USE OF NON-REPLICATED OBSERVATIONS AND FARM TRIALS FOR GUIDING NUTRIENT MANAGEMENT DECISIONS

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SUMMARY

Replication or repetition is the means by which experimental error is estimated in replicated trials. In farm trials, it is sometimes not possible to replicate demonstration and test plots due to manageability and costs. In these situations, non-replicated observations and on-farm trials are used. In addition, nutrient response studies are often needed to calibrate the fertilizer requirement of a field. In this paper, we will define on-farm trials and observations, discuss the nature and underlying principles of non-replicated trials and observations, briefly discuss analysis methods, and provide relevant examples of non-replicated data analyses using SAS procedures.

REPLICATED AND NON-REPLICATED TRIALS AND OBSERVATIONS

Observational studies are comprised of the observation of subjects and measurements of a variable of interest without assigning treatments to the subjects (Benson and Hartz, 2000; Concato, 2004). This approach is not commonly used for nutrient response studies but is useful in nutrient-depletion studies. In nutrient-depletion studies, a farmer or a researcher selects certain fields and then monitors the change in nutrient level over a specified time frame. In contrast, on-farm experimental studies are commonly used for determining major and micro nutrient rates, lime requirements, response of crops to organic amendments, and more. An experimenter deliberately imposes a treatment on a group of subjects (experimental units) with the goal of observing their response (Moore and McCabe, 1989). This is different from an observational study, which involves collecting and analyzing data without changing the existing conditions (Cochran, 1983).

Replication is one of the three pillars of experimental design (the other two are randomization and blocking). It helps to achieve integrity and reliability of experimental results (Kuehl, 2000). Replicating treatments in a trial enables the researcher to separate the true treatment effects from the background noise by absorbing experimental error (Johnson, 2006). However, there are situations where the replication of a treatment in different units is not possible, thereby forcing investigators to conduct non-replicated studies.

A non-replicated trial is an experiment in which a treatment or set of treatments is assigned to only an experimental unit, thereby lacking true replication of treatment. It is not uncommon to find studies that involve no replications (Machado and Petrie, 2006). Often investigators are forced to conduct a study without replication due to physical, financial, or social constraints. Physical limitations include land availability, plot size and inherent variability. Financial limitation arises due to high cost of treatments, units or implementation. Social constraints include a farmer's attitude and preferences toward treatment choice and the size of the area that the farmer can designate for the trial. These factors come into play not only for on-farm studies, but also in other situations (eg., greenhouse and growth chamber studies).

Some trials, such as, system level large scale trials, demonstration trials, and ecological studies are inherently impossible to replicate (Machado and Petrie, 2006). When it comes to nutrient management, historically non-replicated trials have been tremendously useful. Some of the oldest long-term experiments fully or partially dedicated to nutrient management were non-replicated, including the Rothamsted Classical experiment plots (the Braodbalk, the Hoos Barley, and the Exhaustion Land experiments, United Kingdom), the Morrow Plots (Illinois), the Magruder Plots (Oklahoma), and several others (Mitchell et al., 1991). These non-replicated studies were used at some point in the past to evaluate the effect of inorganic fertilizers, organic nutrients, and management practices on crop yield. These long-term studies unequivocally demonstrated that non-replicated plots can be sources and repositories of enormous amount of information.

TYPES AND PRINCIPLES OF NON-REPLICATED ON-FARM RESEARCH

Types of On-Farm Research/Observations

On-farm trials include exploratory, refinement, verification, farmer trials and demonstrations (Concato, 2004). The choice of the type of trial generally depends on the purpose of the study. On-farm nutrient management trials or observations can be conducted by a team of farmers, extension educators, consultants, or researchers depending on the type of study. Trial management and operation are the responsibility of both the farmer and researcher when the trial is initiated jointly. Furthermore, non-replicated on-farm trials must be simple.

The Elements

The key elements of a non-replicated trial include identification of the goal, problem, role, location, and scale. Similarly, it is crucial to understand and identify the necessary experimental design and analysis options. Whether the intention is to conduct an experimental or observational study, it is logical to start with the identification of the problem and then set a goal based on the problem. This process involves developing a series of questions that the researcher, along the farmer, would like to address through experimentation or observation.

Depending on the goal, the role played by the investigator and farmer, as well as their level of involvement, must be delineated as this affects the level of complexity of the intended study. The farmer may take part as a contractor, consultant, collaborator or a combination of any of these. The farmer is the adopter, and it is critical to involve him/her at all stages of the study. A Farmer can be actively or passively involved in on-farm trials. Andrews et al. (2002) applied these different elements of non-replicated studies in an on-farm assessment of soil quality in California's Central Valley. According to the authors, farmers helped in trial site selection, shared knowledge of field variability, suggested trial management options, assisted in implementation, and participated in report preparation.

Likewise, the location of the study is crucial when designing on-farm non-replicated studies. The field conditions, inherent variability, and management options affect where an on-farm study should be positioned within a field. Non-replicated on-farm trial or observation plots should be placed in a field where it is easy to access them, and where other cofounding effects are minimal. The location of the trail must not interfere with the movement of the farmer and cause damage to the soil. Equally important is the size of the area that the grower can dedicate for the observational or on-farm research. The scale of the study area is also related to the location and the specific situation of the farmer. A case in point is fertilizer recommendation demonstration plots for a 5-hectare gardener versus a 1,500-hactare wheat farmer. An on-farm trial for the

gardener could be performed in small area while it would require a large representative area to generate useful information for the wheat grower. The single most limiting factor for non-replicated on-farm research is the difficulty in estimating the experimental error, but this can be overcome by subsampling. The number of sub-samples depends on the scale and size of the field used for on-farm observation or experiment.

DESIGN AND STATISTICAL ANALYSIS OF NON-REPLICATED ON-FARM TRIALS

The conventional methods of variance-covariance parameter estimation cannot be employed to analyze non-replicated trials or observations due to lack of replication. However, thanks to the advances in statistical methods and computation, it is possible to analyze non-replicated data regardless of its nature. There are numerous design, analysis and computing options for non-replicated data depending on the nature of the design of the study. Here, we will review a few of these methods that can be applied to nutrient and crop management studies.

Analysis of Augmented Designs

Augmented designs have traditionally been used to analyze breeding materials at their early stage of development (Khan, 1991) and when seed is limited (Gonçalves et al., 2013). Some investigators included a soil fertility component to the design in order to compare breeding lines with and without fertilization (Lefkovitch, 1992). These procedures are not applicable to on-farm trials because the check used in the augmented design is also used to adjust the yield of other varieties (Khan, 1991). Consequently, the design assumes that all treatments respond similarly regardless of field conditions, which is not true in the case of on-farm studies.

Intraclass Correlation Coefficient Based Analysis

Intraclass correlation coefficients (ICCs) represent the correlation of two subsample units within one experimental unit, such as organic and conventional plots. The principles and mathematical descriptions of ICCs have been discussed at length in the literature (Eg. Perrett, 2004). Perrett and Higgins (2006) applied a modified form of the ICC to analyze non-replicated greenhouse data. This coefficient tests the homogeneity within groups. Their procedure exploited the relationship between the ICC within treatment variance, and between-treatment variance (Perrett, 2004). A pre-selected ICC (prior ICC) forms the basis of analysis of non-replicated studies with sub-sampling. Blair and Higgins (1986) showed that the method allows within treatment unit degrees of freedom to be used in place of experimental unit degrees of freedom for testing the hypothesis between treatment means when the population ICC is known. Perrett (2004) demonstrated the application of ICC in comparing the effect of two different soil preparation methods on the yield of corn. The steps in using ICC to analyze non-replicated trials or observations are as follows.

1) Establish an ICC from previous research using the following relationship (Perrett, 2004).

$$ICC = \frac{\sigma^2(b)}{\sigma^2(b) + \sigma^2(w)}$$

where $\sigma^2(w)$ is the pooled variance within subjects, and $\sigma^2(b)$ is the variance of the trait between subjects. The equation would apply if we knew the true values, $\sigma^2(w)$ and $\sigma^2(b)$. But these parameters can only be estimated from sample data.

2) Use the ICC information to analyze a data (Appendix 1a). This can be done in SAS using the MIXED procedure. However, for PROC MIXED to execute the analysis properly, three steps need to be followed.
    (a) Create global variables and values (see Appendix 1b).
    (b) Create a matrix and a dataset containing the ratios using the IML procedure in SAS (Appendix 1c).
    (c) Specify the GDATA= to suppress the traditional estimation method of the variance of the random effects.
3) Launch the MIXED procedure code (Appendix 1d) using the data (Appendix 1a).

This procedure is demonstrated using an on-farm study conducted in Oklahoma. The trial had three treatments laid on three farms (plots): Manure plus compost, compost and conventional fertilizer. Fourteen microbial respiration measurements were taken from each treatment. Each farm received only a treatment, and treatments were not replicated at each farm. The prior-ICC was identified from previous research to be 0.12. Details of computation, assumptions and reliability of the prior ICC are discussed elsewhere (Perrett and Huggins, 2006). The ICC value should ideally be less than 0.5 (on a scale of 0 to 1). Using this method, the results of the example study analysis are presented in Table 1.Machado et al. (2006) also applied this method to a non-replicated long-term cropping system study in Oregon.

Table1: Least square means and difference of least square means of respiration (ppm CO_2-C) for three non-replicated treatments.

Treatment	Least Squares Means		Treatment Pair		Differences of Least Square Means	
	Estimate	Pr> |t|	trt	trt	Estimate	Pr> |t|†
1-Comp + Manure	108.6	<0.0001	1	2	75.9	<0.0001
2-Inorganic	32.6	<0.0001	1	3	42.4	<0.0001
3- Compost	66.1	<0.0001	2	3	-33.5	0.0015

† Adjusted Tukey

Analysis of 2^k Non-replicated Factorial Designs

When a 2^k non-replicated study is a desired option for conducting a nutrient management study, analysis tools are available. Astatkie et al. (2006) used a non-replicated 2^5 factorial designs to assess the effect of N, P, K, compost and a seaweed extract. The analysis of the 2^k fractional factorials utilizes the pooled variance in the higher-order interactions into an error term and factors deemed to carry low variation (Aboukalam, 2005; Angelopoulos et al., 2012). Decision on which terms to use to construct the error term can be accomplished by following these four steps:

1. Estimate effects for the full factorial but without 3-or 4-way interactions
2. Make a half or full-normal probability plot of the estimates (this is a graphic tool that uses the ordered estimated effects) and identify which factors are important
3. Pool the effects clustered around "0" in the normal plot to construct the error term
4. Use this error term to test significance for the remaining model effects.

Once the effects that go into the error are identified, several analytical procedures can be employed. The SAS procedure ENTROPY or the ADX Interface®[1] (Ramirez and Ramirez, 2001) can be used to generate error term and perform hypothesis tests. Other SAS programs (GLM, ANOVA, REG, MIXED, etc) or analysis procedures in other software can be used for additional analysis. We will demonstrate the 2^4 fractional factorial design analysis using treatment structure and data (Appendix 2) from a hypothetical on-farm N, P, K, and S trial, with two levels for each nutrient (simulating a farmer and no limiting rate) for an average of 4444 kg ha^{-1}winter wheat yield. Nutrients considered were N (112 and 224 kg ha^{-1}), P (56 and 112 kg ha^{-1}), K (45 and 90 kg ha^{-1}) and S (34 ad 67 kg ha^{-1}). The data were subjected to analysis using the ADX Interface® in SAS. The normal plot and ANOVA showed that only five effects were important for further analysis (Figure 1).

The significant effects were the N rate, P rate, S rate, N rate x S rate, and P rate x S rate. All of the effects were highly significant ($p<0.001$), Table 2. The ADX Interface® analysis helped to identify the important factors, provided estimates based on normalized data and significance.

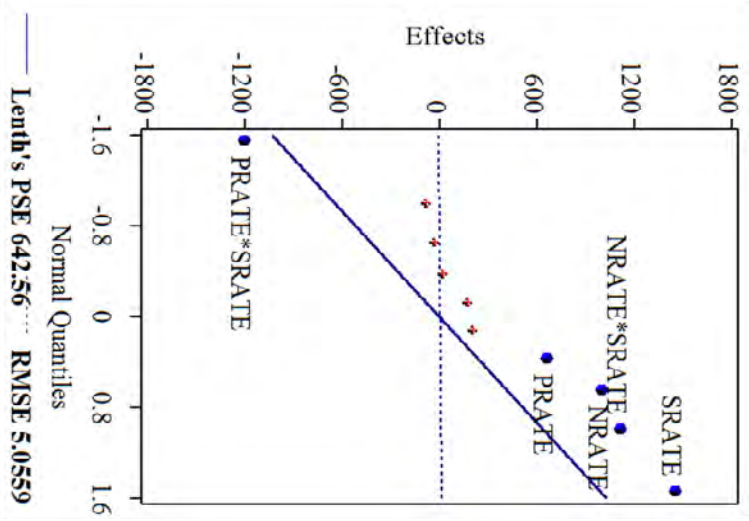

Figure 1. Normal probability plot of 2^4 fractional factorial effects. On the "effects" axis, values close to "0" were not important and thus can be pooled to the experimental error.

This section is not intended to provide statistical theories associated with 2^k fractional factorial experiments; it is provide an applied aspect of these tools for nutrient management research when replications are not possible. The theoretical frame for non-replicated factorial designs is documented (eg., Hamada and Balakrishnan, 1998).

[1] Mention of any company name or software does not constitute endorsement by the authors.

Table 2. Estimated mean differences (kg ha^{-1}) and corresponding probabilities for significant effects.

Term	Estimate	Std Err	t	Pr > |t|
N rate	991	148	6.621	<0.0001
P rate	655	148	4.471	0.0013
S rate	1445	148	9.791	<0.0001
N rate*S rate	1109	148	7.527	<0.0001
P rate*S rate	-1210	148	-8.206	<0.0001

Nutrient Decisions Based on the Management of Spatial and Temporal Variation

Spatial and temporal analysis tools are being used in precision agriculture for on-farm evaluations of nutrient management practices. The use of differential global positioning systems (DGPSs), yield monitors, and computer software allows for a detailed evaluation of treatment effects for different parts of a field (Lawes and Bramley, 2012). Spatial data are analyzed using several methods such as the Monte Carlo simulation (Plant, 2007; Martin et al., 2006). Precision nitrogen management using an optical sensor system is becoming common decision tool. Most of these "on-the-go" methods have built in variability management features and are designed to treat variation in a small area (Raun et al., 2005b). The optical sensor based variable application system is being used to recommend nitrogen fertilizer rates for a variety of crops including corn, wheat, cotton and sorghum (Raun et al., 2005a). There are also simplified forms of sensor based nutrient management tools. Examples of simple tools that manage temporal variability of a field include N-rich strips and Ramp calibration strips (Ran et al., 2005; Girma et al., 2006; Lawes and Bramley, 2012), which are being used by farmers in the United States and elsewhere.

Location as a Pseudo Replication

Multiple locations can be used as replications, but caution should be exercised, particularly when recommendations are developed for a domain where soil and rainfall patters are not uniform. Single plot N, P and K studies have employed this approach to obtain an estimate of yield response and economic optimum rate in the developing world. These trials are non-replicated, but a combined analysis across locations is used to guide fertilization rate recommendations.

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Appendix 1. Data (a), estimation of ICC from prior ICC (b), forming the matrix needed to generate variance (c), and the analysis of the data (d)

```
/** Appendix 1a- data set**/
data respiration;
input plot trt subp resp @@;
cards;
 1  1  1  90   1  1  8  78   2  2  1  45   2  2  9  21   3  3  1  59   3  3  8  71
 1  1  2  92   1  1  9  81   2  2  2  27   2  2  9  30   3  3  2  74   3  3  9  71
 1  1  3  115  1  1  10 106  2  2  3  45   2  2  10 28   3  3  3  59   3  3  10 73
 1  1  4  119  1  1  11 107  2  2  4  45   2  2  11 29   3  3  4  74   3  3  11 60
 1  1  5  134  1  1  12 106  2  2  5  21   2  2  12 23   3  3  5  60   3  3  12 78
 1  1  6  159  1  1  13 107  2  2  6  40   2  2  13 39   3  3  6  64   3  3  13 59
 1  1  7  107  1  1  14 119  2  2  7  33   2  2  14 31   3  3  7  60   3  3  14 64
;
/** Appendix 1b- create global variables**/
%let r0=.12; /** p0 = Plug-in ICC **/
%let pi=1; /** gi = # of classes per treatment **/
%let ti=3; /** ti = # of treatments **/
/** Apendix 1c- Create a matrix and a dataset containing the ratios **/
proc iml;
RATIO=((&r0/(1-&r0))*I(&pi*&ti));
create gratio from RATIO;
append from ratio;
quit;
data gratio;set gratio;row=(_N_);run;
/** Appendix 1d- Perform analysis using IML output and using Mixed Procedure **/
ods rtf;
proc mixed data= respiration ratio;
class plot trt;
model resp=trt/ddfm=kr;
random plot(trt)/ gdata=gratio Ratios;
/*lsmeans trt/pdiff adjust=bon;*/
lsmeans trt/pdiff adjust=tukey;
lsmeans trt/pdiff adjust=simulate(cvadjust report);
run;quit;
```

Appendix 2-Nutrient rates and yield used to demonstrate 2^4 non-replicated factorial experiments.

Run	Rate, kg ha^{-1}				Yield, Mg ha^{-1}
	N	P	K	S	
1	112	56	45	34	2755
2	224	56	45	34	2688
3	112	112	45	34	4301
4	224	112	45	34	4771
5	112	56	90	34	2957
6	224	56	90	34	2755
7	112	112	90	34	5107
8	224	112	90	34	4435
9	112	56	45	67	4502
10	224	56	45	67	6451
11	112	112	45	67	3763
12	224	112	45	67	5510
13	112	56	90	67	4099
14	224	56	90	67	6720
15	112	112	90	67	4099
16	224	112	90	67	6182