DESIGNING A LONG-TERM NITROGEN BALANCE EXPERIMENT

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ABSTRACT

The answers to many agronomic questions can only be obtained from long-term experiments in which the evolution of responses to experimental treatments can be observed for several years. In this paper, we describe our experience in designing and running an experiment set up to study changes in the nitrogen balance associated with a continual rotation of summer and winter crops grown on a Tokomaru silt loam following pasture. We emphasize the importance of careful definition of the objectives of a long-term experiment, and we discuss the role of mechanistic models in such experiments. We point out that it is most necessary to review the progress of the experiment critically, and at regular intervals, lest the objectives slip out of focus and the experiment degenerates into an exercise in data collection.

INTRODUCTION

There is an important category of agronomic experiments where long-term research is necessary. This category embraces experiments in which the effects of treatments are manifest only after periods of time running into years. The classical experiments of this kind are the continual cropping trials which have run in the United States for over ninety years (Russell, 1975; Richards, 1978), and in the United Kingdom for more than a century (Russell, 1961).

On a more modest scale, examples of long-term agronomic experiments in New Zealand include the work of Sears *et al.* (1965) on repeated cropping following pasture, the study of Douglas *et al.* (1972) on crop rotations involving maize, and the investigations of the long-term effects of cultivation techniques by Sims (1978).

The common feature of all these experiments is that they were concerned with the evolution of responses to treatments over relatively long periods of time. In contrast, the great majority of agronomic experiments are concerned with the comparison of treatment effects over a relatively short time period, commonly within a season or a year. Although many of these experiments may be repeated in several years, this amounts to replication of the experiment in time rather than a study of changing responses to experimental treatments. In this paper, we shall take the latter feature, the evolution of response over several years, as the distinguishing characteristic of the long-term experiments we wish to discuss. We note that relatively few of the crop agronomy experiments described in the New Zealand literature in recent years meet our criterion, whilst a number of grassland experiments, particularly those on the farmlet scale, do. However, we shall restrict our discussion to long-term crop agronomy experiments. Our objectives in this paper are to outline the factors we considered in designing such an experiment, and to discuss some of the difficulties we have faced in running it, in the hope that our experience will benefit others.

A LONG-TERM STUDY OF THE NITROGEN BALANCE

A long-term nitrogen balance study was initiated at Palmerston North in 1976. The immediate objective of this experiment is to quantify components of the nitrogen balance of a continual double cropping system. A rotation of summer and winter crops (originally maize/oats, now barley/oats) has been established on a paddock which had previously been under pasture for five years. The soil is a Tokomaru silt loam, an Aeric Fragiaqualf. Three treatments are being used to create long-term changes in nitrogen status: application of nitrogen fertilizer to the summer crop with return of above-ground crop residues, application of nitrogen with minimal return of residues, and no nitrogen and minimal return of residues. The major emphasis at present lies with the second and third treatments, where the components of the nitrogen balance are being monitored at frequent intervals.

Defining the problem and setting objectives

The motivation for the study stemmed initially from the need for increased understanding of the nitrogen requirements of forage cropping systems involving both winter and summer crops. In most New Zealand farming systems, forage crops are used in short-term rotations between periods in pasture, with little consequent need for additional nitrogen. With the prospect of larger-scale, longer-term implementation of forage cropping (Stephen and McDonald, 1978; Taylor and Hughes, 1978), and with evidence of responses to nitrogen appearing after a few years, even on soils with relatively high organic matter status (Sears *et al.*, 1965; Douglas *et al.*, 1972), there is an obvious need to define more precisely the nitrogen fertilizer requirements of double cropping systems. We started, therefore, with the question - how do the nitrogen fertilizer requirements alter during a continual sequence of crops?

To answer this question in a practical fashion, several pieces of information are required. First, we need to know what levels of available N are necessary so that lack of N does not limit crop growth. If we can define this requirement, we must then determine how much N can be supplied from the soil. The difference, suitably weighted to account for incomplete utilization, defines the fertilizer requirement (Parr, 1973; Stanford, 1973) thus:

$$N_f = (N_y - N_{nm} - N_{t1})/E \quad (1)$$

In (1), N_f is the N fertilizer requirement, N_y is the requirement for optimum crop growth, N_{nm} is net mineralization of N from soil organic matter, N_{t1} is the mineral N (NO_3^- and NH_4^+) in the root zone at planting, and E is the fraction of N_f recovered by the crop, an efficiency factor.

Since there are four quantities in (1) which must be known before N_f can be calculated, a worthwhile experimental objective would be to estimate their values. Unfortunately, difficulties immediately become apparent. Although N_y, and possibly E, might be fixed quantities for given crops and soils, N_{nm} and N_{t1} are not. Both the residual N in the profile at planting, and the net amount of mineralization are likely to change with the number of crops after pasture, with season, and with soil type. Furthermore, a measure of N_{t1} at the beginning of the season and an estimate of N_{nm} would only be of use if all mineral N in the profile were available to the crop. This is not likely, particularly for a winter crop where leaching and denitrification can occur.

These considerations led us to a closer examination of what we meant by "mineral N available to a crop". Plants draw their nitrogen from a pool of soluble N in the soil. This pool is subjected to the various losses, gains and exchange processes which are summarized in figure 1. Since the law of conservation of matter must hold for any species of N, we know that

(inputs) – (outputs) = change in storage (2)

for any defined pool. Let us consider the mass balance for the mineral N over the whole of a growing season within the root zone of a crop. We write

$$(N_f + N_m) - (N_v + N_c + N_d + N_l + N_i) = N_{t2} - N_{t1} \quad (3)$$

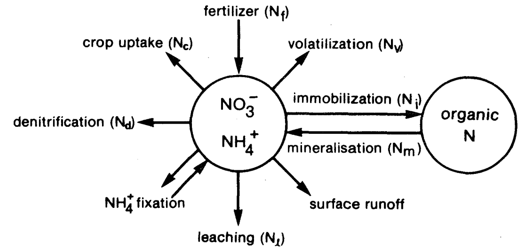
where the terms on the left-hand side are defined in figure 1, and where N_{t1} and N_{t2} are, respectively, the amounts of mineral N in the root zone at the beginning and at the end of the growing season. (In writing (3), we have assumed that the net exchange of NH_4^+ and loss of mineral N by runoff are zero). Matching (3), we can write a mass balance for the organic nitrogen in the root zone:

$$N_i - N_m = N_{ot2} - N_{ot1} \quad (4)$$

Here, N_{ot1} is the soil organic N present at the beginning of the season and N_{ot2} is that present at the end.

Equations (3) and (4) provide a summary of the changes which can occur during a growing season. We expected that the relative importance of the terms in (3) and (4) would vary from summer to winter, and that we would observe trends occuring in the terms as the experiment progressed. We found relatively little information had been published on these changes in New Zealand and knew of no experiment in which a complete N balance had been attempted for a crop. We decided, therefore, that a primary objective of our experiment should be to quantify the N balance of the double cropping system and to measure the changes which occurred in this balance during a sequence of crops.

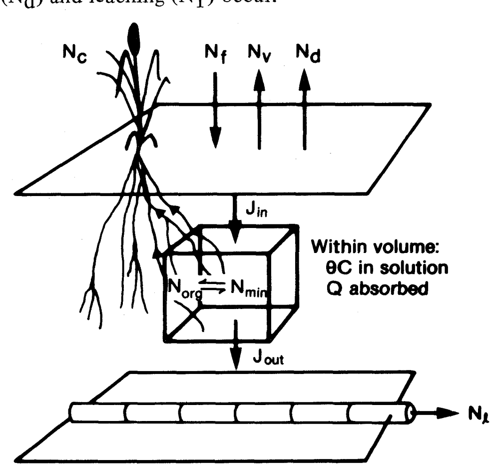
Figure 1: Schematic representation of the possible gains, losses and exchange processes affecting the pool of soluble nitrogen (NO_3^- and NH_4^+) in the soil.



Although equations (3) and (4) summarize the processes which affect the N available to crops, they tell us nothing of the nature of dynamics of these processes. Furthermore, because the equations give only a static accounting of gains, losses and exchanges they have no predictive value – we cannot use them to calculate the fertilizer requirement of a crop. This deficiency made us consider the dynamics of soil N in more detail.

The theoretical basis for models of the dynamics of nitrogen in the soil is well established (Gardner, 1965; Boast, 1973). If we apply the accounting procedure of (2) to a small volume of soil (figure 2),

Figure 2: Processes affecting the nitrogen balance of a small volume of soil. Within the volume, there is an amount Q of soluble N absorbed on soil surfaces and an amount ΘC in solution (where Θ is the volumetric water content and C is the concentration). Soluble N moves through the soil volume with the downwards flow of soil water. The influx, J_{in} (mass per unit area per unit time), may be greater or less than the efflux, J_{out}, with the result that soluble N may accumulate in or be removed from the volume. The activity of roots, and the processes of mineralization and immobilization, also alter the amount of soluble N in the volume. At the soil surface, and at the level of the mole drains, the boundaries of the system, the processes of uptake into above-ground parts (N_c), fertilizer addition (N_f), volatilization (N_v), denitrification (N_d) and leaching (N_l) occur.



we obtain a partial differential equation for the mineral N in the volume:

$$\frac{\partial(\Theta C + Q)}{\partial t} = \frac{\partial J}{\partial z} + S \qquad (5)$$

In equation (5), Θ is the volumetric water content, C is the concentration of mineral N in the solution phase, Q is the concentration of N in the absorbed phase, J is the vertical flux of N, and S is the rate of production or uptake of N per unit volume. This equation states that the time rate of change of N per unit volume (both absorbed and in solution) equals the difference between inflow and outflow (the flux divergence, $\partial J/\partial z$) plus any sink or source term within the volume.

Equation (5) provides a framework within which we can fit all the processes which affect soil N. The important processes which occur *within* any soil volume are downwards movement of N with soil water, root uptake, and mineralization and immobilization (figure 2: as suggested in this figure, the other processes affecting the N balance of equation (1), fertilizer input, and gaseous and leaching losses, can be treated as if they occur at the boundaries of the system and need not concern us here.). These processes can all be described mathematically. Thus, the N flux divergence term of (5) can be written (Boast, 1973) as

$$\frac{\partial J}{\partial z} = -\frac{\partial}{\partial z}\left(D\frac{\partial C}{\partial z}\right) + \frac{\partial(vC)}{\partial z} \qquad (6)$$

In (6), the first term describes the movement of N due to dispersion during water flow and the second, the movement due to simple mass flow of water; D is the dispersion coefficient and v the downward rate of water movement. Equation (6) accounts for movement of N through a soil volume: the production or removal of N within the volume requires another equation which may take the form (Jury *et al.*, 1976)

$$S = (S_{nm} - CS_w)/\Theta \qquad (7)$$

Here, S_{nm} is the net rate of mineralization per unit volume of soil and CS_w is the uptake of N by roots associated with a rate of water uptake per unit volume of S_w. Both these quantities can be described in more detail. For example, Stanford and Smith (1972) related S to a potentially mineralizable fraction of soil organic N, N_p, using the equation

$$S_{nm} = \frac{dN_{nm}}{dt} = k(N_p - N_{nm}) \qquad (8)$$

where k is a temperature and soil moisture dependant rate constant.

Equations (5) to (8) describe the dynamics of some of the processes affecting the availability of N to crops. It was obvious to us that our knowledge of these dynamics was meagre and that relevant data for New Zealand soils were scanty. We therefore set ourselves a second objective - to understand the dynamics of the processes underlying the N balance. By "understand" we mean, ultimately, to be able to specify the correct form of equations such as (6), (7) and (8) and to provide estimates of the parameters (e.g. D and k) contained in them.

Equation (5), in combination with equations for the constituent processes it embodies, is the basis of all models of the N balance of ecosystems. In most cases, these models are solved using a computer (e.g. Jury *et al.*, 1976; Saxton *et al.*, 1977; Watts and Hanks, 1978). The output of the models, the numerical solution of equation (5), is a prediction of the distribution of mineral N as a function of depth and time. In addition, the models lead to estimates of quantities such as crop uptake and leaching as functions of time. The obvious value of the models lies in the opportunity they afford for testing hypotheses, such as those expressed in equations (6), (7) and (8), individually and in combination. For this reason, the third objective we set was to construct a model of the system which incorporated the knowledge we gleaned about the important operative processes in the N balance.

A computer model of the dynamics of N in the cropping system would have educational value, but it would not, of itself, enable us to answer our original, practical question about the amounts of N fertilizer which might be required. However, it is possible to combine the information derived from a model with an estimate of the optimum N uptake of crops and to arrive, thereby, at a prediction of fertlizer requirements (e.g. Barnes *et al.*, 1976). If the processes built into this overall model have been characterised adequately, fertilizer predictions could be made with considerable generality; that is, in a manner which is not restricted to the environment in which the model was developed. This would fulfil the original purpose of the project. Thus, there remains a final objective - to determine the potential N requirement of crops and to combine this information with that derived from our model in order to attempt to make predictions of actual fertilizer needs.

Designing the experiment

In an initial outline written for this project, it was proposed that a straightforward comparative experiment be carried out. The design suggested was a split-plot, with three levels of N applied to the winter cereal in the main plots, and three levels of N applied to the summer cereal in sub-plots, all within four randomized blocks. This gives the statistical model

$$X_{ijk} = \mu + W_i + B_j + \epsilon_{ij} + S_k + SW_{ik} + \partial_{ijk} \qquad (9)$$

where W_i stands for the effect of the ith winter (main) treatment, B_j for the effect of the jth block, S_k for the effect of the kth summer (sub-plot) treatment, and ϵij and ijk are normally distributed errors. The X_{ijk} could be any measured quantity but, in the initial outline, it was assumed that crop yield would be the most important of these quantities.

Equation (9) is an example of the most basic tool of agronomic research, the regression model leading to an analysis of variance. Almost without exception, the work published on N in New Zealand in recent years has been based upon analysis of variance models, or upon closely-related regression models. Despite the popularity of such models, we decided eventually that they were not appropriate.

We had two reasons for this decision. The first arose because we were concerned with the evolution of N responses over several years. Although we could have repeated the split-plot experiment in successive years (as was proposed in the initial outline), it seemed quite unlikely that this would lead us to an understanding of the causes for any responses detected in our analyses of variance. The reason for this opinion lies in the nature of the analysis of variance model. Equation (9) and its ilk do not incorporate hypotheses about the nature of the system being studied, in the manner achieved by equations (5) to (8). As Rose (1975) observes, the analysis of variance model is designed to allow the interpretation of experimental results: it is not designed for studying how a system functions.

It could be argued that the usefulness of an experiment based upon (9) would have been enhanced by the collection of ancillary data on changes occuring within treatments. Thus, had significant differences in crop yields shown up in an analysis of variance, these might have been related to ancillary data, such as soil mineral N within each treatment. The counter to this argument is that equation (9) offers no means of relating ancillary data to primary responses quantitatively. With a statistical, rather than a mechanistic model, we are left with a gap which can be bridged only by qualitative discussion. We note, in passing, that this disjunction between responses and mechanism is often a feature of agronomic research.

The second reason for avoiding a conventional agronomic design was that the results of such experiments are irrevocably site-specific and year-specific (Collis-George and Davey, 1960; Rose, 1975). We had the resources to carry out an experiment at one site for a limited number of years, yet we had the ambition to arrive at conclusions with wider applicability. Since the results of statistically based experiments can be related to other sites and seasons only if conditions are analogous (Rose, 1975), we did not feel the approach to be useful. Although experiments based upon mechanistic concepts are also year- and site-specific, it is possible to build relevant characteristics of site and season into the models which are developed from them. Thus, we chose to base our experiment on process-oriented concepts in the belief that the results from such an experiment are potentially more widely applicable than those from statistically based experiments.

This choice dictated the design of the experiment. Since our objectives are to quantify and to gain a deeper understanding of the N balance, emphasis must be placed upon frequent samplings of the components of the balance. At the same time, the manpower available to run the experiment is limited, so that detailed sampling can only be done on a few treatments. A further limitation arises from the need to measure losses of soluble N through leaching: the most convenient means of measuring these losses is by sampling drainage water, so that experimental blocks must be large enough to accommodate separate mole and tile systems.

For these reasons, the N balance experiment is being carried out on large (0.3ha), unreplicated blocks. One of these blocks has been left in pasture to serve as a long-term control and a second is used for small trials of various sorts. Experimental treatments have been imposed on the remaining three plots. On two of these areas, we mimic a double forage cropping system by cutting and removing all above-ground material; on the third, this material is chopped and returned.

The main emphasis in the experiment lies, at present, with the two plots from which above-ground material is removed. In the first two years of the experiment, these were treated as one unit and we aimed to lower the N status as rapidly as possible. Last summer (1978/79), we split the blocks and began to apply N to one, with the object of creating two areas which gradually diverge in their N fertility. We are attempting to establish a nitrogen balance for each of these blocks; a summary of the measurements made, and of the current state of the experiment is given in Table 1.

Our experiment is extremely simple in design. Because the emphasis of the experiment lies in frequent samplings of components of the N balance for each block, and because we are dealing with a fairly large area within each treatment, we do not see the lack of replication as a disadvantage. In fact, the comparative aspect of the experiment is relatively unimportant. We aim to explain what happens within each of the two main treatments, whether or not they give rise to statistically significant differences in crop production. Thus, we are running, in essence, two separate but contemporaneous experiments.

Our experiment is also flexible. Although we cannot split the main blocks and still measure leaching losses, we can alter what we do within a treatment quite freely. We have already changed the summer crop from maize to barley after experiencing some difficulties growing the former; this matters little because we see the crops primarily as nitrogen-removal mechanisms. In future, we may add N to the block which presently receives none if crop growth becomes too poor. The experiment can accommodate alterations of this sort because we are keeping track of changes in the N balance as they occur. Since long-term experiments are evolutionary in nature, it seems desirable to make them flexible and this is easiest to achieve if their design is kept simple.

Running the experiment

The N balance experiment is a joint Massey University/DSIR project. There are six people, three scientists, two technicians and one Ph.D student, involved in running the N balance experiment, but only the student spends most of his time on the project. In the first two years of the experiment, the efforts of these people were ill-coordinated and some useful data were not collected, or collected poorly. To ensure that the experiment runs more efficiently, one of us (P.E.H.G.) now acts as a coordinator. His job is to assign responsibilities, to check that data are being collected, to ensure that data processing occurs, and that reports were written, and to arrange meetings to assess progress. We hold these meetings at about three-monthly intervals, but should probably hold them at monthly intervals in order to keep the momentum of the experiment going.

Inevitably, in a long-term experiment, changes in personnel occur with staff turnover, periods of leave, and, in the case of universities, the graduation of students. Several difficulties result: sampling techniques may alter, analytical work may vary in degree of precision, samples may be misplaced, and gaps in the collection of data may occur. All these

TABLE 1: Summary of data collected and of present state of knowledge in the N balance experiment. Quantities quoted for terms in the mass balance are based on incompletely processed data.

Quantity in Mass Balance	Method of Measurement	Approximate Values	Details of Process	Present State of Knowledge
Fertilizer input, N_f	–	100kg/ha plot 1/zero, plot 2	Utilization by crop	^{15}N studies initiated
Net mineralization $N_m - N_i$	Change in organic N, N_o over season	75kg/ha/y plot 1/100kg /ha/y, plot 2	Potentially mineralizable N, T and soil Θ dependence	Experiments under design
Volatilization	–	?	–	–
Crop uptake N_c	Yield and N content weekly	Summer: 110kg /ha, plot 1/ 70kg/ha, plot 2	Growth stages; root distribution; LAI; environmental forcing factors	Data collection; models required
Denitrification N_d	–	≈O by crude balance	–	Field measurements required
Leaching N_l	V-notch weir and N content	60kg/ha/y from fallow/ subject to weir calibration	ET water balance deep percolation; movement in cracks; soil physical properties	Data collection; model development
Mineral N N_f	Soil cores weekly	−50kg/ha/y winter/+50kg /ha/y summer	–	Model for C(z, t) under development

things have affected our experiment. In response, we have tried to make sampling as regular, as straightforward, and as standard as possible.

The most serious deficiency in the conduct of the experiment to date is that it has not been subjected sufficiently to regular, critical review. This review should focus upon progress towards objectives and should be the responsibility of all participants in the experiment. Review is only possible if data are processed: we have discovered it is easy to fall into the trap of accumulating quantities of data which are too raw to be used to guide decisions about the next step in the experiment.

It is possible to collect both too few and too many data in a long-term experiment. Data may be too few in number or in kind. We have endeavoured to collect sufficient data by asking ourselves how large samples must be to allow us to detect likely changes. For example, we decided we needed to detect seasonal changes in soil organic N of the order of 250 kg N N/ha. What a guess of 15% for the coefficient variation, we calculated (Snedecor and Cochran, 1967) that at least 120 samples were required in order to detect this level of mineralization at the 5% level of confidence. Unfortunately, the care taken over our sample sizes was, in one case, negated by failure to collect matching bulk density data - a deficiency of kind rather than number.

Collecting too many data occurs because of the natural tendency to cover oneself against the unforseen. However, in an experiment which lasts a long time, and with limited resources, this cannot be justified. We have learnt to ask ourselves why we collect any type of datum. In one case, this question led to a considerable saving in effort. We started by measuring soil NO_3^- and NH_4^+ levels on 20 individual samples taken each month. This gave us adequate data for comparison of treatments, but very little resolution for the time trends which were really more important. Now, we take 10 samples per treatment each week and bulk them. The mineral nitrogen determined on the bulked sample should be within two ppm of the true value, and we have 52 points in the year to show time trends. This alteration reduced the number of NO_3^- and NH_4^+ analyses required by a factor of 0.75.

DISCUSSION

All research appears much more systematic once the dust of planning, execution and analysis has subsided, and the experiment has been written up in the peculiarly terse and ordered prose of science. In this respect, our description of the planning and of the early stages of execution of the N balance study is no exception: there were more blind alleys and a much greater imprecision of thought than is revealed in this paper. In retrospect, it is obvious that there is much we could have done differently and better. There is also much room for improvement at present. However, we have gone far enough to draw some lessons from our experience.

First, we have been reminded of the importance of defining the connections between practical problems and the research questions they spawn. Experiments in agronomy, and long-term experiments in particular, are often set up to answer ill-defined questions which stem directly from some problem. The level at which these problems exist for the farmer, the advisory officer, or the agricultural economist, is often far removed from the level at which the scientist can perform experiments, with the result that his research may seem to be of little direct relevance. To overcome this difficulty, the scientist must be clear on the manner in which his research can be connected to the practical problem. In our experiment, the level at which we can work, and use those scientific skills we have, is that of quantifying and understanding the N balance - our first two objectives. The "how it can be connected" part of the project lies, we hope, in our third and fourth objectives.

The second lesson we have learnt is that there is great value in having process-oriented models to guide research. Long-term experiments are usually concerned with problems which show up at a "whole system" level, and therein lies the difficulty. We have some idea how to apply the analytical methods of science to bits of the system, and yet, if we study these simplified, isolated bits, we lose understanding of the functioning of the whole system. A process-oriented, mechanistic model helps us out of this impasse, for it enables us to place the bits we can study within the context of the whole system. This is not possible with a conventional statistical model. An additional value of a mechanistic model is that it cannot be formulated without precise thinking about the nature of the system being described. We recognise that it is not always easy to formulate a model; the N balance model given in (5), for example, is straightforward compared to the sort of model which would be required for an experiment dealing with the long-term effects of changes in soil structure. Nevertheless, the attempt to construct a mechanistic model would bring much-needed rigour to many agronomic experiements. To this we add a word of caution: we are not advocating modeling by computer simulation; we are suggesting that more use should be made of process-oriented models, which may or may not involve some use of computers, in agronomic research.

The third lesson we have learned is that it is essential to review the progress of a long-term experiment regularly and critically. Without such review, it is all too easy for the experiment to acquire a mindless life of its own as it drifts into data collection for the sake of more data. Regular review of progress keeps the experiment on track and identifies areas where more work is required as knowledge increases. Critical review helps to ensure that most appropriate data are being collected in the right quantity. It is important to avoid the temptation to measure everything in the hope that "it might be useful later", or in the fear that something important may be overlooked. It is equally important not to miss measurements through sloth or oversight: long-term experiments are costly and they are difficult or impossible to repeat. It is not easy to attain this balance between too few and too many data. The key lies in analysing data as they are collected. Allowing data to accumulate without analysis is a certain recipe for diminished success. Thus, in a long-term experiment, if not in all experiments, the desk-work of data analysis is at least as important as the field-work of data collection.

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