OFRA Trial Protocol; January 2015

Background

On 20 July 2013 AGRA confirmed $5.65m funding for a project that will be know as Optimizing Fertilizer Recommendations for Africa (OFRA).

ASHC will be responsible for communications and CABI will be the contract holder for the OFRA project. The University of Nebraska-Lincoln will lead on the research aspects of the program.

In addition the Bill & Melinda Gates Foundation has invited ASHC to apply for a further four years funding. If successful, this will see ASHC working through to 2018. OFRA will facilitate formal and informal collaboration with key initiatives in Africa funded by AGRA, Bill & Melinda Gates Foundation and others.

AGRA's investment in this new project significantly increases the breadth and reach of the ASHC project. The investment also sees the focus moving from four priority countries to 13: Burkina Faso, Ethiopia, Ghana, Kenya, Malawi, Mali, Niger, Nigeria, Mozambique, Rwanda, Tanzania, Uganda and Zambia.

By 2016, fertilizer optimization recommendations will cover the priority cereal-legume cropping systems in these countries. The cereal crops will consist of maize, sorghum, pearl and finger millet, teff and rice. The legumes covered will include beans, groundnuts, soybean, pigeonpea and cowpea. Cassava will also be addressed.

Taking maize as an example, most smallholders in sub-Saharan Africa experience yields of less than 1 tonne per hectare. However, under optimal conditions, yields of over 8 tonnes per hectare are possible.

In Africa, crop yields can often be increased two- to three-fold with very modest levels of fertilizer. This gives smallholders a high net financial return. Optimizing Fertilizer Recommendations for Africa will involve the production of tools and information to allow smallholders to maximize these returns, taking into account the cropping system and other soil management practices.

In sub-Saharan Africa, fertilizer use is very low. It has been estimated that in Africa the average application is around 8 kilograms per hectare per year compared to around 300 kilograms per hectare in the European Union.

OFRA will work with the national agriculture research and extension systems (NARES) to develop better recommendations for efficient and profitable fertilizer use by smallholder farmers in 13 sub-Saharan countries. These recommendations will be within the framework of integrated soil fertility management (ISFM) practices.

In addition to the University of Nebraska Lincoln and national agricultural research systems in 13 sub-Saharan countries, the partnership will be strengthened by links with partners and especially with two initiatives.

The Africa Soil Information Service (AfSIS) has considerable expertise in mapping the soils of sub-Saharan Africa and this information will both be exploited and enhanced through their involvement. OFRA will work with AfSIS to explore how maps and trial data can be integrated to add value and lead to improved recommendations. A science team leader will be appointed using OFRA and AfSIS funds to deliver this work program.

Grameen Foundation works with community knowledge workers. These are locally-based trusted intermediaries with access to information via smartphones, which Grameen develops, to help them provide agronomic information to farmers. They have already received funds to work with OFRA to develop and pilot a mobile application of the Fertilizer Optimization Tool.

The new AGRA-funded Country-level Soil Health Consortiums – currently in 8 countries in East and Southern Africa with the support of IPNI - are also seen as a critical partner in collecting and disseminating information. As the West Africa consortiums come on-stream they will become part of the OFRA project.

In brief the project will:

Improve fertilizer recommendations as part of ISFM

The development of fertilizer recommendation tools within an ISFM framework with local stakeholders for each major agro-ecological zone

Work in partnership with the national research systems

The 13 national agriculture research and extension systems (NARES) will lead activities in their own countries, compiling data from existing research and implementing more field research. Collaborative working between the NARES will ensure they are able to share and make best use of data from similar soil and climate conditions from other countries. OFRA will seek to build capacity to use different tools to improve recommendations. The NARES will play a crucial part in working with national stakeholders through the country-level consortiums to develop a framework in which fertilizer recommendations can be shared.

Collaborate with AfSIS through a jointly funded position to exploit and enhance the soil maps of sub-Saharan Africa

AfSIS soil maps and databases will be used in selection of sites for OFRA field research to ensure appropriate data collection for extrapolation of results across similar agro-ecological zones and with similar soil characteristics.

Improve the use of research data to support fertilizer recommendations

Existing data on fertilizer responses will be supplemented with new data from on-station and on-farm nutrient-response trials (using standard protocols). This will cover the most significant cereal-legume systems in major agro-ecological zones across the 13 countries.
The data will be used to generate fertilizer response curves appropriate for local agro-ecological conditions. These will underpin the agronomic and economic conditions in determining how a farmer should use available finance for fertilizer use for maximizing net returns on the investment.

Improve access to information and communication materials for extension

Drawing on the experience of ASHC and the other partners, a communication strategy will guide development of appropriate materials for different ISFM stakeholders – especially smallholder farmers and their providers of information, including agro-dealers and extension services.
User-friendly decision tools, specific to agro-ecological zones will be adapted to allow farmers and extension staff to determine crop-nutrient-rate combinations that maximize net returns on investment according to financial and agronomic constraints.

In partnership with the Grameen Foundation, a fertilizer optimisation tool will be developed as an application for mobile phone platforms. This app will be piloted in Uganda and government and non-government extension staff will be trained in its use. The application will then be adapted for other participating countries according to their cropping systems and crop response to applied nutrients.

Guides to optimization of fertilizer use will be published. Local capacity to develop information and communication materials will be built in each country – where appropriate, in collaboration with the new AGRA-funded country-level soil health consortiums.

Contents page

1 Introduction

Crop nutrient response functions for major food crops are essential to optimizing fertilizer recommendations for these crops. This requires field research to determine yield with different levels for each nutrient of interest.

This protocol sets out the trials OFRA will conduct to obtain the information needed to determine robust nutrient response functions.

2 Basic definitions and concepts

Block: A full set of treatments and equivalent to one replication. On-farm trials may each have a single block or replication, or, if land is adequate, the trial may be fully replicated as in the case of on-station trials.

Experiment. A test under controlled conditions, often called a trial, to test hypotheses or obtain information on the efficacy of various treatments.

Experimental unit: The lowest level of the experiment to a treatment is applied, e.g. plot or sub-plot.

Experimental design: The plan for grouping experimental units and assigning treatments to them.

Experimental factor: A controlled category of levels or treatments under investigation, e.g. N, P, or farmyard manure. Factors have 2 or more levels set by the experimenter, e.g. 0, 7.5, 15, 22.5 kg/ha P. A tillage factor may have levels of no-till, tie-ridging, or tillage without ridges.

Experimental treatment: A controlled or combination of controlled variables imposed/applied to experimental units by the researcher; e.g. 60N-0P-0K applied to plot 103.

Factor and treatment are words often but erroneously used interchangeably in research. A factor has levels, e.g. an N factor has at least two levels of N. In a factorial set of treatments, there are a minimum of two factors with at least two levels, and the treatments are composed of combinations of these factor levels.

Randomization: The process by which treatments are allocated by chance to experiment units/plots; each treatment has an equal chance to be applied to an experimental unit. The analysis of variance assumes that treatments have been applied randomly. Randomization can also be applied in research siting but more frequently sites should be selected in careful consideration of the research objectives realizing that the unknown climate for the particular season and the climate x site effects result in site-seasons being random.

Replication: This means repetition and the number of complete replications is the number of times a treatment appears in an experiment.

Population: This may be defined as all possible individuals in a specified situation, fields or farmer production situation in a recommendation or extrapolation zone.

Sample: Part of a population drawn to represent the whole population, e.g. treatment applied to an experimental unit, but also plot area harvested for yield determination or soil sample.

Trial site: A research area encompassing the exact land area, delineated by latitude and longitude coordinates, occupied by a trial. A site may have more than one trial addressing more than one crop. A site-season refers to a trial conducted at that site during a given season.

3 Locations and priority crops/systems

To be determined by the national OFRA team with eventual guidance from OFRA-AfSIS using spatial information. The major annual food crops/cropping systems and the major soil units used for the production of these crops within an agro-ecological zone should be addressed. Maize, rice, bean, wheat, cassava, sorghum, pearl millet, finger millet, cowpea, soybean, groundnuts, teff, and pigeon pea.

4 Development of an operational timetable

Determine the HOW, WHEN, WHERE, WHO for each operation early and develop operational plans.

- Site selection (extrapolation potential will eventually be a major consideration)
- Input acquisition and delivery: types and quantities required, deliver to researcher and to project sites
- Weighing of the inputs or calibration of application
- Site characterization will be for the land area occupied by each trial (see data form); soil sampling: see below
- Field/ plot layout, including GPS coordinates
- Land preparation
- Basal fertilizer application
- Planting
- Gapping
- Weeding
- Pesticide application by schedule or as need arises?; purchase and distribute early in ensure availability if and when needed
- Top dressing
- Records of activities and observations in season (see data form); include foliar analysis
- Collection of plot and other data
- Data entry
- Harvesting
- Data sharing

5 Site selection

Sites should represent a large amount of production area in Africa to have good extrapolation value. Sites should not be selected at random but selected to match the OFRA objectives and eventually to-be-defined extrapolation zones.

The trial sites may be at research centers/stations or on-farm. By mid-2014, site selection will consider characteristics of to-be-defined extrapolation zones suggested by AfSIS/GYGA considering logistic and field limitations.

5.1 Logistic limitations to site selection

- Vehicle accessible
- Within 10 km of a 'trials facilitator'
- >50 m and < 5 km from a road
- Site is secure: low risk of animal damage or theft
- Farmer is willingly and known to be reliable and capable
- Suitability for trials of two or more crops (desired; Appendix 6).

5.2 Field limitations

- Land is considered at least moderately productive for the crop of interest
- Field is large and uniform enough to accommodate at least one complete block of a trial with similar soils and past management
- Soil should be within 0.5 of median pH and 15% of clay and sand content of median for the targeted "soil group"
- Field should not be affected by regular disposal of wastes and manure or for other reasons have unusually high nutrient levels, e.g. a former kraal or site of burning
- Field should not have received a total of >5t/ha manure or organic waste (Excludes crop residue produced in the field) in past 2 years
- Field should have been in annual crop production for at least 2 years
- Field should not have conditions likely to render it non-responsive such as no barriers to root growth for 0-90cm depth, rocky or very stony, sand (sometimes justified) or gravel texture, unusually high uncontrollable pest (some cases with Striga) pressure, unless the property is known as typical for the 'soil group'. When OFRA trials are conducted on soils with pH < 5, do a blanket ≥1 t/ha lime application to the whole trial area.

Corners of trial land areas, including corners of single block on-farm trials and all corners of irregularly shaped trials should be geo-referenced. If the block or trial is contained with a simple rectangle, only for geo-referenced points are needed, while more are needed for irregularly shaped and/or dis-jointed trial areas.

6 Community-based facilitators

Quality and efficiency of trial implementation can be greatly improved using local or community based facilitators. These are often young people, farmers, extension workers or others who assist on a part-time basis, are proficient in communicating with the research team as well as with local farmers. They must be able to read and comprehend the protocols, are willing and able to follow your instructions carefully and completely, and are experienced in growing the crops of interest. They assist researchers in the implementation of the trials, especially during their absence and play major roles in planting, supervising and ensuring that farmers carry out planned activities (gap filling, thinning, weeding, pest control and harvesting) on time and as planned. They act as a direct link between the research team and farmers. They organize labor and meetings as needed.

When effectively used local facilitators greatly reduce researcher time and costs for trial implementation. Once they have understood the protocols, they can assume much responsibility, freeing time and resources for the researchers to cover other areas. The facilitations are there with cooperating farmers and can ensure timely land preparation, planting and other operations. They

live near the trials and cooperating farmers and can frequently check trials and keep the researcher team current of trial progress and problems, keep good records of trial activities, and communicate regularly with the cooperating farmers. Facilitators can together with extension and other frontline staff assist in selecting farmers who will successfully implement the trials. One major disadvantage with extension staff is that they often select the same farmers for different projects, with the farmer becoming overwhelmed by the time and farm resources required.

6.1 Training of community-based facilitators

Training of facilitators is commonly in the field such as at the planting or harvest of earlier conducted trials. Learning is much from the researchers' explanations and guidance, and through learn-by doing, while assisting in laying out trials, blocking and avoiding or working around (trees, anthills, burnt charcoal, former kraals, etc.) that disrupt the uniformity of the field, and in conducting other activities.

The facilitators learn the basic practices (e.g. fertilizer placement, planting spacing, etc.) with establishment of initial trials. The research team should take time to explain and go through the trial procedures to ensure that the facilitators understand well the protocol.

6.2 Remuneration of facilitators

Remuneration of facilitators is often according to numbers of trials successfully implemented. Following planting and again after harvest. Within the season, they are paid a monthly supervision fee as agreed between the research team and the facilitators. Payments at planting and harvesting are higher due to the heavy work load, time involved and the need for quality data. High quality work must be required.

7 Site Characterization

Sites will be characterized for the land area of each trial (see Excel record worksheet for site characterization and Appendix 1 and 2). Site characterization includes soil sampling and analysis.

7.1 Plot level soil samples

Obtain composite samples of 3 sub-samples for the 0-20 cm depth from the 0N-0P-0K(or S), the treatment with the second from highest N *or P* rate (e.g. 90N-0P-0K(or S)), and the treatment with the second from highest N and P rate (e.g. 90N-15P-0K(or S)) plots in each block (replication). Heap and use a knife to sub-divide e.g. by coning and quartering, to reduce to about 150 g to when air-dry and process. Therefore with 3 treatments and 3 blocks, nine samples are needed for non-legume and bean trials and six for other legume trials.

7.2 Block level soil samples

Obtain a composite sample of 8 sub-samples. Heap and use a knife to sub-divide, keeping about 400 g to process. Therefore, 3 samples per 3-block trial are needed.

7.3 One sub-soil sample

Collect a composite of 6 sub-samples for the 20-50 cm depth per trial. Heap and use a knife to sub-divide, keeping about 150 g to process. Therefore, one sub-soil sample per trial is needed.

7.4 Reference soil samples

Create a composite sample from soil of the block level 0-20 cm samples, taking about 250 g dry soil from each block-level sample if there are at least three blocks at the site. If there are only one or two blocks at a site, collect enough from the one or two blocks to have about 800 g total before processing. One reference soil sample should represent approximately 10 plot and block soil samples to be analyzed by MIR. In cases of single block on-farm trials, the reference sample can come from up to three trials located in close vicinity on the same farm; the reference sample should not contain soil of or represent more than one farm. After processing, this sample should have about 500 g of soil to be submitted for analyses.

7.5 Manure samples

A sample for each manure source should be analyzed for nutrient content. Analyze manure samples in your NARS laboratory.

7.6 Processing (see Appendix 3)

7.7 Labeling and packaging.

Prepare according to AfSIS specifications and send to Nairobi (see Appendix 3). Most samples will be analyzed by MIR and require only 50 g but the reference sample (one in approximately 10 samples) will require 500 g for analysis by wet chemistry.

8 Experimental design

Crop-nutrient response functions are developed in consideration of variation in response due to agro-ecological zones, soil test properties, previous crop, and yield level. The below treatment combinations allow for at least 4 nutrient levels for the major nutrients. The incomplete factorial design assumes that N is the most limiting nutrient for non-legume crops and common bean, and that P is the next most limiting nutrient for these crops, and then K (another nutrient such as S or Zn if expected to be more limiting than K). See Appendix 4. A diagnostic treatment is included to determine the combined effect of other nutrients once N, P, K is applied.

This gives 16, 14, and 11 fertilizer rate treatments for non-legumes, beans, and other legumes, respectively.

Use randomized complete block designs. Even if there is no indication of gradient, the randomized complete block design is likely to be more efficient than the completely randomized design as the latter compared to the former reduces F-value by only 0.5% for trials of OFRA size.

9 Field Layout

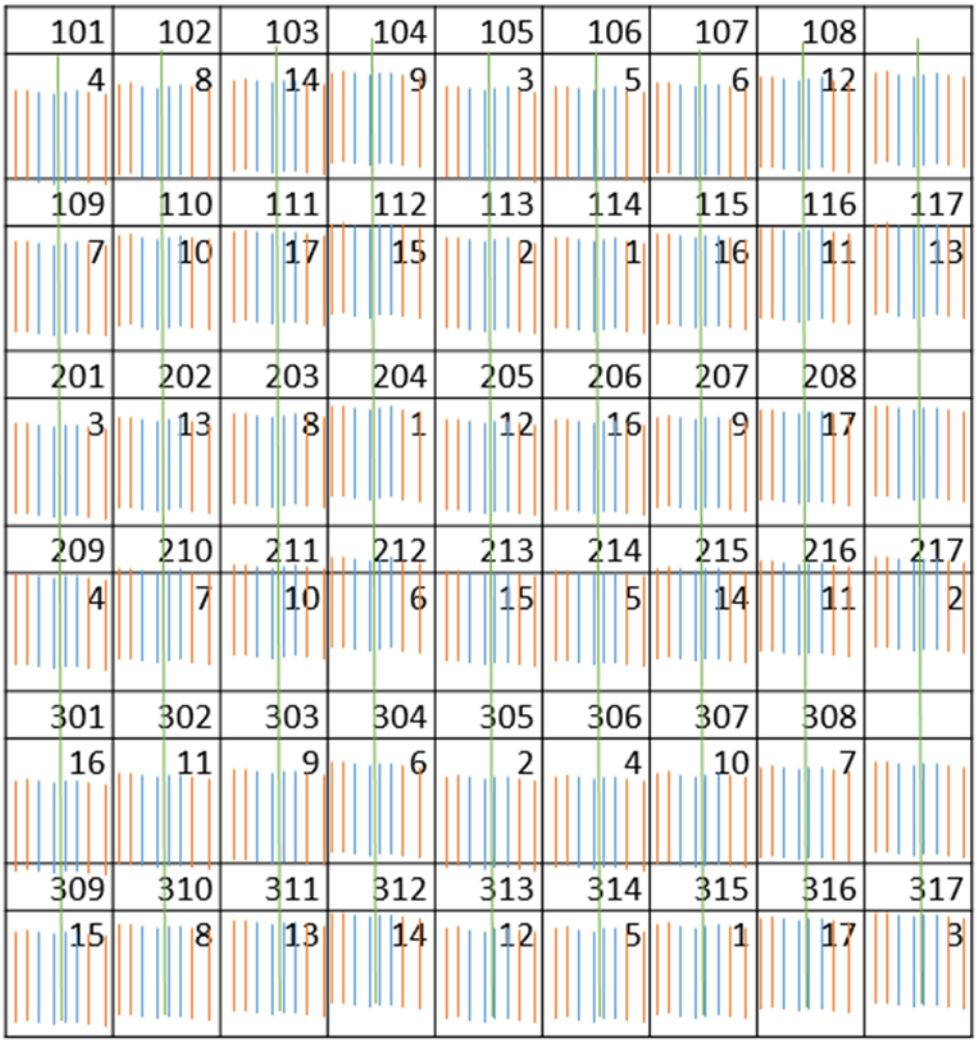
Complete trials will have 3 replications. If on-farm sites are too small for complete trials, conduct single blocks or replication on 5-8 farms.

Plots should be 6 m in length and harvests for yield estimates should be 4 m in length excluding 1-m on ends. Leave 1-m alleys along ends of plots, but preferably with no alleys along sides of plots due to alley effects on microclimate within trials. Enclose all trials with at least 4 border rows or extend the rows beyond the plot area for 2 and 3 m for short and tall statured crops, respectively.

9.1 Maize trials will normally have an OPV and hybrid in a split plot arrangement, without randomized placement of varieties within plots for easier implementation.
Plant maize varieties in 4-row sub-plots with two rows harvested for yield determination, skipping outside two rows. Harvest 4-m length for grain yield.
If only one variety, see for Sorghum and pearl millet below. Maize should be planted according to national recommendations. Trial area, including borders and alleys, is about 0.26 ha for three reps.

9.2 Sorghum and pearl millet trials will have a single variety. Plots should be 6 rows wide and harvest inner 2 rows for grain yield skipping the outside rows. Plant according to national recommendations. Trial area, including borders and alleys, is about 0.2 ha for three reps.

9.3 Rice, finger millet, teff, wheat trials will have a single variety. Plots should be 3 m wide and harvest inside 1-m width for grain yield skipping the outside 1-m areas.
Plant according to national recommendations. Trial area, including borders and alleys, is about 0.15 ha for three reps.



Maize example. 2 varieties; 4 rows of each variety. Plot: 6 m long, 6 m wide. Harvest for yield determination: inside rows (blue) of each variety of 4 m length. The orange rows are buffer rows.

9.4 Cassava trials will have a single variety. Plots can be 4 rows wide if row spacing is ≥1 m, otherwise 6 rows wide, and harvest inner 2 rows for fresh tuber yield skipping the outside 1-rows. Plant according to national recommendations.

9.5 Pulse sole crops should be planted according to national recommendations (e.g. cowpea planted in 50 cm or bean 2 row on 1 m ridges in Zambia) rows.
Plots should be 4 rows wide, except for bean which should be 6 rows wide, and harvest inner 2 rows for grain yield skipping the outside rows. Trial area, including borders and alleys, is about 0.15 ha for three reps.

9.6 Intercropping and manure use response trials. Intercrop and manure use nutrient response functions are expected to be related to sole crop without manure functions. Conduct trials as for the dominant sole crop, e.g. maize or cassava, but add treatments and plots (one maize variety and the 6 m wide plots) with intercrop planting for 5 nutrient rate treatments: 0-0-0, 60-0-0, 60-15-0, 60-15-20, and 90-15-20 treatments or as appropriate for other nutrient increments. Similarly, treatments may be added to compare response with and without manure applied. These treatments will differ for crops/AEZs (see Appendix 7). These additional treatments need to be fully randomized by block with the sole crop treatments.

10 Randomized allocation of treatments, construction of field books, printing of record sheets

We suggest this be done by country project PIs, with assistance of a colleague as needed, using available computer tools such as in SAS, GenStat, Fieldbook, STATBOX, or an Excel example that can be provided. Output should be Excel compatible.

Ideally each replicate should be newly randomized, but at least 12 randomizations should be done for each crop taking care that each rep in a fully replicated trial or each site in a site-season cluster of on-farm trials has a different randomization. Occasionally randomization results in unsatisfactory treatment arrangement such as with the control always in the first plot of the replication; in such cases, re-randomize treatments for one or more blocks.

11 Fertilizer application

Verify the quality of fertilizers to be used. The sources should generally be single nutrient including urea for N, TSP for P, and KCl for K. Sulfur can be from $CaSO_4$, NH_4SO_4 if for non-legumes and urea rates are appropriately adjusted, or elemental S.

The diagnostic treatment will be a blend of secondary and micro-nutrients. Lime at 1 t/ha can be included if soil pH is less than 5.2.

Borax is a good source of B (14.5%).

Greenbelt products are being used in southern Africa: $MgSO_4$ (Kieserite) with 15% Mg and 22% S; Zinc Sulphate Monohydrate = 34% Zn and 18% S; and Boron Granular = 14.5% B.

Therefore, 67 kg/ha of their $MgSO_4$ can supply the 10 kg Mg and 14 kg S.

The diagnostic blend is then composed of 67 parts $MgSO_4$, 3.45 parts Boron Granular and 7.35 parts Zinc Sulphate Monohydrate.

The Mavuno secondary-micro-nutrient product appears satisfactory. If variable quality is suspected, test for both nutrient content as well as solubility, especially for Zn solubility.

The NPK(S) rate with the diagnostic treatment need to be comparable to another treatment. If the third nutrient is S rather than K, include K in the diagnostic package but Mg will need to supplied from a soluble compound other than $MgSO_4$; this is minor issue for $ZnSO_4$ as S supplied is small.

All nutrient application rates are specified in elemental form. Multiply P rate by 2.29 to get P_2O_5 rate and K by 1.2 to get K_2O. See Appendix 4 for the treatment structure and Appendix 5 for plot and replicate level fertilizer requirements for different crop trials.

Basal (pre-plant, planting or post emergence) apply fertilizer nutrients in point or band placement at least 6 cm from the seed as potential for **salt damage** is a concern especially when both N and K applied, and more so with legumes where the tap root may be damaged.

Apply 50% of the N but not more than 30 kg/ha N in the basal application. Top dress application of N can often be done during weeding, but ensure complete coverage with soil.

Cutting an application slot with a ~15cm wide hoe, placing the fertilizer in a 8-cm band to one side of the slot, covering with some soil, and placing the seed to the other side of the slot may be a safe and labor effective approach to basal fertilizer application, or consider point applying basal fertilizer at 2-leaf stage between plants.

The Malawi practice of applying the basal fertilizer after emergence at the 2-leaf stage has merit for reducing salt damage and leaching of nitrate-N, but requires the researchers to make an additional visit, a time and monetary consideration. Apply the remaining N as top dress urea by placing between plants at ~35-45 days after planting; do 2 topping dressing applications where leaching of nitrate-N potential is high. See plot application rates in Appendix 5.

For legumes, apply all of the fertilizer at planting time (or following emergence in Malawi), including the N applied to beans.

For cassava, first application will be at one to two months after growth has started and the second application another two months later or at the beginning of the second rainy season of crop growth in cases where rainfall is bi-modal.

12 Crop management

Good crop management is essential to obtaining results needed for development of good nutrient response functions. A very important pre-caution is consideration of seed quality. Verify seed

quality by conducting germination tests; discard seed if germination is less than 50% and otherwise adjust seed rates according to the germination percentage.

12.1 Land preparation, generally the land will be hoe or plough tilled but reduced tillage or no-till options are acceptable if experience indicates this is a preferred or common practice.

12.2 Planting needs to be timely. Planting practices to be determined nationally.

12.3 Pest control needs to include good weed control. Pesticide application with a systemic insecticide for stem borer control should be routine for maize and sorghum in most cases. Other pesticide applications should be as recommended or based on close monitoring of insect pest and disease pressure.

13 Observations

Data collection can be done recording on field book sheets with entry into Excel worksheets soon after or digitally with synchronized transfer to computer: see OFRA Trial Data spreadsheet.

13.1 Activities such as: date and method of land preparation; planting information including date, variety, seed rate.

13.2 General observations should be made on the condition of the crop and causes of variation across the site. Rate crop damage (disease, pests, weeds, striga, animals, hail, poor stands, etc.) and make other observations on a whole trials basis or where observed if localized.

13.3 Plot data. Plot level measured observations (*use printed labels adhered to all sampling bags to save time in the field and reduce chances of error*) will include:

- days from planting to mid-season growth stage
  - maize R1- 50% of plants with silk showing
  - sorghum and pearl millet-50% of plants have a panicle/head
  - cowpea, groundnut, drybean, pigeonpea-50% of plants have at least 1 open flower/blossom
  - rice, teff, finger millet, wheat: panicle initiation
- number of plants and ears/panicles harvested for yield
- weight of harvested ears/panicles/tubers/plants
- grain or tuber yield
- grain water content measured with a grain moisture tester; determine air-dried grain water content on a block rather plot basis. Report all grain yields at 14% moisture content; the equation for adjusting yield for grain water content to 14% is to multiply yield by the factor (100-%H2O)/86.
- stover yield for maize, sorghum, and pearl millet determined from the harvest area
- stover water contents (sub-sample, weigh air-dry, oven-dry, reweigh, and calculate water content of air-dried stover); determine air-dried stover water content on a block rather plot basis
- collection of grain and stover/straw sub-samples for nutrient analysis is an optional activity if there is interest for further study such as of nutrient use efficiency, or of grain or stover quality. It is not needed by OFRA. Collect 30 g samples for reference sample or MIR analysis. Sample only those treatments that are important to this additional study. See Appendix 3 for instructions regarding handling of plant samples.

13.4 Obtain diagnostic plant tissue samples at or near flowering stage from two plots per block: the highest K rate NPK or PK plots and the control plot. Sample cassava when actively growing and at about 4 months after planting. In addition to sampling the highest K plot,

- foliar sample the control plots to obtain 30 g samples dry weight
- this is for an ICRAF study and they can mill the samples and pay for analytical costs for the control plot sample
- exclude faba bean, finger millet, barley, pigeon pea, and upland rice from the sampling of the control plot.

Sample enough to have 30 g per sample after drying and milling:

- at least 10 leaves if large leaves but more for smaller leaves in order to have enough for 30 g dry weight
- either shoots for "whole plant" or flag leaves can be sampled for rice, teff, and wheat but enough should be collected for 30 g dry weight sample
- sample the trifoliate leaf without petiole for legumes
- collect foliar samples outside the harvest area to be used for grain yield determination.

The sampling leaves and times are

- maize ear leaf at tasseling to early silk
- sorghum and pearl millet: upper fully extended leaf at heading (flag leaf) but if this leaf was torn at head emergence, sample another plant or the leaf immediately below the flag leaf;
- rice, teff, wheat, finger millet: whole plant or flag leaf at heading.
- soybean, bean, other legumes: upper fully expanded leaf at flowering to early pod development.
- cassava: an upper, fully expanded leaves at about 4 months after planting and during active vegetative growth.

Place the leaf samples into clearly labelled paper (e.g., brown) bags and stapled.

- Begin drying as soon as possible
- Put in drying oven as soon as feasible to oven-dried at 60 ºC for 48 hours
- Mill the samples if possible and send 30 g milled samples to ICRAF-Nairobi for IRS analysis (see Appendix 3 for instructions).

Estimates for foliar critical nutrient levels indicating deficiency vary. The following estimates a compromise of estimates from several sources. Further interpretation can be done using nutrient ratios such as by DRIS.

	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B	Mo
	%						ppm					
Maize, silk	2.8	0.22	1.2	0.2	0.1	0.11	20	15	15	3	3	0.1
Sorghum, fl.	2.8	0.22	1.4	0.3	0.16	0.13	20	7	15	3	1	0.1
Rice, p.i.	3.0	0.18	1.5	0.2	0.15	0.15	70	40	20	6	6	0.1
Wheat	3.2	0.22	2.0	0.15	0.12	0.1	25	12	15	3	2	0.05
Groundnut	3.5	0.2	1.7	0.5	0.3	0.2	50	20	20	5	20	0.1
Bean	4.0	0.3	1.4	1.2	0.3	0.14	100	20	15	15	15	0.1
Soybean	3.4	0.25	1.6	0.7	0.22	0.2	25	12	21	4	15	0.05
Cassava	4.0	0.25	0.9	0.25	0.15	0.2	100	30	25	1.5	7	

13.5 **Weather**: Daily rainfall at site or within 5 km for on farm trials, and minimum and maximum temperatures within 10 km. Other information is desired when available.

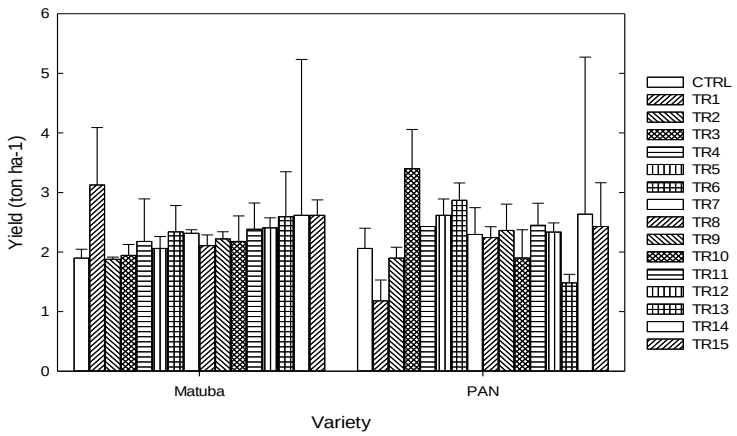
13.6 **Obtain farmer yields** for maize, rice, wheat, sorghum or millet from nearby fields depending on how important the crop is. Yield might be determined using the farmer's estimate of the quantity harvest and your measurement of the field area. Record in the worksheet "Site Description" of Excel file.

14 Data analysis

The following concerns analysis for testing of hypotheses and for the sake of scientific publication; somewhat different analyses are appropriate if the intent is to aid in farmer decision making. The difference arises from the concern that in research we want to have a high probability of not making a Type I error, that is of saying there is a treatment effect when the difference that we see is only due to chance. In real world decision making, we are often at least as much concerned with erroneously saying that there is no difference when if the experiment were repeated under very similar conditions, there would be a treatment effect; this is Type II error. Type II error is important in real life as it implies passing up some favorable opportunities. In OFRA trials, a null hypothesis (H_o) might be that application of a nutrient (N, P, K, S..) does not affect crop yield with a given set of growing conditions; the alternative hypothesis (H_A) is that application of a nutrient does affect yield. In this example, a Type I error is made when we conclude for our growing conditions of interest, with a stated confidence level such as 0.95, that a nutrient application affects yield when it does not. Type II error in this example would occur when we conclude that there is no nutrient effect for our growing conditions of interest when in fact there is a response to the applied nutrient. Chances of a Type II error increase as protection against Type I error is increased

Suggested steps to analysis of OFRA trial data.

i. Determine if your data meets the following assumptions of the analysis of variance (ANOVA)
    1. Homogeneity of variance, or that the variance or standard error for all treatments is not statistically different, as determined by the Bartletts or another test. In the figure below, the high standard error associated with treatment 14 indicates lack of homogeneity of variance.
    2. Normal distribution of residuals (not actual values) as determined by the Shapiro-Wilk or another test is also assumed.



ii. Conduct the analysis of variance (ANOVA) by site-season (trial) using plot data; if there are no treatment effects at $\alpha = 0.05$, we accept H_o and there is no need or justification for additional analysis for that site-season but non-significant results need to be reported. In exceptional cases of much non-treatment variability within blocks, the error variance may be sufficiently reduced by doing the ANOVA with a covariate that is independent of treatments. This might be, for example, distance from a past or current tree, plant stand, or the mean yield of nearest neighbors. If there are significant treatment effects, test for the NxP interaction for non-legumes and the PxK interaction for pulse crops. This can be done by omitting the treatments not concerned with the interaction and repeating the ANOVA as a complete factorial using only the N rate with and without P applied, or P rate with and without K for pulses. This approach does result in reduced degrees of freedom for the error term.

iii. Do means separation (an all pairwise comparison test) such as by LSD 0.05.
   1. Record the value of the least significant difference (critical value for comparison).
   2. Are yield means for nutrient rates significantly different? If not and if for scientific publication, do not determine response functions. If different, determine response functions; see below.
      i. Means separation is also useful in exploring significant interactions.
      ii. Is the mean for the diagnostic treatment different compared with the treatment with similar N-P-K rate?

iv. Conduct the ANOVA combined across site-seasons with site-seasons and replications as random variables and factor-levels (nutrient rates) as fixed variables. Note if the treatment x site-season interaction is significant.

v. When significant nutrient rate effects occur, determine the non-linear asymptotic yield function: Yield (Mg ha^{-1}) = $a - bc^N$, where a is near maximum yield for application of that nutrient, b is the gain in yield due to application of that nutrient, and c^N determines the shape of the curvilinear response where c is a curvature coefficient and N is the nutrient rate. If there is no N x P rate interaction, the N response can be determined across P levels for non-legumes and bean. If there is no P x K rate interaction, the P response will be determined over K levels for other pulses. Where interactions are significant, N response for each level of P and P response for other pulses for each level of K will be determined.

vi. If the treatment x site-season interaction is significant, scrutinize the means such as by using means separation, to determine the inconsistencies of treatment effects across site-seasons that result in the significant interaction.

vii. If there is significant treatment x site-year interaction, try to account for the variation. Conduct regression analyses to relate soil test values and other site information to:
   yield with no nutrient applied,
   response to applied nutrient, and
   yield with nutrient applied.

viii. Calculate the EONR and EOPR, or the nutrient application rates that gave the greatest net return ha^{-1} to fertilizer use, for a range of cost:prices. This can be done stepwise in Excel or with calculus for quadratic functions. Capture the EOR to price relationship with polynomial functions for each crop-nutrient combination to estimate EOR with cost:price ratio as the independent variable.

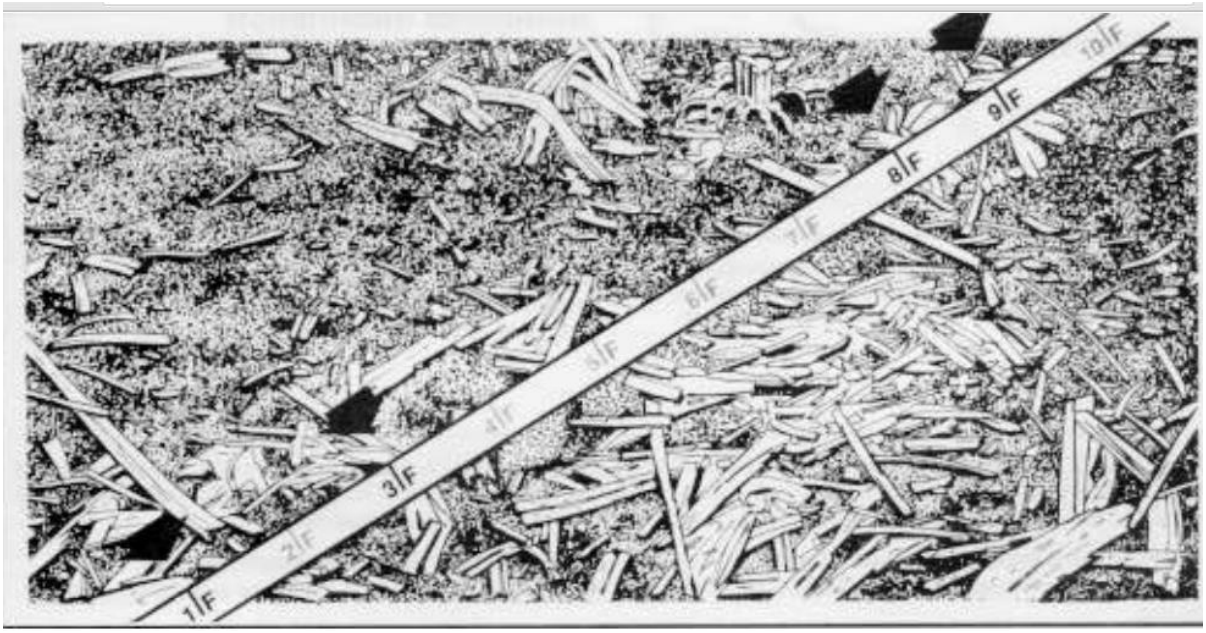
Differences and relationships will be considered significant at $\alpha \leq 0.05$.

APPENDIX 1

Site description for land area of each trial

Country	
Region	
District	
Village	
Farmer/research center	
Crop	
Previous crop	
Lead researcher	
Field assistant (FA)	
FA mobile	
Latitude, corner one	
Longitude, corner one	
Latitude, corner two	
Longitude, corner two	
Latitude, corner three	
Longitude, corner three	
Latitude, corner four	
Longitude, corner four	
Elevation	
Soil class (approximation; FAO or USDA)	
Parent material (alluvium, loess, ancient surface, colluvium, volcanic	
Soil depth if <1M (auger depth restriction)	
Topographic position (upland, ridge/crest, mid-slope, foot slope, bottomland)	
Time since 1 yr/season fallow	
Time since >1 yr fallow	
Water stable soil aggregates (adapt Wortmann and Brubaker 2004); for each rep estimate % soil remaining on sieve; a) soil remaining in aggregates rather than slumped (See Appendix 2).	1= >81%; 2 = 51-80% 3 = 21-50% 4 = <20%))
b) Visual estimate of soil remaining on sieve, %	
Ground cover rating at soil surface: 0 = 0%; 1 = <4%; 2 = 4-15%; 3 = 15-40%; 4 = 40-65%; 5 = >65%; use line transect method of 100 units; for residue cover, pieces should be >1cm length.	
a) Rock/stone cover	
b) Gravel cover	
c) Crop and weed plant residue cover before tillage	
Cereal crop yield (Mz, So, Pm, FM, Ri, Wh, Teff) from neighboring fields of farmers	

(kg/measured area converted to kg/ha); name crop below	
Crop1	
Crop2	
Crop3	



Using the line transect method for estimating soil cover; lay diagonal to crop rows and count points per 100 points with the exact point on the same side of tape overlying plant residue, rock/stone, or gravel. E.g. observations can be made at marks on a measuring tape every 10 cm over a distance of 10 m to get 100 observation points.

Appendix 2.

Water-stable soil aggregation

Crop growth is often constrained by poor root development, by slow water infiltration and water movement through the soil, and by poor soil aeration. These constraints are often associated with poor soil porosity. Soil aggregation is important to developing and maintaining good soil porosity and hence to good root growth and to movement of soil water and gases. With more soil in water stable aggregates, it is expected that:

- The rate of water infiltration and percolation will increase
- Soil crusting will be less
- Resistance to the splash effect of raindrops will increase and soil erodibility will decrease and
- Runoff will decrease, making more water available to the crop.

Quantity	Item	Description
1-3	Tea strainers	Common tea strainers of 5-7cm diameter
1-3	Transparent cup or beaker	Diameter is slightly wider than for the tea strainer and >3cm deep
1	Measuring unit	Can be 1 tablespoon, 1/16 cup, or about 15 g measure
	Water	Water is used for the tests and for cleaning. Squeeze bottles are convenient. One or more liters may be needed.

1. Collect a composite soil sample (4 sample points to 3 cm depth) from each block
2. Fill cups to near brimful with water.
3. Place approximately 15 g (1/16 cup or just over a tablespoon) of soil into a tea strainer.
4. Touch the bottom of the tea strainer to the surface of the water in the cup until the soil is moist.
5. Submerge the soil in strainers in the cups of water 20 times at about 2 seconds per cycle.
6. Carefully dump the wet soil on the paper towel.
7. Observe the quantity and shape (degree of slumping) of the wet soil mass, and the presence of soil aggregates. Rate degree of slumping according to soil remaining in aggregates rather than slumped (1= >80%; 2 = 50-80%; 3 = 20-50%; and 4 = <20%).
8. Estimate the % soil that passed through sieve.

APPENDIX 3 Africa Soil Information Service - Standard Operating Procedure

Soil Sample Processing

1. Introduction

This standard operating procedure covers soil sample preparation and shipping of subsamples to the AfSIS reference soil laboratory at the World Agroforestry Centre (ICRAF) in Nairobi.

2. Air drying

Air dry the soil samples by spreading a sample out as a thin layer into shallow trays or plastic or paper sheets. Drying can be done in large room, a custom-made solar dryer, or a forced-air oven at 40° C. Break up clods as far as possible to aid drying. Contamination from dust, plaster or other potential contaminants should be avoided as soils are subjected to trace element analysis. Great care should be taken at all stages to ensure sample labels remain with the samples. Drying time will depend on the condition of the samples and ambient conditions, but the samples should be thoroughly dried to a constant weight.

3. Crushing and sieving

Spread the sample onto a plastic sheet on a solid table. Using a wooden rolling pin, crush the sample to pass through a 2 mm mesh size certified sieve. While crushing, remove any plant materials (e.g. roots) and any possible pieces of gravel (making sure they are gravel and not soil aggregates) and place in a separate pile (the coarse fraction).

Pass the crushed sample through the 2 mm sieve. DO NOT use the sieve as a grinder; i.e. do not rub or mash the soil on the sieve, but shake the sieve gently to allow the soil to pass through. If a large amount of soil needs to be sieved, it is easier to do it in small batches rather than all at one time.

Place whatever remains on the sieve back onto the plastic sheet and crush again gently. Then pass again through the 2 mm sieve. Make sure that all soil materials are crushed, but do not attempt to crush gravel and rocks.

Transfer anything that now remains on the sieve into the coarse fraction pile. Retain the coarse fraction for subsampling.

The whole sample should be processed and no material should be discarded. You will remain with two fractions:

a. The coarse fraction (>2 mm), which cannot pass through the sieve.
b. The soil fines (<2 mm), which have passed through the sieve.

Weigh the coarse and soil fines sieved fractions and record the weights to 0.1 g on the label.

Clean off the bench with a damp cloth to remove soil dust, so as to prevent contamination from one sample to another.

Subsample the soil fines fraction to give a representative 50 g (500 g and another 50 g for the reference samples) sub-sample for shipping to ICRAF Nairobi for spectral analysis. Place the subsample in a zip-lock polythene bag labeled with the SSN and “Fines”. Adhere printed label to the bag, and place this bag in another bag for security. See below for sample shipping procedures. Retain a second sub-sample for national archiving and in case the shipped sample is lost.

4. Shipping

In advance of shipment, send the details of your samples to the ICRAF Soil-Plant Spectral Diagnostic Lab at ICRAF Headquarters to: Keith Shepherd (k.shepherd@cgiar.org) copied to Elvis Weullow (e.weullow@cgiar.org). The information required is (a) a description of the material (e.g. air-dried 2 mm-sieved soil samples), (b) the number of soil samples, (c) the total weight of the soil in the batch, and (d) name, institutional address and fax number of the scientist shipping the samples. http://worldagroforestry.org/sites/default/files/Soil%20sample%20processing%20at%20ICRAF%20soil%20laboratory_221111.pdf

Appendix 1: Sample Logging Sheet

SSN	Sampling date	Cluster	Plot	Depth_std code	Depth_ top	Depth_ bottom	Total air dried soil weight	Coarse fragments

Obtain a phytosanitary certificate from your country's plant inspectorate authorities or, if this is not possible, a letter from the relevant government authority indicating that the soils are specifically meant for research purposes only and have no commercial value. Send the phytosanitary certificate or letter to the ICRAF laboratory.

Based on the above documentation, the ICRAF laboratory will obtain a Kenya import permit for the samples from the Kenya Plant Health Inspectorate Service. The ICRAF laboratory will email you a scanned copy of the permit[1]. The samples should be shipped together with a copy of the KEPHIS permit and your phytosanitary certificate or government letter. Failure to do so may result in the samples being destroyed by KEPHIS!

The soil samples to be shipped should be carefully double-packed into strong polythene bags that cannot be easily ripped or damaged in transit, and packed into strong shipping cartons. Also have the shipping agent repack the consignment again. Secure packing is critical because if the package arrives damaged the samples will be destroyed by KEPHIS and our agreement may be revoked. Do not underestimate what airlines can do to a package! Complete and include a
ICRAF SOIL-PLANT SPECTRAL DIAGNOSTICS LABORATORY: SAMPLE SUBMISSION AND SERVICES REQUEST FORM.
The shipping address is:

Dr. Keith Shepherd
Att: Elvis Weullow
World Agroforestry Centre (ICRAF).
P. O. Box 30677-00100
Nairobi
KENYA
Tel: +254 20 7224000
Fax: +254 20 7224001

[1] KEPHIS also issue a quarantine (Q) label that the ICRAF Soil Lab will retain for clearance purposes.

Remember that the shipment must be accompanied by the import permit and your phytosanitary certificate.

On shipping you must immediately fax or email the shipping details (e.g. airway bill number) to Samuel Gaturu (s.gaturu@cgiar.org), Elvis Weullow (e.weullow@cgiar.org), and Mercy Nyambura (m.nyambura@cgiar.org), copied to Dr Keith Shepherd (k.shepherd@cgiar.org). This will allow us to alert the shipping agent's Nairobi office about the arrival of the quarantine shipment.

The ICRAF soils lab will arrange clearance of the shipment and inspection of the soils by KEPHIS. Upon clearance by KEPHIS, ICRAF will arrange for collection of the soils and their transport to ICRAF House.

The ICRAF laboratory charges US$100 to cover all the expenses involved in sample clearance protocols, including KEPHIS fee, visits to the KEPHIS office, and clearance when the samples arrive.

5. Plant sample processing shipping

Begin air-drying samples immediately after collection but begin to oven-dried at 60 ºC for 48 hrs as soon as feasible. Mill the samples if possible to < 0.5mm. Store before and after milling to prevent insect and other damage. Sub-sample and put 30 g sub-samples in labeled small zip-lock bag and double bag for security.

Following instructions above for soils, e.g.

- Obtain the photosanitary permit and send a copy to ICRAF
- Have ICRAF obtain the KEPHIS importation permit
- The samples should be shipped together with a copy of the KEPHIS permit, your phytosanitary certificate or government letter, and the completed ICRAF SOIL-PLANT SPECTRAL DIAGNOSTICS LABORATORY: SAMPLE SUBMISSION AND SERVICES REQUEST FORM. Include an inventory list of all samples.
- The shipping address is as above.

Remember that the shipment must be accompanied by the import permit and your phytosanitary certificate.

On shipping you must immediately fax or email the shipping details (e.g. airway bill number) to Samuel Gaturu (s.gaturu@cgiar.org), Elvis Weullow (e.weullow@cgiar.org), and Mercy Nyambura (m.nyambura@cgiar.org), copied to Dr. Keith Shepherd (k.shepherd@cgiar.org) so they can alert the shipping agent's Nairobi office about the arrival of the quarantine shipment. Also, send the inventory list of all samples; see above for soil samples.

6. Analyses to request

The analyses needed are:

- Reference soil samples: wet soil chemistry, LDPSA, and other CN
- MIR soil samples: MIR
- Foliar samples: MIR but some with be selected and composited for wet chemistry analysis following MIR scan.

ICRAF will provide a request form that is something as follows with space to enter identifier and contact information to be added at the top.

Give the number of each sample type submitted and if these are dry and processed (milled and sieved) or unprocessed. The, below Laboratory Tests, indicate the needed analysis: wet soil chemistry, LDPSA, and other CN for reference soil samples, and normally, only MIR for the MIR soil and plant samples.

Submitted by		Scientist	
Organization		Project	
Report to		Country	
Invoice to		Region	
Phone no.		Email	

Quantity	Sample type	Dry	Processed	Unprocessed
	Reference samples			
	Soil, 500 g			
	Foliar, 30 g			
	Grain, 30 g			
	Stover/straw, 30 g			
	MIR samples			
	Soil, 50 g			
	Foliar from high K plot, 30 g			
	Foliar from control plot, 30 g			
	Grain, 30 g			
	Stover/straw, 30g			

Laboratory tests				
		Tick choices		
Reference soil samples	**Wet soil chemistry suite†**	**LDPSA‡**	**Organic CN¶**	**Other††**
Reference foliar samples	**Wet plant chemistry suite**			
Reference grain samples	**Wet plant chemistry suite‡‡**			
Reference stover/straw samples	**Wet plant chemistry suite‡‡**			
MIR soil samples, 50 g		**MIR¶¶**		
MIR foliar samples, 30 g‡‡		**MIR¶¶**		
MIR grain samples		**MIR¶¶**		
MIR stover/straw samples		**MIR¶¶**		

†Current cost is $40 per sample. Includes pH (water), EC, exchangeable acidity, Mehlich 3 (Al, P, K, Ca, Mg, Na, S, Fe, Mn, Cu, B, Zn), and P sorption index.

‡LDPSA is for particle size distribution and soil texture determination. Current cost is $5 per sample.

¶Soil organic C and N are determined as soil organic matter/C are not determined in the wet chemistry suite. Current cost is $16 per sample if pH is ≥ 6.50 per sample; otherwise, $7.5 per sample. For plant samples, cost is $7.5 per sample.

††Generally not needed for OFRA samples.

‡‡ Approximately 1 of 10 plant samples will be selected by ICRAF for wet chemistry analysis. Current cost is $50 per sample. Includes N, P, K, Ca, Mg, Fe, Zn, Mn, Cu, B, Na.

¶¶ Current cost is $1.50 per sample.

APPENDIX 4 Treatment structures

Maize, sorghum†, rice†, finger millet, cassava†				
0P0K	15P,0K	90N,0K	90N,15P	Diagnostic
0N	0N	7.5P	10K‡	90N,15P,20K,15S,2.5Zn,10Mg,0.5B
30N	30N		20K	
60N	60N	22.5P	30K	
90N	90N			
120N	120N			
Common bean				
0P0K	15P,0K	20N,0K	20N,15P	Diagnostic
0N	0N	7.5P	10K	20N,15P,20K,15S,2.5Zn,10Mg,0.5B
10N	10N		20K	
20N	20N	22.5P	30K	
30N	30N			
Groundnuts, soybean, pigeon pea, c/pea				
0K	20K	15P	Diagnostic	
0P	0P	10K	15P,20K, 15S, 2.5Zn,10Mg,0.5B	
7.5P	7.5P			
15P	15P	30K		
22.5P	22.5P			

† For sorghum and pearl millet where severe soil water deficits are common, use N rates of 0, 20, 40, 60 and 80 kg/ha. For cassava use N rates of 0, 20, 40, 60, 80 kg/ha and K rates of 0, 20, 40, 60 kg/ha. Suggested N rates for lowland rice are 0, 40, 80, 120, and 160 kg with 25% applied one week after transplanting, a later top dress of 25%, and then a final topdress of 50% at panicle initiation.

‡If third nutrient is S, rates 0, 5, 10, 15 kg/ha. If Zn, rates are 0, 0.5, 1, 1.5 point applied. Multiply P rate by 2.29 to get P2O5 rate and K by 1.2 to get K2O

APPENDIX 5 Fertilizer to be applied per plot

If S is to be supply from a product other than elemental S, e.g. from CaSO4, the rate and amounts will need to be adjusted.

Treatment	Basal application				Top dressing
	Urea	TSP	KCl or S	Package	Urea
Maize, assumes 6 x 6 m plot, 2 of 3 x 6 m = 36 m^2; g fertilizer per plot					
30N	117	-	-	-	117
60N	235	-	-	-	235
90N	235	-	-	-	469
120N	235	-	-	-	704
0N,15P		270	-	-	-
30N,15P	117	270	-	-	117
60N,15P	235	270	-	-	235
90N,15P	235	270	-	-	469
120N,15P	235	270	-	-	704
90N,7.5P	235	135	-	-	469
90N,22.5P	235	405	-	-	469
90N,15P,10K/5S	235	270	72 or 18	-	469
90N,15P,20K/10S	235	270	144 or 36	-	469
90N,15P,30K/15S	235	270	216 or 54	-	469
Diagnostic	235	270	144	279	469
Total applied kg per replication is: urea 9.62; TSP 2.97; KCl 1.15; S 0.21; 0.279 diagnostic mix					
Sorghum and pearl millet, assumes 4 x 6 m plot =24 m^2; g fertilizer per plot					
30N	78	-	-	-	78
60N	156	-	-	-	156
90N	156	-	-	-	312
120N	156	-	-	-	468
0N,15P		180	-	-	-
30N,15P	78	180		-	78

60N,15P	156	180		-	156
90N,15P	156	180		-	352
120N,15P	156	180		-	528
90N,7.5P	156	90	-	-	352
90N,22.5P	156	270	-	-	352
90N,15P,10K/5S	156	180	48 or 12	-	352
90N,15P,20K/10S	156	180	96 or 24	-	352
90N,15P,30K/15S	156	180	144 or 36	-	352
Diagnostic	156	180	96	186	352
Total applied kg per replication is: urea 7.22; TSP 2.32; KCl 0.87; S 0.16; 0.186 diagnostic mix					

The diagnostic package will be made up of 67 parts MgSO4, 3.45 parts Boron Granular and 7.35 parts Zinc Sulphate Monohydrate.

Treatment	Basal application				Top dressing
	Urea	TSP	KCl or S	Package	Urea
Cassava, assumes 3.6 x 6 m plot (4 rows @ 90 cm)=22 m^2; g fertilizer per plot					
20N	48	-	-	-	48
40N	96	-	-	-	96
60N	96	-	-	-	191
80N	96	-	-	-	287
0N,15P		165	-	-	-
20N,15P	48	165		-	48
40N,15P	96	165		-	96
60N,15P	96	165		-	191
80N,15P	96	165		-	287
60N,7.5P	96	83	-	-	191
60N,22.5P	96	248	-	-	191
60N,15P,20K/5S	96	165	88 or 11	-	191
60N,15P,40K/10S	96	165	176 or 22	-	191
60N,15P,60K/15S	96	165	264 or 33	-	191
Diagnostic	96	165	176	170	191
Total applied kg per replication is: urea 3.93; TSP 1.82; KCl 1.41; S 0.13; 0.170 diagnostic mix					
Finger millet, rice, wheat, teff, assumes 3x6 m plot=18 m^2; g fertilizer per plot					
30N	59	-	-	-	59
60N	118	-	-	-	118
90N	118	-	-	-	235
120N	118	-	-	-	352
0N,15P		135	-	-	-
30N,15P	59	135		-	59
60N,15P	118	135		-	118
90N,15P	118	135		-	235
120N,15P	118	135	-	-	352
90N,7.5P	118	68	-	-	235

90N,22.5P	118	203	36 or 9	-	235
90N,15P,10K/5S	118	135	72 or 18	-	235
90N,15P,20K/10S	118	135	108 or 27	-	235
90N,15P,30K/15S	118	135	72 or 18	-	235
Diagnostic	118	135	72	139	235
Total applied kg per replication is: urea 4.83; TSP 1.49; KCl 0.58; S 0.11; 0.139 diagnostic mix					

The diagnostic package with be made up of 67 parts MgSO4, 3.45 parts Boron Granular and 7.35 parts Zinc Sulphate Monohydrate.

Treatment	Basal application				Top dressing
	Urea	TSP	KCl or S	Package	Urea
Bean, assumes 3 x 6 m plot (e.g. 6 rows @ 50 cm)=18 m^2; g fertilizer per plot					
10N	39		-	-	-
20N	78		-	-	-
30N	117		-	-	-
0N,15P		135	-	-	-
10N,15P	39	135		-	-
20N,15P	78	135		-	-
30N,15P	117	135		-	-
20N,7.5P	78	68	-	-	-
20N,22.5P	78	203	-	-	-
20N,15P,10K/5S	78	135	36 or 9	-	-
20N,15P,20K/10S	78	135	72 or 18	-	-
20N,15P,30K/15S	78	135	108 or 27	-	-
Diagnostic	78	135	72	139	-
Total applied kg per replication is: urea 1.01; TSP 1.35; KCl 0.50; S 0.11; 0.139 diagnostic mix					
Soybean and other pulses, assumes 3 x 6 m plot=18 m^2; g fertilizer per plot					
7.5P	-	68	-	-	-
15P	-	135	-	-	-
22.5P	-	203	-	-	-
0P,20K/10S	-	-	72 or 18	-	-
7.5P,20K/10S	-	68	72 or 18	-	-
15P,20K/10S	-	135	72 or 18	-	-
22.5P,20K/10S	-	203	72 or 18	-	-
15P,10K/5S	-	135	36 or 9	-	-
15P,20K/10S	-	135	72 or 18	-	-
15P,30K/15S	-	135	108 or 27	-	-
Diagnostic	-	135	72	139	-
Total applied kg per replication is: TSP 1.35; KCl 0.50; S 0.11; 0.139 diagnostic mix					

The diagnostic package with be made up of 67 parts $MgSO_4$, 3.45 parts Boron Granular and 7.35 parts Zinc Sulphate Monohydrate.

APPENDIX 6 Trial distribution

Conducting several trials in close proximity can greatly reduce time and costs requirements. Where land is adequate, conduct replicated trials on farm, compensating farmers if necessary although farmers commonly see that the added production gained from fertilizer use in the trials area compensates for the land use. Researchers and facilitators should explain the arrangement clearly to the participating farmers.

Avoid excessive distribution of trials. Consolidating trials in several areas representing much production area can greatly improve the efficiency of time and other resources required for implementation and monitoring of the trials by the research teams and the facilitators.

An approach used in Uganda was to have researcher managed trials at experimental stations, supported by on farm trials within a radius of 10 km. The scientists at the experimental stations were enabled to monitor the on-farm trials within their mandate areas. The research team coordinator received progress reports but trials were also visited periodically and at critical times by a member of the research team.

Appendix 7. AEZ specific adjustments

Modifications of the standard protocol are needed in exceptional cases to develop meaningful crop nutrient response functions.

1. Some trials will include additional treatments to be able to link sole crop response to intercrop response, or to link response with and without manure applied. See 9.6 for more details.
2. Two sets of N levels are needed for sorghum which is produced in a wide range of environments with much variation in potential for response to applied nutrients. This variation is largely due to frequency and severity of periods of soil water deficit which is a function of in-season rainfall amount and distribution, soil water holding capacity, potential evapo-transpiration, and potential rooting depth.
    a. N levels for relatively high yield potential occur in 30 kg ha^{-1} increments.
    b. N levels for relatively low yield potential occur in 20 kg ha^{-1} increments.
3. Nitrogen level increments for pearl millet will differ from the standard for cereals and be in 10 kg ha^{-1} increments, but with all N top-dress applied during weeding.
4. Suggested alternative N rates for lowland and/or irrigated rice are 0, 40, 80, 120, 160 kg with 25% applied one week after transplanting, a top dress of 25%, and then a topdress of 50% at panicle initiation in Rwanda. The higher rates are also for irrigated rice in Mali but with 50:50 application at tillering and panicle initiation. Mali will apply urea in common form but add a treatment for the 120 N rate using split applied super granule urea.
5. In Ghana, maize-cassava intercropping trials will be with planting full stands of crop at the same time with 80-cm maize row spacing and with cassava planted in maize rows at 1 m plant spacing; fertilizer treatments will be as for maize sole crop trials. The maize stover will remain in the field as mulch to cassava which will be harvested about one year after planting.
6. In Nigeria, maize and rainfed rice trials will address Zn response in addition to N,P,K by adding 90N-15P-30K-0.5Zn; 90N-15P-30K-1Zn; 90N-15P-30K-1.5Zn, and for maize by adding 60N-15P-20K-0.5Zn; 60N-15P-20K-1Zn; 60N-15P-20K-1.5Zn.
7. Some bean trials will have added treatments for collaboration with the CIAT-BEAN program to assess biological N fixation and potential response to Mo.
    a. Two treatments may be added to the standard set including BAT 447 nodulation and non-nodulating lines in order to assess BNF by the difference and natural abundance of N15 methods. Preferably, seed will be rhizobia inoculated but not necessarily. Apply N, P, K at rates of 10, 15, and 10 kg/ha on an elemental basis. Sample biomass of the two BAT447 treatments at mid-podfill by cutting the stems of 6 plants at ground level, determining plants/m2, oven-drying the 6 plant samples at 60C and weighing (or obtaining wet weight and then sub-sampling after cutting up the plants and obtain the wet and dry weights of sub-samples). Send the BAT 447 data and 50 g of dry material from the mid-podfill sampling and also 50 g of grain to CIAT-Uganda for milling and analyses. Determine grain yield.
    b. Add Mo at 250 g Mo/ha (1.35g Mo per plot) to the diagnostic treatment preferably as chelated Mo (??% Mo), ammonium molybdate (54% Mo; 2.5 g/plot) or molybdenum trioxide (66%; 2 g/plot), and less preferred Mo sulfate (Mo(SO4)3; only 25% Mo; 5.4 g/plot).
    c. Take a second foliar sample when sampling the NPK treatment in each block to be sent to CIAT-Uganda to arrange for the Mo analysis.

Appendix 8. Naming OFRA trials

Use a standardized format for naming trials and files. Adherence to this is essential for sharing results of OFRA research. An example of a trial in Kenya is as follows with a SITE code used at the trial site/season level that will be the unique identifier for the trial. For example,

KE_KIT_MZ_2014A is for a trial conducted in KE=Kenya at KIT=Kitale for MZ=Maize in 2014 Season A. The trial Excel records file should have this name.

For on-farm trials with just one rep per farm, but also for labeling of soil and plant samples. add the block (rep) identifier e.g. KE_KIT_MZ_2014A_B1.

For samples, also add the plot number e.g. KE_KIT_MZ_2014A_B1_101.

Therefore,

1. Country codes include: BF Burkina Faso; ET Ethiopia; GH Ghana; KE Kenya; ML Mali; MW Malawi; MZ Mozambique; NG Nigeria; NE Niger; RW Rwanda; TZ Tanzania; UG Uganda; ZA Zambia.
2. Location codes: these need to be determined by OFRA country teams.
3. Crop: BE common bean; CA cassava; CP cowpea; GN groundnut; FM finger millet; PM pearl millet; PP pigeon pea; RI, RL, and RU rice, respectively, for irrigated, lowland non-irrigated, and upland non-irrigated; SO sorghum; SB soybean; TE teff; and WH wheat.
4. Year: self-explanatory except where trials span two years, write both such as 2013/4.
5. Season: use A or B as used in your country; use R if mostly or all during the rainy season or D if mostly the dry season such as for dry season irrigated crops or use of residual soil water.
6. Add block number and plot number when needed, such as for on-farm trial or sample identification.
7. For soil samples, add depth if different from 0-20cm.

Appendix 9. Possible OFRA related areas for post-graduate thesis research

OFRA does not have funding for post-graduate education but OFRA trials can present excellent opportunities for thesis research with little or no added operational costs. Possible research areas that have been suggested:

1. Linking nutrient responses with manure application to responses without manure application.
2. Linking nutrient responses for sole crop with cereal-legume intercropping:
    a. maize-cassava intercropping, e.g. in Ghana
    b. cereal-cereal intercropping
    c. cereal-pulse intercropping as proposed by several countries variously for maize-pigeon pea, sorghum-cowpea or groundnuts, pearl millet-cowpea or –groundnut.
3. Determination of nutrient use efficiency and its components with fertilizer application: recovery efficiency, agronomic efficiency, internal or physiological efficiency, etc.
4. Calibration and validation of models for estimation of nutrient response functions, e.g. QUEFTS, DSSAT, APSIM, etc.
5. Extrapolation of information between parts of Africa with similar production conditions using GIS and spatial climate, soil, and crop distribution information
6. Decision tool development, such as alternatives to Uganda Optimizer
7. Account for variation in responses, such as with soil test and other site information
8. Fertilizer use and N fixation by pulses and/or effects on grain nutrition properties
9. Determination of nutrient substitutions values of other ISFM practices, information needed to put fertilizer use in an ISFM framework.
10. Interpretation of foliar diagnostic results such as comparing critical levels and DRIS.

Appendix 10. Template for developing the trial specific protocol for implementation (with example)

The following template and the Niger pearl millet example are to aid in developing trial specific protocols that will serve as guide/instructions to implementation and be pasted in the 'Protocol' worksheet of the trial's Excel file. See appropriate sections and appendixes above for more information.

Trial title:

Location:

Season of implementation:

Trial code name:

Responsible researcher and field assistant:

Trial design

- List of treatments
- Replications
- Plot size

Site characterization:

- Position the trial and get latitude/longitude on each trial corner.
- Approximate soil class, parent material, soil depth if <1M, and topographic position.
- Note time since last seasonal fallow and time since last >1yr fallow.
- 0-20 cm samples of 3 cores each from of these 9 plots.
- 0-20 cm sample of 8 cores each for each rep:
- 20-50 cm sample of 6 cores for the whole trial area
- Line transect determination of ground cover by: plant residue ; living plants ; sand/gravel .
- Determine water stable aggregates
- Previous crop
- Process soil samples

Early implementation

- Obtain and prepare inputs for trials
    - Calculate and weigh fertilizer quantities
    - Specify in the 'FertRateCalc' worksheet the fertilizer amounts to be applied to each plot and the calibration
- Land preparation will include …….. and the last date of completion is ……..
- Mark each plot by ……..
- Record all activities throughout the in the 'Activities' worksheet of the trial Excel file.
- The variety will be …….. Obtain seed and conduct germination test….
- Basal fertilizer applied at least 6 cm from seed and placement will be ……. Basal N application will not exceed 30 kg/ha N.
- Plant with …row spacing and ….. within row spacing. The last acceptable planting date is …..
- Following emergence, thin or gap fill as necessary.
- Weed control will be by ……

- Top dress apply N fertilizer at …… by ……
- Pest control will include routine application of ….. by (method, time of application) to control ……. Address procurement of pesticides. Scout for other pest problems and treat if necessary.
- Record observations on trial condition/health when visiting the trial throughout the season; record in the 'Observations' worksheet.
- Obtain leaf samples at ….
- Collect plot and other data
    - Growth stage
    - Harvest area
    - Count harvested plants
    - Count harvested ears or panicles
    - Obtain harvested ear/panicle …. Wt.
    - Shell/thresh and determine grain weight and grain water
    - Determine stover weight and stover water
    - Obtain and mill grain and stover sub-samples where nutrient content analysis is desired.
- Regularly (at least once a week) enter data into Excel file.
- Send the updated Excel file for this trial to OFRA at the end of the month.

Example of a trial specific protocol translated from French.

REPUBLIQUE DU NIGER
MINISTERE DE L'AGRICULTURE
INSTITUT NATIONAL DE RECHERCHE AGRONOMIQUE DU NIGER (INRAN)

INRAN/CABI-AGRA/SHP/ COLLABORATION PROJECT

OPTIMISING FERTILZER RECOMMENDATIONS FOR AFRICA

OFRA/NIGER

ON- STATION PROTOCOL 2014

CROP : Pearl Millet

Experimental protocol (OFRA/Niger)

Title: Pearl millet **(*Pennisetum glaucum* (L.) R. Br.)** Response Functions to nutriments within ISFM framework

1. **Objective** :

The main objective of this study is to revise and develop within ISFM framework new fertilizer recommendations tools for pearl millet with options which take into account the specific conditions of production; soil and climate as well as economic conditions of the producers and the fertilizer cost/ millet grain price ratio for each major agro-ecological zone

The specific objectives are:

- To compare the effect various fertilizer combinations on the growth and yield of pearl millet in sole crop system and intercropped with cowpea
- To identify the level of the integrated fertilizer rate allowing to obtain the best outputs and income from pearl millet production under the targeted cropping system.

2. Material and Methods

2.1 Experimental sites: the trial will be established during the rainy season in INRAN research station: Tarna/Maradi, Magaria, Bengou, Birnin Konni and Kollo. On-farm trials will be established in some villages neighboring the research stations.

The characterization of the trial sites with soil sampling will be made before the application of fertilizers by a team of INRAN Soil Laboratory / CERRA Niamey in accordance with the OFRA protocol sheet provided for this purpose.

2.2 Vegetable material

Pearl millet variety Zatib will be used for all the trial sites. The seeds must be treated with fungicide to avoid damping-off and to have a good emergency
with vigorous seedlings. The cowpea variety IT99K573-1-1 will be used in the pearl millet/cowpea intercropping system.

2.3 Treatments

Total number of treatments is twenty-six (26) with the structure presented in table 1, next page.

- Treatments **T_1 to T_{16}** will be with pearl millet/cowpea intercropping system with 2.5 t/ha of manure
- Treatments **T_{17} to T_{21}** will be with pearl millet sole crop system with 2.5 t/ha of manure
- Treatments **T_{22} to T_{26}** will be with pearl millet/cowpea intercropping system without manure

The organic manure is 2.5t/ha or 9 **kg/plot of 6mx6m (36 m^2)**

Table 1. Fertilizer rates kg/ha

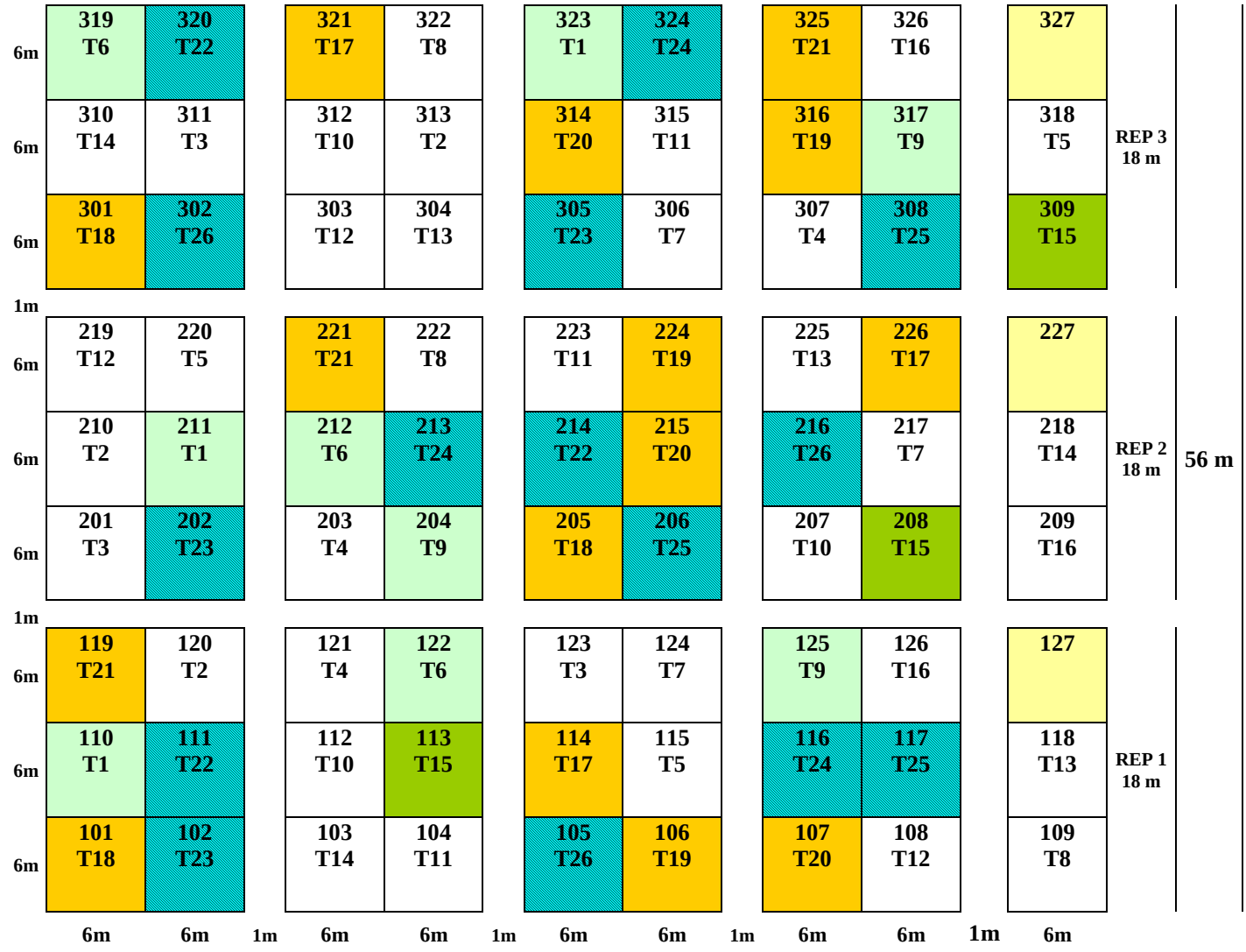
Treatments	N	P	K	S	Zn	Mg	B
	---------- kg /ha ----------						
T_1	0	0	0				
T_2	0	7.5	0				
T_3	0	15	0				
T_4	0	22.5	0				
T_5	0	30	0				
T_6	30	0	0				
T_7	30	7.5	0				
T_8	30	15	0				
T_9	30	22.5	0				
T_{10}	30	30	0				
T_{11}	15	22.5	0				
T_{12}	45	22.5	0				
T_{13}	30	22.5	10				
T_{14}	30	22.5	20				
T_{15}	30	22.5	30				
T_{16}	30	22.5	20	15	2.5	10	0.5
T_{17}	0	0	0				
T_{18}	0	7.5	0				
T_{19}	0	15	0				
T_{20}	15	15	0				
T_{21}	15	15	20				
T_{22}	0	0	0				
T_{23}	0	7.5	0				
T_{24}	0	15	0				
T_{25}	15	15	0				
T_{26}	15	15	20				

2.4 Experimental design

The experimental design is a Randomised Complete Block Design (RCBD) with 3 replications for the on-station trials. For the on-farm trial each of six farmers' field will be a replication.

OFRA/Niger:

Pearl millet On-Station Trial 2014



1 to 16 trts millet/cowpea intercropped with manure at 2.5t/ha

17 to 21 trts pearl millet sole crop with manure at 2.5t/ha

22 to 26 trts millet/cowpea intercropped without manure

soil sampling: 0-0-0;30-0-0; 30-22.5-0 T_1; T_6; T_9

plant sampling T_{15}

2.5 Crop management

Good crop management is essential to obtaining results needed for development of good nutrient response functions. A very important pre-caution is consideration of seed quality. Verify seed quality by conducting germination tests; discard seed if germination is less than 50% and otherwise adjust seed rates according to the germination percentage

2.5.1 Land preparation: generally the land will be hoe or plough tilled but reduced tillage or no-till options are acceptable if experience indicates this is a preferred or common practice.

2.5.2 Semis: Planting needs to be timely from the first decade of June**.**

Crop arrangement:

- ✓ Millet in sole cropping system : T_{17} to $T2_1$ (1m x 1m)
  between row spacing : 1 m and within row spacing 1 m
- ✓ Millet/cowpea intercropped : T_1 to T_{16} ; and T_{22} to T_{26}
  - Millet : 1.5 m x 0.5 m
  - Cowpea : 1.5m x 0.50m with 1 row of cowpea between 2 rows of millet

NB: Plots are 6m x 6m: the number of row are: six (6) for millet in sole crop system with 6 hills/row and 4 rows with millet/cowpea intercropping system with 12 hills/row.
For millet in sole crop system, the first hill will be at 0.5m from the border stake of the plot. For the millet/cowpea intercropping system, the first hill will be at 0.75m from the border stake of the plot. On each row the 1st and the last seed hole will be to 0. 5m of the stake limits of the plot**.**

2.5.3 Crop maintenance

- **Weeding**: it is important to have a good weed control. The 1st weeding must be as soon as possible before the weeds take over the crop. A second weeding must come 10 to 15 days after the 1st weeding. A 3rd weeding may be necessary
- **Thinning:** pearl millet must be thinned to 3 plants per hill and the cowpea to 2 plants per hill after the 1st weeding.
- **Fertilizer application:** P and K fertilizer must be applied and incorporated before planting. N fertilizer will split point application and incorporated; the first half rate application will be during the tilliring growth stage and the 2nd rate application will at the height growth stage (Cf. fertilizer rate per plot sheet).
- **Pest control** needs to include good weed control. Pesticide application with a systemic insecticide for stem borer control should be routine for maize and sorghum in most cases. Other pesticide applications should be as recommended or based on close monitoring of insect pest and disease pressure.

2.6 Data collection:

Data collection can be done recording on field book sheets with entry into Excel worksheets soon after or digitally with synchronized transfer to computer: see OFRA Trial Data spreadsheet

2.6.1 Activities such as: date and method of land preparation; planting information including date, variety, seed rate.

2.6.2 General observations on the condition of the crop. Ratings of damage (disease, pests, weeds, striga, animals, hail, etc.) and other observations on a whole trials basis or where observed if localized should be recorded. The effects of removed or present trees are often great and add to site heterogeneity; where these occur, indicate on the trial layout map and record in the General Observations worksheet the position of tree or trees.

2.6.3 Plot data. Plot level measured observations (*use printed labels adhered to all sampling bags to save time in the field and reduce chances of error*) will include:

- mid-season growth stage
  - sorghum and pearl millet-50% of plants have a panicle/head
- number of plants /panicles harvested for yield
- weight of harvested panicles /plants
- grain yield
- grain water content measured with a grain moisture tester; determine air-dried grain water content on a block rather plot basis
- stover yield for maize, sorghum, and pearl millet determined from the harvest area
- stover water contents (sub-sample, weigh air-dry, oven-dry, reweigh, and calculate water content of air-dried stover); determine air-dried stover water content on a block rather plot basis
- Collect and grind maize and sorghum grain and stover sub-samples for N analysis by IRS (**optional**); 5 g samples needed for analysis. Sample grain samples of legumes for N content (protein) determination. See Appendix 3 for instructions regarding handling of plant samples.

Obtain diagnostic plant tissue samples at near flowering stage from the NPK plots for non-legumes and bean, or PK for other legumes, (highest K rate treatment) applied plot (30 kg/ha). Sampling will be from the plots of treatment 15, T_{15} (Plots 114; 204 and 305)

- 10 flag leaves for pearl millet
- The leaf samples will be placed into clearly labelled paper (e.g., brown) bags and stapled.
- Begin drying as soon as possible, including in the vehicle with windows mostly closed while parked during the day
- Put in drying oven as soon as feasible to oven-dried at 60 ºC for 48 hours
- Mill the samples if possible and send 5 g milled samples to ICRAF-Nairobi for IRS analysis (see Appendix 3 for instructions).
- Sampling times
- Sorghum and pearl millet: upper fully extended leaf at heading

Weather: Daily rainfall at site or within 5 km for on farm trials, and minimum and maximum temperatures within 10 km. Other information is desired when available.

Obtain farmer yields for maize, rice, wheat, sorghum or millet from nearby fields depending on how important the crop is. Yield might be determined using the farmer's estimate of the quantity harvest and your measurement of the field area. Record in the worksheet "Site Description" of Excel file.

Card-index 1 Activities occurred on the level of the test

Please note all the farming operations on the trial with the dates

Date	Activities

Card-index 2 General Observations on the trial site

Please note all the observations (disease. Insects. Damage of animals etc :) dates and actions undertaken

Date	Observations	Carried out actions (please note the means used and the names of products and the rates)

OFRA/NIGER 2014 ON-STATION Pearl millet trial

FICHE DE COLLECTE DES DONNEES

STATION DE :……. **AGENT : ……..**

Rep	Parcelle	Trt	N rate	P rate	Krate	50% panicle	Harvested area (m2)	Number plants	Number panicles	Panicles weight (g)	Grains weight (g)	Stover weight (g)	Grain H_2O content	Stover sample weight (g)	Stover sample oven dried weight (g)
1	101	18	0	7.5	0										
1	102	23	0	7.5	0										
1	103	14	30	22.5	20										
1	104	11	15	22.5	0										
1	105	26	15	15	20										
1	106	19	0	15	0										
1	107	20	15	15	0										
1	108	12	45	22.5	0										
1	109	8	30	15	0										
1	110	1	0	0	0										
1	111	22	0	0	0										
1	112	10	30	30	0										
1	113	15	30	22.5	30										
1	114	17	0	0	0										
1	115	5	0	30	0										
1	116	24	0	15	0										
1	117	25	15	15	0										
1	118	13	30	22.5	10										
1	119	21	15	15	20										
1	120	2	0	7.5	0										
1	121	4	0	22.5	0										
1	122	6	30	0	0										
1	123	3	0	15	0										
1	124	7	30	7.5	0										
1	125	9	30	22.5	0										
1	126	16	30	22.5	20										
1	127														

Rep	Parcelle	Trt	N rate	P rate	Krate	50% panicle	Harvested area (m2)	Number plants	Number panicles	Panicles weight (g)	Grains weight (g)	Stover weight (g)	Grain H_2O content	Stover sample weight (g)	Stover sample oven dried weight (g)
2	201	3	0	15	0										
2	202	23	0	7.5	0										
2	203	4	0	22.5	0										
2	204	9	30	22.5	0										
2	205	18	0	7.5	0										
2	206	25	15	15	0										
2	207	10	30	30	0										
2	208	15	30	22.5	30										
2	209	16	30	22.5	20										
2	210	2	0	7.5	0										
2	211	1	0	0	0										
2	212	6	30	0	0										
2	213	24	0	15	0										
2	214	22	0	0	0										
2	215	20	15	15	0										
2	216	26	15	15	20										
2	217	7	30	7.5	0										
2	218	14	30	22.5	20										
2	219	12	45	22.5	0										
2	220	5	0	30	0										
2	221	21	15	15	20										
2	222	8	30	15	0										
2	223	11	15	22.5	0										
2	224	19	0	15	0										
2	225	13	30	22.5	10										
2	226	17	0	0	0										
2	227														

Rep	Parcelle	Trt	N rate	P rate	Krate	50% panicle	Harvested area (m2)	Number plants	Number panicles	Panicles weight (g)	Grains weight (g)	Stover weight (g)	Grain H_2O content	Stover sample weight (g)	Stover sample oven dried weight (g)
3	301	18	0	7.5	0										
3	302	26	15	15	20										
3	303	12	45	22.5	0										
3	304	13	30	22.5	10										
3	305	23	0	7.5	0										
3	306	7	30	7.5	0										
3	307	4	0	22.5	0										
3	308	25	15	15	0										
3	309	15	30	22.5	30										
3	310	14	30	22.5	20										
3	311	3	0	15	0										
3	312	10	30	30	0										
3	313	2	0	7.5	0										
3	314	20	15	15	0										
3	315	11	15	22.5	0										
3	316	19	0	15	0										
3	317	9	30	22.5	0										
3	318	5	0	30	0										
3	319	6	30	0	0										
3	320	22	0	0	0										
3	321	17	0	0	0										
3	322	8	30	15	0										
3	323	1	0	0	0										
3	324	24	0	15	0										
3	325	21	15	15	20										
3	326	16	30	22.5	20										
3	327														