Interreg
EUROPEAN UNION
North-West Europe
ReNu2Farm
European Regional Development Fund

Annex 4:

Evaluation of the short term N-effect of a recycling-derived fertilizer (RDF) on crop and environment in field trials

Authors: Tomas Van de Sande[1], Anke De Dobbelaere[1], Alain Bouthier[2], Ivona Sigurnjak[3]

[1]Inagro vzw, Research & advice in agriculture & horticulture, Ieperseweg 87, 8800 Rumbeke-Beitem, Belgium, www.inagro.be, tomas.vandesande@inagro.be

[2]Arvalis - Institut du vegetal, 3, rue Joseph et Marie Hackin, 75016 PARIS, www.arvalis-infos.fr

[3]Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium

Co-funder(s):

Partners:

Table of contents

1. Introduction

In this protocol general outlines and principles which should be followed when conducting field trials evaluating the short term N–effect of RDF's for the Renu2farm project are described.

The protocol is not confined to actions and measurements required for the ReNu2Farm project but also mentions extra actions and measurements which supply extra information that can be interesting from a scientific point of view. The protocol is written in order to be able to have a common methodology between project partners though leaving room for local interpretation and discussion on local methods between regions (e.g. chosen protocols for analysis). There where possible however the protocol advises as much as possible to use common standards (e.g. those provided by EPPO, the European and Mediterranean Plant Protection Organization).

Therefore this protocol always clearly distinguishes actions and measurements which are essential for ReNu2Farm and extra research which is optional and which might be interesting (e.g. in the framework of other initiatives like JRC's SafeManure project and the HOR2020 project Systemic for instance).

This protocol is set up to gain insight on nutrient use efficiency and more specific the nitrogen fertilizer replacement value of nutrient recovery products, while at the same time assessing the agronomic performance and risks relating to environmental impact, namely focusing on the risks relating to water pollution while using nutrient recovery products.

From the project proposal we refer to the following deliverables in WP2 'Agro-ecologic assessment of recycling-derived fertilizers' we want to cover with the research coming from the field trials set out in this protocol:

- **Deliverable 3.1**: 'Protocol for the evaluation of the agronomic value of recycling derived fertilizers.'
- **Deliverable 3.2**: 'Report on agronomic performance and suitability of recycling-derived ferilizers'

And also:

- **Deliverable 2.3**: 'Report on environmental impact: nutrient and CO_2 emissions'
- **Deliverable 2.5**: 'Report on environmental impact: nutrient balances'.
- **Deliverable 4.1, 4.2 and 4.3**: Demonstration sites…:

- with recycling derived fertilizers and their best-practice management
- for nutrient efficiency performance of recycling-derived fertilizers
- for carbon footprint of recycling-derived fertilizers

2. Context and state of the art

2.1. Short term N effects on crop: calculation of the nitrogen fertilizer replacement value (NFRV) and apparent nitrogen recovery (ANR)

The ANR (of a specific RDF) is obtained by calculating the relation between the nitrogen supplement absorbed by a crop (all aboveground parts) fertilized with the RDF compared to a non–fertilized (with N) reference (Table 2: treatment 2), and total nitrogen brought by the RDF. P, K and S fertilization will be applied in mineral form on the reference treatment assuring sufficient P, K and S availability on the reference plots as well.

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

$$NFRV^{2}\,(N\ fertilizing\ replacement\ value) = \frac{ANR\ (OWP)}{ANR\ (mineral\ fertilizer)}$$

Both coefficients are interdependent and complementary. NFRV is more directly operational but also dependent on the effectiveness of the reference mineral fertilizer. **The reference mineral fertilizer is pure ammonium nitrate (no added Mg or other nutrients) in granular form and applied broadcast.**

[1] or CAU in France.
[2] or Keq in France.

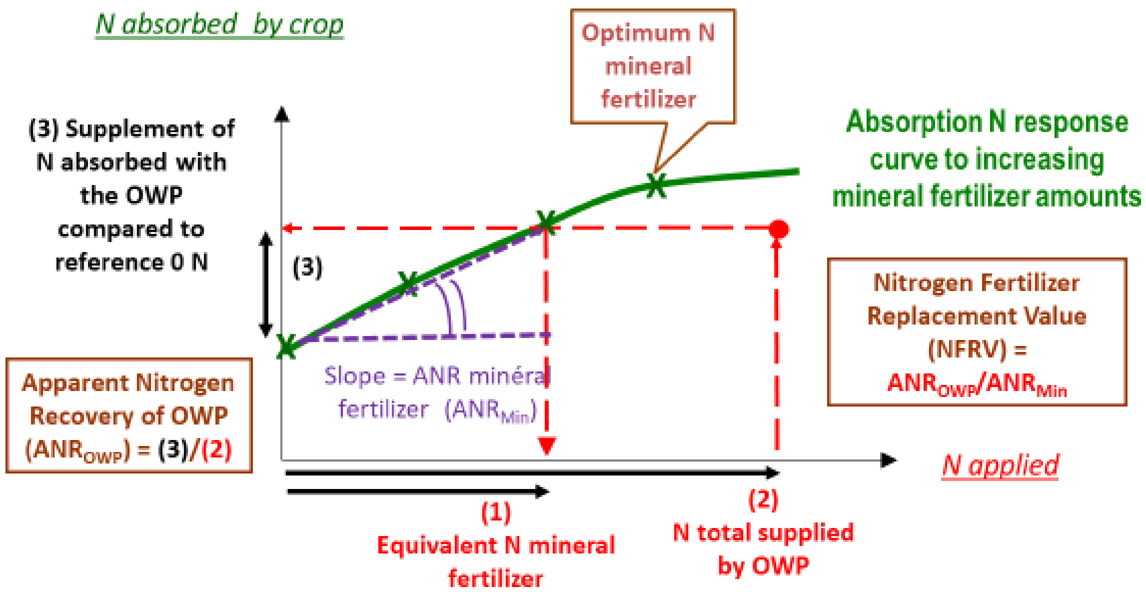

Figure 1: schematic presentation of how ANR and NFRV are calculated.

The calculation of NFRV and ANR requires a crop yield response curve to mineral fertilizer applied, including a treatment without N-fertilization. The treatment without N-fertilization allows to calculate the N-mineralisation. The curve itself allows estimation of the point at which additional N-fertilization will no longer result in additional N-uptake. Ideally CAU and Keq must be calculated for N - fertilization 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 fertilizer 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 fertilizer applied (Feller, 2011)). This can (partially) be explained by higher N-uptake by plant roots, increased immobilization and higher losses (leaching and gaseous losses).

- Crop response to extra N fertilizer decreases with increasing doses of N fertilizer applied.
- N losses during the growing season (leaching or gaseous losses) will generally be higher on the fertilized plots. These losses are also function of the N-mineralisation.

High N-mineralisation, or N-losses due to leaching of volatilization 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 1).

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

Short term N impact of an RDF on the environment mostly comprises (amongst other effects) losses of mineral N below the root zone of crops via N leaching and losses of mineral N to the atmosphere via volatilisation of ammonia and production of N_2O via incomplete denitrification.

Table 1 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.

Table 1: Components of the 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 fertilizer	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 ($M_{tot} = M_{cr} + M_{cc} + M_{so} + M_{o(y-1)} + M_o$)	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 fertilizer applied the year before trial conduct	Via incubation experiment, part of M_S. Separate determination is optional.
M_o	N-mineralisation from organic fertilizer applied shortly before planting/sowing	Essential. Via incubation experiments (WP1)
F_m	Mineral fertilizer	Essential
N-losses		
P_f	Total plant uptake in aboveground biomass (P_f = target gross yield (y) x N requirement per unit of production (b))	Essential
P_r	Total plant uptake in underground biomass (virtually impossible to measure, literature?)	Optional
R_e	Mineral N content in the soil profile accessible to plant roots at harvest.	Essential
N_v	Volatilization of ammonia	Optional, based upon literature, if possible lab scale experiment.
N_l	Nitrogen losses due to leaching	Residual nitrate measurements in the soil at harvest (until a depth of 120 cm) give an indication of leaching and are essential for Renu2farm. More precise measuring of losses due to leaching is very elaborate (lysimeter experiments) and optional.
N_r	Nitrogen losses due to runoff	Nitrogen 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 immobilization	Optional

3. 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 given level of N-fertilization between plots fertilized with different RDFs between themselves and compared to the reference treatment fertilized 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-fertilization between plots fertilized with different RDFs between themselves and compared to the reference treatment fertilized 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 fertilized with the RDFs between themselves and compared to the reference treatment fertilized with mineral N, P, K and S.

4. List of treatments

Table 2: *list of treatments*

Nr	Fertilizer (N)	Dose (X = N-fertilizer advice)	Fertilizer (P,K, S)	Dose (Y = P, K, S fertilizer advice or highest amount applied by RDF following N – fertilizer advice)
1	/	0	0	0
2	Mineral*	0	Mineral	Y
3	Mineral*	X-60%	Mineral	Y
4	Mineral*	X-30%	Mineral	Y
5	Mineral*	X	Mineral	Y
6	Product 1	X-60%	Product 1 + mineral	Y- plant available PKS supplied by product 1
7	Product 1	X-30%	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	X-60%	Product 2 + mineral	Y plant available PKS supplied by product 2
10	Product 2	X-30%	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	…			
* All mineral fertilizers will be applied broadcast and in granular form.				

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

As we are studying N-effects, but a number of the applied RDFs 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 fertilization 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 RDFs is subtracted.

Remark: the fertilization advice for P, K or S may limit the amount of N that can be applied by the RDF. When this is the case, the P, K and S fertilizer 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 RDF (Dose = X) with the highest concentration of P, K or S.

5. Experimental design

5.1. Trial duration

- One–year experiments:

  The duration of the experiment will correspond to the time between the spreading of the RDF and the end of the growing season (November). ANR and NFRV will be calculated at harvest. Residual N_{min} in the soil will be determined at harvest (0-120 cm) and before winter (0-120 cm).

5.2. 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) have to be included.

Preferably the number of replicates in the trial is 4 (or higher), but at least 3. 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 fertilizer 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 RDFs by machine, the net plots within the trial may only be driven on 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.

5.3. Size of elementary plots

In general, plots should be rectangular and of the same size. From practical point of view, the width of the plots should be a multiple of the working width of the machines applied. For fertilizer trials, net plots fertilized with the tested product should have a minimum size of 6 x 8 m.

When applying liquid fertilizers 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 fertilization may be needed (filling of the tubes) before the application of the fertilizer is sufficiently uniform. This area should always be regarded as discard area (figure 2).

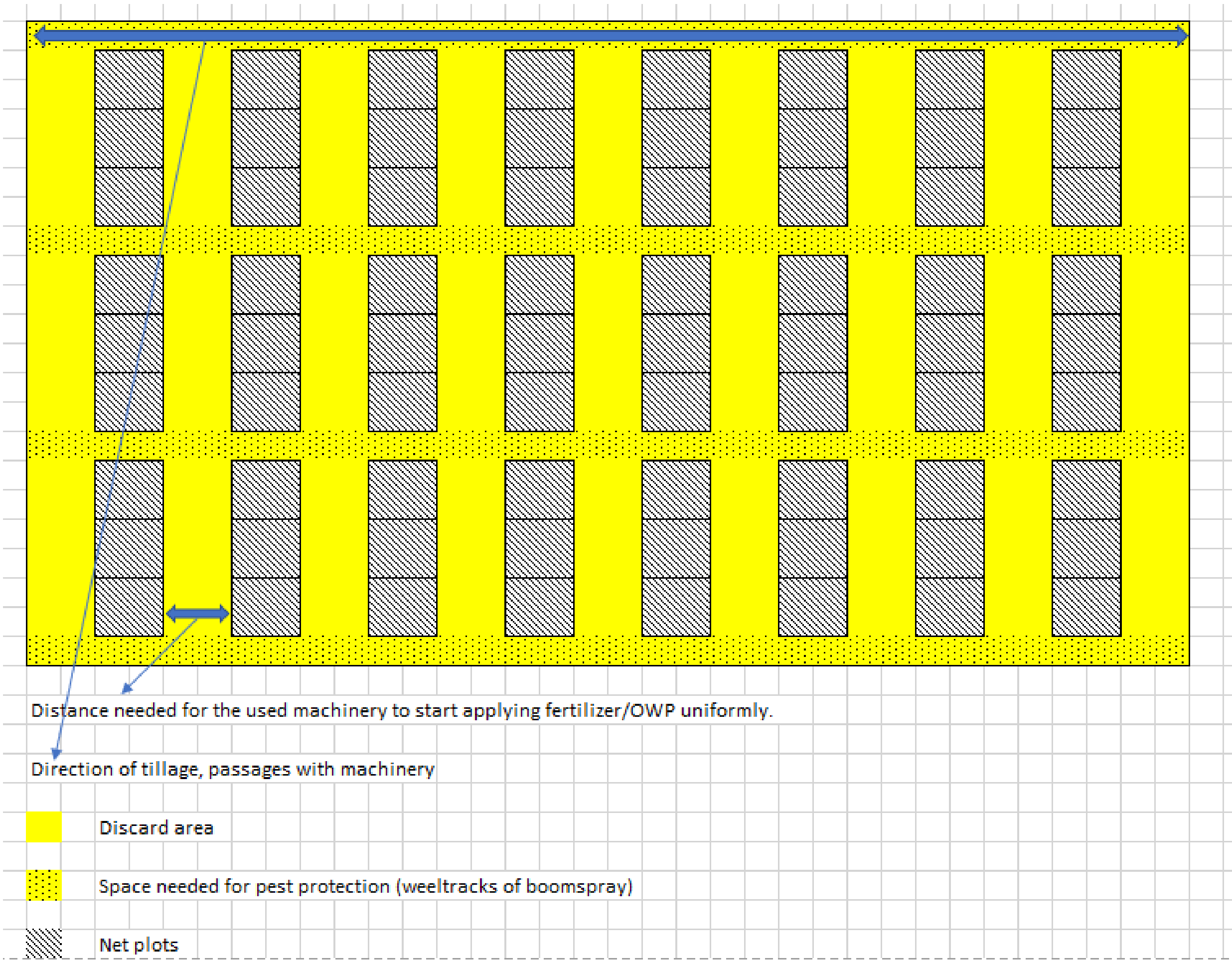

Figure 2: Schematic representation of a possible field trial lay-out.

6. Field selection and fertilization advice

6.1. Location

If fertilizers 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. 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 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 fertilizer 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.

6.2. 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 fertilizer before trial setup. Major shortages should not 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 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.

6.3. Agricultural history

Cropping history should be known for at least 5 years. Also practices regarding the use of organic fertilizers 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.

6.4. 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.

7. N-fertilizer advice

In the following text the principles on which the determination of the N-fertilization advice is based are described. Most N-fertilizer advices issued by research institutes or commercial laboratories follow these principles. For trial conduct within the Renu2Farm framework, locally issued N-fertilizer advices may be followed as long as they comply with the principles described. In any case, the underlying calculations of the N-fertilizer 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-fertilizer advice should be calculated as followed:

$$X = (F_m + M_o) = P_f + P_r + R_l - R_i - N_{irr} - N_{dep} - M_{cr} - M_{cc} - M_{so} - M_{o(y-1)}$$

Most components of this equation are described in table 1.

X = Effective N to be applied

P_f= Theoretical crop uptake

R_l= 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 nitrogen losses are unknown at the beginning of the culture, the N-fertilizer advice should theoretically reduce these losses to zero. As many components of this equation as possible should be used when calculating the N-fertilizer 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 (M_s – P_r).

Theoretical crop N-uptake (P_f) 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). The theoretical

N-uptake is the point at which – with increasing fertilizer doses – the residual nitrate in the soil starts to increase. If insufficient regional data are available for the culture a N-fertilizer advice given by a local laboratory should be followed.

- Figure 3 gives an example of how to calculate the theoretical crop N-uptake for grassland. Data of 30+ different fields with different N-fertilizer regimes are used. When applying extra N–fertilizer, 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 the N-fertilizer advice. The residual nitrate content in the soil at harvest before this point is the R_l (the minimum N concentration which ensures steady growth).

Besides theoretical crop uptake, **Net N-mineralisation (M_s -P_r)** is the most important parameter when calculating a fertilizer advice. Net mineralisation depends – among others – on 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 fertilizer. If available, historical soil samples determining residual nitrate content in the soil after harvest may prove to be interesting when estimating net mineralisation.

Estimation of net N-mineralisation is mostly done using predictive models developed and validated in specific agricultural regions for specific agricultural practices. For this trial every partner shall use his own predictive N-mineralisation model when available. If such a model isn’t available. The N-fertilizer advice shall be obtained from commercial laboratory which uses a locally developed and validated model predicting net N-mineralisation as a component of its N-fertilizer advice.

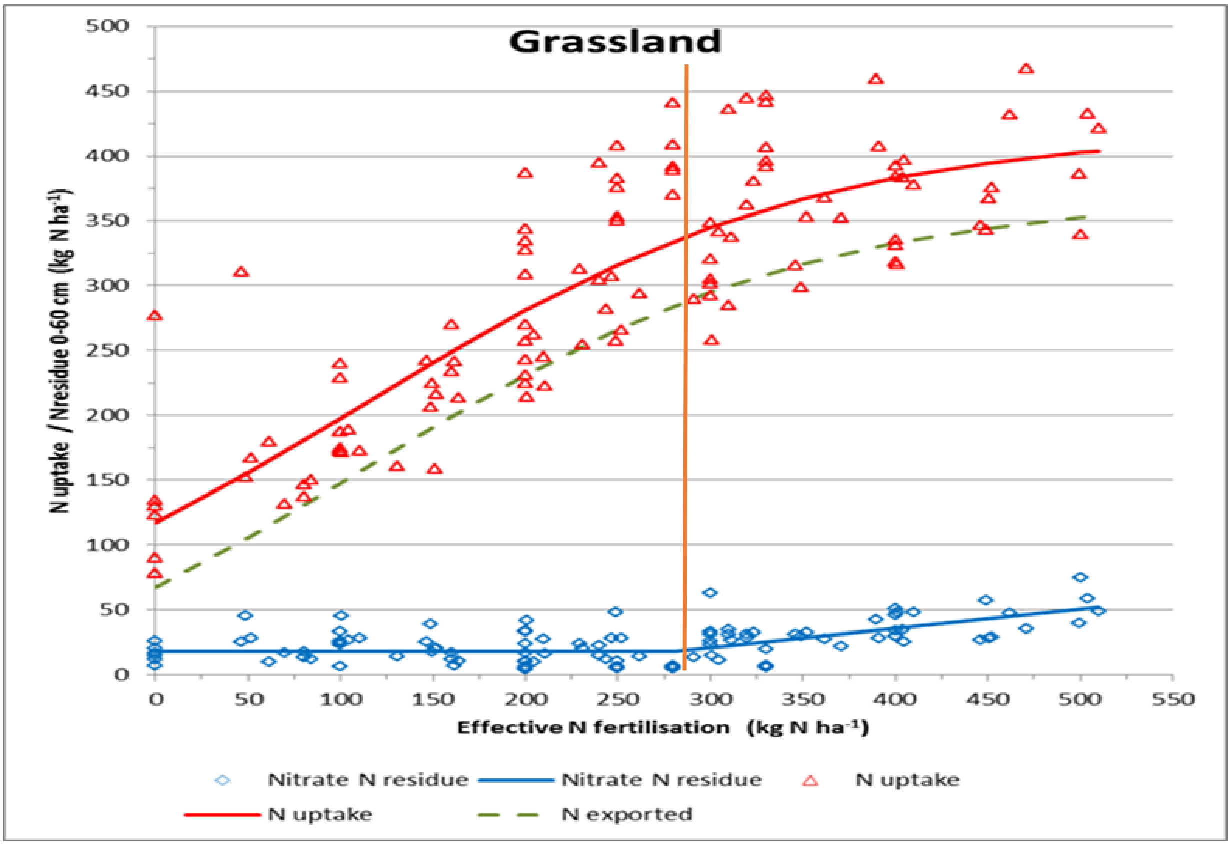

Figure 3: Theoretical N-uptake for grassland in Flanders.

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

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

8. Trial conduct and agricultural practices

8.1. Equipment

Spreading of granular mineral fertilizers will be done manually. The reference mineral fertilizer for mineral N is pure ammonium nitrate (33.5 %) in granular form.

Spreading of liquid mineral fertilizers, scrubber waters will be done using specialized equipment. At any time, the product must be incorporated as soon as possible.

Spreading of solid RDFs 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 RDFs will be done using a slurry injector or a specialized 'trial' injector. If necessary this will be done manually.

After applying fertilizers and RDFs, the applied products must be incorporated into the soil as soon as possible. In Flanders, farmers are legally obliged to incorporate animal manure within 2 hours after application preferably, all RDFs in the trial are injected. If this is not possible, they should be incorporated within 2 hours after application.

8.2. Agricultural practices/tillage

Soil tillage must be uniform on the entire trial field. Moreover, the aligning in which tillage (plowing 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 fertilizer 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.

8.3. Crop rotation

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.

8.4. Trial conduct

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

9. Observations and measurements

9.1. 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. At harvest and after harvest, samples are taken until a depth of 120 cm. On these samples, bulk density should also be determined.

If necessary, uniformity testing of the field is done before plot selection. In this, soil mineral N content in spring is the most important parameter to be assessed. Preferably the field should be homogenous in which case preliminary testing isn't necessary.

Before sowing, one measurement for the whole experiment is sufficient. Collect 15 samples for each block in an X shaped pattern and mix to constitute 1 sample for each horizon (0-30, 30-60 et 60-90 cm).

Measurement of stock N-mineral at harvest shall be done at a sampling depth of at least 90 cm gathered by horizons of 30 cm thickness.

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 RDFs and crop residues after harvest. This last soil sample should be taken as late as possible, but before heavy rain in the fall/winter 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).

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.

9.2. 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 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 nitrogen content
    - Nitrate content
- Quality of the marketable product: sorting in quality classes (especially for vegetables) and choice of parameters to be evaluated takes in 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 Nitrogen content

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

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...

9.3. On fertilizers and RDF's

Samples of the RDF's will be taken (make sure the RDF is properly mixed/the sample is representative) an analysed shortly before applying the RDF. Based upon results of the sample analysis, the dose of RDF to be applied is calculated.

When the RDFs 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
- 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 SafeManure (JRC project):
    - Particle size distribution
    - Presence of humic substances
    - Pathogen
    - VMAs pesticides related to animal husbandry and their degradation products

- N in ground or surface water
- NH_3^- volatilization, N_2O formation
- PKS in water
- Cu, Zn and other heavy metals in water
- CO_2, CH_4
- Soil biodiversity
- Microbial agents, viruses

9.4. 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)
- 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 is not conducted on a non-horizontal field).

10. Data collection and processing

10.1. Data 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.

10.2. 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 RDF, ANR and NFRV should be calculated. The effect of fertilizer dose on ANR and NFRV should be evaluated.

11. References

- Commission Regulation (EU) No 1258/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for nitrates in foodstuffs.
- D'Haene, K., Salomez, J., De Neve, S., De Waele, J., Hofman, G., 2014. Environmental performance of the nitrogen fertiliser limits imposed by the EU Nitrates Directive. Agriculture, Ecosystems and Environment 192, 67-79.
- D'Haene, K., Salomez, J., De Neve, S., Hofman, G., Verhaeghe, M. Van de Sande, T. De Nies, J. 2018. Can optimum yield and quality of vegetables be reconciled with low residual soil mineral nitrogen at harvest? Scientia Horticulturae 223, 78 – 89.
- EPPO PP1/135 (4) Phytotoxicity assessment.
- EPPO PP1/152 (4) Design and analysis of efficacy evaluation trials.
- EPPO PP1/181 (4) Conduct and reporting of efficacy evaluation trials, including good experimental practice.
- Collective of authors. Guide méthodologique Réseau PRO: mise en place d'un essai au champ pour l'évaluation agronomique, environnementale et sanitaire d'un Produit Résiduaire Organique (2014) (http://www.rmt-fertilisationetenvironnement.org/moodle/course/view.php?id=72)
- 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.
- Fink M. & Scharpf H.C. (1993). N-expert – a decision support system for vegetable fertilization in the field. Acta Hort. (ISHS) 339:67-74.
- Ravier. C, Jeuffroy. M, Meynard. J, Mismatch between a science -based decision tool and its use : the case of the balance sheet method for nitrogen fertilization in France (2016). Wageningen Journal of Life Sciences 79 (2016) 31-40.

12. Non-committal information with regard to sample taking and conduct of analysis

All samples and analysis conducted within the scope of the Flemish nitrate directive are described (in Dutch) at following location:

https://emis.vito.be/nl/bam-2010

For field trials investigating the short time N – effect of RDF's the following methods are relevant:

Sampling methods	
Soil sampling	BAM deel 1/01
Soil sampling for organic carbon determination	Compendium BOC
Liquid animal manure sampling	BAM deel 3/01
Solid animal manure sampling	BAM deel 4/01

Analysis on soil		
Sample preparation	BAM deel 1/02	ISO 14464
Moisture content	BAM deel 1/03	ISO 11465
Nitrate	BAM deel 1/04	ISO 14256-1 / ISO 14256-2
Ammonium	BAM deel 1/07	ISO 14256-1 / ISO 14256-2
Organic carbon	BAM deel 1/10 / Compendium BOC	ISO 10694
pH (KCl)	Compendium BOC	ISO 10390
Phosphate (ammonium – lactate extraction)	BAM deel 1/11	ISO 15168-1/ ISO 15168-2/ ISO 6878/ ISO 11885
Analysis on liquid animal manure		
Sample preparation	BAM deel 3/02	NEN 7430
Dry matter content	BAM deel 3/03	NEN 7432
Total P content	BAM deel 3/04	ISO 11885
Ammonium	BAM deel 3/05	ISO 11732 / ISO 7150-1 / NEN 6604
Total N content	BAM deel 3/06	NEN 7437
Analysis on solid animal manure		
Sample preparation	BAM deel 4/02	NEN 7431
Dry matter content	BAM deel 4/03	NEN 7432
Total P content	BAM deel 4/04	ISO 11885

Ammonium	BAM deel 4/05	ISO 11732/ ISO 7150-1/ NEN 6604
Total N content	BAM deel 4/06	NEN 7437

Information with regard to sample collection and analysis of samples as conducted by Arvalis in France can be found in the 'Guide méthodologique Réseau PRO' to be found at the following location.

http://www.rmt-fertilisationetenvironnement.org/moodle/course/view.php?id=72

13. List of abbreviations

RDF = Recycling-derived fertilizer

NFRV (Keq in France) = Nitrogen Fertilizer replacement value

Keq = Coefficient d'équivalence azote

ANR (CAU in France) = Apparent nitrogen recovery

CAU = Coefficient apparent d'utilisation

N_{min} = Mineral Nitrogen

R_i = Mineral N content in the soil profile (accessible to plant roots at harvest) before applying fertilizer

N_{irr} = N from irrigation water

N_{dep} = N from atmospheric deposition

M_{tot} = Total mineralisation ($M_{tot} = M_{cr} + M_{cc} + M_{so} + M_{o(y-1)} + M_o$)

M_s = Total N – mineralisation from soil ($M_s = M_{cr} + M_{cc} + M_{so} + M_{o(y-1)}$)

M_{cr} = N-mineralisation from crop residues

M_{cc} = N-mineralisation from catch crops

M_{so} = N-mineralisation from soil organic matter

$M_{o(y-1)}$ = N-mineralisation from organic fertilizer applied the year before trial conduct

M_o = N-mineralisation from organic fertilizer applied shortly before planting/sowing

F_m = Mineral fertilizer

P_f = Total plant uptake in aboveground biomass (P_f = target gross yield (y) x N requirement per unit of production (b))

P_r = Total plant uptake in underground biomass

R_e = Mineral N content in the soil profile accessible to plant roots at harvest.

N_v = Volatilization of ammonia

N_l = Nitrogen losses due to leaching

N_r = Nitrogen losses due to runoff

N_d = N losses due to denitrification

N_i = N losses due to immobilization