

UNIVERSITY OF COPENHAGEN  
FACULTY OF SCIENCE



# Novel biobased nitrogen fertilizers

## Risks of nitrogen losses, agronomic performance & effects on soil quality

PhD thesis by Lærke Wester-Larsen



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PhD thesis

Lærke Wester-Larsen

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Plant and Soil Science Section

Department of Plant and Environmental Sciences

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Front page picture of biobased fertilizers, from top ECO, FEK, BIO, BA6, MO13 and PAL, photo by Lærke Wester-Larsen

### **Novel biobased nitrogen fertilizers**

### **Risks of nitrogen losses, agronomic performance & effects on soil quality**

PhD thesis 2024

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## **Preface**

The work presented in this PhD thesis was conducted during the period from 2020 to 2023 at the University of Copenhagen, Department of Plant and Environmental Sciences. Part of the work took place at the Research Institute of Organic Agriculture (FiBL) and at Aarhus University, Department of Agroecology.

This PhD was part of, and received funding from, the European Union's Horizon 2020 research and innovation program under grant agreement No. 818309 (LEX4BIO).

This PhD thesis is based on three scientific papers (studies I-III), which I wrote as first author. One paper is published (study I) and the other two have been submitted (studies II and III). I am also contributing to three scientific papers as a co-author. One of these papers can be found in Appendix IV of this PhD thesis. The other two are currently being prepared. Apart from the three scientific papers on which this PhD is based (studies I-III), data from Agostini et al. (submitted, Appendix IV) and a Master's thesis by Esben Pedersen, whom I supervised, are presented and discussed in the general discussion chapter.

## Acknowledgement

First and foremost, I would like to thank my two supervisors, Lars Stoumann Jensen and Dorette Sophie Müller-Stöver, for your amazing support during the last few years. Even though you are often busy, you always took the time to answer my questions when I came and knocked on your doors and you were always quick to reply to my emails. You have always given thorough and good feedback on my work and you were always ready to join me in the field when I needed some help. I am very grateful to have had the two of you as my supervisors.

I would like to thank the Soil Fertility group for great social and academic company. Thanks to all of my colleagues for always being helpful when I asked for method guidance or help in the field or the lab. I would especially like to thank Line Vinther Hansen and Tine Engedal, with whom I shared an office throughout my PhD research. You were always there for me to share both the happy, sad, and nerdy moments and I cannot imagine better office mates than you two. Also, a special thanks to the PhD students of the Soil Fertility group as well for your great company and support. Whether we were having fun, brainstorming how to turn our research topics into silly business ideas, or acknowledging the tough parts of PhD life, I have appreciated our PhD community a lot. A special thanks as well to our lab technician Jannie Jessen. Thanks for your endless patience, great company, support, and guidance. Thanks also to the technical staff at the Plant and Soil Science Section and at Højbakkegård in Taastrup for your help and guidance in the lab and in the field.

I would also like to thank my colleagues in the LEX4BIO project for great discussions and collaborations. It has been inspiring being part of a bigger project. Special thanks to Else Bünemann-König, Lucilla Agostini, and Sarah Symanczik for welcoming me at the Research Institute of Organic Agriculture (FiBL). I enjoyed our academic discussions and your company during my stay and also our remote collaborations. Also, thanks to the European Union's Horizon 2020 research and innovation program for funding this study.

Last, but not least, I would like to thank my very patient partner, Casper, who has listened to a lot of complaining in the tough periods. Thank you for cooking dinners for me after long nights in the lab and for giving me a hug when I needed it. Also, thank you for listening to all of my nerdy stories when I was over-excited. You have been a great support.

## Summary

The agricultural sector is currently facing the challenges of reducing climate and environmental impacts, like all other sectors. Biobased fertilizers (BBFs), produced from organic waste and side-streams, could be one of the means to tackle this challenge by recycling otherwise wasted nutrients and substituting mineral fertilizers. Lately, political interest in recycling nutrients, in a circular economy context, has been increasing. Moreover, the commercial BBFs market is growing and many novel BBFs are now being introduced. However, little knowledge exists on the pros and cons of using these novel BBFs. The aim of this PhD research project was therefore to investigate novel BBFs as a substitute for mineral nitrogen (N) fertilizers and i) Evaluate the main N loss risks from the use of BBFs ii) determine the agronomic performance of field crops fertilized with BBFs and iii) evaluate the effect of BBF application on soil quality.

The environmental risks of using BBFs were assessed in a laboratory study (study I) investigating the ammonia ( $\text{NH}_3$ ) volatilization risk. The  $\text{NH}_3$  volatilization risk was found to vary greatly among BBFs both regarding temporal patterns and the accumulated amount of  $\text{NH}_3$  volatilized. It was affected by soil type and the incorporation of BBFs into soil effectively reduced the  $\text{NH}_3$  volatilization risk. A laboratory study conducted by Pedersen (2021) assessing the nitrous oxide ( $\text{N}_2\text{O}$ ) fluxes from the application of BBFs to soil showed a higher emission of  $\text{N}_2\text{O}$  from BBFs compared to mineral N ( $\text{NH}_4\text{NO}_3$ ) fertilizer. The N leaching risk was assessed from soil mineral N (sum of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) content (0-90 cm) 18 days after harvest in a field trial (study II). The soil mineral N content indicated that BBFs do not have a higher risk of N leaching than mineral N ( $\text{NH}_4\text{NO}_3$ ) fertilizer.

The agronomic performance of BBFs was assessed in field trials at different European sites (study II). The study found that most of the BBFs performed equally well as the mineral N fertilizer reference. A tendency of a second year residual fertilization effect, higher than the mineral reference, was found for some BBFs at some sites, though it was not significant. However, the performance of individual BBFs was not consistent across sites and years. No good explanations were found for this inconsistency. In a comparison of BBFs with more traditional organic fertilizers, e.g. manures, with the same source materials, the BBFs generally had a higher agronomic performance.

Biobased fertilizer effects on soil quality were assessed in a laboratory study simulating long-term application (study III). The study showed that BBFs had a neutral to improving effect on soil quality indicators overall when compared to mineral N ( $\text{NH}_4\text{NO}_3$ ) fertilizer. Soil quality was improved to the greatest extent by the compost (BVC), followed by the plant-based (PAL) and the digestate (SDG).

In conclusion, BBFs overall seem to be promising substitutes for mineral N fertilizers. However, the underlying mechanisms determining BBF behavior were not fully uncovered. Thus, more research is needed, particularly regarding the controlling factors for the agronomic performance of BBFs.

## Resumé

Landbrugssektoren står, ligesom alle andre sektorer, overfor store udfordringer ift. at nedbringe påvirkningen på miljø og klima. Biobaserede gødninger (BBG'er), produceret af organisk affald og restprodukter, kunne bidrage til at imødekomme disse udfordringer. Ved at bruge BBG'er, i stedet for mineralsk gødning, genanvendes næringsstoffer, som ellers kunne være gået tabt. På det seneste er der kommet en stigende politisk interesse for genanvendelse af næringsstoffer, i konteksten af øget fokus på cirkulær økonomi. Markedet for kommercielle BBG'er er også stigende og mange nye BBG'er er blevet introduceret. Der er dog ikke særlig stor viden om hvilke fordele og ulemper der kan være ved at bruge disse nye BBG'er. Formålet med dette Ph.d. studie var derfor at undersøge nye BBG'er som erstatning for mineralsk kvælstof (N) gødning. Dette blev undersøgt ved i) at evaluere de primære risici for N tab ved anvendelsen af BBG'er, ii) at bestemme den agronomiske effektivitet af BBG'er og iii) at evaluere BBG'ers effekt på jordkvalitet.

Miljørisici forbundet til brugen af BBG'er blev undersøgt i et laboratoriestudie (studie I) som undersøgte ammoniak ( $\text{NH}_3$ ) fordampningsrisikoen. Risikoen for fordampning af  $\text{NH}_3$  varierede meget imellem de forskellige BBG'er, både over tid, og i akkumuleret mængde  $\text{NH}_3$  fordampet. Desuden var  $\text{NH}_3$  fordampningsrisikoen påvirket af jordtype, og indblanding af BBG'erne i jorden reducerede risikoen for  $\text{NH}_3$  fordampning betydeligt. I et laboratoriestudie udført af Pedersen (2021) var lattergas ( $\text{N}_2\text{O}$ ) emissionerne højere, efter tilførsel til jord, for BBG'erne sammenlignet med mineralsk N (ammonium ( $\text{NH}_4^+$ ) nitrat ( $\text{NO}_3^-$ )) gødning. Risikoen for udvaskning af N blev vurderet ud fra indholdet af mineralsk N (summen af  $\text{NH}_4^+$  og  $\text{NO}_3^-$ ) i jorden (0-90 cm) målt 18 dage efter høst i markforsøget (studie II). Indholdet af mineralsk N i jorden indikerede at risikoen for udvaskning var den samme for BBG'er som for mineralsk N ( $\text{NH}_4\text{NO}_3$ ) gødning.

Den agronomiske effektivitet af BBG'erne blev undersøgt i markforsøg på forskellige lokaliteter i Europa (studie II). Studiet viste at de fleste af BBG'erne havde samme effektivitet som den mineralske referencegødning. Der var en tendens til at BBG'erne havde en andet-års gødningseftervirkning, som var højere end for den mineralske reference på nogle lokaliteter for nogle af BBG'erne. Men det var ikke signifikant. Effektiviteten af de enkelte BBG'er var ikke konsistent på tværs af lokaliteter og år, og der kunne ikke findes nogen forklaringer på dette. Når BBG'erne blev sammenlignet med mere traditionelle organiske gødninger, såsom gylle, baseret på samme udgangsmateriale, havde BBG'erne generelt en højere agronomisk effektivitet.

Effekten af BBG'er på jordkvalitet blev undersøgt i et laboratoriestudie der simulerede lang tids tilførsel (studie III). Studiet viste at BBG'erne havde en neutral til forbedrende effekt på jordkvaliteten, overordnet set, når de blev sammenlignet med mineralsk N ( $\text{NH}_4\text{NO}_3$ ) gødning. Jordkvaliteten blev forbedret mest ved tilførsel af komposten (BVC), efterfulgt af den plantebaserede BBG (PAL) og digestaten (SDG).

Opsummerende virker BBG'er, overordnet set, som lovende erstatninger for mineralsk N gødning. Dog mangler underliggende mekanismer stadig at blive belyst, især for at forstå hvad der styrer BBG'ers agronomiske effektivitet.

# List of publications and manuscripts

## *Main publications and manuscripts:*

Study I:

**Potential ammonia volatilization from 39 different novel biobased fertilizers on the European market – A laboratory study using 5 European soils.**

Authors: Wester-Larsen, Lærke, Müller-Stöver, Dorette Sophie, Salo, T., & Jensen, Lars Stoumann.

Published in the Journal of Environmental Management (2022). 323, 12 s., 116249.

Supplementary material can be found in appendix I.

Study II:

**Agronomic performance of novel biobased fertilizers across European field trial sites.**

Authors: Benedikt Müller<sup>a</sup> and Lærke Wester-Larsen<sup>a</sup>, Lars Stoumann Jensen, Tapio Salo, Ramiro Recena Garrido, Mustapha Arkoun, Aurélien D'Oria, Iris Lewandowski, Torsten Müller, and Andrea Bauerle

<sup>a</sup>*equal contribution as first authors*. Submitted to Field Crops Research, December 2023.

Supplementary material can be found in appendix II.

Study III:

**Effects of biobased fertilisers on soil physical, chemical and biological indicators – A long-term incubation study.**

Authors: Lærke Wester-Larsen, Lars Stoumann Jensen, Johannes Lund Jensen, Dorette Sophie Müller-Stöver.

Submitted to Soil Research, October 2023.

Supplementary material can be found in appendix III.



*Written work which I have contributed to in the LEX4BIO project, but not included in the thesis:*

Appendix IV:

**Testing and evaluation of laboratory methods for the assessment of mineralizable nitrogen from bio-based fertilizers.**

Authors: Agostini, L., Bünnemann, E. K., Jakobsen, C., Salo, T., Wester-Larsen, L., Symanczik, S.  
Submitted to Soil Biology and Biochemistry, December 2023. This manuscript has been included in the appendix, so readers can access this yet unpublished study, because I make references to it in the general discussion.

**Quantifying the agronomic and environmental effects of biobased nitrogen fertilizers in European agriculture through modeling.**

Authors: Rashid, Muhammad Adil, Wester-Larsen, Lærke & Jensen, Lars Stoumann.  
Draft manuscript, not yet published or submitted to a journal. Not included in appendix as no reference is made to it in the general discussion.

**Predicting behavior of biobased fertilizers from Fourier transform infrared-photoacoustic and Near infrared spectroscopy.**

Authors: Cantero, Juan Nieto, Jensen, Lars Stoumann, Wester-Larsen, Lærke & Delgado, Antonio.  
Draft manuscript, not yet published or submitted to a journal. Not included in appendix as no reference is made to it in the general discussion.

**Assessment of N<sub>2</sub>O emissions from biobased fertilizers.**

Author: Pedersen, E.

Master's thesis, 2021.

Supervisors: Jensen, Lars Stoumann (main supervisor) & Wester-Larsen, Lærke (co-supervisor).

Plant and Environmental Sciences. University of Copenhagen, Frederiksberg, Denmark.

Data from this Master's thesis is referenced in the general discussion.

The Master's thesis is openly available at the repository of University of Copenhagen, ERDA, following this link: <https://doi.org/10.17894/ucph.26532e4e-a2c4-45c3-bf70-63f780390497>

## List of abbreviations

AE: Agronomic efficiency

BBF: Biobased fertilizer

C: Carbon

CEC: Cation exchange capacity

$\text{CHCl}_3$ : Chloroform

CMC: Component material categories

$\text{CO}_2$ : Carbon dioxide

DM: Dry matter

EOM: Effective organic matter

ESPP: European Sustainable Phosphorus Platform

FW: Fresh weight

$\text{H}_2\text{SO}_4$ : Sulfuric acid

$\text{HCO}_3^-$ : Bicarbonate

K: Potassium

MAOM: Mineral-associated organic matter

N: Nitrogen

$\text{N}_2$ : Dinitrogen

$\text{N}_2\text{O}$ : Nitrous oxide

NaOH: Sodium hydroxide

NFRV: Nitrogen fertilizer replacement value

$\text{NFRV}_{\text{AE}}$ : Nitrogen fertilizer replacement value based on agronomic efficiency

$\text{NFRV}_{\text{NUE}}$ : Nitrogen fertilizer replacement value based on nitrogen use efficiency

$\text{NH}_3$ : Ammonia

$\text{NH}_4^+$ : Ammonium

$\text{NH}_4\text{HCO}_3$ : Ammonium bicarbonate

$\text{NO}_3^-$ : Nitrate

NUE: Nitrogen use efficiency

OM: Organic matter

P: Phosphorus

PFC: Product function categories

PM<sub>x</sub>: Particulate matter  
POM: Particulate organic matter  
TAN: Total ammonical N (sum of NH<sub>4</sub><sup>+</sup> and NH<sub>3</sub>)  
TOC: Total organic carbon  
TOF: Traditional organic fertilizer  
TRL: Technological readiness level  
SMB-C: Soil microbial biomass carbon  
SOC: Soil organic carbon  
SOM: Soil organic matter  
WFPS: Water filled pore space  
WHC: Water holding capacity

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# Introduction

Up until the 20<sup>th</sup> century, farmers have used organic wastes and residues as fertilizers. These mainly consisted of animal manures from on-farm animals and human wastes. Thus, nutrients were recycled on farm level. These traditional organic fertilizers (TOFs) were, largely, non-processed.

In 1913, the Haber-Bosch technology was invented. This enabled the conversion of non-reactive atmospheric dinitrogen ( $N_2$ ) into plant-available forms (ammonium ( $NH_4^+$ ) and nitrate ( $NO_3^-$ )) (Buckwell and Nadeu, 2016). However, it was not until around 1950, at the beginning of the green revolution, that the use of mineral nitrogen (N) fertilizers, produced via the Haber-Bosch technology, started to increase rapidly (Buysse and Cardona, 2020). Moreover, developments in the mining of phosphorus (P) and potassium (K) led to the increased use of minerals P and K as fertilizers (Buysse and Cardona, 2020). As mineral fertilizers became the new standard, the appreciation of manures declined and manures were increasingly perceived as wastes (Wadman et al., 1987). The increase in fertilizer use, together with the development of plant breeding and plant protection, led to great increases in food production (Buckwell and Nadeu, 2016).

However, the fixation of  $N_2$  using the Haber-Bosch process has led to an acceleration of the N cycle. The planetary boundary for N load in natural ecosystems has also been transgressed (Richardson et al., 2023). Globally, we are currently fixing the same amount of atmospheric  $N_2$ , or even more, as all natural systems (Rockström et al., 2009; Sutton et al., 2011). The fixed N is primarily used as mineral fertilizer (Sutton et al., 2011). Moreover, the Haber-Bosch process is highly energy demanding (Sutton et al., 2011). Thus, the global production of mineral N fertilizers constitutes 1 % of global climate gas emissions and 1 % of global energy consumption (Capdevila-Cortada, 2019; Menegat et al., 2022). Additionally, the planetary boundary for P load in natural ecosystems, driven by P fertilizer application, has been transgressed (Richardson et al., 2023). Using mineral P and K as fertilizers also leads to the depletion of non-renewable resources.

A means to reduce the climate impact from mineral N fertilizer production and decrease dependency on non-renewable nutrient resources is by recycling otherwise potentially wasted nutrients using biobased fertilizers (BBFs) instead of mineral fertilizers. Biobased fertilizers (BBFs) were defined in Wester-Larsen et al. (2022) as “materials or products derived from biomaterials (plant, animal or microbial origin, often wastes, residues, or side-streams from agriculture, industry, or society) with a content of bioavailable plant nutrients suitable to serve as a fertilizer for crops”. They can also consist of mineral components, as long as the mineral fraction was originally derived from organic components. Furthermore, novel BBFs were defined in Wester-Larsen et al. (2022) as “a BBF produced by processes beyond simple biogas digestion of animal manures and simple composting. The processes involved in producing novel BBFs can e.g. be drying, pelletizing or mineral extraction.”

The increased focus on reducing the environmental and climate impact of fertilizers has led to increased interest in organic wastes for fertilizer production (Chew et al., 2019). In the European Union (EU), there is political interest in using waste or residue-based BBFs in a circular economy context (EC, 2015a). However, BBFs have been hindered from entering the free-trade market for fertilizers (Schoumans et al., 2019). The new EU Fertilizing Products Regulation (EU, 2019), which applied from July 2022, gives BBFs the same status as mineral fertilizers. The regulation contains common rules on quality, safety, and labelling requirements, which should be controlled by national bodies to avoid fraud (EU, 2019). However, the scientific evaluation of the wide range of BBFs is very limited compared to mineral fertilizer (Abbott et al., 2018; Case and Jensen, 2019). Moreover, previous studies have focused

on traditional organic fertilizers/amendments, such as manure, sewage sludge, and compost (Abbott et al., 2018; Nkoa, 2014). Therefore, little knowledge about the novel BBFs exists. Thus, there is a need for knowledge on the agronomic potential and risks of novel BBFs for political decision-making (Schoumans et al., 2019).

## **Objectives**

The overall objective of this PhD research project was to investigate how a selected range of novel BBFs can serve as a substitute for mineral N fertilizers and to compare their impacts with synthetic mineral and traditional organic fertilizers. The specific objectives were to:

- i) Evaluate the main N loss risks from the use of BBFs:
  - a. Quantify the potential ammonia (NH<sub>3</sub>) volatilization under varying environmental conditions from the use of BBFs.
  - b. Assess the importance of other gaseous and aquatic N losses from BBFs applied to soil.
- ii) Determine the agronomic performance of field crops fertilized with BBFs:
  - a. Quantify their first-year mineral N fertilizer replacement value and second-year residual effects.
  - b. Assess the impact of crop, soil and climatic setting on their agronomic performance.
- iii) Evaluate the effect of BBF application on soil quality:
  - a. Quantify effect of standard and accelerated single application of a range of BBF on soil biological, physical and chemical indicators
  - b. Compare soil quality effects of a specific BBF application annually in a long-term field trial and a single large dose in a laboratory incubation.

## **Context of the PhD research in the LEX4BIO project**

This PhD project was part of the EU Horizon 2020 funded research project LEX4BIO “Optimizing Bio-based Fertilizers in Agriculture – Providing a Knowledge Basis for New Policies” (LEX4BIO, 2023; [www.lex4bio.eu](http://www.lex4bio.eu)). The overall aim of LEX4BIO is to create a knowledge base and provide scientific underpinning for the new EU Fertilizer Regulation (EU, 2019).

The LEX4BIO project includes research on different aspects of production, utilization and impacts of the use of BBF products already on the market or market-ready. The research is organized in seven work packages, the first work package assesses the availability of source materials for BBFs. The second work package evaluates BBFs’ impact on soil quality, particularly C dynamics. The third work package determines the agronomic P use efficiency of BBFs and develops compliance methods to ensure P fertilizer quality. The fourth work package determines the N use efficiency of BBFs and develops compliance and standard test methods for assessing crop N availability along with remote sensing techniques. The fifth work package defines risks related to food safety, human health, and the environment of BBF use. The sixth work package performs life cycle assessments of BBFs comparing them to mineral- and traditional organic fertilizers. Finally, the seventh work package will deliver recommendations on the optimal use of BBFs and socioeconomic improvements for the rural population.

The output of the LEX4BIO project thereby covers a wide range of aspects related to the use of BBFs from the socioeconomic to the environmental and agronomic aspects. Partners involved in the project includes private companies, research institutes and universities across Europe.

Many other research projects on the topic of BBFs focus on the development of new products and the optimization of existing products by post processing, e.g. separation, drying, acidification and fermentation. The LEX4BIO project, on the other hand, has its primary focus on investigation of existing products already on the European or national markets in their final product form or market-ready innovation prototypes at a very high technological readiness level (TRL).

This PhD project was part of the work package four focusing on N, but also covers work I have done on soil quality (related to work package two). The other aspects investigated in the LEX4BIO project are not included in this PhD, but will be briefly visited in the general discussions section.

### ***Selection of biobased fertilizers***

The focus of the LEX4BIO project is indeed on Europe and therefore only BBF products available on the European market or local BBFs of European countries at the time of project initiation were considered. In addition, promising novel BBFs in development were also considered (Bauerle et al., 2021). Consequently, BBFs outside the borders of Europe are not covered in the project, and neither in this PhD. The availability and use of BBFs has been found to differ among geographical regions globally (Charles et al., 2017). Thus, if this study had focused on a different region of the world, the BBFs selected for the studies would likely have differed.

At the initial stage of the project, a systematic approach was taken jointly in the LEX4BIO project to evaluate and rank the suitability of different existing and novel European BBF products for crop production. The selection protocol was based on the following consecutive steps (Bauerle et al., 2021):

- Step 1. An online survey was created and distributed to all partners and a wide range of stakeholders and networks within science, fertilizer industry and agriculture. Answers to the survey were analyzed.
- Step 2. The first level of evaluation of the survey results for relevant BBFs was done. It was complemented by scouting ongoing relevant projects for promising BBF sources, literature search, and additional information from partners or partners' industrial and business contacts.
- Step 3. Beside agronomic and environmental aspects, rules and priorities of the new EU fertilizer regulation (2019/1009) were also taken into consideration, including coverage of the PFC/CMC criteria amongst the selected BBFs.

As a result of the selection process, a relatively wide range of BBFs were found relevant for laboratory, greenhouse, container and field trials planned in the different work packages of LEX4BIO and thus, included on the longlist of around 40 BBFs with relatively high N contents and around 40 BBFs with relatively high P contents. These cover the variety of BBFs available on the European market at the time of collection (2020) and as such, the selection of BBFs on the list was also partly based on commercial availability of them, thus favoring BBFs produced in large amounts, as these were argued to be more representative for what is available on the market (Bauerle et al., 2021). Moreover, the selected BBFs were chosen based on the criteria to represent the relevant component material categories (CMCs) and product function categories (PFCs) (see Table I-1 in study I for an overview of CMCs and PFCs of the selected N BBFs) of the EU Fertilizer Regulation. The CMCs classify main categories of raw materials the BBFs are composed of, and which processing technologies have been used to produce them (EU, 2019). The PFCs describe the physical characteristics in terms of whether BBFs are liquid or solid, and whether the BBF contains organic, mineral or mixed components (EU, 2019). The work

on selecting the BBFs was undertaken by LEX4BIO work package two with inputs from other project partners.

Almost all of the around 40 N BBFs were evaluated in a laboratory setup in study I. For studies II and III, ten BBFs were selected from the list to represent the variety of commercial BBFs (seven BBFs) and some local products (three BBFs) representative of Danish conditions. Thus, these same ten BBFs were included in all three scientific papers (studies I-III) included in this PhD. This enabled comparisons across these studies.

### ***Context of the three individual studies included in the thesis***

#### ***Study I: Part of LEX4BIO Task 4.2 - Standard test protocol development***

As a wide range of BBFs exists, and new ones are introduced frequently, the scientific evaluation of them is complicated and it is unlikely that research on all of the BBFs will become available (Abbott et al., 2018) or evaluations be done in the same way. Today no systemic framework for evaluation of BBFs regarding product quality, fate in the soil system and the expected agronomic and environmental impact exists (Schoumans et al., 2019). Product quality in relation to N fertilizers is here defined as the N fertilizer replacement value (NFRV), N mineralization profile, physical form, spread ability, stability, N content, manageability and content of impurities and contaminants. Such a framework would enable rapid scientific and practical evaluations of BBFs and is essential for farmers as well as policy makers (Schoumans et al., 2019). Therefore, the LEX4BIO project work package 4 task 4.2 aimed to create a set of standard test protocols to be used for analysis of BBFs, both existing and new ones emerging on the market. Study I of this PhD contributed to this part of the project by developing a standard test protocol for NH<sub>3</sub> volatilization potential of BBFs. Moreover, the project has focused on developing methods that can predict, or constitute proxies for, behavioral properties of BBFs, e.g. N mineralization patterns, from fast and low cost measurements such as spectroscopy measurements and simple chemical analysis.

#### ***Study II: Part of LEX4BIO Task 4.1 - Test of agronomic N performance***

A fertilizers main purpose is to provide nutrients to the crop. Therefore an important aspect of the work conducted in work package 4 was to determine the agronomic performance of the BBFs. This was the aim of task 4.1 in work package 4. The agronomic N performance of selected BBFs was determined by conducting field trials at five locations in Europe (Finland, Denmark, Germany, France and Spain). Each partner was responsible for the field trial in their respective country and I was the main responsible for the field trial conducted in Denmark. For this task a short-list consisting of seven of the around 40 N BBFs were selected. The seven short-list common BBFs were selected to represent the variety of BBFs on the long-list. In addition 2-4 local BBFs were selected for each field location to represent relevant local BBFs. The main results of all field trials were compiled and analyzed collectively by me and a PhD student from the University of Hohenheim, Benedikt Müller. The results of the field trials are presented in study II. Similar field trials were conducted to test the agronomic P performance of a selection of the P rich BBFs in work package 3.

#### ***Study III: Part of LEX4BIO Task 2.2 – Potential effects of BBFs on soil quality and C sequestration***

In task 2.2 in work package 2 BBFs effects on soil properties, particularly C sequestration, was investigated through a literature review. The results of study III on BBFs effects on soil quality indicators investigated in a laboratory incubation contributed to this task indirectly. This PhD project is officially part of the work package 4 and therefore the results of study III did not contribute directly to the work in work package 2.

# Background

## Nitrogen fertilizer use in Europe

Nitrogen is the most used nutrient applied in agriculture, accounting for 70 % of applied nutrients in the EU (Buckwell and Nadeu, 2016). In 2021, the European consumption of mineral N fertilizers was 9.8 million tonnes (Eurostat, 2023). Mineral N accounts for 51 % of the applied N, whereas manure N is the second largest source constituting 34 % of applied N in the EU and the remaining 15 % stems from crop residues, atmospheric deposition and biological N fixation (Buckwell and Nadeu, 2016).

Currently in the EU, there is an unbalanced distribution of organic fertilizers both across regions, but also within regions (Tröster, 2023). Nutrients accumulate in agriculture due to import of external nutrients, e.g. animal feed, and the disappearance of mixed farming systems (Buysse and Cardona, 2020). Today most farms in the Global North are highly specialized. Moreover, animal production is concentrated in specific regions leading to surplus of manures in these regions. Due to costs of transportation it is not economically sound to transport manures from animal intensive regions to arable farming regions.

## Traditional organic fertilizers and biobased fertilizers

It is very rare that authors define what is meant by terms such as organic fertilizers (Lazcano et al., 2021), recycling-derived fertilizers (Postma et al., 2020) or organic amendments (Charles et al., 2017). However, clear and common definitions are necessary for market clarity about their effects and environmental impacts (ESPP, 2023), and to avoid greenwashing (EC, 2022b).

Nkoa (2014) proposed redefining organic fertilizers based on their fertilizer value. In line with this, Veeken et al. (2018) argues that it is important to distinguish fertilizers from soil amendments/soil improvers when it comes to biobased products. They propose a definition where soil amendments should contain a high amount of effective organic matter (EOM) contributing to SOM and a low amount of nutrients, whereas a fertilizer should be the opposite, with high amounts of nutrients and a low EOM.

Moreover, BBFs could be defined as either local or global based on their N content, transportability, and durability. Thus, BBFs with low N content and/or short-term durability, e.g. digestates and potato cell water, would be defined as local BBFs, whereas BBFs with long-term durability and high N content, thus supporting ease of transportation and logistics, would be defined as more globally tradeable BBFs.

ESPP (2023) argues that fertilizers produced partly from biobased materials should be termed partly biobased fertilizers. These could be struvites, which contain magnesium rock, or stripping products, which contain sulfuric acid derived as a by-product from oil refinery (ESPP, 2023).

Thus, many aspects are worth considering regarding definitions, particularly of the new products. The European Sustainable Phosphorus Platform (ESPP) is currently working on a common definition of BBFs (ESPP, 2023).

In this PhD thesis the term traditional organic fertilizers (TOFs) is used to describe well studied organic waste- or side streams such as manures, which are either non-processed, or only processed to a limited degree, and not with the aim of changing the nutrient availability. Whereas, the definition by Wester-Larsen et al. (2022) is used to define BBFs: “Biobased fertilizers can be defined as materials or products derived from biomaterials (plant, animal or microbial origin, often wastes, residues, or side-streams from agriculture, industry or society) with a content of bioavailable plant nutrients suitable to serve as a fertilizer for crops.” Furthermore, a definition of novel BBFs was proposed in study I as “a BBF produced by processes beyond simple biogas digestion of animal manures and simple composting. The



processes involved in producing novel BBFs can e.g. be drying, pelletizing or mineral extraction”. Novel is a term that relates to time, as a BBF which is novel now will not be so in 20 years. Thus, the term novel is difficult to use for definition purpose. What is meant with the term novel in the PhD project is BBFs which are novel at the current point in time.

Traditional organic fertilizers are well known and have been used by farmers in agriculture for a very long time. In contrast, commercial BBFs remain relatively unknown and un-used by farmers, despite recent advances and expansions in the market of BBFs (Postma et al., 2020). The scientific evaluation of the wide range of BBFs being introduced more recently is very limited compared to mineral fertilizer (Abbott et al., 2018; Case and Jensen, 2019). Among the most studied types of TOFs are animal manure and compost of both plant and animal origin (Abbott et al., 2018).

One thing that differentiates the non-processed TOFs from the processed BBFs is the physical form. Most BBFs studied in the LEX4BIO have been dried and pelletized; however, also liquid but homogenized and concentrated forms like biogas digestate or potato cell water have been included. The physical form of BBFs can be of high importance for its properties. Yang et al. (2020) compared the N release of the same manure fertilizer in granular and powder form and concluded that the granular form was optimal for most crops, as the N release was delayed one month, and therefore more synchronized with crop uptake.

Traditional organic fertilizers vary substantially in their N loss risks, agronomic performance, and effects on soil quality. Composts have been found to have the lowest agronomic performance with NFRV sometimes as low as 0 % and urine the highest agronomic performance with NFRV of up to 100 % (Gutser et al., 2005). This highlights the importance of distinguishing different types of TOFs and BBFs. Some of the products studied in this PhD, namely those locally sourced, such as digestates and composts, have been known for a long time and much research has already been done on some of these products. However, some are very new. Most of the organic waste- and side-streams used as source materials for these novel BBFs are not new. What is new is how they are processed.

Several processing techniques are used to produce BBFs. The majority of the N rich BBFs studied in the LEX4BIO project have been dried and pelletized with or without prior processing. The locally available BBFs are generally less processed compared to the BBFs traded across Europe. The processing of BBFs may lead to loss of N to varying degrees. During the pelletizing of BBFs, high temperatures combined with high moisture content can lead to mineralization and loss of organic N (Brod et al., 2018). Almost all N is recovered when biomass is anaerobically digested (Chojnacka et al., 2020), whereas, during composting, a lot of the N in the input source material is lost as gaseous N (Chen et al., 2023).

In study II it was found that the compost (BVC) could not be defined as a fertilizer due to its inability to supply nutrients to the crops. In line with the proposals by Nkoa (2014) and Veeken et al. (2018), it was argued that composts should be defined as soil amendments or soil improvers and not as fertilizers.

Moreover, the definition of BBFs by Wester-Larsen et al. (2022) states that BBFs should be derived from wastes, residues, or side-streams. The origin of the source material is also an attention point which should be considered for definitions. As has been discussed for other biobased products, e.g. biobased plastics, whether the biomass is waste- and side-stream derived or derived from primary biomass directly produced for its use in the given product matters in terms of the environmental impact of the feedstock (EC, 2022a). Thus, for biobased plastics, the European Commission has argued that the priority should be to use waste- or by-product biomass for the production of biobased plastics (EC, 2022a). In line with this, the European Commission has worked towards decreasing the share of biofuels produced from crops and focus on biofuels produced from organic waste or side-streams (EC, 2012). The same arguments could be used for biobased fertilizers.

### **Risk of nitrogen loss from biobased fertilizer use**

On a global scale, only 46 % of applied N is taken up and removed with the harvested crop, which leaves a surplus of N in the soil that, in many cases, can readily be lost to the environment (Zhang et al., 2021) and if not, will accumulate in the soil, increasing the long-term potential for loss. Rockström et al. (2023) stress that the current surplus of N should be reduced by 51 % on a global scale in order to stay within the safe earth system boundaries.

Nitrogen losses from agriculture in the EU consist of approximately 50 % losses to groundwater, or surface run-off, and 50 % gaseous losses to the atmosphere, mainly as  $\text{NH}_3$  or  $\text{N}_2$  (Buckwell and Nadeu, 2016). The losses of N to the environment from using BBFs are important to estimate, as these not only imply economic losses for the farmer from lost fertilizer use, but also environmental impacts such as acidification, eutrophication and climate change (UNEP, 2019).

### ***Ammonia volatilization***

Ammonia volatilization is complex and involves both physical, chemical and biological processes (Cameron et al., 2013). Ammonia is an electroneutral base which is highly water-soluble and when dissolved in water, it is in equilibrium with ammonium ( $\text{NH}_4^+$ ). Ambient concentrations of  $\text{NH}_3$  in the atmosphere are very low, about 1 ppb (Sommer and Feilberg, 2013).

Volatilization of  $\text{NH}_3$  is affected by the  $\text{NH}_3$  to  $\text{NH}_4^+$  equilibria, which are affected by the proton ( $\text{H}^+$ ) activity, with higher  $\text{NH}_3$  volatilization at high pH. An equilibrium between  $\text{NH}_3$  in the aqueous and gaseous phase exists and this equilibrium changes with temperature. At higher temperatures, relatively more  $\text{NH}_3$  will be in the gaseous phase compared to the aqueous phase. Moreover,  $\text{NH}_3$  volatilization is affected by diffusion and advection. Diffusion is driven by the concentration gradient, whereas advection occurs due to movements in air or liquid. Both processes transport  $\text{NH}_3$  (Sommer and Feilberg, 2013).

At high pH in a liquid, the  $\text{NH}_3$  concentration in the liquid is also high and the volatilization potential increases at increasing pH. However, volatilization of  $\text{NH}_3$  increases oxonium ( $\text{H}_3\text{O}^+$ ) concentrations which decreases the pH. This depletion of bases at the surface where  $\text{NH}_3$  volatilize, will in turn initiate an upward diffusive and advective movement of pH buffer components from layers below the depleted layer (Sommer and Feilberg, 2013). If the solution contains a pH buffer component, e.g. Bicarbonate ( $\text{HCO}_3^-$ ), it can react with the  $\text{H}^+$  ion to form carbon dioxide ( $\text{CO}_2$ ) and water (Husted et al., 1991).  $\text{CO}_2$  will then volatilize and if  $\text{CO}_2$  and  $\text{NH}_3$  volatilize at equal rates, pH will not change (Husted et al., 1991). However,  $\text{CO}_2$  is less soluble than  $\text{NH}_3$  and will therefore volatilize at a higher rate initially, which increase the pH, until  $\text{NH}_3$  and  $\text{CO}_2$  will volatilize in equal rates (Husted et al., 1991).  $\text{HCO}_3^-$  can be formed by dissolution of carbonates (Borggard and Elberling, 2013) or from  $\text{CO}_2$  by biological decomposition of organic material.

BBFs differing in pH, total ammoniacal N (TAN ( $\text{NH}_4^+ + \text{NH}_3$ )) concentration and mineralization potential can therefore be expected to differ in their  $\text{NH}_3$  volatilization potential. Moreover, physical properties of the BBF such as the specific surface area will affect the  $\text{NH}_3$  volatilization potential. Which physical form the BBF is in, e.g. pelletized, liquid or powder, will also affect the  $\text{NH}_3$  volatilization potential as it affects the dissolution and diffusion of the BBF into the soil solution, even for BBFs with the same chemical composition (Sommer et al., 2004).

As the BBFs in the LEX4BIO project vary in source material and some are composed of several different source materials, they could be expected to behave different from well-studied organic fertilizers, such as manure. A BBF made from manure which has been further processed, e.g. by anaerobic digestion or drying could have a very different  $\text{NH}_3$  volatilization potential compared to the non-processed manure. pH buffers, cations and anions added with a fertilizer will affect the soil pH and thereby the  $\text{NH}_3$  volatilization (Sommer et al., 2004). Moreover, if the BBFs consist of mixed sources, interactions may change the  $\text{NH}_3$  volatilization potential. This could be if a BBF is composed of a source

material, which in itself has a high  $\text{NH}_3$  volatilization potential, combined with a source material which has a high content of pH buffer or components which sorb  $\text{NH}_4^+$ . Such interactions have been observed by Steiner et al. (2010) who found reduced  $\text{NH}_3$  volatilization from poultry litter during composting when biochar was added. Moreover, separation of BBFs into solid and liquid fractions may reduce the  $\text{NH}_3$  volatilization as has been found for animal manure (Zhang and Westerman, 1997). Furthermore, incorporation or injection of the BBF can be expected to decrease the  $\text{NH}_3$  volatilization as has also been shown for manure (Feilberg and Sommer, 2013).

Apart from the BBF properties, the soil on which the fertilizer is applied, will also affect the  $\text{NH}_3$  volatilization potential. Volatilization of  $\text{NH}_3$  from soil is very complex and involves chemical, physical and biological factors (Cameron et al., 2013). Zhenghu and Honglang (2000) stated that no single soil property dominates the  $\text{NH}_3$  volatilization from soil nor does any soil property control  $\text{NH}_3$  volatilization consistently. Zhenghu and Honglang (2000) therefore stress the need to perform systematic multifactorial analysis in order to determine processes and mechanisms of  $\text{NH}_3$  volatilization.

In a study including 22 soils differing in soil properties Duan and Xiao (2000) found  $\text{NH}_3$  volatilization rates to be positively correlated with soil pH,  $\text{CaCO}_3$  and salt content and negatively correlated with soil organic matter (SOM) content, cation exchange capacity (CEC) and clay content. In acidic soils or soils with a low content of pH buffer components, the  $\text{NH}_3$  volatilization will rapidly decline (Sommer et al., 2004). Indirectly  $\text{NH}_3$  volatilization has been found to be affected by biological processes e.g. TAN immobilization (Sommer et al., 2004).

Soil moisture affects  $\text{NH}_3$  volatilization potential in various ways (Haynes and Sherlock, 1986). The concentration of TAN in solution is lower at higher soil moisture, which may lead to lower  $\text{NH}_3$  volatilization at high soil moisture content (Haynes and Sherlock, 1986). However, if soil moisture is initially high and followed by moisture loss,  $\text{NH}_3$  volatilization can be high (Haynes and Sherlock, 1986).

To sum up, the soil properties which should be taken into consideration in the experimental design are pH, pH buffer components, salt content, CEC, SOM and texture. Moreover, soil moisture, temperature, air movement and application method should be considered.

### ***Emissions of nitrous oxide***

Nitrous oxide ( $\text{N}_2\text{O}$ ) is a very potent greenhouse gas with a climate warming potential that is ~ 300 times higher than  $\text{CO}_2$  over a 100 years period (IPCC, 2007). Fertilizer use and management of manure is estimated to constitute almost one third of global  $\text{N}_2\text{O}$  emissions (Charles et al. 2017).

In a literature review by Walling and Vaneeckhaute (2020), no consistency was found as to whether the application of organic fertilizers led to higher, lower, or similar  $\text{N}_2\text{O}$  emissions, compared to mineral fertilizers. The risk of  $\text{N}_2\text{O}$  emissions increases with the use of organic fertilizers if they contain readily available N and degradable C (Hansen et al., 2019; Velthof et al., 2003). Charles et al. (2017) conducted a meta-analysis and found that TOFs/BBFs could be divided in three groups based on their  $\text{N}_2\text{O}$  emissions. The high-risk group consisted of slurries, wastewater and biosolids, a medium risk group of solid manure and a low risk group of composts, crop residues, paper mill sludge and pellets (Charles et al. 2017). The authors found that the average  $\text{N}_2\text{O}$  emission for TOFs/BBFs was 0.6 % of applied N. Moreover, the authors highlighted the skewness in studies regarding types of TOFs/BBFs, where very few studies exist on pelleted BBFs. In a study on coarse textured soils, representative for Denmark, Petersen et al. (2023) found higher  $\text{N}_2\text{O}$  emissions for TOFs compared to mineral N fertilizers. This is in contrast to the guidelines by IPCC (2019) that suggest a lower emission factor for TOFs of 0.006 kg  $\text{N}_2\text{O}$ -N kg  $\text{N}^{-1}$  compared to 0.016 kg  $\text{N}_2\text{O}$ -N kg  $\text{N}^{-1}$  for mineral fertilizers in wet climates.

### ***Leaching of nitrate***

Concerning N pollution of aquatic ecosystems, the majority of N enters the ecosystems through leaching and to some extent, surface runoff. Nitrogen entering surface waters through leaching and runoff can cause eutrophication. Moreover leaching may cause accelerated concentrations of nitrate ( $\text{NO}_3^-$ ) in the groundwater related to negative effects on human health from drinking the polluted groundwater. Schullehner et al. (2018) found increased risk of colorectal cancer from drinking water with concentrations of  $\text{NO}_3^-$  above  $3.87 \text{ mg L}^{-1}$ . In a Danish study by Jacobsen et al. (2024) it was found that by lowering the upper limit of  $\text{NO}_3^-$  in drinking water from  $50 \text{ mg L}^{-1}$  to  $9.25 \text{ mg L}^{-1}$  the annual colorectal cancer cases could be reduced by 72 %.

Leaching of N is primarily caused by  $\text{NO}_3^-$ , as  $\text{NO}_3^-$  does not adsorb in the soil and is therefore found in the soil solution. Leaching of soluble organic N and  $\text{NH}_4^+$  may also occur, but its contribution to leaching is minor compared to  $\text{NO}_3^-$ . Thus, organic N needs to be mineralized and  $\text{NH}_4^+$  needs to be nitrified to  $\text{NO}_3^-$  to constitute a risk for leaching (Sørensen and Jensen, 2013).

Leaching of  $\text{NO}_3^-$  may be of special concern when using organic nutrient sources (Torstensson et al., 2006). This is related to conditions where the mineralization of N from TOFs/BBFs is not synchronized with crop N uptake and N might thus be lost to the environment as  $\text{NO}_3^-$  by leaching (Jensen, 2013a). For manure it has been demonstrated that the risk of  $\text{NO}_3^-$  leaching is higher than for mineral N fertilizers based on the same N application levels (Bergström and Kirchmann, 2006).

### **Agronomic nitrogen performance of BBFs**

Apart from environmental implications, gaseous N losses from BBFs may also decrease the inorganic N content in the soil applied with BBFs and thereby lead to economic losses for the farmer from lost N, necessitating increased N fertilizer use (Kirchmann and Lundvall, 1993). The agronomic performance of BBFs is therefore of high importance for farmers and agricultural systems ability to produce food in general.

### ***Important factors for agronomic nitrogen performance***

For mineral N fertilizers, all N is available for the plant as the N is on mineral form. As BBFs are based on organic material, a variable fraction of the N is in organic form in most BBFs (except those consisting of recovered inorganic N only, e.g. in ammonia scrubber concentrate). The organic N contained in the BBFs therefore needs to be mineralized in order to become plant available (Celestina et al., 2019). Thus, N mineralization potential has been found to be an important factor for the agronomic performance of TOFs. Westerik et al. (2023) found a positive correlation between  $\text{NFRV}_{\text{NUE}}$  and potential mineralizable N for TOFs/BBFs in a pot trial ( $R^2$  of 0.75), but they found an even stronger correlation to total N content ( $R^2$  of 0.86).

The composition and degree of stabilization, and thereby capacity to release nutrients, varies greatly among TOFs/BBFs (Flavel and Murphy, 2006). This variation is due to great differences in source materials and processing of these materials covering very stable composts to processed easily degradable animal-based by-products. Flavel and Murphy (2006) examined N mineralization of pelletized poultry manure, green waste-based composts, a straw waste-based compost and a vemi-cast. They found that gross N mineralized was positively correlated to total N, total C, cellulose and lignin contents of poultry manure. Whereas the gross N mineralized was negatively correlated to total ash and  $\text{NO}_3\text{-N}$  content of poultry manure.

Case and Jensen (2019) upgraded organic waste materials by heating and pressure, alkalization and sonification but found no single upgrading treatment to increase N release of the materials consistently. They found composted, dried and raw organic waste materials to release 11%, 45% and 47% of total N

respectively. These materials released less N compared to digestates, industry derived organic fertilizers and Struvites, which released 58%, 78% and 100% of total N respectively.

They also observed that maximum N release generally was negatively correlated with C:N ratio of the organic waste material ( $r=-0.6$ ). In line with this, Westerik et al. (2023) found a negative correlation between  $NFRV_{NUE}$  and the C:N ratio for TOFs/BBFs in a pot trial ( $R^2$  of 0.42).

Apart from BBFs containing high amounts of mineral N or with large net N mineralization (Øvsthus et al., 2017), BBFs can be expected to give lower yields at the same N application rate compared to mineral N fertilizers, at least if compared on a single application basis.

Therefore, concerning BBFs' agronomic performance, both the initial content of available N, synchronization of N mineralization with crop N needs, N gaseous losses, and residual fertilizer effect in subsequent crops are important to take into account.

### ***Nitrogen fertilizer replacement values***

Often, crop N supply from BBFs is expressed as the nitrogen fertilizer replacement value (NFRV) of the BBF (Hijbeek et al., 2018). The NFRV is defined as the amount of mineral N fertilizer saved when using BBFs attaining the same yield (Jensen, 2013a). The NFRV can be calculated in several ways and two different methods are commonly used (Westerik et al., 2023). The most commonly used method is based on the same N application rates for both TOFs/BBFs and mineral N fertilizers (Westerik et al., 2023). Whereas, the other method is based on equal N uptake (Westerik et al., 2023).

The NFRV has been demonstrated to become higher over a longer period with repeated application of BBF compared to the NFRV of a single application (Hijbeek et al., 2018). The form of N in the BBF, the crop type, soil type, application method, timing of BBF application, and the organic amendment history of the soil are all factors known to affect the NFRV (Hijbeek et al., 2018).

### **Effects on soil quality from using BBFs**

Postma et al., (2020) conducted a farmer survey and found that the OM content of BBFs was the second most important feature, next to nutrient content and balance, when farmers choose BBFs. However, nutrient recycling via TOFs/BBFs mainly focuses on the macro-nutrients: N, P and K, whereas OM and its benefits for soil quality are often overlooked (Veeken et al., 2018). Soil quality has been defined by Karlen et al. (1997) as “the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation”.

Other terms related to the same topics as soil quality are soil fertility, soil health and soil security (Lehmann et al., 2020). The term soil fertility is focused on the soils ability to provide nutrients and water for the crop and the term is thus the narrowest of the concepts (Lehmann et al., 2020). Soil health can be said to be a broader term than soil quality, and soil security broader than soil health (Lehmann et al., 2020). Soil health also includes broader sustainability goals and therefore broader ecosystem services, expanding beyond those related directly to humans, which soil quality focus on (Lehmann et al., 2020). Soil quality and soil health have been used interchangeably (Bünemann et al., 2018) and the term soil health originates from soil quality (Lehmann et al., 2020). Soil security is the newest term and treats soil as a common good in line with water and air (Lehmann et al., 2020).

In study III the functional soil quality is evaluated, mainly related to the soils ability to support crop growth. Other aspects of pollutants and pathogens are also important for soil quality evaluation.



However, these were not considered in this PhD study as they were thoroughly evaluated in work package 5 of the LEX4BIO project.

The use of TOFs is known to improve soil physical parameters, such as water holding capacity (WHC), infiltration, aggregate stability, and to decrease the risk of compaction, but also soil chemical parameters, such as increased soil organic matter (SOM) and CEC, and soil biological parameters, such as the mineralization of organically bound nutrients and xenobiotic degradation (Abbott et al., 2018; Gómez-Muñoz et al., 2017). These benefits can potentially enhance soil quality and thereby improve the agronomic performance of the soil in the long term.

Moreover, the application of OM sources may lead to disease suppression, which supports plant growth (Veeken et al., 2018). Additionally, the OM input can lead to an increase in soil organic carbon (SOC). This counteracts climate change by storing C in the soil instead of emitting it into the atmosphere. This increase in SOM, and its associated effect on the climate, may however, require several years and an immediate effect can therefore, not be expected (Abbott et al., 2018).

The more stable products, e.g. composts, have been shown to have positive effects on soil quality in the longer term (Alvarenga et al., 2017). Zavattaro et al. (2015) looked at multiple long-term experiments in Europe comparing TOFs with mineral fertilizers at the same N application rate. The authors concluded that the slight reduction in yield is counteracted by benefits to soil quality and climate change mitigation when using BBFs. Thus, improved soil quality may be an important asset in the use of BBFs.

## Study I

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Supplementary material can be found in appendix I

### **Potential ammonia volatilization from 39 different novel biobased fertilizers on the European market – A laboratory study using 5 European soils**

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#### **Highlights**

- NH<sub>3</sub> volatilization potential from 39 EU marketed biobased fertilizers varied vastly.
- Highest NH<sub>3</sub> volatilization potential from digestates.
- Lowest NH<sub>3</sub> volatilization potential from composts and struvites.
- Incorporation of biobased fertilizers reduced potential NH<sub>3</sub> volatilization.
- Soil characteristics affected biobased fertilizers' potential NH<sub>3</sub> volatilization.

## Abstract

Current political focus on promoting circular economy in the European Union drives great interest in developing and using more biobased fertilizers (BBFs, most often waste or residue-derived). Many studies have been published on environmental emissions, including ammonia ( $\text{NH}_3$ ) volatilization from manures, but there have only been a few such studies on BBFs. Ammonia volatilization from agriculture poses a risk to the environment and human health, causing pollution in natural ecosystems when deposited and formation of fine particulate matter ( $\text{PM}_{10}$ ). Furthermore,  $\text{NH}_3$  volatilization results in removal of plant-available N from agricultural systems, constituting an economic loss for farmers.

The aim of this laboratory study was to determine the potential  $\text{NH}_3$  volatilization from 39 different BBFs commercially available on the European market. In addition, this study aimed to investigate the effect of incorporation, application rate, soil type, and soil moisture content on potential  $\text{NH}_3$  volatilization in order to derive suggestions for the optimal field application conditions. Results showed a great variation between BBFs in potential  $\text{NH}_3$  volatilization, both in terms of their temporal pattern of volatilization and amount of  $\text{NH}_3$  volatilized. The potential  $\text{NH}_3$  volatilization varied from 0 % of applied total N (olive oil compost) to 64 % of applied total N (manure and crop digestate) during a 27- or 44-day incubation period. Characteristics of BBFs (pH,  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, DM, C:N) and their interaction with time could explain 89 % of the variation in accumulated potential  $\text{NH}_3$  volatilization. Incorporation of BBFs into an acidic sandy soil effectively reduced potential  $\text{NH}_3$  volatilization by 37 % to 96 % compared to surface application of BBFs. Potential  $\text{NH}_3$  volatilization was not significantly affected by differences in application rate or soil moisture content, but varied between five different soils (with different clay and organic matter content), with the highest  $\text{NH}_3$  volatilization potential from the acidic sandy soil.

**Keywords:** (Potential) ammonia volatilization, Biobased fertilizers, Incorporation, Surface application, Incubation, Soil type.

## 1 Introduction

Biobased fertilizers (BBFs) can be defined as materials or products derived from biomaterials (plant, animal or microbial origin, often wastes, residues or side-streams from agriculture, industry or society) with a content of bioavailable plant nutrients suitable to serve as a fertilizer for crops. Producing and using BBFs is therefore a means to recycle otherwise potentially wasted nutrients. They can replace mineral fertilizers, lowering the environmental and climate impacts of mineral nitrogen (N) fertilizer production, and the environmental and resource depletion impacts of phosphorus (P) and potassium (K) mining and extraction.

Therefore, there is a lot of political interest in promoting waste- or residue-based BBFs in a circular economy context in the European Union (EU) (EC, 2015b). A new EU Fertilizing Products Regulation (EU, 2019) has been approved by the European Commission and will be implemented in the member states in the coming years. This is expected to lead to an increase in the use of novel BBFs, as the new fertilizer regulation will allow various BBFs to enter the free trade market for fertilizers, provided they comply with certain production and quality standards (Schoumans et al., 2019). The recent instability of mineral fertilizer markets, with rapidly fluctuating fertilizer prices (Baffes and Koh, 2021), will also increase interest in alternative and less energy market-sensitive nutrient sources.

However, knowledge about the N fertilizer effect and environmental impacts of the use of novel BBFs is currently very limited. An important aspect is ammonia ( $\text{NH}_3$ ) volatilization, which is of growing environmental concern (Li et al., 2020). Globally, 80 % of  $\text{NH}_3$  volatilization is associated with human activities, mainly related to animal husbandry and the application of fertilizers (UNEP, 2019).

Once volatilized,  $\text{NH}_3$  reacts with acidic gasses which impacts the formation, transformation, and deposition of aerosols (Akiyama et al., 2004). Re-deposition of  $\text{NH}_3$  may contribute directly or

indirectly to soil acidification, eutrophication of aquatic environments, and biodiversity loss. Furthermore,  $\text{NH}_3$  may be oxidized to nitrate ( $\text{NO}_3^-$ ) and further transformed to the potent greenhouse gas  $\text{N}_2\text{O}$  through denitrification (Ferm, 1998). In addition to environmental concerns, secondary ultrafine particles in the atmosphere formed by  $\text{NH}_3$  and other reactive compounds pose a significant human health risk (Sanz-Cobena et al., 2014). Moreover,  $\text{NH}_3$  volatilization may also reduce the inorganic N content in the soil applied with BBFs, thereby causing decreases in yields, with consequential economic losses for the farmer (Kirchmann and Lundvall, 1993).

Theoretically, BBFs with a high pH and/or high ammonium ( $\text{NH}_4^+$ ) and/or high uric acid content will be expected to have higher  $\text{NH}_3$  volatilization potential compared to BBFs with a low pH and/or low  $\text{NH}_4^+$  and/or low uric acid content (Jensen and Sommer, 2013; Sommer and Feilberg, 2013). However, BBFs with a low  $\text{NH}_4^+$  content may contain a substantial pool of easily mineralizable organic N, which upon soil microbial decomposition of the BBF will become mineralized and provide  $\text{NH}_4^+\text{-N}$ , increasing the potential for  $\text{NH}_3$  volatilization. Moreover, the physical form, e.g. pelletized, liquid or powder, may affect the  $\text{NH}_3$  volatilization potential, as it affects the dissolution and diffusion of the  $\text{NH}_4^+$  content of the BBF into the soil (Sommer et al., 2004).  $\text{NH}_3$  may also be lost during the BBF production process, as observed e.g. during the composting (Usmani et al., 2020) and drying of anaerobic digestate (Awiszus et al., 2018).

Apart from the BBF properties, the soil type to which the BBF is applied may affect the  $\text{NH}_3$  volatilization potential. In a study including 22 soils differing in properties, Duan and Xiao (2000) found  $\text{NH}_3$  volatilization rates to be positively correlated with soil pH,  $\text{CaCO}_3$ , and salt content and negatively correlated with soil organic matter (SOM) content, cation exchange capacity (CEC), and clay content. In acidic soils, or soils with a low content of pH buffer components,  $\text{NH}_3$  volatilization will rapidly decline (Sommer et al., 2004). Moreover, soil moisture may affect  $\text{NH}_3$  volatilization potential, as the concentration of  $\text{NH}_4^+$  content in solution is lower at higher soil moisture, which may lead to lower  $\text{NH}_3$  volatilization (Haynes and Sherlock, 1986).

Previous studies on  $\text{NH}_3$  volatilization from BBFs have focused on a limited variety and number of BBFs, including mainly manure (Akiyama et al., 2004; Bernal and Kirchmann, 1992; Mkhabela et al., 2006) and to some extent digestates (Nkoa, 2014; Verdi et al., 2019). Ammonia volatilization from both livestock and green manure has been shown to be generally higher compared to mineral N fertilizers (Ma et al., 2021). Therefore, studies on  $\text{NH}_3$  volatilization from novel BBFs covering a wide range of source material as well as chemical and physical properties are strongly needed.

Thus, the overall objective of this study was to determine the potential  $\text{NH}_3$  volatilization from the use of 39 different BBFs under variation of soil type and moisture conditions, as well as application rate and method, and to derive suggestions for the optimal application conditions under practical settings.

The following hypotheses were tested: i) Novel BBFs are expected to behave in the same way as more traditional BBFs, e.g. manure, where a high pH and/or high  $\text{NH}_4^+$  content will lead to high  $\text{NH}_3$  volatilization potentials; ii) Biobased fertilizers produced partly or fully from poultry manures are expected to have high  $\text{NH}_3$  volatilization potentials, due to their high content of uric acid, rapidly hydrolyzing to  $\text{NH}_4^+$ ; iii) Application of BBFs to soils with a high pH will increase their  $\text{NH}_3$  volatilization potential, whereas soils with a high organic matter (OM) content, high clay content, and/or high CEC will decrease potential  $\text{NH}_3$  volatilization of BBFs; and iv) Incorporation of BBFs into soil or sand will decrease the potential  $\text{NH}_3$  volatilization.

The method used in this study was developed as a standard test method for the EU H2020 LEX4BIO project. The method is meant to be used as an assessment of whether the BBF poses a risk of  $\text{NH}_3$  loss and does not take into account varying environmental conditions that can affect the actual  $\text{NH}_3$  emissions in the field. It allows estimation of the potential  $\text{NH}_3$  loss from a BBF under conditions of constant removal of  $\text{NH}_3$  and  $\text{CO}_2$ , creating a maximum gradient for both gasses because they interact in enhancing  $\text{NH}_3$  volatilization (Husted et al., 1991). Due to the maximized gas gradients, these conditions simulate field conditions with high wind speeds and no vegetation cover. Moreover, to assess the  $\text{NH}_3$  volatilization potential from the 39 BBFs independently from their interactions with the soil,

they were also applied to pure sand, imitating the physical properties of soil, but lacking the chemical and biological characteristics that may affect  $\text{NH}_3$  volatilization.

## 2 Materials and methods

### 2.1 Materials

#### 2.1.1 Biobased fertilizers

39 BBFs, produced from various waste- and side streams of agricultural, urban or industrial origin, were included in this study. A novel BBF is here defined as a BBF produced by processes beyond simple biogas digestion of animal manures and simple composting. The processes involved in producing novel BBFs can e.g. be drying, pelletizing or mineral extraction. Of the BBFs included in this study, 90 % are defined as novel BBFs, with GRF, HDG, OOC and SDG defined as non-novel BBFs. All BBFs were available on the European market or on regional/national markets at the time of writing. A description of raw materials and technologies used, along with product function categories (PFC) and component material categories (CMC) according to the new EU Fertilizing Products Regulation (EU, 2019), is provided in Table I-1. The CMCs refer to the processing of BBFs, e.g. compost and digestate and the input material, e.g. industry by-products. The PFCs are related to the main BBF functions; subcategories relate to whether it is liquid or solid and the amount of mineral and organic components it contains. An extensive review of all technologies and processes involved in producing BBFs is beyond the scope of this study and readers are referred to Meers et al. (2020).

Moreover, the BBFs in this study were also grouped based on more classical terms used in the literature (Table I-1). Standard categories were digestate and compost. Meat and bone meal and other products from the meat industry were grouped together as animal by-products. Struvites and other BBFs derived from mineral extractions were grouped together as mineral precipitates. Potato fruit juice and other BBFs produced solely from plant materials were grouped together as plant-based BBFs. BBFs in a pelletized form produced from various materials were lumped in a group called “mixed”.

Before application to soil or sand, all BBFs were homogenized. For this purpose, non-fluid BBFs were carefully crushed using a mortar until they could pass through a 2-mm sieve. For BBFs with a high-fiber content, which could not be crushed using the mortar, fibers were cut with scissors and passed through a 2-mm sieve. Dry BBFs with particle sizes  $< 2$  mm and homogenous liquid BBFs were not further processed. Non-homogenous liquid and moist BBFs, e.g. digestates and composts, were homogenized using a blender for approximately one minute. Comparisons of  $\text{NH}_3$  volatilization potential from homogenized and non-homogenized BBFs are included in Appendix A, Fig. I-A1.



Table I-1: Raw material(s) and technologies used in production, along with their product function category (PFC) and component material categories (CMC) (EU, 2019), and grouping of the biobased fertilizers (BBFs) included in this study. Properties of the 39 BBFs included pH (1:5 in Milli-Q water), total N,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , Dry-matter (DM) content, total C, and C:N ratio. N, DM, and C are reported as % of fresh weight (FW). Values are means, N=2, except for C and N of liquid BBFs where N=5 and pH where N = 3. All the BBFs are presented here with their acronyms (three letters/numbers) Full product names and manufacturers are shown in the supplementary material (Table I-A1).

BBF	Raw material	Technology	PFC**	CMC*	Group	pH	N (% of FW)	$\text{NH}_4^+\text{-N}$ (% of total N)	$\text{NO}_3^-\text{-N}$ (% of total N)	DM (g kg <sup>-1</sup> of FW)	C (% of FW)	C/N
ASL	Product of nutrient-recycle-plant	Anaerobic digestion	1 A-II	4	Mineral precipitate	7.42	4.76	100	<0.1	216	0.11	0.02
AV4	Broiler litter	Drying, granulating, and pelletizing	1 A-I	10	Poultry manure	6.27	3.98	25	<0.1	893	38.9	9.77
AV8	Broiler litter, blood meal, and potassium sulfate	Drying, granulating, and pelletizing	1 B-I	10	Poultry manure	6.51	7.24	8.4	<0.1	920	38.9	5.38
BA6	Plant-based residues (wheat and maize)	Fermentation and distillation	1 A-II	4(6)***	Plant-based	4.85	5.57	1.4	<0.1	907	43.6	7.83
BIH	Animal horns	Pelletizing (Sphero technology)	1 A-I	10	Animal by-product	6.75	13.8	2.0	<0.1	892	42.5	3.08
BIL	Broiler litter and seaweed	Drying and pelletizing	1 A-I	10	Poultry manure	6.32	4.11	19	0.4	894	38.9	9.47
BIO	Meat and bone meal, apatite, vinasse, poultry manure, and potassium sulfate	Pelletizing	1 B-I	10	Animal by-product	5.69	7.39	3.5	<0.1	941	35.9	4.85
BIP	Different plant wastes, e.g. leftovers from sugar production	Liquidization	1 A-II	6	Plant-based	5.21	6.50	49	0.7	569	21.0	3.24
BLM	Blood meal		1 A-I	10	Animal by-product	6.85	14.7	0.2	<0.1	917	49.6	3.37
BO1	Potato cell water	Evaporation	1 A-II	6	Plant-based	5.88	1.46	20	<0.1	184	7.53	5.16
BO2	Molasses	Anaerobic digestion	1 A-II	6	Plant-based	6.59	1.66	0.9	0.9	521	20.5	12.3
BO4	Vinasse (sugar production)	Anaerobic digestion	1 A-II	6	Plant-based	6.23	3.56	1.3	0.6	620	21.2	5.96
BVC	Municipal organic food waste	Anaerobic digestion and composting	1 A-I	3	Compost	8.56	1.57	8.1	<0.1	557	14.7	9.37

CGR	Wastewater supernatant	Struvite precipitation	1 C-I	12	Mineral precipitate	8.38	5.53	0.7	<0.1	605	0.20	0.04
ECO	Blood and feather meal	Pelletizing	1 B-I	10	Animal by-product	5.48	11.6	2.7	<0.1	892	45.3	3.89
FEK	Poultry manure	Drying and processing (extrusion process)	1 A-I	10	Poultry manure	6.43	3.94	20	<0.1	901	34.7	8.81
FEL	Poultry manure	Drying in low temperature and pelletizing	1 A-I	10	Poultry manure	6.70	4.51	3.3	<0.1	925	36.0	7.98
GRF	Manure and crop digestate	Digestion	1 A-II	5	Digestate	8.19	0.37	75	0.3	45	2.02	5.46
HDG	75 % slurry, 25% source-separated Organic household waste plus organic industrial wastes	Digestion	1 A-II	5	Digestate	8.15	0.44	61	0.1	46	1.57	3.57
ILF	Shavings of treated hides and skins	Enzymatic hydrolysis	1 A-II	10	Animal by-product	6.37	8.84	2.1	<0.1	561	25.1	2.83
MAL	Mixture of malt germ, malt, minerals, and vinasse	Drying and pelletizing	1 A-I	6	Plant-based	5.02	4.37	28	<0.1	955	35.0	8.02
MB2	Meat and bone meal	Pelletizing (Sphero technology)	1 B-I	10	Animal by-product	6.39	8.01	0.6	<0.1	959	35.6	4.45
MO13	Feather meal	Pelletizing	1 A-I	10	Animal by-product	5.07	14.2	1.0	<0.1	927	49.0	3.45
NAD	Liquid manure and vegetables residue	Fermentation, drying, and pelletizing	1 B-I	4	Mixed	8.43	2.49	0.4	3.6	881	37.8	15.2
NE4	Sugar, molasses, syrup, mycelium from <i>Aspergillus niger</i>	Drying and pelletizing	1 A-I	6	Plant-based	8.41	3.99	0.2	<0.1	925	24.8	6.22
NE7	Plant-based organic raw material and crude phosphate, <i>Bacillus amyloliquefaciens</i>	Drying and pelletizing	1 B-I	6	Plant-based	6.51	6.65	1.7	<0.1	936	35.9	5.39
OG1	Meat and bone meal	Pelletizing	1 B-I	10	Animal by-product	5.73	10.2	0.7	<0.1	949	43.4	4.26

OG2	Horn meal (pig bristles)	Hydrolysis	1 A-I	10	Animal by-product	5.29	13.9	1.0	<0.1	940	48.3	3.47
OOC	Olive oil production residues	Composting	1 A-I	3	Compost	8.13	1.24	0.3	<0.1	900	40.8	32.9
OPU	Poultry manure	Pelletizing	1 B-I	10	Poultry manure	8.44	2.69	11	<0.1	893	33.3	12.4
PAL	Fermented biochar and high-quality clay and rock flour	Pyrolysis and fermentation "Terra Preta"	1 A-I	4	Plant-based	5.55	4.89	19	0.1	907	38.8	7.92
PCS	Sewage water	P extraction via struvite	1 C-I	12	Mineral precipitate	9.21	5.61	1.1	<0.1	614	0.27	0.05
PCW	Potato cell water	Evaporation	1 B-II	6	Plant-based	4.70	1.53	15	1.1	339	11.2	7.29
PRI	Organic material animal and/or vegetal origin + mineral granules	Compost into granules, then blending with mineral granules	1 B-I	3(6)***	Mixed	5.80	17.4	51	47	968	5.46	0.31
SDG	Agro and food waste + seaweed	Digestion	1 A-II	6	Digestate	8.40	0.41	68	<0.1	38	1.08	2.63
SIF	Animal and vegetal raw materials	Granulating	1 A-I	6(10)***	Mixed	5.87	9.92	4.3	0.3	950	42.0	4.23
SYS	Ammonium-nitrogen and potassium	Digestion using reverse osmosis	1 C-II	6	Mineral precipitate	7.06	8.07	95	0.5	384	0.12	0.01
TRS	Fish soluble, 78 % proteins, 16 % ash	Enzymatic hydrolysis	1 A-I	10	Animal by-product	5.87	13.6	5.0	<0.1	975	37.2	2.74
VEC	Residues of the digestion of horse manure	Composting	1 A-I	3	Compost	8.00	0.59	0.3	3.7	262	7.76	13.2

\* CMC 3: Compost, CMC 4: Fresh crop digestate, CMC 5: Digestate other than fresh crop digestate, CMC 6: Food industry by-products, CMC 10: Derived products within the meaning of Animal By-products Regulation, and CMC 12: Precipitated phosphate salts and derivatives (EU, 2019). \*\* PFC 1 A-I: Solid organic fertilizer:  $\geq 15$  % organic C, PFC 1 A-II: Liquid organic fertilizer:  $\geq 5$  % organic C, PFC 1 B-I: Solid organo-mineral fertilizer:  $\geq 7.5$  % organic C, 1 B-II: Liquid organo-mineral fertilizer:  $\geq 3$  % organic C, 1 C-I: Straight solid inorganic macronutrient fertilizer, and 1 C-I: Compound solid inorganic macronutrient fertilizer (EU, 2019). \*\*\* For BBFs which belong to more than one CMC group, the secondary CMC group is included in brackets.

### 2.1.2 Soil and sand matrix

For the incubation experiments on potential  $\text{NH}_3$  volatilization, either pure sand or different soils were used as the matrix to which the BBFs were added. The sand used was in the size range 0.4 – 0.9 mm, contained 0.2 % OM, and had been washed with water (Dansand A/S, Brødstrup, Denmark). Five different soils were used in the different experimental runs. These soils were selected from the experimental field sites of the EU H2020 LEX4BIO project, representing some of the variation in soil types present in Europe (Table I-2). The soil samples were collected from the top 0-30 cm. Acidic sandy soil was air-dried and sieved to 2 mm. Acidic clay soil, Alkaline loamy soil, Neutral loamy soil, and Alkaline clay soil were sieved to 4 mm and air-dried.

Table I-2: Soil characteristics of the soils used in the experiment. Values are means  $\pm$  Standard error. For N, C, pH Milli-Q water, pH  $\text{CaCl}_2$ , water-holding capacity (WHC), and cation exchange capacity (CEC), N = 3, except WHC for Alkaline loamy soil, where N = 2. For organic matter (OM), clay, silt, fine sand, and coarse sand, N = 1.

	<b>Acidic sandy soil</b>	<b>Acidic clay soil</b>	<b>Neutral loamy soil</b>	<b>Alkaline loamy soil</b>	<b>Alkaline clay soil</b>
Soil geography	Eastern Denmark	Southern Finland	Southern Germany	Southern France	Southern Spain
Coordinates	55°40'28.6"N 12°17'17.8"E	60°48'15.6"N 23°27'06.5"E	48°42'53.8"N 9°12'52.2"E	43°29'35.6"N 1°12'07.6"E	37°24'06.6"N 5°35'45.9"W
N (%)*	0.16 $\pm$ <0.01	0.27 $\pm$ <0.01	0.19 $\pm$ <0.01	0.12 $\pm$ <0.01	0.16 $\pm$ 0.01
C (%)*	1.39 $\pm$ 0.01	2.89 $\pm$ 0.02	1.31 $\pm$ <0.01	0.83 $\pm$ 0.03	4.07 $\pm$ 0.03
pH Milli-Q water	6.64 $\pm$ 0.07	6.79 $\pm$ 0.03	7.75 $\pm$ 0.01	7.89 $\pm$ 0.01	8.35 $\pm$ 0.02
pH $\text{CaCl}_2$	5.73 $\pm$ 0.11	5.74 $\pm$ 0.01	7.04 $\pm$ 0.02	7.20 $\pm$ 0.05	7.66 $\pm$ 0.01
WHC (% of dry mass)	35.5 $\pm$ 0.3	46.8 $\pm$ 0.4	41.4 $\pm$ 1.0	39.1 $\pm$ 1.7	46.7 $\pm$ 0.3
CEC <sub>pH7</sub> (cmol/kg)**	7.1 $\pm$ 0.4	15.4 $\pm$ 4.0	10.4 $\pm$ 3.1	11.0 $\pm$ 0.7	34.9 $\pm$ 4.6
OM (%)***	2.1	4.8	2.2	1.3	1.9
Clay <0.002 mm (%)***	9.6	48.8	33.2	14.2	61.7
Silt 0.002-0.02 mm (%)***	8.4	26.1	30.2	20.9	25.9
Fine sand 0.02-0.2 mm (%)***	44.7	14.5	33.0	47.7	9.2
Coarse sand 0.2-2 mm (%)***	35.2	5.9	1.4	15.9	1.4

\* Total C and N was determined by Dumas combustion. \*\* CEC determined by the  $\text{NH}_4^+$  acetate method, quantifying  $\text{NH}_4^+$ -N with flow injection analysis. \*\*\* Texture and OM content determined at Agrolab Sarstedt by sieving and sedimentation for texture and by Dumas combustion for total OM.

## 2.2 Incubation setup for determining potential ammonia volatilization

The experiment was performed using a static diffusion enclosure method, developed by modifying the procedures described by the following authors (Husted et al., 1991; Ndegwa et al., 2009; Mandal et al., 2016). The method is meant to be used as an assessment of whether the BBF poses a risk of  $\text{NH}_3$  loss and does not take into account varying environmental conditions that can affect the actual  $\text{NH}_3$  emissions in the field. A detailed methods description is openly available online in ERDA, the repository of the University of Copenhagen:

<https://doi.org/10.17894/ucph.456e08b8-e902-49a5-a51c-9ecd65216df0>

One hundred g (oven dry weight) of air-dry sieved soil/sand was added to 120 mL plastic containers. The soil was compacted to 1.25 g  $\text{cm}^{-3}$  (Acidic sandy soil and Alkaline loamy soil), 1.11 g  $\text{cm}^{-3}$  (Neutral loamy soil and Alkaline clay soil), and 1 g  $\text{cm}^{-3}$  (Acidic clay soil). The sand was not compacted. Milli-Q water was added to each container with a pipette to adjust the water content to 60 % of the soil's water-holding capacity (WHC, determined modified according to ISO 14238). For the sand, it was

assessed that 20 mL of Milli-Q water was suitable for 100 g (dry weight) of sand. The moisture content of the air-dry soil/sand and the water content of the BBFs were subtracted from the amount of water to be added to ensure an equal moisture content in all setups. The containers with moist soil/sand were pre-incubated for five days at 15 °C in a dark incubator (Termaks, A/S Ninolab, Solrød, Denmark) before addition of the BBF.

To mimic areas of a field receiving high BBF applications on its soil surface, e.g. a strip of liquid digestate from a trailing hose or the area around a solid BBF pellet, 400 kg total-N ha<sup>-1</sup>, corresponding to 0.004 g total-N cm<sup>-2</sup>, was applied as the standard rate for all 39 BBFs in experiment 1 (Table I-3). In a different trial, eight selected BBFs were either surface applied and distributed evenly on the soil/sand surface (standard application method) or they were applied and thoroughly incorporated by mixing into the soil/sand before addition of Milli-Q water (see Table I-3, experiment 2). Furthermore, to test the implication of the application rate, selected BBFs were applied at both the standard rate (1\* application rate), which corresponds to 720 mg N kg<sup>-1</sup> soil; half the rate (½ \* application rate), corresponding to 360 mg N kg<sup>-1</sup> soil (200 kg BBF N ha<sup>-1</sup>); and double the rate (2\* application rate), corresponding to 1440 mg N kg<sup>-1</sup> soil (800 kg BBF N ha<sup>-1</sup>) (see Table I-3, experiment 3). The effect of the soil type was tested with five different BBFs (see Table I-3, experiment 4). Moreover, the effect of the soil moisture was tested with three BBFs (see Table I-3, experiment 5).

Table I-3: Overview of treatments in the experiment.

Experiment	No of BBFs	Soil/sand	BBF application	Water content (% of WHC)	BBF application rate (mg N kg <sup>-1</sup> soil)
1	39	Sand	Surface	60	720
2	8	Acidic sandy soil; Sand	Surface; Incorporated	60	720
3	3	Acidic sandy soil	Surface	60	360; 720; 1440
4	5	Acidic sandy soil; Acidic clay soil; Neutral loamy soil; Alkaline loamy soil; Alkaline clay soil	Surface	60	720
5	3	Acidic sandy soil	Surface	30; 60; 90	720

Three replicates of each treatment were included in all experimental runs. Three replicates of controls with only sand/soil and no fertilizer were also included, as well as two blank tests of the setup without any sand/soil or fertilizer. Both no-treatment controls and blanks generally had NH<sub>3</sub> concentrations below the detection limit and were therefore not accounted for. Moreover, a reference fertilizer of ammonium bicarbonate (NH<sub>4</sub>HCO<sub>3</sub>) dissolved in Milli-Q water was included in each experimental run. This was, firstly, to serve as a reference for maximum potential NH<sub>3</sub> loss (due to all N being present as NH<sub>4</sub><sup>+</sup> and bicarbonate promoting pH rise upon application) and, secondly, to allow comparisons between experimental runs.

Each 120 mL plastic container with soil/sand and applied BBF, surface area of 18 cm<sup>2</sup>, was incubated in a glass canning jar (0.75 L) with an airtight lid. A trap consisting of 5 mL 0.2 M sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) in an open plastic container (26 mL airtight plastic container, Frisenette ApS, Knebel, Denmark) was placed in the canning jar. The amount and concentration of H<sub>2</sub>SO<sub>4</sub> was calculated stoichiometrically so that the H<sub>2</sub>SO<sub>4</sub> was able to trap the double amount of NH<sub>3</sub> that could potentially volatilize (Ndegwa et al., 2009). The surface area of the acid in the acid trap was 7 cm<sup>2</sup>. In a pre-trial, the efficiency of the acid trap was tested with NH<sub>4</sub>HCO<sub>3</sub> added to sand. The result showed that 82 % of the N added as NH<sub>4</sub>HCO<sub>3</sub> was recovered in the acid trap during an experimental period of 44 days (Fig. I-1). This is within the range of previously reported NH<sub>3</sub> recovery values of 73 – 103 % in laboratory setups (Woodward et al., 2011) and indicates that the setup did not have any significant leakage. The trap

collected after the last trap exchange in the test trial with  $\text{NH}_4\text{HCO}_3$  contained  $\text{NH}_3$  and, thus,  $\text{NH}_3$  was still volatilizing at the end of the trial period. If the experimental period had been extended, the recovery percentage would likely have approached 100 %.

A base trap consisting of 10 mL of 1 M sodium hydroxide (NaOH) in a plastic shot glass was additionally inserted in the canning jar to trap any  $\text{CO}_2$  evolved during the incubation. Furthermore, a plastic shot glass containing 10 mL of deionized water was placed in the canning jar to maintain high humidity and prevent desiccation of the BBF and soil sample over time.

Canning jars were placed in a dark incubator (Termaks, A/S Ninolab, Solrød, Denmark) set to 15 °C to reflect a mean European air temperature.

#### *Trap exchange and quantification of $\text{NH}_3$*

The  $\text{H}_2\text{SO}_4$  and NaOH traps were exchanged 1, 2, 5, 9, 14, 20, and 27 days after the start of the experiment at the same time of day  $\pm$  30 minutes. For the experimental run with all BBFs surface-applied to sand (Experiment 1 Table I-3), two additional sampling times (35 and 44 days after the start) were applied. However, four of the 39 BBFs (MAL, BLM, ASL, and GRF) were included in a different experiment which only ran for 27 days. The base traps with NaOH were replaced, but not analyzed for  $\text{CO}_2$  content. The acid traps with  $\text{H}_2\text{SO}_4$  were stored at ambient temperature with air-tight lids for a maximum of two weeks until analysis of the  $\text{NH}_4^+$ -N content. The stability of the  $\text{NH}_4^+$  content in the traps was assessed to be at least three weeks by analyzing the  $\text{NH}_4^+$ -N in the same traps at different time points. The  $\text{NH}_3$  trapped in the  $\text{H}_2\text{SO}_4$  was quantified by analyzing the  $\text{NH}_4^+$ -N content in the traps using flow injection analysis (FIAstar 5000 flow injection analyser (Foss Analytical, Hillerød, Denmark)). The lowest detected concentration was 0.0045 mg  $\text{NH}_4^+$ -N  $\text{L}^{-1}$ . This method of quantifying  $\text{NH}_3$  was tested against the titration method (see Fig. I-A2).

### **2.3 Analysis of soil and BBFs**

At the end of each experimental run, all samples of soil/sand with BBFs applied were mixed thoroughly and a subsample of five g was collected to determine the pH value. The pH was determined on the last day of the experiment (day 27 or day 44) as 1:5 in Milli-Q water.

Dry matter (DM) of solid BBFs was determined at 105°C by TGA701 (Leco) and that of liquid BBFs by drying in a ventilated oven (105°C) until the weight did not change. The total C and N of BBFs was determined by the Dumas dry combustion method (Trumac or CHN628, Leco). The water-soluble  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N of the BBFs were analyzed in 1:60 water extracts with a continuous flow analyzer (Skalar San ++ System). All analyses of BBFs were carried out on their original dry matter content.

### **2.4 Statistics**

All illustrations were made in SigmaPlot 14.00 (Systat Software, Inc.). All statistical analyses were performed in R version 4.0.2 (R Core Team). A minimum significance level of  $p < 0.05$  was applied for all tests. Data were visually assessed with diagnostic plots to check the homogeneity of variance and data were square-root transformed for all statistical analyses due to heterogeneity of variance.

The analyses were performed on the accumulated  $\text{NH}_3$ -N volatilized as a percentage of total added N for all days. The repeated measurements of accumulated  $\text{NH}_3$  volatilization of each sample were accounted for by setting the sampling day as a repeated variable. This transformation was applied for all statistical analyses. All analyses were performed using linear mixed models and pairwise comparisons of least square means.  $R^2$  values and correlation coefficients were retrieved from the linear mixed models. A quadratic term of measurement day was included in the linear mixed models to account for non-linearity. Moreover, the sample number was always included as a random effect. BBF was included as a random effect for experiment 1 (Table I-3) and for the correlation coefficients and  $R^2$  value for experiment 4 (Table I-3), but not for the remaining analyses, as these were performed separately for each BBF treatment. See Table I-A2 for further information on statistics.

### 3 Results

#### 3.1 BBF properties

The properties of the 39 BBFs varied substantially (Table I-1). The greatest variations were found in  $\text{NH}_4^+$ -N content and dry matter (DM) content.

#### 3.2 Experiment 1: Potential $\text{NH}_3$ volatilization from all BBFs

The 39 BBFs surface applied to sand varied greatly in terms of accumulated potential  $\text{NH}_3$  volatilized at the end of the incubation period (Fig. I-1). However, all the BBFs showed some potential  $\text{NH}_3$  volatilization during the incubation period, except for OOC (data not shown), where volatilization was below the detection limit on all days. Moreover, the temporal pattern of potential  $\text{NH}_3$  volatilization during the incubation period differed between BBFs. Some BBFs had a high initial potential  $\text{NH}_3$  volatilization and had concave shaped curves of accumulated potential  $\text{NH}_3$  volatilization. For other BBFs, however, the initial potential  $\text{NH}_3$  volatilization was very low and increased towards the end of the incubation period, resulting in convex curves of accumulated potential  $\text{NH}_3$  volatilization.

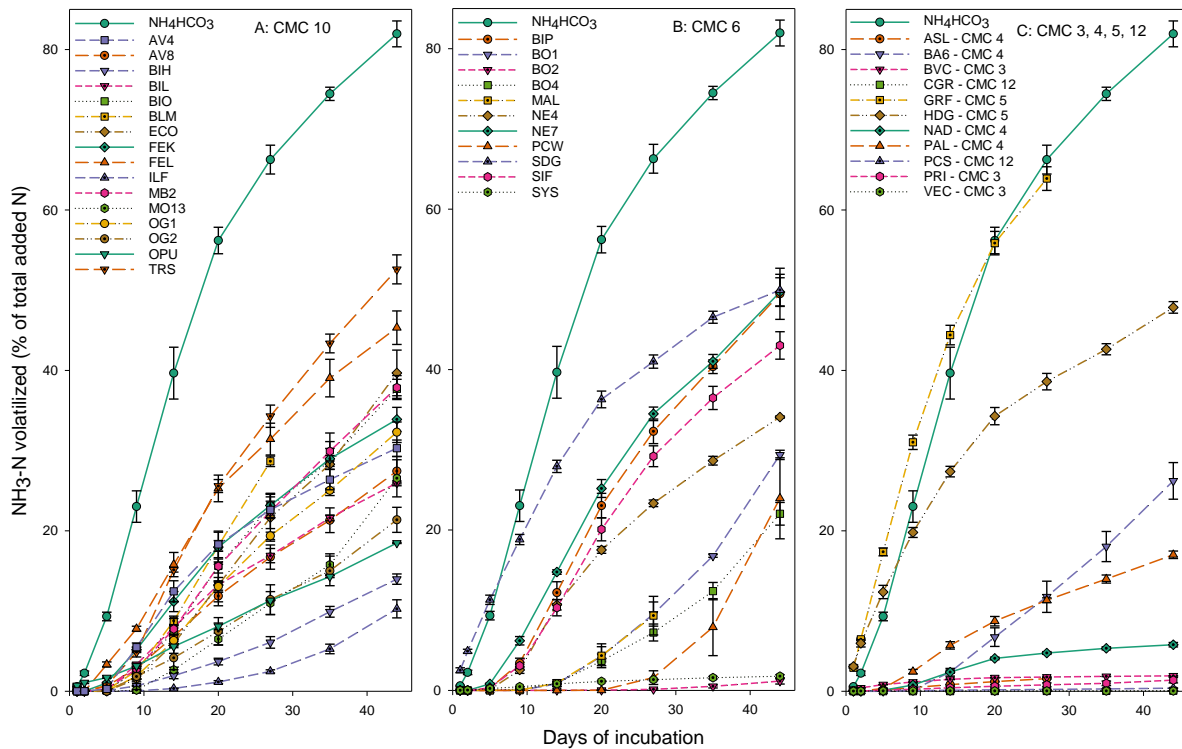


Fig. I-1: Mean accumulated  $\text{NH}_3$ -N volatilized (% of total added N)  $\pm$  standard error during 44 (a few with only 27) days of incubation from 38 different biobased fertilizers and a reference of  $\text{NH}_4\text{HCO}_3$ . A) BBFs from CMC 10 (Animal by-products). B) BBFs from CMC 6 (Food industry by-products). C) BBFs from CMC 3, 4, 5, and 12 (Composts, crop digestate, other digestates, salts & derivatives respectively). All BBFs were surface applied on sand at an application rate of  $0.72 \text{ g N kg}^{-1} \text{ DW soil}$ .  $N = 3$ .

Among the six CMCs, the accumulated  $\text{NH}_3$  volatilization on day 27 was lowest for BBFs in CMC 12, followed by  $\text{CMC } 3 < 4 < 6 < 10 < 5$ . Among the six PFCs, the accumulated  $\text{NH}_3$  volatilization on day 27 was lowest for BBFs in PFC 1 A-II, followed by  $1 \text{ C-I} < 1 \text{ C-II} < 1 \text{ B-II} < 1 \text{ A-I} < 1 \text{ B-I} < 1 \text{ A-II}$ . Among the seven BBF groups, the accumulated  $\text{NH}_3$  volatilization on day 27 was lowest for Compost

followed by Mineral precipitate < Mixed < Plant based < Animal by-products < Poultry manure < Digestate.

Based on the number of significant differences between categories/groups in the three different categorizations included, CMCs and PFCs were not as good at predicting accumulated potential  $\text{NH}_3$  volatilization compared to the groups based on traditional categorizations (Table I-A3).

A linear mixed model including BBF properties (pH,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , DM, C:N) and their interaction with time explained 89 % of the variation in accumulated potential  $\text{NH}_3$  volatilization during the experimental period of 44 days. Potential  $\text{NH}_3$  volatilization was positively correlated with  $\text{pH}_{\text{Milli-Q water}}$  (0.312) and  $\text{NH}_4^+\text{-N}$  content (0.013) and negatively correlated with  $\text{NO}_3^-\text{-N}$  content (-0.007), DM content (-0.01), and C:N ratio (-0.002).

### **3.3 Experiment 2: Soil vs. sand and BBF application technique**

The potential  $\text{NH}_3$  volatilization was significantly lower for soil (Acidic sandy soil) compared to sand for the  $\text{NH}_4\text{HCO}_3$  reference and all BBFs, except BO1 ( $P < 0.0001$ ) (Fig. I-2). Moreover, potential  $\text{NH}_3$  volatilization was significantly lower for incorporation compared to surface application for all BBFs ( $P < 0.0001$ ), except BO1 (Fig. I-2). For the digestate HDG, the  $\text{NH}_3$  volatilization after one day was reduced by 88 % as a result of incorporation into soil relative to surface application. For the remaining BBFs, no noteworthy  $\text{NH}_3$  had volatilized during the first day of incorporation. Looking at the accumulated  $\text{NH}_3$  volatilization after 27 days of incubation, incorporation into soil reduced  $\text{NH}_3$  volatilization by 37 % and 64 % for the  $\text{NH}_4\text{HCO}_3$  reference and HDG respectively compared to surface application on soil. For AV4, SIF, PAL, ECO, NAD, and BVC, incorporation reduced  $\text{NH}_3$  volatilization by more than 96 %.



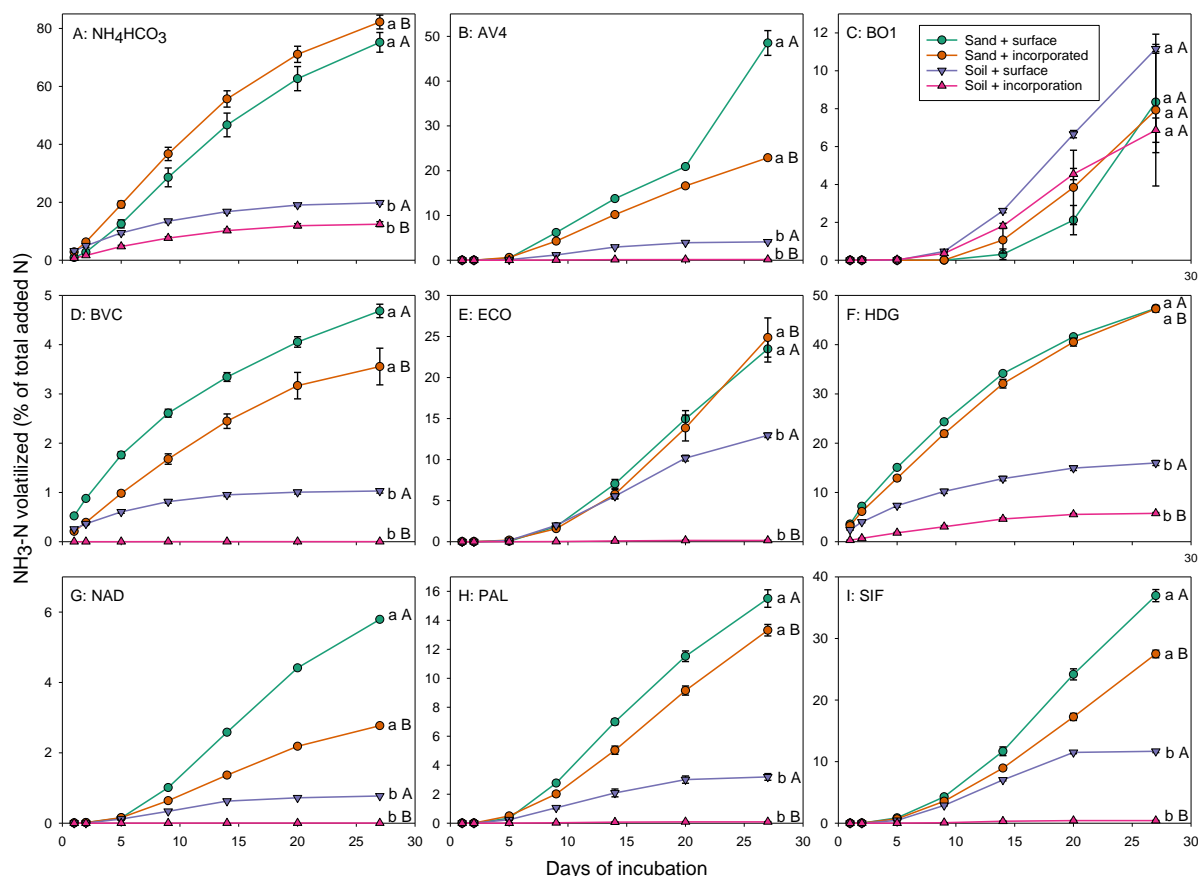


Fig. I-2: Mean accumulated  $\text{NH}_3\text{-N}$  volatilized (% of total added N)  $\pm$  standard error during 27 days of incubation from biobased fertilizers (B-I) and a reference of  $\text{NH}_4\text{HCO}_3$  (A) surface applied or incorporated into soil or sand. Application rate of  $0.72 \text{ g N kg}^{-1}$  DW soil and a soil moisture content at 60 % of WHC. Note different scales on vertical axes. N = 3. Lower case letters indicate significant different  $\text{NH}_3$  volatilization potential between sand and soil within each BBF, Capital letters indicate significant different  $\text{NH}_3$  volatilization potential between incorporation and surface application within each BBF.

### 3.4 Experiment 3: BBF application rate

For the  $\text{NH}_4\text{HCO}_3$  reference and AV4 (Fig. I-3 A+B), the accumulated  $\text{NH}_3$  volatilized was approximately doubled at the end of the incubation (day 27) when doubling the application rate. For ECO and HDG (Fig. I-3 C+D), the differences between application rates were smaller, but showed a similar trend. Therefore, the potential  $\text{NH}_3$  volatilized did not generally increase linearly with an increasing application rate. However, there were no overall significant differences between the three application rates. Only  $\frac{1}{2}$  the application rate was significantly different from both 1 and 2 times the application rate for AV4 and the  $\text{NH}_4\text{HCO}_3$  reference (Fig. I-3 A and B).

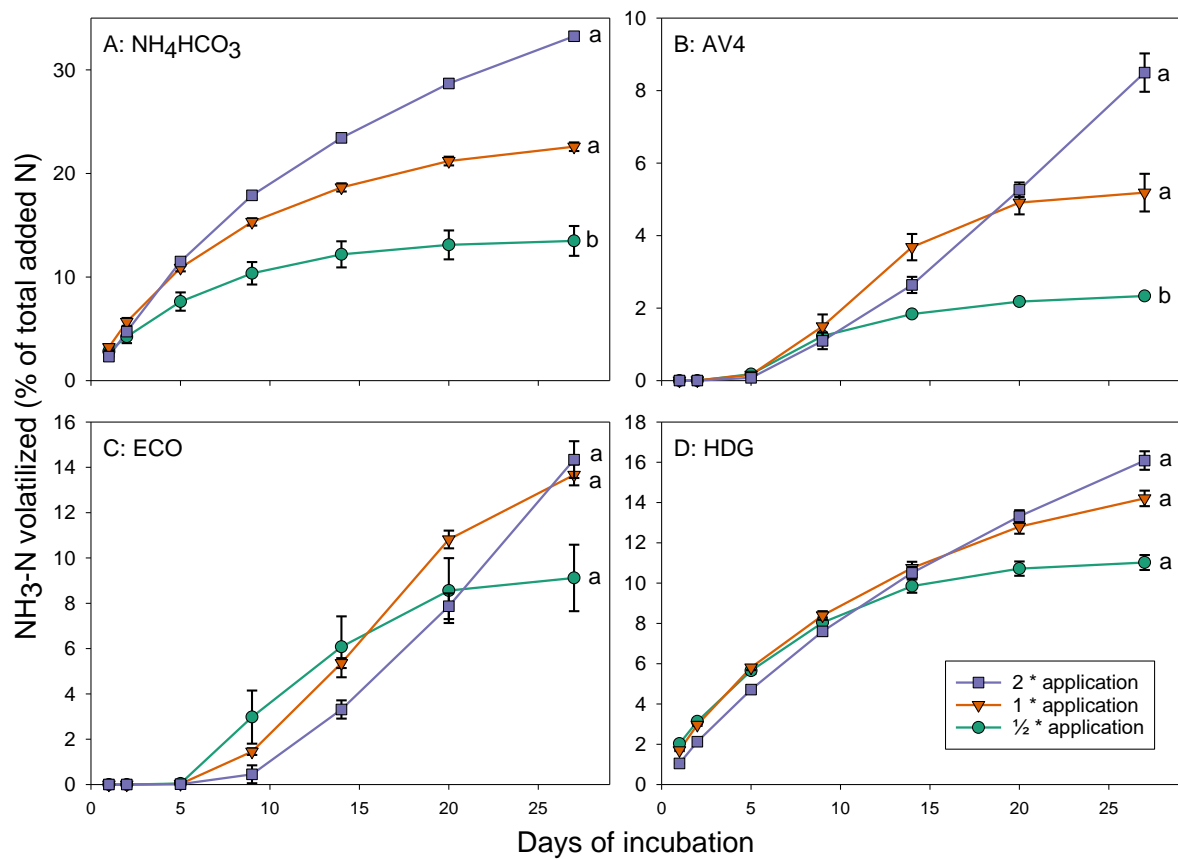


Fig. I-3: Mean accumulated  $\text{NH}_3\text{-N}$  volatilized (% of total added N)  $\pm$  standard error during 27 days of incubation from biobased fertilizers and a reference of  $\text{NH}_4\text{HCO}_3$  surface applied to soil (Acidic sandy soil) at application rates of 1/2:  $0.36 \text{ g N kg}^{-1} \text{ DW soil}$ , 1:  $0.72 \text{ g N kg}^{-1} \text{ DW soil}$ , and 2:  $1.44 \text{ g N kg}^{-1} \text{ DW soil}$ . Soil moisture content at 60 % of WHC. Note different scales on vertical axes. Letters indicate significant different  $\text{NH}_3$  volatilization potential between application rates within each BBF.

### 3.5 Experiment 4: Effect of soil type

The accumulated potential  $\text{NH}_3$  volatilization was significantly higher for Acidic sandy soil for all BBFs and the  $\text{NH}_4\text{HCO}_3$  reference at the end of the incubation period compared to Acidic clay soil, Neutral loamy soil, and Alkaline clay soil (Fig. I-4). The accumulated potential  $\text{NH}_3$  volatilization was lowest for Acidic clay soil and Alkaline clay soil.

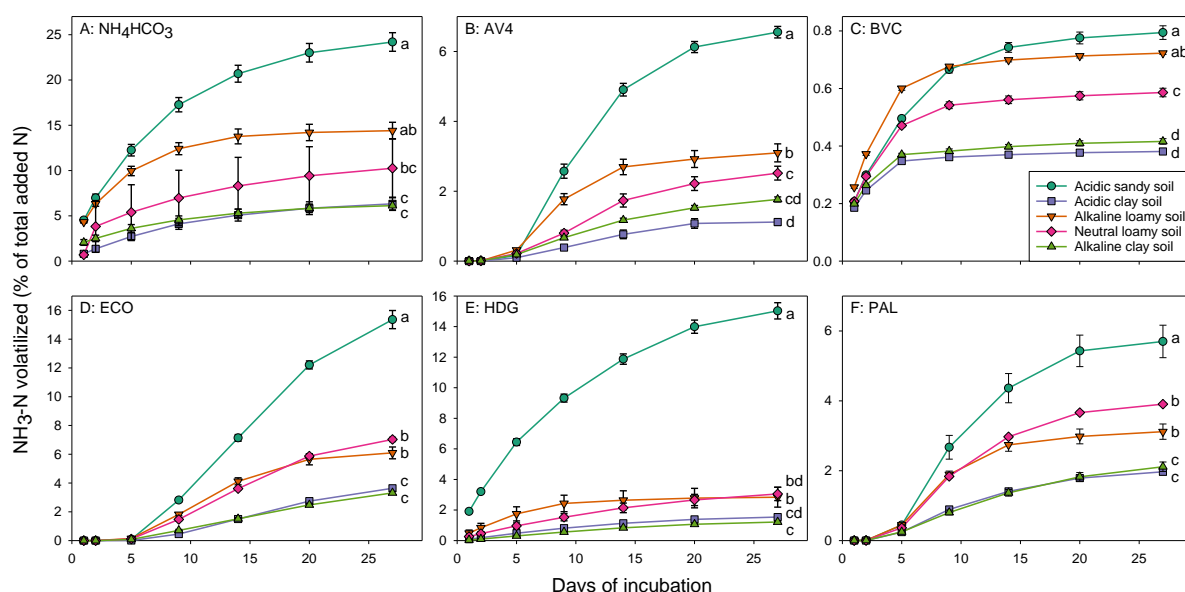


Fig. I-4: Mean accumulated  $\text{NH}_3\text{-N}$  volatilized (% of total added N)  $\pm$  standard error during 27 days of incubation from biobased fertilizers (B-F) and a reference of  $\text{NH}_4\text{HCO}_3$  (A) surface applied to five different soils (Acidic sandy soil, Acidic clay soil, Alkaline loamy soil, Neutral loamy soil, and Alkaline clay soil). Application rate of  $0.72 \text{ g N kg}^{-1} \text{ DW soil}$  and a soil moisture content at 60 % of WHC. Note different scales on vertical axes. Letters indicate significant different potential  $\text{NH}_3$  volatilization between soils within each BBF.  $N = 3$ , except for D: ECO on day 20 for Alkaline loamy soil where  $N = 2$  and B: AV4 on day 27 for Acidic clay soil where  $N = 2$ .

In a linear mixed model, soil characteristics ( $\text{pH}_{\text{CaCl}_2}$ , OM, and clay content) and their interactions with time explained 88 % of the variation in potential  $\text{NH}_3$  volatilization for all days, all five soils and all BBFs +  $\text{NH}_4\text{HCO}_3$  reference. Accumulated potential  $\text{NH}_3$  volatilization was negatively correlated with  $\text{pH}_{\text{CaCl}_2}$  (-0.15), OM (-0.13), and clay content (-0.002).

### 3.6 Experiment 5: Effect of soil moisture

No clear pattern across BBFs was observed for the differences in soil moisture (WHC levels) (Fig. I-5). There were no marked differences in  $\text{NH}_3$  volatilization between WHC levels for ECO and HDG. For AV4, 90 % WHC resulted in the highest volatilization and 30 % WHC in the lowest; whereas, the opposite was true for the  $\text{NH}_4\text{HCO}_3$  reference.

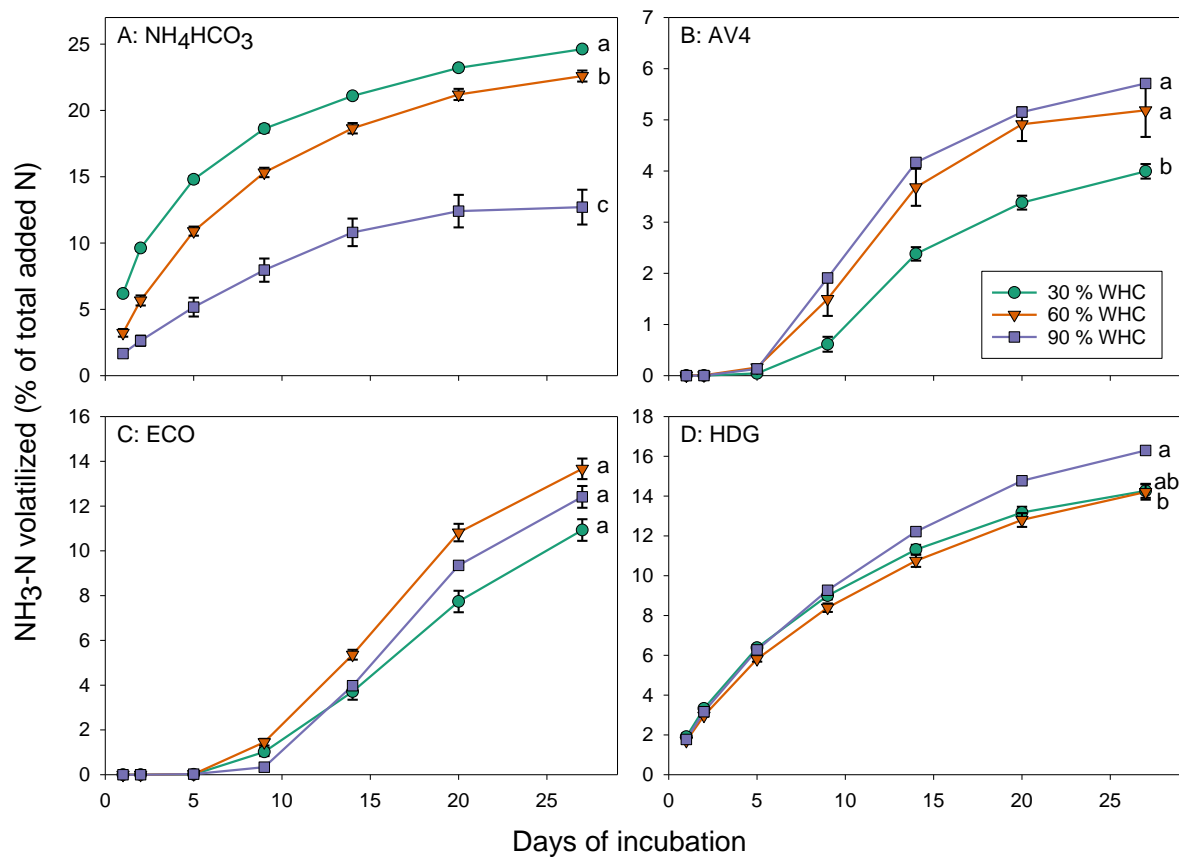


Fig. I-5: Mean accumulated  $\text{NH}_3\text{-N}$  volatilized (% of total added N)  $\pm$  standard error during 27 days of incubation from biobased fertilizers and a reference of  $\text{NH}_4\text{HCO}_3$  surface applied to soil (Acidic sandy soil); soil moisture content was at 30 %, 60 % or 90 % of WHC. Application rate of  $0.72 \text{ g N kg}^{-1} \text{ DW soil}$ , except for D: HDG 30 % WHC with an application rate of  $0.48 \text{ g N kg}^{-1} \text{ DW soil}$ . Note different scales on vertical axes. N = 3.

## 4 Discussion

### 4.1 Potential $\text{NH}_3$ volatilization from biobased fertilizers

The great variability in the quantity and temporal pattern of potential  $\text{NH}_3$  volatilization from the 39 BBFs included in this study (Experiment 1) highlights the importance of accounting for the fertilizer type when predicting  $\text{NH}_3$  volatilization upon application.

#### 4.1.1 Temporal pattern in $\text{NH}_3$ volatilization

The Michaelis-Menten or Monod type equation has often been used to describe  $\text{NH}_3$  volatilization from soil upon application of slurry (Monaco et al., 2012). Liao et al. (2019) observed high initial  $\text{NH}_3$  volatilization during the first 2-3 days from digestates applied to soil using a dynamic flow-through setup. After the initial days,  $\text{NH}_3$  volatilization was very low. Thus, the pattern of  $\text{NH}_3$  volatilization followed a concave/Michaelis-Menten type equation. The findings of Liao et al. (2019) are in agreement with this study regarding the three digestates SDG, GRF, and HDG (Fig. I-1). However, for the majority of BBFs, the pattern of potential  $\text{NH}_3$  volatilized followed a convex shape in the initial period of 20 days, where a lag phase in the potential  $\text{NH}_3$  volatilization was observed (Fig. I-1). This is in agreement with the findings of Erwiha et al. (2020), who also found convex shaped curves of accumulated  $\text{NH}_3$  volatilization during the initial 2-3 weeks after application of blood meal and feather meal on a fine clay

loam in a field trial in Colorado, USA. This convex curve of  $\text{NH}_3$  volatilization potential could indicate that  $\text{NH}_3$  volatilization from the majority of BBFs is controlled by the rate of BBF N mineralization. However, this was not measured in the present study. Another explanation for this delay in  $\text{NH}_3$  volatilization, resulting in convex shaped curves, could be an increase in pH during the experimental period, which was not measured in the present study; pH was only measured, after thoroughly mixing the sand with BBF applied, at the end of the incubation. At the end of the incubation period (day 27/44) nearly all samples with BBFs surface applied to sand had a pH above 7 (Table I-A4). Only SYS had a pH below 7 (6.7) and only three treatments (ASL, MAL, and SYS) had a pH below the no treatment control of pH 7.9. Thus, pH was not the factor limiting  $\text{NH}_3$  volatilization at this last stage, where close to all  $\text{NH}_4^+$ -N could potentially volatilize as  $\text{NH}_3$ . However, earlier in the incubation period, low pH might have limited  $\text{NH}_3$  volatilization.

#### *4.1.2 BBF properties' ability to predict $\text{NH}_3$ volatilization potential*

89 % of the variation in accumulated  $\text{NH}_3$  volatilization potential could be explained by initial BBF properties (pH,  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, DM, and C:N) and their interaction with time. The ammonium-N content and pH of the BBFs showed a weak positive correlation with potential  $\text{NH}_3$  volatilization on the first day. This confirms the hypothesis that BBFs with a high  $\text{NH}_4^+$ -N content and/or high pH will have a high  $\text{NH}_3$  volatilization potential.

#### *4.1.3 Digestates*

Generally, digestates with animal manure and slurry as source material have a higher  $\text{NH}_3$  volatilization potential upon application in the field compared to non-digested animal manure and slurry (Moeller and Stinner, 2009; Nkoa, 2014). In this study, the three digestates (HDG, SDG, and GRF) (Table I-1) were also the BBFs with the highest  $\text{NH}_3$  volatilization potential. Rabiger et al. (2020) found  $\text{NH}_3$  volatilization of 7.6-18.3 % of total applied N from digestate applied at five different sites in Germany, using a standardized dynamic closed chamber method. The results of  $\text{NH}_3$  volatilization from digestates surface applied to sand in this study were higher (39-64% of applied N). However, the results of Rabiger et al. (2020) are comparable, although slightly higher, compared to the results in this study on  $\text{NH}_3$  volatilization potential from the digestate HDG surface applied to five different soils (1.2-15% of applied N).

#### *4.1.4 Poultry manure containing BBFs*

This study included six BBFs containing poultry manure or poultry litter (Table I-1). In a previous laboratory incubation study, using a sandy loam soil,  $\text{NH}_3$  volatilization from application of poultry manure was found to range from 3.2% - 6.1% of applied N (Akiyama et al., 2004). For AV4 surface applied to the Acidic sandy soil, accumulated  $\text{NH}_3$  volatilization on day 27 was only 4.1 % of applied N, which is comparable to the results of Akiyama et al. (2004). The hypothesis that BBFs produced from poultry manure would have a high  $\text{NH}_3$  volatilization potential can neither be completely confirmed nor completely rejected, as the poultry manure BBFs were the group with the second highest accumulated  $\text{NH}_3$  volatilization potential after digestates, but very similar to the animal by-product BBFs (see Fig. I-A4). The comparatively low  $\text{NH}_3$  volatilization potential may be due to the production process of the BBFs containing poultry manure, since this typically includes a drying step and thereby already promotes a potential  $\text{NH}_3$  loss during production. Lockyer et al. (1989) found lower  $\text{NH}_3$  volatilization of air-dried poultry manure (6.5 % of applied N) compared to poultry slurry (45.5 % of applied N). The air-dried poultry manure contained 30 %  $\text{NH}_4^+$ -N of total N compared to 55 %  $\text{NH}_4^+$ -N of total N for the poultry slurry (Lockyer et al., 1989). Moreover, Lockyer et al. (1989) found a high initial  $\text{NH}_3$  volatilization from poultry, pig, and cattle manure and slurry, with 80 % or more of the  $\text{NH}_3$  volatilization occurring within the first 48 hours after application. This rapid initial  $\text{NH}_3$  volatilization was not observed for the poultry manure containing BBFs included in this study, which all had a delayed response in  $\text{NH}_3$  volatilization (Fig. I-1). This might be explained by a lower average concentration of 15 %  $\text{NH}_4^+$ -N of total N in the poultry manure containing BBFs compared to the air-dried poultry manure in Lockyer et al. (1989). Marshall et al. (1998) conducted a study using broiler litter with similar average  $\text{NH}_4^+$ -N of total N, 14 %, as in this study. Comparable to the observations of this study, Marshall et al. (1998) observed a delayed response in  $\text{NH}_3$  volatilization with a sharp increase in the  $\text{NH}_3$

volatilization rate 1-3 days after broiler litter application in a field study, using the micrometeorological method.

#### *4.1.5 Animal by-product BBFs*

This study included nine BBFs containing animal by-products (Table I-1). Accumulated  $\text{NH}_3$  volatilization on day 27 from these BBFs surface applied to sand ranged from 6.1 % - 34.3 % of applied N and was generally in the upper range of  $\text{NH}_3$  volatilization potential from all BBFs (see Fig. I-A4). However, the animal by-products containing BBFs was the group with the lowest  $\text{NH}_4^+\text{-N}$  content with 2 %  $\text{NH}_4^+\text{-N}$  of total N. Thus, the relatively high accumulated  $\text{NH}_3$  volatilization could not be explained by a high  $\text{NH}_4^+\text{-N}$  content. Therefore, a rapid mineralization of N and subsequent volatilization of the  $\text{NH}_4^+\text{-N}$  likely explains the relatively high  $\text{NH}_3$  volatilization. Erwiha et al. (2020) conducted a field experiment on a fine clay loam soil using a semi-static chamber and found  $\text{NH}_3$  volatilization of 25 % and 32.1 % of applied N for blood meal and feather meal respectively. The  $\text{NH}_3$  volatilization potential from animal by-product derived BBFs in this study is thus comparable, although lower than that found by Erwiha et al. (2020) especially considering that BBFs were applied to sand in this study and to a fine clay loam in the study conducted by Erwiha et al. (2020).

### **4.2 Implication of BBF application technique and rate**

#### *4.2.1 Application technique*

The incorporation of all BBFs reduced potential  $\text{NH}_3$  volatilization significantly, except for BO1 (Experiment 2, Fig. I-2). This is in agreement with numerous previous studies conducted on other BBFs in both field and laboratory trials (Svensson, 1994; Nyord et al., 2012; Feilberg and Sommer, 2013; Herr et al., 2019; Liao et al., 2019; Rocha et al., 2019; Maris et al., 2020).

For the digestate HDG, which was the only BBF with a substantial  $\text{NH}_3$  volatilization potential during the first day that was tested for the effect of incorporation, the incorporation reduced the potential  $\text{NH}_3$  volatilization markedly by 88 % during the first day. Thus, incorporation was an effective way of preventing initial  $\text{NH}_3$  volatilization. Looking at the accumulated  $\text{NH}_3$  volatilization for the 27-day period for HDG, it was overall reduced by 64 % though, showing that some  $\text{NH}_3$  still volatilized. From these results, it was concluded that incorporation is an effective way to reduce  $\text{NH}_3$  volatilization from BBFs, as is also often assumed for more commonly studied BBFs, e.g. manure. Thus, this confirms the hypothesis that incorporation reduces the potential  $\text{NH}_3$  volatilization from BBFs.

#### *4.2.2 Application rate*

Although a tendency towards a non-linear increase in potential  $\text{NH}_3$  volatilization was visually observed for AV4, HDG, and the  $\text{NH}_4\text{HCO}_3$  reference, it was not clearly significant (Experiment 3, Fig. I-3). This is in agreement with results from a study conducted by Erwiha et al. (2020), who found no clear response of  $\text{NH}_3$  volatilization to the BBF application rate of blood meal, feather meal, fish emulsion, and cyano-fertilizer applied on a fine clay loam in a field trial in Colorado, USA. This suggests that the overall trends found in this study are valid over a wider range of application rates than those tested here.

### **4.3 Effect of soil type and soil moisture**

#### *4.3.1 Soil properties*

Soil characteristics and their interaction with time explained 88 % of the variation in accumulated potential  $\text{NH}_3$  volatilization from BBFs applied to different soils (Experiment 4, Fig. I-4). Potential  $\text{NH}_3$  volatilization was found to negatively correlate with soil  $\text{pH}_{\text{CaCl}_2}$  and soil OM. Moreover, a very weak negative correlation was found between potential  $\text{NH}_3$  volatilization and soil clay content. The negative correlation observed between soil pH and potential  $\text{NH}_3$  volatilization is the opposite of what was expected. This is likely attributed to the fact that the soils expected to have a high  $\text{NH}_3$  volatilization

due to their alkaline pH also had a high content of clay, which sorbs  $\text{NH}_4^+$ . Even though Alkaline clay soil had the highest pH, Acidic clay soil and Alkaline clay soil overall had the lowest  $\text{NH}_3$  volatilization potentials. Alkaline clay soil had, though, by far the highest CEC and clay content, which must have counteracted the effect of the high pH on the potential  $\text{NH}_3$  volatilization. However, the hypothesis that high OM and high clay content decrease the potential  $\text{NH}_3$  volatilization can be confirmed.

The results of this study indicate that it is important to consider the potential interactions of soil characteristics that impact  $\text{NH}_3$  volatilization when assessing the impact of the soil on potential  $\text{NH}_3$  volatilization. This viewpoint is in agreement with Zhenghu and Honglang (2000), who stress that no single soil property alone can explain  $\text{NH}_3$  volatilization. However, it should be emphasized that only five soils were included in this study. Thus, results on the impact of the soil type are not comprehensive, as only a small fraction of variation in soil types was considered here. Despite the incomplete set of soil types included in this study, the effect of the soil type was consistent between BBFs, with the same soils resulting in the highest and lowest  $\text{NH}_3$  volatilization potentials, although the magnitude of the difference between soils differed between the different BBFs.

#### 4.3.2 Soil moisture

Mkhabela et al. (2006) found that  $\text{NH}_3$  volatilization increased with increasing water filled pore space (WFPS) (50, 70, and 90 % WFPS) from hog slurry applied to a fine loam soil in a laboratory study. However, this was not the case for all BBFs in this study. Only AV4 and partly HDG showed a tendency to increase  $\text{NH}_3$  volatilization with increasing soil moisture level (Experiment 5, Fig. I-5). This study's finding that there is no clear relationship between  $\text{NH}_3$  volatilization potential and soil moisture level is, however, in agreement with other previous studies conducted by Akiyama et al. (2004) and Liao et al. (2019). Liao et al. (2019) found soil moisture to have relatively little impact on  $\text{NH}_3$  volatilization, using a dynamic flow-through setup. Akiyama et al. (2004) found no significant effect of different WFPS (40, 60, and 80 %) levels on  $\text{NH}_3$  volatilization on a sandy loam soil, using a flow-through chamber technique.

## 5 Conclusions

The 39 novel BBFs surface applied to sand varied immensely in potential  $\text{NH}_3$  volatilization during the 27-day incubation period. CMC and PFC grouping systems for BBFs could not adequately explain the differences in potential  $\text{NH}_3$  volatilization from BBFs. However, a grouping of BBFs based on more traditional concepts was markedly better in explaining differences in potential  $\text{NH}_3$  volatilization. The highest accumulated  $\text{NH}_3$  volatilization potential was found for digestates (48 % of applied N volatilized) while the lowest was found for composts and struvites (<1 % of applied N volatilized). Poultry manure, animal by-products, plant-based, and mixed source BBFs had intermediate accumulated  $\text{NH}_3$  volatilization potentials in a similar range (12 % to 20 % of applied N). The temporal pattern of potential  $\text{NH}_3$  volatilization also varied greatly between BBFs, with most BBFs showing a delayed response in  $\text{NH}_3$  volatilization. The initial BBF characteristics measured in this study and their interaction with time explained 89 % of the variation in accumulated potential  $\text{NH}_3$  volatilization from the 39 novel BBFs surface applied to sand. Potential  $\text{NH}_3$  volatilization was affected significantly by soil type, with highest  $\text{NH}_3$  volatilization potential for the sandy soil (acidic) and lowest for the two clayey soils (acidic and alkaline). The incorporation of BBFs in soil could effectively reduce potential  $\text{NH}_3$  volatilization. However, when incorporation is not an option, e.g. when applying BBFs in growing crops,  $\text{NH}_3$  volatilization may pose a significant risk of yield loss and environmental impact for certain BBFs. It should be emphasized that under field conditions where crops will take up some of the  $\text{NH}_4^+$ , and the gas gradient of  $\text{NH}_3$  and  $\text{CO}_2$  might be less steep,  $\text{NH}_3$  volatilization might not be as severe as in this laboratory incubation where conditions favoring  $\text{NH}_3$  volatilization were applied.

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## Author contributions

**Lærke Wester-Larsen:** Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. **Dorette Sophie Müller-Stöver:** Conceptualization; Methodology; Resources; Supervision; Writing - review & editing. **Tapio Salo:** Data curation; Formal analysis; Methodology; Resources; Validation; Writing - review & editing. **Lars Stoumann Jensen:** Conceptualization; Funding acquisition; Methodology; Project administration; Resources; Supervision; Writing - review & editing.

## Declaration of competing interest

The authors declare that they have no known competing interests, neither financially nor personally.

## Data availability

All data is openly available online in ERDA, the repository of the University of Copenhagen: <https://doi.org/10.17894/ucph.65b73d07-af5f-42f3-9d9e-d6aeab183224>



## Study II

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Supplementary material can be found in appendix II

### **Agronomic performance of novel biobased fertilizers across European field trial sites**

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#### **Highlights**

- Field trials testing 18 biobased fertilizers (BBF) at 4 sites across Europe for 2 years
- Generally similar yield with BBF as mineral reference at same total N application
- Overall average first-year BBF mineral nitrogen fertilizer replacement value of 71%
- Second-year residual effect of BBF detected, but not higher than mineral reference
- No significant differences in BBF performance between the four sites

## Abstract

**Context or problem:** Substituting mineral fertilizers with novel biobased fertilizers (BBFs) produced from various organic waste and side streams could contribute to a reduction in the environmental and climate impacts of fertilizer production and use and the recycling of otherwise potentially wasted nutrients. For the substitution to be beneficial for farmers, the environment, and food security, the BBFs need to be effective, reliable and safe. However, the agronomic performance of novel BBFs has not yet been well studied.

**Objectives or research question:** The main objective of this study was to determine the agronomic efficiency of a relevant range of commercially available BBFs. We hypothesized that they can function as effective substitutes for mineral N fertilizers, independent of agricultural and geographic setting.

**Methods:** The agronomic performance (crop yield and N offtake, first and second year) of seven commercial BBFs was tested for two years at four field sites across Europe covering different climates, soil types and crop rotations. Seven common BBFs were tested at all sites, with an additional two or three local BBFs at individual sites.

**Results:** The BBFs showed an average nitrogen fertilizer replacement value (NFRV) of 71% across sites and years, with variations in the agronomic performance between the trial sites and years. Compared with the mineral N fertilizer reference applied at the same total N level, no consistent ranking of BBF and no significant differences in yields were found. The BBFs tended to have a higher NFRV when incorporated compared to surface application. Of the 18 BBFs tested, 8 had a NFVR above 75%, 6 were in the range 60-75% and 4 were in the low range of 10-60%. The residual effect of BBFs in the year after application was not significantly higher for any of the BBFs than that of the mineral N fertilizer.

**Conclusions:** Generally, the BBFs performed similar to the mineral reference applied at the same total N level. The performance of BBFs was not significantly affected by climate or soil type. The BBFs appeared to have higher agronomic performance when incorporated into soil compared to surface application. The second year residual effect of BBF was not significantly higher than that of the mineral reference fertilizer.

**Implications or significance:** In general, most of the investigated BBFs can be considered suitable substitutes for mineral N fertilizers. The results suggest that soil incorporation of BBFs will result in better agronomic performance than surface application.

**Keywords:** Residual fertilizer effect, Agronomic efficiency, Nitrogen fertilizer replacement value, Nitrogen use efficiency, Crop yield.

# 1 Introduction

The United Nations project that the earth's population will grow to 9.7 billion by 2050 (United Nations, 2022). To feed the increasing human population, future food production needs to be more efficient and less harmful to the environment. This can be achieved by changing to more plant-based diets, expanding cultivated areas or increasing the productivity of existing agricultural land, e.g., by applying higher amounts of fertilizers (Tilman et al., 2011). Since the invention of the Haber-Bosch technology to produce mineral N fertilizers, use of and dependency on mineral fertilizers has increased, as have crop yields. However, the production of mineral N fertilizers through Haber-Bosch synthesis is very energy-intensive (Sigurnjak et al., 2017; Svanbäck et al., 2019), resulting in a high climate impact as the energy sources used are mainly based on fossil fuels. Moreover, current highly fluctuating energy (gas) prices have led to increased price volatility for mineral N fertilizers (Schnitkey et al., 2023).

One way to substitute mineral N fertilizers could be to reuse otherwise poorly utilized organic materials to produce biobased fertilizers (BBFs). BBFs have been defined by (Wester-Larsen et al., 2022) as “materials or products derived from biomaterials (plant, animal, or microbial origin, often wastes, residues, or side-streams from agriculture, industry, or society) with a content of bioavailable plant nutrients suitable to serve as a fertilizer for crops”. The recovery of nutrients from organic wastes is a central part of the EC bioeconomy strategy. As part of the transition towards optimal use of resources, the European Union aims to make greater use of recycled organic resources to satisfy consumer and industrial demands, thus mitigating climate change and combating the depletion of non-renewable rock phosphate. The European Commission’s “Europe 2020” strategy to develop a sustainable low-carbon economy by 2050 (EC 2011) regards the substitution of mineral fertilizers in agricultural production with BBFs an important pathway for the recovery of materials and energy (Christel et al., 2014).

Due to their novelty, little is known about the fertilizing efficiency of novel BBFs and their potential to replace Haber-Bosch synthesized mineral fertilizers (Sigurnjak et al., 2019; Vaneckhaute et al., 2014). Biobased fertilizers with high organic N contents could be used as single-application N fertilizers. Due to their organic composition, the release of nitrogen depends on microbial decomposition, which is affected by soil moisture and soil temperature (Abbott et al., 2018). Thus, BBFs may act as slow-release fertilizers, providing adequate nutrients for the crop during the growing season. However, if the nutrients do not become available until after the crop has stopped taking up nutrients, this will lead to a reduction in yield and potential loss of these nutrients, if no other crop is there to take them up. If this delay in nutrient availability is sufficiently long, the nutrients applied with BBFs can be available for subsequent crops, i.e., BBFs could have a residual fertilizing value. Moreover, variations in BBF fertilizer efficiency are to be expected between European sites with varying climates and soil types. With the hitherto inconsistent European legislative systems regulating BBFs (Vaneckhaute et al., 2014), the new EU fertilizing product regulation (EU, 2019) aims to make BBFs enter the European fertilizer market on equal terms with mineral fertilizers. Therefore, it is necessary to investigate novel BBFs in different European settings to generate knowledge on the efficiency of BBFs and their potential to replace mineral fertilizers.

The overall objective of this study was to determine the agronomic efficiency of a relevant range of commercially available BBFs. The specific objectives were to: i) determine their mineral N fertilizer replacement value; ii) examine the impact of agricultural (crop) and geographic (soil type and climate) setting on their agronomic efficiencies; and iii) determine their second-year residual fertilizing effect.

The following hypotheses were tested: i) BBFs can function as substitutes for mineral N fertilizers; ii) BBFs have a second-year residual fertilizing effect significantly above that of mineral N fertilizers; and iii) the agronomic efficiency of individual BBFs varies with agricultural and geographic setting.

## 2 Materials and methods

### 2.1 Sites and soils

Field experiments were conducted at five sites across a climate gradient in Europe from Spain (Seville) and France (Toulouse) in the south, Germany (Stuttgart) in the centre, to Denmark (Copenhagen) and Finland (Forssa) in the north (Table II-1). Thus, covering Mediterranean (Spain), Atlantic (France), Continental (Germany and Denmark) and Boreal (Finland) biogeographical regions (EEA, 2017). The trials in Spain however failed in both years due to drought and data is therefore not reported in this paper (see 2.4.4), why soil data for Spain is omitted from Table II-1. To ensure a response to N fertilization, sites were selected based on certain criteria: no organic fertilizer application five years prior to the start of the experiment and no N-rich (leguminous) crops or cover crops included in the previous crop rotation. Soil samples were collected prior to the start of the experiment to determine soil properties (Table II-1).

Table II-1: Coordinates and soil properties of the four sites (France, Germany, Denmark and Finland). See supplementary material for description of methods used for soil analysis.

	<b>France</b>	<b>Germany</b>	<b>Denmark</b>	<b>Finland</b>
Latitude N	43.492826	48.715024	55.674204	60.804564
Longitude E	1.202851	9.2144937	12.287907	23.454594
WRB soil type	Luvisol	Haplic Luvisol	Arenosol	Vertic Cambisol
Clay % (<0.002 mm)	17	30	6.2	45
Silt % (0.002-0.02 mm)	26	68	5.5	25
Sand % (>0.02 mm)	57	2	85.3	30
Organic C (g kg <sup>-1</sup> )	9.2	14	30	26
pH H <sub>2</sub> O	7.38	6.99	6.01	6.60
Olsen P (mg kg <sup>-1</sup> )	23	-	34	72
CAL-P (mg kg <sup>-1</sup> )	-	15.5	-	-
Exchangeable K (mg kg <sup>-1</sup> )	128	-	103	208
CAL-K (mg kg <sup>-1</sup> )	-	20	-	-

## 2.2 Weather data

Monthly average temperature and monthly sum of precipitation were obtained from the nearest weather station for each site for the two experimental years 2021 (Fig. II-1A) and 2022 (Fig. II-1B). Climate norms (average of last 30 years) were acquired from climate-data.org and values were subtracted from the 2021 and 2022 values to display the monthly deviations from the climate norm for each site for these years (Fig. II-1C and D).

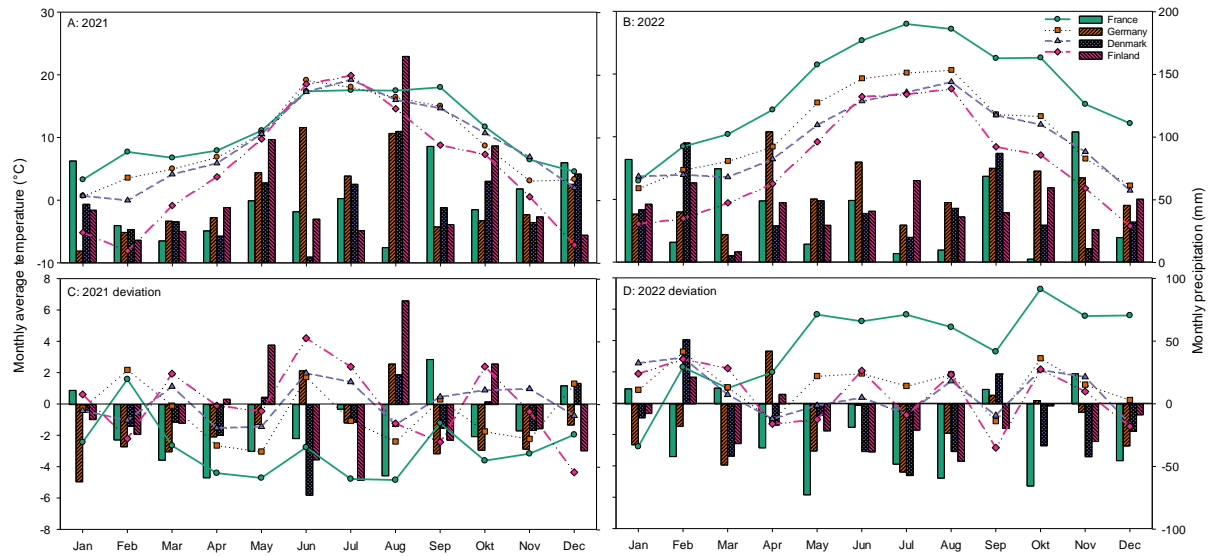


Fig. II-1: Weather data from weather stations at the experimental field sites (France, Germany, Denmark, and Finland) for 2021 and 2022 (A and B). C and D show the deviation of the 2021 and 2022 weather data from the long-term mean at the respective location (France: Toulouse 1991-2021, Germany: Stuttgart 1999-2019, Denmark: Taastrup 1999-2019, and Finland: Forssa 1991-2021) (climate-data.org). Negative values demonstrate colder or drier months compared to the climate norm, positive values demonstrate warmer or wetter months compared to the climate norm. The monthly average temperature (°C) is displayed as points (left axis) and the sum of monthly precipitation (mm) as columns (right axis).

## 2.3 Biobased fertilizers

All the BBFs investigated in the current study were commercially available on European or regional/national markets at the time the experiments were conducted (Table II-2). Seven of the BBFs tested in this study were available across Europe as pellets or granules and tested in each trial site. These seven “common” BBFs were taken from a list of 39 (Wester-Larsen et al., 2022) and selected to represent the variation in BBFs on the list in terms of source materials and processing technology. Another selection criteria was that they had a high technology readiness level, were available in sufficiently large quantities in Europe (Bauerle et al, 2021). Partners at each site additionally selected two to three BBFs that were of local (national, regional) relevance and interest in terms of either traditional fertilizer distribution logistics, availability on regional markets or their physical form (e.g. liquid/moist) impeding long distance transport. Because the BBFs ranged from liquid, semi-liquid to pelletized BBFs the fertilizer application technique was also correspondingly different.

Table II-2: Biobased fertilizer (BBF) component(s) and properties; pH (1:5 in Milli-Q water), total N,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , dry matter (DM) content, total C, and C:N ratio. N and C are reported as % of fresh weight (FW). Values are means,  $n=2$ , except for C and N of liquid BBFs where  $n=5$  and pH where  $n=3$ . The BBFs are presented here by acronym, with local BBFs in italics. Full product names and list of manufacturers can be found in the supplementary material (Table II-S1). The data is derived from Wester-Larsen et al. (2022).

	BBF	Component(s)	pH	N (% of FW)	NH <sub>4</sub> <sup>+</sup> -N (% of total N)	NO <sub>3</sub> <sup>-</sup> -N (% of total N)	DM (g kg <sup>-1</sup> of FW)	C (% of FW)	C/N
Common	BA6	Fermented and distilled plant-based residues	4.85	5.57	1.4	<0.1	907	43.6	7.83
	BIO	Pelletized meat and bone meal, apatite, vinasse, poultry manure, and potassium sulfate	5.69	7.39	3.5	<0.1	941	35.9	4.85
	ECO	Pelletized blood and feather meal	5.48	11.6	2.7	<0.1	892	45.3	3.89
	FEK	Pelletized poultry manure	6.43	3.94	20	<0.1	901	34.7	8.81
	MO13	Pelletized feather meal	5.07	14.2	1.0	<0.1	927	49.0	3.45
	OG2	Hydrolysed horn meal (pig bristles)	5.29	13.9	1.0	<0.1	940	48.3	3.47
	PAL	Fermented biochar and high-quality clay and rock flour	5.55	4.89	19	0.1	907	38.8	7.92
France	FR13	Pelletized sterilized feather and pig bristle powders	5.74	11.9	3.45	<0.1	901	45.3	3.8
	FR8	Pelletized sterilized animal proteins, bone powders, feather and pig bristle powders	6.30	7.67	3.17	<0.1	946	34.2	4.5
Germany	ASL *	Product of nutrient recovery plant. Ammonium sulfate (liquid)	7.42	4.76	100	<0.1	216	0.11	0.02
	GRF	Anaerobically digested manure and crops	8.19	0.37	75	0.3	45	2.02	5.46
	MAL	Pelletized malt germ and minerals, and vinasse	5.02	4.37	28	<0.1	955	35.0	8.02
Denmark	BVC	Anaerobically digested and composted municipal organic food waste	8.56	1.57	8.1	<0.1	557	14.7	9.37
	PCW	Potato cell water	4.70	1.53	15	1.1	339	11.2	7.29
	SDG	Anaerobically digested agro and food waste + seaweed	8.40	0.41	68	<0.1	38	1.08	2.63
Finland	AV8	Pelletized poultry manure, blood meal, and potassium sulfate	6.51	7.24	8.4	<0.1	920	38.9	5.38
	BO4	Anaerobically digested vinasse	6.23	3.56	1.3	0.6	620	21.2	5.96
	FEL	Pelletized poultry manure	6.70	4.51	3.3	<0.1	925	36.0	7.98

\*ASL was applied in two different ways; ASL1 same as all other BBF, ASL2 was directly injected in the soil in both years.

## 2.4 Trial design

The field trials were established at each site using a complete randomized block design, except for Finland where a row-column design was used. Individual plot sizes varied from 30 to 150 m<sup>2</sup> among the sites.

The trials were conducted during two cropping seasons (2021 and 2022) as two separate one-year trials. In 2021, spring crops were grown at all sites (Table II-3). In 2022, winter crops were grown at all sites,

except in Finland (spring crop) where spring cereals are more common due to climatic limitations for winter types. BBFs were incorporated for spring-sown crops and surface-applied in spring in the growing crop for autumn-sown crops. The first-year fertilization effect was evaluated for 2021 in one area of the field, and in 2022 in another area adjacent to the first area, except in Finland where the two trials were conducted in different fields 600 m apart. In 2022, the residual effect of the 2021 fertilization was determined in the area fertilized in 2021, without additional fertilization in 2022. Additionally, at the German site in 2023, the residual fertilization effect of the 2022 fertilization was determined in the area fertilized in 2022, without additional fertilization in 2023.

The trials were designed and run in accordance with a commonly agreed and detailed field trial protocol, but with local fertilizing recommendations and common cropping practices of each country and therefore trial design and plot sizes varied slightly among the trial sites. Table II-3 lists the crops grown, the total BBF N quantity applied, the application method used, and the mineral N reference application levels for all trial sites and years.

#### *2.4.1 Fertilizer management*

The BBFs were applied based on total BBF N content at the level recommended by local advisory services and based on experience for the specific crops each year (Table II-3). The fertilizers were all applied on the same day, or in two days, in the spring prior to sowing for the spring-sown crops and at the onset of crop spring growth for autumn-sown crops. The BBFs were applied in a manner as close to common farming practice as possible, e.g., in strips simulating a trailing hose for liquid BBFs and broadcast applied for solid pelletized BBFs. For spring-sown crops, BBFs were incorporated to a depth of 10 cm within 24 hours of application, shortly before sowing of the crop. For autumn-sown crops, BBFs were surface-applied in the spring in the growing crop. The sites were fertilized with sufficient amounts of all nutrients other than N, according to local common practice, to ensure that only N was growth-limiting.

Mineral N fertilizer (ammonium-nitrate based) was applied at four to five gradually ascending levels with the lowest at zero and the highest slightly above the level at which the BBFs were applied (Table II-3). Exceptions to this rule were made in France and Finland, where the second-highest reference received the same amount of N as the BBFs (at all other sites, it was lower). In Finland, another exception was a 6<sup>th</sup> reference (Fig. II-S1), which was used for fitting equations for the references (2.5) but left out for further evaluations made in this study for comparability reasons. In Germany, it is common practice to take the initially measured soil mineral N content into account when calculating the fertilizer application amount; therefore this was done for the German site, but not for any of the other sites.

The BBFs were applied on different dates depending on the site and year (Table II-3). At the Danish site, the application of the mineral references was split into two doses for winter wheat in 2022, as this is common practice. The second dose of mineral references was applied on 2 May 2022. However, the BBFs were all applied at one time and in one dose. At the French site, the application of the mineral references was split into three doses for winter wheat in 2022, as this is common practice.

Table II-3: Type of mineral fertilizer used as reference at the four sites. Total N applied for BBFs and mineral references in the categorized reference groups (Ref\_1 – Ref\_5) for each site and crop. The second lowest references at the Finnish site (30 kg N ha<sup>-1</sup> in 2021 and 25 kg N ha<sup>-1</sup> in 2022) were left out of statistical analysis and are not shown in the table. Application method used and crops grown at each site and year.

Year	2021				2022			
Trial site	France	Germany	Denmark	Finland	France	Germany	Denmark	Finland
Crop	Corn maize	Silage maize	Spring barley	Spring barley	Winter wheat	Winter wheat	Winter wheat	Spring wheat <sup>¶</sup>
Mineral Reference	AN †	CAN ‡	AN §	AN *	AN †	CAN ‡	AN §	AN *
Applic. Method	Incor-porated	Incor-porated	Incor-porated	Incor-porated	Surface applied	Surface applied	Surface applied	Incor-porated
Date of BBF application	6/4	26/3-28/3	29/3	24/5-25/5	7/2	1/3-2/3	30/3	19/5
BBF application (kg N ha <sup>-1</sup> )	<b>200</b>	<b>116</b>	<b>137</b>	<b>90</b>	<b>200</b>	<b>160</b>	<b>181</b>	<b>100</b>
Ref_1 (kg N ha <sup>-1</sup> )	0	0	0	0	0	0	0	0
Ref_2 (kg N ha <sup>-1</sup> )	-	33	-	30	-	45	-	50
Ref_3 (kg N ha <sup>-1</sup> )	100	66	50	70	75	90	70	75
Ref_4 (kg N ha <sup>-1</sup> )	200	99	100	90	150	135	140	100
Ref_5 (kg N ha <sup>-1</sup> )	250	132	150	110	225	180	210	125

¶ AN: Ammonium nitrate; CAN: Calcium ammonium nitrate. †16.8% NH<sub>4</sub><sup>+</sup>-N, 16.7% NO<sub>3</sub><sup>-</sup>-N; ‡13.5% NH<sub>4</sub><sup>+</sup>-N, 13.5% NO<sub>3</sub><sup>-</sup>-N, 12% CaO, 4% MgO; § 13.5% NO<sub>3</sub><sup>-</sup>-N, 13.5% NH<sub>4</sub><sup>+</sup>-N, 3.7% S, 0.6% MgO; \* 14.6% NH<sub>4</sub><sup>+</sup>-N, 12.2% NO<sub>3</sub><sup>-</sup>-N, 1% K<sub>2</sub>O, 4% S.

#### 2.4.2 Crop management

Crops were mainly harvested using an experimental combine harvester on a representative area varying in size from 15-25 m<sup>2</sup> (depending on site) for each treatment. At the German site, maize (2021) and wheat straw (2022) were harvested by hand while wheat grain yield was determined using a combine. The dry matter of grain and straw was determined by drying the samples at max 65 °C for three days. Grain yield at 15% moisture was calculated. The total N and C contents of straw and grain, or whole crop (silage maize), samples were determined using an elemental analyser (France, Germany and Denmark). At the Finnish site, grain N was determined using near infrared spectroscopy calibrated with Kjeldahl N measurements and with Kjeldahl N measurements for straw N content. The crop N offtake was calculated as the sum of the grain and the straw N content (dry matter x N concentration) per ha.

#### 2.4.3 Site-specific adaptations to external effects

At the Spanish site, extreme drought led to failure of the experiment in both 2021 and 2022, since irrigation was not available at the site and not common practice in the area. Therefore, the data was not included in this study. At the Finnish site in 2021, heavy rain immediately after sowing and a following one-month drought period led to the failure of seed emergence and to total yield loss. Therefore, only the first-year fertilization effect of 2022 and the residual fertilization effect of the 2021 trial were included in this study for this site. However, no data for straw N offtake was collected for the second-year residual fertilization trial for the Finnish site. Due to transportation issues during the COVID-19 pandemic, the BBF 'PAL' arrived too late to be included in the Danish trial in 2021. It was therefore



only included in the 2022 trial here. At the German site, fertilization with ‘GRF’ in 2021 was unsuccessful due to technical problems and this treatment was thus excluded in the first year. At the French site, the fields were irrigated both years.

## 2.5 Calculations of mineral fertilizer equivalent values

The grain yield (DM), or aboveground biomass for silage maize, of the mineral references was plotted as a function of the application rate and linear equations of 2<sup>nd</sup>-order and 3<sup>rd</sup>-order were fitted to the points. The R<sup>2</sup> values of the fitted equations were assessed and the equation with the highest R<sup>2</sup> value was chosen. In all cases, a 2<sup>nd</sup>-order equation was chosen (Table II-S3). The equation was then used to calculate the yield at the level of BBF N application. Then the mean grain yield (DM) of the unfertilized reference (REF1) and the grain yield (DM) for the mineral reference at the BBF application level (REF) were plotted and the slope of the linear regression line between these points was used as the agronomic efficiency (AE) of the mineral reference. The AE of each BBF replicate was calculated using Equation 1.

$$\text{Eq1: } \text{BBF}_{\text{AE}} = \frac{\text{Yield}_{\text{BBF}_x} - \text{Yield}_{\text{REF}_1}}{\text{N applied}_{\text{BBF}_x}}$$

Subsequently, the N fertilizer replacement value (NFRV) based on AE was calculated using Equation 2:

$$\text{Eq2: } \text{BBF}_x \text{ NFRV}_{\text{AE}} (\%) = \left( \frac{\text{AE}_{\text{BBF}_x}}{\text{AE}_{\text{REF}}} \right) * (100)$$

Likewise, the calculation of NFRV based on N use efficiency (NUE) was performed in the same way as for NFRV<sub>AE</sub> where the crop N offtake (grain and straw) was used instead of grain yield and linear equations were fitted to the mineral N references. The equations used, and R<sup>2</sup> values are provided in the supplementary material (Tables S2 and S3).

## 2.5 Statistics

Data were split into trials fertilized in the current year and trials fertilized in the year prior to data sampling (residual value). In both cases, data were analyzed using a two-stage analysis, where means and their standard error were estimated per location and year first and then subjected to the across-location and across-year analysis in the second stage (Möhring and Piepho et al., 2009). In the first stage, trials were analyzed according to the randomized complete block design used in the trials in Denmark, Germany, and France by:

$$(1) \ y_{ij} = \mu + \tau_i + \varphi_j + e_{ij},$$

where  $y_{ij}$  is the observation of fertilization treatment  $i$  in block  $j$ ,  $\mu$  is the intercept,  $\tau_i$  is the fixed effect of treatment  $i$ ,  $\varphi_j$  is the random effect of block  $j$ , and  $e_{ij}$  is the confounded effect of plot effect and error of  $y_{ij}$ . In Finland, a non-resolvable row-column design was used, therefore data were analyzed by:

$$(2) \ y_{ijk} = \mu + \tau_i + \varphi_j + f_k + e_{ijk},$$

where  $y_{ijk}$  is the observation of fertilization treatment  $i$  in block  $j$  and column  $k$ ,  $\mu$  is the intercept,  $\tau_i$  is the fixed effect of treatment  $i$ ,  $\varphi_j$  is the random effect of block  $j$ ,  $f_k$  is the random effect of column  $k$ , and  $e_{ijk}$  is the compounded effect of plot effect and error of  $y_{ijk}$ . Afterwards, treatment means  $\bar{y}_i$  of location  $m$  in year  $l$  were used in the second-stage model given by:

$$(3) \ \bar{y}_{ilm} = \mu + \tau_i + a_l + l_m + (al)_{lm} + (\tau a)_{il} + (\tau l)_{im} + (\tau al)_{ilm} + f_{ilm}$$

where  $y_{ilm}$  is the observation of treatment  $i$  at the  $m$ th trial site of year  $l$ ,  $\mu$  is the intercept,  $\tau_i$  is the fixed effect of treatment  $i$ ,  $l_m$  is the fixed effect of location  $m$ ,  $a_l$  is the fixed effect of year  $l$ ,  $(al)_{lm}$  is the random effect of the trial in location  $m$  and year  $l$ ,  $(\tau l)_{im}$  is the fixed interaction effect of treatment  $i$  and location  $m$ ,  $(\tau a)_{il}$  is the random interaction effect of treatment  $i$  and year  $l$ ,  $(\tau al)_{ilm}$  is the random

interaction effect of treatment  $i$  with year  $l$  and location  $m$ , and  $f_{ilm}$  is the approximated error of mean  $\bar{y}_{ilm}$ . The errors of  $\bar{y}_{ilm}$  were approximated with the diagonal element of the R matrix fitted in stage I. The model was extended by accounting for the application method. Note that all trials in 2021 used the application method ‘incorporated’, while in 2022 fertilizers were surface applied in France, Germany, and Denmark, but again incorporated in Finland (Table II-3). Therefore, application methods and year can be separated, but their interactions with locations were confounded. Arbitrary, year-by-location and year-by-location-by-treatment effects were fitted, but the effects estimate the confounded effect. Note further that crop and year effects were also confounded. Again, year effects and their interaction effects were completely confounded with the corresponding crop effects.

Pre-requirements for analysis (normal distribution and homogeneous variances of residuals) were checked graphically via residual plots. Where significant differences were found for one term, corresponding least square means were compared using Fisher’s LSD test. A letter display was used for description of results of multiple comparisons. For the statistical analysis, the program SAS 9.4 TS Level 1M7 was used. For graphical work, SigmaPlot 14.0, Systat, was used.

## 3 Results

### 3.1 Agronomic performance of novel BBFs

#### 3.1.1 First-year effect of BBFs

Fertilization with BBFs and mineral fertilizers had a significant impact on yield at all sites in both years (Table II-4). In 2021, fertilization with BBFs and mineral fertilizers had no significant effect on N offtake in Germany but did affect N offtake significantly in France and Denmark (Table II-S4). In 2022, fertilization had a significant effect on N offtake at all sites (Table II-S4).

In the combined analysis of all trials, fertilization was close to being a significant factor ( $p=0.0509$ ) for yield (means are shown in (Table II-S7)). The factors of trial site, year, and interaction of trial site and treatment were not significant. In the analysis of crop N offtake, the fertilization treatment was significant in the combined analysis of all trials. Here, the two highest mineral references differed significantly from the lowest (0), but not from the majority of the BBFs, which also did not differ significantly from each other (Table II-S7).

At the French site, all BBFs had yields similar to that of the highest mineral reference in both 2021 and 2022 (Table II-4 and Fig. II-2). At the German site, all BBFs except ECO had yields similar to the two highest references in 2021. However, the yields for BA6, ECO, FEK, and MO13 did not differ significantly from the unfertilized reference, which had a relatively high yield and hence a relative small response to increasing mineral N input (Fig. II-S1). In 2022, all BBFs had significantly lower yields compared to the two highest references at this site, but significantly higher yields than the unfertilized reference. At the Danish site, all of the BBFs except BVC resulted in yields similar to the highest reference in 2021. In 2022 however, all BBFs except FEK, PAL, PCW, and SDG had significantly lower yields than the highest reference. By contrast, compared with the second-highest reference, only BIO and BVC had significantly lower yields, all others had yields similar to the second-highest reference. At the Finnish site, only BA6, OG2, and AV8 had a significantly lower yield compared to the second-highest reference in 2022. However, only PAL had a similar yield to the highest reference. All BBFs had a significantly higher yield than the unfertilized reference. Data for the yield of the mineral fertilizer references for both years and all sites can be found in the supplementary material (Fig. II-S1).

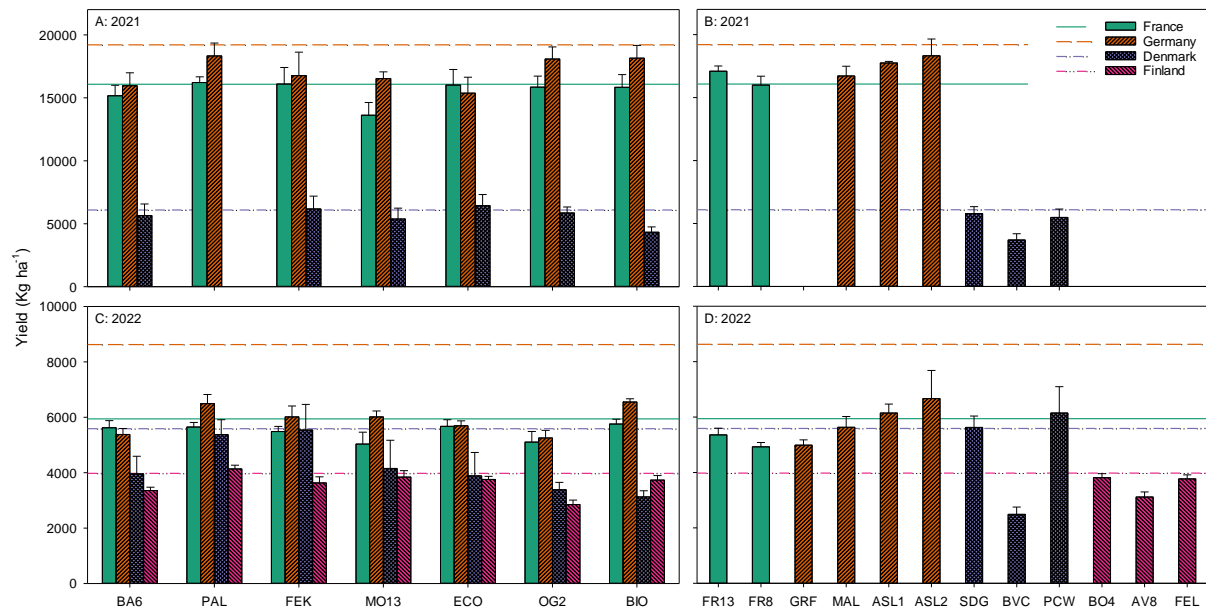


Fig. II-2: Means  $\pm$  standard error of recorded yield (kg ha<sup>-1</sup>) of silage maize (Germany 2021) and grain yield (kg ha<sup>-1</sup>) of grain maize (France 2021), spring barley (Denmark 2021), winter wheat (France, Germany and Denmark 2022) and spring wheat (Finland 2022). Values are given for dry matter for silage maize and grain maize and at 15% moisture content for all cereal grains. The yields obtained with the seven common BBFs tested at all sites are shown as bars in A (2021) and C (2022). Yields from the local BBFs are shown in B (2021) and D (2022). The horizontal lines indicate the site-specific mean yield of the mineral N reference fertilizers at the same application rate as the BBFs. Application rates differed between sites and years.  $n = 4$ . Note different scale on the vertical axis for the two years.

Table II-4: ANOVA of first-year fertilization treatments followed by Fisher's LSD test separately for each site and each year (2021 and 2022). LSD means of grain yields estimated from the model (kg ha<sup>-1</sup>) (grain maize, spring barley, winter wheat, and spring wheat), whole-crop yield (silage maize), and p-values of tests. Means followed by a common letter are not significantly different in the LSD test at the 5% level of significance. Local BBFs are displayed in italics. "SE" is the modelled pooled standard error for all treatments. "REF\_1-5" are mineral references with ascending N application.

	France (p-value: <0.0001)				Germany (p-value: <0.0001)				Denmark (p-value: 0.0002)			
	Yield <sup>¶</sup>		LSD		Yield <sup>¶</sup>		LSD		Yield <sup>†</sup>		LSD	
Fertilization in 2021	SE	916			SE	1078			SE	747		
	BA6	15152	a b		BA6	15964	b c d e		BA6	5651	a b c	
	BIO	15835	a b		BIO	18153	a b c		BIO	4327	a b c d	
	ECO	16010	a b		ECO	15372	d e		ECO	6446	a	
	FEK	16093	a b		FEK	16765	c d e		FEK	6174	a	
	MO13	13620	a		MO13	16528	c d e		MO13	5375	a b c d	
	OG2	15847	a b		OG2	18081	a b c		OG2	5854	a b	
	PAL	16211	a b		PAL	18329	a b c		PAL	.		
	<i>FR13</i>	17092	a		<i>ASL1</i>	17748	a b c d		<i>BVC</i>	3694	c d	
	<i>FR8</i>	15992	a b		<i>ASL2</i>	18319	a b c		<i>PCW</i>	5487	a b c	
					<i>GRF</i>	.			<i>SDG</i>	5792	a b c	
					<i>MAL</i>	16716						
	Ref_1	8400		d	Ref_1	14484	e		Ref_1	3257	d	
	Ref_2	.			Ref_2	17083	a b c d e		Ref_2	.		
Fertilization in 2022	Ref_3	14047	b c		Ref_3	17436	a b c d		Ref_3	3793	b c d	
	Ref_4	16571	a b c		Ref_4	19528	a		Ref_4	5216	a b c d	
	Ref_5	15016	a b		Ref_5	19098	a b c		Ref_5	6449	a	
	France (p-value: <0.0001)				Germany (p-value: 0.0303)				Denmark (p-value: <0.0001)			
	Yield <sup>†</sup>		LSD		Yield <sup>†</sup>		LSD		Yield <sup>†</sup>		LSD	
Fertilization in 2022	SE	263			SE	378			SE	662		
	BA6	5620	a b c d		BA6	5378	e f		BA6	3957	b c d	
	BIO	5755	a b		BIO	6551	c d		BIO	3123	d e	
	ECO	5672	a b c		ECO	5692	d e		ECO	3884	b c d e	
	FEK	5485	a b c d		FEK	6012	d e		FEK	5538	a b	
	MO13	5033	a b c d		MO13	6013	d e		MO13	4154	b c d	
	OG2	5103	b c d		OG2	5254	e f		OG2	3385	c d e	
	PAL	5648	a b c		PAL	6492	c d		PAL	5374	a b	
	<i>FR13</i>	5355	c d		<i>ASL1</i>	6145	d e f		<i>BVC</i>	2487	d e	
	<i>FR8</i>	4924	d		<i>ASL2</i>	6665	c d		<i>PCW</i>	6144	a	
					<i>GRF</i>	4984	e		<i>SDG</i>	5625	a b	
					<i>MAL</i>	5629	d e					
	Ref_1	2776		e	Ref_1	3107	g		Ref_1	2047	e	
	Ref_2	.			Ref_2	5233	e f		Ref_2	.		
	Ref_3	5453	a b c d		Ref_3	7350	b c		Ref_3	5037	a b c	
	Ref_4	5815	a		Ref_4	8317	a b		Ref_4	5030	a b c	
	Ref_5	5551	a b c d		Ref_5	8706	a		Ref_5	6305	a	

<sup>¶</sup>Yield displayed as dry matter, <sup>†</sup>Yield displayed at 15% moisture content.

### 3.1.2 Residual fertilizer effect of BBFs in the second year

The evaluation of the residual fertilizer effect in 2022 showed the lowest yields for the unfertilized 2021 reference (Ref\_1) at all sites (Table II-5) and a clear residual fertilizer response for the trial sites France, Germany, and Denmark of the gradually increasing fertilizer levels of the other references (Fig. II-S1-C). Although the residual effect is clearly evident in the mineral references (Fig. II-S1), the 2021 fertilization (mineral references and BBFs) had no statistically significant effect on 2022 yields obtained at the sites in France, Denmark, or Finland (Fig. II-3, Table II-5), i.e. the residual effects of mineral fertilizer and BBFs are not significantly different when applied at the same N level. However, in Germany, the residual effect of fertilization in 2021 had a significant effect on the winter wheat yield in 2022 (Table II-5). Here, OG2 and both the highest and second-highest mineral references obtained significantly higher yields than the unfertilized reference. Yet no BBFs had higher residual fertilizer effects than the mineral reference applied at the same N level. Additionally, the 2022 fertilization had a significant effect on spring barley yield in 2023 at the German site (Table II-S5). However, none of the treatments in Germany differed from the unfertilized reference in 2023. When comparing the trial sites, there was a tendency for the BBFs to have higher yields than the corresponding mineral reference in the second year at the French and Danish sites (Fig. II-3). However, in Germany, the residual fertilizer effect was lower than that of the corresponding mineral reference.

The fertilization in 2021 had no significant effect on residual N offtake in 2022 at any of the sites (Table II-S6). A comparison of the sites shows that the BBFs in France resulted in comparable high N offtake as the mineral references. In Germany, the N offtake was lower for BBFs than for the corresponding mineral reference.

Table II-5: ANOVA of residual second-year fertilization treatments using Fisher's LSD test separately for each site in 2022 (fertilization in 2021) in winter wheat. LSD means (kg ha<sup>-1</sup>) of grain yields estimated from the model at 15% moisture content and p-values of tests. Means followed by a common letter are not significantly different in the LSD test at the 5% level of significance. Local BBFs are displayed in *italics*. "SE" is the modelled pooled standard error for all treatments. "REF\_1-5" are mineral references with ascending N application.

France (p-value: 0.41)			Germany (p-value: <0.02)			Denmark (p-value: 0.41)			Finland (p-value: 0.19)		
	Yield	LSD		Yield	LSD		Yield	LSD		Yield	LSD
SE	605	-	SE	342		SE	269	-	SE	264	-
BA6	3450	-	BA6	3081	e f	BA6	2404	-	BA6	4282	-
BIO	3188	-	BIO	3263	b c d e f	BIO	2348	-	BIO	4234	-
ECO	3499	-	ECO	3445	b c d e f	ECO	2523	-	ECO	4482	-
FEK	2976	-	FEK	3472	a b c d e f	FEK	2329	-	FEK	3629	-
MO13	4031	-	MO13	3372	b c d e f	MO13	2677	-	MO13	4085	-
OG2	3008	-	OG2	3730	a b	OG2	2542	-	OG2	4215	-
PAL	3832	-	PAL	3256	b c d e f	PAL	.	-	PAL	3546	-
<i>FR13</i>	3288	-	<i>ASL1</i>	3607	a b c d	<i>BVC</i>	1998	-	<i>AV8</i>	4143	-
<i>FR8</i>	3458	-	<i>ASL2</i>	3671	a b c	<i>PCW</i>	2594	-	<i>BO4</i>	3489	-
			<i>GRF</i>	.		<i>SDG</i>	2078	-	<i>FEL</i>	3819	-
			<i>MAL</i>	3508	a b c d e						
Ref_1	2518	-	Ref_1	2979	f	Ref_1	1794	-	Ref_1	3832	-
Ref_2	.	-	Ref_2	3151	d e f	Ref_2	.	-	Ref_2	4018	-
Ref_3	2999	-	Ref_3	3183	c d e f	Ref_3	2032	-	Ref_3	4132	-
Ref_4	3024	-	Ref_4	3556	b c d e	Ref_4	2082	-	Ref_4	4211	-
Ref_5	3393	-	Ref_5	3980	a	Ref_5	2481	-	Ref_5	3845	-

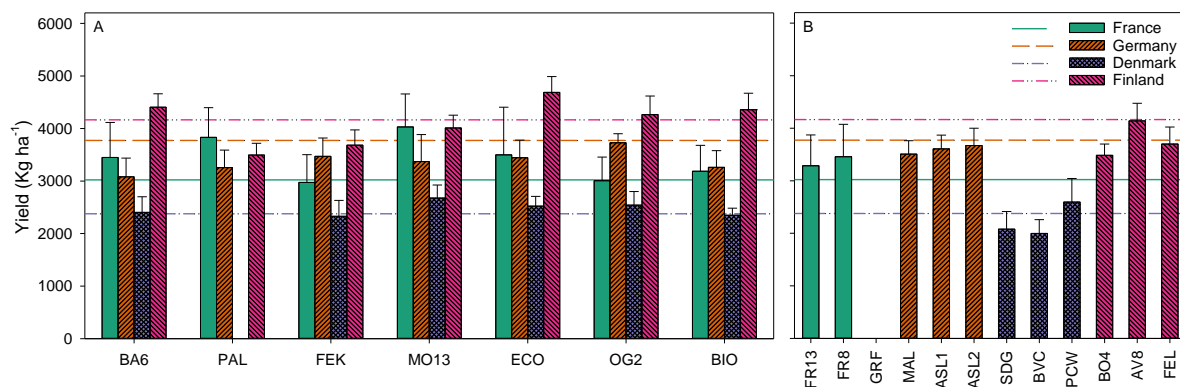


Fig. II-3: Means  $\pm$  standard error of recorded yield (kg ha<sup>-1</sup>) (residuals of 2021) at 15% moisture content for winter wheat (France, Germany and Denmark) and spring wheat (Finland) in 2022. The yields obtained with the seven common BBFs tested at all sites are shown as bars in A. The local BBFs are shown as bars in B. The horizontal lines indicate the mean yield of the mineral N reference fertilizers at the same application rate (2021) as the BBFs. The yields were obtained in 2022 from the plots fertilized in 2021 to measure the second-year residual fertilizer value. Application rates differed between sites.  $n = 4$ .

### 3.2 Nitrogen fertilizer replacement values

In the year of application, the  $\text{NFRV}_{\text{AE}}$  varied greatly from 9% (BVC 2022 Denmark) to 113% (FR13 2021 France) (Fig. II-4). The average  $\text{NFRV}$  across sites and years for all BBFs was higher when based on agronomic efficiency,  $\text{NFRV}_{\text{AE}}$  (71%) compared to when estimated from N use efficiency,  $\text{NFRV}_{\text{NUE}}$  (58%) (data shown in Fig. II-S4). Across sites and years and for all BBFs, the average  $\text{NFRV}_{\text{AE}}$  was higher (76%) when BBFs were incorporated than when surface applied (64%).

Of the 18 BBFs tested (19 different treatments, since ASL was applied in two different ways), nine had high agronomic performances with an  $\text{NFRV}_{\text{AE}}$  above 75% (ranking FR13>BO4>FEL>PAL>PCW>FR8>ASL2>FEK>SDG), six had an intermediate  $\text{NFRV}_{\text{AE}}$  of 60-75% (ranking ECO> ASL1> MO13> OG2>BA6>BIO) and four had a low  $\text{NFRV}_{\text{AE}}$  of 10-60% (ranking BVC>GRF>AV8> MAL).

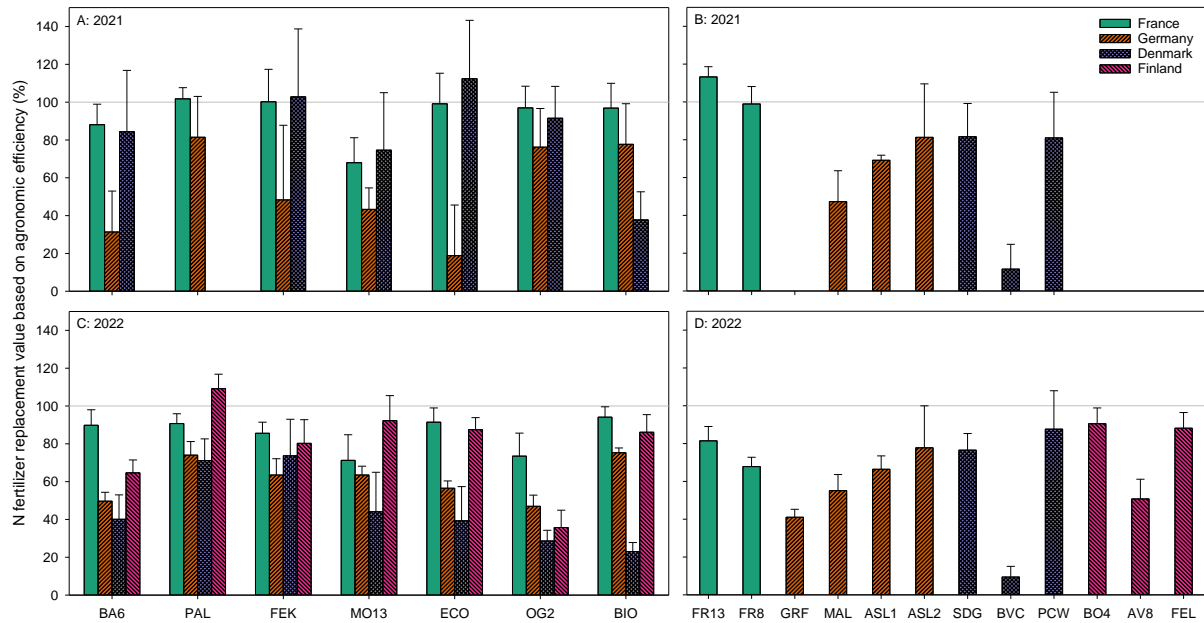


Fig. II-4: Means  $\pm$  standard error of  $\text{NFRV}_{\text{AE}}$  (%) in the year of application. The seven common BBFs tested at all sites are shown as bars in A (2021) and C (2022). The local BBFs are shown in B (2021) and D (2022). The horizontal grey line indicates 100%  $\text{NFRV}_{\text{AE}}$ , i.e. where the BBF performs equally well as the mineral N reference at the same application rate. Crops and application rates differed between sites and years.  $n = 4$ .

In general, the BBFs had a positive residual  $\text{NFRV}_{\text{AE}}$  in the second year after application (Fig. II-5). In France and Denmark, the residual  $\text{NFRV}_{\text{AE}}$  of the BBFs was mainly higher than the mineral N reference applied at the same total N rate, and in the range 17-40%. At the German site however, the values of the residual  $\text{NFRV}_{\text{AE}}$  of the BBFs were all below that of the mineral N reference applied at the same total N rate. In Finland, some of the BBFs have higher, some have lower residual value than the mineral references, two even negative values (but not significantly different from nil).

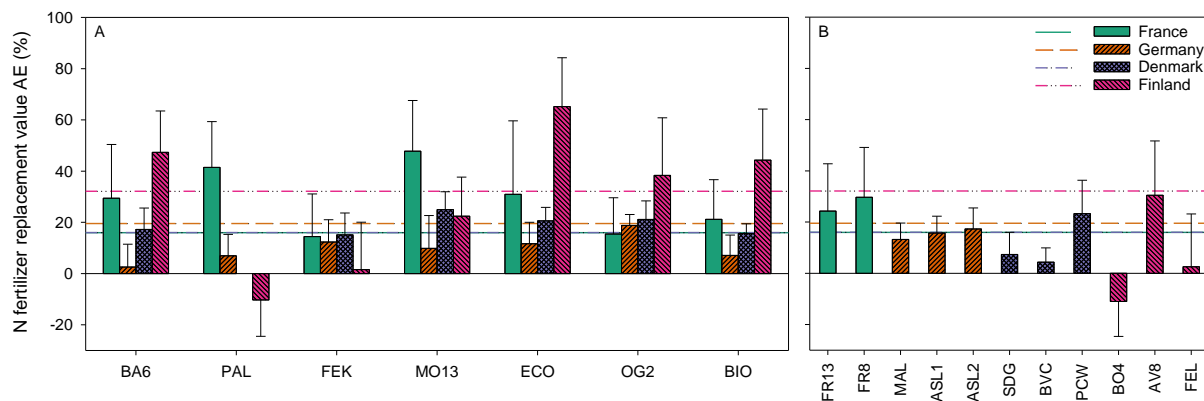


Fig. II-5: Means  $\pm$  standard error of residual (second year)  $\text{NFRV}_{\text{AE}}$  (%) in the year following BBF application. The seven common BBFs tested at all sites are shown in A, the local BBFs in B. The horizontal lines indicate the mean residual (second-year)  $\text{NFRV}_{\text{AE}}$  (%) of the mineral N reference fertilizers at the same application rate (2021) as the BBFs. The crop is winter wheat (France, Germany and Denmark) and spring wheat (Finland) in 2022. Application rates differed between sites.  $n = 4$ .

## 4 Discussion

### 4.1 First-year agronomic performance of BBFs in 2021 and 2022

Overall, BBFs generally resulted in high and comparable yields relative to mineral references at most sites, but also somewhat lower relative yields compared with mineral references in Germany (Fig. II-2), where the yield level was very high even at none or low mineral N application (Fig. II-S1), due to generally high soil fertility of the German site. The combined analysis of data of all trials showed that the BBFs did not differ from each other with regard to yield or N offtake (Table II-S7). A closer look at the trial sites reveals that nearly all the common BBFs had high yields comparable to the mineral reference applied at the same total N level for the French, Danish, and Finnish sites in both years (Table II-4).

In this two-year multisite trial, no consistency in ranking of individual fertilizers over the years or between sites was found. However, it should be noted, that the factors “site” and the interaction of “site and fertilization treatment” were not significant, and that “site” is partly confounded with application methods in this study. Overall though, eight of the 18 BBFs had high average agronomic performances with  $\text{NFRV}_{\text{AE}}$  above 75% and six had an intermediate  $\text{NFRV}_{\text{AE}}$  of 60-75%. For the ASL, direct injection (ASL2 treatment) actually increased the  $\text{NFRV}_{\text{AE}}$  from the intermediate to high range. This suggests that the majority of the BBFs can act as potential good substitutes for mineral N fertilizers (hypothesis I confirmed). However, their fertilization effect varies between agricultural settings of the trial sites, but not in a consistent way (hypothesis III partly rejected).

Zandvakili et al., (2019) stated that the C/N ratio of organic fertilizers determines the release of plant-available N and that BBFs with C/N ratios above 7-8 release far less plant-available N. This contrasts with the findings in the current study where no relation between C/N ratio of the BBFs (Table II-2) and the agronomic performance of BBFs was found. As an example, FEK with a C/N ratio of almost 9 had a high average  $\text{NFRV}_{\text{AE}}$  of 79%. Additionally, the agronomic performance of the BBFs did not correlate with any of the other assessed properties of the BBFs (pH, total N,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , DM, total C content) (data not shown). Even though BBFs with a high total N content did not have a higher agronomic performance than those with low total N content, a high BBF total N content is an advantage for transport, storage and application, as smaller fertilizer quantities need to be handled and field applied. Furthermore, BBFs with a high mineral N content ( $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ ) did not perform better than those with a low mineral N content. Thus, the data in this study suggest that BBFs should be applied based on their total N content and not mineral N content ( $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ ), as is common for more traditional organic fertilizers like animal manures and slurries.

The compost (BVC) was the only (local) BBF that did not consistently differ from the unfertilized reference and therefore had no significant fertilizer effect (Table II-4). Composts have also in previous studies been found to give lower yields compared to mineral N fertilizers based on total N addition (Chalk et al., 2013; Gutser et al., 2005; Ronga et al., 2019). Based on the definition in Wester-Larsen et al. (2022), BVC can thus not be defined as a BBF, since it does not produce a fertilizing effect; rather it should be considered a soil amendment or amelioration product.

Apart from the low agronomic performance of the compost, no general statement on agronomic performance can be made based on the BBF source materials and production technologies. The two digestates included in this study performed very differently, with higher yield responses for SDG (Danish site) compared to GRF (German site). This may be due to differences between the two sites and weather conditions on the day of application (precipitation in Denmark, sunshine in Germany). But the two digestates also differed in origin and compositions as GRF is the liquid fraction of mechanically separated digestate from an anaerobic digestion (AD) plant using manure and crop feedstocks, whereas SDG consists of unprocessed whole digestate from an AD plant using seaweed, agro and food waste as feedstocks. However, previous studies have reported agronomic performances equal to mineral references both for digestates and liquid-fraction digestates (Cavalli et al., 2016; Luo et al., 2022), as was found for SDG at the Danish site.



## 4.2 Second-year residual fertilizer effect of BBFs

Although there is no significant difference in residual fertilizer effects of BBF and mineral reference in the second year after application in France, Denmark, and Finland, the strong responses of the increasing levels of mineral N references at all sites indicates that fertilization had a marked residual effect on the following crop in this study. The large modelled standard errors for the residual effect data for France, Denmark and Finland (Table II-5) are mainly due to a high variance of the BBF data from the experimental fields. Even in Germany where the residual fertilizer effect was significant in 2022 and 2023, the differences between the BBFs were not useful for interpretations (Table II-5, Table II-S5; Fig. II-3). The lack of a significantly higher residual fertilization effect of the BBFs compared to the mineral N reference can possibly be attributed to the relatively high average first-year fertilization effect, with the first-year crop utilizing the largest proportion of the N in the BBF, and residual mineralization of remaining BBF organic N being correspondingly low. Based on this BBFs that did not have a high agronomic performance in the first year could be expected to have a higher residual effect. However, this was not found to be the case in this study (Fig. II-2 vs. Fig. II-3), but we can only speculate if it is due to lack of synchrony of the residual N availability from these BBF and subsequent crop demand, leading to N losses. When comparing the relatively low yields (Fig. II-3) and N offtake (Fig. II-S3) per site, it can be noted that most BBFs achieved higher yields and N offtake compared to the mineral reference at the same total N level, even though at the German site they all were lower. Therefore, we conclude that even if BBFs have a second-year fertilizing effect which tends to be higher than that of mineral fertilizers, this was not found to be significant in this study (hypothesis II rejected).

Zavattaro et al., (2015) performed a meta-analysis on European long-term trials and found that the effect of organic fertilizers (manure, slurry and compost) improved with the duration of repeated application. Schröder (2005) carried out a model simulation of N mineralization with repeated annual applications of manure and found that the N mineralization increases due to previous applications. Moreover, after several years of repeated manure application an equilibrium is reached between the amount of N applied annually and the amount of N mineralized annually. In the current study, the residual fertilization effect of a single application only was studied. Thus, if the trial had been continued for more years and had been designed with continuous application of the BBFs, a residual fertilization effect from previous years may have been detected as significant.

## 4.3 Implications of agricultural and geographic setting

At the German high soil fertility trial site, the seven common BBFs generally resulted in lower yield response than the corresponding mineral reference. This stands in contrast to the findings at the other sites with overall comparable yield responses to the corresponding mineral reference. Based on a meta-study of European long-term trials with compost, manure and slurry, Zavattaro et al. (2015) found that organic fertilizers result in higher yields in cooler climates and on coarse-textured soils. With a texture of 30% clay, 68% silt and only 2% sand (Table II-1) the German soil had the finest texture of the four sites. Together with the high background fertility (low N responsiveness) of the soil, this could possibly explain the lower yield responses to the BBFs. However, BBFs do not generally appear to result in higher yields in cooler climates in this study. This could be due to drier summers compared to the climate norm in 2021 and 2022 at the Danish and Finnish sites (Fig. II-1). Despite variation in agronomic efficiency between years and sites, soil type and climate could not be identified as significant factors for the agronomic efficiency of the BBFs across sites.

Compared to the mineral reference at the same N application rate, the BBFs resulted in higher yields for the spring crops (2021 all sites, 2022 Finnish site) with immediate BBF soil incorporation than the winter crops with BBF surface application (2022 French, German and Danish sites). Overall, the BBFs had a  $NFRV_{AE}$  which was on average 16% higher when incorporated than when surface applied. When incorporated, the BBFs are less prone to environmental influences, and it may reduce the risk of ammonia volatilization (Müller et al., unpublished; Wester-Larsen et al., 2023). However, it should be remembered that the effect of application was partly confounded with site in this study.

The three local liquid BBFs applied both years (SDG, PCW and ASL) differed less in  $\text{NFRV}_{\text{AE}}$  between the two years compared to the solid BBFs. As suggested by Ehmann et al. (2018) and (Luo et al., 2022), it could be speculated that the liquid BBFs would not be affected by the application method to the same extent as the solid BBFs, as they are better able to infiltrate the soil. However, the digestate SDG has previously been shown to have a high potential  $\text{NH}_3$  volatilization compared to the other BBFs (Wester-Larsen et al., 2022). Thus, the slightly lower  $\text{NFRV}_{\text{AE}}$  of 77% for SDG in 2022 when surface applied compared to 82% in 2021 when incorporated may be due to  $\text{NH}_3$  volatilization. Solid BBFs applied on the soil surface rely on precipitation for their disintegration, dissolution and infiltration into the soil root zone. The physical properties of the BBFs were not thoroughly assessed in this study, but a visual assessment of the surface applied solid BBFs at the Danish and German trial in 2022 found that they were all still visible on the soil surface two weeks after application (Fig. II-S6 in supplementary material). Moreover, the BBFs can be more prone to being eaten by wild animals when they are surface applied compared to incorporation (Fig. II-S5).

## 5 Conclusions

The 18 BBFs were found to have a high average mineral N fertilizer replacement value,  $\text{NFRV}_{\text{AE}}$ , of 71% across all sites and years. The  $\text{NFRV}_{\text{AE}}$  varied greatly between BBFs, ranging from 41 to 113% (excluding BVC at only 10%). Eight of the BBFs tested had a  $\text{NFRV}_{\text{AE}}$  above 75%, and another six were in the range 60-75%. Overall, most of the BBFs can therefore be considered good potential substitutes for mineral N fertilizers, as they performed more or less equally well as the mineral reference applied at the same total N level. Despite variation in agronomic efficiency between years and sites, soil type and climate were not found as significant explanatory factors for BBF yield effects or  $\text{NFRV}_{\text{AE}}$  across sites.

The application of BBFs resulted in a positive residual fertilizing effect in the second year, which tended to be higher than that of mineral fertilizer references; however almost none of the BBF residual N effects were found significantly higher than for the mineral fertilizers in this study.

The BBFs tended to have a higher agronomic performance when incorporated into the soil compared to soil surface application. This suggests that BBFs are better suited for spring crops, as it is currently not technically feasible to incorporate solid fertilizers in growing crops. However, field trials designed specifically to test the application method are necessary to confirm this.

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## Declaration of competing interests

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

## Author Contributions

**Benedikt Müller:** Conceptualization, methodology, formal analysis, investigation, data curation, writing - original draft. **Lærke Wester-Larsen:** Conceptualization, methodology, investigation, data curation, writing - original draft, visualization. **Lars Stoumann Jensen:** Conceptualization, methodology, writing - review and editing, project administration, funding acquisition. **Tapio Salo:** Conceptualization, methodology, investigation. **Ramiro Recena Garrido:** Investigation. **Mustapha Arkoun:** Conceptualization, methodology. **Aurélien D'Oria:** Writing - review and editing. **Iris Lewandowski:** Writing - review and editing. **Torsten Müller:** Writing - review and editing. **Andrea Bauerle:** Conceptualization, methodology, investigation, writing - review and editing, project administration, funding acquisition.

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## Data Availability

All data is openly available online in ERDA, the repository of the University of Copenhagen: <https://doi.org/10.17894/ucph.74c9271c-1346-4a57-9c8e-fb854dbb3500>

## Study III

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Supplementary material can be found in appendix III

### **Effects of biobased fertilisers on soil physical, chemical and biological indicators – A long-term incubation study**

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#### **Highlights:**

- This lab study investigated the effects of novel biobased fertilizers on soil quality indicators.
- Overall, the application of biobased fertilizers improved soil quality.
- Compost improved soil quality most, followed by a plant-based fertilizer and a biogas digestate.
- The amount of total and easily degradable carbon added was only related to some positive effects.
- Some but not all effects of long-term field application of compost could be predicted in the lab.

## Abstract

Soil quality is declining in Europe and globally due to agricultural practices and climate change. The European market for novel biobased fertilizers (BBFs) is growing and the new EU fertilizer regulation promotes their use. However, knowledge about the effects of many novel BBFs on soil quality is currently very limited. In a one-year laboratory incubation experiment, this study aimed to test the effect on biological (microbial biomass carbon (C)), physical (clay dispersibility and water-holding capacity) and chemical (pH, cation exchange capacity (CEC), total C and C in soil size fractions (<250  $\mu\text{m}$ , 50-250  $\mu\text{m}$  and >50  $\mu\text{m}$ )) soil quality indicators of ten BBFs applied at two different rates on two soil types: an Arenosol and a Luvisol. The set-up also included a soil that was subjected to long-term annual application of the compost used in the incubation. The application of BBFs generally improved soil quality, with the compost material improving soil quality most, followed by a plant-based fertilizer and a biogas digestate. The effect of BBF application on CEC, total C and particulate organic matter (POM) was related to the amount of total C added with the BBF. Furthermore, the effect on total C and POM fractions was also related to easily decomposable C added with the BBF. Comparing the single accelerated application with annual application under field conditions indicated that the long-term incubation trial is a reasonable predictor of compost long-term effects in the field. Whether this applies to BBFs with very different properties remains to be shown though.

**Keywords:** Soil quality, Soil fertility, Soil health, Soil type, Compost, Accelerated application.

## 1. Introduction

The main focus of European farmers is on primary crop productivity (Schröder et al., 2020) and economic profitability. However, soil also provides a range of highly important services, such as regulating climate, water and air quality and supporting biodiversity (Schröder et al., 2020). The provision of these services depends on the soil's ability to perform its functions. Soil functions can be linked to soil quality, which is defined by Karlen et al. (1997) as “the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation”. Soil quality indicators can be divided into three groups: biological, physical and chemical (Bünemann et al., 2018).

Despite the decline in soil quality in the EU (Stolte et al., 2015), European farmers do not invest in improving soil quality because they perceive it to be more costly than technological measures, such as deeper tillage, artificial drainage or increased irrigation, that are implemented to compensate for the effects of soil quality degradation (Schröder et al., 2020). However, soil quality is likely to become even more important in future due to climate and environmental change. Soil structural stability and water-holding capacity (WHC) may become crucial in future climates experiencing more frequent weather extremes, such as droughts and heavy rainfall (Allan & Soden, 2008). In addition, there is an increasing focus on the ability of soils to store carbon (C) and thus mitigate climate change (Moinet et al., 2023).

Soil quality is strongly linked to soil organic matter (SOM) (Amundson et al., 2015). The decline in SOM has been identified as one of the main threats to soil quality in the EU and globally (Amundson et al., 2015; EC, 2021; Stolte et al., 2015). The problem of SOM loss from agricultural soils is likely to increase in future as climate change leads to higher soil temperatures, accelerating SOM decomposition by microbes (Ofiti et al., 2021). This, in turn, will lead to increased soil degradation and hasten climate change through C emissions to the atmosphere, forming a feedback loop. Microbes are the main drivers of organic matter (OM) decomposition, and microbial activity is affected by the availability of OM added to soil (Bardgett, 2005). Therefore, the microbial biomass will increase following the addition of OM to soil. The addition and decomposition of OM has also been shown to improve soil structure and thus water, oxygen and nutrient availability (Allan & Soden, 2008). Moreover, increased SOM could counteract the predicted increase in drought stress caused by climate change (IPCC, 2022) by boosting the plant-available water capacity (Jensen et al., 2020). In addition, OM contributes to the soil cation exchange capacity (CEC) (Martinez et al., 2008).

Biobased fertilizers (BBFs) were defined by Wester-Larsen et al. (2022) as “materials or products derived from biomaterials (plant, animal or microbial origin, often wastes, residues or side-streams from agriculture, industry or society) with a content of bioavailable plant nutrients suitable to serve as a fertilizer for crops”. Novel BBFs were further defined in Wester-Larsen et al. (2022) as “a BBF produced by processes beyond simple biogas digestion of animal manures and simple composting. The processes involved in producing novel BBFs can e.g. be drying, pelletising or mineral extraction”. Biobased fertilizers often contain OM, and as soil quality is related to OM input to the soil, BBFs can be expected to enhance soil quality. The use of BBFs and organic amendments has also previously been shown to improve soil quality in long-term field experiments (Domingo-Olive et al., 2016; Sanden et al., 2018; Zavattaro et al., 2015). However, previous studies have focused on less processed products, such as manure and crop residues. The effect of novel BBFs on soil quality is less well known and they have not yet been evaluated in long-term field experiments (Schoumans et al., 2019). Therefore, little is known about the long-term effects of these novel BBFs on soil quality and, due to the nature of long-term field trials, results are unlikely to be available in the near future. Shorter-term laboratory incubation studies could potentially fill this gap, however, studies simulating the long-term application of BBF on soil quality in laboratory conditions are very scarce. Consequently, it is unclear whether laboratory set-ups can mimic such long-term processes.

Therefore the overall aim of the present study was to investigate the effects of selected BBFs on soil quality indicators. The specific objectives of the experiment were, firstly, to investigate how a single application of various BBFs at one year standard application and accelerated application affects i) a soil biological indicator (microbial biomass), ii) soil physical indicators (water-holding capacity and clay dispersibility), and iii) soil chemical indicators (cation exchange capacity, pH, total C and C in different soil particle-size fractions). The second objective was to compare the effects on soil properties of the long-term application of a specific BBF, a compost material, in a field trial with those obtained after a single accelerated application of the same material in the laboratory incubation.

The following hypotheses were tested: i) the decomposability of the C added with the BBF has a major effect on several soil quality indicators, with the effects more pronounced for BBFs containing a large amount of easily decomposable C, and ii) a single accelerated addition of compost in a laboratory incubation will have similar effects on soil quality indicators to the small annual addition of compost for 17 years in a long-term field trial with the same accumulated input of compost.

## **2. Materials and Methods**

### **2.1 Initial soil properties**

All the soils used in this experiment were collected from the University of Copenhagen’s experimental farm in Taastrup six months prior to the start of the experiment. A sandy soil was collected from a non-treated area in one of the fields (55°40'28.6"N 12°17'17.8"E) and was classified as an Arenosol based on data from Grønning et al. (unpublished). A soil with a higher clay content classified as a Luvisol by Peltre et al. (2015) was collected from the field used for the long-term CRUCIAL trial (Gómez-Muñoz et al., 2017). At the time of collection, the trial had been running for 17 years. The soil was collected from a non-fertilized plot, and the FIELD soil for the field/lab comparison was collected from an adjacent plot that had been amended annually with the same compost material as that used in the laboratory incubation (BVC) at a rate supplying a moderate amount of N for the crop. Soils were sampled from the 0-30 cm layer and stored in moist field-like conditions and at ambient temperatures until the start of incubation. The properties of the three soils used in the experiment are shown in Table III-1.

Table III-1: Characteristics of the soils used in the experiment. Values are means  $\pm$  standard error. For N, C, pH CaCl<sub>2</sub> and water-holding capacity (WHC) N = 3. For clay, silt, fine sand and coarse sand N = 1.

	<b>Arenosol</b>	<b>Luvisol</b>	<b>Luvisol FIELD</b>
N (%)†	0.15 $\pm$ <0.01	0.14 $\pm$ <0.01	0.34 $\pm$ 0.01
C (%)†	1.67 $\pm$ 0.02	1.23 $\pm$ 0.02	3.10 $\pm$ 0.16
C:N	10.9	9.0	9.0
pH CaCl <sub>2</sub>	6.17 $\pm$ 0.03	6.35 $\pm$ <0.01	6.65 $\pm$ 0.07
WHC (% of dry mass)	37.1 $\pm$ 0.5	33.5 $\pm$ 1.2	40.7 $\pm$ 1.3
Clay <0.002 mm (%)‡	9.2	18.4	20.9
Silt 0.002-0.02 mm (%)‡	7.5	14.0	15.3
Fine sand 0.02-0.2 mm (%)‡	42.4	37.0	33.8
Coarse sand 0.2-2 mm (%)‡	38.5	28.6	25.6

† Total C and N was determined by Dumas combustion. ‡ Texture was determined by sieving and sedimentation (ISO 11277).

## 2.2 Biobased fertilizers

Ten BBFs produced from different waste and side streams of agricultural, urban or industrial origin were included in this study. The ten BBFs were selected because they represented the variety of commercially available BBFs at the time of acquisition in terms of the raw materials and processing technologies used. All the BBFs were available on the European or regional/national market at the time the study was conducted. A description of the raw materials, processing technologies used and properties of the BBFs is provided in Table III-2; further details can be found in Wester-Larsen et al. (2022).

All ten BBFs were applied to the Arenosol, while four of them (BVC, OG2, PAL and SDG) were applied to the Luvisol (Table III-1). For both soils, a negative (no amendment) and a positive control (mineral reference fertilizer, standard ammonium-nitrate-sulfate, NS 27-4, Yara Ltd.) were included. Prior to their application, all BBFs and the reference mineral fertilizer were homogenized. To do this, the dry BBFs were carefully crushed, but not pulverized, using a mortar until they could pass through a 2-mm sieve. The BBFs with a high fiber content that could not be crushed with the mortar were cut with scissors and passed through a 2-mm sieve. The compost (BVC) was homogenized using a blender for approximately one minute. The two liquid BBFs (PCW and SDG) were dried at 50 °C to reach a moisture content of 44 % for PCW and 77 % for SDG in order not to exceed the water-holding capacity (WHC) of the incubated soil with the planned application rate. Prior to application, NH<sub>4</sub><sup>+</sup>-N lost as NH<sub>3</sub> during the drying was added as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> in solid powder form to the partially dried BBFs.

Table III-2: Biobased fertilizer (BBF) group, raw material(s) and technologies used in the production of the BBFs included in this study. Properties of the 10 BBFs included pH (1:5 in Milli-Q water), total N,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , dry matter (DM) content, total C, easily decomposable C (ED C) (see 2.10) and C:N ratio. N and C are reported as % of fresh weight (FW). Values are means, N=2, except for C and N of liquid BBFs where N=5 and pH where N = 3. All the BBFs are presented here with their acronyms (three letters/numbers). Full product names and manufacturers are shown in the Supplementary Material (Table III-S1). Data were derived from (Wester-Larsen et al., 2022); calculation of easily decomposable C was based on data from Agostini et al. (submitted, Appendix IV).

BBF	Group	Raw material	Technology	pH	N (% of FW)	$\text{NH}_4^+\text{-N}$ (% of total N)	$\text{NO}_3^-\text{-N}$ (% of total N)	DM (g kg <sup>-1</sup> of FW)	C (% of FW)	ED C (% of total C)	C/N
BA6	Plant-based	Plant-based residues (wheat and maize)	Fermentation and distillation	4.85	5.57	1.4	<0.1	907	43.6	60	7.8
BIO	Animal by-product	Meat and bone meal, apatite, vinasse, poultry manure, and potassium sulfate	Pelletising	5.69	7.39	3.5	<0.1	941	35.9	62	4.9
BVC	Compost	Municipal organic food waste	Anaerobic digestion and composting	8.56	1.57	8.1	<0.1	557	14.7	11	9.4
ECO	Animal by-product	Blood and feather meal	Pelletising	5.48	11.6	2.7	<0.1	892	45.3	67	3.9
FEK	Poultry manure	Poultry manure	Drying and processing (extrusion process)	6.43	3.94	20	<0.1	901	34.7	46	8.8
MO13	Animal by-product	Feather meal	Pelletising	5.07	14.2	1.0	<0.1	927	49.0	48	3.5
OG2	Animal by-product	Horn meal (pig bristles)	Hydrolysis	5.29	13.9	1.0	<0.1	940	48.3	45	3.5
PAL	Plant-based	Fermented biochar and high-quality clay and rock flour	Pyrolysis and fermentation “Terra Preta”	5.55	4.89	19	0.1	907	38.8	35	7.9
PCW	Plant-based	Potato cell water	Evaporation	4.70	1.53	15	1.1	339	11.2	99	7.3
SDG	Digestate	Agro and food waste + seaweed	Digestion	8.40	0.41	68	<0.1	38	1.08	65	2.6



## 2.3 Incubation setup

One week before the start of the experiment, the soil was sieved to 4 mm in moist field-like conditions. Batches of the sieved Arenosol and Luvisol were air-dried to a moisture content of 28 % and 25 % of WHC, respectively. These batches were used for the treatments receiving liquid BBFs to avoid moisture contents exceeding 60 % of WHC (see 2.2.5) after BBF application. For the treatments with dry BBFs, the soil was not air-dried. Soil equivalent to  $600 \pm 0.1$  g dry weight was transferred to 1 L round plastic containers. The BBFs were added based on total N content in amounts corresponding to a single application of  $0.137 \text{ g N kg}^{-1}$  soil (standard application rate), equivalent to  $164.4 \text{ kg N ha}^{-1}$  to a depth of 10 cm with an average bulk density of  $1.2 \text{ g cm}^{-3}$ . The accelerated application was 20 times this amount, i.e.  $2.74 \text{ g N kg}^{-1}$  soil. An additional treatment with a single accelerated application was set up to be compared to a soil that had been subjected to 17 years of annual application of the same compost product (BVC) as that used in the incubation study. This treatment with BVC thus received 17 times the annual application rate, i.e.  $2.33 \text{ g N kg}^{-1}$  soil, on the Luvisol. Thus, comparable amounts of C and N were added to the FIELD soil and in the 17 years accelerated treatment. Both the FIELD soil and the accelerated treatment were incubated under the same conditions. The mineral fertilizer reference was applied at the same total N rates as the BBFs.

After application of the BBFs or the reference mineral fertilizer, the soil was thoroughly mixed and then compacted to achieve bulk densities of  $1.20 \text{ g cm}^{-3}$  for the Arenosol,  $1.24 \text{ g cm}^{-3}$  for the Luvisol and,  $1.15 \text{ g cm}^{-3}$  for the Luvisol field soil. Bulk densities differed due to differences in the clay and OM content of the soils (Table III-1) (Keller & Håkansson, 2010). Milli-Q water was then added to the soil to achieve a water content of 60 % WHC (see 2.2.5), taking into account the water content of the added BBF. The containers were covered with a piece of black plastic with ventilation holes to minimize evaporation and germination of seeds. The headspace was 0.5 L.

Three replicates of each treatment were prepared. Moreover, the incubation set-up was prepared with three identical batches due to destructive sampling after two weeks of incubation (batch 1), after four months of incubation (batch 2) and after one year of incubation (batch 3). The incubation temperature was a room temperature of  $22.5 \pm 3$  °C. The water content was checked by weighing and adjusted throughout the experiment so that the samples did not lose more than 5 g of water (corresponding to 3.7 %, 4.2 % and 3.4 % of the total water content in the sample for the Arenosol, Luvisol and Luvisol field, respectively). The WHC was measured after 14 and 120 days of incubation. The water content of the samples was then adjusted according to any changes in WHC that occurred during the incubation period.

## 2.4 Overview of assessed indicators

Table III-3 provides an overview of the soil quality indicators assessed at different time points during the incubation period.

Table III-3: Overview of different soil quality indicators assessed throughout the incubation period.

	Before BBF application <sup>†</sup>	14 days after application	120 days after application	365 days after application
Total C	X			X
C fractions		X		X
CEC		X		X
pH	X	X	X	X
Water-holding capacity	X	X	X	X
Clay dispersibility				X
Microbial biomass		X	X	X

<sup>†</sup> Measurements on the three soils with three replicates without any fertilizer added.

## 2.5 Microbial biomass

Soil microbial biomass C (SMB-C) was determined by the direct chloroform extraction method first proposed by (Gregorich et al., 1990) and further developed, tested and compared with the traditional chloroform fumigation-extraction by (Setia et al., 2012). Fresh soil equivalent to 5 g dry weight was transferred to 50 mL Falcon tubes. Two subsamples were prepared: one as a non-fumigated control and one for fumigation. Soil-free blanks with and without  $\text{CHCl}_3$  were also included. Twenty mL of freshly prepared 0.5 M  $\text{K}_2\text{SO}_4$  was added to each Falcon tube and 0.5 mL of ethanol-free  $\text{CHCl}_3$  was added to one of the Falcon tubes. The samples were shaken for one hour in an end-over-end shaker. The extracts were then filtered into new Falcon tubes through Whatman no. 42 filter paper. The extracts were bubbled with air for 30 minutes to ensure removal of all  $\text{CHCl}_3$ . Samples were stored at  $-18^\circ\text{C}$  until analysis for total organic C (TOC) (Shimadzu TOC-Vcpn, Kyoto, Japan). The microbial biomass C was calculated using a conversion factor of 0.45 (Joergensen, 1996).

Extreme outlier values in the dataset were removed and the assessment of outliers was based on the relative difference between replicates. Single replicates (due to the loss of samples) with a very high SMB-C value, compared with the other time points, were also removed. Moreover, replicates where the non-fumigated sample had a higher concentration of TOC compared with the fumigated sample were removed from the dataset.

## 2.6 Clay dispersibility

Clay dispersibility was determined on fresh samples after one year of incubation using the procedure described in Jensen et al. (2019) and based on the method proposed by Pojasok and Kay (1990). The fresh soil was gently crumbled by hand so it could pass through an 8 mm sieve. A soil sample equivalent to 10 g DW soil was transferred to a plastic tube and artificial rainwater was added to obtain a soil-to-water ratio of 1:8. The tube was shaken in an end-over-end shaker (Stuart Tube Rotator model SB3) for 2 min at 40 rpm and 25 cm diameter rotation. After shaking, the suspension was left to settle for 3 hours and 50 min. Then 60 mL of the solution containing  $\leq 2\ \mu\text{m}$  particles was siphoned off and transferred to a beaker. While stirring the solution, two analytical replicate subsamples of 10 mL each were transferred to pre-weighed glass vials. All water in the glass vials was removed by drying at  $105^\circ\text{C}$  and the weight was recorded again. Any OM was then removed by igniting the glass vials with  $\leq 2\ \mu\text{m}$  particles for three hours at  $550^\circ\text{C}$  in a muffle furnace. After ignition, the glass vials were cooled in a desiccator and weighed. The OM estimated from loss-on-ignition was subtracted from the weight of particles  $\leq 2\ \mu\text{m}$  to obtain the amount of dispersed clay. The sediment in the tube was corrected for particles  $>2\ \text{mm}$  by sieving. Particles  $>2\ \text{mm}$  and the total OM content were subtracted from the total soil weight to express the results as g dispersed clay  $\text{kg}^{-1}$  mineral soil without stones.

## 2.7 Water-holding capacity

Water-holding capacity was determined on air-dry soil using a modified ISO 14238 method. Briefly, open plastic cylinders with a mesh in the bottom were two thirds filled with soil and left overnight in a container with water to saturate the soil from the bottom. The tubes with soil were left to drain on a water-saturated sand bath for one day and then the weight of the central part of the soil column was recorded before and after drying at  $105^\circ\text{C}$ . The WHC was measured on the soil samples prior to incubation and a water content of 60 % of WHC was assumed to be biologically optimal and used in the incubation set-up. As the WHC of the soil may change due to BBF addition and further during the incubation period, the water content in the incubations was adjusted according to the measured changes in WHC after 14 and 120 days of incubation.

## 2.8 pH

Soil pH was measured on air-dry soil with the addition of 0.01 M CaCl<sub>2</sub> solution at a soil-to-solution ratio of 1:5 (ISO 10390).

## 2.9 Cation exchange capacity

Cation exchange capacity was determined by the neutral ammonium acetate method. Ten g of air-dry soil was transferred to 50 mL Falcon tubes and 30 mL 1 M ammonium acetate pH 7 was added. The sample was shaken briefly and then left overnight at 5 °C. The next day the sample was filtered through Whatman no. 5 filter paper into Erlenmeyer flasks using a vacuum pump. A volume of 30 mL 1 M ammonium acetate pH 7 was then added four times to the soil on the filter paper. Afterwards the soil was rinsed with four separate additions of 20 mL ethanol 96 %. The NH<sub>4</sub><sup>+</sup>-N in the soil was then extracted by adding a volume of 20 mL 1 M KCl to the soil four times, collecting the extract and transferring it to a volumetric flask, and adjusting the final volume to 100 mL with 1 M KCl. The extract was kept at -18 °C until analysis of NH<sub>4</sub><sup>+</sup>-N.

## 2.10 Total C and easily decomposable C

Total C was determined by Dumas combustion using an elemental analyzer (CNS vario Macro Cube, Elementar). The amount of easily decomposable C in the BBFs (Table III-2) was calculated as the fraction of respired C relative to total C from data on CO<sub>2</sub>-C emissions (Agostini et al. submitted, Appendix IV), and the BBF total C content (Table III-2). The CO<sub>2</sub>-C was quantified by trapping respired CO<sub>2</sub>-C in 0.1 M NaOH at five time points during 84 days of BBFs.

## 2.11 Carbon in soil particle size fractionation

Soil C particle size fractionation was performed using procedures based on (Amelung & Zech, 1999). Air-dry soil (20 ± 0.05 g) was transferred to a beaker and 100 mL of Milli-Q water was added at a soil-to-water ratio of 1:5. The soil aggregates were disintegrated by sonication at 450 J mL<sup>-1</sup> using a probe-type sonicator. During sonication, the temperature did not exceed 60 °C to avoid loss of C. The dispersed samples were sieved at 250 µm and 50 µm, leaving three size fractions: > 250 µm, 50-250 µm and < 50 µm. The fractions were dried at 60 °C, their weight was recorded and they were ball-milled for total C analysis by Dumas combustion. The recovery of sample mass was in the range of 103±7 % and the recovery of C was 99±16 %. The fraction smaller than 50 µm was regarded as mineral-associated organic matter (MAOM) (Lavalley et al., 2020), while the fraction larger than 50 µm was regarded as particulate organic matter (POM) (Lavalley et al., 2020) and further divided into large POM > 250 µm (POM<sub>large</sub>) and small POM 50-250 µm (POM<sub>small</sub>).

## 2.12 Statistics

All graphs were produced in SigmaPlot 14.00 (Systat Software, Inc.). All statistical analyses were performed in R version 4.1.2 (R Core Team). Data were visually assessed with diagnostic plots to check the homogeneity of variance. In the event of heterogeneity of variance, the data were log<sub>10</sub> transformed. The dataset was divided into two according to the two soil types included in the experiments, except for correlations between soil quality indicators.

To detect differences between treatments for the soil quality indicators (microbial biomass C, clay dispersibility, WHC, pH, CEC, total C and the three C fractions) on the different sampling dates, linear models were used. BBF was included as a categorical factor in these models. The interactions between BBF, application rate and batch were included as fixed effects. Comparisons of Bonferroni-adjusted pairs of estimated marginal means were used to assess the differences between treatments. The

Bonferroni-adjusted significance levels used were calculated based on the number of comparisons made, and were  $0.05/5 = 0.01$  for the Luvisol dataset and  $0.05/11 = 0.0045$  for the Arenosol dataset.

To test for changes in C fractions during the incubation period, linear models were used. Interactions between the treatments and batches were included. Comparisons of Tukey-adjusted pairs of estimated marginal means were used to assess the differences in measurements between the two time points (batches). A minimum significance level of  $p < 0.05$  was applied.

To assess the effect of applied total and easily decomposable C on the soil quality indicators (microbial biomass C, clay dispersibility, WHC, pH, CEC, total C and the three C fractions), linear regression models for each soil and each batch were used. A minimum significance level of  $p < 0.05$  was applied and correlations with coefficients less than or equal to  $\pm 0.1$  were not included. To assess the correlations between the measured soil quality indicators (microbial biomass C, clay dispersibility, WHC, pH, CEC, total C and the three C fractions), linear regression models including both soils at the accelerated application rate were used. A minimum significance level of  $p < 0.05$  was applied and correlations with coefficients less than or equal to  $\pm 0.1$  were not included.

To compare the experimental set-up with the long-term field trial soil for the soil quality indicators (microbial biomass C, clay dispersibility, WHC, pH, CEC, total C and the three C fractions), linear models were used. Interactions between the treatments and batches were included. Comparisons of Tukey-adjusted pairs of estimated marginal means were used to assess the differences between accelerated application and long-term field application treatments and the negative control. A minimum significance level of  $p < 0.05$  was applied.

## 3 Results

### 3.1 Biological soil quality

#### 3.1.1 Microbial biomass

The results for SMB-C were not very consistent and did not reveal very clear trends. Due to the high variability and many missing values (2.2.3), statistics were not performed for the SMB-C data. Only the results for the accelerated application are shown in Fig. III-1 as the treatments did not differentiate from the negative control at the standard application rate (see Fig. III-S1 in the Supplementary Material). Overall, there was a trend showing an increase in SMB-C after the addition of BBFs compared with the negative control in the Arenosol (Fig. III-1 B), while in the Luvisol, this was mainly visible on the first sampling date. There were also no clear differences between the BBFs, but the potato cell water (PCW) and plant-based BBF (BA6) treatments had a high SMB-C compared with the other treatments in the Arenosol (Fig. III-1 B), and the addition of compost (BVC) resulted in a relatively high SMB-C in the first part of the experiment in both soils (Fig. III-1 A and B).

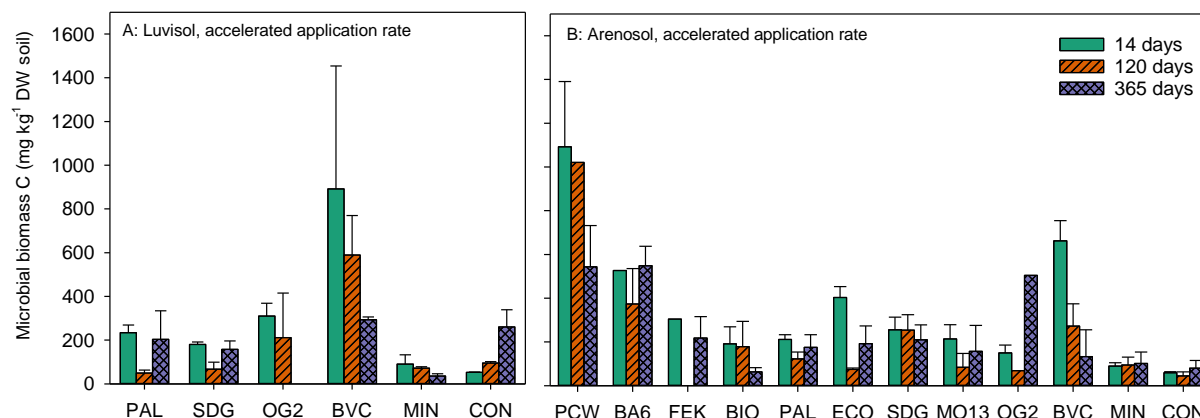


Fig. III-1: Microbial biomass C ( $\text{mg kg}^{-1} \text{DW soil}^{-1}$ ). Luvisol accelerated application rate (A), Arenosol accelerated application rate (B). Values are means  $\pm$  standard error for samples taken at three time points during the incubation period: 14 days, 120 days and 365 days after the start of incubation. BA6, BIO, BVC, ECO, FEK, MO13, OG2, PAL and SDG are biobased fertilizers, MIN is a reference mineral fertilizer and CON is the negative control. N=1 for OG2 120 days, PCW 120 days, OG2 365 days (B). N=2 for MIN 14 days, SDG 14 days, OG2 120 days (A), MO13 14 days, SDG 14 days, ECO 120 days, PAL 120 days, SDG 120 days, BVC 365 days, ECO 365 days (B). For all others, N=3.

## 3.2 Soil physical quality

### 3.2.1 Clay dispersibility

The negative control treatment with the Luvisol had much higher clay dispersibility than its Arenosol counterpart (Fig. III-2). For the Luvisol, the plant-based BBF (PAL) and digestate (SDG) treatments resulted in significantly lower clay dispersibility, and thus increased soil structural stability, at both standard and accelerated application rates, compared with the negative control (Fig. III-2 A and C). Furthermore, the animal-based BBF (OG2) and compost (BVC) treatments significantly reduced clay dispersibility compared with the negative control at the accelerated application rate (Fig. III-2 C). The mineral reference treatment (MIN) also reduced clay dispersibility significantly compared with the negative control at both application rates (Fig. III-2 A and C). For the Arenosol, only one animal-based treatment (MO13) had significantly lower clay dispersibility compared with the negative control at the standard application rate (Fig. III-2 B). For all other treatments, clay dispersibility was either increased or unchanged compared with the negative control (Fig. III-2 B and D).

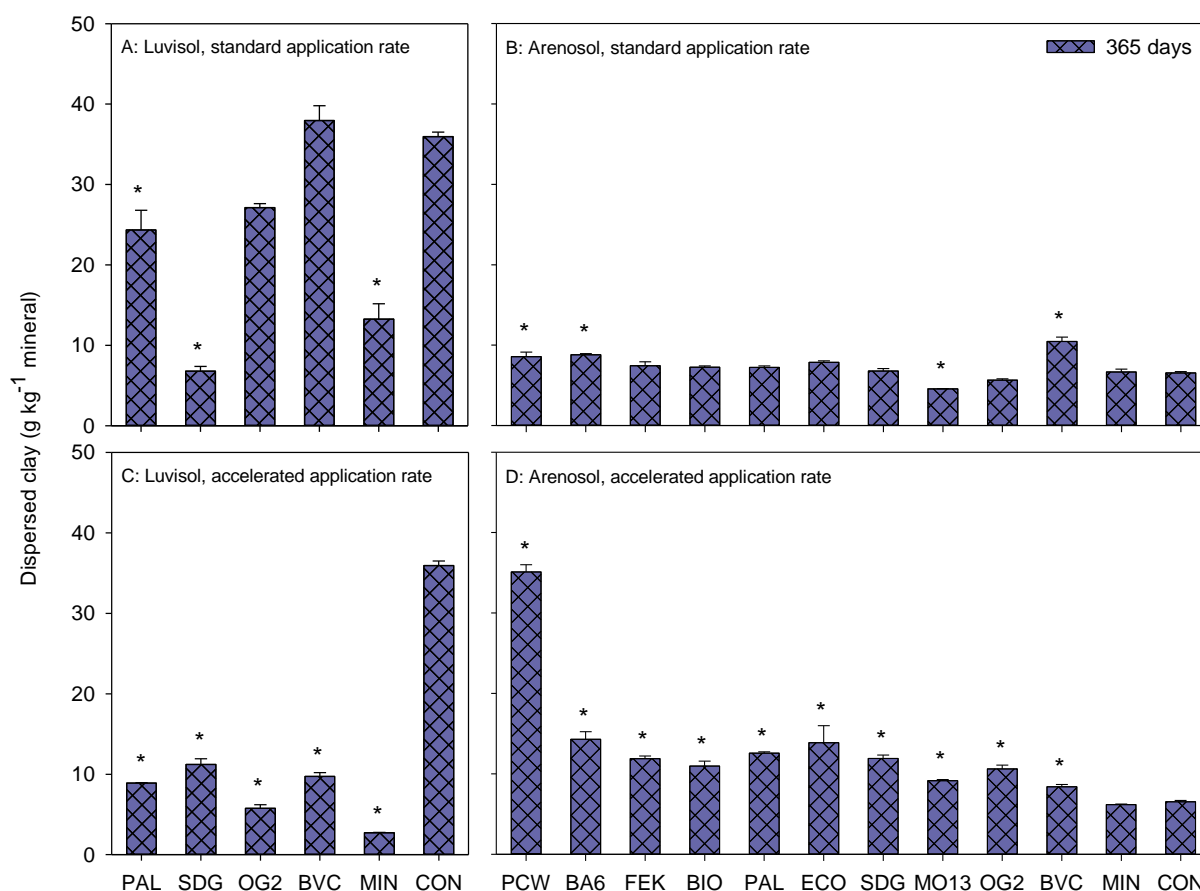


Fig. III-2: Clay dispersibility (g clay kg minerals<sup>-1</sup>). Luvisol standard application rate (A), Arenosol standard application rate (B), Luvisol accelerated application rate (C), Arenosol accelerated application rate (D). Values are means  $\pm$  standard error for samples taken out 365 days after the start of incubation. BA6, BIO, BVC, ECO, FEK, MO13, OG2, PAL and SDG are biobased fertilizers, MIN is a reference mineral fertilizer and CON is the negative control. Asterisks indicate significant differences between CON and treatments. N=2 for OG2 (A), for all others N=3.

### 3.2.2 Water-holding capacity

The accelerated application of the compost (BVC) significantly increased WHC compared with the negative control for both soils at all time points, except after 14 days for the Luvisol (Fig. III-3 C and D, Tables III-S2 and III-S3). For the Arenosol, after 365 days the lower rate application of one animal-based BBF (MO13) significantly increased WHC compared with the negative control (Table III-S2), however after 120 days the WHC was very low for MO13 compared with the negative control (Fig. III-3 B). The accelerated application of MO13 on the Arenosol after 365 days significantly decreased WHC compared with the negative control (Fig. III-3 D, Table III-S2).

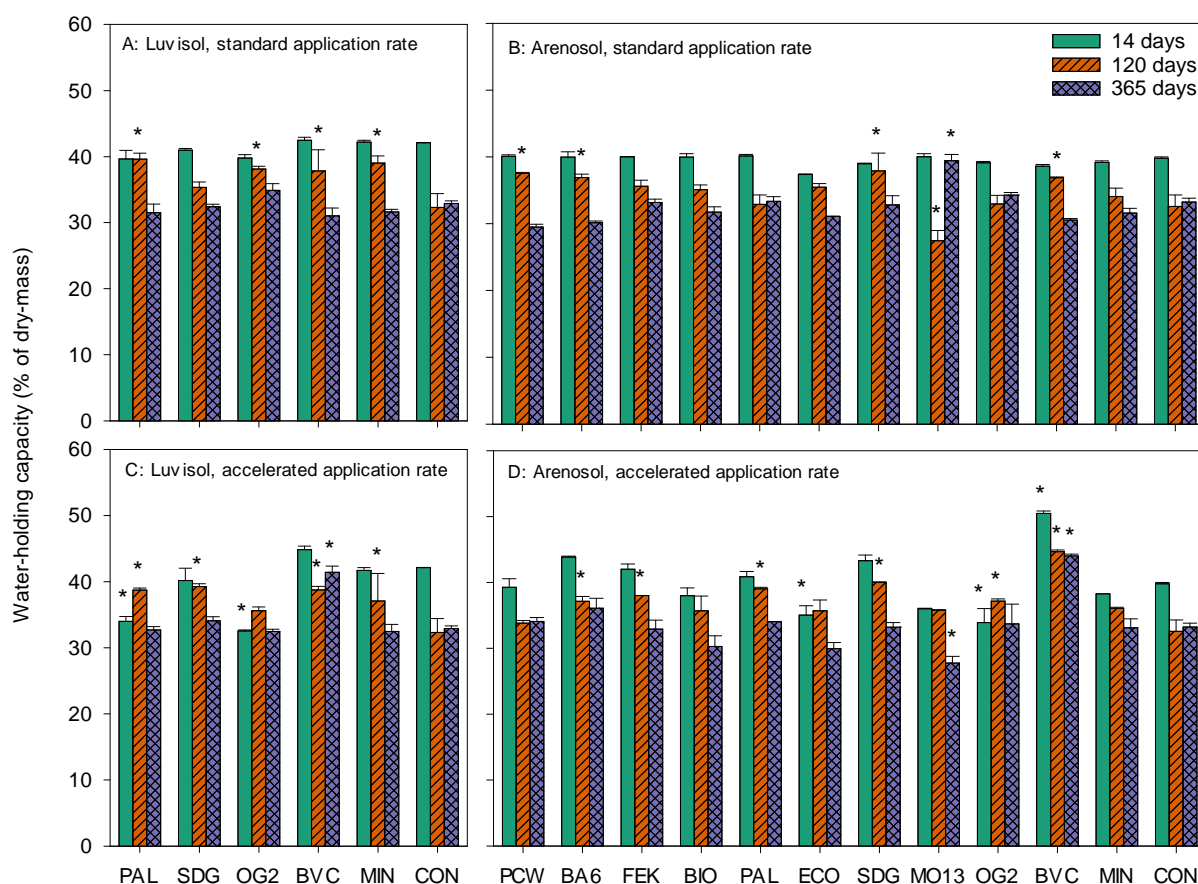


Fig. III-3: Water-holding capacity (% of dry mass). Luvisol standard application rate (A), Arenosol standard application rate (B), Luvisol accelerated application rate (C), Arenosol accelerated application rate (D). Values are means  $\pm$  standard error for samples taken at three time points during the incubation period: 14 days, 120 days and 365 days after the start of incubation. BA6, BIO, BVC, ECO, FEK, MO13, OG2, PAL and SDG are biobased fertilizers, MIN is a reference mineral fertilizer and CON is the negative control. Asterisks indicate significant differences between CON and treatments for each time point. N=1 for MIN 14 days (D), for all others N=2.

### 3.3 Soil chemical quality

#### 3.3.1 pH

The accelerated applications of the potato cell water (PCW) and compost (BVC) were the only treatments that increased pH after 365 days of application compared with the negative controls (Fig. III-4 C and D). For the accelerated application of all other BBF treatments, except the poultry manure-based BBF (FEK), the pH after 365 days was significantly lower than for the negative control for both soils (Fig. III-4 C and D, Tables III-S2 and III-S3). For the standard rate application of the digestate (SDG) and one animal-based BBF (OG2), the pH after 365 days was significantly lower than for the negative control for both soils (Fig. III-4 A and B, Tables III-S2 and III-S3).

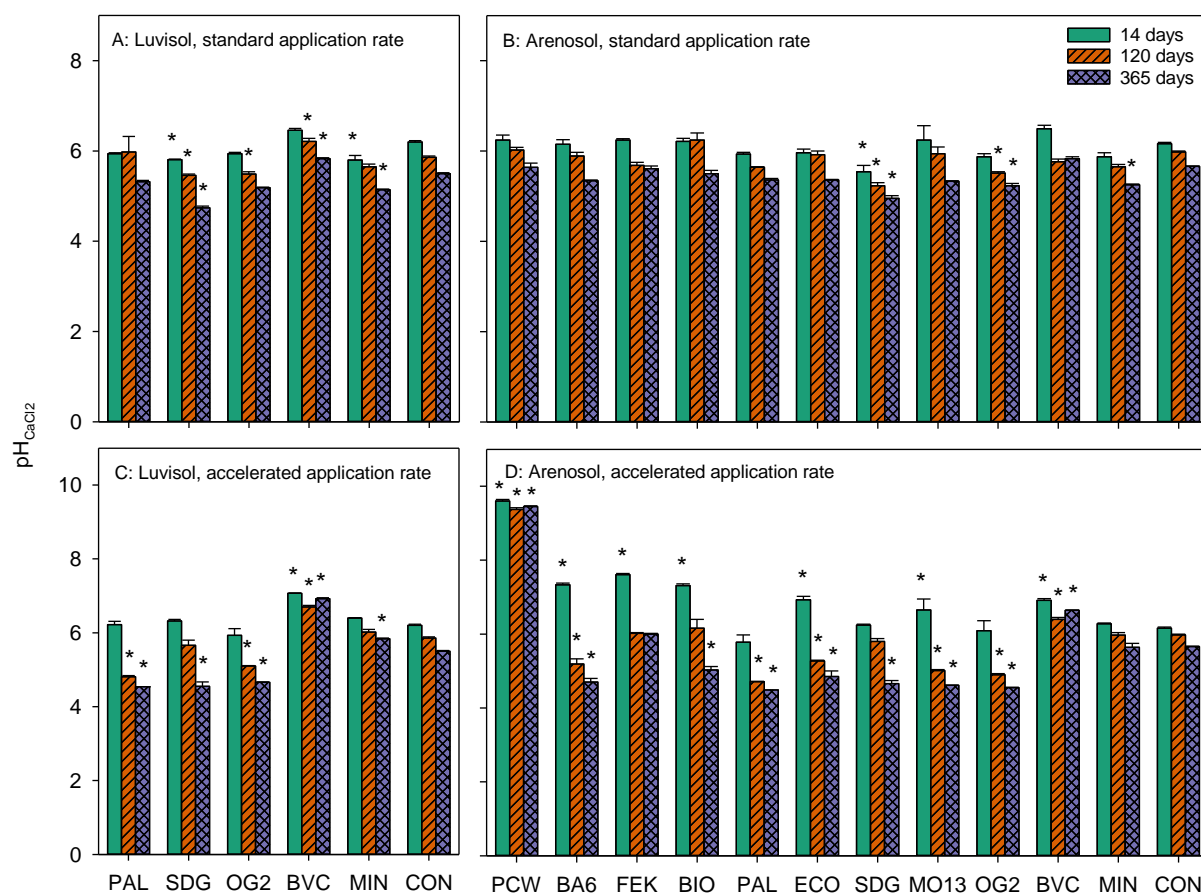


Fig. III-4: pH 1:5 CaCl<sub>2</sub>. Luvisol standard application rate (A), Arenosol standard application rate (B), Luvisol accelerated application rate (C), Arenosol accelerated application rate (D). Values are means  $\pm$  standard error for samples taken at three time points during the incubation period: 14 days, 120 days and 365 days after the start of incubation. BA6, BIO, BVC, ECO, FEK, MO13, OG2, PAL and SDG are biobased fertilizers, MIN is a reference mineral fertilizer and CON is the negative control. Note the different scales on the vertical axes. Asterisks indicate significant differences between CON and treatments for each time point. N=3.

### 3.3.2 Cation exchange capacity

The standard application treatments did not change CEC compared with the negative control for any of the soils (Fig. III-5A and B). The accelerated application treatments of the potato cell water (PCW), plant-based BBF (BA6) and compost (BVC) treatments significantly increased CEC after 14 days compared with the negative control (Fig. III-5 C and D, Tables III-S2 and III-S3). However, only the accelerated BVC treatment on the Luvisol still showed a significantly high CEC after 365 days compared with the negative control (Fig. III-5 C, Table III-S3), while the accelerated rate BA6 treatment on the Arenosol had a significantly lower CEC compared with the negative control after 365 days (Fig. III-5 D, Table III-S2).



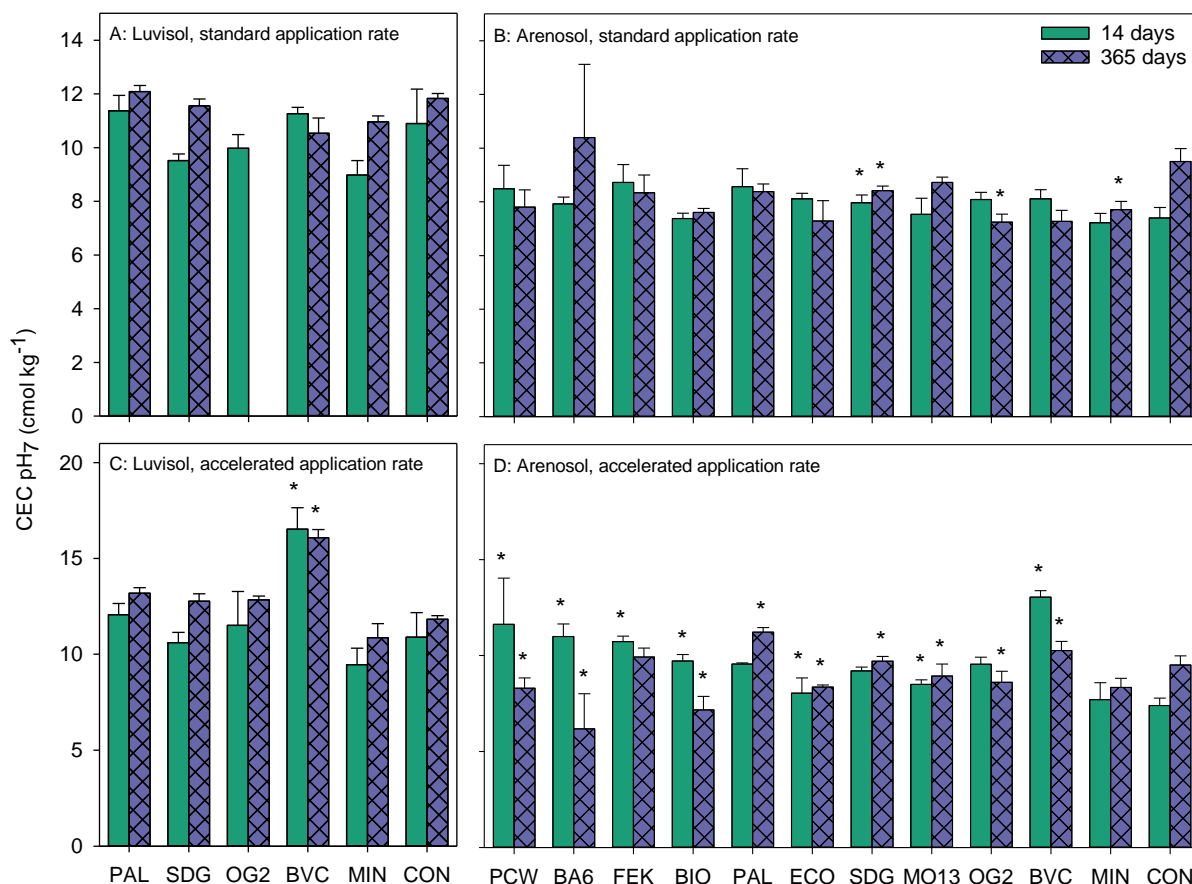


Fig. III-5: Cation exchange capacity at pH 7 (cmol kg<sup>-1</sup>). Luvisol standard application rate (A), Arenosol standard application rate (B), Luvisol accelerated application rate (C), Arenosol accelerated application rate (D). Values are means  $\pm$  standard error for samples taken at two time points during the incubation period: 14 days and 365 days after the start of incubation. BA6, BIO, BVC, ECO, FEK, MO13, OG2, PAL and SDG are biobased fertilizers, MIN is a reference mineral fertilizer and CON is the negative control. Note the different scales on the vertical axes. Asterisks indicate significant differences between CON and treatments for each time point. N=2 for CON (B and D), for all others N=3.

### 3.3.3 Total C

The initial total C content was 1.2 % and 1.7 % for the Luvisol and Arenosol, respectively (Table III-1), and it decreased in the negative controls by 16 % and 4 % during 365 days of incubation for the Luvisol and Arenosol, respectively. For the Luvisol, all accelerated rate application BBF treatments and the compost (BVC) standard application treatment significantly increased total C content compared with the negative control (Fig. III-6 A and C, Tables III-S2 and III-S3). For the Arenosol, accelerated rate application of the poultry manure-based BBF (FEK), plant-based BBF (PAL), animal-based BBF (MO13), digestate (SDG), and compost (BVC) treatments significantly increased total C content compared with the negative control (Fig. III-6 B and D, Table III-S2).

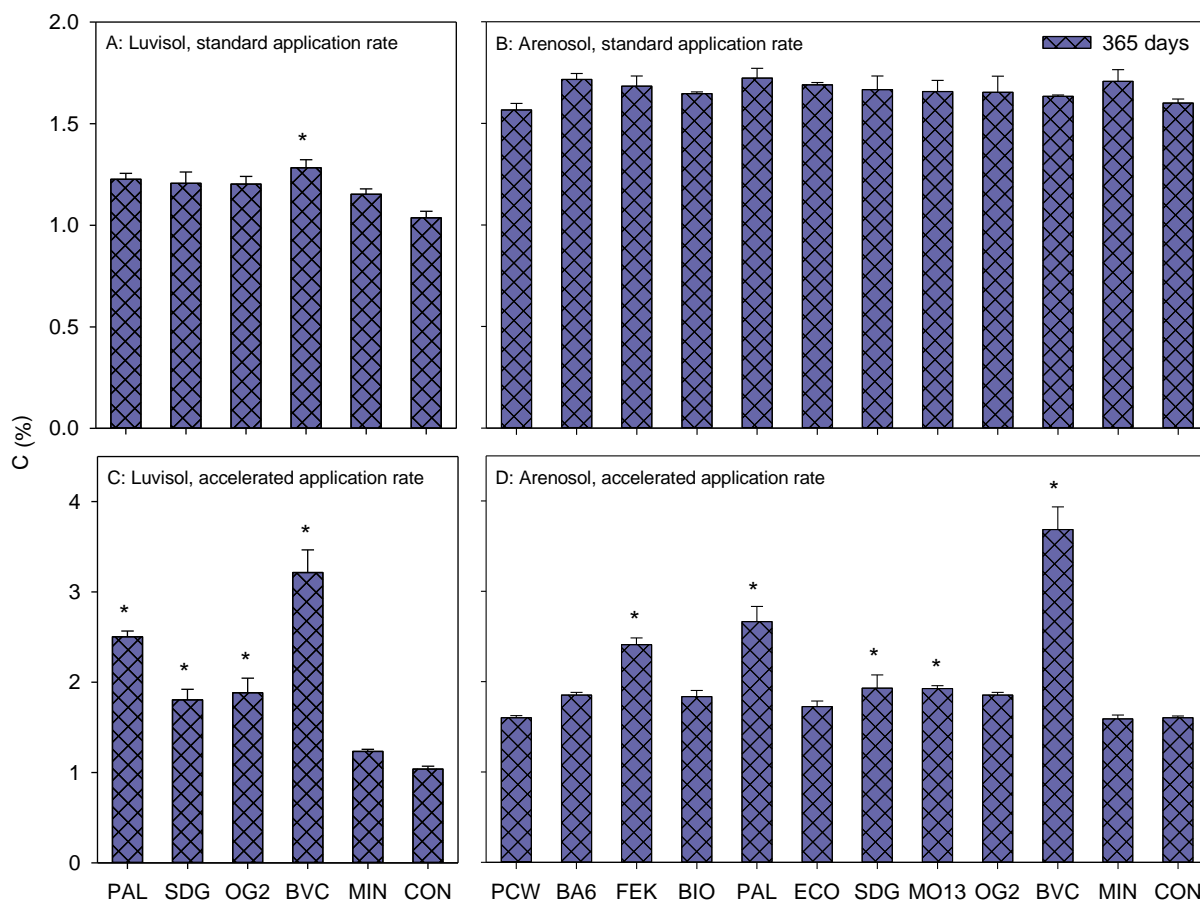


Fig. III-6: Total C (%). Luvisol standard application rate (A), Arenosol standard application rate (B), Luvisol accelerated application rate (C), Arenosol accelerated application rate (D). Values are means  $\pm$  standard error for samples taken 365 days after the start of incubation. BA6, BIO, BVC, ECO, FEK, MO13, OG2, PAL and SDG are biobased fertilizers, MIN is a reference mineral fertilizer and CON is the negative control. Asterisks indicate significant differences between CON and the treatments. Note the different scales on the vertical axes. N=3.

### 3.3.4 Carbon fractions

The MAOM fraction was by far the largest of the three C fractions for both soils and all treatments (Fig. III-7). During the incubation period, the  $POM_{large}$  fraction decreased significantly and the MAOM fraction increased significantly for the accelerated application of all BBFs, except the digestate (SDG) on the Luvisol (Fig. III-7 C, Table III-S4). For the Arenosol, the same tendency was observed during the incubation period for all BBFs at the accelerated application rate, except for the potato cell water (PCW) and the two animal-based BBFs, BIO and ECO (Fig. III-7 D). However, only the accelerated application of the plant-based BBFs BA6 and PAL and the digestate (SDG) showed a significant decrease in the  $POM_{large}$  fraction and only BA6, PAL and the compost (BVC) demonstrated a significant increase in the MAOM fraction (Table III-S4). Generally, the C fractions did not change significantly during the incubation period for the standard application rate (Fig. III-7 A and B, Table III-S4). However, the standard application rate of the digestate (SDG) led to a significant decrease in POM and a significant increase in MAOM in both soils (Table III-S4).

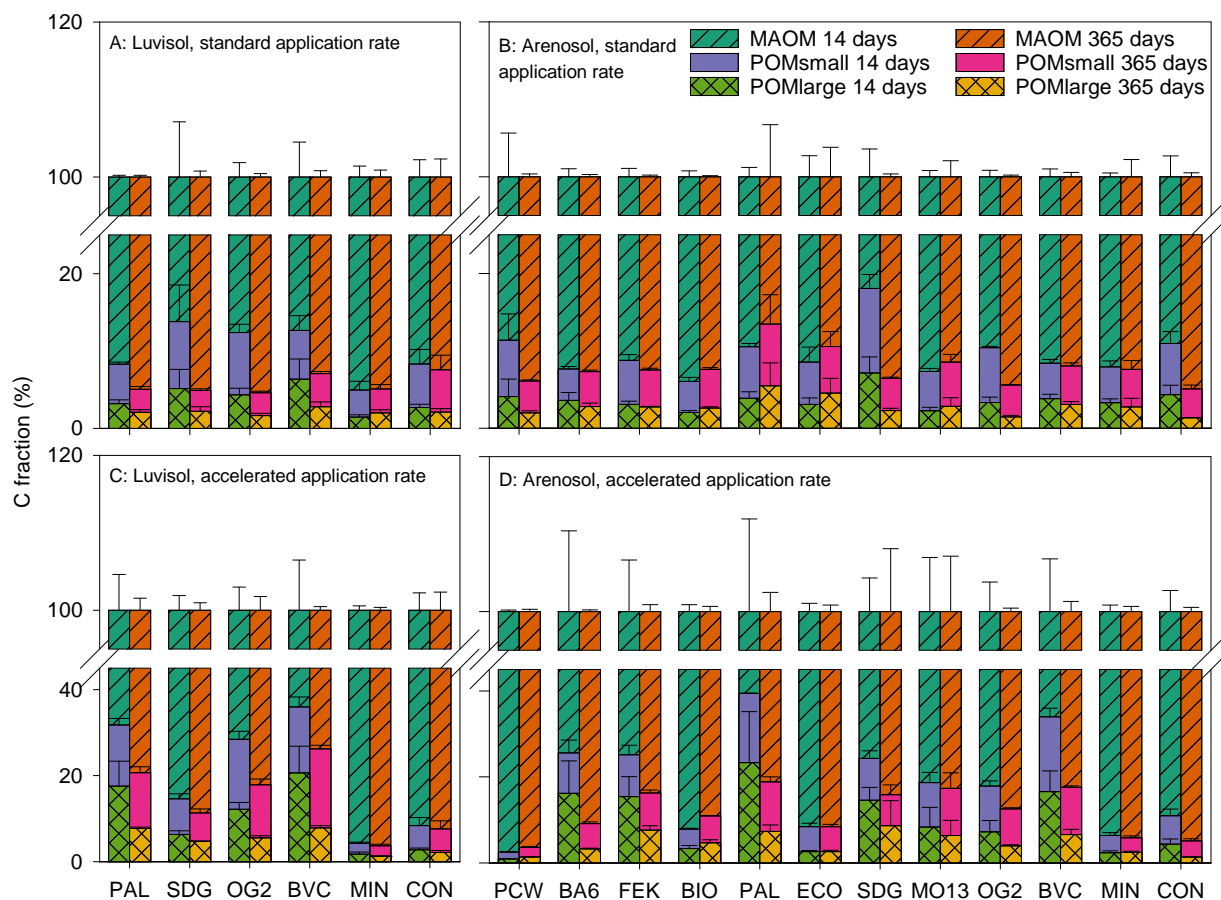


Fig. III-7: POM<sub>large</sub> (C fraction >250 μm (%)), POM<sub>small</sub> (C fraction 50-250 μm (%)) and MAOM (C fraction <50 μm (%)). Luvisol standard application rate (A), Arenosol standard application rate (B) Luvisol accelerated application rate (C), Arenosol accelerated application rate (D). Values are means ± standard error for samples taken at two time points during the incubation period: 14 days and 365 days after start of incubation. BA6, BIO, BVC, ECO, FEK, MO13, OG2, PAL and SDG are biobased fertilizers, MIN is a reference mineral fertilizer and CON is the negative control. Note the break in the vertical axes. N=3.

### 3.4 Correlations between soil quality indicators and C input

Total C content correlated significantly with WHC, CEC and the two POM C fractions (Table III-4), but the correlations were rather weak. Only three strongly significant correlations were found: between clay dispersibility and CEC (-0.87), WHC and CEC (0.73), and POM<sub>large</sub> and POM<sub>small</sub> (0.96).

Table III-4: Coefficients of correlations between soil quality indicators for both soils at the accelerated application rate. Only significant ( $p < 0.05$ ) correlations with coefficients  $\geq \pm 0.1$  are included.

	WHC	pH	CEC	Total C	POM <sub>large</sub>	POM <sub>small</sub>	MAOM
Clay dispersibility			-0.87			-0.57	
WHC	-		0.73			0.58	0.14
pH	0.10	-					
CEC	0.17		-		0.18	0.30	
Total C	0.10		0.11	-	0.13	0.10	
POM <sub>large</sub>	0.53				-	0.96	
POM <sub>small</sub>	0.48				0.54	-	
MAOM							-

For both soils, the applied easily decomposable C and total C correlated significantly with total C and POM<sub>large</sub> 365 days after the start of incubation (Table III-5). For both soils, total C correlated significantly with POM<sub>small</sub> 365 days after the start of incubation. Moreover, for the Arenosol, the applied easily decomposable C correlated significantly with POM<sub>small</sub> 365 days after the start of incubation. For the Luvisol, total C applied correlated significantly with CEC 365 days after the start of incubation. All correlation coefficients were rather weak (0.1-0.3), except for correlations with total C (0.6-0.9).

Table III-5: Coefficients of correlations between the amount of applied easily decomposable C (ED C) or applied total C and soil quality indicators 365 days after the start of incubation. Only significant ( $p < 0.05$ ) correlations are included.

Applied	Soil	Clay dispersibility	WHC	pH	CEC	Total C	POM <sub>large</sub>	POM <sub>small</sub>	MAOM
ED C	Luvisol					0.73	0.14		
	Arenosol					0.60	0.19	0.16	
Total C	Luvisol				0.32	0.90	0.17	0.10	
	Arenosol					0.80	0.22	0.19	

### 3.5 Implication of soil type

The effect of applying the different BBFs was the same for the biological and chemical soil quality indicators regardless of soil type. However, the BBFs had different effects on the two soil types for the two physical soil quality indicators. BBFs had a structure-improving effect on the Luvisol, with the application of BBFs decreasing clay dispersibility, while BBFs increased clay dispersibility in the Arenosol compared with the negative control. In contrast, the BBFs generally increased WHC more in the Arenosol than in the Luvisol.

### 3.6 Comparison of laboratory accelerated application with annual field application

The laboratory incubation experiment was generally able to mimic real long-term effects of annual compost (BVC) applications on WHC, pH, CEC and total C (Fig. III-8 C, D, E and F). This is evident from the lack of significant differences between the field (FIELD – soil from a real long-term field experiment with BVC application for 17 years) and the LAB (one time accelerated application of BVC corresponding to 17 years of annual application), although this was not consistently significant for WHC and pH (Fig. III-8). For the MAOM fraction, the experimental set-up could partly mimic the real long-term application of BVC (Fig. III-8 I). However, for microbial biomass, clay dispersibility and the two POM C fractions (Fig. III- 8 A, B, G and H), the experimental set-up overestimated the effect of BVC application and was therefore not able to mimic the real long-term application of BVC.

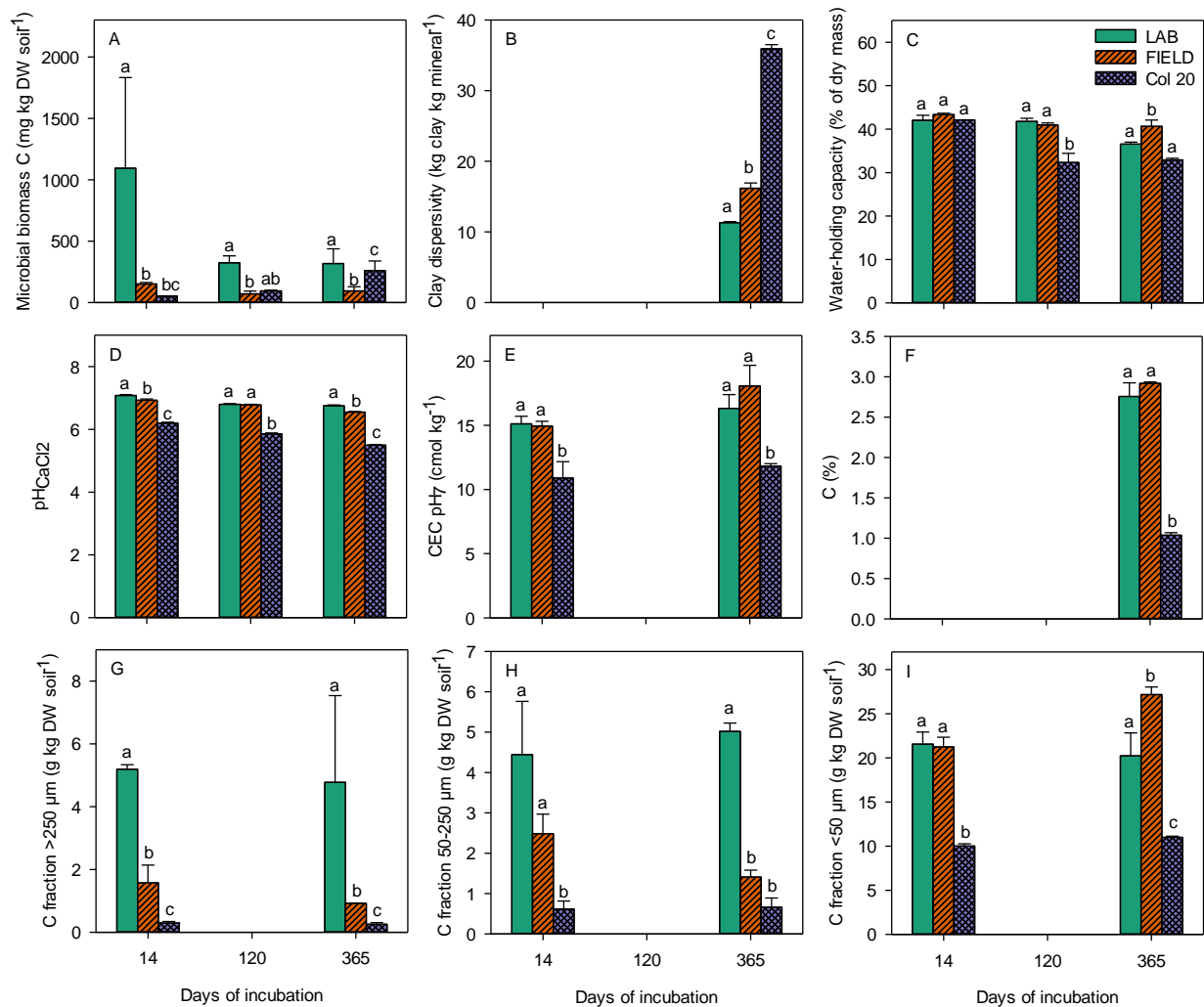


Fig. III-8: Microbial biomass C (mg kg dry soil<sup>-1</sup>) (A), clay dispersibility (g clay kg minerals<sup>-1</sup>) (B), water-holding capacity (% of dry mass) (C), pH 1:5 CaCl<sub>2</sub> (D), cation exchange capacity at pH 7 (cmol kg<sup>-1</sup>) (E), total C (%) (F), C fraction >250 μm (g kg dry soil<sup>-1</sup>) (G), C fraction 50-250 μm (g kg dry soil<sup>-1</sup>) (H) and C fraction <50 μm (g kg dry soil<sup>-1</sup>) (I). Values are means ± standard error for samples taken at three time points during the incubation period. FIELD is soil from real life long-term field experiment with BVC application for 17 years, LAB is the one time accelerated application of BVC corresponding to 17 years of annual application and CON is the negative control. Note the different scales on the vertical axes. N=2 for FIELD 14 days, LAB 120 days, CON 120 days (A) and all samples (C). For all others, N=3. Lower case letters indicate significant differences between treatments within each incubation time point.

## 4 Discussion

Overall, the application of BBFs improved soil quality indicators. As hypothesized, soil quality was also influenced by the decomposability of C added with the BBFs, but this was far from the only factor influencing their effects on soil quality indicators. Contrary to expectations, total applied C and applied easily decomposable C were equally good predictors of effects on soil quality (Table III-5). A strong correlation between SMB-C and easily degradable added C was anticipated, but this could not be assessed due to the high variability in SMB-C data. In contrast, CEC was predicted more effectively by total C application than by easily decomposable C application, which is in agreement with earlier findings concerning relationships between soil total organic C and CEC (Brady, 2014). Furthermore, C application appeared to be far from the only factor controlling the effects of BBF application on soil quality, as most soil quality indicators did not correlate with either total C application or application of easily decomposable C (Table III-5).

### 4.1 Soil biological quality

In general, The SMB-C was low in the negative control samples compared with previous findings for agricultural soils (Bhattacharyya et al., 2003; Cai et al., 2016). This was probably due to the soil being stored before the start of the incubation. Compared with a freshly sampled soil from the same field, the SMB-C for the negative control was 40 % lower after 14 days of incubation for the Arenosol (Fig. III-S2 in the Supplementary Material).

Cai et al. (2016) found an increase in SMB-C of 46-196 % after 32 years of manure application compared with a non-fertilized control and mineral fertilizer application. In this study, the decrease/increase in SMB-C compared with the negative control was in the range of -65 to +11 % and -27 to +85 % for the Luvisol and Arenosol, respectively, after 365 days at the accelerated application rate. Pezzolla et al. (2013) also found increases in SMB-C after the addition of organic amendments to two different soils, with the highest SMB-C after 12 and 20 days from measurements on days 0, 5, 12, 20, 30 and 45. This is in agreement with the results obtained in the present study, where SMB-C showed the highest increase compared with the negative control after 14 days of application, with values declining after 120 and 365 days of application, indicating higher initial decomposition rates of the added organic materials.

The plant-based BBFs, PCW and BA6, increased SMB-C most, which may be related to the high amounts of easily degradable C in them. However, the compost BVC also caused a relatively high increase in SMB-C compared with the negative control, despite BVC containing the lowest amount of easily degradable C. This may be related to the BVC containing microbes from the composting process, unlike most of the other BBFs that have been hygenised and therefore do not contain living microbes.

### 4.2 Soil physical quality

In a meta-analysis of 251 European long-term field experiments, soil physical quality was improved by the application of organic fertilizers and amendments (Sanden et al., 2018). However, in the present study, the effects varied depending on soil type, BBF and quality indicator.

All accelerated applications and some standard applications of BBFs and the mineral reference (MIN) decreased clay dispersibility in the Luvisol, and thus increased soil structural stability, compared with

the negative control (Fig. III-2). The mineral fertilizer treatment had not been expected to improve soil structural stability and no explanation for this could be found. The negative control treatment of the Arenosol had much lower clay dispersibility than its Luvisol counterpart. This could be explained by the high clay/carbon ratio of 15 for the Luvisol compared with 5.5 for the Arenosol (Table III-1), as well as the difference in clay content per se (Schjønning et al., 2012).

The effect on clay dispersibility of the high BBF application on the Arenosol showed the opposite to what had been expected, with most BBFs significantly increasing the amount of dispersible clay (Fig. III-2 D). The samples were combusted at 550 °C to remove all OM, leaving only the clay, as it was suspected that a high amount of OM in the suspension could have caused the clay dispersibility to become higher in the BBF treatment samples, compared with the negative control for the Arenosol. However, the combustion step did not change the difference between the treatments and the negative control. The numerical differences between the treatments and the negative control for the Arenosol were not very large, except for the potato cell water (PCW). When comparing clay dispersibility with soil pH, clay dispersibility increased with rising pH for the standard application rate on the Arenosol (Fig. III-S3 B in the Supplementary Material). However, for the accelerated application rate there was no general correlation, although the accelerated application of the potato cell water (PCW) resulted in a marked increase in both pH and clay dispersibility compared with the other treatments (Fig. III-S3 A). This increase in pH and the associated increase in the net negative charge of the clay particles may explain the increase in clay dispersibility following accelerated application of PCW (Chorom et al., 1994).

While there was an effect of the accelerated application rate on clay dispersibility for all BBFs, at least in the Luvisol, only the accelerated application rate of the compost (BVC) significantly increased WHC compared with the negative control for both soils after 365 days (Fig. III-3 C and D). In a long-term field trial, Gómez-Muñoz et al. (2017) measured volumetric water content near saturation and found that all organic amendments, except human urine, increased soil water content compared with the negative control after 11 years of application. In accordance with our study, high amounts of added compost resulted in a significantly higher water content than in all other treatments.

### 4.3 Soil chemical quality

The compost (BVC) increased soil pH compared with the negative control (Fig. III-4 D), which is in agreement with some previous studies (D'Hose et al., 2014; Sanden et al., 2018). On the Arenosol, the accelerated application of PCW, which is a potato cell water-based fertilizer, also increased soil pH significantly compared with the negative control (Fig. III-4 D). PCW has a low pH of 4.7 (Table III-2), so the high pH caused by its application must be related to decomposition products. The exact chemical composition of PCW was not analyzed, but it may contain organic acids that led to this pH increase when oxidized (Yan et al., 1996; Zwijnenberg et al., 2002). The pH increase caused by the application of BVC and PCW would be a benefit in agricultural practices and humid climates where regular lime addition is common practice, e.g. in Denmark (Borggard & Elberling, 2013). Except for BVC and PCW, BBF application decreased the pH of both soils compared with the negative control. This contrasts with the findings of Sanden et al. (2018), who found no change in soil pH after application of farmyard manure, slurry or crop residues, or of Lin et al. (2016), who performed a meta-analysis and even found that poultry manure increased soil pH. The decrease in soil pH after the application of BBFs could be caused by the decomposition of the high amounts of easily degradable C added with the BBFs (Alvarenga et al., 2017).

The application of BBFs did not have a pronounced or very consistent effect on soil CEC. In a meta-analysis, Lin et al. (2016) found that poultry manure increased soil CEC compared with mineral fertilizer. In the present study, the poultry manure-based BBF, FEK, also increased CEC, although not

significantly so. Only a significant correlation between total added C and CEC for the Luvisol was found, which suggests that CEC is related more to other C pools than the easily decomposable C pool. Previous studies have also found a relationship between soil total C and CEC (da Costa et al., 2020; Sinoga et al., 2012).

van der Bom et al. (2019) found a 10-32 % increase in total soil C content after 21 years of slurry application of 11-22 tons C per ha per year. In the present study, the annual rate of C application varied from 0.4-1.4 tons C per ha. The results of van der Bom et al. (2019) are lower than the findings in the present study where the increase in total soil C content was higher, albeit more variable, ranging from 0-68 % for BBFs applied at the accelerated rate corresponding to 20 years of application, despite lower annual C input in the present study. This could be attributed to the incubation lasting for just one year. Therefore the decomposition of OM is probably lower compared with 20 years of decomposition of the same total amount of OM added in smaller quantities per year. The greatest increase in total C was found for the compost (BVC) on the Luvisol with a 68 % increase, whereas the potato cell water (PCW) application did not lead to an increase in total C content. This is consistent with the PCW almost exclusively containing easily degradable C and the compost containing the least amount of easily degradable C due to stabilization of the C during composting (Gutser et al., 2005) (Table III-2). Sanden et al. (2018) also found the greatest increase in SOC after compost addition, compared with the increase from farmyard manure and slurry, which led to lower and similar increases in SOC.

Most BBF treatments showed an increase in MAOM and a decrease in one or both of the POM fractions from 14 to 365 days after application, although not significantly so for all of them (Fig. III-7). This indicates that a stabilization of C took place during the 365 days of incubation. However, the negative control of the Arenosol showed a non-significant decrease in POM and an increase in MAOM, which was greater than all standard application rate treatments, except the digestate SDG and half of the accelerated application treatments. Nevertheless, all the treatments that significantly decreased the POM fractions and increased the MAOM fraction demonstrated larger changes than the negative control. For the potato cell water (PCW) and animal-based BBF (BIO) there was a non-significant decrease in MAOM, implying either that stable soil C is lost through priming or that very small POM, <50 µm, was added with the BBFs. Both of these BBFs contain high proportions of easily degradable C (Table III-2), which could indicate that C may have been lost through priming. Moreover, no density fractionation was performed due to a large number of samples, therefore it is also plausible that small POM may have ended up in the MAOM fraction at the first sampling date (Lavalée et al., 2020).

#### **4.4 Comparison of laboratory accelerated application with annual field application**

Soil microbial biomass C was significantly higher for the single accelerated rate application at all time points compared with the long-term annual application of the compost (BVC) (Fig. III-8 A), suggesting that BVC application initially increases SMB-C, but that this effect does not last long. This could be related to the decomposition of easily degradable C, which is supported by the higher amount of POM C in the accelerated application sample compared with the field sample (Fig. III-8 G and H). Comparing the effect of BVC application on SMB-C with the effect of other BBFs (Fig. III-1), some BBFs have longer-lasting effects on SMB-C during the incubation period, e.g. the plant-based BA6 and PAL and the digestate (SDG). This suggests that the differences identified between FIELD and LAB are specific for BVC and would not necessarily be observed with other BBFs.

Soil structural stability was significantly higher in the incubation study than in the long-term field trial (Fig. III-8 B). This could be due to the lack of tillage after application of BVC in the incubation study compared with the soil sampled from the long-term experiment, which was subjected to tillage after BVC application in all 17 years (Schröder et al., 2016). Thus, it cannot be assumed that the increase in soil structural stability found in the present study would be as great if the BBFs were applied every year



at a normal fertilizer rate in the field. Water holding capacity was initially similar for the field and the laboratory sample, but significantly different from each other after 365 days of incubation (Fig. III-8 C). This suggests that the effect on WHC of long-term application of BVC was mimicked by the single accelerated application, although the effect of the single accelerated BVC application on WHC was slightly underestimated 365 days after the start of the incubation compared with the soil receiving an annual application for several years.

When comparing the pH of the accelerated application of compost (BVC) samples with the soil from the long-term annual field application, the accelerated samples were slightly, but significantly higher (Fig. III-8 D). This may suggest that annual long-term compost application will lead to a slightly smaller increase in soil pH than the single accelerated application, although the results were in the same range as those for the untreated control soil.

The accelerated application resulted in the same levels as those obtained after long-term application of BVC in the field for CEC and total C (Fig. III-8 E and F). The lack of difference in total C between the field soil and accelerated samples could be due to the C in BVC being very stable and therefore not greatly degraded, as the C content would otherwise be expected to be higher in the accelerated lab sample than in the field soil. The latter assumption is more in line with the results obtained for the C fractions that suggest that the C is decomposed to a greater extent in the field sample than in the accelerated application sample after 365 days (Fig. III-8 G and H). The two POM fractions contained significantly more C for the accelerated application sample compared with the field sample. However, the two POM fractions decreased and the MAOM fraction increased during the incubation period for the field sample, which suggests that some C transformation also occurred in the field soil.

In conclusion, pH and the soil quality indicators that depend on stable C (CEC, WHC and total C) were mimicked quite well by the accelerated application, in contrast to soil quality indicators related more to microbial activity and soil management. This may be specific to BVC, which contains a high proportion of stable C. It cannot be assumed with any certainty that the results of the comparison between the one-time accelerated application and the long-term annual application are valid for the other BBFs. As the other studied BBFs contain a higher proportion of easily degradable C, the process of C stabilization may take longer than for BVC. In addition, more C may be lost through respiration from application of the BBFs containing a higher proportion of easily degradable C.

## 5 Conclusions

Overall, the BBFs improved soil quality, although the effects observed were not significant in most cases. Some BBFs, however, produced a decrease in pH, which would be perceived as negative in agricultural environments with acidic soils. Generally, the compost (BVC) improved the soil quality indicators most, particularly WHC, total C and CEC. The plant-based BBF (PAL) and digestate (SDG) treatments also improved soil quality to a greater extent than the other BBFs. The effect of application of the BBFs on the soil quality indicators depended on both the decomposability of the C and the total C added with the different BBFs for some, but not all, of these indicators. Their impact was expected to depend more on the decomposability of the added C than on the total C added, but this could not be confirmed. The different soil types tested in this study did not influence the effect that the application of BBFs had on biological and chemical soil quality indicators. However, soil type did affect the impact on soil physical quality indicators, with an improvement in soil structural stability for the Luvisol, but not for the Arenosol. In contrast, WHC was improved more for the Arenosol than for the Luvisol. The effects on soil quality indicators of a single accelerated application of compost (BVC) in a laboratory incubation compared with a standard annual application in a long-term field trial indicated that the laboratory incubation could mimic long-term yearly field application for WHC, pH, CEC and total C. However, it is unclear whether this applies to BBFs containing less stable carbon than BVC.

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## Author contribution

**Lærke Wester-Larsen:** Conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing – original draft preparation. **Lars Stoumann Jensen:** conceptualization, funding acquisition, methodology, project administration, supervision, writing – review & editing. **Johannes Lund Jensen:** supervision, writing – review & editing. **Dorette Sophie Müller-Stöver:** conceptualization, methodology, supervision, writing – review & editing.

## Declaration of competing interests

The authors declare that they have no financial or personal competing interests.

## Data availability

All data presented in this paper are openly available online in ERDA, the repository of the University of Copenhagen: <https://doi.org/10.17894/ucph.f4ed5a3a-af63-4253-99f5-ba0cbd2f31dc>

## General discussion

The previous sections presented and discussed the results of the individual studies on ammonia ( $\text{NH}_3$ ) volatilization (study I), agronomic performance (study II), and soil quality (study III). This general discussion section will first provide a critical discussion on the methods used. Next, this section will present and discuss the interaction between studies I-III and some additional results from associated studies to which I have contributed and discuss all of the work in the context of the existing literature.

### A critical assessment of methods applied

This section will discuss the methods used in the three studies (I-III). The methods will be related to other potential methods and it will be discussed how the choices of methods may have affected the results obtained in studies I-III.

#### *Study I*

When assessing  $\text{NH}_3$  volatilization one should decide whether to determine the actual or potential  $\text{NH}_3$  volatilization. Actual  $\text{NH}_3$  volatilization is determined in the field where the environmental conditions of pressure, temperature, humidity and wind speed affect the measurements. Determining actual  $\text{NH}_3$  volatilization thus gives a more accurate picture of the  $\text{NH}_3$  volatilization under these specific conditions with the given soil type and weather conditions during the cropping season.

The potential  $\text{NH}_3$  volatilization is determined in the laboratory under controlled conditions. The controlled conditions limit variable factors such as wind and temperature to inflict on the results. The advantage of using these controlled conditions is that it enables comparisons between biobased fertilizers (BBFs) and provides a risk assessment of maximum  $\text{NH}_3$  volatilization. In addition, it is useful to gain an understanding of the temporal pattern of the  $\text{NH}_3$  volatilization. This is useful information to determine whether the  $\text{NH}_3$  volatilization occurs initially or later, and thus, whether the  $\text{NH}_3$  volatilization risk can be reduced effectively by incorporation of the BBF or not.

When determining potential  $\text{NH}_3$  volatilization in the laboratory, two overall methods exist. One is dynamic flow through systems and the other is static diffusion chambers. The difference between these two overall methods is how air circulation occurs in the setups. In the dynamic flow through system air is passed through the system and exchanged. Whereas, the static diffusion chambers rely on passive diffusion of air. When using the static diffusion chambers, a closed (enclosure method) and a semi open (venting method) exist.

For the enclosure method, the soil sample is enclosed in an airtight container wherein the  $\text{NH}_3$  volatilized is captured, usually with an acid trap, serving as a sink for gaseous  $\text{NH}_3$ . The  $\text{NH}_3$  that has volatilized and trapped, can then subsequently be quantified (Yang et al., 2018). The advantage of the enclosure method is that it is simple and easy to operate. Moreover, by selecting the right trap capture matrix a quantitative recovery of all gaseous  $\text{NH}_3$  can be achieved, if sufficient exposure time is allowed. Therefore, the static enclosure method was used in study I, to measure potential  $\text{NH}_3$  volatilization under controlled laboratory conditions, where  $\text{NH}_3$  volatilization was maximized.

One shortage of the experimental design used in study I was that no reference of mineral N fertilizer with a well-documented  $\text{NH}_3$  volatilization risk was included; only a positive control with maximum  $\text{NH}_3$  volatilization potential ammonium bicarbonate ( $\text{NH}_4\text{HCO}_3$ ) was included. In hindsight, it would have been very useful to have included a reference of ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ), representing the reference used in study II and III, and representing a mineral N fertilizer with low  $\text{NH}_3$  volatilization

risk. Moreover, it could have been interesting to have included a reference of urea, which has a well-known high  $\text{NH}_3$  volatilization risk. If these two additional references had been included the BBFs could have been related both to a mineral counterpart with high (urea) and low ( $\text{NH}_4\text{NO}_3$ )  $\text{NH}_3$  volatilization risk.

Measuring actual  $\text{NH}_3$  volatilization in a field experiment is much more complicated and demanding than determining potential  $\text{NH}_3$  volatilization in the laboratory. When determining actual  $\text{NH}_3$  volatilization in the field it can be done using either wind tunnels or micrometeorological techniques. The advantage of wind tunnels is that they can be used to measure  $\text{NH}_3$  volatilization from several treatments in the field, as they do not require large sampling areas. The drawback is that the setup disturbs the environment and they therefore tend to overestimate  $\text{NH}_3$  measurements compared to micrometeorological methods. The drawback of micrometeorological methods is that they require large and homogenous areas for measurements and thereby limit the number of treatments and replicates (Pedersen et al., 2020). If the actual  $\text{NH}_3$  volatilization of the BBFs should have been assessed in study I, it would not have been possible to include all 39 BBFs unless more time and resources had been invested in the study.

## *Study II*

The agronomic efficiency of the BBFs was tested in a field trial (study II). To relate the agronomic N efficiency of BBFs to mineral N fertilizers in field experiments, N fertilizer replacement values (NFRV) are often calculated. The NFRV can be calculated in different ways. In study II the NFRV was calculated based on equal total N application between BBFs and the mineral reference. Westerik et al., (2023) argue that it is more accurate to use the method for NFRV calculations that is based on equal N use efficiency (NUE) as the NFRV is overestimated when the method based on same N application level is used. Schils et al. (2020) also argue that it is more correct to calculate NFRV based on several levels of both BBF and mineral reference fertilizer application to produce response curves for both the mineral reference and the BBFs. Schils et al. (2020) state that the next best thing is to conduct field experiments with several levels of mineral fertilizer application and one moderate level of BBF application. Thus, the data that study II is based on complies with this second best option according to Schils et al. (2020). If several levels of BBF application should have been included in study II, it would have meant that fewer BBFs could have been tested in the experimental design. Unless more time and space had been invested in the trial, as both increase with an increasing number of treatments.

Another point of discussion on NFRV calculations is whether it should be calculated based on agronomic efficiency (AE) or NUE. The advantage of using NUE for NFRV calculations is that the response curve for the mineral reference is usually linear over a relatively wide range of N application levels, which makes estimating the value for the N application level corresponding to BBF application level easier, compared to the non-linear response curves for AE. However, it can also be argued that calculations based on NUE are further away from practice, where farmers would compare yields. Therefore, NFRV based on AE are more representative in this regard. In study II, data on N offtake at the German site were inexplicably very random. Therefore, emphasis on NFRV values based on AE was made, though both were calculated.

An alternative method to determine the agronomic efficiency, not based on NFRV calculations, is by using  $^{15}\text{N}$  labelled fertilizers. It has the advantage that the fertilizer N can be tracked in the system quantifying how much ends up in the plant parts and in the soil respectively and thus indirectly, how much fertilizer N has been lost to the environment. However, the  $^{15}\text{N}$  labelling method requires that the fertilizer can be produced with labelled  $^{15}\text{N}$  and is also more costly compared to methods based on NFRV calculations (Dobermann, 2005).

Conducting field trials is always related to great variability as conditions, such as soil texture and soil moisture within the field vary substantially, even in the most homogeneous fields. This variability transfers to the results obtained in the trial with great variation between replicates and large standard errors as a result. This “background” variability can potentially mask differences between treatments either “hiding” them within the background variability or resulting in non-significant differences when testing the differences between treatments statistically.

The drawback of “background” variability in field trials can be, at least partly, accommodated when conducting experiments under more controlled conditions. Testing the agronomic efficiency of the BBFs under more controlled conditions could have been obtained in pot experiments in greenhouses where soil and air moisture and temperature is controlled and equal among replicates and treatments. Moreover, the soil could have been mixed, sieved and packed so differences between samples become smaller than what is actual conditions in the field. Pot experiments or laboratory experiments can be used to determine the relative NFRV (Schils et al., 2020). The advantage of these relative NFRV are that many different BBFs can be compared (Schils et al., 2020). However, if results are to be extrapolated to actual field conditions, references of field experiments must be conducted (Schils et al., 2020).

### *Study III*

Soil quality can be assessed in many different ways, as many different parameters are attributed to soil quality. In study III a set of different soil quality indicators were chosen. As described in the background chapter, no pollution indicators were assessed in study III due to coverage of this topic in work package 5 in the LEX4BIO project. Additionally, the chosen soil quality indicators were focused on functionality related to crop production, and thus do not cover the entire concept of soil quality (Lehmann et al., 2020). In this regard it could be argued that study III focused on soil fertility rather than soil quality (Lehmann et al., 2020). However, soil C fractions were assessed in study III, which relate to other ecosystem services, apart from crop production, more focused on C sequestration potential. Therefore, the term soil quality was used in study III. Assessment of soil quality tends to focus on chemical soil quality indicators, whereas biological indicators are underrepresented in the literature (Bünemann et al., 2018). In study III it was chosen to include both biological, physical and chemical soil quality parameters.

One biological soil quality indicator was chosen, namely soil microbial biomass C (SMB-C). Soil microbial biomass can be highly fluctuating over time and therefore the measurements of SMB-C at three different time points only provides snapshots. Moreover, the fumigation method used to quantify SMB-C used in study III, often produces results with great variability, which made interpretation of the results challenging. To get more in depth information of the soil microbiology over time, microbial respiration could have been assessed with a setup that allowed to have traps capturing the respired CO<sub>2</sub> or by collecting gas samples frequently. Other methods could have been used to look into the functional diversity and if it changed during the incubation period due to the different BBFs applied, e.g. the MicroResp method (MicroResp, 2023). This could have given an insight into whether the microbial communities adopted to the different components of the BBFs. Moreover, it could have been interesting to see at what pace this adoption occurred to evaluate whether it is relevant for the first cropping season after application or if it takes several applications for the adoption to take place.

While biological soil indicators may change fast due to changes in soil management, the build-up of soil structural stability occurs slowly. Therefore, it was chosen to assess clay dispersibility, as this is an indicator that is known to be sensitive to small differences in soil structural stability (Munkholm, 2021) and thereby give an indication of the differences between treatments despite the experimental period being one year.

I originally planned to conduct a study on soil quality in the field using accelerated application as well in field mini plots. However, this was not feasible due to the delay of the field trial activities caused by the covid-19 pandemic. In field conditions the “natural” soil architecture for agricultural soil, would have been preserved. Moreover, crops would have grown and normal farming practices would have been conducted. As discussed above for study II, the drawbacks would have been an expected larger variability between plots.

Assessment of soil quality indicators in a long-term field trial would have been highly interesting. However, for obvious reasons it was not possible to conduct a long-term field trial within the period of this PhD study; and no existing long-term field trial exist including the range of BBFs in the project. Luckily, a long-term field trial testing application of one of the selected BBFs, the exact same compost as studied in this PhD, the BVC compost, had been on-going with annual applications for 17 years at the time study III was conducted. This enabled a comparison between the results obtained in study III with the results of long-term application under field conditions for this compost BBF.

### **Nitrogen loss risks of using BBFs**

In order to evaluate the main N loss risks from the use of BBFs, I conducted Study I to quantify the variation in potential  $\text{NH}_3$  volatilization from the use of BBFs under different environmental conditions. Furthermore, I will try to assess the importance of other gaseous and aquatic N losses from BBFs applied to soil. This will be done by discussing results from a MSc thesis project (Pedersen, 2021) I supervised on  $\text{N}_2\text{O}$  emissions from some of the same BBFs as in study I. Furthermore I will interpret residual soil mineral-N data from the Danish field trial in Study II as an indicator for  $\text{NO}_3^-$  leaching risk. Finally, an overall risk score for N loss will be presented.

#### ***Variation in potential $\text{NH}_3$ volatilization from BBFs under different environmental conditions***

In Study I we found that the BBFs investigated varied immensely in potential  $\text{NH}_3$  volatilization when surface applied to a non-adsorbing matrix (sand), with the highest accumulated potential  $\text{NH}_3$  volatilization for digestates (48 % of applied N) and the lowest for composts and struvites (<1 % of applied N). Many BBFs derived from animal by-products, poultry manure, plant-materials and mixed sources had intermediate potential  $\text{NH}_3$  volatilization in a similar range (12 % to 20 % of applied N). This corresponds with other studies, e.g. Rabiger et al. (2020) found actual  $\text{NH}_3$  volatilization of 8-18 % of applied N from digestates applied to soil (in study I: 1.2-15% of applied N), Lockyer et al. (1989) who found  $\text{NH}_3$  volatilization for air-dried poultry manure of 6.5 % of applied N and Erwiha et al. (2020) who found  $\text{NH}_3$  volatilization of 25 and 32 % of applied N for blood meal and feather meal respectively.

The temporal pattern of potential  $\text{NH}_3$  volatilization also varied greatly between BBFs, with most BBFs showing a delayed response in  $\text{NH}_3$  volatilization. For some of them a high initial volatilization rate followed by a concave decline was found, similar to studies of slurry (Monaco et al., 2012) and digestates (Liao et al., 2019). However, for the majority of BBFs a convex loss curve with a substantial lag-phase (up to 20 days) in  $\text{NH}_3$  volatilization was observed, in agreement with Erwiha et al. (2020). The temporal patterns are useful for assessing the  $\text{NH}_3$  volatilization risk when BBFs are field applied. If  $\text{NH}_3$  volatilization occurs initially, as found for the digestates in study I, there is a risk of  $\text{NH}_3$  volatilization, even when BBFs are incorporated, as application and incorporation are two separate activities in agriculture. Whereas, for BBFs with a lag-phase in  $\text{NH}_3$  volatilization potential, incorporation will be an effective measure to reduce  $\text{NH}_3$  volatilization risk.

The initial BBF characteristics measured in this study and their interaction with time explained a large fraction (89 %) of the variation in accumulated potential  $\text{NH}_3$  volatilization. However, the differences in potential  $\text{NH}_3$  volatilization from BBFs, could not be explained from component material category (CMC) and product function category (PFC) grouping systems, but a grouping of the BBFs based on more traditional concepts (digestate, manure, animal-byproduct based) worked markedly better.

Soil type affected potential  $\text{NH}_3$  volatilization significantly, with highest  $\text{NH}_3$  volatilization potential for a sandy soil (acidic) and lowest for two clayey soils (acidic and alkaline). Zhenghu and Honglang (2000) also suggest that no single soil property alone can explain  $\text{NH}_3$  volatilization.

Incorporation of the BBFs in the soil could effectively reduce potential  $\text{NH}_3$  volatilization. However, when incorporation is not an option, e.g. when applying BBFs in growing crops,  $\text{NH}_3$  volatilization may pose a significant risk of yield loss and environmental impact for certain BBFs. This is in line with several studies conducted on other TOFS/BBFs in both field and laboratory trials (Svensson, 1994; Nyord et al., 2012; Feilberg and Sommer, 2013; Herr et al., 2019; Liao et al., 2019; Rocha et al., 2019; Maris et al., 2020).

### ***Correlation between N mineralization and $\text{NH}_3$ volatilization potential***

Zandvakili et al. (2019) found low  $\text{NH}_3$  volatilization for compost and cow manure and high  $\text{NH}_3$  volatilization for blood- and feather meal fertilizers. They stated that this was related to the higher N mineralization of the blood- and feather meal, compared to the low mineralization for the compost and cow manure. In study I,  $\text{NH}_3$  volatilization of the 39 BBFs was found to be correlated with initial  $\text{NH}_4^+$ -N content. However, this was not the only factor determining  $\text{NH}_3$  volatilization potential. Thus, N mineralization may be a factor controlling the  $\text{NH}_3$  volatilization potential of the BBFs studied, but this could not be included in study I.

To test whether a correlation between  $\text{NH}_3$  volatilization potential (study I) and the N mineralization from the BBFs exists, additional N mineralization data were included. The data on N mineralization was derived from an unpublished study by Agostini et al., to which I contributed. As it is not yet published it was included in appendix IV. The N mineralization data of Agostini et al. (submitted, Appendix IV) was interpolated between sampling days to enable comparisons with  $\text{NH}_3$  volatilization data (study I) for the same sampling days. The correlation was tested individually for each sampling date using mean values of N mineralization and  $\text{NH}_3$  volatilization. When including all BBFs tested in both study I and in the N mineralization incubation study (Agostini et al. submitted, Appendix IV), no significant correlations were found. However, when excluding the few BBFs which consist of pure mineral N, significant correlations were found (Fig. IV-1). The correlations coefficients increased towards the later part of the incubation period. This indicates that the  $\text{NH}_3$  volatilization potential of the BBFs is more controlled by N mineralization dynamics in the last part of the incubation period. If this period coincides with the period of N uptake by plants in a field situation, e.g. for spring sown crops fertilized immediately prior to sowing, the  $\text{NH}_3$  volatilization risk decreases. However, in a field situation where plants do not take up the N mineralized, e.g. BBF fertilization in the autumn, or with very early spring application before main crop demand, it may more readily volatilize as  $\text{NH}_3$ .

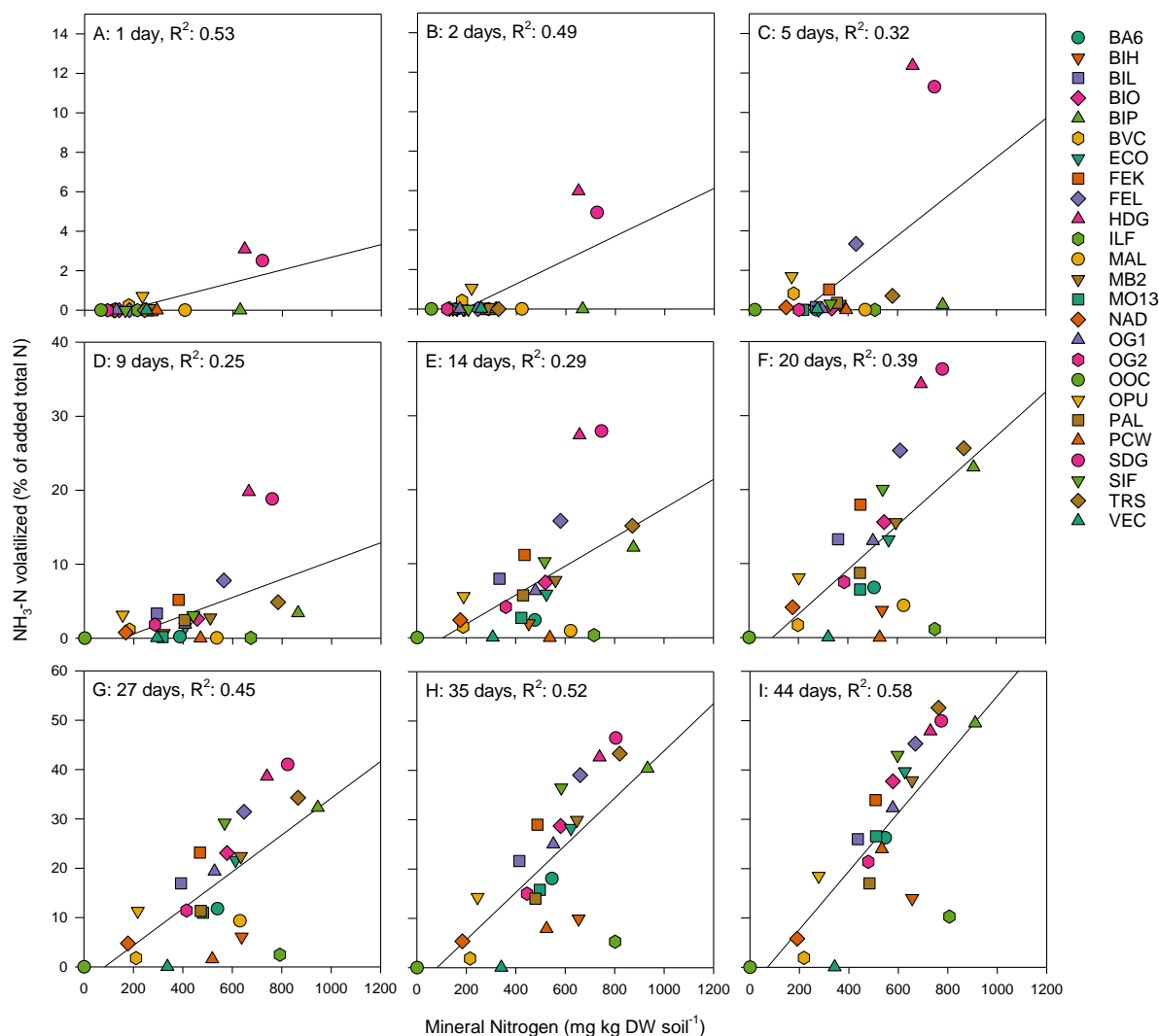


Fig. IV-1: Correlation between mean accumulated  $\text{NH}_3\text{-N}$  volatilization potential (% of added total N) (Study I) and mean mineral N ( $\text{mg kg DW soil}^{-1}$ ) (Appendix IV) for each time point (days after start of incubation). The linear regression line is shown for each plot as a black line. Three mineral precipitate BBFs (CGR, PCS, and SYS) and one BBF containing mineral fertilizer (PRI) were excluded. Note different scales on the vertical axis.  $N=3$ .

### ***Risk of $\text{N}_2\text{O}$ emissions from BBFs***

When N fertilizers are applied, some of the N may also be lost to the atmosphere due to denitrification, either as  $\text{N}_2$ ,  $\text{N}_2\text{O}$  or  $\text{NO}$ . As  $\text{N}_2\text{O}$  is a very potent greenhouse gas (see Background chapter) it is of main interest to know whether the application of BBF instead of synthetic N fertilizers result in higher, lower, or similar  $\text{N}_2\text{O}$  emissions, as indicated by Walling and Vaneeckhaute (2020).

A MSc student I co-supervised (Pedersen, 2021) conducted a laboratory incubation study assessing  $\text{N}_2\text{O}$  fluxes of five of the BBFs included in study I. Briefly, the study consisted of the following 5 BBFs: poultry manure (AV4), compost (BVC), animal based (ECO), digestate (HDG), and plant based (PAL). In the study, a mineral reference of ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) (MIN) and a negative control (CON) were also included. The fertilizers were mixed into soil sampled from a non-treated area in the field of the Danish trial (study II) and incubated at 60 % or 90 % of total water holding capacity (WHC). Samples of  $\text{N}_2\text{O}$  were taken on days 0, 2, 5, 10, 15, 20, 25, and 35 and daily fluxes were calculated based on the change in  $\text{N}_2\text{O}$  concentration from a sample taken at time zero and a sample taken two hours after closing the incubation jar. To test differences between treatments, a linear model with



pairwise comparisons of estimated marginal means across WHC levels was made on accumulated N<sub>2</sub>O fluxes with a minimum significance level of  $p < 0.05$  using R version 4.1.2 (R Core Team).

The accumulated N<sub>2</sub>O emissions, as an average of 60 % and 90 % WHC, was highest for PAL > ECO > AV8 > BVC > HDG > MIN > CON and was 3.2, 1.7, 1.3, 1.3, 0.9, 0.1 and 0.01 % of applied N respectively. Four of the five BBFs tested (AV4, ECO, HDG, and PAL) had significantly higher N<sub>2</sub>O emissions compared to the mineral reference (MIN) (Fig. IV-2). This is in agreement with a Danish study on coarse textured soils by Petersen et al. (2023) who found higher N<sub>2</sub>O emissions for TOFs compared to mineral N fertilizers. The compost (BVC) did not differ significantly from the mineral reference (MIN) (Fig. IV-2). Moreover, the N<sub>2</sub>O emissions were higher with higher WHC of the soil, which is in agreement with previous findings (Akiyama et al., 2004). Additionally, the N<sub>2</sub>O emissions decreased with time, due to the soil drying out during the incubation period.

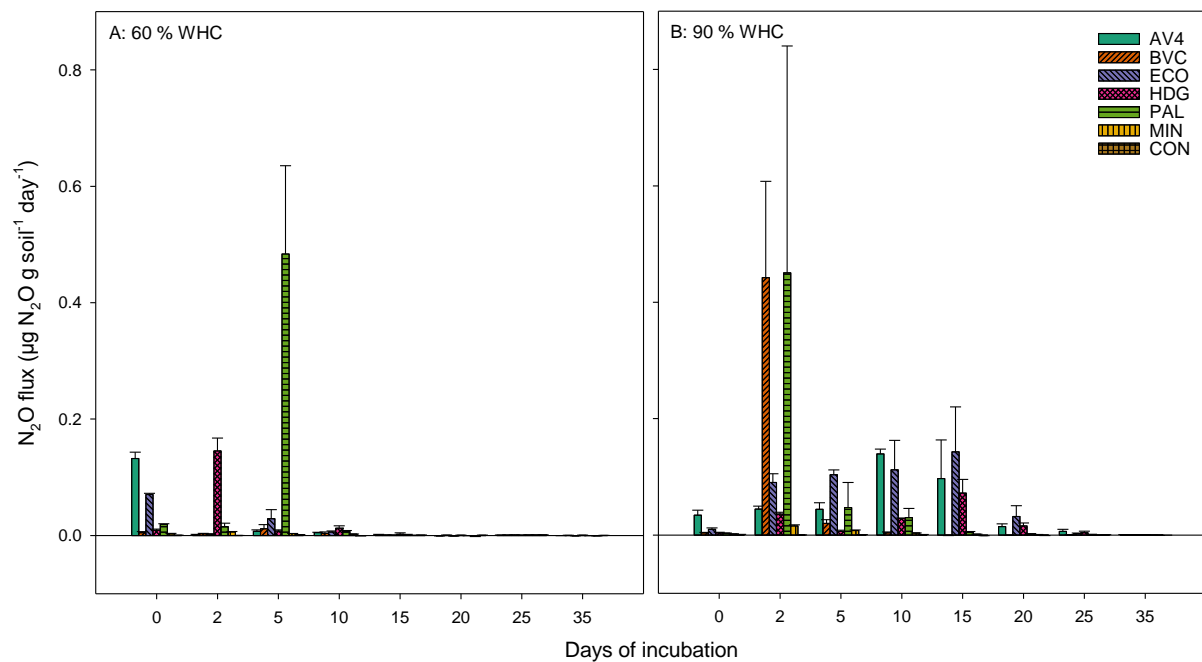


Fig. IV-2: Mean daily fluxes of N<sub>2</sub>O (µg N<sub>2</sub>O g soil<sup>-1</sup> day<sup>-1</sup>) ± standard error from BBFs (AV4, BVC, ECO, HDG, and PAL), a reference of ammonium nitrate (MIN), and a negative control (CON) on incubation days (0, 2, 5, 10, 15, 20, 25, and 35). A) Soil at 60 % of water holding capacity at the start of incubation. B) Soil at 90 % of water holding capacity at the start of incubation. Soil water content decreased throughout incubation in both A and B. N=3. Data from Pedersen (2021).

The data from Pedersen (2021) presented in Fig. IV-2 is from a short-term laboratory experiment and does therefore not necessarily resemble the N<sub>2</sub>O fluxes of BBFs applied in the field. Moreover, as the soil dried out during the incubation period, the N<sub>2</sub>O emissions could be expected to be higher if the soil moisture had been maintained constant throughout the incubation.

As N<sub>2</sub>O emissions originate from organic N which has been mineralized, a delay from the time of application to N<sub>2</sub>O emissions has often been observed for manures (Sommer et al., 2013). Moreover, the N<sub>2</sub>O emission has been found to increase with increasing content of readily available N in TOFs (Hansen et al., 2019; Velthof et al., 2003). Thus, the N mineralization patterns of the BBFs could be expected to influence N<sub>2</sub>O emissions. The compost BVC had the overall lowest N<sub>2</sub>O emission, despite a very high emission on day 2 at 90 % WHC. This might be attributed to the low mineralization of BVC found in Agostini et al. (submitted, Appendix IV). The plant-based BBF (PAL) had a high emission on

day 2 (at 90 % WHC) and 5 (at 60 % WHC). This could be due to the rapid mineralization of PAL during the first seven days of incubation after which it levels off (Agostini et al. submitted, Appendix IV). The poultry manure BBF (AV4) and the animal-based BBF (ECO) had the highest N<sub>2</sub>O emissions on day 0 at both WHC levels and showed continuous emissions until day 15 at the high 90 % WHC level. AV4 was not included in the mineralization study, but ECO showed high initial mineralization, which continued until day 28 before flattening off (Agostini et al. submitted, Appendix IV). Thus, the continued N<sub>2</sub>O emissions at 90 % WHC might be attributed to the mineralization pattern of ECO. The digestate (HDG) had a substantial N<sub>2</sub>O emission on day 2 at 60 % WHC and lower, but more continuous, emissions at the 90 % WHC level. While HDG had a high initial mineral N content, it did not mineralize further in the mineralization incubation study (Agostini et al. submitted, Appendix IV). Thus, overall, it seems that the N<sub>2</sub>O emissions (Fig. IV-2) might be related to the BBF mineralization patterns (Agostini et al. submitted, Appendix IV). However, observations are few and general conclusions cannot be made.

### ***Mineral N leaching risks***

Applied N in fertilizers may be lost to aquatic ecosystems through NO<sub>3</sub><sup>-</sup> leaching and surface runoff, potentially causing eutrophication or contamination groundwater. Therefore it is of interest whether BBF result in higher NO<sub>3</sub><sup>-</sup> leaching risk than mineral N fertilizers, as indicated by (Torstensson et al., 2006).

The N leaching risk was assessed using the soil mineral N (sum of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N) content sampled 18 days after harvest in the field of the Danish field trial (study II). Briefly, soil samples were taken in two areas of each plot with three samples, one meter apart, at each area. Samples were divided in three depths (0-30 cm, 30-60 cm, and 60-90 cm) and were bulked together into one composite sample per plot. To test differences between treatments, a linear model with pairwise comparisons of estimated marginal means across years was made on soil mineral N in 0-90 cm depths with a minimum significance level of p<0.05 using R version 4.1.2 (R Core Team).

Soil mineral N content was generally higher after harvest in 2022, compared to 2021, particularly in the top soil layer (0-30 cm) (Fig. IV-3). This seems likely to be attributed to the lower yield obtained in 2022 compared to 2021, thus indicating reduced crop N offtake in 2022. The highest mineral N reference (REF 4) had significantly higher soil mineral N content compared to the compost and the lowest mineral N reference (REF 1). The plant-based BBFs PAL and PCW had mineral N content at the higher end of the ten BBFs, though not significantly higher. The PCW, in particular, had a higher mineral N content in the deeper soil layers (30-90 cm). The BBFs did not have higher soil mineral N content compared to the mineral reference at the same N application level (corresponding to between references 3 and 4). This could indicate that the leaching risk is not higher for the BBFs than for the mineral N fertilizer, which is in contrast to earlier findings for TOFs (Bergström and Kirchmann, 2006).

The soil mineral N content 18 days after harvest is a snapshot and does not provide information about how the mineral N content will change during the autumn and winter. Thus, it cannot be used as an absolute measure for the risk of N leaching, though it can give some kind of indication. The mineral N content in the plots fertilized with BBFs could increase further due to mineralization of organic N and leaching risk could therefore be higher than what the soil mineral N samples after harvest indicate (Sørensen and Jensen, 2013). If the actual leaching were to have been assessed, samples should have been collected at several time-points during the autumn and winter. The actual root depth was not measured, but in Danish conditions the usual root depth of cereals is at least one meter, and thus the soil sampling down to 90 cm should be within the root zone.

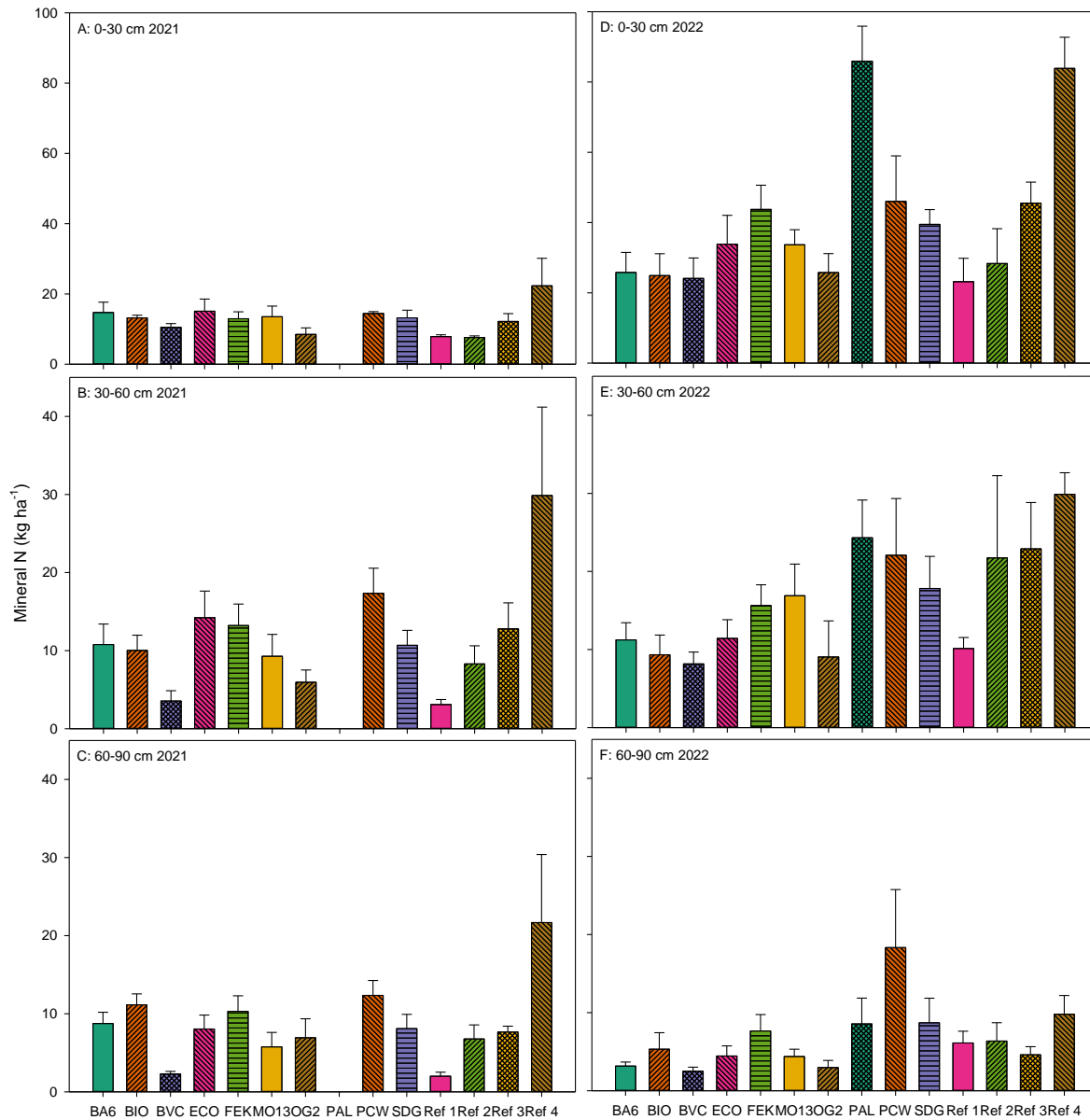


Fig. IV-3: Soil mineral N (sum of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ ) ( $\text{kg ha}^{-1}$ ) of soil samples from 0-30 cm (A and D), 30-60 cm (B and E), and 60-90 cm (C and F) for samples taken 18 days after harvest in 2021 (A, B, and C) and 2022 (D, E, and F) at the Danish site. BBFs are displayed with three letter acronyms and mineral references (Ref 1-4). N=4, except for PAL, MO13, ECO, and REF 2 (D, E, and F). Note different scales on the vertical axis.

The BBF soil mineral N content was positively correlated with the crop N uptake for both years, however, only significantly ( $p < 0.05$ ) in 2022 ( $R^2$ : 0.65) (Fig. IV-4). Thus, the soil mineral N content does not indicate that the BBF N release is non-synchronized with crop N demand. It indicates that the higher N mineralization rate leads to higher contents of mineral N in the soil. Therefore, either the crop did not efficiently utilize the available N or the mineralization continued in the period after the crop had stopped taking up N. It therefore seems that the risk of mineral N loss through leaching increases with increased fertilizer performance. Moreover, the correlation between soil mineral N and crop N uptake for the mineral references, only significant ( $p < 0.05$ ) in 2022 ( $R^2$ : 0.74) (Fig. IV-4), indicates that with an increased N application rate both the crop N uptake and the mineral N content in the soil increase. This is a known phenomenon that leads to increased risk of N loss with increased N application rate (Goldan et al., 2019).

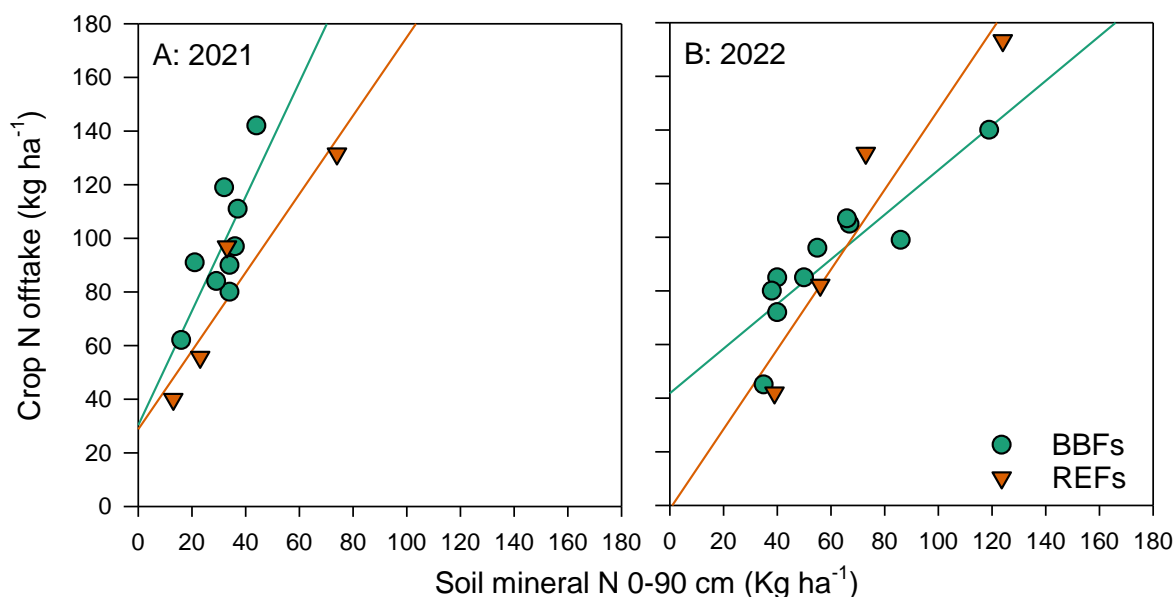


Fig. IV-4: Correlation between mean crop N offtake (N content in grain and straw at harvest) ( $\text{kg ha}^{-1}$ ) and mean soil mineral N (sum of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ ) ( $\text{kg ha}^{-1}$ ) of soil samples from 0-90 cm from Danish field trial in 2021 (A) and 2022 (B). The ten BBFs, nine in 2021, are shown by green-blue dots with a green-blue solid regression line. The four mineral N references are shown by orange triangles with a solid orange regression line.  $N=4$ .

#### ***Nitrogen loss risk score of BBFs***

Based on study I and Fig. IV-2, it seems that BBFs have a higher risk of gaseous ( $\text{NH}_3$  and  $\text{N}_2\text{O}$ ) N losses compared to mineral  $\text{NH}_4\text{NO}_3$  fertilizers. However, the mineral N content in the soil after harvest (Fig. IV-3) does not indicate that BBFs result in a higher risk of N leaching than mineral fertilizers. Table IV-1 summarizes the N loss risks from leaching,  $\text{NH}_3$  volatilization, and  $\text{N}_2\text{O}$  emissions.

The soil mineral N values shown in Table IV-1 were calculated as the average of the measurements in 2021 and 2022 of soil mineral N 18 days after harvest 0-90 cm for the ten BBFs and the four mineral references (data presented in Fig. IV-3). For the  $\text{NH}_3$  loss risk (Table IV-1), the accumulated  $\text{NH}_3$  volatilization potential on day 20 was selected. This was chosen, as the initial  $\text{NH}_3$  volatilization is more closely related to the initial properties of the BBFs (pH and  $\text{NH}_4^+\text{-N}$  content), whereas the  $\text{NH}_3$  volatilization in the later stages of the incubation seems to be related to N mineralization (Fig. IV-1). The  $\text{NH}_4^+\text{-N}$  mineralized from the organic N can, to a greater extent, be expected to be utilized by the growing crops in field conditions. Thus, 20 days was selected to best represent an intermediate time point comprising both the initial and the mineralization induced  $\text{NH}_3$  volatilization potential. The  $\text{N}_2\text{O}$  emissions (Table IV-1) are calculated as the mean of the accumulated daily  $\text{N}_2\text{O}$  flux, negative control subtracted, of the two moisture contents (60 % and 90 % of WHC) for the ten BBFs and a mineral reference.

The leaching loss score, ranging from 0-10, was calculated as the soil mineral N (Table IV-1) of each BBF divided by the highest BBF soil mineral N value (PAL) multiplied with 10. The  $\text{NH}_3$  loss score, ranging from 0-10, was calculated as the soil  $\text{NH}_3$  loss risk (Table IV-1) of each BBF divided by the highest BBF  $\text{NH}_3$  loss risk value (SDG) multiplied with 10. Finally, an N loss risk score was calculated as the sum of the leaching loss score and the  $\text{NH}_3$  loss score, omitting  $\text{N}_2\text{O}$  emissions, as only data for three BBFs was available.

The reference of  $\text{NH}_4\text{HCO}_3$  included in study I cannot be compared to the mineral references included in the studies of  $\text{N}_2\text{O}$  emissions and soil mineral N (study II). Therefore, no mineral reference is shown for the  $\text{NH}_3$  volatilization potential in Table IV-1. The risk of  $\text{NH}_3$  volatilization potential from mineral N fertilizers varies and is high for urea, for example, whereas the mineral N reference included in the other studies ( $\text{N}_2\text{O}$  study, study II, and study III) will have a low potential for  $\text{NH}_3$  volatilization.

The N loss risk score shows that the compost (BVC) had the lowest risk of N loss, whereas the digestate (SDG) had the highest risk. The two plant-based BBFs (PCW and PAL) had the highest leaching risks, though relatively low  $\text{NH}_3$  loss risks. This was contrary to the animal-based BBF (BIO), which had relatively low N leaching risk and relatively high  $\text{NH}_3$  volatilization risk.

Table IV-1: Sum of soil mineral N 18 days after harvest 0-90 cm, average of measurements in 2021 and 2022 for the ten BBFs and the four mineral references (soil mineral N). The accumulated  $\text{NH}_3$  volatilization potential (% of added N) on day 20 assessed in study I for the ten BBFs ( $\text{NH}_3$  loss risk). Accumulated daily  $\text{N}_2\text{O}$  flux, negative control subtracted, values are means of the two moisture contents (60 % and 90 % of water holding capacity) of the ten BBFs and a mineral reference ( $\text{N}_2\text{O}$  emissions). N loss score for leaching (soil mineral N (Nmin)) and  $\text{NH}_3$  volatilization potential ranking BBFs from 0-10, and a summarized score of Nmin and  $\text{NH}_3$  loss scores. Color shading from low (blue) to high (red) is used.

	BA6	BIO	BVC	ECO	FEK	MO13	OG2	PAL	PCW	SDG	Ref 1	Ref 2	Ref 3	Ref 4
Soil mineral N	37	37	26	44	52	42	30	119	65	49	26	40	53	99
$\text{NH}_3$ loss risk	6.7	16	1.7	13	18	6.5	7.5	8.7	0.0	36				
$\text{N}_2\text{O}$ emissions			1.3	1.7				3.2					0.1	
Leaching loss score	3.1	3.1	2.2	3.7	4.4	3.5	2.5	10	5.5	4.1	2.2	3.3	4.4	8.3
$\text{NH}_3$ loss score	1.9	4.3	0.5	3.7	5.0	1.8	2.1	2.4	0.0	10				
<b>Overall N loss risk score</b>	5.0	7.4	2.6	7.3	9.3	5.3	4.6	12	5.5	14				

## Agronomic nitrogen performance of BBFs

To determine the agronomic performance of field crops fertilized with BBFs I conducted study II in collaboration with partners in the LEX4BIO project. The results of study II will in this section be compared to data on  $\text{NH}_3$  volatilization from study I and data on N mineralization from (Agostini et al., submitted, Appendix IV). Moreover, I will in this section discuss the temporal effects on NFRV of the BBFs.

### *First-year mineral N fertilizer replacement value of BBFs*

For the first-year fertilization effect, the BBFs generally resulted in high yields comparable to the mineral N references at most of the sites. However, the BBFs resulted in somewhat lower relative yields compared to the mineral N references at the German site.

Across sites and years the average  $\text{NFRV}_{\text{AE}}$ , at the year of application, of all BBFs was 71%. Great variation was found in the  $\text{NFRV}_{\text{AE}}$  of 9% (BVC 2022 Denmark) to 113% (FR13 2021 France). The average  $\text{NFRV}_{\text{NUE}}$  based on the NUE of BBFs was 58%, and thus lower than the NFRV based on AE. Nine of the 18 BBFs included had high agronomic performances with an  $\text{NFRV}_{\text{AE}}$  above 75%, six had an intermediate  $\text{NFRV}_{\text{AE}}$  of 60-75% and four had a low  $\text{NFRV}_{\text{AE}}$  of 10-60%.

No consistency, across or within sites or across years, in regards to the ranking of the BBFs was found. Previously composts have been found to result in low agronomic performance (Chalk et al., 2013; Gutser et al., 2005; Ronga et al., 2019), which was confirmed in study II. Apart from the compost (BVC) resulting in low agronomic performance, no statements can be drawn on BBF agronomic performance based on the production technologies or source materials used for the BBFs. Additionally, the agronomic performance of BBFs were not found to correlate with BBF properties assessed (pH, total N,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , DM, total C content). This is in contrast to earlier findings by Zandvakili et al. (2019) who claimed that the release of plant-available N of TOFs is related to their C:N ratio.

### ***Second-year residual N fertilizer effects of BBFs***

A tendency of higher second-year residual N fertilizer effect for BBFs compared to the corresponding mineral reference was found at the French and Danish sites. However, at the German site the second-year residual N fertilizer effect tended to be lower than the corresponding mineral reference. Nevertheless, the residual fertilization effect on the second-year yield did not differ significantly between BBFs and the mineral N reference.

### ***Impact of crop, soil and climatic setting on BBF agronomic performance***

In study II no significant impact of soil type climatic setting (co-founded) or crop type on BBF agronomic performance of BBFs were found. This is contradictory to previous findings by Zavattaro et al. (2015) where TOFS were found to result in higher yields in cooler climatic conditions and on coarse textures soils. A tendency of the BBFs resulting in higher yields for the spring crops compared to winter crops was found. However, this was not significant and was confounded with other factors.

### ***Correlation between agronomic performance and ammonia volatilization potential***

Nitrogen loss may lead to decreases in the agronomic performance of a BBF if the N loss is substantial and occurs during the period where crops take up N. Therefore, one could expect there to be a correlation between the  $\text{NH}_3$  volatilization potential (study I) and the agronomic performance, particularly for surface-applied BBFs in study II. However, there were no general correlations between the potential  $\text{NH}_3$  volatilization and the agronomic performance of the ten BBFs included in all studies.

### ***Correlation between agronomic performance and N mineralization***

Synchronization of N release by mineralization of organic N and crop N uptake is crucial in order for BBFs to result in high crop yields (Chen et al., 2014). Pinto et al. (2020) found that N mineralization patterns of farmyard manure and green manure differed between a field trial and a laboratory incubation. Whereas, the authors found that mineralization patterns in the field trial and laboratory incubation were similar for a BBF composed of poultry manure, molasses and feathers. Thus, one would expect the BBF agronomic performance (study II) to be highly related to their N mineralization patterns (Agostini et al., submitted, Appendix IV). However, this was not the overall trend found for the BBFs included in this thesis when assessing the average agronomic performance across field sites and years.

Correlations between agronomic performance, represented by the NFRV based on agronomic efficiency ( $\text{NFRV}_{\text{AE}}$ ) and N mineralization, were better when only including data of  $\text{NFRV}_{\text{AE}}$  from single field sites. However, correlations were still not very good overall (Fig. IV-5). However, at the Danish site in 2021, where BBFs were incorporated, a good correlation was found between  $\text{NFRV}_{\text{AE}}$  and the late N mineralization (day 84) (Fig. IV-5B).

The fact that the correlations between  $\text{NFRV}_{\text{AE}}$  and N mineralization are better for each site, as opposed to the average  $\text{NFRV}_{\text{AE}}$  for all sites, could indicate that climate conditions, particularly soil moisture and temperature, were crucial for the mineralization of N in the field trials. It could also be related to differences in soil types. However, the effect of the site was not found to affect the BBF agronomic performance significantly in study II. Moreover, in Agostini et al. (submitted, Appendix IV), the mineralization of BBFs did not differ between soil types, except for the soil at the Danish site, which showed lower N mineralization compared to the other soils.

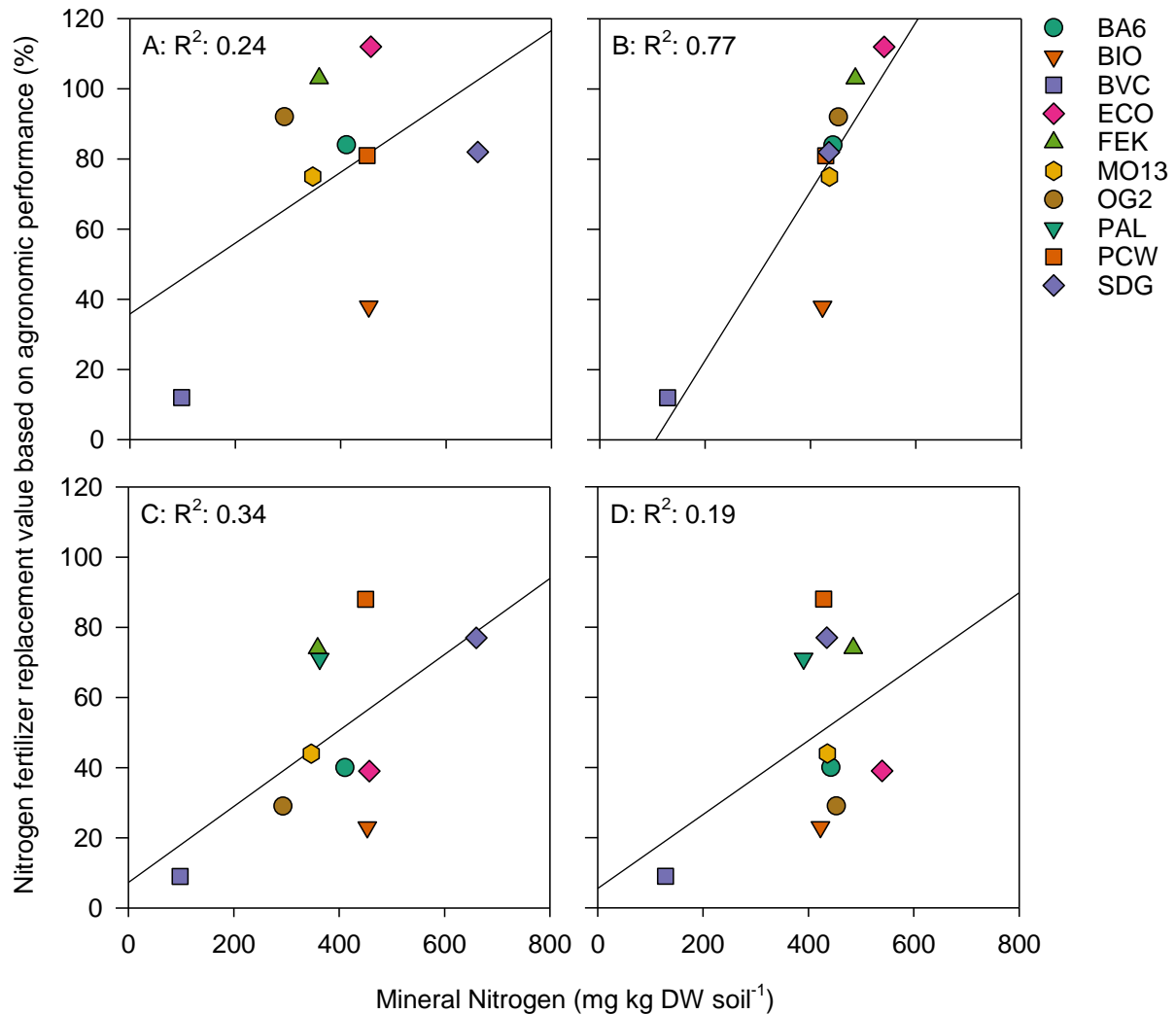


Fig. IV-5: Correlations between mean N fertilizer replacement value ( $\text{NFRV}$ ) based on agronomic efficiency (AE) at the Danish field site (study II) ( $N=4$ ) and mean mineral N ( $\text{mg kg DW soil}^{-1}$ ) (Appendix IV) ( $N=3$ ). A)  $\text{NFRV}_{\text{AE}}$  of 2021 and mineral N after 14 days of incubation. B)  $\text{NFRV}_{\text{AE}}$  of 2021 and mineral N after 84 days of incubation. C)  $\text{NFRV}_{\text{AE}}$  of 2022 and mineral N after 14 days of incubation. D)  $\text{NFRV}_{\text{AE}}$  of 2022 and mineral N after 84 days of incubation.

#### ***Short-term and long-term agronomic performance of BBFs***

The annual  $\text{NFRV}$  of TOFs increase with the duration of annual applications (Gutser et al., 2005; Zavattaro et al., 2015). This increase is higher for TOFs with low short-term  $\text{NFRV}$ , which may double in  $\text{NFRV}$  in the long term (Gutser et al., 2005). The higher the short-term  $\text{NFRV}$ , the lower the increase in annual  $\text{NFRV}$  in the long term (Gutser et al., 2005). The long-term  $\text{NFRV}$  of the BBFs studied in this

PhD have not been assessed. However, the second year residual fertilizer value of the BBFs, assessed in study II, was higher than the mineral reference at the same N application level for some BBFs at some sites. However, it was not significantly higher. Thus, the BBFs studied do not have a high short-term residual NFRV, but the long-term accumulated NFRV of BBFs with low short-term NFRV, e.g. the compost (BVC) could become substantial (Jensen, 2013a). This can be attributed to the accumulated nutrient release increasing with time, but it may also be attributed to the more indirect effect of improving soil quality and fertility with repeated application of BBFs. Westerik et al. (2023) argued that it is not possible to predict the long-term agronomic efficiency of BBFs and therefore, new long-term field experiments are necessary to determine the long-term NFRV of new BBFs.

### **Effects of BBFs on soil quality**

To evaluate the effect of BBF application on soil quality, I conducted study III where ten BBFs were applied to two different soils (an Arenosol and a Luvisol) at a standard and accelerated rate. In the following I will present and discuss a soil quality index that will condense the data presented in study III enabling easier comparisons between BBFs and a mineral N fertilizer reference.

#### ***The effect of application of BBF on soil biological, physical and chemical indicators***

In study III we found that the BBFs overall improved soil quality, despite the lack of significance in most cases. Soil quality indicators were in general affected by the accelerated application to a greater extent than the standard application. Soil quality was improved most by application of the compost (BVC) in general. Moreover, the plant-based BBF (PAL) and digestate (SDG) applications also improved soil quality to a greater extent, compared to the remaining BBFs.

Generally the accelerated application of BBFs increased SMB-C compared to the negative control, and to the greatest extent 14 days after application. These findings are in agreement with previous findings by Cai et al. (2016) and Pezzolla et al. (2013). The SMB-C was increased the most by application of the plant-based BBFs, (PCW and BA6), and the compost (BVC).

Previously, TOFs have been shown to improve soil physical quality indicators (Sanden et al. 2018). In study III, the BBF application led to an increase in soil structural stability on the Luvisol, but not the Arenosol where the apparent effect was the opposite, with BBF application decreasing soil structural stability. Only the accelerated compost (BVC) treatment increased WHC significantly compared to the negative control. Composts ability to increase soil water content has previously been demonstrated by Gómez-Muñoz et al (2017).

In agreement with previous studies (D'Hose et al. 2014; Sanden et al. 2018) the compost (BVC) increased soil pH compared with the negative control. Soil cation exchange capacity (CEC) was not affected substantially by BBF application. However, the poultry manure-based BBF (FEK) showed a tendency of increasing CEC which agrees with previous findings by Lin, van Santen, and Watts (2016). Total soil C was increased to the greatest extent by application of the compost (BVC), while the potato cell water (PCW) application did not increase soil total C content. Increase in soil total C has previously been found for TOFs van der Bom et al. (2019). Moreover, in agreement with the findings in study III, Sanden et al., (2018) found greatest increase in soil total C from application of compost relative to other TOFs. The increase in mineral-associated organic matter (MAOM) and decrease in particulate organic matter (POM) during the year of incubation in study III indicates that C has been stabilized in most BBF treatments.



### Comparison of BBF annual long-term application and a single large dose application

The long-term effects from application of compost (BVC) on the soil quality indicators pH, CEC, WHC and total C could be predicted in the laboratory. Whereas, the soil quality indicators related more to microbial activity and soil management were not well predicted in the laboratory incubation. However, the results of the study do not necessarily apply for the other studied BBFs which contain less stable carbon than BVC.

### Soil quality score

In order to be able to compare the BBFs with each other and the mineral N fertilizer reference in terms of overall effect on soil quality indicators, a soil quality score was calculated. A description of how the soil quality score was calculated is provided in Appendix V. Indication of whether treatments increased or decreased the assessed soil quality indicators, relative to the negative control, is shown in Table IV-2.

In study III, it was concluded that the compost (BVC) was the BBF that improved the soil quality indicators to the greatest extent. This is also reflected in the soil quality score. Furthermore, the plant-based BBF (PAL) and the digestate (SDG) were also highlighted as particularly increasing soil quality in study III, which is again confirmed by the soil quality score.

Table IV-2: Indications of change in soil quality indicators by treatments relative to the negative control for each soil quality indicator assessed in study III. The indications of increase are marked by blue shaded upward facing arrows, decreases are indicated by red shaded downward facing arrows, and un-changed indicators are marked by light color shaded horizontal arrows. Significant increases/decreases are shaded in dark colors while non-significant increases/decreases are shaded in light colors. Horizontal arrows indicate no change relative to the negative control. The calculated soil quality score is shown on the bottom line and color graded from low (red) to high (blue).

		BA6	BIO	BVC	ECO	FEK	MO13	OG2	PAL	PCW	SDG	MIN
CEC	365	↓	↓	↑	↓	↑	↓	↓	↑	↓	↑	↓
SMB-C	14	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
pH	365	↓	↓	↑	↓	↑	↓	↓	↓	↑	↓	↑
Total C	365	↑	↑	↑	↑	↑	↑	↑	↑	↔	↑	↑
WHC	365	↑	↓	↑	↓	↓	↓	↔	↑	↑	↑	↓
Soil structure	365	↓	↓	↑	↓	↓	↓	↑	↑	↓	↑	↑
C size fractions	14 - 365	↑	↓	↑	↓	↑	↓	↑	↑	↓	↑	↓
Soil quality score		1.5	0	4.5	-0.5	2	-0.5	1	3	0	3	-0.5

Generally, the animal-based BBFs (ECO, MO13, BIO, and OG2) are at the lower end of the soil quality score together with the mineral reference (MIN) ranging with a score of -0.5 to 1. Thus, the animal-based BBFs only have relatively minor to no effects on soil quality (Table IV-3). The plant-based, poultry manure, digestate, and compost are found at the higher end of the soil quality score. Thus, most BBFs improved soil quality to a greater extent compared to the mineral reference, though a few had a similarly low effect as the mineral reference. Compared to applying mineral N fertilizers, this study indicates that applying BBFs will therefore have a neutral to positive effect on soil quality.

Table IV-3: Soil quality score color graded from low (red) to high (blue) for the ten BBFs and the mineral reference (MIN). The group to which the BBFs were assigned, based on a more traditional classification (study I), is shown with color coding for each group.

	MIN	ECO	MO13	BIO	PCW	OG2	BA6	FEK	PAL	SDG	BVC
Soil quality score	-0.5	-0.5	-0.5	0	0	1	1.5	2	3	3	4.5
Group	Reference	Animal based	Animal based	Animal based	Plant based	Animal based	Plant based	Poultry manure	Plant based	Digestate	Compost

### Overall most favorable BBFs

To be able to draw some general conclusions about the effect of the BBFs across the three studies included in this PhD, the main results from each study were summarized in Table IV-4. The N loss risk score (presented in Table IV-1) was used to represent the combined risk of NH<sub>3</sub> volatilization (study I) and N leaching (Fig. IV-3). No value is shown for the N loss score for the mineral reference in Table IV-4. This is due to the fact that the mineral N reference included in studies II and III was not included in study I. To represent the agronomic performance of the BBFs, the average NFRV<sub>AE</sub> for all sites and both years was selected for the seven common BBFs tested at all sites and for both years at the Danish site for the three local BBFs. To represent the effect of the BBFs and the mineral reference on soil quality, the soil quality score (presented in Table IV-2) was selected to sum up the overall effect.

Table IV-4: The N loss score based on NH<sub>3</sub> volatilization potential and soil mineral N after harvest (Table IV-1). Average NFRV<sub>AE</sub> (%) for all sites and both years (BA6, BIO, ECO, FEK, MO13, OG2, and PAL) and for both years at the Danish site (BVC, PCW, and SDG). Soil quality score taken from Table IV-2. Numbers are color shaded from low (blue) to high (red) for NH<sub>3</sub> volatilization potential and from low (red) to high (blue) for NFRV<sub>AE</sub> and soil quality indicators.

	BA6	BIO	BVC	ECO	FEK	MO13	OG2	PAL	PCW	SDG	MIN
N loss score	5	7	3	7	9	5	5	12	5	14	-
Agronomic performance	64	70	11	72	79	65	64	88	84	79	100
Soil quality score	1.5	0	4.5	-0.5	2	-0.5	1	3	0	3	-0.5

The plant-based BBF (PAL), the digestate SDG, and the poultry manure BBF (FEK) are the BBFs with the highest N loss risk scores (Table IV-4). All these three BBFs have a high agronomic performance, with PAL having the highest of all ten BBFs (NFRV<sub>AE</sub> of 88 %). Furthermore, these three BBFs were also among the BBFs that improve soil quality to the greatest extent, only exceeded by the compost (BVC). So, for these three BBFs, it seems that there is a trade-off, as they are beneficial for yield and soil quality, but lead to high N loss risks.

A somewhat opposite trade-off trend was found for the potato cell water (PCW), which had a low N loss score and a high agronomic performance. However, it did not have a substantial effect on soil quality based on the soil quality score.

The animal-based BBFs BIO and ECO have an N loss score of 7, which is slightly higher than the average of the ten BBFs. They have an average agronomic performance with a NFRV<sub>AE</sub> of 70 % (BIO) and 72 % (ECO). However, they are among the BBFs that did not improve soil quality based on the soil quality score.

The two animal-based BBFs (MO13 and OG2) and the plant-based BBF (BA6) all had relatively low N loss risk scores of 5. They all resulted in agronomic performance at the lower end for the ten BBFs included in all studies with  $\text{NFRV}_{\text{AE}}$  of 64-65 %. Moreover, they were at the lower end of the BBFs regarding the soil quality score (OG2 and BA6) and MO13 did not improve soil quality.

The compost (BVC) has the lowest N loss score and it was the BBF that improved soil quality indicators to the greatest extent. However, it was also the BBF that performed poorest among those studied in the field trial with an average  $\text{NFRV}_{\text{AE}}$  of only 11%. Thus, for the BVC, there was a clear trade-off between soil quality improvement and agronomic performance. Composts are also mainly used by farmers as soil improvers for SOM build-up and soil quality improvements (Veeken et al., 2018), which is very much in line with the findings in this PhD.

Sanden et al. (2018) conducted a meta-analysis of long-term field studies on the effects of management practices (tillage, crop rotations, and TOFs (farmyard manure, slurry, composts, and crop residues)) on soil quality, yield, and greenhouse gas (GHG) emissions. They found that none of the management practices could benefit all of the assessed parameters, i.e. reducing GHG emissions, improving soil quality, and resulting in high yields. They found that soil quality was improved and yield reduced from the use of traditional organic fertilizers. This general trade-off trend cannot be confirmed based on the data presented in this PhD. It was the case for the compost (BVC), but not for the remaining BBFs. Furthermore, for the potato cell water (PCW), the trade-off was even the opposite of this as observed for the BVC. Thus, PCW was more comparable with the mineral reference fertilizer with high agronomic performance and no effect on soil quality.

Regarding high agronomic performance, which is an important aspect for farmers, FEK, PAL, PCW, and SDG ( $\text{NFRV}_{\text{AE}}$  of 79-88 %) can be said to be the most favorable in this regard. Moreover, all of these BBFs, except PCW, have positive effects on soil quality. However, for all of them, except PCW, N loss risk is an issue. However, it does not seem to be higher than for the mineral reference for N leaching risk and only SDG has a substantial  $\text{NH}_3$  volatilization risk. To highlight just one BBF as the most favorable fertilizer, the most obvious choice seems to be PAL, as it has the highest agronomic performance: a positive effect on soil quality and its N loss risk is mainly based on leaching risk, which is not higher than for its mineral counterpart. The compost (BVC) is, without a doubt, the best soil improver and the least effective fertilizer, whereas the remaining, mainly animal-based BBFs, all rank somewhere in between, although they resemble fertilizers, not soil amendments/improvers.

## **Comparison of novel BBFs with TOFs**

This section will compare the agronomic performance and impacts of TOFs and novel BBFs based on common source materials and, for some, also processing technology.

### ***Poultry manure***

When comparing previous findings for dried poultry manure with the poultry manure-based BBFs in study I, the  $\text{NH}_3$  volatilization was similar. However, when comparing raw poultry slurry in previous findings with the dried poultry manure-based BBFs in study I, the pattern and amount of  $\text{NH}_3$  volatilization is different with higher and more initial  $\text{NH}_3$  volatilization for the raw poultry slurry (study I).

Compared to earlier findings on the  $\text{NFRV}_{\text{NUE}}$  of non-processed poultry manure ranging from 50%-65% (Jensen, 2013a), the dried and pelletized poultry manure-based BBFs included in study II (FEK and FEL) resulted in higher  $\text{NFRV}_{\text{NUE}}$  of 58%-80%. The findings in study II are more in line with the findings of Gutser et al. (2005) for dried poultry excrements with  $\text{NFRV}_{\text{NUE}}$  of 60-70 %. Thus, drying

of poultry manure might increase its agronomic performance. Lin et al. (2016) did a meta-analysis on poultry manure and found that poultry manure resulted in lower yields compared to mineral fertilizers for field studies of 1-2 years' duration, whereas poultry manure resulted in higher yields compared to mineral fertilizers for field studies with durations of 3-13 years.

Moreover, Lin et al. (2016) found that poultry manure increased soil C content, CEC, and pH compared to mineral fertilizers. This is in agreement with the findings of study III, where the poultry manure-based BBF (FEK) had a soil quality score above average for the ten BBFs (Table IV-2).

### ***Digestates***

Ammonia volatilization is generally higher from application of digestates compared to raw manures due to the increase in  $\text{NH}_4^+$  content and pH during anaerobic digestion (Nkoa, 2014). Contrarily,  $\text{N}_2\text{O}$  emissions are generally lower from application of digestates compared to raw manures possibly due to the lower content of easily degradable OM in digestates compared to raw manures (Nkoa, 2014). The digestates included in study I were also the group with the highest  $\text{NH}_3$  volatilization potential.

Digestates generally have an NFRV 10-15 % higher than their raw source material (Jensen et al., 2020b). The high agronomic performance of digestates is associated with their high  $\text{NH}_4^+$ -N content (Alburquerque et al., 2012). The digestate (SDG) included in study II at the Danish site had an average  $\text{NFRV}_{\text{NUE}}$  of 72 %. This is thus in the range and at the high end of the  $\text{NFRV}_{\text{NUE}}$  of 40-70 % reported for digestates in Gutser et al. (2005).

### ***Plant-based***

Plant-based commercial BBFs are a diverse group. Of traditional organic fertilizers, green manures are maybe the most common plant-based type. Gutser et al. (2005) reported  $\text{NFRV}_{\text{NUE}}$  for green manures to range from 10-40 % and brewery and distillery residues 25-35 %. In study II, five plant-based BBFs were included and their  $\text{NFRV}_{\text{NUE}}$  ranged from 55–86 % and was thus higher than both green manures and brewery and distillery residue values reported in Gutser et al. (2005).

### ***How BBFs differ from traditional organic fertilizers***

The comparison shows that the processing of the raw source materials can increase the agronomic performance. This is in line with Sradnick and Feller (2020), who found that plant- and animal-based commercial organic fertilizers had high net N mineralization, whereas slurries and composts were dominated by immobilization. Moreover, it seems that the processing can decrease the risk of  $\text{NH}_3$  volatilization for some of the source material groups and increase it for others, namely anaerobic digestion. However, the comparisons are complicated by the mixing of different source materials for many of the novel BBFs.

### **Practical implications of BBF use**

This PhD study has focused on the agronomic efficiency, N loss risks and effects on soil quality from the use of BBFs. However, other more practical aspects of BBF use are also important to consider when evaluating their use.

According to data from surveys of various stakeholders from four European countries, Hou et al. (2018) found that governmental pressure, in the form of regulations, is the key driver of BBF production.

However, farmers lacking knowledge, confidence, and acceptance with respect to BBFs could hinder their use despite ambitious political targets set by the EU (Hidalgo et al., 2020).

### ***Transportability, storage and spreadability of BBFs***

One aspect that could hinder the use of BBFs across regions is their transportability. The higher the nutrient concentration of a BBF, the further it can be transported without the costs exceeding the fertilizing value (Jensen, 2013b). As an example, digestate can be tradeable fertilizer in a local context, as the transport costs will otherwise exceed the fertilizer value (Jensen, 2013b). Thus, converting waste- and side-streams with low nutrient concentrations into BBFs with high nutrient concentrations could enable trade across European regions (Tur-Cardona et al., 2018).

All of the commercial, non-local BBFs studied in this PhD are dried and pelletized. This greatly reduces the amount of product to be transported and handled for many of the BBFs. Moreover, it increases the durability of the products and they can be produced when the source material is there and then kept until used. This combats the issues of storing animal manures, which is related to large impacts on the environment and losses of nutrients, e.g. through  $\text{NH}_3$  volatilization during storage (Hirel et al., 2011). Furthermore, the pelletized form makes it easy for farmers to apply them to their fields using the same machinery as for spreading mineral fertilizers. In a survey Postma et al., (2020) found that, granular BBFs were preferred over liquid, powder and paste textured BBFs by both farmers and advisors in North West Europe. However, the temperature and moisture content of bio-wastes and side-streams during pelletizing are crucial for the physical properties of the BBF produced, determining whether they can be used by conventional mineral spreading machines (Brod et al., 2018).

### ***Economic price***

Another aspect of farmers' willingness to use a given fertilizer is its price. In the EU, the fertilizer price is the most important operational price, regarding total production, for some crops (Buysse and Cardona, 2020). Traditional organic fertilizers have often been valued less than their actual nutrient values and sometimes the receiving party has even been paid to receive them (Tröster, 2023). The NUE is responsible for the price farmers are willing to pay for BBFs (Tröster, 2023). Based on a discrete choice experiment in seven European countries, Tur-Cardona et al. (2018) concluded that BBFs with similar properties as mineral fertilizers should be sold at a price that is 65 % lower than mineral fertilizers if farmers were to choose them. However, the BBF industry currently has higher production costs compared to the mineral fertilizer industry (Buysse and Cardona, 2020). This is due to high manufacturing costs and underdevelopment of the industry, as it is very new (Fava et al., 2015). However, the price of input materials is low to none (Fava et al., 2015) and, thus, with development of the industry, prices of BBFs would be expected to decrease. Moreover, the high prices of BBFs could be offset by economic interests in environmentally friendlier alternatives to mineral fertilizers by political regulations, e.g. taxation (Fava et al., 2015). However, no policies have currently confronted the issue of the price of BBFs (Kurniawati et al., 2023).

### ***Nutrient contents of BBFs***

Processing of the source materials into high nutrient concentrated BBFs may also lead to losses of nutrients and environmental and climate impacts. Nutrient losses vary substantially between different processing technologies, as described in the background chapter. Moreover, the energy consumption from processing of BBFs is important to account for when evaluating BBFs.

Another feature that is important for farmers is the ratio of nutrients in a given fertilizer. Often, organic waste- or side-streams do not contain nutrients in ratios appropriate for crops. This is a drawback of

TOFs when compared to mineral fertilizers. However, with the highly processed BBFs, it is possible to combine different organic source materials, or supplement mineral nutrients, to comply with appropriate nutrient ratios. This is an advantage of BBFs compared to raw organic waste- or side-stream materials, e.g. animal manure.

Another aspect of farmers' willingness to use a given fertilizer is its reliability to supply nutrients. Many farmers perceive manure as an unreliable nutrient source, particularly regarding N (Schröder, 2005). Tur-Cardona et al. (2018) found that the stability of the N content was a determining factor regarding whether or not farmers are willing to use BBFs. The lack of consistency in individual BBFs' performance across sites and years (study II) and the lack of general correlations between N mineralization and agronomic performance highlight that there are still some things to uncover regarding the reliability of BBF agronomic performance.

### ***Application technique***

One aspect of BBFs' reliability to supply nutrients is likely related to the application technique. Based on the results of the study on NH<sub>3</sub> volatilization potential (study I), it is generally a good idea to incorporate the BBFs and this should always be done for digestates. Furthermore, some BBFs are not very prone to NH<sub>3</sub> volatilization and these could therefore, in this regard, be surface applied. However, based on the results obtained in the field study (study II), there was a tendency for the agronomic performance of the BBFs to be lower for the dry BBFs when surface applied compared to incorporated. Based on this, it therefore seems recommendable to incorporate BBFs of all types. However, problems arise with winter crops, where we currently do not have appropriate techniques for the incorporation of fertilizers in the spring. The plants are prone to root damage when incorporating BBFs in growing crops, which can counteract the benefits of incorporation over surface application (Schröder, 2005).

### ***Different farming systems' perception of BBFs***

As described above, many barriers could still potentially hinder the widespread use of BBFs in conventional farming systems. However, the benefits of BBFs over TOFs might promote their use in other farming systems. Usually, OM matter is not highly valued by conventional farmers, whereas private gardeners and vineyard owners, among others, appreciate it (Jensen, 2013b). The combined effect of a reasonably high fertilizer efficiency, and OM input, with some of the BBFs studied in this PhD may therefore make them popular in specific farming systems. Certified organic BBFs may be used by organic farmers, who currently lack N fertilizers in particular (Øvsthus et al., 2017). Also, with political targets to increase the share of organic agriculture in Europe (EC, 2023), this could increase the use of BBFs. However, currently, BBFs need to appeal to conventional farmers, if they are to be widely used.

## Conclusion

Biobased fertilizers showed great variation in  $\text{NH}_3$  volatilization potential, both in terms of temporal patterns and accumulated amount of  $\text{NH}_3$  volatilized. However, the  $\text{NH}_3$  volatilization potential could be effectively reduced when BBFs were incorporated into soil. Ammonia volatilization potential also varied greatly depending on soil type with the highest potential observed for the coarsest textured soil. If the pure mineral BBFs were excluded, the  $\text{NH}_3$  volatilization potential was correlated with the N mineralization and the correlation improved with increasing incubation time. The BBFs seem to have a higher risk of  $\text{N}_2\text{O}$  emissions compared to mineral ( $\text{NH}_4\text{NO}_3$ ) fertilizers. Moreover, the  $\text{N}_2\text{O}$  emissions seem to increase with an increasing mineralization rate of the BBFs. Based on the soil mineral N (sum of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ ) content 18 days after harvest, the BBFs do not seem to be more prone to N loss through leaching. Moreover, the risk of N leaching loss seems to increase with increased agronomic performance of the BBFs. Thus, overall, BBFs seem to have a higher risk of gaseous N loss ( $\text{NH}_3$  and  $\text{N}_2\text{O}$ ), but similar risk of N leaching loss, compared to mineral ( $\text{NH}_4\text{NO}_3$ ) fertilizers.

Most BBFs performed equally well as the mineral reference in terms of yield in the field experiments. The 18 BBFs had a high average  $\text{NFRV}_{\text{AE}}$  of 71 %, though it varied greatly. The second year residual fertilizer value of the BBFs, tended to be higher than the mineral reference at the same N application level for some BBFs at some sites, though not significantly higher. The agronomic performance of the individual BBFs differed among the field sites. This could not be correlated to soil type or climate conditions. Thus, crop type, soil type and climatic setting was not found to affect the BBF agronomic performance significantly. The BBF agronomic performance ( $\text{NFRV}_{\text{AE}}$ ) could generally not be predicted based on their N mineralization. However, correlations between N mineralization and  $\text{NFRV}_{\text{AE}}$  improved slightly when accessing the individual sites and years, as opposed to the average  $\text{NFRV}_{\text{AE}}$  across sites and years. Moreover, the  $\text{NH}_3$  volatilization potential of BBFs did not correlate with their  $\text{NFRV}$ . When comparing the BBFs with TOFs based on source materials, the BBFs generally have higher agronomic performance.

Application of the BBFs studied had the ability to improve soil quality indicators overall, though not significantly in most cases. In general the accelerated application rate of BBFs had a greater effect on soil quality indicators compared to the standard application rate. Moreover, the BBFs differed in their ability to improve soil quality indicators with the compost (BVC) improving soil quality to the greatest extent, followed by the plant-based (PAL) and the digestate (SDG). However, most animal-based BBFs and the potato cell water (PCW) did not improve soil quality and thus had similar effects on soil quality as the mineral reference. The effects on soil quality indicators were compared between a single accelerated application of compost (BVC), in the laboratory incubation, with a standard annual application in the long-term field trial. The comparison suggests that the laboratory incubation could mimic the effects on soil quality of long-term yearly field application for WHC, pH, CEC and total C. However, this does not necessarily apply for other BBFs containing less stable carbon compared to BVC.

Overall, the poultry manure BBF (FEK), the plant-based BBFs (PAL and PCW), and the digestate (SDG) were assessed to be the most favorable BBFs, whereas the compost (BVC) is the most favorable soil improver. Biobased fertilizers overall seem to be promising substitutes for mineral N fertilizers. However, the underlying mechanisms for BBF behavior were not fully uncovered and more knowledge is needed to understand and predict their behavior. Moreover, for some BBFs, certain measures should be taken in order to optimize their use, namely incorporation to avoid  $\text{NH}_3$  volatilization, and potentially increase agronomic performance.

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## Appendix I

*Supplementary material for*

### **Potential ammonia volatilization from 39 different novel biobased fertilizers applied to soil – A laboratory study using European soils**

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Tables I-A1 to I-A7

## Effect of homogenizing biobased fertilizers

The effect of homogenizing three different biobased fertilizers (BBFs) was tested on the Acidic sandy soil. A linear mixed model and pairwise comparisons of least square means carried out individually for each BBF over the entire incubation period showed that there was no significant difference in accumulated  $\text{NH}_3$  volatilization between homogenization and non-homogenization for any of the BBFs (Fig. I-A1).

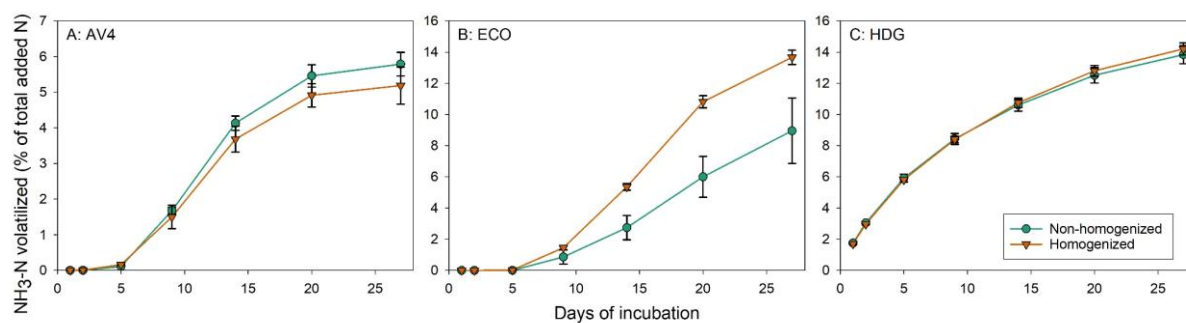


Fig. I-A1. Accumulated  $\text{NH}_3$ -N volatilized (% of total added N)  $\pm$  standard error during 27 days of incubation from biobased fertilizers surface applied to the Acidic sandy soil at an application rate of  $0.72 \text{ g N kg}^{-1}$  DW soil and a soil moisture content at 60 % of WHC. Note different scales on vertical axes. N = 3.

## Titration vs colorimetric quantification of $\text{NH}_3$

In a pre-trial, titration using boric acid traps was tested against the colorimetric method using sulfuric acid traps and flow injection analysis. This was done using  $\text{NH}_4\text{HCO}_3$  applied to Acidic sandy soil. The results showed no differences between the two methods (Fig. I-A2). The colorimetric method was chosen, as it requires less work, can detect lower concentrations of  $\text{NH}_3$  and has less operator variability.

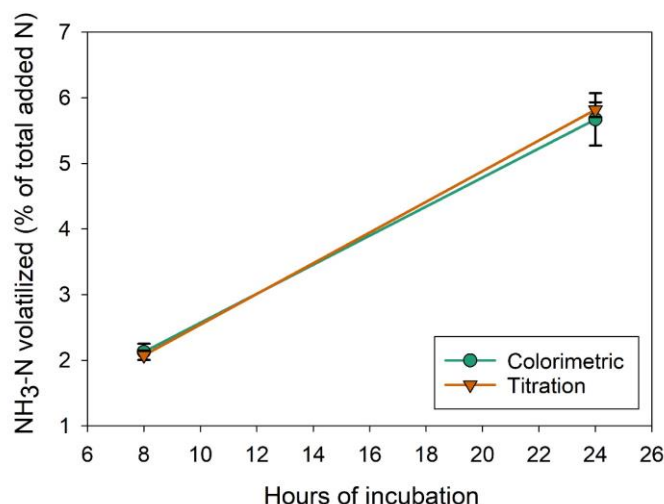


Fig. I-A2: Accumulated  $\text{NH}_3$ -N volatilized (% of total added N)  $\pm$  standard error during 24 hours after surface application of  $\text{NH}_4\text{HCO}_3$  to the Acidic sandy soil. Turquoise round symbols represent data where the colorimetric method was applied, whereas orange triangle symbols represent data where titration was applied. N = 3.

### **NH<sub>3</sub> volatilization potential related to grouping of biobased fertilizers**

The grouping of BBFs based on more traditional grouping criteria is presented in Fig. I-A3 and I-A4.

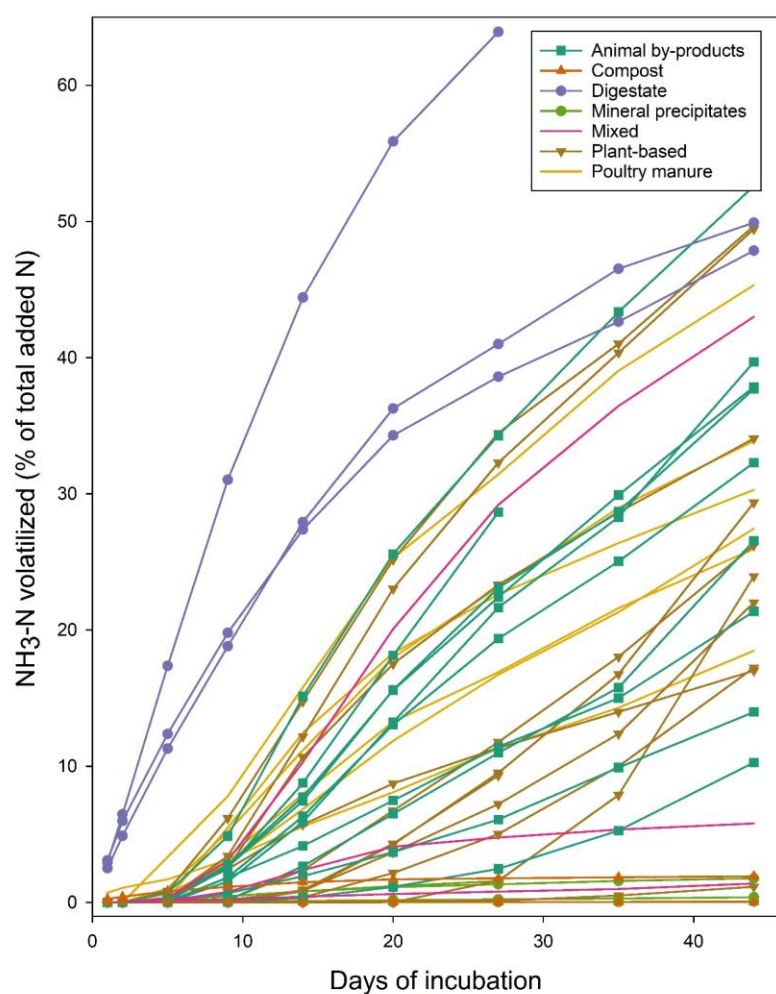


Fig. I-A3: Accumulated NH<sub>3</sub>-N volatilized (% of total added N) during 27/44 days of incubation from 39 different biobased fertilizers. Values are means, N=2. The lines are colored according to the group to which they were assigned.

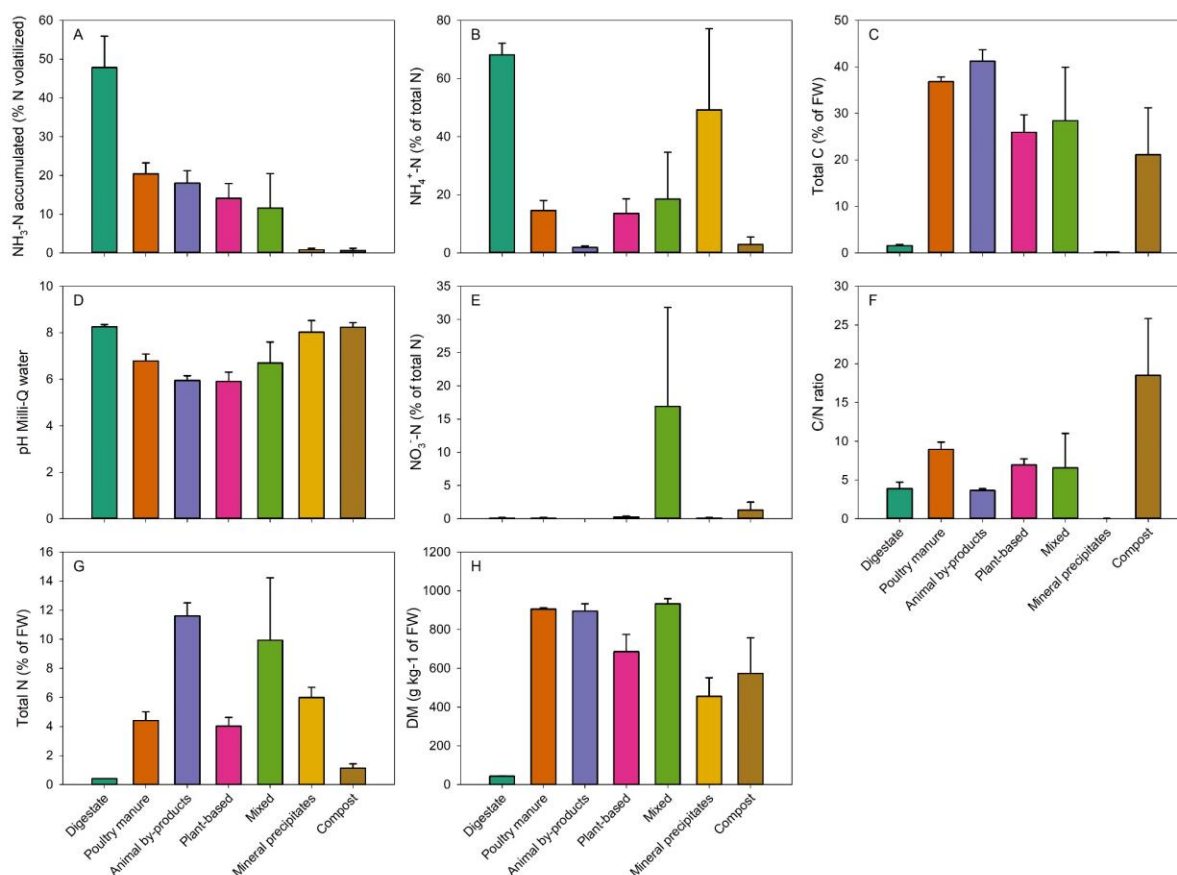


Fig. I-A4: A: The accumulated  $\text{NH}_3$  volatilization on day 27 for the different groups and the BBF characteristics of the different groups (B:  $\text{NH}_4\text{-N}$ , C: Total C content, D: pH, E:  $\text{NO}_3\text{-N}$ , F: C/N ratio, G: Total N content, and H: dry-matter (DM) content). Values are means  $\pm$  SE. No = 3 (digestates, mixed, and compost), no = 4 (mineral precipitate), no = 6 (poultry manure), no = 10 (animal by-products), and no = 11 (plant based).



Table I-A1: BBF acronyms (three letters/numbers), full product names, and name of manufacturer.

<b>BBF</b>	<b>BBF</b>	<b>Manufacturer's name</b>
ASL	ASL UHOH	NA *
AV4	Arvo 4-1-3-1	Biolan Oy (marketed by Novarbo Oy)
AV8	Arvo 8-1-5-2	Biolan Oy (marketed by Novarbo Oy)
BA6	Bioagenasol	Agrana, A
BIH	Biorga Hornmeal	Hauert, CH
BIL	Biolan luonnonlannoite	Biolan Oy
BIO	Bio 8-4-2 yara	YARA, FI
BIP	Bioplant	Bioplant
BLM	Blood meal Spain	NA *
BO1	Boost NPK	Soilfood Oy
BO2	Boost NK	Soilfood Oy
BO4	Boost NKS	Soilfood Oy
BVC	Biovækst compost	Biovækst
CGR	Crystal green ostara struvite	Ostara
ECO	Ecolan	Ecolan Oy
FEK	Fertikal	Fertikal, BE
FEL	Fertilex	Fertilex Oy
GRF	Digestate UHOH	NA *
HDG	Hashøj digestate	Hashøj biogas
ILF	Ilsadrip forte	Ilsagroup
MAL	Maltaflor BIO	MALTaflor
MB2	Biorga vianos	Hauert, CH
MO13	Monterra bio 13-0-0	MeMon, NL
NAD	Nadu	Agro Energie Hohenlohe GmbH & Co KG, DE
NE4	Neko luonnonlannoite	Biofert /AU (Distribution in Finland by NEKO OY)
NE7	Neko luomu NPK 6-1-3 + Bact	DCM /Belgium (Distribution in Finland by NEKO OY)
OG1	Øgro 10-3-1	Daka
OG2	Øgro N14	Daka
OOC	Olive oil compost	NA *
OPU	Optisol universal	Optisol, CH
PAL	Palaterra	Palaterra Betriebs- und Beteiliungsgesellschaft mbH, DE
PCS	Århus vand struvite	Århus vand
PCW	AKV K2	AKV
PRI	Primax kemisor 16-4-10	MeMon, NL
SDG	Solrød digestate	Solrød Biogas
SIF	Siforga bio 10-3-1	MeMon
SYS	Systemic Scrubber water	SYSTEMIC biogas plant
TRS	Triple S	Sopropeche
VEC	Vermicompost	NA *

\* NA: Unknown manufacturer.

Table I-A2: Statistical models and tests with input fixed effects, interactions, and random effects for each experiment.

	<b>Model and test</b>	<b>Response variable</b>	<b>Fixed effects</b>	<b>Interactions</b>	<b>Random effects</b>
Exp. 1: Differences between CMCs and PFCs	Lmer + lsmeans comparisons	Sqrt(NH <sub>3</sub> )	CMC; PFC; pH; NH <sub>4</sub> ; NO <sub>3</sub> ; DM; C:N; time; I(time <sup>2</sup> )	CMC*time; PFC*time; pH*time; NH <sub>4</sub> *time; NO <sub>3</sub> *time; DM*time; C:N*time	BBF; Sample number
Exp. 1: Differences between BBF groups	Lmer + lsmeans comparisons	Sqrt(NH <sub>3</sub> )	BBF group; pH; NH <sub>4</sub> ; NO <sub>3</sub> ; DM; C:N; time; I(time <sup>2</sup> )	BBF group*time; pH*time; NH <sub>4</sub> *time; NO <sub>3</sub> *time; DM*time; C:N*time	BBF; Sample number
Exp. 1: Initial BBF properties' ability to explain BBF NH <sub>3</sub> volatilization potential	Lmer + correlation coefficients and R <sup>2</sup> retrieved from model	Sqrt(NH <sub>3</sub> )	pH; NH <sub>4</sub> ; NO <sub>3</sub> ; DM; C:N; time; I(time <sup>2</sup> )	pH*time; NH <sub>4</sub> *time; NO <sub>3</sub> *time; DM*time; C:N*time	BBF; Sample number
Exp. 2: Effect of application method	Lmer + lsmeans comparisons	Sqrt(NH <sub>3</sub> )	Application; Media; time; I(time <sup>2</sup> )	Application*Media; Application*Day; Media*Day	Sample number
Exp. 3: Effect of application rate	Lmer + lsmeans comparisons	Sqrt(NH <sub>3</sub> )	Application rate; time; I(time <sup>2</sup> )	Application rate*time	Sample number
Exp. 4: Effect of soil type	Lmer + lsmeans comparisons	Sqrt(NH <sub>3</sub> )	Soil; time; I(time <sup>2</sup> )	Soil*time	Sample number
Exp. 4: Soil properties' ability to explain BBF NH <sub>3</sub> volatilization potential	Lmer + correlation coefficients and R <sup>2</sup> retrieved from model	Sqrt(NH <sub>3</sub> )	pH; OM; Clay; time; I(time <sup>2</sup> )	pH*time; OM*time; Clay*time	BBF; Sample number
Exp. 5: Effect of soil moisture level	Lmer + lsmeans comparisons	Sqrt(NH <sub>3</sub> )	Soil moisture; time; I(time <sup>2</sup> )	Soil moisture*time	Sample number
Effect of homogenization	Lmer + lsmeans comparisons	Sqrt(NH <sub>3</sub> )	Homogenized or not; time; I(time <sup>2</sup> )	Homogenized or not*time	Sample number

Table I-A3: BBF groups, CMCs, and PFCs which were significantly different from each other based on a linear mixed model including all time points. No. of groups = 7, no. of CMCs = 6, and no of PFCs = 6.

Group, CMC, and PFC			p-value	Estimates of least square means*
Animal by-product	≠	Digestate	0.002	-3.46
Animal by-product	≠	Mineral extract	0.018	2.16
Compost	≠	Digestate	0.002	-3.19
Compost	≠	Mineral extract	0.031	2.43
Digestate	≠	Mineral extract	<0.001	5.62
Digestate	≠	Mixed	0.014	3
Digestate	≠	Plant based	0.002	3.13
Digestate	≠	Poultry manure	0.009	2.76
Mineral extract	≠	Mixed	0.010	-2.62
Mineral extract	≠	Plant based	0.003	-2.49
Mineral extract	≠	Poultry manure	0.0004	-2.87
CMC 5	≠	CMC 3	0.007	-3.95
CMC 5	≠	CMC 4	0.002	-3.65
CMC 5	≠	CMC 6	0.037	2.47
CMC 5	≠	CMC 10	0.024	-2.89
PFC 1 C-II	≠	PFC 1 A-I	0.033	3.58
PFC 1 C-II	≠	PFC 1 B-I	0.034	3.75

\* Estimates of differences in least square means are square-root transformed.

Table I-A4: pH (1:5 in Milli-Q water) of incubated samples from Experiment 1 at the end of the experimental period (day 27 or 44). Samples were mixed before a subsample was taken out for pH measurement. All BBFs were surface applied to sand. pH values are means, N=3.

<b>BBF</b>	<b>pH</b>
ASL	7.06
AV4	9.35
AV8	8.98
BA5	8.69
BA6	8.77
BIH	8.61
BIL	9.42
BIO	8.73
BIP	8.55
BLM	8.31
BO1	9.61
BO2	9.36
BO4	9.56
BVC	9.15
CGR	9.61
ECO	8.68
FEK	9.49
FEL	9.36
GRF	9.66
HDG	9.63
ILF	8.58
MAL	7.56
MB2	8.59
MO13	8.57
NAD	9.64
NE4	9.36
NE7	8.98
OG1	8.59
OG2	8.63
OOC	9.77
OPU	9.65
PAL	8.30
PCS	9.69
PCW	9.42
PRI	8.04
SDG	8.39
SIF	8.88
SYS	6.72
TRS	8.71
VEC	9.01
NH <sub>4</sub> HCO <sub>3</sub>	8.89
Control	7.94

Table I-A5: pH (1:5 in Milli-Q water) of incubated samples from Experiment 2a at the end of the experimental period (day 27). Samples were mixed before a subsample was taken out for pH measurement. BBFs were surface applied or incorporated into sand or soil (Acidic sandy soil). pH values are means, N=3.

<b>BBF</b>	<b>Media</b>	<b>Application</b>	<b>pH</b>
AV4	Sand	Surface	9.20
AV4	Sand	Incorporated	9.24
AV4	Soil	Surface	6.38
AV4	Soil	Incorporated	5.71
BO1	Sand	Surface	9.08
BO1	Sand	Incorporated	8.46
BO1	Soil	Surface	8.03
BO1	Soil	Incorporated	7.63
BVC	Sand	Surface	9.36
BVC	Sand	Incorporated	8.99
BVC	Soil	Surface	6.49
BVC	Soil	Incorporated	6.55
ECO	Sand	Surface	8.48
ECO	Sand	Incorporated	8.72
ECO	Soil	Surface	6.63
ECO	Soil	Incorporated	5.98
HDG	Sand	Surface	9.46
HDG	Sand	Incorporated	9.40
HDG	Soil	Surface	7.25
HDG	Soil	Incorporated	6.76
NAD	Sand	Surface	9.65
NAD	Sand	Incorporated	9.68
NAD	Soil	Surface	7.04
NAD	Soil	Incorporated	7.05
PAL	Sand	Surface	8.28
PAL	Sand	Incorporated	8.26
PAL	Soil	Surface	6.17
PAL	Soil	Incorporated	5.64
SIF	Sand	Surface	8.69
SIF	Sand	Incorporated	8.52
SIF	Soil	Surface	6.66
SIF	Soil	Incorporated	5.99
NH <sub>4</sub> HCO <sub>3</sub>	Sand	Surface	8.94
NH <sub>4</sub> HCO <sub>3</sub>	Sand	Incorporated	8.96
NH <sub>4</sub> HCO <sub>3</sub>	Soil	Surface	7.05
NH <sub>4</sub> HCO <sub>3</sub>	Soil	Incorporated	7.08
Control	Sand		7.09
Control	Soil		6.14

Table I-A6: pH (1:5 in Milli-Q water) of incubated samples from Experiment 3a at the end of the experimental period (day 27). Samples were mixed before a subsample was taken out for pH measurement. BBFs were surface applied to the five different soils (Acidic sandy soil, Alkaline loamy soil, Neutral loamy soil, Alkaline clay soil, and Acidic clay soil). pH values are means, N=3.

<b>BBF</b>	<b>Soil</b>	<b>pH</b>
AV4	Acidic sandy soil	6.70
AV4	Alkaline loamy soil	6.82
AV4	Neutral loamy soil	7.02
AV4	Alkaline clay soil	8.04
AV4	Acidic clay soil	6.35
BVC	Acidic sandy soil	6.75
BVC	Alkaline loamy soil	7.91
BVC	Neutral loamy soil	7.60
BVC	Alkaline clay soil	8.42
BVC	Acidic clay soil	6.79
HDG	Acidic sandy soil	7.29
HDG	Alkaline loamy soil	7.20
HDG	Neutral loamy soil	7.72
HDG	Alkaline clay soil	8.11
HDG	Acidic clay soil	6.67
ECO	Acidic sandy soil	6.86
ECO	Alkaline loamy soil	7.14
ECO	Neutral loamy soil	7.62
ECO	Alkaline clay soil	8.34
ECO	Acidic clay soil	6.80
PAL	Acidic sandy soil	6.33
PAL	Alkaline loamy soil	6.23
PAL	Neutral loamy soil	6.99
PAL	Alkaline clay soil	7.99
PAL	Acidic clay soil	6.10
NH <sub>4</sub> HCO <sub>3</sub>	Acidic sandy soil	7.59
NH <sub>4</sub> HCO <sub>3</sub>	Alkaline loamy soil	7.14
NH <sub>4</sub> HCO <sub>3</sub>	Neutral loamy soil	8.27
NH <sub>4</sub> HCO <sub>3</sub>	Alkaline clay soil	8.19
NH <sub>4</sub> HCO <sub>3</sub>	Acidic clay soil	7.26
Control	Acidic sandy soil	6.34
Control	Alkaline loamy soil	8.08
Control	Neutral loamy soil	7.76
Control	Alkaline clay soil	8.90
Control	Acidic clay soil	6.87

Table I-A7: pH (1:5 in Milli-Q water) of incubated samples from Experiments 2b and 3b at the end of the experimental period (day 27). Samples were mixed before a subsample was taken out for pH measurement. BBFs were surface applied soil (Acidic sandy soil) with three different soil moisture levels (30, 60, and 90 % WHC) and three different application rates (360, 720, and 1440 mg N kg<sup>-1</sup> soil). pH values are means, N=3.

<b>BBF</b>	<b>Application</b>	<b>WHC (%)</b>	<b>BBF application rate (mg N kg<sup>-1</sup> soil)</b>	<b>pH</b>
AV4	homogenized	90	720	6.81
AV4	non-homogenized	60	720	6.82
AV4	homogenized	60	720	6.84
AV4	homogenized	30	720	7.40
AV4	homogenized	60	360	6.01
AV4	homogenized	60	1440	8.42
ECO	homogenized	90	720	7.24
ECO	non-homogenized	60	720	6.69
ECO	homogenized	60	720	7.03
ECO	homogenized	30	720	7.84
ECO	homogenized	60	360	6.13
ECO	homogenized	60	1440	8.76
HDG	homogenized	90	720	7.73
HDG	non-homogenized	60	720	7.52
HDG	homogenized	60	720	7.56
HDG	homogenized	30	720	7.74
HDG	homogenized	60	360	6.52
HDG	homogenized	60	1440	9.01
NH <sub>4</sub> HCO <sub>3</sub>	homogenized	90	720	7.21
NH <sub>4</sub> HCO <sub>3</sub>	homogenized	60	720	7.63
NH <sub>4</sub> HCO <sub>3</sub>	homogenized	30	720	8.64
NH <sub>4</sub> HCO <sub>3</sub>	homogenized	60	360	6.39
NH <sub>4</sub> HCO <sub>3</sub>	homogenized	60	1440	9.22
Control		90		6.87
Control		60		6.35
Control		30		6.22

## Appendix II

*Supplementary material for*

### **Agronomic performance of novel biobased fertilizers across European field trial sites**

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### **Methods used for soil characteristics**

**Soil texture:** For the French site, soil texture was determined using the sedimentation-pipette method according to standard NFX 31-107. At the German, Finnish and Danish sites, soil texture was determined by sieving and sedimentation using the ISO 11277 method. **Soil organic carbon:** At the French site, the soil organic carbon content was determined using spectrometry on air-dried soil samples after oxidation in a sulfochromic medium, as described in standard NF ISO 14235. At the Danish and Finnish sites, organic C content was determined by Dumas combustion (CNS vario Macro Cube, elemental in Denmark and Leco Trumac in Finland). **Soil pH:** For the French and Danish sites, soil pH was measured on air-dry soil by addition of Milli-Q water at a soil-to-solution ratio of 1:5 (ISO 10390). In Finland, the national water-pH method used has a soil-to-solution ratio of 1:2.5. **Soil available phosphorus:** At the French site, soil phosphorus soluble in a sodium hydrogen carbonate solution was determined using the Olsen P extraction method (NF ISO 11263) after heating to a high temperature and colour development at ambient temperature. In Germany, plant-available P was extracted with calcium acetate lactate before using a flame photometer and FIA. At the Danish and Finnish sites, Olsen P was determined by extraction of P with sodium bicarbonate solution pH 8.5. **Soil available potassium:** At the French site, exchangeable K was determined using the NFX 31-108 method agitating the soil sample with an ammonium acetate solution, followed by spectrophotometric analysis (ICP AES). In Germany, plant-available K was extracted with calcium acetate lactate before using a flame photometer and FIA. At the Danish site, exchangeable K was determined by extraction using ammonium acetate solution with photometrical quantification. The



exchangeable K in the Finnish soil was determined by the extraction of acid ammonium acetate (pH 4.65) with a ratio of 1:10. Potassium concentration was then determined by ICP AES.

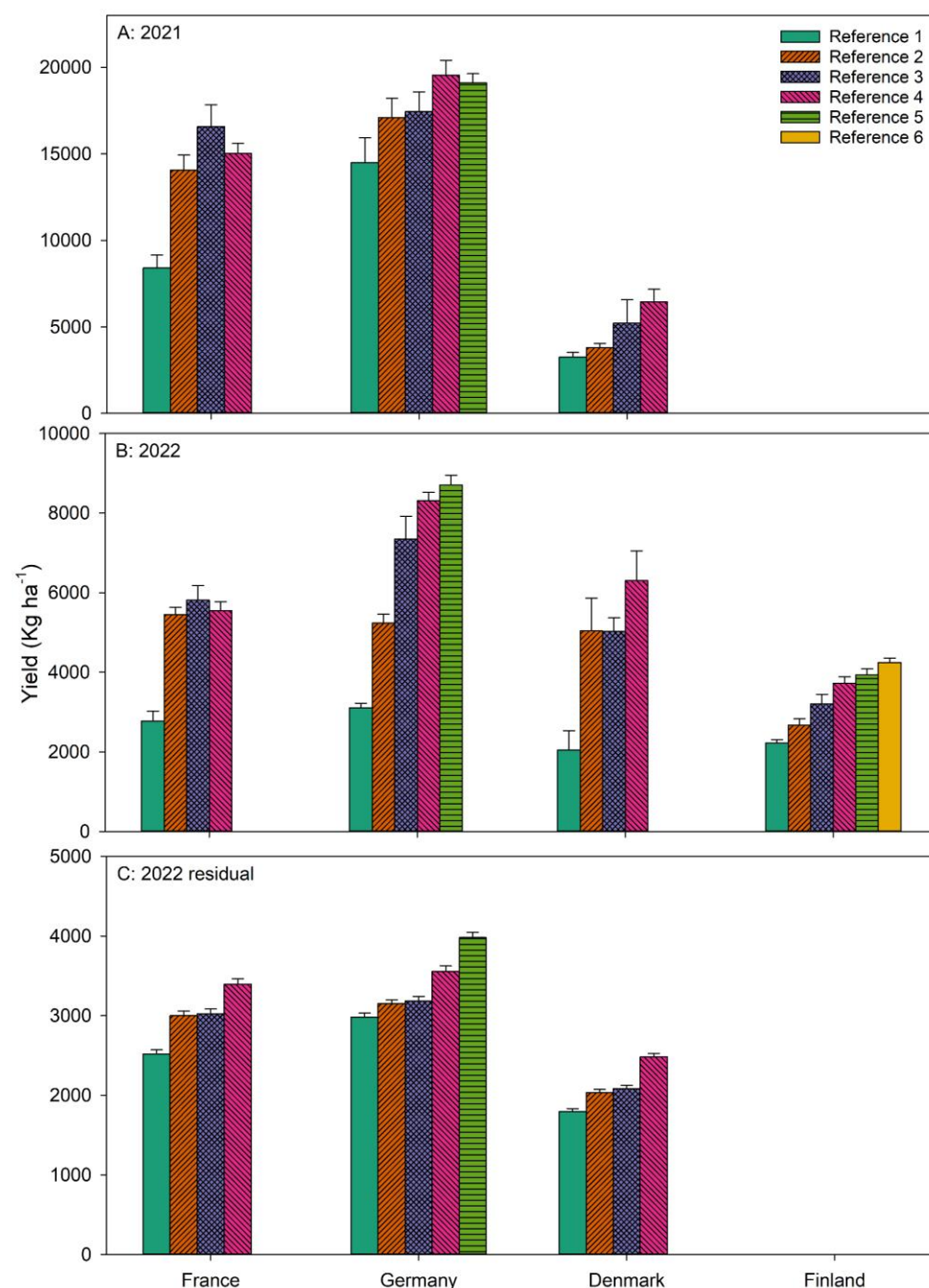


Fig. II-S1: Mean yield recorded  $\pm$  standard error for the reference plots treated with mineral N fertilizer applied in 2021 (A) and 2022 (B), as well yield response to residual effect in 2022 of mineral fertilizer applied in 2021 (C). Yields ( $\text{kg ha}^{-1}$ ) of silage maize (Germany 2021) and grain maize (France 2021), spring barley (Denmark 2021), winter wheat (France, Germany and Denmark 2022) and spring wheat (Finland 2022), in dry matter for silage maize and grain maize and at 15% moisture content for all cereal grains. The references received incremental increasing fertilizer amounts starting with Reference 1 (0  $\text{kg N/ha}$ ), according to Table II-3.  $n = 4$ .

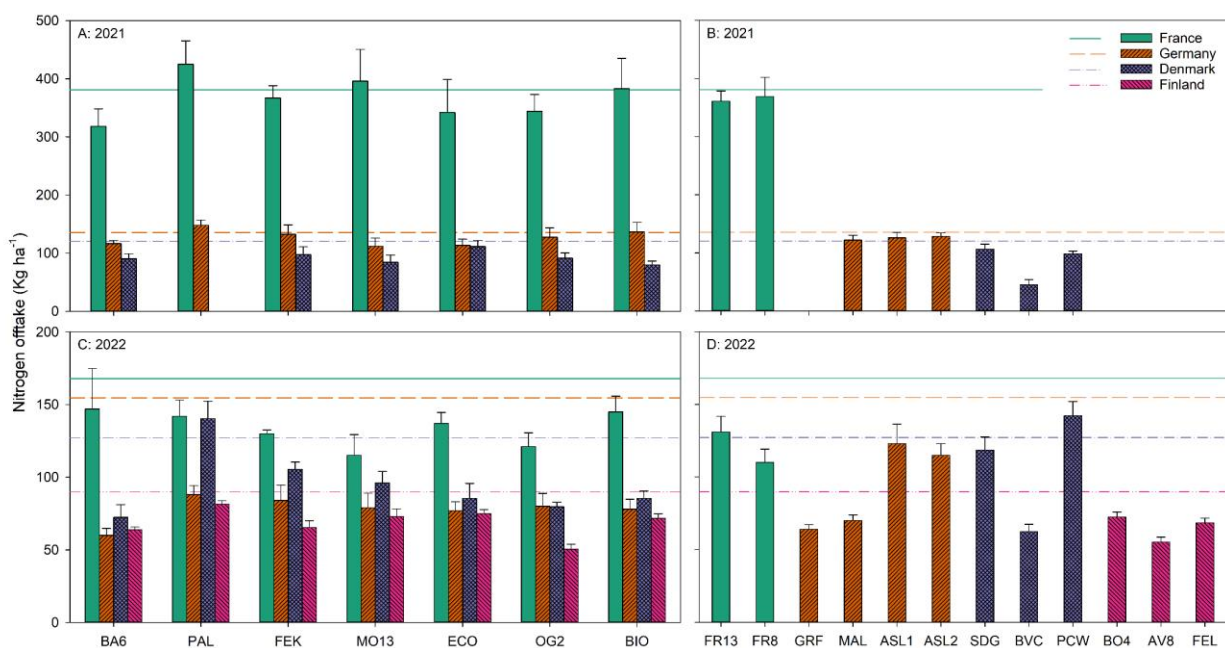


Fig. II-S2: Means  $\pm$  standard error of N offtake recorded (kg N ha<sup>-1</sup>) of silage maize (Germany 2021), grain maize (France 2021), spring barley (Denmark 2021), winter wheat (France, Germany and Denmark 2022) and spring wheat (Finland 2022). Values are reported for the aboveground crop biomass. Nitrogen offtake in the plots treated with the seven common BBFs tested at all sites are shown as bars in A (2021) and C (2022). The local BBFs are shown in B (2021) and D (2022). The horizontal lines indicate the mean N offtake of the mineral N reference fertilizers at the same N application rate as the BBFs. Application rates differed between sites and years.  $n = 4$ , except for OG2 and BVC for the Danish site in 2021 (A and B), BA6, FEK and BIO for the French site (C), PAL for the German site (C) where  $n = 3$ . Note different scales on the vertical axis.

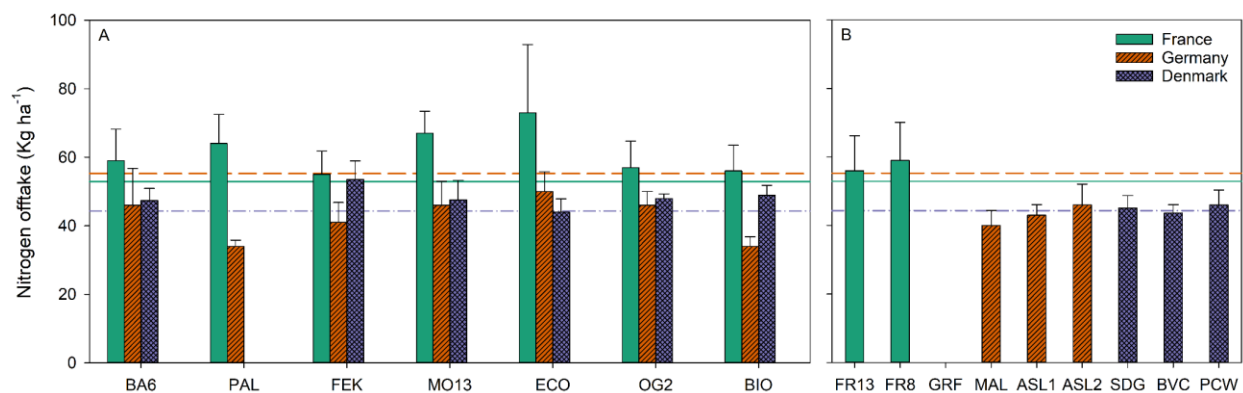


Fig. II-S3: Means  $\pm$  standard error of recorded crop N offtake (kg ha<sup>-1</sup>) of winter wheat in 2022 in the experiment where BBF and reference fertilisers were applied in 2021 (residual value). Values are reported for the aboveground crop biomass. The N offtake in the plots treated with the seven common BBFs tested at all sites are shown as bars in A and the local BBFs in B. The horizontal lines indicate the calculated mean N offtake of the mineral N reference at the same N application rate (2021) as the BBFs. Application rates differed between sites.  $n = 4$ .

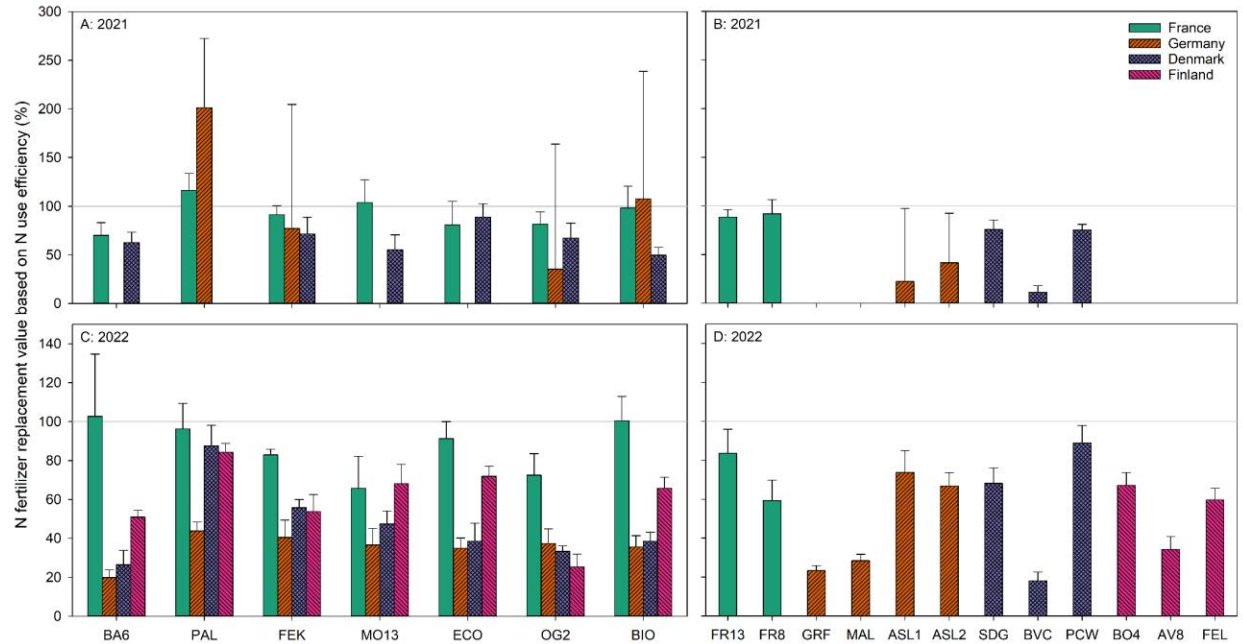


Fig. II-S4: Means  $\pm$  standard error of  $\text{NFRV}_{\text{NUE}}$  (%). The seven common BBFs tested at all sites are shown as bars in A (2021) and C (2022). The local BBFs are shown in B (2021) and D (2022). The horizontal grey line indicates 100%  $\text{NFRV}_{\text{AE}}$ , i.e. where the BBF performs equally well as the mineral N reference at the same application rate. For Germany 2021 (A),  $\text{NFRV}$  were below 0 for BA6, MO13 and ECO. Crops and N application rates differed between sites and years.  $n = 4$ , except for OG2 and BVC for the Danish site in 2021 (A and B), BA6, FEK and BIO for the French site (C), PAL for the German site (C) where  $n = 3$ . Note different scales on the vertical axis.

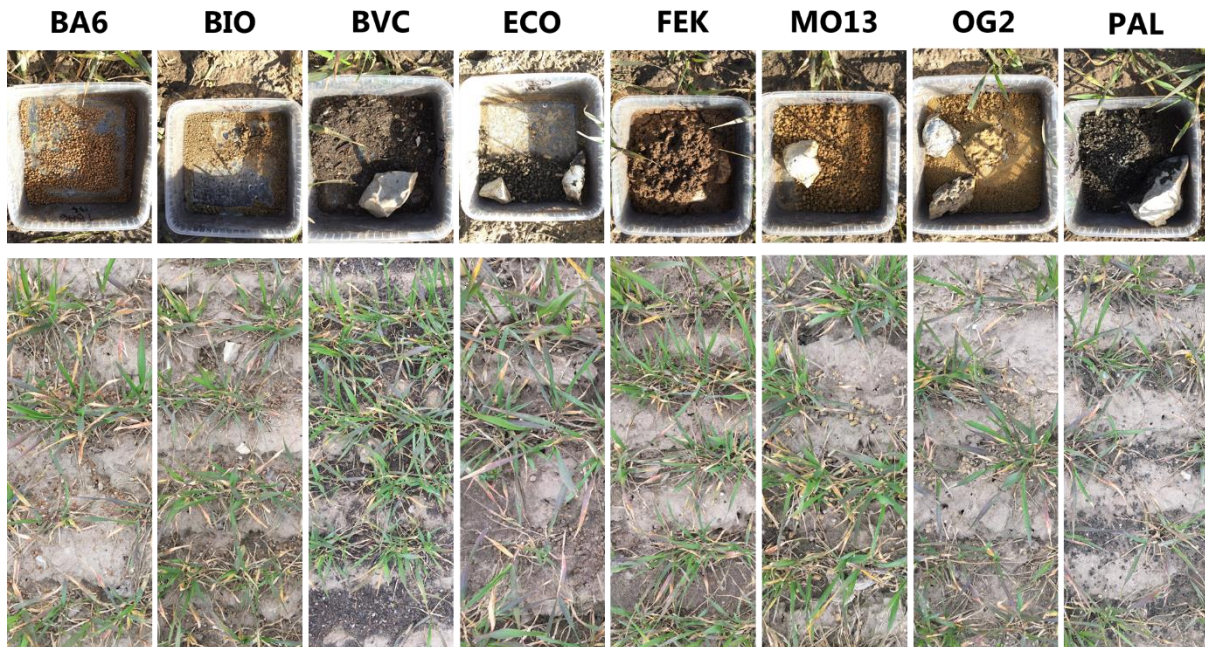


Fig. II-S5: Photos of the seven common BBFs and the local BVC at the Danish site in 2022 two weeks after BBFs were surface-applied to winter wheat. BBF acronyms are given above each photo. The top photos show the BBFs added to a plastic box with drainage holes inserted into the soil in the corner of the plots. The loss of BBF in dry

weight after two weeks in the plastic boxes varied from 0 (BVC) – 87 (BIO) %. The bottom photos were taken inside the plots.

Table II-S1: BBF acronyms (three letters/numbers), full product names, and name of manufacturer. Information derived from Baurle et al. (2021) and Wester-Larsen et al. (2022).

<b>BBF</b>	<b>BBF</b>	<b>Manufacturer's name</b>
BA6	Bioagenasol	Agrana, A
BIO	Bio 8-4-2 yara	YARA, FI
ECO	Ecolan	Ecolan Oy
FEK	Fertikal	Fertikal, BE
MO13	Monterra bio 13-0-0	MeMon, NL
OG2	Øgro N14	Daka, DK
PAL	Palaterra	Palaterra Betriebs- und Beteiliugngsgesellschaft mbH, DE
ASL	Ammonium sulphate (liquid)	Geltz Umwelt-Technologie GmbH, DE
GRF	Digestate	University of Hohenheim, DE
MAL	Maltaflor BIO	MALTaflor, DE
FR13	13-0-0 France	¶
FR8	8-10-5-5 France	¶
BVC	Biovækst compost	Biovækst, DK
PCW	AKV K2	AKV, DK
SDG	Solrød digestate	Solrød Biogas, DK
AV8	Arvo 8-1-5-2	Biolan Oy (marketed by Novarbo Oy), FI
FEL	Fertilex	Fertilex Oy, FI
BO4	Boost NKS	Soilfood Oy, FI

¶ NA: Unknown manufacturer.



Table II-S2: Functions and  $R^2$  values for N reference levels used to calculate NFRV based on N use efficiency for each site and each year.

	2021		2022	
	Function	$R^2$	Function	$R^2$
France	$y = 1.0523x + 176.81$	0.68	$y = 0.4159x + 61.828$	0.60
Germany	$y = 0.1273x + 120.76$	0.07	$y = 0.7627x + 32.573$	0.87
Denmark	$y = 0.6298x + 33.897$	0.82	$y = 0.6288x + 40.971$	0.94
Finland	-	-	$y = 0.5509x + 34.727$	0.97

Table II-S3: Functions and  $R^2$  values for N reference levels used to calculate NFRV based on agronomic efficiency for each site and each year.

	2021		2022	
	Function	$R^2$	Function	$R^2$
France	$y = -0.219x^2 + 82.677x + 8300.8$	0.79	$y = -0.0996x^2 + 35.621x + 2801.7$	0.88
Germany	$y = -0.2832x^2 + 72.758x + 14574$	0.45	$y = -0.1632x^2 + 61.107x + 3025.2$	0.93
Denmark	$y = 0.0698x^2 + 11.538x + 3202.9$	0.54	$y = -0.0773x^2 + 34.639x + 2291.9$	0.61
Finland	-	-	$y = -0.0573x^2 + 23.619x + 2187.2$	0.87

Table II-S4: ANOVA of first-year fertilization treatments using Fisher's LSD test separately for each site and each year (2021 and 2022). LSD means of N offtake (kg N ha<sup>-1</sup>) estimated from the model (grain maize, spring barley, winter wheat and spring wheat), whole-crop yield for silage maize and p-values of tests. Means followed by a common letter are not significantly different according to the LSD test at the 5% level of significance. Local BBFs are displayed in italics. "SE" is the modelled pooled standard error for all treatments. "REF\_1-5" are mineral references with ascending N application.

Fertilization in 2021	France (p-value: 0.0004)				Germany (p-value: 0.21)				Denmark (p-value: <0.0001)								
	N offtake		LSD		N offtake		LSD		N offtake		LSD						
	SE	39			SE	11.8			SE	10.7							
	BA6	318	b		BA6	117	-		BA6	103	b	c					
	BIO	383	a	b	BIO	136	-		BIO	91		c d					
	ECO	342	a	b	ECO	114	-		ECO	127	a	b					
	FEK	367	a	b	FEK	133	-		FEK	111		b c					
	MO13	396	a	b	MO13	112	-		MO13	96		c					
	OG2	344	a	b	OG2	127	-		OG2	104		b c					
	PAL	425	a		PAL	148	-		PAL	.							
	FR13	361	a	b	ASL1	125	-		BVC	60		d e					
	FR8	369	a	b	ASL2	128	-		PCW	113		b c					
					GRF	.			SDG	121	a	b c					
				MAL	122	-											
Ref_1	156		c	Ref_1	123	-		Ref_1	45		e						
Ref_2	.			Ref_2	125	-		Ref_2	.								
Ref_3	319		b	Ref_3	118	-		Ref_3	63		d e						
Ref_4	381	a	b	Ref_4	146	-		Ref_4	111		b c						
Ref_5	430	a		Ref_5	134	-		Ref_5	150	a							
Fertilization in 2022	France (p-value: 0.0007)				Germany (p-value: 0.0001)				Denmark (p-value: <0.0001)				Finland (p-value: <0.0001)				
	N offtake		LSD		N offtake		LSD		N offtake		LSD		N offtake		LSD		
	SE	12.5			SE	8.3			SE	8.1			SE	2.9			
	BA6	146	a	b c	BA6	60		d e	BA6	74			g h	BA6	66		e f
	BIO	144	a	b	BIO	78		c d	BIO	87			e f g	BIO	72		d e
	ECO	137	a	b	ECO	77		c d	ECO	87			e f g	ECO	77		c d
	FEK	129		b c	FEK	84		c	FEK	107			d e	FEK	67		e f
	MO13	115		b c	MO13	79		c d	MO13	97			e f	MO13	73		c d e
	OG2	121		b c	OG2	80		c d	OG2	81			f g h	OG2	52		g
	PAL	142	a	b	PAL	88		c	PAL	143			b c	PAL	80		b c
	FR13	131		b c	ASL1	124		b	BVC	63			e f g	AV8	55		g
	FR8	110		b	ASL2	115		b	PCW	144			b	BO4	71		d e
					GRF	63		c d	SDG	121			c d	FEL	67		e f
					MAL	70		c d									
	Ref_1	58		d	Ref_1	38		e	Ref_1	42			i	Ref_1	36		h
	Ref_2	.			Ref_2	69		c d	Ref_2	.				Ref_2	59		f g
	Ref_3	126		b c	Ref_3	86		c	Ref_3	84			f g h	Ref_3	75		c d
	Ref_4	168	a		Ref_4	147	a		Ref_4	134			b c	Ref_4	87		b
	Ref_5	139	a	b	Ref_5	168	a		Ref_5	176	a			Ref_5	106	a	

Table II-S5: ANOVA of residual second-year fertilization treatments using Fisher's LSD test for Germany in 2023 (fertilization in 2022) in spring barley. LSD means (kg ha<sup>-1</sup>) of grain yields estimated from the model at 15% moisture content and p-values of tests. Means followed by a common letter are not significantly different according to the LSD test at the 5% level of significance. Local BBFs are displayed in italics. "SE" is the modelled pooled standard error for all treatments. "REF\_1-5" are mineral references with ascending N application.

<b>Germany</b> (p-value: <0.0023)						
	Yield	LSD				
SE	264					
BA6	4006	a	b			
BIO	4084	a	b			
ECO	4221	a	b			
FEK	3962		b	c		
MO13	4258		b			
OG2	4172	a	b			
PAL	3608		b	c	d	
<i>ASL1</i>	3275			c	d	e
<i>ASL2</i>	2844					e
<i>GRF</i>	3575		b	c	d	
<i>MAL</i>	3742		b	c	d	
Ref_1	3523	a	b	c	d	e
Ref_2	3073				d	e
Ref_3	3245			c	d	e
Ref_4	3618		b	c	d	
Ref_5	4083	a	b			

Table II-S6: ANOVA of the residual effect of fertilization treatments on N offtake (kg N ha<sup>-1</sup>) estimated from the model fertilized in 2021 using Fisher's LSD test separately for each site in 2022 in winter wheat. LSD means (kg N ha<sup>-1</sup>) of grain yields and p-values of tests. Local BBFs are displayed in italics. "SE" is the modelled pooled standard error for all treatments. "REF\_1-5" are mineral references with ascending N application.

France (p-value: 0.237)			Germany (p-value: 0.313)			Denmark (p-value: 0.231)		
	N offtake	-		N offtake	-		N offtake	-
SE	10.1		SE	5.9		SE	3.6	
BA6	59		BA6	46		BA6	48	
BIO	56		BIO	34		BIO	50	
ECO	73		ECO	50		ECO	45	
FEK	55		FEK	41		FEK	54	
MO13	67		MO13	46		MO13	48	
OG2	58		OG2	46		OG2	49	
PAL	64		PAL	34		PAL	.	
<i>FR13</i>	57		<i>ASL1</i>	43		<i>BVC</i>	44	
<i>FR8</i>	59		<i>ASL2</i>	45		<i>PCW</i>	47	
			<i>GRF</i>	.		<i>SDG</i>	46	
			<i>MAL</i>	40				
Ref_1	47		Ref_1	41		Ref_1	39	
Ref_2	.		Ref_2	34		Ref_2	42	
Ref_3	52		Ref_3	46		Ref_3	42	
Ref_4	53		Ref_4	50		Ref_4	43	
Ref_5	60		Ref_5	51		Ref_5	46	



Table II-S7: ANOVA of the effect of fertilization treatments on N offtake and Yield fertilized in 2021 and 2022 at all sites and crops using Fisher's LSD test. LSD means are shown in kg N ha<sup>-1</sup> for N offtake and kg ha<sup>-1</sup> for grain yield with p-values of tests. Means followed by a common letter are not significantly different according to the LSD test at the 5% level of significance. "SE" is the standard error for all treatments. "REF\_1-5" are mineral references with ascending N application.

<b>Combined analysis of all trials fertilized in 2021 and 2022</b>				
	N offtake (p-value: 0.0015)		Yield (p-value: 0.0509)	
	kg N ha <sup>-1</sup>	LSD	kg ha <sup>-1</sup>	-
SE	56.7		165	
<b>BA6</b>	143	c d e f g h i j k	8313	
<b>BIO</b>	152	b c d e f g	8525	
<b>ECO</b>	155	f g h i j k l	8614	
<b>FEK</b>	154	d e f g h i j	8823	
<b>MO13</b>	149	a b c d e f g h	8287	
<b>OG2</b>	142	f g h i j k	8258	
<b>PAL</b>	172	e f g h i j	9171	
<b>Ref_1</b>	95	j k l	5887	
<b>Ref_2</b>	.		.	
<b>Ref_3</b>	146	h i j	8537	
<b>Ref_4</b>	187	a b c d e	9394	
<b>Ref_5</b>	203	a b c d e f	9645	

## Appendix III

### *Supplementary Material for*

#### **Effects of biobased fertilisers on soil physical, chemical and biological indicators – A long-term incubation study**

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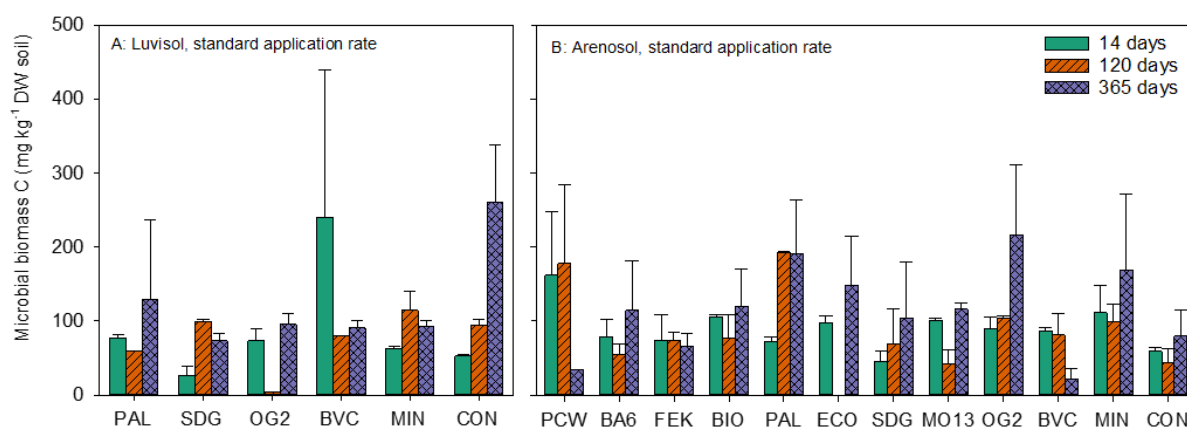


Fig. III-S1: Microbial biomass C (mg g dry-weight soil<sup>-1</sup>). Luvisol soil standard application rate (A), Arenosol soil standard application rate (B). Values are means  $\pm$  standard error for samples taken at three time points during the incubation period: 14 days, 120 days and 365 days after the start of incubation. BA6, BIO, BVC, ECO, FEK, MO13, OG2, PAL and SDG are biobased fertilisers, MIN is a reference mineral fertiliser and CON is the negative control. N=1 for BVC 120 days, OG2 120 days, PAL 120 days (A), PCW 365 days (B). N=2 for OG2 14 days, SDG 14 days, SDG 120 days, MIN 120 days, CON 120 days, OG2 365 days (A), SDG 14 days, MIN 14 days, BVC 120 days, FEK 120 days, OG2 120 days, PAL 120 days, SDG 120 days, SDG 365 days (B). For all others, N=3.

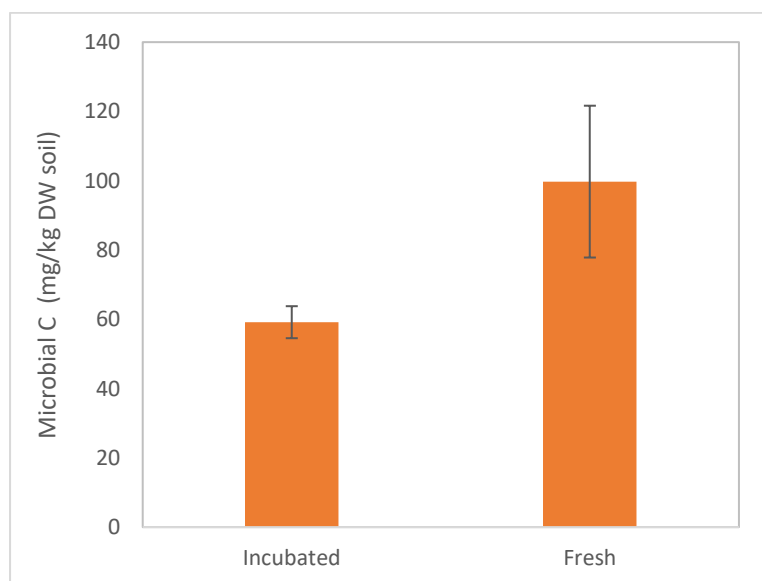


Fig. III-S2: Microbial biomass C (mg g dry-weight soil<sup>-1</sup>) for the Arenosol soil negative control (CON) 14 days after the start of incubation (Incubated) and a non-treated freshly sampled Arenosol soil from the same field. Values are means  $\pm$  standard error, N=3.

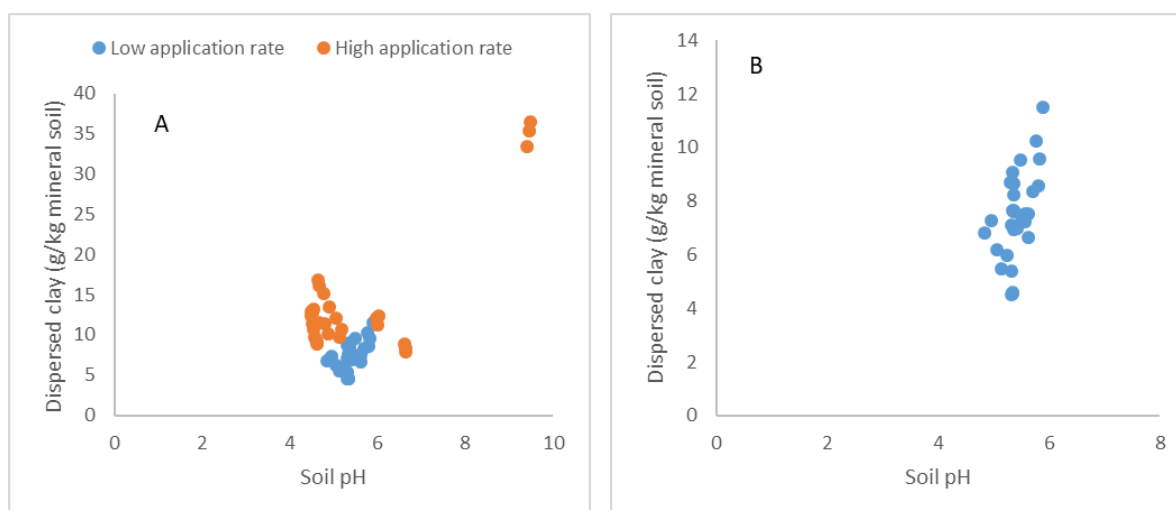


Fig. III-S3: Relationship between clay dispersibility (g clay / kg mineral soil) and soil pH (1.5 in CaCl<sub>2</sub>) for the BBF treatments at the accelerated and standard application rate (A) and the standard application rate only (B).

Table III-S1: BBF acronyms (three letters/numbers), full product names and manufacturer names. The data are from Wester-Larsen et al. (2022).

BBF	BBF	Manufacturer
BA6	Bioagenasol	Agrana, A
BIO	Bio 8-4-2 yara	YARA, FI
ECO	Ecolan	Ecolan Oy
FEK	Fertikal	Fertikal, BE
MO13	Monterra bio 13-0-0	MeMon, NL
OG2	Øgro N14	Daka
PAL	Palaterra	Palaterra Betriebs- und Beteiligungsgesellschaft mbH, DE
BVC	Biovækst compost	Biovækst
PCW	AKV K2	AKV
SDG	Solrød digestate	Solrød Biogas

Table III-S2: Output of statistical analyses showing p-values of Bonferroni-adjusted pairs of estimated marginal means to test differences between treatments 14 days, 120 days and 365 days after the start of incubation on the Arenosol soil for the standard and accelerated application rate. Only significant (P<0.05) results are shown.

		14 days						120 days				365 days									
		Standard application rate			Accelerated application rate			Standard application rate		Accelerated application rate		Standard application rate						Accelerated application rate			
		CEC	pH	WHC	CEC	pH	WHC		pH	WHC	pH	WHC	CEC	Clay disper-sibility	pH	WHC	Total C	CEC	Clay disper-sibility	pH	WHC
CON	PCW				0.001	<0.001			0.001	<0.001			0.001					<0.001	<0.001		
CON	BA6				0.003	<0.001			0.003	<0.001	0.002		<0.001				0.002	<0.001	<0.001		
CON	FEK					<0.001					<0.001							<0.001			<0.001
CON	BIO					<0.001												<0.001	<0.001		
CON	PAL									<0.001	<0.001							<0.001	<0.001		<0.001
CON	ECO					<0.001	0.001			<0.001								<0.001	<0.001		
CON	SDG		<0.001					<0.001	<0.001		<0.001			<0.001				<0.001	<0.001		0.001
CON	MO13					0.001			<0.001	<0.001			<0.001		<0.001			<0.001	<0.001	<0.001	0.001
CON	OG2						<0.001	0.001		<0.001	0.002			0.002				<0.001	<0.001		
CON	BVC				<0.001	<0.001	<0.001		0.003	0.002	<0.001		<0.001					0.001	<0.001	<0.001	<0.001
CON	MIN													0.004							
PCW	BA6					<0.001	0.002			<0.001								<0.001	<0.001		
PCW	BIO					<0.001				<0.001								<0.001	<0.001		
PCW	BVC					<0.001	<0.001			<0.001	<0.001							<0.001	<0.001	<0.001	<0.001
PCW	ECO				0.001	<0.001	0.004			<0.001								<0.001	0.004		
PCW	FEK					<0.001				<0.001	0.004							<0.001	<0.001		<0.001
PCW	MIN				<0.001	<0.001				<0.001			0.001					<0.001	<0.001		
PCW	MO13				0.004	<0.001			<0.001	<0.001			<0.001		<0.001			<0.001	<0.001	<0.001	0.001
PCW	OG2					<0.001	<0.001	<0.001	0.002	<0.001			<0.001	0.004	0.001			<0.001	<0.001		
PCW	PAL					<0.001			0.001	<0.001	<0.001							<0.001	<0.001		<0.001
FEK	BA6									<0.001							0.001	<0.001	<0.001		<0.001
FEK	BIO							<0.001										0.003	<0.001		<0.001
FEK	BVC					<0.001	<0.001				<0.001		<0.001					<0.001	<0.001	<0.001	<0.001
FEK	ECO					<0.001	<0.001			<0.001								<0.001	<0.001		<0.001
BIO	BA6						<0.001	<0.001		<0.001								0.001		<0.001	
PAL	BA6					<0.001				0.001							<0.001	<0.001			<0.001
PAL	BIO					<0.001		<0.001		<0.001							<0.001	<0.001	<0.001		<0.001
PAL	BVC		<0.001		0.002	<0.001	<0.001			<0.001	<0.001		<0.001	0.001				<0.001	<0.001	<0.001	<0.001
PAL	ECO					<0.001	<0.001			<0.001											<0.001
PAL	FEK					<0.001				<0.001									<0.001		
PAL	MIN					<0.001				<0.001								<0.001	<0.001		<0.001
PAL	MO13					<0.001	0.001		<0.001				<0.001		<0.001			<0.001		<0.001	<0.001
PAL	OG2						<0.001						0.001								<0.001
ECO	BA6						<0.001											<0.001		<0.001	

ECO	BIO							<0.001						<0.001				
ECO	BVC	<0.001	<0.001	<0.001				<0.001	<0.001		<0.001	0.001		<0.001	<0.001	<0.001	<0.001	
SDG	BA6	<0.001		<0.001				<0.001			0.001		0.001	<0.001				
SDG	BIO	<0.001		<0.001	<0.001	<0.001			0.003			<0.001		0.002				
SDG	BVC	<0.001	0.001	<0.001	<0.001	<0.001		<0.001	0.001		<0.001	<0.001			<0.001	<0.001	<0.001	
SDG	ECO	0.003		<0.001	<0.001	<0.001		<0.001	0.003			0.004						
SDG	FEK	<0.001		<0.001		0.001						<0.001			<0.001		<0.001	
SDG	MIN					0.003								<0.001	<0.001		<0.001	
SDG	MO13	<0.001		0.004	<0.001	<0.001	<0.001	<0.001	0.004		<0.001		<0.001	0.001		<0.001		
SDG	OG2				<0.001		0.001	<0.001										
SDG	PAL			0.001		0.004	0.001	<0.001				0.003						<0.001
SDG	PCW	<0.001		<0.001		<0.001		<0.001	<0.001		0.003	<0.001		<0.001	<0.001		0.001	
MO13	BA6			<0.001	<0.001		<0.001				<0.001		<0.001	<0.001		<0.001		
MO13	BIO			<0.001			<0.001	<0.001			<0.001		<0.001	<0.001		0.003		
MO13	BVC		<0.001		<0.001		<0.001	<0.001	<0.001		<0.001	<0.001	<0.001		<0.001	<0.001	<0.001	
MO13	ECO						<0.001				<0.001		<0.001					
MO13	FEK			<0.001	<0.001		<0.001	<0.001			<0.001		<0.001		0.001	<0.001	<0.001	<0.001
MO13	MIN						<0.001	<0.001			<0.001		<0.001		<0.001	<0.001	<0.001	<0.001
OG2	BA6			<0.001	<0.001					0.00	<0.001			0.001				
OG2	BIO			<0.001		<0.001		<0.001			0.001			<0.001	0.001			
OG2	BVC	<0.001	0.001	<0.001	<0.001			<0.001	<0.001		<0.001	<0.001			<0.001	<0.001	<0.001	<0.001
OG2	ECO			<0.001		0.004					<0.001							
OG2	FEK			<0.001	<0.001			<0.001			0.001				<0.001			<0.001
OG2	MIN							<0.001						<0.001	<0.001		0.003	
OG2	MO13			<0.001		0.002	<0.001						<0.001				<0.001	
BVC	BA6			0.003	<0.001			<0.001	<0.001	0.00		0.001		<0.001	<0.001	<0.001	<0.001	<0.001
BVC	BIO		0.002		<0.001	0.001			<0.001		<0.001			<0.001	<0.001	<0.001	<0.001	<0.001
MIN	BA6		0.003	<0.001	0.002			<0.001			<0.001			<0.001	<0.001		0.003	
MIN	BIO			<0.001		<0.001								<0.001	<0.001			
MIN	BVC	<0.001	<0.001	<0.001	<0.001			0.002	<0.001		<0.001	<0.001			<0.001	<0.001	<0.001	<0.001
MIN	ECO			<0.001				<0.001							<0.001			
MIN	FEK			<0.001										<0.001			<0.001	

Table III-S3: Output of statistical analyses showing p-values of Bonferroni-adjusted pairs of estimated marginal means to test differences between treatments 14 days, 120 days and 365 days after the start of incubation on the Luvisol soil for the standard and accelerated application rate. Only significant (P<0.05) results are shown.

		14 days						120 days				365 days									
		Standard application rate			Accelerated application rate			Standard application rate		Accelerated application rate		Standard application rate				Accelerated application rate					
		CEC	pH	WHC	CEC	pH	WHC	pH	WHC	pH	WHC	CEC	Clay dispersibility	pH	WHC	Total C	CEC	Clay dispersibility	pH	WHC	Total C
CON	PAL					<0.001		<0.001	<0.001	0.001		0.001					<0.001	<0.001		<0.001	
CON	SDG		0.002					0.001		<0.001		<0.001	<0.001				<0.001	<0.001		<0.001	
CON	OG2					<0.001		0.002	0.002	<0.001							<0.001	<0.001		<0.001	
CON	BVC				<0.001	<0.001		0.004	0.003	<0.001	0.001			0.007		0.004	<0.001	<0.001	<0.001	<0.001	
CON	MIN		0.001						0.001		0.010		<0.001	0.003			<0.001	0.004			
PAL	BVC		<0.001		<0.001	<0.001	<0.001			<0.001		<0.001	<0.001			0.005		<0.001	<0.001	0.001	
PAL	MIN					<0.001		0.007		<0.001		<0.001					<0.001	<0.001		<0.001	
PAL	OG2							<0.001									<0.001			<0.001	
SDG	BVC		<0.001		<0.001	<0.001		<0.001		<0.001		<0.001	<0.001			0.001		<0.001	<0.001	<0.001	
SDG	MIN									0.003		<0.001	0.001				<0.001	<0.001		<0.001	
SDG	OG2					0.002	<0.001			<0.001		<0.001	<0.001				<0.001				
SDG	PAL						0.001	<0.001		<0.001		<0.001	<0.001							<0.001	
OG2	BVC		<0.001		<0.001	<0.001	<0.001	<0.001		<0.001		0.006	<0.001			0.002	<0.001	<0.001	<0.001	<0.001	
OG2	MIN					<0.001	<0.001			<0.001		<0.001					<0.001	<0.001		<0.001	
MIN	BVC		<0.001		<0.001	<0.001		<0.001		<0.001		<0.001	<0.001			<0.001	<0.001	<0.001	<0.001	<0.001	

Table III-S4: Output of statistical analyses showing p-values of Tukey-adjusted pairs of estimated marginal means to test differences between C fractions 14 days and 365 days after the start of incubation. Only significant ( $P < 0.05$ ) results are shown.

		Standard application rate			Accelerated application rate		
		POM <sub>large</sub>	POM <sub>small</sub>	MAOM	POM <sub>large</sub>	POM <sub>small</sub>	MAOM
Luvisol soil	PAL				<0.01		0.02
	SDG		0.01	0.04			
	OG2	0.02	<0.01		<0.01		0.04
	BVC				<0.01		0.02
	MIN						
Arenosol soil	PCW						
	BA6				<0.01		0.04
	FEK						
	BIO						
	PAL				0.05		<0.01
	ECO						
	SDG	0.01	<0.01	<0.01	0.03		
	MO13						
	OG2	0.04					
	BVC						0.04
	MIN						



## Appendix IV

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### Testing and evaluation of laboratory methods for the assessment of mineralizable nitrogen from bio-based fertilizers

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**Keywords:** bio-based fertilizers, chemical predictors, soil incubation, N mineralization, C mineralization

## Abstract

Bio-based fertilizers (BBFs) are an increasingly popular source of nutrients in agriculture and enable closing nutrient cycles on the regional scale. However, predicting their mineralization-dependent nutrient release is critical for the appropriate use of these fertilizers. Thus, we assessed mineralizable nitrogen (N) and carbon (C) of a representative selection of 32 BBFs and evaluated a set of chemical extraction methods to predict their N mineralization dynamics. In 84-day aerobic incubations, cumulative mineral N release varied between -134 and 1056 mg N<sub>min</sub>-N g<sup>-1</sup> amended N. Mineralized C ranged from 101 to 1172 mg CO<sub>2</sub>-C g<sup>-1</sup> amended C. Based on the results of the biological mineralization assays, BBFs were classified into five significantly different groups. Tested chemical indicators included C:N ratio and N extractable by cold water, hot water, hot potassium chloride and hot sulfuric acid. Cold and hot water extractions presented the lowest extraction intensities, followed by hot potassium chloride and hot sulfuric acid extractions. Cold water extractable N was most strongly correlated with mineralizable N at the start of incubation ( $r = 0.99$ ), while hot sulfuric acid extractable N was correlated with mineralizable N after the first month of incubation ( $0.73 < r < 0.85$ ). The C:N ratios showed the highest correlations to mineralizable N at the end of the incubation ( $-0.83 > r > -0.86$ ). However, the combination of those three indicators was insufficient to discriminate BBFs into the five N and C mineralization classes without ambiguity. Given the diversity of N release patterns of BBFs, a combination of chemical N extraction methods nevertheless appears to provide a cost-effective yet accurate estimation of their N mineralization dynamics.

## 1. Introduction

Bio-based fertilizers (BBFs) are a fertilizer category that is increasingly gaining popularity, offering the opportunity to close nutrient cycles on a regional level, especially between urban and rural areas, but their handling is more knowledge-intensive than traditional fertilizers. BBFs include all fertilizers derived from animal or vegetal bio-waste as well as derivatives from bio-waste processing. Recent legislation to support the use of BBFs efforts culminated in the current European Fertilizer Regulation (Fertilizing Products Regulation (EU) 2019/1009), which entered into force in June 2022. In Europe, they are currently classified based on their component materials, state of matter, and organic material content (European Council, 2019). However, their international commerce and environmentally friendly application have been constrained by a scarcity of scientific knowledge to support international guidelines and policies (European Council, 2016), but the scientific knowledge basis needed for practical guidelines has to be further extended. In the context of nitrogen (N) fertilization, the development of holistic yet simple, fast and low-cost methods for the assessment of N release from BBFs is crucial in order to efficiently and suitably employ these fertilizers. One of the central aspects controlling N availability from BBFs is their N mineralization dynamic, but its assessment is challenging due to the complexity of both the turnover of organic matter fractions in soil and the biogeochemistry of N. For this reason, in-vitro biological mineralization assays are often carried out to determine mineralizable N of BBFs (Calderón et al., 2005; Lashermes et al., 2010; Lazicki et al., 2020; Morvan et al., 2006; Parnau et al., 2004; Thuriès et al., 2001). The resulting mineralizable N value is defined as the sum of the mineral N species ammonium (NH<sub>4</sub>-N) and nitrate (NO<sub>3</sub>-N) extractable after aerobically incubating a soil-BBF mixture for a defined period of time. The representativeness of this value is commonly accepted as it derives directly from biologically driven mineralization processes (Ros et al., 2011).

However, mineralization assays have a number of drawbacks. These include the tendency to overestimate mineralizable N due to ideal conditions in which incubations are carried out, potential artifacts generated by handling (Jarvis et al., 1996), and the time-consuming experimental setup. Even though there is no evidence, it cannot be excluded that mineralizable N values are dependent on the characteristics of the soils chosen for the mineralization assays (Lazicki et al., 2020; Noirot-Cosson et al., 2017). Additionally, the ongoing development of new organic fertilizer products makes it desirable to search for alternative methods to estimate N mineralization dynamics. Although satisfactory results have been obtained using modeling approaches for several organic fertilizers (Noirot-Cosson et al., 2017), it is advantageous to further reduce the analytical workload to characterize N mineralization of BBFs. To predict the mineralization of N from soil organic matter (SOM), a range of chemical extraction methods have been developed since the 1970s. So far, only stepwise chemical digestion

(SCD) and basic CN parameters such as total N (TN), total carbon (TC) and C:N ratios are usually used for BBFs, although soil extractions could perhaps also be applied to BBFs to better predict N mineralization.

In the present study, four chemical N extraction methods were selected, which correlate with mineralizable N at different stages of mineralization. Mild extraction methods extracting less than 20% SOM-N were selected for their potential to target highly labile organic N pools. EN 13652 defines a standard method to determine water-soluble nutrients from soil improvers and growing media. Hot water extractable N (HW-N) has been used to extract labile organic SOM-N (Curtin et al., 2006; Gregorich et al., 2003) and was originally proposed as an index for N availability in soils (Keeney and Bremner, 1966). Curtin et al. (2006) found a weak correlation ( $R^2 = 0.30$ ) between HW-N and soil mineralizable N and a stronger correlation with plant N uptake ( $R^2 = 0.50$ ), which was later confirmed by Braos et al. (2016) ( $R^2 = 0.90$ ). Hot potassium chloride extractable N (hKCl-N) is one of the most widespread methods to assess highly labile organic N pools (Ros et al., 2011). Previous studies found strong correlations between hKCl-N and soil mineralizable N (Curtin and Wen, 1999,  $R^2 = 0.65$ ; Villar et al., 2014,  $R^2 = 0.63$ ), but hKCl-N values obtained by direct extraction of manures showed no significant correlation with mineralizable N (Chadwick et al., 2000). Sulfuric acid extractable N ( $H_2SO_4$ -N) has been proposed by Martínez and Galantini (2017) as a fast and low-cost method to assess the moderately labile organic N pool of particulate organic matter (POM-N), which is considered a good predictor for mineralizable N (Curtin and Wen, 1999). The highest correlations with POM-N ( $R^2 = 0.94$ ) and mineralizable N ( $R^2 = 0.90$ ) were obtained at  $H_2SO_4$  concentrations of 0.5 M, and  $H_2SO_4$ -N values were reported to increase proportionally with the acid concentration.

The suitability of chemical extractions to quantify organic N pools as predictors for mineralizable N has been typically evaluated based on the strength of correlations between N extractable by a given method and mineralizable N. However, it has been pointed out that the prediction power of chemical proxies is greatly improved when several predictors addressing differently labile organic N pools are considered simultaneously, as this allows to better represent the complexity of N mineralization (Braos et al., 2016; Lashermes et al., 2010; Ros et al., 2011). Statistically, this has been achieved with multi-variate clustering methods (Lashermes et al., 2010; Morvan et al., 2006; Parnaudeau et al., 2004). Ultimately, this approach generates solid multi-predictor decision trees (Lashermes et al., 2010), allowing to characterize BBFs based on multiple defining N features rather than a single indicator. However, the number of established chemical indicators for N mineralization from BBF categories is currently limited compared to those available for SOM.

The objective of the present study was to test laboratory-based methods to assess mineralizable N and chemically extractable N fractions for a set of 32 BBFs in order to evaluate the ability of the chemical extraction methods to predict N mineralization as assessed in a laboratory incubation.

## 2. Materials and methods

### 2.1 Bio-based fertilizers (BBFs)

Thirty-two commercial or prototyped BBFs were selected to represent the range of products currently or soon available in Europe. This selection includes fertilizing products from 10 European countries, six out of eight component material categories (CMCs) excluding virgin materials (CMC 1) and plant-related products (CMC 2), as they are not typically used as commercial fertilizers, and all six product function categories (PFCs) in accordance with the European Fertilizing Product Regulation (EC) No 1009/2019 (European Council, 2019) (**Error! Reference source not found.**). As storage conditions can affect N properties of BBFs, products were stored following manufacturers' recommendations to keep them unaltered. Fresh samples, such as digestates, were stored at  $-20^{\circ}\text{C}$ . The storage period did not exceed one year. In preparation for incubation and extraction experiments, subsamples of BBFs were homogenized and ground to less than 2 mm using a pestle and mortar if necessary.

Basic characterization of BBFs included dry matter content ( $105^{\circ}\text{C}$ , EN 13040), organic matter content (combustion for 1 hour at  $350^{\circ}\text{C}$  + 5 hours at  $480^{\circ}\text{C}$ ), C:N ratio (Trumac or CHN628, Leco), nitrate-N and ammonium-N (1:60 (v:v) water extracts analyzed with Skalar San++ system).

## 2.2 Biological characterization of mineralizable nitrogen and carbon

Apparent net mineralizable N and microbial respiration activity induced by the amendment of BBFs were assessed using aerobic incubation assays carried out over 84 days in three replicates. C and N mineralization dynamics were assessed in separate samples, which were incubated simultaneously at 15°C and 80% air humidity in loamy topsoil (Loamy1) with a pH of 6.2 (Table 2). In addition to BBFs, unfertilized soil (UNF), ammonium sulfate (AMS) and potassium nitrate (PTN) were included as controls. Apparent net mineralizable N was assessed drawing from the reference method of the Swiss Federal Agricultural Research Station's "Investigation of N dynamics of soils and substrates in aerobic incubation experiments" (Agroscope, 1998a). In a nutshell, BBFs were amended to the equivalent of 120 g dry weight (DW) soil in a ratio of 800 mg N kg<sup>-1</sup> DW soil. N mineralization was quantified at six points (day 0, 7, 14, 28, 56, 84) by extracting the BBF-soil mixture in 1 M KCl. Extracts were stored at -20°C until photometrical analyses with an automated discrete analyser (Smartchem 450 Discrete Analyser, [AMS Alliance](#) - Alliance Instruments, Frépillon, FR). Nitrate content of the extracts was determined according to Keeney and Nelson (1983), while ammonium was determined using the modified indophenol blue reaction (Krom, 1980). The cumulative apparent N mineralization of the incubated soil-BBF mixture expressed in N<sub>min</sub>-N g<sup>1</sup> amended TN was calculated as the sum of nitrate (NO<sub>3</sub>-N) and ammonium (NH<sub>4</sub>-N) in the sample minus the sum of NO<sub>3</sub>-N and NH<sub>4</sub>-N in the unfertilized control:

$$N_{min} = (NO_3 - N + NH_4 - N)_{sample} - (NO_3 - N + NH_4 - N)_{UNF} \quad (\text{Eq. 1})$$

To test the influence of soil type and long-term organic amendment on apparent net mineralizable N, an additional incubation experiment was conducted with BIO, ECO, PAL and OG2, including UNF and ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) as controls, with five additional soils (Table 2, supplementary information S11).

In parallel, microbial respiration was measured based on a modified version of the reference method of the Swiss Federal Agricultural Research Station's "Determination of basal respiration" (Agroscope, 1998c). Essentially, BBFs were amended to the equivalent of 20 g DW soil in a ratio of 800 mg N kg<sup>-1</sup> DW soil and incubated in a closed system. Respired CO<sub>2</sub>-C was trapped in 0.1 M NaOH and quantified at five points in time (day 1, 7, 14, 56, 84) by the addition of 1 mL BaCL<sub>2</sub> followed by inverse titration with 0.1 M HCl on an automated titrator (complete Titrand modular setup, Metrohm AG, Herisau, CH). Emitted CO<sub>2</sub>-C was expressed in mg CO<sub>2</sub>-C g<sup>-1</sup> amended TC. Values of unfertilized soil were subtracted in analogy to equation 1 to obtain cumulative apparent C release from BBFs.

A detailed description of biological characterization methods is given in the supplementary information (S11).

Dynamics of N<sub>min</sub>-N and respiration rates of UNF, mineral controls (AMS and PTN) and internal organic references are given in supplementary information S12 and Fig. S2, S3 and S4.

## 2.3 Chemical characterization of BBFs

Four chemical extraction methods developed for the extraction of N from soil samples were adapted to extract N from BBFs: 1:60N<sub>min</sub> modified from EN 13652, hot water extractable N (HW-N) originally described by Curtin et al. (2006), hot potassium chloride extractable N (hKCl-N) originally described by Chadwick et al. (2000) and sulfuric acid extractable N (H<sub>2</sub>SO<sub>4</sub>-N) originally described by Martínez and Galantini (2017). In pre-tests, different BBF-extractant ratios, incubation times and temperatures (Table S1) were tested on a subset of eight BBFs to select final protocols based on the minimum requirement of average extractable N values above 5% of total N. All final protocols followed the same extraction scheme with different settings (Table 3). In brief, a pre-defined amount of homogenized BBF with its original water content was weighted into 50 mL polypropylene centrifuge tubes and mixed with an aliquot of extractant. The suspensions were incubated at a given temperature for a specific period in an oven (Binder GmbH, Tuttlingen, DE). For 1:60 CW-N<sub>min</sub>, HW-N and hKCl-N, a pre-extraction was

performed in order to differentiate between N released during chemical extraction and easily soluble mineral N species already present in the BBFs. Extracts were separated from the residue by centrifugation (Sigma Laborzentrifugen GmbH, Osterode am Harz, DE, settings: 2600 x g, 15°C, 10 min) and stored at -20°C until further analysis. All extractions were run in duplicate reactions with coefficients of variance mostly below 5% and never exceeding 10%. Extracted N was quantified by measuring Total Nitrogen bound (TNb) in the extracts with a multi-N/C analyzer (Analytik Jena GmbH, Jena, DE) calibrated with Glycine. Extractable N values were expressed as mg TNb g<sup>-1</sup> TN of BBFs. In addition to chemical extractions, we performed SCD but only for a subset of eleven solid and fully organic BBFs. A detailed description is given in the supplementary information SI3.

## 2.4 Statistical analysis

All statistical analyses were performed using R, version 4.1.0 or 4.1.2 (R Core Team, 2021). BBFs were classified according to their N and C mineralization dynamic using a multivariate approach. In detail, a principal component analysis (PCA, R Core Team, 2021) was performed jointly on N<sub>min</sub>-N and CO<sub>2</sub>-C data, and the obtained principal components served as input data for the agglomerative clustering algorithm (euclidean distance, ward.D2 method) provided by the “eclust” function of package “factoextra” (Kassambara and Mundt, 2020). This approach represents a simplification of the clustering technique used by Parnaudeau et al. (2004) and Morvan et al. (2006) and enables to reduce the effect of multicollinearity on the clustering result. The suitability of predictors for N mineralization estimation was evaluated based on linear correlations (Pearson) between predictors and N<sub>min</sub>-N values performed with packages “PerformanceAnalytics” (Peterson et al., 2020) and “Hmisc” (Harrell, 2021). To avoid an underestimation of correlation coefficients caused by differences in moisture contents of BBFs (Lazicki et al., 2020), all values used for correlation analysis were expressed on the basis of the dry weights of the BBFs. The suitability of selected N mineralization predictors to uniquely characterize previously generated clusters was evaluated based on significance levels obtained from analyses of variance followed by Tukey tests (ANOVA, R Core Team, 2021). To test the effect of soil type and long-term organic amendment history, linear models were used separately for each BBF. Interaction between the soil and incubation time was included. Tukey adjusted pairs of estimated marginal means were used to determine differences between soils at given incubation times. An alpha level of p<0.05 was used to determine statistical significance. Visual assessments of the data with diagnostic plots were used to check for homogeneity of variance.

## 3. Results

### 3.1 Basic BBF properties

Overall, analyzed BBFs showed a total N range from 9 mg TN g<sup>-1</sup> DW BBF to 210 mg TN g<sup>-1</sup> DW BBF and total C values between 3 mg TC g<sup>-1</sup> DW BBF and 528 mg TC g<sup>-1</sup> DW BBF (Figure 1). Composts accounted for the lowest TN values and highest C:N ratios. Animal by-products other than manures presented the lowest C:N ratios. For digestates, and the industrial by-products BIP, PRI and SYS, cold water soluble NH<sub>4</sub>-N constituted over 50% of TN. PRI was the only BBF containing substantial amounts of cold-water-soluble NO<sub>3</sub>-N.

PRI, SYS, CGR and PCS had very low organic matter contents, but an accurate assessment by calcination was not possible, as some substances in their mineral fraction had boiling temperatures below the calcination temperature. Composts and chicken manures had lower OM contents than BBFs derived from animal by-products. Dry matter content varied from 56% to 97% for solid BBFs and from 4% to 56% for liquid ones.

### 3.2 Biological characterization of mineralizable N

Apparent net mineralizable N was driven by increasing NH<sub>4</sub>-N values during the first seven days of incubation (Figure S6), by increasing NO<sub>3</sub>-N values and proportionally decreasing NH<sub>4</sub>-N values in the period between the 7<sup>th</sup> and the 28<sup>th</sup> day of incubation (Figures S6 and S7), and by stagnating NO<sub>3</sub>-N values for the remaining incubation time (Figure S7). Mineralizable N<sub>min</sub>-N followed four main patterns: stagnation throughout incubation (e.g., VEC), stagnation followed by a decrease towards day 84 of

incubation (e.g., SDG), increase or decrease following a log-type curve (e.g., SIF, OOC), increase following a linear curve (e.g., CGR).

Composts (CMC 3) showed very low or negative apparent net N mineralization, achieving  $N_{\min}$ -N values between  $-134.2 \pm 0.3$  mg  $N_{\min}$ -N g<sup>-1</sup> amended TN (OOC) and  $260.6 \pm 3.7$  mg  $N_{\min}$ -N g<sup>-1</sup> amended TN (VEC) on the last sampling day (Figure 2). Fresh crop digestates (CMC 4) achieved higher final  $N_{\min}$ -N values than composts (from  $15.5\% \pm 0.0\%$  to  $52.9\% \pm 2.0\%$  of amended TN) either as a result of higher initial  $N_{\min}$ -N values or higher net N mineralization rates (Figure 2). In contrast, non-fresh crop digestates (CMC 5) showed very low net N mineralization rates, with  $N_{\min}$ -N values tending to decrease towards the end of the incubation as a result of decreasing  $NH_4$ -N. Nonetheless,  $N_{\min}$ -N values of these BBFs remained high throughout incubation period because of initial  $NH_4$ -N values equivalent to an average of 50% of amended TN. Similar to non-fresh crop digestates, TRS and BIP showed considerable negative  $N_{\min}$ -N trends after day 28.

Industrial by-products (CMC 6) and precipitated phosphate salts and derivatives (CMC 12) produced very diverse apparent net N mineralization patterns. Especially for BBFs low in organic matter, cumulative  $N_{\min}$ -N increased linearly or remained constant throughout the incubation period, reaching  $N_{\min}$ -N values from  $87.3\% \pm 7.3\%$  to  $105.6\% \pm 0.3\%$  of amended TN after 84 days of incubation. This pattern was mainly  $NO_3$ -N driven, as  $NH_4$ -N values only increased by a maximum of 12.8% in the first 2 weeks of incubation. For the remaining BBFs in these categories, the highest increases of  $N_{\min}$ -N were recorded during the first seven days of incubation, achieving apparent net  $N_{\min}$ -N values from  $42.9\% \pm 3.6\%$  to  $54.7\% \pm 1.6\%$  of amended TN on the 84<sup>th</sup> day of incubation. BBFs derived from animal by-products (CMC 10) presented similar N mineralization curves, with an accentuated  $N_{\min}$ -N increase in the first seven days of incubation and steady values between  $34.1\% \pm 1.0\%$  and  $58.0\% \pm 0.7\%$  of amended TN from day 28 onwards. Exceptions were SWG and OPU, which showed lower  $N_{\min}$ -N values until day 56 of incubation in the case of SWG or until the end of incubation for OPU.

### 3.3 Nitrogen mineralization in different soils

When comparing N mineralization of four BBFs (BIO, ECO, PAL, OG2) in different soils, only the sandy soil showed significantly lower mineralization rates than the other tested soils (Figure 3, Table S2). In addition, BIO and OG2 showed significantly different N mineralization patterns when incubated in the Loamy2\_OA and Loamy3 soils (Figure 3, Table S2). No significant differences in N mineralization were observed between Loamy2\_NPK and Loamy2\_OA, which are the same soil type but with different fertilization histories during the past 18 years, comparing mineral fertilization (NPK) with compost fertilization (OA).

### 3.4 Biological characterization of mineralizable C

Respiration rates representing apparent C mineralization from BBFs covered a wide range of patterns overall and even within each CMC group (Figure 4). For most BBFs, the highest respiration rates were recorded within the first 24 h of incubation, with a maximum value of  $13.2 \pm 0.3$   $\mu$ g  $CO_2$ -C g<sup>-1</sup> DW soil h<sup>-1</sup> for BA6 (data not shown). BBFs inducing the highest respiration rates in the period between one and seven days were either liquid and fully organic (BIP, PCW, ILF), based on meat and bone meal (ECO, OG1) or on chicken manure (FEK, OPU, BIL). After the initial rapid mineralization phase,  $CO_2$ -C values stabilized at a constant level until day 84 of incubation for all BBFs.

Over the entire incubation period, between  $13.7\% \pm 0.7\%$  and  $111.6\% \pm 2.2\%$  of total amended C was respired. For BBFs very low in TC (CGR, PCS, SYS), average respiration rates remained within  $\pm 0.3$   $\mu$ g  $CO_2$ -C g<sup>-1</sup> DW soil h<sup>-1</sup> from UNF values throughout incubation. For those BBFs, an apparent respired C value of 0 mg  $CO_2$ -C g<sup>-1</sup> amended TC was assumed but not considered for statistical analyses. Only two liquid BBFs (BIP and ILF) achieved apparent respired C values significantly higher than 100% of amended TC.

### 3.5 Classification of BBFs based on mineralizable N and C

Bio-based fertilizers were classified according to their N and C mineralization data using a multivariate approach. This classification produced five strongly data-supported BBF clusters as indicated by bootstrapping *p*-values lower than or equal to 0.05 (Figure 5, S10, S11).

Cluster 1 consisted of ten fully organic, solid BBFs and one organo-mineral, liquid BBF. Animal protein (meat and bone meal) and vegetal by-products from the food industry represented the main component

materials (Table 4). Despite the mainly low initial  $N_{\min}$ -N levels ( $<50 \text{ mg } N_{\min}\text{-N g}^{-1}$  amended TN), BBFs of cluster 1 showed high initial N mineralization dynamics, achieving the overall highest  $N_{\min}$ -N levels of tested BBFs. Cluster 2 encompassed seven fully organic or organo-mineral, solid BBFs based mainly on animal protein (hairs or feather meal) or chicken manure.  $N_{\min}$ -N values from cluster 2 showed similar dynamics to cluster 1 but were about 30% lower than those of cluster 1. Cluster 3 contained three liquid or easily soluble BBFs based on animal proteins and vegetal by-products from the food industry. These BBFs were characterized by high initial  $N_{\min}$ -N values (logarithmic-type  $N_{\min}$  curves) and negative N mineralization rates from day 28 onwards. Cluster 4 exclusively included solid, composted BBFs characterized by the overall lowest  $N_{\min}$ -N levels on day 84 of incubation. N mineralization dynamics in this cluster presented almost linear curves dominated by very low or even negative N mineralization rates within the first seven days of incubation. Cluster 5 comprised only digestates. These liquid BBFs were characterized by the overall highest initial  $N_{\min}$ -N values and constant negative N mineralization rates from day 28 onwards.

### 3.6 Chemical characterization

Based on the results of the pre-tests (Supplementary Information SI4, Figure S8), HW-N.1, hKCl-N.1 and  $\text{H}_2\text{SO}_4$ -N.3 were chosen for the definitive N extraction of all BBFs included in the present study. Permanganate oxidizable N protocols were excluded from this selection, as they had even weaker extraction intensities than HW-N protocols.

Across all BBFs, cold water extractable mineral N (1:60 CW- $N_{\min}$ ) yielded the lowest extractable N values (Table 5) and reflected the mineral N contents of BBFs (Figure 1). Also HW-N yielded low extractable N values ( $2 \pm 2 \text{ mg TNb g}^{-1}$  TN BBF to  $248 \pm 7 \text{ mg TNb g}^{-1}$  TN BBF), followed by hKCl-N ( $0 \text{ mg TNb g}^{-1}$  TN BBF to  $466 \pm 11 \text{ mg TNb g}^{-1}$  TN BBF) and  $\text{H}_2\text{SO}_4$ -N ( $95 \pm 5 \text{ mg TNb g}^{-1}$  TN BBF to  $1222 \pm 10 \text{ mg TNb g}^{-1}$  TN BBF). Liquid and easily soluble BBFs (TRS) achieved the lowest HW-N ( $< 90 \text{ mg TNb g}^{-1}$  TN BBF) and hKCl-N ( $< 200 \text{ mg TNb g}^{-1}$  TN BBF) values coupled with highest  $\text{H}_2\text{SO}_4$ -N levels ( $> 790 \text{ mg TNb g}^{-1}$  TN BBF), whereas solid BBFs yielded extractable N values in between those extremes. 1:60 CW- $N_{\min}$  was highest for liquid BBFs low in organic matter. Cumulatively, SOL, CEL and LIG fractions constituted between 68% and 91% of the BBFs' OM, with composts and fermented BBFs achieving the highest values and animal-protein-based BBFs (e.g., MO13) the lowest (Figure S9). Individual fractions, particularly SOL and LIC, appeared to vary considerably between BBFs of different CMCs. Further details are given in supplementary information SI3.

The prediction power of chemical extraction methods in relation to cumulative apparent  $N_{\min}$ -N values at different stages of incubation was evaluated based on Pearson correlations (Table 6). In this regard, dry matter content and 1:60 CW- $N_{\min}$  presented the strongest correlations to  $N_{\min}$ -N values measured at the start of the incubation, with 1:60 CW- $N_{\min}$  explaining 97% of the variance of  $N_{\min}$ -N on day 0. At the late stage of incubation, the C:N ratio was the predictor showing the strongest correlation with  $N_{\min}$ -N levels on day 56 ( $R^2 = 0.68$ ) and day 84 ( $R^2 = 0.73$ ). A similar pattern was found for TN but with lower correlation strengths. HW-N and hKCl-N did not strongly correlate with  $N_{\min}$ -N values at any incubation stage, explaining less than 11% of  $N_{\min}$ -N variance. In contrast,  $\text{H}_2\text{SO}_4$ -N showed high correlation coefficients for days 7 and 14 of the incubation, explaining 72% and 63% of  $N_{\min}$ -N variance, respectively. Similarly, SOL and the index for residual organic carbon showed the highest correlation coefficients for days 7 and 14 of the incubation, explaining 62% and 57% of  $N_{\min}$ -N variance, respectively, even though only 11 BBFs were included (Table S3).

### 3.7 Chemical indicators as cluster predictors

Referring to the results of Pearson correlations between single predictors and  $N_{\min}$ -N values, 1:60 CW- $N_{\min}$ ,  $\text{H}_2\text{SO}_4$ -N and C:N ratio were selected to be evaluated as explanatory variables for BBF classes defined by  $N_{\min}$ -N and  $\text{CO}_2$ -C mineralization patterns. When projecting these three chemical predictors in the multivariate space defined by the principal components of the PCA conducted with cumulative apparent  $N_{\min}$ -N and  $\text{CO}_2$ -C values over the entire mineralization period, 1:60 CW- $N_{\min}$  strongly separated BBFs from cluster 5 from the others (Figure 6). The trends of  $\text{H}_2\text{SO}_4$ -N and C:N ratio were almost opposite to each other and discriminated between BBFs of clusters 1, 2, 3 and 4. Tukey tests were conducted to individually assess significant differences between the BBF clusters for the three selected chemical predictors (Table 7). Cluster 5 had significantly higher 1:60 CW- $N_{\min}$  values

compared to all other clusters ( $p < 0.001$ ), cluster 3 and 5 had significantly higher  $\text{H}_2\text{SO}_4\text{-N}$  values compared to clusters 1, 2 and 4 ( $p < 0.05$ ), and cluster 4 showed significantly higher C:N ratios compared to the other clusters ( $p < 0.01$ ). Combining all selected  $\text{N}_{\min}\text{-N}$  predictors, cluster 4 could be significantly discriminated based on its high C:N ratios, cluster 3 based on its high  $\text{H}_2\text{SO}_4\text{-N}$  values, and cluster 5 by taking into consideration its high 1:60 CW- $\text{N}_{\min}$  levels combined with high  $\text{H}_2\text{SO}_4\text{-N}$  values. Clusters 1 and 2 could not be significantly distinguished from each other with any of the selected predictors, despite a measurable difference in value distribution of 1:60 CW- $\text{N}_{\min}$ ,  $\text{H}_2\text{SO}_4\text{-N}$  and C:N ratio.

## 4. Discussion

### 4.1 Biological characterization

In 84-day aerobic incubations, overall cumulative mineral N releases from -13 to 106% amended N were achieved (Figure 2). A similar range was found by Levavasseur et al. (2022) in their database containing N mineralization data for 663 exogenous organic materials and in previous studies (Calderón et al., 2005; Cordovil et al., 2005; Morvan et al., 2006).

Composts (CMC 3) showed low respiration rates (0% - 20%), in some cases combined with net N immobilization (e.g., OOC). As suggested by several studies, this N limitation results from high initial C:N ratios of composted materials rather than from the composting process itself (Amlinger et al. 2003, Lazicki et al., 2020; Sradnick and Feller, 2020). In fact, the animal by-product-based VEC (C:N ratio: 13.2) achieved net mineralization levels of 26% of amended N, while for OOC (C:N ratio: 32.9) net N immobilization occurred. Similarly to Pansu and Thuriès (2003), no remineralization of immobilized N was observed, confirming that BBFs with high C:N ratios need to be regarded as mineral N sinks rather than sources during short- or medium-term incubation periods.

Non-fresh crop digestates (CMC 5), mainly including digested slurries, presented considerably higher mineralizable N values than similar materials in the study by Levavasseur et al. (2022), where up to 50% of N was mineralized after a 1-year incubation under field conditions. However, the initially high mineral N contents and the rapidly decreasing  $\text{NH}_4\text{-N}$  values coupled with low respiration rates indicate the predominance of  $\text{NH}_4\text{-N}$  removing processes (nitrification, volatilization, immobilization) over ammonification. Indeed, potential volatilization has been shown to account for losses of up to 15% of added N for digestates in laboratory incubation experiments (Wester-Larsen et al., 2022).

Precipitated phosphate salts and derivatives (CMC 12) did not show pronounced logarithmic first-order-kinetic-type mineralization curves (Thuriès et al., 2001), achieving cumulative  $\text{N}_{\min}\text{-N}$  values higher than 80% of amended N after 84 days of incubation despite very low respiration rates throughout the incubation period. These results resemble N-release patterns of controlled release fertilizers (Du et al. 2006). Given the low organic matter content of CGR and PCS,  $\text{N}_{\min}$  release from these BBFs is primarily driven by the solubilization of the coating of the granules and not by mineralization processes. Similar N release patterns were observed for SYS and PRI. For these reasons, CGR, PSC, PRI and SYS should be considered similar to mineral or slow-release fertilizers. Therefore, they are not included in the evaluation of chemical proxies nor the BBF classification proposed in the present study.

Animal by-products (CMC 10) and fresh crop digestates (CMC 4) presented a first-order kinetic-type N mineralization in accordance with previously reported results (Lazicki et al., 2020; Levavasseur et al., 2022). Based on the collected cumulative  $\text{N}_{\min}\text{-N}$ , no evidence for N immobilization or volatilization was found. However, Wester-Larsen et al. (2022) observed cumulative potential ammonia volatilization above 10% of added TN for all animal BBFs after 44 days of incubation when applied on the soil surface. When incorporated into the soil, volatilization decreased. This highlights the importance of assessing ammonia volatilization potential in a given system to achieve a comprehensive estimation of mineralizable N from BBFs. Particularly TRS and SIF recorded low respiration and high  $\text{NH}_3$  volatilization potentials (Wester-Larsen et al. 2022) after the 14<sup>th</sup> day of incubation which could indicate  $\text{NH}_4$  or  $\text{NH}_3$  toxicity (Monaghan and Barraclough, 1992). However, it is not possible to draw further conclusions from these studies on the relationship between  $\text{NH}_3$  volatilization and nitrification inhibition through reduced forms of N.



The comparison of N mineralization from selected BBFs in five different soils revealed lower N mineralization rates in sandy soils than in loamy and clayey soils (Figure 3). In agreement with our results, Amato et al. (1987) found only small differences between decomposition patterns of plant material in different Australian soil types and observed that clay content affected decomposition, whereas SOM and pH did not. Although mineralization of organic N depends on soil microbiology (Schröder et al. 2016), no significant difference between the Loamy2\_OA and the Loamy2\_NPK soils was found in the present study, despite the higher microbial biomass of Loamy2\_OA resulting from regular compost amendments over 18 years. Similarly, Nett et al. (2010) and Nett et al. (2012) found only minor effects of soil amendment history on N mineralization of newly added organic materials.

#### 4.2 Chemical characterization

Concerning CMCs, C:N ratios and original mineral N values, BBFs in the present study show similar ranges as recently reported by Lazicki et al. (2020) and Levavasseur et al. (2022). In contrast to other incubation studies (Levavasseur et al., 2022; Parnaudeau et al., 2004), precipitated phosphate salts and derivatives in the present study (CGR, PCS) were very low in organic matter and total C content, resulting in C:N ratios close to zero.

To the best of our knowledge, no published studies assess HW-N or H<sub>2</sub>SO<sub>4</sub>-N of exogenous organic matters (EOMs) as potential predictors for their mineralizable N values. hKCl-N extractions have been applied to EOMs before (Chadwick et al., 2000), with similar results as observed in the present study. HW-N and hKCl-N included a pre-extraction in order to separate readily available N from highly labile N. Thus, for liquid or highly soluble BBFs (e.g., BIP, TRS) the pre-extraction step removed up to 99% of TN from the BBFs, resulting in very low HW-N and hKCl-N. On the other hand, H<sub>2</sub>SO<sub>4</sub>-N (no pre-extraction) values of these BBFs were close to 100% of the BBF's TN.

For HW-N, hKCl-N and H<sub>2</sub>SO<sub>4</sub>-N, standard errors were under 5% and never exceeded 10%, attesting that the protocols used can be considered reliable. However, sample homogenization in preparation for extractions is a critical step, especially for suspended BBFs such as digestates or BBFs based on mixed animal and vegetal by-products (e.g., MO13), which showed the highest standard errors despite careful sub-sampling.

Extraction intensity and leaching mechanisms are generally critical features that can influence the prediction power of chemical N extraction methods. Of particular relevance is the thermal hydrolysis of heat-labile organic N compounds to NH<sub>4</sub>-N, which can be expected to occur during extraction procedures at elevated temperatures (Curtin et al., 2006; Gregorich et al., 2003). This implies that mineral-to-organic N ratios measurable in the extracts are not fully representative of the original ratios of untreated samples. To compensate for this effect, for the screening intentions of the present study, extractable N was quantified as TN<sub>b</sub> rather than by N<sub>min</sub> or Kjeldahl N assessment methods. However, this choice implied the lack of a further subdivision of extracted N into mineral and organic species, which could give a deeper insight into the nature of extracted N and lead to different correlation patterns with mineralizable N. Nonetheless, even with TN<sub>b</sub> quantification NH<sub>4</sub>-N remained a critical N species to assess because especially at high concentrations losses could not be avoided, as was clearly visible from the H<sub>2</sub>SO<sub>4</sub>-N value of the fully mineral and liquid BBF "SYS" (Table 4).

The comparison of extraction intensities made by Ros et al. (2011) for SOM-N extraction methods shows that the HW-N, hKCl-N and H<sub>2</sub>SO<sub>4</sub>-N protocols tested in the present study leached more TN from BBF than from SOM. When applied to BBFs rather than SOM, HW-N appeared to achieve systematically lower values than hKCl-N, with an average extraction difference of 9%. However, this is likely a result of the higher extraction temperature of hKCl-N (100°C) compared to HW-N (80°C). In fact, extraction temperature has been reported as a critical parameter influencing the extraction intensity (Ros et al., 2011).

For SOM-N, it has been proposed to classify extraction procedures that extract up to 5% TN as mild methods, those extracting up to 35% as moderate methods, and the rest as strong methods (Ros et al., 2011). However, based on the results of the present study, thresholds for extractable N for the classification of BBF-adapted methods need to be reconsidered in relation to the intensity of N extraction. As a matter of fact, all tested extraction methods yielded average extractable N equivalents above 9.5% of TN in the BBFs, except H<sub>2</sub>SO<sub>4</sub>-N, which leached on average above 50% TN. Building

on the comparison of different extraction intensities, in the context of SOM-N mineralization studies, some authors observed stronger correlations between mineralizable N and mild chemical indicators, explaining this result with the argument that such extractions are less prone to extracting recalcitrant organic N pools (Braos et al., 2016; Curtin et al., 2006). However, neither Ros et al. (2011) nor the present study confirm this observation.

In fact, extraction intensity did influence  $N_{\min}$ -N prediction power, but methods with low extraction intensities (HW-N and hKCl-N) correlated only weakly with mineralizable N, supporting the findings of Chadwick et al. (2000). In contrast,  $H_2SO_4$ -N showed a considerably higher correlation strength with  $N_{\min}$ -N values (Table 6). SCD-derived variables showed strong correlations with mineralizable N in the mid-stages of incubation (Table S3). Trends of  $r$ -values for different stages of mineralization are comparable to those reported in former studies (Jensen et al., 2005; Lashermes et al., 2009; Morvan et al., 2006). In particular, SOL fractions were reported to be linked by a significant negative correlation to LIC fractions and mostly explained mineralization patterns in the early to mid-stages of incubation, whereas cellulose-related fractions better explained mineralization dynamics at later stages of incubation. Compared to SOL and LIC, correlation coefficients achieved by CEL were lower. This is probably linked to the high C content, which has been observed to be associated with this fraction, making it a more suitable predictor of C rather than N mineralization dynamics (Jensen et al., 2005).

The prediction power of C:N ratios and TN for mineralizable N has been widely studied, and the highest correlations have been reported in relation to  $N_{\min}$ -N achieved in the late stages of incubation (Chadwick et al., 2000; Jensen et al., 2005; Lashermes et al., 2010; Lazicki et al., 2020). Similarly to Lazicki et al. (2020), C:N ratios appeared to be better indicators of  $N_{\min}$ -N than TN, as C:N ratios are more decisive than TN alone for the affinity of EOMs to mineralization.

The strong correlation between 1:60 CW- $N_{\min}$  and  $N_{\min}$ -N levels at the start of the incubation can be explained by considering the methodological aspects of these N quantifications. In fact, both variables were obtained following similar protocols, which are designed to target readily available N pools exclusively. The correlation between  $N_{\min}$ -N and DMC can be explained by the fact that the total N of liquid BBFs often has a higher proportion of inorganic N. Also, physical degradation properties (e.g., affinity to solubilization or crumbling) of BBFs could serve as explanatory variables of C and N mineralization dynamics in the first week of incubation, but they were not considered in the present study.

### 4.3 Evaluation of chemical predictors

In the present study, the suitability of a number of chemical BBF characterization methods was evaluated with respect to i) the estimation of cumulative apparent  $N_{\min}$ -N levels in different incubation phases and ii) the discrimination of significantly different C and N mineralization patterns. Based on correlation coefficients, coefficients of determination and levels of significance, three predictors were considered suitable for estimating  $N_{\min}$ -N at given incubation stages. Threshold values of the correlation coefficient  $r > 0.8$ ,  $R^2 > 0.6$  and  $p < 0.001$  were applied as evaluation criteria for the selection of suitable chemical indicators. In contrast, for fertilizer recommendations based on mineralizable SOM-N it has been suggested that only characterization methods associated with  $R^2 > 0.83$  can be reliably used to extrapolate mineralizable SOM-N levels (Malley et al., 2004). However, even the best predictors for mineralizable SOM-N did not achieve  $R^2 > 74\%$  (Ros et al., 2011), suggesting that  $R^2 \geq 0.6$  can be considered indicative of reasonably high prediction power for the estimation of  $N_{\min}$ -N from BBFs.

In this regard, it is important to notice how the reliability of predictors changes in relation to different phases of N mineralization. The C:N ratio, for example, could be considered a reliable predictor of cumulative  $N_{\min}$ -N at the end of the incubation, as reported by several authors (Chadwick et al., 2000; Lazicki et al., 2020; Morvan et al., 2006), but has been reported as only a poor proxy for  $N_{\min}$ -N levels in the first stages of incubation (Jensen et al., 2005). Thus, the C:N ratio is not a suitable candidate for a holistic single-predictor-based estimation of BBFs' mineralization dynamics.

Ros et al. (2011) suggested that the combination of multiple predictors could lead to higher confidence levels of  $N_{\min}$ -N prediction through a more comprehensive characterization of the mineralized materials. Furthermore, considering the incubation stage-dependent correlation patterns, a multi-predictor approach comprising a combination of reliable proxies for different stages of N mineralization could further improve prediction power. A number of EOM classifications build on this principle, using

Multivariate approaches to generate and evaluate chemical predictor-based EOM clusters (Bareha et al., 2018; Lashermes et al., 2010; Morvan et al., 2006; Parnaudeau et al., 2004).

Following this approach, C:N ratios,  $\text{H}_2\text{SO}_4\text{-N}$  and 1:60 CW- $\text{N}_{\min}$  were selected as chemical predictors for the reconstruction of the  $\text{N}_{\min}$ -N and  $\text{CO}_2$ -C-based BBF clustering (Figure 6). Based on the analyses of variance, C:N ratios could significantly discriminate BBF classes characterized by low N mineralization or immobilization (cluster 4) from those with medium to high cumulative  $\text{N}_{\min}$ -N values, with similar thresholds to those observed by other authors for plant materials (Trinsoutrot et al., 2000), wastewater sludges (Parnaudeau et al., 2004) and EOMs (Lashermes et al., 2010; Pansu et al., 2003). Values of 1:60 CW- $\text{N}_{\min}$  discriminated BBFs contained in cluster 5 mainly as a result of their high initial  $\text{N}_{\min}$ -N but did not remarkably contribute to subdividing BBFs based on their N and C mineralization patterns in the mid to late stages of incubation. This finding is related to the rapidly decreasing influence of initial mineral N concentrations on N mineralization within the first seven days of incubation. Amongst selected chemical indicators,  $\text{H}_2\text{SO}_4\text{-N}$  was the predictor significantly discriminating the highest number of BBF clusters due to its high prediction power for mid-incubation  $\text{N}_{\min}$ -N values.

#### 4.4 Classification of BBFs

Classification of BBFs based on principal components of  $\text{N}_{\min}$ -N and  $\text{CO}_2$ -C data alone generated five strongly data-supported clusters, which presented similar compactness as those generated by  $\text{N}_{\min}$ -N data alone (data not shown), but with a higher number of significantly different clusters. In fact, the consideration of C mineralization dynamics in the establishment of N mineralization classes of EOMs has been reported to increase the number of generated classes (Lashermes et al., 2010), thus representing a mean to discern N mineralization patterns in more detail. The number of  $\text{N}_{\min}$ -N- and  $\text{CO}_2$ -C-based BBF clusters in our study is similar to other studies on EOMs where multivariate clustering approaches generated five (Parnaudeau et al., 2004, 17 EOMs) to six (Lashermes et al., 2010, 273 EOMs; Morvan et al., 2006, 47 EOMs) clusters. Similarly to these publications, BBF clusters generally discriminated between i) very high and fast N mineralization associated with high C mineralization (cluster 3), ii) high N mineralization associated with medium-high C mineralization (cluster 1), iii) medium N mineralization associated with medium-low C mineralization (cluster 2), iv) low or negative N mineralization associated with medium-low C mineralization (cluster 4), and v) low or negative N mineralization associated with medium C mineralization (cluster 5).

Similar to studies discriminating six clusters by further dividing between low and negative N mineralization patterns (Lashermes et al., 2009; Morvan et al., 2006), in the present study, cluster 4 could have been further subdivided by assigning OOC to a sixth cluster. However, given the low representation of this type of N mineralization dynamic amongst the analyzed BBFs, a six-cluster classification was not data-supported.

In terms of CMCs (i.e., raw materials and their processing), only cluster 4 and cluster 5 could be assigned uniquely to one category, whereas clusters 1, 2 and 3 all included a combination of fully organic or organo-mineral BBFs based on animal, vegetal or mixed raw materials. Thus, even within the same CMC, BBFs differ with respect to their C and N mineralization patterns. In this respect, it should be mentioned that the CMCs are primarily intended to simplify trading and are less suitable for application recommendations. In particular, BBFs from CMC 10 formed three sub-groups: animal proteins (e.g., meat and bone meal) represented in cluster 1, animal proteins high in beta-keratin content (e.g., feather meal, animal hairs) and (chicken) manures represented in cluster 2, easily soluble animal proteins (e.g., fish soluble) represented in cluster 3. Wester-Larsen et al. (2022) proposed a more detailed CMC grouping (especially CMC10), which could potentially better fit clusters in our study, which groups BBFs according to a combination of feedstock and processing technology. Similarly to the CMC distribution amongst clusters of the present study, also Parnaudeau et al. (2004) and Morvan et al. (2006) discriminated municipal wastewater sludges and composted materials, respectively, into four significantly different N mineralization classes. Thus, it appears unlikely that CMC is a suitable predictor for C and N mineralization dynamics of BBFs.

## 5. Conclusion and outlook

The 32 bio-based fertilizers investigated here presented a wide span of mineralizable N and C patterns in biological mineralization assays. Based on their mineralization dynamics, BBFs were divided into

five significantly different clusters which did not match the CMC classification specified by the European Fertilizing Products Regulation (EC) No 1009/2019.

Tested chemical BBF characterization methods covered a spectrum of extraction intensities. Their prediction power for mineralizable N levels of BBFs varied depending on the considered stage of N mineralization. The correlations between mineralizable N and cold water extractable mineral N, sulfuric acid extractable N and C:N ratios were satisfactory for the estimation of mineralizable N from BBFs at different mineralization stages.

However, the combination of the three chemical indicators was insufficient to classify BBFs according to their N and C mineralization patterns in five groups without ambiguity. SCD-fractions and near-infrared spectroscopy could potentially be used as additional indicators to address this uncertainty.

This study shows that a multiple-predictor approach exclusively based on chemical indicators targeting differently labile organic N pools of BBFs is regarded as a realistic alternative to biological characterization methods, provided that a sufficient number of suitable predictors, e.g. three as in this study, is available. Thus, we suggest chemical characterization methods as promising tools to achieve a holistic characterization of N and C mineralization dynamics of BBFs, which is a key prerequisite for the appropriate use of organic fertilizers. Thus, we recommend to use those indicators for the development of a novel BBF classification system to facilitate the agronomical application of BBFs and ensure a link between N release dynamics of BBFs and their context-specific application (pedoclimate, crop, growth stage).

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This study was financially supported by the EU Horizon 2020 project, LEX4BIO. We thank all BBF manufacturers for providing samples for this study (Agrana, Agro Energie Hohenlohe GmbH & Co KG, Biofert, AKV, Biolan Oy, Bioplant, Biovækst, Daka, DCM, Ecolan Oy, Fertikal, Fertilex Oy, Hashøj biogas, Hauert, Ilsagroup, MALTaflor, MeMon, Optisol, Ostara, Palaterra Betriebs- und Beteiliungsgesellschaft mbH, Solrød biogas, Sopropêche SYSTEMIC biogas plant, YARA and Århus vand).” The authors also thank A. Kuhn, A. Wiget for chemical analysis of samples and methodological advice; A. Munyangabe for assistance in lab work; A. Steiner for support during performance of stepwise chemical digestion; M. Krauss for advice on statistical analyses.

## Tables

*Table 1: Description, country of origin, component material category (CMC) and product function category (PFC) of bio-based fertilizers (BBFs) included in the present study. Component material numbers in parentheses only apply to a minor portion of component materials of the BBF. The table was modified from Wester-Larsen et al., (2022) and additionally includes SWG and SLA20. For information on BBF properties see Wester-Larsen et al., (2022).*

BBF	Origin	Description	CMC	PFC
BA5	AT	Organic fertilizer based on bioethanol residues amended with molasses, potato fruit water, maize steeping water and wheat gluten.	4, (6)	1 A-I
BA6	AT	Organic fertilizer based on bioethanol residues amended with molasses, potato fruit water and maize steeping water.	4, (6)	1 A-I
BIH	CH	Organic fertilizer based on horn meal.	10	1 A-I
BIL	FI	Organic fertilizer based on broiler manure and seaweed.	10	1 A-I
BIO	FI	Organo-mineral fertilizer containing meat and bone meal, apatite, vinasse, chicken manure and potassium sulphate.	10	1 B-I
BIP	DK	Liquid organic fertilizer based on by-products from sugar beets' and potatoes' processing.	6	1 A-II
BVC	DK	Organic fertilizer based on composted municipal organic food waste.	3	1 A-I
CGR	UK	Mineral fertilizer based on recovered nutrients from wastewater supernatants with a special coating for nutrient release optimization.	12	1 C-I-a
ECO	FI	Organic fertilizer based on meat and bone meal.	10	1 B-I
FEK	BE	Organic fertilizer fully based on chicken manure.	10	1 A-I
FEL	FI	Organic fertilizer based on chicken manure.	10	1 A-I
HDG	DK	Liquid organic fertilizer based on digestated pig's slurry	5	1 A-II
ILF	IT	Liquid organic fertilizer based on gelamin produced by the enzymatic hydrolysis of shavings from treated hides and skins.	10	1 A-II
MAL	DE	Organo-mineral fertilizer based on malt sprouts, vinasse from sugar beets processing and minerals.	6	1 A-I
MB2	CH	Organic fertilizer based on meat and bone meal.	10	1 B-I
MO13	NL	Organic pelleted fertilizer based on feather meal and vegetal by-products.	10	1 A-I
NAD	DE	Organic fertilizer based on fermented manure and vegetal by-products.	4	1 B-I
OG1	DK	Organo-mineral fertilizer based on meat and bone meal.	10	1 B-I
OG2	DK	Organic fertilizer based on horn meal from pigs' bristles.	10	1 A-I
OOC	ES	Composted by-products from the processing of olives.	3	1 A-I
OPU	CH	Organic fertilizer based on chicken manure.	10	1 B-I
PAL	DE	Organo-mineral fertilizer based on vegetal and animal by-products amended with fermented biochar, clay and stone powder.	4	1 A-I
PCS	DK	Liquid mineral fertilizer based on wastewater treatment by-products.	12	1 C-I-a
PCW	DK	Liquid organic fertilizer based on cell water from potato protein production.	6	1 B-II
PRI	NL	Organo-mineral fertilizer integrated with animal and vegetal by-products.	3, 6	1 B-I
SDG	DK	Liquid organic fertilizer based on fermented agricultural and food industry by-products, amended with seaweed.	5, 6	1 A-II
SIF	NL	Organic fertilizer based on hair meal, feather meal, meat and bone meal and vegetable by-products from the food industry.	6, (10)	1 A-I
SLA20	CH	Liquid organic fertilizer based on digested slurry.	5	1 A-II
SWG	CH	Organic fertilizer based on sheep wool.	10	1 A-I

SYS	NL	Liquid mineral fertilizer based on scrubber water from a biogas facility.	6	1 C-II-b
TRS	FR	Organo-mineral fertilizer based on fish soluble, proteins and ash.	10	1 A-I
VEC	ES	Vermicompost from horse manure.	3	1 A-I

Table 2: Characteristics of soils used for aerobic incubation assays.

Soil property	Loamy1	Loamy2_OA	Loamy2_NPK	Loamy3	Sandy	Clayey
pH 1:5 CaCl <sub>2</sub>	6.5	6.94	6.87	7.04	5.16	5.75
WHC (% of DM)	39.3	44.1	42.4	39.5	42.7	50.1
Total N (% of DM)	0.16	0.35	0.2	0.09	0.13	0.24
Total C (% of DM)	1.58	3.18	1.82	0.79	1.45	2.9
C:N ratio	10.06	9.1	9.12	8.46	10.85	11.92
Clay <0.002 mm (%)	14	18.3	16.3	14.5	6.9	44.4
Silt 0.002-0.02 mm (%)	26	15	13.6	22.9	6.8	22
Fine sand 0.02-0.2 mm (%)	56	61.9	67.6	60.8	82.3	29
Coarse sand 0.2-2 mm (%)	19.8	2.86	1.5	1.04	2.3	2.72

Total carbon (C) and nitrogen (N) were determined by Dumas combustion. Water holding capacity (WHC) was determined based on a modification of ISO 14238; pH was determined in 0.01 M CaCl<sub>2</sub> in the ratio 1:5; analysis of texture (sieving and sedimentation) was performed at Agrolab Sarstedt.

Table 3: Overview of extraction methods, references and incubation settings for the final protocols to determine hot water extractable nitrogen (HW-N), hot potassium chloride extractable N (hKCl-N), sulfuric acid extractable N (H<sub>2</sub>SO<sub>4</sub>-N) and cold water extractable mineral N (1:60 CW-N<sub>min</sub>) in bio-based fertilizers (BBFs).

Extraction method	Original protocol	BBF fresh weight	Extractant	Extractant volume	Pre-extraction	Incubation settings
HW-N	Curtin et al., 2006	4 g	Demineralized water	30 mL	Cold demin. water, 30 min shaking at room temperature	16 h, 80°C
hKCl-N	Chadwick et al., 2000	4 g	2 M KCl	20 mL	Cold 2 M KCl, 2 h shaking at room temperature	4 h, 100° C
H <sub>2</sub> SO <sub>4</sub> -N	Martínez and Galantini, 2017	2 g	0.1 M H <sub>2</sub> SO <sub>4</sub>	24 mL	None	4 h, 100° C
1:60 CW-N <sub>min</sub>	EN13652 (Modified from 1:5 to 1:60 extraction ratio)	Equivalent of 2 ml	Demineralized water	120 ml	Cold demin. water, 1 h shaking at room temperature	None

Table 4: Characterization of bio-based fertilizers (BBF) clusters based on nitrogen (N) and carbon (C) mineralization data (Figure 5) with respect to component material categories (CMCs), product function categories (PFCs), average initial  $N_{min}$ -N ( $N_{min}$ - $N_{d0}$ ), average cumulative apparent  $N_{min}$ -N at the end of incubation ( $N_{min}$ - $N_{d84}$ ), average apparent mineralizable N represented by  $N_{min}$ -N differences between days 0 and 84 of incubation ( $\Delta N_{min}$ ), average cumulative apparent  $CO_2$ -C at the end of incubation ( $CO_2$ - $C_{d84}$ ) and a qualitative description of N mineralization dynamics ( $N_{min}$  curve type). UNF values were subtracted.

Cluster	CMCs	PFCs	Mean $N_{min}$ - $N_{d0}$	Mean $N_{min}$ - $N_{d84}$	Mean $\Delta N_{min}$	Mean $CO_2$ - $C_{d84}$
			(mg $N_{min}$ -N g <sup>-1</sup> amended TN)	(mg $N_{min}$ -N g <sup>-1</sup> amended TN)	(mg $N_{min}$ -N g <sup>-1</sup> amended TN)	(mg $CO_2$ -C g <sup>-1</sup> amended TC)
1	(4), 6, 10	1A-I, 1B-I, 1B-II	71 ± 28	491 ± 17	419 ± 36	654 ± 35
2	(3), 4, 10	1A-I, 1B-I	107 ± 32	404 ± 26	297 ± 51	415 ± 20
3	6, 10	1A-I, 1A-II	223 ± 120	516 ± 21	292 ± 109	1038 ± 65
4	3	1A-I, 1B-I	85 ± 30	103 ± 73	18 ± 47	118 ± 7
5	5, (6)	1A-II	583 ± 24	480 ± 19	-102 ± 41	445 ± 95

Table 5 Mineralizable nitrogen (N) fractions of bio-based fertilizers (BBFs) obtained using the following chemical extraction methods: hot-water extractable N (HW-N, mg TNb g<sup>-1</sup> TN BBF), hot-KCl extractable N (hKCl-N, mg TNb g<sup>-1</sup> TN BBF), sulfuric acid extractable N (H<sub>2</sub>SO<sub>4</sub>-N, mg TNb g<sup>-1</sup> TN BBF) and cold water extractable mineral nitrogen (1:60 CW-N<sub>min</sub>, mg N<sub>min</sub>-N g<sup>-1</sup> TN BBF). Data were collected at the Research Institute of Organic Agriculture (FIBL) and the Natural Resources Institute of Finland (LUKE).

BBF	HW-N	hKCl-N	H <sub>2</sub> SO <sub>4</sub> -N	1:60 CW-N <sub>min</sub>	BBF	HW-N	hKCl-N	H <sub>2</sub> SO <sub>4</sub> -N	1:60 CW-N <sub>min</sub>
BA5	32.1	100.6	403.7	12.6	NAD	112.4	226.1	203.1	40.2
BA6	96.9	260.8	435.7	14.4	OG1	95.6	249.2	485.3	6.9
BIH	159.2	200.5	257.1	21.0	OG2	48.8	97.8	242.2	10.1
BIL	132.2	355.7	513.9	199.5	OLC	87.3	105.7	95.1	0.0
BIO	200.3	393.5	502.1	35.2	OPU	218.3	375.8	355.4	111.5
BIP	10.6	24.8	1137.9	495.4	PAL	93.3	221.5	410.6	196.3
BVC	147.3	174.1	254.7	82.8	PCS	18.6	196.0	502.6	10.7
CGR	23.7	290.4	346.9	7.2	PCW	40.6	129.0	1040.6	156.9
ECO	48.8	114.5	332.4	26.7	PRI	105.1	229.2	871.9	973.5
FEK	303.3	466.1	438.4	203.0	SDG	77.9	177.0	891.7	682.9
FEL	156.3	174.2	280.0	33.3	SIF	139.1	270.7	391.5	46.4
HDG	81.8	6.0	941.9	613.6	SLA20	59.2	103.5	790.7	558.1
ILF	7.0	22.8	921.3	21.5	SWG	64.2	102.1	174.5	2.6
MAL	26.6	366.2	706.4	279.2	SYS	0.0	0.3	963.8	951.7
MB2	248.3	401.0	558.3	5.0	TRS	6.4	18.1	989.8	50.1
MO13	59.6	161.3	308.7	9.9	VRC	148.3	328.9	428.6	33.9

Table 6: Pearson correlation coefficients between mineral N ( $N_{\min}$ -N) values obtained from biological incubation assays and potential chemical predictors. Bold values indicate corresponding  $R^2 > 0.6$ , p-values of correlation coefficients are indicated with asterisks ( $p < 0.001$  \*\*\*,  $p < 0.01$  \*\*,  $p < 0.05$  \*).

Predictor <sup>#</sup>	$N_{\min}$ -N (mg $N_{\min}$ -N g <sup>-1</sup> amended TN)					
	Day 0	Day 7	Day 14	Day 28	Day 56	Day 84
DMC	-0.85***	-0.53***	-0.38*	-0.35*	-0.20	-0.11
TN	-0.09	0.29	0.45**	0.59***	0.67***	0.62***
TC	-0.46**	-0.28	-0.13	-0.03	0.15	0.22
C:N ratio	-0.15	-0.49**	-0.58***	-0.69***	<b>-0.83***</b>	<b>-0.86***</b>
HW-N	-0.20	-0.34	-0.36*	-0.31	-0.28	-0.13
hKCl-N	-0.19	-0.32	-0.29	-0.29	-0.22	-0.12
H <sub>2</sub> SO <sub>4</sub> -N	0.67***	<b>0.85***</b>	<b>0.80***</b>	0.73***	0.62***	0.42*
1:60 CW- $N_{\min}$	<b>0.99***</b>	0.63***	0.49**	0.46**	0.32	0.2

<sup>#</sup> Listed predictors are: dry matter content (DMC), total nitrogen content (TN), total carbon content (TC), carbon to nitrogen ratio (C:N ratio), hot water extractable N (HW-N), hot potassium chloride extractable N (hKCl-N), sulfuric acid extractable N (H<sub>2</sub>SO<sub>4</sub>-N) and cold water extractable mineral N (1:60 CW- $N_{\min}$ ). BBFs with fully mineral behaviour (PRI, SYS, CGR, PCS) not included in correlation analyses.

Table 7: Results of the Tukey test performed for each selected chemical indicator individually to test the ability of chemical predictors to discriminate BBFs into clusters generated by aggregative hierarchical clustering (Figure 5). Selected predictors are cold water extractable mineral N (1:60 CW- $N_{\min}$ ), sulfuric acid extractable N (H<sub>2</sub>SO<sub>4</sub>-N) and carbon to nitrogen ratio (C:N ratio).

Chemical indicator	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	p-value
1:60 CW- $N_{\min}$	a	a	a	a	b	$1.4 \times 10^{-6}$
H <sub>2</sub> SO <sub>4</sub> -N	a	a	b	a	b	$6.6 \times 10^{-6}$
C:N ratio	a	a	a	b	a	$5.3 \times 10^{-4}$



## Figures

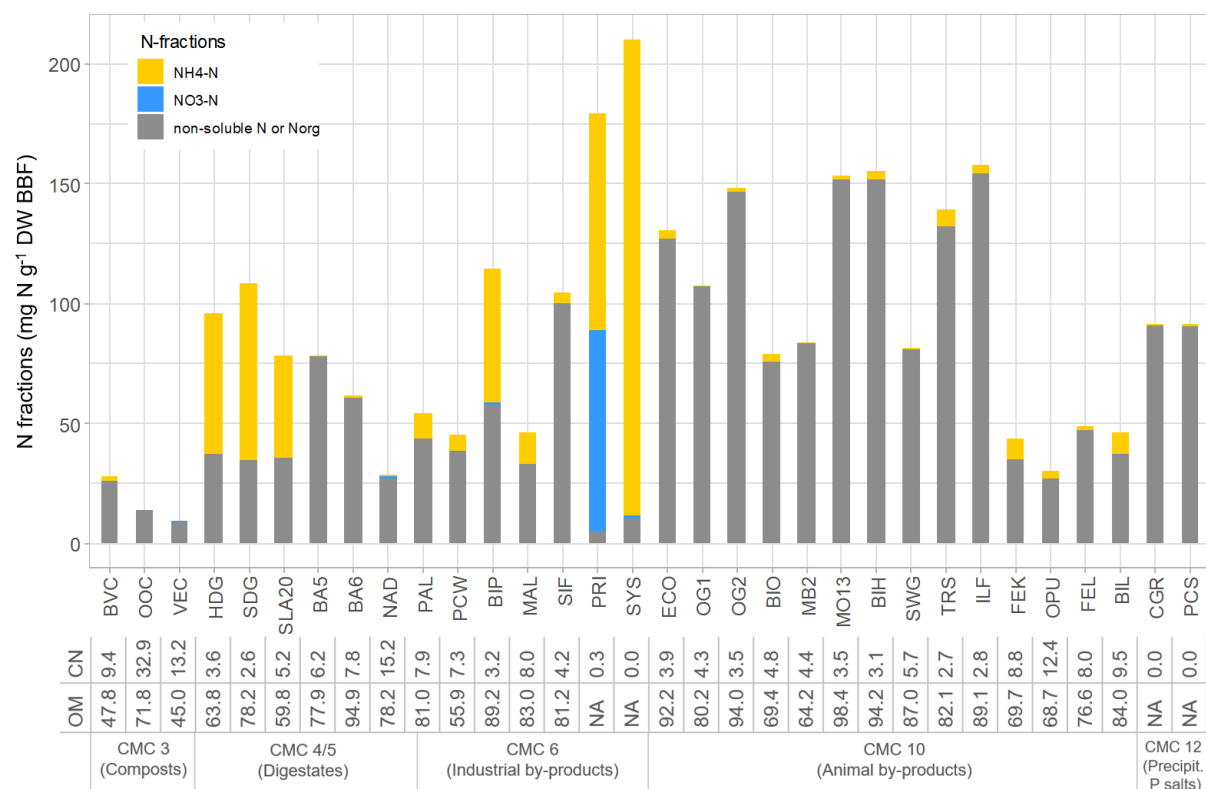


Figure 1: Nitrogen (N) content of analyzed bio-based fertilizers (BBFs) expressed relative to their dry matter content (DW). Total N measured by dry combustion is further divided into cold-water soluble ammonium ( $\text{NH}_4\text{-N}$ ), cold-water soluble nitrate ( $\text{NO}_3\text{-N}$ ) and cold-water insoluble or organic N. Organic matter (OM) content expressed in percent DW, CN ratios of each BBF and component material categories (CMC) are noted in the table underneath the plot. Data presented here are partially redrawn from those presented in Wester-Larsen et al., (2022).

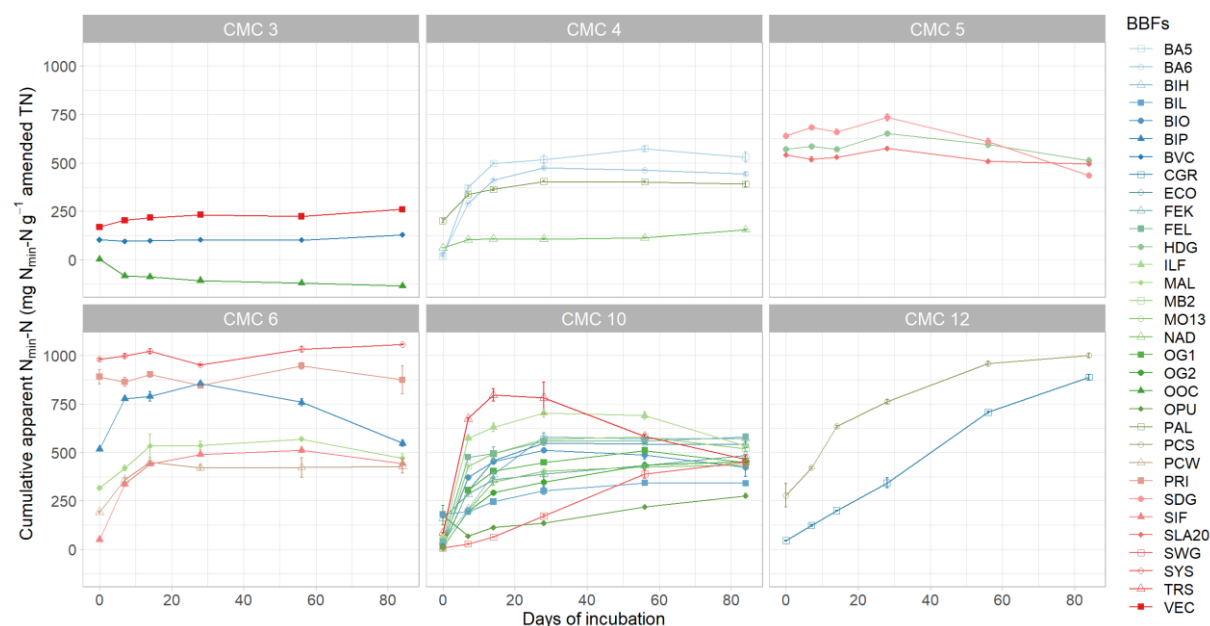


Figure 2: Cumulative apparent mineralized nitrogen ( $N_{\min}\text{-N}$ ) values (means and standard errors) expressed relative to total amended nitrogen (TN) ( $800 \text{ mg N kg}^{-1} \text{ DW soil}$ ) and shown in sub-groups based on the component material category (CMC) of bio-based fertilizers (BBFs). Values of unfertilized soil were subtracted. The  $N_{\min}\text{-N}$  value on day 0 is not derived from mineralization, but reflects the intrinsic mineral nitrogen content of the BBFs. Assuming linear mineralization rates between time points, the slope of the lines connecting measured values represent  $N_{\min}\text{-N}$  mineralization rates.

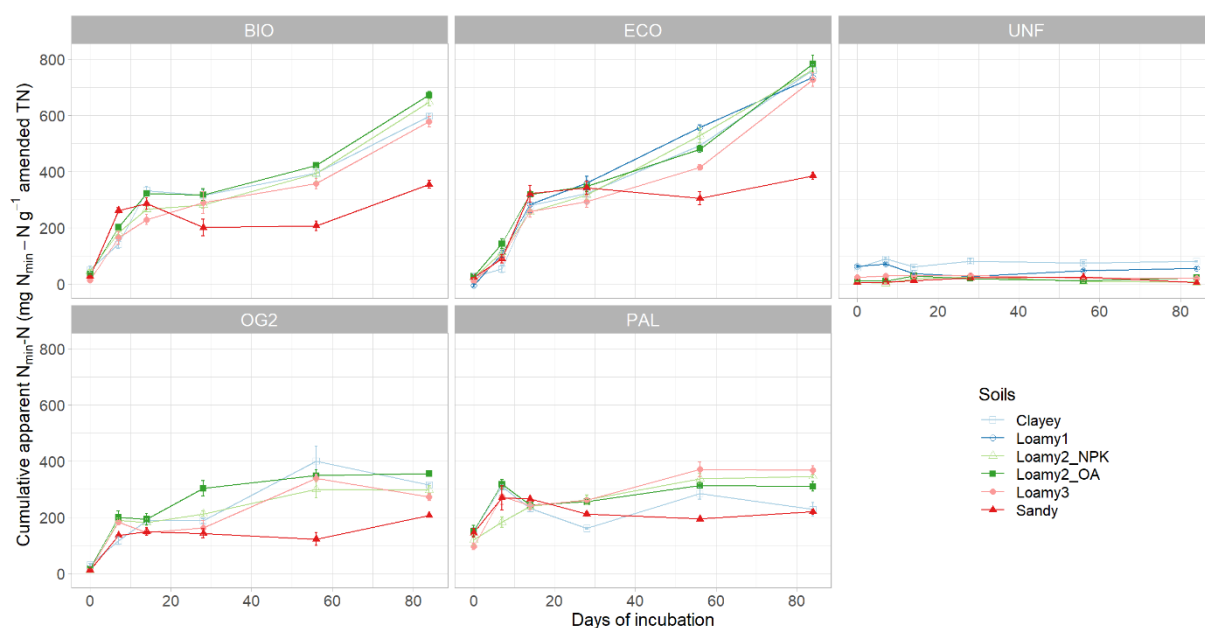


Figure 3 Cumulative apparent mineralized nitrogen ( $N_{min-N}$ ) values (means and standard errors) expressed relative to total amended nitrogen (TN) ( $800 \text{ mg N kg}^{-1} \text{ DW soil}$ ) of bio-based fertilizers (BBFs) in soils with different soil textures (see Table 1 for details on soil properties). Values of unfertilized soil were subtracted. The BBFs BIO, PAL and OG2 were tested in five soils, ECO was tested in six soils. Values of unfertilized soil were not subtracted. The  $N_{min-N}$  value on day 0 is not derived from mineralization, but reflects the intrinsic mineral N content of the BBFs. Assuming linear mineralization rates between time points, the slope of the lines connecting measured values represents  $N_{min-N}$  mineralization rates.

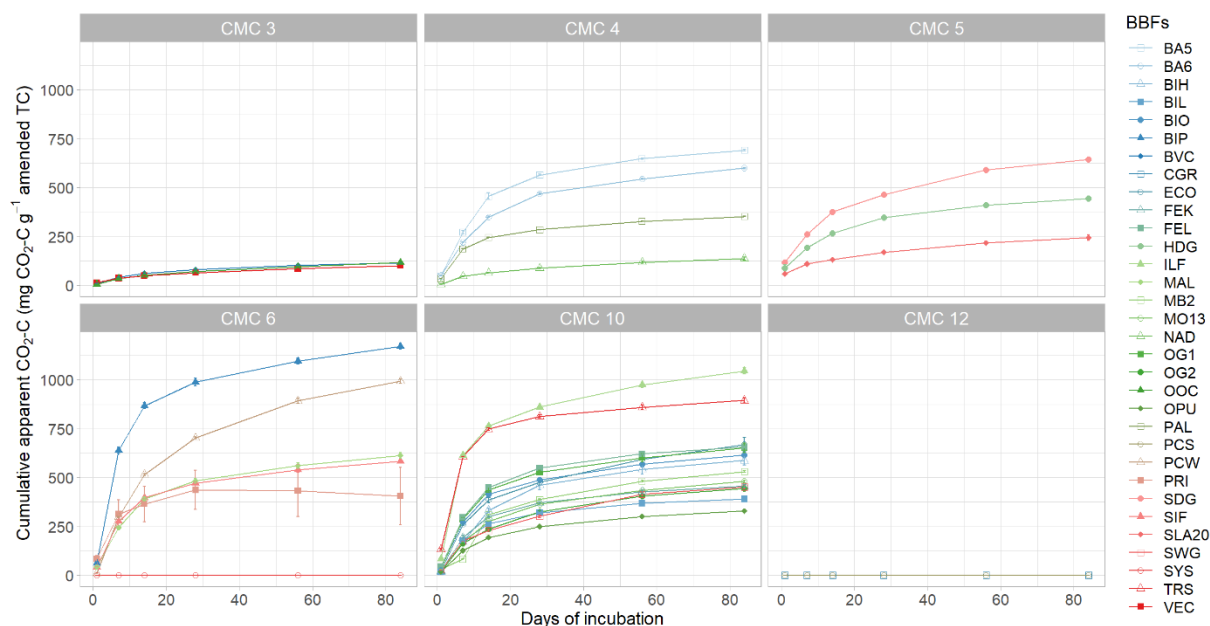


Figure 4: Cumulative apparent respired carbon ( $\text{CO}_2\text{-C}$ ) values (means and standard errors) expressed relative to the amount of amended total carbon (TC) and shown in sub-groups based on the component material category (CMC) of bio-based fertilizers (BBFs). Values of the unfertilized soil were subtracted and values of CGR, PCS and SYS set to zero. Assuming linear respiration rates between time points, the slope of the lines connecting measured values represent C mineralization rates.

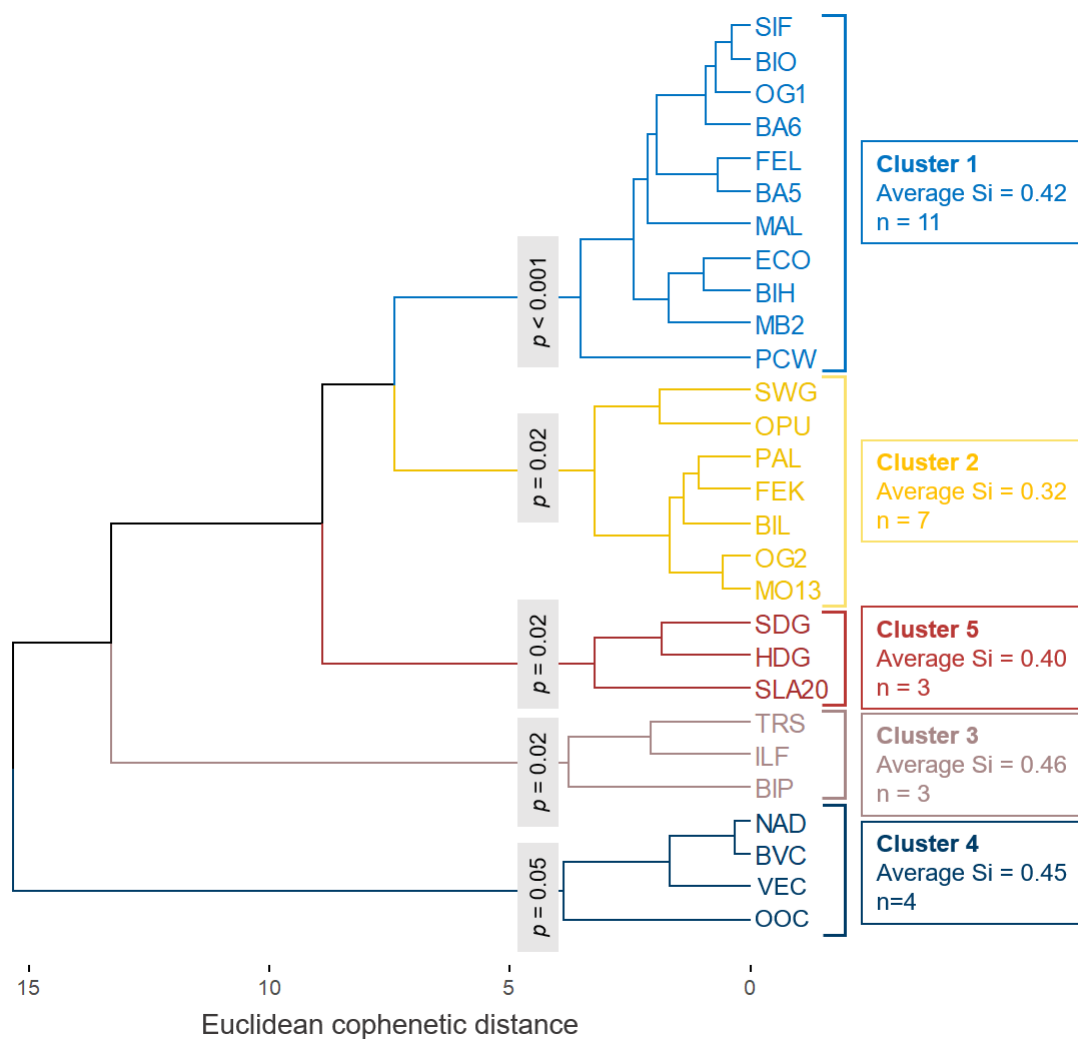


Figure 5: Classification of bio-based fertilizers (BBFs) based on  $N_{min}$ -N and  $CO_2$ -C data. Clusters result from aggregative hierarchical clustering applied to principal components of  $N_{min}$ -N and  $CO_2$ -C data. Cluster information includes cluster number, average silhouette width (Average Si), number of included BBFs (n) and bootstrapping-generated p-values. Note that clusters are not ordered numerically ascending.

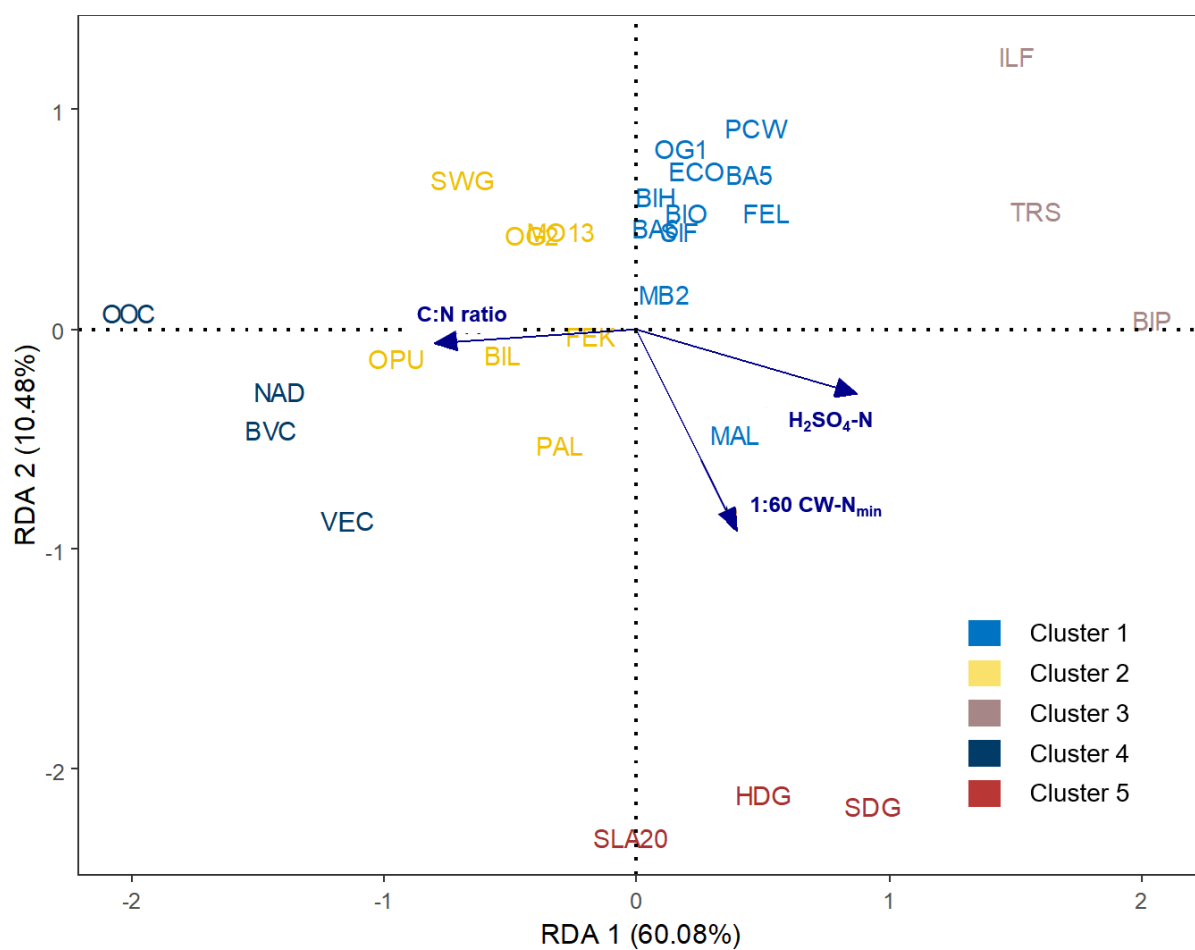


Figure 6: Redundancy analysis (RDA) based on principal components of mineral nitrogen ( $N_{min}$ -N) and respired carbon ( $CO_2$ -C) data (dependent variables) displaying selected chemical predictors as explanatory variables. Selected predictors are cold water extractable mineral N (1:60 CW- $N_{min}$ ), sulfuric acid extractable N ( $H_2SO_4$ -N) and carbon to nitrogen ratio (C:N ratio). Bio-based fertilizers (BBFs) names are colored according to the cluster they are assigned to by aggregative hierarchical clustering (Figure 5).

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## Appendix V

### Calculation of soil quality score

The “background” effect represented by the negative control was subtracted from the samples treated with BBFs or mineral N fertilizer (MIN). The assessment was based on the high application treatments for both soils for BVC, OG2, PAL, SDG, and MIN and for the Arenosol for BA6, BIO, ECO, FEK, MO13, and PCW. If the soil quality indicator was increased by a treatment on one soil and decreased on the other soil, a horizontal arrow representing no change was used. If the soil quality indicator was markedly increased or decreased by a treatment on one soil and only subject to a minor change in the opposite direction on the other soil, the marked change was selected to indicate the direction of change. Data for SMB-C was from 14 days after the start of incubation. Data for soil structure (clay dispersibility), WHC, pH, CEC, and total C was from 365 days after the start of incubation. For the C size fraction, the change in the relative contribution of MAOM from 14 to 365 days was used. The bottom line summarizes the soil quality score for each treatment where significant increases/decreases counted as 1 and non-significant increases/decreases counted as 0.5. pH and soil structure were not included in the summarized score. Whether an increase or decrease in pH is perceived as beneficial in agricultural settings depends on the specific soil. In regions with acid soils where liming is used, e.g. in Denmark, an increase in pH from BBF application would be perceived as a positive effect. Whereas, on alkaline soils, a decrease in pH would, instead, be preferable. Thus, pH was not included in the calculation of the soil quality score in Table IV-2. Moreover, as the BBFs had opposing effects on soil structure on the two soils (study III), this soil quality indicator was not included in the calculation of the soil quality score (Table IV-2).