VIRTUAL TRAINING IN THE USE OF ^{15}N IN DATE PALM

Enhancing Nutrient Use Efficiency using ^{15}N Fertilizer Studies

(Part I of II)

17 August 2020, 11:00-13:00 Vienna time (CET)

Part I

SOME FUNDAMENTALS OF ^{15}N STUDIES

1. **Introduction**

Nitrogen is the foremost of inorganic fertilizers used to enhance growth and production of crops. Nitrogenous fertilizers are expensive and account for 30-40% of cost of cultivation. Its gaseous oxides produced from interaction with soil are green house gases while soluble nitrates lead to pollution of ground waters and therefore of ecological consequences. The use efficiency among crops is 20-50% and there is an ample scope to enhance it by refining fertilizer N management. The use of ^{15}N provides a direct and simple method of developing ways of improving N use efficiency.

Urea [$CO(NH_2)_2$, 46%N] is the most widely used N fertilizer. When applied to soil, it hydrolyses to NH_4^+ and CO_2 in the presence of moisture and urease enzyme. The part of NH_4^+ not adsorbed by cation exchange complex of the soil or absorbed by the plant roots is subjected to loss as gaseous ammonia from moist soil surface or is nitrified to soluble NO_3^- by nitrifying bacteria and leached underground. These transformations in the soil have to be managed by proper time and depth of placement so as to minimize losses and improve N use efficiency during crop growth.

^{15}N is a heavy and stable isotope that is identical to the naturally abundant ^{14}N and safe for researchers as well as to the crop plant since the isotope does not emit any radiation. It is biologically and chemically identical to ^{14}N and is free from isotope effect normally associated with radioactive isotopes and from priming effect, significant is herbaceous crops (insignificant in large plants like date palm tree). ^{15}N is physically heavier than ^{14}N and it can be measured accurately and precisely using mass spectrometry even in very small amounts. Enrichment of nitrogenous fertilizers with ^{15}N is achieved during production of ammonia from atmospheric N by Haber's process for manufacture of nitrogenous fertilizers.

When the plant is confronted with both soil and fertilizer sources of N, the use of ^{15}N fertilizer source does not cause any quantitative discrepancy in the measure the relative N use by the crop. The isotope dilution principle propounded by G. Hevesy and R. Hobbie (1932): "The dilution of the radioactive isotope by its non-radioactive counterpart results in the reduction of specific activity, defined as the activity per unit volume or mass, in a conserved manner proportional to the original specific activity and the amount of the analyte" becomes applicable. The words corresponding to radioactive isotope, however, have to be adopted for stable isotope and its atom percent excess. In other words, the presence of naturally present soil N (^{14}N) does not interfere with the absorption of fertilizer (^{15}N) and later measurements. This is a robust assumption that stands scrutiny in fertilizer use investigations.

1.2. N use efficiency studies:

Quantitative approaches of assessing an optimal N rate are usually based on fertilizer response trials in the area of interest. Rates of application are then a function of: (i) N uptake of the crop to give a desired yield level, (ii) the N supplied by the soil, and (iii) recovery of the applied fertilizer N or N use efficiency.

Alternatively, easiest target of supplying the crop need of N would be to replenish the N removed by harvested produce. This approach, however, does not take into account current stocks of available N and, depending on the supply of soil N, the applied fertilizer N may be used with varying levels of efficiency. Moreover, if a nutrient balance study indicates a deficit (i.e. an overall removal of the nutrient of interest), then simply supplying that amount of nutrient in the form of mineral fertilizer will not lead to a balanced nutrient budget. Particularly in the case of N, applied fertilizer is subject to loss, particularly through leaching. In perennial fruit crops, the permanent frame work of the tree, acts as the buffer between the active current season growth (CSG consisting of foliage, flowers and fruits) and the roots. It is evident that CSG depends for energy and nutrient needs almost completely on the former to the extent that such depletion will have to be made good by uptake of soil/ fertilizer nutrient absorption by roots subsequently. While such a cycle may be perpetual in fruit crops, the exact role of this phenomenon needs to be studied and understood to aid overall nutrient requirement in general and of N requirement in particular. The basic approach of an efficient fertilizer practice would be to apply appropriate quantities of less mobile nutrients (P, K and S) at or soon after planting with small quantities of N and then applying the rest of N as top-dressing to suit the crop needs.

1.3. Measurement of Fertilizer N Use Efficiency

The application of fertilizer N and the efficiency of its use by a crop require the consideration of several distinct criteria. Basic definitions related to N and N use efficiency is provided below.

1.3.1. Agronomic efficiency

The agronomic efficiency (AEN) is the amount of harvestable product, i.e. kg of cereal grain, potato tubers, tomato fruit, etc., per kg of applied nutrient (N). Most fertilizer studies focus on this parameter. When determined at various levels of application, the values are called fertilizer–response ratios and are used to evaluate crop response to fertilizer application and the profitability of fertilizer use. This, the classical method for evaluating fertilizer use, is defined by the following equation:

$$\text{AEN (kg produce/kg N)} = Y_N - Y_0 / F_N$$

where Y_N and Y_0 are crop yields (kg/ha) at a certain level of fertilizer application (F kg/ha) and in the control treatment, respectively.

1.3.2. Uptake efficiency

The N uptake efficiency (UEN) is the total amount of N absorbed (including that present in the roots, often disregarded) per kg of applied N. This eco-physiological parameter, also referred to as recovery efficiency (REN), is defined by the equation:

$$UEN\ (kg\ N/\ kg\ N) = U_N - U_0/F_N$$

where U_N and U_0 are total plant N uptake (kg/ha) in the above ground biomass at a certain level of fertilizer application (F_N kg/ha), and in the control, respectively. It depends largely on the synchrony between plant N demand and the quantities of N supplied by the fertilizer and by the soil. Consequently, REN is strongly affected by N management methods as well as by crop management practices (e.g. genotype, tillage, water supply).

1.3.3. Plant nitrogen use efficiency

This is the total dry matter or grain yield produced per unit of N absorbed. This physiological parameter, also called physiological efficiency (PEN), is defined as:

$$PEN\ (kg\ produce/\ kg\ N) = Y_N - Y_0\ /\ U_N - U_0$$

The PEN represents the ability of a plant to transform a given amount of acquired fertilizer N into produce and thus depends on genotype characteristics (e.g. harvest index) and environmental and management factors, particularly during reproductive growth. Low PEN usually suggests suboptimal growth conditions, often caused by nutrient deficiencies other than N and/or by drought stress, insect predation and disease. Plant N use efficiency is related to the other parameters as follows:

$$AEN = PEN \times UEN$$

1.3.4. Fertilizer nitrogen use efficiency

Fertilizer N use efficiency (FNUE) is the amount of fertilizer N taken up by the plant per kg of N applied as fertilizer. It is often expressed as a percentage and termed “fertilizer N plant recovery” or “coefficient of utilization.”

1.4. Parameter relationships

These parameters are interrelated. However, the selection of one or more of them for the evaluation of fertilizer N and overall N use efficiency depends on the objectives of the study. In the present guidelines, the main focus is on the use of isotopic N tracers, in particular the stable isotope ^{15}N, to measure and improve FNUE. The full concept of FNUE is broader and more complex than the simple recovery of the fertilizer N by a crop, since it involves: (i) maximizing plant uptake of the applied fertilizer N; (ii) minimizing losses of fertilizer N from the soil; (iii) maximizing plant system; and (iv) providing/promoting favourable conditions (soil, climate, plant, management, water, etc.) preventing applied N from becoming unavailable to the crop and the cropping system as a whole.

Fertilizer N research has traditionally been concerned with the first aspect, namely obtaining economic yield responses from the crop of interest. Recent research has focused on the second and third aspects, because financial support has been allocated primarily to environmentally oriented projects, in particular in developed countries. However, an integrated approach to all these aspects of fertilizer N management is needed at the cropping system level in selected agro-ecological zones.

1.5. Measurement methods

Agronomic approaches have traditionally involved the examination of the value of interventions such as fertilizer management practices (timing, placement, fertilizer sources, etc.) and soil/crop management on the uptake of the applied fertilizer N by a crop during a single growing season. Estimates of fertilizer N uptake (FNU) can be made by the non-isotopic difference method as well as by the isotopic method.

1.5.1. Isotopic method

The isotopic method is the only direct means of measuring N uptake from applied fertilizer. The recovery data are known as the 'real coefficient of utilization'. The more or less constant ^{14}N:^{15}N ratio in the atmosphere and natural substances makes it possible to use N products artificially enriched in ^{15}N as tracers in ecological systems. Thus, much research work on N in the recent past has used the stable isotope ^{15}N.

The following studies related to FNUE have been done using ^{15}N:

— FNUE by annual crops worldwide in a number of environments;
— FNUE by perennial pastures and by fruit and plantation trees;
— FNUE by a crop sequence or the same crop over a number of years;
— Fate of applied fertilizer N or fertilizer N balance/budget in crop and soil, to assess the unaccounted for fraction considered as loss;
— Evaluation of amount and movement of water and associated nitrate leaching;
— Assessment of gaseous N losses (denitrification, ammonia volatilization);
— Interactions with other N sources (soil N, organic sources, biological nitrogen fixation, etc.);
— Fertilizer and soil-N cycling in cropping/farming systems.

1.6. ^{15}Nitrogen techniques in fertilizer nitrogen use efficiency studies

^{15}Nitrogen techniques, though relatively expensive, usually provide results that have lower variability and are of higher sensitivity, resulting in more precise information in a shorter period of time. In addition, their use and applications require scientific and technical staff with adequate skills and expertise, adequate financial resources and functional laboratory facilities to properly conduct the experiments, perform the isotope measurements and interpret the results.

1.6.1. Direct approach

In this case, the fertilizer is labelled with ^{15}N. This requires a commercial source of high quality ^{15}N-labelled fertilizers, i.e. uniform labelling. Thus, direct quantitative measurements of ^{15}N and total N contents can be made in selected samples, usually plant material. These measurements can also be made for the soil mass (solid phase), soil solution (liquid phase) or soil atmosphere (gaseous phase), depending on the type of study. The following types of FNUE study are possible using the isotope method:

— Fertilizer N management practices (timing, placement, sources);
— Genotypic differences in uptake and use efficiency of N;

— Interactions of fertilizer N with crop/water/soil management variables (irrigation, tillage, cropping system, plant population, soil amendments, plant spacing, etc.);
— Fertilizer N balance/budget determinations (accounted/unaccounted for);
— Direct measurement of labelled fertilizer N losses (leaching/gaseous losses).

In all of these studies, the main goal is to improve FNUE through several approaches. In a first instance, the initial objective is to measure the uptake of the applied fertilizer N and to determine the actual level of FNUE in the system in order to devise ways and means of improvement. Thus, the main goal is to supply N as fertilizer to the plant in the correct amount at the right time and place, matching the crop's needs. The ^{15}N labelled single-treatment fertility design is a variant of the direct approach to measure FNUE without plant–fertilizer interaction.

The significance of changes in climatic conditions over time needs to be taken into account when FNUE studies are conducted in multi-location trials in selected agro-ecological zones and cropping systems to further undertake fertilizer N budget studies by measuring inputs and outputs (losses) and to develop appropriate strategies to improve FNUE in the context of ARASIA.

1.7. Conclusion

Experiments with ^{15}N labelled fertilizers, using either the direct approach, provide precise and quantitative data on the efficiency of use, residual effect, movement and transformation of fertilizer N. The use of the isotopic method provides a direct and quick means to obtain the needed information, resulting in higher economic return. This information is valuable both for the design of better fertilizer N strategies and for the provision of sound recommendations for the application of fertilizer N.

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Part II of II

18 August 2020, 11:00-13:00 Vienna time (CET)

Field Experimentation Techniques for the ^{15}Nitrogen Method

2. Introduction

Isotope aided studies involve the use of labelled materials as tracers for quantitative determination of the fate of specific nutrient elements in a specific component or the whole soil–plant system. In the case of N, the isotope ^{15}N can be traced in each or all of the components of the soil–plant system, such as plants (and their parts), soil mass (soil phase), soil solution (liquid phase) or soil gas (gaseous phase), depending on the objectives of the study.

At the planning stage it is advisable that the research team establish connections with appropriate research institutions, extensions and farmers' associations/communities to ensure broad input on the definition of the problems to be solved, gathering of background information, and implementation of the studies and dissemination of the results to beneficiaries and end users. Experience gained in developing countries through FAO/IAEA Technical Cooperation Projects has demonstrated that the best results are obtained when collaborative agreements are established between specialized groups of national agricultural institutes/universities and nuclear experts from atomic energy institutions. Similarly, in FAO/IAEA Coordinated Research Projects, such collaboration and networking is established between agricultural groups of developing and industrialized countries to promote the sharing of knowledge and exchange of experience enhancing synergies to develop new technologies.

Since the isotopic method is normally complementary to conventional or classical methods in agricultural investigations, the research team should ideally consist of scientists not only trained in the use of the method but also skilled and experienced in field experimentation. When conducting isotope aided fertilizer studies, first we must consider the current status of implementation of a national, regional or local fertilizer programme.

Although it is known that isotopic techniques are a powerful tool in agricultural research, in deciding to use them to full advantage, one must consider if the following criteria are met: (i) the isotopic method is the only way to solve a particular question or to obtain a particular piece of information, and (ii) if other methods are available, the isotopic method is a quick and cost effective means to obtain the needed information.

In the context of fertilizer studies, it is essential to determine first when and where the isotope method will be applied during the experimentation phase. Therefore, correct application of the ^{15}N techniques is absolutely necessary to obtain high quality data and the valuable information desired. This, in turn, demands that adequate field experimentation techniques (field experiment layout, plot design, application of N labelled products, chemical and isotopic analyses, data calculations, etc.) be utilized.

2.1. Experimental guidelines

Detailed planning of an experiment using isotopically labelled fertilizers should include preparation of an experimental guideline, which, after review by the research team, is distributed among all staff. Main points to be taken into account in planning and implementing any isotope aided FNUE experiment are the following:

Aspect highlighted	Action to be taken
Identify the specific fertilizer-N related problem(s) to be studied, define the topic(s) of research and establish priorities of the work to be done. These aspects should be reflected in the title of the study.	Done already by KISR; please revise as needed.
Compile background information (recent and relevant work) on the topic(s) from the scientific literature,	The role of isotopic technique in the study needs to be highlighted. ^{15}N use gives direct, accurate and undisputable

databases, reports, etc. Perform a bibliographic search, utilizing key words from the above sources. In particular, define the role of the isotopic techniques in the study. Prepare a list of selected key references.	evidence. It also obviates long-term experimentation.
Define the objective(s) of the experiment/question(s) to be answered/hypotheses to be tested. A golden rule is to design simple experiments with concrete and well defined objectives.	Revise as needed.
Define type and location of the experiment(s): Establish the experimental treatments and select the appropriate experimental design, replicates, statistical analysis (software), and tests for comparisons of means and error estimates. Determine plot layout design, greenhouse, on-station, farmer's field, sequence.	Done already at KISR; please revise as needed for other Centres.
Estimate the approximate amount of ^{15}N product(s) and enrichment.	To be done. Pilot study is suggested to be taken up at KISR and this holds key to all the aspects of the left-hand-column. The recovery of the isotope in the target tissues of date palm is adequate to achieve the objective. Other experimental details of different Centres can then be standardized in the pilot study. This will mean: one year of initial work need be taken up at KISR which is suggested to act as the Nodal Agency for ARASIA. Based on the evaluation of the pilot study the overall programme
Define sampling/harvesting times and procedures. Estimate the total number of samples to be analysed.	
Analytical methods, laboratory standards, quality control, data reporting.	
Calculation of data and selection of the evaluation parameters in relation to the objectives of the study.	
Compile all information and develop guidelines for the experiment.	
Assess the resources (physical, human and financial) needed to conduct the	

experiment, including a budget and sharing among institutions.	has to be finalized for the total network project. It is also suggested that tracking the ^{15}N presence in date palm plant as a means of studying the exact role of permanent plant part in the overall N dynamics may be confined to KISR only. This valuable information will serve the crop true in all other locations.
Revise the draft experimental guidelines (working copy) with relevant staff members.	
Distribute the final experimental guidelines among all participating staff.	

2.2. Practical experimental procedures

2.2.1. Experimental site

Select a representative location for the problem/topic to be studied and the predominant cropping system in the agro-ecological zone of interest. In a regional or national programme, multilocational trials are established to obtain information on FNUE. This is normally done through the conduct of ʼon-stationʻ and ʼon-farmʻ trials, to avoid significant ʼyield gapsʻ due to differences in soil fertility and management practices between experimental stations and farmers’ fields, and to facilitate the transfer of the generated technologies to beneficiaries and end users.

The normal approach is to proceed stepwise, starting with detailed on-station experiments followed by on-farm field trials with a simplified experimental design (reduced experimental treatments, with each farmer considered as one replication within a particular location) with farmer participation to facilitate adoption. Sometimes, when time is too short to generate fertilizer recommendations, both can be performed simultaneously; however, this requires more resources, and tighter control and supervision on the part of the local staff.

2.2.2. Treatments and experimental design

The experimental design should be established in direct relation to the objectives of the study. The number of treatments and replications and the statistical design are a function of each experiment. The final decision on the total number of experimental units should be based both on technical and economic considerations.

Basic principles of statistical analysis and biometrics should be considered in selecting the appropriate experimental design. Past experimental plans of cooperative research projects on FNUE included a core of 4–6 mandatory treatments (common to all investigators participating in the project) and 2–4 additional treatments (as an option of each investigator to address local factors/issues). Randomized block arrangements with 4–6 replications per treatment were the most commonly used statistical design. Also, the split-plot design has been used in several FNUE experiments. Select appropriate software for statistical analyses (ANOVA) and tests for comparisons of means. A statistician's advice may be useful.

2.2.3. Plot layout

In isotope aided experiments, two types of plot are required: (i) yield (control) and (ii) isotope plots. The plot layout depends on the plant species/variety and the cropping system. Isotope plots are the smallest possible area (usually called microplots) to obtain a representative sample for isotope enrichment measurements while reducing the amount of isotope utilized due to its cost. Microplot in some cases may have just one plant (e.g. a tree in the lysimeter). Yield (control) plots must be sufficiently large to obtain precise information on yield parameters (total biomass and economic crop yield) and for other additional observations (crop growth measurements, physiological parameters, soil water measurements, plant and soil samplings, etc.) to be made throughout the growth cycle of the crop.

2.2.4. Field layout

The experimental layout or the spatial arrangement of the plots (yield and isotope plots) depends to a large extent on the objective of the experiment and on local field conditions (available area, field orientation, slope conditions, etc.). A common approach is to have main plots, subdivided for yield, and isotope subplots with lysimeters each located adjacently or within it.

Preparation of diagrams illustrating the field and plot layout is the key for conducting field observations by a research team and for ensuring efficient establishment, maintenance, sampling and harvesting operations by the field staff.

2.2.5. Requirements for ^{15}N labelled materials

In most FNUE experiments to define the best fertilizer management practices or fertilizer-N balance studies using the isotope method, only one rate of application of fertilizer N is utilized, namely that normally recommended to obtain optimum yield. Where split applications are recommended, that single rate of application, for instance 100 kg N/ha, can be divided into two (50 kg N/ha), three (33 kg N/ha) or four (25 kg N/ha) equal fractions to be applied at selected stages in the growth cycle to study the time course of N uptake (and recovery) by the crop. In other cases, the splitting can also be made in different amounts to best match crop needs, depending on the objectives of the study and the local conditions.

In some experiments where interactions between fertilizer N and other factors are being studied — irrigation, planting density, tillage, residual effects, etc. — different N applications may be utilized, including the recommended rate as intermediate.

When utilizing the direct approach, a control or check treatment (without fertilizer N application) is, in principle, not needed because fertilizer N uptake is measured directly using the ^{15}N labelled fertilizer. However, in some cases, a control is included in order to gather additional information (e.g. comparing difference-method data) and for economic evaluations.

The amount of ^{15}N applied as fertilizer must be sufficient to be detected eventually in the plant samples collected. It depends both on the rate of application and the enrichment (^{15}N% atom excess) of the labelled fertilizer/material used and is determined by several factors such as the objective of the study, type of crop, duration of the experiment, and primarily the available equipment for measuring the N–isotope ratio.

As a general guideline, fertilizer N studies (fruit/plantation trees like date palm) may mean 100 kg N/ha dosage that may require 1, 2 or 5 atom % ^{15}N excess.

Pilot study will take care of the suitable ^{15}N atom % excess needed as the proposed project is first of its kind study on date palm.

2.2.6. Calculation of requirements for ^{15}N labelled fertilizer

It should be noted that isotopically labelled fertilizers are chemically pure compounds and not simple commercial fertilizers. Thus, calculations must be made following basic N isotope terminology and stoichiometry.

2.2.7. Calculations of ^{15}N labelled fertilizer requirements

Following the guidelines above, the total ^{15}N labelled fertilizer requirements can be calculated from total number of lysimeters or experimental plants/ treatment units (number of trees/treatment) required for the experiment. With this information and number of replications in the experiment it is possible to further estimate the ^{15}N requirements and make a cost estimate based on recent bid quotations from commercial suppliers.

2.2.8. Application of the ^{15}N labelled materials

The application of ^{15}N labelled materials in the field has a profound influence on sampling procedures and experimental results. The procedures should be described in detail when publishing the data.

When utilizing the direct approach for FNUE studies, the ^{15}N should be applied in a form that reflects the standard practice to be tested (e.g. solid fertilizer). Therefore, in order to draw conclusions about fertilizer N uptake and recovery, the isotopically labelled fertilizer should be chemically (carrier) and physically (form) identical to the commercial fertilizer. Most fertilizers are applied as homogeneously as possible, on per row or per plot basis, in solid, dry form, broadcast or banded, with or without incorporation. Other application practices are left up to the research team for the development of fertilizer management practices.

2.2.9. Field observations

Field visits should be made regularly, to follow the development of the crop and any differences among treatments. The experimental field book should be kept up to date, with detailed records of experimental designs and procedures, crop observations, planting, cultural practices and applications, weed and pest control, crop growth, changes in climatic conditions, etc. Details of harvesting and sampling procedures should also be recorded.

2.2.10. Harvesting and sampling

This activity, though laborious and time consuming, is critical to the validity of the ^{15}N recovery data. It is necessary to plan in detail the sampling strategy, considering the objectives of the experiment and the parameters of evaluation.

Most FNUE studies include plant samplings for quantitative estimates of plant recovery of fertilizer N and to compare fertilizer management practices. Therefore, times should be set for sampling, to follow biomass produced and the total amount of nutrient taken up during the course of the experiment. Sometimes several sampling harvests may be necessary within a single growth/yield cycle of a crop.

During a single-season experiment, the final harvest of the isotope plots should not be later than physiological maturity, to minimize leaf shedding, seed shattering and other physiological phenomena of advanced maturity, which greatly increase the experimental error. Remember that harvestable products (grain, root, tubers, etc.) should be collected from the yield plots at full maturity.

The harvesting procedure consists of gathering all above ground plant material in the lysimeter or treatment plant/s of the isotope plot and treating it as a sample. Avoid contamination of plant samples with labelled soil.

In studies of fertilizer N balance sheets, roots and soil must be sampled along with plants. The roots must be washed carefully. Some practical considerations for plant harvesting and sampling techniques are: (i) Plan detailed harvesting/sampling operations and allocate necessary resources. Whenever possible, visit the field in advance. Similarly, prepare in advance bags, labels and a field book to record results. (ii) Careful labelling and organization of samples per treatment and replication are essential. (iii) Before leaving the field, check that all samples have been collected.

2.2.11. Sample preparation

Sample preparation is an essential step in all isotope aided experiments, but often it is not given enough attention. The ultimate goal is to obtain a representative sample for chemical and isotopic analyses.

The two basic considerations for sub-sampling are:

— The size of the sample required for chemical and isotopic analyses is usually very small (10–1500 mg). However, the amount of harvested plant material is often bulky (several kilograms) and the entire sample is too large for processing.

— When approaching maturity, differences in physical consistency but also non-uniformity in ^{15}N content among plant organs, thus often requiring fractionation or separation into parts (reproductive and vegetative), e.g. tops and roots including the crown to obtain a representative sample . The sequence to be followed in the sampling and sub-sampling procedures may involve stepwise:

- Sample (e.g. plant material);
-Separation into different plant parts;
-Total fresh weight per plant harvested (TFW);
-Cutting 1-3cm;
-Mixing; Quartering;
-Sub-sample;
-Sub-sample fresh weight (SFW);
-Drying at 60 C;
-Sub-sample fresh weight (SDW);
-Grinding sub-sample; and
-Analysis.

Before harvesting an isotope aided experiment, the procedures to be followed must be established taking into account the type of information to be obtained in relation to the objectives of the experiment and the availability of resources (personnel, sample preparation equipment, transportation, funding, analytical facilities, etc.).

In order to reduce the sample size, it is necessary to quarter the chopped sample by saving two opposite quarters. Quartering is repeated until the sample size has decreased to 200–300 g fresh weight. The subsamples are weighed fresh and then placed in a draft circulation oven (for about 24 hours) at 70°C until constant dry weight is reached. The dry weight of the subsample is recorded and the final step is the grinding of the subsample to pass through a 1 mm sieve. It is important that the weights of the total fresh sample (TFW) and its subsample (SFW) be taken within a short period, to avoid significant water loss between these two weighings; otherwise an inaccurate estimate of dry matter yield will be obtained. Particular care should be taken to obtain correct dry matter data when various plant parts (organs) are sub-sampled.

Once the finely ground subsamples have been obtained, it is usually not necessary to analyze them (from plant parts) separately for FNUE studies. On the other hand, scientists must realize that, in the application of the isotopic method, the analytical process is laborious, time consuming and expensive. One should remember that the ultimate goal is to obtain a representative sample for chemical and isotopic analyses and fertilizer-N recovery data. Therefore, re-composing the plant samples of the experimental treatments (and replications) is recommended. This will avoid explaining differences between the replicates that may not be feasible. The 3-4 repetitions of analysis of the same sample may serve as replication. The counting statistics need to be followed as required in counting ^{15}N abundance. Utilizing the ratios of the total dry matter weights of the samples, one can again obtain a composite sample (for each treatment and replication) by mixing the corresponding amounts of the dry subsamples (plant parts). It is important that these two subsamples be dried, finely ground and well mixed (homogenized). These composite samples, duly identified, will be sent to the laboratory for analysis. The remaining materials are retained as spares for additional analyses.

Sample preparation techniques are reported to be the main source of error in isotope aided experiments. The following precautions are necessary to obtain precise analytical data and facilitate the interpretation of results:

— Careful organization of samples during preparation procedures (chopping and grinding) is essential to avoid cross-contamination problems. This is done by starting routine sample processing with the samples of expected lowest ^{15}N enrichment followed by increasing ^{15}N enrichments.

— Proper identification of field samples (treatment, replication and plant part) coming from the harvested plots and of the obtained subsamples, which are sent to the laboratory for chemical and isotopic analyses. All of this information should be entered into the experimental field book. When the subsamples are analyzed elsewhere, the complete information should be provided in duplicate: one report included in the parcel and the other mailed separately to the laboratory rendering the analytical services, together with the plant subsamples and fertilizer standard(s) used in the experiment. Every sample should have proper identification (treatment, replication, plant part). It is the responsibility of the Nodal Officer to prepare the reports, check the parcel contents for correctness and completeness, and to send them to the laboratory for analysis. Past experience with IAEA projects shows that many mistakes can arise if these precautions are neglected.

2.2.12. Total-N and ^{15}N analyses

In this laboratory phase, it is necessary to analyze the plant and soil samples for total N and for the ^{15}N:^{14}N ratio or ^{15}N abundance. All samples collected in the field must be properly codified by treatment and replication. Samples of ^{15}N fertilizer standards and labelled materials (solutions) used in the experiment must also be included.

The ^{15}N abundance (or the stable N isotope ratio, ^{15}N:^{14}N) is determined in N_2 gas generated from the samples, by either mass spectrometry or optical emission spectrometry.

Major established laboratories have their own routine analytical and quality control procedures. It is advisable that all laboratory facilities performing these services participate in the annual inter-comparison exercises organized by the IAEA Seibersdorf Laboratory to ensure compliance with quality standards and the production of good quality analytical data.

2.3. Calculations for experiments with ^{15}N

2.3.1. Basic primary data

For all field and greenhouse experiments with ^{15}N labelled materials, the following basic primary data need to be recorded for each plot and treatment:

— Dry matter yield for the whole plant or subdivided into parts. This parameter is utilized to estimate the amounts of total N uptake, and is determined from the isotope plot. Agronomic yield data are obtained from the corresponding yield plots. Sometimes the dry matter yields are calculated separately by plant part (vegetative and reproductive) and then summed to obtain the total biomass or total dry matter produced by the crop at harvesting time.
— Total N concentration (% total N in dry matter) of the whole plant or plant parts, as in point one. This is done by chemical methods, e.g. Kjeldahl, or by dry combustion (Dumas).
— Plant % ^{15}N abundance, which is determined by emission or mass spectrometry.
— Fertilizer % ^{15}N abundance, which is determined by the same method and equipment as the plant samples.
— ^{15}N labelled fertilizer(s) used and N rate(s) of application.

2.3.2. Quantification of fertilizer nitrogen use efficiency

The first parameter to be determined when studying fertilizer N uptake by the isotopic method is the N in the plant derived from the ^{15}N labelled fertilizer (Ndff). The information to calculate this parameter is obtained from the plant % ^{15}N abundance and fertilizer % ^{15}N abundance data (third and fourth points above). Nitrogen-15 abundance data must be converted into at.% ^{15}N excess by subtracting the natural abundance (0.3663 at.% ^{15}N) from the % ^{15}N abundance of the sample. Next, a series of calculations is made, as shown below.

The following calculations are needed to estimate FNUE in field experiments with ^{15}N labelled fertilizers:

Nitrogen derived from the fertilizer (Ndff) and from the soil (Ndfs): isotopic parameters:

$$\%Ndff = \text{atom } \%\ ^{15}N \text{ excess plant} / \text{atom } \%\ ^{15}N \text{ excess} \times 100$$

$$\%Ndfs = 100 - \%Ndff$$

Biomass produced or dry matter yield per hectare (kg/ha):

$$\text{Dry matter yield} = [\text{Fresh weight (kg)} \times 10{,}000 m^2/ha)] / \text{Harvested area} (m^2) \times [SDW/ SFW]$$

where FW is the fresh weight of the harvested area and SFW and SDW are the subsample fresh and dry weights (in kg or g), respectively,

Total N uptake or N yield (kg/ha):

$$\text{N yield} = (\text{Dry matter yield (kg/ha)} \times \%\ \text{total N}) / 100$$

Fertilizer N uptake or fertilizer N yield (kg/ha):

$$\text{Fertilizer N yield (FNU)} = (\text{N yield (kg/ha)} \times \%Ndff) / 100$$

Fertilizer N use efficiency (FNUE); fertilizer N recovery; real coefficient of utilization:

$$\%FNUE = (\text{Fertilizer}-\text{N yield} / \text{Applied N rate}) \times 100$$

2.3.3. Calculation exercises for experiments with nitrogen-15:

To illustrate the calculations to be made and the potential of using ^{15}N techniques, examples utilizing actual data from N fertilization experiments are presented below.

2.3.3.1. Example 1

Greenhouse experiment

In pots containing 2 kg soil, 100 mg N/kg as ammonium sulphate (1.39% 15N abundance) was applied to flooded rice. At harvesting, the plant dry matter yield per pot was 14 g and the plant samples had 0.70% 15N abundance and 2.2% total N.

Questions

(1) What fraction of N in the plant was derived from the fertilizer?
(2) What fraction of N in the plant was derived from the soil?
(3) What was the total N uptake or yield of the crop?
(4) What was the fertilizer N uptake?
(5) What was the FNUE or recovery by the crop?

Calculations and results (see fuller explanation in Example 2)

at. % ^{15}N excess plant = 0.70 – 0.37 = 0.33

at. % ^{15}N excess fertilizer = 1.39 – 0.37 = 1.02

(1) Ndff = 0.33/1.02 = 0.324 or %Ndff = 32.4

(2) Ndfs = 1 – 0.324 = 0.676 or %Ndfs = 67.6

(3) Total N uptake or yield of the crop = $(14 \times 2.2)/100 = 0.308$g

(4) Fertilizer N uptake by the crop = $(0.308 \times 32.4) / 100 =$ = 0.1 g or 100 mg

(5) FNUE or % recovery by the crop = $(100 / 200) \times 100 = 50$

2.3.3.2. Example 2

In a field experiment, 80 kg N/ha was applied as labelled urea (1.37% ^{15}N abundance) to a maize crop. Plants were harvested at tasseling. Dry matter yield was 4000 kg/ha, and the plant samples had 0.67% ^{15}N abundance and 3% total N.

Questions

(1) What fraction of N in the plant was derived from the fertilizer?
(2) What fraction of N in the plant was derived from the soil?
(3) What was the total N uptake or yield of the crop?
(4) What was the fertilizer N uptake? (5) What was the FNUE or recovery by the crop?

Calculations and results

$$\text{at. \% }^{15}\text{N excess plant} = 0.67 – 0.37 = 0.30$$

$$\text{at. \% }^{15}\text{N excess fertilizer} = 1.37 – 0.37 = 1.00$$

(1) %N derived from the fertilizer:

$$\%\text{Ndff} = 0.30/1.00 \times 100 = 30$$

(2) %N derived from the soil: Since the crop had only two sources of nutrients, the %N derived from the soil is obtained by difference as follows:

$$\%\ \text{Ndfs} = 100 – \%\text{Ndff and}$$

$$100 – 30 = 70\%$$

(3) N yield of the crop: The total amount of N contained in the crop during the experimental period is obtained by recording the dry matter yield and multiplying it by the % total N in the crop as follows:

$$(4000 \times 100) / 3 = 120 \text{ kg N/ha}$$

(4) Fertilizer N uptake by the crop: The amount of fertilizer N taken up by the crop is calculated by multiplying the total N yield by the fraction of Ndff:

$$120 \times (100 / 30) = 36 \text{ kg N/ha}$$

(5) Fertilizer N use efficiency or recovery by the crop: The fraction of the fertilizer nutrient taken up by the plant in relation to the rate of fertilizer nutrient applied is commonly expressed as a percentage:

$$\text{FNUE} = (36 /80) \times 100 = 45\%$$

2.3.3.3. Example 3

In a field experiment, 60 kg N/ha as ^{15}N labelled ammonium sulphate was applied to hybrid sorghum. The ^{15}N treated plots were harvested at the milk stage of grain development. The harvest consisted of gathering all above ground material in the harvesting area of the isotope plots and separating it into shoots and panicles.

Fresh weights of both components were recorded. Adequate subsamples were taken, and chemical and isotopic analyses were performed on each subsample separately.

Question

What was the fertilizer N utilization of sorghum?

Calculations

As shown in Table below, the total N uptake and fertilizer N yield of each plant part have to be calculated separately. Thereafter, the data from the plant parts are summed to obtain the total N uptake or yield and total fertilizer N yield for the entire crop.

The next step is to estimate by back-calculation a weighted average %Ndff for the entire crop:

$$\%Ndff = (25.5 / 106) \times 100 = 24$$

CALCULATION SHEET FOR FERTILIZER NITROGEN USE EFFICIENCY

Plant part	Dry matter yield (t/ha)	Total N (%)	N yield or uptake (kg/ha)	Ndff (%)	Fertilizer N yield (kg/ha)
Shoots	5.0	1.2	60	27	16.4
Panicles	2.2	2.1	46	20	9.10
Total	7.7		106		25.5

Finally, %FNUE is calculated using the total fertilizer N uptake or yield and the rate of fertilizer N application as (25.5 / 60) × 100 = 42.5%

2.4. Factors affecting efficiency and losses: Environmental issues related to N fertilizer application

2.4.1 Fertilizer efficiency

The efficiency of fertilizer sources is usually not very high. The use of ^{15}N labelled fertilizers allows direct quantification of use efficiency and of losses. Plant uptake of fertilizer N is usually less than 50%. An important reason for the low efficiency is loss of the applied fertilizer from the plant–soil system. In order to minimize these losses, all effort should be made to increase the fertilizer N efficiency.

2.4.2. Factors affecting and measures to increase fertilizer nitrogen efficiency

Fertilizer N efficiency depends on type and amount of fertilizer, mode of application, and soil and crop characteristics as well as weather conditions. It is generally accepted that fractional recovery decreases with increasing fertilizer N rate because of increased chances of N loss through runoff, erosion, leaching and gaseous emissions. These loss processes mainly depend on soil, climate and agricultural practices; a number of measures can be taken to minimize them and to increase N use efficiency:

— no excess inorganic or organic N fertilizer should be applied, and excess mineral N should be avoided during fallow periods;
— nitrogen fertilization should be synchronized with plant needs.

In practice, these conditions can be fulfilled through:
— application of fertilizer N at optimal rates, taking into consideration all N sources (applied as well as mineralized);
— when appropriate, fertilization should be split-applied, in order to be timed with the crop needs and development stage (multiple applications); when irrigation is used, there is opportunity to supply fertilizer N along with the irrigation water in accordance with crop requirements.
— avoiding fertilizer application outside the growing period and certainly before a fallow period;

— adjustment of the fertilization plan for conditions whereby unexpected losses might occur (e.g. excessive rainfall) or with deviations from the expected crop development;
— nitrogen uptake by the crop should be fostered by balanced fertilization with other nutrients; application techniques should be as professional as possible (e.g. precision farming, subsurface application, band or point application). For example, deep placement of urea or ammonium-containing fertilizers has long been known to substantially reduce N losses from paddies. Nitrogen loss is retarded both by placement in the reduced zone and by increasing the granule size, which gives a relatively smaller active surface area and a higher NH_4^+ concentration in the micro-site. Also, to avoid excessive ammonia losses and maximize N use efficiency, liquid manure (slurry) should be injected below the soil surface. The use of urease as well as nitrification inhibitors may retard the hydrolysis of urea and regulate nitrate accumulation. Under these conditions, fertilizer use efficiency can be increased and gaseous emissions decreased.

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