because sulphur is only added in the first two fertilisations in Dutch practice. For each of these harvests, the fresh matter was weighed directly and a random

sample was taken to determine dry matter content. Soil mineral N was only measured following the conclusion of the 2022 experiment. A more complete description of methodology can be found in *Rietra et al., 2024*.

(iii) Statistics

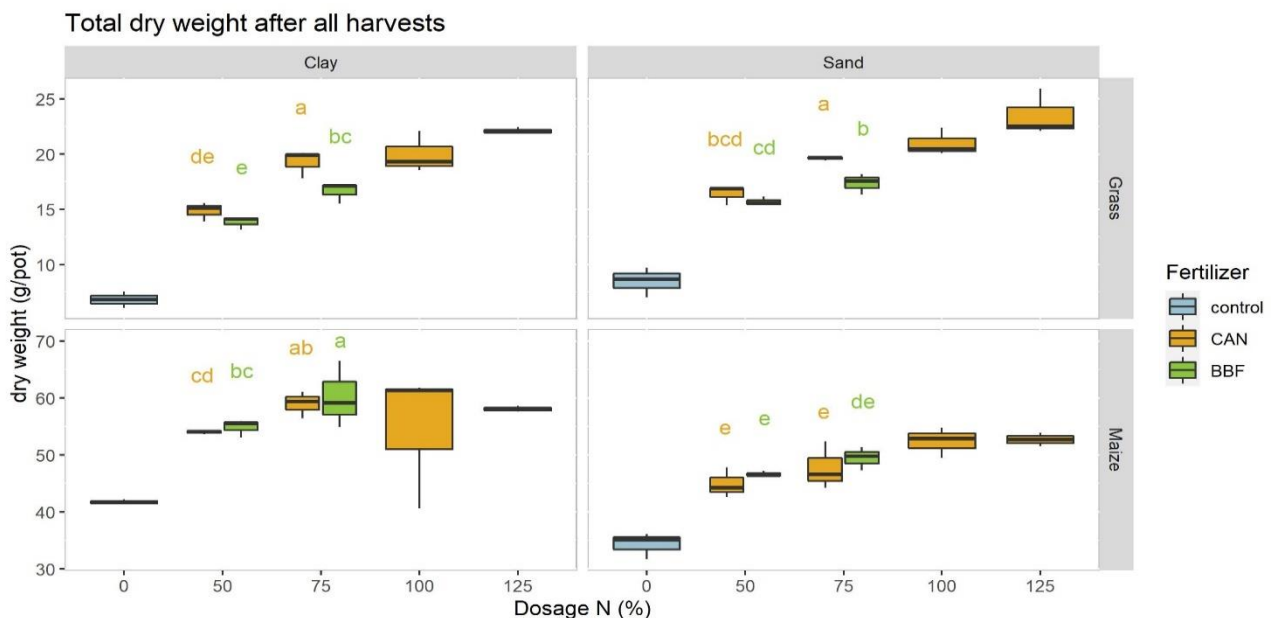
In general, maize and grass were tested separately for each trial, and treatments that were not fully balanced or replicated were left out of the statistical tests. This means that there was no control treatment in the field trials, so the results of the field trial should mainly be considered as supportive of the pot trial. Linear models followed by an ANOVA test and LSD test were used to determine statistically significant treatments. A full description of statistical models can be found in *Rietra et al., 2024*.

3.3.3 Results and discussion

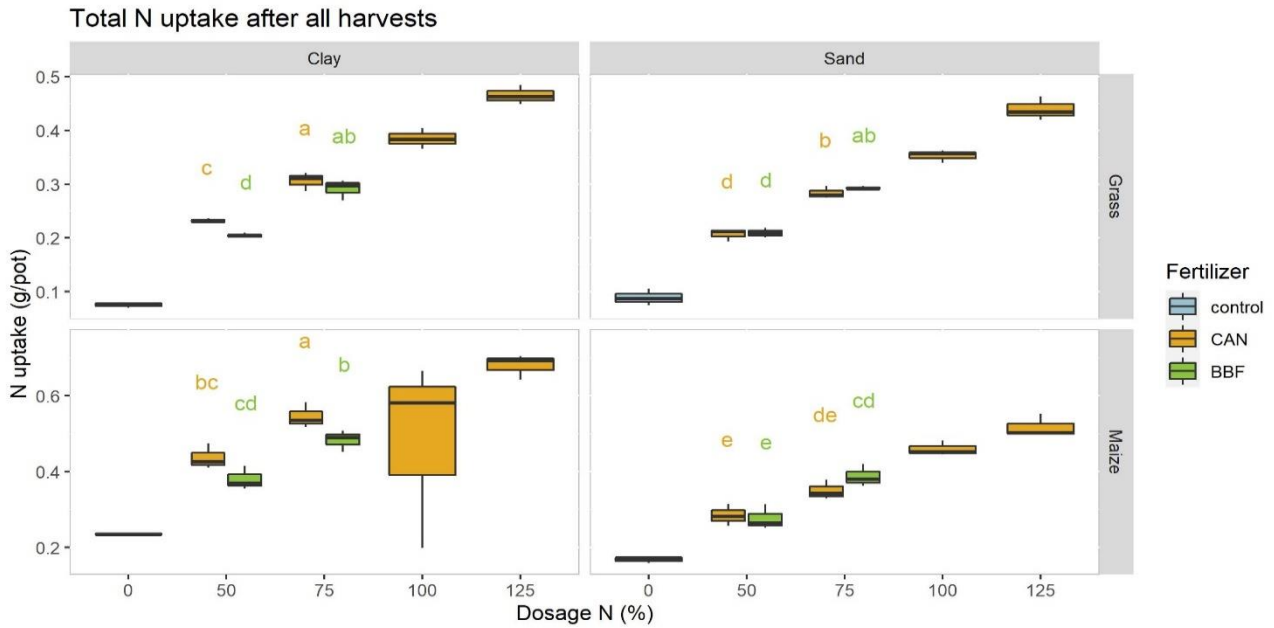
(i) Pot experiment 2021

The results of the pot experiment showed no significant effect of fertiliser on dry matter yield of grass, except for in the 75% N treatment, where a yield loss of 11.5% on sand and 13.7% on clay were observed. For crop N uptake of grass, fertiliser showed a significant interaction effect with soil, which can be seen in the 50% treatment on clay from the LSD groupings (Figure 18), though fertiliser alone was not significant. These results imply there may be a small effect on the dry matter yield of grass, but there is no evidence for reduced quality at the higher N dosage. The overall yields were similar to what can be expected in Dutch conditions (CBS 2022), thus the yield decrease can be considered a minor difference.

The dry matter of maize showed no significant effect of fertiliser, but crop N uptake had a significant effect from fertiliser and the interaction of soil and fertiliser. This mainly affected the 75% dosage of maize on clay soil (Figure 17). This implies that the quality of maize might be slightly reduced by the use of NL-AS, but there is no evidence for this affecting the dry yield. The yields were high compared to the Dutch national harvest of silage maize, especially considering that the maize plants were cropped before reaching maturity. This can be explained by the high planting density, which was almost 13 times higher than what is conventional in Dutch practice.

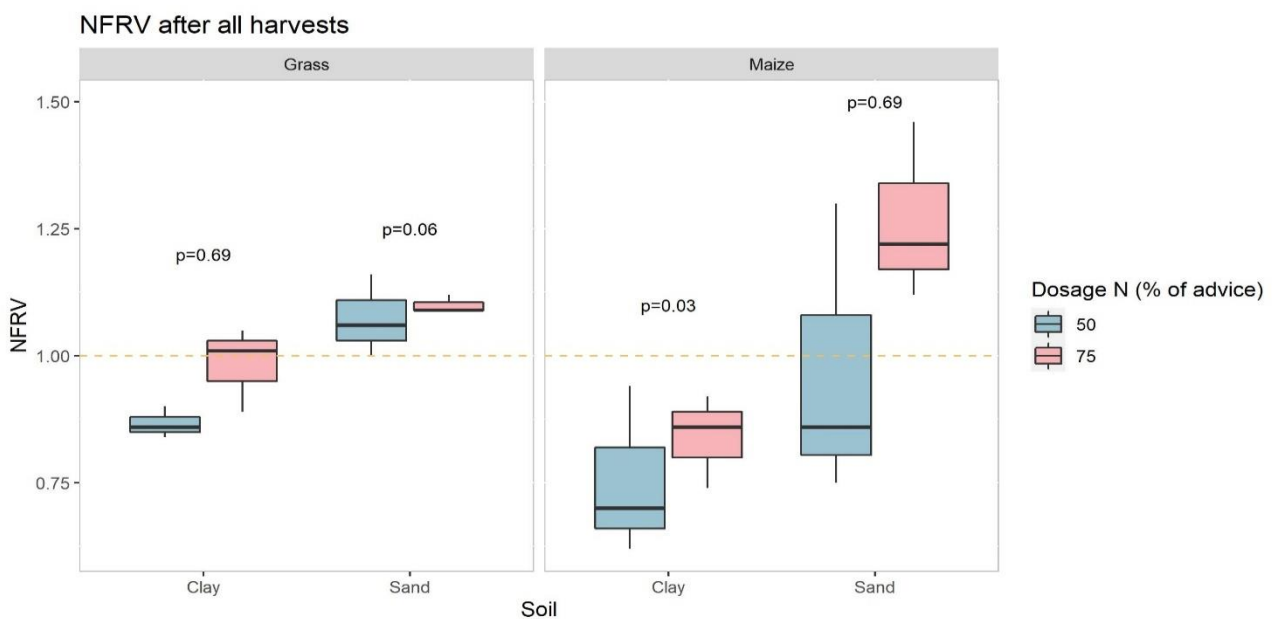


**Figure 17.** Total dry weight of NL-AS and CAN treatments after all harvests by soil, crop, and dosage. BBF refers to NL-AS. Letters indicate groupings of the treatments determined by the LSD test: two treatments with the same letter are not significantly different from each other. Grass and maize were analysed separately and only 50% and 75% of treatments were included in statistical tests. LSD is 10.61 g pot<sup>-1</sup> for grass and 4.71 g pot<sup>-1</sup> for maize.



**Figure 18.** Total N uptake of NL-AS and CAN treatments after all harvests by soil, crop, and dosage. BBF refers to NL-AS. Letters indicate groupings of the treatments determined by the LSD test: two treatments with the same letter are not significantly different from each other. Grass and maize were analysed separately and only 50% and 75% of treatments were included in statistical tests. LSD is 0.02 g pot<sup>-1</sup> for grass and 0.31 g pot<sup>-1</sup> for maize.

Figure 19 shows the NFRV results and significance test (sign-test of values where 75% and 50% treatment are combined, but crop/soil combination are tested separately). The NFRV was only significantly different than one for maize on clay, where it was 0.75 for the 50% dose and 0.84 for the 75% dose (standard deviation 0.17 and 0.92 respectively). This is supported by similar results from the linear models, where fertiliser showed an effect on maize N uptake, especially on clay soil.



**Figure 19.** NFRV of NL-AS by crop, soil, and dosage with CAN as reference. P value determined by two sides sign test. Data of 50 and 75% combined and tests performed per crop/soil combination. A p-value <0.05 is considered significant.

The mineral N in the soil following both the grass and maize pot experiments was very low. This leads to the conclusion that all available N was fully utilised for plant growth, or lost as gas during fertilisation. The possibility of gaseous losses is further examined in section **Error! Reference source not found.** on environmental monitoring campaigns.

#### (ii) Field trials 2021 and 2022

In 2021, the fertiliser treatment was not a significant factor for any of the tested factors of either the maize or grass field experiments (fresh and dry matter, and content of N, P, K, S, Mg, Na, and Ca, N residue in the soil). Soil type did have a significant effect in most cases.

In 2022, fertiliser did not have a significant effect on the total dry matter harvest or total N uptake over two cuts. It did affect N residue after the 2 cuts in the grass experiment. In addition, fertiliser had an effect on the individual cuts for the P, S, and Ca content of the first cut, and the fresh matter, S, Mg, and Ca content of the second cut.

These results give no evidence of a significant yield or N uptake difference between CAN and NL-AS treatments at a dosage of 100% in field conditions. However, sulphur content may be affected by the high sulphur dosage when using NL-AS as the sole N fertiliser, thus NL-AS should primarily be used to top-up a fertilisation of animal manure. It should be noted that when enough fertiliser or manure is applied to supply 100% of advised available N, no differences in crop yield are expected.

#### 3.3.4 Conclusion and recommendation

For most of the tested soil-crop combinations, there was no evidence found to indicate that NL-AS is less effective than CAN as a N fertiliser. There were two main exceptions in the pot experiments: dry matter yield of grass on both clay and sandy soil, and crop N uptake for maize on clay soil. For grass, this may imply a small reduction of yield while quality is maintained. For maize, this indicates that a quality reduction may be expected for use on clay soils. However, these effects did not result in a reduction of harvest in the field experiments. In all of the experiments, dry matter yields followed or surpassed expectations compared to national harvest levels of 2019.

The 2022 grass field trial showed that sulphur uptake may be increased when using high amounts of NL-AS. In order to prevent toxicity to cattle when used as feed, these increased values must be accounted for in their entire diet or NL-AS must only be used to top-up the use of animal manure, instead as the sole N fertiliser. This usage is also more comparable to the use of CAN in practice for Dutch dairy systems, where it is used to top up the N dosage after fertilisation with animal manure in accordance with the Nitrates Directive.

### 3.4. Silage maize field cultivation (CRAB, France): FR-AS

*For more information on this study, please contact the author from Chambre d'agriculture de Bretagne: Mariana Moreira ([mariana.moreira@bretagne.chambagri.fr](mailto:mariana.moreira@bretagne.chambagri.fr)).*

#### 3.4.1 Introduction

Silage maize is one of the most representative crops in France. Depending on the farming system type, maize can be fertilized using synthetic mineral fertilisers or organic manure. In Brittany, this crop represented 17% of utilised agricultural area (UAA) in 2021 and is usually fertilised with animal manure. Since FR-AS contains N in a fully water-soluble form (directly available for crops), it is expected that this BBF could replace synthetic mineral fertilisers and manure for silage maize fertilisation. To assess the short-time silage maize response to N for FR-AS and compare it with the response for synthetic mineral fertiliser and a pig slurry, a 2-year trial has been set-up in Brittany in 2021 and 2022.

### 3.4.2 Methodology

The maize trial was located in Bignan (Morbihan, Brittany) at the Kerguéhennec Experimental Station (latitude: 47.882389, longitude: -2.739355) on two adjacent plots. The soils of both plots are sandy clay loam (pH=6.4; Org C=31.7 g/kg; total N=3.3 g/kg in 2021; pH=6.6; Org C=26.9 g/kg; total N=2.6 g/kg in 2022). The minimum and maximum average temperatures are 3.2°C and 8.8°C in January and 12.6°C and 23.5°C in August; the total annual precipitation is 945 mm (for the 1981-2010 period). The meteorological conditions in 2021 were wetter than usual in June and July (60% more than an average year); the year 2022 was marked by abnormally high maximum temperatures in July and August (3.5°C higher than in an average year). The trial was conducted as a randomised complete block design with 3 replicates (elementary plot size = 3m x 15 m). After soil preparation, 7 fertiliser treatments were applied within each block on the 5<sup>th</sup> May (2021 and 2022):

- A control without N-fertilisation that enables the estimation of the soil N-mineralisation: C;
- Three treatments with synthetic mineral fertiliser (Min) applied at incremental rates (30%X, 60%X and X, where X represents the N fertiliser advice): *Min-30*, *Min-60*, *Min-100*;
- Three treatments with bio-based ammonium sulphate FR-AS (BBF) applied at incremental rates (30%X, 60%X and X, where X represents the N fertiliser advice): *BBF-30*, *BBF-60*, *BBF-100*;
- Three treatments receiving incremental rates of pig slurry (raw manure used to produce the BBF ammonium sulphate) in 2021: *PS-30*, *PS-60* and *PS-100*. In 2022, only one treatment was applied (*PS-106 kg N/ha*), which corresponds to a 20 m<sup>3</sup>/ha application.

The N fertiliser advice (=X dose), calculated from crop needs and N soil supply, was 119 kg N/ha in 2021 and 92 kg N/ha in 2022. In 2021, the balance sheet method (plant N requirements – soil N supply). In 2022, to better estimate soil N supply, the Sol-Aid® tool, a web application to estimate soil N mineralisation available for crops in Brittany, was used. This tool takes into account all factors having an impact on soil organic N mineralisation – soil, climate and cultural system data. The used reference synthetic mineral fertiliser was a pure ammonium nitrate in granular form (33.5% N). Since pig slurry contains P and K, in 2021 these nutrients were also applied in a mineral form (triple superphosphate - 45% P<sub>2</sub>O<sub>5</sub> - and potassium oxide - 50% K<sub>2</sub>O) to make the other treatments reach the same level. In 2022, no complement on P or K was made since the soil had already the necessary supplies (90 ppm P<sub>2</sub>O<sub>5</sub> Olsen, 380 ppm K<sub>2</sub>O). Also in 2022, pig slurry was applied at only one rate (20 m<sup>3</sup>/ha, which corresponds to 106 kg/ha of total N). The product characterisation can be found in Table 13.

**Table 13.** Physio-chemical characterisation of fertilisers applied on the field trials in 2021 and 2022.

Fertiliser	Trial	Dry matter (%)	pH	Total N (%)	N-NH <sub>4</sub> (%)	P <sub>2</sub> O <sub>5</sub> (%)	K <sub>2</sub> O (%)
Ammonium nitrate	Both years	NA	NA	33.5	16.5	0	0
BBF FR-AS	Maize 2021	19.8	1.9	4.4	4.4	<0.07	<0.05
BBF FR-AS	Maize 2022	19.8	1.9	3.73	3.73	<0.07	<0.05
Pig slurry	Maize 2021	4.85	NA	0.48	0.33	0.24	0.42
Pig slurry	Maize 2022	5.8	7.8	0.53	0.37	0.32	0.38

NA – not analysed

Mineral fertilisers (solid) were manually broadcasted. Liquid fertilisers were applied using a graduated watering can. Subsequently, fertilisers were incorporated by shallow soil tillage. Sown took place hereafter. Maize was harvested on 7<sup>th</sup> October 2021 and 14<sup>th</sup> September 2022 (Figure 20). Three sub-plots (2 rows of 10 m) by elementary plot were collected manually and weighed (fresh yield). A sub-sample of 5 representative maize plants by elementary plot was grinded and placed into an oven to obtain dry matter values and perform N content analyses. The DM yield and N uptake from each plot were then calculated for each plot on a kg ha<sup>-1</sup> basis. Soil sampling was made per 30 cm layer until a depth of 90 cm after harvest to analyse soil N residue. Data on yield, calculated N-uptake and soil N residues were expressed as the mean value of the 3 replicates with the standard error by treatment. When the conditions were set (normality - Shapiro Wilk test - and homogeneity of variance - Bartlett test), an ANOVA followed by a Tukey HSD post hoc was performed to test significant differences between treatments. If the conditions were not set, non – parametric testing was



performed (Kruskal-Wallis). All tests were performed using R version 4.2.1 and R packages *RVAideMemoire* and *multcomp*. ANR and NFRV values were calculated.



**Figure 20.** BBF application and maize harvest in 2021.

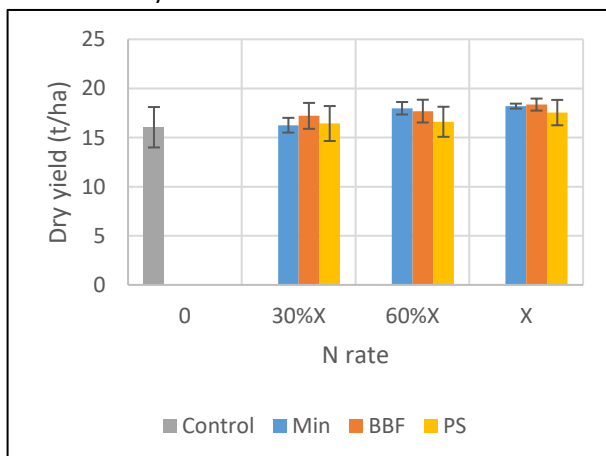
### 3.4.3 Results and discussion

#### (i) Maize yield and N-uptake

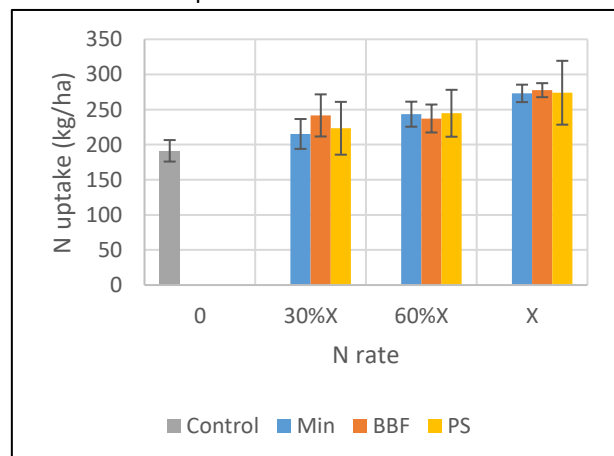
Silage maize average yield in the Brittany region is usually around 12.6 t/ha (dry matter), and 15 t/ha is the average yield for the last 10 years in the experimental plots of Fertimanure field trials. For this experiment, more specifically, the average yield was 17.2 t/ha (13.8 t/ha to 18.9 t/ha) in 2021 and 18.1 t/ha (15.4 t/ha to 22.0 t/ha) in 2022 (Figure 21). In both years, the N incremental rates did not generate incremental yields. In addition, yields obtained for the control plots were not significantly different from those obtained in the fertilised plots (Min, BBF and PS). This means that N was not a limiting factor to maize production in these trials. Past manure inputs to the soil can partly explain these results. In addition, the regular precipitations in May, June and July, coupled with mild temperatures in early summer, contributed to a higher soil N mineralisation in 2021. The use of an accurate tool to estimate soil N supply in 2022 was not enough to ensure maize response to N. In both years, soil N supply highly contributed to satisfying maize N needs during the crop cycle which could explain the high yields obtained for the control plots. In that situation, at a given level of N-fertilisation, there are no significant differences in crop yields between plots fertilised with BBF FR-AS compared to plots fertilised with ammonium nitrate (Min) or pig slurry (PS).

Regarding maize N-uptake, the lowest average N uptake values were obtained for the control plots without N fertilisation ( $191 \pm 15$  kg/ha in 2021 and  $196 \pm 19$  kg/ha in 2022) (Figure 21). In 2021, the maize N uptake between N incremental rates followed the same pattern as yield - no significant differences were observed. In that situation, at a given level of N-fertilisation, there is no significant difference in N-uptake between plots fertilised with BBF FR-AS compared to plots fertilised with ammonium nitrate (Min) or pig slurry (PS). In 2022, BBF at a 60% rate led to less N uptake than synthetic mineral fertiliser (Figure 21). This has not led to higher soil N residues in these plots, assuming that N was probably lost by volatilisation at the time of spreading.

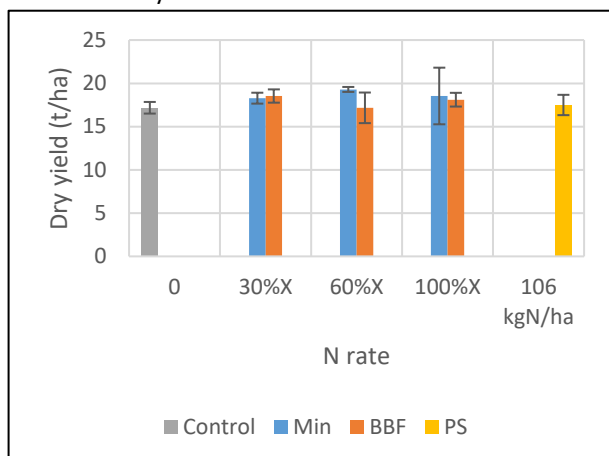
2021 - Maize yield



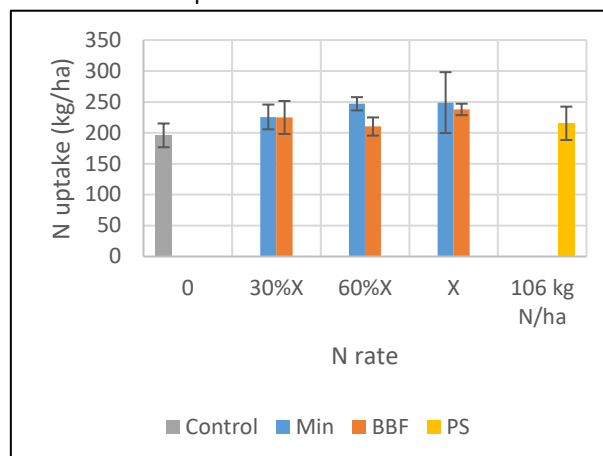
2022 - Maize N uptake



2022 - Maize yield



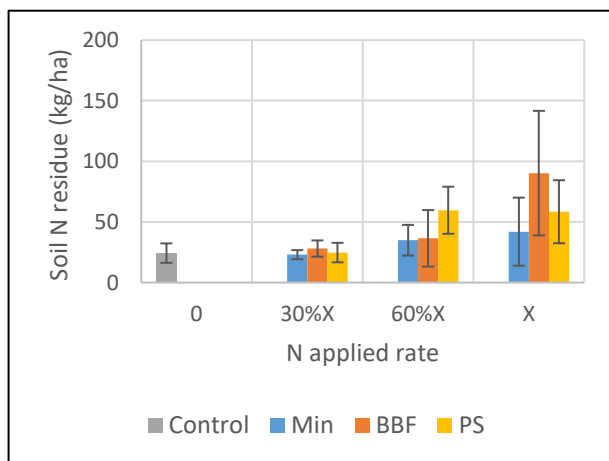
2022 - Maize N uptake



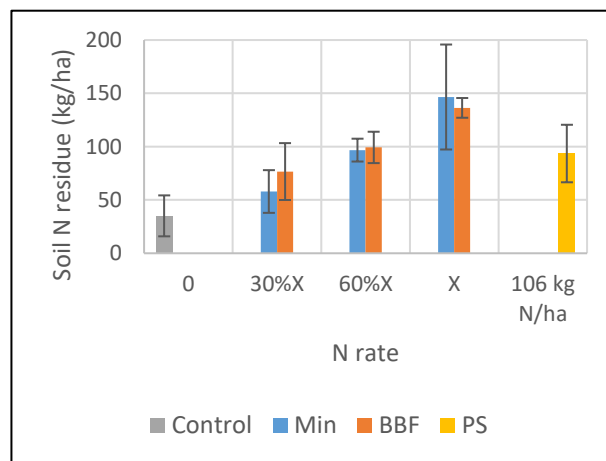
**Figure 21.** Maize yield and N uptake (kg/ha) for the different treatments (average  $\pm$  standard deviation, n=3). Min – synthetic mineral fertiliser; BBF – biobased fertiliser FR-AS; PS – pig slurry; X – N fertiliser advice for maize (119 kg/ha in 2021 and 92 kg/ha in 2022). No statistically significant differences were observed among treatments for both parameters (ANOVA and post hoc Tukey HSD, p-value<0.001).

Since N fertilisation was not a limiting factor to maize production in this trial, it was expected that the N incremental rates would generate incremental soil residual N. This soil parameter, measured after maize harvest, indicates the N quantity that remains in the soil and is at risk of leaching during autumn. However, in both years, no significant differences were observed between treatments due to a high variability of the values (indicated by the high standard deviation) (Figure 22). In this sense, N leaching is not expected to be different between treatments.

2021



2022



**Figure 22.** Soil N residue at harvest (kg/ha) for the different treatments (average  $\pm$  standard deviation, n=3). Min – synthetic mineral fertiliser; BBF – biobased fertiliser FR-AS; PS – pig slurry; X – N fertiliser advice for maize (119 kg/ha in 2021 and 92 kg/ha in 2022). No statistically significant differences were observed among treatments (ANOVA and Kruskal-Wallis test, p-value<0.001).

(ii) ANR and NFRV index

Since additional N-fertilisation did no longer result in additional crop yield, the index values for 60%X and 100%X could not be interpreted. For a 30%X N rate, the FR-AS led to a higher N efficiency than the synthetic mineral fertiliser in 2021 and an equal efficiency in 2022 but with extraordinarily high standard deviation values (Table 14). Since pig slurry (PS) was applied considering a 70% factor, the N efficiency was expected to be equivalent to those from ammonium nitrate. Nevertheless, pig slurry had a relatively higher N efficiency than



synthetic mineral fertiliser. The NFRV of PS could not be calculated in 2022 because the applied dose was not equivalent to any dose of the Min treatments.

**Table 14.** ANR and NFRV values calculated for the different fertilised treatments. No significant difference.

Treatments	ANR 2021	ANR 2022	NFRV 2021	NFRV 2022
Min-30	0.67±0.44	0.99±1.25	-	-
Min-60	0.73±0.22	0.85±0.22	-	-
Min-100	0.69±0.19	0.59±0.58	-	-
BBF-30	1.40±0.97	0.96±1.51	2.10±1.46	0.97±1.52
BBF-60	0.65±0.10	0.24±0.11	0.88±0.14	0.28±0.12
BBF-100	0.73±0.18	0.47±0.31	1.06±0.26	0.79±0.53
PS-30	0.89±1.08	-	1.34±1.62	-
PS-60	0.75±0.55	-	1.03±0.75	-
PS-100	0.70±0.48	-	1.01±0.70	-
PS-2022	-	0.18±0.39	-	-

### 3.4.4 Conclusion and recommendation

In general, BBF ammonium sulphate (FR-AS) led to the same maize yield and N uptake compared to ammonium nitrate (Min). Additional N-fertilisation did not result in additional yield because the soil had a high N mineralisation due to past organic inputs. The soil N residue at harvest was not different between treatments. Since this parameter indicates the N quantity that remains in the soil and is at risk of leaching during autumn, N leaching is not expected to be different between treatments. Since N was not a limiting factor to maize production in this plot, care must be taken when interpreting the data. ANR and NFRV were only interpreted for the 30% rate.

## 3.5. Winter wheat field cultivation (CRAB, France): FR-AS

For more information on this study, please contact the author from Chambre d'agriculture de Bretagne: Mariana Moreira ([mariana.moreira@bretagne.chambagri.fr](mailto:mariana.moreira@bretagne.chambagri.fr)).

### 3.5.1 Introduction

Winter wheat is one of the most representative crops in France. Depending on the farming system type, winter wheat can be fertilised using synthetic mineral fertilisers or organic manure. In Brittany this crop represented 18% of UAA in 2021 and is usually fertilised with pig slurry or digestate completed with synthetic mineral fertilisers, for a total of 2 to 4 N applications. By splitting the fertilisation, the dose and date of fertilisation can be adjusted to optimise the efficiency of the N applied and ensure wheat yield and quality (particularly protein levels). Since FR-AS contains N in a fully water-soluble form (directly available for crops), it is expected that this BBF could replace synthetic mineral fertilisers and slurry or digestate for winter wheat fertilisation. An experiment was conducted in 2023 in Brittany to investigate the short-term winter wheat response to N for FR-AS and compare it to the response to synthetic mineral fertiliser, pig slurry, and digestate.

### 3.5.2 Methodology

The winter wheat trial was located in Crédin (Morbihan, Brittany) (latitude: 48.025261, longitude: -2.762415) on a loam soil (pH=6.8; Org C=21.5 g/kg; total N=2.17 g/kg). The minimum and maximum average temperatures are 3.2°C and 8.8°C in January and 12.6°C and 23.5°C in August; the total annual precipitation

is 945 mm (for the 1981-2010 period). The year 2023 was marked by abnormally high rainfall in July (twice that of a normal year), which made harvesting particularly difficult. Winter wheat was sown in November 2022. The trial was conducted as a randomized complete block design with 3 replicates (elementary plot size = 3m x 18 m). Seven fertiliser treatments were applied within each block:

- A control without N-fertilisation that enables the estimation of the soil N-mineralisation: C;
- Five treatments with synthetic mineral fertiliser (Min) applied at incremental rates (50 kg N/ha, 90 kg N/ha, 130 kg N/ha, 170 kg N/ha (the N fertiliser advice) and 210 kg N/ha: *Min-1*, *Min-2*, *Min-3*, *Min-4*, *Min-5*;
- One treatment with bio-based ammonium sulphate FR-AS (BBF) applied at 90 kg N/ha (equivalent to Min 3);
- Two treatments receiving organic fertilisers: pig slurry (PS) or digestate (D) applied at 194 kg N/ha and 147 kg N/ha (or 117 and 88 kg N/ha theoretic effective N) respectively, which corresponds to a 30 m<sup>3</sup>/ha application.

The used reference synthetic mineral fertiliser was a pure ammonium nitrate in granular form (33.5% N). On the 9<sup>th</sup> March (wheat tillering stage), the total volume of PS and D were applied. For the Min and BBF treatments, a first dose of 30 kg N/ha was applied on the same day. The following Min and BBF applications were carried out at the end of the wheat tillering stage (24<sup>th</sup> March – 60 kg N/ha for BBF and every Min treatment), 2 weeks later (11<sup>th</sup> April – 40 kg N/ha for BBF, Min 3, Min 4 treatments and 80 kg N/ha for Min5) and the end of wheat stem extension (2<sup>nd</sup> May – 40 kgN/ha for Min4 and Min5), as recommended (Figure 23). The PS, D and BBF (liquid) were applied using a graduated watering can. Mineral fertiliser (solid) was manually broadcasted. The product characterisation can be found in Table 15 and the N fertilisation scheme by treatment in Table 16.

**Table 15.** Physio-chemical characterisation of fertilisers applied on the field trial in 2023.

Fertiliser	Dry matter (%)	pH	Total N (%)	N-NH <sub>4</sub> (%)	P <sub>2</sub> O <sub>5</sub> (%)	K <sub>2</sub> O (%)
Ammonium nitrate (Min)	NA	NA	33.5	16.5	0	0
FR-AS (BBF)	20.2	2.3	4.53	4.53	<0.07	<0.05
Pig slurry (PS)	5.3	7.85	0.49	0.31	0.30	0.32
Digestate (D)	6.81	8.13	0.65	0.43	0.20	0.31

NA – not analysed

**Table 16.** Fertilisation scheme for wheat trial and the corresponding quantity of nutrients (N, P and K) applied for each treatment.

Treatment	N source	Total quantity applied (kg/ha or l/ha)	Number of applications	Total N dose (kg/ha)	Total P <sub>2</sub> O <sub>5</sub> dose (kg/ha)	Total K <sub>2</sub> O dose (kg/ha)
C	None	0	0	0	0	0
Min-1	Ammonium nitrate	149	2	50	0	0
Min-2	Ammonium nitrate	268	2	90	0	0
Min-3	Ammonium nitrate	388	3	130	0	0
Min-4	Ammonium nitrate	507	4	170	0	0
Min-5	Ammonium nitrate	627	4	210	0	0
BBF	Ammonium sulphate (FR-AS)	2869	3	130	<2	<2
PS	Pig Slurry	30000	1	147**	93	96
D	Digestate	30000	1	194*	60	93

\* 194 x 0.6 = 117 kg/ha effective N; 147 x 0.6 = 88 kg/ha effective N.

To ensure more accurate sampling of the wheat, harvest sampling was carried out in 2 steps:

- (i) “Hand harvest” - Two weeks before harvest (26<sup>th</sup> July), three sub-plots (2 rows of 1 m) by elementary plot were collected manually and weighed (fresh yield). The number of ears was counted for each sample (three sub-plots by treatment). The ears were passed through the threshing machine to check the grain/straw ratio. At the laboratory dry matter and N content of grain and straw were analysed. The DM yield and N uptake by the crop were calculated for each plot on a kg ha<sup>-1</sup> basis.
- (ii) “Machine harvest” - On the 8<sup>th</sup> of August, using a combine harvester with automatic indication of fresh yield.

To analyze soil N residual, soil samples were collected every 30 cm layer to a depth of 90 cm following harvest. Data on yield, calculated N-uptake, and soil N residues were reported as the mean of three replicates with standard error by treatment. The ANR and NFRV values for BBF, PS, and D were calculated using the N uptake response curve to mineral fertiliser. When the conditions were set (normality - Shapiro Wilk test - and homogeneity of variance - Bartlett test), an ANOVA followed by a Tukey HSD post hoc was performed to test significant differences between treatments. If the conditions were not set, non – parametric testing was performed (Kruskal-Wallis). All tests were performed using R version 4.2.1 and R packages *RVAideMemoire* and *multcomp*. ANR and NFRV values were calculated.

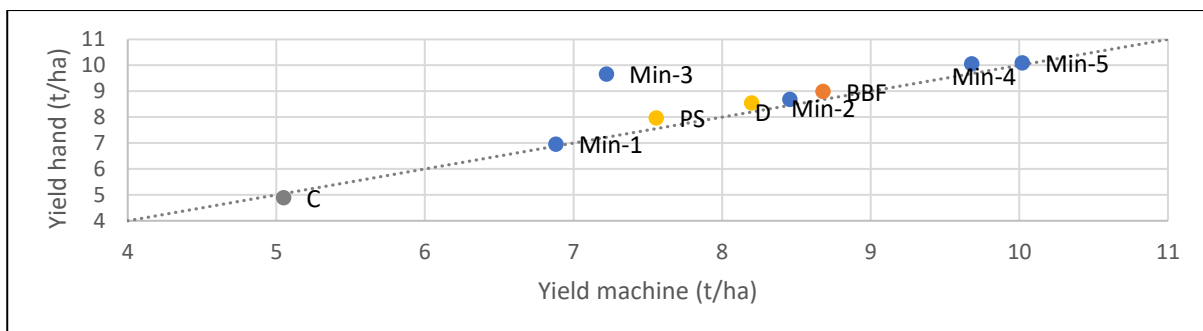


**Figure 23.** Digestate application, BBF (second application) and winter wheat harvest.

### 3.5.3 Results and discussion

#### (i) Wheat yield and N-uptake

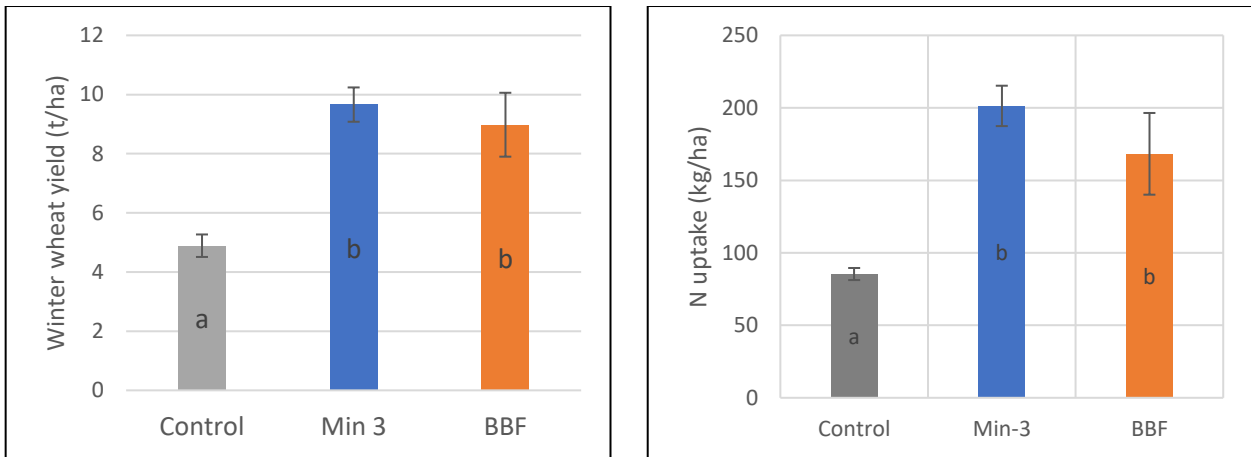
At harvest, there were technical complications with the harvesting machine getting blocked on 2 blocks of the Min-3 treatment (the only one that received the same dose of N as the BBF treatment – 130 kg/ha). A comparison between the yield data obtained with the machine (“Machine harvest”) and those obtained by manual harvesting (“hand harvest”) revealed yield differences only for this treatment (Figure 24). As the machine yields for the other treatments were equivalent to the manual yields, it was decided to use the manual yields (“hand harvest”) as the basis for analysing the data of the whole trial and to allow a direct comparison between BBF and Min-3.



**Figure 24.** Comparison between average machine yield and average hand yield for all treatments (n=3). Min – synthetic mineral fertiliser at increasing doses; BBF – biobased fertiliser FR-AS; PS – pig slurry, D - digestate.



Winter wheat average yield in the region is usually around 7.2 t/ha (fresh wheat grain yield). For this experiment, all treatment combined, the average yield was 8.4 t/ha (4.5 t/ha to 10.4 t/ha, “hand harvest”). For the same level of fertilisation (130 kg/ha), the average yields were 9.7 t/ha for the mineral treatment (Min-3) and 9.0 kg/ha for the BBF but no statistical differences were observed between both treatments (Figure 25).

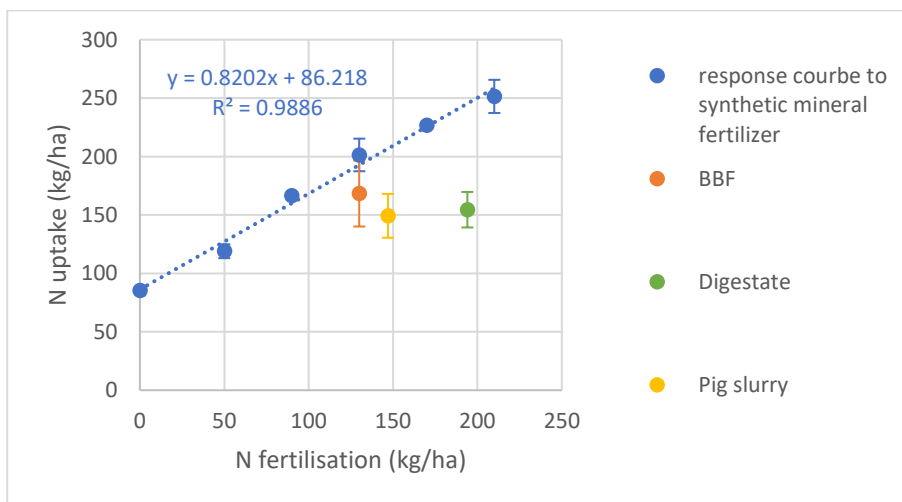


**Figure 25.** Winter wheat fresh yield (t/ha) and N uptake (kg/ha) for the different treatments (average ± standard deviation, n=3). Min-3 – synthetic mineral fertiliser at 130 kg N/ha; BBF – biobased fertiliser FR-AS at 130 kg N/ha.

For the N uptake, the same treatments were also not statistically differences despite a gap of 30 kg/ha in the N absorbed by the wheat of BBF treatment. This gap can be explained by the fact that following the second application of BBF burning was observed in some wheat leaves, which may have induced stress in the plant. However, during the development cycle, the plants were able to recover, at least partially, from this stress.

(iii) ANR and NFRV index

Two response curves were produced using the yield and N uptake data in relation to the amount of N applied. The latter was more precise ( $r^2=0.99$  vs  $r^2=0.89$  for a linear model) (Figure 26) and was therefore chosen to calculate the ANR and NFRV indicators (Table 17). The fact that increasing doses of N produced increasing quantities of N uptake by the crop, confirms the validity of this trial.



**Figure 26.** Response curve representing N uptake (average ± standard deviation, n=3) as a function of the increasing doses of N applied with a synthetic mineral fertiliser.

**Table 17.** ANR and NFRV values calculated for the different fertilised treatments.

Treatment	ANR	NFRV
Min1	0.65±0.12 ab	-
Min2	0.89±0.03 b	-
Min3	0.89±0.11 b	-
Min4	0.83±0.01 b	-
Min5	0.79±0.07 b	-
BBF	0.63±0.22 a	0.77±0.26 a
D	0.35±0.08 a	0.43±0.10 a
PS	0.43±0.13 a	0.52±0.16 a

With a NFRV of 77%, the BBF led to a lower N efficiency than the synthetic mineral fertiliser. There are two possible explanations: a lower N uptake due to leaf burning at the time of the second BBF application and/or a greater susceptibility to N volatilisation than the synthetic mineral fertiliser. As expected, organic fertilisers (digestate and pig slurry) led to a lower N efficiency than FR-AS and mineral fertilisers. For pig slurry, a NFRV of 52% is very close to the current regional references for winter wheat (60%). The NFRV of the plots that received digestate (43%) was slightly lower than that of the plots that received pig slurry (52%) because the proportion of ammonia (N-NH<sub>4</sub>) and pH value in the digestate is higher, making it more susceptible to N volatilisation at spreading. Indeed, given that wheat plants are already in place at the time of fertilisers application, unlike other crops such as maize, it is not possible to incorporate the product into the soil after spreading, which could limit N losses through volatilisation. The soil N residue at harvest varied between 30.7 (control) and 54.9 (Min-5) kg/ha; and 37.1 kg/ha for BBF. The average values were significantly different between treatments. Since this parameter indicates the N quantity that remains in the soil and is at risk of leaching during autumn, N leaching is not expected to be different between treatments.

#### 3.5.4 Conclusions and recommendations

Even if there is no significant differences for yield and N uptake between BBF ammonium sulphate (FR-AS) and ammonium nitrate (Min-3), the large standard deviations observed for BBF suggest a higher heterogeneity for this treatment, certainly related to the burning of the leaves observed on the second application or to a greater susceptibility of BBF to N volatilisation. NFRV for BBF is 77%, it can replace ammonium nitrate in wheat if attention is paid to the weather conditions on the day of application (avoid windy or hot days) and that the spreading equipment is carefully chosen (keep it as close to the ground as possible) in order to limit N losses through volatilisation and the burning of young wheat leaves. N fertilisation is well valorised by this crop, which is reflected by the low levels of N residues in the soil at harvest. The use of the BBF does not increase the level of soil N residue and therefore does not constitute a risk for the leaching of N from the soil.

### 3.6. Spinach field cultivation (CRAB, France): FR-AS

For more information on this study, please contact the author from Chambre d'agriculture de Bretagne: Mariana Moreira ([mariana.moreira@bretagne.chambagri.fr](mailto:mariana.moreira@bretagne.chambagri.fr)).

#### 3.6.1 Introduction

Spinach is a crop with high N demands and for which the fertilisation programme is generally completely based on synthetic mineral fertilisers. Since FR-AS contains N in a mineral form, it is expected that this BBF could replace synthetic mineral fertilisers for spinach fertilisation. With the aim of assessing the short-time spinach

response to N for FR-AS and compare it with the response for synthetic mineral fertiliser, a 2-year trial has been set-up in Brittany in 2021 and 2022.

### 3.6.2 Methodology

The spinach trial was located in Auray (Morbihan, Brittany) at Bretagne Sud Experimentation Station (latitude: 47.658722, longitude: -2.970543), on two adjacent plots. The soils of both plots are sandy clay loam (pH=6.3; Org C=18.6 g/kg; total N=1.8 g/kg in 2021). The minimum and maximum average temperatures are 3.9°C and 9.6°C in January and 14°C and 23.6°C in August. The total annual precipitation is 1011 mm (for the 1981-2010 period). In 2021 rainfall was low at the beginning of the year, but in May, and during the crop cycle, it was high, as in a normal year; the year 2022 was marked by high maximum temperatures in April and May (2°C – 3°C higher than in an average year) and low rainfall. The trial was conducted as a randomised complete block design with 3 replicates (elementary plot size = 3 m x 15 m). After soil preparation, 7 fertiliser treatments were applied within each block in April (27<sup>th</sup> in 2021 and 14<sup>th</sup> in 2022):

- A control without N-fertilisation that enables the estimation of the soil N-mineralisation: C;
- Three treatments with synthetic mineral fertiliser (Min) applied at incremental rates (30%X, 60%X and X, where X represents the N fertiliser advice): *Min-30*, *Min-60*, *Min-100*;
- Three treatments with bio-based ammonium sulphate FR-AS (BBF) applied at incremental rates (30%X, 60%X and X, where X represents the N fertiliser advice): *BBF-30*, *BBF-60*, *BBF-100*;

The N fertiliser advice (=X dose) was 160 kg N/ha in both years. Thus, X corresponds to 160 kg N/ha, 60%X to 100 kg N/ha and 30%X to 48 kg N/ha applied with synthetic mineral fertiliser or BBF. Only crop needs were taken into account for dose calculation in spite of a 35 kg/ha soil N residue in 2021. In 2022, the soil N residue was nearly zero. The used reference synthetic mineral fertiliser was a pure ammonium nitrate in granular form (33.5% N). No complement on P or K was made since the soil already had the necessary supply. The product characterisation can be found in Table 18.

**Table 18.** Physio-chemical characterisation of fertilisers applied in the field trials in 2021 and 2022.

Fertiliser	Trial	Dry matter (%)	pH	Total N (%)	N-NH <sub>4</sub> (%)	P <sub>2</sub> O <sub>5</sub> (%)	K <sub>2</sub> O (%)
Ammonium nitrate	Both plots	NA	NA	33.5	16.5	0	0
BBF ammonium sulphate	Spinach 2021	19.8	1.9	3.8	3.8	<0.07	<0.05
BBF ammonium sulphate	Spinach 2022	19.8	1.9	3.82	3.82	<0.07	<0.05

NA – not analysed

Ammonium nitrate was manually broadcasted. Ammonium sulphate (FR-AS) was applied using a graduated watering can with a ramp. Subsequently, fertilisers were incorporated by shallow soil tillage and a mulch film was installed. Sown took place hereafter. Spinach was harvest on 3<sup>rd</sup> June in 2021 and 19<sup>th</sup> May in 2022 (Figure 27). Three sub-plots (1 m<sup>2</sup>) by elementary plot were collected manually and weighed (fresh yield). A sub-sample of 1 kg by elementary plot was collected to analyse dry matter values and perform N content analyses (total N and nitrate). The DM yield and N uptake from each plot was then calculated for each plot on a kg ha<sup>-1</sup> basis. Soil sampling was made until a depth of 30 cm after harvest to analyse soil nitrate residue. Data on yield, calculated N-uptake, nitrate concentration on leaves and soil N residues were expressed as the mean value of the 3 replicates with the standard error by treatment. When the conditions were set (normality - Shapiro Wilk test - and homogeneity of variance - Bartlett test), an ANOVA followed by a Tukey HSD post hoc was performed to test significant differences between treatments. If the conditions were not set, non – parametric testing was performed (Kruskal-Wallis). All tests were performed using R version 4.2.1 and R packages *RVAideMemoire* and *multcomp*. ANR and NFRV values were calculated as stated in Chapter 1.





**Figure 27.** Spinach sown and harvest in 2021.

### 3.6.3 Results and discussion

#### (i) Spinach yield and N-uptake

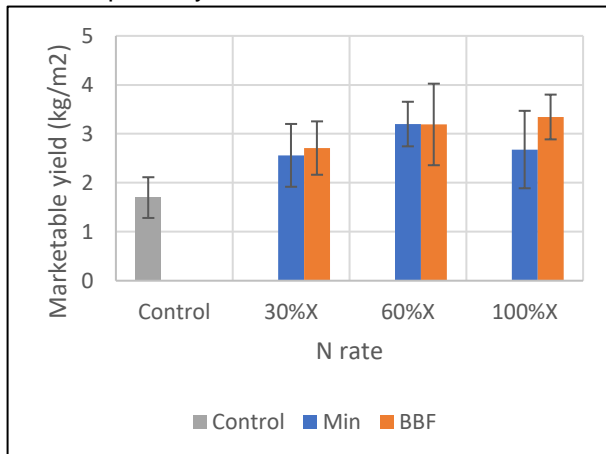
Spinach's average yield in the region is usually around 2 kg/m<sup>2</sup>. Depending on the climatic conditions of the year, one to 3 cuts are possible. In 2021, satisfactory climatic conditions enabled the good growth of spinach, with no irrigation. However, at the beginning of June, spinach started going to seed and consequently, only one cut was achievable. In 2022, even if sowing took place two weeks earlier, 2<sup>nd</sup> cut was not achievable either.

For this experiment, the average yield was 2.8 kg/m<sup>2</sup> (1.3 kg/m<sup>2</sup> to 3.9 kg/m<sup>2</sup>) in 2021 and 2.2 kg/m<sup>2</sup> (0.8 kg/m<sup>2</sup> to 3.8 kg/m<sup>2</sup>) in 2022. In both years, there is no significant difference in spinach yields between treatments (Figure 28). The N incremental rates did not generate incremental yields either. This indicates that N was not a limiting factor to spinach production in this trial. Plantation took place late in the season, by the end of April when temperatures started to increase, which increased soil N mineralisation. Thus, spinach N needs were satisfied by N soil supply. Therefore, the 100%X rate was probably overestimated and the 60%X rate should have been taken as the N fertiliser advice in 2021. The earlier plantation in 2022 did not produce any differences either.

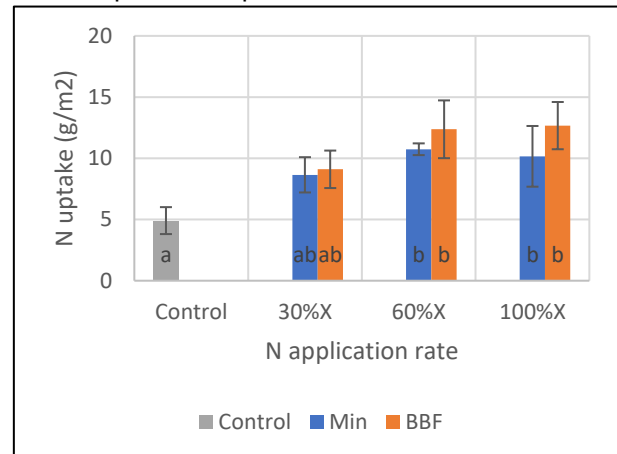
At a given level of N-fertilisation, there is no significant difference in N uptake between BBF ammonium sulphate plots compared to ammonium nitrate plots (Min) (Figure 28). The lowest N uptake values were obtained for Control, without N fertilisation (4.9 g/m<sup>2</sup> in 2021 and 3.5 g/m<sup>2</sup> in 2022). The highest values were obtained for BBF at the highest application rate (12.7 g/m<sup>2</sup> in 2021 and 13.8 g/m<sup>2</sup> in 2022). The N incremental rates did not generate incremental N uptake. Consequently, N incremental rates generate incremental soil residual N. As expected, the highest values were observed in the fertilised plots (Min and BBF). However, at a given level of N-fertilisation, no significant differences were found between fertilisers.



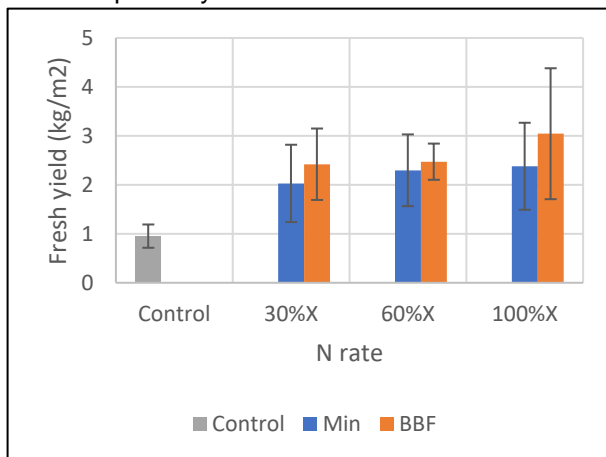
2021 - Spinach yield



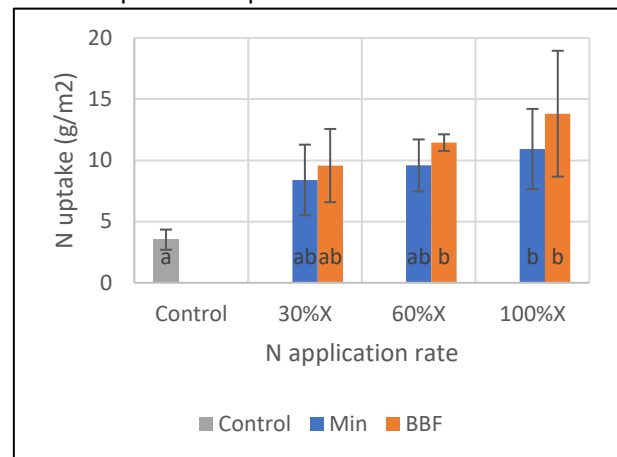
2021 - Spinach N uptake



2022 – Spinach yield



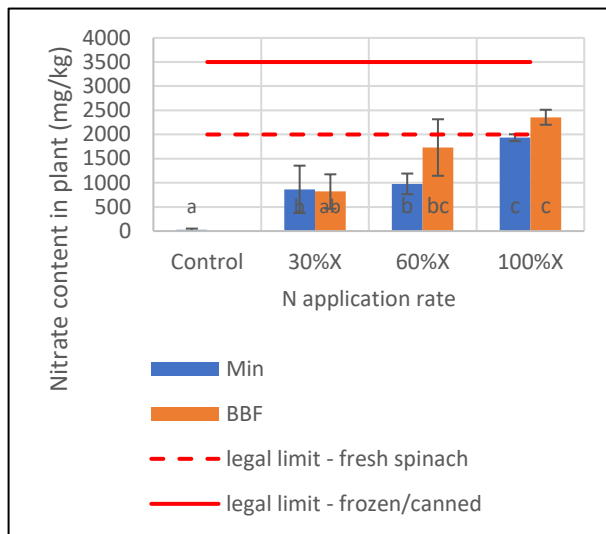
2022 – Spinach N uptake



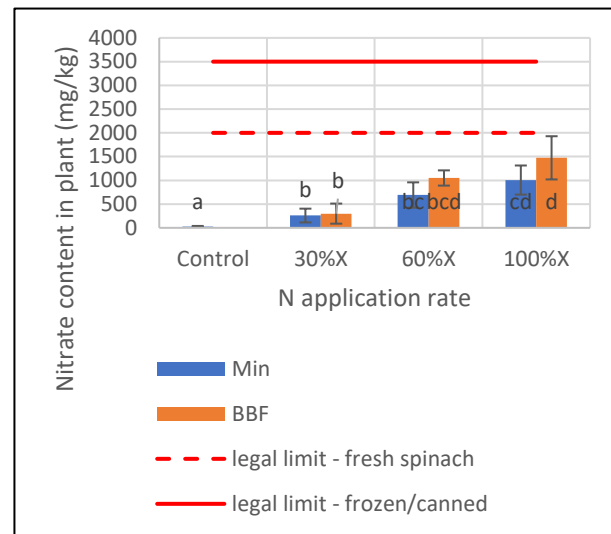
**Figure 28.** Spinach yield and N uptake (kg/ha) for the different treatments (average  $\pm$  standard deviation, n=3). Small letters refer to statistically significant differences among treatments (ANOVA and post hoc Tukey HSD, p-value<0.001). The lack of small letters indicates no statistically significant differences among treatments. Min – synthetic mineral fertiliser; BBF – biobased fertiliser FR-AS; X – N fertiliser advice for spinach (160 kg/ha).

Regarding nitrate concentration on the leaves, spinach fertilised with BBF or with ammonium nitrate had a higher concentration compared to those that did not receive N fertilisation (Control) (Figure 29). Even if the N incremental rates did not generate incremental N uptake, nitrate concentration on leaves was still increasing. Spinach can store N in its vacuoles without increasing yield. At a given level of N-fertilisation, nitrate concentration on spinach fertilised with BBF ammonium sulphate was not different from nitrate concentration on spinach fertilised with ammonium nitrate (Min). None of the treatments exceeded the legal limits for frozen/canned spinach commercialisation (3500 mg/kg). For fresh spinach, the highest N rate application of the BBF (BBF-100%X) and mineral fertiliser (Min-100%X) exceeded the legal limits of nitrates (2000 mg/kg) in 2021 (not observed in 2022 probably due to lower humidity condition in May 2022, with 40 mm less cumulative rainfall than in 2021).

2021



2022



**Figure 29.** Nitrate concentration (mg/kg) in spinach leaves for each treatment (average  $\pm$  standard deviation,  $n=3$ ). Red lines represent the legal limits for fresh spinach and canned spinach. Small letters refer to statistically significant differences among treatments (ANOVA and post hoc Tukey HSD,  $p$ -value $<0.001$ ). Min – synthetic mineral fertiliser; BBF – biobased fertiliser; X – N fertiliser advice for spinach (160 kg/ha).

(ii) ANR and NFRV index

The index values for 60%X and 100%X could no longer be interpreted, as additional N-fertilisation did not yield any further results. However, in 2021 and 2022, for a 30%X N rate, the BBF resulted on average in higher ANR compared to synthetic mineral fertiliser (Table 19).

**Table 19.** ANR and NFRV values calculated for the different fertilised treatments. No significant differences.

Treatment C	ANR 2021	ANR 2022	NFRV 2021	NFRV 2022
Min-30	0.78 $\pm$ 0.30	1.02 $\pm$ 0.50		
Min-60	0.58 $\pm$ 0.05	0.61 $\pm$ 0.13		
Min-100	0.33 $\pm$ 0.15	0.46 $\pm$ 0.16		
BBF-30	0.87 $\pm$ 0.32	1.26 $\pm$ 0.62	1.12 $\pm$ 0.41	1.24 $\pm$ 0.61
BBF-60	0.75 $\pm$ 0.24	0.79 $\pm$ 0.01	1.28 $\pm$ 0.40	1.30 $\pm$ 0.02
BBF-100	0.49 $\pm$ 0.12	0.64 $\pm$ 0.31	1.48 $\pm$ 0.37	1.39 $\pm$ 0.68

3.6.4 Conclusion and recommendation

At a given level of N-fertilisation (30%X, 60%X and 100%X), BBF ammonium sulphate (FR-AS) led to the same spinach yield, N uptake, nitrate concentration on leaves and soil N residue compared to ammonium nitrate (Min). Additional N-fertilisation did not result in additional yield because spinach needs are probably overestimated and do not take into account soil N mineralisation, which is high in Brittany soils and may impact spinach nutrition especially when the weather conditions of the year are favourable (as in 2021).

Nitrate concentration in spinach leaves exceeded the legal limits for canned commercialisation (2000 mg/kg) for the BBF and synthetic mineral fertiliser applied at the highest rates (Min-100%X and BBF-100%X) in 2021. This was not the case in 2022 where legal thresholds were respected in all treatments. In any case, the N fertilisation advice for spinach are probably overestimated. A N dose of less than 160 kg/ha applied using the BBF should not cause an excess of nitrates in the leaves. Since additional N rates did not generate additional

N plant uptake, care must be taken when interpreting the data. Thus, to be able to draw conclusions, only ANR and NFRV for the 30% rate were considered.

### 3.7. Sauerkraut cabbage field cultivation (CRAGE, France): FR-AS

For more information on this study, please contact the author from Chambre d'agriculture du Grand Est: Clement Munier ([clement.munier@grandest.chambagri.fr](mailto:clement.munier@grandest.chambagri.fr)).

#### 3.7.1 Introduction

Ammonium sulphate (FR-AS) was tested in 2021, 2022 and 2023 on sauerkraut cabbage in Alsace (Figure 30). The tested BBF was produced by French pilot. The main goals of the sauerkraut cabbage trials were:

- To assess the short-time crop response to N for BBF and compare them with the response for synthetic mineral fertiliser (Basammon 26S (26%N; 32.5%SO<sub>3</sub>)).
- To estimate the impact of the BBF on soil N leaching compared to a synthetic mineral fertiliser.
- To assess the impact of the BBF on crop N balance

The research hypotheses were the following:

- At a given level of N-fertilisation, there is no difference in crop yields and N-uptake between plots fertilised with FR-AS fertilisers compared to a reference treatment fertilised with synthetic mineral fertiliser.
- At a given level of N-fertilisation, there is no difference in N-environmental losses by leaching between plots fertilised with FR-AS compared to a reference treatment fertilised with synthetic mineral fertiliser.

#### 3.7.2 Methodology

The field trials were located in Alsace on 3 different sites on loamy, sandy-clay soil (around 33 % clays / 33 % loam / 33 % sands ; Organic matter ~ 4 % ; pH~7-8 depending on the sites). Weather conditions were very different for the 3 years. In 2021 after planting, average temperatures were close to 15 degrees and rainy periods in spring and summer were frequent with low intensity (except in June and July). In 2022, spring and summer were very sunny and dry. In 2023, weather conditions were initially mild and very dry, then rainy in summer, before becoming very dry again from mid-August. The trials were conducted as a randomised complete block design with 3 replicates (elementary plot size = 3 m x 9m). When the conditions were set (normality - Shapiro Wilk test - and homogeneity of variance - Bartlett test), an ANOVA followed by a Newman Keuls was performed to test significant differences between treatments. All tests were performed using XLstats. ANR and NFRV values were calculated as stated in Chapter 1. For each trial, different treatments were applied:

- A control without N fertilisation that enables the estimation of the soil N-mineralisation (*Control*);
- Synthetic mineral fertiliser (Basammon 26S) applied at incremental rates (30%X, 60%X and X, where X represents the N fertiliser advice) : *100%min, 60%min, 30%min*
- FR-AS applied at the same incremental rates (30%X, 60%X and X for, where X represents the N fertiliser advice) (*100%BBF, 60%BBF, 30%BBF*)

The winter soil N residues were analysed in February, before planting in order to calculate the N fertiliser advice. The recommended N application (X) was 176 kg N/ha in 2021, 150 kg N/ha in 2022 and 210 kg N/ha in 2023. Fertilisers characteristics and measured crop parameters are detailed in Tables 20 and 21.



**Figure 30.** Spreading of ammonium sulfate

**Table 20.** Fertiliser characteristics.

Fertiliser	Form	Dry matter (%)	pH	Total N (%)	N-NH <sub>4</sub> (%)	P <sub>2</sub> O <sub>5</sub> (%)	K <sub>2</sub> O (%)	SO <sub>3</sub> (%)
Triple superphosphate	Granular	NA	NA	0	0	45.2	0	0
Potassium sulphate	Granular	NA	NA	0	0	0	50	45
Potassium chloride	Granular	NA	NA	01.89	0	0	60	0
Ammonium nitrate (Ammonitrate)	Granular	NA	NA	27 (2021) 33.5 (2022)	27 (2021) 33.5 (2022)			
BBF Ammonium sulphate (FR-AS)	Liquid	19.8	1.9	4.4	4.4	0	0	12.6
Mineral N (Basammon 26s)	Granular	NA	NA	26	19	0	0	32.5

NA = not analysed

**Table 21.** Measured parameters in soil and crops

Description of measured parameters	Scale/unit
Plant vigour (Leaves / plot)	0 to 10
Photosynthetic activity (by using N tester) (Leaves / plot)	Transmittance in nm
Weight at harvest (Heart / 20 cabbages)	kg
Weight after trimming (Heart / 5 cabbages)	kg
Observation after cross section (Heart / 2 cabbages)	visual
Leaf N content	%/00 of dry weight
Soil N Residue after harvest 3 horizons in 2021 (0-30 cm; 30-60 cm; 60-90 cm), 2 horizons in 2022 and 1 horizon in 2023. The sampling depth depends on the depth of soil in the plot; less deep soil in 2022 and 2023)	Kg N/ha

### 3.7.3 Results and discussion

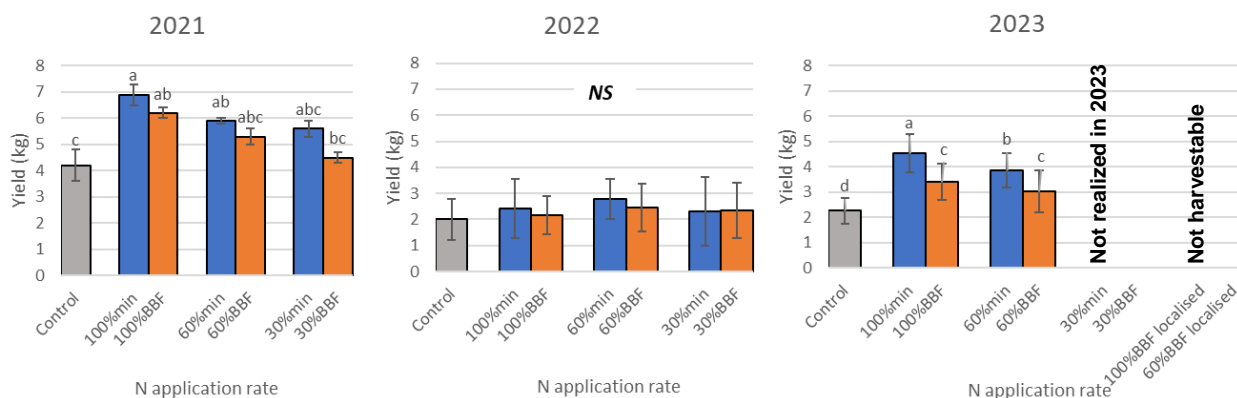
#### (i) Crop yield

To evaluate the effect of fertilisers on yield, the average weight of cabbages was calculated at the harvest for each treatment (Figure 31). In 2021 and 2023, a gradient was always observed between the average weight of the cabbage at harvest and the amount of N applied. In 2021, fertilisation with 100% and 60% BBF resulted in weights that were not significantly different from those of cabbages fertilised with 100% and 60% Mineral fertiliser. The treatments fertilised with 60% and 30% BBF did not differ significantly in weight from the unfertilised control. In 2023, the FR-AS treatments were significantly less productive than the synthetic mineral fertiliser treatment.

In 2022, the cabbages were smaller due to the dry and hot weather conditions during the summer. Usually, the average weight of a cabbage is 5 kg, whereas it was only 2.3 kg on average in this trial. Statistically, there were no significant differences in cabbage weight between treatments. With both mineral and BBF fertilisation,



there was no effect of the amount of N applied on cabbage weight. In overall, yields with BBF were slightly lower than those with synthetic mineral fertiliser. In 2023, 2 treatments with BBF ammonium sulphate in localised application were tested but were not conclusive. The localized applications on the cabbage generated burns which caused the death of the cabbages (yield=0) (Figure 32).



**Figure 31.** Average weight of cabbages (in kg) at the harvest for each treatment. Control ; 100%, 60% and 30%min = mineral fertilisation at 100%, 60% and 30% N crop needs ; 100%, 60% and 30%BBF = ammonium sulphate BBF fertilisation at 100%, 60% and 30% of N crop needs ; 100 and 60% BBF localised = ammonium sulphate BBF fertilisation in localised application. Small letters refer to statistical treatment ; NS= statistically not significant



**Figure 32.** Picture 1 = Cabbage burns due to ammonium sulphate application (June 21st, 2023) ; Picture 2: treatment with 100% BBF ammonium sulphate (September 21st, 2023) ; Picture 3: treatment with 100% localized application of ammonium sulphate (September 21st, 2023)

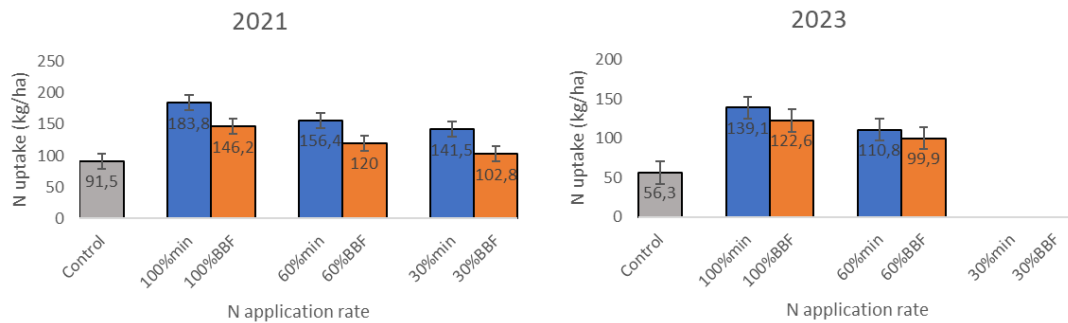
The cabbages were trimmed to evaluate the percentage of waste before processing in sauerkraut factories. For the 3 years, no significant differences were found on the percentage of waste between treatments. In 2021 and 2023, a gradient was observed between the dose of N applied and the vigour of the cabbages. For the 3 years, treatments with synthetic mineral fertiliser had better vigour than BBF treatments. Vigor scores are variable in 2022 and it is therefore difficult to draw conclusions. These observations are confirmed by measurements of photosynthetic activity using an N-tester, which produces the same trends.

(ii) N Uptakes

At a given level of N-fertilisation, there is no significant difference in N uptake between BBF ammonium sulphate plots compared to ammonium nitrate plots (Min) (Figure 33). The lowest N uptake values were obtained for Control, without N fertilisation. The highest values were obtained for synthetic mineral fertilisation

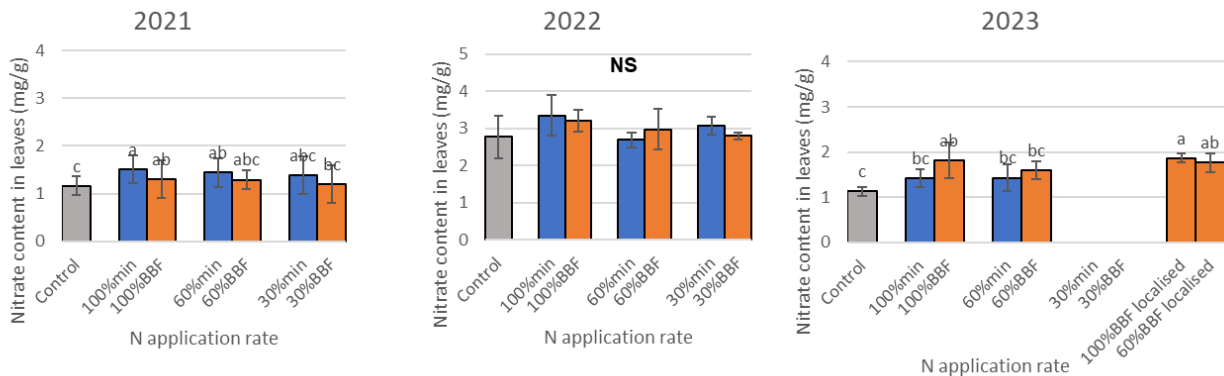


at the highest application rate. The N incremental rates generate slight incremental N uptake. In 2022, no significant differences were observed between control and BBF or mineral fertiliser



**Figure 33.** N Uptake. Control ; 100%, 60% and 30%min = mineral fertilisation at 100%, 60% and 30% N crop needs ; 100%, 60% and 30%BBF = ammonium sulphate BBF fertilisation at 100%, 60% and 30% of N crop needs ; 100 and 60% BBF localised = ammonium sulphate BBF fertilisation in localised application.

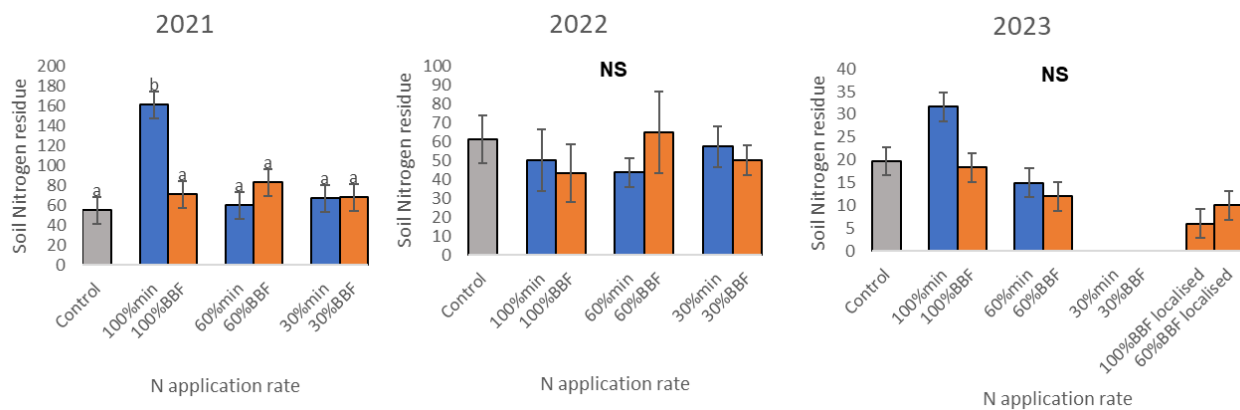
Fertilised treatments show higher nitrate concentrations in leaves than the control (significant differences in 2021 and 2023; not significant in 2022). Nevertheless, there are no significant differences between mineral fertilisation and BBF fertilisation (Figure 34).



**Figure 34.** Cabbage nitrate content in leaves. Control ; 100%, 60% and 30%min = mineral fertilisation at 100%, 60% and 30% N crop needs ; 100%, 60% and 30%BBF = ammonium sulphate BBF fertilisation at 100%, 60% and 30% of N crop needs ; 100 and 60% BBF localised = ammonium sulphate BBF fertilisation in localised application. Small letters refer to statistical treatment. NS=statistically not significant.

(iii) Nitrate leaching

Consequently, N incremental rates usually generate incremental soil residual N. Soil residual N are highly variable for the 3 years (Figure 35). In 2021, the very high values in the treatment with 100% synthetic mineral fertiliser resulted in a total residue of 161 kg N/ha, significantly higher than the other treatments. It may be due to sampling variability or analysis error, rather than the real leaching of N. Like the 60% and 30% synthetic mineral fertiliser, the ammonium sulphate fertilisation did not result in significantly higher N leaching than the unfertilised control. In 2023, there also tends to be more N residues in the treatment with 100% synthetic mineral fertiliser (like in 2021). In 2022, no significant differences were found on soil N residues between treatments.



**Figure 35.** Soil N residue on three horizons (0-30 cm; 30-60 cm; 60-90 cm) (in kg/ha). Control ; 100%, 60% and 30%min = mineral fertilisation at 100%, 60% and 30% N crop needs ; 100%, 60% and 30%BBF = ammonium sulphate BBF fertilisation at 100%, 60% and 30% of N crop needs ; 100 and 60% BBF localised = ammonium sulphate BBF fertilisation in localised application. Small letters refer to statistical treatment. Small letters refer to statistical treatment. NS=statistically not significant.

(ii) ANR and NFRV index

In 2021 and 2023, at equivalent dose applied on cabbages, ammonium sulphate efficiency is always lower than the synthetic mineral fertiliser efficiency (Table 22). NFRV is higher in 2023, than in 2021. In 2022, no significant differences were observed between control and BBF or mineral fertiliser for all the measured parameters. That is why these coefficients could not be calculated.

**Table 22.** ANR and NFRV values calculated for the different fertilised treatments in 2021.

Treatments	ANR 2021	NFRV 2021	ANR 2023	NFRV 2023
100%min	0.52		0.39	
60%min	0.62		0.43	
30%min	0.95		Treatment not tested in 2023	
100%BBF	0.31	0.59		0.8
60%BBF	0.27	0.44		0.8
30%BBF	0.22	0.23	Treatment not tested in 2023	

### 3.7.4 Conclusion and recommendation

In conclusion, ammonium sulphate is a source of N for cabbages. Nevertheless, it seems less efficient than synthetic mineral fertiliser at the same dose. In trends, yields with BBF are slightly lower than those with synthetic mineral fertiliser. N content in leaves and nitrate leaching are overall equivalent for synthetic mineral and BBF fertiliser. Nevertheless, N uptake and NFRV are lower with BBF than with synthetic mineral fertiliser.

In practice, the use of ammonium sulphate as an N fertiliser for cabbage could be limited by the technical feasibility of the application (more than 2000 L/ha required) and by the very high amount of sulphur applied (more than 700 kg with the organic product compared to 300 kg usually). In 2023 the localization of fertilisation is tested, to bring N as close as possible to the plants, to evaluate if it is possible to reduce the quantities of BBF applied. Two treatments were tested but were not conclusive. The localized applications generated burns which caused the death of the cabbages. In view of the damage caused by localized application of ammonium sulphate, this method of fertilisation is not an option for farmers.



### 3.8. Sugar beet field cultivation (CRAGE, France): FR-AS

For more information on this study, please contact the author from Chambre d'agriculture du Grand Est: Clement Munier ([clement.munier@grandest.chambagri.fr](mailto:clement.munier@grandest.chambagri.fr)).

#### 3.8.1 Introduction

Field trials were set-up in Champagne-Ardennes in 2021 and 2022 to test FR-AS. This BBF was produced by French pilot and was tested in sugar beet crops. The goals and hypothesis for FR-AS are the same as for cabbage trial (reported in section 3.6).

#### 3.8.2 Methodology

The field trials are located on the experimental platform of Terralab in Champagne-Ardennes (Latitude: 49.317387 / Longitude: 4.043506) on chalk soil (6.6 % clays / 11.2% loam / 5.9 % sands ; Organic matter: 3.3% ; pH: 8.3) (Figure 36). Weather conditions were relatively different in 2021 and 2022. In 2021, the mild but not scorching conditions in summer and the frequent rains allowed a good development of the crops. In 2022, there was very sun and dry conditions in spring and summer. The trials were conducted as a randomised complete block design with 3 replicates (elementary plot size = 5.4 x 8 m). When the conditions were set (normality - Shapiro Wilk test - and homogeneity of variance - Bartlett test), an ANOVA followed by a Newman Keuls was performed to test significant differences between treatments. All tests were performed using XLstats. ANR and NFRV values were calculated as stated in Chapter 1. The experimental set-up included several treatments with 3 replicates:

- A control without N fertilisation.
- 2 treatments with synthetic mineral fertiliser (*Ammonium nitrate 27 or 33.5%*) applied at incremental rates (50%X, and X, where X represents the N fertiliser advice): 50% min, 100% min
- 2 treatments with bio-based ammonium sulphate (*BBF*) applied at incremental rates (50%X, and X, where X represents the N fertiliser advice): 50% BBF, 100% BBF.

The recommended N application (X) was 120 kg N/ha in 2021, and 90 kg N/ha in 2022. Fertilisers characteristics and measured crop parameters are detailed in Tables 23 and 24.

**Table 23.** Fertiliser characteristics.

Fertiliser	Form	Dry matter (%)	pH	Total N (%)	N-NH <sub>4</sub> (%)	P <sub>2</sub> O <sub>5</sub> (%)	K <sub>2</sub> O (%)	SO <sub>3</sub> (%)
Ammonium nitrate	Granular	NA	NA	27 (2021) 33.5 (2022)	27 (2021) 33.5 (2022)			
BBF (FR-AS)	Liquid	19.8	1.9	4.4	4.4	0	0	12.6
Mineral N (Basammon 26s)	Granular	NA	NA	26	19	0	0	32.5

NA = not analysed

**Table 24.** Measured parameters in soil and crops (sugar beets).

Description of measured parameters	Scale/unit
Population	Plants/ha
Yield	t/ha
saccharine richness	%
Soil N Residue after harvest	Kg N/ha
Leaf and root N content	g/kg
Leaf and root K content	g/kg
Leaf and root P content	g/kg

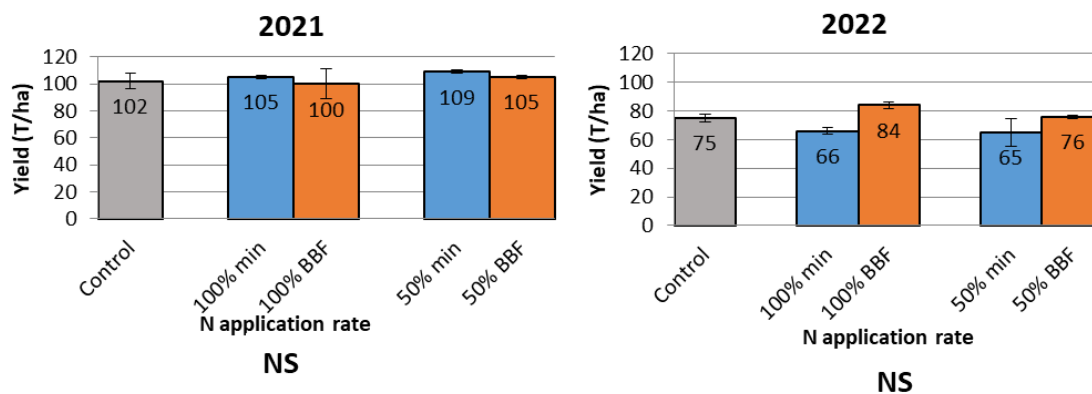


**Figure 36.** Location of the sugar beet test site (left) and beet leaf analysis (right).

### 3.8.3 Results and discussion

#### (i) Crop yield

At a given level of NPK -fertilisation, there were no significant differences in sugar beets yield in 2021 and 2022 (Figure 37). There were no differences with control either. In 2021 and 2022, N was not the limiting factor and it raises the question of N efficiency for this crop.

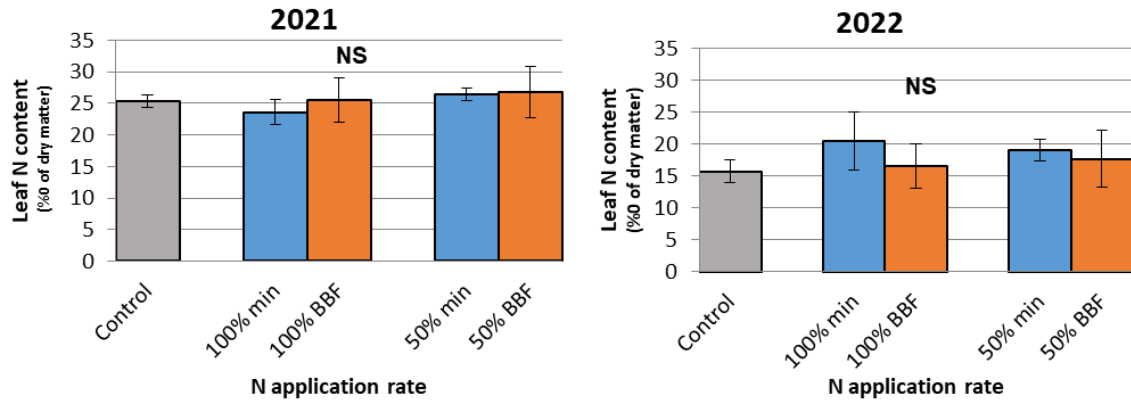


**Figure 37.** Sugar beets yield in t/ha for the N P K treatments. Control = unfertilised control (without N or PK); 50%N mineral and 100% mineral = N mineral fertilisation at 100% and 50% crop needs; 50% and 100% ammonium sulphate = BBF ammonium sulphate fertilisation at 100% and 50% of crop needs. NS=statistically not significant.

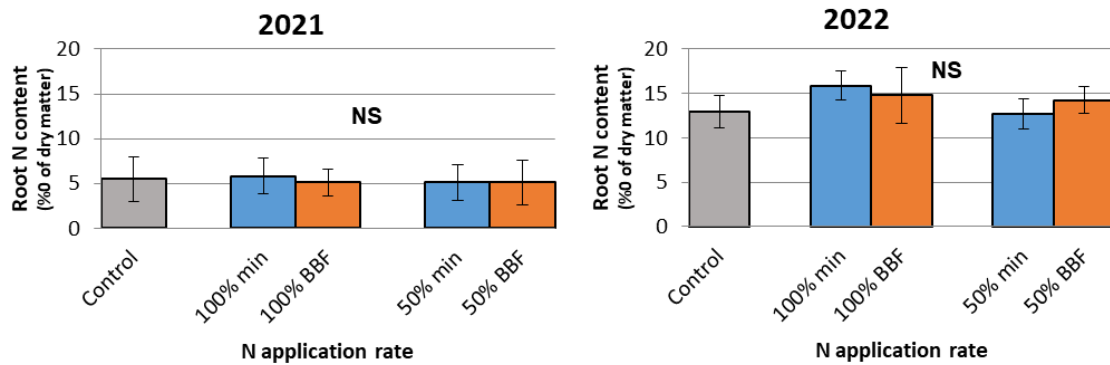
In 2021, no significant difference in saccharin content was observed between mineral and bio-based fertilisation. The saccharin content on the 100% BBFs seems to be slightly higher compared to the other treatments, but not significant. For sugar beet, good value depends on yield and sugar content. A high sugar content is required (over 16%). In 2022, saccharin content was abnormally low (13-14%, instead of 16%). This phenomenon may be due to an excess of N. However, the winter soil N residue with 72.87 kg N/ha on 120 cm and the dose applied were not excessive. Given the level of saccharin content, the validity of the trial is questionable.

#### (ii) Leaf and root N content

In 2021, the treatments with the highest N doses of synthetic mineral fertiliser appear to have slightly higher N leaf content. It's the opposite in 2022 (Figure 38). There is no trends for roots N content (Figure 39). Nevertheless, there is no significant differences in nitrate concentration on beet leaves and roots.



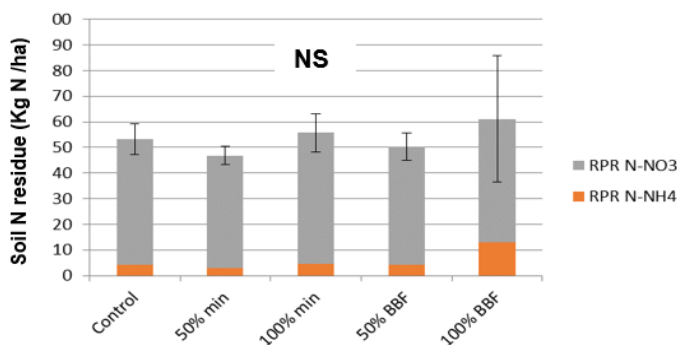
**Figure 38.** Amount of N in leaves in % of dry matter. Control ; 100% and 50%min = mineral fertilisation at 100%, and 50% N crop needs ; 100% and 50%BBF = ammonium sulphate BBF fertilisation at 100% and 50% of N crop needs. NS=statistically not significant



**Figure 39.** Amount of N in roots in % of dry matter. Control ; 100% and 50%min = mineral fertilisation at 100%, and 50% N crop needs ; 100% and 50%BBF = ammonium sulphate BBF fertilisation at 100% and 50% of N crop needs. NS=statistically not significant.

(iii) Nitrate leaching

In 2021, treatments with 100% Nmin or 100% ammonium sulphate seem to leave more residues than treatments with 50% Nmin or 50% ammo sulphate. But no significant difference in soil N residue was observed between mineral and bio-sourced fertilisation. (Figure 40). In 2022, no soil N residue was sampled after harvest (technical issues).



**Figure 40.** Soil N residue after harvest (2021). 100% and 50%min = mineral fertilisation at 100%, and 50% N crop needs ; 100% and 50%BBF = ammonium sulphate BBF fertilisation at 100% and 50% of N crop needs. NS=statistically not significant.

(iv) ANR and NFRV index

Calculations of ANR, NFRV, APR and PFRV are explained in the introduction (Chapter 1). These calculations are based on a difference from the control. But for the 2021 and 2022 trials, no significant differences were observed between the control and BBF or mineral fertiliser for all the measured parameters. That is why ANR, NFRV, APR and PFRV cannot be calculated for these trials.

### 3.8.4 Conclusion and recommendation

No significant differences were observed between the control and FR-AS or mineral fertiliser for all the measured parameters. In 2021 and 2022, N was not the limiting factor and it raises the question of N efficiency for this crop. It is difficult to draw robust conclusions for this crop. There were strong effects of dry conditions in 2022. In practice, the use of ammonium sulphate for sugar beet could be limited by the technical feasibility of the application (more than 2000L/ha required). The 2 years of experimentation did not produce convincing results, hence the trial was not repeated in 2023.

## 3.9. Potato field cultivation (CA80, France): FR-AS

For more information on this study, please contact the authors from *Chambre d'agriculture de la Somme*: Pierre-Baptiste Blanchant ([pb.blanchant@somme.chambagri.fr](mailto:pb.blanchant@somme.chambagri.fr)) and Matthieu Preudhomme ([m.preudhomme@somme.chambagri.fr](mailto:m.preudhomme@somme.chambagri.fr)).

### 3.9.1 Introduction

Potatoes are the most representative crop in the north of France. Since it is a crop with high N demands, it was chosen to evaluate the short term response to ammonium sulphate performance (FR-AS) in 2021 and 2022. The research hypotheses were the following:

- For a given level of N fertilisation, there is no difference in yield and N uptake between plots fertilised with FR-AS and those fertilised with synthetic mineral fertiliser.
- For a given level of N fertilisation, there is no difference in N loss on the soil through leaching between plots fertilised with FR-AS and those fertilised with synthetic mineral fertiliser.

### 3.9.2 Methodology

The potatoes trial was located in Aizecourt le Haut (Somme department, Hauts de France) in the Ferme 3.0 (pilot farm for many trials) on a clay loam soil (pH=8.2; Org C=11.3 g/kg; ; CaCO<sub>3</sub><10 g/kg; P<sub>2</sub>O<sub>5</sub>=85 mg/kg and K<sub>2</sub>O=239 mg/kg). The average temperatures range from 0.6 to 5.5°C in January (coldest month) and from 12.5 to 23.5°C in August (warmest month). In 2021, precipitation was normal with 788 mm, but high during summer. And the year 2022 was marked by high maximum temperatures in April and May (2°C – 3°C higher than in an average year) and low rainfall. The trial was conducted as a randomised complete block design with 4 replicates (elementary plot size = 3.6 m x 10 m (360 m<sup>2</sup>). After soil preparation (Table 25), 7 fertiliser treatments were applied within each block in April (2021 and 2022):

- A control without N-fertilisation that enables the estimation of the soil N-mineralisation: C;
- Three treatments with synthetic mineral fertiliser (Min) applied at incremental rates (30%X, 60%X and X, where X represents the N fertiliser advice): *Min-30*, *Min-60*, *Min-100*;
- Three treatments with bio-based ammonium sulphate (BBF) applied at incremental rates (30%X, 60%X and X, where X represents the N fertiliser advice): *BBF-30*, *BBF-60*, *BBF-100*;

**Table 25.** Soil N residue and N fertiliser advice in 2021 and 2022

	2021	2022
<b>Soil N residue before planting</b>	51 kg/ha	58 kg/ha
<b>Recommended N balance dose to be applied (X)</b>	170 kg/ha (the crop needs 230 kg/ha)	185 kg/ha (the crop needs 230 kg/ha)

The used reference synthetic mineral fertiliser was a pure ammonium nitrate in solution form (39% N). No complement on P or K was made since the soil had already the necessary supplies. The product characterisation can be found in Table 26 and the N fertilisation scheme by treatment in Table 27.

**Table 26.** Physio-chemical characterisation of fertilisers applied on the field trials in 2021 and 2022.

Fertiliser	Dry matter (%)	pH	Total N content (%)	NH <sub>4</sub> content (%)	NO <sub>3</sub> -content (%)	Urea N content (%)	P <sub>2</sub> O <sub>5</sub> content (%)	K <sub>2</sub> O content (%)
<b>Synthetic mineral fertiliser (solution N39)</b>	NA	6.5	39	9.75	9.75	18.5	0	0
<b>BBF (FR-AS)</b>	19.8	1.9	4.68	4.68	0	0	0	0

NA – not analysed

**Table 27.** Fertilisation scheme for potatoes trial and the corresponding quantity of N applied for each treatment.

Treatment	N source	2021		2022	
		The quantity applied (kg/ha or l/ha)	N dose (kg/ha)	The quantity applied (kg/ha or l/ha)	N dose (kg/ha)
C	None	0	0	0	0
Min-30	Ammonium nitrate	131	51	142	55.5
Min-60	Ammonium nitrate	261	102	285	111
Min-100	Ammonium nitrate	435	170	474	185
BBF-30	Ammonium sulphate (FR-AS)	1090	51	1427	55.5
BBF-60	Ammonium sulphate (FR-AS)	2188	102	2854	111
BBF-100	Ammonium sulphate (FR-AS)	3638	170	4757	185

Ammonium nitrate and ammonium sulphate (FR-AS) were applied using an experimental sprayer just before ridging to avoid N losses through volatilisation. Potatoes were harvested on the 20<sup>th</sup> September 2021 and on the 13<sup>th</sup> September 2022 (Figure 41). All the harvest was realised manually.



**Figure 41.** Field trial just before the harvest after aerial biomass was removed (left) and potato harvest (right).

Before planting, a complete soil analysis was carried out to make sure that there were no other limiting factors that would bias the trial. A water balance controls irrigation throughout the cycle to ensure that the plant is always hydrated. Yields were calculated for each elementary plot and averaged (2 rows (1.8m) on 8 meters was sampled for each microplot). Fresh weight, dry matter content (with specific tool on CA80, densimetric method) and N content of the tubers were measured in the laboratory for each sample. N uptake was obtained



by multiplying the N content of tubers by dry matter yield. N short term response for the crop was evaluated using the ANR and NFRV index for which equations are presented before (Chapter 1).

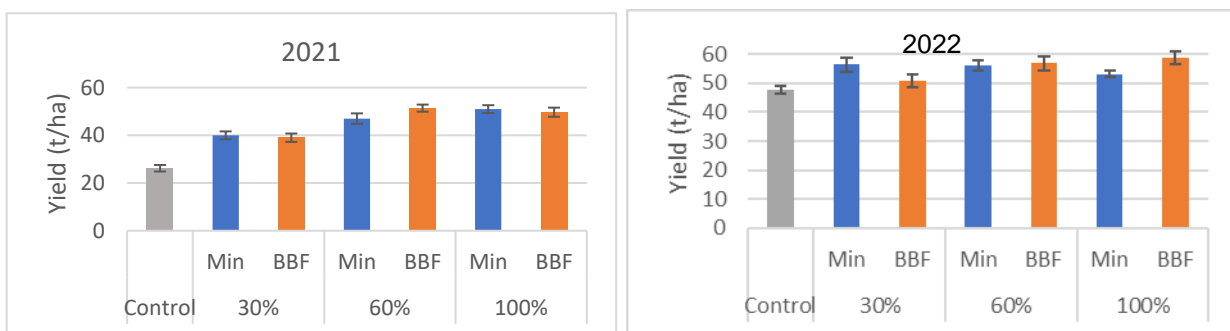
After harvest, soil N content (ammonium and nitrate N) was measured (end of October), before winter N leaching, from 0 cm to 90 cm depth. N balance was calculated to provide additional information on N mineralisation or environmental losses (through leaching and/or volatilisation), which could have an impact on the ANR and the NFRV values. For each factor, the results are the average of the 4 replications. The software used to process the statistical data is Smartbox (ANOVA and Tukey test).

### 3.9.3 Results and discussion

#### (i) Potatoes yield and N-uptake

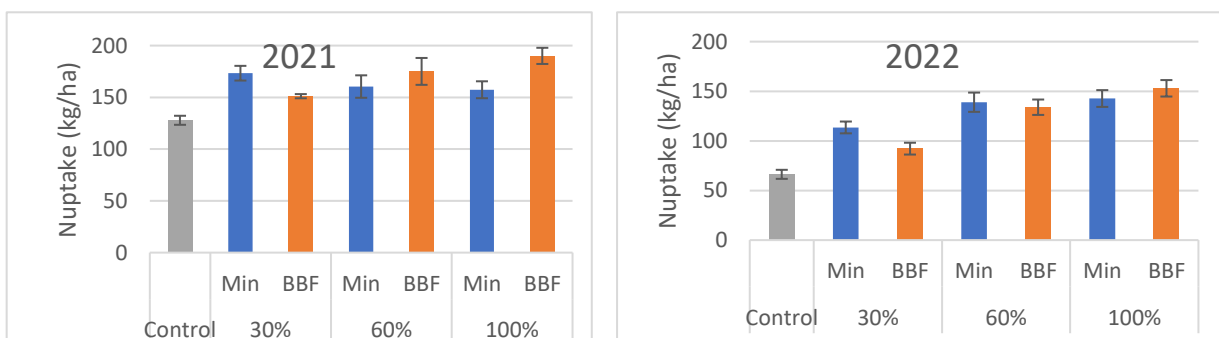
Potatoes average yield in the region is usually around 45 tonnes/ha (with irrigation). In 2021, satisfactory climatic conditions enabled the normal growing of potatoes, with less irrigation (very little water added). In 2022, with irrigation and high temperatures, the yield was very high.

For this experiment, the average yield was 43.5 t/ha (26.2 t/ha to 51.4 t/ha) in 2021 and 54.3 t/ha (47.9 t/ha to 58.8 t/ha) in 2022. In both years, at the same level of N fertilisation, there were no significant differences in crop yields between plots fertilised with ammonium sulphate BBF compared to plots fertilised with synthetic mineral fertiliser (Min). However, in 2021, there was a significant difference depending on the amount of N applied. A yield response curve could be observed for the amount of N applied (Figure 42).



**Figure 42.** Potatoes N-uptake for the different treatments (average +/- standard deviation ; n=4.) MIN – Synthetic mineral fertiliser ; BBF – Biobased fertiliser FR-AS ; X represents 30%, 60% and 100%– N fertiliser advice for potatoes (170 kg/ha in 2021 & 185 kg/ha in 2022).

At a given level of N-fertilisation, there is no significant difference in N uptake between BBF ammonium sulphate plots compared to ammonium nitrate plots (Min) (Figure 43). However, in 2021 there was a significant difference depending on the amount of N applied.



**Figure 43.** Potatoes N-uptake (kg/ha) for the different treatments (average +/- standard deviation ; n=4. Min – Synthetic mineral fertiliser ; BBF – Biobased fertiliser FR-AS ; X represents 30%, 60% and 100%– N fertiliser advice for potatoes (170 kg/ha in 2021 & 185 kg/ha in 2022).

The lowest N uptake values were obtained for Control, without N fertilisation (66.27 kg/ha in 2021 and 127.86 kg/ha in 2022). The highest values were obtained for BBF at the highest application rate (152.96 kg/ha in 2021 and 190.07 in 2022).

(ii) ANR and NFRV index

ANR is quite similar between mineral fertiliser and BBF (difference only for x = 30% (Table 28). So both products are assimilated in the same way. It is possible to conclude that both products have the same efficiency in terms of assimilation by the plant. Regarding NFRV, it increases when N dose applied increases, as expected in our prerequisites. For the X dose, a NFRV value around 1 indicates that BBF can replace the synthetic mineral fertiliser.

**Table 28.** ANR and NFRV values calculated for the different fertilised treatments.

Treatment	ANR 2021	ANR 2022	NFRV 2021	NFRV 2022
Min-30	0.79	0.82		
Min-60	0.64	0.50		
Min-100	0.38	0.16		
BBF-30	0.43	0.42	0.55	0.51
BBF-60	0.56	0.43	0.89	0.86
BBF-100	0.43	0.35	1.13	2.11

### 3.9.4 Conclusion and recommendation

In both years, at the same level of N fertilisation, there were no significant differences (although the results are more marked in 2021) in crop yields between plots fertilised with ammonium sulphate BBF compared to plots fertilised with synthetic mineral fertiliser (Min). BBF ammonium sulphate can replace ammonium nitrate.

## 3.10. Ryegrass pot cultivation (RITTMO, France): FR-AS

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

### 3.10.1 Introduction

This experiment compared the N bioavailability of BBF generated from the French pilot: ammonium sulphate (FR-AS), to raw manure pig slurry (PS) and mineral N reference (calcium ammonium nitrate: CAN). The final goal was to determine the NFRV of FR-AS on the Italian ryegrass in pot cultivation.

### 3.10.2 Methodology

A pot experiment was conducted using agricultural soil. The test plant was ryegrass grown in pots of 2 kg of dried-air soil. The soil characteristics are given in Table 29. Mineral fertilisation was provided to ensure that solely N is the limiting plant nutrient in the system. Then pots were sowed with Italian ryegrass seeds (at a seeding density of 2 g per pot). The pots were placed in cultivation greenhouses. Pots were watered in such a way that the humidity was optimal. Studied treatments (5 replicates) were control: unfertilised soil, FR-AS, pig slurry and CAN (Table 30). For each product three fertilisation rates were used: 30% (51 kg/ha); 60% (102 kg/ha), 100% (170 kg/ha). The ryegrass was harvested 3 times during the 14 weeks trial to deplete the





substrate and evaluate the amount of biomass produced. The biomass was dried at 40°C for 4 days and later analysed to quantify the amount of N in the biomass. ANR and NFRV were calculated as stated in Chapter 1.

**Table 29.** Soils characteristics of standard and agricultural soil.

Parameters	Units	Agricultural soil
pH water	-	6.4
Cation exchange capacity	(meq/100g)	ND
Organic matter	(%)	5.49
Total carbon	(%)	ND
Organic carbon	(%)	3.17
C/N	-	9.70
Total N	(%)	0.33
N-NH <sub>4</sub>	(mg/kg)	ND
N-NO <sub>3</sub>	(mg/kg)	ND
Total P <sub>2</sub> O <sub>5</sub>	(%)	0.01
Available P (P-Olsen)	(mg/kg)	50
K <sub>2</sub> O	(%)	ND
MgO	(%)	ND
SO <sub>3</sub>	(%)	ND
CaO	(g/kg)	<0.1
Exchangeable K <sub>2</sub> O	(g/kg)	0.32
Exchangeable MgO	(g/kg)	0.014

ND: not determined.

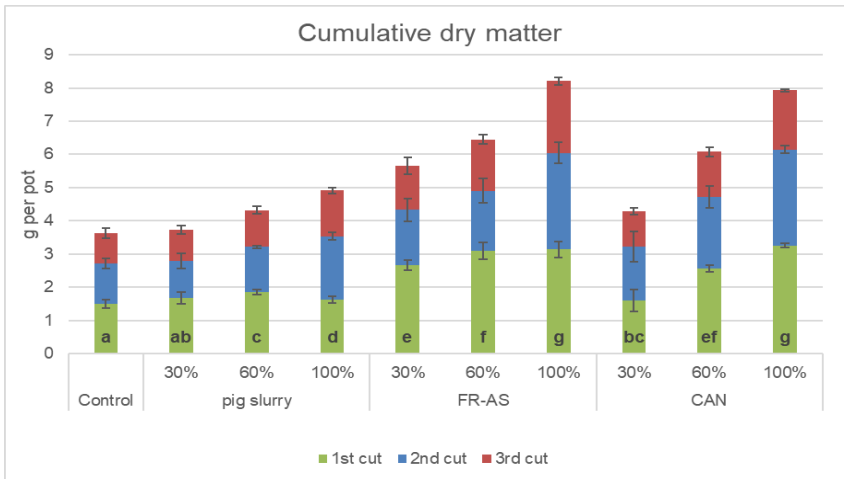
**Table 30.** Products characteristics on (g/kg) on fresh weight basis.

Parameters	FR-AS	Pig slurry	CAN
Dry matter	207	13.3	9980
Total carbon	0.0	2.7	ND
Total N	43.8	1.8	160
NH <sub>4</sub> -N	43.8	1.4	10*
NO <sub>3</sub> -N	0.0	<0.2	150*
Total P	0.0	0.11	0.0
Total potassium	0.0	2.57	0.0
Total sulphur	163.5	0.12	0.0

ND: not determined.

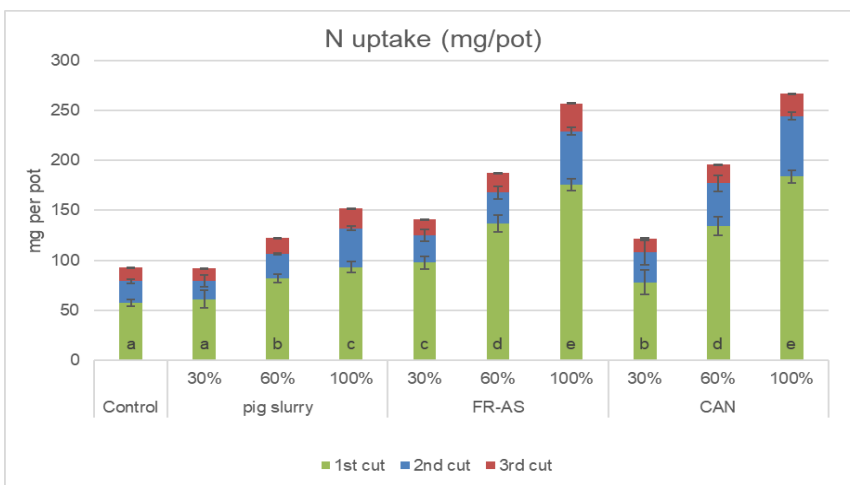
### 3.10.3 Results and discussion

For the dry matter of ryegrass (Figure 44) the ANOVA test showed that cumulated dry matter yield was not significantly different in pots fertilised with FR-AS and CAN, except when combined with the 30% N treatment, in which FR-AS 30% produced more dry biomass (+32%). All treatments are significantly different from the control except of PS 30%. For all treatments, we can observe a dose response, with an increase in dry biomass production as the N dose increases. Plant N uptake (Figure 45), as measured by dry matter biomass, increases with the dose of N supplied by the tested products. There is a significant difference between dose applications, but no difference was observed between FR-AS and CAN.



**Figure 44.** 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.

FR-AS and CAN present a very good ANR with a value equal to or higher than 100%, regardless of dosage application, except ANR for CAN 30% (Table 31). This could be due to the form of the product. CAN comes in the form of solid granules. It is possible that these granules took time to dissolve in the soil, and that even at the lowest dose, i.e. 30%, the concentration of N available in the soil was insufficient for the growth of the ryegrass. The harvested dry matter and N export are significantly higher in the FR-AS 30% modality than in the CAN 30%. This difference fades away at 60% and 100% doses. Concerning NFRV (Table 31), results have shown that FR-AS presents fertiliser effect similar to CAN with FR-AS 60% and FR-AS 100% exhibiting NFRVs of 92% and 94% respectively.



**Figure 45.** N uptake (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.

**Table 31.** Results of ANR and NFRV calculation.

Treatments		ANR	NFRV
Pig slurry	30%	-2%	-
	60%	31%	-
	100%	38%	-
FR-AS	30%	103%	166%
	60%	100%	92%
	100%	105%	94%



FERTIMANURE

CAN	30%	62%	-
	60%	110%	-
	100%	111%	-

#### 3.10.4 Conclusion and recommendation

For tested treatments, results demonstrated that FR-AS presents N fertiliser efficiency at least equivalent that of mineral reference CAN. This is not surprising because it is also a mineral BBF and in liquid form. Moreover, FR-AS also provides sulphur which is a secondary fertiliser element.

## 4. Biochar

### 4.1. Potato field cultivation (CA80, France): FR-BC

For more information on this study, please contact the authors from Chambre d'agriculture de la Somme: Pierre-Baptiste Blanchant ([pb.blanchant@somme.chambagri.fr](mailto:pb.blanchant@somme.chambagri.fr)) and Matthieu Preudhomme ([m.preudhomme@somme.chambagri.fr](mailto:m.preudhomme@somme.chambagri.fr))

#### 4.1.1 Introduction

In 2021, CA80 evaluated biochar performance (FR-BC) like a P fertiliser. The main objective of this trial was to evaluate the phosphorous short-term bioavailability of the biochar (FR-BC) compared to a synthetic mineral fertiliser. The research hypotheses were as follows :

- There is no difference in yield at a given level of P fertilisation between plots fertilised with BBF Biochar and plots fertilised with synthetic mineral fertiliser.
- There is no difference in P uptake at a given level of P fertilisation between BBF biochar fertilised plots and plots fertilized with synthetic mineral fertiliser.

#### 4.1.2 Methodology

The trial was carried out on the potato crop, one of the most representative crops in the sector due to the added value it generates and the presence of a very favourable soil. This crop is generally fertilised with synthetic mineral fertiliser. The variety used was “Hermes”, suitable for the industrial market (processing into chips). The trial was located at Aizecourt-le-Haut (Somme department, Haut de France) in the Ferme 3.0 (a pilot farm for many trials on a clay-limestone soil with low levels of available P (30 ppm P<sub>2</sub>O<sub>5</sub> Olsen method). Average temperatures range from 0.6 to 5.5 °C in January (coldest month) and from 12.5 to 23.5°C in August (warmest month). In 2021, the total precipitation was normal with 788 mm, but with a different distribution on the year (high during summer). The products characterisation can be found in Table 32 and the N fertilisation scheme by treatment in Table 33.

**Table 32.** Fertilisation applied according to tested treatments.

Treatment	Source of P	Quantity applied (kg/ha)	P <sub>2</sub> O <sub>5</sub> Dose (Kg/ha)
Control	None	0	0
S45 – 30% X	Triple superphosphate	104	47
S45 – 60% X	Triple superphosphate	209	94
S45 – 100% X	Triple superphosphate	347	156
BBF – 30% X	BBF Biochar (FR-BC)	927	47
BBF – 60% X	BBF Biochar (FR-BC)	1854	94
BBF – 100% X	BBF Biochar (FR-BC)	3077	156

**Table 33.** Fertilisers characteristics used for the trial in 2021

Fertiliser	Dry matter (%)	pH	Total N content (%)	P <sub>2</sub> O <sub>5</sub> content (%)	K <sub>2</sub> O content (%)
Triple superphosphate S45	NA	NA	0	45	0
Biochar (FR-BC)	97.7	NA	2.07	5.07	8.82

NA: not analysed

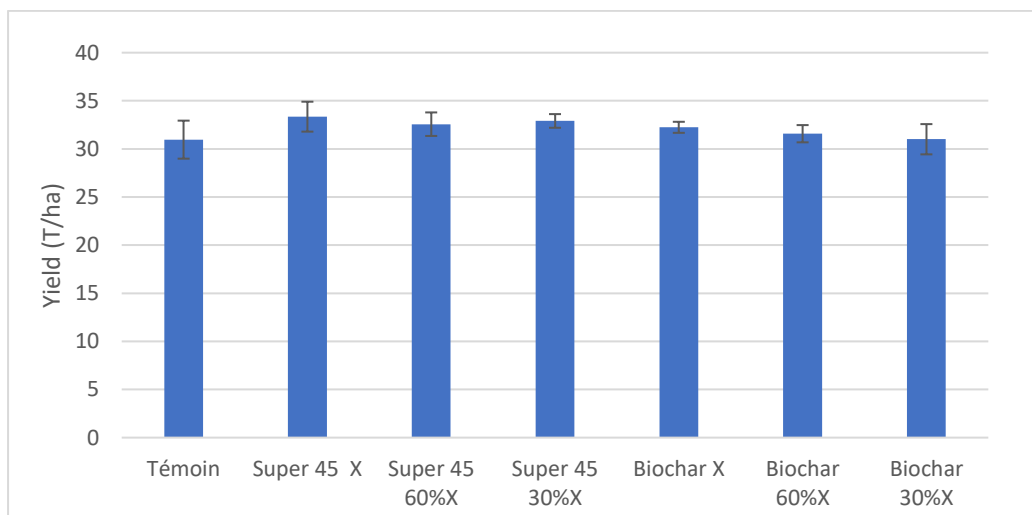
To ensure that N is not a limiting factor 200 kg/ha of N39 solution was applied before planting as recommended. Potatoes were harvested manually on the 20<sup>th</sup> of September in 2021. Yields were calculated on each microplot

and averaged (2 rows on 8 meters were sampled for each microplot). Fresh weight, dry matter content and N content of the tubers were evaluated in the laboratory for each sample.

For each factor, the results are the average of the 4 replications. The software used to process the statistical data is Smartbox. P-uptake is obtained by multiplying the P content in the tubers by the dry matter yield per hectare. Yield data and calculated P-uptake data of the different treatments were compared. P short-term response (APR and PFRV) to the crop is evaluated according to the equations stated in Chapter 1.

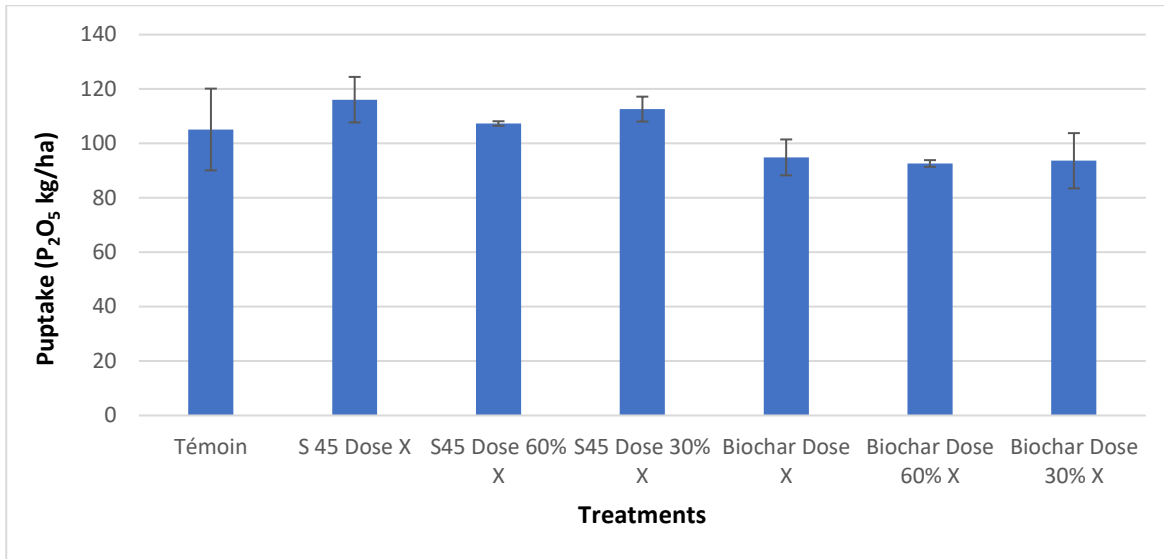
#### 4.1.3 Results and discussion

Figure 46 shows yield as a function of the type of fertiliser applied and dose. Yields varied between 31 t/ha (Control/Témoin) and 33.4 t/ha (Super 45 X). The variety obtained low results in terms of yield, well below the expected target of 45 t/ha. The FR-BC led to slightly lower yields than mineral P treatments (-1,2 t/ha for dose X, -1 t/ha for dose 60%X at -1,9 t/ha). Moreover, there were no significant differences between the different treatments.



**Figure 46.** Gross yield (t/ha) for the different treatments (average +/- standard deviation ; n=4. The differences between means are not statistically significant (p-value>0.05) Témoin – Control, Super45 – Synthetic mineral fertiliser, Biochar – Biobased fertiliser FR-BC, X – P fertiliser advice for potatoes (156 Kg/ha).

P uptake is approximately the same between treatments (Figure 47). Differences between treatments are not statistically different (Bonferonni & Newman-Keuls test). APR of BBF is low (and negative) in this trial, indicating that BBF would not be suitable to supply P to the crop during crop growth. APR is close to 0, so there is no luxury consumption of P in this test.



**Figure 47.** P-uptake (kg/ha) for different treatments (average +/- standard deviation ; n=4. The differences between means are not statistically significant (p-value>0.05) Témoin – Control, Super45 – Synthetic mineral fertiliser, Biochar – Biobased fertiliser FR-BC, X – P fertiliser advice for potatoes (156 Kg/ha).

**Table 34.** APR and PFRV of tested treatments.

Treatment	P <sub>2</sub> O <sub>5</sub> dose (kg/ha)	P uptake (kg)	APR	PFRV
Control	0	105.12		
S45 (Super45) X	156.75	116.08	0.07	
S45 (Super45) 60%X	94.05	107.31	0.02	
S45 (Super45) 30%X	47.03	112.62	0.16	
BBF X	156.75	94.88	-0.07	-0.94
BBF 60%X	94.05	92.63	-0.13	-5.71
BBF 30%X	47.03	93.64	-0.24	-1.53

#### 4.1.4 Conclusion and recommendation

For the same P fertilisation level, biochar led to slightly lower yields than synthetic P treatments, but the differences were not statistically different. The APR of BBF is low in this experiment, indicating that BBF would not be suitable for supplying P to crops during plant growth. However, the low average yields obtained in this trial do not allow to conclude on P efficiency of FR-AS in potatoes (even when using soil with low P levels). We tried to use biochar as a positive response in N retention capacity in 2022 but there were no differences between treatments with and without biochar.



## 4.2. Sauerkraut cabbage field cultivation (CRAGE, France): FR-BC

For more information on this study, please contact the author from Chambre d'agriculture du Grand Est: Clement Munier ([clement.munier@grandest.chambagri.fr](mailto:clement.munier@grandest.chambagri.fr)).

### 4.2.1 Introduction

The biochar (FR-AS) was tested in 2022 in Alsace under the same experimental conditions as in section 3.7. The FR-AS was also tested on sugar beet, and these results are reported in section 4.3. The FR-AS was produced by French pilot. The main goals of the sauerkraut cabbage trials were:

- To assess the short-time crop response to P for FR-AS and compare it with the response for synthetic mineral fertiliser (Super 46 (46%P<sub>2</sub>O<sub>5</sub>)).
- To assess the impact of FR-AS on crop P balance

The research hypotheses were the following:

- At a given level of P-fertilisation, there is no difference in crop yields and P-uptake between plots fertilised with BBF fertilisers compared to a reference treatment fertilised with synthetic mineral fertiliser.
- At a given level of P-fertilisation, there is no difference in P-environmental losses by leaching between plots fertilised with BBF compared to a reference treatment fertilised with synthetic mineral fertiliser.

### 4.2.2 Methodology

The field trials were located in Alsace on 3 different sites on loamy, sandy-clay soil (around 33 % clays / 33 % loam / 33 % sands ; Organic matter ~ 4 % ; pH~7-8 depending on the sites). Weather conditions were very different for the 3 years. In 2021 after planting, average temperatures were close to 15 degrees and rainy periods in spring and summer were frequent with low intensity (except in June and July). In 2022, spring and summer were very sunny and dry. In 2023, weather conditions were initially mild and very dry, then rainy in summer, before becoming very dry again from mid-August. The trials were conducted as a randomised complete block design with 3 replicates (elementary plot size = 3m x 9m). When the conditions were set (normality - Shapiro Wilk test - and homogeneity of variance - Bartlett test), an ANOVA followed by a Newman Keuls was performed to test significant differences between treatments. All tests were performed using XLstats. APR and PFRV values were calculated as stated in Chapter 1. Fertilisers characteristics and measured crop parameters are detailed in Tables 35 and 36. The following treatments were applied:

- A control without P fertilisation (*Control A*);
- Two treatments with Biochar and ammonium sulphate were applied in 2022 at incremental rates (60%X and X, where X represents the N fertiliser advice + 2T/ha Biochar)

**Table 35.** Fertiliser characteristics

Fertiliser	Dry matter (%)	pH	Total N content (%)	P <sub>2</sub> O <sub>5</sub> content (%)	K <sub>2</sub> O content (%)
Triple superphosphate S45	NA	NA	0	45	0
Biochar (FR-BC)	97.7	NA	2.07	5.07	8.82

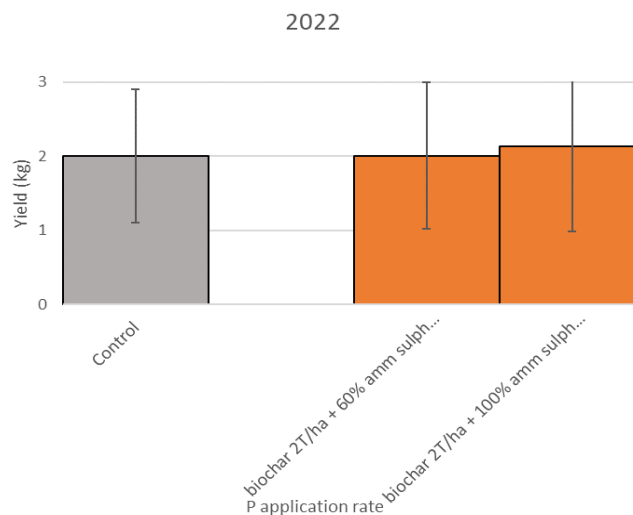
NA = not analysed

**Table 36.** Measured parameters in soil and crops

Description of measured parameters	Scale/unit
Plant vigour (Leaves / plot)	0 to 10
Photosynthetic activity (by using N tester) (Leaves / plot)	Transmittance in nm
Weight at harvest (Heart / 20 cabbages)	kg
Weight after trimming (Heart / 5 cabbages)	kg
Observation after cross section (Heart / 2 cabbages)	visual
Leaf N content	%00 of dry weight
Soil N Residue after harvest 3 horizons in 2021 (0-30 cm; 30-60 cm; 60-90 cm), 2 horizons in 2022 and 1 horizon in 2023. The sampling depth depends on the depth of soil in the plot; less deep soil in 2022 and 2023)	Kg N/ha

#### 4.2.3 Results and discussion

In 2022, the cabbages were smaller due to the dry and hot weather conditions during the summer. Usually, the average weight of a cabbage is 5 kg, whereas it was only 2.3 kg on average in this trial. Statistically, there was no significant difference in cabbage weight between treatments. Biochar applications did not have an effect on crop yield (Figure 48). A biochar dose of 2t/ha is probably too low to have an effect on yield.



**Figure 48.** Average weight of cabbages (in kg) at the harvest for each treatment. Control; 60% amm sulph + Biochar 2t/ha = 60% ammonium sulphate + Biochar 2t/ha ; 100% amm sulph + Biochar 2t/ha = 100% ammonium sulphate + Biochar 2t/ha.

No significant effect of fertilisation on P content in cabbage leaves or in vigour were found. Measurements of photosynthetic activity using an N-tester show a slight effect of biochar combined with ammonium sulphate, but no significant. No significant differences were observed between control and BBF or mineral fertiliser for all the measured parameters. That is why APR and PFRV could not be calculated.

#### 4.2.4 Conclusion and recommendation

Finally, whatever the variable observed, no effect of biochar could be observed under the trial conditions. A biochar dose of 2t/ha is probably too low to have an effect on cabbages. Weather conditions were not favourable for cabbage development in 2022. N and P were very poorly valorised, and it would have been necessary to continue the experiment for another year.

### 4.3. Sugar beet field cultivation (CRAGE, France): FR-BC

For more information on this study, please contact the author from Chambre d'agriculture du Grand Est: Clement Munier ([clement.munier@grandest.chambagri.fr](mailto:clement.munier@grandest.chambagri.fr)).

#### 4.3.1 Introduction

Field trials were set-up in Champagne-Ardennes only in 2022 to test biochar (FR-BC) (Figure 49). FR-BC was tested under the same experimental conditions as in part 3.8. The tested BBF was produced by French pilot. The main goals of the sauerkraut cabbage trials were:

- To assess the short-time crop response to P for BBF and compare them with the response for synthetic mineral fertiliser (Super 46 (46%P<sub>2</sub>O<sub>5</sub>)).
- To assess the impact of BBF on crop P balance

The research hypotheses were the following:

- At a given level of P-fertilisation, there is no difference in crop yields and P-uptake between plots fertilised with BBF fertilisers compared to a reference treatment fertilised with synthetic mineral fertiliser.
- At a given level of P-fertilisation, there is no difference in P-environmental losses by leaching between plots fertilised with BBF compared to a reference treatment fertilised with synthetic mineral fertiliser.

#### 4.3.2 Methodology

The field trials are located on the experimental platform of Terralab in Champagne-Ardennes (Latitude: 49.317387 / Longitude: 4.043506) on chalk soil (6.6 % clays / 11.2% loam / 5.9 % sands ; Organic matter: 3.3% ; pH: 8.3). Weather conditions were relatively different in 2021 and 2022. In 2021, the mild but not scorching conditions in summer and the frequent rains allowed a good development of the crops. In 2022, there was very sun and dry conditions in spring and summer. The trials were conducted as a randomised complete block design with 3 replicates (elementary plot size = 5.4 x 8 m). When the conditions were set (normality - Shapiro Wilk test - and homogeneity of variance - Bartlett test), an ANOVA followed by a Newman Keuls was performed to test significant differences between treatments. All tests were performed using XLstats. APR and PFRV values were calculated as stated in Chapter 1. Fertilisers characteristics and measured crop parameters are detailed in Tables 37 and 38. FR-BC was tested under the same experimental conditions as in part 3.8. The experimental set-up included several treatments with 3 replicates:

- 1 treatment without P fertilisation
- 2 treatments with bio-based Biochar (BBF) applied at 2 doses: Biochar 2t ; Biochar 5t

The doses applied are not based on the product's composition, because there are no references on the efficiency of biochar. Two different doses were tested to observe an hypothetical effect of biochar.

**Table 37.** Fertiliser characteristics.

Fertiliser	Dry matter (%)	pH	Total N content (%)	P <sub>2</sub> O <sub>5</sub> content (%)	K <sub>2</sub> O content (%)
Triple superphosphate S45	NA	NA	0	45	0
Biochar (FR-BC)	97.7	NA	2.07	5.07	8.82

NA = not analysed

**Table 38.** Measured parameters in soil and crops (sugar beets).

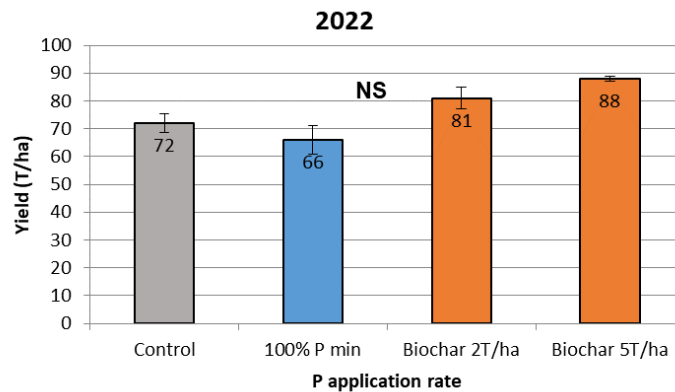
Description of measured parameters	Scale/unit
Population	Plants/ha
Yield	t/ha
Leaf and root P content	g/kg



**Figure 49.** Spreading of biochar.

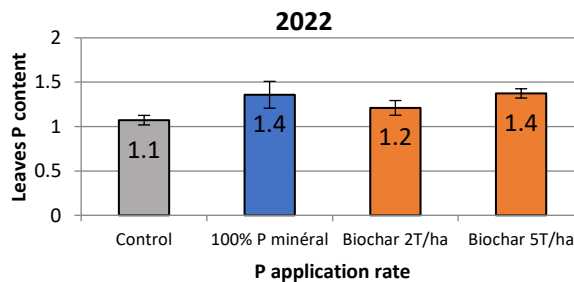
### 4.3.3 Results and discussion

At a given level of P fertilisation, plants treated with biochar showed greater biomass when compared to control and mineral P. However, there were no significant differences in yield in 2022. There were no differences with control either. A yield difference appears on the graph, but the trial was heterogeneous, explaining no significant differences (Figure 50).

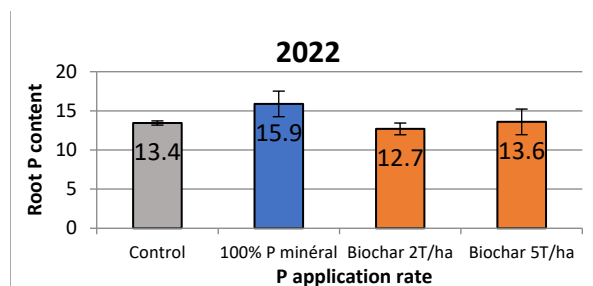


**Figure 50.** Sugar beets yield in T/ha for the P treatments. Control ; 100% Pmin = mineral fertilisation at 100%, crop needs ; Biochar 2T/ha and 5T/ha are the dose of Biochar applied.

There was no significant difference in leaf and roots contents for the P treatments (Figure 51 and 52). It seems to have more P in leaves and roots, when the dose applied with biochar increase.



**Figure 51.** Amount of P in leaves in %0 of dry matter. Control = unfertilised control ; 100% Pmin = mineral fertilisation at 100%, crop needs ; Biochar 2T/ha and 5T/ha are the dose of Biochar applied.



**Figure 52.** Amount of P in roots in %0 of dry matter. Control = unfertilised control ; 100% Pmin = mineral fertilisation at 100%, crop needs ; Biochar 2T/ha and 5T/ha are the dose of Biochar applied.

No significant differences were observed between control and BBF or mineral fertiliser for all the measured parameters. That is why APR and PFRV could not be calculated.

#### 4.3.4 Conclusion and recommendation

No significant differences were observed between the control and BBF or mineral fertiliser for all the measured parameters. There were strong effects of dry conditions in 2022, so these trials do not allow us to assess the performance of FR-AS. Nevertheless, there seems to be a dose effect. Even if the results are not significant, the 5t/ha treatment tends to have a better yield and a higher P concentration in the leaves and roots. It might be interesting to repeat this trial in more favourable conditions to conclude on the effects of biochar.

#### 4.4. Ryegrass pot cultivation (RITTMO and CRAB, France): FR-BC and FR-AS

For more information on this study, please contact the author *Chambre d'agriculture de Bretagne: Mariana Moreira* ([mariana.moreira@bretagne.chambagri.fr](mailto:mariana.moreira@bretagne.chambagri.fr)), and from RITTMO Agroenvironnement: *Lionel Ruidavets* ([lionel.ruidavets@rittmo.com](mailto:lionel.ruidavets@rittmo.com)) or *Fiona Ehrhardt* ([fiona.ehrhardt@rittmo.com](mailto:fiona.ehrhardt@rittmo.com)).

##### 4.4.1 Introduction

P in biochar can be in mineral or organic form and its short-term bioavailability to crops depends on the characteristics of the pyrolysis process (duration and temperature) and the soil properties. The objective of these trials was to evaluate the P use efficiency of the P-rich biochar produced by the French pilot (FR-BC) as compared to fossil reserve-based mineral fertiliser (TSP) and raw manure (poultry manure).

##### 4.4.2 Methodology

A first pot trial was carried out using 14-litre containers left in a field at the Kerguéhennec experimental station (Morbihan, France) (Figure 53). The test crop was an Italian ryegrass (*Lolium multiflorum*). To ensure a P deficit to ryegrass, soil from a plot not received P fertilisation since 1985 was used ( $P_2O_5$  - Olsen content of 0.04 g/kg). Ten treatments were established on a randomised complete block design with 4 replicates: 2 doses of biochar FR-BC (BBF-40 and BBF-80), 2 doses of poultry manure (RM-40 and RM-80), 4 doses of synthetic mineral fertiliser (TSP20, TSP40, TSP80, TSP100), 1 control without P application (P0) and 1 blank without NPK fertilisation (C). The reference mineral fertiliser was triple superphosphate (TSP). Since BBF biochar and poultry manure also contain N and K, and to be sure that they were not limiting factors, all plots received the



same amount of N and K in a mineral form. Mineral N and K fertilisers were ammonium nitrate (33,5% N) and potassium oxide (50% K<sub>2</sub>O). Three ryegrass cuts were made on 18th May and 26<sup>th</sup> July 2021 (68 days). At each cut, the total aboveground biomass of each pot was cut down and weighed to obtain the fresh weight. The dry matter and P-uptake were measured. Dry yield and calculated P-uptake data of the different treatments were compared to the reference treatment (P0) for each cut and the cumulative of all cuts. The APR was calculated as stated in Chapter 1.

Data were expressed as the mean value of the 4 replicates with the standard error by treatment. When the conditions were set (normality - Shapiro Wilk test - and homogeneity of variance - Bartlett test), an ANOVA followed by a Tukey HSD post hoc was performed to test significant differences between treatments. If the conditions were not set, non – parametric testing was performed (Kruskal-Wallis). All tests were performed using R version 4.2.1 and R packages RVAideMemoire and multcomp.

Due to technical issues during this experimentation, data on mineral treatments (TSP20, TSP40, TSP80 and TSP100) were not exploitable. This is why it was necessary to set up a second trial in 2022. This time, a greenhouse trial was preferred in order to better control the trial conditions. The same crop and the same soil as in 2021 were used. The trial was carried out in RITTMO Agroenvironnement facilities, using 1-litre containers and according to the protocols described in D4.5. The results of this trial should make it possible, on the one hand, to confirm the APR results obtained in the 2021 trial for biochar and, on the other, to calculate the PFRV for biochar.

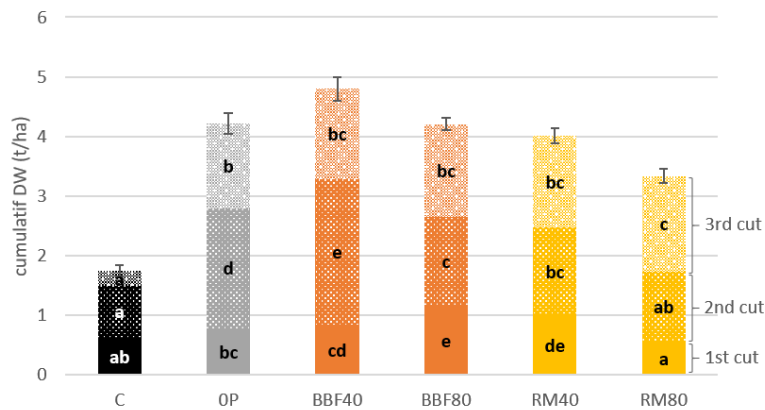


**Figure 53.** Set-up of the 2021 trial (soil containers weighting, Italian ryegrass sown) view of the trial on the 2<sup>nd</sup> June 2021 and on the 8<sup>th</sup> July 2021 (2<sup>nd</sup> cut).

#### 4.4.3 Results and discussion

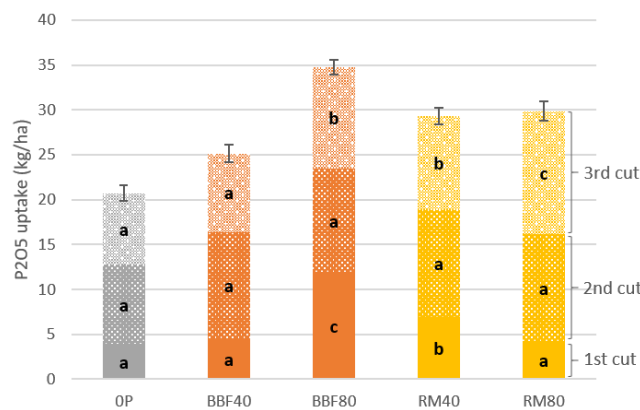
Globally, for the 3 cuts, the average dry weight of the ryegrass varied from 1.7 t/ha (for the Control: 0P) to 4.8 t/ha (for the BBF-40) (Figure 54). Cumulative dry weight on fertilised treatments (BBF or RM) was not significantly different from that in soil that did not receive P fertiliser (0P). The lack of response to P fertilisation and the low dry weight values (5-8 t/ha is the average dry weight in the region) indicate an almost certain N deficiency in the soil. Probably, the quantity of N applied to the plots was not sufficient to ensure good crop nutrition. In fact, the N uptake by ryegrass for all 3 cuts (3.3 g/kg for 0P treatment and 3.2 g/kg for the BBF40 treatment) was far below the expected level for this grass (20-25 g/kg). In terms of each cut, the highest dose of raw manure (RM80) resulted in a lower dry weight on the first cut than the other treatments, whereas the third cut had a greater dry weight. We could presume that P will gradually become available to crops on those pots, and globally on plots receiving organic fertilisers. However, the same trend was not observed for the other treatments where organic fertilisers were applied (RM40, BBF40 and BBF80).





**Figure 54.** Rye-grass dry weight (t/ha) on every treatment for each cut (average) and the cumulative dry weight for the three cuts (average  $\pm$  standard deviation, n=4). For each cut, small letters refer to statistically significant differences among treatments (ANOVA and post hoc Tukey HSD, p-value<0.001). For cumulative yields, capital letters are used. C- no NPK fertilised control; OP – no P fertilised control; BBF - biobased fertiliser; RM – raw manure.

Even if we cannot observe a net effect of P fertilisation on ryegrass cumulative yields, the fertilised treatments (BBF and RM) led to higher crop P uptake than plots not receiving P (OP) (Figure 55). For plots receiving biochar (BBF40 and BBF80), an incremental P application rate led to an incremental P uptake. However, for plots receiving poultry manure, there were no statistically significant differences between the 2 doses.



**Figure 55.** Rye-grass P uptake on every treatment for each cut (average) and the cumulative P uptake for the three cuts (average  $\pm$  standard deviation, n=4). For each cut, small letters refer to statistically significant differences among treatments (ANOVA and post hoc Tukey HSD, p-value<0.001). For cumulative yields, capital letters are used. C- no NPK fertilised control; OP – no P fertilised control; BBF - biobased fertiliser; RM – raw manure.

APR calculated for the two fertiliser treatments indicates a low P bioavailability during this experiment (Table 39). The low APR value indicates that BBF would not provide enough P during the early growth stages of crops. This could be explained by the pyrolysis conditions, which produced a high amount of ashes and probably affected the different forms of P on the used biochar (FR-BC).

**Table 39.** Apparent phosphorous recovery (APR) for the different fertiliser treatments based on crop phosphorous uptake (average  $\pm$  standard deviation, n=4).

Trial	Treatment	Fertiliser product	P <sub>2</sub> O <sub>5</sub> applied (kg/ha)	APR (%)
Field conditions	BBF-40	FR-BC	40	11.1 $\pm$ 2.3 a
Field conditions	BBF-80	FR-BC	80	17.6 $\pm$ 1.0 b

Field conditions	RM-40	RM	40	21.6 ± 2.4 c
Field conditions	RM-80	RM	80	11.5 ± 1.4 a
Greenhouse*	FR-BC1 – 200 U P/ha	FR-BC	200	6.5 ± 1.2 A
Greenhouse*	Min. Reference – 200 U P/ha	TSP	200	13.6 ± 2.9 B

*Small letters refer to statistically significant differences among treatments (ANOVA and post hoc Tukey HSD, p-value<0.001) for the trial carried out at field conditions. Capital letters refer to statistically significant differences among treatments (ANOVA and post hoc Tukey HSD, p-value<0.05) for the \* trial carried out in RITTMO Agroenvironnement facilities (D4.5).*

For the trial carried out in greenhouse conditions, APR values were also low. Nonetheless, a PFRV of 47.6% could be calculated for the FR-BC. The results of all the biochar P-effect tests carried out in greenhouse conditions are detailed in D4.5.

#### 4.4.4 Conclusion and recommendation

From the pot trial carried out at field conditions, we can conclude that the FR-BC led to similar or slightly higher yields than the no-fertilised plots or plots fertilised with poultry manure. Apparent P recovery of BBF was low indicating that BBF FR-BC would not be suitable to provide P to crops during early growth stages anyway. The results in greenhouse conditions indicates that FR-BC1 (D4.5) could replace a synthetic mineral fertiliser by 48% but the APR values still low. The benefits of biochar for fertilising ryegrass or other arable crops are also still low, given the large quantities to be applied, the difficulty of spreading and mixing it with the soil. This BBF would be best suited to fertilising high added value crops (given the potential cost of the product) and would be ideally used in a formulation with other fertilisers.

## 5. Liquid potassium fertiliser

### 5.1. Sugar beet field cultivation (CRAGE, France): FR-LK

For more information on this study, please contact the author from Chambre d'agriculture du Grand Est: Clement Munier ([clement.munier@grandest.chambagri.fr](mailto:clement.munier@grandest.chambagri.fr)).

#### 5.1.1 Introduction

Field trials were set-up in Champagne-Ardennes in 2021 and 2022 to test BBF liquid K-fertiliser (FR-LK). The FR-LK was tested under the same experimental conditions as in section 3.8. The main goal of the FR-LK trial was to assess the short-time crop response to FR-LK to compare it with the response for synthetic mineral fertiliser (KCl (60%K<sub>2</sub>O)). The research hypotheses was that at a given level of K-fertilisation, there is no difference in crop yields and uptakes between plots fertilised with FR-LK compared to a reference treatment fertilised with mineral fertiliser.

#### 5.1.2 Methodology

The field trials are located on the experimental platform of Terralab in Champagne-Ardennes (Latitude: 49.317387 / Longitude: 4.043506) on chalk soil (6.6 % clays / 11.2% loam / 5.9 % sands ; Organic matter: 3.3% ; pH: 8.3). Weather conditions were relatively different in 2021 and 2022. In 2021, the mild but not scorching conditions in summer and the frequent rains allowed a good development of the crops. In 2022, there was very sun and dry conditions in spring and summer. The trials were conducted as a randomised complete block design with 3 replicates (elementary plot size = 5.4 x 8 m). When the conditions were set (normality - Shapiro Wilk test - and homogeneity of variance - Bartlett test), an ANOVA followed by a Newman Keuls was performed to test significant differences between treatments. All tests were performed using XLstats. AKR and KFRV values were calculated as stated in Chapter 1. Fertilisers characteristics and measured crop parameters are detailed in Tables 40 and 41. Different treatments were applied:

- A control without K fertilisation;
- Two treatments with mineral K fertiliser (potassium sulphate or potassium chloride) were applied in 2021 and 2022 at incremental rates (50%X and X, where X represents the K fertiliser recommended dose)
- Two treatments with BBF liquid K fertiliser were applied in 2021 at incremental rates (50%X and X, where X represents the K fertiliser recommended dose)
- One treatments with BBF liquid K fertiliser were applied in 2022 at incremental rates (37.5%X where X represents the K fertiliser recommended dose) : lack of liquid K fertiliser, not possible to apply the desired dose of K solution

**Table 40.** Fertiliser characteristics

Fertiliser	Form	Dry matter (%)	pH	Total N (%)	N-NH <sub>4</sub> (%)	P <sub>2</sub> O <sub>5</sub> (%)	K <sub>2</sub> O (%)	SO <sub>3</sub> (%)
Potassium sulphate	Granular	NA	NA	0	0	0	50	45
Potassium chloride	Granular	NA	NA	01.89	0	0	60	0
BBF Liquid K fertiliser	Liquid	NA	NA	NA	NA	1.1	2.3	NA

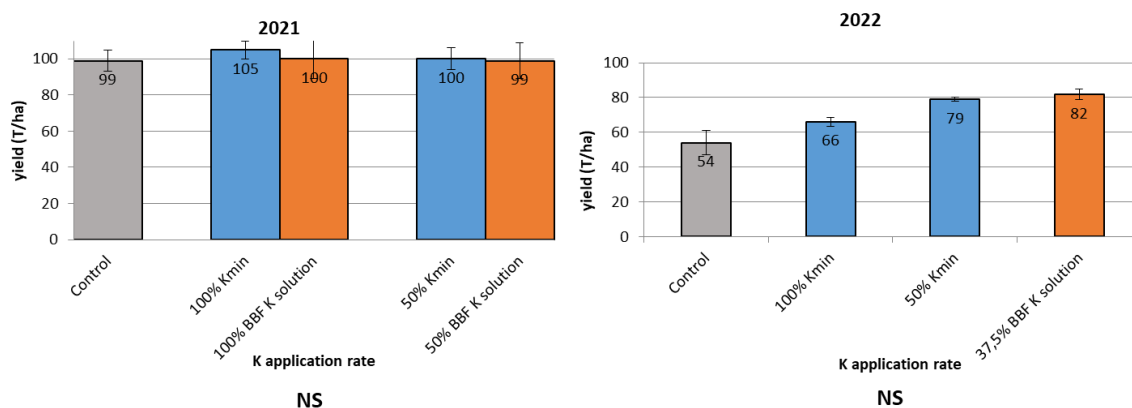
NA = not analysed

**Table 41.** Measured parameters in soil and crops (sugar beets).

Description of measured parameters	Scale/unit
Population	Plants/ha
Yield	t/ha
Leaf and root K content	g/kg

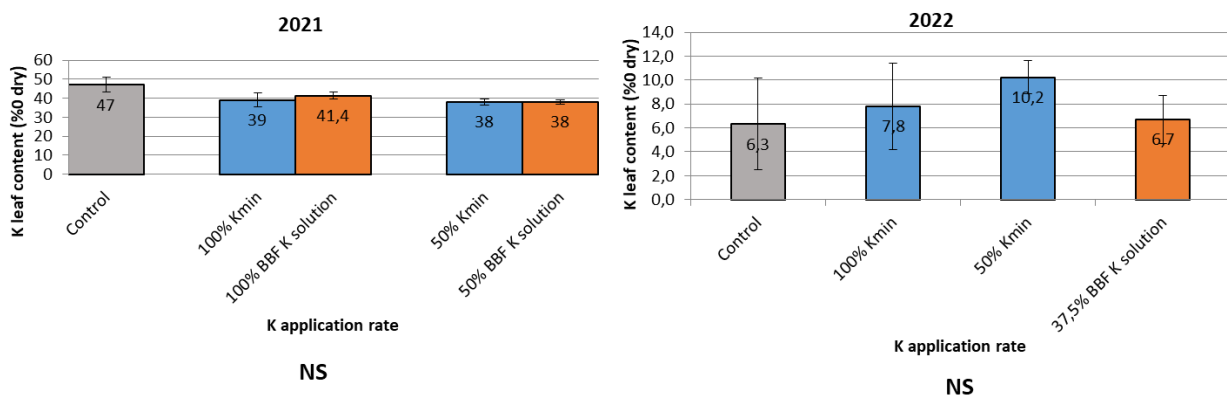
### 5.1.3 Results and discussion

There were no significant differences for yield, with control either (Figure (56)). In 2022, yield decreased with increasing K doses, which is questionable.

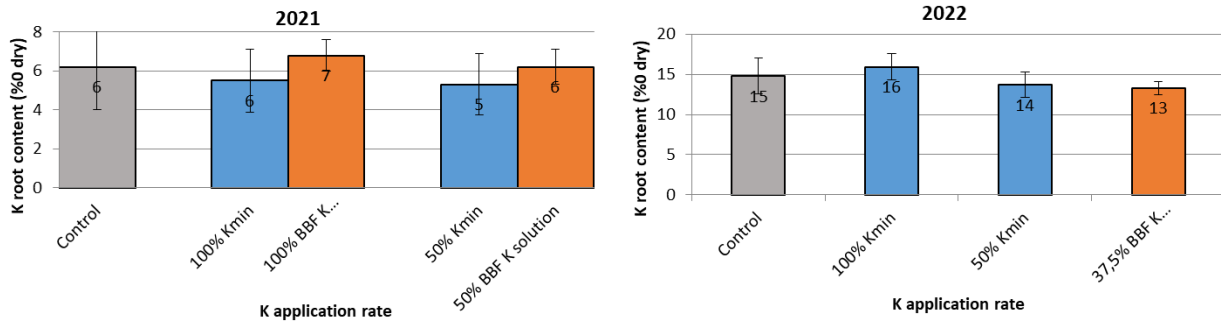


**Figure 56.** Yield for different K treatments. Control; 100% and 50% Kmin = K mineral fertilisation at 100% and 50% crop needs; 100%, 50% and 37.5% BBF K solution = K solution fertilisation at 100%, 50% and 37.5% crop needs ; NS=statistically not significant

There is no significant difference in leaf and roots contents for the K treatments (Figure 57 and 58). In 2022, K contents in the leaves are very low, with no clear trend. In 2021, K content in the roots seems to be higher in the K solution treatments than in the mineral treatments. In 2022, K content in roots were much higher than in 2021 and it is difficult to draw conclusions because it was not possible to apply the desired dose of K solution.



**Figure 57.** Amount of K in leaves in g/kg. Control; 100% and 50% Kmin = K mineral fertilisation at 100% and 50% crop needs; 100%, 50% and 37.5% BBF K solution = K solution fertilisation at 100%, 50% and 37.5% crop needs ; NS=statistically not significant



**Figure 58.** Amount of K in roots in g/kg. Control; 100% and 50% Kmin = K mineral fertilisation at 100% and 50% crop needs; 100%, 50% and 37.5% BBF K solution = K solution fertilisation at 100%, 50% and 37.5% crop needs ; NS=statistically not significant

No significant differences were observed between control and BBF or mineral fertiliser for all the measured parameters. That is why AKR and KFRV could not be calculated.

#### 5.1.4 Conclusion and recommendation

No significant differences were observed between the control and BBF or mineral fertiliser for all the measured parameters. There were strong effects of dry conditions in 2022, so these trials do not allow us to assess the performance of liquid K fertiliser. It is difficult to draw robust conclusions for this crop and we can conclude that liquid K fertiliser is currently not efficient and must be improved to be used by farmers.

## 6. Environmental monitoring campaigns

### 6.1. Ammonium sulphate (FR-AS), liquid potassium fertiliser (FR-LK) and 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](mailto:lionel.ruidavets@rittmo.com)) or Fiona Ehrhardt ([fiona.ehrhardt@rittmo.com](mailto:fiona.ehrhardt@rittmo.com)).

#### 6.1.1 Introduction

Ammonia (NH<sub>3</sub>) volatilisation is responsible for major losses of N supplied in agrosystems by N-fertilisers. Beyond the economic impact of N losses reducing crop N use efficiency, NH<sub>3</sub> volatilisation constitutes also an important negative environmental externality due to the polluting fine particles emitted that are accumulating in the atmosphere. Furthermore, the application of mineral or organic N fertilisers in agrosystems constitutes the main source of nitrous oxide (N<sub>2</sub>O) emissions in agriculture (Paustian et al., 2016). A significant increase in N<sub>2</sub>O emissions is generally observed with the application of N fertilisers (Bouwman et al., 2002) with, however, different levels depending on the type of the fertiliser employed, given the N and C contents, as well as the forms it contains (Butterbach-Bahl et al., 2013, Hénault et al. al., 2012). Therefore, this study aimed to investigate the effect of biochar addition to commonly used N-fertilisers applied to soil, in order to estimate whether any NH<sub>3</sub> volatilisation reduction is possible with this practice. Also, mitigation of N<sub>2</sub>O emissions was explored.

#### 6.1.2 Methodology

##### (i) NH<sub>3</sub> emissions

The objective was to estimate N losses by volatilisation of ammoniacal N from agricultural soil after having been fertilised with mineral N and mixed with biochar. The method employed was adapted from Le Cadre et al (2005) and conducted under controlled laboratory conditions (T°= 20 ± 3°C). The experimental device is based on a closed dynamic flow system with a randomized block design (Figure 59). Blocks comprise eight volatilisation chambers connected by pumps that allow ammonia-free air flow. The outgoing air flow, loaded with ammonia resulting from volatilisation, is captured by an 0.05M H<sub>2</sub>SO<sub>4</sub> solution which is then dosed for ammoniacal N concentrations (N-NH<sub>4</sub> in mg/L). Each treatment (Table 43) was replicated three times and incubated for 16 days. The quantity of ammoniacal N volatilised is dosed at successive times (0, 1, 2, 3, 7, 11, 14 and 16 days) to determine the kinetics of emissions. The quantity of N-NH<sub>3</sub> emitted (expressed in mg N) was calculated with the following formula:

$$N-NH_4^+ \text{ trapped} = [N-NH_4^+]_{\text{acid solution}} \times \text{Volume}_{\text{acid solution}} \quad (\text{Eq. 5})$$



**Figure 59.** Experimental device for ammonia volatilisation (here, one block constituted of eight measurement chambers).



To evaluate the potential of biochar to reduce NH<sub>3</sub> volatilisation from N fertilisers, all treatments were fertilised (surface application) with a single dose of N equivalent of 170 kg N/ha, using different sources of N (including organic and mineral forms). One biochar produced by pyrolysis of poultry manure has been tested (FR-BC) and incorporated into the soil at an equivalent dose of 10 t/ha. Product characteristics are shown in Table 42 and the compared treatments are shown in Table 43. The soil pH was 6.7 and FR-BC pH was 11.8.

**Table 42.** Products characteristics on (g/kg) on fresh weight basis.

Parameters	FR-AS	Pig slurry (PS)	FR-LK	CAN	FR-BC
Dry matter	207	13.3	14.3	9980	977
Total carbon	0.0	2.7	1.2	ND	ND
Organic carbon	ND	ND	ND	ND	544.0
Total N	43.8	1.8	0.5	160	20.7
NH <sub>4</sub> -N	43.8	1.4	<0.2	10*	<0.05
NO <sub>3</sub> -N	0.0	<0.2	<0.2	150*	ND
Total P	0.0	0.11	0.10	0.0	22.1
Total K	0.0	2.57	2.3	0.0	73.2
Total S	163.5	0.12	0.15	0.0	ND

ND: not determined; \*estimated

**Table 43.** List of tested treatments for the assessment of ammonia volatilisation.

Treatment	Reference	N supplied (Kg N/ha)	Biochar supplied (T/ha)
Ammonium sulphate	FR-AS	170	-
Calcium Ammonium Nitrate	CAN	170	-
Pig Slurry	PS	170	-
Exhausted pig slurry	FR-LK	170	-
Biochar FR-BC + Ammonium sulphate	FR-BC+FR-AS	170	10
Biochar FR-BC + Calcium Ammonium Nitrate	FR-BC+CAN	170	10
Biochar FR-BC + Pig Slurry	FR-BC+PS	170	10
Biochar FR-BC + Exhausted pig slurry	FR-BC+FR-LK	170	10

An analysis of variance (ANOVA) was performed using XLSTAT BASIC (v.2023.3.0) in order to determine significant differences in NH<sub>3</sub> emissions among treatments.

#### (ii) N<sub>2</sub>O emissions

For this test, a simplified approach has been conducted under laboratory controlled conditions to compare immediate N<sub>2</sub>O emissions following the application of two selected N-fertilisers (FR-LK and FR-AS) combined or not with FR-BC addition. The experiment has been voluntarily designed to maximize potential N<sub>2</sub>O emissions (continuous soil water saturation) and to perform a comparative analysis of these N-fertilisers. In line with the N release behavior considering these types of fertilisers, measurements have been focused on the days following the application (T<sub>0</sub> ; T+3 days and T+9 days), in order to capture the potential effect of biochar on immediate N<sub>2</sub>O emissions. The method consisted of microcosms (250 mL glass bottles with a surface area of 0.0034 m<sup>2</sup>) containing soil and N-fertilisers, mixed or not with FR-BC in glass bottles. An equivalent of 150 g of 2 mm-sieved dried soil were first mixed with biochar (equivalent dose of 10 T/ha) and pre-incubated for a week at 70 % of the maximal water capacity retention (pF=2.5) to guarantee microbial activities. After 7 incubation days at 25°C, N-fertilisers were added to the soil surface (at an equivalent dose of 170 kgN/ha) and soils were fully saturated with water to reach maximal soil water retention capacity (100 % CR<sub>max</sub>). Bottles were sealed and gas samples were immediately analysed (T<sub>0</sub>) up to 24 hours (0, 30, 60 and 1440 minutes) to capture the change in dynamics induced by soil saturation in water. This moisture rate has been maintained until the end of the test (day 9).

For gas analysis, bottles were directly connected to a gas chromatograph (MICROGC 490, Agilent) equipped with a column BF 10 m PPU with heated injector and a Micro TCD detector. Helium (99.9995% purity, 200 kpa) was used as the carrier gas, and the injector and column temperatures were 110°C and 80°C, respectively. Modalities were repeated three times to assess daily N<sub>2</sub>O emissions expressed in µg N<sub>2</sub>O.m<sup>-2</sup>.h<sup>-1</sup> using the equation:

$$N_2O \text{ flux} = (dC/dt)(M/V_m)V/A \quad (\text{Eq. 6})$$

where  $dC/dt$  is the change in  $N_2O$  concentration (in  $\mu\text{L L}^{-1}$ ) in the chamber after the incubation time (in hours);  $M$  is the molecular weight;  $V_m$  is the molecular volume of  $N_2O$  at the sampling temperature,  $V$  is the volume of the chamber in litres and  $A$  the area in  $\text{m}^2$ .

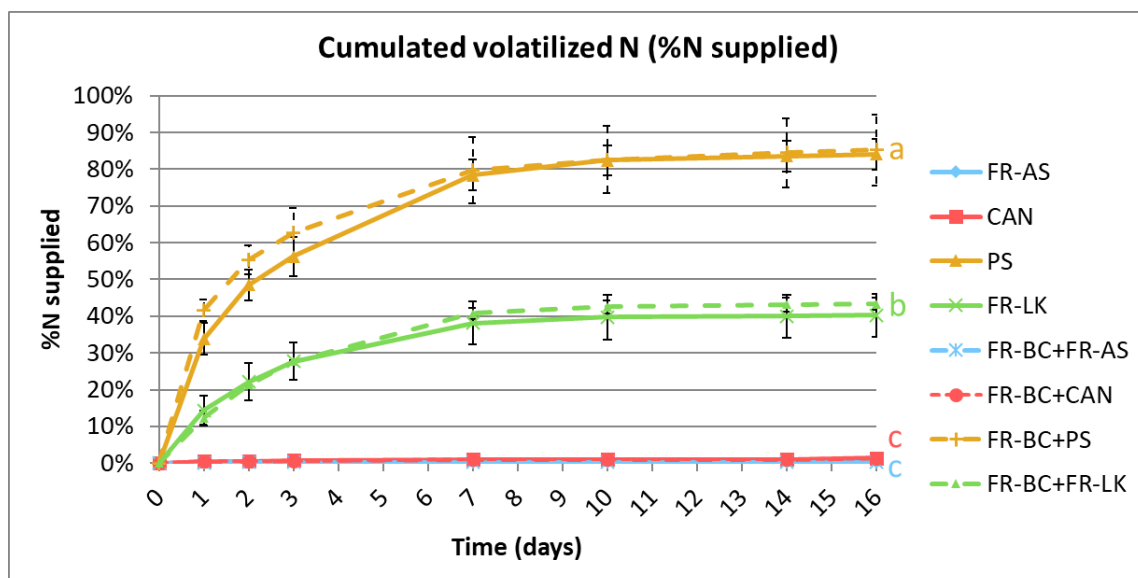
### 6.1.3 Results and discussion

#### (i) $NH_3$ volatilisation

The cumulated proportions of N losses through  $NH_3$  volatilisation from the different fertilisers supplied, mixed or not with FR-BC biochar, are shown in Figure 60. Values are presented in Table 44. Globally, the average  $NH_3$  volatilisation losses ranged from 0.3 to 85.2% of the supplied N, depending on the N-fertiliser type. The percentage of N loss as  $NH_3$  was the highest in treatments containing pig slurry (PS and FR-BC+PS), reaching an average of 84.7 and 85.2% of the initial amount of N supplied respectively. Treatments with exhausted pig slurry show a significant emissions, reaching 40 % without biochar addition (FR-LK) and 43 % with biochar (FR-BC+FR-LK), representing half of the  $NH_3$  emissions observed with PS. These results are consistent considering the reduction of the initial N concentration in both FR-LK samples compared to the PS.

Results obtained with CAN treatments showed much lower N losses, with an average value of 1.3 % of the initial N supplied, for both treatments (with and without biochar addition). The reduced emission rate is consistent with the literature and can be explained by the specificities of CAN compared to pig slurry. Indeed, CAN is in granular form and contains  $Ca^{2+}$ , which, over the duration of the experiment, has not been totally solubilised due probably to a lack of sufficient soil humidity.

The lowest percentage of N loss as  $NH_3$  was observed with ammonium sulphate (FR-AS) treatments, with 0.3 % (with biochar) and 0.4 % (without biochar) of the initial N supplied volatilized. This result can be explained by the acidity of ammonium sulphate, which balanced the global pH of the system and probably avoided exacerbated volatilisation.



**Figure 60.** Kinetics of cumulated proportions of volatilized N from the fertiliser supplied, mixed or not with FR-BC biochar, during 16-days incubations.

Results obtained for the different N-fertilisers tested showed significant differences between types of N-fertiliser, but no significant effect of biochar addition. Based on a meta-analysis comparing multiple N-fertilisers, Sha et al. (2019) also showed no impact of biochar addition on ammonia volatilisation, with some variations depending on soil and biochar types (especially related to pH impact), as well as environmental conditions. In our experiment, soil and biochar were similar and only N-fertiliser type was varied. By comparing pairs of samples for a given N-fertiliser, we showed that biochar addition has not reduced N volatilisation in our case. Conversely, we showed slightly higher volatilisation rates with treatments containing the biochar, although these observations were not statistically significant. These observations can be attributed to the pH value of

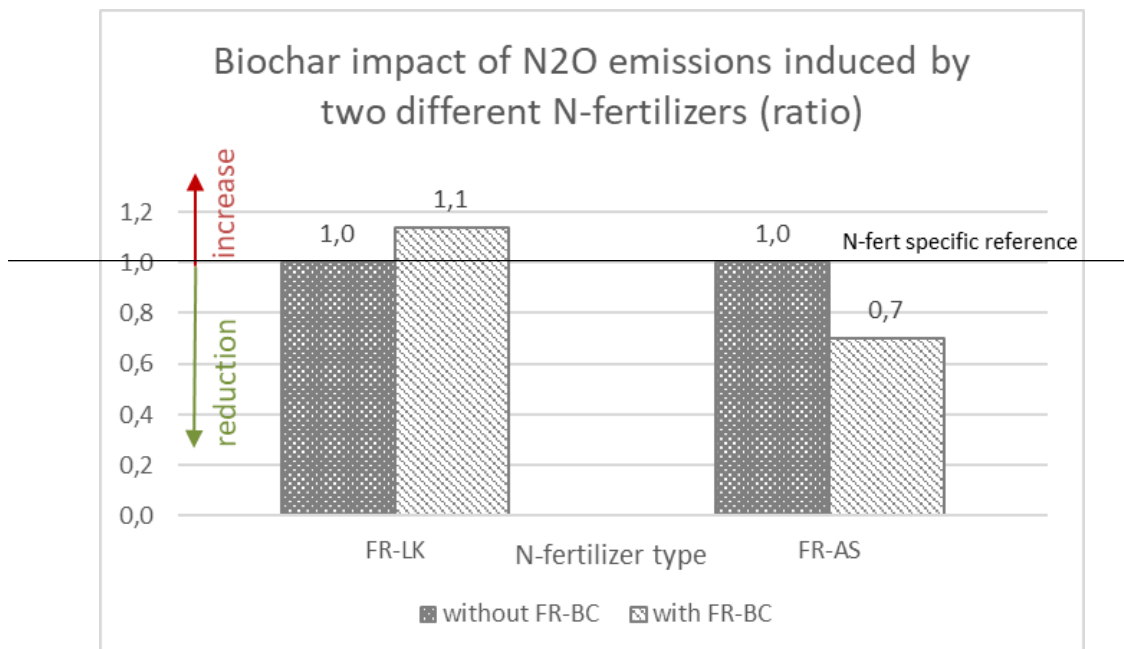
the tested biochar (pH=12.3). According to Sha et al. (2019), one of the major explanatory variables on the response of NH<sub>3</sub> volatilisation to biochar addition are attributed to pH (both soil and biochar). The authors highlighted the potential of high pH biochars in stimulating NH<sub>3</sub> volatilisation, particularly in soils with pH values >6.

**Table 44.** Cumulated volatilized N (%N supplied).

%N supplied	Days						
	1	2	3	7	10	14	16
FR-AS	0.3	0.3	0.3	0.3	0.4	0.4	0.4
CAN	0.5	0.5	0.7	1.0	1.0	1.0	1.3
PS	33.8	48.6	56.3	78.4	82.4	83.6	84.1
FR-LK	14.4	22.1	27.7	38.2	39.7	40.0	40.2
FR-BC+FR-AS	0.2	0.3	0.3	0.3	0.3	0.3	0.3
FR-BC+CAN	0.5	0.6	0.6	1.0	1.1	1.2	1.3
FR-BC+PS	41.6	55.3	62.7	79.8	82.5	84.5	85.2
FR-BC+FR-LK	12.3	21.3	27.7	40.8	42.6	43.2	43.4

(ii) N<sub>2</sub>O emissions

The effect of FR-BC addition on N<sub>2</sub>O emissions caused by various N-fertilisers was investigated. The contribution of soil emissions was removed and fluxes for each treatments containing biochar were normalised with the similar treatments with no biochar addition. Thus, the potential of biochar to reduce or increase N<sub>2</sub>O emissions is assessed by relative comparison with the similar N-fertiliser form without biochar addition. The results obtained are indicative and showed that for treatments having exhausted pig slurry as N-fertiliser, FR-BC addition increased N<sub>2</sub>O daily emissions by 10%, but for treatments with ammonium sulphate as N-fertiliser, biochar reduced N<sub>2</sub>O emissions by 30% (Figure 61). Thus, these contrasted results highlight the fact that the impact of biochar on N<sub>2</sub>O emissions induced by N-fertilisation is dependent on the type of N-fertiliser employed.



**Figure 61.** Impact of biochar addition on N<sub>2</sub>O emissions induced by N-fertilisers in soil water saturation conditions. The emissions attributed to soil were firstly removed and daily fluxes with treatments containing biochar were normalized with regard to fluxes measured with similar (1). Thus, values <1 show a mitigation impact (reduction) while values >1 show an amplification impact (increase).

6.1.4 Conclusion

In regard to the ammonia, results obtained for the different N-fertilisers tested showed significant differences between types of N-fertiliser, but no significant effect of biochar addition. For N<sub>2</sub>O results showed that for treatments having exhausted pig slurry as N-fertiliser, FR-BC addition increased N<sub>2</sub>O daily emissions by 10%, but for treatments with ammonium sulphate as N-fertiliser, biochar reduced N<sub>2</sub>O emissions by 30%. Thus, these contrasted results highlight the fact that the impact of biochar on N<sub>2</sub>O emissions induced by N-fertilisation is dependent on the type of N-fertiliser employed.

## 6.2. Ammonium sulphate (NL-AS) from Dutch pilot (WENR)

*This study was published as Rietra, René, Kimo van Dijk, and Oscar Schoumans (2024). "Environmental Effects of Using Ammonium Sulfate from Animal Manure Scrubbing Technology as Fertilizer" Applied Sciences 14, no. 12: 4998. <https://doi.org/10.3390/app14124998>*

### 6.2.1 Introduction

NL-AS from the Arjan Prinsen Farm (APF) was tested for its yield performance as a N fertiliser for grass and maize as detailed in section 3.3. During the pot experiment (2021) and the field experiment of 2022, the gaseous emissions were also measured to determine the environmental performance of the product. These experiments were followed by ammonia emissions measurements of multiple products from APF added to one standard soil mimicking low-emission techniques.

### 6.2.2 Methodology

In the pot experiment, a photo-acoustic infrared gas analyser (Innova 1512) was used to measure emissions of N<sub>2</sub>O and CH<sub>4</sub> in the first 38 days after fertilisation. The grass and maize, and the soil surface were covered with a polyvinyl chloride (PVC) flux chamber 30 min before the measurement (Lubbers et al., 2011): a flux chamber of 8-L and a height of 20 cm was used to accommodate grass and small maize plants. The total emissions (over 31 (grass) or 38 (maize) days ( $\mu\text{g m}^{-2}$ ) is N<sub>2</sub>O and CH<sub>4</sub> is calculated according to Lubbers et al. (2011), assuming a linear emission during the closure time and a surface area of 0.0314 m<sup>2</sup> in the pot.

The pot experiment as detailed in section 3.3 was completely replicated to measure NH<sub>3</sub> emissions using acid traps. Closed-chamber acid traps were used to measure emissions of NH<sub>3</sub> in the first two weeks after fertilisation. The acid traps were changed twice resulting in two measurements per pot over 10 days, and total emissions across the two measurements were further analysed for the results. For the measurement, a 100 ml vessel with 50 ml 0.5 M H<sub>2</sub>SO<sub>4</sub> was placed on the soil, and a PVC chamber (8 L) was placed over each pot in the replicate experiment. After 10 days these pots were discarded. Each acid solution was analysed after 1:10 dilution using automated segmented flow spectroscopy (SFA) (ISO/TS-14256-1, 2003). The total ammonia emissions ( $\text{g pot}^{-1}$ ) are calculated by adding up the emissions from each of the measurements.

In the field experiment of 2022, N<sub>2</sub>O emissions were measured repeatedly following the first fertilisation using the Innova gas analyser in the same manner as described for the pot experiment, except that the PVC tube was pressed into the grass sod. There was also a zero measurement a few weeks prior to the first fertilisation. Each measurement was measured at two locations within the plot, one on top of the injection strokes, and one in between the injection strokes (Figure 62). On the CAN plots two random locations were chosen, since this fertiliser is spread and not injected. The N<sub>2</sub>O emissions were measured 5 times over 36 days after fertilisation. The method was the same as the method used for N<sub>2</sub>O and CH<sub>4</sub> in the pot experiment.

The ammonia emissions multiple products from APF were determined using a standard method in which a grass-sod is placed in air chamber, and the air above the grass-sod (2 liter) is drawn from each chamber and is drawn through 100 ml 0.05 M H<sub>2</sub>SO<sub>4</sub> using methods described earlier (De Ruijter et al., 2010). The flow rate per chamber is 2 liters per minute resulting in an air refresh rate of one per minute, and the experiment is carried out in open air under a shelter. The tested products from APF are: 1. Untreated cattle slurry (CS), (2)

digestate of CS, (3) liquid fraction of CS, (4) potassium liquid fertiliser after stripping the liquid fraction of CS, (5) AS resulting from stripping the liquid fraction of CS.

As in the yield experiments, maize and grass were tested separately. First, linear models and ANOVA tests were attempted for all measures. However, this had to be replaced by non-parametric Kruskal-Wallis tests for N<sub>2</sub>O emissions in the pot experiment and for CH<sub>4</sub> emissions on maize pots because of a large amount of 0s in the data. A full description of the statistical procedure can be found in Rietra et al. (2024).



**Figure 62.** Injection of ammonium sulphate.

### 6.2.3 Results and discussion

#### (i) Ammonia

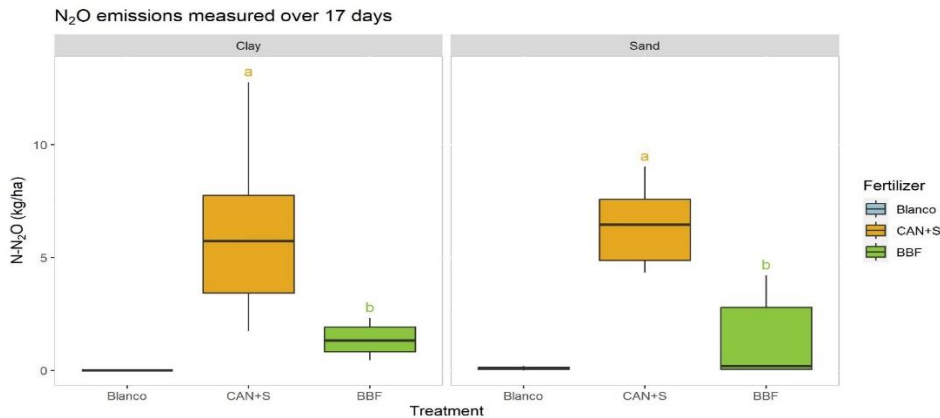
Very low NH<sub>3</sub> emissions were measured in the pot experiment of 2021, and no effect from fertilisers was shown. Soil did have a significant effect for the maize pots, sandy soil had higher emissions than the clay soil.

Emissions were far lower than what would have been estimated using the corresponding emission factor from the Dutch National Emission Model Agriculture (NEMA), which is 2.5% of applied N for CAN and 1.8% of applied N for air scrubber effluent (van Bruggen et al. 2021). While there are questions about the applicability and accuracy of these emission factors for small scale estimations, they give an indication of the range that might be expected. The measured ammonia was a factor of 7 or more lower. Because the experiment was carried out with closed chambers, the wind effect was missing from the experiment. Several examples in the literature show that ammonia emissions are consistently underestimated when wind is not allowed to pass over the measuring area (Schlossberg et al. 2018, Alexander et al. 2021). These experiments work around this by placing an artificial wind source, such as a fan, blowing at realistic speeds near the acid trap.

To make more reliable ammonia emissions, addition trials were performed using a technique in which all air above the fertilised soil is refreshed every minute (De Ruijter et al. 2010), and all products from APF.

#### (ii) Greenhouse gases

The N<sub>2</sub>O emissions measured in the pot experiment were extremely low and contained many zero values (values under the detection limit). Therefore, they could not be tested with a linear model and were tested with a Kruskal-Wallis non parametric test. This showed no significant effects of soil, dosage, or fertiliser type. The N<sub>2</sub>O emissions from the field experiment were much higher than the pot experiment, which may be related to the wet weather that was occurring at the time of fertilisation and measurement, since anaerobic conditions caused by wet weather facilitate N<sub>2</sub>O emission. In this trial, a linear model was used and the fertiliser was found to have a significant effect on the emissions, namely that NL-AS had significantly lower N<sub>2</sub>O emissions than CAN+S across both soil types (Figure 63).



**Figure 63.** Total N<sub>2</sub>O emissions from grass field trial of 2022 in 17 days after fertilisation. Emissions were measured from plots fertilised with CAN+S and NL-AS (BBF) at multiple intervals over 17 days after fertilisation. Cumulative emissions on the last day of measurement are reported as total emissions. LSD is 2.94 kg N-N<sub>2</sub>O/ha.

The CH<sub>4</sub> emissions in the pot experiment did not show any significant effects for maize (Kruskal-Wallis test) or grass (Linear model).

#### 6.2.4 Conclusion and recommendation

The pot experiment provided no evidence for significant differences in NH<sub>3</sub>, N<sub>2</sub>O, or CH<sub>4</sub> emissions caused by fertiliser type. However, this may have been caused by the very low amount of emissions that were measured overall. The low amount of N emissions aligns with the N balance, which shows that the amount of uptake by the plant was higher than the application rate by approximately the amount taken up by the control, implying that the crop was able to utilise all or most of the applied N in addition to some N already in the soil and that there was very little loss. This experiment was done under controlled conditions; in contrast the field trials of 2022 and 2023 lend insight to emissions in a less controlled environment. Here, the N<sub>2</sub>O emissions measured by Innova showed a higher emission from CAN fields compared to NL-AS fields after rainfall. Ammonia emissions from all products from APF show high emissions from solid and liquid fraction of digestate and from liquid product with a high pH: potassium fertiliser after stripping liquid fraction of digestate.

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

*The part of this section has been redrafted from – “Shrivastava, V., Sigurnjak, I., Edayilam, N., & Meers, E. (2023). Ammonia water as a biobased fertiliser: Evaluating agronomic and environmental performance for Lactuca sativa compared to synthetic fertilisers. Biocatalysis and Agricultural Biotechnology, 102907.”*

*The part of 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”*

#### 6.3.1 Introduction

The objective of this study was to examine the immediate emissions of methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>), nitrous oxide (N<sub>2</sub>O) and ammonia (NH<sub>3</sub>) arising from fertilisers produced from manure through nutrient recovery technologies available at the Belgian pilot plant within the FERTIMANURE project. Specifically, we collected samples of BE-AS, BE-AN, and BE-AW from the Belgian pilot plant and subjected them to characterisation. The characterisation involved assessing various parameters including pH levels, dry matter content, organic matter content, total carbon content, total N content, and mineral N content.



### 6.3.2 Methodology

#### (i) Incubation study

CH<sub>4</sub>, CO<sub>2</sub>, N<sub>2</sub>O emissions were measured over 18 days using a Gasera One Multi-gas analyser with a photo-acoustic infrared analyser. Soil mesocosms were 1L Duran bottles with modified Smart caps. Conducted in November 2021, the experiment included one unfertilised control, two mineral fertilisers (urea and calcium ammonium nitrate), raw pig manure, and three BBFs (BE-AN, BE-AS, BE-AW). Following the Nitrates directive, fertilisers were mixed and applied to 568 g of pre-incubated soil at 170 kg total N/ha. Moisture levels were kept at 80% WFPS. CH<sub>4</sub>, CO<sub>2</sub>, N<sub>2</sub>O fluxes were calculated based on concentration changes over time, considering the headspace volume, tubing, and soil surface area.

#### (ii) Lettuce pot trial

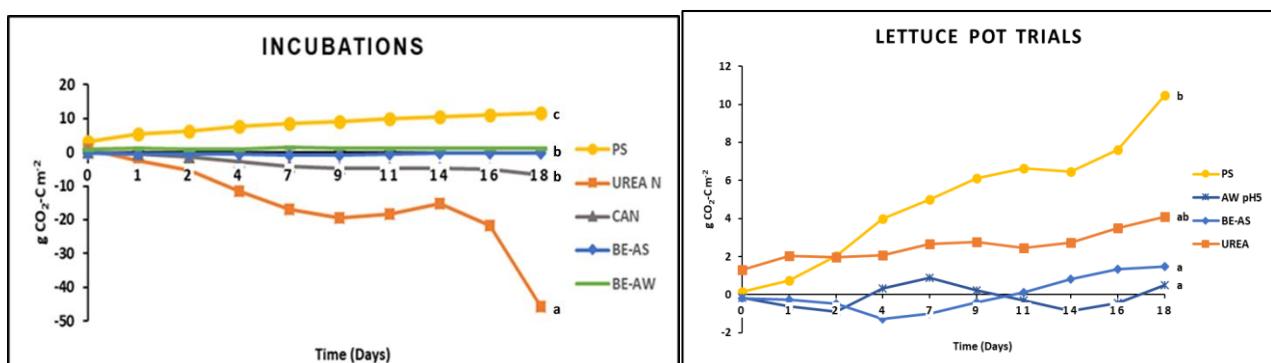
Incubation experiments were conducted using lettuce pots designed as 300 ml PTFE bottles (cut from the bottom and inserted into the soil) equipped with GL45-thread Smart Caps (model: SW45-2A). These pot trials were carried out over the period from January 2022 to June 2022. The experimental setup included BE-AS, BE-AN, BE-AW at two pHs (acidified pH 5 and initial pH 11), one control group with unfertilized soil, and two positive control groups using mineral fertilisers (urea and calcium ammonium nitrate - CAN). All fertilisers were applied at an equivalent rate of 200 kg of total N per hectare to 1.2 kg of pre-incubated soil and thoroughly mixed. The moisture content in each pot was maintained at 70% of the water holding capacity and was consistently monitored throughout the experiment.

During the 18-day gas monitoring period, emissions of CH<sub>4</sub>, CO<sub>2</sub>, N<sub>2</sub>O and ammonia (NH<sub>3</sub>) were assessed using the Gasera One Multi-gas analyser (Turku, Finland), which is equipped with a photo-acoustic infrared analyser. To determine NH<sub>3</sub> fluxes, changes in NH<sub>3</sub> concentration over time were analysed, taking into consideration the volume of the headspace, tubing, and the soil surface area.

### 6.3.3 Results and discussion

#### (i) Carbon dioxide (CO<sub>2</sub>)

In both cases, the respiration of soil microbes and dead plant roots accounts for the majority of the CO<sub>2</sub> flux produced in the soil. The initial organic carbon (OC) content of the BBFs and mineral fertilisers directly affects CO<sub>2</sub> emissions from the mesocosms and pot trials (Figure 64). Within the first five days of the experiment, 60% of the CO<sub>2</sub> emissions were caused by urea, demonstrating rapid mineralisation from hydrolysis. Due to their increased soil OC content, which facilitates microbial respiration, organic fertilisers generally produce noticeably higher emissions. The released C from BBFs in soils is regarded as biogenic C and is therefore considered to be C-neutral. Any CO<sub>2</sub> emissions that are present in these products result from the soil's pre-existing carbon having a positive priming effect.

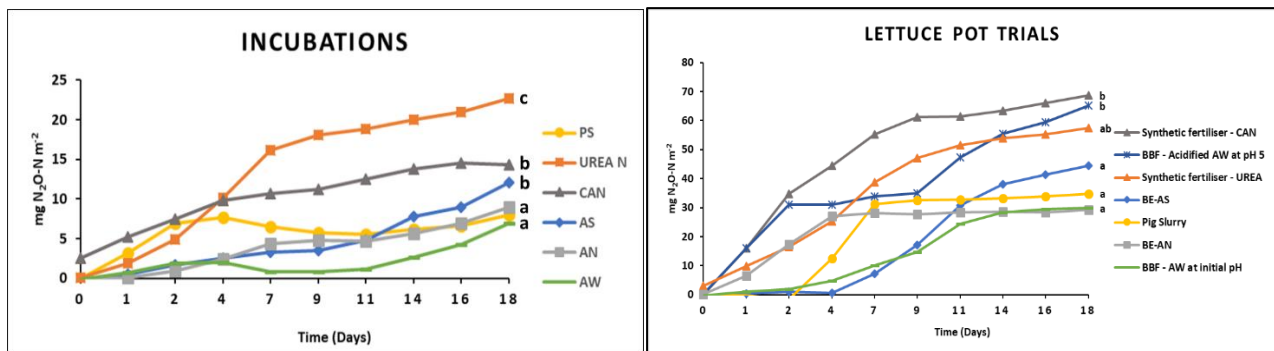


**Figure 64.** Cumulative emissions of carbon dioxide (mg CO<sub>2</sub>-C per m<sub>2</sub> of soil) from incubation and pot study. Legend: BE-AN: ammonium nitrate, BE-AS—ammonium sulphate, BE – AW: ammonia water. UREA– 46%

urea, CAN— 16% calcium ammonium nitrate, BE-AW<sub>ph5</sub> – BE-ammonia water at pH 5, PS – pig slurry and BE-AW<sub>phini</sub> – BE-ammonia water at initial pH. The values are obtained after subtracting control from all the treatments. Lowercase letters “a, b, c, d” represent significant difference among the treatments according to Tukey HSD.

(ii) Nitrous oxide (N<sub>2</sub>O)

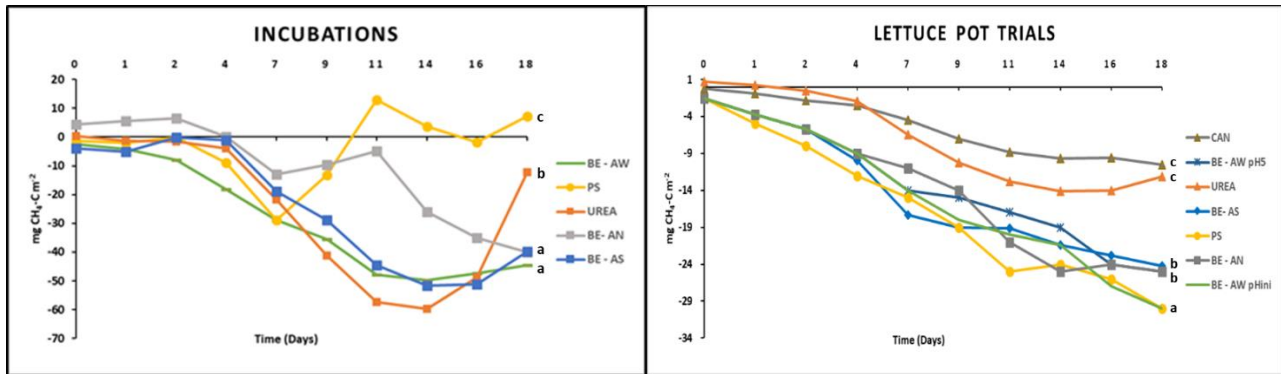
The synthetic fertilisers UREA (0.15%) and CAN (0.12%) resulted in the highest N<sub>2</sub>O emissions among the treatments examined (Figure 65), followed by bio-based fertilisers: BE-AS (0.10), BE-AN (0.08), and BE-AW (0.07). Similarly, in pot trials, the cumulative average N<sub>2</sub>O emissions ranged from 30 to 65 mg N<sub>2</sub>O-N m<sup>-2</sup> and peaked within the first seven days post-fertiliser application across all treatments. CAN (0.40% of applied N), BE-AW<sub>ph5</sub> (0.38% of applied N), and UREA (0.34% of applied N) showed the highest cumulative N<sub>2</sub>O emissions among the treatments. Compared to synthetic fertilisers, BBFs derived from primary and secondary manure/digestate processing are likely to emit less N<sub>2</sub>O (Figure 65). This is attributed to rapid hydrolysis in the soil within hours of application, increasing NH<sub>4</sub> availability followed by nitrification and N<sub>2</sub>O production (van der Weerden et al., 2016). N<sub>2</sub>O emissions from BBFs, however, were linked to the availability of OC, which provides energy for denitrifying bacteria. This increased bacterial activity lowers soil oxygen levels, promoting the denitrification of initially nitrified NH<sub>4</sub> and releasing more N<sub>2</sub>O (Velthof and Rietra, 2019). It is also important to note that the peak N<sub>2</sub>O emissions are likely due to nitrification, suggesting that a longer-duration experiment might be required.



**Figure 65.** Cumulative emissions of nitrous oxide (mg N<sub>2</sub>O-N per m<sub>2</sub> of soil) from incubation and pot study. Legend: BE-AN: ammonium nitrate, BE-AS—ammonium sulphate, BE – AW: ammonia water. UREA– 46% urea, CAN— 16% calcium ammonium nitrate, BE-AW<sub>ph5</sub> – BE-ammonia water at pH 5, PS – pig slurry and BE-AW<sub>phini</sub> – BE-ammonia water at initial pH. The values are obtained after subtracting control from all the treatments. Lowercase letters “a, b, c, d” represent significant difference among the treatments according to Tukey HSD.

(iii) Methane (CH<sub>4</sub>)

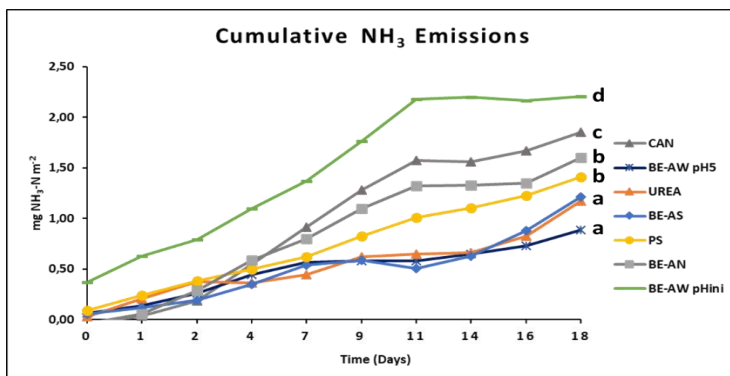
Due to the existence of aerobic conditions during the incubations, CH<sub>4</sub> emissions were noticeably low throughout the experiment. Additionally, it was discovered that applying manure-derived products to soil improved soil aeration, which in turn decreased CH<sub>4</sub> emissions (Figure 66). The net soil CH<sub>4</sub> flux is a result of methanogenesis and methanotrophism. All BBFs and mineral fertilisers (CAN and urea) cause negative methane emissions from the soil because their CH<sub>4</sub> intake is greater than their CH<sub>4</sub> production.



**Figure 66.** Cumulative emissions of methane ( $\text{mg CH}_4\text{-C per m}_2$  of soil) from incubation and pot study. Legend: BE-AN: ammonium nitrate, BE-AS—ammonium sulphate, BE – AW: ammonia water. UREA– 46% urea, CAN— 16% calcium ammonium nitrate, BE-AW<sub>ph5</sub> – BE-ammonia water at pH 5, PS – pig slurry and BE-AW<sub>phini</sub> – BE-ammonia water at initial pH. The values are obtained after subtracting control from all the treatments. Lowercase letters “a, b, c, d” represent significant difference among the treatments according to Tukey HSD.

(iv) Ammonia ( $\text{NH}_3$ )

For the cumulative  $\text{NH}_3$  emissions, the BE-AW<sub>phini</sub> ( $1.13 \text{ mg NH}_3\text{-N/m}^2$ ) showed highest  $\text{NH}_3$  emissions closely followed by PS ( $0.99 \text{ mg NH}_3\text{-N/m}^2$ ) and CAN ( $0.96 \text{ mg NH}_3\text{-N/m}^2$ ). However, the acidified BE-AW showed the lowest emissions among all the treatments compared (BE-AW<sub>ph5</sub> =  $0.34 \text{ mg NH}_3\text{-N/m}^2$ ). The emissions for BE-AW<sub>ph5</sub> were significantly lower in comparison to other treatments (Figure 67). In case of BE-AW<sub>phini</sub>, the ammonia emissions were significantly higher than rest of the treatments except CAN.



**Figure 67.** Cumulative emissions of ammonia ( $\text{mg NH}_3\text{-N per m}_2$  of soil). Legend: BE-AN: ammonium nitrate, BE-AS—ammonium sulphate, UREA– 46% urea, CAN— 16% calcium ammonium nitrate, BE-AW<sub>ph5</sub> – BE-ammonia water at pH 5, PS – pig slurry and BE-AW<sub>phini</sub> – BE-ammonia water at initial pH. The values are obtained after subtracting control from all the treatments. Lowercase letters “a, b, c, d” represent significant difference among the treatments according to Tukey HSD.

For the  $\text{NH}_3$  emissions, BE-AW<sub>phini</sub> and CAN showed significantly higher emissions as comparison to other treatments. Additionally, a reverse trend to  $\text{N}_2\text{O}$  emissions could be seen in this case – where BE-AW<sub>ph5</sub> and BE-AW<sub>phini</sub> are producing lowest and highest  $\text{NH}_3$  emissions, respectively. It has been well documented in the previous studies that acidification is commonly used to decrease the  $\text{NH}_3$  emissions from manure/slurry based applications (Sørensen and Eriksen, 2009; Fangueiro et al., 2009; 2010; 2013). Cumulative  $\text{NH}_3$  emissions throughout the period of measurement decreased by 40.9% in the pots of BE-AW<sub>ph5</sub> compared to the highest emissions observed for BE-AW<sub>phini</sub>. The similar results have also been found out in recent studies (Pedersen et al., 2022; Giolli et al., 2022; Pereira et al., 2022) where acidification of pig/cattle slurry resulted in 20-70%

decrease in NH<sub>3</sub> emissions. Additionally, it is also justified in some studies that acidification of manure derived fertiliser could outperform injection as a NH<sub>3</sub> mitigation measure (Keskinen et al., 2022; Silva et al., 2023). This reduction of NH<sub>3</sub> occurs due to basic nature of ammonia, can react with acidic compounds to form ammonium salts, which are less volatile and less likely to be emitted into the atmosphere (Schlesinger and Bernhardt, 2020). By lowering the pH of the environment, acidification can promote the formation of these ammonium salts, which can then remain in the soil or water and not be released into the air (Schlesinger and Bernhardt, 2020). Additionally, a successful NH<sub>3</sub> reduction was observed because the acidification caused a delay in the nitrification activities of microbes. This delay prevented the excessive formation of NO<sub>3</sub><sup>-</sup> that could have resulted in high leaching tendencies (Petersen and Sommer, 2011; Fanguerio et al., 2016).

#### 6.3.4 Conclusion and recommendation

This study aimed to assess soil GHG emissions following the use of bio-based fertilisers produced at Belgian pilot plants. N<sub>2</sub>O emissions were lowest for ammonium salts and raw pig manure. In comparison to urea or CAN, none of the bio-based fertilisers had more cumulative N<sub>2</sub>O emissions. The CO<sub>2</sub> emissions were directly proportional to the amount of OM content applied to the soil. Following this trend, pig slurry and urea showed the highest cumulative CO<sub>2</sub> emissions.

The acidification of BE-AW to pH 5 resulted in significantly lower NH<sub>3</sub> emissions compared to BE-AW<sub>pHini</sub>. In general, the comprehensive evaluation of BE-AW considering various agronomic and environmental factors reveals its promise as a sustainable alternative to synthetic N fertilisers. However, the thorough field validation of BE-AW across diverse environmental conditions, soil types, and crops is of utmost importance to gather comprehensive data on its agronomic attributes and environmental impacts in future studies.

### 6.4. Bio-dried fraction (ES-DSC) and ammonium sulphate (NL-AS) from Spanish pilot (UVIC-UCC) and Dutch pilot (WENR)

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

#### 6.4.1 Introduction

In this experiment, the risk for ammonia volatilisation of pig slurry, commercial ammonium sulphate and a TMF consisting of ammonium sulphate (NL-AS) and bio-dried fraction (ES-DSC) was assessed. The objective was to evaluate and compare the effectiveness of the TMF as alternative fertilisation strategy for reducing ammonia emissions.

#### 6.4.2 Methodology

The set-up used a volatilisation system, following the methodology adapted from Le Cadre et al. (2005) in a temperature-controlled laboratory (23 ± 3°C) with a randomized block design (Figure 68). A stream of clean air was injected into the enclosures. The outgoing air flow was bubbled into an acid trap allowing NH<sub>3</sub> dissolution. Before arriving to the final acid trap, air was humidified in 150 mL of water and cleaned from residual ammonia with a first acid trap with 0.05M H<sub>2</sub>SO<sub>4</sub>. Then it continued into the sample enclosure and finally arrived to the final acid trap, which consist of 5 mM H<sub>2</sub>SO<sub>4</sub>, only containing ammonia from the sample volatilisation. The airstream was adjusted to pump an airflow of 3L/min.



**Figure 68.** Set-up of the volatilisation system with acid traps used in the ammonia emission trial.

Sample blends were composed of 150g (dry matter) of soil in which products were incorporated. Blends were then watered to 50% WHC and incubated for 11 days. Tested treatments were unamended soil (negative control), bio-dried fraction and ammonium sulphate (TMF treatment), commercial ammonium sulphate (MINERAL treatment) and pig slurry (PS treatment). Each treatment was replicated 4 times. The N input was a single dose of 170 kg N/ha in each treatment. In the case of the TMF, the N input was incorporated from both bio-dried fraction and ammonium sulphate in equal parts. Table 45 sums up the different fertilisation treatments, while products characteristics are shown in Table 46.

For measuring the N volatilized as  $N-NH_4^+$ , the acid traps were sampled at days 1, 2, 3, 5, 8 and 11, after the beginning of the assay. Results were expressed in percentage of the N applied volatilized as  $N-NH_4^+$ . The data set obtained was statistically analysed using the IBM SPSS® 28 program. Analysis of variance (one-way ANOVA) and post-hoc analysis (DMS test) were conducted with a significance level of  $p \leq 0.05$ , to evaluate differences between the fertilisation treatments.

**Table 45.** Treatments applied in the ammonia volatilisation experiment.

Treatment	Compounds
CONTROL	No fertilisers added
MINERAL	Commercial ammonium sulphate
TMF	Bio-dried fraction (ES-DSC) + ammonium sulphate (NL-AS)
PS	Pig slurry

**Table 46.** Characteristics of the products used in the ammonia volatilisation experiment.

Parameter	Bio-dried fraction (ES-DSC)	Ammonium sulphate (NL-AS)	Pig slurry	Commercial ammonium sulphate
pH	7.19	-	-	-
Electrical Conductivity ( $\mu\text{s}\cdot\text{cm}^{-1}$ )	6.5	-	-	-
Organic matter ( $\text{g}\cdot\text{kg}^{-1}$ )	410.82	<1.8	-	-
NTK ( $\text{g N-total}\cdot\text{kg}^{-1}$ )	14.59	77.53	4.77	210
Ammonium ( $\text{g NH}_4^+\cdot\text{kg}^{-1}$ )	1.80	72.50	3.25	210
Total P ( $\text{g P}\cdot\text{kg}^{-1}$ )	5.45	<0.07	-	-
P soluble on water ( $\text{g P}\cdot\text{kg}^{-1}$ )	1.58	-	-	-
Total potassium ( $\text{g P}\cdot\text{kg}^{-1}$ )	11.64	<0.83	-	-
Copper ( $\text{g Cu}\cdot\text{kg}^{-1}$ )	0.078	-	-	-
Zinc ( $\text{g Zn}\cdot\text{kg}^{-1}$ )	0.62	-	-	-

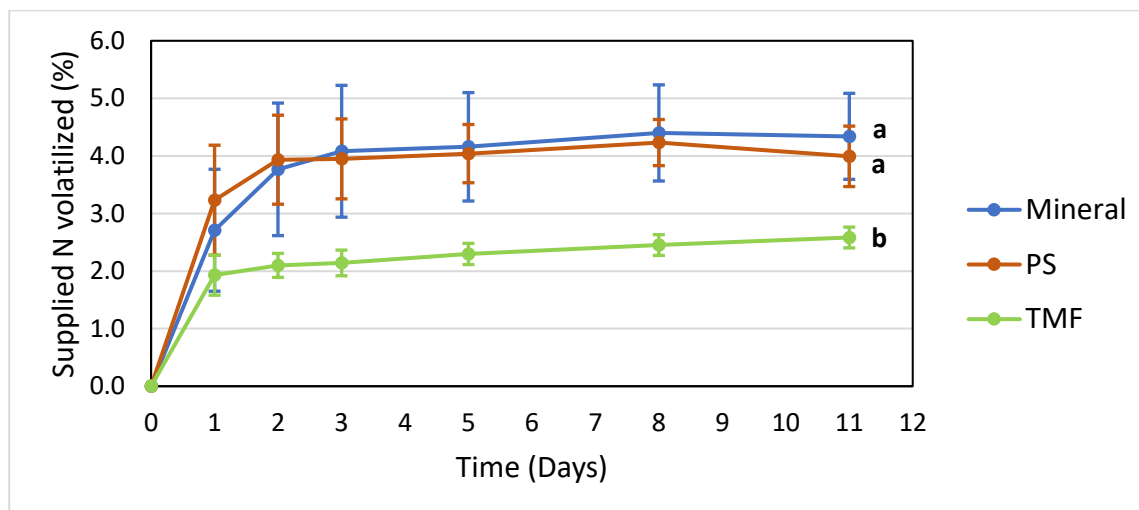


### 6.4.3 Results and discussion

The volatilised N fraction from the different treatments is shown in Figure 69. PS and TMF treatments exhibited high levels of ammonia volatilisation from day 1, whereas the mineral treatment volatilised ammonia more slowly, over the initial days. However, the percentage of N volatilised as ammonia was significantly lower in the soil fertilised with the TMF, while no differences were found between the mineral and PS treatments. Most of the N applied was  $\text{NH}_4^+$  by commercial sulphate and pig slurry, so similar results were expected between them. In the case of TMF, part of the N was applied with the bio-dried fraction, which contains mainly organic N, leading to slower volatilisation rate.

Thus, the TMF treatment volatilised 2.5% of the applied N by day 11, whereas the Mineral and PS treatments volatilised 4.3% and 4.0%, respectively, which is 72% and 60% higher than the TMF, respectively. Maximum volatilisation percentage was reached by the PS treatments at day 3, while mineral treatments reached it at day 8 and TMF treatments at day 11.

In general, values from all treatments were low compared to others reported in previous studies, but the range of N volatilisation is very wide. According to Powlson and Dawson (2021), in soils with  $\text{pH} > 7.0$ , ammonium sulphate losses can range from 0% to 66% of the total N applied, being the 20%-40% the most common range in laboratory experiences. Also, Bosch-Serra et al. (2014) estimated  $\text{NH}_3$  volatilisation in Spanish soils to be in the range of 7%-78% of the total ammonium N applied with mineral fertilisers or pig slurries. The emission of ammonia can vary depending on several factors such as soil moisture, temperature, or pH, in addition to the method used for capturing and determining ammonia (Huang et al., 2006).



**Figure 69.** Cumulated percentage of the supplied N volatilized (mean values  $\pm$  SE) throughout the experimental time, from the fertilisation with commercial ammonium sulphate (Mineral), the mixture of bio-dried fraction and ammonium sulphate (TMF) and the pig slurry (PS). Different letters represent significance differences between treatments according to DMS test ( $p \leq 0.05$ ;  $n = 4$ ).

### 6.4.4 Conclusion and recommendation

Based on the results of the experiment, TMF application resulted in significantly lower ammonia volatilisation, not only enhancing nutrient efficiency but also providing an environmental benefit by mitigating harmful gas emissions. Thus, the TMF can be used as a good alternative to the mineral fertilisers in order to promote more responsible and sustainable agronomic practices.



## 7. General conclusions and recommendations

### 7.1. Tailor-made fertilisers (Fertinagro, UVIC-UCC)

#### 7.1.1 TMF (ES-DSC + NL-AS + ES-AA) (UVIC-UCC)

Based on the results of the Spanish field trial with winter wheat, in general none of the tested treatments (i.e. TMF, Raw Manure and Mineral fertiliser treatment) were able to increase crop yield in the 2-year duration of the experiment in comparison to unfertilised control. Comparing the crop yield obtained in FERTIMANURE trial with an average winter wheat yield values that are expected per harvest and the initial soil characterisation, we can probably conclude that the soil already had enough nutrients to cover the crop requirements.

Results on soil  $\text{NO}_3\text{N}$  residue showed significantly higher values in Mineral fertiliser treatment. Therefore, if necessary to use a Mineral fertiliser, its application should be done at low doses and with a very strict control of the crop and the soil. Furthermore,  $\text{NH}_3$  volatilisation was also found higher in Mineral and Raw Manure treatments than in TMF (ES-DSC + NL-AS) treatments. This may indicate that the TMF can be a good alternative to mineral fertilisers in order to optimise nutrient use in agriculture, reducing the risk of nitrate pollution by leaching and the  $\text{NH}_3$  emissions to the atmosphere.

The findings of this study suggest that the use of TMF formulation, and the conventional Mineral fertilisation, may be interchangeable in agricultural practice for some crops, providing farmers with an additional choice in fertiliser selection without significantly compromising crop performance. This is particularly relevant in a context where fertiliser choice may be influenced by economic and environmental considerations.

#### 7.1.2 TMF (ES-AS + ES-NC) (UVIC-UCC)

The TMF was effective in increasing crop yield of spinach cultivation, but not in lettuce. The salinity of the ES-NC could be the cause of the poor results obtained for lettuce cultivation, since this is a crop very sensitive to increases in soil salinity. Despite this, in accordance with the results obtained in the field trial, TMF fertilisation resulted in lower soil  $\text{NO}_3\text{N}$  residue after harvest, indicating a reduction in leaching risk in comparison with Mineral treatment.

#### 7.1.3 TMF (Fertinagro)

For the Fertinagro's TMF in potato cultivation, yield results in traditional mineral fertilisation and TMF approaches were similar. Therefore, slurry can be improved on-farm and expected yield can be achieved. In this TMF fertilisation plan, due to improvements in the adequacy of the product for the potato cash crop, more pig slurry could be used, effectively replacing mineral fertilisers, so that the amount of mineral N fertiliser applied was lower than in the reference case (sheep manure + mineral fertiliser). Therefore, the TMF practice reduces the amount of chemical fertilisers used and also helps mitigate any possible toxic effects of heavy metals and antibiotics on the crop and soil.

### 7.2. Ammonium sulphate solution (UGent, WENR, CRAB, CRAGE, C80, RITTMO)

At a given level of N fertilisation, in general, there were no significant differences observed in respect to the crop yield between tested ammonium sulphate solutions (FR-AS, BE-AS and NL-AS) and synthetic N fertiliser references (Table 47). In Dutch experiments, sometimes an effect of the soil (clay vs. sand) was observed by reporting lower grass and maize yield on clay soil.

In regard to NFRV, application of FR-AS led to higher N efficiency (NFRV: 1.12 in 2021 and 1.24 in 2022 for spinach, and 0.97 in 2022 and 2.10 in 2021 for maize) than synthetic N fertiliser at a 30% N rate for spinach and maize. However, since additional N-fertilisation did no longer result in additional N-uptake, the ANR and NFRV values for 60% and 100% treatments could not be interpreted for these crops. For potato trial in France also higher N efficiency was observed for FR-AS at a 100% rate (NFRV: 1.13 in 2021 and 2.11 in 2022), whereas for BE-AS in Flanders higher N efficiency was observed only at a 70% N rate (1.04 in 2021 and 1.29 in 2022). For cabbage and winter wheat trial lower efficiency of FR-AS was observed (NFRV at 100% $\times$  = 0.59 in 2021 and 0.80 in 2023 for cabbage, and 0.77 for winter wheat). In Dutch pot trial, NL-AS performed similarly to CAN reference (NFRV at 75% N rate mean sand+clay maize 1.05, grass 1.04). However, lower NFRV was observed in clay soil as compared to NFRV in sand soil. In sugar beet field trial N efficiency could not be determined as there were no differences as compared to the unfertilised control. For ryegrass pot trial the NFRV of FR-AS at 30%, 60% and 100% N rate was 1.66, 0.92 and 0.94 respectively.

In all scientific field trials, at a given level of N fertilisation there was no significant difference observed in regard to soil nitrate residue between ammonium sulphate solutions and used references of synthetic N fertiliser.

For gaseous emissions, FR-AS and the respective synthetic N fertiliser reference (CAN) showed no significant effect on NH<sub>3</sub> volatilisation. Similar results were observed in WENR's pot experiment which provided no evidence for significant differences in NH<sub>3</sub>, N<sub>2</sub>O, or CH<sub>4</sub> emissions caused by fertiliser type (NL-AS vs. CAN). However, this may have been caused by the very low amount of emissions that were measured overall. The low amount of N emissions aligned with the N balance, which shows that the amount of uptake by the plant was higher than the application rate by approximately the amount taken up by the control, implying that the crop was able to utilise all or most of the applied N in addition to some N already in the soil and that there was very little loss. This experiment was done under controlled conditions; in contrast the field trials of 2022 and 2023 that lend insight to emissions in a less controlled environment. Here, the N<sub>2</sub>O emissions measured by Innova showed a higher emission from CAN fields compared to NL-AS fields after rainfall. Similar was observed by UGent on incubation level where mineral fertilisers (CAN and urea) showed highest N<sub>2</sub>O emissions due to the rapid hydrolysis of products after application, resulting in increased NH<sub>4</sub> availability. Finally, RITMO investigated the effect of FR-BC addition to FR-AS on N<sub>2</sub>O emissions. Results have shown that addition of FR-BC has reduced the N<sub>2</sub>O emissions of FR-AS by 30%.

In regard to the recommendations for practical use or future tests, ammonium sulphate has a potential to be used as a replacement for synthetic N fertilisers. The practical challenges can be seen in low N content and hence large volumes of the BBF that should be applied to satisfy crop N needs. In 2023 cabbage field trial the localization of fertilisation was tested, to bring N as close as possible to the plants, to evaluate if it is possible to reduce the quantities of FR-AS applied. Two treatments were tested but were not conclusive. The localized applications generated burns which caused the death of the cabbages. In view of the damage caused by localized application of ammonium sulphate, this method of fertilisation is not an option for farmers. To reduce ammonia and GHG emissions, the BBF should be injected and hence more specific application machinery might be needed (as it was done in Belgian and Dutch field trials). Moreover, pH of ammonium sulphate can vary from acidic to neutral. In order to mitigate potential ammonia and GHG emissions, the pH of ammonium sulphate should be advised to keep in acidic range.

Finally, this BBF is not only N rich, but also contains significant amount of S. The 2022 grass field trial showed that S uptake may be increased when using high amounts of NL-AS. In order to prevent toxicity to cattle when used as feed, these increased values must be accounted for in their entire diet or NL-AS must only be used to top-up the use of animal manure, instead as the sole N fertiliser. This usage is also more comparable to the use of CAN in practice for Dutch dairy systems, where it is used to top up the N dosage after fertilisation with animal manure in accordance with the Nitrates Directive.

**Table 47.** Overview of field trial results for ammonium sulphate solution as compared to synthetic N reference (MinRef).

Description		Yield	ANR / NFRV	Nitrate leaching	
FR	Spinach (field trial – 2021 and 2022)	FR-AS vs. MinRef (Ammonium nitrate 33.5% N)	<ul style="list-style-type: none"> <li>- No significant difference</li> <li>- No visible effect of incremental rates</li> </ul>	<ul style="list-style-type: none"> <li>- For a 30%X N rate, the N efficiency of FR-AS &gt; MinRef (in 2021 ANR: FR-AS 0.87 vs. MinRef 0.78; NFRV of FR-AS = 1.12; in 2022 ANR: FR-AS 1.26 vs. MinRef 1.02; NFRV of FR-AS = 1.24)</li> <li>- ANR and NFRV values for 60%-X and 100%-X treatments could not be interpreted.</li> </ul>	- No significant differences
	Silage maize (field trial – 2021 and 2022)	FR-AS vs. MinRef (Ammonium nitrate 33.5% N)	<ul style="list-style-type: none"> <li>- No significant difference</li> <li>- No visible effect of incremental rates</li> </ul>	<ul style="list-style-type: none"> <li>- For a 30%X N rate, the N efficiency of FR-AS &gt; MinRef (in 2021 ANR: FR-AS 1.40 vs. MinRef 0.67; NFRV of FR-AS = 2.10; in 2022 ANR: FR-AS 0.96 vs. MinRef 0.99; NFRV of FR-AS = 0.97)</li> <li>- ANR and NFRV values for 60%-X and 100%-X treatments could not be interpreted.</li> </ul>	- No significant differences
	Winter wheat (field trial 2023)	FR-AS vs. MinRef (Ammonium nitrate 33.5% N)	<ul style="list-style-type: none"> <li>- No significant difference</li> </ul>	<ul style="list-style-type: none"> <li>- NFRV of FR-AS was 0.77 for a 53%X N rate (X = 170 kg N/ha)</li> </ul>	- No significant differences
	Sauerkraut cabbage (field trial – 2021, 2022 and 2023)	FR-AS vs. MinRef (Basammon 26% N)	<ul style="list-style-type: none"> <li>- No significant difference in 2021 and 2022, but lower yield observed in 2023 with FR-AS</li> </ul>	<ul style="list-style-type: none"> <li>- At equivalent dose applied on cabbages, FR-AS efficiency is always lower than the MinRef efficiency.</li> <li>- In 2021 NFRV of FR-AS at 30%, 60% and 100% applied N dosage was respectively 0.23, 0.44 and 0.59. In 2023, NFRV of FR-AS at 60% and 100% was 0.80. 30% dose was not tested in 2023, and no determination of NFRV in 2022</li> </ul>	- No significant differences
	Sugar beet (field trial – 2021 and 2022)	FR-AS vs. MinRef (Ammonium nitrate 27% N)	<ul style="list-style-type: none"> <li>- No significant difference</li> </ul>	<ul style="list-style-type: none"> <li>- No significant differences were observed between control and FR-AS or MinRef for N uptake. Therefore, ANR and NFRV are not calculated for this trial.</li> </ul>	- No significant differences
	Potato (field trial – 2021 and 2022)	FR-AS vs. MinRef (N39 solution)	<ul style="list-style-type: none"> <li>- No significant difference</li> </ul>	<ul style="list-style-type: none"> <li>- In 2021 NFRV of FR-AS at 30%, 60% and 100% applied N dosage was respectively 0.55, 0.89 and 1.13. In 2022 NFRV of FR-AS at 30%, 60% and 100% applied N dosage was respectively 0.51, 0.86 and 2.11.</li> </ul>	- No significant differences
	Ryegrass (pot trial in 2023)	FR-AS vs. MinRef (calcium)	<ul style="list-style-type: none"> <li>- No significant difference</li> </ul>	<ul style="list-style-type: none"> <li>- NFRV of FR-AS at 30%, 60% and 100% was 1.66, 0.92 and 0.94 respectively.</li> </ul>	- Not applicable as it is a pot trial



		ammonium nitrate 16% N)			
NL	Grass and Maize (field trial – 2021 and 2022; pot trial 2021)	NL-AS vs. MinRef (calcium ammonium nitrate 26% N)	<ul style="list-style-type: none"> <li>- No significant difference</li> <li>- More the effect of the soil (sand vs. clay)</li> <li>- Sulphur uptake in grass might be increased with NL-AS</li> </ul>	<ul style="list-style-type: none"> <li>- No significant difference between treatments. However, results indicate lower NFRV values on clay soil.</li> </ul>	<ul style="list-style-type: none"> <li>- Not performed in field trial.</li> </ul>
BE	Potato (field trial 2021 and 2022)	BE-AS vs. MinRef (ammonium nitrate 30%)	<ul style="list-style-type: none"> <li>- No significant difference between treatments</li> <li>- No visible effect of incremental rates</li> </ul>	<ul style="list-style-type: none"> <li>- NFRV of BE-AS in 2021 at 40%, 70% and 100% applied N dosage was respectively 0.51, 1.04 and 0.76.</li> <li>- NFRV of BE-AS in 2022 at 40%, 70% and 100% applied N dosage was respectively -0.08, 1.29 and 0.71.</li> </ul>	<ul style="list-style-type: none"> <li>- No significant differences</li> </ul>

### 7.3. Ammonium nitrate solution (UGent)

In case of BE-AN obtained from Belgian pilot, the field trial results showed a slightly reduced average yield in comparison to BE-AS and synthetic N fertiliser. However, no significant differences could be observed between treatments for all the three incremental N dosages.

NFRV of BE-AN was lower than the one of synthetic N fertiliser, resulting in 0.47, 0.85 and 0.82 in 2021 and 0.51, 1.04 and 0.76 in 2022 for dose of 40%, 70% and 100% N. As compared to BE-AS, whose mineral N is fully in  $\text{NH}_4\text{-N}$  form, the mineral N in BE-AN is a sum of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ . The  $\text{NO}_3\text{-N}$  is more prone to leaching than  $\text{NH}_4\text{-N}$  and heavy rain at the start of the potato trial probably leaked  $\text{NO}_3\text{-N}$  from the top layer, making it unavailable for the crop and hence leading to lower yield and NFRV as compared to BE-AS and synthetic N fertiliser. The synthetic N fertiliser used was ammonium nitrate 30%, but in granulated form – which probably delayed the loss of  $\text{NO}_3\text{-N}$  in that treatment.

In regard to environmental performance, there was no significant difference between BE-AN, BE-AS and synthetic N fertiliser when it comes to nitrate residue in post-harvest period. For the GHG emissions, the BE-AN showed the lower amount of  $\text{N}_2\text{O}$  emissions in comparison to synthetic N fertiliser and pig slurry.

### 7.4. Ammonium water (UGent)

The lettuce pot experiment with ammonium water (BE-AW) has shown no significant differences between treatments fertilised with BE-AW and synthetic N fertiliser reference in regard to crop yield. Comparing the performance of BE-AW at tested pH levels (BE-AW<sub>phini</sub> and BE-AW<sub>ph5</sub>) in terms of lettuce yield, on average the BE-AW<sub>ph5</sub> exhibited lower performance at 30% and 100% dose in comparison to BE-AW<sub>phini</sub>. No significant differences were observed in respect to incremental rates.

For NFRV, a reduction was seen for BE-AW at both pHs. The BE-AW<sub>ph5</sub> resulted in NFRV of 0.03, 0.60 and 0.36 at 30%, 60% and 100% N dose as compared to BE-AW<sub>phini</sub> (NFRV = 0.64, 0.71 and 0.72 for the respective incremental dose). A significant decrease could be noted for BE-AW<sub>ph5</sub> at 30% and 100% of the dose. At 30%, the lower NFRV could be the result of reduced N-uptake (even less than unfertilised control). Nevertheless, the standard deviation was too high at both doses to draw conclusions on NFRV. Therefore, FUE and FRUE indicators were calculated (by excluding unfertilised control). According to these results BE-AW<sub>phini</sub> performed similarly to CAN (FRUE = 0.93, 0.92 and 0.92 at 30%, 60% and 100%), and for BE-AW<sub>ph5</sub> slightly lower FRUE (0.82, 0.89 and 0.81 at 30%, 60% and 100%) was observed.

For the GHG emissions, the BE-AW showed the lowest amount of  $\text{N}_2\text{O}$  emissions out of all the treatments compared. The BE-AW exhibited merely half  $\text{N}_2\text{O}$  emission (7 mg  $\text{N}_2\text{O-N m}^{-2}$ ) in comparison to the CAN for the same N-applied.

Following these results, the recommendation would be to test BE-AW in full scale field trial conditions. However, due to the concerning problem of ammonia emissions, UGent plans to assess this aspect in the following period. Additionally, due to the societal and agronomic concerns, the pH moderation of ammonium water by acidification or dilution might be recommended.

### 7.5. Biochar (CRAB, CA80, CRAGE, RITTMO)

Field trials on using FR-BC as a P source in potato, cabbage and sugar beet cultivation were conducted in France. In none of the field trials an effect of biochar on crop yield was observed. Moreover, there were no significant differences observed between FR-BC, TSP and unfertilised control. Therefore, APR and PFRV of FR-BC could not be assessed in the field trials. In ryegrass pot trial carried out at field conditions the FR-BC led on average to similar or slightly higher yields than the unfertilised plots or plots fertilised with poultry

manure. Due to the technical error the performance of FR-BC against TSP could not be assessed, and APR value of 0.11 and 0.18 was recorded for FR-BC at 40% and 80% P dose. If we look at the RITTMO's results of ryegrass pot trial in greenhouse conditions (D4.5), also low APR values were recorded. Moreover, in greenhouse conditions the PFRV was calculated and it amounted to 48%. As such, FR-BC could partially replace the mineral P source.

Furthermore, French partners made an attempt to assess the use of FR-BC for N retention capacity in potato trial, but there were no differences observed between treatments with and without biochar. For NH<sub>3</sub> and GHG emissions, the effect of adding biochar (FR-BC) to other fertilisers in order to reduce emissions was investigated. The effect was not visible in terms of NH<sub>3</sub>, but was observed for N<sub>2</sub>O. The results showed that for treatments having exhausted pig slurry as N-fertiliser, FR-BC addition increased N<sub>2</sub>O daily emissions by 10%, but for treatments with ammonium sulphate (FR-AS) as N-fertiliser, biochar reduced N<sub>2</sub>O emissions by 30% (Figure 48). Thus, these contrasted results highlight the fact that the impact of biochar on N<sub>2</sub>O emissions induced by N-fertilisation is dependent on the type of N-fertiliser employed.

The benefits of biochar for fertilising ryegrass or other arable crops are also still low, given the large quantities to be applied, the difficulty of spreading and mixing it with the soil. This BBF would be best suited to fertilising high added value crops (given the potential cost of the product) and would be ideally used in a formulation with other fertilisers.

## 7.6. Liquid potassium fertiliser (CRAGE)

At a given level of K-fertilisation (50%, 100% K dose) in sugar beet cultivation, no significant differences were observed between the unfertilised control and FR-LK or mineral fertiliser for all the measured parameters. There were strong effects of dry conditions in 2022, so these trials do not allow us to assess the performance of liquid K fertiliser (FR-LK). It is difficult to draw robust conclusions for this crop and we can conclude that liquid K fertiliser is currently not efficient and must be improved to be used by farmers..

In practice, as for the ammonium sulphate (FR-AS), the use of FR-LK for sugar beet could be limited by the technical feasibility of the application (more than 2000L/ha required).



## References

1. Alexander, J. R., Spackman, J. A., Wilson, M. L., Fernández, F. G., & Venterea, R. T. (2021). Capture efficiency of four chamber designs for measuring ammonia emissions. *Agrosystems, Geosciences & Environment*, 4(3), e20199.
2. Bosch-Serra A.D., Yagüe M.R., Teira-Esmatges M.R. (2014). Ammonia emissions from different fertilizing strategies in Mediterranean rainfed winter cereals. *Atmos Environ* 84: 204-212. <https://doi.org/10.1016/j.atmosenv.2013.11.044>
3. Bouwman, A. F., Boumans, L. J. M., & Batjes, N. H. (2002). Modeling global annual N<sub>2</sub>O and NO emissions from fertilized fields. *Global Biogeochemical Cycles*, 16(4), 28-1.
4. Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R. et Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils : how well do we understand the processes and their controls ? *Philosophical Transactions of the Royal Society B : Biological Sciences*, 368(1621):20130122.
5. CBS (2022). Grasland; oppervlakte en opbrengst. StatLine. URL <https://opendata.cbs.nl/statline/#/CBS/nl/dataset/7140gras/table?dl=6F976>
6. DACC. (2019) Taula d'extraccions de cultius extensius. <https://ruralcat.gencat.cat/oficina-de-fertilitzacio/taules-dades/extraccions-dels-cultius>
7. De Ruijter, F. J., Huijsmans, J. F. M., & Rutgers, B. (2010). Ammonia volatilization from crop residues and frozen green manure crops. *Atmospheric Environment*, 44(28), 3362-3368.
8. El-Bassyouni, M. S. S. (2016). Effect of Different Nitrogen Sources and Doses on Lettuce Production. *Middle East Journal of Agriculture Research*, 05(04), 647-654.
9. Fangueiro, D., Ribeiro, H., Coutinho, J., Cardenas, L., Trindade, H., Cunha-Queda, C., ... & Cabral, F. (2010). Nitrogen mineralization and CO<sub>2</sub> and N<sub>2</sub>O emissions in a sandy soil amended with original or acidified pig slurries or with the relative fractions. *Biology and fertility of soils*, 46, 383-391.
10. Fangueiro, D., Ribeiro, H., Vasconcelos, E., Coutinho, J., & Cabral, F. (2009). Treatment by acidification followed by solid-liquid separation affects slurry and slurry fractions composition and their potential of N mineralization. *Bioresource technology*, 100(20), 4914-4917.
11. Fangueiro, D., Surgy, S., Fraga, I., Monteiro, F. G., Cabral, F., & Coutinho, J. (2016). Acidification of animal slurry affects the nitrogen dynamics after soil application. *Geoderma*, 281, 30-38.
12. Gioelli, F., Grella, M., Scarpeci, T. E., Rollè, L., Pierre, F. D., & Dinuccio, E. (2022). Bio-Acidification of Cattle Slurry with Whey Reduces Gaseous Emission during Storage with Positive Effects on Biogas Production. *Sustainability*, 14(19), 12331.
13. Gulser, F. (2005). Effects of ammonium sulphate and urea on NO<sub>3</sub> and NO<sub>2</sub> accumulation, nutrient contents and yield criteria in spinach. *Scientia Horticulturae*, 106, 330-340.
14. Hénault, C., Grossel, A., Mary, B., Roussel, M., & Léonard, J. (2012). Nitrous oxide emission by agricultural soils: a review of spatial and temporal variability for mitigation. *Pedosphere*, 22(4), 426-433.
15. Huang B.X., Fang S.U., Ding X.Q., et al. 2006 J. German wind-tunnel system for measuring ammonia volatilization from agricultural soil. *Soils*. 38 712-716.



16. 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.
17. Jones, J. B., (2016). Hydroponics: a Practical Guide for the Soilless Grower. CRC press, Boca Raton, 0-8493-3167-6.
18. Keskinen, R., Termonen, M., Salo, T., Luostarinen, S., & Rätty, M. (2022). Slurry acidification outperformed injection as an ammonia emission-reducing technique in boreal grass cultivation. *Nutrient Cycling in Agroecosystems*, 122(2), 139-156.
19. Krężel, J., & Kołota, E. (2010). The effect of nitrogen fertilization on yielding and biological value of spinach grown for autumn harvest. *Acta Scientiarum Polonorum, Hortorum Cultus*, 9(3), 183-190.
20. Le Cadre E., Géniermont S., Decuq C., Recous S., Cellier P . « A laboratory system to estimate ammonia volatilization » in *Agronomy for Sustainable Development* 25 (2005), 101-107.
21. Machado, R. M. A., Alves-Pereira, I., Lourenço, D., & Ferreira, R. M. A. (2020). Effect of organic compost and inorganic nitrogen fertigation on spinach growth, phytochemical accumulation and antioxidant activity. *Heliyon*, 6(9), e05085.
22. Olaniyi J.O.; Comparative Effects of the Source and Level of Nitrogen on the Yield and Quality of Lettuce, *Am.-Eurasian J. Sustain. Agric.*, 2(3): 225-228, 2008
23. Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P., & Smith, P. (2016). Climate-smart soils. *Nature*, 532(7597), 49-57.
24. Pedersen, J., Feilberg, A., & Nyord, T. (2022). Effect of storage and field acidification on emissions of NH<sub>3</sub>, NMVOC, and odour from field applied slurry in winter conditions. *Journal of Environmental Management*, 310, 114756.
25. Pereira, J. L., Perdigão, A., Marques, F., Wessel, D. F., Trindade, H., & Figueiro, D. (2022). Mitigating Ammonia and Greenhouse Gas Emissions from Stored Pig Slurry Using Chemical Additives and Biochars. *Agronomy*, 12(11), 2744.
26. Petersen, S. O., & Sommer, S. G. (2011). Ammonia and nitrous oxide interactions: roles of manure organic matter management. *Animal Feed Science and Technology*, 166, 503-513.
27. Powlson, D. S., & Dawson C. J. (2022). Use of ammonium sulphate as a sulphur fertilizer: Implications for ammonia volatilization. *Soil Use and Management*, 38, 622–634. <https://doi.org/10.1111/sum.12733>
28. Rietra, René, Kimo van Dijk, and Oscar Schoumans (2024). "Environmental Effects of Using Ammonium Sulfate from Animal Manure Scrubbing Technology as Fertilizer" *Applied Sciences* 14, no. 12: 4998. <https://doi.org/10.3390/app14124998>
29. Schiils, R., Schröder, J., & Ve thof, G. (2020). Fertiliser Replacement Value. In *Biorefinery of Inorganics* (pp. 189-214).
30. Schlesinger, W.H. and Bernhardt, E.S, (2020). Biogeochemistry: An Analysis of Global Change. 4th edition. Academic Press. <https://doi.org/10.1016/C2017-0-00311-7>

31. Schlossberg, M. J., McGraw, B. A., & Hivner, K. R. (2018). Comparing closed chamber measures of ammonia volatilization from Kentucky bluegrass fertilized by granular urea. *Journal of Environmental Horticulture*, 36(3), 85-91.
32. Sha, Z., Li, Q., Lv, T., Misselbrook, T., & Liu, X. (2019). Response of ammonia volatilization to biochar addition: a meta-analysis. *Science of the Total Environment*, 655, 1387-1396.
33. Shrivastava, V., Sigurnjak, I., Edayilam, N., & Meers, E. (2023). Ammonia water as a biobased fertiliser: Evaluating agronomic and environmental performance for *Lactuca sativa* compared to synthetic fertilisers. *Biocatalysis and Agricultural Biotechnology*, 102907.”
34. Silva, A. A., Fangueiro, D., & Carvalho, M. (2022). Slurry acidification as a solution to minimize ammonia emissions from the combined application of animal manure and synthetic fertilizer in no-tillage. *Agronomy*, 12(2), 265
35. Sørensen, P., & Eriksen, J. (2009). Effects of slurry acidification with sulphuric acid combined with aeration on the turnover and plant availability of nitrogen. *Agriculture, ecosystems & environment*, 131(3-4), 240-246.
36. Van der Weerden, T. J., Luo, J., Di, H. J., Podolyan, A., Phillips, R. L., Saggar, S., ... & Rys, G. (2016). Nitrous oxide emissions from urea fertiliser and effluent with and without inhibitors applied to pasture. *Agriculture, Ecosystems & Environment*, 219, 58-70.
37. Velthof, G. L., & Rietra, R. P. J. J. (2018). *Nitrous oxide emission from agricultural soils* (No. 2921). Wageningen Environmental Research.

## 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)

Dorset Green Machines (The Netherlands)

Prinsen Dairy Company (The Netherlands)

French Chamber of Agriculture (France)

Cooperativa Plana de Vic (Spain)

AlgaEnergy S.A. (Spain)

Fertinagro Biotech (Spain)

RITTMO Agroenvironnement (France)

Agrifutur (Italy)

Departament d'Agricultura, Ramaderia, Pesca I Alimentació (Spain)

Fertilisers Europe (Belgium)

Instituto Nacional de Tecnología Agropecuaria (Argentina)

### PROJECT WEBSITE:

<https://www.fertimanure.eu>

## 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

**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.