Ref. Ares(2023)1906762 - 16/03/2023

D4.1 – Good experimental practice protocol

WP4, T4.1 Towards harmonisation / standardisation of Good Experimental Practices in assessment of recovered nutrients for biobased fertiliser utilisation

[Version 1.0 – 28/02/2023]

Authors: Ivona Sigurnjak (UGent), Sarah Moreno Sayavedra (UGent), Mathilde Blanc (APCA), Marie Castagnet (APCA), Jean-Philippe Bernard (APCA), Massimo Pugliese (UNITO), Erik Meers (UGent)

Website: walnutproject.eu

Twitter: @walnut_project

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

Disclaimer

Copyright notice

Technical references

Grant Agreement n°	101000752
Project Acronym	WalNUT
Project Title	Closing waste water cycles for nutrient recovery
Project Coordinator	Francisco Corona, PhD FUNDACIÓN CARTIF fraenc@cartif.es
Project Duration	Sep 2021 – Feb 2026

Deliverable No.	D4.1
Dissemination level*	Public
Work Package	WP4 – Demonstration and validation of biofertilisers at relevant scale
Task	T4.1 – Towards harmonisation / standardisation of Good Experimental Practice in assessment of recovered nutrients for biobased fertiliser utilisation
Lead beneficiary	UGent
Contributing beneficiary/ies	APCA-CA17 & CA34, UNITO
Due date of deliverable	28/02/2023
Actual submission date	28/02/2023

v	Date	Author(s)	Reviewers	Comments
0.1	11/01/2023	Ivona Sigurnjak	-	1st draft
0.2	06/02/2023		Marie Castagnet, Mathlide Blanc, Jean-Philippe Bernard	Small improvements and suggestions on the content
0.3	15/02/2023	Ivona Sigurnjak	-	Comments incorporated and 2nd draft available
0.4	27/02/2023		Massimo Pugliese, Georgianna Anthanasoulia	Small improvements and suggestions on the content
1.0	28/02/2023	Ivona Sigurnjak	-	Comments incorporated and final draft for submission available

Table of Contents

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

List of Tables

List of Figures

List of Abbreviations

ANR: Apparent Nitrogen Recovery

BBF: Bio-based Fertiliser

CMC: Component Material Category

EU: European Union

FPR: Fertilising Products Regulation

GEP: Good Experimental Practice

NFRV: Nitrogen Fertiliser Replacement Value

NRUE: Nitrogen Replacement Use Efficiency

NUE: Nitrogen Use Efficiency

PFC: Product Function Category

TMF: Tailor-made Fertiliser

WP: Work Package

Executive Summary

Deliverable 4.1 *''Good experimental practice protocol'* is a part of WalNUT work package (WP) 4. The WP4 aims to demonstrate and validate the use of bio-based fertilisers (BBFs; produced in WP2 and WP3) for their ability to substitute conventional synthetic mineral fertilisers whose production is based on finite fossil-based resources. The D4.1 reports on actions made under Task 4.1 '*Towards harmonisation /standardisation of Good Experimental Practices in assessment of recovered nutrients for biobased fertiliser utilisation'*, which concerns the following two sub-tasks:

Sub-task 4.1.1. Lexicon – standardized definitions of NUE / NFRV terminology

Sub-task 4.1.2. Good Experimental Practice (GEP) protocol

Therefore, the deliverable is divided into 4 chapters. The introduction is given in **Chapter 1**. **Chapter 2** focuses on standardisation of common terminology in a form of Lexicon. More specifically, the standardisation of 16 terms took place in a joint understanding with other European projects that are active in the field of BBF assessments. The harmonisation was done in the frame of a joint webinar with other relevant projects from Nutrient Recycling Community. The webinar took place online on 9th of February 2023. **Chapter 3** provides a harmonised protocol for field evaluation of the agronomic efficiency of BBFs in the WalNUT project. The protocol consists of an experimental design for future field trials and instructions on how to collect and process the data. Finally, **Chapter 4** provides current conclusions on the presented work.

Keywords: Lexicon, Field Trial Protocol, Bio-based Fertiliser, Apparent Nitrogen Recovery, Nutrient Use Efficiency, Fertiliser Replacement Value.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

1 Introduction

1.1 Purpose, scope and target group

The European Union (EU) is highly dependent on imports of raw materials for fertilising purposes. According to Fertilizers Europe (2022), in 2021 the EU imported 32% of N, 65% of P_2O_5 and 88% of K_2O of the total nutrients that were consumed as fertiliser products. Some of them such as P or Mg have been qualified as Critical Raw Materials by the European Commission (COM(2017)490). These are crucial for EU growth, competitiveness and especially for a sustainable food industry.

Emerging technologies to recover nutrient from biomass sources such as animal manure, by-products of the agri-food, fisheries, aquaculture or forestry sectors and waste water and sewage sludge, are considered as sustainable alternatives for energy demanding and fossil-based processes (e.g. Haber-Bosch) to produce synthetic mineral fertilisers. Large-scale nutrient recovery from these streams and their processing into bio-based fertilisers (BBFs) could offer a new, circular and sustainable model tackling both, the limited nutrient-mineral reserves, and their crucial environmental issues.

The WalNUT project aims to develop the necessary concepts and technological solutions to re-design the value and supply chains of nutrients from waste water and brine. Specifically, the project will tailor 5 pilot plants for the nutrient recovery from waste water and brine by combining multiple process units selected from the pool of technologies studied at lab scale and from partners previous relevant projects. The pilot plants will target not only high nutrient recovery efficiencies but also minimisation of negative environmental impacts. Thorough quality assessment of the resulting BBFs shall be made in order to establish a concrete view of their market positioning.

Therefore, one of the project aims (scientific and technological objective nr. 4) is to evaluate agronomic efficiency of produced WalNUT BBFs and their potential to replace conventional, non-renewable minerals obtained via more sustainable processes. As a first step towards achieving this goal, a harmonised Good Experimental Practice (GEP) protocol for field scale evaluation of the agronomic efficiency of WalNUT BBFs is reported in this deliverable (Sub-task 4.1.2; Chapter 3). Moreover, a standardisation of the terms used in the assessment of BBFs (e.g. nutrient use efficiency, fertiliser replacement value) is valorised in a form of a Lexicon (Sub-task 4.4.1; Chapter 2). The GEP protocol is developed with the aim to be used by WP4 partners (APCA, UNITO and UGent) involved in the field assessment of BBFs (Task 4.4), whereas the Lexicon will be used by the WalNUT consortium to align communication and reporting with the harmonised terminology. As both the GEP and the Lexicon are publicly available, they are open for use by WalNUT stakeholders, predominantly researchers and policy makers.

Finally, it should be noted that next to the planned 2-year field trials in Belgium, Italy and France (Task 4.4), also CETAQUA plans to conduct a 1-year field trial to assess the use of their Smart BBF. Similar is expected to be done by 3R in Hungary, where 3R pilot BBFs will be assessed in demonstration trials. As CETAQUA and 3R aim to assess their BBF as demonstration field trials (3R) and in different time scale (CETAQUA), these trials might not comply fully with the proposed GEP protocol that covers more aspects of the scientific field trials which will take place in France, Italy and Belgium.

1.2 Contribution partners

This deliverable has been made in collaboration between University of Ghent (UGent), University of Turin (UNITO) and French Chambers of Agriculture (APCA). Other partners, such as CARTIF Technology Center

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

(CARTIF), 3R-BioPhosphate Ltd (3R), National Technical University of Athens (NTUA), Fundacion Centro Gallego de Investigaciones del Agua (CETAQUA), Universidade de Comibra (UC) and Kobenhavns Universitet (UCPH) provided their input only on Chapter 2, by revising the Lexicon. The contribution of core partners is described in Table 1-1.

Table 1-1: Contribution of main partners

Partner	Contribution
UGent	Writing the deliverable
APCA	Writing of text parts, revision of first draft
UNITO	Writing of text parts, revision of second draft

1.3 Relation to other activities in the project

Since deliverable 4.1 provides a GEP protocol for field assessment of WalNUT BBFs, it is closely related to Task 4.4 '*Field validation of agronomic performance in quadruplicate-randomised block design utilisation*' that aims to conduct field trials, collect and process the plant and soil data. The harmonised terminology that is valorised in the form of a Lexicon will be used in all WPs and tasks of the WalNUT project.

Table 1-2: Relation to other activities in the project

Task	Description
Task 4.4 *Field validation of agronomic performance in quadruplicate-randomised block design utilisation*	This task aims to assess the agronomic efficiency of the WalNUT BBFs on field scale level. The assessment will take place in France, Belgium and Italy. The assessment will be done in line with the GEP protocol and harmonised efficiency indicators from the Lexicon.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

2 Lexicon

The Lexicon aims to reach a joint understanding with other projects on the correct use of terminology and definitions on commonly used terms, such as Nutrient Use Efficiency (NUE), Bio-based Fertilisers (BBFs), Fertiliser Replacement Value (FRV), etc. With that aim, WalNUT consortium proposed the harmonisation of 16 terms (sections 2.1 – 2.16) whose definitions are based on the Interactive Terminology for Europe (IATE) and other available scientific literature. In the case if no suggestion is made by WalNUT consortium, then consortium is in agreement with IATE and a definition is called 'EU definition'. If certain changes are made by the WalNUT consortium and new definition is proposed, then it is called 'Proposed definition'. For the proposed definitions they are always based on existing literature that, along with its references, is cited under the proposed definition.

The Lexicon was revised by WalNUT consortium and 5 researchers from UGent who work in the field of nutrient recovery, but are not part of WalNUT consortium. Afterwards, the Lexicon was shared with members of Nutrient Recycling Community. The Community is built by H2020 Fertimanure project and Biorefine Cluster Europe, and brings together the following 13 projects in the field of nutrient recovery: BioDen, FertiCycle, Fertimanure, Lex4Bio, Novafert, NutriBudget, Nutri2Cycle, ReNu2Farm, Run4Life, Sabana, Sea2Land, Systemic, WalNUT. To reach a joint understanding with these projects on the correct use of terminology and definitions on commonly used terms, a webinar on Lexicon was organized on February 9th 2023. During the webinar 36 participants from 13 projects were present. Agenda and speaker overview is available Annex 1.

The overall discussion was quite active, interesting and engaging. In general, on 14 out of 16 terms no disagreement came forward during the webinar. On the other hand, with terms 'organic fertiliser' and 'bio-based fertiliser' agreement was not achieved. The challenge is the term 'bio-based' which assumes based on biological origin, and therefore all organic fertilisers in reality are also bio-based. Furthermore, suggestions were made to replace term 'bio-based fertilisers' by 'recovered fertilisers' or 'recovered nutrients fertilisers'. However, not all participants supported this idea.

Currently the term 'bio-based fertiliser' is being used a lot in R&D publications, and is mostly used with aim to distinguish between 'raw' and 'refined' products, as being currently done in WalNUT Lexicon. As this is a problem on European level, both from scientific and legal perspective, other actions towards harmonisation of 'bio-based fertiliser' term are being currently taken independent from the WalNUT project. One of them is an action of European Sustainable Phosphorus Platform (ESPP) that is currently developing a Position Paper on the definitions of 'bio-based fertiliser' or 'bio-based nutrient'. Therefore, the current version of the Lexicon did not achieve a harmonisation of 'bio-based fertiliser' term and WalNUT consortium will further collaborate with the Nutrient Recycling Community and ESPP, and any other initiative that might become active in future, to reach common understanding on the use of this term.

2.1 Fertilising product

EU definition: Substance, mixture, micro-organism or any other material, applied or intended to be applied on plants or their rhizosphere or on mushrooms or their mycosphere, or intended to constitute the rhizosphere or mycosphere, either on its own or mixed with another material, for the purpose of providing the plants or mushrooms with nutrient or improving their nutrition efficiency.

Reference:

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

(1) Interactive Terminology for Europe (IATE) (2019). *Fertilising product*. IATE - Entry ID 3567753 (europa.eu). Retrieved from: Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003, OJ L170 114 (2019)].

2.2 Fertiliser

EU definition: Fertilising product aimed at providing nutrients to plants or mushrooms.

Reference:

(1) Interactive Terminology for Europe (IATE) (2019). *Fertiliser*. IATE - Entry ID 757051 (europa.eu). Retrieved from: Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003, OJ L170 114 (2019)].

2.3 Synthetic fertiliser

Proposed definition: Chemical (synthetic) fertiliser containing only inorganic plant nutrients (with the exception of urea) or mined minerals, obtained from the transformation of primary raw materials.

The definition is proposed on the basis of the following existing definitions:

(1) High quality plant nutrition products obtained from the transformation of primary raw materials, such as air, natural gas and mined ores (Fertilizers Europe, n.d.a).
(2) Chemical (synthetic) fertilizer containing simple, inorganic plant nutrients or mined minerals. Note that though urea is technically an organic material, it is referred to within this Fertiliser Code as an inorganic fertiliser (IATE, 2020).

References:

(1) Fertilizers Europe. (n.d.a). *Types of Fertilizer*. Retrieved November 8, 2022, from https://www.fertilizerseurope.com/fertilizers-in-europe/types-of-fertilizer/,
(2) IATE. (2020). *Synthetic Fertiliser*. IATE - Entry ID 329954 (europa.eu). Retrieved from COM-Terminology Coordination, based on Royal Horticultural Society (2020).

2.4 Organic fertiliser

Proposed definition: Fertilisers containing carbon (C) and nutrients of solely biological origin (biomass) (*with exception of additives that might be added during the processing step) that are either by-products or end-products of biological processes that may be further processed thermally, physico-chemically, or biologically, excluding material which is fossilized or embedded in geological formations. They may exhibit varying nutrient bioavailability and properties.

** 'with exception of additives that might be added during the processing step' has been added to the existing definition: otherwise products obtained from the processes that use synthetic chemicals, such as the phosphorus-poor solid fraction of manure or digestate after acidification step with sulphuric acid, might not fall under the definition of the organic fertiliser.*

The definition is proposed on the basis of the following existing definitions:

(1) Fertilisers with varying nutritional values, obtained by recycling the nutrients and organic carbon in materials generally present on the farm, such as crop residues and animal manures and slurries. The latter cover a wide range of nutrient sources with different physical

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

properties and nutrient contents, depending on the region, type of livestock and the farm management system (Fertilizers Europe, n.d.b).
(2) Organic fertilisers contain plant- or animal-based materials that are either a by-product or end product of naturally occurring processes, such as animal manure and composted organic materials (Wei et al., 2020).
(3) An organic fertiliser shall contain carbon (C) and nutrients of solely biological origin, excluding material which is fossilized or embedded in geological formations (with the exception of peat, leonardite and lignite) (EU 1009/2019).

References:

(1) Fertilizers Europe. (n.d.b). *Types of Fertilizer*. Retrieved November 8, 2022, from https://www.fertilizerseurope.com/fertilizers-in-europe/types-of-fertilizer/,
(2) Wei, X., Chen, J., Gao, B., Wang, Z., 2020. Chapter 39 - Role of controlled and slow release fertilizers in fruit crop nutrition, in: Srivastava, A.K., Hu, C. (Eds.), Fruit Crops. Elsevier, pp. 555–566. https://doi.org/10.1016/B978-0-12-818732-6.00039-3
(3) Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003, OJ L170 114 (2019).

2.5 Bio-based fertiliser (BBF)

Proposed definition: Fertiliser derived from biomass using the nutrient recovery and re-use (NRR) technologies (physical, thermal, chemical and/or biological, excluding anaerobic digestion and composting*), in order to up concentrate nutrients from the initially treated biomass and hence improve its nutrient efficiency.

**Anaerobic digestion and composting are excluded as they do not aim to up concentrate nutrients. Therefore, digestate, manure and compost fall under organic fertilisers and not biobased fertilisers. On the other hand, mechanical separation aims to up concentrate nutrients, and hence liquid fractions of manure and digestate are considered as BBFs.*

The definition is proposed on the basis of the following existing definitions:

(1) "Biobased fertilisers" are defined as organic or organo-mineral fertiliser products derived from renewable biomass-related resources rather than synthetic products which require fossil resources for their production (including anaerobic digestion (EIP-AGRI Focus Group, 2017).
(2) Bio-based fertilisers (BBFs) are fertilising products or a component to be used in the production of (Tailor-Made) Fertilisers that are derived from biomass-related resources. The BBFs of FERTIMANURE are "obtained through a physical, thermal/thermo-chemical, chemical, and/or biological processes for the treatment of manure or digestate that result into a change in composition due to a change in concentration of nutrients and their ratios compared to the input material(s) in order to get better marketable products providing farmers with nutrients of sufficient quality". However, just separation of manure in a solid and liquid fraction (as first processing step) is excluded. These products are not conceived as a BBF, although they are valuable sources to supply nutrients on agricultural land (Fertimanure, n.d).
(3) 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 (Wester-Larsen et al., 2022).

References:

(1) EIP-AGRI Focus Group. (2017). *EIP-AGRI Focus Group on Nutrient recycling: Final report* [Text]. https://ec.europa.eu/eip/agriculture/en/publications/eip-agri-focus-group-nutrient-recycling-final
(2) Fertimanure. (n.d.). The project's response—Fertimanure. Fertimanure. https://www.fertimanure.eu/en/the-project-s-response
(3) Wester-Larsen, L., Müller-Stöver, D. S., Salo, T., & Jensen, L. S. (2022). Potential ammonia volatilization from 39 different novel biobased fertilizers on the European market – A laboratory study

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

using 5 European soils. Journal of Environmental Management, 323, 116249. https://doi.org/10.1016/j.jenvman.2022.116249

2.6 Tailor-made fertiliser (TMF)

Proposed definition: A tailor-made fertiliser (TMF) is a customised fertiliser that meets with the nutrient requirements of a specific crop by taking into account the soil type, soil fertility status, and growing conditions and fertilisation practises. It may consist of a mixture of BBFs and/or synthetic fertilisers and/or organic fertilisers and/or other compounds (eg. biostimulants).

The definition is proposed on the basis of the following existing definitions:

(1) A tailor-made fertiliser (TMF) is a customized fertiliser that meets with the nutrient requirements of a specific crop by taking into account the soil type, soil fertility status, and growing conditions and fertilisation practises. The TMFs obtained in FERTIMANURE are produced from BBFs (produced from manure or digestate and/or other recovered fertilising products that are available) and/or mineral fertilisers (MF) (and/or biostimulants).

Reference:

(1) Fertimanure. (n.d.). The project's response—Fertimanure. Fertimanure. https://www.fertimanure.eu/en/the-project-s-response

2.7 Smart BBF

Proposed definition: 'Smart' is a prefix that can be given to BBFs whose formulation can adapt the timing of nutrient release (eg. slow release, controlled release) to the plant nutrient demand, enhancing the agronomic yields and reducing the environmental impact at sustainable costs when compared to conventional synthetic fertilisers.

The definition is proposed on the basis of the following existing definitions:

(1) Smart fertilisers introduce conditionality into the fertilizing product as they are equipped with monitoring equipment which can sense fluctuations in the temperature, moisture or acidity of their surroundings and make subsequent adjustments to how quickly they release their nutrients. This allows farmers to create tailored plans for each of their crops, optimising the use of their fertilisers and ensuring minimal adverse effects from their application (Envirotech Online, 2019).
(2) Smart fertilizer is any single or composed (sub)nanomaterial, multi-component, and/or bioformulation containing one or more nutrients that, through physical, chemical, and/or biological processes, can adapt the timing of nutrient release to the plant nutrient demand, enhancing the agronomic yields and reducing the environmental impact at sustainable costs when compared to conventional fertilizers (Raimondi et al., 2021).

References:

(1) Envirotech Online. (2019, July 21). *What Are Smart Fertilisers?* Envirotech Online. https://www.envirotech-online.com/news/environmental-laboratory/7/breaking-news/what-are-smart-fertilisers/49762
(2) Raimondi, G., Maucieri, C., Toffanin, A., Renella, G., & Borin, M. (2021). Smart fertilizers: What should we mean and where should we go? *Italian Journal of Agronomy*, *16*(2), Article 2. https://doi.org/10.4081/ija.2021.1794

2.8 Nutrient Use Efficiency (NUE)

Proposed definition: An indicator for the utilisation of nutrients that is based on mass balance principle (i.e. nutrient input (applied; kg ha^{-1} yr^{-1}) and nutrient output (taken up by crop; kg ha^{-1} yr^{-1})). In crop production systems, NUE is determined as follows:

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

$$Nutrient\ Use\ Efficiency\ (NUE) = \frac{Nutrient\ uptake\ by\ crop\ (kg\ ha^{-1}\ yr^{-1})}{Nutrient\ applied\ (kg\ ha^{-1}\ yr^{-1})}$$

High NUE indicates high nutrient output via harvested products and low nutrient surplus in soil, whereas low NUE can be used as an indication for the potential nutrient loss to the environment. This indicator is applicable to most of nutrients, but predominantly is applied for macronutrients such as nitrogen (Nitrogen Use Efficiency, NUE), phosphorous (Phosphorous Use Efficiency, PUE) and potassium (Potassium Use Efficiency, KUE). Note, NUE is similar but not the same as Apparent Nutrient Recovery (ANR; section 1.10) since ANR takes the effect of unfertilised soil into account and NUE does not.

The definition is proposed on the basis of the following existing definitions:

(1) Fertilizer use efficiency (FUE) is equal to nutrient uptake (in kg/ha) divided by the total nutrient applied (in kg/ha). It is not compared to an unfertilised treatment and it is applicable to N, P and K (Sigurnjak, 2017).
(2) The concept for NUE used here is based on the mass balance principle, i.e. using N input and N output data for its calculation: NUE = N output of harvested products / N input. NUE should always be reported along with the nutrient surplus/deficiency = nutrient input – nutrient output (EU Nitrogen Expert Panel, 2015).
(3) The NUE indicator is based on nitrogen input and nitrogen output at different levels and provides information about resource use efficiency, the economy of food production (nitrogen in harvested yield), and the pressure on the environment (nitrogen surplus) (Fertilizers Europe, n.d.).

References:

(1) Sigurnjak, I. (2017). *Animal manure derivatives as alternatives for synthetic nitrogen fertilizers*. Gent University. Faculty of Bioscience Engineering, Ghent Belgium. https://biblio.ugent.be/publication/8541469
(2) EU Nitrogen Expert Panel (2015). Nitrogen Use Efficiency (NUE) - an indicator for the utilization of nitrogen in agriculture and food systems. Wageningen University. http://www.eunep.com/wp-content/uploads/2017/03/Report-NUE-Indicator-Nitrogen-Expert-Panel-18-12-2015.pdf
(3) Fertilizers Europe. (n.d.). *EU Nitrogen Expert Panel (EUNEP)*. Retrieved December 13, 2022, from https://www.fertilizerseurope.com/initiatives/eu-nitrogen-expert-panel-eu-nep/

2.9 Nutrient Replacement Use Efficiency (NRUE)

Proposed definition: An indicator that compares the nutrient use efficiency (NUE) of a tested treatment (eg. organic or bio-based fertilisers) against the reference treatment (eg. synthetic fertiliser, animal manure, or combination of both), as follows:

$$Nutrient\ Replacement\ Use\ Efficiency\ (\%) = \frac{NUE_{tested\ treatment}}{NUE_{reference\ treatment}} \times 100$$

NRUE indicates the percentage of nutrient in reference treatment (eg. synthetic fertiliser) that can be replaced by a nutrient provided from the tested treatment (eg. BBF). This indicator is applicable to most of nutrients, but predominantly is applied for macronutrients such as nitrogen (NRUE), phosphorous (PRUE) and potassium (KRUE).

The definition is proposed on the basis of the following existing definitions:

(1) Fertilizer replacement use efficiency (FRUE; %) is equal to FUE of bio-based treatment divided by the FUE of the reference treatment (Sigurnjak, 2017).

References:

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

(1) Sigurnjak, I. (2017). *Animal manure derivatives as alternatives for synthetic nitrogen fertilizers*. Gent University. Faculty of Bioscience Engineering, Ghent Belgium. https://biblio.ugent.be/publication/8541469

2.10 Apparent Nutrient Recovery (ANR)

Proposed definition: An indicator for the utilisation of nutrients that is based on mass balance principle (i.e. nutrient input (applied; kg ha^{-1} yr^{-1}) and nutrient output (taken up by crop; kg ha^{-1} yr^{-1})) by taking into consideration the unfertilised control treatment as nutrient uptake baseline. In crop production systems, ANR is determined as follows:

$$Apparent\ Nutrient\ Recovery\ (ANR) = \frac{nutrient\ uptake\_fertilised\ treatment\ (kg\ ha^{-1}\ yr^{-1}) - nutrient\ uptake\_unfertilised\ treatment\ (kg\ ha^{-1}\ yr^{-1})}{Nutrient\ applied\_fertilised\ treatment\ (kg\ ha^{-1}\ yr^{-1})}$$

High ANR indicates high nutrient output via harvested products and low nutrient surplus in soil, whereas low ANR can be used as an indication for the potential nutrient loss to the environment. This indicator is applicable to most of nutrients, but predominantly is applied for macronutrients such as nitrogen (Apparent Nitrogen Recovery, ANR), phosphorous (Apparent Phosphorous Recovery, APR) and potassium (Apparent Potassium Recovery, AKR).

The definition is proposed on the basis of the following existing definitions:

(1) There are similarities between ANR and NUE, and between NFRV and NRUE indicators. The main difference rises from the presence or absence of unfertilized treatment (i.e. control) in experimental design. ANR can also stand for apparent nitrogen recovery, APR: apparent phosphorus recovery; AKR: apparent potassium recovery.
(2) Apparent recovery efficiency of applied nitrogen is equal to the difference of the total N uptake in aboveground biomass at maturity, with applied fertilizer N (kg per ha) and the total N uptake in aboveground biomass at maturity without applied fertilizer N (kg per ha) divided by the amount of fertilizer N applied (kg per ha) (EU Nitrogen Expert Panel, 2015).

References:

(1) Sigurnjak, I. (2017). Animal manure derivatives as alternatives for synthetic nitrogen fertilizers. Gent University. Faculty of Bioscience Engineering, Ghent Belgium. https://biblio.ugent.be/publication/8541469
(2) EU Nitrogen Expert Panel (2015). Nitrogen Use Efficiency (NUE) - an indicator for the utilization of nitrogen in agriculture and food systems. Wageningen University.

2.11 Nutrient Fertiliser Replacement Value (NFRV)

Proposed definition: An indicator that compares the apparent nutrient recovery (ANR) of a tested treatment (eg. organic or bio-based fertilisers) against the reference treatment (eg. synthetic fertiliser, animal manure, or combination of both), as follows:

$$Nutrient\ Fertiliser\ Replacement\ Value\ (\%) = \frac{ANR_{tested\ treatment}}{ANR_{reference\ treatment}} \times 100$$

NFRV indicates the percentage of nutrient in reference treatment (eg. synthetic fertiliser) that can be replaced by a nutrient provided from the tested treatment. This indicator is applicable to most of nutrients, but predominantly is applied for macronutrients such as nitrogen (nitrogen fertiliser replacement value, NFRV),

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

phosphorous (phosphorous fertiliser replacement value, PFRV) and potassium (potassium fertiliser replacement value, KFRV).

The definition is proposed on the basis of the following existing definitions:

(1) The extent to which a nutrient (N, P) in a manure or in a compost is as plant-available as that nutrient in a common mineral equivalent applied according to good agricultural practices, usually expressed as kg per 100 kg applied [= fertilizer equivalency = ratio of apparent recoveries* (or of apparent efficiencies**) of a nutrient (often N) from manure and from a commonly used mineral fertilizer equivalent] (Landmark Horizon 2020 project, n.d.).
(2) The fertilizer replacement value (FRV) of an organic manure – given crop type, soil type, application time, and application method – specifies how much standard mineral fertilizer – given formulation, application time, and application method – is needed for a similar crop response measured over a given period. The NFRV is expressed as kilograms of mineral fertilizer-nutrient per 100kg manure-nutrient. (Schils et al., 2020).
(3) Plant availability of nutrients (N, P, K) in bio-based fertilizers compared to their availability in mineral fertilizers (Forrestal et al., n.d.)
(4) An indicator that compares the apparent nutrient recovery (ANR) of nitrogen from mineral and organic/bio-based fertiliser treatment. It is the ratio of the organic/bio-based fertiliser ANR to the mineral fertiliser ANR, expressed as percentage: $Nutrient\ fertiliser\ replacement\ value = \frac{ANR_{organic\ or\ bio-based\ fertiliser}}{ANR_{mineral\ fertiliser}} \times 100$

References:

(1) Landmark2020. (n.d.). *Fertilizer replacement value*. Landmark2020. Retrieved December 13, 2022, from Landmark Glossary - Landmark2020 (landmarkproject.eu)
(2) Schils, R., Schröder, J., & Velthof, G. (2020). Fertilizer Replacement Value. In Biorefinery of Inorganics (pp. 189–214). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118921487.ch5-1
(3) Forrestal, P. J., Adani, F., Snauwaert, E., Veeken, A., Bernard, J.-P., & Jensen, L. S. (n.d.). Mini-paper—Towards increasing the mineral fertiliser replacement value of bio-based fertilisers. https://ec.europa.eu/eip/agriculture/sites/default/files/fg19_minipaper_4_nue_en.pdf
(4) Sigurnjak, I. (2017). Animal manure derivatives as alternatives for synthetic nitrogen fertilizers. Gent University. Faculty of Bioscience Engineering, Ghent Belgium. https://biblio.ugent.be/publication/8541469

2.12 Nutrient Recovery

Proposed definition: A biological, physico-chemical, thermal or thermo-physical process through which a nutrient is extracted, purified or concentrated from a biomass.

The definition is proposed on the basis of the following existing definitions:

(1) A process through which a nutrient is extracted, purified or concentrated from a substrate (Buckwell and Nadeu, 2016).
(2) Recovery of waste means any operation the principal result of which is waste serving a useful purpose by replacing other materials which would otherwise have been used to fulfil a particular function, or waste being prepared to fulfil that function, in the plant or in the wider economy (Eurostat, 2008).
(3) Recovery is meant as an energy recovery only (European Environment Agency, n.d.).

References:

(1) Buckwell, A., & Nadeu, E. (2016). *Nutrient Recovery and Reuse (NRR) in European agriculture. A review of the issues, opportunities, and actions.* RISE Foundation. https://risefoundation.eu/wp-content/uploads/2020/07/2016_RISE_NRR_Full_EN.pdf
(2) Eurostat. (2008). *Glossary: Recovery of waste*. https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Glossary:Recovery_of_waste
(3) European Environment Agency. (n.d.). *EPER Pollution register glossary* [Glossary]. Retrieved December 15, 2022, from https://www.eea.europa.eu/help/glossary/eper-pollution-register-glossary

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

2.13 Nutrient Recycling

Proposed definition: The re-introduction of nutrients recovered from biomass, into productive sectors such as agriculture.

The definition is proposed on the basis of the following existing definitions:

(1) A general term which can refer to the reuse in agriculture of collected or recovered nutrients (Buckwell & Nadeu, 2016)
(2) Recycling of waste is defined in the Waste Framework Directive as any recovery operation by which waste materials are reprocessed into products, materials or substances whether for the original or other purposes. It includes the reprocessing of organic material but does not include energy recovery and the reprocessing into materials that are to be used as fuels or for backfilling operations (Eurostat, 2008).
(3) A resource recovery method involving the collection and treatment of a waste product for use as raw material in the manufacture of the same or a similar product. Recycling meant as a material recycling (as opposed to organic recycling [digestion and composting]), only, and with a reference to structural changes in products (European Environment Agency, n.d.).

References:

(1) Buckwell, A., & Nadeu, E. (2016). *Nutrient Recovery and Reuse (NRR) in European agriculture. A review of the issues, opportunities, and actions.* RISE Foundation. https://risefoundation.eu/wp-content/uploads/2020/07/2016_RISE_NRR_Full_EN.pdf
(2) Eurostat. (2008). *Glossary: Recycling of waste* (as retrieved from Article 3 of the Waste Framework Directive 2008/98/EC). https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Glossary:Recycling_of_waste
(3) European Environment Agency. (n.d.). *EPER Pollution register glossary* [Glossary]. Retrieved December 15, 2022, from https://www.eea.europa.eu/help/glossary/eper-pollution-register-glossary

2.14 Nutrient Reuse

Proposed definition: The application of un-modified, nutrient-rich biomass (eg. animal manure, crop residues) in agriculture.

The definition is proposed on the basis of the following existing definitions:

(1) The act of applying recovered or collected nutrients to agricultural production or some other non-agricultural use (Buckwell and Nadeu, 2016).
(2) Reuse of waste means any operation by which products or components that are not waste are used again for the same purpose for which they were conceived (Eurostat, 2008).
(3) Reuse is material reuse without any structural changes in materials (European Environment Agency, n.d.).

References:

(1) Buckwell, A., & Nadeu, E. (2016). *Nutrient Recovery and Reuse (NRR) in European agriculture. A review of the issues, opportunities, and actions.* RISE Foundation. https://risefoundation.eu/wp-content/uploads/2020/07/2016_RISE_NRR_Full_EN.pdf
(2) Eurostat. (2008). *Glossary: Reuse of waste* (as retrieved from Article 3 of the Waste Framework Directive 2008/98/EC). https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Glossary:Reuse_of_waste
(3) European Environment Agency. (n.d.). *EPER Pollution register glossary* [Glossary]. Retrieved December 15, 2022, from https://www.eea.europa.eu/help/glossary/eper-pollution-register-glossary

2.15 Biorefinery / Biofactory

EU definition: Facility that integrates biomass conversion processes and equipment to produce fuels, power, and chemicals from biomass.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

Reference:

(1) IATE. (2007). Biorefinery. https://iate.europa.eu/entry/result/2213495/en-en

2.16 Nutrient balance

EU definition: Nutrient balance is the difference (surplus or deficit) between the nutrients entering a farming system (mainly through livestock manure and fertilisers) and the nutrients leaving the system (through uptake of nutrients by crops and pasture production).

Reference:

(1) IATE. (2022). *Nutrient Balance*. https://iate.europa.eu/entry/result/2245210/en-en

3 Good Experimental Practice (GEP) protocol for WalNUT field trials

The Good Experimental Practice (GEP) protocol aims to provide a guideline on how to evaluate agronomic efficiency of the WalNUT BBFs. The BBFs will be assessed under controlled (i.e. laboratory and greenhouse pot trial level) and uncontrolled (i.e. field scale level) experimental conditions (e.g. temperature, rainfall, etc.). The assessment under controlled laboratory conditions is covered in Task 4.3 '*Demonstration of biofertilisers under controlled conditions utilisation'* (M13-M45) which consist of the following sub-tasks:

Sub-task 4.3.1 Assessment of nitrogen release patterns via soil incubation assay (UGent)

Sub-task 4.3.2 Assessment of phosphorus plant availability via dedicated plant growth assay (UGent)

Sub-task 4.3.3 Assessment of BBF efficacy in pot trials (UNITO, CETAQUA)

Sub-task 4.3.4 Carbon footprint & ammonia emissions (UGent)

Sub-task 4.3.5 Micronutrient assessment (NTUA)

As the sub-tasks of Task 4.3 have different aims and mostly only one partner (as indicated above between brackets) participates in the certain sub-task, there is no need to harmonize a protocol for BBF assessment under controlled conditions. For each specific 4.3 sub-task an oriented protocol will be eventually available as a part of D4.4 *Report on agronomic performance of BBFs in pot trials*. Therefore, the GEP protocol in D4.1 focuses solely on BBFs assessment in uncontrolled field conditions. Moreover, it focuses on assessment of N-rich BBFs, and not for example on P-rich fertilisers. However, most parts of the protocol can be applied also in P-field trials. In general, assessment of P-rich BBFs is extremely challenging to be conducted in field trials due to P-rich soils, and hence there is a need for field trials that were not fertilised for decades. As it is not easy to have an access to unfertilised fields, P-rich BBFs are usually assessed in controlled conditions by using P-poor growing medium such as river sand. This type of assessment falls under *Sub-task 4.3.2 Assessment of phosphorus plant availability via dedicated plant growth assay*. Finally, preliminary overview of WalNUT BBFs (Table 3-1) to be produced in WP2 and WP3 indicates that from 13 currently identified BBFs, only 5 of them might contain P (still not certain in case of CETAQUA). The majority of identified BBFs are N-rich.

Table 3-1: Preliminary list of WalNUT BBFs to be produced on lab (WP2) and pilot (WP3) scale level

Partner / pilot	No.	BBF name	Form	Description
Aquafin + UGENT	1	Ammonium sulphate	Liquid	NS fertiliser
	2	Ammonium-rich irrigation water	Liquid	N fertiliser
	3	Ammonium-loaded natural adsorbent	Solid	N fertiliser (and maybe K)
CARTIF + VEOLIA	4	Algae-based biofertiliser	Solid (powder)	NPK fertiliser + C presence
3R	6	ABC Animal Bone Char adsorber (CMC14 - PFC1. A1. I.)	Solid	High nutrient density P fertiliser
	7	Solid fermented ABC adsorbent for compound BBF	Solid	BIO-NPK-C compound bioertiliser
CETAQUA	8	Ammonium nitrate/ sulphate	Liquid	N and/or S fertiliser
	9	Smart BBF	Liquid	N-P and/or S fertiliser + PGBs
	10	Smart BBF	Gel	N-P and/or S fertiliser + PGBs
NTUA	11	Potassium (K) – KCl	Powder	K fertiliser
	12	Magnesium (Mg) - Mg(OH)2	Powder	Mg fertiliser
	13	Calcium (Ca)-CaCO3	Powder	Ca fertiliser

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

Field testing of BBFs in WP4 concerns the work that will be done in Task 4.4 *'Field validation of agronomic performance in quadruplicate-randomised block design utilisation'* (M25-M54), with aim to assess nutrient recovery efficiency of novel BBFs compared to mineral fertilisers. Field trials will take place in several regions of Europe which are soil/climate defined to build solid results and access the variability of novel fertiliser efficiency on studied crops. To this end, field trials are envisaged in Belgium (sub-task 4.4.1), France (sub-task 4.4.2) and Italy (sub-task 4.4.3). As stated in DOA (page 27 of 66): '*Field trials will be set with the same basic protocol and it will be adapted on crop conditions (size of plots, amount of nutrient targeted). Each treatment will be replicated in randomised complete block design (RBCD) with four replications. Fertiliser amounts will be applied at incremental rates (eg. (i) sub equilibrium fertilisation (i.e. at N level 25% below crop requirement), (ii) at crop requirement and (iii) above predicted crop requirement (+25%)) to determine the potential fertiliser replacement value of BBF and TMF. In addition, an unfertilised blank is included as a separate treatment.'*

Initially the aim was to include in the GEP protocol also the determination of the BBF quality (nutrient content, physical-chemical properties) and safety parameters (metals/ metalloids, persistent organic pollutants, microbial and eco-toxicological parameters) with consideration of EU 1009/2019. These aspects are covered in Annex 2, by providing a list of parameters per Product Function Category (PFC, type of product/fertiliser) and the Component Material Category (CMC, allowed input materials) for conformity of bio-based fertilisers (BBFs) to the Fertilising Product Regulation. The actual methods for determination of the listed parameters are being currently developed by the European Committee for Standardization (CEN). In connection with the new (EU) 2019/1009, CEN already published 82 new Technical Specifications on fertilising products for CE marking and continuously developing new technical specifications. Once WalNUT BBFs are generated by the 5 pilots that are operating under stable conditions, they will be fully assessed in regard to the conformity with Fertilising Product Regulation. This will done as a part of Task 4.2 *Quality requirements & QAS for WWTP derived recycled fertilising products utilisation*.

In this chapter, the presented GEP protocol is strongly based on the protocol *'Evaluation of the short term N-effect of a recycling-derived fertiliser (RDF) on crop and environment in field trials'* that has been developed by Inagro (BE), Arvalis (FR) and UGent (BE) within Interreg NWE project ReNu2Farm (2018 -2023) (Van de Sande et al., 2019). Since the protocol deals with assessment of BBFs, it is seen as a good starting point to adapt its structure towards the requirements of the WalNUT project.

3.1 Context and state of the art

Knowledge of the N content in BBFs is not sufficient to determine their efficacy as well as their performance on plant development and growth. The N contained in BBFs can come in several forms (Figure 3-1):

- Mineral nitrogen (NO_3-N and/or NH_4-N)
- Rapidly mineralisable organic N
- Slowly mineralisable organic N

Mineral nitrogen from a BBF is fully available for a crop if it is applied during the sowing. The N losses of this form can be by NH_3 volatilisation into the air during spreading or by NO_3-N leaching into surface and ground waters after transformation of the NH_4^+ ions into NO_3^- (nitrification process).

The rapidly mineralisable organic nitrogen from a BBF is comparable to mineral nitrogen. Its availability depends on the mineralisation conditions in the soil.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

Slowly mineralisable organic nitrogen joins the slowly mineralising organic matter compartment of the soil. In the case of large and repeated fertiliser applications containing a large proportion of slowly mineralisable organic nitrogen, there is an increase in the organic matter stock in the soil and the amount of mineralised nitrogen each year. This efficacy will not be evaluated in WalNUT since the project focuses on short term N effects in the frame of 2 years.

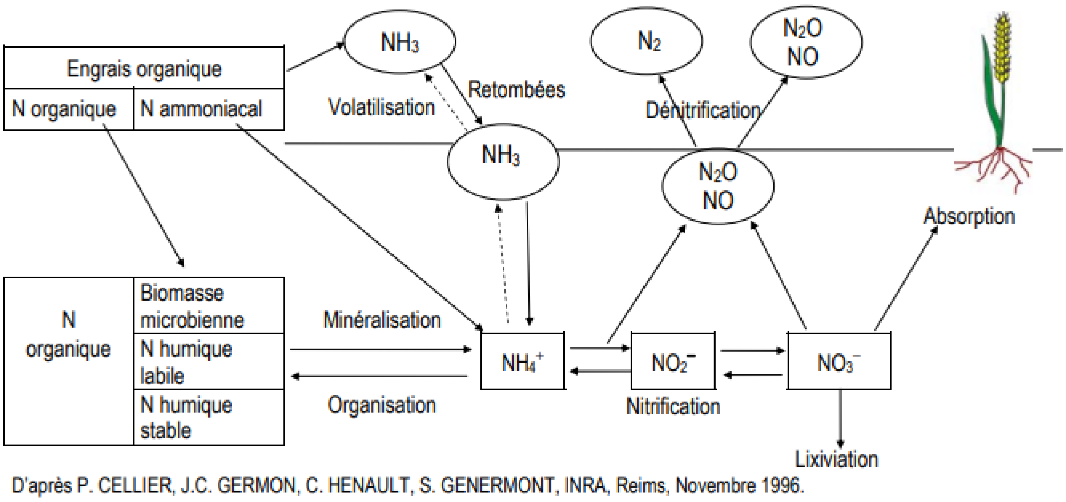

Figure 3-1: Evolution of the nitrogen contained in an organic fertiliser applied to a winter wheat

3.1.1 Short term N effects on crop: calculation of the N fertiliser replacement value (NFRV) and apparent N recovery (ANR)

The apparent N recovery (ANR) of BBFs is obtained by calculating the relation between the N supplement absorbed by a crop (all aboveground parts) fertilised with the BBF compared to a non–fertilised reference and total N brought by the BBF. P, K and S fertilisation will be applied in mineral form on the reference treatment assuring sufficient P, K and S availability on the reference plots as well.

$$\mathrm{ANR}^{1} = \frac{\text{N absorbed (by a crop fertilised with the BBF)} - \text{N absorbed by the non–fertilised crop}}{\text{total N amount brought by the BBF}}$$

$$NFRV^{2} = \frac{ANR\ (BBF)}{ANR\ (mineral\ fertilizer)}$$

Both coefficients are interdependent and complementary (Figure 3-2). NFRV is more directly operational but also dependent on the effectiveness of the reference mineral fertiliser. The reference mineral fertiliser can depend on the regional aspects and business as usual of farmers in certain region. In Flanders for example, pure ammonium nitrate (no added Mg or other nutrients) in granular form is used and applied broadcast. On the other hand, in Italy urea might be used.

[1] or CAU in France.

[2] or Keq in France.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

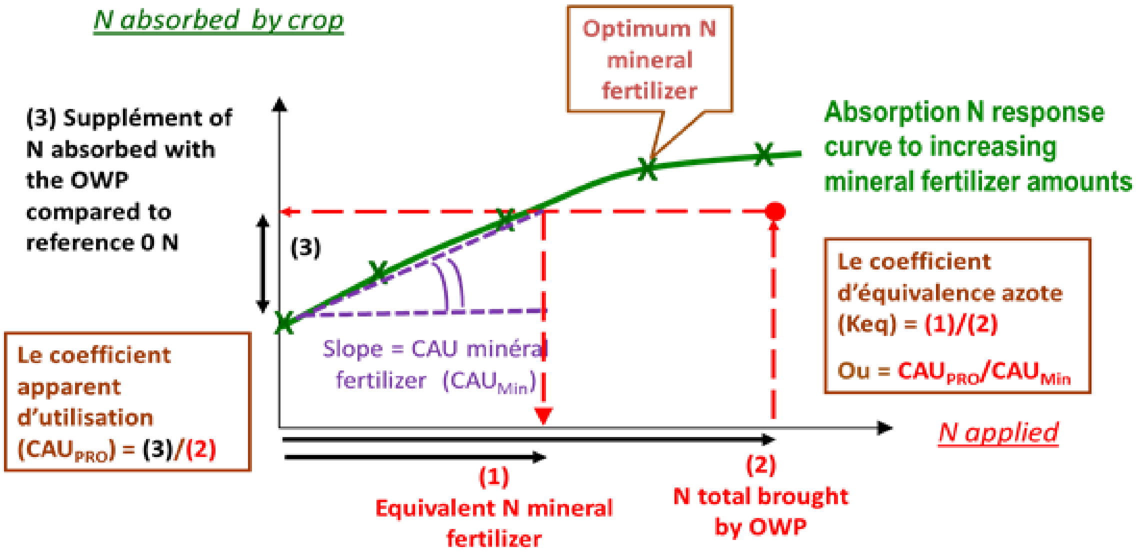

Figure 3-2: Schematic presentation of how ANR and NFRV are calculated

The calculation of NFRV and ANR requires a crop yield response curve to mineral fertiliser applied, including a treatment without N-fertilisation. The treatment without N-fertilisation allows to calculate the N-mineralisation. The curve itself allows the estimation of the point at which additional N-fertilisation will no longer result in additional N-uptake. Ideally CAU and Keq must be calculated for N -fertilisation doses below this point. Having a calculation of the N balance for different N doses applied also supplies extra information regarding environmental losses in different situations. ANR and NFRV values vary with the amount of fertiliser applied but also with the amount of mineral N released by N-mineralisation. N-losses during the growing period also influence the ANR and NFRV. High N-mineralisation or N-losses during the growing season should therefore be avoided (selection of the trial field).

Net N-mineralisation ((N_{min} in the soil before planting + N applied) – (N_{min} in the soil at harvest + N uptake)) will decrease with increasing amounts of fertiliser applied (Feller, 2011). This can (partially) be explained by higher N-uptake by plant roots, increased immobilisation and higher losses (leaching and gaseous losses). Crop response to extra N fertiliser decreases with increasing doses of N fertiliser applied. N losses during the growing season (leaching or gaseous losses) will generally be higher on the fertilised plots. These losses are also function of the N-mineralisation. High N-mineralisation, or N-losses due to leaching of volatilisation during the growing season can also interfere with the calculated ANR and NFRV values. Therefore it is interesting to calculate or measure as many components of the N-balance as possible (Table 3-2).

3.1.2 Short term N - effect of an organic waste product on the environment is determined calculating the complete N - balance for every treatment

Table 3-2 lists the components of the N-balance (according to the French balance sheet method + extra terms). It is interesting to measure or calculate as many components of the balance as possible. Because of budgetary or practical constraints, a number of these components can be based upon literature or an indicative value can be obtained via incubation experiments or other experiments in laboratory conditions.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

Table 4-2: Components of N - balance

N -supply		Relevance + proposed method of acquisition
R_i	Mineral N content in the soil profile (accessible to plant roots at harvest) before applying fertiliser	Essential
N_{irr}	N from irrigation water	Essential if irrigation is significant.
N_{dep}	N from atmospheric deposition	Essential. Based upon literature. If possible, measure in the field.
M_{tot}	Total mineralisation (M tot = Mcr + Mcc + Mso + Mo (y-1) + Mo)	Optional, assumption based upon N – uptake in the reference treatment or literature.
M_s	Total N – mineralisation from soil ($M_s = M_{cr} + M_{cc} + M_{so} + M_{o(y-1)}$)	Essential, Via incubation experiments (WP1)
M_{cr}	N-mineralisation from crop residues	Essential. Estimation from literature, local experience/data.
M_{cc}	N-mineralisation from catch crops	Via incubation experiment, part of M_s. Separate determination is optional.
M_{so}	N-mineralisation from soil organic matter	Via incubation experiment, part of M_s. Separate determination is optional.
$M_{o(y-1)}$	N-mineralisation from organic fertiliser applied the year before trial conduct	Via incubation experiment, part of M_s. Separate determination is optional.
M_o	N-mineralisation from organic fertiliser applied shortly before planting/sowing	Essential. Via incubation experiments (WP1)
F_m	Mineral fertiliser	Essential
N-losses		
Pf	Total plant uptake in aboveground biomass (Pf = target gross yield (y) x N requirement per unit of production (b))	Essential

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

P_r	Total plant uptake in underground biomass (virtually impossible to measure, assumption to be taken from the literature)	Optional
R_e	Mineral N content in the soil profile accessible to plant roots at harvest.	Essential
N_v	Volatilisation of ammonia	Optional, based upon literature, if possible lab scale experiment.
N_l	N losses due to leaching	Residual nitrate measurements in the soil at harvest (preferably until a depth of 60 to 90 cm) give an indication of leaching. More precise measuring of losses due to leaching is very elaborate (lysimeter experiments) and optional.
N_r	N losses due to runoff	N losses due to runoff should be avoided (field selection and cultural practices).
N_d	N losses due to denitrification	Optional, based upon literature, if possible lab scale experiment.
N_i	N losses due to immobilisation	Optional

3.2 Research hypothesis

- For every treatment the NFRV, ANR and as many components of the N balance as possible are measured or calculated.
- H_0: there is a clear difference in N – uptake at a given level of N-fertilisation between plots fertilised with different BBFs between themselves and compared to the reference treatment fertilised with mineral N, P, K and S.
- $H_{0,bis}$: there is a clear difference in crop yield (in harvestable biomass) at given level of N-fertilisation between plots fertilised with different BBFs between themselves and compared to the reference treatment fertilised with mineral N, P, K and S.
- $H_{0,bis1}$: there is a clear difference with regard to environmental losses of N (atmospheric and soil and surface waters) between the plots fertilised with the BBFs between themselves and compared to the reference treatment fertilised with mineral N, P, K and S.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

3.3 List of treatments

Table 5-3: Example of treatments in field trials

Nr	Fertiliser (N)	Dose (X = N-fertiliser advice)	Fertiliser (P,K, S)	Dose (Y = P, K, S fertiliser advice or highest amount applied by BBF following N – fertiliser advice)
1	/	0	0	0
3	Mineral*	30%X	Mineral	Y
4	Mineral*	60%X	Mineral	Y
5	Mineral*	X	Mineral	Y
6	Product 1	30%X	Product 1 + mineral	Y- plant available PKS supplied by product 1
7	Product 1	60%X	Product 1 + mineral	Y-plant available PKS supplied by product 1
8	Product 1	X	Product 1 + mineral	Y plant available PKS supplied by product 1
9	Product 2	30%X	Product 2 + mineral	Y plant available PKS supplied by product 2
10	Product 2	60%X	Product 2 + mineral	Y plant available PKS supplied by product 2
11	Product 2	X	Product 2 + mineral	Y plant available PKS supplied by product 2
12	…			
* It is advised to apply all mineral fertiliser broadcast and in granular form. Of course, this can depend on the choice of the mineral fertiliser and the tested crop.				

The application dates or plant growth stages for N, P, K and S mineral fertiliser are those which are recommended by advisory services for that specific crop. The application date or plant growth stage of the BBFs are chosen in order to maximize fertilising value and will take into account legal spreading constraints.

As we are studying N – effects, but a number of the applied BBFs also contain P, K and S (and micronutrients). P, K and S may never be limiting factors. If needed, mineral P, K and S is applied based upon a P, K, S fertilisation advice (dose = Y) given by specialist advisory services. The amount applied is the amount required by the crop from which the amount of P, K and S that is expected to become plant-available from the applied BBFs is subtracted.

Remark: the fertilisation advice for P, K or S may limit the amount of N that can be applied by the BBF. When this is the case, the P, K and S fertiliser advice shall be ignored. The dose of P, K and S applied will be increased until all plots receive the same amount of P, K and S as the amount given when applying the BBF (Dose = X) with the highest concentration of P, K or S.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

3.4 Experimental design

i. Trial duration

- One–year experiments :

  The duration of the experiment will correspond to the time between the spreading of the BBF and the end of the growing season (November). ANR and NFRV will be calculated at harvest. Residual Nmin in the soil will be determined at harvest (0-90 cm) and before winter (0-90 cm). If not feasible due to problematic soil type (eg. too hard, too dry), then lower depth can be sampled.

- Multi–year experiments :

  The duration of the experiment will correspond to the period between the first spreading of the BBF and the end of the last growing season (November). Of course, the last growing season depends on the test crop (eg. winter wheat).

ii. Type of design

Randomized complete block design or completely randomized design depending on the circumstances (uniformity of the field and presence/absence of gradients, See EPPO PP 1/152 (4) for further clarification). The control(s) should preferably be included.

Preferably the number of replicates in the trial is 4 (or higher). In any case the amount of degrees of freedom (df) should not be lower than 12 (EPPO PP 1/152 (4)) and preferably higher as the EPPO protocols were designed for efficacy evaluation trials and not for fertiliser tests.

Variability of the N – mineralisation from soil organic matter and differences in moisture and nutrient availability create a high residual interference compared to efficacy evaluation trials. Ideally, the power of the trial should be higher compared to efficacy evaluation trials.

When applying BBFs by machine, the net plots within the trial may only be driven upon once in order to avoid compaction of the upper soil layer. Therefore, the discard area (difference between gross and net plot) around the plots should in any case be broad enough for the used machinery to manoeuvre upon. Furthermore, it is important that wheel tracks of the boom spray providing pest protection are always situated in the discard area or outside of the gross plots and never inside the net plots (Figure 3-3).

iii. Size of elementary plots

In general, plots should be rectangular and of the same size. From a practical point of view, the width of the plots should be a multiple of the working width of the machines applied. For fertiliser trials, net plots fertilised with the tested product should have a minimum size of 6 x 8 m. However, plot size can also depend on the quantity of the tested product (i.e. low quantity, smaller plot) and on the crop type and nutrient need.

When applying liquid fertilisers using a manure injector or boom spray (or any other machine) the properties of the machine should be taken in account. A certain distance to drive over while starting fertilisation may be needed (filling of the tubes) before the application of the fertiliser is sufficiently uniform. This area should always be regarded as discard area (Figure 3-3).

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

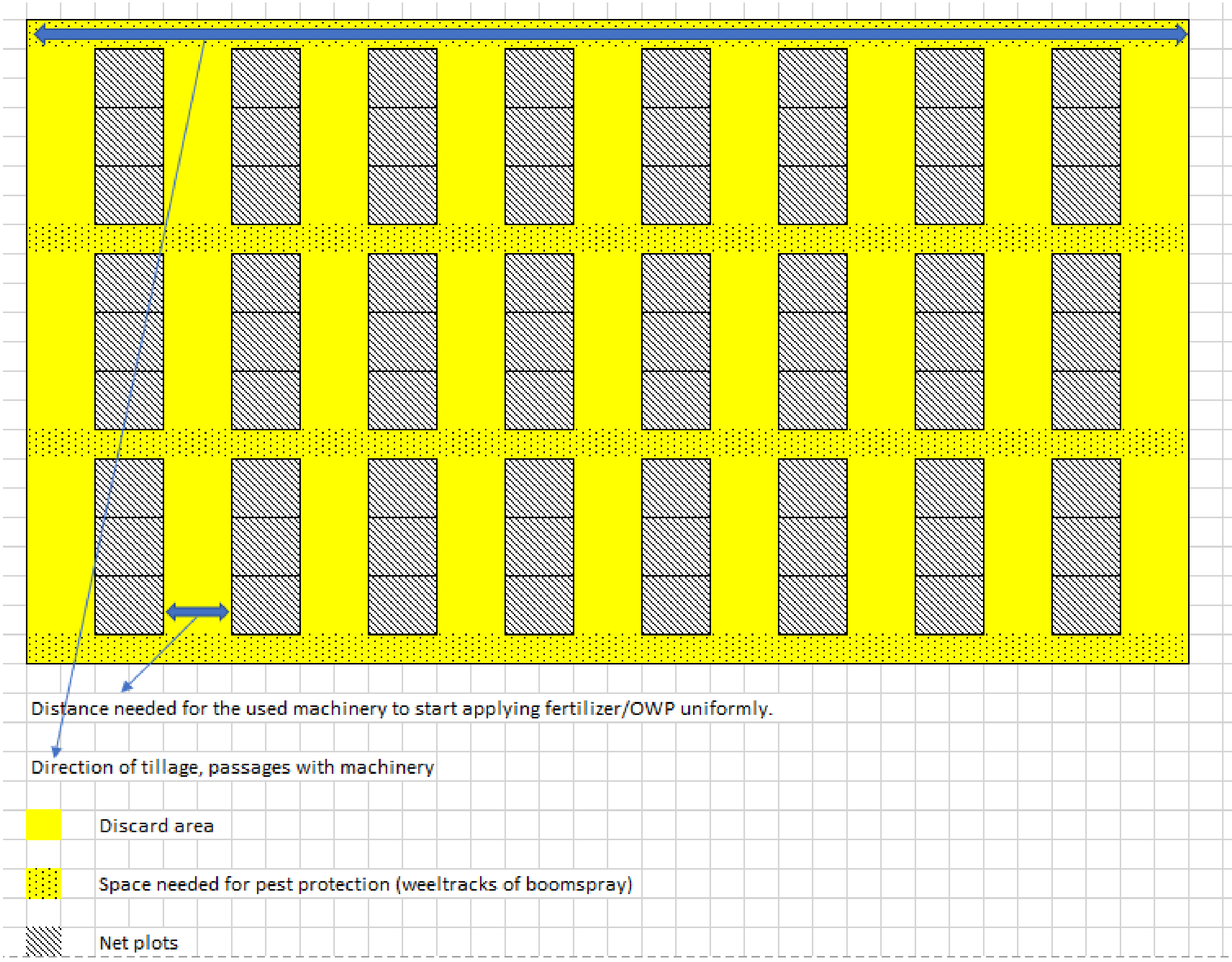

Figure 3-3: Schematic representation of a possible field trial lay-out for field trials

3.5 Field selection

i. Location

If fertilisers are applied by machine (manure injector, …), the chosen field should be sufficiently accessible. The history (at least over a period of 10 years) of the entire trial field should be the same (i.e. the field should be homogenous). Fields which were recently merged into a larger field should be excluded or the trial should be performed completely on one of the smaller fields with a uniform cultivation history. The cultivation history recorded of the period before merging the fields should be that of the original field on which the trial is performed.

Fields on which a significant amount of crop residues from the previous culture or catch crop is left should be avoided. Also, fields on which organic fertiliser was applied frequently the past few years should be avoided. In general, fields on which high N-mineralisation is expected should be avoided. Runoff and erosion should be prevented choosing a sufficiently level field and using adequate tillage and cultivation practices.

ii. Soil

The soil type should be representative for the region in which the trials are conducted. Soil fertility indicators (pH, %C, macronutrients, micronutrients) should be within the target zones defined by local advisory services for each indicator. Minor shortages of micro– or macronutrients can be adjusted applying mineral fertiliser before trial setup. Major shortages shouldn't occur. Also, in order to prevent nutrient uptake problems due to antagonisms, fields in which some macro- or micronutrients heavily exceed the target value should also be

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

refused. Soil structure should be optimal. No topsoil or subsoil compaction should be present. Wet patches in the field should also be excluded from the trial.

iii. Agricultural history

Cropping history should be known for at least 5 years. Also practices regarding the use of organic fertilisers should be known as detailed as possible. As well as the use of catch-crops. If results of earlier soil analysis are available, they should be collected.

iv. Culture

Crops which are representative for or common in the region are preferred. Preferably crops with a high N - demand are chosen. N – fixating crops (Leguminosae) should be excluded.

3.6 Fertilisation advice

In the following text the principles on which the determination of the N-fertilisation advice is based are described. Most N- fertiliser advices issued by research institutes or commercial laboratories follow these principles. For trial conduct within the WalNUT framework, locally issued N- fertiliser advices may be followed as long as they comply with the principles described. In any case, the underlying calculations of the N- fertiliser advice should be known by the researchers conducting the field trial and the researchers must check whether the issued advice complies with these principles.

The N- fertiliser advice should be calculated as followed:

$$X = (Fm + Mo) = Pf + Pr + Rl - Ri - Nirr - Ndep - Mcr - Mcc - Mso - Mo(y-1)$$

Most components of this equation are described in Table 3-2.

X = Effective N to be applied

Pf= Theoretical crop uptake

Rl= Minimum mineral N content in the soil profile accessible to plant roots at harvest needed to ensure optimal growth (depends on the crop). For vegetables, theoretical values are given by Feller et al (2011).

Expected N losses are unknown at the beginning of the culture, the N- fertiliser advice should theoretically reduce these losses to zero. As many components of this equation as possible should be used when calculating the N-fertiliser advice. Expected N-mineralisation from different components and N-uptake in plant roots are difficult to calculate separately. If impossible to predict, the sum of these terms can be estimated as net N-mineralisation (Ms – Pr).

Theoretical crop N – uptake (Pf) should be determined as followed:

- If regional data regarding crop yield and residual nitrate content in the soil after harvest are in sufficient quantities available, theoretical N-uptake should preferably be calculated using these data (Figure 3-4). The theoretical N – uptake is the point at which – with increasing fertiliser doses – the residual nitrate in the soil starts to increase. If insufficient regional data are available for the culture a N – fertiliser advice given by a local laboratory should be followed.
- Figure 3-4 gives an example of how to calculate the theoretical crop N – uptake for grassland. Data of 30^{+} different fields with different N- fertiliser regimes are used. When applying extra N– fertiliser, crop N-uptake increases linearly until a certain point at which additional mineral N no longer solely results in extra N uptake by the crop. At that point, residual nitrate measured in the soil after harvest starts to increase. The crop N – uptake at this point should be used as theoretical crop N–uptake when calculating

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

the N- fertiliser advice. The residual nitrate content in the soil at harvest before this point is the Rl (the minimum N concentration which ensures steady growth).

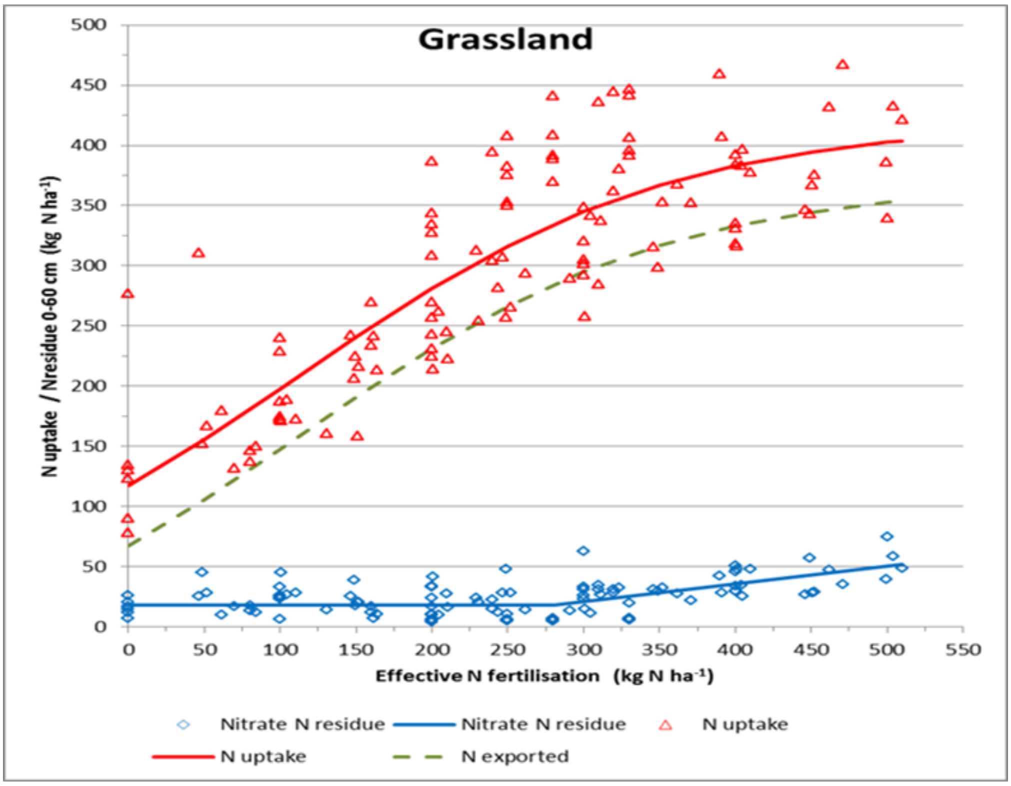

Figure 3-4: Theoretical N – uptake for grassland in Flanders (Belgium)

Besides theoretical crop uptake, **Net N-mineralisation (Ms -Pr)** is the most important parameter when calculating a fertiliser advice. Net mineralisation depends - among others - upon weather conditions and is hard to predict. Nevertheless, a prediction should be made using all available information about soil fertility parameters (pH, %C …) crop rotation and historical use of organic fertiliser. If available, historical soil samples determining residual nitrate content in the soil after harvest may prove to be interesting when estimating net mineralisation.

For all applied fertilisers, the total amount of N (organic and inorganic) in the product will be accounted for when calculating the dose of fertiliser to be applied.

Note: for fertilisation trials conducted over multiple years, starting from the second year fertilisation advices will be calculated per object (4 parallels) and not per plot individually.

3.7 Trial conduct and agricultural practices

i. Equipment

Spreading of granular mineral fertilisers will be done manually. The reference mineral fertiliser for mineral N is pure ammonium nitrate (33.5 %) in granular form. Spreading of liquid mineral fertilisers, ammonium salts will be done using specialized equipment. At any time, the product must be incorporated as soon as possible.

Spreading of solid BBFs will be done manually or using a small solid manure spreader (frequently used in greenhouses). In this case, plots must be sufficiently long and experienced driving is required in order to guarantee a uniform spreading of the manure. Spreading of liquid BBFs will be done using a slurry injector or a specialized ‘trial’ injector. If necessary this will be done manually.

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement 101000752.

After applying fertilisers and BBFs, the applied products must be incorporated into the soil as soon as possible. In Flanders (Belgium), farmers are legally obliged to inject their manure and liquid fertilisers. If this isn't possible, they should be incorporated within 2 hours after application. In Italy there is no such obligation, but by taking these types of measures (i.e. injection or incorporation) the risk for ammonia emissions is reduced and consequently incorporation of BBFs is recommended in all trials.

ii. Agricultural practices / tillage

Soil tillage must be uniform on the entire trial field. Moreover, the aligning in which tillage (ploughing but also soil preparation with rotary harrow) is done must always be the same. In this direction, there should be a sufficiently large discard area before and after each plot. If subsequent passages with the rotary harrow (or other machinery) are needed to prepare the field for sowing or planting, try to perform subsequent passages in opposite directions (180 °). This avoids excessive soil and fertiliser displacement.

Crop protection will be carried out uniformly over the entire trial field and following 'good agricultural practice'. The studied crop should be kept in good health. Pests and diseases should be avoided.

iv. Crop rotation

The crop rotation should be representative for the region. If possible crops producing large amounts of instable crop residue (vegetables) should be avoided (to avoid N – mineralisation).

If possible - depending on the time of harvest and weather conditions - a catch crop is sown after harvest. Before winter a determination of aboveground biomass production and N – uptake is done on the catch crop.

v. Trial conduct

All actions and interventions made during the trial shall be recorded. Unforeseen circumstances (pests, game damage…) shall be recorded.

3.8 Observations and measurements

i. On soil

Needed:

Evaluation of soil fertility should be done using a chemical characterisation of the top soil layer (0 -30 cm). At least soil pH, soil texture, total N content and organic carbon content should be assessed. Preferably the trial is conducted on a level field. If performed on a slant field, the inclination should be measured and runoff and erosion should be prevented using proper tillage and cultivation practices.

Before sowing/planting and after harvest mineral N content (ammonia and nitrate) in the soil is measured. This is done per 30 cm layer. Before sowing, samples are taken until a depth of 90 cm, if feasible. At harvest and after harvest, samples are taken until a depth of 120 cm, if feasible. On these samples, bulk density should also be determined.

After harvest (depending on crop), a third soil sample is taken shortly before winter. This sample is also taken until a depth of 120 cm and provides information regarding N-mineralisation from soil, applied BBFs and crop residues after harvest. This last soil sample should be taken as late as possible, but before heavy rain in the fall/winter that can cause N-leaching.

Optional:

If possible a soil analysis making a general assessment of chemical soil fertility (plant available macro– and micronutrients) is useful. If possible, an assessment of soil porosity is made (can be calculated from bulk density).

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

Measurements of gaseous N-losses from the field is relevant calculating the different terms of the N-balance. These measurements however are very elaborate and require vast amounts of space which are not compatible with this trial. Moreover, most partners do not have experience using these methods.

ii. On crop

Needed:

In any case, following parameters should be assessed.

- Delay in emergence
- Thinning
- Effects on yield: separation in harvestable and non-harvestable plant parts is made depending on the culture. EPPO standards (e.g. those made for herbicide effectivity) can be helpful in determining how parameters like yield should be assessed in specific crops[3].

    - Fresh weight of yield in kg ha -1, taken from the centre of the plots.
    - Dry matter content
    - Total N content
    - Nitrate content

- Quality of the marketable product: sorting in quality classes (especially for vegetables) and choice of parameters to be evaluated takes into account regional specifications (standards of auctions, regional purchasers …). The parameters to be evaluated are chosen to be representative for the local market circumstances. Furthermore, parameters evaluating legal constraints regarding crop quality are evaluated (e.g. nitrate content in spinach ((EU) 1258/2011)).
- On the catch crop sown after harvest (if relevant), a determination of aboveground biomass and N – uptake is done before winter (but as late as possible).
    - Fresh weight in kg ha^{-1}, taken from the centre of the plots.
    - Dry matter content
    - Total N content

Optional:

Evaluation of phytotoxicity effects on crops is evaluated following guidelines in EPPO PP 1/135 (4). Relevant assessment parameters should be chosen on a case by case basis depending on the product tested, the mode of action, application time, culture etc…

iii. On fertilisers

Samples of the BBFs will be taken (make sure the BBF is properly mixed/the sample is representative) an analysed shortly before applying the BBF. Based upon results of the sample analysis, the dose of BBF to be applied is calculated. When the BBFs are applied, a second representative sample is taken to determine the actual applied nutrients. On both samples as many of the following parameters as possible should be analysed:

Needed:

- Dry matter content

[3] See also: https://pp1.eppo.int/standards/herbicides, e.g. description for yield determination in maize explained in EPPO PP1/050 (3).

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

- Organic matter
- Organic carbon
- pH
- EC
- Total N
- Organic N
- NH_4^+
- NO_3^-
- Total P, K, S content

Optional:

- Mg, Ca, Na and Cl content
- Cu, Zn, B, Mo and Fe content
- Not measured but relevant compared to selection of parameters in Fertilising Products Regulation (FPR; EU (2019/1009). The list of parameters can be found in Annex 2.
    -

iv. Meteorological conditions and general info

Acquisition of climatic data will preferably be done placing a mobile weather pole at the trial site or by extracting the climatic data from the nearest weather station. Necessary data to be collected in function of time are:

- Precipitation (l/m^2)
- Average daily temperature (°C)
- Soil temperature at a depth of 15 cm (°C) (optional)
- Maximum daily temperature (°C)
- Minimum daily temperature (°C)
- Relative humidity (%) (optional)

When a suitable location is chosen, the trial field will be georeferenced (GPS, latitude, longitude, altitude) on the basis of the 4 corners of the trial. When the trial is performed on a non-horizontal field, the slope of the field should be recorded (preferably the trial isn't conducted on a non-horizontal field).

3.9 Data collection and processing

Statistical analysis is described in EPPO PP1/152 (4).

If the trial is successful, yield data and calculated N-uptake data of the different treatments should be compared to the reference treatment.

When the conditions are met (homogeneity of variance and normality and independence of the error) a one way ANOVA should be performed. If not, non – parametric testing is more suited.

ii. Calculation of ANR, NFRV and components of the N – balance

For every plot, as many components of the N-balance as possible should be calculated. If a sufficiently complete N – balance can be calculated for all plots, a statistical comparison (ANOVA) of the N – losses per plot might be interesting.

For every treatment and for every BBF, ANR and NFRV should be calculated. The effect of fertiliser dose on ANR and NFRV should be evaluated.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

4 Conclusions

The first part of this deliverable, harmonisation of 16 terms in the form of the Lexicon, was successful as a first step towards achieving the harmonisation and agreement across different projects in the field of the nutrient recovery. However, the harmonisation is not fully completed and certain aspects still need to be clarified, not only on level of projects, but also on European legal level. One of the terms that that raises quite some controversy is the term ‘bio-based fertiliser’. WalNUT project will continue to contribute to its clarification, along with help of other projects that are part of the Nutrient Recycling Community. And of course, support other initiatives such as the one of ESPP that is currently developing a Position Paper on the definitions of ‘bio-based fertiliser’ or ‘bio-based nutrient’.

The second part of the deliverable concerns the GEP protocol. The protocol aims to provide a guideline on how to evaluate agronomic efficiency of the WalNUT BBFs. Of course, some degree of freedom remains for partners in order to adapt to certain regional or experimental situations. For example, if quantity of the BBF is low then plot size can be reduced accordingly. Or if injection is obligatory incorporation technique of BBFs in one region, it does not have to be obligatory for other region if there regional legislation does not impose it.

In general, the Lexicon and GEP protocol are first outputs of WP4 towards providing the needed elements to design the proper way to test and use the BBFs in medium term field with different climatic & soil conditions.

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement 101000752.

Annexes

<u>Annex 1</u>: Agenda and Keynote speakers of the webinar on Lexicon, on February 9th 2023.

Our Agenda

14:00 Welcome and opening, *Ana Robles Aguilar, UVIC*

Keynote speeches:

- European regulators' perspective: *Ludwig Hermann, Proman*
- Academic standpoint: *Ivona Sigurnjak, Ghent University (WalNUT)*

Discussion with participating projects, *moderated by Kari Ylivianio, LUKE*

- BioDen
- FertiCycle
- Fertimanure
- Lex4Bio
- Novafert
- Nutribudget
- Nutri2Cycle
- ReNu2Farm
- Run4Life
- Sabana
- Sea2Land
- Systemic
- Walnut

16:00 End of the meeting

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

Annex 2: The list of parameters based on Product Function Category (PFC, type of product/fertiliser) and the Component Material Category (CMC, allowed input materials) for conformity of bio-based fertiliser (BBF) to Fertilising Product Regulation.

Category	Parameter
Product Function Category	Dry matter
	Organic carbon
	Total nitrogen
	Ammonium nitrogen
	Nitrate nitrogen
	Total P_2O_5
	Total K_2O
	Total SO_3
	Total MgO
	Total CaO
	Total Na_2O
	Ni
	Pb
	Cr IV
	As
	As inorganic
	Cd
	Cu
	Zn
	Biuret C2H5N3O2
	Perchlorate (ClO4-)
	Salmonella (absence in 25g or 25ml)
	E. coli or Enterococcaceae (in 1g or 1 ml)
Component Material Category	PAH 16
	PCB (Sum of congeners PCB 28, 52, 101, 138, 153, 180)
	H / Corg
	sum Al + Fe
	Cr total
	Thallium
	Chlorine
	Vanadium
	WHO toxicity equivalents of PCDD/F/kg DM
	macroscopic impurities above 2 mm in any of the following forms: glass, metal or plastics
	sum of glass, metal or plastics
	oxygen consumption rate: — definition: indicator of the degree of decomposition of biodegradable organic matter in a given period of time. The method is not suitable for materials consisting of more than 20% of particles of size > 10 mm; OR residual biogas production potential: — definition: indicator of the quantity of gas released by a digestate in 28 days and measured according to the volatile solids contained in the sample. The test is carried out three replicates, and the average result is used to demonstrate compliance with the criterion. Volatile solids are those solids contained in a sample of material which undergo a loss on ignition when heated to 550°C in the dry matter;

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.

References

Cellier, P., Germon, J.C., Henault, S. & Genermont, S. (1996). Les émissions d'ammoniac (NH_3) et d'oxydes d'azote (NO_x et N_2O) par les sols cultivés : mécanismes de production et quantification des flux. *Maîtrise de l'azote dans les agrosystèmes*. Reims (France), 19-20 novembre 1996. Ed. INRA, Paris 1997 (Les Colloques, n°83).

EC (2011). Commission Regulation (EU) No 1258/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for nitrates in foodstuffs.

EPPO (2014). PP1/135 (4) Phytotoxicity assessment. Bulletin OEPP/EPPO Bulletin(2014)44(3), 265–273. DOI: 10.1111/epp.12134

EPPO (2012). PP1/152 (4) Design and analysis of efficacy evaluation trials. Bulletin OEPP/EPPO Bulletin (2012) 42 (3), 367–381. DOI: 10.1111/epp.2610

EPPO (2021). PP1/181 (4) Conduct and reporting of efficacy evaluation trials, including good experimental practice. EPPO Bulletin. 2021;00:1–13. DOI: 10.1111/epp.12788

European Commission (2017). COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS on the 2017 list of Critical Raw Materials for the EU. Available on: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52017DC0490

European Commission (2019). Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003 (Text with EEA relevance) http://data.europa.eu/eli/reg/2019/1009/oj

Feller, C., Fink M., Laber, H., Maync, A., Paschold, P., Scharpf, H.C., Schlaghecken, J., Strohmeyer, K., Weier, U. & Ziegler, J. (2011) Düngung im Freilandgemüsebau. In: Fink, M. (Hrsg.): Schriftenreihe des Leibniz-Instituts für Gemüse- und Zierpflanzenbau (IGZ), 3. Auflage, Heft 4, Großbeeren.

Fertilizers Europe (2022). Industry facts and figures 2021. Available on: https://www.fertilizerseurope.com/wp-content/uploads/2022/09/Industry-Facts-and-Figures-2022.pdf

Van de Sande, T., De Dobbelaere, A., Bouthier, A. & Sigurnjak, I., 2019. Evaluation of the short term N-effect of a recycling-derived fertiliser (RDF) on crop and environment in field trials. Interreg NWE ReNu2Farm project. Available from: https://www.biorefine.eu/publications/annex-4-evaluation-of-the-short-term-n-effect-of-a-recycling-derived-fertilizer-rdf-on-crop-and-environment-in-field-trials/

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 101000752.