A QUANTITATIVE ANALYSIS OF THE VARIABILITY IN THE ACTIVITY OF NITRIFYING ORGANISMS IN A SOIL UNDER PASTURE

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ABSTRACT

Variability in the inputs, outputs and transformations of mineral N under field conditions makes the predictive modelling of the leaching of soil nitrate very difficult. In an attempt to understand and quantify this variability, the activity of nitrifying organisms in the Tokomaru silt loam (a Typic fragiaqualf) under pasture was measured using a short-term nitrification assay (SNA). Spatial dependence of the variability in SNA was examined using geostatistical methods, and the effect on SNA of soil pH change through liming, and of seasonal changes in soil temperature and moisture were investigated.

Nitrifier activity and associated soil properties such as the amount of exchangeable ammonium and the soil nitrate concentration, were found to decrease in value with depth between 0-24 cm. The greatest decrease in SNA was observed between 0-9 cm depth, but due to the need for sufficient quantities of soil to make SNA measurements, and the desire to avoid the possibility of inhibitory effects of grass roots on nitrification, soil was sampled from the 3-9 cm depth range for the bulk of the work reported here. Results indicated that the technique of sieving and mixing samples was satisfactory for removing depth-dependence from the results for spatial variability and other analyses.

The spatial variability of SNA, soil NO_3^-, soil moisture content and the pH of the SNA incubation, which was assumed to approximate the field soil pH, was investigated over areas of 9 m^2 and 625 m^2 using a regular 11 x 11 square grid sampling design with minimum sample separations of 30 cm and 2.5 m respectively. However, the results of these analyses proved inconclusive, apparently due to the lack of samples separated by lags that were sufficiently short in relation to the overall dimensions of the sampling area. Accordingly, spatial analysis of the above properties, together with exchangeable ammonium, was carried out over 625 m^2 using a *nested* sampling design that permitted an adequate number of observation points at lags ranging from 12.5 cm to 25 m. This design was a considerable improvement on the regular square design, although it had a number of shortcomings, notably bias caused in the estimation of the sample variance due to the nesting of a large number of data points

within a small area, and bias caused in the estimation of values of the semivariance at some lags due to missing sampling points at some positions in the sampling grid.

The values of SNA, NO_3^- and exchangeable ammonium were all highly variable and conformed to lognormal distributions. The range of spatial dependence in the variability of SNA, soil NO_3^- and incubation pH was 2.4, 5.4 and 6.1 m respectively. Exchangeable ammonium, SNA, soil NO_3^- and incubation pH varied isotropically within the sampling area but Ex-NH_4^+ showed no spatial dependence. Soil moisture content was strongly anisotropic, and showed no spatial dependence in one direction, but clear evidence of drift in a perpendicular direction. These results are discussed in relation to the most efficient sampling strategy for estimation of the mean field NO_3^- concentration. It was concluded that sufficient small localized clusters of samples should be taken to give a low standard error of the mean, with each cluster separated by at least 5 m. In the case of the Tokomaru silt loam, 20 clusters, each comprising 5 samples (bulked), would be required for estimation of the mean field nitrate concentration with 95% probability of $\hat{\mu}$ being within ± 5% of μ, the true mean. This represents a large sampling effort.

The activity of nitrifiers was studied in relation to soil pH and seasonal changes in soil moisture and temperature over two consecutive years in an attempt to explain the spatial variability in SNA values. The pH optimum for nitrifier activity (pH_{opt}) was defined for four variates of the Tokomaru silt loam with different liming histories. Values of pH_{opt} which varied between the four soils in the range 5.92-6.45 did not vary markedly with season, and it was found that the form of the relationship between SNA and pH remained constant with time. It was further observed that the addition of lime in 1987 had the effect of raising the mean soil pH and pH_{opt} in previously unlimed soil, but had negligible effect on either the soil pH or pH_{opt} in soil that had been limed in 1982. The significance of heterotrophic relative to autotrophic nitrification could not be discerned.

No significant relationships could be found for the four soils between soil pH, pH_{opt}, SNA, soil moisture content and soil temperature at 30 cm depth. Values of SNA (μmol N g^{-1} soil h^{-1}) at pH_{opt} (SNA_{opt}) were calculated from equations fitted to plots of SNA *vs.* the pH of SNA incubation, and these show a more obvious seasonal trend. SNA values calculated for the prevailing soil pH (SNA_{pH}) were never very different from values of SNA_{opt} and follow a 1:1 relationship over a range of values from 0.015-0.110 μmol g^{-1} h^{-1}; that is, the nitrifier activity in the soil, irrespective of variations that were random (unknown influences) or associated with seasonal variables (temperature and moisture), was near the optimum with respect to the soil pH at the time of sampling.

The effect of soil moisture variation on nitrifier activity was further investigated in an experiment in which soil samples were stored for 124 days at different soil moisture tensions. The optimum moisture conditions for nitrifier activity in the Tokomaru silt loam prevailed at pF 3.39. However, this optimum was less clearly defined than was the pH_{opt}. Since the soil moisture status changes considerably with season, whilst soil pH does not, it was concluded that nitrifiers were more tolerant of changes in pF than changes in pH.

Comparison of these with published results indicates that not only is the soil nitrifier population dynamic, and changes in response to changes in its environment, but the degree to which nitrifier activity is affected by various soil properties is soil-specific. It is therefore concluded that the spatial variability of nitrifier activity will also be soil-specific, and that different soils are likely to have different ranges of spatial dependence for the parameters of mineral N. Furthermore, the fact that SNA is not the only factor governing the soil NO_3^- concentration, and that other factors such as plant uptake and leaching are also important, indicates that SNA variability is not necessarily a good estimator of soil NO_3^- variability. This conclusion is certainly supported by the geostatistical aspects of this work.

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CHAPTER 1

INTRODUCTION AND THE AIMS OF THE PROJECT

i. Background to the project - the nitrate leaching problem

The annual world consumption of fertilizer nitrogen has been variously estimated at 60 M t (Hauck, 1988) and 73 M t (Douglas & Cochrane, 1989). Douglas and Cochrane (1989) estimated that an increase in consumption of fertilizer nitrogen of 17-18 % was needed annually to maintain world food supply, whilst Hauck (1988) more conservatively estimated that consumption by the year 2000 would be 100 M t. On top of this, 90 M t of N is added to agricultural and pastoral systems annually through biological fixation of nitrogen (White, 1989b) which comprises 79 % by volume of the Earth's atmosphere. The distribution on a global scale of this addition of fertilizer N is by no means uniform, 58 % of world consumption being in developed western countries, 35 % in countries with centrally controlled economies, and 7 % in the developing countries. Although the N-fertilizer market is stable in some parts of the world such as Europe, consumption in others is increasing rapidly - in China for example, by as much as 27 % for the year 1987/88 (Douglas & Cochrane, 1989). Against this background of increasing and uneven N consumption, the environmental consequences of high fertilizer application, in particular, are gaining increasing prominence in the public eye, to the extent that in Western Europe, the eutrophication of streams and waterways and nitrate pollution of potable water supplies due to leaching has become something of a *cause célèbre*.

Figures for N consumption and nitrate leaching in New Zealand are scarce, although the use of fertilizer-N in New Zealand is only small, being tied to *user-prosperity* more than any other factor (Douglas & Cochrane, 1989). Indeed, most N.Z bull beef farmers, for example, do not as a rule apply N fertilizer to their pasture, and tend only to apply it as a rescue

mechanism in times of low production (Baars *et al.*, 1989). In contrast, during 1978 in Britain, the total input of *new nitrogen* to agriculture was about 2 M t for the year, comprising 0.2-0.4 M t from biological N_2 fixation, 1.15 M t as fertilizer, at least 0.28 M t from precipitation and 0.18 M t from imported animal feedstuffs (Thomson, 1985). All of this except the animal feedstuffs (i.e. 91 %) can be regarded as a direct application to land; the feedstuffs represent an indirect application (Ball & Ryden, 1984). In the U.K, the fertilizer component applied to arable crops is almost all applied in spring, whilst pastures are fertilized through the summer aswell. It is therefore not surprising that the level of nitrate in water supplies often exceeds the so-called *acceptable* concentration. This is especially the case when one considers that 37 % of the grassland in England and Wales now receives more than 200 kg N ha^{-1}, and 5 % receives more than 400 kg N ha^{-1} (Ryden *et al.*, 1984). The acceptable range of N in drinking water according to the World Health Organisation is 11.3-22.6 mg N dm^{-3} (W.H.O., 1970) whilst the European Community treat 11.3 mg N dm^{-3} as a permissible maximum but have a *guide level* of 5.7 mg N dm^{-3} (E.E.C., 1980). All these figures were greatly exceeded by the concentration of nitrate in water draining from the experimental plots of Ryden *et al.* (1984) and were somewhat similar to the mean annual concentration of $\overline{NO_3}$-N in water draining from the lysimeters of Dowdell *et al.* (1984) of 11.8-26.7 mg N dm^{-3}, a concentration which was unaffected by the rate of fertilizer application in the range 0-120 kg N ha^{-1}. The latter suggests that the source of the leachable nitrate may not in fact be applied fertilizer.

Ryden *et al.* (1984) measured nitrate leaching from grazed and cut swards, and found that leaching losses of NO_3^- from the grazed (uncut) sward far exceeded (by a factor of 5) the loss from cut swards despite a common input of fertilizer, and also exceeded the losses from arable land. The enhanced nitrate movement observed below grazed pastures was attributed to the return in urine and dung of as much as 90 % of the N in herbage consumed by the cattle. This casts considerable doubt on the previously held view that arable land was the main source of leached $\overline{NO_3}$ (White, 1984). The problem of enhanced leaching due to the presence of ruminants is exacerbated by the spatial heterogeneity of dung and urine depositions. The result of this is that *hotspots* (White, 1984) of very

high inorganic N occur, and since the salt concentration in the urine is high, plants suffer from scorch. Plant uptake is low as a result and so does not significantly reduce the pool of inorganic N (Ball & Ryden, 1984).

Despite the many advances in agricultural practice, both technological and otherwise, the efficiency of use of fertilizer nitrogen by crops and pastures is poor and the consequent loss due to leaching is likely to be high. White (1989b) noted that wet and dry depositional inputs of N are insignificant in New Zealand, and that the biological input of N is much more important than the fertilizer input on a kg N ha^{-1} basis. The results of Ryden *et al.* (1984) and Dowdell *et al.* (1984) suggested that fertilizers applied to arable land may not be the major source of leached nitrate, but that grasslands, especially those grazed by ruminant animals were the source of much of the leached nitrate. One is therefore led to conclude that since the use of N fertilizer in New Zealand is so low, if a New Zealand study were to show significant leaching of NO_3^- from grasslands, it would be confirmed that the predominant source of leached NO_3^- was indeed grazed grassland, and the continuing debate on the source of the leached nitrate could be ended. Thus, there is a very real need for a model (or models) for the accurate prediction of nitrate leaching, so that steps may be taken to minimise its effects.

ii. Nitrate leaching models

In the absence of a direct application of NO_3-N fertilizer, the size of the nitrate pool available for leaching in a defined volume of soil at a given time depends on the balance between the nitrification rate and the rate of nitrate removal by plants and micro-organisms. The nitrification rate is determined by the activity of the ammonium oxidase enzyme system in nitrifying organisms which in soil is affected primarily by the supply of NH_4-substrate, soil temperature, moisture and pH (Barber, 1984; Gilmour, 1984). These factors are discussed in Chapter 2. Given a pool of leachable nitrate, the leaching process is governed by patterns of rainfall and evaporation, soil texture and structure, irrigation management (if any) and land use (White, 1988).

A first attempt at modelling nitrate leaching was presented by Burns (1980a). The model was based on the concept of the *effective rooting depth* above which all the inorganic N in the soil was considered equally available, and below which, all N was considered totally unavailable. NO_3^- release from the soil organic matter was ignored and all of the ammonium fertilizer added was assumed to have been converted to nitrate, except for the condition when most of the drainage occurred within six weeks of fertilizer application, in which case only 50 % of the NH_4^+ was considered to have been nitrified. In addition, the leaching of nitrate was assumed to occur only over the winter period between fertilizer application and the beginning of March, by which time all top dressings had been applied, that is, it was assumed that most of the leaching had taken place before there was significant crop uptake. It was found that the slow release of mineral N from organic sources, and the differences between the distributions of mineralized and freshly applied-N, affected the rate at which nitrate from the two sources was lost, and since no account was taken of mineralization, it was clear that a more complex model was required. A similar conclusion was drawn by Burns (1980b) who tested his model against data from published N response experiments in the Netherlands and U.K. Predictions of the influence of winter rainfall on the fertilizer residues in spring tended to overestimate the spring NO_3^- concentration observed in Dutch experiments, and underestimate that for the English data. However, the predictions were broadly correct, and the difference between the predicted and measured effects appeared to be caused by errors in the estimation of the amount and distribution of autumn nitrate in the soil, and by other losses of nitrate (e.g. by denitrification) which may have occurred during the winter. Again, a more complex model was required.

Bresler and Laufer (1974) and Bresler *et al.* (1982) used the *convection dispersion equation* in conjuction with a nitrate production term to model nitrate movement, whilst White (1985a; 1985a,b; 1987; 1989a) used the equations developed by Jury (1982) and modelled nitrate leaching as a stochastic process using transfer functions. The former approach is based on the mechanisms governing the spread (or dispersion) of a solute injected into a liquid moving through homogenous porous media (Dyson &

White, 1987). The latter approach acknowledges that the various processes governing solute transport are poorly known, especially in heterogeneous media such as soil, but it can be used to make predictions of nitrate leaching based on probability, providing that the nitrate concentration prior to leaching is known, and that estimates of gains or losses of mineral N by other processes can be made (White, 1987). Dyson and White (1987) compared these two approaches for the leaching of chloride through a structured clay soil, and on the basis of comparisons between experimental results and model predictions were unable to conclude that one model approach was better than the other. However, since the mechanisms of solute transport are poorly understood, the transfer function approach seems preferable from a philosophical point of view.

In spite of the relative success of the transfer function model for nitrate leaching (White, 1989a), spatial and temporal variability in the inputs, outputs and transformations of mineral N under field conditions make the predictive modelling of soil nitrate difficult (White & Bramley, 1986; White, 1988; 1989a). White (1985a) found that a change of ± 1 standard deviation in the mean NO_3^- concentration in the soil solution had a relatively large effect on the prediction of the amount of nitrate leached. Thus, the estimate of this (initial) nitrate concentration is crucial to the successful use of the model, but as indicated above is made very difficult by the spatial variability of nitrate in the field. White (1985a) took soil cores over an area of 4 m^2 and found that their nitrate concentrations ranged from 30 to 226 $\mu g\ N\ cm^{-3}$, whilst White *et al.* (1987) conducted an elementary spatial analysis of soil nitrate and found that almost all the variance was short-range, occurring within 0.4 m.

iii. The aim of the project

The aim of the work reported in this thesis was to try to quantitatively analyse spatial (and other) variability in soil mineral nitrogen, so that the nitrate concentration in the soil solution could be estimated more accurately over relatively large areas (e.g. a field). The areal average NO_3^- concentration can then be used as an input parameter in field-scale

nitrate leaching models. In a departure from previous work on this problem (e.g. White *et al.*, 1987), the emphasis was placed on studying the system that generates the nitrate in an attempt to understand why the concentration is so variable. In the following two chapters, the literature on nitrification and its modelling is reviewed and a *precis* of geostatistical theory is presented. In further chapters, the variability of nitrifier activity over area, depth and time is investigated, together with the way in which factors such as pH and soil moisture content may affect soil nitrate concentrations.

CHAPTER 2

NITRIFICATION IN SOILS: A REVIEW

The *mineralization* of nitrogen in soil comprises two separate processes - *ammonification*, which is the release of NH_4^+ from the soil organic matter, and *nitrification*, which is the oxidation of NH_4^+ to NO_3^-. A review of the biochemistry of these processes is given by Focht and Verstraete (1977). Despite the pre-occupation of the work reported in this thesis with nitrification, it was considered pertinent to review previous work on the whole mineralization process, in addition to the nitrification literature, prior to carrying out further research, for two reasons. Firstly, as Addiscott (1983) pointed out, it is not possible to measure nitrification in the soil using added ammonium substrates in isolation from either ammonification or *immobilization* (the re-conversion of inorganic to organic N). Secondly, nitrification depends on a supply of NH_4-N substrate, but because the source of N for the ammonification process, the soil organic matter, comprises many substances some of which are only loosely defined, ammonification has not been extensively researched in its own right. As a result, much of the earlier work on soil nitrogen transformations dealt instead with the mineralization process as a whole (e.g. Stanford & Smith, 1972). Since the rate-limiting step in soil N mineralization is the conversion of organic N to NH_4-N, under conditions of adequate aeration over a broad range of temperatures and soil moisture contents, soil derived NH_4^+ is oxidized to NO_3^- rapidly enough to prevent NH_4^+ accumulation (Stanford & Epstein, 1974; Wild & Cameron, 1980; Schmidt, 1982). NO_2^-, the intermediary in the conversion of NH_4^+ to NO_3^- is not normally detected in excess of $^1/_3$ ppm (e.g. Reichman *et al.*, 1966), and so the rate of NO_3^- accumulation generally reflects the rate of N mineralization. Thus, in the following discussion both nitrification and mineralization as a whole are considered.

Rosswall (1982) suggested that there was a problem regarding the identification of the specific functions of the various nitrifying bacteria. *Nitrosomonas* has always been accredited with the conversion of NH_4^+ to NO_2^-, with *Nitrobacter* completing the transformation to NO_3^-.

However, *Nitrosolobus* and *Nitrospora* are also common in soils and Rosswall (1982) claimed that *Nitrosolobus* was the dominant NH_4^+ oxidizer in agricultural soils. Schmidt (1982) even suggested that methane oxidizing bacteria may be involved, despite acknowledging that the only micro-organisms known to be linked directly to nitrification in natural environments are the *gram -ve* chemosynthetic autotrophs which comprise the family *Nitrobacteriaceae*. Since the purpose of this study was to investigate the variability and magnitude of the *net* production of leachable nitrate, the types and differences between the various nitrifying organisms was considered to be of secondary importance and it was assumed that autotrophic organisms were predominantly responsible for the production of nitrate in the particular soil studied (see Chapter 9). Throughout, the generic term *nitrifiers* will be used to denote all soil nitrifying organisms (both autotrophic and heterotrophic); similarly, *ammonifiers* will be used to denote those organisms responsible for ammonification.

Barber (1984) suggested that 2 % of the total soil nitrogen could be mineralized each year. Dowdell and Webster (1984) applied ^{15}N-labelled fertilizer to soils in lysimeters. From the amounts of fertilizer derived ^{15}N that remained at the beginning of each cropping season, they estimated that 5-6 % of the residual ^{15}N applied *turned over* each year. Shen *et al.* (1982) estimated that the amount of nitrogen contained in the soil microbial biomass represented about 6 % of the total soil N, and that about 30 % of the ^{15}N residual from labelled fertilizer applied to the soil was associated with the microbial biomass. Using these figures, and their own results for the change in residual ^{15}N, Dowdell and Webster (1984) suggested that about 20 % of the biomass turned over each year. They distinguished between more readily available organic matter (microbial biomass and roots) and the total soil organic nitrogen, and calculated that if the estimate of biomass N turnover was correct, and the total soil organic matter turned over at the same rate, it would be equivalent to a release of 100-120 kg N ha^{-1} per year. The output of total nitrogen from the fertilizer treated lysimeters exceeded inputs by 76-94 kg N ha^{-1} per year and in the unfertilized lysimeters by 129 kg N ha^{-1} per year. Thus, if these differences were due to net mineralization, then there is good agreement between them and the N turnover rates estimated from the ^{15}N results.

i. The factors affecting nitrification and mineralization

Macduff and White (1985) found that the rate of nitrification was never less than the rate of ammonification, whilst Kowalenko (1978) found that nitrification proceeded very rapidly after the addition of NH_4-substrate with only background levels of extractable-NH_4 remaining after a period of 42 days. In view of these findings, and those detailed above, it is a reasonable assumption that the factors affecting nitrification are those affecting mineralization.

A number of factors have been identified as rate-determining for ammonification and nitrification. These include the amount, type and availability of substrate, the size of the ammonifier and nitrifier populations, which in the case of the ammonifiers is very diverse and therefore difficult to investigate, and the environmental conditions under which these organisms live; i.e. mineral nutrients, temperature, aeration, soil moisture content and pH must all be at satisfactory levels (Harmsen & Kolenbrander, 1965; Legg & Meisinger, 1982).

Organic substrate

In a series of field and laboratory experiments, Campbell and Biederbeck (1982) identified the importance of crop residues for microbial proliferation. For an appreciable net mineralization of soil organic nitrogen to occur, the C:N ratio of the decomposing substrate must be below 20-25 (Harmsen & Kolenbrander, 1965) or 20 (Barber, 1984). When plant residues with a C:N ratio of greater than 20 were added to soil, nitrate and ammonium levels in the soil decreased as micro-organisms used up carbon from the residues, and nitrogen was immobilized as a result (Bartholomew, 1965). If the C:N ratio was less than 20, nitrogen was released at the rate of decomposition as micro-organisms decomposed the residues. In the field, this rate is usually between 1 and 3 % per year when calculations are based on the total amount of soil organic matter (Barber, 1984). Thus, providing soil organic matter has a C:N ratio of less than 20, under otherwise non-limiting soil conditions, the supply of

ammonium for nitrification will depend on the size of the ammonifier population and the amount of decomposable substrate. The effect of the concentration of available ammonium is discussed in Chapter 4.

pH

There have been a number of studies on the effect of pH on nitrification (e.g. Frederick, 1956; Aleem & Alexander, 1960; Morrill & Dawson, 1961; Dancer *et al.*, 1973; Darrah *et al.*, 1986b), but commonly these have involved either long-term perfusion experiments or pure culture studies of nitrifiers growing under laboratory conditions. However, Schmidt (1982) noted that nitrification "proceeds at soil reactions far below the pH limits observed for the nitrifying bacteria in pure culture", and that most observations had indicated an "arbitrary lower limit" for nitrification of pH 4, with obvious nitrification between pH 4 and 6, and pH independent nitrification in the range pH 6 to 8. The optimum pH for mineralization of soil organic nitrogen was stated by Harmsen and Kolenbrander (1965) to be on the alkaline side of neutrality, following an increase of 100-1500 kg N ha^{-1} per year when acid sandy soils with high humus content were limed. Dancer *et al.* (1973) studied ammonification and nitrification over a range of soil pH values using limed plots in which the soil pH had been constant for a number of years, and found that soil pH did not affect rates of ammonification appreciably but significantly affected nitrification; Weier and Gilliam (1986) had similar results. Frederick (1956) found that there was a marked decrease in the nitrification rate as the pH dropped below neutrality, whilst Gilmour (1984) found that there was a linear increase in the nitrification rate over the range pH 4.9 to 7.2, and by setting relative NO_3^- production to unity at pH 7.2, derived the following equation to describe the effects of pH on the level of soil NO_3-N:

$$NO_3^- = (E \times pH) - F \qquad (2.1)$$

where E and F are constants to which the values 0.33 and 1.36 were assigned respectively. Bhat *et al.* (1980) used 0.4 and 1.6 suggesting that such a relationship is unlikely to be the same for all soils. Whilst equation (2.1) will (correctly) predict the cessation of nitrification below pH 4, it does not allow for the effects of an alkaline pH on nitrification (Darrah *et al.*, 1986b). Evidence from the literature suggests that pH has a major controlling effect on soil nitrification but it also appears that nitrification rates in different soils will be affected to differing degrees by soil pH. The work of Pang *et al.* (1975) confirmed that the difference in nitrifying capacity amongst soils was related to the initial nitrifier numbers whose activities were affected by the initial soil pH. It therefore seems likely with respect to this study, that variability in the nitrification rate may be in part due to variability in the soil pH. This is investigated in Chapter 7.

All the above relates to the effects on nitrification rates of pH as measured in bulk solutions. Little account has been taken in the literature of the possibility that the pH of the immediate environment of the nitrifiers may be rather different to that of the bulk solution. Evidence suggests (Fletcher, 1985) that soil bacteria tend to be attached to solid surfaces and do not just drift about in the soil solution. Keen and Prosser (1987) found that the degree of attachment of *Nitrobacter* to an anion-exchange resin increased with pH over the range 5.5-8.0. No such increase was observed when the attachment was to glass coverslips, but attached cells grew approximately 20 % faster than free cells. Fletcher (1985) attributed this to different H^+ ion concentrations in the mucilage of attached organisms compared to the bulk solution, although why this should be different to the H^+ concentration in the mucilage of unattached bacteria in the same medium is unclear. Nevertheless, assuming that nitrifiers exist in the soil in clusters of cells (Molina, 1985; Darrah *et al.*, 1987b - see below), and that the clusters of cells are attached to surfaces in the soil, the idea that nitrifying organisms can generate a pH environment different to that of the bulk soil solution is acceptable. The effects of change in the pH of bulk solutions on nitrifiers must therefore be due to altering the pH gradient between the bulk solution and the microbial mucilage; when this gradient is steep, the pH of the mucilage may be altered sufficiently to affect the activity of nitrifiers covered by it.

Temperature

Very little work has looked at the effect of temperature on nitrogen mineralization *per se* although Harmsen and Kolenbrander (1965) attributed the seasonal fluctuation in mineralization rate to changes in soil temperature. Kowalenko and Cameron (1978) found an increase in soil mineral N in spring and considered that it was due to higher temperatures enabling mineralization of substrates which had presumably accumulated during winter when low temperatures prevailed. Anderson and Purvis (1955) studied the effects of low temperatures on nitrification in incubated soils, and found that whilst nitrification began sooner in some soils than others following the addition of ammonium, and maximum rates varied, the differences tended to decrease with increasing temperature. In all but one of their soils, the accumulation of NO_3^- at least doubled between 5.5° and 8.3 °C (42°-47 °F), although Stanford *et al.* (1973) found that Q_{10} for the mineralization process as a whole was approximately equal to 2. Frederick (1956) found the greatest increase in nitrification between 7° and 15 °C, but noted that temperatures fluctuating in a 24 hour cycle generally resulted in an increased rate of nitrification at temperatures below 15.5 °C. Harmsen and Kolenbrander (1965) found that nitrification was inhibited at the upper end of the *mesophilic range* and that none took place above 45 °C, whilst below their stated optimum range of 25°-30 °C, it decreased slowly and practically ceased near freezing point. Schmidt (1982) stated that cold and wet soils were effectively inactive with respect to nitrification, whilst Mahli and M^{c}Gill (1982) thought it likely that microbes in cool climates would adapt to those conditions. Schmidt (1982) noted that there were indeed geographical differences in the optimum temperature for nitrification - 20°-25 °C in northwestern U.S.A; 30°-40 °C in southwestern U.S.A and 60 °C in tropical Australia. Mahendrappa *et al.* (1966) added $(NH_4)_2SO_4$ to different soils from western U.S.A and incubated them at a range of temperatures between 20° and 40 °C at 0.3 bar moisture tension. In all the soils from northern regions, nitrification was faster at 20° and 25 °C than at 35° and 40 °C, whilst the reverse was true in the case of the southern soils which nitrified fastest at 35 °C. Under conditions where the temperature was "unfavourable", nitrite accumulated. These results suggest that microbial populations in different soils will adapt to their specific environmental conditions. The work of Nakos (1984) further supports this.

Gilmour (1984) described the effect of temperature on nitrification by the equation:

$$K_{max} = \exp \{ A/T + B\} \qquad (2.2)$$

where T is the temperature, K_{max} is the zero-order rate constant at optimum soil moisture, and A and B are constants. The exp(B) term is the *frequency factor* and A is equal to the ratio of the activation energy of the reaction to the gas constant in the original form of the Arrhenius equation. However, Macduff and White (1985) found that nitrification was limited by the supply of NH_4^+ irrespective of temperature and Rosswall (1982) agreed that this was probably the "main factor" controlling nitrification. With respect to experimental technique, it seems likely from the above, that when studying soils from a temperate climate (such as New Zealand), incubation temperature is not going to be critical, so long as it is kept constant and extreme temperatures are avoided.

Moisture content, aeration and osmotic stress

It has been well known for many years that the autotrophic nitrifiers are strictly aerobic organisms (Meiklejohn, 1953), and it is therefore to be expected that the soil moisture content and degree of aeration would be of fundamental importance to nitrification. Indeed, Bresler and Laufer (1974) found that the rate of NH_4^+ oxidation was directly related to the degree of oxygen availability by gaseous diffusion, which was in turn inversely proportional to soil moisture content.

Much of the work on the effects of moisture content on mineralization was carried out in the context of the effects of drying (and storing) soils on their rates of mineralization; this is discussed in Chapter 4. With respect to nitrification, Bresler *et al.* (1982) stated that under isothermal conditions when the soil water content varies considerably during infiltration, redistribution and evaporation, the effect of soil moisture content on nitrate production will be of prime importance. Yadvinder-Singh and Beauchamp (1988) found that nitrifier activity

increased with increasing soil water potential, and Sindhu and Cornfield (1967a) found that the optimum moisture content for ammonification and nitrification in the soils they studied was equivalent to 50 % of the maximum water holding capacity. Reichman *et al.* (1966) found that the rate of both ammonification and nitrification of soil N were almost directly proportional to the soil water content at suctions between 0.2 and 15 bars. At 15 bars there was still measurable nitrification. Dubey (1968) found that the nitrification rate in a sandy loam increased as the soil moisture tension decreased from 15 to 2 bars and then decreased at lower tensions, although marked nitrification (presumably heterotrophic) occurred even under flooded conditions. In contrast, he found practically no difference in the nitrification rate between 15 and 0.3 bars in a loamy sand. Justice and Smith (1962) found that at the optimum incubation temperature for nitrification in their calcareous soil, the start of nitrification following addition of substrate was delayed at tensions greater than 7 bars, although nitrification did occur at the permanent wilting point. A maximum level of nitrifier activity was noted by Miller and Johnson (1964) in the range 0.1-0.2 bars, although there was variation in microbial behaviour with respect to the ammonifiers - those producing NH_4^+ at zero tension did not function either when under tension, or with more aeration than was found at zero tension. Those producing NH_4^+ at higher tensions did not function with less aeration. Stanford and Smith (1972) stated that the moisture content after vacuum suction at 60 cm Hg was near optimal for mineralization and they regarded the oxygen concentration under these conditions as similarly near optimal. Sanchez (1976) reported that accumulation of NO_3^- in the upper horizons of some tropical soils could be explained by the existence of nitrification at soil moisture tensions of 15-80 bars since the crumbs of such soils can hold water at these very high tensions due to their *microaggregate* structure. All this suggests that the response of nitrifiers to changing moisture stress is soil-specific.

Khyder and Cho (1983) measured the partial pressure of O_2 in the soil atmosphere at several depths and found that when the air-porosity was 10.5 % (30 % moisture) the boundary between the aerobic and anaerobic soil layers occurred at approximately 20 cm depth, but when the air-porosity was increased to 16 % (25 % moisture), this boundary occurred at

40 cm depth. i.e. a small change in the air porosity led to a considerable change in the position of the boundary between the aerobic and anaerobic zones. It may well be therefore, that N flushes (Birch, 1958; 1960) may occur on a micro-scale as the groundwater table rises and falls, allowing for significant oxidation of soil organic matter below the surface horizon.

Gilmour (1984) observed a linear decline in nitrification rate as the soil moisture content decreased over the range 0.2-0.12 g g^{-1} and he described the moisture relations of nitrification by the equation:

$$K/K_{max} = (C \times WC) + D \qquad (2.3)$$

where K_{max} is the maximum nitrification rate, K is the nitrification rate at a specific gravimetric moisture content, WC, and C and D are constants which Gilmour (1984) found to be equal to 4.8 and 0.3 respectively. There is no theoretical basis for C and D, however, and it seems likely that a linear model is inappropriate to decribe the relationship between moisture content and nitrification rate since the evidence is that both very high and very low moisture contents are inhibitory to nitrification. This is investigated in Chapter 8.

The factors discussed above relate primarily to matric potential effects on nitrifier activity. In addition, the osmotic effects of particular soil solutes may be important. The effects of osmotic stress on nitrification and mineralization have not been extensively studied although Darrah *et al.* (1985a; 1986c; 1987a) looked at the effects of high concentrations of $(NH_4)_2SO_4$ and NH_4Cl on nitrification rates. This work is discussed in Chapter 4. Sindhu and Cornfield (1967b) studied the effects of chlorides and sulphates of Na, K, Ca and Mg added in solution at concentrations of 0.1-2.0 % (Na equivalent) on N mineralization and nitrification. Cl^- in concentrations between 0.5 and 1 % caused almost complete suppression of nitrification, but mineralization was only reduced when a concentration of more than 1 % salt was added. SO_4^{2-} only reduced mineralization and nitrification when added as 2 % Na_2SO_4. In some cases, both sulphates and chlorides of all cations except Na resulted in small but significant increases in N mineralization. Whether

or not the sodium response is a toxic effect is unclear, but Harris (1980) noted that Na as NaCl (along with sucrose) was the most important solute with respect to osmotic stress in soils. Here it is enough to say that high osmotic stress is inhibitory to nitrifiers and presumably ammonifiers too. One might therefore conclude that equation (2.3) is either incomplete, or that C and D are in some way dependent on either osmotic stress, degree of aeration or both.

Other factors

In addition to the factors discussed above which may be thought of as most important, there may be other less obvious factors which will influence nitrification rates in certain situations. For example, Purchase (1974) found that P deficiency affected nitrification to the extent that NO_2^- would accumulate if P levels were low enough. Loveless and Painter (1967) demonstrated that the effect of deficiencies of copper, sodium, calcium and magnesium on the growth of *Nitrosomonas europaea* were such that the effect of pH was dependent on the metal ion concentration. pH was also found to strongly influence copper toxicity.

The literature on the effects of pH on nitrification rates suggests that the nitrification rate is highest at neutral or mildly alkaline pH (see above). Some of the mildly acidic New Zealand soils (Yellow brown loams) studied by Steele *et al.* (1980) were found to have surprisingly high nitrifier activity (2-3 $\mu g\ N\ g^{-1}\ h^{-1}$), this activity being of the order of, or higher than, that of soils of near neutral pH. Sarathchandra (1978) noted that the dominant clay mineral in these soils is allophane, and thought it probable that at pH 5.5 while negative charges on the allophane surface retain some NH_4^+, the positive charges present may in fact repel H^+ ions, establishing a micro-site containing fewer H^+ ions than the bulk soil, and thus a higher pH than the bulk solution. This explanation is based on the assumption that pH 5.5 is close to the point of zero charge, that is, at this pH allophane has approximately equal numbers of positive and negative surface charges (Sarathchandra, 1978). However, at the point of zero charge the pH measured in water is similar to that measured in salt solution, and so the difference between the pH

at adsorption surfaces and the bulk solution is likely to be small. Sarathchandras' explanation for high nitrifier activity in allophanic soils may therefore not be correct.

ii. Modelling of Nitrification

At the simplest level, Gilmour (1984) took equations (2.1) to (2.3) and by combining them and adding an expression for the effect of substrate concentration, N_c, (see Chapter 4), calculated the absolute nitrification rate NR, according to the equation:

$$NR = \{[\exp(A/TB)] \times [(C \times WC) + D] \times \frac{[(E \times pH) - F]}{0.95}\}N_c \qquad (2.4)$$

where the symbols are as before. However, this seems little more than an elaborate curve fitting exercise; in any case, the value of equations (2.1) to (2.3) is doubtful. Clearly something more sophisticated is required.

In all the models describing the nitrification process which are outlined below, it has been assumed that the oxidations involved are zero-order reactions (e.g. McLaren, 1976). Addiscott (1983) questioned whether nitrification was truly zero-order on the grounds that nitrification rates are not independent of the initial NH_4^+ concentration (see Chapter 4). Molina *et al.* (1979) suggested that the kinetics of NH_4^+ oxidation are the resultant averages of pulses of activity from small isolated and asynchronous clusters of NH_4^+ oxidizers, and a theoretical consideration of cell clustering (Darrah *et al.*, 1987b) supported this. The oxidation of ammonium around each cluster, which may comprise a few hundred cells (Molina *et al.* (1979), is very rapid and the exponential (first-order) model applies. Thus, if the soil can be regarded as a single large aggregate with only one cluster, in the non-steady state the kinetics of nitrification will be first-order and will follow the kinetics of microbial growth, and it may be assumed that the rate of ammonium oxidation will not be constant until steric saturation is achieved (Molina *et al.*, 1979). At this point, steady state conditions apply and

NO_3^- production is constant. Thus, zero-order kinetics apply. In addition, Molina *et al.* (1979) found that there was no synchronization of the beginning of nitrification amongst the aggregates tested, despite the fact that they all came from the same field sample; indeed, some particles took eight weeks to exhibit their nitrifying potential. Thus, even under non-steady state conditions, zero-order kinetics will appear to apply due to the averaging effect of cluster asynchronicity. McLaren (1969) ignored this problem on the assumption that the whole microbial population will grow uniformly until a maximum population is achieved, and that this population continues to carry out nitrification with very little multiplication. As outlined below, this assumption is not really acceptable, although it enabled initial progress to be made in the modelling of nitrification. Burns (1980a) modelled with a deliberate lack of analysis of N interactions so that the "main effects of the slow release of nitrate and the distribution of rainfall on the leaching of nitrified-N" could be considered. Whether or not this is a helpful approach is debatable.

McLaren (1969) modelled the concentrations of NH_4^+, NO_2^- and NO_3^- under conditions of microbial steady state (i.e. with no growth in the microbial population) as NH_4^+ was applied to the top of a soil column by the equations:

$$[NH_4^+] = [NH_4^+]_o e^{-k_1 x} \qquad (2.5)$$

$$[NO_2^-] = \frac{\{k_1 (NH_4^+)_o\}}{(k_2 - k_1)} \{\exp(-k_1 x) - \exp(-k_2 x)\} \qquad (2.6)$$

$$[NO_3^-] = [NH_4^+]_o - \{[NH_4^+] + [NO_2^-]\} \qquad (2.7)$$

where x is the distance of flow down the column, k_1 is the rate of the NH_4^+ to NO_2^- oxidation divided by the flow rate down the column, k_2 is the rate of the NO_2^- to NO_3^- oxidation divided by the flow rate down the column, and $[NH_4^+]_o$ is the concentration of ammonium in solution applied at the top of the column. McLaren (1970, 1971) used these equations as inputs to the main model which gave the rate of either oxidation at small substrate concentrations by:

$$\frac{-\partial[S]}{\partial t} = A\forall m + \alpha m + \frac{(\eta\beta m[S])}{(km + [S])} \qquad (2.8)$$

where S is the substrate concentration, m is the biomass, A is a proportionality constant (the reciprocal of growth yield) equal to N oxidized per unit weight of biomass synthesized, α is the N oxidized per unit weight of biomass per unit time for *cell maintenance*, β is the amount of enzyme per unit biomass involved in *waste metabolism*, $\forall m = \partial m/\partial t$ for *growth metabolism*, η is a proportionality constant and *k*m is a saturation constant. Thus, the first term relates the disappearance of substrate due to microbial growth, the second term provides for maintenance of the population in the absence of growth, and the third term represents the rate of change of substrate in addition to growth and maintenance, the waste metabolism. According to M^{c}Laren (1970; 1971) it is this which provides NO_3^- for plant uptake and may be regarded as occurring simply because the enzyme system is present and active. However, this partitioning of NH_4^+ oxidation into growth, maintenance and waste metabolism is misleading because the only reason that nitrifying organisms oxidize NH_4^+ is to gain energy for growth. According to Wild (1988), the *Nitrosomonas* group of bacteria oxidize 35-70 moles of NH_4^+ for every mole of carbon assimilated, and *Nitrobacter* oxidize 70-100 moles of NO_2^- for every mole of carbon assimilated. If we assume that the C:N ratio of nitrifying bacteria is approximately 6 (Brady, 1984), it follows that the amount of N that is assimilated by the organisms is so small in relation to the amount oxidized, that virtually all the NO_3^- produced is available for release to the medium (i.e. assuming for the sake of argument that the mass of a mole of C and N is approximately the same, 6 moles of C assimilated gives 420 moles of oxidized N of which only 1 is retained for protein synthesis). Nevertheless, M^{c}Laren (1970) persisted with the α, β and $\forall$ terms and presented sub-models for a range of scenarios with varying degrees of substrate concentration and microbial enrichment.

M^{c}Laren (1976) acknowledged that, in fact, the steady state situation is never achieved due to ion exchange and leaching of nutrients. In a flowing solution, hydrodynamic dispersion, equations for which were given by Kirkham and Powers (1972), may also be important. Thus, for a microbial oxidation in a column of soil with the substrate moving at a flow rate *f*, the change in concentration with time is described by the equation (M^{c}Laren, 1976):

$$\frac{\partial[S]}{\partial t} = -\frac{f(\partial[S])}{\partial x} + \frac{D(\partial^2[S]}{\partial x^2} - \xi[S] \qquad (2.9)$$

where D is hydrodynamic dispersion and $\xi[S]$ is some function of [S] that represents change, i.e. loss of substrate by microbial oxidation. M^{c}Laren (1976) presented equations to describe $\xi[S]$ under conditions of different growth, flow and substrate concentration in terms of α, β and γ. Despite the fact that M^{c}Laren (1970; 1971; 1976) presented a nice theoretical model with respect to these terms, they cannot in practice be in any way separated or identified, and as indicated above, there seems to be little justification in distinguishing between them. An alternative treatment was therefore required.

The rate of growth of nitrifying biomass, *m*, was modelled by Darrah *et al.* (1985b) as:

$$\frac{\partial m}{\partial t} = \frac{\mu_{max}\{[C_1]_t\}m}{[C_1]_t + k_s} \qquad (2.10)$$

where μ_{max} is the maximum specific growth rate (h^{-1}), k_s is an affinity constant (μmoles cm^{-3}), and C_1 is the ammonium concentration in solution, where at any time t:

$$[C_1]_t = [C_1]_o - \int \frac{\partial C_2}{\partial t} + \int \frac{(\partial[C_N])\partial t}{\partial t} \qquad (2.11)$$

where $[C_1]_o$ is the NH_4^+ concentration at time zero and C_2 is the NO_3^- concentration per unit soil volume. The last term describes the rate of ammonification for native soil organic N.

The rate of NO_3^- formation is given by the equation (Darrah *et al.*, 1985b):

$$\frac{\partial C_2}{\partial t} = \left\{\frac{1}{Y}\right\}\left\{\frac{\partial m}{\partial t}\right\} \qquad (2.12)$$

where Y is the yield constant defined as μg biomass formed per μmole of NH_4^+ transformed. Substitution from equation (2.10) gives:

$$\frac{\partial C_2}{\partial t} = \mu_{max}\left\{\frac{[C_1]_t}{[C_1]_t + k_s}\right\}\left\{\frac{m}{Y}\right\} \qquad (2.13)$$

Then dividing both sides of equation (2.10) by Y gives (Darrah *et al.*, 1985b):

$$\frac{\partial (m/Y)}{\partial t} = \mu_{max}\left\{\frac{[C_1]_t}{[C_1]_t + k_s}\right\}\left\{\frac{m}{Y}\right\} \qquad (2.14)$$

and thus the formation of NO_3^- can be expressed in terms of equations (2.13) and (2.14) containing the three parameters (m/Y), μ_{max} and k_s. When C_1 is less than, or of similar magnitude to k_s, equations (2.13) and (2.14) are solved numerically, but where C_1 is greater than k_s, they can be integrated to give the nitrate formed in terms of the two constants (m_o/Y) and μ_{max} (Darrah *et al.*, 1985b).

Using this theory as a basis, in addition to equations describing diffusion of NH_4^+ and NO_3^- in soil (Darrah *et al.*, 1983), adjustments were made to enable the modelling of simultaneous nitrification and diffusion in soil with respect to the addition of ammonium sulphate (Darrah *et al.*, 1986a), pH (Darrah *et al.*, 1986b), and osmotic potential (Darrah *et al.*, 1987a). A simplification of the model is presented by Darrah *et al.* (1986d).

Models such as those of McLaren (1970; 1971; 1976) and Darrah *et al.* (1985b) require some estimation of the size of the microbial population. Many workers have used the most probable number (MPN) technique of estimating microbial populations (Cochran, 1950; Schmidt, 1982). However, this technique shows a high degree of variability and cannot be regarded as accurate (Schmidt, 1982). Morrill and Dawson (1961) initiated the development of an alternative indirect method of measuring the nitrifier population when they noted that advantage could be "taken of the facts that chemoautotrophs are the major, if not the sole agents concerned with NO_3^- production in nature, and that the oxidation of ammonium and nitrite compounds is growth linked. Hence by measuring the quantity of ammonium- or nitrite-N oxidized, the rate of growth of the respective bacteria can be ascertained." However, if the time period over which NH_4^+ oxidation occurs is limited, then the assumption can be made that microbial growth is minimal and the nitrification rate measured is an index of the size and activity of the nitrifier population. By optimizing conditions in terms of substrate, O_2, temperature and moisture, so that each organism can function optimally, the nitrification rate in a *short-term nitrification assay*, SNA, should be an index of the number of organisms present. This was the basis of the work of Sarathchandra (1978) and Steele *et al.* (1980) who found that the main benefit of the SNA was that the short time of perfusion or incubation meant that the results were unaffected by microbial proliferation, and thus a good reflection of nitrification activity was obtained. The technique employed in the SNA (Sarathchandra, 1978; Steele *et al.*, 1980, Darrah *et al.*, 1986b; 1987a) gives a high degree of reproducibility (P.R. Darrah - personal communication; see also chapters 4-9) and may thus be used to give useful input data to nitrification models.

Molina (1985) looked at nitrification in a completely different and novel way. As already mentioned, he assumed that nitrification proceeded from pulses of ammonium oxidation generated by microbial clusters, and in this connection carried out his experiments with individual soil micro-aggregates rather than soil columns. He noted that for every NH_4^+ ion oxidized, two H^+ ions are released, resulting in a pH decrease:

$$NH_4^+ + 3/2\ O_2 \rightarrow 2H^+ + NO_2^- + H_2O \qquad (2.15)$$

and therefore used an experimental procedure involving bromothymol blue indicator which allowed the pH change to be monitored by transmittance through a spectrophotometer. This method may be potentially useful in investigations of variability of nitrification on a micro-scale, especially in relation to the differences in nitrifier activity between the inside and outside of clods.

iii. A comment on measured nitrification rates

Brandt *et al.* (1963) found that there were large discrepancies in their results between NH_4^+ disappearance and NO_3^- accumulation and as a result, had to distinguish between *nitrification* as the biological oxidation of reduced forms of N and *net nitrification* as the observed accumulation of NO_3^-. With respect to experimental techniques such as the SNA, this is of great importance. It was stated at the beginning of this chapter that it is not possible to measure nitrification in soil in isolation from either mineralization or immobilization (Addiscott, 1983) and it is implied above that the SNA measures *net* nitrate production irrespective of the mode of production. It is not within the scope of either this thesis or this review to consider the vast literature on denitrification, immobilization, and volatilization of soil N. However, it is well known (e.g. Starr *et al.*, 1974; Kowalenko, 1978) that nitrification can occur simultaneously with these processes. Indeed, Colbourn *et al.* (1984) demonstrated that in a drying soil, nitrification was rate-determining for denitrification. Furthermore, when NH_4^+ substrates are added in incubation experiments, some may be fixed by clays (Mogilevkina & Lebedeva, 1982) depending on the amount of available NH_4^+ and the clay percentage; Darrah *et al.* (1985b) attributed their incomplete N recovery to the fixation of NH_4^+ by mica-type clays. Thus, until such time that measurements of these various processes can be made in isolation from one another, net measurements will have to suffice. There is no indication in the literature to suggest that any interpretation is lost as a result. Moreover, if abundant NH_4^+ is added, the fixation of NH_4^+ by clays will

be unimportant; if the system is well aerated, denitrification will be insignificant; if incubation times are short so that growth of the organisms is minimal, then NO_3^- immobilization should also be insignificant, and the concentration of NH_4^+ should ensure that the requirements of both the autotrophic nitrifiers and the heterotrophs are satisfied.

iv. Conclusion

Overall, it appears that the variability in nitrifier activity may be a function of variabilty in the factors discussed in Section i (above), most importantly soil pH, moisture content and availability of NH_4^+ substrate. Since the effects of the first two of these factors appear to be soil-specific, they clearly merit attention in this study. For a study of variability in nitrifier activity, the SNA appears to have the most potential since it is much quicker than the other techniques, gives readily reproducible results, and in view of the small amount of soil required (Darrah *et al.*, 1986b) may be very suitable for spatial studies involving large numbers of samples. In addition, the problem of different nitrifying species can be ignored with this technique (P.R. Darrah - personal communication).

CHAPTER 3

A THEORETICAL CONSIDERATION OF SPATIALLY DEPENDENT VARIABILITY

The main objective of the work reported in this thesis was to investigate the variability of nitrifier activity. As was indicated in the introductory chapter, in addition to investigating this variability in relation to factors which might be expected to control nitrification, it was of major interest to investigate and quantify the spatial variability of nitrifier activity so as to improve estimates of the initial nitrate concentration as an input parameter for nitrate leaching models. This can not be readily done using classical statistics. The following discussion explains why this is so, and outlines the means by which spatial variability may be investigated.

i. Why do we need geostatistics ?

The traditional means of statistical analysis that soil scientists have used to corroborate the hypotheses which inspired their experiments - described by Nielsen (1987) as "aggie statistics" - involved calculation of the mean and variance of sets of data collected from regions, or under conditions, that were perceived to be homogeneous. Often this was done, and still is today, with total disregard for the distribution of the data about the mean, and with results explained in terms of cause and effect; when an effect could not be attributed to a cause, it was explained away by an error term (usually the residual mean square or variance) which was commonly ascribed to the inadequacy of sampling, the inaccuracy of an analytical technique or simply to random variation. In fact, a large part of such error is most likely to be due to measurements being made in *non-homogenous* areas, but this possibility has either been ignored, or the worker has been ignorant of the possibility of some spatial dependence in the data. Given this background of an experimental and statistical *status quo*, D.R. Nielsen speaking to the Dutch Soil Science Society on their 50th anniversary, argued that

> "....[incorporated into] the next page of soil science should be regionalized variable theory....If we are to achieve greater success, we must take advantage of spatial and temporal variability instead of avoiding it. If we acknowledge its existence, it will enhance our research efforts even when we subject experimental sites to selected treatments."

He commented further:

> "....The mean value of a soil property, which we have become so accustomed to seek and appreciate, may not, in the final analysis, be as important as its spatial and temporal variance or the identification and possible significance of its perturbed values." (Nielsen, 1987).

Thus, the need has arisen for quantitative spatial and temporal analysis of soil properties, to complement the investigation of cause and effect relationships between them.

In the following sections, the rationale behind geostatistics is presented. Since much of the theory is not new and has been extensively detailed in a soil science context previously (Webster, 1985; Trangmar *et al.*, 1985; Oliver, 1987), the topic will be developed here in relation to the work presented in this thesis, and thus some aspects, such as interpolation by kriging, are omitted. In this discussion, geostatistical techniques are looked at from a different angle to that presented in the literature with the intention of explaining to the uninitiated, and the non-mathematician in particular, the potential of these powerful statistical tools.

For the purpose of this discussion, a data set comprising 25 soil pH values will be considered; the data are listed in Table 3.1. It will be assumed that they represent measurements taken along a transect within an area which is assumed to be homogeneous, with equal spacing between samples; samples *No.* 1 and 25 represent the two ends of the transect.

Table 3.1 25 values of soil pH measured at equal spacings along a transect

Sample No.	pH	Sample No.	pH
1	5.38	14	4.93
2	4.38	15	4.80
3	5.00	16	4.88
4	5.10	17	4.88
5	4.65	18	4.90
6	5.20	19	4.93
7	4.78	20	5.05
8	5.38	21	4.85
9	4.95	22	4.85
10	4.78	23	4.95
11	4.70	24	4.80
12	4.53	25	4.85
13	4.68		

ii. Some preliminary data analysis using classical statistics

Any kind of analysis requires a starting point, and one useful way to begin to analyse a data set is to find out about its distribution; i.e. one needs to know the mean and variance, and the way the data are distributed about the mean. It is assumed that the sample has been drawn from a population with true mean μ, and variance σ^2 (Clarke, 1980). The true mean value of a property Z, is estimated by $\hat{\mu}$, the *arithmetic* mean, where (Clarke, 1980):

$$\hat{\mu} = \frac{\Sigma\ Z(x_i)}{n} \qquad (3.1)$$

and the population variance σ^2 is estimated by s^2, the sample variance, by (Clarke, 1980):

$$s^2 = \frac{1}{n-1}\ \Sigma\ \{Z(x_i) - \hat{\mu}\}^2 \qquad (3.2)$$

where n is the number of realizations or values of the property Z, and x defines the location, in cartesian coordinates with i=1,2,3...n, at which the individual values of the property Z are observed or measured. Applying these equations to the data in Table 3.1, values of 4.89 and 0.0517 are obtained for the arithmetic mean and sample variance respectively. White *et al.* (1987) noted the dangers of assuming that the simple arithmetic mean and variance were the best estimates of the population mean and variance when sample data are *skewed* and do not conform to a normal distribution. When this is the case, an estimate of the arithmetic mean derived from the parameters of a log-normal distribution, or one based on Sichel's estimator (Sichel, 1952) may give better estimates of the population mean. Thus, the distribution of the data must be checked.

The 25 pH data were grouped into classes (0.2 pH units wide), the frequency of each class normalized, and the resultant distribution plotted (Figure 3.1). This was shown by means of a least-squares fitting

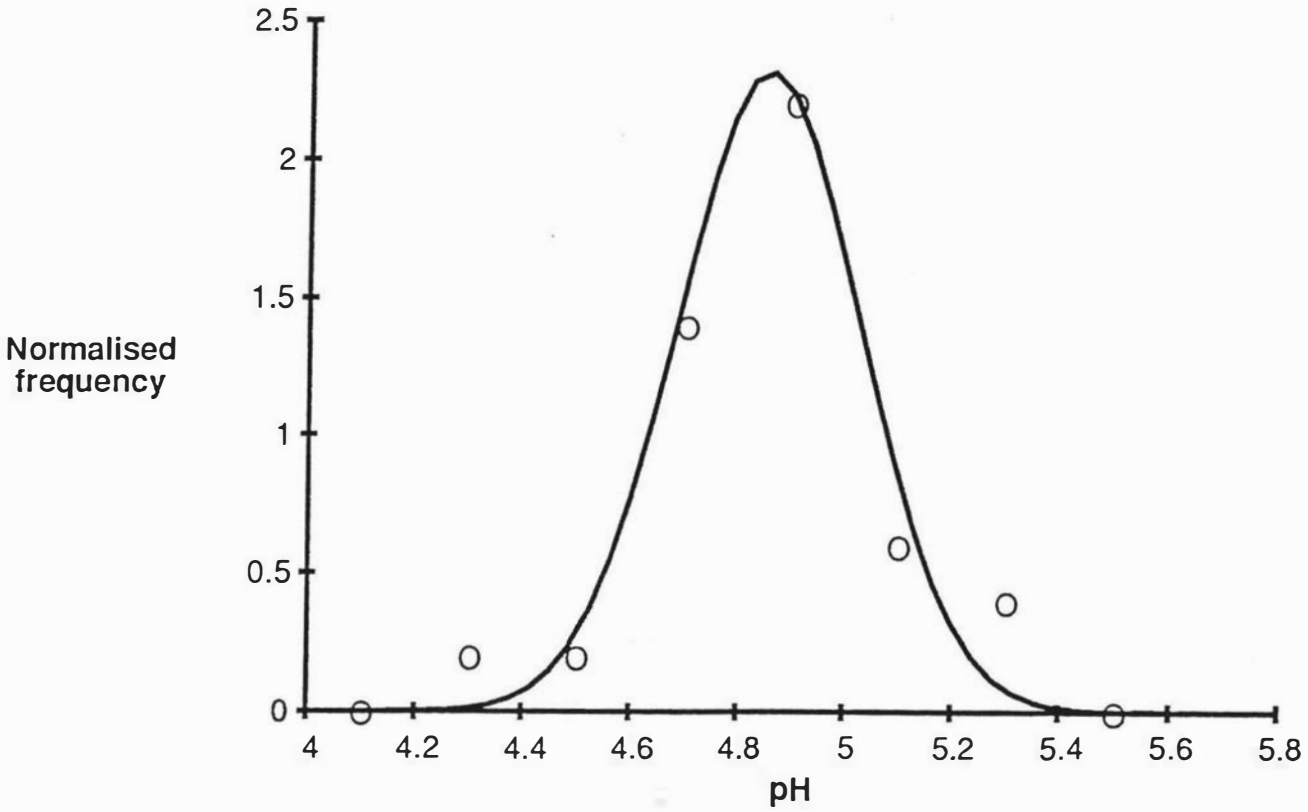


Figure 3.1 Distribution of a set of 25 pH data

procedure (CFIT - Dept. Soil Science, Massey University) to conform to the normal distribution $f(x)$ ($R^2 = 0.94$, $p<0.1\%$) where (Clarke, 1980):

$$f(x) = \frac{1}{\sqrt{2\pi\tau^2}} \exp\{-\frac{(w - \phi)^2}{2\tau^2}\} \qquad (3.3)$$

Here, ϕ and τ^2 are best estimates of the population mean and sample variance, μ and σ^2, and w is the mid-value for each pH class. $\hat{\mu}$ is calculated as an average of all the values of $Z(x_i)$, and it should give a good estimate of the property Z, in this case soil pH, when that is measured at any point x_i along the transect. We therefore say that the *expected value* of Z at any point x_i along the transect is given by (Webster, 1985):

$$E\,[Z(x_i)] = \mu \qquad (3.4)$$

where E denotes expectation. Taking this idea to its logical extension and considering just the first point on the transect, it can be argued that if the value of Z at this first point is unknown, it could be expected to be equal to $\hat{\mu}$ which is an estimate of μ in equation (3.4); i.e 4.89. Conversely, if the mean was unknown but the value of Z at this point was known, and assuming that the distribution of pH values along this transect was normal, $\hat{\mu}$ would be expected to be equal to the value of Z i.e. 5.38. Using this argument, and equation (3.1) to calculate the value of $\hat{\mu}$ when n is greater than 1, the change in the estimated mean and variance with increasing sample number was investigated by starting at one end of the transect and moving along it, one sample separation at a time to include the other points, until the whole data set was included. The results are shown in Figure 3.2 as a plot of the mean and variance *vs.* the number of values used to calculate them. Since the sample variance of a single realization is zero, the change in s^2 was calculated for n=2 to n=25.

By definition, the variance σ^2 is a measure of the scatter or dispersion of the values of $Z(x_i)$ about the mean μ (Clarke, 1980). s^2 and $\hat{\mu}$ are also assumed to be good estimates of the true variance and mean of the population from which the sample data are drawn. The size of s^2 tells us

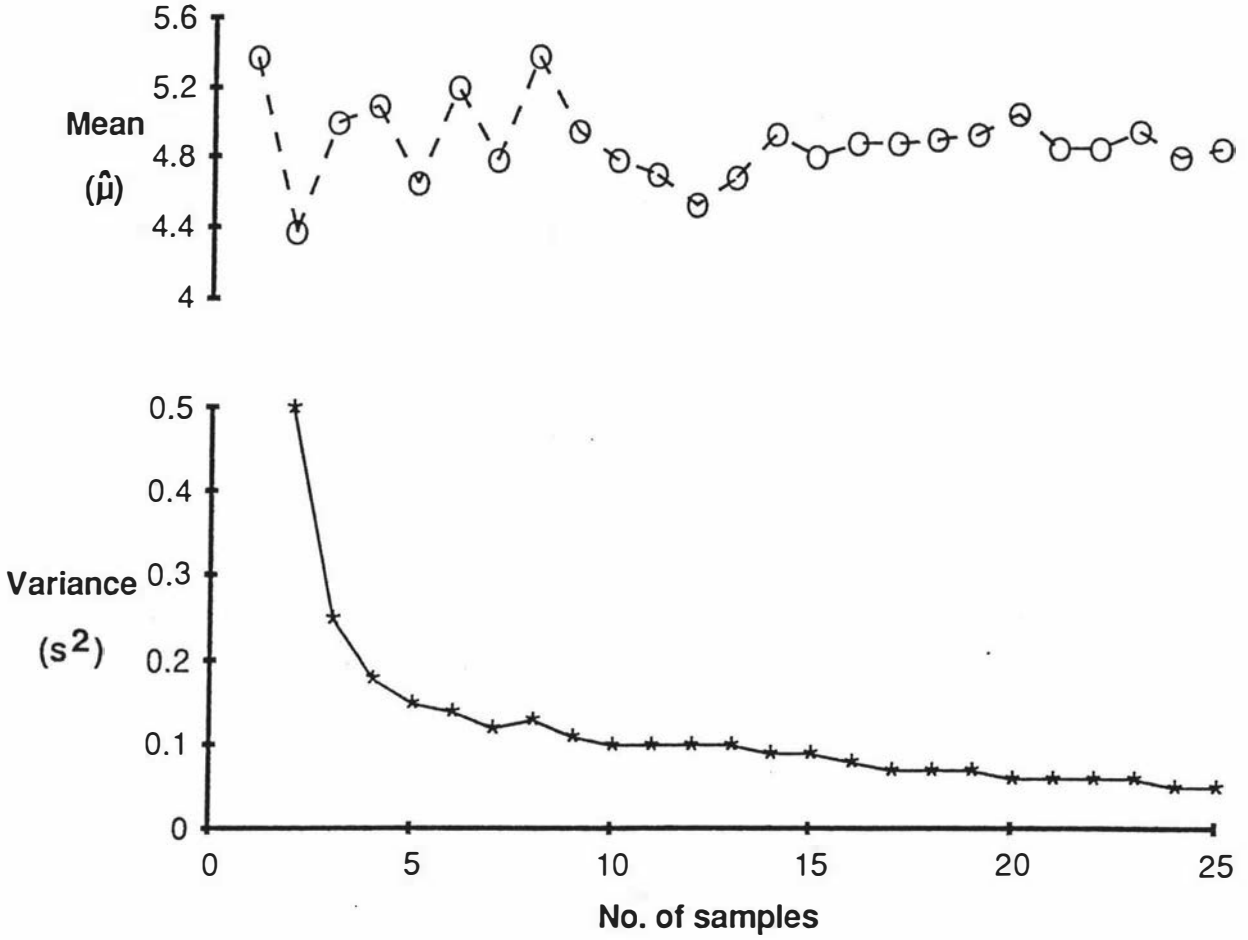


Figure 3.2 Change in the mean ($\hat{\mu}$) and variance (s^2) with increasing sample number for a set of 25 pH data

something about the precision with which $\hat{\mu}$ is measured and we can assume that the more precisely $\hat{\mu}$ is measured, the nearer it will approach to μ. It is therefore interesting to note from Figure 3.2 that as n increases, the change in the value of $\hat{\mu}$ calculated for n and n+1 realizations of $Z(x_i)$ decreases, to the extent that for values of n greater than 15, there is very little fluctuation in the estimated value of $\hat{\mu}$ relative to the fluctuation when n is less than 9. That is, the greater the value of n, the lower the value of s^2 (Figure 3.2), and consequently the more precise the estimate of μ by $\hat{\mu}$. This idea of precision will be seen to be important in the following sections when the spatial distribution of the $Z(x_i)$ is considered.

In addition to the concepts of the mean and variance, it is also an important pre-requisite of spatial analysis to understand the concept of *covariance*. Supposing that in addition to soil pH, the C.E.C had been measured at each site, x_i, along the transect. As part of the data analysis, it may be useful to have a measure of the correlation between the two properties, denoted here by Z and Y. This can be estimated by the covariance, COV, where (Clarke, 1980):

$$\text{COV}\ [Z(x_i),Y(x_i)] = \frac{1}{n-1} \sum \{[Z(x_i) - \hat{\mu}_z]\ [Y(x_i) - \hat{\mu}_y]\} \qquad (3.5)$$

where $\hat{\mu}_z$ and $\hat{\mu}_y$ are the mean values of the sample data of Z and Y. It is shown below that the concept of covariance is important to geostatistical theory.

iii. Stationarity and the semi-variance

Although equation (3.4) states that the expected value of Z at any point x_i is μ, it is clear from Table 3.1 and implicit in Figure 3.2 that the value of Z will *in fact* vary from place to place. This would more obviously be the case if, for example, the transect crossed the boundary between two distinct soil types. Thus, $Z(x_i)$ is called a *random variable* - geostatistics are concerned with identifying its spatial structure. By spatial structure is meant the spatial correlation of the variable with itself, which can be described by properties of its probability distribution. The first property is the mean, defined by equation (3.4); the second is the spatial covariance, defined below.

When the mean value of $Z(x_i)$ does not vary along the transect, the condition of *first-order stationarity* is said to hold (Webster, 1985):

$$E\,[Z(x_i)] = \mu = \text{constant} \qquad (3.6)$$

and it would be expected that a plot like Figure 3.2 would show a straight horizontal line corresponding to a pH of 4.89. If equation (3.6) holds, the expected difference between any two values of $Z(x_i)$ separated by a distance or *lag*, h, would be zero (Trangmar *et al.*, 1985):

$$E\,[Z(x_i) - Z(x_i + h)] = 0 \qquad (3.7)$$

If the mean does vary, *drift* is said to be present and the changing value of the mean can be described by the *drift function*, $d(x_i)$ (Starks & Fang, 1982), and equation (3.6) can be re-written more generally as:

$$E\,[Z(x_i)] = \mu = d(x_i) + \omega(x_i) \qquad (3.8)$$

where $\omega(x_i)$ is a random function of zero mean and finite, fixed variance. $\omega(x_i)$ depends on the variation between values of $Z(x_i)$ and $Z(x_i + h)$, for all values of h.

One of the aims of geostatistics is to quantify the degree of spatial correlation between the values of $Z(x_i)$ and $Z(x_i + h)$. This can be done using the concept of covariance, expressed mathematically in equation (3.5). Thus, the *spatial covariance* of $Z(x_i)$, C(h), is given by:

$$C(h) = [Z(x_i) - \hat{\mu}]\,[Z(x_i + h) - \hat{\mu}] \qquad (3.9)$$

Unlike (3.5), equation (3.9) has no denominator because 1/(n-1) = 1 when there are only two observation points, x_i and $(x_i + h)$. *Second-order stationarity* exists if the value of C(h) for each pair of property values $Z(x_i)$ and $Z(x_i + h)$ is the same, and independent of its position in the sampling region; that is, C(h) depends only on h (Trangmar *et al.*, 1985), and the variability of Z is the same throughout the region (Russo & Bresler, 1981). By implication, when h is zero, C(h) must be equivalent

to the variance of Z, often denoted by C(0). The ratio of the spatial covariance to the sample variance is called the *spatial auto-correlation coefficient*, P(h) given by:

$$P(h) = C(h) / C(0) \qquad (3.10)$$

Thus, under second order stationarity, the mean and variance do not vary. P(h) = 1 when h = 0 and the spatial covariance decreases as h increases and so P(h) becomes a useful geostatistical tool since a plot of P(h) against h will give an indication of the size of h for which values of Z remain correlated, or are *spatially dependent*.

The assumption of second-order stationarity upon which P(h) and C(h) depend is regarded by many geostatisticians as too strong for many spatial variables because of the tendency of estimates of the variance to vary without limit as the size of the area under investigation is extended (Oliver, 1987). As an alternative to assuming second-order stationarity, the *intrinsic hypothesis* of *regionalized variable theory* may be used. This assumes that equation (3.4) holds *and* that for a given value of h, the difference between $Z(x_i)$ and $Z(x_i + h)$ has a finite variance which is independent of x_i, the position of the sample (Webster, 1985):

$$\begin{aligned} \text{VAR}\,[Z(x_i) - Z(x_i + h)] &= E\,\{[Z(x_i) - Z(x_i + h)]^2\} \\ &= 2\,\gamma(h) \end{aligned} \qquad (3.11)$$

where γ is the *semi-variance*.

Implicit in the assumptions underlying equations (3.4) and (3.11) is that the soil property follows the following model of variation:

$$Z(x_i) = \mu_v + \varepsilon(x_i) \qquad (3.12)$$

where μ_v is the mean value of Z in a region, v, and $\varepsilon(x_i)$ is a spatially dependent random component with zero mean, and a variance defined by:

$$\text{VAR}\,[\xi(x_i) - \xi(x_i + h)] = E\,\{[\xi(x_i) - \xi(x_i + h)]^2\} = 2\,\gamma(h) \qquad (3.13)$$

Thus, under the constraints of the intrinsic hypothesis, variables need only be *locally* stationary. It will be assumed for the rest of the analysis of the 25 pH data that local stationarity applies.

iv. The variogram

The semi-variance, $\gamma(h)$, is estimated by $\hat{\gamma}(h)$ for each value of h where (Webster, 1985):

$$\hat{\gamma}(h) = \frac{1}{2m(h)} \sum \{Z(x_i) - Z(x_i + h)\}^2 \qquad (3.14)$$

$\hat{\gamma}(h)$ is equivalent to half the sum of the squared difference between pairs of values of $Z(x_i)$ and $Z(x_i + h)$ averaged according to the number of pairs, m, at each value of the lag h.

A plot of $\hat{\gamma}(h)$ against h for a range of separation distances is the *semi-variogram*, which for simplicity will henceforth be called the *variogram*. The variogram represents the average rate of change of a property with distance (Oliver, 1987). Figure 3.3 shows the experimental variogram for the 25 pH data from the transect. Although there is much fluctuation in the value of $\hat{\gamma}(h)$, it can be seen that the trend is for $\hat{\gamma}(h)$ to increase as the lag increases; i.e. samples closer together have a lower semi-variance than those farther apart, such that the variance of the property is said to be spatially dependent.

Figure 3.3 also shows how the number of pairs of points declines with increasing lag. From Figure 3.2 and the preliminary analysis described in section (ii), it would seem likely that the values of $\hat{\gamma}(h)$ at large lags have low precision compared with those at small lags. Oliver (1987) noted that the precision of the variogram depends on the effective degrees of

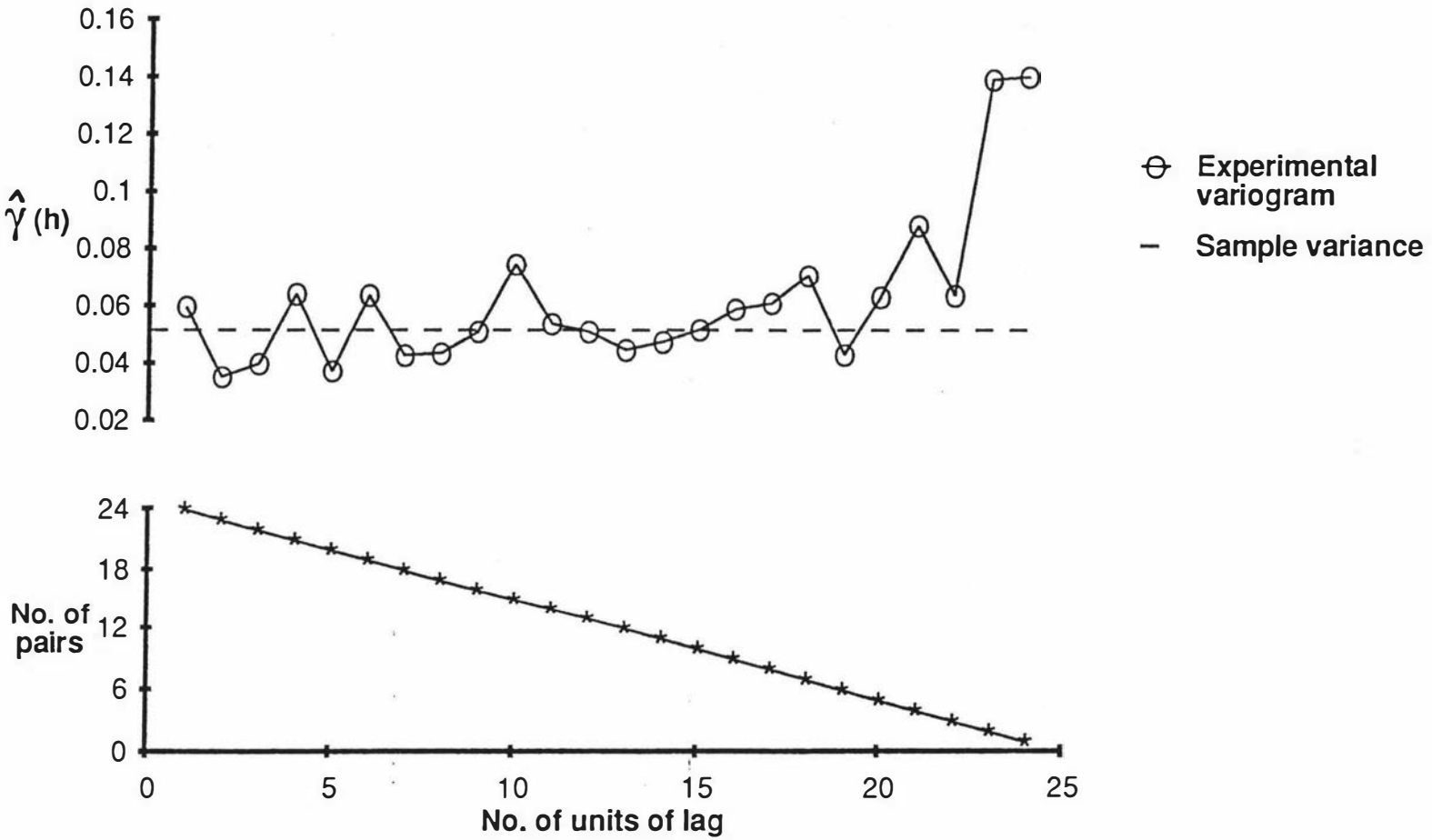


Figure 3.3 Experimental variogram for the 25 pH data, and the number of pairs of points separated by each lag

freedom at each lag, which are a function of the number of pairs at each point, and also on the sampling interval, and degree of spatial variation. The dependence of variogram precision on the effective degrees of freedom is demonstrated by Figure 3.4, which shows the change in $\hat{\gamma}(h)$ as the number of pairs used to calculate it increases. From Figure 3.3 it can be concluded that as the distance separating samples increases, so does the value of $\hat{\gamma}(h)$; i.e. the variance between close samples is less than the variance between points far apart. This, as has already been suggested, could be due to the decreased precision of $\hat{\gamma}(h)$ at large h, so there may be a double effect; increased $\hat{\gamma}(h)$ due to increased h (Figure 3.3), and increased $\hat{\gamma}(h)$ due to a decrease in m (Figure 3.4). The former is the *spatial effect* and one could reasonably conclude that the value of h at which $\hat{\gamma}(h)$ ceases to increase, called the *range*, marks the limit of spatial dependence, the finding of which in many cases may be the objective of the geostatistical analysis. The value of $\hat{\gamma}(h)$ at this and greater values of h is known as the *sill*. The significance of both sill and range is discussed more fully in section (vi). Nevertheless, to find the sill and range, a model must be fitted to the experimental variogram so that its value can be interpolated. However, the increase in $\hat{\gamma}(h)$ due a possible lack of precision at large values of h presents a problem since clearly, $\hat{\gamma}(h)$ at large h should carry less weight than $\hat{\gamma}(h)$ at small h. Thus, to account for the different value of m at different values of h, weighted least squares must be used for the fitting of variogram models (the types of which are outlined below). This point is discussed extensively by Kitanidis (1983), Armstrong (1984), Cressie (1985), and McBratney and Webster (1986).

Figure 3.5 shows the experimental variogram for pH along the transect with two linear models fitted - one, by weighted, and the other by ordinary least squares. As the models are linear, the range cannot be identified since no point is reached where $\hat{\gamma}(h)$ ceases to increase with increasing h. Given that the slope of the variogram is a measure of the degree or intensity of spatial dependence (Oliver, 1987), it can be seen that weighted and non-weighted models differ markedly; the intensity of spatial dependence is shown by the weighted model to be much less than is indicated by the model fitted by ordinary least squares. The weighted variogram model takes the form:

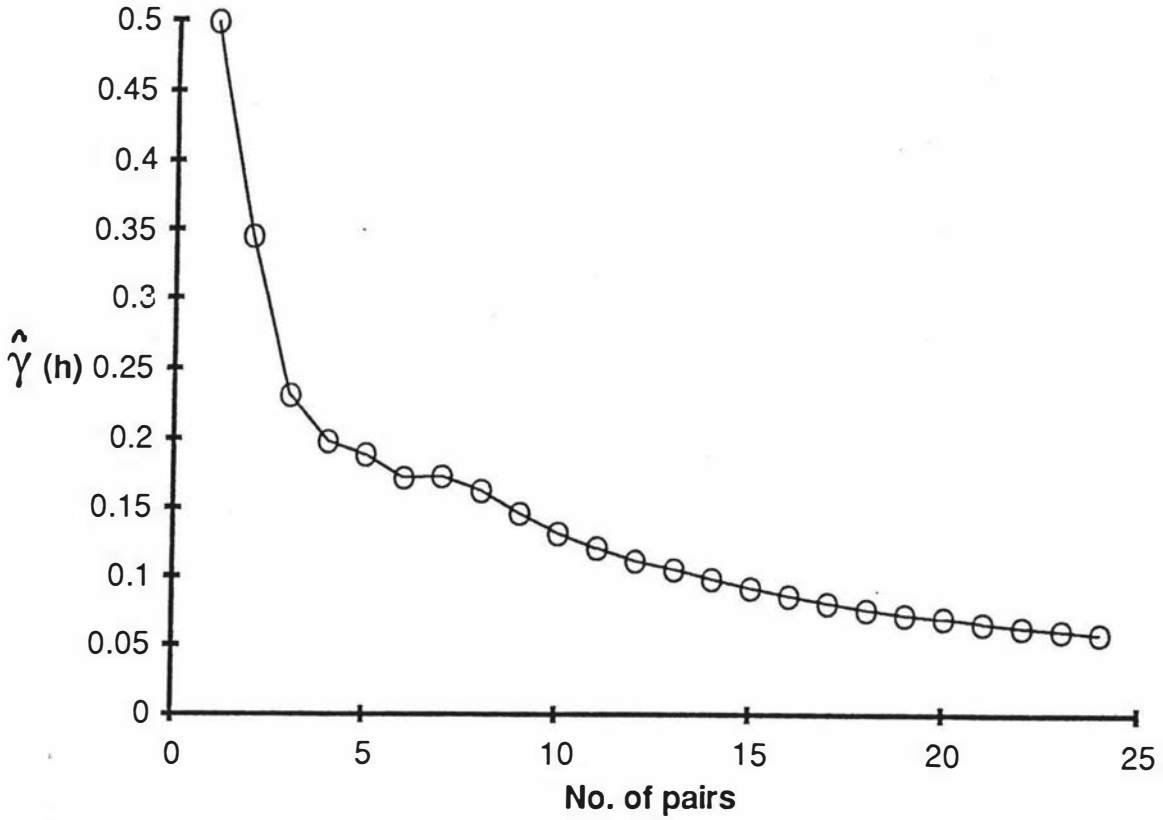


Figure 3.4 Change in the value of $\hat{\gamma}(h)$ as the number of pairs of data points used to calculate it increases. Here h = 1 lag unit; the maximum number of pairs separated by this value of h is 24.

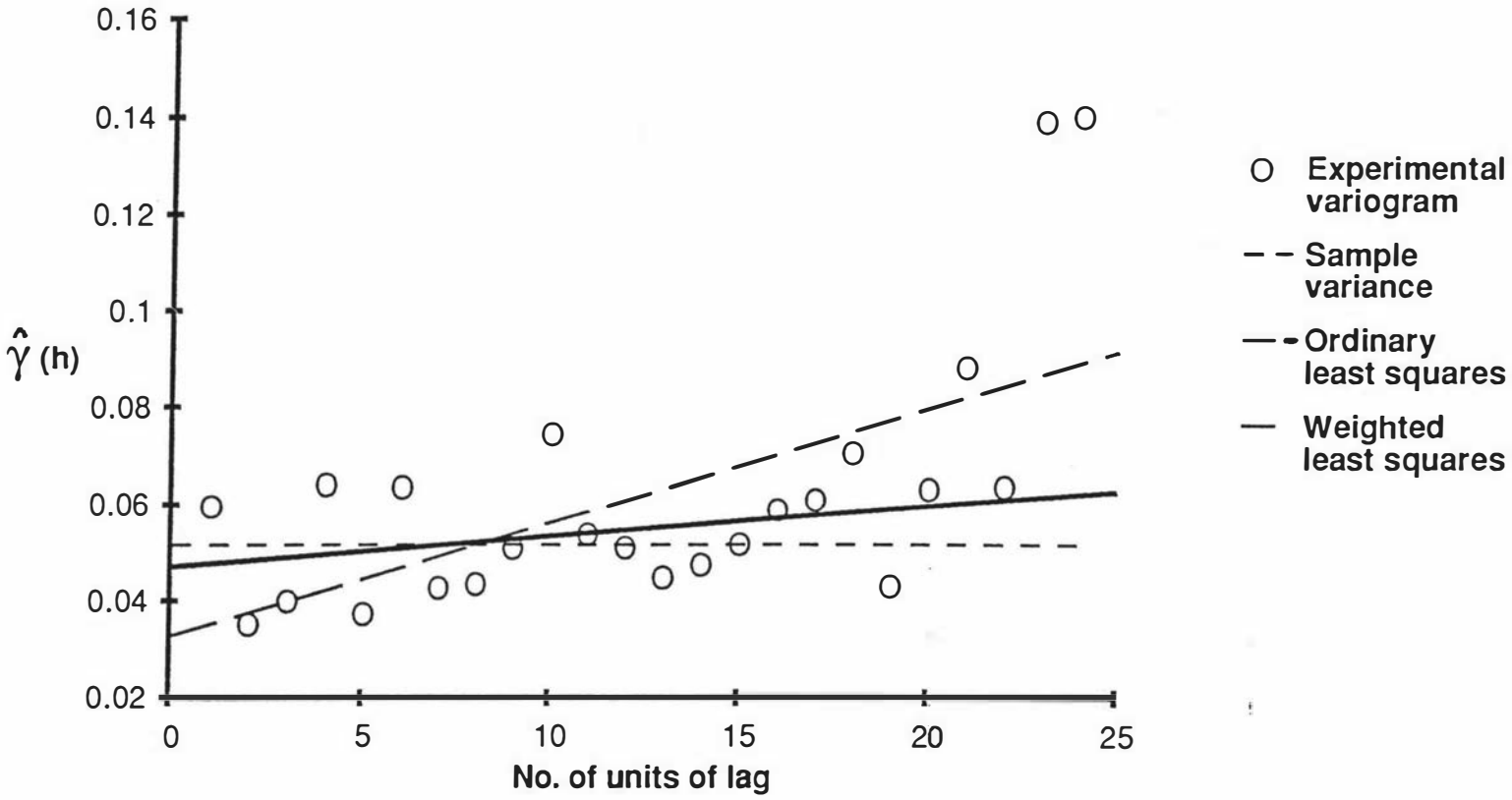


Figure 3.5 Experimental variogram for soil pH with linear models fitted by ordinary and weighted least squares optimization

$$\hat{\gamma}(h) = 0.0470 + 0.0006\ h \qquad (3.18)$$

By definition, $\gamma(h)$ at $h = 0$ must be zero (Webster, 1985). However, as can be seen in Figure 3.5, the fitted model used here to approximate the sample semi-variance does not pass through the origin. This fact that the fitted variogram intersects the ordinate at a value of $\hat{\gamma}(h)$ greater than zero is one of the more important aspects of the experimental variogram. The value of $\hat{\gamma}(h)$ at this point, denoted by C_o is known as the nugget variance, and corresponds to either unexplained error or variability of Z which is undetected at the scale of sampling, or both. In Figure 3.5, the value of C_o (0.0470) is not much less than either s^2 (0.0517) or the value of $\hat{\gamma}(h)$ at $h = 25$ units of lag (0.0620), and thus, this variogram is said to have a high nugget variance; i.e. the degree of spatial dependence is low. This point is discussed in more detail in section (vi).

v. Spatial variation in two dimensions

Since a transect will only show variation in one dimension, data sampled in the same area in two dimensions might be expected to show different spatial variation, and it is therefore useful to repeat the analysis outlined above on data sampled on a grid. Figure 3.6 shows the spatial arrangement of a data set comprising 121 pH measurements made on soil samples taken from the same area as the transect, this time on an 11 x 11 sampling grid with a grid spacing of 2.5 m.

The 121 data points were found to be normally distributed (Figure 3.7; $R^2 = 0.91$, $p<0.1\%$). The mean, $\hat{\mu}$, was 4.92 and the sample variance, s^2, was 0.0335. Instead of analysing the data one point at a time as for the transect (Figure 3.2), the data were analysed in two dimensions by starting in the top left corner and moving in stages along both the *north-south* and *east-west* axes simultaneously, one lag at a time. Throughout this analysis, *isotropy* was assumed, that is, it was assumed that the spatial variation (if any) is the same in both the north-south and east-west directions (and in all other directions). When the data are isotropic the lag becomes a scalar (Oliver & Webster, 1987), and the data for all directions may be grouped, which is what has been done here.

	A	B	C	D	E	F	G	H	I	J	K
a	5.38	4.53	4.95	4.90	4.75	4.80	4.75	4.80	5.00	5.18	4.98
b	4.38	4.68	4.80	4.98	4.65	4.90	4.80	4.85	5.00	4.95	4.95
c	5.00	4.93	4.85	4.68	4.68	4.70	4.98	4.80	5.03	4.83	4.95
d	5.10	4.80	4.90	4.53	4.80	4.73	4.93	4.90	4.98	4.63	4.58
e	4.65	4.88	4.70	4.85	4.90	4.95	4.93	5.05	5.05	4.78	4.70
f	5.20	4.88	5.08	5.05	4.78	4.93	4.90	4.90	5.20	5.30	5.08
g	4.78	4.90	4.83	5.20	4.80	5.20	5.10	4.70	5.13	5.13	5.28
h	5.38	4.93	4.75	5.10	4.98	5.20	5.05	4.93	5.05	5.10	5.35
i	4.95	5.05	5.03	5.05	5.03	4.93	4.93	4.95	4.75	4.78	5.05
j	4.78	4.85	4.90	5.00	5.05	5.05	4.83	4.80	5.13	5.05	5.20
k	4.70	4.85	4.78	4.78	4.98	5.05	4.70	4.70	4.70	5.00	4.93

Figure 3.6 121 equally spaced pH data. (N.B. The ak and AK axes were of equal length in the field sampling grid.)

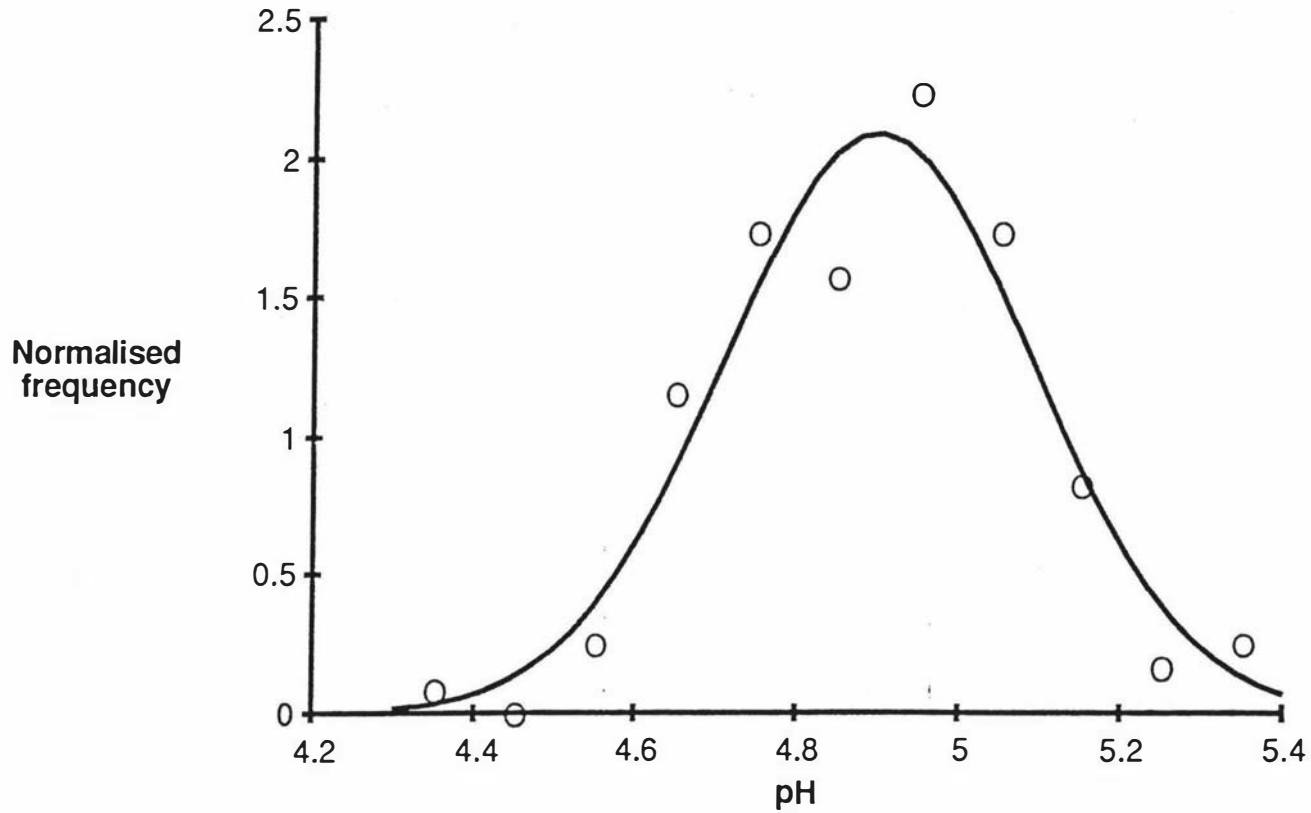


Figure 3.7 Distribution of the 121 pH data shown in Figure 3.6

The data were assessed for changing mean and variance as the number of samples increased with increasing lag, this time in a progression dependent on the square of the lag; 1,4,9,16,25....121, rather than in a simple arithmetic progression as was the case with the transect. As Figure 3.8 shows, the precision of the mean again increased with increasing sample number. Figure 3.9 shows the change in the number of pairs of points, m, with increasing lag, h. As in Figure 3.3, m decreases markedly as the lag increases. However, the degree of precision is seen to be much greater at high lags in the case of the grid compared to the transect (Figure 3.10). In the case of the transect, there were only 24 pairs of points at the smallest lag. With the grid, there are 22 pairs at the largest, and 220 pairs at the smallest lag. Obviously, a much greater sampling effort is needed for 121 samples as compared to 25, but these two figures illustrate the value of sampling in two dimensions; in the case of a transect-based sampling strategy, this could be done by sampling along two intersecting transects. Assuming isotropy, this would have the effect of doubling the precision of a variogram based on a single transect since there would be twice as many pairs of observations. i.e. samples would have to be taken from 49 points instead of 25 (the point at which the transects intersect would occur on both transects). The effect of changing the point of intersection on the variogram is not clear and will not be investigated here, although where the data are *anisotropic* the point of intersection and the angle of one transect to the other may be important.

Figure 3.10 shows how the variogram changes as more samples are included by increasing the length of the side of the square grid to include more lags. In Figure 3.4, $\hat{\gamma}(h)$ was shown to decrease as m increased when samples separated by a single unit of lag were considered. Here, $\hat{\gamma}(h)$ is shown to decrease as m at all lags increases, as is indicated by the development of the variogram from 2 lags (4 samples) to 10 lags (121) samples. It is clear that the precision of the variogram for all 10 lags is significantly greater than when h is less than 10; one infers, because $\hat{\gamma}(h)$ decreases at intermediate lags as m for any one lag increases, that the precision of the variogram is increasing. If there were spatial dependence, increasing m through bringing in more data points might be expected to increase $\hat{\gamma}(h)$ at intermediate lags.

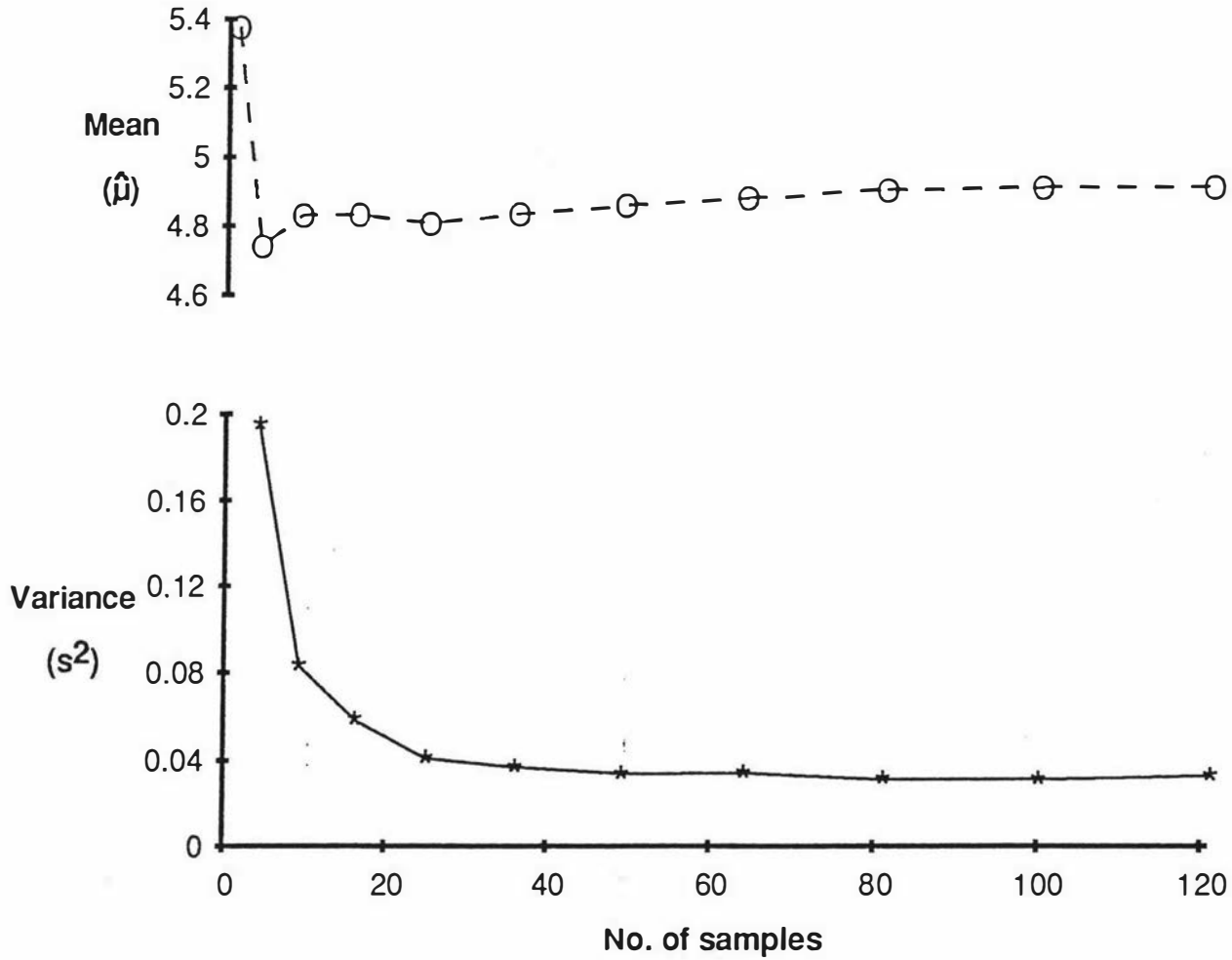


Figure 3.8 Change in the mean ($\hat{\mu}$) and variance (s^2) with increasing sample number for the set of 121 pH data

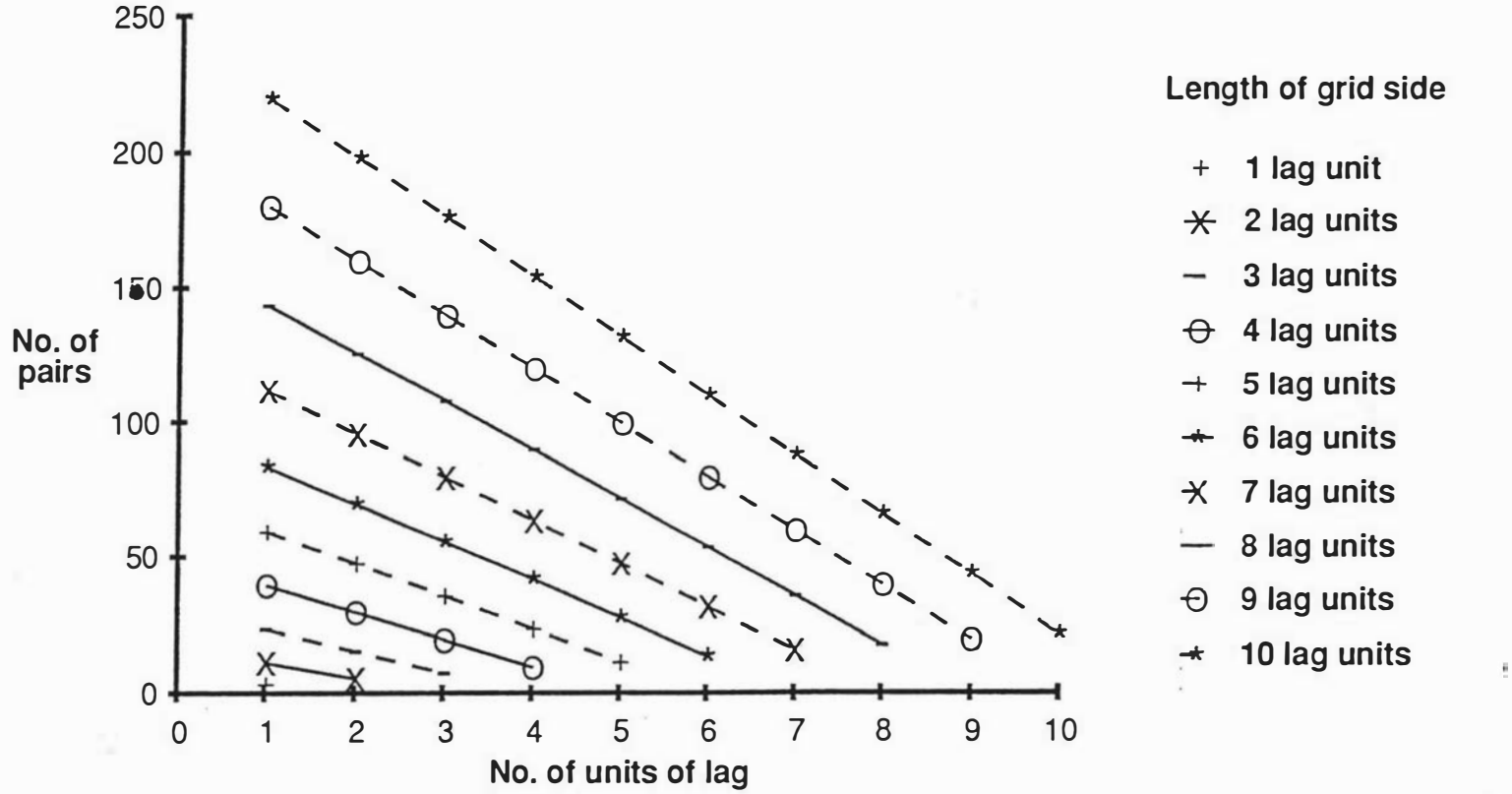


Figure 3.9 Change in the number of pairs of points separated by each lag as the length of the grid side increases from 1 to 10 lag units

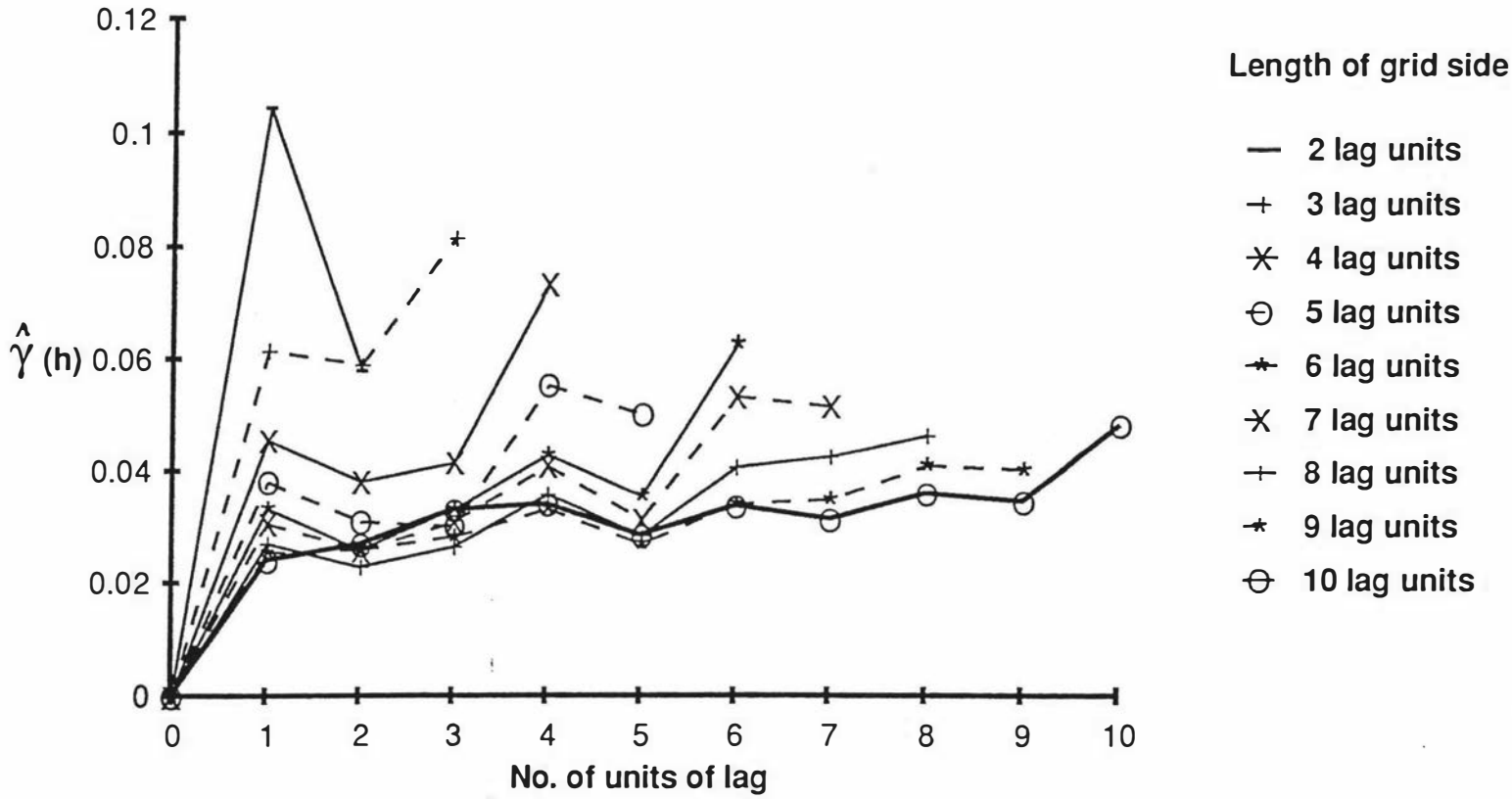


Figure 3.10 Development of an experimental variogram as the number of samples used to estimate it increases as the length of the grid side increases from 2 (4 samples) to 10 lag units (121 samples)

vi. Other variogram models

Unlike the variogram in Figure 3.5, the complete variogram for the grided data (Figure 3.10) has a curvilinear form. It is therefore appropriate to investigate some other possible variogram models.

Armstrong (1984) noted that the efficacy of geostatistics was essentially dependent on the quality of the estimate obtained for the variogram. Thus, getting the best fit for the variogram is very important, whether it is to be used for interpolating values of Z at unsampled sites (kriging), or simply to find the values of h for which values of Z are related. It is not intended here to discuss kriging since it was not necessary to use this technique in any of the work presented in this thesis. Suffice to say that kriging involves minimizing the estimation variance of the interpolated values of a property. One of its principle advantages over other interpolation methods is that it gives a measure of precision, but this is only good if the model for the spatial structure (ie. the variogram) is at least approximately correct (Starks & Fang, 1982). Kriging therefore relies heavily on the form and goodness of fit of the variogram model, especially at small lags, since interpolation is invariably used to give information on the spaces between existing sampling points.

The rationale which determines whether a particular model is suitable to describe a variogram is complicated and will not be dealt with here. It is discussed extensively by Armstrong and Jabin (1981), Christakos (1984), and M^{c}Bratney & Webster (1986). Here it is enough to say that linear, spherical, and exponential models are permissible functions for variograms and will describe most.

As indicated by equation (3.18), the linear model takes the general form:

$$\gamma(h) = C_o + kh \qquad \text{for } h > 0 \qquad (3.19)$$

where C_o is the nugget variance, and k is the slope. Note that this model has no sill, and therefore no range. In the case where k = 0, the variogram is said to show a *pure nugget effect* (Webster, 1985); that is, there is no spatial dependence at the scale of sampling.

The spherical model takes the form:

$$\gamma(h) = C_o + C\{[3h/2a] - [(h/a)^3/2]\} \quad \text{for } 0 < h < a$$
$$\gamma(h) = C_o + C \quad \text{for } h > a \qquad (3.20)$$

and its tangent at h = 0 cuts the sill at 2a/3. Here, a is the range, and h, and C_o are the lag, and nugget variance respectively. Just as C_o represents variability which *is not* detected at the scale of sampling, C represents variability which is detected at the scale of sampling; the sill variance is given by $C_o + C$. In theory, the spherical model is three-dimensional, yet Webster (1985) noted that it nearly always fits experimental results from soil sampling better than one and two-dimensional analogs, such as the circular model (which is not recommended for describing variograms).

The exponential model takes the form:

$$\gamma(h) = C_o + C[1 - \exp(-h/r)] \quad \text{for } h > 0 \qquad (3.21)$$

Here, r is a distance parameter which controls the spatial extent of the function (Webster, 1985). In the exponential model, $\gamma(h)$ approaches the sill asymptotically and there is no such thing as a definable finite range. However, since the semi-variance must cease to increase beyond a certain point, the range is taken to be equal to 3r, where $\gamma(h)$ is approximately equal to $C_o + 0.95C$ (Webster, 1985). Oliver and Webster (1987) have noted however, that taking the range as equal to 3r tends to overestimate it, and where this appears to be the case, the spherical model will probably give a better fit because it curves more tightly. Whichever model is chosen, it is pertinent to bear in mind that

> "to serve us well, the model has to adequately portray the behaviour of the measurements as they really are. It is not enough to represent how we wish the measurements had been (but were not)." (Tukey (1973) quoted by Armstrong (1984).)

Accordingly, McBratney and Webster (1986) recommend the Akaike Information Criterion (AIC) for determining which is the best model for the experimental variogram. The AIC is calculated according to the equation:

$$AIC = n[\ln(R)] + 2p \qquad (3.22)$$

where n is the number of observations (or points on the variogram), p is the number of estimated parameters (sill, range etc...), and R is the residual mean square of deviations from the fitted model. The model with the smallest AIC value is the best. Where the models being compared have the same number of parameters (spherical and exponential), there is no need to calculate the AIC since a simple comparison of the residual sum of squares will reveal which model has the best fit.

The three variogram models described above were fitted to the experimental variogram for the 121 grided data and the AIC calculated for each. The value of AIC was lowest when the spherical model was used; this model is shown fitted to the experimental variogram by weighted least squares in Figure 3.11. Here, $\hat{\gamma}(h)$ increases from a nugget variance of 0.0183 to a sill of 0.0509 at h = 3.96 lag units, the range. Thus, the pH data show spatial dependence at sample separations less than 3.96 lag units which is equivalent to 9.9 m.

In Figure 3.11, and also in the other figures showing the variograms for both the transect and grided data, the sample variance has been plotted as well as the experimental variogram, and in Figure 3.11 it is seen to be approximately equivalent to the sill. Trangmar *et al.* (1985) stated that the sill corresponds to the maximum variabilty of Z which is detected at the scale of sampling. When the sample variance is almost *pure nugget*, that is $C_o \approx s^2$, the sill will be approximately equal to the sample variance (Webster, 1985). Generally the sill is expected to be somewhat larger than the sample variance (Webster, 1985). One reason that this might be expected to be so is that the fitted model represents the *best* fit to a set of *experimental* data, although it might equally be expected that the sill would under-estimate the sample variance for the

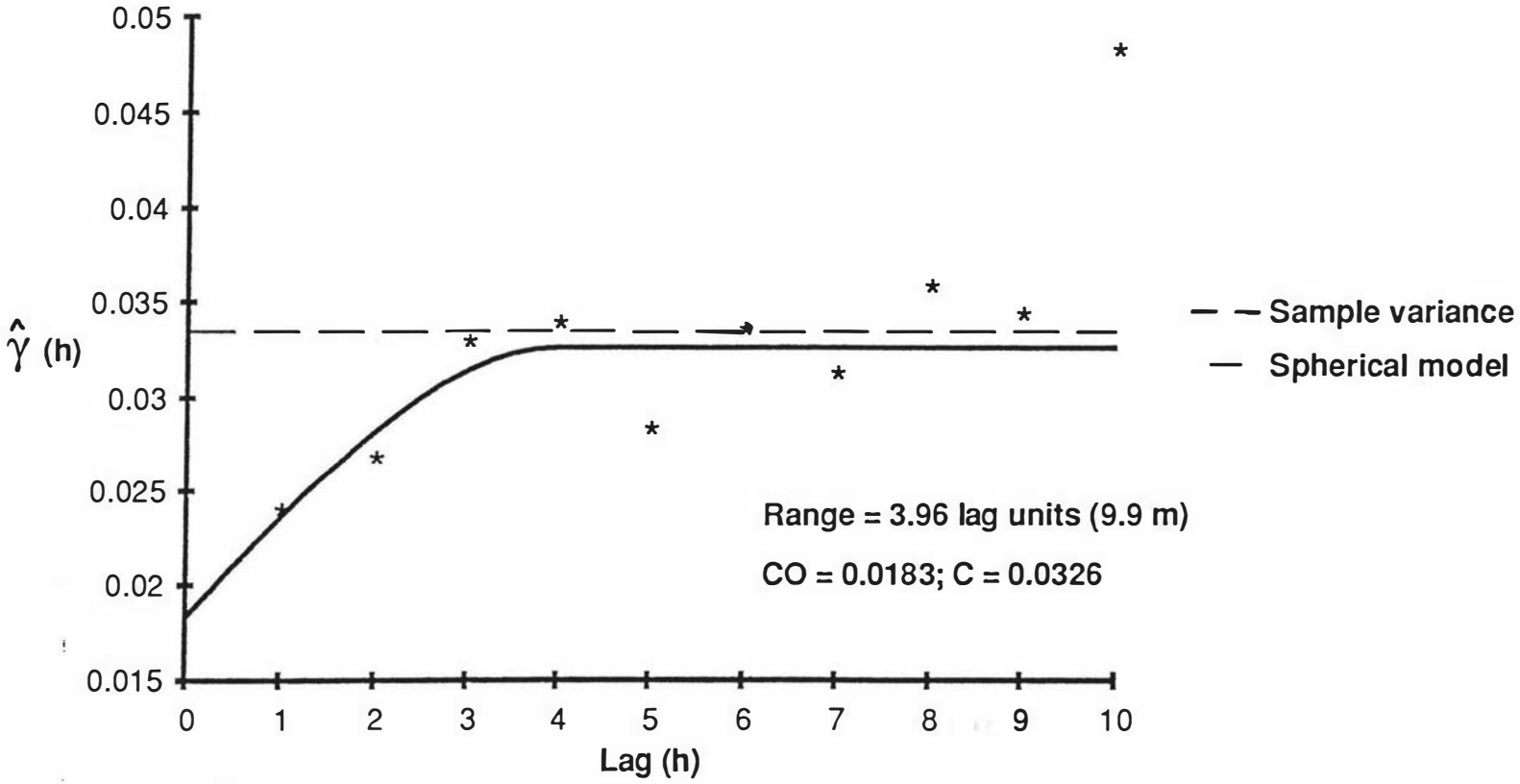


Figure 3.11 Experimental variogram for the 121 pH data fitted with a spherical model by weighted least squares optimization

same reason. Another is that the estimated variance s^2 must be smaller when more samples are taken within a given area, especially when there is some spatially dependent variability. This point is discussed in some detail by Webster (1985) and will be returned to in Chapter 10. For now, it may be concluded that the spherical variogram shown in Figure 3.11 is a good model of the experimental data as sampled on the grided design as described.

vii. Conclusions

Using "aggie" statistics (Nielsen, 1987), the conclusions made as to the 121 pH data would have been confined to comments on the estimated mean and sample variance, and in isolation these would tell very little. Of course, if measurements of another property had also been made at each x_i, then correlations, comparisons and relationships between them could have been investigated, but the often large error term may have occurred. By performing a spatial analysis using geostatistical techniques as detailed above, a large portion of this error term can be accounted for in terms of the spatial effect. When the nugget-variance is low as a proportion of the sample variance, then the spatial effect at the scale of sampling is large and may merit more intensive investigation. If C_o is large in comparison with s^2, the error term may not be due to a high degree of spatial dependence; at least, not at the scale of sampling used. This would either merit further spatial analysis at a different sampling scale, or alternatively may point to an inadequacy in the analytical techniques used or to the use of an insufficient number of samples. The other important information gained from a spatial analysis is the identification of the range (if present) since it indicates the minimum sample separation for which samples are unrelated. In other words, knowing the range to be equal to λ, any further sampling should be done with samples taken at intervals of $h \geq \lambda$ so as to avoid spatial dependence in the data, and thus to eliminate error due to sampling design. This is the object of the analysis of spatial variability in nitrifier activity which forms the bulk of the work described in this thesis.

CHAPTER 4

EXPERIMENTAL METHODOLOGY

A. THE SHORT-TERM NITRIFICATION ASSAY

Studies of nitrification and the effects on it of various soil parameters such as pH (e.g. Frederick, 1956; Aleem & Alexander, 1960; Morrill & Dawson, 1961; Darrah *et al.*, 1986b), or soil moisture and temperature (e.g. Sindhu & Cornfield, 1967a; Kowalenko & Cameron, 1976; Addiscott, 1983; Nakos, 1984; Macduff & White, 1985) have been undertaken for a number of years. Commonly these have involved either long-term perfusion experiments, pure culture studies of nitrifiers growing under laboratory conditions, or incubation experiments of several days or weeks duration. Thus, little attention has been paid to monitoring the *in situ* nitrifier activity, either in isolation, or in response to changes in one or more of the soil parameters. The short-term nitrification assay, **SNA**, of Sarathchandra (1978) and Schmidt and Belser (1982), as modified by Darrah *et al.* (1986b), permits the study of *in situ* nitrifier activity without the complication of microbial growth. Consequently, this technique, with the modifications described below, was chosen as the basis of the experimental work reported in this thesis.

The rate of nitrate production by nitrifiers growing under non-limiting substrate conditions can be described by the equation (Darrah *et al.*, 1985b):

$$\frac{\partial NO_3}{\partial t} = \frac{m\mu_{max}}{Y} \cdot \exp(\mu_{max} t) \qquad (4.1)$$

where $\partial NO_3/\partial t$ is the instantaneous rate of nitrate production, μ_{max} is the maximum specific growth rate (h^{-1}), m is the nitrifier biomass (μg g^{-1} soil) and Y is the growth yield constant (μg biomass formed per μmol of ammonium oxidized). When the incubation time t is short, m may be assumed to be constant, and equation (4.1) simplifies to (Darrah *et al.*, 1986b):

$$\frac{\partial NO_3}{\partial t} = \frac{m\mu_{max}}{Y} \qquad (4.2)$$

This constant rate of nitrate production can be measured in the short-term nitrification assay. The rate of nitrate production in a steady-state population should depend on the size of the nitrifier population and the physiological activity of the organisms making up that population. Thus, the measured nitrification rate in a short-term assay reflects these features of the population and will be referred to by the all-encompasing term, *nitrifier activity*; the SNA value is an index of nitrifier activity.

Since the test of no measurable growth in a nitrifier population is linearity of nitrification, the successful use of equation (4.2) in laboratory incubation experiments depends upon the selection of a time interval for incubation such that nitrification proceeds linearly over the whole period t. It also depends on the use of a non-limiting incubation medium and substrate concentration. Thus, the SNA technique has to be tailored to suit the particular soil under investigation.

i. Selection of incubation medium for SNA analyses

The observations of Darrah *et al.* (1987a) of the adverse effect on nitrifiers of solutions of low osmotic potential suggest that incubation media should have an ionic strength approximately equivalent to that of soil solutions in the field. Edmeades *et al.* (1985) studied the chemical composition and ionic strength of a range of New Zealand topsoils under grassland and found that ionic strengths ranged from 0.003-0.016 mol dm^{-3} with a mean value of 0.005. Dolling and Ritchie (1985) found that the ionic strengths of soil solutions from 20 soils from Western Australia had very similar values. They also noted the marked effect of differing ionic strength on the measurement of soil pH. pH measurements made in solutions with an ionic strength of 0.005 differed least from measurements made at the ionic strength of soil solutions at field capacity, whilst the differences that occurred in comparisons with

distilled water or $CaCl_2$ at an ionic strength of 0.03 (0.01 M) were much greater ($\geq$ 0.4 pH units). In view of the dependence of nitrification on pH (Darrah *et al.*, 1986b) and the fact that the relationship between pH and nitrifier activity was to be a major area of interest in this study, it was clear that all SNA measurements had to be made in solutions of ionic strength close to that of the soil solution in the field. The mean ionic strength of the topsoil of Tokomaru silt loam was measured by Edmeades *et al.* (1985) as 5.4×10^{-3} mol dm^{-3} (with a range of $2.1\text{-}11.3 \times 10^{-3}$ mol dm^{-3}). On this basis, it was decided that 0.005 M KCl would be suitable for SNA analyses and as a medium for all the experiments reported here (unless otherwise stated); the difference in ionic strength between 0.005 M KCl and the expected extremes in ionic strength of field soil solutions was assumed to be sufficiently small to cause neither dispersion of the soil nor inhibition of the nitrifiers.

ii. Linearity of nitrification rate in the Tokomaru Silt Loam

Aleem and Alexander (1960) found that the minimum generation time for *Nitrobacter agilis* was about 7 hours, whilst Sarathchandra (1978) found no significant change in the most probable number of soil nitrifiers during 17 hours incubation. Accordingly, Sarathchandra (1978) and Steele *et al.* (1980) sampled incubating media after 1 and 17 hours and calculated SNA values as the difference between the amount of NO_3-N produced between these times per g soil per hour. Darrah *et al.* (1986b) calculated SNA values in the same way but used shorter incubations, sampling after 1 and 8 hours following the addition of NH_4-N substrate. In view of this range of incubation times for SNA measurements, an experiment was conducted to investigate the linearity of the nitrification rate in the Tokomaru silt loam.

Methods and Materials

A bulk soil sample (approx. 5 kg) was dug from the 3-9 cm depth range of a randomly selected site in field No. 6 (see Section B, below). The soil was sieved (<2 mm), thoroughly mixed, and a 200 g subsample was placed in a Buchner funnel fitted with a Whatman No. 1 filter paper, and leached overnight with 1 dm^3 0.005 M KCl to remove any nitrate present. At the end of leaching, excess moisture was removed from the soil by suction filtration for 90 minutes, after which 38 replicate 5 g samples (oven-dry equivalent - determined by oven-drying overnight at 105 °C) were placed into 50 cm^3 incubation tubes containing 20 cm^3 0.005 M KCl with 0.3 % $^w/_v$ agar. In later experiments the dilute agar suspension allowed the incubating media to be sub-sampled after 1 hour without affecting the soil:solution ratio for the rest of the incubation (Darrah *et al.*, 1987a).

10 cm^3 0.01 M $(NH_4)_2SO_4$ was added to each tube and the tubes were shaken at 22 °C in an enclosed end-over-end shaker fitted with a thermostat. (The choice of 22 °C as a suitable incubation temperature was entirely arbitrary, and was governed by the fact that since the temperature inside the shaker was maintained by two 100 watt light bulbs, 22 °C was a temperature that was easily maintained at a constant, at all times of the year.) At hourly intervals up to 19 hours after the start of incubation, two tubes were removed, shaken and duplicate 5 cm^3 samples of suspension were quickly taken by pipette from each tube. These were centrifuged at 3000 r.p.m for 10 minutes and the supernatant frozen and stored. The solutions were later analysed for NO_3-N on a Technicon Autoanalyser following the method of Downes (1978).

Results and Discussion

Figure 4.1 shows the amount of NO_3-N produced in each incubation plotted as a function of the time of incubation. By expressing the data on an hourly basis, it was clear that the nitrification rate over the first hour was significantly higher than it was during the remaining eighteen hours of the incubation. Thus, the assumption of linear nitrification did

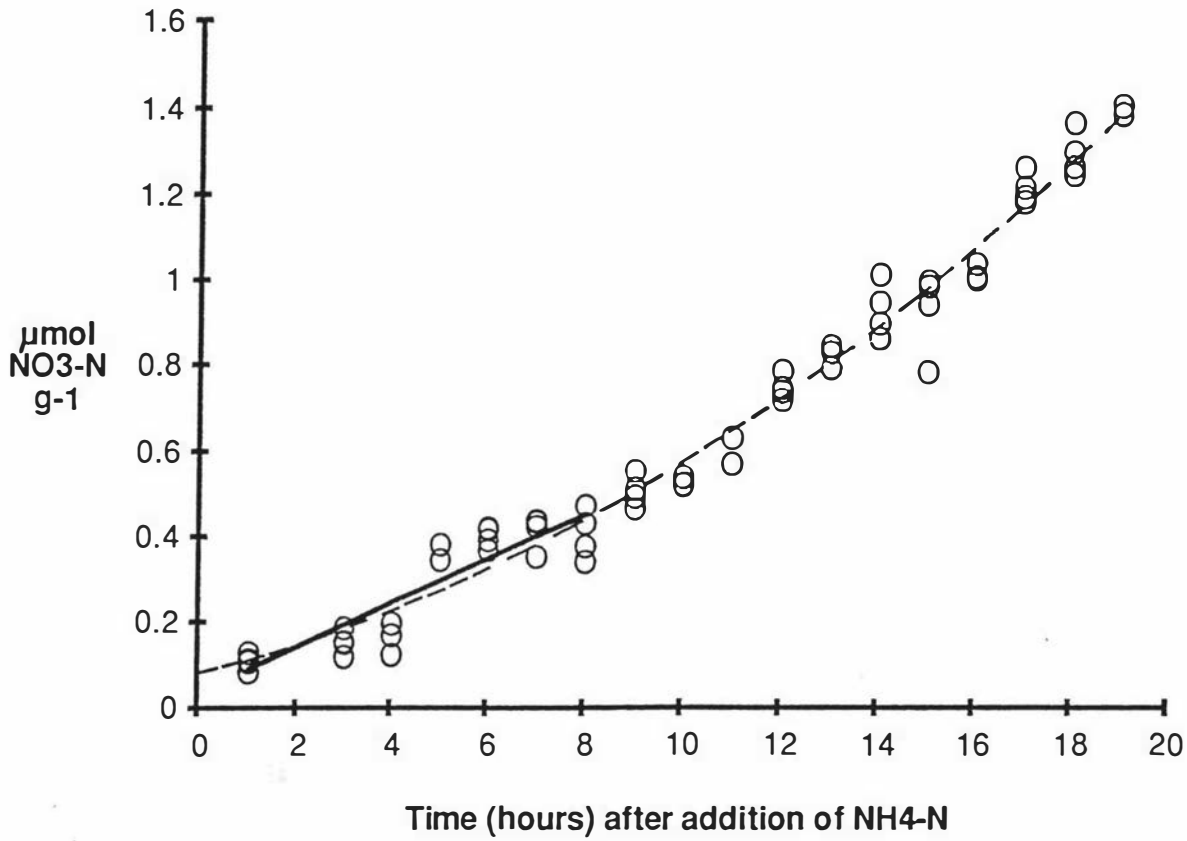


Figure 4.1 Nitrification rate in the Tokomaru silt loam measured at 22 °C for 19 hours after the addition of ammonium substrate

not seem to be a good one. In fact the data were best-fitted by an upwards curvilinear model (Figure 4.1, dotted line; R^2 = 0.98, $p<0.1\%$) which is what would be expected for a growing population. It was therefore concluded that the incubation time selected would have to be a compromise which satisfied three basic requirements; (a) that nitrate production should be approximately linear with time; (b) the incubation time would have to be long enough to allow for production of a measurable increase in NO_3^- concentration; and (c) not too long that significant population growth occurred, or the experiment became physically impossible to do. A linear model was fitted to the data between 1 and 8 hours (Darrah *et al.*, 1986b) and since this gave a good fit (Figure 4.1 solid line; R^2 = 0.82, $p<0.1\%$) these times seemed suitable for SNA measurements on the Tokomaru silt loam.

As indicated above, the time taken to complete an SNA analysis was an important consideration. It was apparent that the maximum number of tubes that could be dealt with at a time without incurring a time error whilst sampling the suspensions, was between 15 and 20. (In fact with practice, 15 tubes could be sampled in 2 minutes. i.e. the time of sampling given as 1 or 8 hours is accurate to ± 3% after 1 hour and ± 0.4 % after 8 hours.) By starting the Buchner suction at 6.30 am (and allowing for a period of 5 hours for the samples to equilibrate following the addition of acid or alkali - see Chapter 7 and Darrah *et al.* (1986b)), and separating the tubes into four groups of 15 with a stagger of half an hour between the first and second and the third and fourth groups, and 45 minutes between the second and third, it was possible to complete 60 SNA analyses by midnight if an incubation of 8 hours was used. Accordingly, in all SNA analyses reported here, the incubating media were sampled after 1 and 8 hours, and the SNA value calculated as the difference between NO_3-N produced at these times per g soil per hour.

iii. Selection of ammonium substrate concentration for SNA analyses

Macduff and White (1985) measured nitrification rates over a range of soil moisture contents and incubation temperatures, and found that irrespective of temperature, nitrification was limited by the supply of NH_4-N. Gilmour (1984) predicted that nitrification rates following zero-order kinetics will increase as the initial concentration of NH_4^+ increases according to the equation:

$$NR = k \times NX_t \qquad (4.3)$$

where NR is the absolute nitrification rate, k is the rate constant, and NX_t is the initial NH_4^+ concentration at the start of the time period t. Molina (1985) asserted that nitrification proceeded from pulses of ammonium oxidation generated by microbial clusters. The size of the pulse must be subject to a negative feedback system, however, because if large applications of ammonium are supplied, the effect of low osmotic potential will inhibit nitrification (Darrah *et al.*, 1987a). Thus equation (4.3) must be treated with circumspection because it suggests that NR will continue to increase linearly with increasing initial NH_4 concentration even at very high concentrations. Furthermore, Clay *et al.* (1985) noted that if clusters of NH_4^+ oxidizers were to generate a pulse of NO_2^- large enough, their *microniche* may be acidified to toxic levels and nitrification would be reduced (Chapter 7). Nevertheless equation (4.3) does indicate that nitrification rates can be limited by inadequate NH_4-N substrate concentrations. Thus, the concentration of NH_4-N substrate supplied in SNA incubations must be such that it is non-limiting in the sense that it is in excess, but not so much so as to generate conditions which are toxic to the nitrifiers.

The form in which the ammonium was to be supplied for SNA measurements was also an important consideration, the obvious choice being between NH_4Cl and $(NH_4)_2SO_4$ since these are readily available forms of ammonium. Darrah *et al.* (1985a) monitored the response of soil nitrifiers to additions of both NH_4Cl and $(NH_4)_2SO_4$. Additions of more than 7.3 μmoles N g^{-1} soil as NH_4Cl were found to inhibit nitrification, but a similar effect was not found with $(NH_4)_2SO_4$ suggesting that the chloride ion

rather than osmotic potential was the cause of the inhibition. Further work (Darrah *et al.*, 1987a) demonstrated that the inhibitory effect of the Cl^- ion was disproportionate to its contribution to the osmotic potential of the soil solution. i.e. Cl^- is toxic to nitrifiers. Accordingly, $(NH_4)_2SO_4$ was chosen as the substrate to be used for SNA measurements, but an experiment was required to establish what concentration should be used, since Darrah *et al.* (1985a; 1987a) had shown that this salt was also inhibitory to nitrifier activity in high concentrations.

Methods and Materials

A bulk soil sample was collected as for the linearity experiment (Section ii. above) and leached overnight with 0.005 M KCl. After the removal of excess moisture, 60 incubations were set up following the method described above, except that the tubes were split into 6 groups and $(NH_4)_2SO_4$ substrate added as 10 cm^3 of 0.001, 0.005, 0.008, 0.010, 0.015 or 0.020 M (10 replicates each). The incubations were carried out as described, with sampling of the suspension after 1 and 8 hours.

Results and Discussion

The mean SNA values for each concentration of $(NH_4)_2SO_4$ added to the incubations are shown in Table 4.1. An analysis of variance showed that there was no significant difference in SNA value ($p<0.1\%$) over the range of NH_4-N concentrations tested. i.e. there was either no inhibition of nitrifier activity in any treatment, or the amount of inhibition was the same for each treatment. Direct comparison between these results and those of Darrah *et al.* (1987a) is made difficult by the fact that (a) in this experiment there was no means of measuring the osmotic potential (an osmometer was not available); (b) the ionic strengths of the incubation media were quite different (0.01 M $CaCl_2$ compared to 0.005 M KCl); and most importantly (c) the ionic strength of the soil solution in the field in the soil studied by Darrah *et al.* (1987a) was probably different from that in the Tokomaru silt loam, with the consequence that the soil

Table 4.1 Effect of $(NH_4)_2SO_4$ substrate concentration on SNA value

Concentration of $(NH_4)_2SO_4$	Mean SNA (μmol NO_3-N g^{-1} h^{-1})	Standard error
0.001 M	0.016	0.0010
0.005 M	0.015	0.0008
0.008 M	0.015	0.0011
0.010 M	0.015	0.0015
0.015 M	0.013	0.0010
0.020 M	0.015	0.0017

nitrifier response to changes in osmotic potential might be quite different between the two soils, certainly in terms of its magnitude. Nevertheless, if one assumes that the differences between the incubating media were insignificant in terms of their effect on the overall osmotic potential in the two experiments, simple comparisons can be made.

Osmotic pressures were calculated for each substrate concentration using the equation (Hillel, 1980):

$$\Gamma = MRT \qquad (4.4)$$

where Γ is the osmotic pressure (Pa; 1 bar = 10^5 Pa), M is the total molar concentration of solutes (mol m^{-3}), T is the temperature (degrees Kelvin) and R is the gas constant (8.314 J K^{-1} mol^{-1}). Using equation (4.4), it was apparent that the osmotic potential of the substrates used was high (osmotic potential = - osmotic pressure) in relation to those of Darrah *et al.* (1987a), and therefore one was led to conclude that there was no inhibition of nitrification in any of these incubations. In view of this, and mindful that no account had so far been taken of the possible seasonal fluctuation in SNA value (see Chapter 7) and the consequent possibilty of much higher values at other times of the year than those measured here, 0.01 M $(NH_4)_2SO_4$ was chosen as the substrate to be used in all future SNA analyses. Since the SNA value for 0.01 M $(NH_4)_2SO_4$ was 0.015 ± 0.0015 µmol N g^{-1} h^{-1} indications were that this concentration of NH_4^+ was well in excess of the nitrifier requirements, but not too high to cause toxicity problems.

iv. Analysis of exchangeable ammonium

In several of the experiments described in the following chapters, exchangeable ammonium was analysed. This was a particularly important analysis in the spatial variability experiment (Chapter 6) since one possibilty was that variability in nitrifier activity followed variability in Ex-NH_4^+.

Ex-NH_4^+ was analysed by the following method (A.D.A.S., 1981):

6 g soil (oven-dry equivalent, sieved <2 mm) was weighed into 50 cm^3 plastic tubes (the same as those used for SNA measurements). To these, 30 cm^3 2 M KCl was added and the tubes were shaken in an end-over-end shaker for 2 hours. The suspensions were then filtered through Whatman No. 32 filter paper, and the filtrate frozen and stored. This was later analysed for NH_4-N on a Technicon autoanalyser following the standard method (Technicon Users Manual, 1976).

B. FIELD SAMPLING

i. Site Details

Unless otherwise indicated, all the experimental work reported here was carried out using soil sampled from the Massey University No. 4 Dairy Farm, specifically from two adjacent fields in the Soil Science Research Area - No. 6, which had no previous history of fertilizer trials or other experimental work, was used for the bulk of the study, and No. 2, a former lime trial, whose history is detailed in Chapter 7, was used for the pH related work. Both fields had been under a ryegrass-white clover pasture for several years and for the duration of this study were periodically grazed by sheep (3-4 days each grazing) at approximately 140 stock units ha^{-1}; one stock unit is equivalent to a 55 kg ewe (weight at mating) which consumes sufficient dry matter to produce one weaned lamb per year (Cornforth & Sinclair, 1984).

The soil at this site, the Tokomaru silt loam (Cowie, 1974) is classified as a Yellow Grey Earth (Taylor & Pohlen, 1968) or Typic fragiaqualf (Soil Survey Staff, 1974). It is a poorly drained soil of low nutrient status and was not recommended by Cowie (1974) for cropping or horticultural use. He recommended regular dressings of phosphate, lime and potash to achieve optimum pasture growth. Accordingly, land on this soil is typically used for town dairy supply (serving Palmerston North) and the fattening of sheep, although production can be limited by the wet conditions in winter and spring, and by the drying out of the soil in summer.

Mean annual rainfall at the site, which is approximately 75 m above sea level, is 995 mm and mean monthly temperatures range from 8 °C in July to 17 °C in February (New Zealand Meteorological Service; Figure 4.2).

ii. Soil Sampling

Soil samples for all experiments except the pH work (Chapter 7) were taken in sections using a core auger 3 cm deep with a diameter of 5 cm. For all SNA measurements (see below), the top 3 cm layer was discarded to minimise any inhibitory effects that grass roots may have on the rate of nitrification (Molina & Rovira, 1964; Neal, 1969; Moore & Waid, 1971), and the 3-9 cm layer retained for analysis. The rationale behind using this depth range for experimental work is more fully explained in Chapter 5. On all sampling occasions, samples were sieved (<2 mm) as soon as possible after sampling and stored in sealed plastic bags at 3 °C (see Section C below).

Prior to soil sampling, there was always a period of three weeks during which there was no grazing. This was done in an attempt to minimise grazing effects such as *hotspots* of high nitrate concentration caused by the urine and excreta of grazing animals (Ryden *et al.*, 1984; Ball & Ryden, 1984; White, 1984).

iii. Correlation between moisture contents of sieved and unsieved soil

As outlined in section A (above), the amount of soil required for a single SNA analysis was approximately 5 g (oven-dry equivalent) of sieved soil. Each soil sample was analysed in duplicate for nitrifer activity, and in addition, two further sub-samples of 6 g each (oven-dry equivalent) were needed for analysis of exchangeable ammonium, and a further 10 g needed for measurement (in duplicate) of the soil moisture content so that results could be calculated on a per g dry soil basis. Overall, approximately 35 g sieved soil was needed for analysis. Since the purpose of this work was to study field nitrifier activity, each

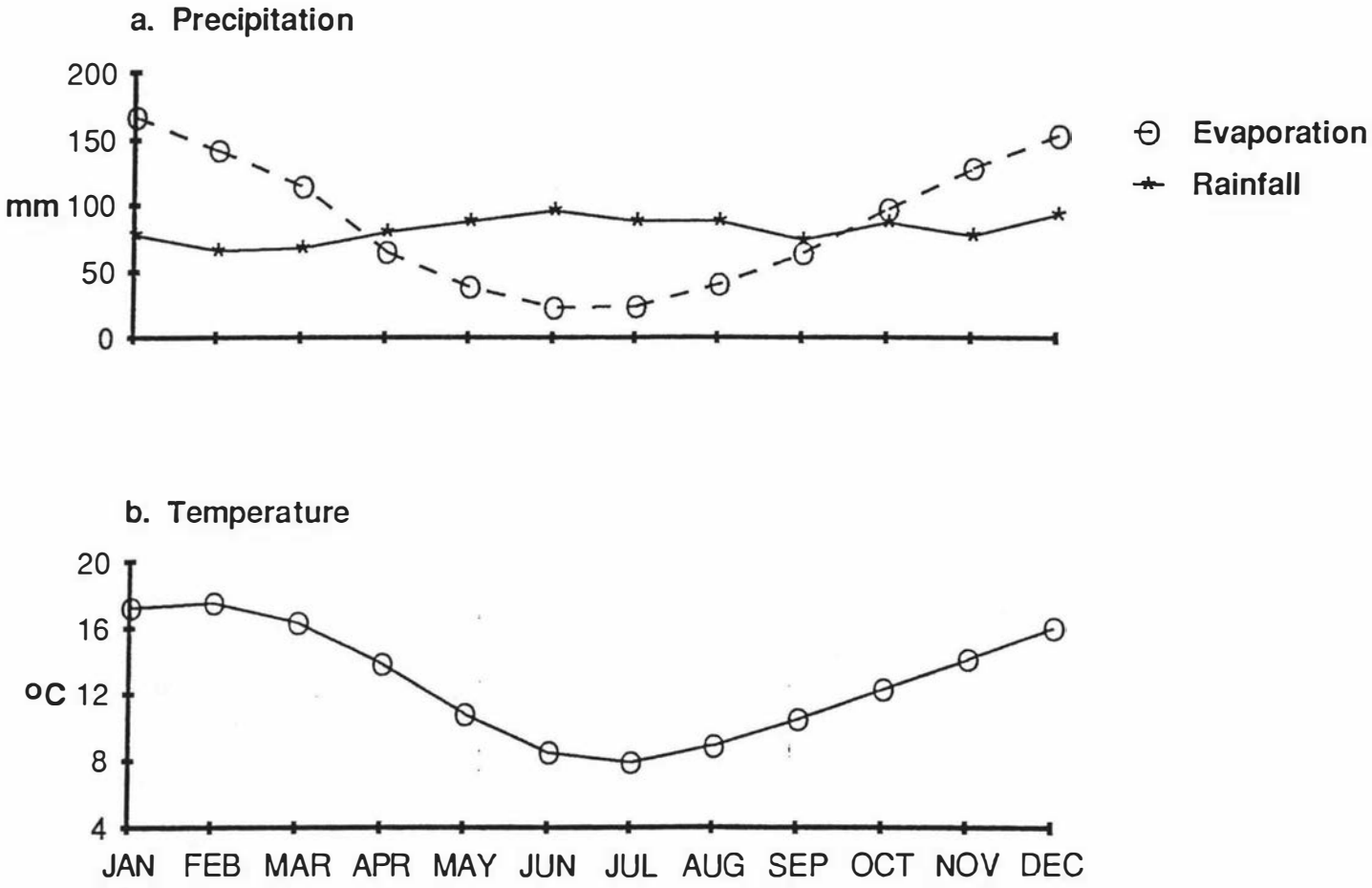


Figure 4.2 Mean monthly weather data (1928-1980) for the D.S.I.R Grasslands weather station, Palmerston North (N.Z. Meteorological Service, 1983)

sample had to be sieved in the field-moist state to maintain field conditions, and as a result, the amount of sieved soil gained from a 5 cm diameter core from the 3-9 cm depth range was often not far in excess of the amount needed for analysis, although this depended on the moisture content of the soil. Therefore in order to *conserve* soil, it was decided to investigate the possibility of a relationship between the moisture content of sieved and unsieved soil samples in the hope that field moisture contents could be inferred from the sieved moisture content. This was particularly important for the spatial variability analyses since the variability in moisture content was considered a possible cause of variability in nitrifier activity.

Methods and Materials

Fifty soil samples were taken from sampling sites randomly arranged at roughly 5 m intervals in field No. 6 at a range of depths to 24 cm using the core auger. Sub-samples were assayed immediately for gravimetric moisture content by oven drying overnight at 105 °C. The remainder of each sample was sieved (<2 mm) and this too, was dried by the same method.

Results and Discussion

The moisture content of sieved samples was plotted against that of unsieved samples and an equation fitted by linear regression (Figure 4.3; $R^2 = 0.91$, $p<0.1\%$). The equation took the form:

$$S = 0.0624 + 0.74911U \qquad (4.5)$$

where S and U were the moisture contents of the sieved and unsieved samples respectively.

In view of the good fit of equation (4.5) to the data over a wide range of moisture contents (0.2-0.5 g g^{-1}), this equation was used in all subsequent experiments to calculate the field soil moisture content on the basis of the moisture content of the sieved soil.

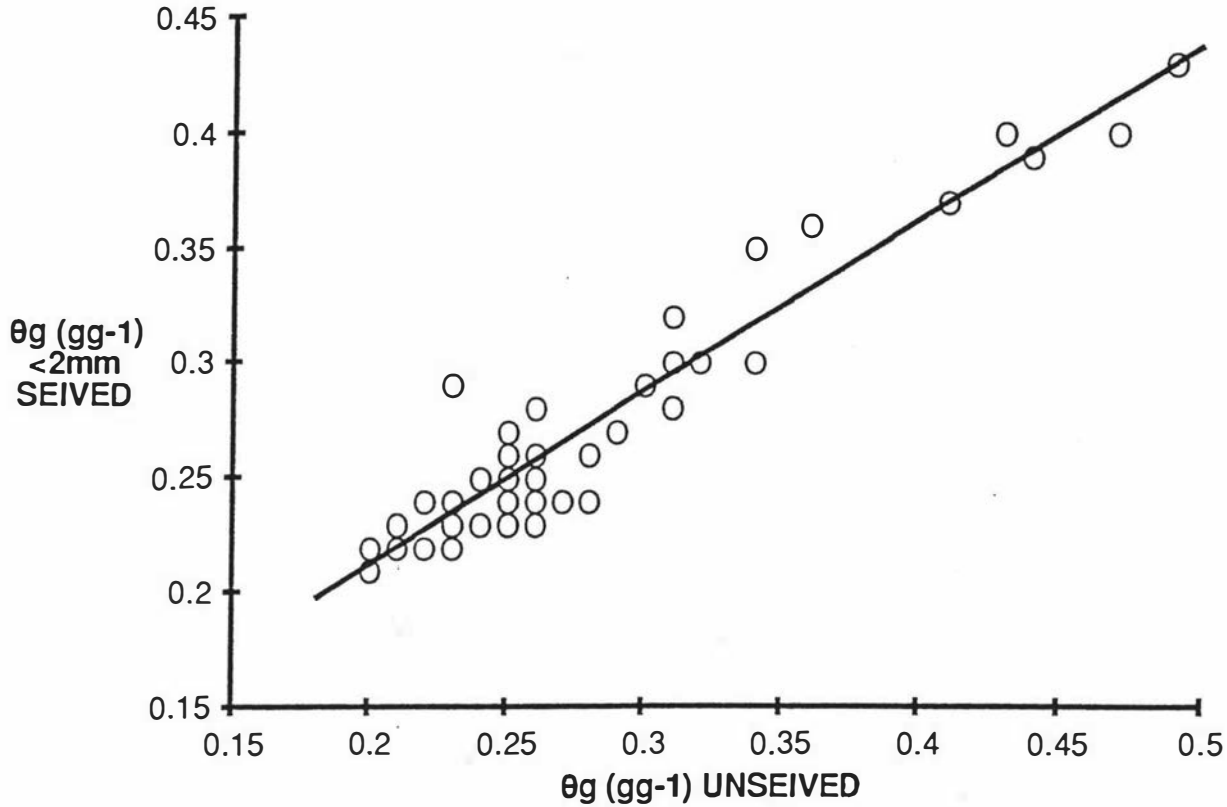


Figure 4.3 Correlation between the gravimetric moisture contents of sieved (<2 mm) and unsieved Tokomaru silt loam

C. STORAGE OF SAMPLES PRIOR TO SNA MEASUREMENTS

It was frequently necessary to collect soil samples several days before laboratory measurements were made. Therefore the samples had to be stored. The term *storage* often encompasses drying, pulverization and sieving (Bartlett & James, 1980), and soils are often stored with little regard for the possible effects of storage on the soil's properties. The physical properties of soils are well known to be affected by drying (and wetting); the contraction of pores is known to occur and further structural alterations may also arise. In extreme cases, such as in Vertisols, the development of large cracks and fissures upon drying is likely, and will be especially significant in larger, more massive samples. In view of these known physical changes, it appears unlikely that the microbial and chemical characteristics of a soil would remain unaltered from the field state during storage. Since the purpose of this study was to investigate the activity of nitrifiers in the field, the method of sample storage and preparation was obviously of great importance.

i. Effects of drying and storage on mineral nitrogen

It has often been observed that variation in pretreatments, particularly degree of drying, affects the amount of soil nitrogen mineralized during short periods, thereby complicating the use of the results as indices of soil nitrogen availability (Stanford & Smith, 1972). Patten *et al.* (1980) studied the effects of drying and air-dry storage on the soil's capacity for denitrification under anaerobic incubation. Their results indicated that the drying of soils markedly increased their capacity for denitrification, and this effect increased as the drying temperature increased. Partial drying and air-drying had a similar but slightly smaller effect. Agarwal *et al.* (1971) found that the temperature of drying as well as the drying-rewetting cycles enhanced both nitrogen and carbon mineralization in "practically all soils" studied. The N release increased if incubation followed drying. In all but one of their soils, air-drying caused a greater N release than heating at 60 °C regardless of

the number of rewetting-drying cycles. Ross *et al.* (1979) found that sample sieving led to a slight increase in all mineral N fractions as did storage of the sieved samples for 24 hours at 4° or -20 °C. Frye and Hutchinson (1981) found a pronounced increase in exchangeable NH_4^+ on drying, this increase being greater after oven-drying. However, the source of this NH_4^+ was unknown although the effect was less in the subsoil suggesting that the NH_4^+ was released in the topsoil. Thus, either humus or microbes killed by drying (or both) were a possible source of mineralizable N. These results agree with those of Soulides and Allison (1961), who found a similar increase in available ammonium. Gasser (1961) found that drying led to an increase in both NH_4^+ and NO_3^- and found that on rewetting, most extra mineralization had occurred after 10 days and all by 42 days. He also found that the increase in nitrogen mineralization became more marked with time of storage although a clear trend was not established for 12-16 weeks before which, values fluctuated. Munro and Mackay (1964) found that incubation of soils at a humidity of less than 85 % severely restricted NO_3^- production. This effect was due to drying as the soil moisture content at 85 % relative humidity was half that at a relative humidity of 100 %. However, on rewetting the drier samples, the NO_3^- production increased significantly. They also found that air-drying the soil from field capacity to wilting point had little or no effect on NO_3^- production, but further drying due to air-dry storage caused a marked increase on rewetting.

Tests on CO_2 evolved during the experiments of Patten *et al.* (1980) indicated that the increase in the soil's capacity for denitrification was due to an increase in soil organic matter which was readily utilised by denitrifying organisms. This was thought to be due to humus breakdown although there is good evidence (Jenkinson, 1966; Jenkinson & Powlson, 1976a; 1976b) to suggest that much of this increase in organic matter is in the form of dead organisms that were killed off by drying. This is supported by the results of Agarwal *et al.* (1971), who with respect to mineralization, proposed that in addition to microbial stimulation in rewetted samples following drying, heat was directly responsible for the amount of N and C released in unincubated samples through chemical alteration of otherwise unavailable organic matter, and by the "killing off" of organisms. When incubation followed the drying and heating

treatment, the direct effect of heat together with increased microbial activity and associated changes during incubation accounted for C and N mineralization.

Addiscott (1983) noted that the net amount of N ammonified in time t, N_t, measured as the increase in the sum of ammonium and nitrate, increased approximately linearly with t. However, this zero-order relationship was found to be dependent to some extent on whether the soil was pre-dried. Many authors (e.g. Stanford & Smith, 1972; Tabor *et al.*, 1985; Clay *et al.*, 1985) used soils that had been dried and sieved and only a very few (e.g. Addiscott, 1983) used soils in the field state. It was thought conceivable that the flush of mineral N caused by rewetting the dry soil could change a linear relationship with t into an apparent $\sqrt{t}$ relationship, or even a first-order (exp(t)) relationship (Addiscott, 1983).

Despite all this apparently conclusive work, it has been found that the effect of storing soils on their subsequent ability to mineralise nitrogen depends on the individual soil (Harding & Ross, 1964). Investigations of four soils showed the effect to be much more pronounced in three but not so great in the fourth. These differences were related to the moisture and carbon contents of the soils but it was found that for a given drying period, the amounts of carbon and nitrogen mineralised were proportional to the carbon content of the soil, while for a given soil, they were found to be a significant linear function of the logarithm of the time the soil was in an air-dry state prior to moistening.

ii. The effects of drying and storage on soil biomass

From the above, it is clear that the major effects of soil drying and storage are governed by the effects of storage on the microbial populations since dead organisms add to the pool of mineralizable substrate. Thus, the mode and time of storage were especially important to this study, given that it was the activity of the nitrifiers that was under investigation.

Chao and Alexander (1982) studied the influence of drying on the survival of *Rhizobium*. They found that the numbers of both *R. meliloti* and *R. phaseoli* fell markedly as the soils dried, but their abundance only declined slowly in soils maintained in the air-dry state. Further, the number of surviving cells increased if the bacteria were added to sterile soil and allowed to grow before desiccation was extensive. The work of Stevenson (1956) on the respiration of air-dried and fresh soils showed that a higher level of metabolic activity is attained in air-dried soils on remoistening than occurs in fresh soil. The degree by which the metabolic activity increased varied directly with the concentration of free amino acids and other nitrogenous materials released by the air-drying process. Salonius (1983) allowed microbial populations from dried, remoistened and undried forest organic horizons to recolonise sterilised forest horizons and concluded that samples of forest organic soil material designated for the study of microbially driven processes should not be air-dried. White (1964) reached a similar conclusion with regard to the study of soil phosphate potential. He found that when air-dried samples were used for measurements of phosphate potential, microbial uptake of phosphate interfered with the results if the samples were shaken for more than two hours. i.e. re-wetting led to increased microbial activity and thus uptake of P, this effect being significant when samples were shaken for more than two hours.

Harding and Ross (1964) added ammonium to dried stored soils and obtained results suggesting that numbers of nitrifying organisms decreased after six months of storage. Soulides and Allison (1961) found that drying was more destructive to organisms than freezing and so the latter would seem preferable if storage is required. Far more desirable however, would be to use fresh soil. Salonius (1983) reported the work of Sneath (1962) who studied dormant populations in dry soil stored for up to three hundred years and estimated that these soils would reach sterility in a thousand years at ambient temperature and humidity. The difference between six months and a thousand years storage is obvious, but this hypothesis would nevertheless seem both reasonable, and with clear implications.

Investigations on the effect of storage on soil biomass estimated by biochemical techniques were carried out by Ross *et al.* (1980). For determination of biomass by CO_2 evolution from chloroform fumigated soils incubated for fixed periods, the differences in patterns of CO_2 evolution between soils stored for 28 and 56 days at 25°, 4°, and -20 °C were negligible and not significantly different from those calculated from individually determined incubation periods for each treatment and soil. However, biomass C values could change significantly at all storage temperatures but generally least at -20 °C, the temperature of storage which was best for maintaining ATP contents. Overall, no storage temperature was satisfactory for all indices of microbial biomass tested, but 4 °C was adequate for short periods. It is clear therefore that assays of fresh soil are preferable.

Mention has been made of the effect of drying on soil organic components such as amino acids. Birch (1958) showed that C and N mineralization occurred rapidly on rewetting dried soil, the effect being greater for oven-dried than for air-dried soil. He therefore concluded that drying of any kind leads to humus decomposition. The work of Stevenson (1956) has already been discussed, but this assertion of Birch (1958) would explain Stevenson's correlation of metabolic activity increase on remoistening dried soil, with availability of free amino acids and "other nitrogenous materials" released by the air-drying process. Soulides and Allison (1961) showed that when either drying or freezing was followed by a period of incubation, there was an increase in the decomposition of soil organic matter, this being substantially greater for drying than for freezing. Prolonged drying increased the rate of decomposition, and multiple dryings had a cumulative effect. Multiple freezings had no effect. It would appear that the increased decomposition of organic matter following intermittent drying or freezing is due primarily to the release of nutrients, especially energy sources that can be rapidly oxidized by the soil flora. From the above, much of these are likely to be derived from microbes killed by the drying process. Providing the C/N ratio is sufficiently low to allow for the net mineralization of N, a *snowball effect* results, leading to further breakdown, and thus the burst of CO_2 production and release of NH_4^+ following drying is enhanced by the

youthful state of a growing (feeding) microbial population (Soulides & Allison, 1961); the microbes which survive the drying process are provided with an excess of substrate, and so grow rapidly. The key to succesful storage of soils therefore lies in the maintenance of microbial populations at field levels.

From the above it is obvious that experiments - especially those such as SNA measurements - should be carried out on soil in the field state. If storage is necessary, then the period of storage should be as short as possible and under conditions of low temperature and high humidity. Conditions of high humidity are easily attained when samples are stored in sealed plastic bags at low temperature, especially when the soil in the field is moist or even wet. Accordingly, all soil samples collected in this study were stored at 3 °C in sealed plastic bags. However, it was considered desirable to check on the effects of a period of storage under these conditions on SNA values, and gain some idea as to how long samples could be stored.

Methods and Materials

This experiment was carried out in conjunction with the second experiment of the first year of the work on the pH relations of nitrifier activity (Chapter 7). Despite modification of the SNA technique to accommodate the adjustment of incubation pH, the experimental procedure lent itself to the study of storage effects since in addition to investigating the effect of storage on the SNA value, it was also of interest to see if the relationship between nitrifier activity and pH was affected by storage.

A bulk soil sample (approx. 5 kg) was dug from one of the control plots from field No. 2 on August 25, 1986. The soil was sieved (<2 mm), thoroughly mixed, and a 200 g sub-sample leached overnight with 1 dm^3 0.005 M KCl as before. At the end of leaching, excess moisture was removed from the soil by suction filtration for 90 minutes after which, 30 replicate 5 g samples (oven-dry equivalent) were placed into 50 cm^3 incubation tubes containing 20 cm^3 0.005 M KCl with 0.3 % $^w/_v$ agar. The pH of the suspensions was then adjusted in triplicate by adding small

amounts of 0.1 M HCl or KOH. A preliminary experiment (Chapter 7) established that the pH of the suspensions attained an approximately steady value within five hours, and accordingly, after five hours, 10 cm^3 0.01 M $(NH_4)_2SO_4$ was added to each tube and the incubations begun as before. After the eight hour sampling, the incubation pH was measured by glass electrode and pH meter.

Three weeks after this experiment, a further 200 g sub-sample of soil was taken from the original bulk sample which had been stored in a sealed plastic bag at 3 °C and the experiment was repeated.

Results and Discussion

For both experiments, SNA values were plotted against pH and the data were fitted with a quadratic equation by a least-squares fitting procedure (Figure 4.4). By differentiating the fitted equations, a pH optimum for nitrification pH_{opt} was calculated. The fitted equations for fresh soil (Figure 4.4a; $R^2 = 0.85$, $p<0.1\%$) and stored soil (Figure 4.4b; $R^2 = 0.53$, $p<0.1\%$) were:

$$SNA = -0.17622 + 0.06864pH - 0.00583pH^2 \qquad (4.6)$$

for fresh soil, and

$$SNA = -0.15600 + 0.06494pH - 0.00577pH^2 \qquad (4.7)$$

for stored soil. Predicted values of pH_{opt} were 5.89 and 5.63 for the fresh and stored samples respectively. The calculated SNA values at pH_{opt}, SNA_{opt} were 0.026 µmol NO_3-N g^{-1} h^{-1} for the fresh, and 0.027 µmol NO_3-N g^{-1} h^{-1} for the stored soil sample. Looking at Figure 4.4, there appeared to be very little difference between the fitted curves for fresh and stored soils. By combining analysis of variance and analysis of covariance (Freund & Minton, 1979) it was found that the SNA and pH data for both experiments could be grouped and fitted with a common quadratic equation (Figure 4.5, $R^2 = 0.62$, $p<0.1\%$), which predicted values of pH_{opt} and SNA_{opt} of 5.76 and 0.026 respectively. i.e. the 3 week period of storage had no effect on the SNA or the response of the nitrifiers to pH. Accordingly it was concluded that samples could be stored for 3 weeks without affecting experimental results.

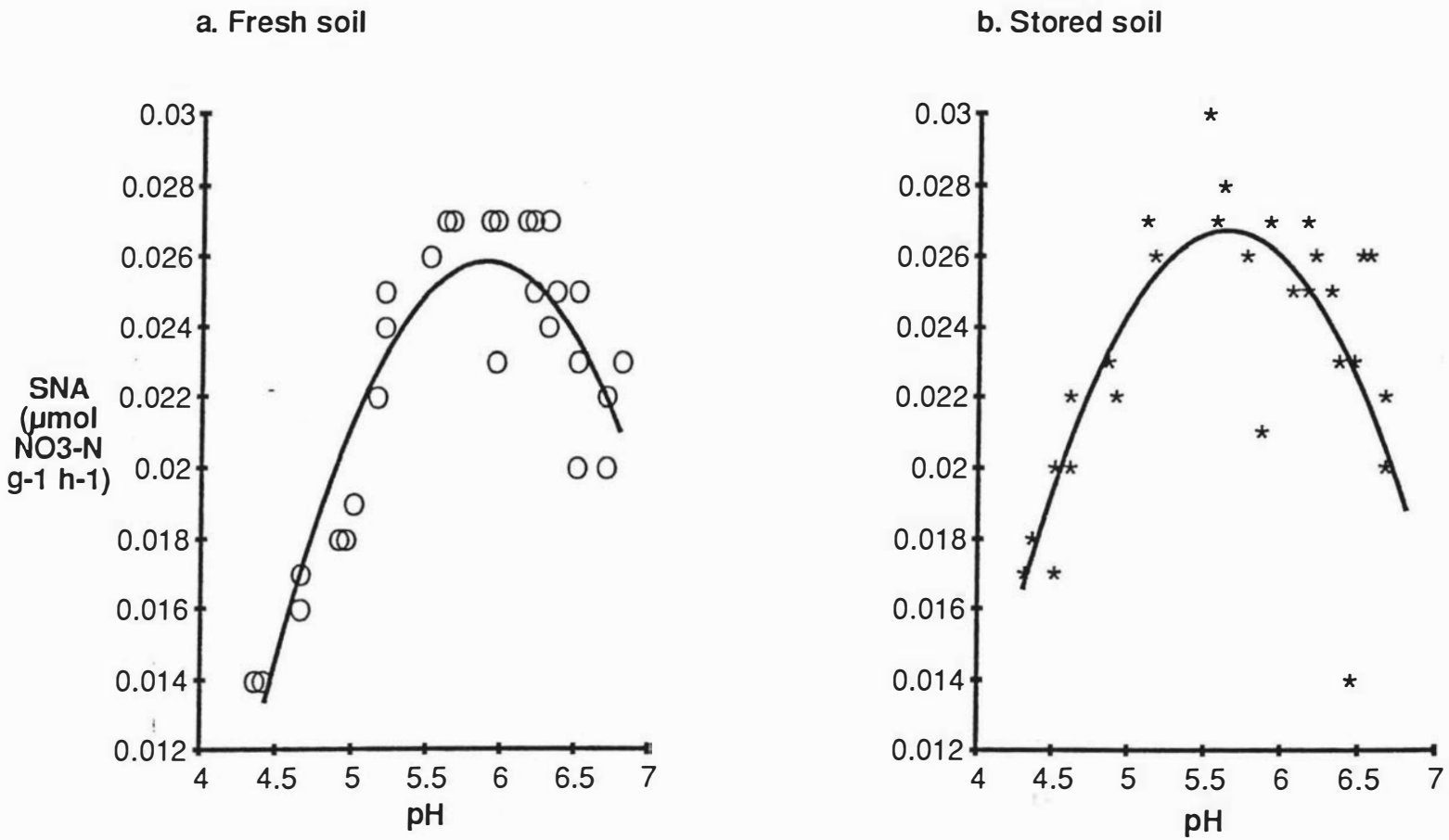


Figure 4.4 pH optima curves for nitrifier activity in (a) fresh soil and (b) soil that had been stored for 3 weeks

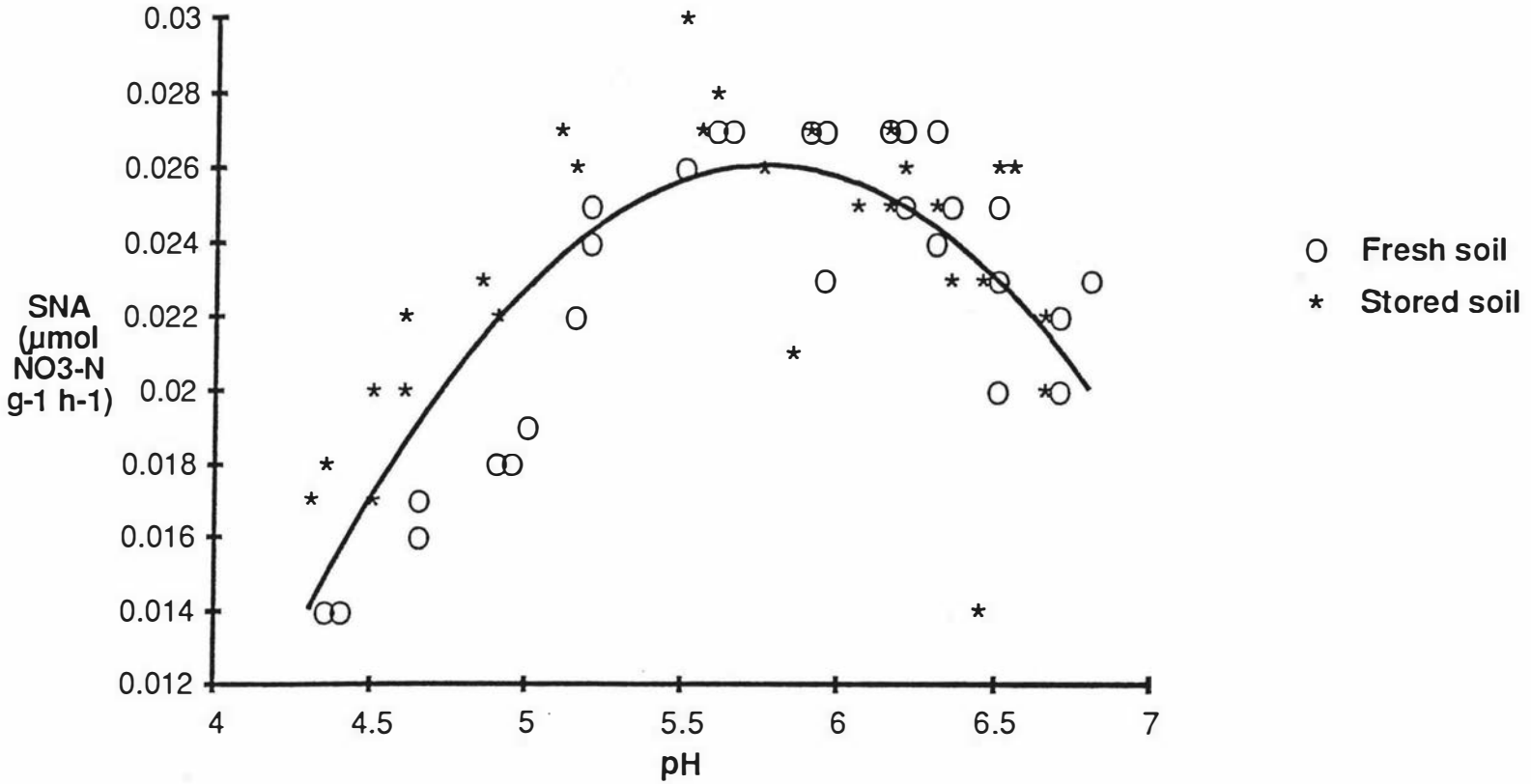


Figure 4.5 Common pH optimum curve fitted to SNA data for fresh and stored soil

As mentioned above, Ross *et al.* (1980) found no temperature to be satisfactory for storage of soil where maintenance of the biomass was a priority, although 4 °C was adequate for short periods. In the light of this and other findings (detailed above), storage periods were clearly to be avoided if possible. The period of storage for samples used in the work reported in this thesis was never longer than 10 days, and was generally confined to 3 days. Thus, on the assumption that any microbial, chemical or biochemical changes undergone by the soil as a result of sieving and storage were either negligible, or would have been manifested over periods of storage much longer than three weeks, it was concluded that SNA measurements were unaffected by storage, and therefore represented field nitrifier activity. Of course, there may have been an immediate effect on nitrifer activity caused by sieving; this was not investigated, but was unavoidable since the soil had to be sieved to obtain a homogeneous bulk sample for the incubation experiments.

D. CONCLUSIONS

The preliminary experimental work outlined in this chapter was done with the aim of tailoring the SNA technique to the Tokomaru silt loam, and ensuring that the logistics of the various parts of the technique were compatible with the intended lines of research. As a result, the following was drawn up as a standard procedure for SNA measurements on the Tokomaru silt loam, and was used as the basis of experimental technique for the research reported in the following chapters of this thesis:

1. Following sampling, each soil sample was sieved (<2 mm) and stored at 3 °C in a sealed plastic bag. The period of storage was never greater than 10 days.

2. Each sample was subsampled for moisture content and Ex-NH_4^+ (where applicable), and leached with 0.005 M KCl in a Buchner funnel fitted with a Whatman No.1 filter paper at a soil:solution ratio of 1:5. (In the spatial variability experiments (Chapters 5 & 6) the leachate was retained and analysed for NO_3-N)

3. Excess moisture was removed by suction filtration for 90 minutes.

4. 5 g samples (oven-dry equivalent) were placed into 50 cm^3 plastic tubes containing 20 cm^3 0.005 M KCl with 0.3 % $^w/_v$ agar.

5. To each tube, 10 cm^3 0.01 M $(NH_4)_2SO_4$ was added. The tubes were sealed and placed in an enclosed end-over-end shaker fitted with a thermostat, and incubated at 22 °C for 8 hours.

6. After 1 and 8 hours, a 5 cm^3 sample of suspension was taken from each tube, centrifuged at 3000 r.p.m for 10 minutes and the supernatant frozen and stored for NO_3-N analysis at a later date.

7. After the 8 hour sampling, the pH of the suspension was measured by glass electrode and pH meter.

SECTION II. AN ANALYSIS OF SPATIAL VARIABILITY IN NITRIFIER ACTIVITY

CHAPTER 5

VARIABILITY IN NITRIFIER ACTIVITY WITH DEPTH AND DISTANCE

A. DEPTH DEPENDENT VARIABILITY

It is generally accepted that microbial activity is higher in the upper relative to the lower layers of soil profiles (e.g. Speir *et al.*, 1984, Higashida & Takao, 1985). This is due to the fact that organic matter (i.e. substrate) enters the soil system at or near the surface, and consequently occurs predominantly in the surface layers, and the oxygen needed by aerobic micro-organisms decreases in availability with depth (Khyder & Cho, 1983, Colbourn *et al.*, 1984). When N is being mineralized at a rate in excess of that of NO_3^- loss by leaching, one might expect the distribution of NO_3^- down a profile to follow that of NH_4^+ which, because it is readily adsorbed by soil colloids, might in turn be expected to follow the distribution of organic-N substrate. Cameron *et al.* (1978, 1979) found that both NO_3^- and NH_4^+ tended to decrease with depth in a clay loam in the range 0-60 cm, whilst Young and Aldag (1982) noted that only a very small proportion of the total soil N occurred as readily available mineral N. Stevenson (1982a) stated that over 90 % of the N in the surface layer of most soils was organically combined, much of it as amino acid-N or amino sugar-N (Khan & Sowden, 1971, Stevenson, 1982b), although the amount of amino-N as a proportion of the total N tends to decrease with depth due to greater humification.

For the purposes of the preliminary experimental work outlined in Chapter 4, soil samples were taken from the 3-9 cm depth range. As explained previously, the top 3 cm were avoided to minimise any inhibitory effects that grass roots may have on the rate of nitrification (Molina & Rovira, 1964, Neal, 1969, Moore & Waid, 1971). However, the assumption that this would be the most suitable depth range of sampling for an investigation of spatial variability in nitrifier activity in the Tokomaru silt loam

may not have been a good one because (a) grass roots may not affect nitrification in this soil; (b) nitrification may occur at higher (or lower) rates in the 0-3 cm range regardless of the effect of grass roots; and (c) the degree of any spatial dependence in nitrifier activity may not be the same at all depths. As a consequence, a field-scale estimate of the mean soil nitrate concentration, made for use as an input parameter to a nitrate leaching model may not be a *best estimate* due to depth dependence. Thus, it was considered important to investigate the vertical distribution of nitrifier activity and its associated parameters in addition to any spatial analysis.

i. Methods and Materials

Soil sampling

Ten sampling sites were selected in field No. 6 using random numbers to generate the coordinates of each site. Following adjustment (where necessary) of the position of some of the sites to ensure a minimum site separation of 3 m, the soil at each site was sampled to a depth of 24 cm in 3 cm layers using the corer described in Chapter 4. Two immediately adjacent cores were taken at each site. The soil from the two cores was sieved and bulked on a depth basis to give 80 samples representing the 10 sites at 8 depths. The samples were stored as described previously prior to analysis for SNA and exchangeable-NH_4. The leachate from the SNA pre-leaching was retained for analysis of NO_3-N to give the quantity of NO_3-N present initially per g soil.

A further five sampling sites were randomly sampled in the manner described above (only one core per site) and the soil sieved and stored as before. These samples were analysed for their total nitrogen, carbon and phosphorus contents following the methods described below.

Six additional sites were randomly selected for the measurement of soil bulk density. (A minimum of four samples are required for this measurement (D.R. Scotter, Dept. Soil Science, Massey University - personal communication), but six sites gave an easily manageable number

and allowed for more precise estimation of mean values - see Chapter 3.). At each of these sites, cores measuring 5 cm deep with a diameter of 4.8 cm (i.e. 90.48 cm^3 soil) were taken to a depth of 25 cm, oven-dried overnight at 105 °C, and the bulk density calculated on a g dry soil cm^{-3} basis.

Sampling was carried out during mid-May; there was a period of 9 days between the sampling for SNA measurements and that for analysis of C, N and P. It was assumed that any change in the latter soil properties between the two sampling dates was insignificant.

Analysis

SNA, exchangeable-NH_4 and NO_3-N were analysed by the methods described in Chapter 4.

The analysis of total nitrogen and phosphorus (4 replicates per sample) was carried out by Kjeldahl digestion following the method of Bolan & Hedley (1987). 1 g finely-ground air-dry soil was placed in a pyrex tube and 4 cm^3 digest acid (250 g K_2SO_4 and 2.5 g Se powder dissolved in 2.5 dm^3 conc. H_2SO_4) were added, and the tube heated at 350 °C for 4 hours. After cooling, the contents of each tube were diluted to 50 cm^3 with deionised water, thoroughly mixed in a vortex shaker, and the solutions simultaneously analysed by autoanalyser for their N and P contents, following the method of Twine and Williams (1971).

The carbon content of each sample was analysed by dry combustion in a stream of O_2 using a Leco furnace following the method of Bolan and Hedley (1987); a copper oxide catalyst was used to promote the conversion of CO to CO_2, and an MnO_2 trap used to remove any halogens present. The amount of carbon in the soil was calculated as the mass of CO_2 produced (mg) x 0.2729 (the proportion by mass of C in CO_2) per mg air-dry soil.

ii. Results

Figure 5.1a shows the bulk density at each site plotted as a function of the depth at the centre of each core (the centre of the 0-5 cm depth core for example, was taken to be at 2.5 cm depth). Since the dimensions of the bulk density corer were different from those of the corer used for all other soil sampling, it was necessary to find an expression relating bulk density to soil depth so that the bulk density at the depth at which the other samples were taken could be interpolated. The data followed a curvilinear trend with depth and were best fitted using least squares optimization by the equation ($R^2 = 0.88$; $p<0.1\%$):

$$\rho_b = 0.93611 + 0.0355D - 0.00064D^2 \qquad (5.1)$$

where ρ_b is the bulk density and D denotes depth (cm). It should be noted that this equation is valid only over the depth range 0-25 cm.

The soil moisture content at the time of sampling for SNA analysis was calculated from the moisture content of the sieved soil using equation (4.5), and these gravimetric data were converted to volumetric moisture contents, θ_v, using values of ρ_b calculated from equation (5.1). The data at each depth were assumed to be normally distributed (see section B, and also Chapter 6) and the mean values are plotted against depth in Figure 5.1b.

The spatial analysis (see section B and also Chapter 6) indicated that SNA, initial NO_3^- and Ex-NH_4^+ conformed to log-normal distributions. White *et al.* (1987) suggested that for lognormally distributed data, when the variance of the natural logarithms of the property values is less than 0.5, and the number of samples is large, the best estimate, $\hat{\mu}$, of the mean, μ, of the population from which the sample is drawn is given by:

$$\hat{\mu} = X_a = \exp(\xi + v/2) \qquad (5.2)$$

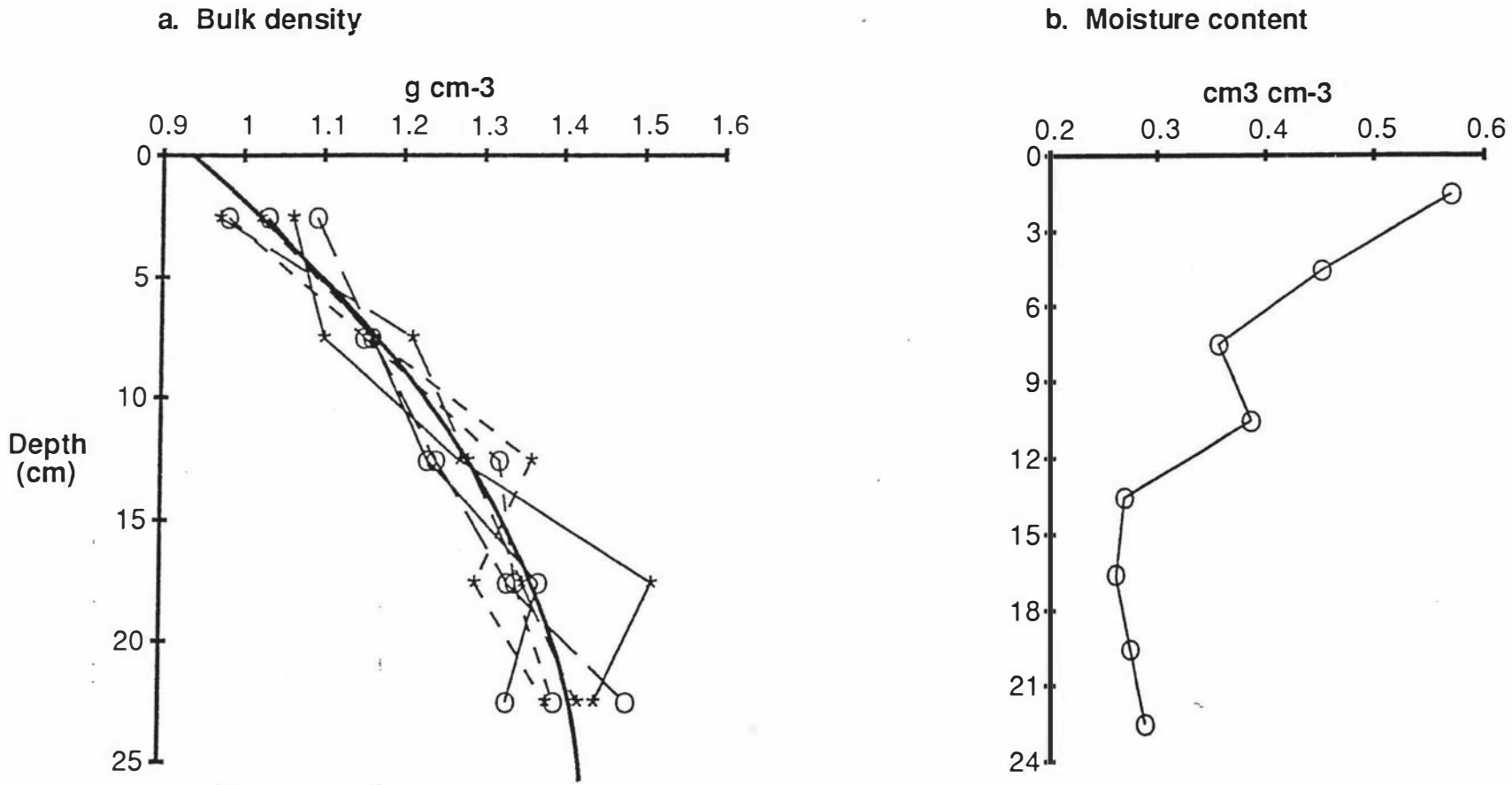


Figure 5.1 Change in (a) bulk density and (b) mean volumetric moisture content with depth in the Tokomaru silt loam sampled in mid May

where X_e is the estimate of the mean value of the sampled property, and ξ and υ are the arithmetic mean and variance of the natural logarithms of the property. Both SNA and soil NO_3^- fulfilled the criteria for use of equation (5.2) with respect to υ and despite there being only 10 samples for each depth, this equation was used to estimate the mean values. In contrast, the value of υ in the case of Ex-NH_4^+ exceeded 0.5 at all depths and so the mean value was estimated using Sichel's estimator, X_s (Sichel, 1952) where:

$$X_s = \{\exp(\xi)\}\{1 + \frac{\upsilon}{2} + \frac{(n-1)\upsilon^2}{2^2 2!(n+1)} + \frac{(n-1)^2\upsilon^3}{2^3 3!(n+1)(n+3)} + \ldots\} \qquad (5.3)$$

The data for C, N, P and C/N were assumed to conform to normal distributions as did the incubation pH (see section B and also Chapter 6). The distributions of all the measured properties and their standard error at each depth are shown in Figure 5.2a-h. In the case of the normally distributed properties, the standard error, S.E, was calculated using the equation (Clarke, 1980):

$$S.E = \sqrt{s^2/n} \qquad (5.4)$$

where s^2 is the sample variance, and n is the number of samples; the variance of the sample mean is given by s^2/n. White *et al.* (1987) presented equations for the variance of both X_e and X_s and used these to infer the reliability of X_s relative to X_e. However, it is suggested that for the purpose of estimating standard errors of lognormally distributed properties, distinguishing between the variance of X_e and X_s may not be necessary. Sichel (1952) stated that the variance of the estimate of the arithmetic mean X_e, denoted here by Var(X), could be calculated using the equation:

$$Var(X) = \{\exp(2\xi + \frac{\upsilon}{n})\}\{[\exp(\frac{\upsilon}{n})][1 - \frac{2\upsilon}{n}]^{-(n-1)/2} - [1 - \frac{\upsilon}{n}]^{-(n-1)}\} \qquad (5.5)$$

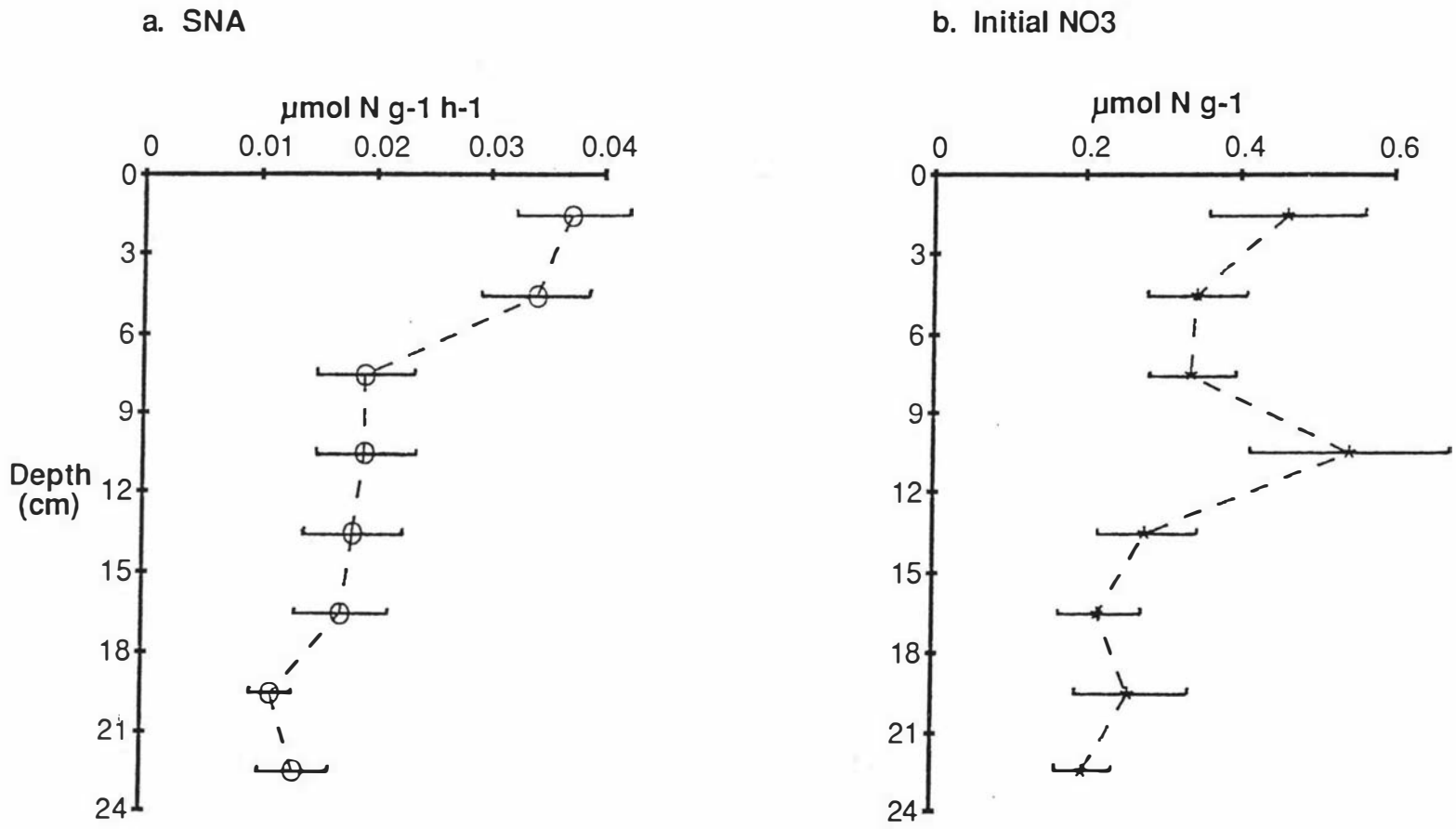


Figure 5.2 Depth profiles of (a) SNA, (b) NO_3^-, (c) Ex-NH_4^+, (d) incubation pH, (e) total carbon, (f) total nitrogen, (g) C/N ratio, (h) total phosphorus and (i) % mineral N in the Tokomaru silt loam sampled in mid May

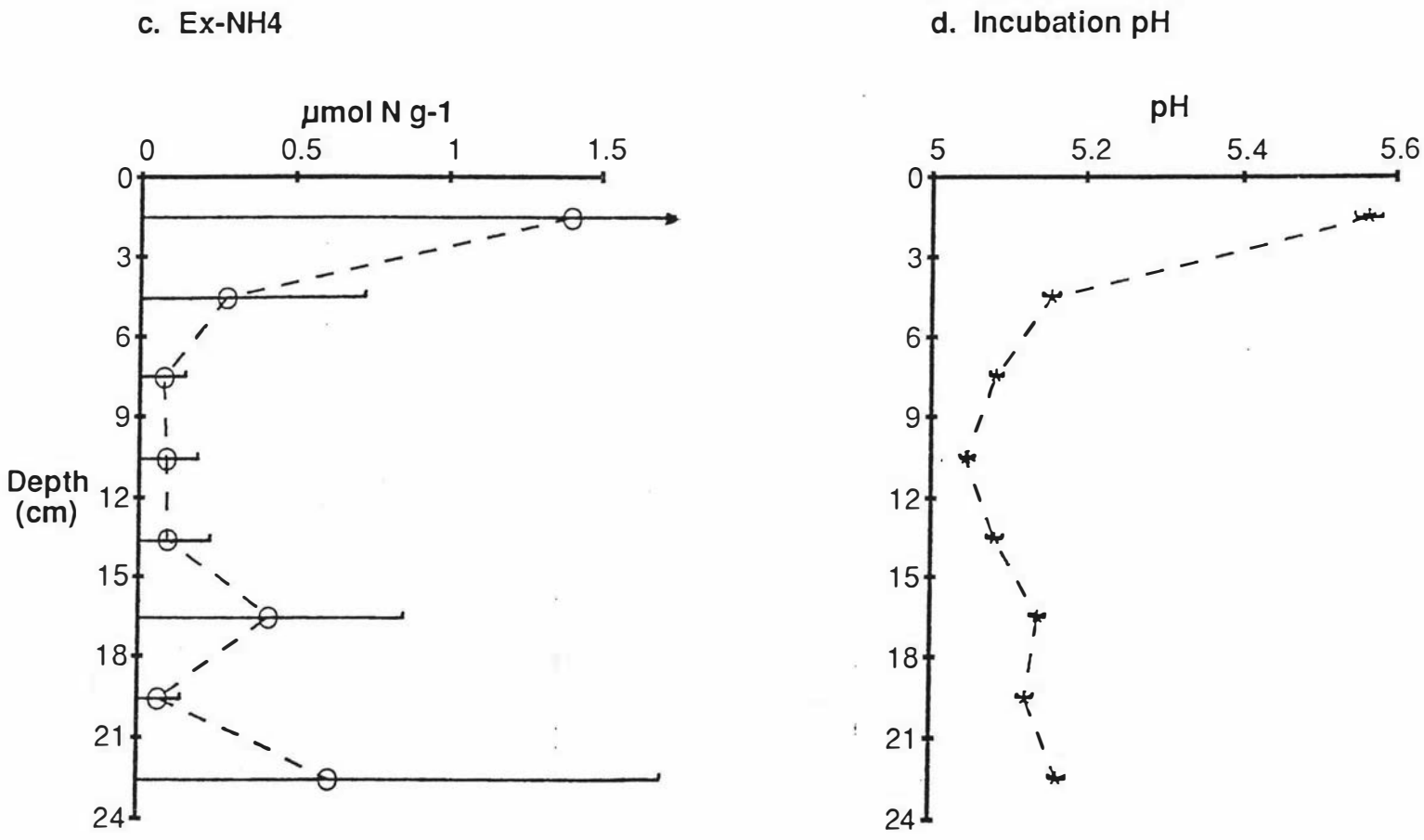


Figure 5.2 (Contd)

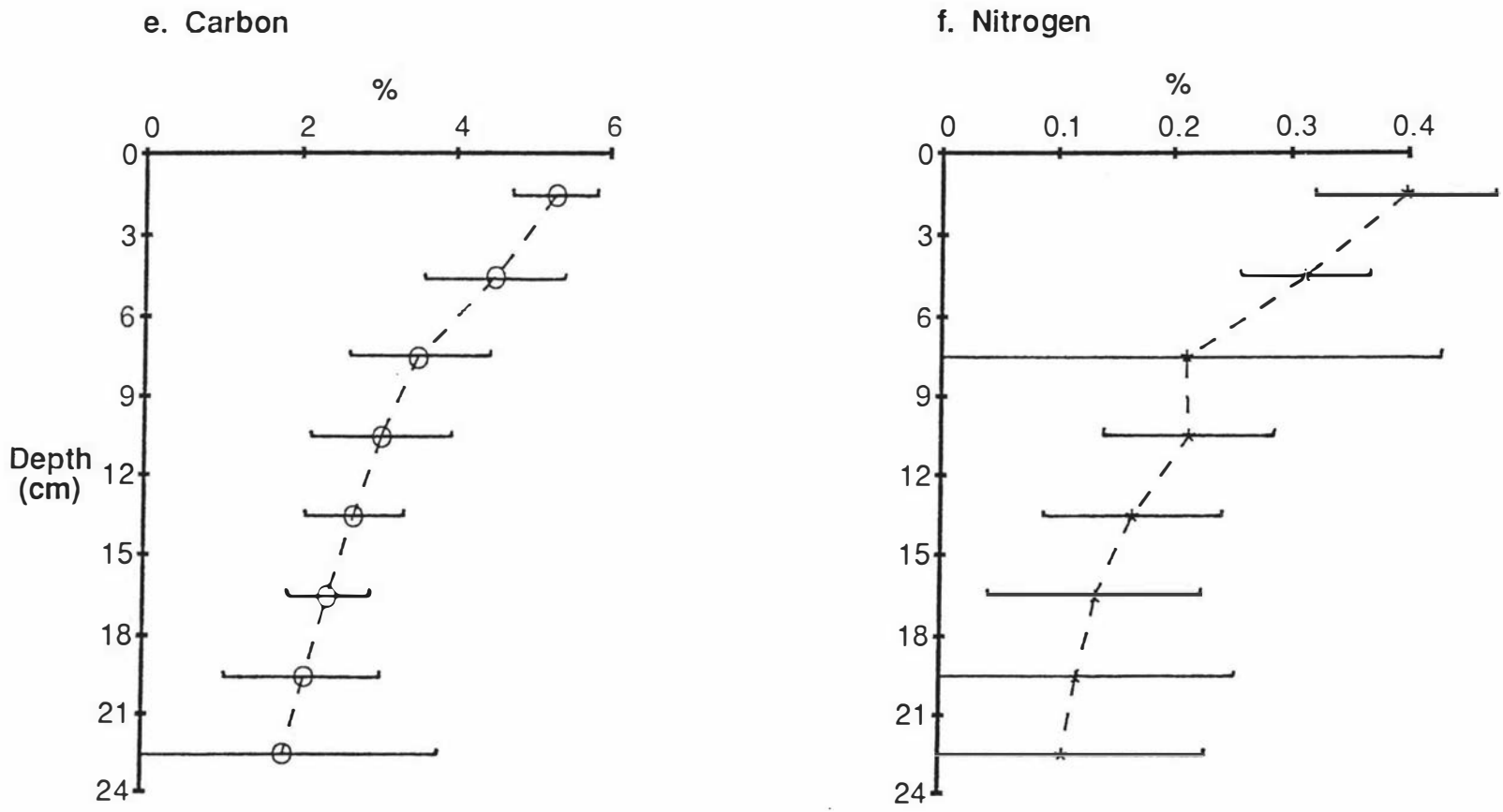
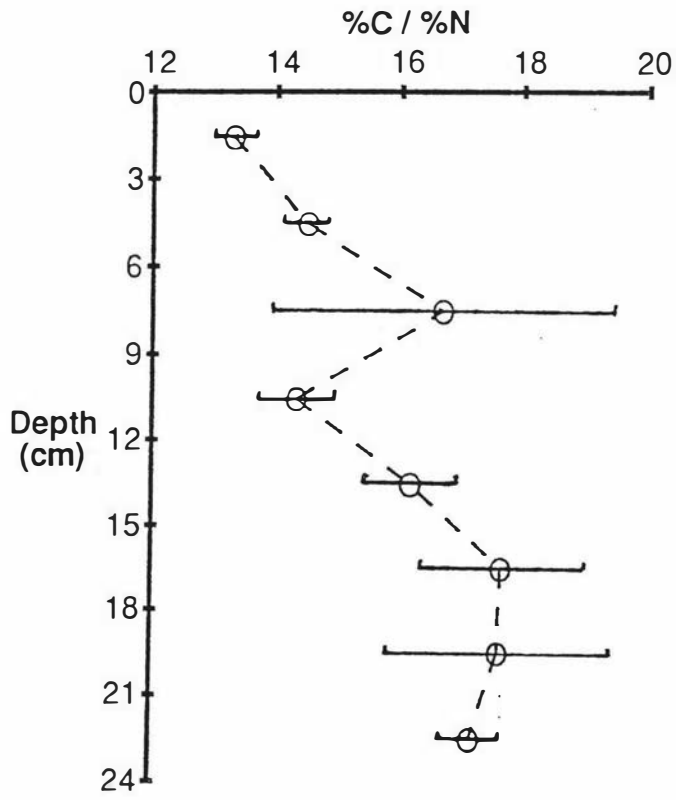


Figure 5.2 (Contd)

g. C:N ratio



h. Phosphorus

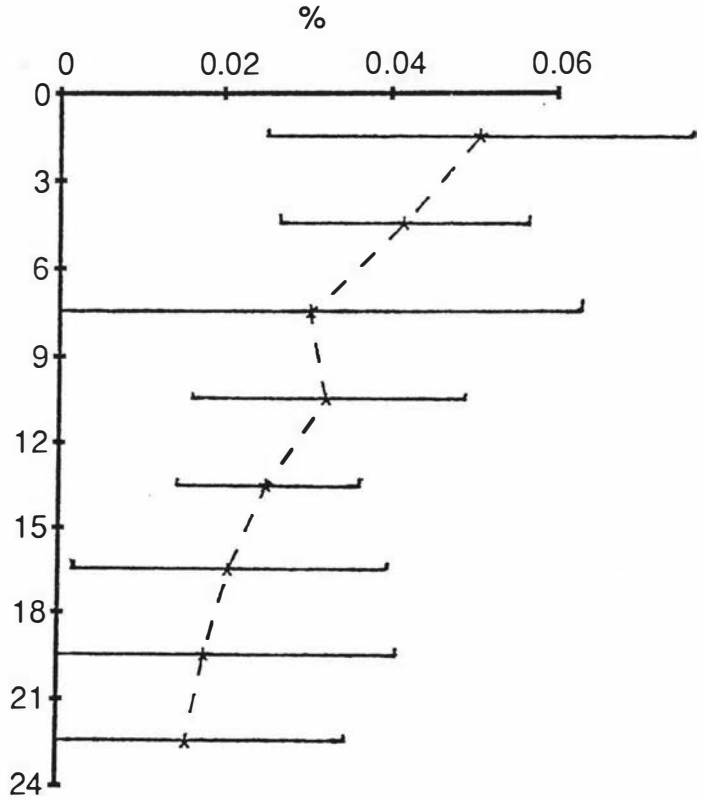


Figure 5.2 (Contd)

i. % Mineral N

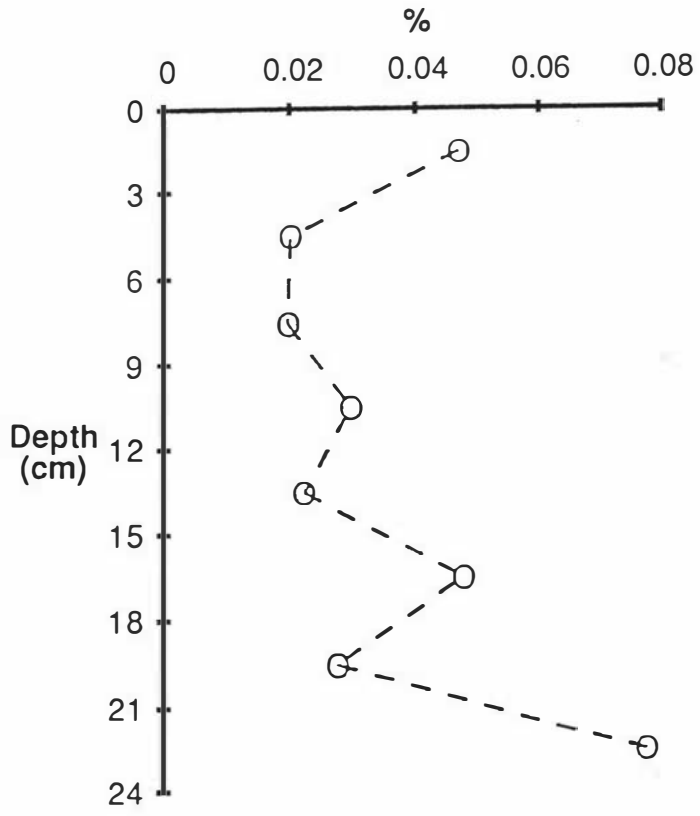


Figure 5.2 (Contd)

Since Var(X) is an estimate of the variance of the mean of a population, it can be considered analogous to the s^2/n term in equation (5.4). Assuming that both X_a and X_g are good estimates of the arithmetic mean and therefore closely approximate μ, it follows from equation (5.4) that the standard error of a lognormally distributed data set, denoted here by $S.E_{ln}$, should be calculated by the equation:

$$S.E_{ln} = \sqrt{[Var(X)]} \qquad (5.6)$$

irrespective of whether X_a or X_g is used as the estimator of the mean. Equation (5.6) was used for the calculation of the standard errors of the means of SNA, initial NO_3^- and Ex-NH_4^+ values.

The % mineral N was calculated at each depth by expressing the sum of the mean soil NO_3^- and Ex-NH_4^+ values at each depth as a percentage of mean total N at that depth (Figure 5.2i). Young and Aldag (1982) distinguished between available or exchangeable-NH_4 and fixed or unavailable-NH_4. The latter tends to increase down a profile with only about 10 % occurring in the "plough layer" and is generally correlated with the presence of illites, vermiculites and micas in the soil parent material. Regardless of whether the amount of fixed-NH_4 is large or small, it will have little bearing on the variability of exchangeable ammonium-N measured here since fixed-NH_4 is not extracted by the extraction technique used (Mogilevkina & Lebedeva, 1982). The possible occurrence of fixed (i.e. unexchangeable) ammonium was ignored in the calculation of % mineral N; any fixed-NH_4 present was therefore included with the total N, which is predominantly organic N. Thus, the values of % mineral N may be a little low.

Figure 5.2a shows that nitrifier activity was highest in the upper two depth samples, being slightly higher in the 0-3 cm layer. A rapid decline was observed between the 3-6 and 6-9 cm layers, and below 9 cm SNA values declined slowly with increasing depth. In the light of this result, one is tempted to conclude that there was no inhibition of nitrification by grass or other plant roots. However, it may well be that such inhibition did occur, but the rate of nitrification was still higher in the 0-3 cm layer, which consists of a dense root mat, than lower down the profile,

in spite of any inhibition. Although potentially of great importance, especially regarding variability of nitrifier activity on the micro-scale, this problem will not be considered further.

Generally, all the properties measured except ρ_b and C/N ratio declined with depth. C, N and P which were strongly positively correlated ($p<0.1\%$) with each other, were also strongly negatively correlated ($p<0.1\%$) with depth. SNA ($p<1\%$) was also negatively correlated with depth, whilst ρ_b ($p<0.1\%$) and C/N ($p<5\%$) were positively correlated with depth. The complete correlation matrix is given in Table 5.1.

In view of the correlation of many of the properties with depth, it is difficult in some cases to decide whether or not the relationships between them are important irrespective of their statistical significance. For example, SNA and P are closely correlated. Although Purchase (1974) found that a deficiency of P caused suppression of nitrification, there is no evidence to suggest that higher levels of P would lead to higher nitrifier activity. Since both are closely correlated with depth and carbon, the correlation between these two properties may simply be a function of depth and/or C content. Nevertheless, a number of relationships were found. The close correlation of C, N, and P was not surprising in view of their occurrence in organic combination, and the negative correlation between these properties and ρ_b was similarly predictable since organic matter generally has low bulk density. The fact that SNA was strongly positively correlated with these organic properties (Figure 5.3) supports the view expressed above, that the distributions of NO_3^-, NH_4^+ and organic substrate might follow one another. However, Ex-NH_4^+ was not correlated ($p<5\%$) with any other property except incubation pH ($p<0.1\%$), whilst initial NO_3^- was correlated ($p<1\%$) only with the C/N ratio. The latter (negative) correlation would also support the suggestion that soil N parameters should be similarly distributed since initial NO_3^- was large when C/N ratio was small. i.e. NO_3^- production is dependent on a favourable C/N ratio. Indeed, SNA and C/N ratio were similarly correlated. It is therefore surprising that no significant correlation was found between SNA and either Ex-NH_4^+ or initial NO_3^- or between Ex-NH_4^+ and initial NO_3^-.

Table 5.1 Correlation matrix for a range of soil properties measured at different sampling depths.

	Depth	θ_v	ρ_b	pH*	SNA	NO_3	NH_4	C	N	C/N
θ_v	-0.869									
ρ_b	**-0.934**	**0.982**								
pH	-0.485	**0.744**	-0.612							
SNA	-0.897	**0.914**	**-0.940**	0.683						
NO_3	-0.703	**0.716**	-0.666	0.259	0.532					
NH_4	-0.351	0.681	-0.488	**0.956**	0.600	0.179				
C	**-0.967**	**0.943**	**-0.995**	0.659	**0.964**	0.632	0.535			
N	**-0.942**	**0.970**	**-0.981**	**0.707**	**0.968**	0.675	0.594	**0.991**		
C/N	**0.828**	**-0.902**	0.847	-0.539	-0.847	-0.850	-0.473	-0.849	-0.898	
P	**-0.964**	**0.987**	**-0.984**	0.642	**0.955**	**0.731**	0.528	**0.987**	**0.994**	-0.918

Significant correlations are indicated by: ($p<0.1\%$) **bold and underlined**
($p<1.0\%$) **bold**
($p<5.0\%$) underlined

* pH after 8 hours incubation

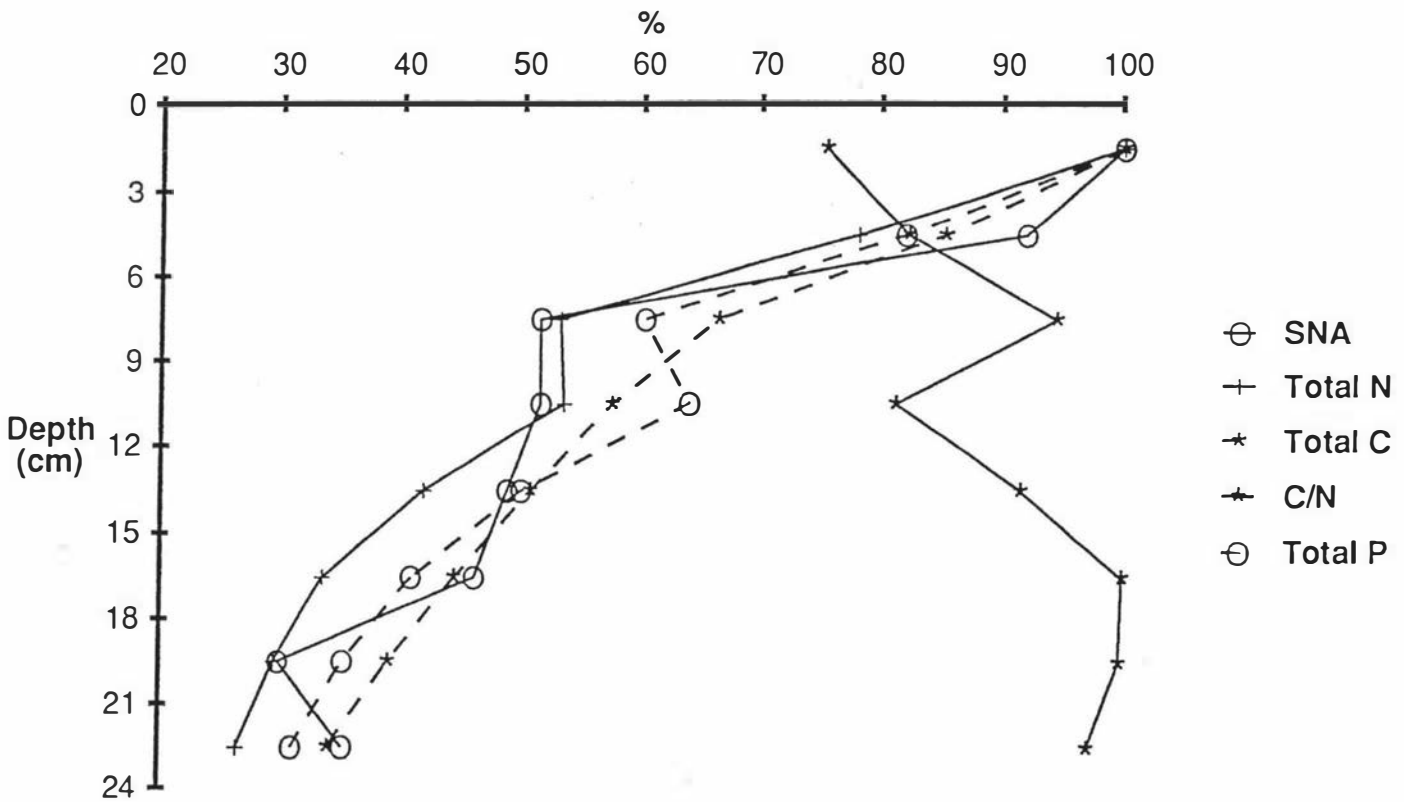


Figure 5.3 Distribution with depth of total carbon, nitrogen and phosphorus contents, and C/N ratio and SNA expressed as a % of their maximum values

The strong negative correlation between SNA and ρ_b suggests a possible relationship between SNA and soil porosity. Porosity was not measured in this experiment, but the pore space ratio (PSR) was calculated for 7.5 and 20 cm depth on the basis of the bulk density (equation (5.1)) and particle density data supplied by D.R. Scotter (Dept. Soil Science, Massey University - personal communication). At 7.5 and 20 cm depth, PSR was equal to 0.57 and 0.49 respectively, which was taken to indicate that the PSR generally declined with increasing depth. It is therefore likely that a dependence of SNA on O_2 availability (Khyder & Cho, 1983) occurred here, which is what would be expected for nitrifiers since they are aerobic organisms.

iii. Discussion

Speir *et al.* (1984) found that most indices of biochemical activity in soil were correlated with the total carbon and nitrogen contents, although they found that biochemical activities declined more rapidly with depth than the C and N contents which suggested that they were affected by factors additional to organic matter. Figure 5.3 shows that in the case of the Tokomaru silt loam, the decline in depth of SNA matches that of C, N and P. Macduff and White (1985) measured rates of mineralization and net nitrification in a clay soil and found that both decreased with increasing depth, a result which they attributed to decreasing microbial populations and substrate concentration with depth. Thus, the results of this study are in agreement with the findings of Speir *et al.* (1984) and Macduff and White (1985), and also those of Hadas *et al.* (1986) and Doran (1987). However, the lack of a significant relationship between SNA and incubation pH was unexpected (see Chapter 2 and Chapter 7), as was the lack of a relationship between SNA and Ex-NH_4^+. Nevertheless, the mean values of these properties do follow the same trend.

A further curious result was the apparent *kink* in the distribution of N and P between the 6-9 and 9-12 cm layers (Figures 5.2f, 5.2h and 5.3). The reason for this uneven decline with depth is not clear; it is unlikely to be due to a sudden change in organic matter distribution, such as might be expected if an old plough layer was present at this depth, because the C distribution (Figure 5.2e) is smooth, as is the change in bulk density with depth. A possible explanation for the kink is that it may represent an aberration in loess accretion at this site (R.C. Wallace, Dept. Soil Science, Massey University) such as has been observed in some soils containing andesitic ash in the Wairarapa (A.S. Palmer, Dept. Soil Science, Massey University), although the uneven distribution of N and P in these soils tends to be mirrored by a similarly uneven distribution of C. It is interesting to note that the standard errors of N and P are very large for the 6-9 cm samples, although the standard errors of the mean values of C, N and P are generally larger than the parameters of mineral N. The latter may be due to a deficiency in the techniques used for analysis of C, N and P, but is more likely to be a reflection of the fact that the standard errors of C, N and P represent the error of means of 5 sample values, whilst the standard errors of the parameters of mineral N represent the error of means of 10 sample values.

The rapid decline in SNA in the second and third depth samples is in agreement with the finding of Speir *et al.* (1984) who observed a similar decline in biochemical activity down to 7.5 cm; at depths greater than 7.5 cm biochemical activity declined gradually. With respect to soil sampling for the analysis of spatial variability in nitrifier activity, this variation with depth presents something of a problem. Youden and Mehlich (1937) were amongst the first to identify the problem of soil spatial variability in soil surveying, and stated that:

> "Since the purpose of any soil survey is to characterize the area as fairly as possible within the limitations of the number of samples that it is possible to take, the efficiency of the sampling plan adopted is a major concern"

In order to study the spatial variability of soil properties it follows that for maximum precision, samples need to be taken from *points* within a given area rather than from small areas within the larger area under investigation; i.e. the areal dimensions of the individual samples must be as small as possible. In this regard, the 5 cm diameter x 3 cm deep corer seemed ideal for this study. However, as outlined in Chapter 4, the analysis of moisture content, initial NO_3^-, SNA and Ex-NH_4^+ required that more sieved soil was needed than that normally obtained from a single 5 x 3 cm core. Hence, the soil was sampled twice; that is, between 3 and 9 cm. However, the results of the analysis of the change in these parameters with depth indicated that variation over this depth range was considerable. The possible effects of grass roots have been discussed previously, but another objection to using the 0-3 cm core was that being essentially a dense root mat, it was very difficult to sieve in the field-moist state, and in any case, yielded very little sieved soil. It seemed unavoidable therefore, that in order to get sufficient soil for the experiments, some averaging over depth must occur. The standard errors for SNA, NO_3^- and incubation pH (which is assumed to be closely correlated with soil pH) were generally less than 5 % of the mean at each depth, which suggested that averaging over the 3 cm depth of each individual core did not result in a large standard error. The standard error of SNA values was also low in the experiment to determine a suitable substrate concentration for SNA measurements (see Chapter 4. In fact the S.E for SNA values was low in all experiments.) It therefore appears that the sieving and mixing process was thorough enough to avoid confusion of spatial (i.e. areal) variation with variation that is depth dependent. Thus, throughout this study, soil sampling was done in the 3-9 cm depth range.

B. SPATIALLY DEPENDENT VARIABILITY

Nearly fifty years ago it was observed that samples taken only 1 $^{1}/_{2}$ inches apart could have nitrate concentrations which varied by as much as 90 ppm between samples (Thompson & Coup, 1940). Despite this finding, of all the work done since then on nitrate leaching and the distribution of soil nitrate with depth (see section A), very little attention has been paid to the spatial variability of soil nitrate, other than noting its existence (e.g. Beckett & Webster, 1971; Cameron *et al.*, 1971; Hunt *et al.*, 1979). White & Bramley (1986) concluded that in order to overcome the problems caused by the large spatial variability in nitrate concentration in soil under grazed fertilized pasture, areal averages had to be calculated from large sample sets of the order of fifty samples per hectare to get the *best estimate* of soil nitrate concentration for input into a nitrate leaching model. However, in the absence of any knowledge of the nature of the variability of nitrate concentrations in a field soil, specifically the distance over which adjacent samples are spatially dependent, much of this sampling effort may be wasted. To eliminate such sampling inefficiency, a sampling strategy is required that is *optimal* in that the sampling error is minimised (McBratney *et al.*, 1981) such that both short and long range variability is accounted for. Therefore, to further improve estimates of soil nitrate concentration, information is required as to where samples should be taken, especially in relation to their nearest neighbours.

Maclusky (1960) found that in a pasture grazed by cattle, the area which was affected by urine and faeces was approximately equivalent to 1.5 m^2 per cow per day. White *et al.* (1987) found that much of the spatial variability in nitrate concentration in their clay soil under pasture grazed by sheep was short range, occurring within 0.4 m. Thus, under grazed pastures, soil nitrate variability is likely to be high. It was for this reason that the fields sampled for the experimental work described in this thesis were ungrazed for three weeks prior to sampling so that grazing effects might be ameliorated. Nevertheless, the preliminary experimental work done to tailor the SNA to the Tokomaru silt loam (Chapter 4) suggested that irrespective of grazing effects, any variability in nitrifier activity was likely to be soil specific (the pH

work detailed in Chapter 7 supports this view), and thus it was considered unwise to use 0.4 m as the sample separation in the initial spatial analysis and that a larger scale investigation was therefore appropriate. Accordingly, the first attempt to quantify the spatial variability in the Tokomaru silt loam under pasture was carried out over an area of 625 m^2 and the assumption was made that any spatial dependence would occur within a range of 25 m. By sampling at this scale it was intended to obtain an indication of the likely range of any spatial dependence of nitrifier activity, so that a more precise analysis could be carried out at a more appropriate scale.

i. Methods and Materials

Soil sampling

A square grid 25 m x 25 m with a grid spacing of 2.5 m was marked out in field No. 6. Following the three week period of no grazing (see Chapter 4), the soil was sampled at each of the 121 nodes of the grid between 3 and 9 cm depth. The samples were sieved and stored as before. Sampling was carried out on 29 October, 1986.

On 15 June, 1987, the soil was re-sampled and the experiment repeated. On this occasion the soil was sampled as described, but over a 3 m x 3 m grid with a grid spacing of 30 cm. This smaller grid was randomly sited in field No. 6, using random numbers to generate the coordinates of the top left corner, and the grid was marked out with the same orientation as the larger grid (above). Its position was close to the centre of the larger grid.

Analysis

SNA measurements were made in duplicate on all samples following the method given in Chapter 4. Thus 242 incubations were done at a rate of 60 incubations every other day (62 on the last day), beginning on the day after sampling. It was assumed that storage had no effect on the samples analysed on the 4^{th}, 6^{th} and 8^{th} days after sampling, in relation to

those analysed the day after sampling (see Chapter 4). NO_3-N in the leachate collected from the SNA pre-leaching was also analysed and the pH of the incubating medium was measured as usual after the eight hour sampling.

ii. Results

Field soil moisture contents were calculated using equation (4.5) and their distribution together with that of SNA, initial NO_3^- and incubation pH was investigated; the data were grouped into suitable class intervals, and the normalized frequency for each class plotted against the mid-value for that class. The data were fitted by least squares with equations for the normal and log-normal distributions (White *et al.*, 1987) and the best fit determined on the basis of minimizing the residual sum of squares for the regression. The distributions are shown in Figure 5.4. Incubation pH and moisture content conformed to a normal distribution (R^2 = 0.91 and 0.96 respectively; $p<0.1\%$) whilst SNA and initial NO_3^- were lognormally distributed (R_2 = 0.96 and 0.91; $p<0.1\%$). Accordingly, values of SNA and initial NO_3^- were log-transformed (ln) prior to further analysis so as to stabilise their variances (White *et al.*, 1987). In order to remove the effects of negative ln values which were found to seriously affect the variogram, the units of SNA and initial NO_3^- were changed from μmol N g^{-1} h^{-1} (μmol N g^{-1} in the case of initial NO_3^-) to ng N g^{-1} (h^{-1}) by multiplying by 14000. Semivariances were calculated for the *north-south*, *east-west* and both directions combined using a simplified version of the FORTRAN programme GAMMAH (see Appendix 1), and the experimental variogram plotted for each property. Isotropy was assumed, and weighted least squares was used to fit linear, spherical and exponential models to the experimental variograms using NAG routine E04FDF (Numerical Algorithms Laboratory, Oxford, U.K). The weights for each lag, $\lambda(h)$, were calculated by the equation:

$$\lambda(h) = \frac{m(h)}{\Sigma\, m(h)} \qquad (5.7)$$

such that

$$\Sigma\, \lambda(h) = 1 \qquad (5.8)$$

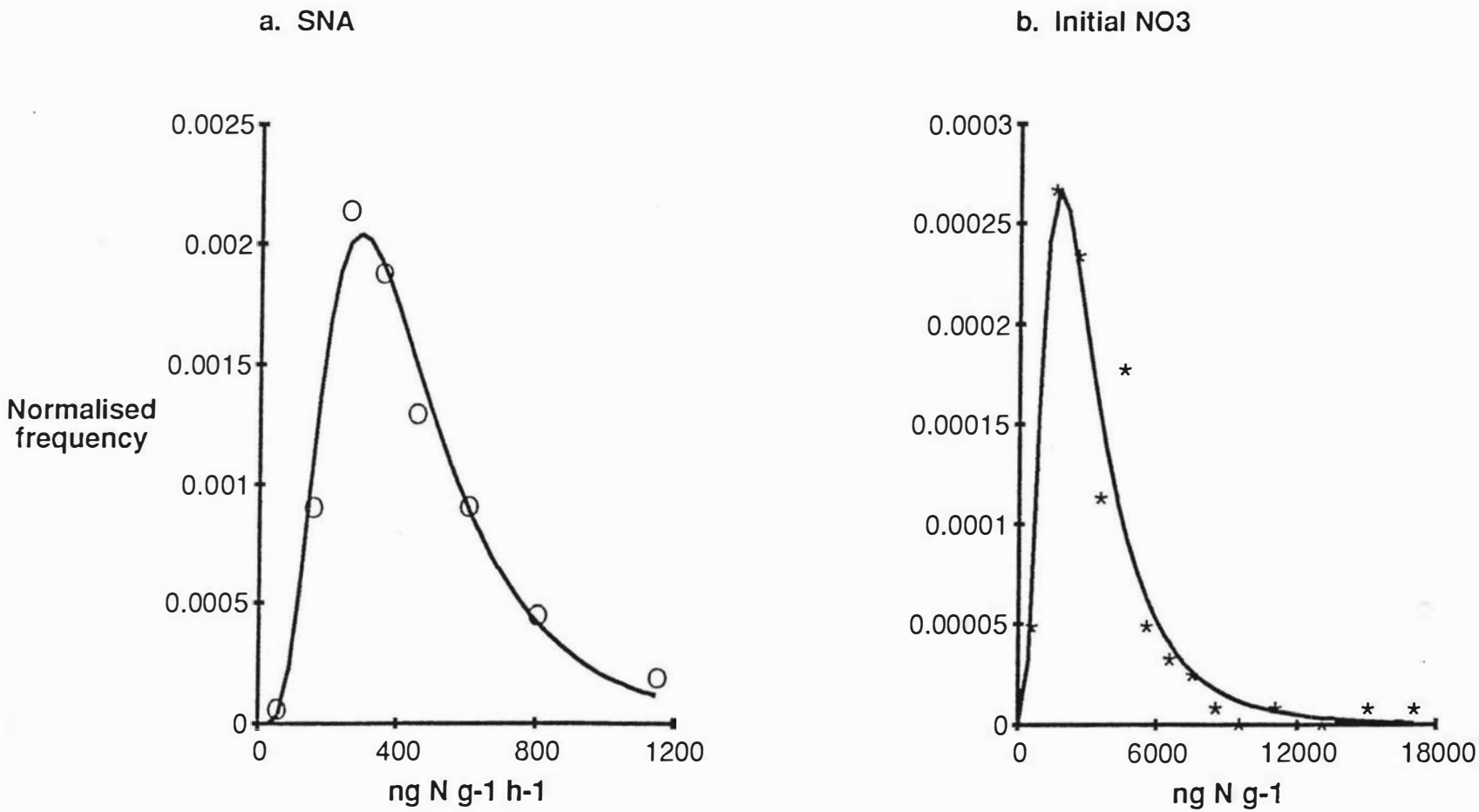
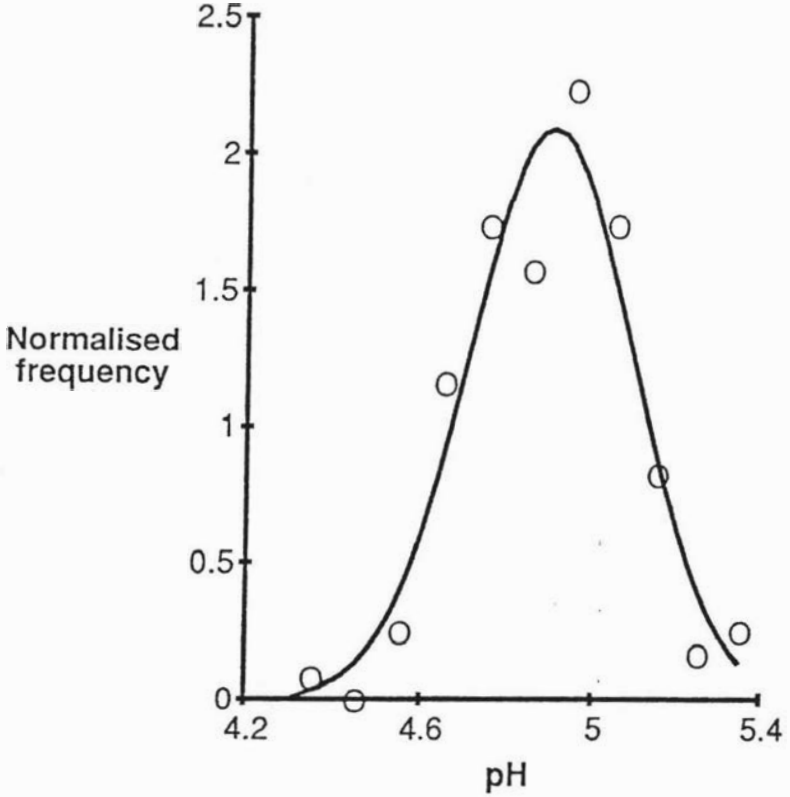


Figure 5.4 Distribution of (a) SNA, (b) NO_3^-, (c) incubation pH and (d) gravimetric moisture content sampled on a regular 11 x 11 square grid between 3-9 cm over 625 m^2

c. Incubation pH



d. Moisture content

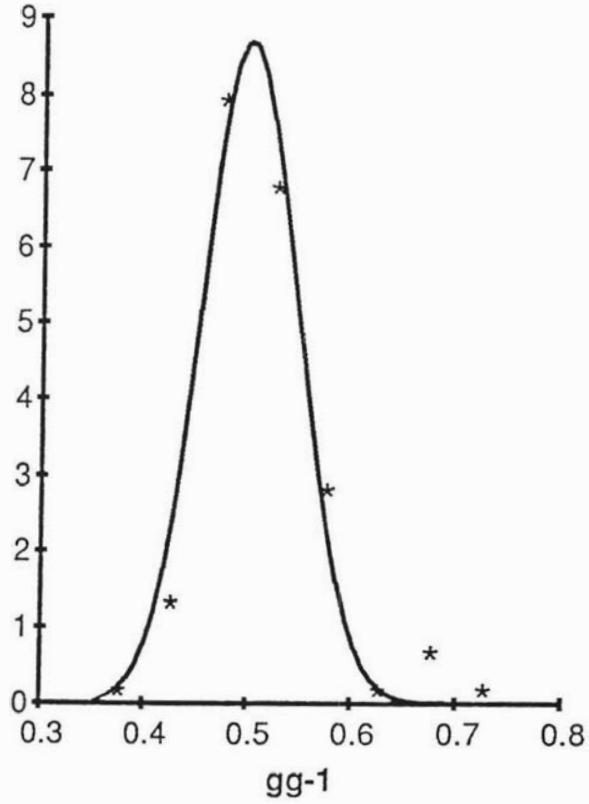


Figure 5.4 (Contd)

The best fit model was chosen by calculating its AIC value using equation (3.22). The variograms for SNA, moisture content and initial NO_3^- were best fitted by linear models, whilst incubation pH was best fitted by a spherical model (Figure 5.5).

The intention in this experiment was to gain some idea as to the approximate range of spatial dependence (if any) so that further analysis of that dependence could be carried out at a more appropriate scale. The variogram for SNA appeared to show a pure nugget effect (see Chapter 3); the fitted linear model showed a slight decline in $\gamma(h)$ with increasing h, although the difference between this fitted model and the sample variance was only very small for all values of h. Thus, any spatial dependence of nitrifier activity was undetectable at this scale of sampling. In contrast, the variograms for initial NO_3^-, moisture content and incubation pH all suggested spatial dependence in the data, and in the case of incubation pH, this could be defined within a range of 9.9 m. As the best fitted models for moisture content and initial NO_3^- were linear, the scale of spatial dependence could not be discerned. However, taking into account the results of White *et al.*(1987) and the pure nugget variance of SNA, together with the fact that by definition $\gamma(h) = 0$ at $h = 0$, it was considered that the scale of sampling used here was probably too large. Hence the experiment was repeated with the same sampling design, but this time over an area of 9 m^2 as opposed to the 625 m^2 sampled in the first experiment.

The distribution of values of the various properties sampled over 9 m^2 was investigated as for the 625 m^2 experiment. Incubation pH and moisture content again conformed to the normal distribution (R^2 = 0.89 and 0.99 respectively; $p<0.1\%$) whilst SNA and initial NO_3^- followed lognormal distributions (R^2 = 0.88 and 0.96; $p<0.1\%$)as before (Figure 5.6). Semivariances were again calculated using GAMMAH and the experimental variograms were plotted. Isotropy was assumed, and the variograms were fitted with models by weighted least squares as before. The best fit models are shown in Figure 5.7.

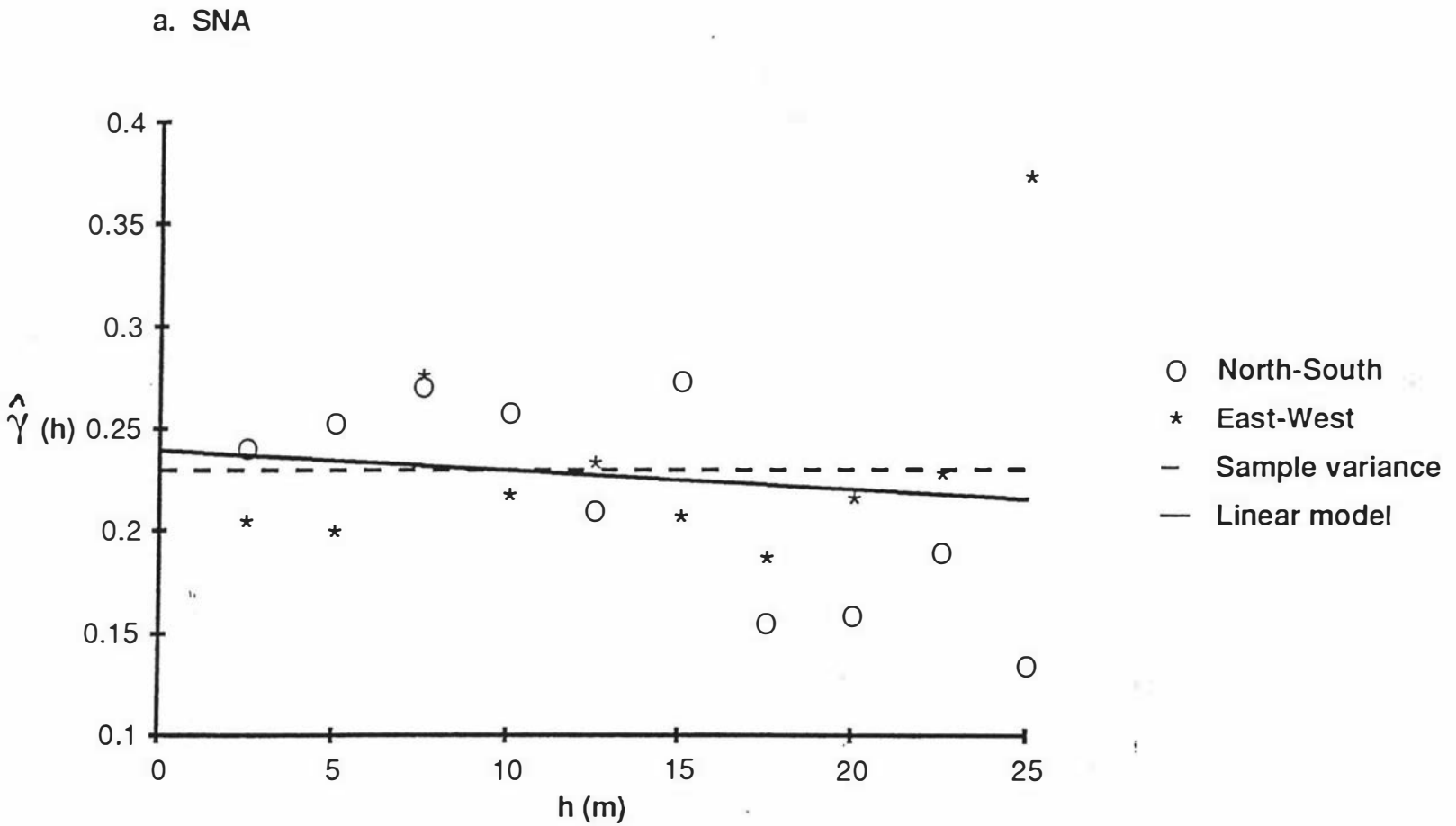


Figure 5.5 Experimental variograms of (a) SNA, (b) NO_3^-, (c) incubation pH and (d) moisture content sampled on a regular 11 × 11 square grid between 3-9 cm depth over 625 m^2 with models fitted by weighted least squares optimization

b. Initial NO3

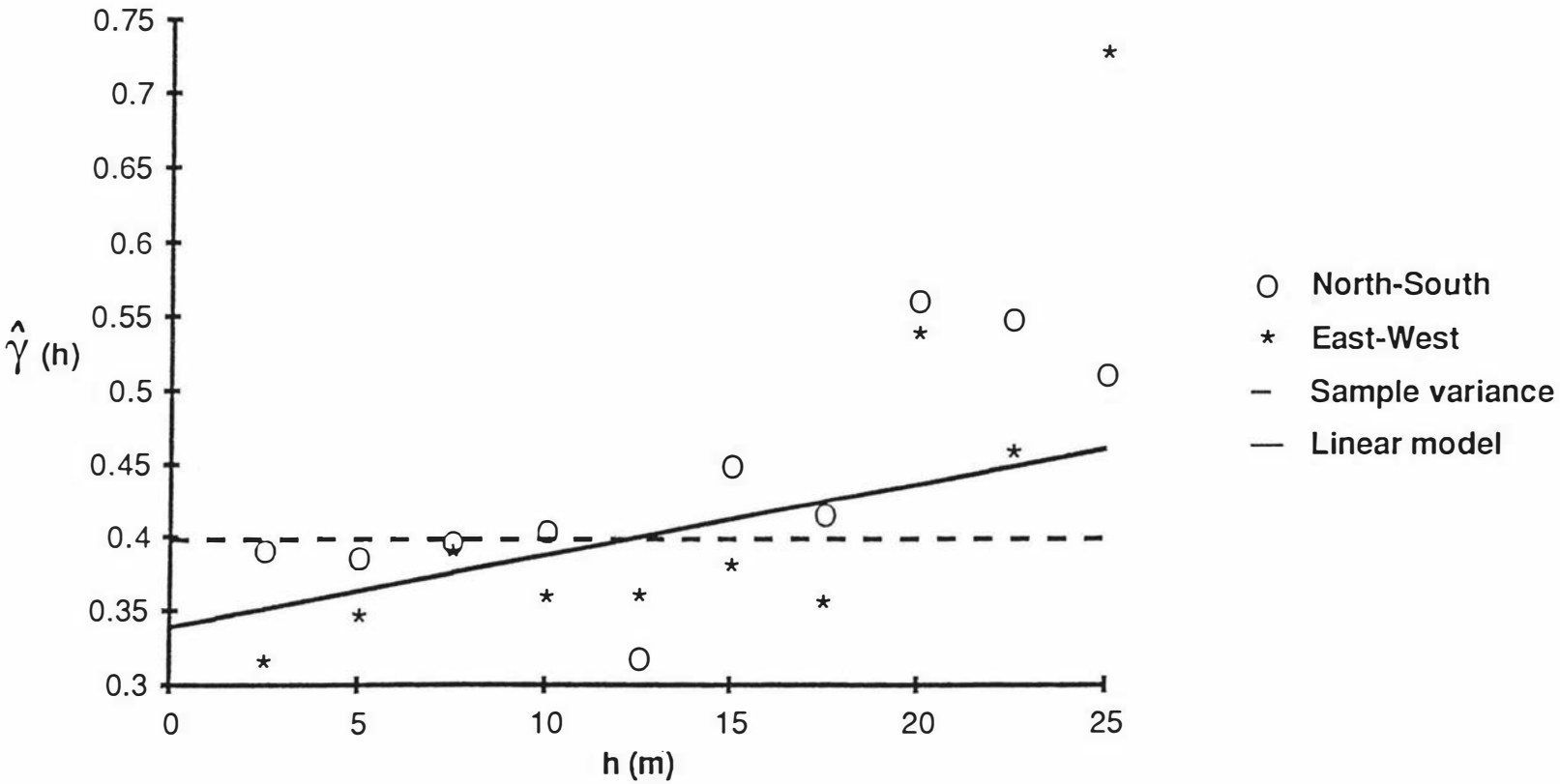


Figure 5.5 (Contd)

c. Incubation pH

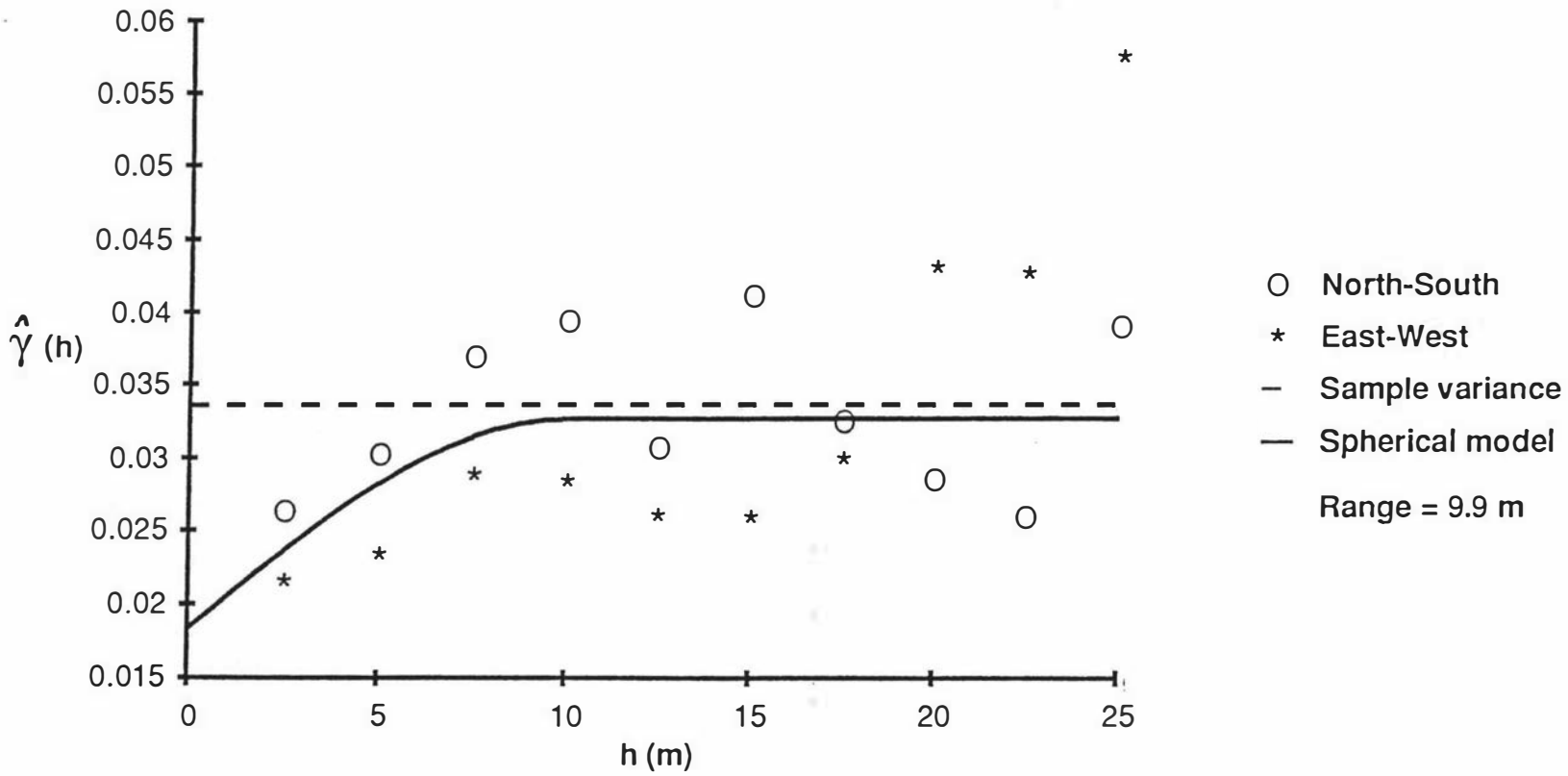


Figure 5.5 (Contd)

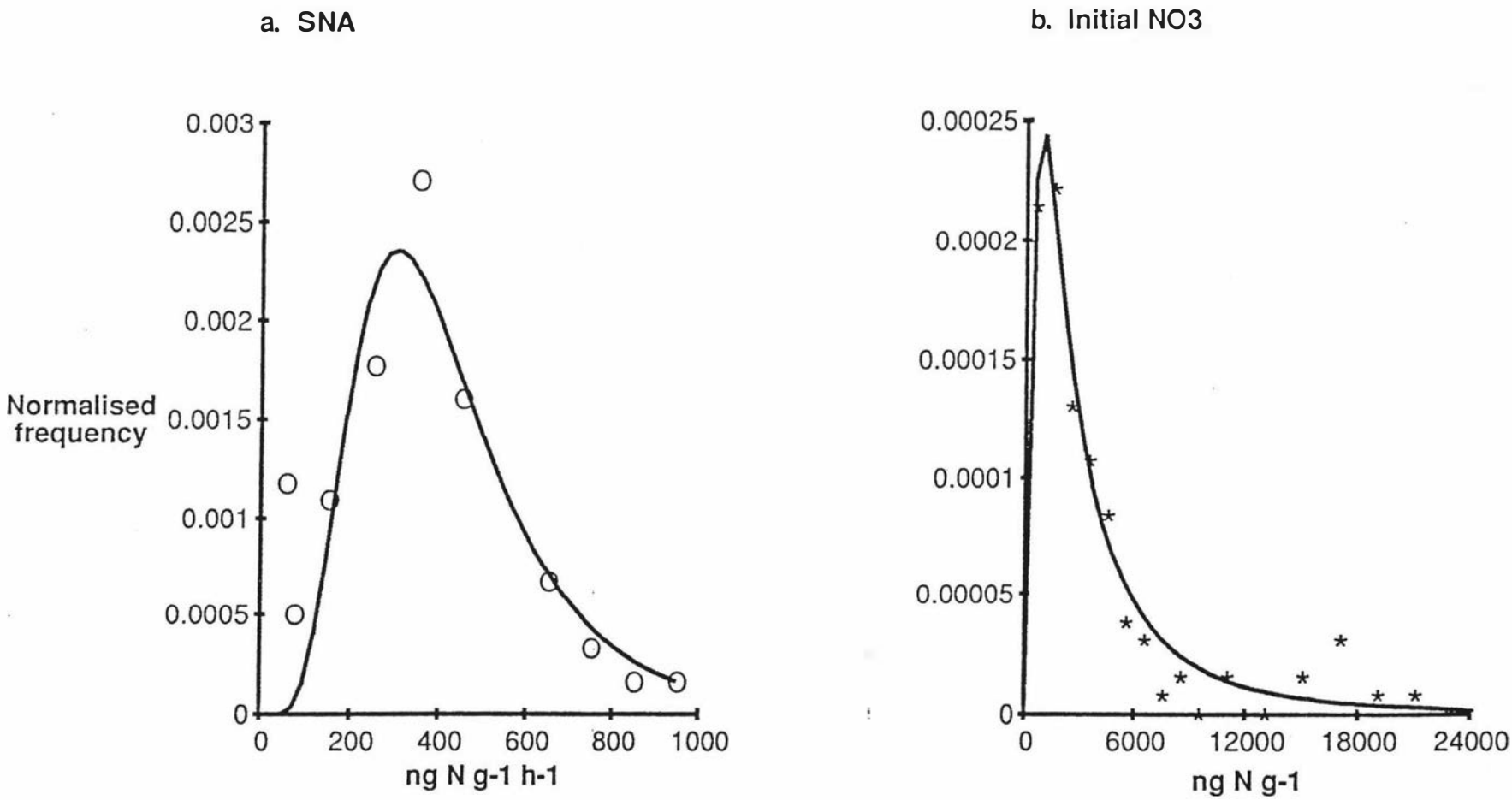
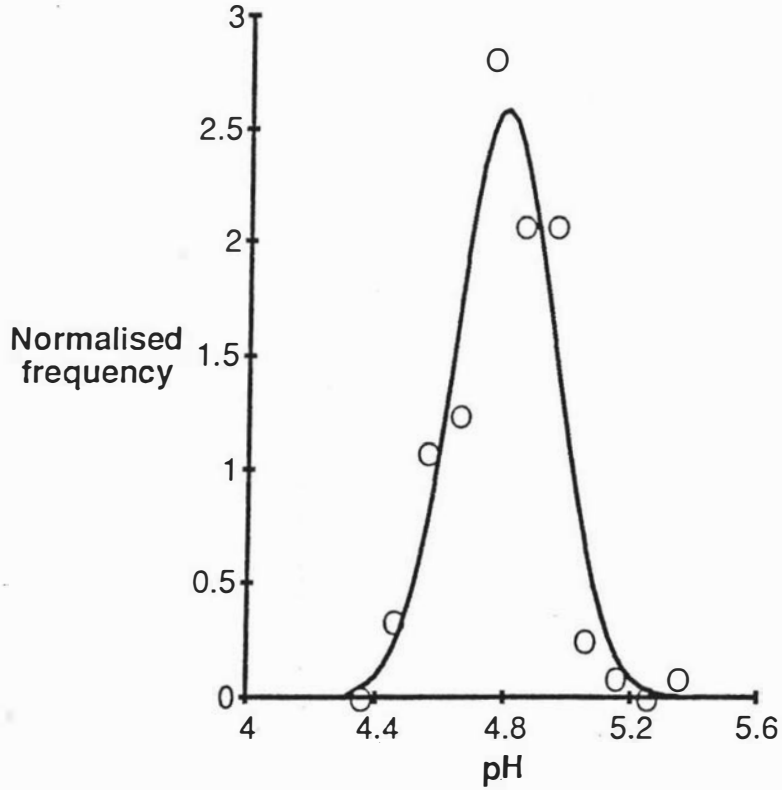


Figure 5.6 Distribution of (a) SNA, (b) NO_3^-, (c) incubation pH and (d) moisture content sampled on a regular 11 x 11 square grid between 3-9 cm over 9 m^2

c. Incubation pH



d. Moisture content

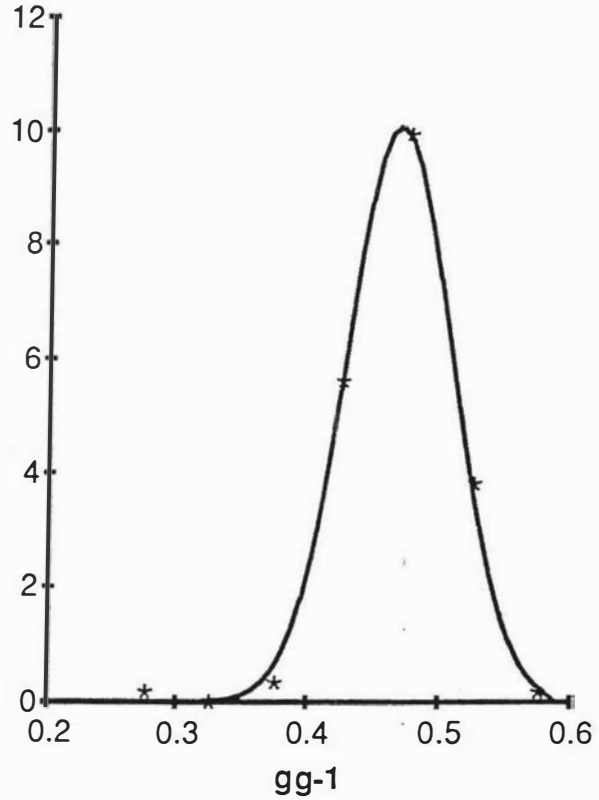


Figure 5.6 (Contd)

A number of differences between the variograms for the two experiments were immediately apparent. In contrast to the pure nugget variance shown by SNA for lags ranging from 2.5-25 m, spatial dependence was found in the 9 m^2 experiment, with a range calculated from the fitted spherical model equal to 0.6 m. Incubation pH which previously showed spatial dependence within 9.9 m showed pure nugget variance over the smaller sampling area. Since the largest lag in the second experiment (3 m) was much less than the range of spatial dependence found in the first experiment, spatial variability of incubation pH was expected to conform to a linear model. i.e. it was expected that there would be spatial dependence in the data, but not within a defined range. The fact that there appeared to be a complete absence of spatial dependence in the second experiment suggests that the spatial dependence of a soil property, in addition to the actual property values, may be subject to seasonal variation. Initial NO_3^- showed a pure nugget effect in the second experiment, whilst moisture content showed only slight spatial dependence, again with undefinable range (Figure 5.7).

iii. Discussion

In both experiments, isotropy was assumed following the advice of A.B. McBratney (C.S.I.R.O Division of Soils, Brisbane - personal communication) who considered that unless anisotropy was obvious, it was difficult to identify with any certainty due to the tendency for values of $\hat{\gamma}(h)$ to fluctuate with increasing h, as is the case for example, in the variogram for incubation pH sampled over 625 m^2 (Figure 5.5c). However, the variogram for moisture content over 625 m^2 (Figure 5.5d) is clearly anisotropic as the differing trends in the variograms for the north-south and east-west directions indicate. Over 9 m^2 such anisotropy in moisture content is not so obvious. There is also a suggestion of anisotropy in the SNA data from the 9 m^2 experiment (Figure 5.7a). The question of anisotropy is discussed more fully in Chapter 10, but at this stage, the doubts as to whether or not it is present may call into question the value of these experimental variograms.

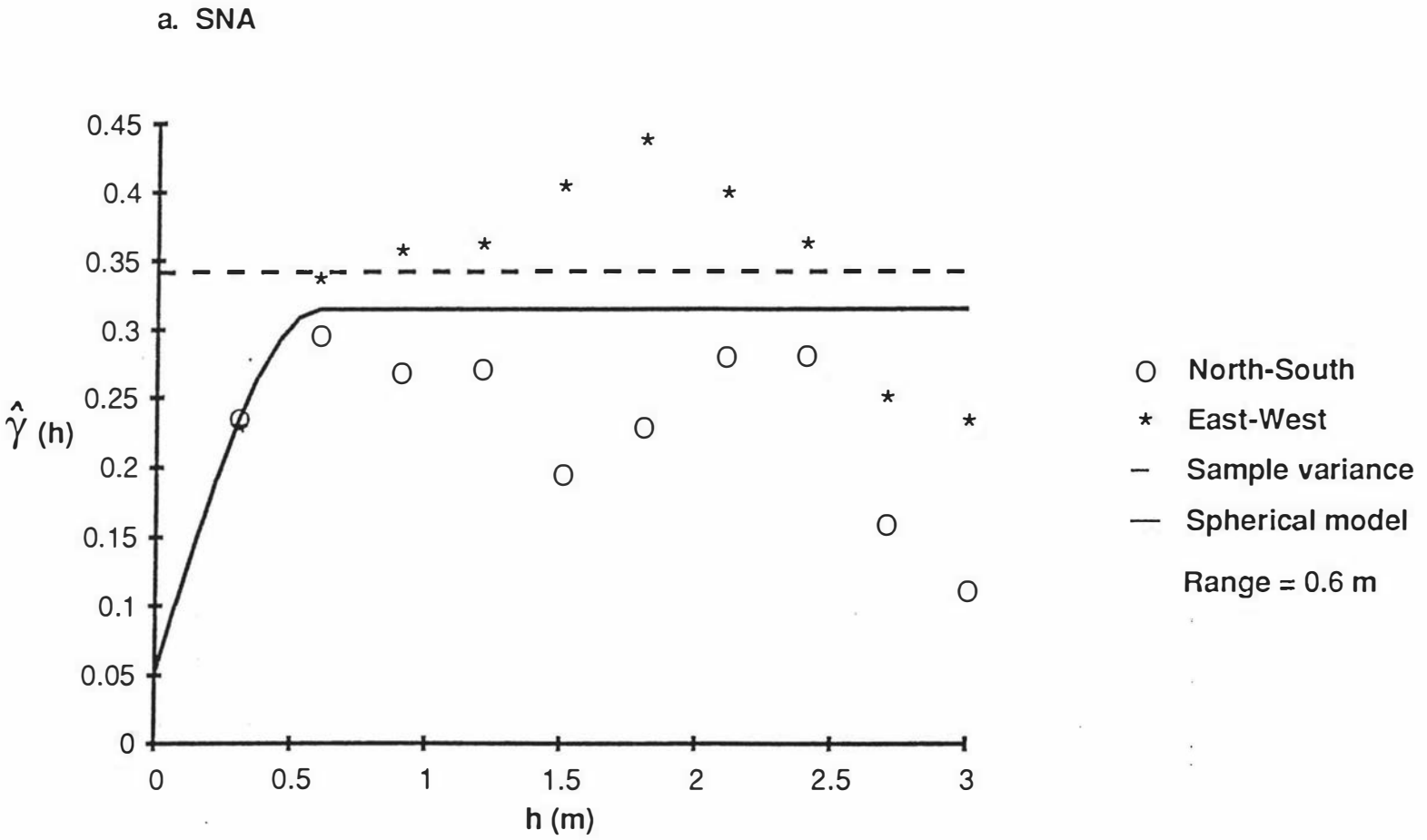


Figure 5.7 Experimental variograms of (a) SNA, (b) NO_3^-, (c) incubation pH and (d) moisture content sampled on a regular 11 × 11 square grid between 3-9 cm depth over 9 m^2 with models fitted by weighted least squares optimization

b. Initial NO3

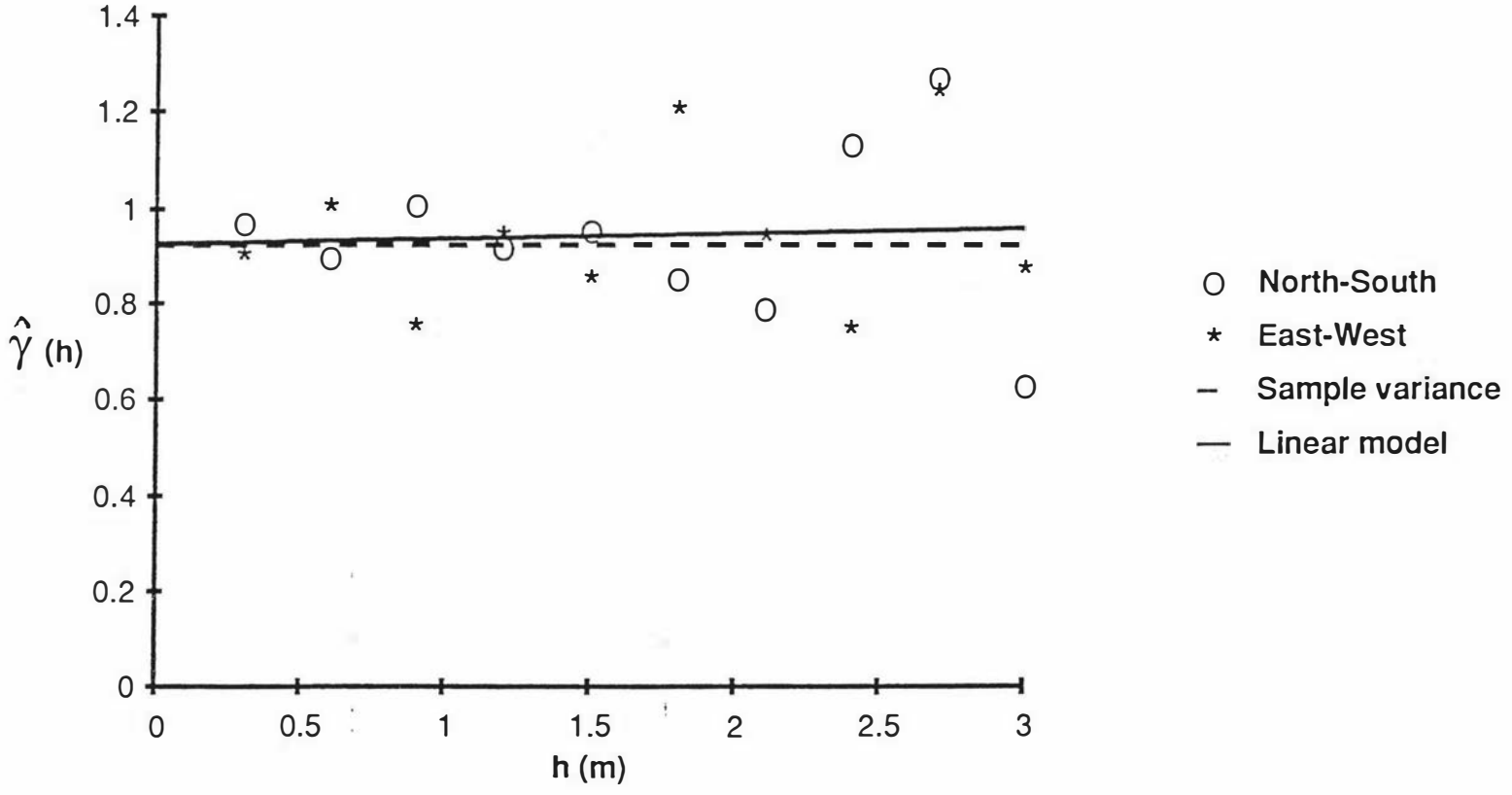


Figure 5.7 (Contd)

c. Incubation pH

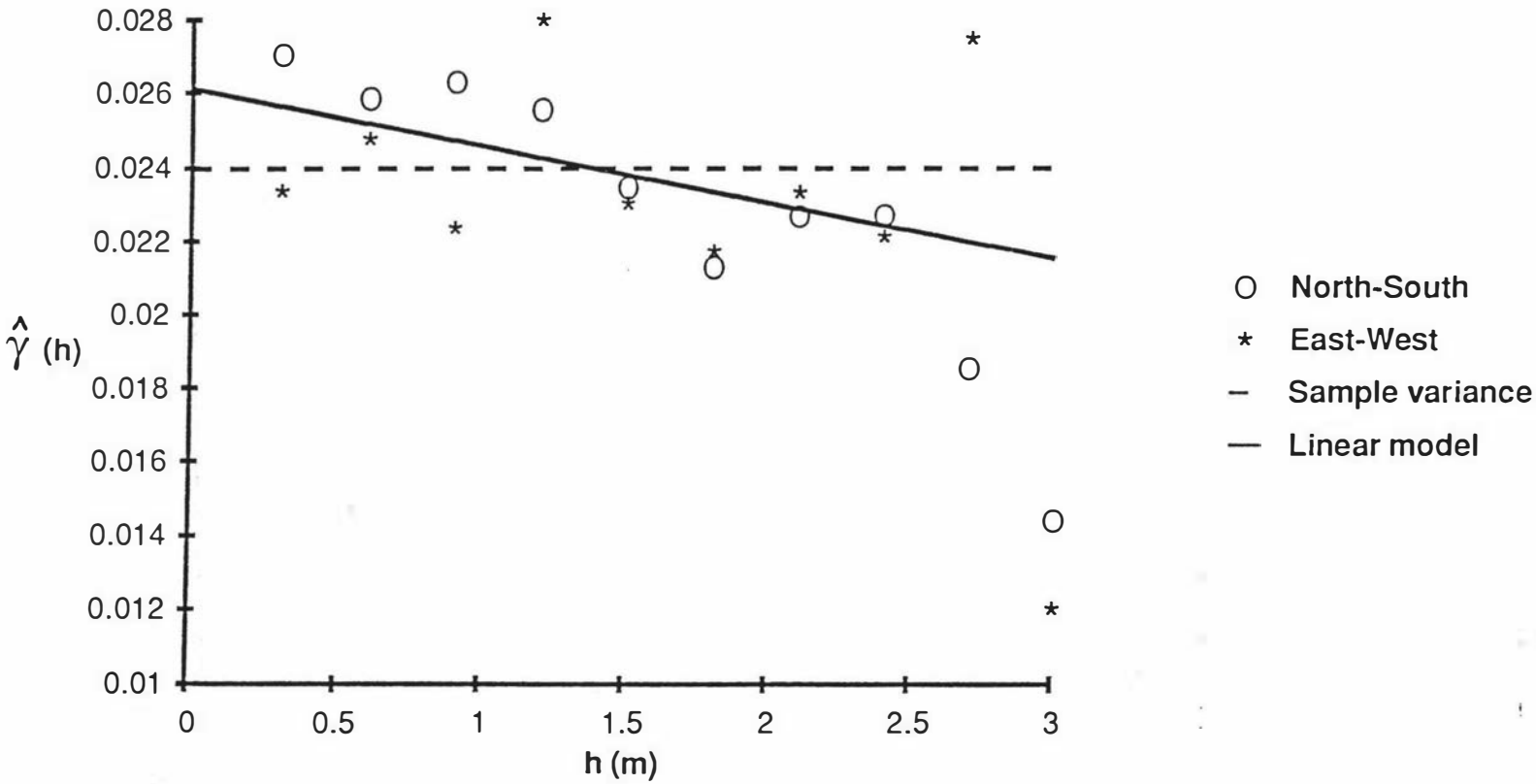


Figure 5.7 (Contd)

d. Moisture content

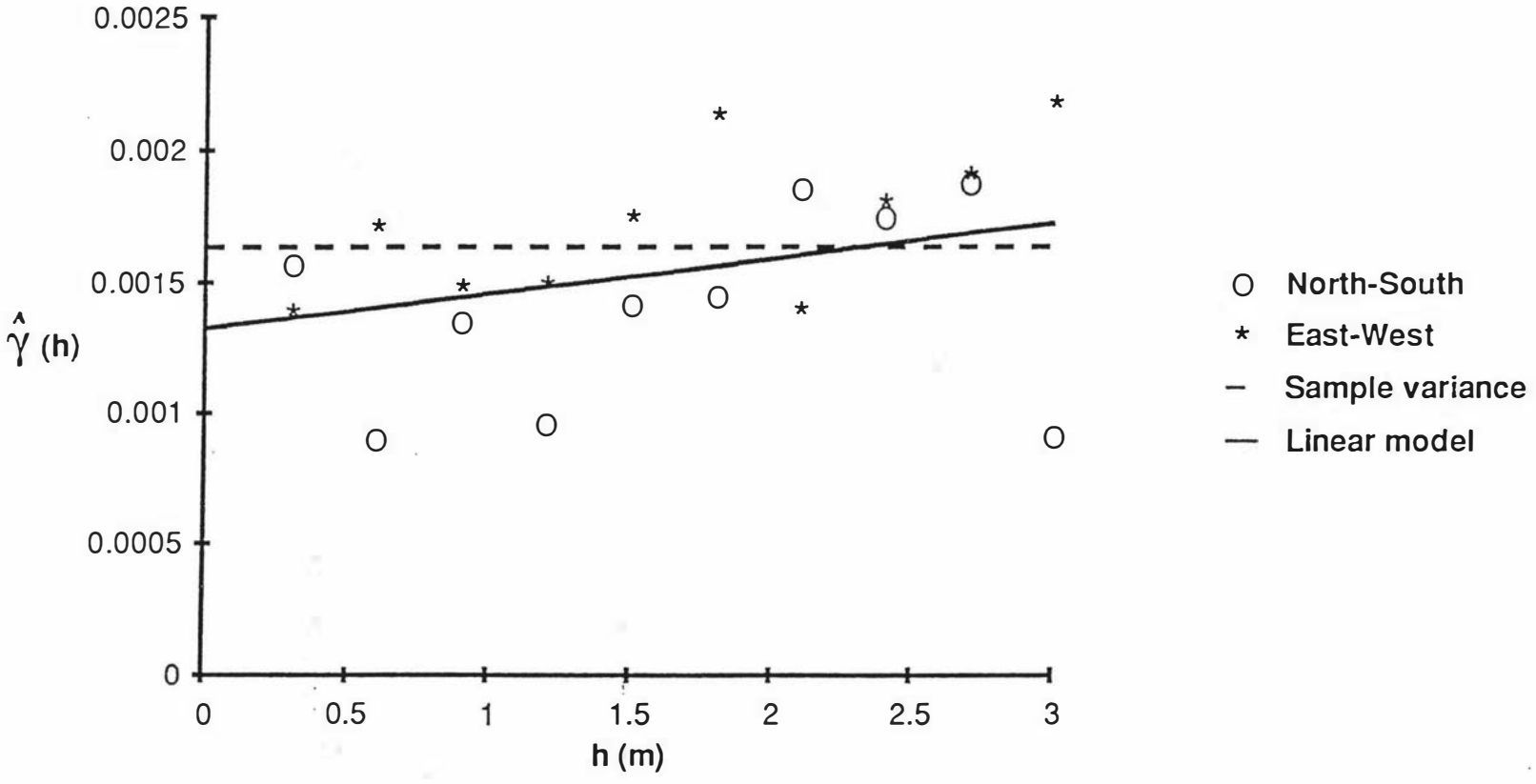


Figure 5.7 (Contd)

The differences between the two sets of variograms highlighted an additional problem with respect to the interpretation of spatial dependence in the data. It was clear that it was not possible to redraw the two sets of variograms as a single one for each property by simply combining them, because of obvious temporal variation between the properties as sampled at the two different dates (the first experiment was carried out in October (i.e. spring) whilst the second was done in June (i.e. winter)). Comparing the two sets of variograms, it can be seen that the sample variances, and therefore the likely position of the sill, were different between the two dates. Thus, the variograms in Figures 5.5 and 5.7 had to be considered separately. Since the variograms for NO_3^- and θ_g over 9 m^2 showed pure nugget effects, it was concluded that any spatial dependence for these properties was again undetected at the smaller sampling scale, which was unexpected since the spatial analysis over 625 m^2 suggested that there was spatial dependence, but that information was lacking for lags less than 2.5 m. It therefore seemed probable that the sampling design was not a good one, and that a more sophisticated sampling strategy was required. One might also deduce that the variances and variograms, even of log transformed soil property values, were dependent on the time of measurement; the fact that a property could show complete nugget variance when measured eight months after showing some spatial dependence was a bit disturbing.

A further problem in the interpretation of the variograms was found in the case of the properties whose experimental variograms were best fitted by linear models. In fact, the experimental variograms for incubation pH over 625 m^2 and SNA over 9 m^2, were the only ones which were not best fitted by linear models. The interpretation of linear variogram models was not discussed in Chapter 3, and has recieved scant attention in the literature. The idea of pure nugget variance has been discussed previously and deals satisfactorily with the problem of interpreting the linear model when it is horizontal. However, when $\hat{\gamma}(h)$ appears to increase without limit as h increases, interpretation is not so straightforward. Obviously there must be some spatial dependence, otherwise $\hat{\gamma}(h)$ would not increase with increasing h. However, the fact that no sill is reached and that as a result no range is defined,

suggests that linear models show evidence of drift in the data. In other words, local stationarity, which is required under the constraints of the intrinsic hypothesis of regionalized variable theory, does not apply at the scale of sampling, and so the mean value of the property varies over the sampling area. On the basis of the variograms for initial NO_3^-, it appeared that whether or not drift was present depended on the scale of sampling and/or the sampling design. Initial NO_3^- appeared to show evidence of drift over 625 m^2, whilst over 9 m^2 the variance was pure nugget. In the case of moisture content drift appeared to be present in the data at both scales of sampling. This problem, and the related observation that the range may be a function of the scale of sampling (Russo & Jury, 1987a, 1987b) is considered further in Chapter 10.

M^{c}Bratney *et al.* (1981) and M^{c}Bratney and Webster (1981) presented a theoretical consideration of sampling design and concluded that providing the data were isotropic, an equilateral triangular grid was an optimal design with respect to minimizing the error of interpolating unsampled points (kriging) on the basis of the experimental variogram produced by that sampling strategy. The increase in sampling efficiency over a square grid was estimated at 10 % but the computational difficulties of using such a sampling strategy, not the least of which is getting the computer to read triangularly spaced data, make this design unattractive in practice. Indeed, Burgess *et al.* (1981) and M^{c}Bratney and Webster (1983) found a square grid with the same sampling density as the triangular grid to be more convenient. In the case where the data are anisotropic, an equilateral triangular grid has no advantage over a square grid (M^{c}Bratney and Webster, 1981) and thus, one might conclude a square grid to be adequate in all cases because unless a preliminary survey is done prior to detailed sampling, the presence or otherwise of anisotropy will be unknown. The results of the two experiments described above suggest that sampling may have to be very detailed to identify anisotropy with any degree of certainty, especially as there is still no statistical test for it (Laslett *et al.*, 1987). Thus, the sampling design used in the experiments described above might be considered satisfactory. However, Laslett *et al.* (1987) contended that regularly grided data without observations at close or intermediate spacings were not suitable for regionalized variable theory, although they did not elaborate why. Russo

(1984a) outlined a scheme whereby the total number of sampling points and the number of points separated by a given lag are chosen, and the position of randomly situated points is adjusted sufficiently to accommodate the required number of pairs separated by given lags. From a theoretical point of view, this method has certain attractions for isotropic data since the different pairs are randomly orientated. However, it is somewhat complicated, and as with the equilateral triangular grid, one would would need some prior sampling to determine whether or not isotropy applied.

In the light of the results of the spatial analysis described above, it seemed probable that the deficiency of the sampling design used was that small lags of the order of a few centimetres were not considered simultaneously with those of the order of several metres; i.e the ratio of the smallest to the largest lag was not small enough. If a strategy which allowed for such a range of lags to be included could be designed, it seemed probable that the resultant experimental variogram would provide more precise information than that provided by Figures 5.5 and 5.7. A *nested* sampling design satisfies this strategy. Oliver and Webster (1986; 1987) and Oliver (1987) used a series of nested transects based on the sampling strategy used by Youden and Mehlich (1937) to examine soil pH and particle size variability in the Wyre Forest in England. In contrast, Wopereis *et al.* (1988) used a nested square grid to investigate the spatial variability of heavy metals in soil over an area of one hectare. With respect to the analysis of nitrifier activity in field No. 6, a square grid seemed preferable to a series of transects because the sampling area is relatively small compared, for example, to the 60 km^2 surveyed by Oliver (1987) and Oliver and Webster (1986). Furthermore, the computation of semivariances in different directions is made easier by the symmetry of the design. In the following chapter, a more refined spatial analysis of nitrifier activity is described, whereby samples were taken on a nested square grid with a much smaller ratio of smallest to largest lag.

CHAPTER 6

SPATIAL VARIABILITY OF NITRIFIER ACTIVITY - A MORE REFINED ANALYSIS

The initial analysis of spatial variability in nitrifier activity and associated soil properties proved unsatisfactory because of the lack of information provided on variability over short distances, especially relative to larger distances. On the basis of the results of the experiments described in the previous chapter, it appeared that the efficacy of a sampling design was a function of the ratio of the smallest to the largest lag which, it was concluded, should be as small as possible. Indeed, Laslett *et al.* (1987) warned against the situation where there are insufficient data at small lags. Thus, a sampling design was needed which allowed for sample separations to vary between a few cm and 25 m or more. One way to achieve this is to use a design whereby the sample separations form a logarithmic progression (A.B. McBratney C.S.I.R.O, Division of Soils, Brisbane - personal communication); evidently logarithms to base 2 are particularly suitable, although such a design is better suited to large sampling areas. Alternatively, a nested design of the type used by Wopereis *et al.* (1988) may be used. However, for the spatial analysis of nitrifier activity as measured by the SNA, a constraint is put on the sampling design by the number of samples that it is (a) possible to take from the field in a day; and (b) possible to analyse in the laboratory at any one time. With respect to the former, it follows that since the nitrifier activity being measured by the SNA is assumed to be a good estimate of the nitrifier activity in the field at the time of sampling, the samples must be taken all at once so that error due to temporal variability is avoided. In this study, *all at once* was taken to mean *on the same day*. With respect to the laboratory analysis, 60 incubations per day were feasible, that is, 30 field samples could be assayed per day allowing for duplicate incubation of each sample. In view of the time required for preparation both of samples and incubation tubes, this effectively meant 60 SNA measurements could be made every other day. This in turn, put a constraint on the total number field samples that could be analysed over the few days following sampling in view of the possibility of storage effects, even though it was

established in Chapter 4, that these were unlikely to be significant within 3 weeks of sampling. Thus, a desirable sampling design was deemed to be one which gave the maximum number of samples that could be analysed under the logistic constraints of the analysis, but which allowed all the field sampling to carried out in one day.

In the two previous experiments, 121 samples were taken on a regular square grid. The number of pairs of points separated by each lag under this design for both the north-south and east-west directions combined (i.e. assuming isotropy) is given in Table 6.1. Russo (1984a) stated that

> "most experienced geostatisticians would regard 100 values evenly spaced as a minimum requirement for estimation of the variogram...(and that)...it is generally recommended that (the number of pairs of points separated by a given lag) should exceed 30."

Thus, one criticism of the regular square design is that the number of pairs of points separated by each lag is uneven and decreases markedly as the size of the lag increases. One would think that if weighted least squares is used for fitting models to the experimental variogram, uneven and decreasing m(h) with increasing h should not present a problem, especially if the range of spatial dependence is much less than the largest lag. Cressie (1985) also argued that 30 should be a minimum number of pairs at each lag. The reason given was that the weighted least squares procedure is Gaussian-based, that is, it depends on the points to which a model is fitted being normally distributed. However, the values of the parameter $\Sigma \{Z(x_i) - Z(x_i + h)\}^2$ (equation 3.14) are skewed (Cressie, 1985), and so although weighted least squares is a convenient way of dealing with inequality in the number of pairs of points at each lag, it may not be satistfactory for small values of m. Cressie (1985) described it as a *true compromise between simplicity and statistical efficiency*.

Table 6.1 The number of pairs of points separated by a given lag (h) under the two sampling designs used for spatial analysis over 625 m² assuming isotropic data*

Regular design		Nested design	
h (metres)	No. pairs	h (metres)	No. pairs
25.0	22	25.0	18
22.5	44	22.5	20
20.0	66	20.0	26
17.5	88	17.5	36
15.0	110	15.0	38
12.5	132	12.5	56
10.0	154	10.0	68
7.5	176	7.5	56
5.0	198	5.0	54
2.5	220	2.5	78
		2.0	20
		1.875	26
		1.75	28
		1.625	32
		1.5	32
		1.375	32
		1.25	36
		1.125	36
		1.0	36
		0.875	40
		0.75	44
		0.625	82
		0.5	60
		0.375	76
		0.25	84
		0.125	92

Mean No. pairs per lag = 121 ± 21 (Regular grid)
46 ± 4 (Nested grid)

No. lags with more than 30 pairs = 9 (Regular grid)
20 (Nested grid)

* In the case of anisotropy, the number of pairs that may be considered at each lag will be half the value given here.

The laboratory constraints put on the sampling design meant that consideration of the requirement that m(h) should be greater than 30 at all values of h was almost impossible because the number of samples required in a design which fulfills this requirement is too great. In any case, 30 seems to be an arbitrary value which Cressie (1985) chose without much theoretical basis as sufficiently large to avoid skewness in $\Sigma \{Z(x_i) - Z(x_i + h)\}^2$. For this study, it was decided that the number of samples to be taken for SNA measurement could be increased from 121 to approximately 150 (i.e. by a further 30 samples, or 60 incubations). By removing some of the sampling points in the original square grid design and placing them in a smaller nested grid at the centre of a large grid (henceforth called the nested and main grids respectively), and including the additional 30 points gained by increasing the total number to be taken in the nested grid, a sampling design was produced which (a) had a much more even spread of points over all lags; (b) had a very small ratio of smallest:largest lag; and (c) fitted the constraints of the laboratory analysis. This design is shown in Figure 6.1 and the number of pairs of points at each lag is listed in Table 6.1. The total number of samples required under this design was 154 and lags ranged from 12.5 cm to 25 m. Symmetry was maintained about the top-right to bottom left diagonal of the grid which, as in the original square grid design, allowed for equal consideration of the data in both the north-south and east-west directions, and assuming isotropy, for both directions combined. Table 6.1 also illustrates the more even distribution of the number of pairs per lag in the nested design as compared to the regular square grid. The benefits of the nested grid are further indicated (assuming isotropy) by the increased number of lags with greater than 30 pairs of points, despite a large reduction in the mean number of pairs per lag, and also by the reduction in the ratio of smallest:largest lag - from 0.1 in the case of the regular square grid to 0.005 for the nested grid. One might infer that under this design, the fitting of models to the experimental variogram should be more precise than was the case in the previous two experiments.

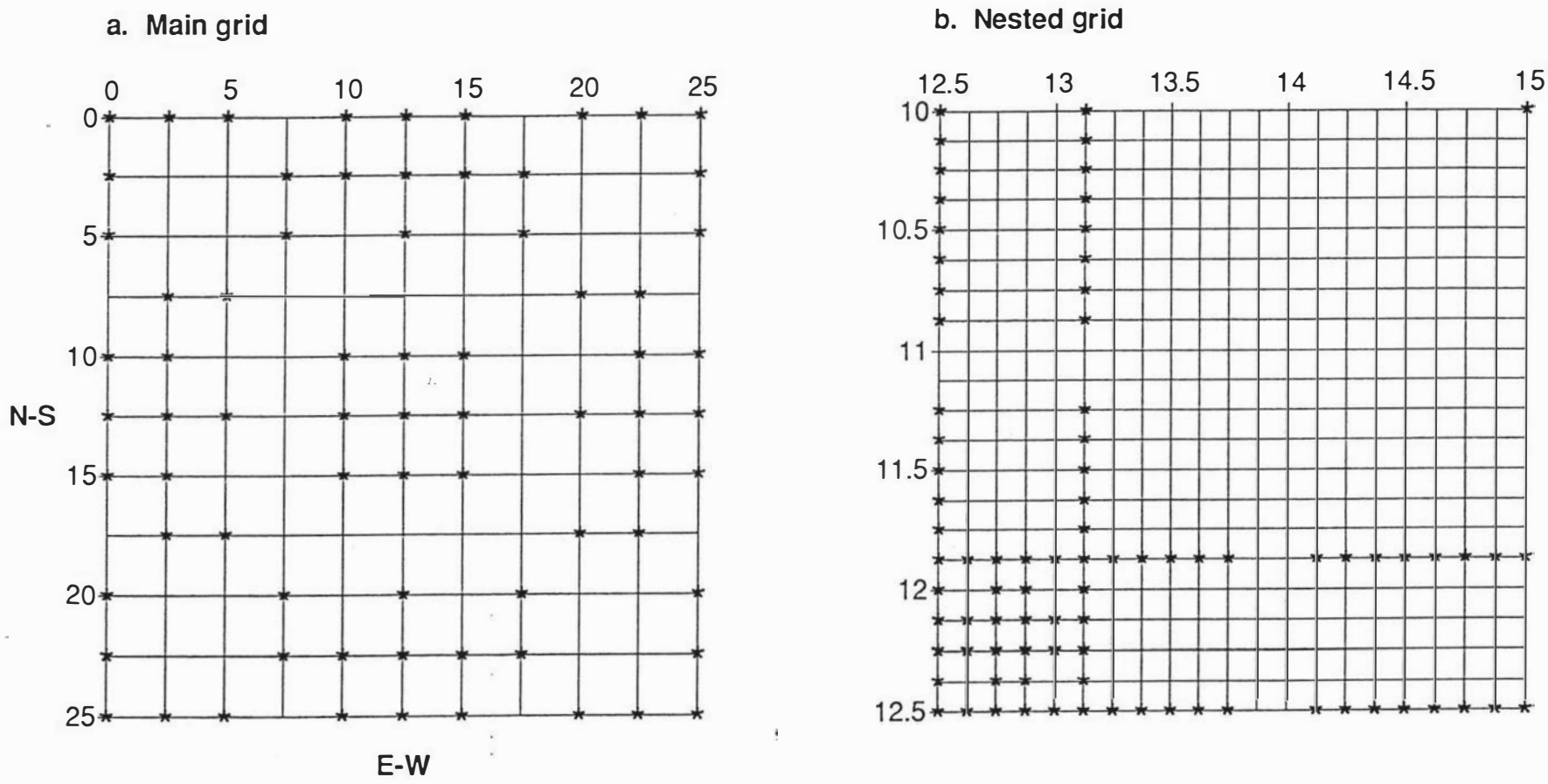


Figure 6.1 Sampling design for a nested spatial analysis over 625 m^2

i. Methods and Materials

Soil sampling

The grid (Figure 6.1) was marked out over 625 m^2 in field No. 6, sufficiently displaced from the original grid (Chapter 5) to ensure that the nodes of the new grid did not coincide with those of the old. On 5 October, 1987 (i.e. almost a year after the first spatial anaysis), soil samples were taken at the points shown in Figure 6.1 between the 3-12 cm depth range and were sieved and stored as before. The additional 3 cm core (9-12 cm) was taken to ensure that sufficient sieved soil was obtained to allow for analysis of exchangeable ammonium in addition to SNA, and initial NO_3^- as before. It was assumed that the sieving and mixing process would remove the effects of depth-dependent variability (see Chapter 5), and that the only effect of taking the additional core would be to lower the mean SNA and Ex-NH_4 values in relation to those measured for samples taken between 3 and 9 cm depth.

Analysis

SNA measurements were made in duplicate on all soil samples. Ex-NH_4 was also analysed following the method detailed in Chapter 4. The leachate from the SNA pre-leaching was retained for analysis of NO_3-N, and incubation pH was measured following the eight hour sampling. The gravimetric moisture content of the samples was determined by oven-drying a subsample overnight at 105 °C as before.

ii. Results

Soil moisture contents were calculated on the basis of the moisture content of sieved soil using equation (4.5), and the distributions of all the properties measured were investigated as before. Incubation pH and moisture content were again normally distributed (R^2 = 0.99 and 0.93 respectively, $p<0.1\%$; Figure 6.2d,e), whilst SNA and initial NO_3^-

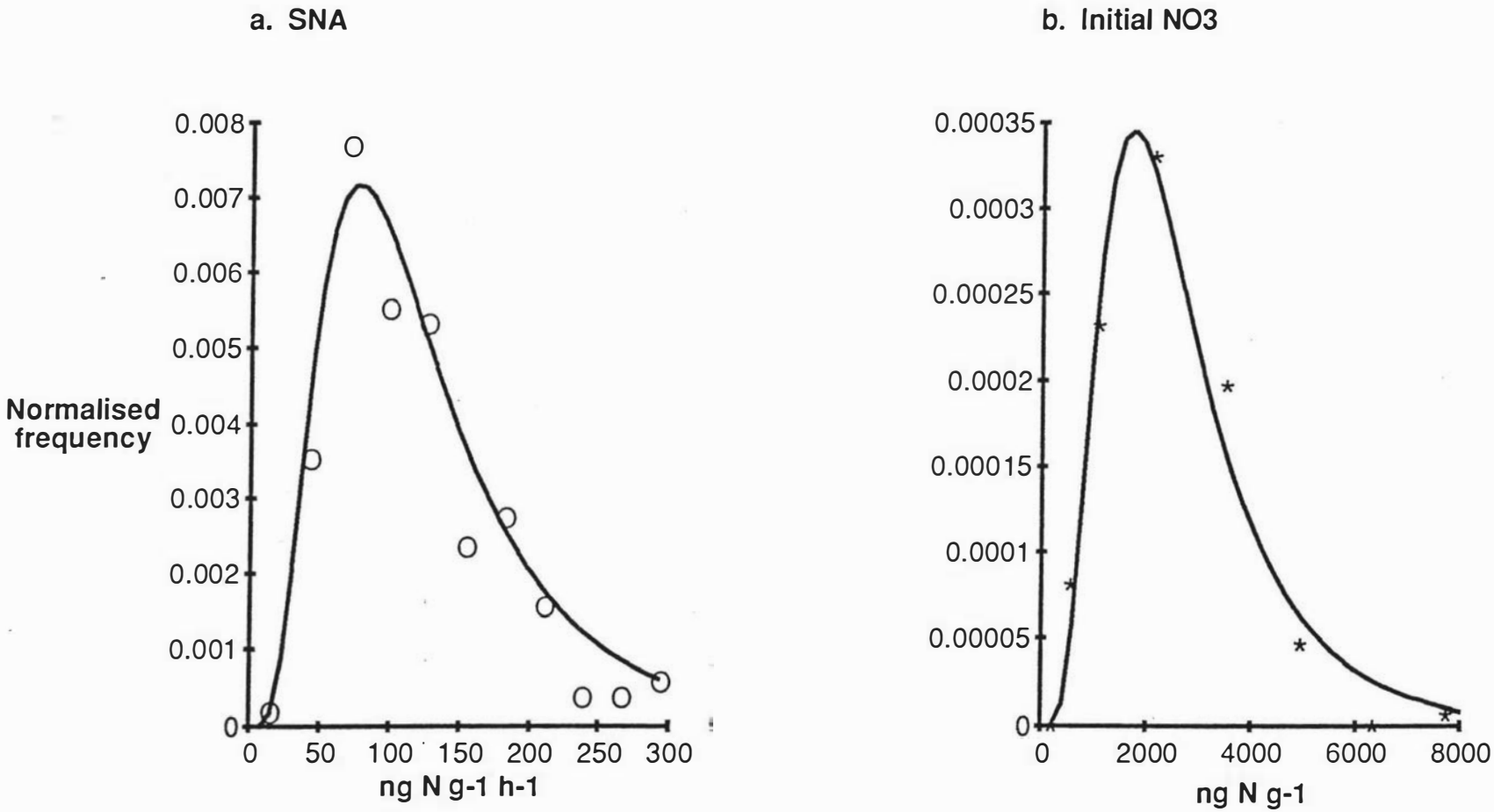


Figure 6.2 Distribution of (a) SNA, (b) NO_3^-, (c) Ex-NH_4^+, (d) incubation pH and (e) gravimetric moisture content sampled between 3-12 cm depth over 625 m^2 using the nested sampling design (Figure 6.1)

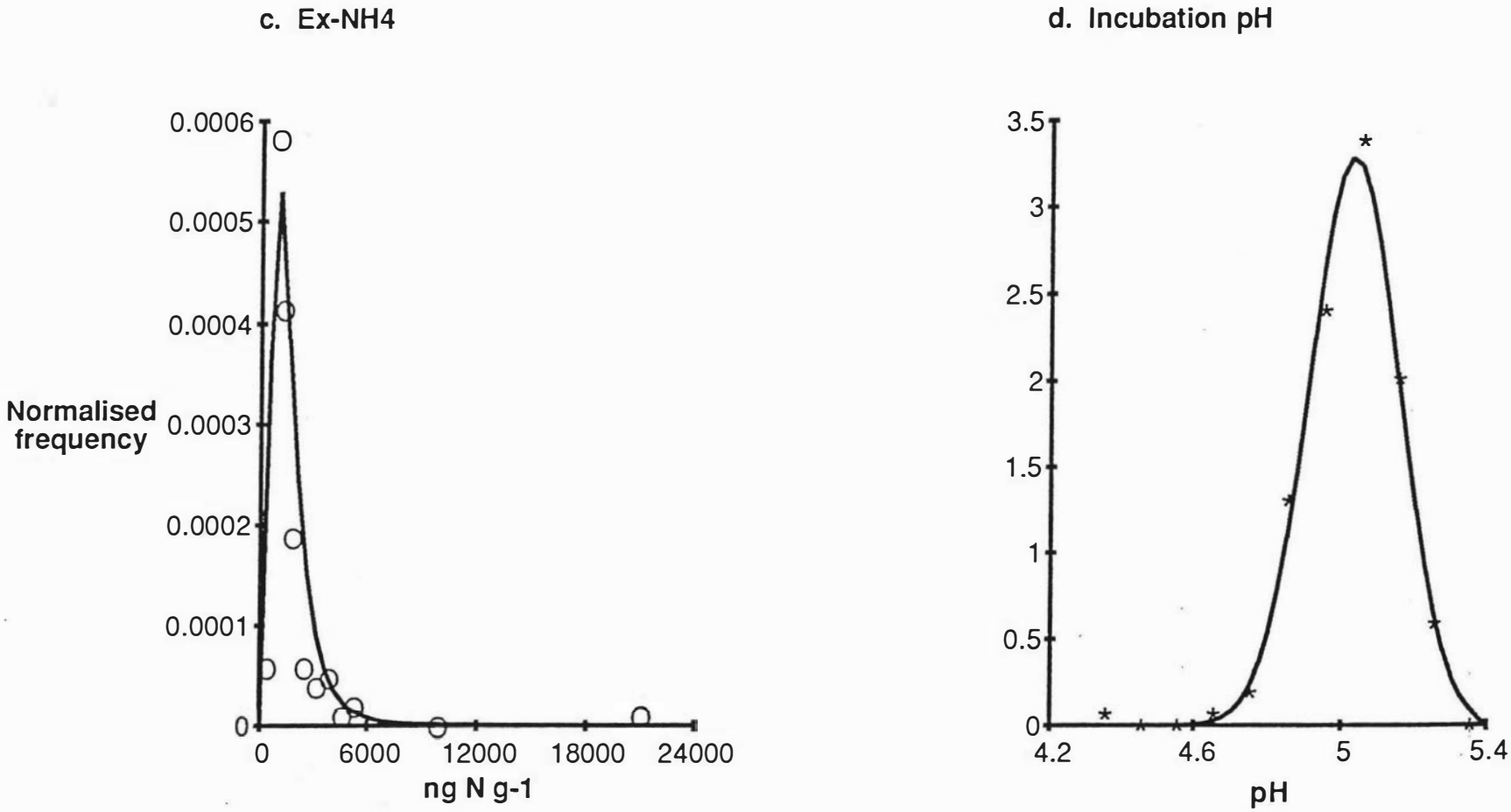


Figure 6.2 (Contd)

e. Moisture content

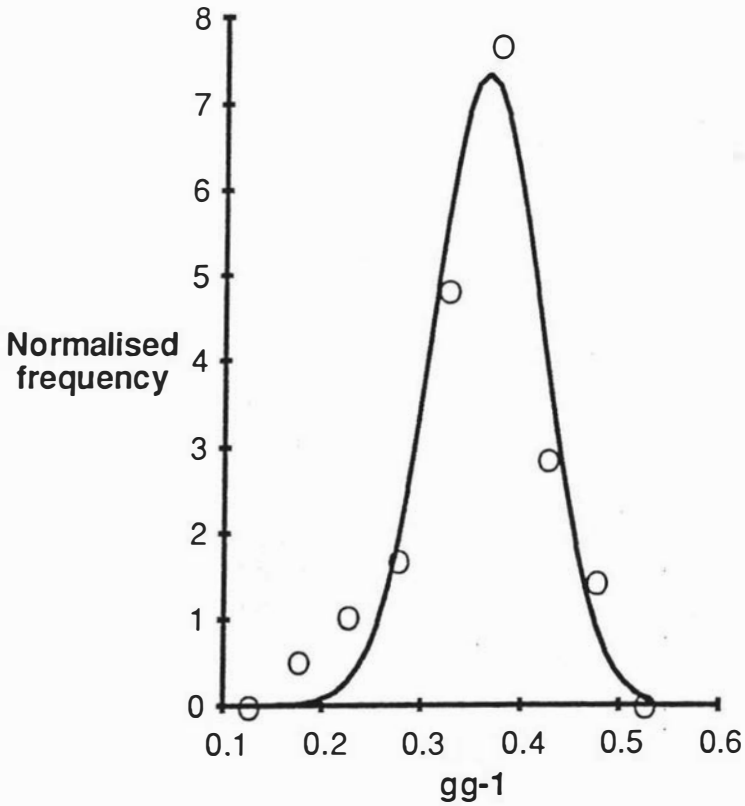


Figure 6.2 (Contd)

conformed to lognormal distributions as in the previous experiments (R^2 = 0.91 and 0.96, $p<0.1\%$; Figure 6.2a,b). Exchangeable ammonium, which showed very large variability was also lognormally distributed (R^2 = 0.84, $p<0.1\%$; Figure 6.2c). Accordingly, the units of SNA, initial NO_3^- and Ex-NH_4^+, μmol N g^{-1} were converted to ng N g^{-1} and the data were log transformed (ln) prior to further analysis.

Semivariances were calculated for both the north-south and east-west directions using GAMMAH (Appendix 1) and the experimental variograms were plotted (Figure 6.3). Weights for model fitting by weighted least squares were calculated using equation (5.7) and linear, spherical and exponential variograms were tested as before. Isotropy was assumed for all properties except moisture content which was clearly anisotropic (Figure 6.3e). The best-fit models, which were determined on the basis of their AIC values (equation 3.22), are shown in Figure 6.3.

The experimental variograms for SNA and incubation pH were best-fitted by exponential models (Figure 6.3a,d), whilst that for initial NO_3^- was best-fitted by the spherical model (Figure 6.3b). The experimental variograms for both Ex-NH_4^+ and moisture content were best-fitted by linear models, and in the case of moisture content, there was obvious anisotropy in the data, so separate models were fitted for the north-south and east-west directions (Figure 6.3e); the slope of the east-west model was greater (by a factor of 44) than that of the north-south model.

On the basis of the fitted models, spatial dependence was identified for initial NO_3^- within a range of 5.4 m, and taking the range of an exponential model to be equal to 3r (equation 3.21), spatial dependence of SNA and incubation pH was identified within ranges of 2.4 m and 6.1 m respectively. Values for the various parameters of the fitted models are given in Table 6.2.

iii. Discussion

In the experiments described in Chapter 5, spatial dependence was defined for incubation pH within 9.9 m, and for SNA within 0.6 m. However, the contrasting results from the 625 m^2 and 9 m^2 experiments, together with the large nugget variances shown in many of the variograms, suggested that the results were unreliable. In contrast, the experiment described

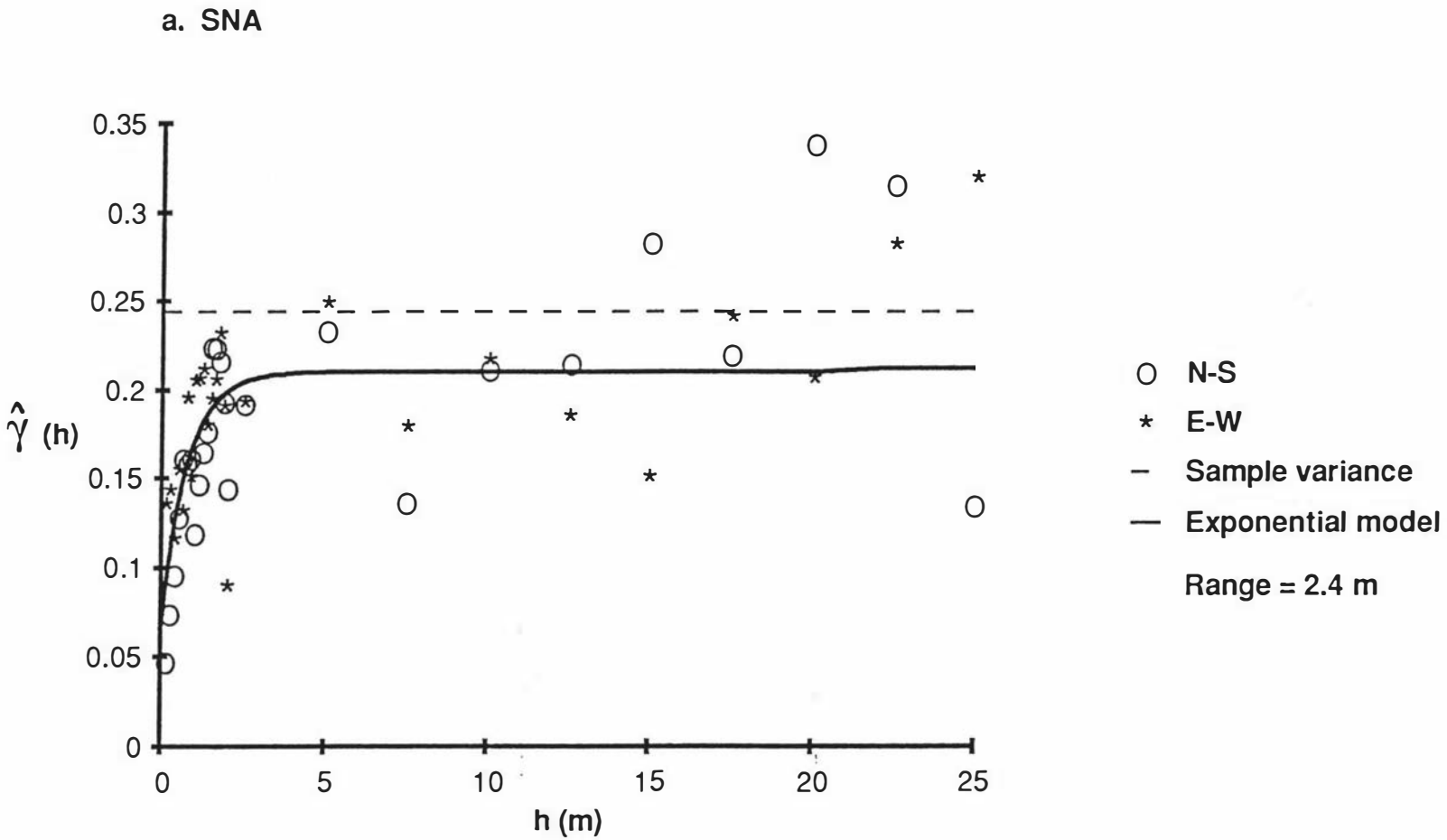


Figure 6.3 Experimental variograms of (a) SNA, (b) NO_3^-, (c) Ex-NH_4^+, (d) incubation pH and (e) moisture content sampled between 3-12 cm depth over 625 m^2 using the nested sampling design (Figure 6.1) with models fitted by weighted least squares optimization

b. Initial NO3

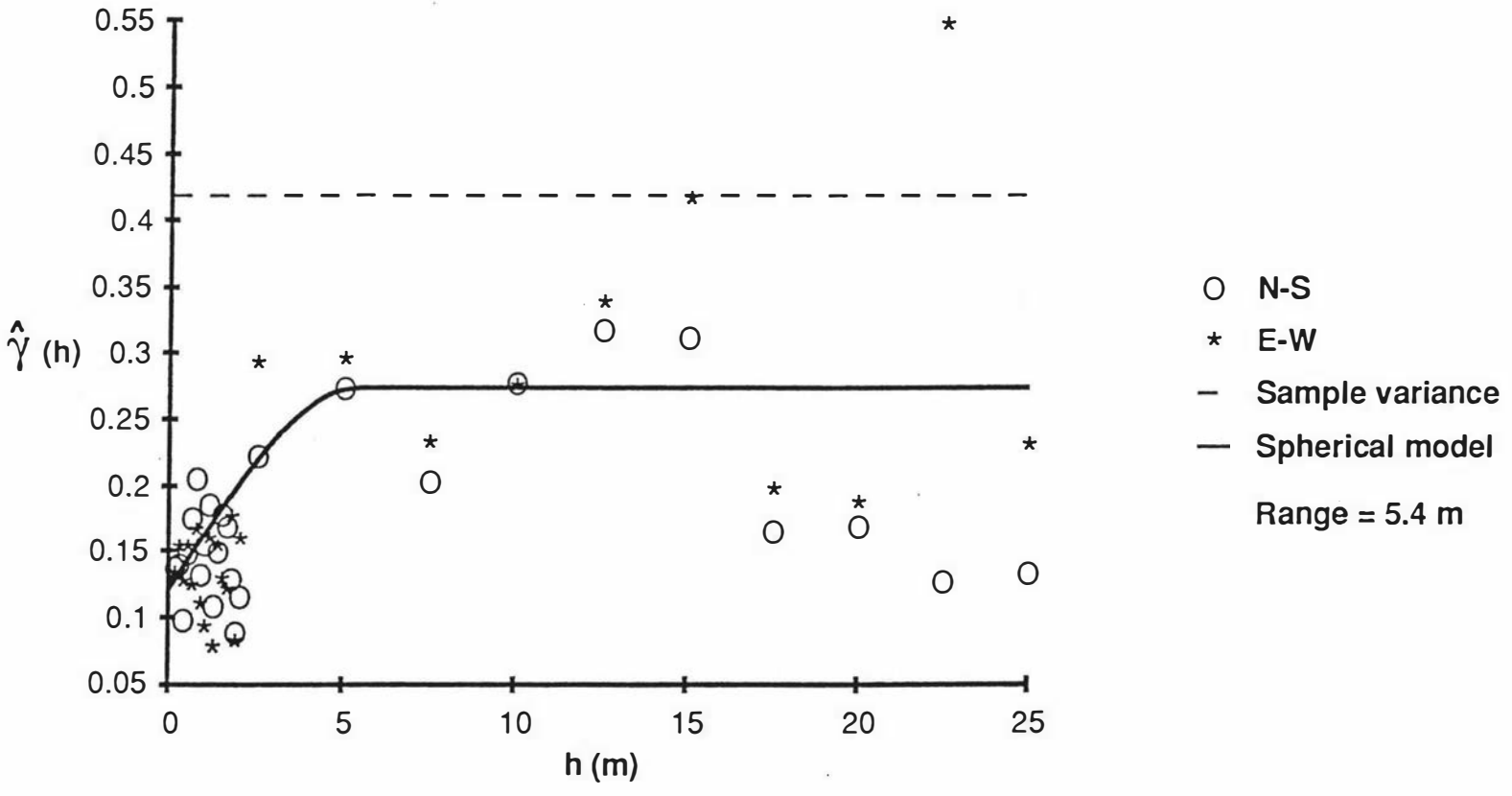


Figure 6.3 (Contd)

c. Ex-NH4

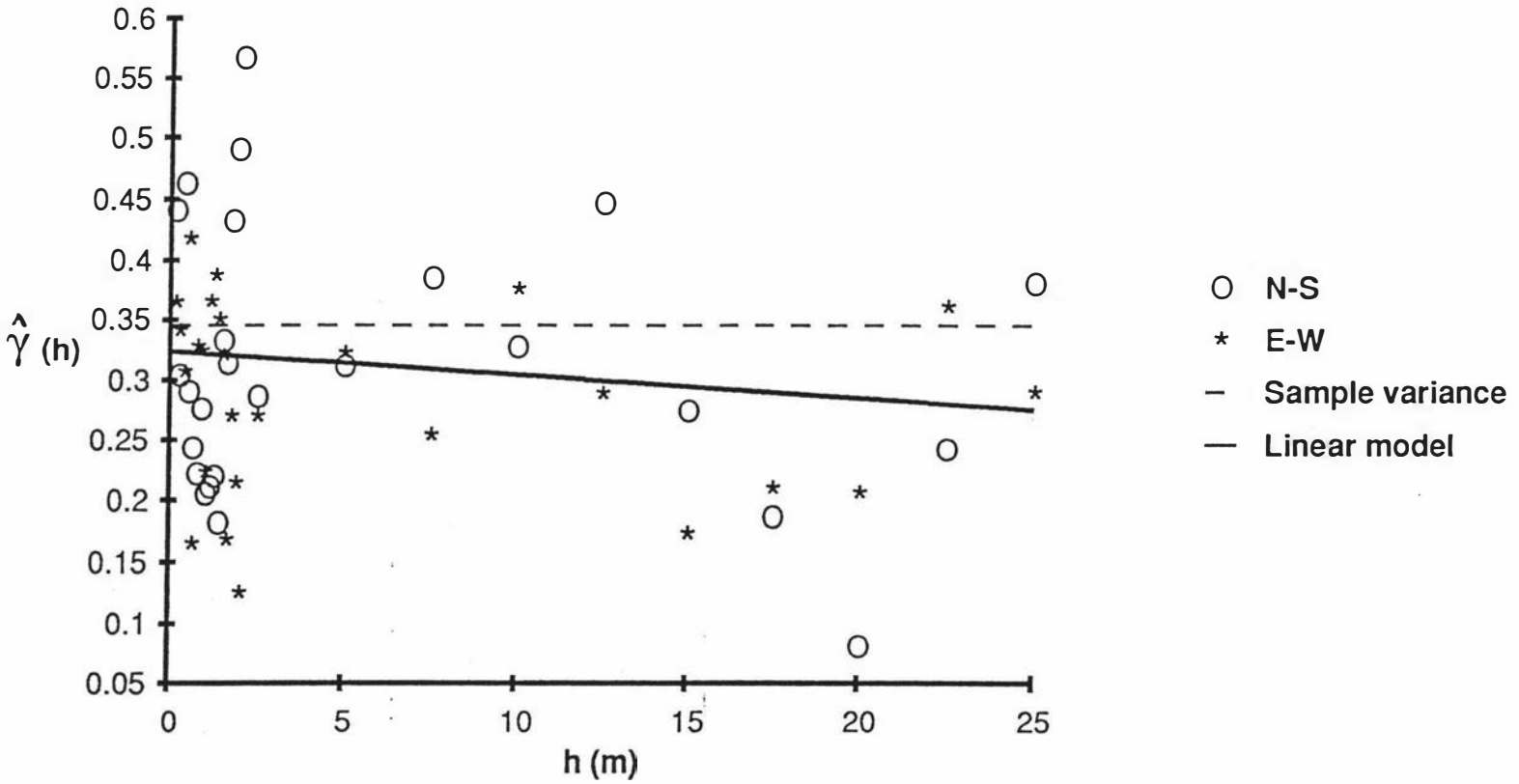


Figure 6.3 (Contd)

d. Incubation pH

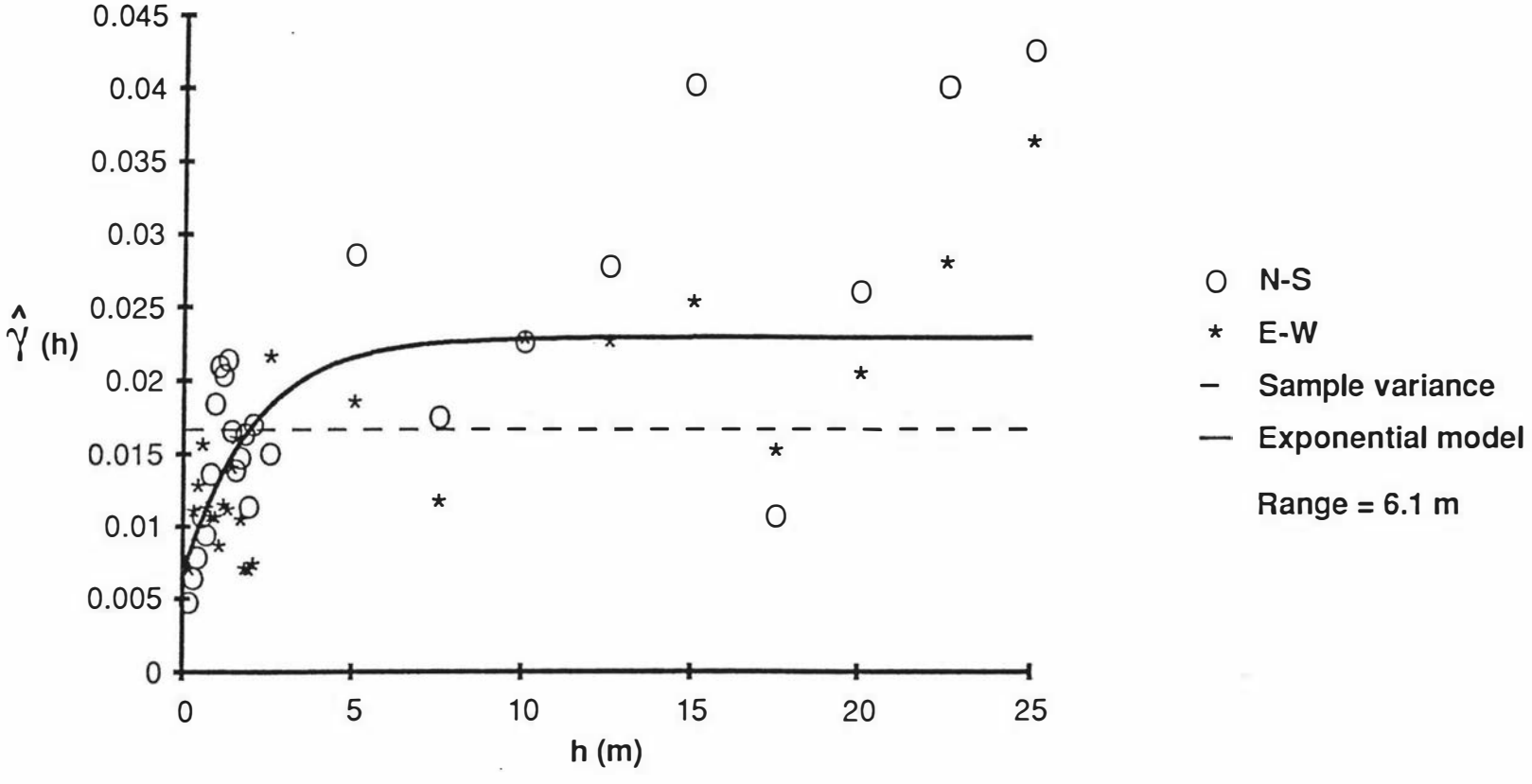


Figure 6.3 (Contd)

e. Moisture content

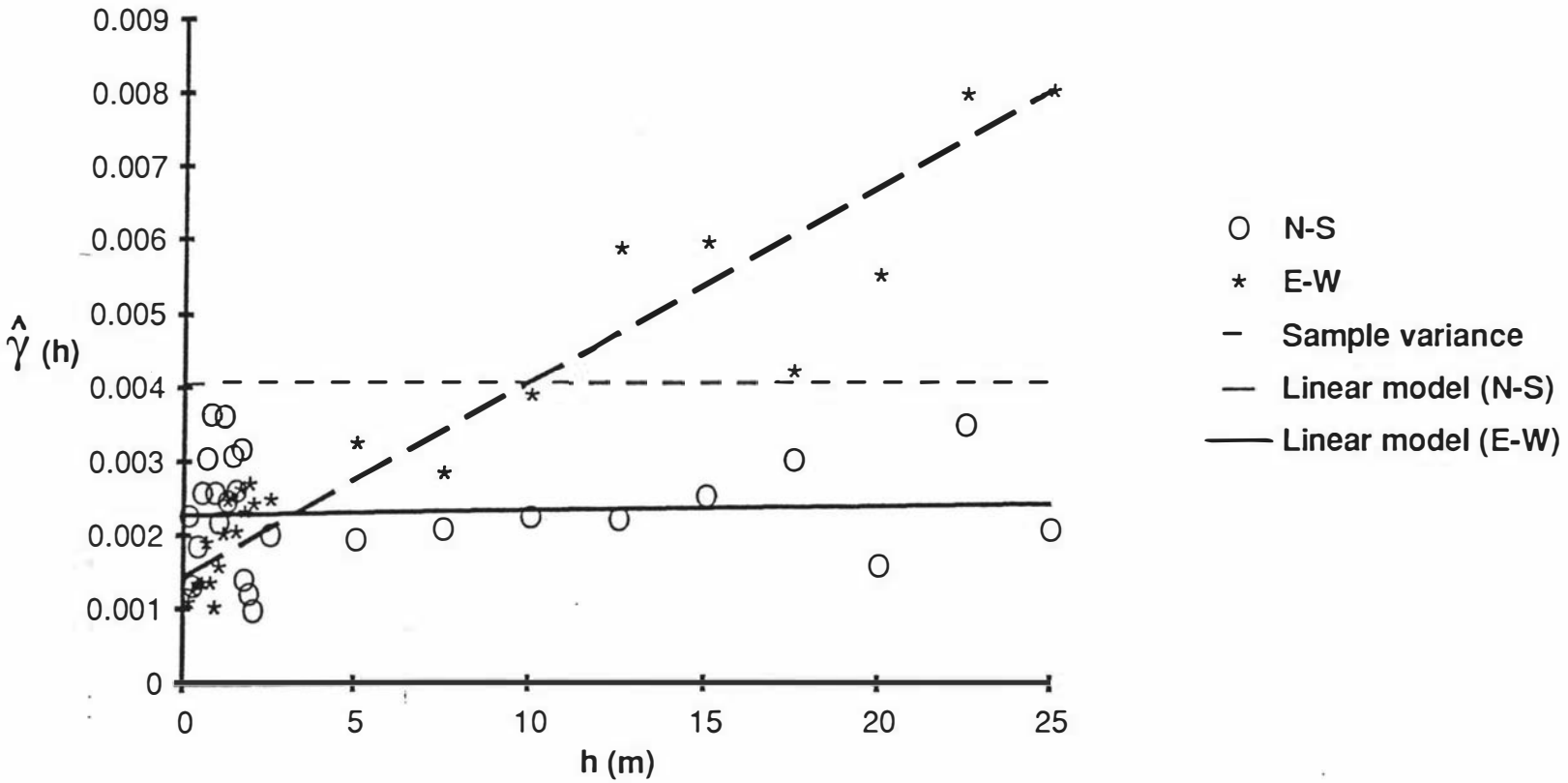


Figure 6.3 (Contd)

Table 6.2 Sample variance (s^2) and the parameters of models fitted by weighted least squares optimization to the experimental variograms of SNA, initial NO_3^-, Ex-NH_4^+, incubation pH and soil moisture content

Soil Property	Variogram Model	s^2	C_o	C	k	r	a
SNA	Exponential	0.24441	0.06819	0.14203		0.784	2.35
Initial NO_3^-	Spherical	0.41914	0.11919	0.16403			5.44
Ex-NH_4^+	Linear	0.34653	0.32393		-0.00194		
pH*	Exponential	0.01675	0.00668	0.01629		2.022	6.07
Moisture content	Linear N-S	0.00407	0.00164		0.000006		
	Linear E-W		0.00131		0.000264		

* pH of the incubation medium following the eight hour sampling

s^2 is the sample variance
C_o is the nugget variance
C is the spatially dependent variance
k is the slope of a linear variogram
r is the distance parameter of an exponential variogram
a is the range (metres)

above has produced variograms which appear more meaningful in the sense that they account for variability over both long and short lags. Thus, the decision to select a sampling design with a small ratio of smallest:largest lag seems to have been vindicated.

As was the case in the first experiment over 625 m^2, incubation pH was spatially dependent, but here the range occurred at 6.1 m; in the first experiment the range was 9.9 m. The difficulty of explaining changes in the sample variance and range with time was noted in the previous chapter. Considering just the experiments where samples were taken over an area of 625 m^2, the difference in the range could be due to sampling error, temporal variability, or differences in actual sampling technique between the two experiments - a result of sampling over 3-12 cm rather than 3-9 cm. Since the nested experiment was carried out almost exactly a year after the first experiment, temporal variability seems an unlikely cause of the difference between the range in the two experiments, especially if one assumes that the seasonal variation in factors which affect soil pH and by implication, incubation pH, follow the same seasonal trend from year to year. A more likely reason for the change in the range between experiments is that the nested sampling design has led to a more precise variogram, and that as a result, the range is better defined and can therefore be taken to occur at 6 m rather than 10 m. Nevertheless, the fact that the range changes raises important questions as to the degree of reliability that can be placed on the experimental variogram, and also its reproducibility. These problems, which do not seem to have been properly addressed in the literature, are considered in Chapter 10.

In addition to observing differences in the variograms and fitted models between the two experiments, it was also noted that in spite of the fact that the sample sets were drawn from the same area, the sample variances differed considerably between the two experiments. In the case of incubation pH, the sample variance in the second experiment was approximately half its value as determined for samples taken on the regular square grid. This may have been caused by an increase in precision resulting from the calculation of s^2 based on 154 rather 121 samples (Chapter 3). However, it seemed unlikely that this increase in n

would lead to such a large decrease in s^2 because as Figure 3.8 shows, once n becomes greater than 20, the decrease in s^2 with further increases in n is not significant for incubation pH. It therefore appeared that the decrease in the sample variance between the regular and nested experiments was a function of either the depth or the scale of sampling.

Russo and Jury (1987a) noted a tendency for the range to vary with the scale of sampling. In terms of the various parameters of the variogram, such a phenomenon could be due to a change in the value of the sill (i.e. a change in the spatially dependent variance and/or the nugget variance) as the scale of sampling changes, or alternatively could be due to a change in the intensity of the spatial dependence, which is defined by the slope of the tangent of the variogram model at h=0. The relationship between the sill and the sample variance is uncertain (Chapter 3) and is considered further in Chapter 10, especially with regard to the fact that in many of the variograms produced in this study, the position of the sample variance appears to be inconsistent with the values of $\hat{\gamma}(h)$. However, since the sill is effectively a measure of the maximum variation in the data (Trangmar *et al.*, 1985), it seems logical that if the sill is a function of the scale of sampling then so too must be the sample variance, because this is also a measure of the variation within the sampling area.

A fundamental tenet of geostatistics is that for spatially dependent properties, samples taken close together have similar values, whilst those taken at separations greater than the range of spatial dependence may show a greater degree of variation. In the first 625 m^2 experiment, the 121 samples were evenly spaced at separations increasing from 2.5 m to 25 m in multiples of 2.5 m. The mean value for incubation pH was 4.9 and the sample variance was 0.0334. Under the nested sampling design used one year later, samples were not evenly spaced; those in the main grid (Figure 6.1a) were separated by distances which were multiples of 2.5 m, whilst those in the nested grid (Figure 6.1b) had sample separations which were multiples of 12.5 cm. The latter were confined to an area measuring 2.5 m x 2.5 m. The mean value of incubation pH under this sampling design (n=154) was 5.0 and the sample variance was 0.0165. However, if the samples taken from the nested grid (Figure 6.1b) are

removed from the calculation, and just those samples separated by multiples of 2.5 m considered (n=73), the mean value remains equal to 5.0, but the sample variance rises to 0.022. The difference between this value and 0.0334 (the value of s^2 in the first experiment) suggests that some factor in addition to the change in the scale of sampling is responsible for the change in s^2 between the two experiments. However, given that there is no significant change in s^2 when n increases above a value of 20, the decrease in the value of s^2 when the nested data are included (n=154, s^2=0.0165) compared to the value of s^2 when they are excluded (n=73, s^2=0.022), indicates that the scale of sampling does indeed affect the sample variance of property values measured over the same given area. Thus, the sample variance must to some extent be a function of the range of spatial dependence and therefore the scale of sampling. This conclusion is further supported by the fact that the mean value of the nested data alone (n=85) was again 5.0, but the sample variance was only 0.012.

Similar changes to those observed for the sample variance of incubation pH over the three experiments were observed for the other properties. The reason that the above discussion was centred on incubation pH rather than any of the other measured properties, was that the literature suggests that there is a strong seasonal dependence (e.g Sarathchandra & Perrott, 1984; Higashida & Takao, 1985; Sarathchandra *et al.*, 1988) of the values of SNA, soil NO_3^- and by implication, EX-NH_4^+. It would therefore seem unwise to make judgements as to the efficacy of sampling design on the basis of highly variable biological properties. Incubation pH was assumed to be closely related to the actual soil pH, and under the conditions in field No. 6 (i.e. no fertilizer or other management other than periodic grazing - see Chapter 4), pH was not expected to fluctuate widely. Seasonal variation is discussed in the following chapter.

Given that the nested sampling design was a good one, it was considered of interest to look at the spatial relationships (if any) between the measured properties. In Chapter 5, it was shown that SNA was not closely correlated over depth with either incubation pH or initial NO_3^-. However, one might expect them to be correlated over space *at the same depth*. In the case of pH and SNA, this is because there is plenty of evidence in

the literature (Chapter 2) to suggest that pH plays an important role in regulating the degree of nitrifier activity. In the case of SNA and initial NO_3^-, one might expect the distribution of NO_3^- to follow that of SNA since the former may be regarded as the result of the latter. However, since NO_3^- is readily leached, some displacement with depth is expected, and spatial variability in the soil hydraulic properties (Russo & Bresler, 1981; Sisson & Wierenga, 1981) may lead to a lack of spatial correlation at any given depth. Nevertheless, it was felt important in this study to establish the form of the relationship over space between SNA and initial NO_3^-.

SNA was spatially dependent within 2.4 m, whilst for incubation pH, dependence occurred within 6.1 m; initial NO_3^- showed dependence within 5.4 m. Given that the spatial dependence of these three properties occurred within distances of similar orders of magnitude, it seemed feasible to investigate their spatial correlation, or *the spatial dependence of one property on another* (Webster, 1985; Trangmar *et al.*, 1985) by plotting their *crossvariogram* (Webster, 1985). The *cross semivariance* $\hat{\gamma}_{zy}(h)$ is estimated in a similar way to the semivariance, except that instead of taking the sum of the squared differences between values of Z at x_i and $x_i + h$, the sum of the product of the differences between $Z(x_i)$ and $Z(x_i + h)$ and $Y(x_i)$ and $Y(x_i + h)$ is calculated, where Y represents some soil property other than Z. Thus, (Webster, 1985):

$$\hat{\gamma}_{zy}(h) = \frac{1}{2m(h)} \sum [Z(x_i) - Z(x_i + h)][Y(x_i) - Y(x_i + h)] \qquad (6.1)$$

where m(h) is the number of pairs of observations of either Z or Y at lag h. An important feature of crossvariograms is that values of $\hat{\gamma}_{zy}(h)$ may be negative, and when this is so, they reflect a negative correlation between Z and Y (Davidoff & Selim, 1988). One would therefore presume that when values of $\hat{\gamma}_{zy}(h)$ are positive, Z and Y *are* correlated with each other.

Values of $\hat{\gamma}_{zx}(h)$ were calculated for SNA and incubation pH, and for SNA and initial NO_3^- using the FORTRAN program COVGM (Appendix 1) which is an altered version of GAMMAH. The crossvariograms were fitted with models by weighted least squares and the best fit determined on the basis of the AIC value. Both crossvariograms were best fitted by linear models, and are shown in Figure 6.4.

The interpretation of crossvariograms has not received much attention in the literature. If it assumed that crossvariograms should be interpreted in the same way as semivariograms (Webster, 1985), then the spatial dependence of SNA on pH and soil NO_3^- can be described as pure nugget. However, it is not clear what this means. Russo (1984b) used crossvariograms to investigate the spatial dependence of soil water pressure, salinity, crop yield and two soil hydraulic properties on each other in a trickle irrigation system. As was the case here, he found that the crossvariograms were pure nugget despite the fact that the variograms for individual properties had clearly defined ranges. Davidoff and Selim (1988) presented crossvariograms for soil moisture and temperature and concluded that the range of spatial correlation was defined by the point at which values of $\hat{\gamma}_{zx}(h)$ changed from being positive to negative, or *vice versa*, with increasing h. In both Figure 6.4a and Figure 6.4b, the values of $\hat{\gamma}_{zx}(h)$ are predominantly positive which suggests that SNA was generally correlated over space with both soil NO_3^- and incubation pH irrespective of the lag; the fact that the fitted models in both crossvariograms were horizontal linear, and that there were both positive and negative values at similar values of h, may simply indicate that the correlations are not particularly strong. Alternatively, it might be concluded that since the fluctuation in $\hat{\gamma}_{zx}(h)$ increases considerably as h increases beyond a lag of approximately 5 m, the correlation between the properties is stronger at lags of less than 5 m than it is for lags greater than 5 m. This conclusion is not inconsistent with the results of the variogram analysis for individual properties which indicated a range for NO_3^- of approximately 5 m and for incubation pH of approximately 6 m. Furthermore, this conclusion is supported by the fact that the differences between values of $\hat{\gamma}_{zx}(h)$ in the north-south and east-west directions generally increase as h increases beyond a value of

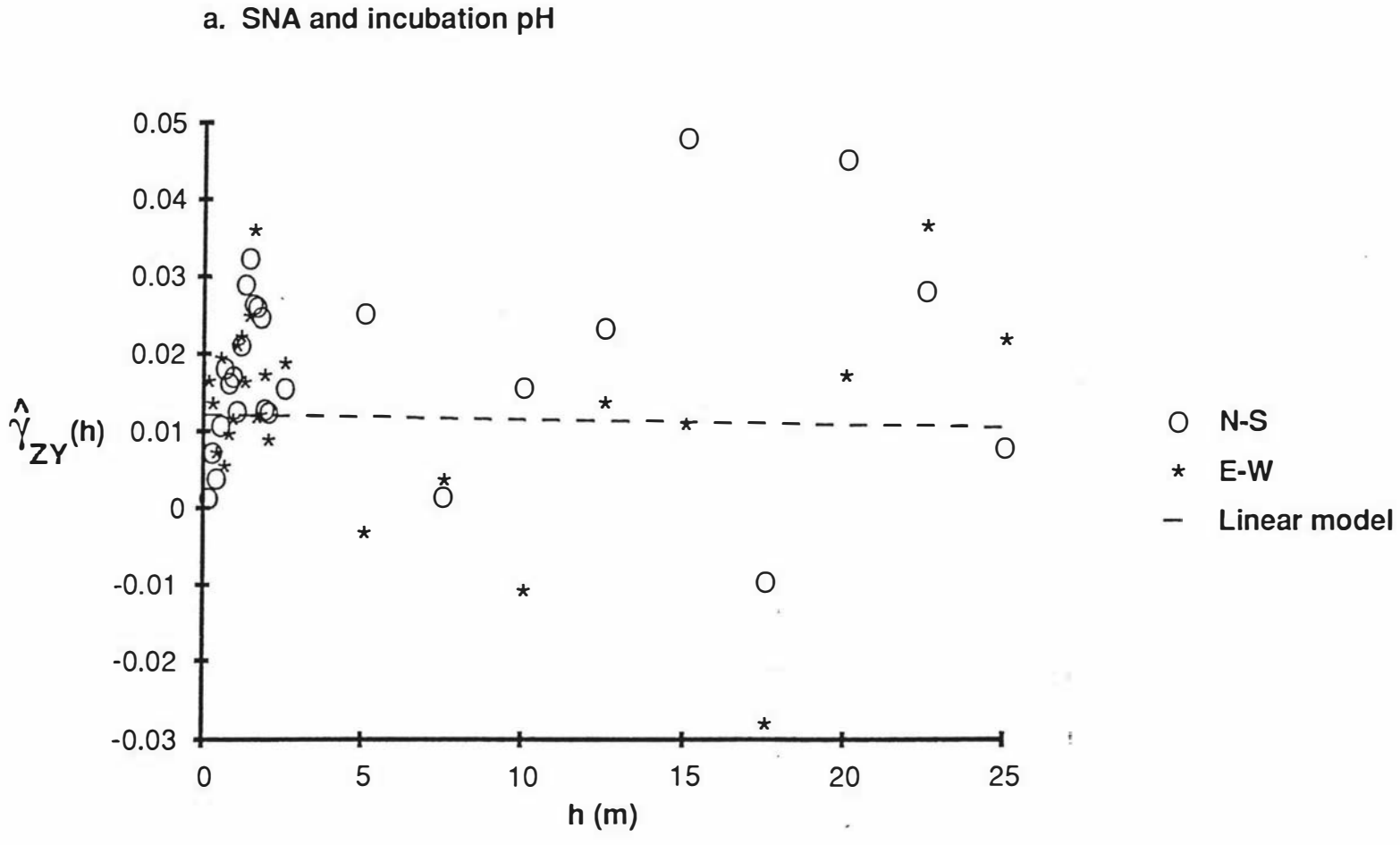


Figure 6.4 Crossvariograms of (a) SNA and incubation pH and (b) SNA and NO_3^- sampled between 3-12 cm depth over 625 m^2 using the nested sampling design (Figure 6.1) with linear models fitted by weighted least squares optimization

b. SNA and initial NO3

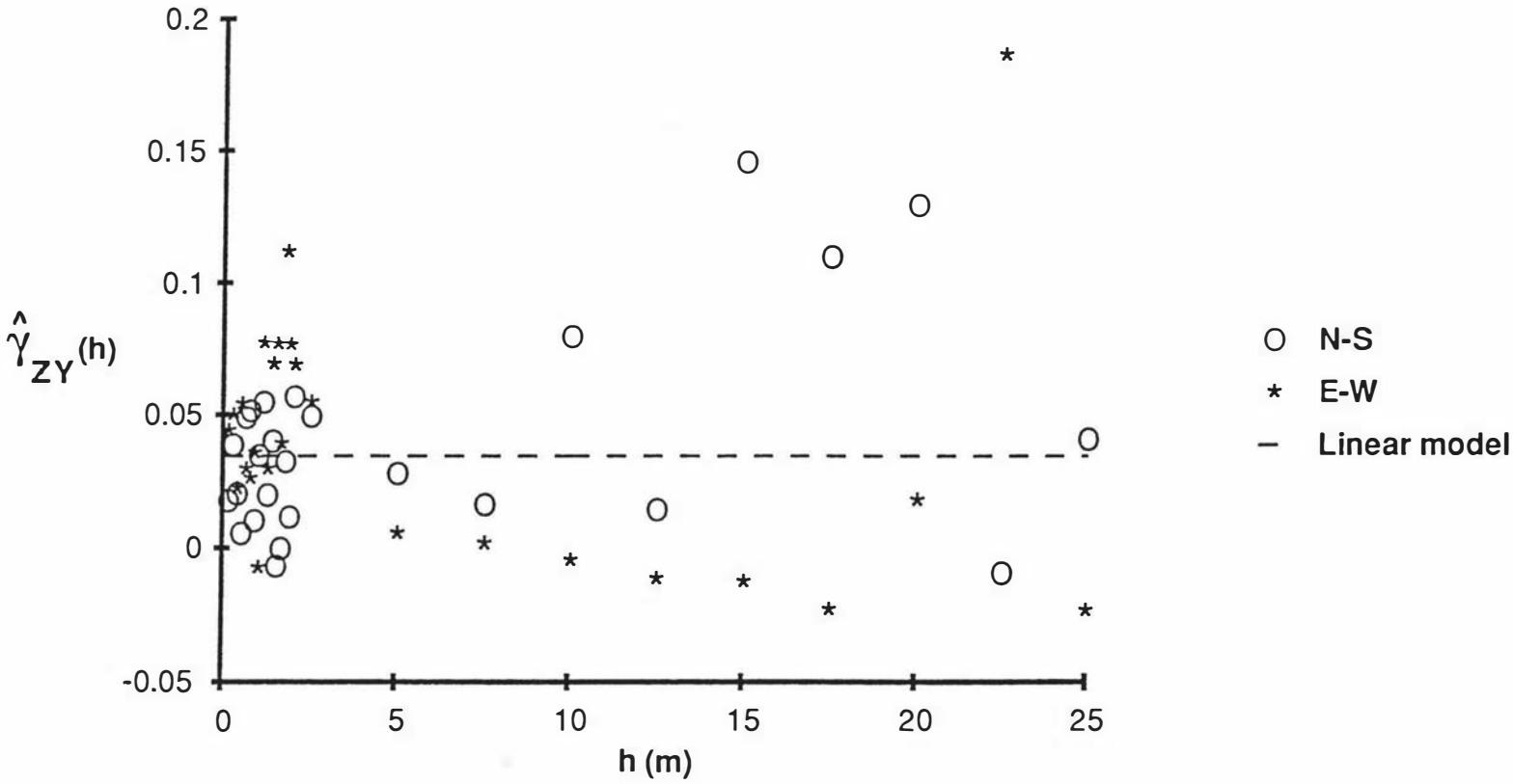


Figure 6.4 (Contd)

approximately 5 m. It is also consistent with the observation that the sample variance may be a function of the scale of sampling (see above). Over larger areas, that is, at large sample separations, the variation in individual properties tends to be greater than at small sample separations. One would therefore expect any correlation between two properties to decrease as the sample separation increases.

The value (if any) of crossvariogram analysis over and above straightforward tests of correlation using classical methods is discussed in Chapter 10. However, on the assumption that crossvariograms can give potentially useful information, those in Figure 6.4 raise an important question with wide implications to the whole of this project. If it was correct to conclude that SNA and NO_3^- are not strongly correlated over space, one wonders whether the SNA has provided a useful means of investigating the variability of soil mineral N. This question is all the more important when it is remembered that the aim of this work was to provide information which would enable more precise and efficient estimation of the mean soil NO_3^- concentration for use as an input parameter to a nitrate leaching model. Since it is shown in the following chapter that SNA is highly pH dependent, the lack of a strong correlation over space between SNA and pH as indicated by Figure 6.4a would seem a curious result, and the lack of a strong correlation between SNA and NO_3^- as indicated by Figure 6.4b may similarly be open to question. Figure 6.4 may therefore reflect serious shortcomings in the geostatistical analysis used here. Burrough (1983) pointed out that even if spatial variability can be defined in terms of a model such as the variogram, it does not mean that it can as a consequence be defined in physical terms. Geostatistics may therefore not necessarily offer any improvement over other techniques in the interpretation of such variability. This question is discussed in Chapter 10.

The literature contains no report of the spatial analysis of nitrifier activity. However, a few workers have investigated the spatial variability of soil nitrate concentration. White *et al.* (1987) noted spatial dependence of soil nitrate concentration within 0.4 m. However, in view of the apparent dependence of the results of the experiments

described here and in Chapter 5 on the sampling design used, the reliability of this result may be in doubt since only 50 sampling sites were used. Dahiya *et al.* (1985) found NO_3^- variability to be pure nugget, although this was not a surprising result in view of the fact that their smallest lag was 30 m. Tabor *et al.* (1985) drew the curious conclusion that soil NO_3^- concentration was spatially dependent at sample separations *greater* than 150 m. Their interpretation of the linear model fitted to their variogram is open to serious question however, and White *et al.* (1987) further questioned their results on the grounds that drift was present in their data. In the light of the discussion in Chapter 5 relating to the interpretation of linear variogram models, the conclusions of Tabor *et al.* (1985) do indeed seem open to question.

iv. Conclusions and recommendations for future sampling strategy

Despite the apparent shortcomings of the geostatistical analysis, especially the crossvariograms, in view of the general conclusion that the sampling design used in this experiment was a good one, it will be assumed here that the degree of importance that may be attached to the fitted variogram models was sufficient to make definite conclusions. It is therefore concluded that *under the management conditions of field No. 6*, nitrifier activity in the Tokomaru silt loam as measured by the SNA is spatially dependent within a range of 2-3 m; soil nitrate concentration is spatially dependent within a range of 5-6 m; and pH is spatially dependent within approximately 6 m. There is no spatial dependence of exchangeable ammonium, and the distribution of soil moisture content shows spatial variation, but the extent of this can not be defined within 25 m, probably due to the presence of drift in the data. However, because the dependence of nitrification rates on factors such as pH, moisture content and temperature appears to be soil-specific (Chapter 2), it seems logical that the measured *spatial* dependence is similarly soil-specific, which is why the conditions of the sampling area, or *support* (Webster, 1985), were stressed above. Thus, one might infer that until similar spatial analyses are made over a range of soil types (and also over a range of management conditions) so that general patterns and trends (if any) in the spatial dependence of the parameters of mineral N can be

identified, the results of this spatial analysis can only be considered of use when sampling in this particular soil. For estimation of the initial soil nitrate concentration as an input parameter to a nitrate leaching model, the knowledge that samples should be taken at separations greater than 2-3 m (or 5-6 m depending on whether SNA or soil NO_3^- is considered the more important property), is only of use if the leaching model is to be developed for the Tokomaru silt loam and tested in the same region as that in which this spatial analysis was carried out. (The leaching model may also, of necessity, be soil-specific). Alternatively, one could take the view that the difference in the range between SNA, initial NO_3^- and incubation pH is not important in a practical context. Since there should be no increase in the sill variance of SNA between 2.4 m (the range for SNA) and 5.4 m (the range for soil NO_3^-), taking samples at a minimum separation distance of 5 m would be satisfactory in so far as minimising the sampling error and obtaining samples which are representative of the whole field is concerned. In addition, the fact that the range of spatial dependence measured here is of a similar order of magnitude to that found by White *et al.* (1987) (even though their variogram may not be as reliable as that produced here), suggests that 5 m should be a satisfactory sample separation when sampling for mineral N in any soil.

Knittel and Fischbeck (1979) calculated that in order to obtain a representative sample with respect to soil NO_3^- in an arable brown earth, the soil must be sampled at nine positions, although they commented that in practice, a larger number of samples would be necessary. They did not qualify this comment, but presumably the greater number of samples was needed because they failed to take account of the position of their sampling points. M^{c}Bratney and Webster (1983) presented a means of estimating how many samples should be taken for estimation of areal mean values, but as this involves kriging, and therefore knowledge of the variogram prior to sampling, it may not be useful for planning a general sampling strategy for the future sampling of soil mineral N, unless it is assumed that the variograms shown in Figure 6.3 closely approximate those for the same properties measured in different soils. Given that sampling

sites should be at least 5 m apart, it is suggested that it is quite acceptable to estimate the number of samples, N, that are required for good estimation of the areal mean by more classical means using the equation (e.g. Cline, 1944):

$$N = \frac{t^2 s^2}{L^2} \qquad (6.2)$$

where s^2 is the sample variance of a sample data set already taken, t is the value from Student's t-distribution depending upon the degrees of freedom for s^2 and the desired level of significance, and L represents the tolerable deviation of $\hat{\mu}$ from μ.

M^{c}Bratney and Webster (1983) noted that the precision attained by random sampling is almost always bettered by systematic sampling. Since the variation in SNA and soil NO_3^- concentration is made up of both short and long range components, it is concluded that the most efficient sampling strategy for estimating mean soil nitrate concentrations is to take small clusters of samples with a lag of at least 5 m between the clusters, which should be systematically positioned, and calculate an areal mean from these. In this way, both short and longer range variation is accounted for. Using the SNA data from the first 625 m^2 experiment (3-9 cm depth), and equation (5.2) to estimate $\hat{\mu}$, values of N were calculated with L fixed at ± 5 % using equation (6.2). At significance levels of 99, 95, 90, 80 and 50 %, N equals 31, 22, 19, 15, and 11 respectively. If L is fixed at ± 10 %, the value of N at the 95 % level of significance is only 6 samples. Performing a similar calculation for the initial NO_3^- data with L again fixed at ± 5 %, at significance levels of 99, 95, 90, 80 and 50 %, the number of samples required is 27, 20, 16, 13 and 9.

Whether or not N should refer to the total number of samples or the number of samples in each cluster depends on the preferred method of sample analysis. Bulking and mixing the soil samples in each cluster will reduce the amount of laboratory analysis. However, it will also mean that the soil from each cluster will make up one bulk sample and therefore N should be taken to refer to the number of clusters required. Since SNA and NO_3^- values measured on samples taken close together are known to be

spatially dependent, the variance of samples taken in a cluster should be smaller than the overall sample variance. Therefore, taking clusters separated by at least 5 m with 5 samples in each cluster should give a good estimate of the areal mean value of either SNA or soil NO_3^-. The number of clusters to be taken will depend on the desired level of significance and the cost of sampling. Since the estimate of NO_3^- concentration for use as an input parameter to a leaching model needs to be accurate, it is suggested that 20 clusters will be needed. Obviously this represents a large sampling effort.

SECTION III. FACTORS AFFECTING NITRIFIER ACTIVITY

CHAPTER 7

THE EFFECT OF pH, MOISTURE AND TEMPERATURE ON NITRIFIER ACTIVITY

The crossvariogram analysis described in Chapter 6, suggested that nitrifier activity and soil pH were not strongly correlated over space. However, in view of the work reported in the literature on the effects of pH on nitrification rates (Chapter 2), especially that of Darrah *et al.* (1986b), it seemed inconceivable that variability in SNA was not at least in part due to variability in soil pH. Furthermore, the literature on the effects of temperature and moisture on nitrification rates (Chapter 2) together with the observation that mean SNA values and sample variances changed between the three spatial analyses (Chapters 5 & 6), suggested that there was a seasonality in nitrifier activity which was most likely a function of climatic factors such as soil temperature and moisture content. These effects and the possible pH dependence of nitrifier activity in the Tokomaru silt loam were investigated in the work reported below.

i. Methods and Materials

Site selection and soil sampling

Soil samples were collected from plots (25 m^2) in field No. 2. This field had previously been used for a lime trial which was laid down in 1982. Sampling was confined to plots representing two of the treatments in the lime trial, specifically from a control plot (no lime) and from a plot which had received 5000 kg $CaCO_3$ ha^{-1}. Both plots had also received 40 kg P ha^{-1} as superphosphate in 1982. These plots were designated T and TL respectively. On each sampling occasion, bulk soil samples of approximately 5 kg were taken from the 3-9 cm depth range. Soil was repeatedly sampled from the same plots to eliminate the effect of spatial

variability on the nitrification rate (Chapters 5 & 6). The bulk soil sample was subsampled for measurement of pH and moisture content (Chapter 4), and was sieved and stored as before. In the first year, soil was sampled on 3 June, 25 August, 4 October and 28 November, 1986, and on 2 February and 12 April, 1987. As in the spatial variability experiments, there was no grazing for at least 3 weeks prior to sampling, in order to minimize any effects of urine patches on nitrifier activity (Ryden *et al.*, 1984; Ball & Ryden, 1984; White, 1984).

On 14 April, 1987, the plots of the original trial were divided in half and one half of each (T and TL) top-dressed with $CaCO_3$ at the rate of 2500 kg ha^{-1}. The half plots with extra lime were designated **TX** and **TLX**. These plots were subsequently sampled as described above on 19 May, 20 July, 14 September and 27 November, 1987, and on 3 February and 30 March, 1988.

Analyses

The pH change during the SNA incubation was assumed to be negligible considering that the amount of NO_3^- produced and therefore the amount of H^+ released was so small. Since the intention in this experiment was to modify the incubation pH by adding small amounts of acid or alkali, a preliminary experiment was carried out to determine the length of time required for equilibration of the medium following the addition of acid or alkali (Darrah *et al.*, 1986b). This was necessary in order that the pH as measured at the end of the incubation could be inferred to be a fair measure of the pH throughout the incubation.

100 g sieved soil was leached with 0.5 dm^3 0.005 M KCl following which excess moisture was removed by suction filtration for 90 minutes. Approximately 5 g soil (oven-dry equivalent) was placed into each of 36 incubation tubes containing the standard incubation medium (Chapter 4) but with no $(NH_4)_2SO_4$ added. The pH of the suspension was modified by adding (in duplicate) the following amendments; (a) 1.0 cm^3 0.1 M HCl; (b) 0.5 cm^3 0.1 M HCl; (c) 3.0 cm^3 0.01 M HCl; (d) no amendment; (e) 2.0 cm^3 0.01 M KOH; (f) 4.0 cm^3 0.01 M KOH; (g) 0.5 cm^3 0.1 M KOH; (h) 0.75

cm^3 0.1 M KOH; and (i) 1.0 cm^3 0.1 M KOH. The tubes were shaken, and the pH of the suspensions measured immediately. The pH was also measured after 50 minutes, 3 h 20 min, 5 h 5 min and 20 h 20 min. The change in pH is shown as a function of time for soils T and TL in Figure 7.1, from which it was concluded that following the addition of small volumes acid or alkali, the pH of the suspensions attained an approximately steady value within about 5 hours.

SNA measurements were made on the bulk soil samples following the method described in Chapter 4 with the modifications outlined below. Approximately 200 g of sieved soil was placed in a Buchner funnel fitted with a Whatman No. 1 filter paper and leached overnight with 1 dm^3 0.005 M KCl as before. At the end of leaching, excess moisture was removed from the soil by suction filtration for 90 minutes, after which 30 replicate 5 g samples (oven-dry equivalent) were placed into incubation tubes which were prepared in the manner described in Chapter 4. The pH of the soil suspensions was adjusted in triplicate by adding small volumes of 0.1 M HCl (0.2-0.6 cm^3) or KOH (0.5-2.5 cm^3), to give 10 pH values within the range 4.5-7.5. (The range of pH values for the unlimed and limed soil was 5.1 to 6.4). The tubes were shaken and then left to stand for 5 hours, after which 10 cm^3 0.01 M $(NH_4)_2SO_4$ was added and the incubation of the suspensions commenced. The incubation pH was measured following the 8 hour sampling.

Soil temperatures were not measured at the time of sampling, but monthly data for the soil temperature at 10 and 30 cm depth were obtained from the nearby D.S.I.R Grasslands weather station (New Zealand Meteorological Service, May 1986-April 1988). Temperature at 30 cm depth was used in preference to 10 cm depth, despite soil samples being taken at 3-9 cm, because the soil temperature data for 10 cm depth are strongly influenced by diurnal temperature fluctuations; the measurements were made at 9.00 am and therefore are likely to be quite different from soil temperatures during the middle and later part of the day. This component of error is not present in the 30 cm data because diurnal variation in soil temperature is negligible at this depth (D.R. Scotter, Dept. Soil Science, Massey University. - personal communication). The data were fitted with sine curves for the two years of the experiment (R^2 = 0.99; Figure 7.2), and the soil temperatures on the actual dates of sampling were interpolated using the fitted equations.

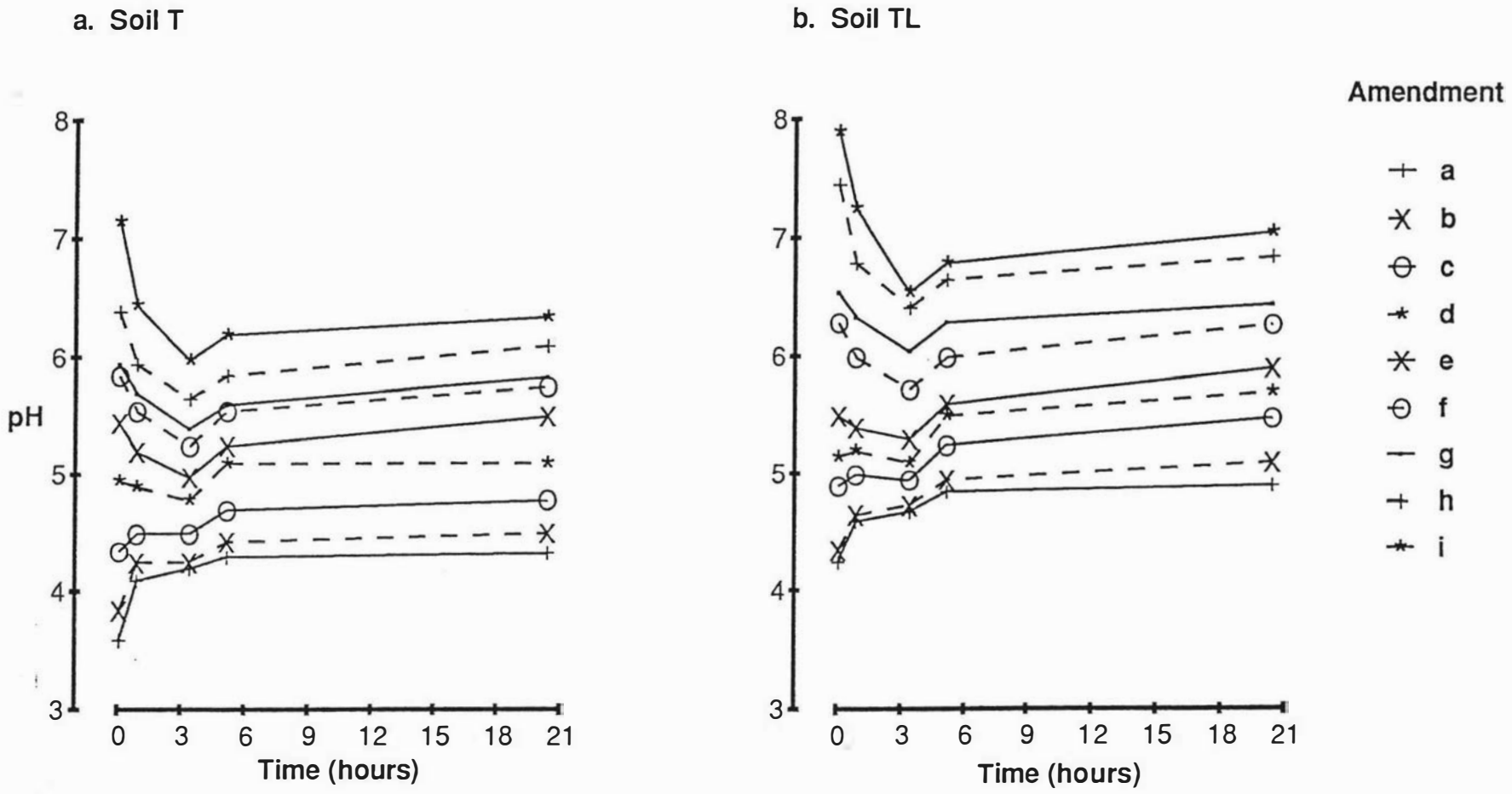


Figure 7.1 Change in the pH of suspensions of soil in agar solution with time following addition of acid or alkali

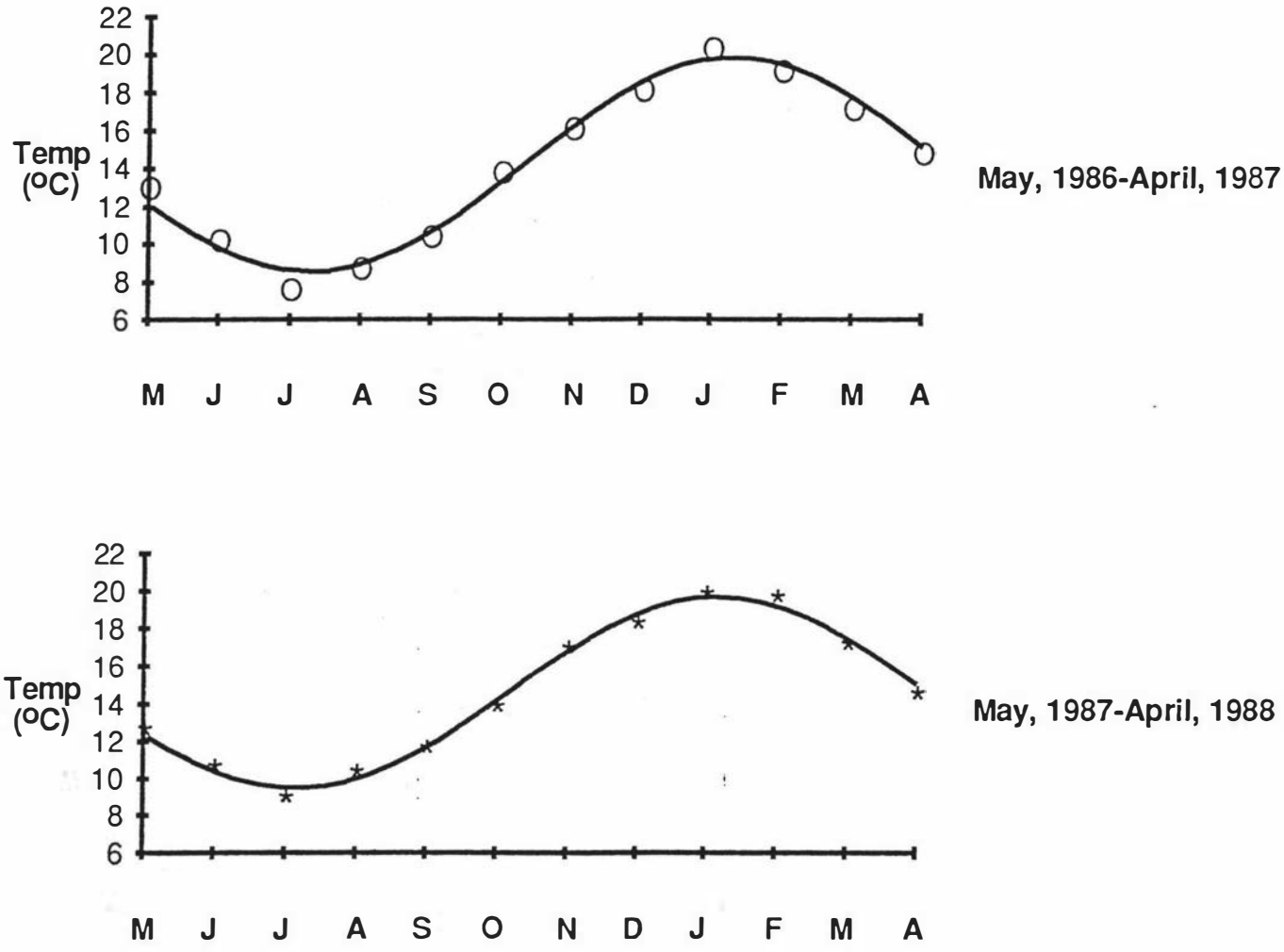


Figure 7.2 Mean monthly temperatures for the years (a) 1986/87 and (b) 1987/88 with sine curves fitted by ordinary least squares optimization

ii. Results

The effect of pH on nitrifier activity

SNA values were plotted against pH for each sampling date, and except for soil **T** sampled on 2 February, 1987, the data were fitted by least squares optimization with quadratic curves. Plots for each experiment (Soils **T** and **TL**) are shown in Figure 7.3a-f. Soil **T** data for the assay on 2 February, 1987 (Figure 7.3e) could not be fitted with a quadratic curve because there were insufficient points towards the alkaline side of the expected pH optimum. An optimum pH for the SNA (pH_{opt}) was obtained for all sampling times except soil **T** on 2 February, 1987 by differentiating the fitted equations. The trend in pH_{opt} with time is shown in Figure 7.5a,b.

pH_{opt} did not change markedly for either soil **T** or **TL** over the first 320 days of observation, the respective mean values being 5.91 ± 0.08 and 6.31 ± 0.09. The mean soil pH values over the same period were 5.18 ± 0.05 and 6.14 ± 0.09 respectively. The lower pH_{opt} for the unlimed Tokomaru soil suggests that the nitrifier population had adjusted to the acid soil conditions (mean pH 5.18), whereas the nitrifiers in the same soil four years after the original lime application seemed to have adjusted to the more alkaline conditions (mean pH 6.14). There was no obvious correlation between pH_{opt} and either the gravimetric soil moisture content (θ_g) or temperature at 30 cm depth (Figure 7.5a,b).

Following the application of lime on 14 April, 1987, pH_{opt}, θ_g, soil temperature and SNA_{opt}, the SNA value at pH_{opt}, calculated from the fitted quadratic curve, were monitored for a further 350 days (Figure 7.5c,d). The plots of SNA *vs.* incubation pH for these experiments (Soils **TX** and **TLX**) are shown in Figure 7.4a-f. Comparing Figure 7.5a with 7.5c, and Figure 7.5b with 7.5d, it is seen that the pH of the previously unlimed soil **T** rose to a new steady value (mean for soil **TX** of 5.51 ± 0.06), while the pH of soil **TL**, previously limed in 1982, at first rose to *c.*6.4 but then fell so that the mean value for soil **TLX** over the year was 6.18 ± 0.09. The value of pH_{opt} for soil **TX** and **TLX** remained

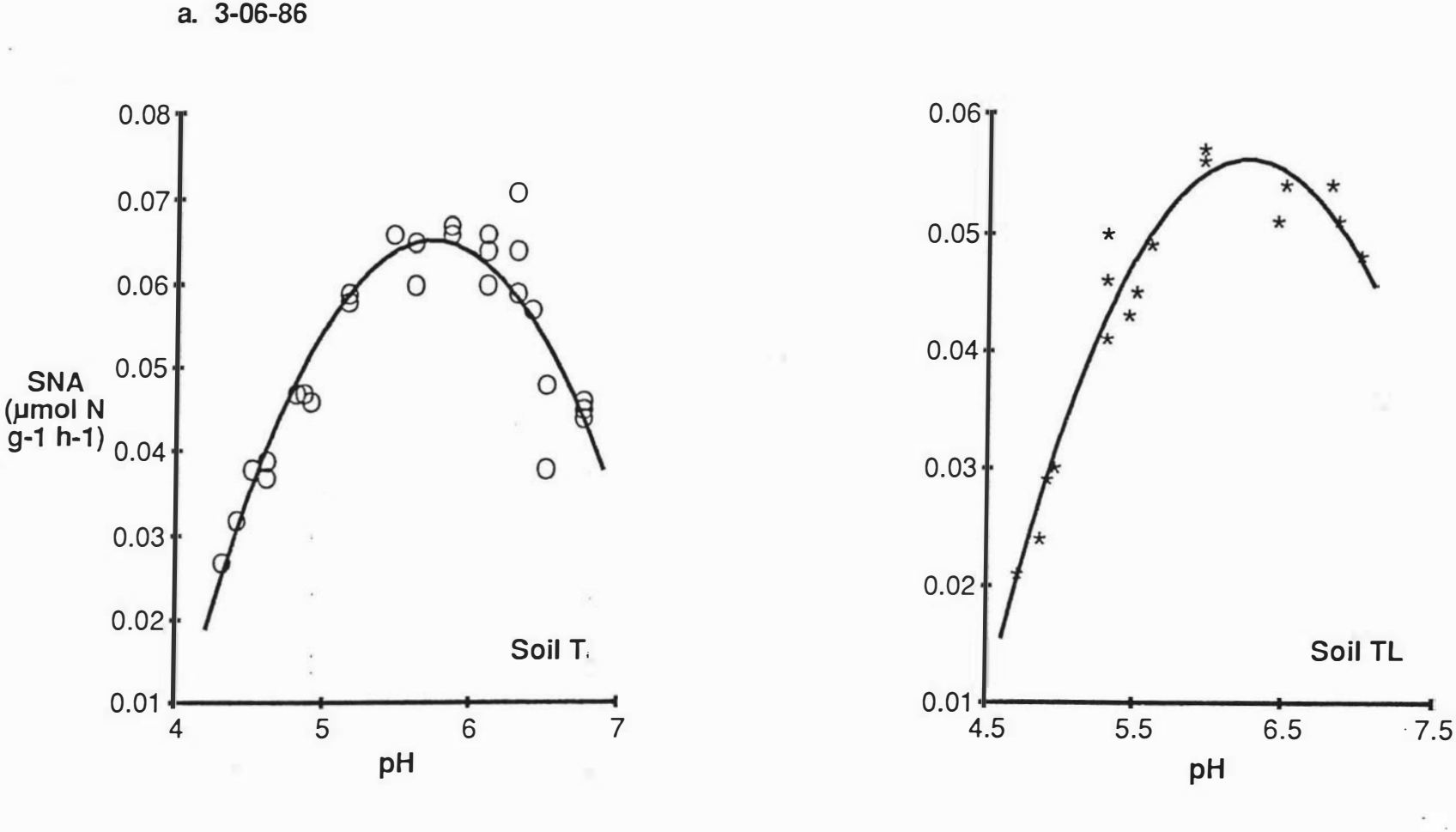


Figure 7.3 pH optima curves for nitrifier activity in soils T and TL

b. 25-08-86

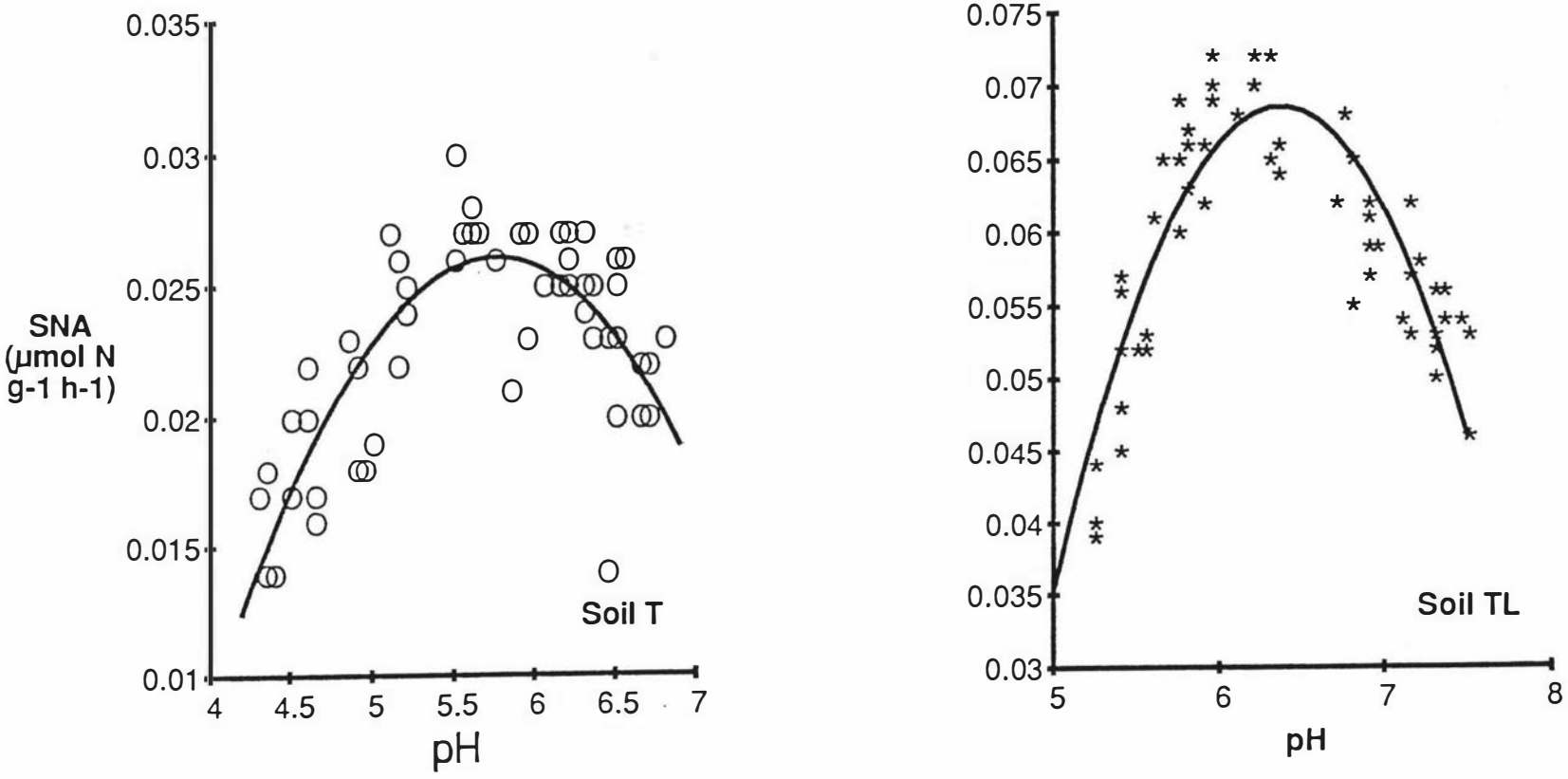


Figure 7.3 (Contd)

c. 4-10-86

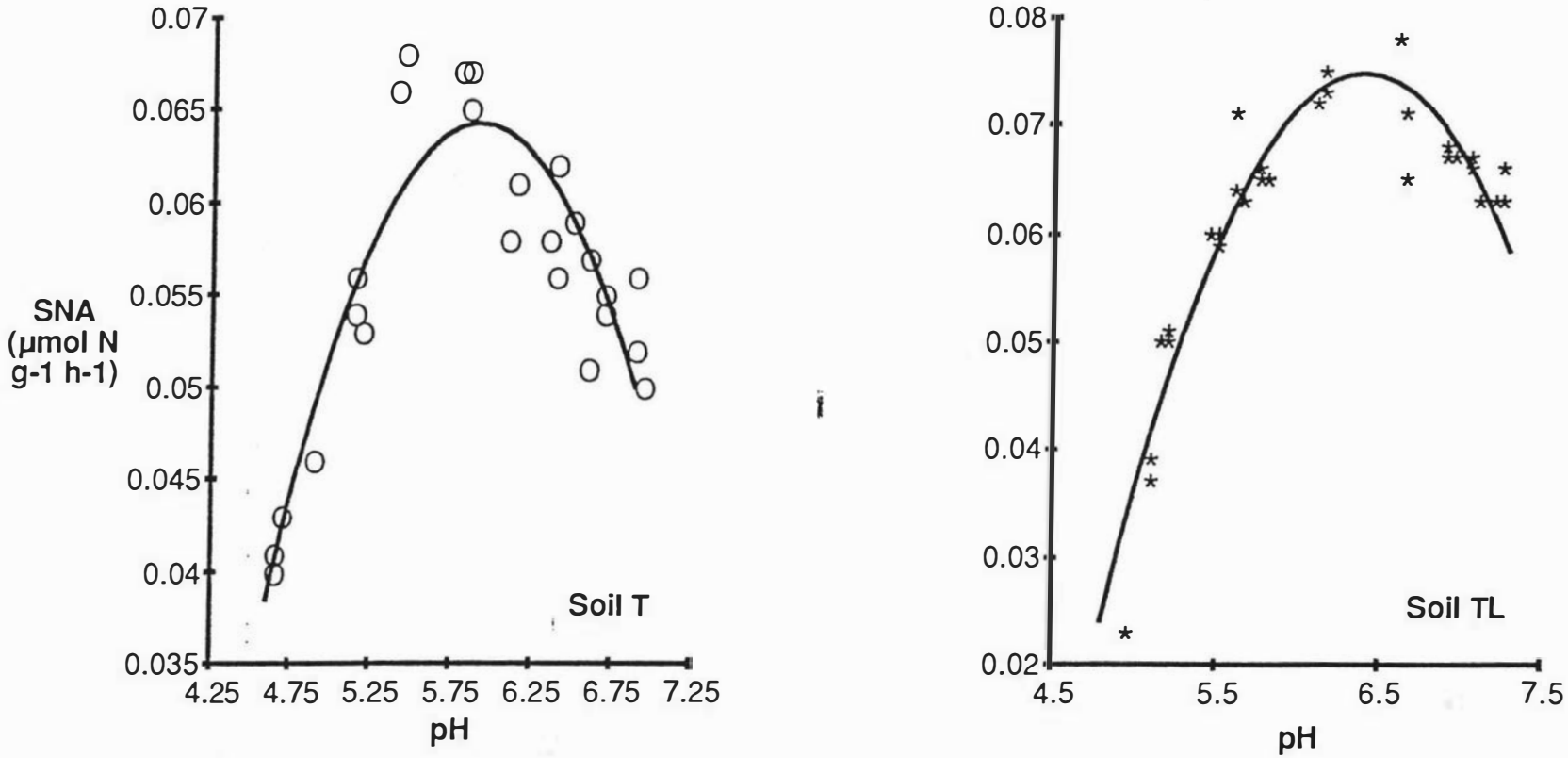


Figure 7.3 (Contd)

d. 28-11-86

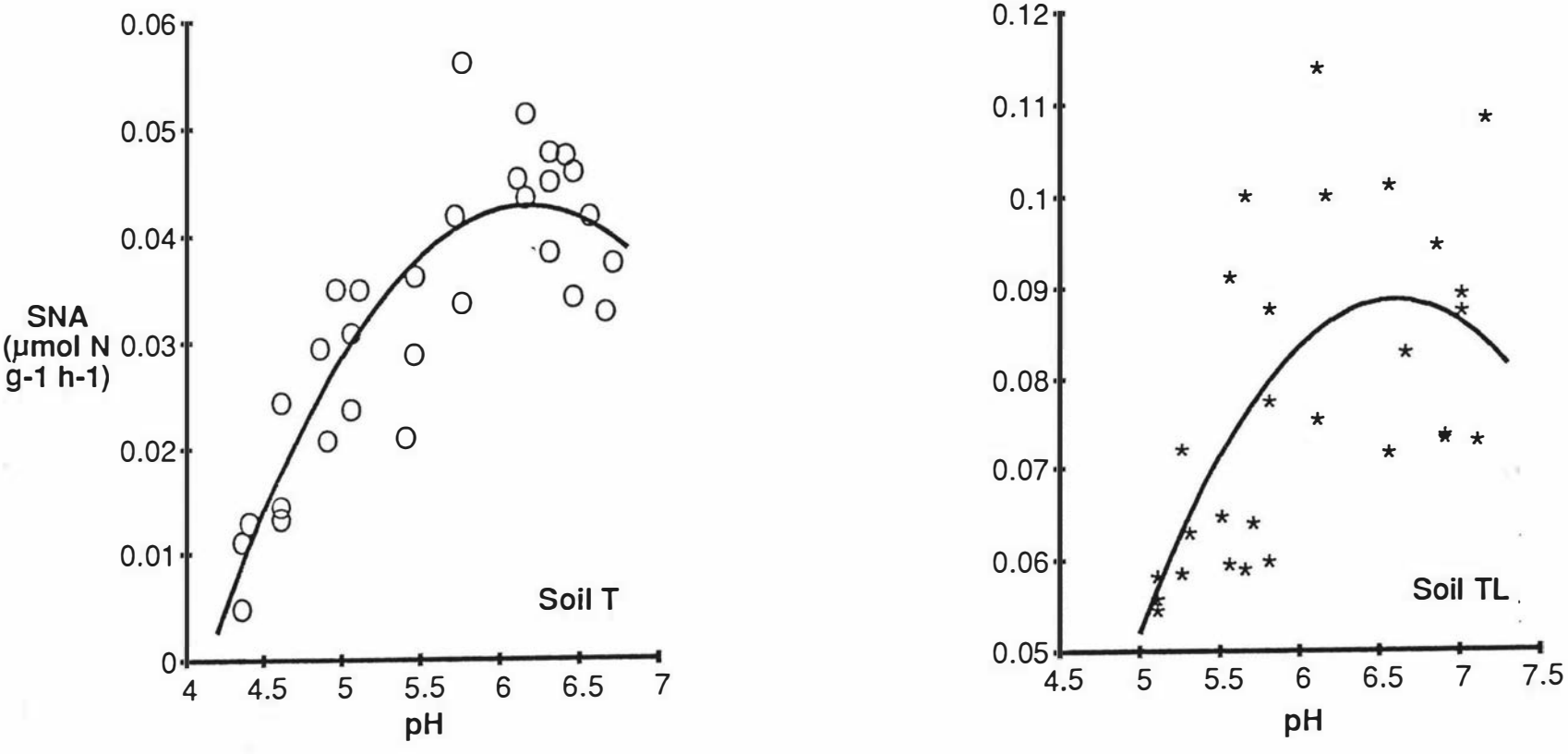
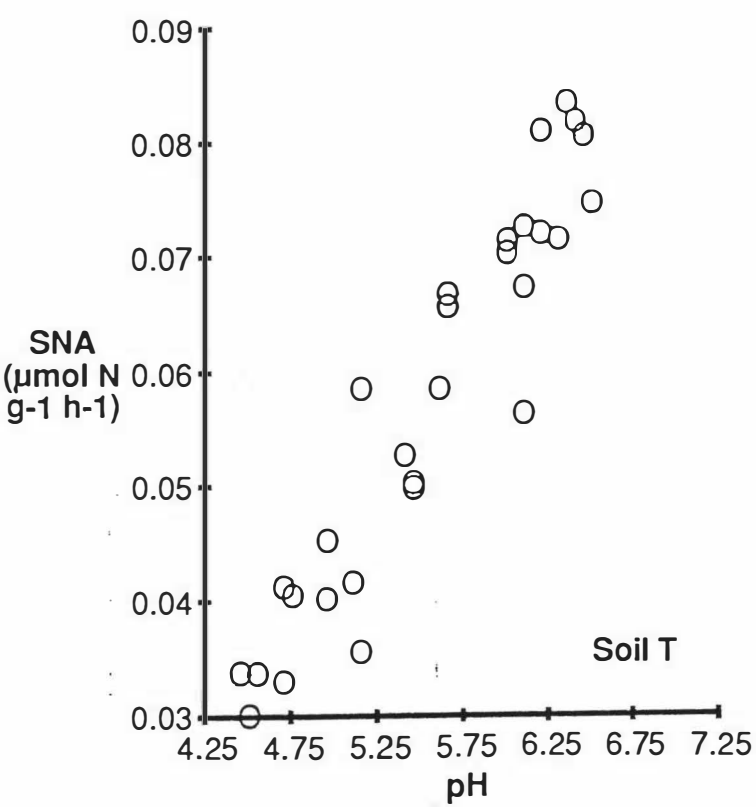


Figure 7.3 (Contd)

e. 2-02-87



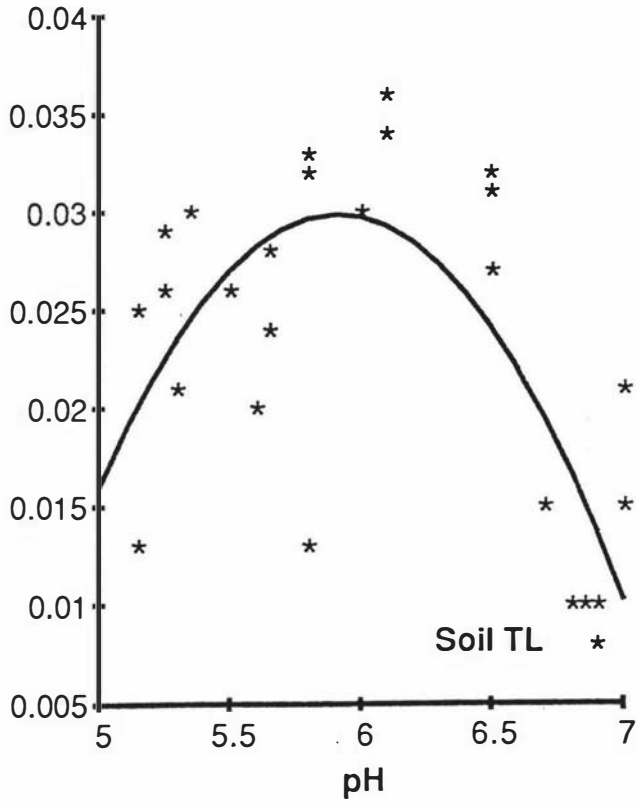
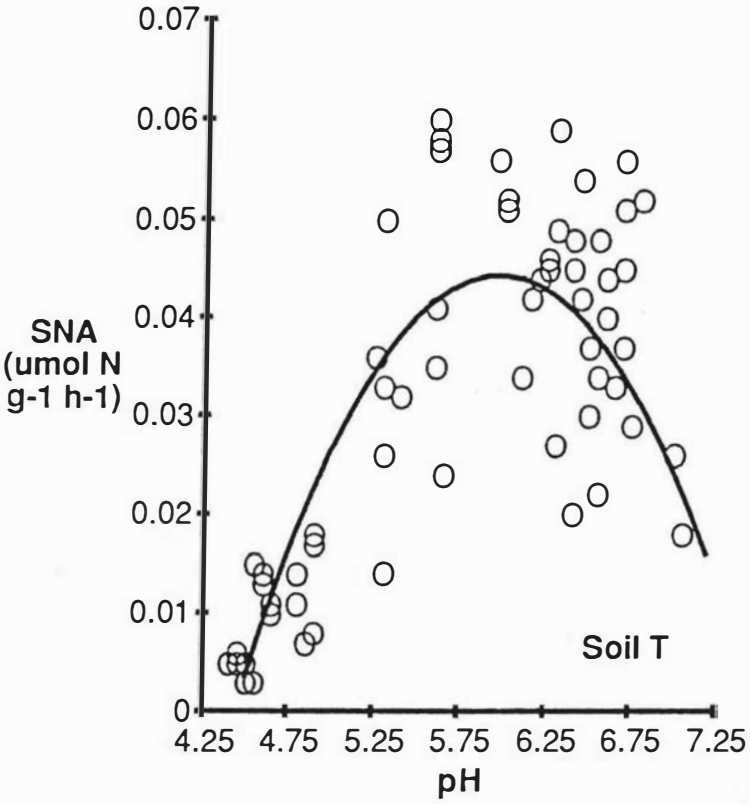


Figure 7.3 (Contd)

f. 12-04-87



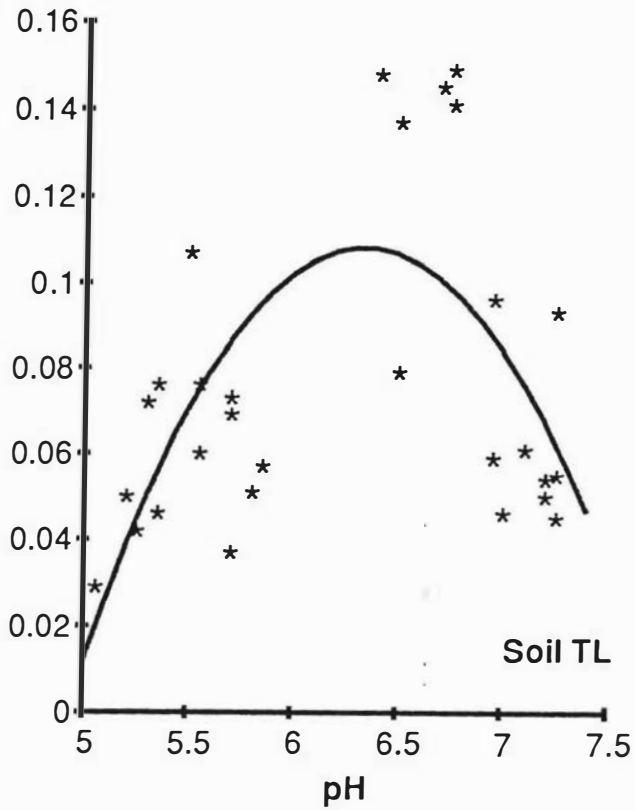


Figure 7.3 (Contd)

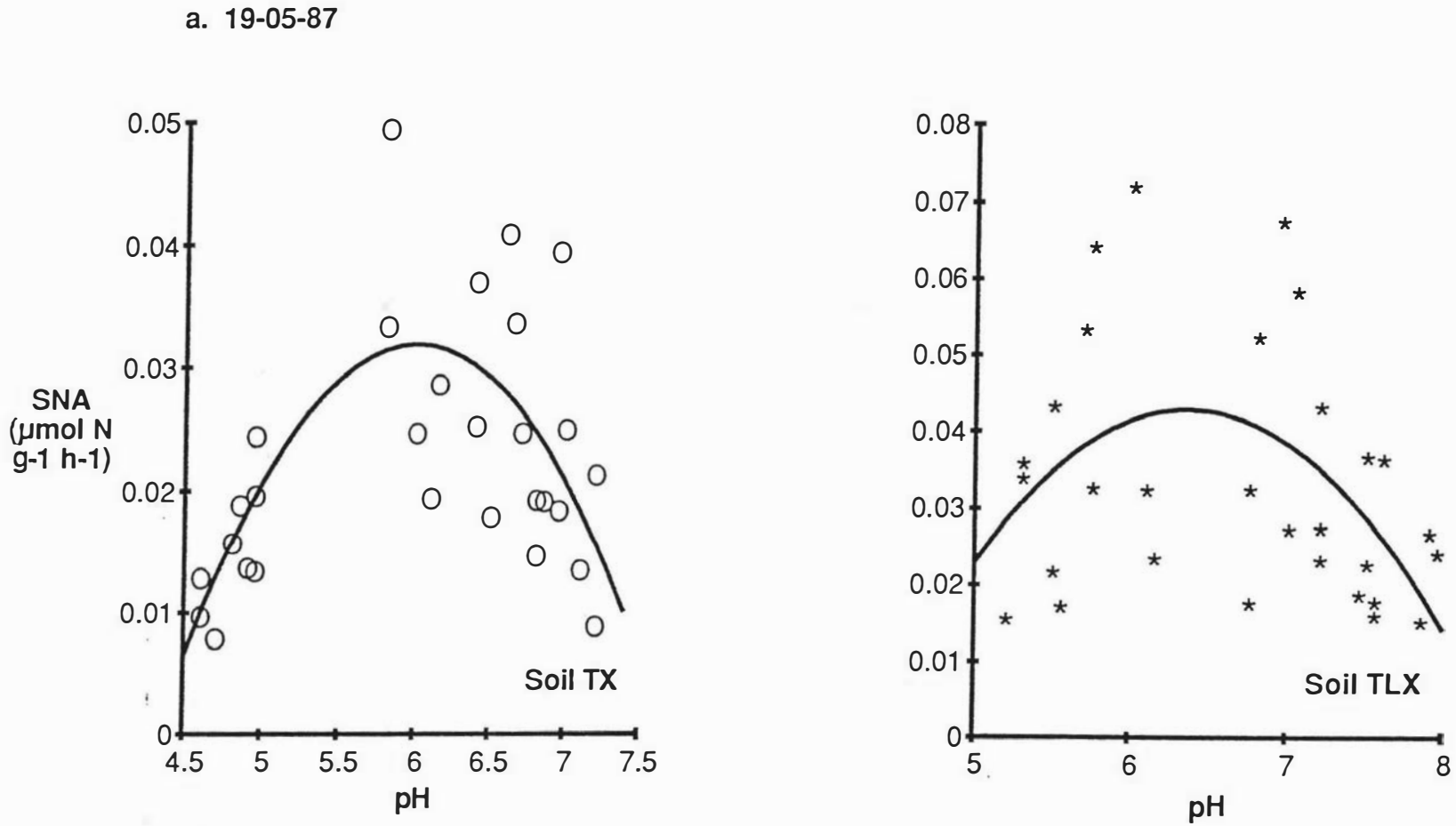


Figure 7.4 pH optima curves for nitrifier activity in soils TX and TLX

b. 20-07-87

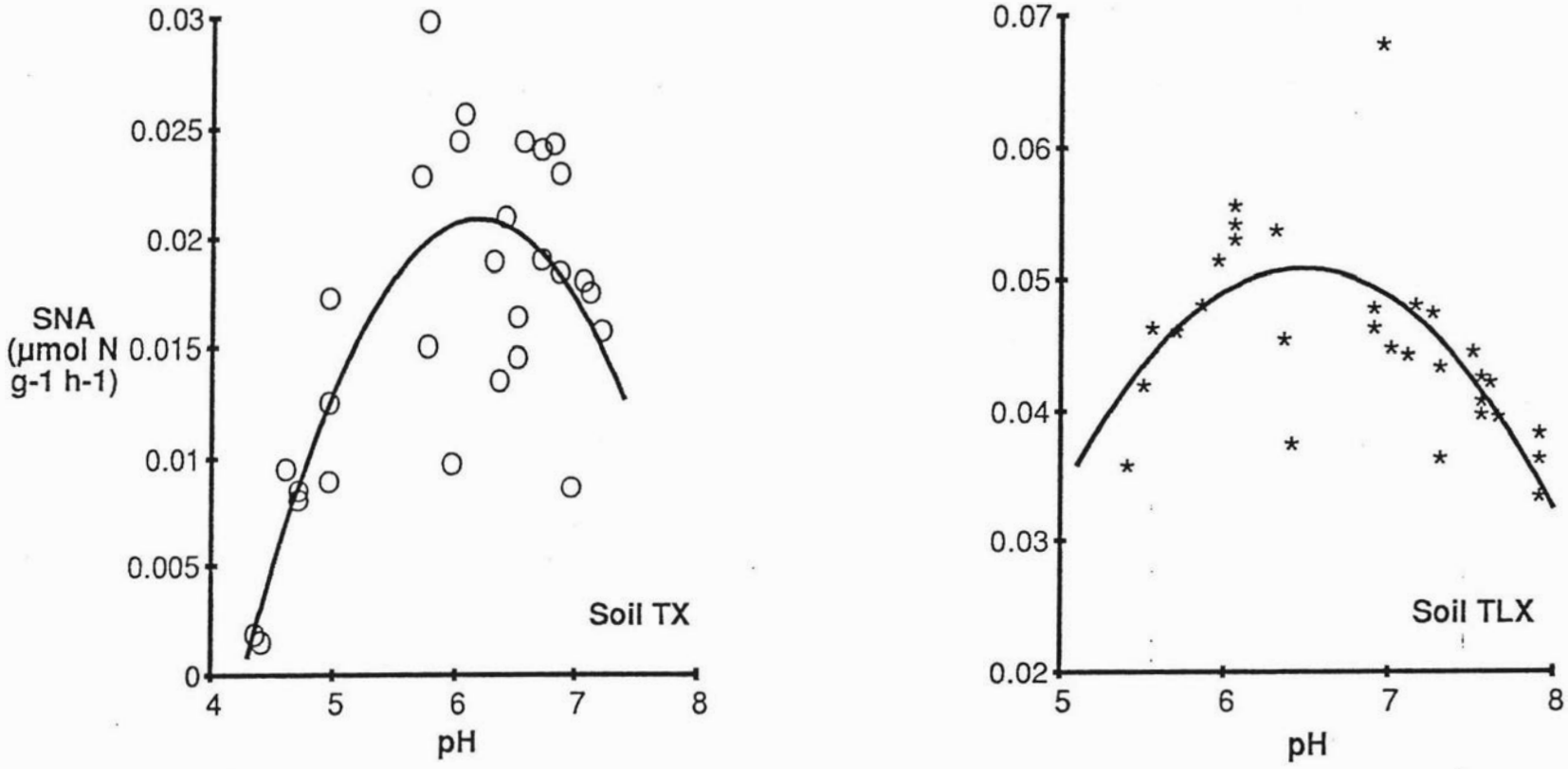


Figure 7.4 (Contd)

c. 14-09-87

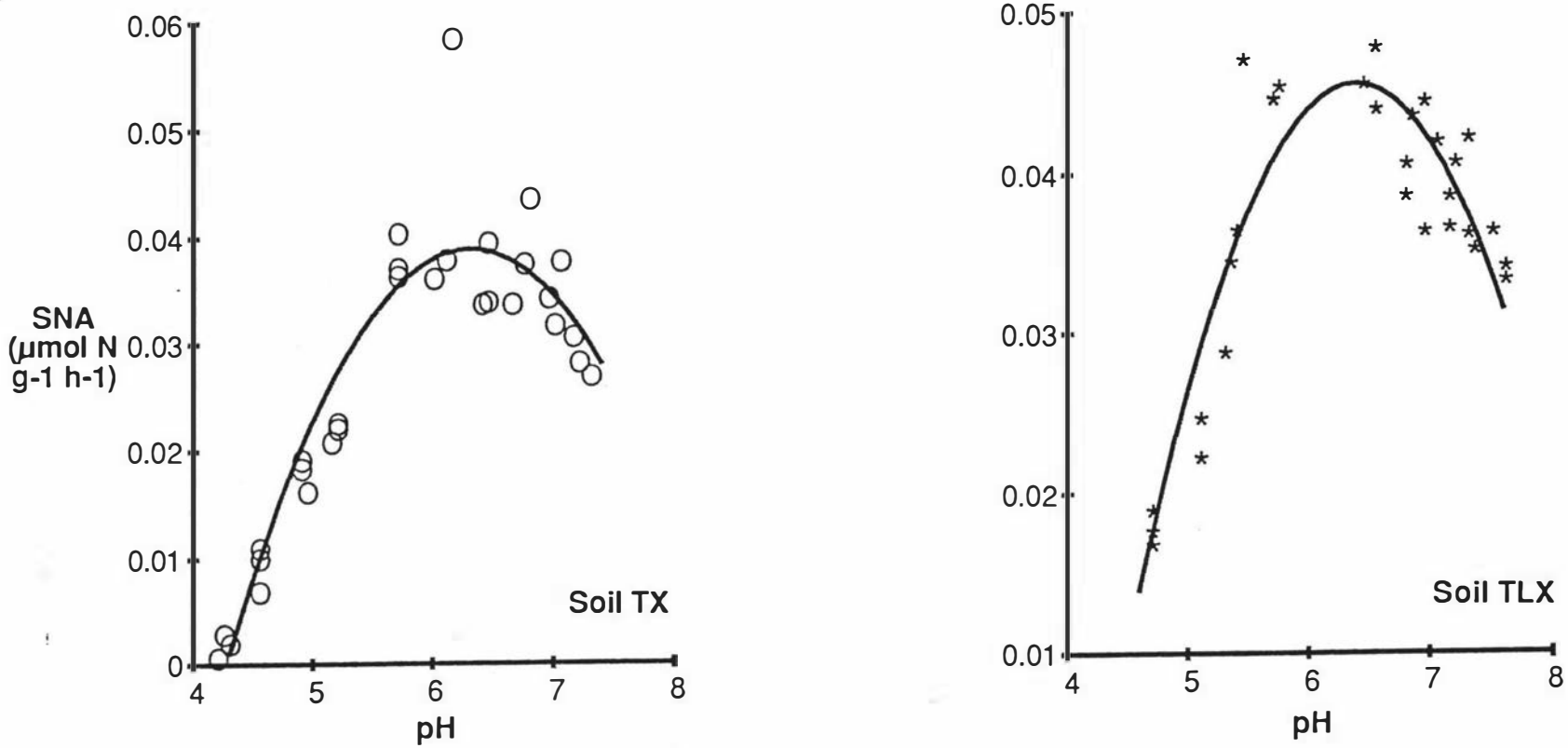
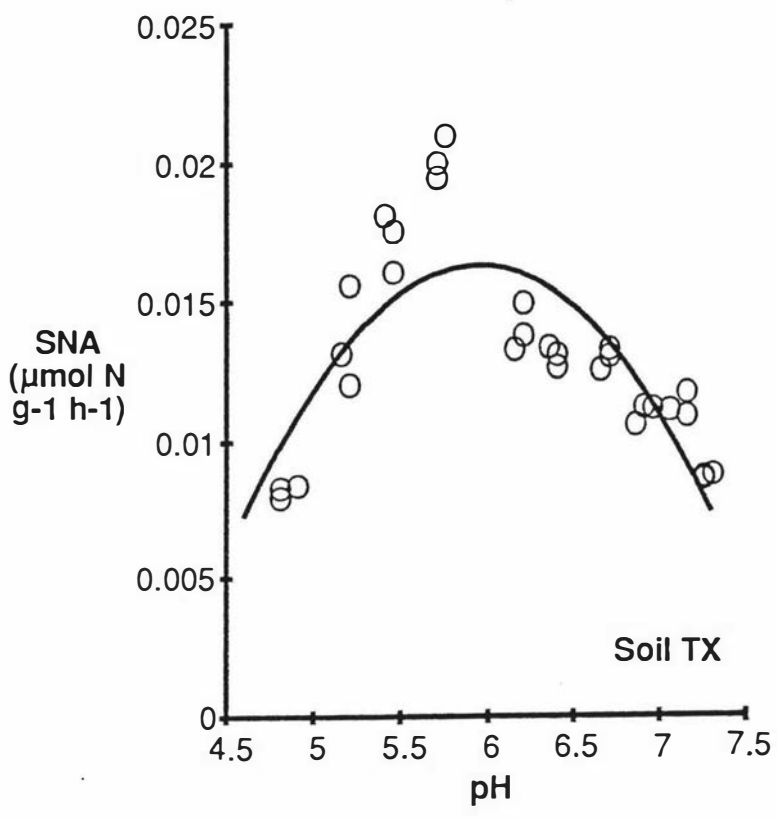


Figure 7.4 (Contd)

d. 27-11-87



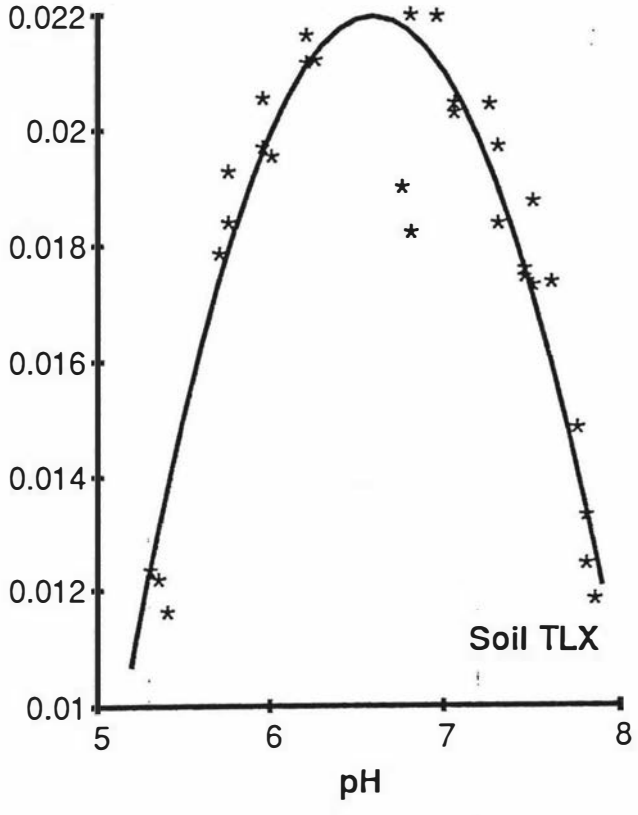


Figure 7.4 (Contd)

e. 3-02-88

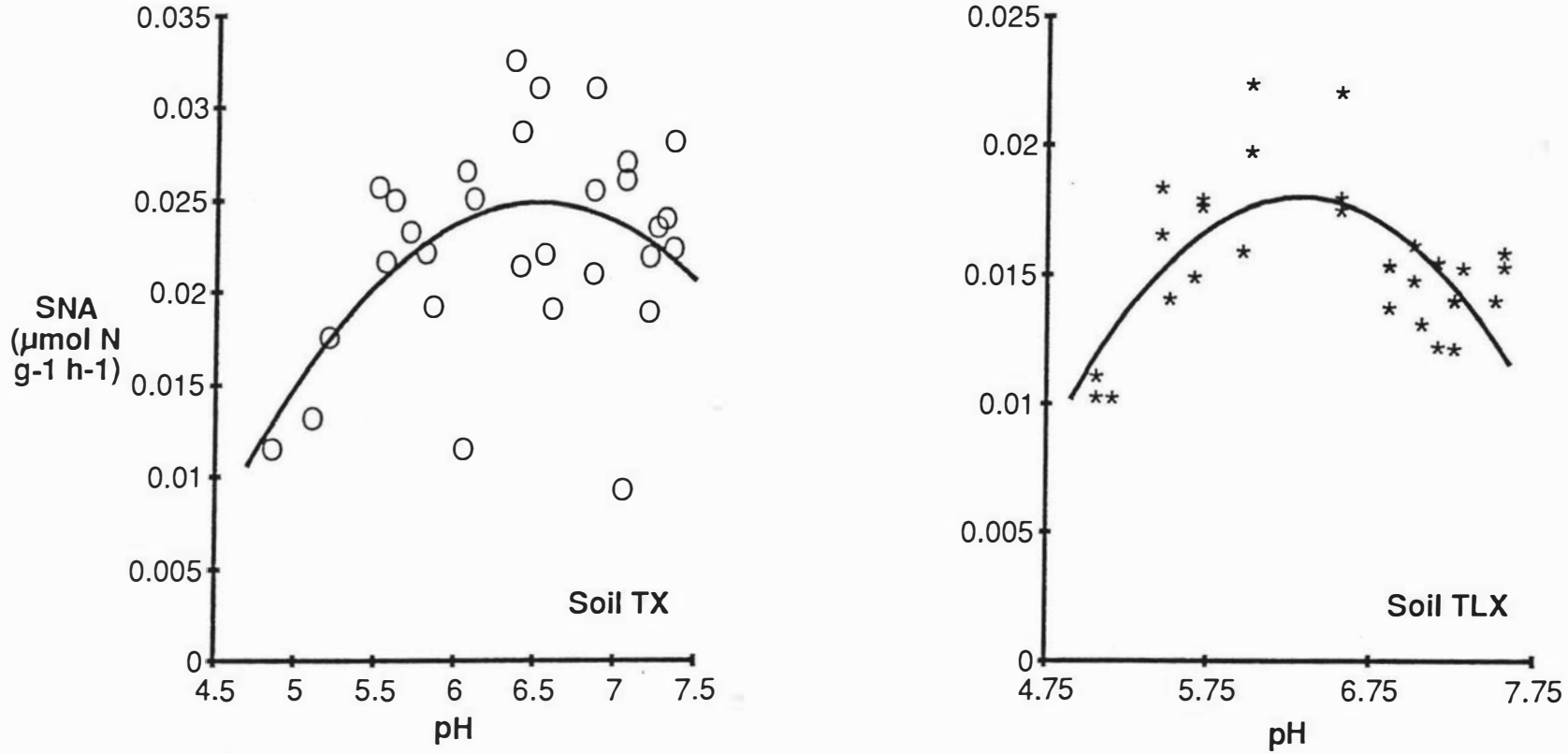


Figure 7.4 (Contd)

f. 30-03-88

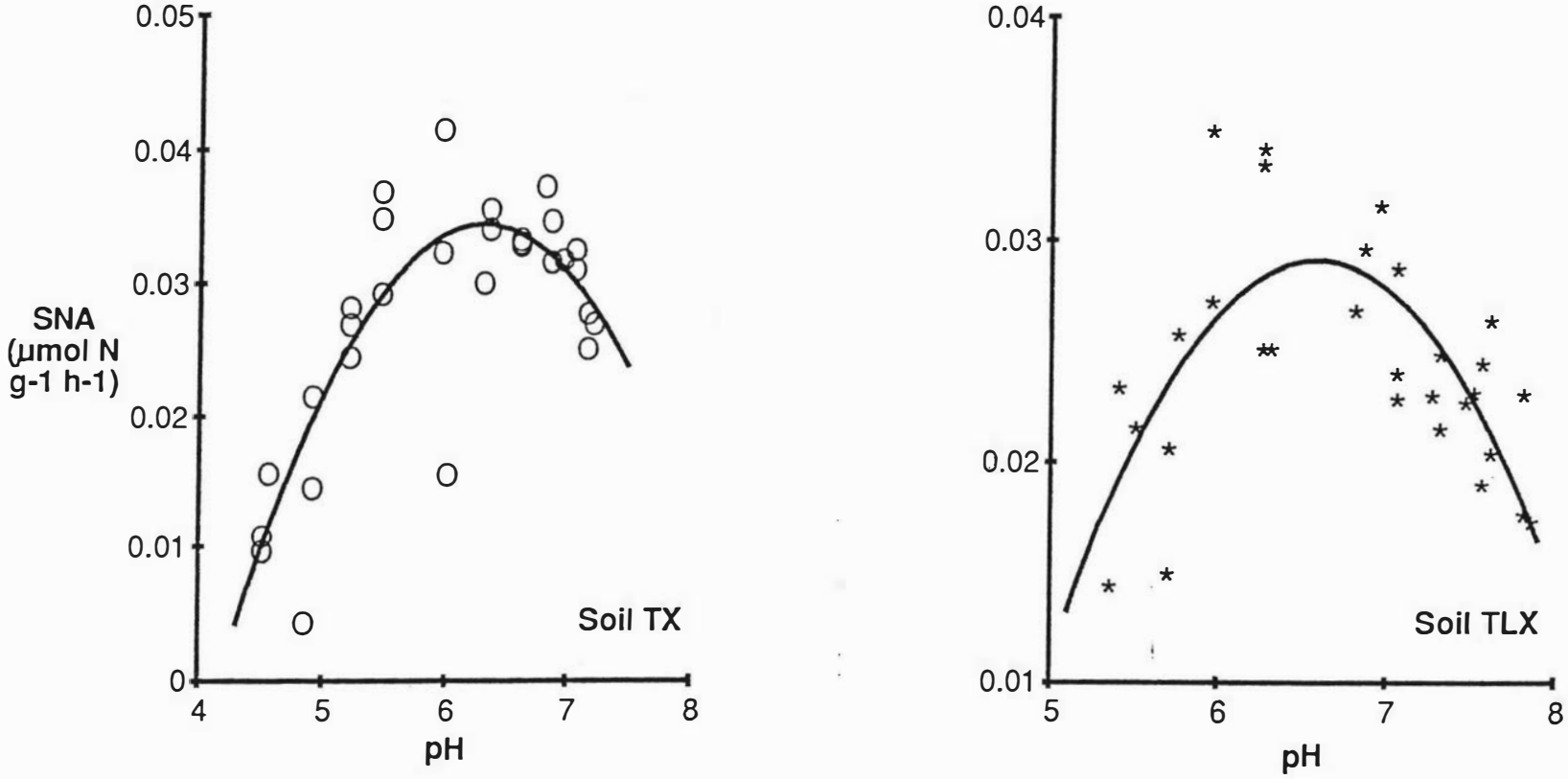


Figure 7.4 (Contd)

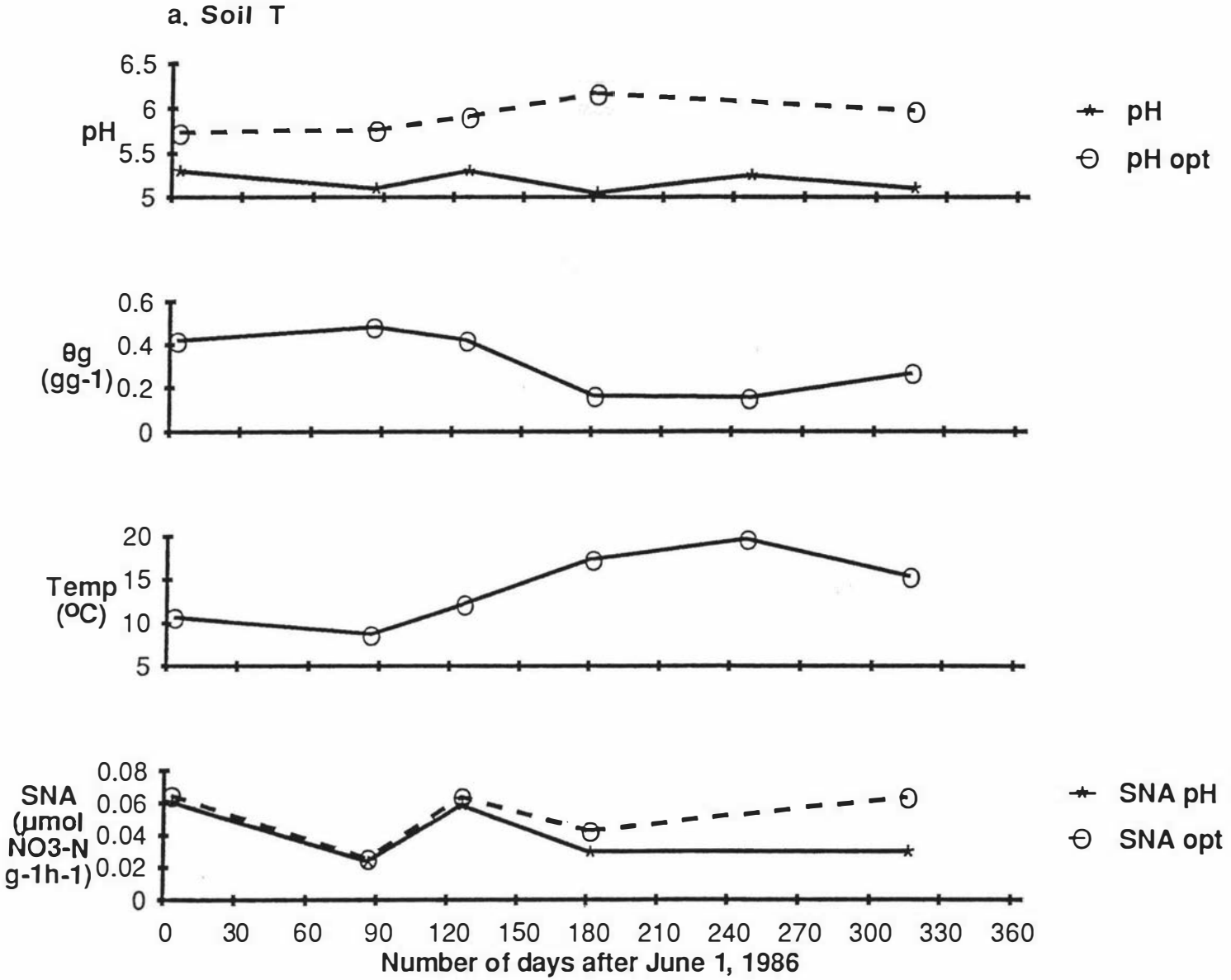


Figure 7.5 Seasonal variation in pH, pH_{opt}, SNA_{pH}, SNA_{opt}, soil moisture content and soil temperature

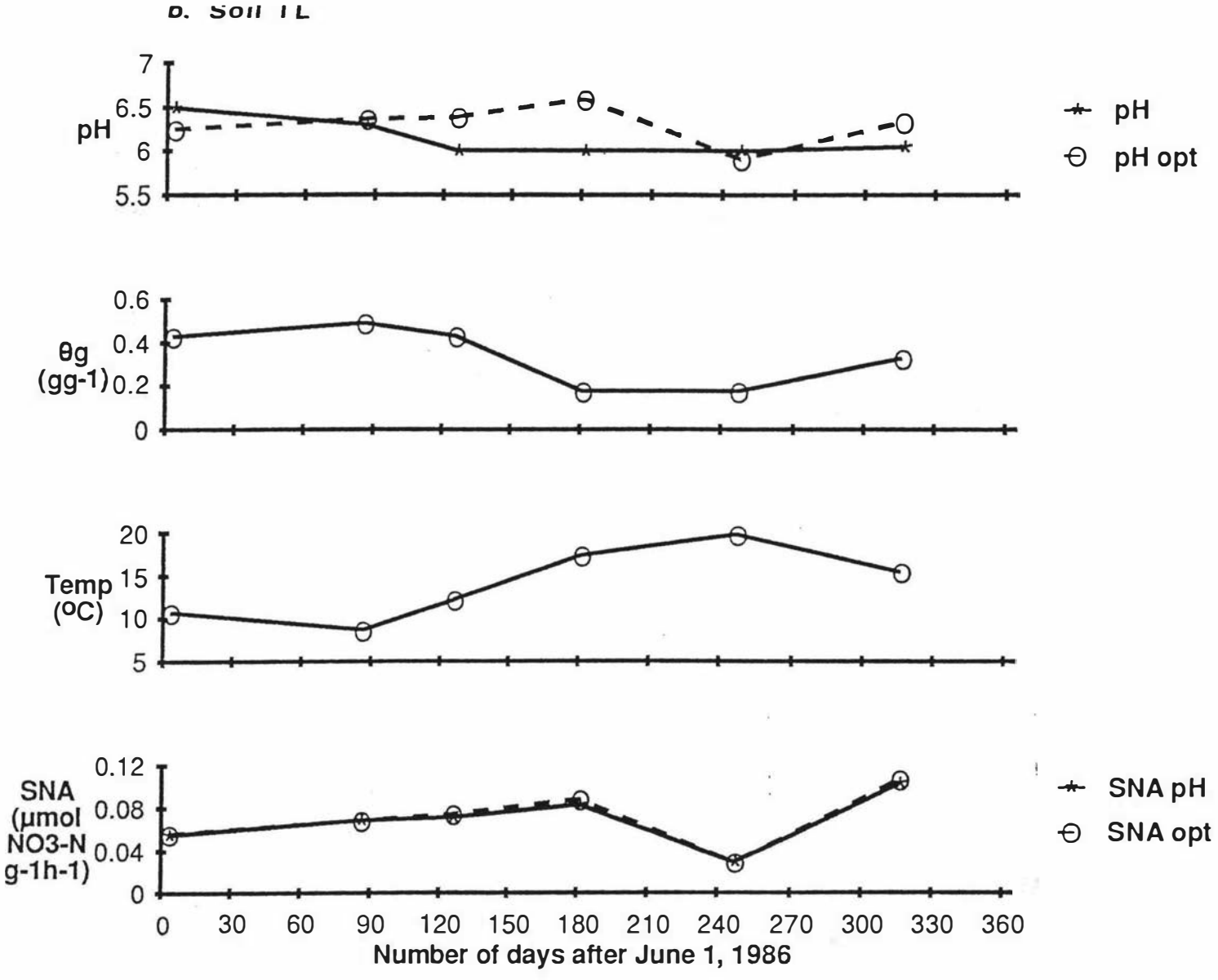


Figure 7.5 (Contd)

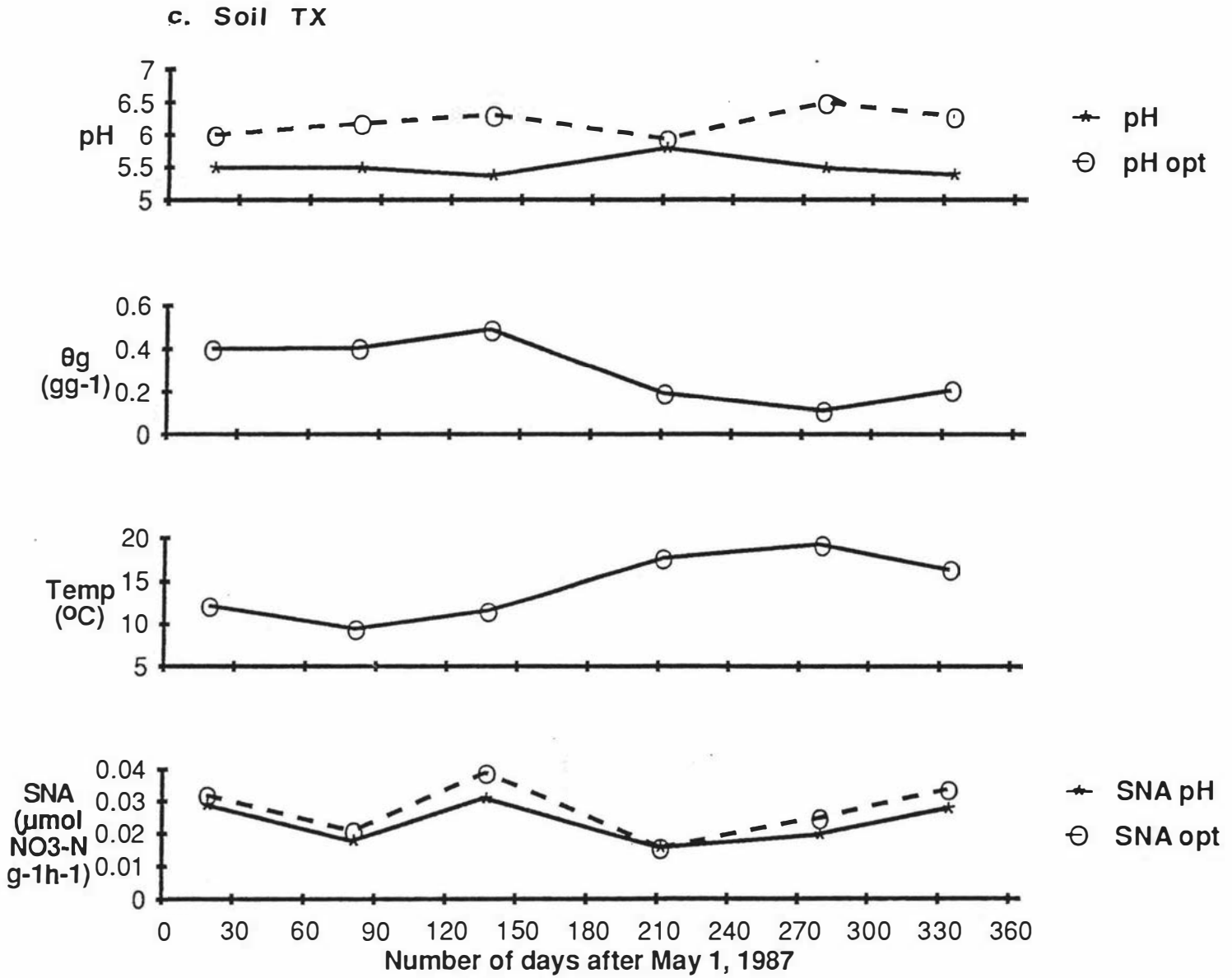


Figure 7.5 (Contd)

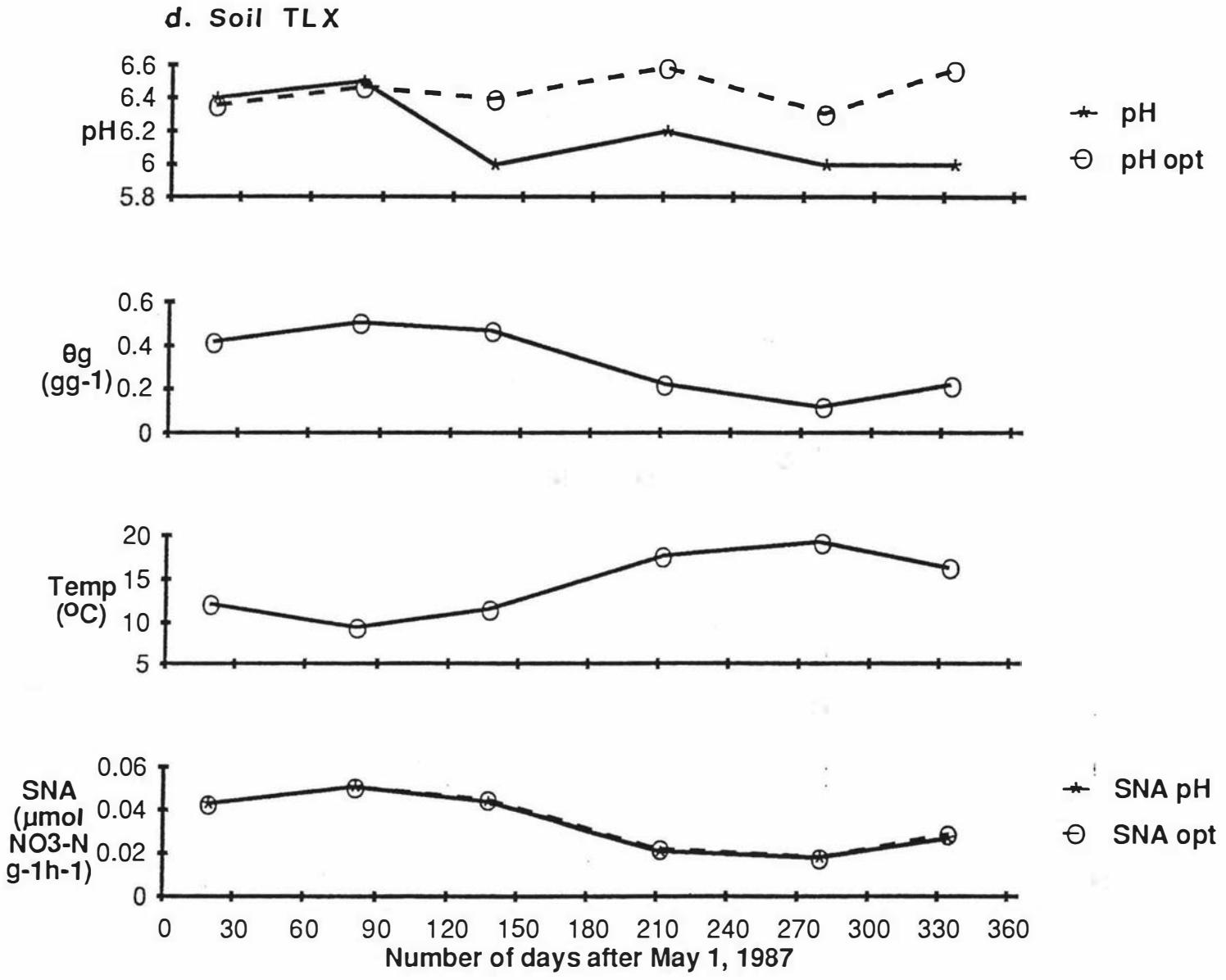


Figure 7.5 (Contd)

approximately constant over the year, averaging 6.21 ± 0.09 and 6.45 ± 0.05 respectively. Thus, the addition of 2500 kg $CaCO_3$ ha^{-1} on 14 April, 1987 had the effect of raising the mean soil pH and optimum pH for nitrification in the unlimed soil by 0.33 and 0.29 pH units respectively, but had a smaller effect on the mean soil pH and pH_{opt} in the soil limed 5 years previously in which the values of soil pH and pH_{opt} rose by 0.04 and 0.14 pH units respectively. This seems to suggest that although the nitrifiers in soil T had adjusted to low pH, addition of lime to raise the soil pH still lifts the value of pH_{opt}. There was no obvious correlation between pH_{opt} and either θ_g or soil temperature at 30 cm depth (Figure 7.5c,d).

Seasonal variation in nitrifier activity

The value of SNA (μmol N g^{-1} soil h^{-1}) at pH_{opt} for each sampling occasion (SNA_{opt}) could be calculated from the equations given in Table 7.1. These values, plotted in Figure 7.5a-d, show more obvious seasonal variation than the pH_{opt} values. Also plotted are the values of SNA at the soil pH for each sampling time (SNA_{pH}), which show very similar seasonal trends to the SNA_{opt} values (Figure 7.5a-d). They demonstrate that the SNA value at the soil pH was generally close to the estimated SNA value at the optimum pH for nitrifier activity, the greatest divergence being for soil T between 28 November, 1986, and 12 April, 1987. Indeed, the plot of SNA_{opt} against SNA_{pH} for all soil treatments at all sampling times showed a near 1:1 relationship, except for the aberrant observation on 12 April, 1987 (Figure 7.6). The fitted line had the equation (R^2 = 0.91, $p<0.1\%$):

$$SNA_{opt} = 0.005 + 0.975SNA_{pH} \qquad (7.1)$$

The small positive intercept (0.005 μmol N g^{-1} h^{-1}) indicates that at low SNA values, that is, generally at low soil pH, SNA_{pH} was slightly less than SNA_{opt}, but at high SNA values, that is, higher soil pH, SNA_{pH} and SNA_{opt} were approximately equal.

Table 7.1 Summary of SNA results for soils T, TL, TX, and TLX

Soil	Date of Sampling	Soil pH	pH_{opt}	Equations for SNA_{opt} & SNA_{pH}	R^2
T	03-06-86	5.30	5.73	$SNA = -0.58651 + 0.22763pH - 0.01988pH^2$	0.86
T	25-08-86	5.10	5.76	$SNA = -0.15995 + 0.06455pH - 0.00560pH^2$	0.62
T	04-10-86	5.30	5.91	$SNA = -0.45471 + 0.17539pH - 0.01482pH^2$	0.80
T	28-11-86	5.05	6.17	$SNA = -0.34973 + 0.12717pH - 0.01030pH^2$	0.75
T	02-02-87	5.25	n.d	No curve fitted	n.d.
T	12-04-87	5.10	5.97	$SNA = -0.63139 + 0.22631pH - 0.01895pH^2$	0.60
	Mean	5.18	5.92		
	S.E.	0.05	0.08		
TL	03-06-86	6.50	6.25	$SNA = -0.52582 + 0.18618pH - 0.01489pH^2$	0.93
TL	25-08-86	6.30	6.37	$SNA = -0.65247 + 0.22646pH - 0.01778pH^2$	0.73
TL	04-10-86	6.00	6.39	$SNA = -0.74308 + 0.25587pH - 0.02001pH^2$	0.88
TL	28-11-86	6.00	6.59	$^{a}SNA = -0.53658 + 0.18958pH - 0.01437pH^2$	0.34
TL	02-02-87	6.00	5.91	$SNA = -0.55348 + 0.19728pH - 0.01668pH^2$	0.49
TL	12-04-87	6.05	6.33	$^{a}SNA = -2.07189 + 0.68832pH - 0.05433pH^2$	0.33
	Mean	6.14	6.31		
	S.E.	0.09	0.09		
TX	19-05-87	5.50	6.00	$SNA = -0.37253 + 0.13473pH - 0.01122pH^2$	0.39
TX	20-07-87	5.50	6.19	$SNA = -0.19500 + 0.06979pH - 0.00564pH^2$	0.60
TX	14-09-87	5.38	6.32	$SNA = -0.33405 + 0.11810pH - 0.00935pH^2$	0.86
TX	27-11-87	5.80	5.95	$SNA = -0.15877 + 0.05882pH - 0.00494pH^2$	0.58
TX	03-02-88	5.50	6.51	$^{b}SNA = -0.15975 + 0.05668pH - 0.00435pH^2$	0.18
TX	30-03-88	5.40	6.30	$SNA = -0.26452 + 0.09482pH - 0.00752pH^2$	0.63
	Mean	5.51	6.21		
	S.E.	0.06	0.09		
TLX	19-05-87	6.40	6.36	$^{b}SNA = -0.38971 + 0.13600pH - 0.01069pH^2$	0.14
TLX	20-07-87	6.50	6.47	$SNA = -0.28116 + 0.10256pH - 0.00792pH^2$	0.40
TLX	14-09-87	6.00	6.40	$SNA = -0.35368 + 0.12477pH - 0.00975pH^2$	0.82
TLX	27-11-87	6.20	6.59	$SNA = -0.23065 + 0.07662pH - 0.00581pH^2$	0.86
TLX	03-02-88	6.00	6.31	$SNA = -0.13819 + 0.04949pH - 0.00392pH^2$	0.39
TLX	30-03-88	6.00	6.58	$SNA = -0.28753 + 0.09629pH - 0.00732pH^2$	0.45
	Mean	6.18	6.45		
	S.E.	0.09	0.05		

pH_{opt} = pH optimum for nitrification found by differentiating the fitted quadratic equations.

All equations are significant at the 0.1 % level except as indicated by the superscripts: [a] significant at the 1.0 % level; [b] significant at the 5 % level.

Comparisons of the changes in SNA_{pH} with changes in θ_g and soil temperature during the winter and summer periods of 1986-87 for soils T and TL (Figure 7.5a,b) do not suggest any obvious correlations, except for the inverse relationship between soil temperature and soil moisture content, which reflects the rise in the evapotranspiration rate during the warmer summer months. The same inverse relationship between soil temperature and moisture held for the 1987-88 period, but there was also a more definite tendency for SNA_{pH} to vary directly with θ_g, particularly in soil TLX (Figure 7.5c,d). The cyclical pattern of soil temperature and moisture change was very similar for the two observation periods, allowing for the fact that one started on 3 June, 1986, and the second on 19 May, 1987. However, the second year's results indicate more conclusively than the first that soil nitrifier activity may decline by as much as 100 % between mid-winter and mid-summer in limed Tokomaru soil.

Evaluation of the effect of liming (current or historic) on the soil nitrifier activity is to some extent confounded by the variable seasonal trend in SNA_{pH} in soils T and TL during the 1986-87 period. Nevertheless, if the SNA_{pH} values are averaged over time, the results shown in Table 7.2 are obtained. As expected for this acid soil, liming increased the activity of nitrifying organisms, the effect being most marked due to the residual effect of lime applied in 1982 which, even after 4-5 years, kept the pH of the limed soil at 6.14 compared to the unlimed soil pH of 5.18. In the year following the application of additional lime in April, 1987, to both unlimed and limed soils, the pH rose to 5.51 and 6.18 respectively, but the differential effect on the SNA value was smaller than in the preceding year.

iii. Discussion

The fact that SNA_{opt} and SNA_{pH} are so similar (Figure 7.6) over an approximately seven-fold range of values (0.015-0.110 $\mu mol\ g^{-1}\ h^{-1}$) suggests that the nitrifier activity in the soil, irrespective both of variations that are random and unknown and those associated with seasonal

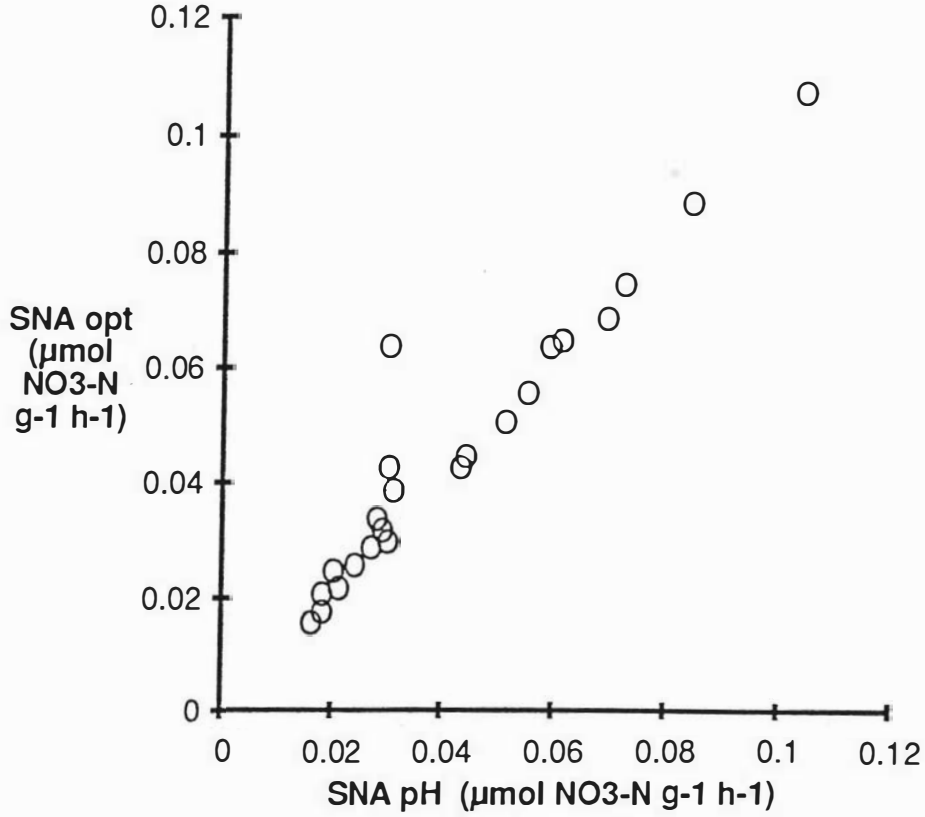


Figure 7.6 Relationship between SNA_{opt} and SNA_{pH} for soils T, TL, TX, and TLX

variables (temperature and moisture), is near the optimum with respect to pH on each sampling occasion. Given the liming history of the four soils, it seems that the nitrifier population is fairly adaptable to changes in its environment and that as a result, pH_{opt} for an indigenous population is never far from the prevailing soil pH. Darrah *et al.* (1986b) studied a sandy loam soil (Begbroke series) with a pH of 6.1 and found a value for pH_{opt} of 6.67, whilst the heavy clay soil (Evesham series) studied by White *et al.* (1983) which had a soil pH of 7.3, was found in a preliminary study (Bramley, unpublished) to have a pH_{opt} of 6.72. During the early stages of this work a pH optimum experiment was also done on the Patua soil, a very strongly leached yellow brown loam (N.Z Soil Bureau, 1968), sampled from the lower slopes of M^t Egmont. This acid soil (pH 4.9) had a pH_{opt} of 5.09. (This result is discussed further in Chapter 9.) These data, together with those for soils **T**, **TL**, **TX** and **TLX** are shown in Figure 7.7, which indicates a curvilinear relationship between pH_{opt} and soil pH for this limited range of soil types (R^2 = 0.92, $p<0.1\%$):

$$pH_{opt} = 6.61 - 1.49 \quad exp\{-2.33 \quad (pH - 4.90)\} \qquad (7.2)$$

From Figure 7.7 and equation (7.2), it might be concluded that the highest pH optimum likely to be observed for nitrifier activity in pasture soils lies at a soil pH of approximately 6.6-6.7, even though populations of indigenous nitrifiers in soils more acid than those included in Figure 7.7 appear to adapt, so that the optimum for short-term measurements of nitrification activity is not much less than the prevailing soil pH.

Comparison of SNA values between soils **T** and **TL**, and soils **TX** and **TLX** cannot be regarded as meaningful since the two pairs of soils were studied in different years, and as Figure 7.5 shows, the seasonal trends in SNA differed between 1986-87 and 1987-88. Sarathchandra *et al.* (1988) reported a similar problem when comparing data for a range of microbial properties in a New Zealand Typic Vitrandept between 1983 and 1984. However, the mean SNA values given in Table 7.2 generally show the beneficial effect of liming (i.e. raising the soil pH) on nitrifier activity in this soil, both when the effect of lime is residual and when

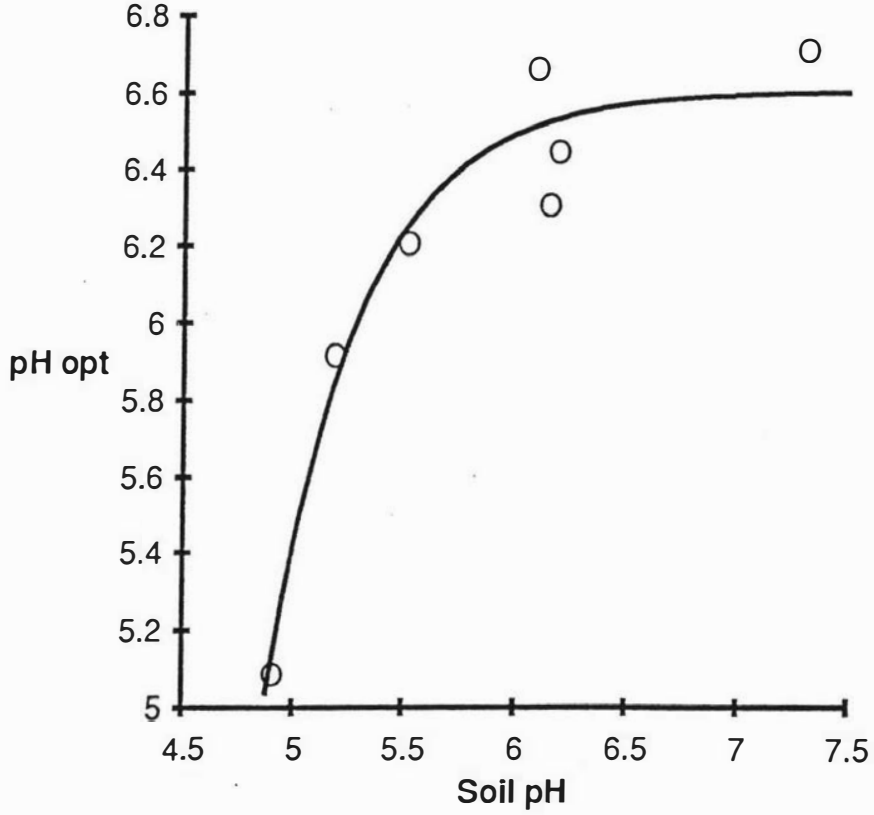


Figure 7.7 Relationship between pH_{opt} and soil pH for a range of soils (see text)

Table 7.2 Effect of liming on nitrifier activity in the Tokomaru Silt Loam (3-9 cm depth) under pasture

SNA_{pH} (μmol N $g^{-1}h^{-1}$)[a]			
Period 1986-87		Period 1987-88	
Soil T no lime	Soil TL 5t lime ha^{-1} 1982	Soil TX no lime 1982 2.5t ha^{-1} 1987	Soil TLX 5t lime ha^{-1} 1982 2.5t lime ha^{-1} 1987
0.041[b]	0.069	0.024	0.034
± 0.008	± 0.010	± 0.003	± 0.006

[a] Calculated from the equations given in Table 7.1
[b] Mean of 5; others means of 6

it is immediate, although this result is not necessarily consistently reported in the literature. For example, Pang *et al.* (1975) found during a six week incubation that the addition of lime to a Canadian soil, to raise the pH from 5.4 to 6.5, decreased the nitrification rate, this decrease being ascribed to the adverse effect of lime on the nitrifier population initially present, and especially on the *Nitrosomonas* spp. Further, it was found that the difference in nitrifying capacity among the soils studied was related to the size of the initial nitrifier population whose activity was affected by the initial pH prior to incubation. Hojito *et al.* (1987) found that the effects of liming on an orchard grass sward in Japan were to increase microbial numbers and activity in proportion to the amount of lime added up to 4 t ha^{-1}. At higher rates of lime application, microbial activities and numbers decreased. These effects were confined to the top 5 cm of the profile since the pH did not change appreciably in the 5-10 cm layer during the six months after lime application. One would therefore presume that the reason why the effect of the 1987 lime application was small was probably that the soil at 3-9 cm was not markedly affected by the lime - certainly the pH data reflect this (Table 7.1).

Regression analyses were performed on data for soil pH, SNA_{pH}, SNA_{opt}, pH_{opt}, soil temperature and soil moisture content, but no significant relationships were found consistently for the four soils other than between SNA_{opt} and SNA_{pH} (Figure 7.6), and the unsurprising inverse relationship between soil temperature and moisture content. Morrill and Dawson (1967) identified three patterns of nitrification, and found that only those factors associated with soil pH were significantly correlated with nitrification pattern. Sarathchandra *et al.* (1984) performed incubation experiments on a range of New Zealand soils and studied rates and amounts of CO_2 production, N-mineralized, total N, Org-C, microbial biomass, microbial P, mineral-N flush, SNA, soil pH, and the activities of phosphatase, arylsulphatase, urease and protease. They did a principal components analysis on these properties and found that two components explained 71 % of the total variance, but neither component was strongly correlated with pH. The work of Steele *et al.*(1980) also failed to show any close relationships between SNA, soil pH, Org-C, total N and C/N ratio. One of the soils studied by Sarathchandra *et al.* (1984) was the Tokomaru silt loam under a productive dairy pasture which had a soil pH of 6.4 and an SNA value of 0.054 µmol N g^{-1} h^{-1} - values comparable to soil TL.

By combining analysis of variance and analysis of covariance (Freund & Minton, 1979), it was found that for each soil, the SNA and pH data for all sampling dates could be grouped over the year and the relationship between the two variables described by a common quadratic equation, except that the intercept parameter C (i.e. the SNA value at pH 0) changed for each sampling date. The fitted equations were as follows:

Soil T; $SNA = C + 0.17088pH - 0.01443pH^2$ (7.3a)

Soil TL; $SNA = C + 0.21993pH - 0.01716pH^2$ (7.3b)

Soil TX; $SNA = C + 0.08340pH - 0.00671pH^2$ (7.3c)

Soil TLX; $SNA = C + 0.07934pH - 0.00614pH^2$ (7.3d)

The fact that these common equations could be fitted for each of the four soils leads to the important conclusion that the response of the soil nitrifier population to pH change was constant and did not vary with season, though the level of activity was displaced up or down depending on environmental factors. Understanding the role of these is not helped by the results reported here, although despite the lack of significant correlations, there is a suggestion that moisture content exerts a controlling influence on nitrifier activity. Soil drying in summer appeared to depress nitrifier activity and it is clear from Table 7.1, that those sampling dates for which the quadratics fit least well tend to be in the summer, i.e. the controlling effect of pH on nitrifier activity is being outweighed by some other factor, most probably soil moisture content. However, the work of Kowalenko and Cameron (1976) suggests that the interaction between soil temperature and moisture content makes it very difficult to isolate the effect of one of these factors alone. They found that due to the importance of the temperature-moisture interaction, the optimum moisture content for the nitrifiers appeared to be dependent on temperature.

Higashida and Takao (1985) showed that meteorological factors were not the exclusive cause of changes in microbial numbers in the Japanese soils they studied. Generally, microbial numbers fluctuated in response to the supply of substrates from vegetation. Peaks in bacterial numbers occurred in May when dead winter grass became available for decomposition and mineralization, and in September when roots decayed following the cutting of heading tillers for forage. These results seem to be confirmed by those of Steele *et al.* (1980) who studied a range of New Zealand soils under pasture and suggested that the increased rate of ammonium oxidation in a perfusion experiment, as compared with rates measured by a field technique, was due to the correction of a nitrification-limiting factor in the perfusion system. They suggested that the most probable limiting factor was substrate. Robinson (1963) noted that mineral-N tended to remain at low levels under grass and although this might be explained by the quick removal of mineral-N by grass roots, the low nitrification rates measured were not so easily understood. A series of perfusion experiments with Craigieburn soil, a Yellow Brown Earth, which was amended in the laboratory and field with lime, urea and "enriched" garden soil, indicated that lack of substrate was responsible for the small nitrifier population, and therefore the low nitrification rate. Robinson (1963) commented further that since grass roots remove mineral-N from soil very rapidly, the nitrifiers have to compete for an already low supply of NH_4-N. Total soil N for soil T between 3-9 cm depth was 2.60 mg N g^{-1} or 0.26 % N (Chapter 5) with mineral-N amounting to 0.51 µg N g^{-1}, or only 0.02 % of the total N. This compares, for example, with 0.94 % total N for the Evesham soil studied by Macduff and White (1985), so that the Tokomaru soil must be considered to be low in soil N, both organic and inorganic, i.e. low in substrate available for nitrifiers. Soil T had SNA_{pH} values which ranged from 0.024-0.061 µmol NO_3-N g^{-1} h^{-1}. In contrast, the Evesham soil, sampled from under pasture on the Oxford University farm in England in January, had a soil pH of 7.30 and a SNA_{pH} value of 0.313 µmol NO_3-N g^{-1} h^{-1} (Bramley, unpublished). Thus, even ignoring seasonal effects, the Evesham soil had a nitrification rate at least five times, and up to 13 times, that measured for the Tokomaru silt loam by an identical technique.

iv. Conclusions

Overall it is clear that whilst the factors governing levels of mineral-N and nitrifier activity in soils are broadly the same throughout the world, the degree to which one factor is important in relation to others can vary markedly from one soil to another. This, together with the fact that the nitrifier population is dynamic, leads to the somewhat depressing conclusion that for progress to be made in modelling various aspects of the soil nitrogen cycle, and more importantly, in order to test any models that are produced, specific information on the nitrifying characteristics of the particular soils being modelled is needed. By implication it seems likely that it may not be possible to produce a definitive model which works for all soil types, a view supported by the results of the analysis of spatial variability (Chapter 5 & 6), and also by many of the results reported in the literature (Chapter 2) of experiments which examined the factors known to control nitrification rates.

CHAPTER 8

A FURTHER INVESTIGATION OF THE EFFECT OF MOISTURE ON NITRIFIER ACTIVITY

The results reported in the previous chapter confirmed the suggestion from the literature (Chapter 2) that soil nitrifier activity is largely dependent on the soil pH. However, the spatial analysis (Chapter 6) indicated that SNA and pH were not, in fact, strongly correlated over space. This, together with the seasonality of SNA values (Chapter 7), suggested that variability in some other factor, most likely the soil moisture status, influenced the variability in nitrifier activity in the Tokomaru silt loam. The results of the investigation of spatial variability in nitrifier activity (Chapter 6) suggested that there was little to be gained from crossvariogram analysis of SNA and θ_g in view of the marked difference between their variogram models, and the strongly anisotropic nature of θ_g variability in field No. 6. It was therefore concluded that there was unlikely to be a strong correlation over space between SNA and θ_g. However, as indicated in Chapter 6, the value of these various variogram models may be open to question, and it was therefore considered worthwhile to conduct an experiment to further investigate the effect of soil moisture on nitrifier activity.

It was suggested in Chapter 2 that microbial populations in different soils are adapted to their specific environments. This appeared to be particularly so in the case of soil temperature (Mahendrappa *et al.*, 1966; Nakos, 1984). In Chapter 7 it was found that nitrifiers in different soils were adapted to the prevailing pH of the soil which they inhabited (Figures 7.6 and 7.7) to the extent that they operated at near-optimum levels at the ambient soil pH, providing substrates were in satisfactory supply. In view of these findings, it seemed likely that the nitrifiers might readily adapt to changes in the soil moisture status. Mindful that the SNA technique involves wet incubation, it was considered that rather than investigating the moisture relations of nitrifier activity using a new technique which would account for the possibility of a flush of microbial activity upon rewetting dry soil samples (Birch, 1958; 1960), more useful information would be gained if soil samples

could be maintained at specific moisture contents for a considerable period of time before measuring their nitrifier activities by the usual SNA technique. By using such an experimental design, it was hoped to give the soil nitrifiers long enough to come to equilibrium with the moisture status of soil samples which had been maintained at constant moisture contents for a considerable period of time. Thus, any differences between the measured nitrifier activities in the various samples should reflect the true response of the nitrifiers to their moisture environment.

The literature contains a number of reports of the effect of soil moisture content on nitrification and mineralization (e.g. Kowalenko & Cameron, 1976; Higashida & Takao, 1985) but as has been pointed out by numerous authors (e.g. Miller & Johnson, 1964; Dubey, 1968; Sabey, 1969), the usefulness of these to other workers is limited in the absence of accompanying information on the moisture characteristics of the specific soils being studied. For this reason, in addition to the main experiment, the soil moisture characteristic curve for the Tokomaru silt loam, as sampled from field No. 6, was also determined.

i. Methods and Materials

Soil sampling

On 6 July, 1988, a bulk soil sample (approx. 10 kg) was taken from a randomly chosen site in field No. 6 in the 3-9 cm depth range. The soil was sieved and stored as described in Chapter 4.

Analyses

Soil moisture characteristic. Approximately 30 g sieved soil was loosely packed into a Haynes apparatus and the head of water adjusted to 70 cm (equivalent to -0.07 bars or -7 kPa). After equilibration for 24 hours, duplicate samples (approx. 5 g) were taken, and their moisture contents were determined on a gravimetric basis by oven drying overnight at 105 °C. Further samples (approx. 10 g each) were placed on pressure

plates (3 replicates each). The pressure was adjusted to 1, 2, 5, and 15 bars. The apparatus was left to equilibrate for 2 days in the case of the 1 bar plate, for 4 days in the case of the 2 and 5 bar plates, and for a week in the case of the 15 bar plate. Following equilibration the gravimetric moisture content of each sample was determined as before.

Moisture relations of nitrifiers. Immediately following field sampling, the nitrifier activity of a subsample (St) was determined by SNA measurement (10 replicates) following the standard method (Chapter 4). Two subsamples (approx. 200 g) were placed in plastic jars. The soil in one of these was saturated (Sat), and the soil in the other left in the field-moist state (F). The mass of each jar plus soil was recorded, and they were then tightly sealed. The lid of each jar was pierced with two pin holes to allow for gaseous exchange, and both jars were stored as detailed below. The remainder of the bulk sample was air-dried in a drying room kept at a constant temperature (20 °C). At intervals of approximately twelve hours, the drying process was stopped by placing the soil in a sealed plastic bag at 3 °C, and the moisture content of a subsample was determined. When drying had taken place long enough to obtain moisture contents of approximately 0.30, 0.21, 0.19, 0.17 and 0.16 g g^{-1} (treatments 6,5,4,3 and 2 respectively), a 200 g sample of soil at each of these moisture contents was placed in jars as described. The remainder of the sample was allowed to become completely air-dry, its moisture content was determined and a further 200 g subsample placed in a sealed jar. Thus, there were eight jars containing soil samples with moisture contents ranging from saturation to air-dryness.

The jars were stored next to the outside wall of an unheated and little-used laboratory for 124 days, which was assumed to be sufficient time for the nitrifiers to adjust to the moisture status of the various soil samples. It was further assumed that the temperature of storage approximated the field air temperature, and that there was no significant difference in the soil temperature between treatments . At weekly intervals the jars were weighed, and when necessary, the mass was adjusted to its original level by adding water drop-wise from a pipette. It was never necessary to add more than 0.08 g water to any jar.

At the end of the 124 day storage period, the moisture content of each sample was determined. Four subsamples (approx. 6 g each) were removed from each jar, and were extracted with 2 M KCl for analysis of exchangeable ammonium (Chapter 4). The NO_3-N concentration of each extract was also determined. The remainder of each sample was leached overnight with 1 dm^3 0.005 M KCl and following removal of the excess moisture by suction filtration for 90 minutes, SNA measurements (15 replicates per sample) were made in the usual way (Chapter 4).

ii. Results

Soil moisture characteristic

Figure 8.1 shows the plot of moisture content *vs.* suction (bars). The data were fitted by least squares optimization with a power function of the form (Figure 8.1; $R^2 = 0.99$):

$$\Gamma = -(0.241/\theta_g)^{5.878} \qquad (8.1)$$

where Γ is the suction (bars) and θ_g is the gravimetric moisture content as before.

Moisture relations of nitrifiers

In view of the good fit of equation (8.1) over the range of values measured, it was used to calculate the moisture tension of the soil in the eight jars on the basis of their gravimetric moisture content at the end of the 124 day period. However, because of the exponential nature of the curve, it did/could not give a good estimate of the moisture tension in the air-dry sample ($\theta_g = 0.035$ g g^{-1}), predicting a value of the order of -80,000 bars ! An estimate was made of the relative humidity of the drying room (D.R. Scotter, Dept. Soil Science, Massey University - personal communication), and the moisture tension of the air-dry sample was calculated using the equation:

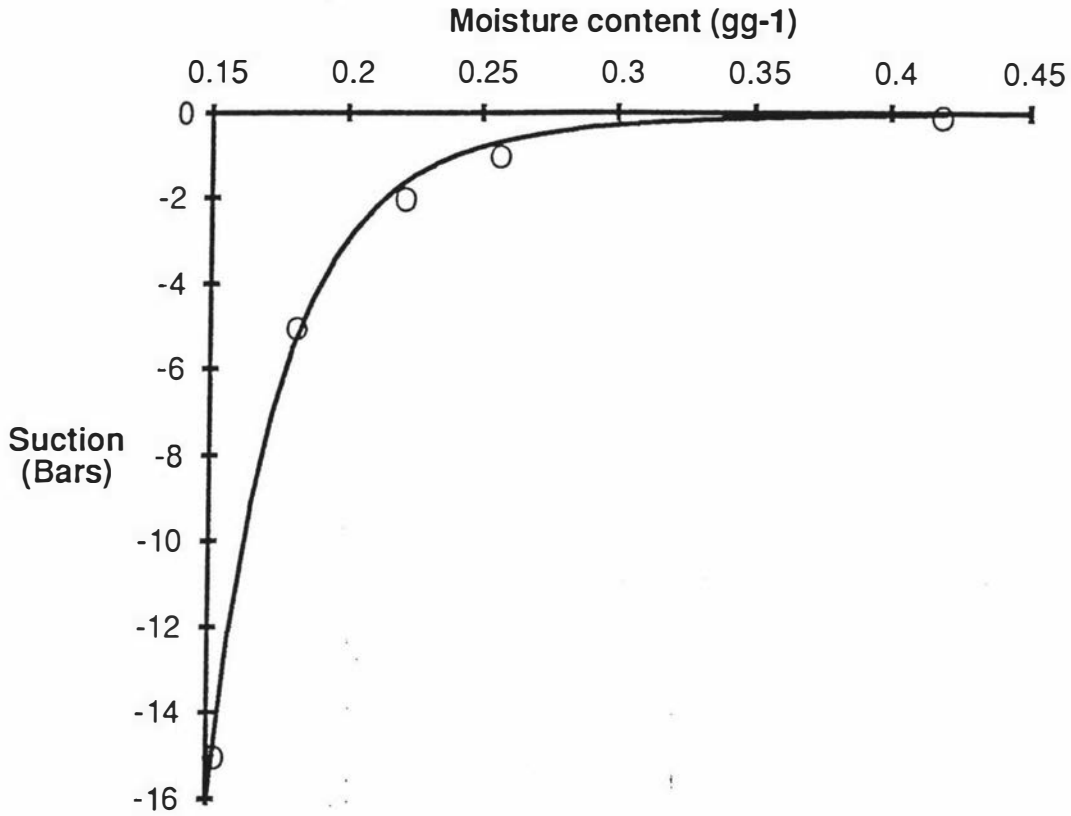


Figure 8.1 Moisture characteristic curve for the Tokomaru silt loam (sieved < 2 mm)

$$\Gamma = \frac{\{\rho RT\}\{\ln(e/e_o)\}}{M} \qquad (8.2)$$

where ρ is the density of water (1000 kg m^{-3}), R is the gas constant (8.31 J K^{-1} mol^{-1}), T is the temperature (Kelvin), M is the molar mass of water (0.018 kg mol^{-1}), e/e_o is the relative humidity and Γ is the water potential (Pascals; 1 bar = 10^5 Pa). Using this equation, the moisture tension of the air-dry sample was estimated as approximately -980 bars. It was assumed that the moisture content of the air-dry sample did not change in the time between the completion of air-drying following field sampling and the end of the 124 day storage period (there was no change in the mass of the jar + soil over the 124 days). The values of the moisture tension (bars) for each of the eight samples were converted to units of pF, where F denotes the free energy of the soil water in cm (1 bar = 1020 cm) and pF = $\log_{10}$F. Values of pF for the different soil moisture treatments are shown in Figure 8.2a together with the values for SNA, NO_3^-, Ex-NH_4^+, θ_g and incubation pH (Figure 8.2b-f).

In view of the difference in incubation pH between the saturated sample and the others, the SNA values of all treatments were adjusted to account for the effect of pH change using the concept of the relative nitrification rate RNR (Darrah *et al.*, 1986b). A mean value for C (the SNA value at pH 0) was calculated from the values obtained by fitting equation (7.3a) to the six pH experiments done on soil T (Chapter 7). RNR values were then determined by dividing the values of SNA_{pH} calculated over a range of pH using equation (7.3a), by the value of SNA at pH 5.92, the pH optimum for soil T, calculated from the same equation, that is:

$$RNR_{pH} = \frac{SNA_{pH}}{SNA_{opt}} = \frac{-0.45826 + 0.17088pH - 0.01443pH^2}{0.04763} \qquad (8.3)$$

and thus:

$$RNR_{pH} = -9.62125 + 3.58765pH - 0.30296pH^2 \qquad (8.4)$$

Measured SNA values were then divided by the value of RNR appropriate to the measured incubation pH. The mean values are shown in Figure 8.2b.

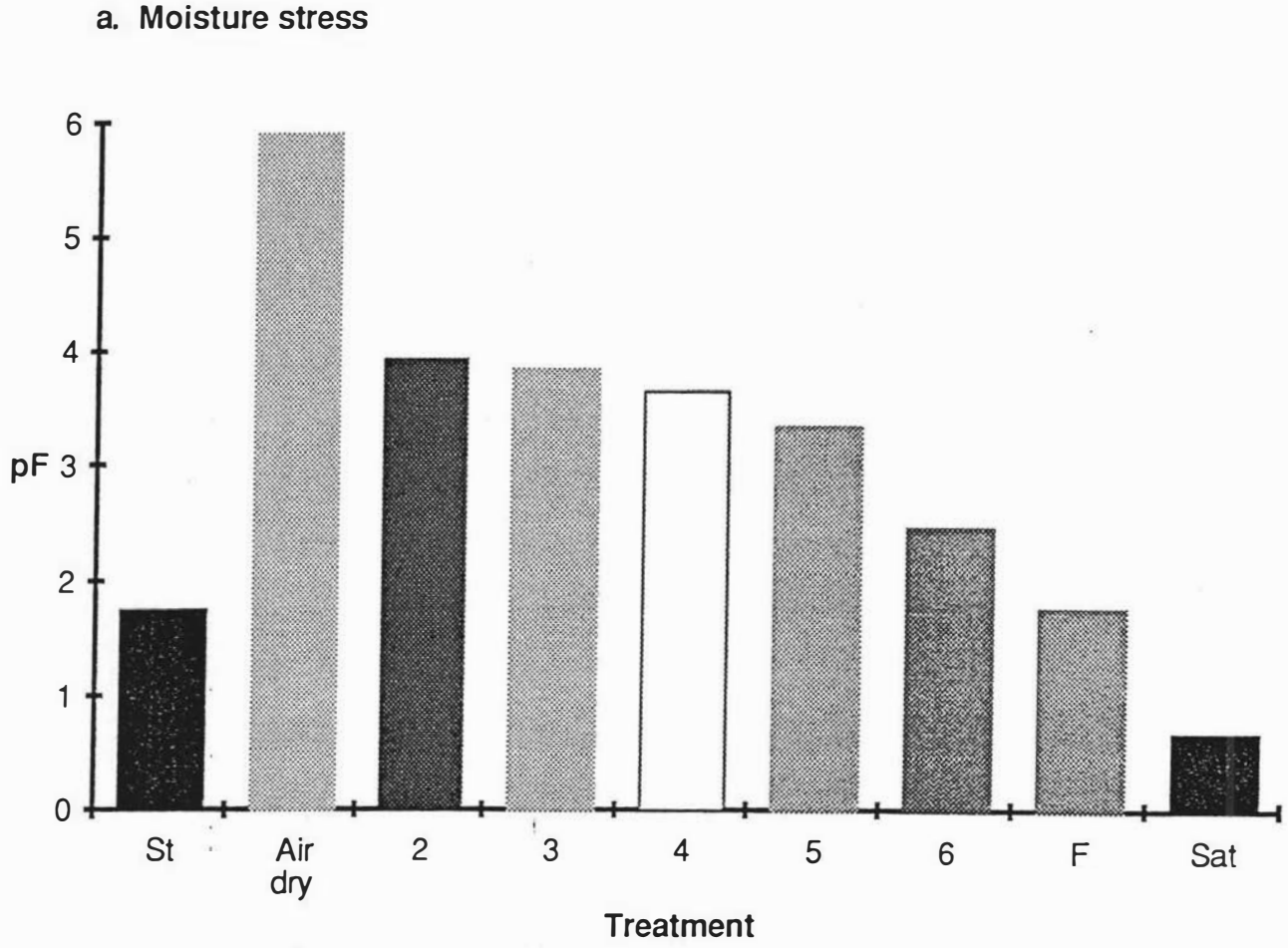


Figure 8.2 Levels of (a) soil moisture stress, (b) nitrifier activity, (c) NO_3^-, (d) $Ex\text{-}NH_4^+$, (e) gravimetric moisture content and (f) incubation pH in the eight samples stored for 124 days

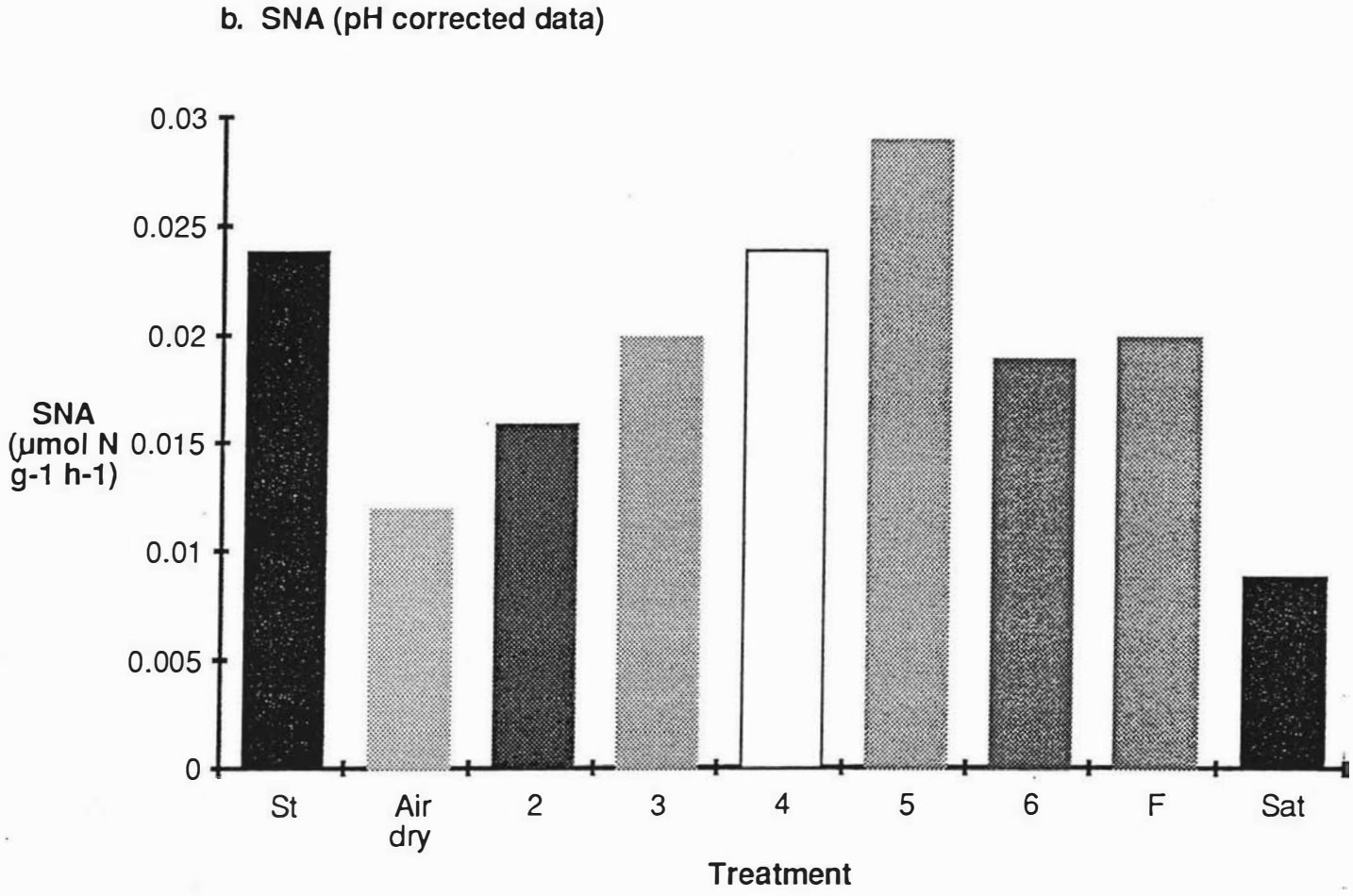
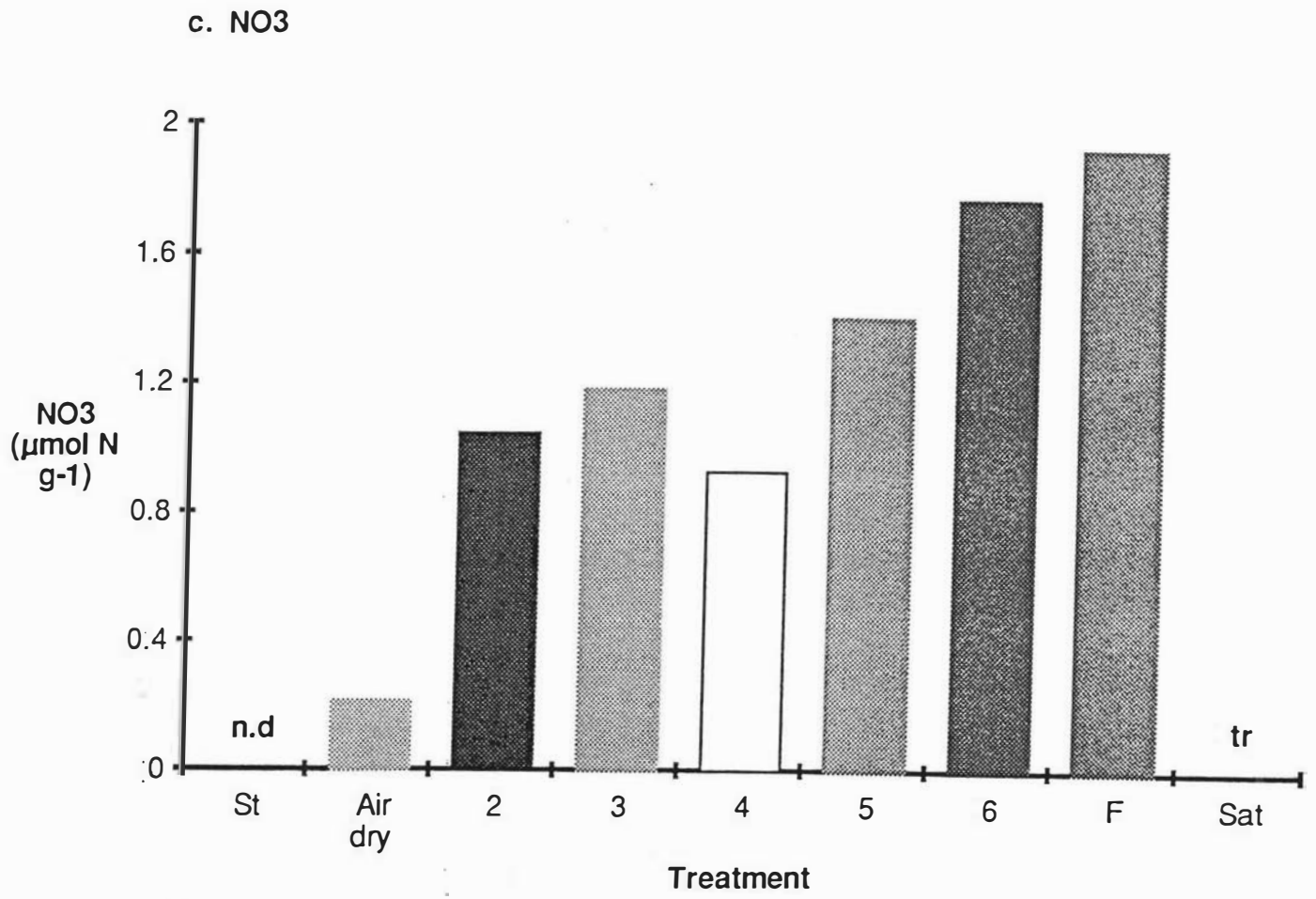


Figure 8.2 (Contd)



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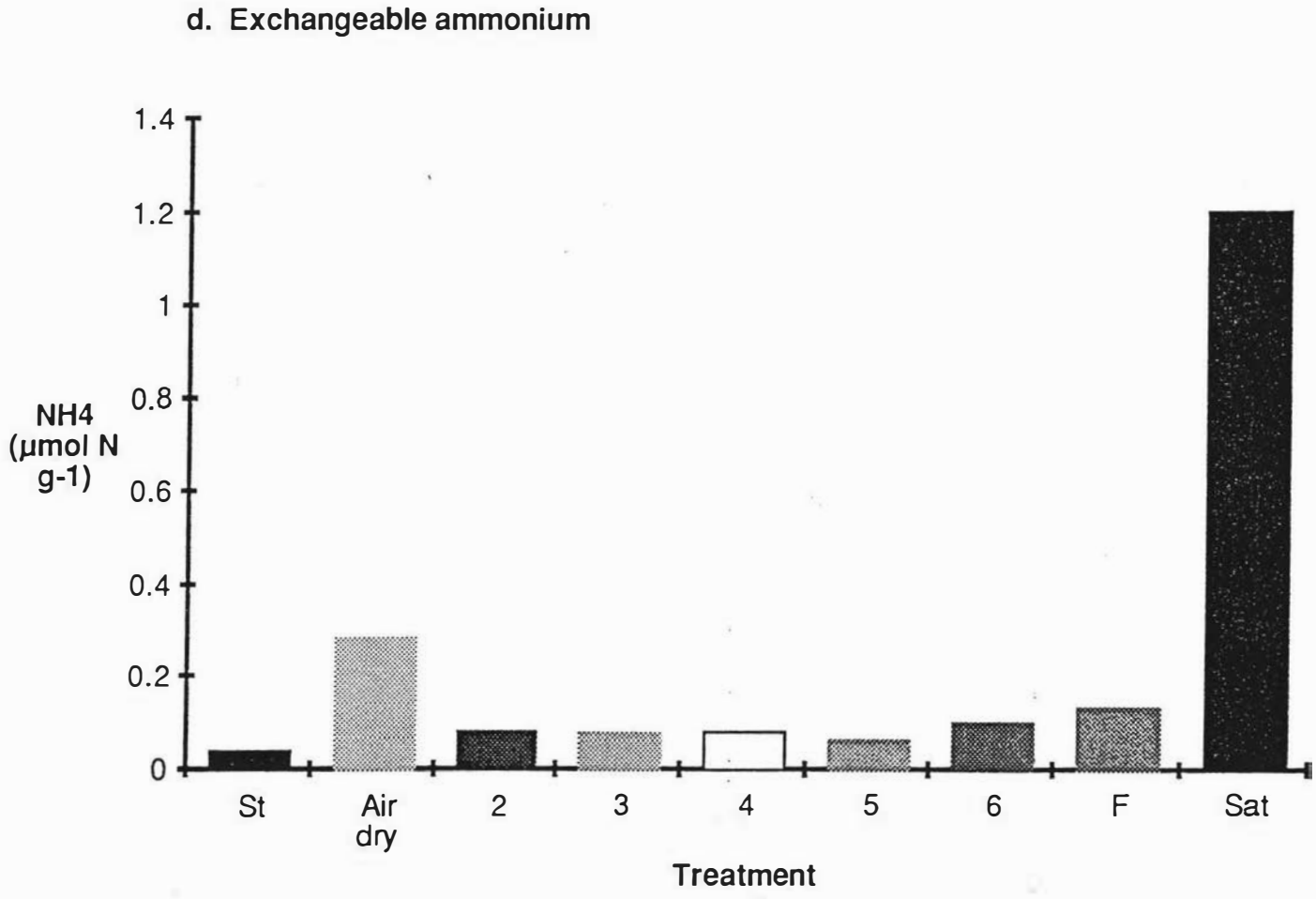

Figure 8.2 (Contd)

e. Moisture content

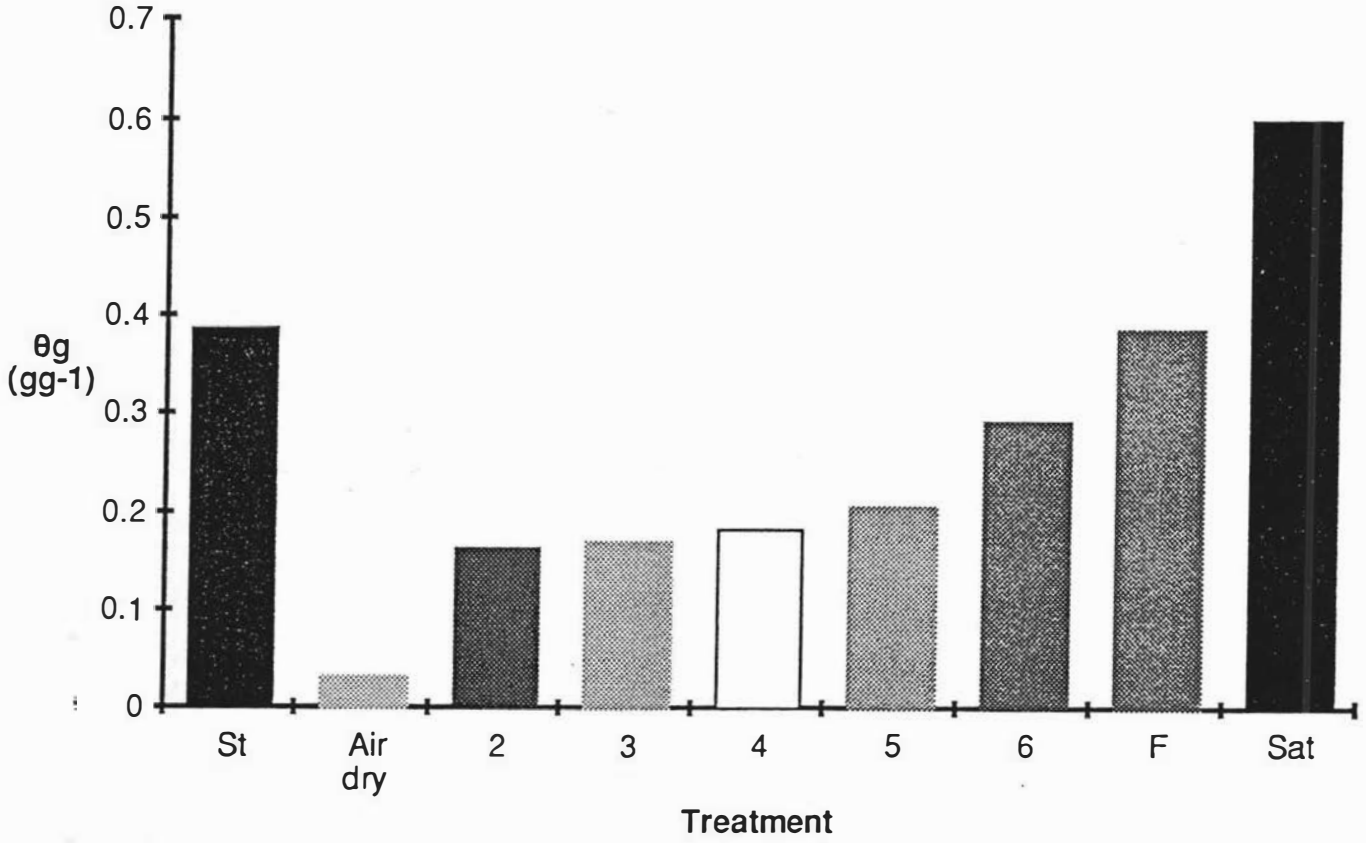

Figure 8.2 (Contd)

f. Incubation pH

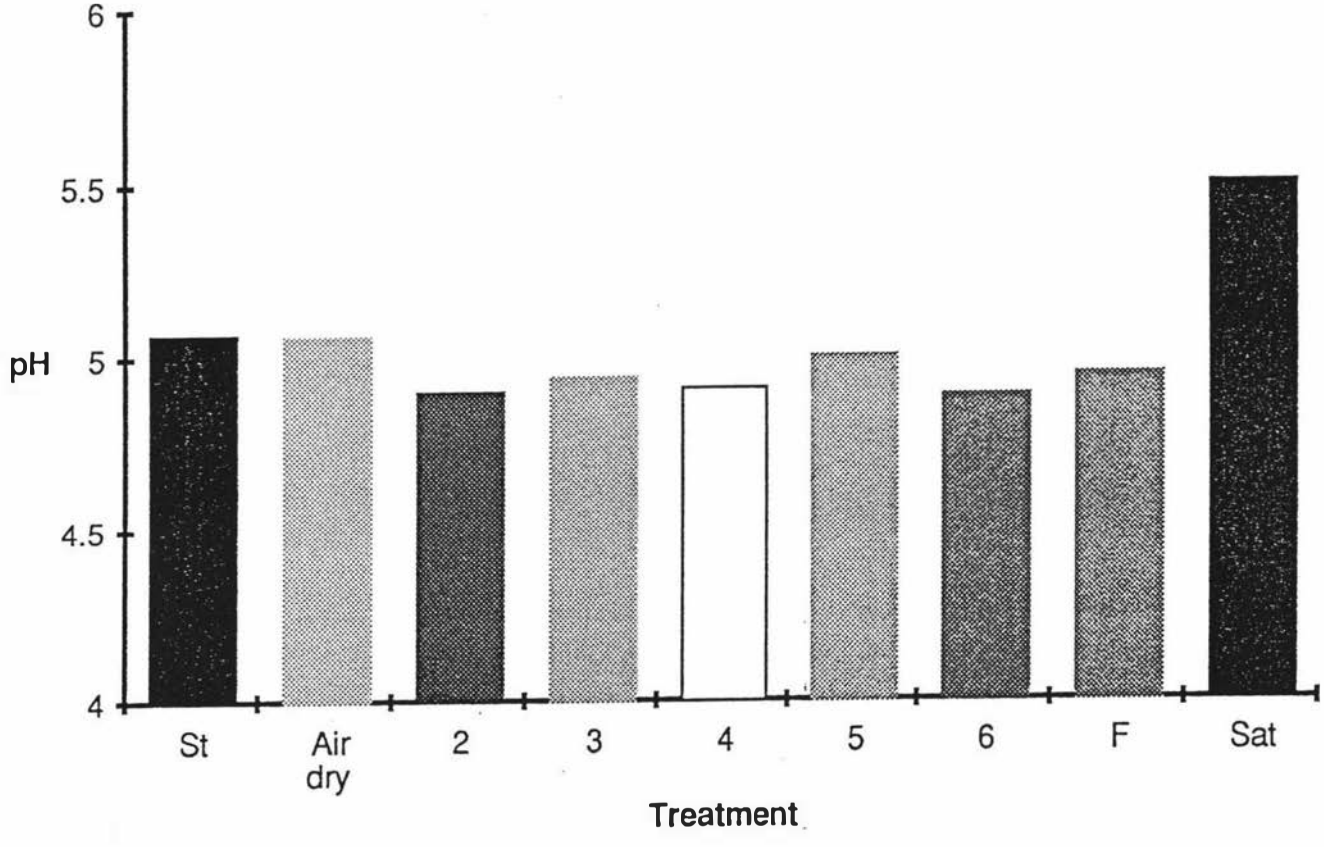

Figure 8.2 (Contd)

At the end of the 124 day period it was observed that all the samples except the saturated one had maintained the appearance of sieved soil. The saturated sample in contrast, had lost all structure and had become a pungent black mud. Nitrate had accumulated in all but the saturated sample. Since there could have been no leaching under the conditions of the experiment, the absence of NO_3^- in the saturated sample was probably due to a very low nitrifier activity and also the occurrence of denitrification, reflecting the anaerobic nature of the sample. Indeed, the suggestion of low nitrifier activity (relative to ammonifier activity) in the saturated sample is supported by the accumulation of NH_4^+ although this could also be due to low immobilization rates caused by the lack of microbial growth. In view of the lack of true replication of treatments in this experiment, paired sample t-tests were used to identify any significant differences between the mean values of SNA, NO_3^- and NH_4^+ for each treatment. Mean values and the levels of significant differences ($p<0.1$, 1 and 5%) between them are given in Table 8.1.

Other than for the difference between the mean SNA values of the air-dry treatment and treatment 2, which were significantly different at $p<1.0\%$, mean values of SNA, NO_3^- and Ex-NH_4^+ in the air-dry and saturated treatments were significantly different ($p<0.1\%$) from all other treatments in addition to each other. Mean SNA and NO_3^- values were generally significantly different between treatments, and in the case of NO_3^- the level of the significant difference was greatest between samples whose difference in moisture status was greatest. Ex-NH_4^+ was not significantly different ($p<0.1\%$) between any treatments other than the saturated and air-dry samples, and differences amongst treatments tended to be either insignificant or only significant at the $p<5.0\%$ level. This observation supports previous findings (Chapter 2) that the nitrification rate tends to follow the rate of mineralization. If this were not the case, then those treatments which showed higher (or lower) SNA values might have been expected to have lower Ex-NH_4^+ contents.

Table 8.1 Mean values of SNA (μmol N g^{-1} h^{-1}), initial NO_3^- and Ex-NH_4^+ (μmol N g^{-1}) in soil samples kept for 124 days at different moisture tensions and the significance of differences between the means

Treatment	Air-dry	2	3	4	5	6	F	Sat
Mean SNA	0.012	0.016	0.020	0.024	0.029	0.019	0.020	0.009
Mean NO_3^-	0.225	1.054	1.190	0.935	1.415	1.718	1.936	0.040
Mean NH_4^+	0.290	0.087	0.084	0.085	0.068	0.107	0.138	1.213
Air dry		b a a	a a a	a a a	a a a	a a a	a a a	a a a
2			b c n.s	a c n.s	a c c	n.s a n.s	b a c	a a a
3				a a n.s	b n.s n.s	n.s a n.s	n.s a b	a a a
4					n.s c n.s	b a n.s	a a c	a a a
5						b n.s b	b c b	a a a
6							n.s b n.s	a a a
F								a a a

Differences between the mean values are significant at: a, p<0.1%
b, p<1.0%
c, p<5.0%

n.s denotes no significant difference at p<5%

The finding that the SNA value in treatment 5 (θ_g = 0.209 g g^{-1}, pF = 3.37) was significantly different from the SNA values of all other treatments (except treatment 4) at a level of significance of at least $p<1.0\%$ (the only treatment for which this was so), together with the observation from Figure 8.2b that treatment 5 appeared to approximate the optimum moisture conditions for nitrifier activity, suggested that such an optimum could be identified in the same way that it was possible to identify an optimum pH for nitrifier activity (Chapter 7). Values of SNA were therefore plotted against pF for all treatments (not including the SNA measurements made immediately after sampling), and the data were fitted by least squares optimization with the quadratic equation (Figure 8.3; R^2 (adjusted for the degrees of freedom of the regression) = 0.43, $p<0.1\%$):

$$SNA = 0.00222 + 0.01195pF - 0.00176pF^2 \qquad (8.5)$$

which predicted maximum nitrifier activity at pF 3.39 (i.e. at a moisture status approximately equivalent to that of treatment 5). The data for the St sample were not included in fitting equation (8.5) because the difference between its mean SNA value and that of treatment F suggested that storage for 124 days had affected the nitrifier activities of the stored samples; clearly this effect was not present in soil assayed immediately after field sampling.

In spite of the strong significance ($p<0.1\%$) of equation (8.5), the wide range of SNA values at each value of pF (Figure 8.3) suggested that soil moisture stress was not as critical in regulating nitrifier activity as was soil pH (Chapter 7). Indeed, the low value of R^2 indicated that only 43 % of the variance was accounted for in the fitting of equation (8.5) to the experimental data points. Furthermore, as Figure 8.3 shows, the nitrifier activity as predicted by equation (8.5) is expected to be appreciable at moisture levels well outside the limits defined by the wilting point and field capacity.

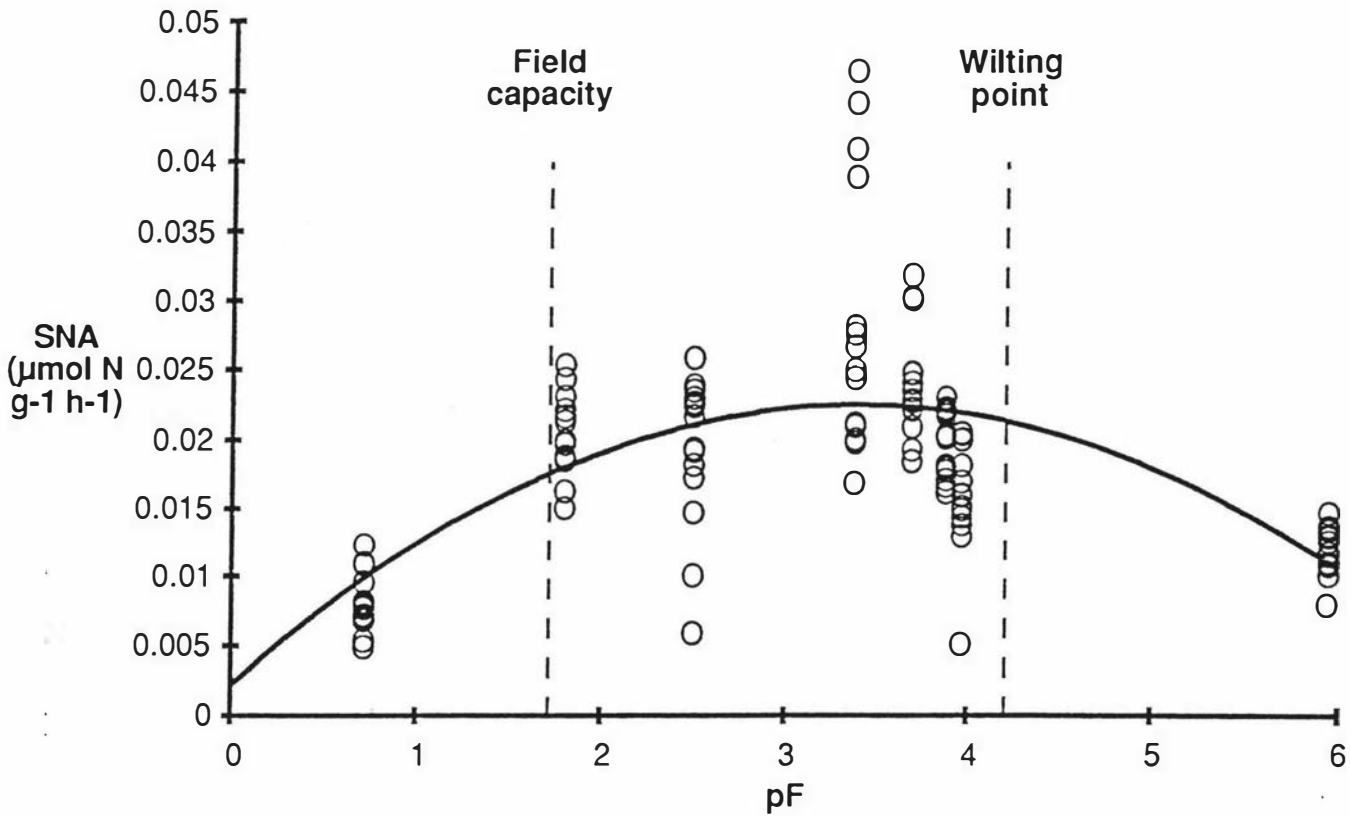

Figure 8.3 pF optimum curve for nitrifier activity in the Tokomaru silt loam

The possibility of relationships between the various properties measured in this experiment was investigated by multiple regression. In some instances strong correlations were found, but the physical and biochemical significance of these appeared to be in doubt regardless of their statistical significance, on account of the extreme values measured in the air-dry and saturated soil samples. For example, as indicated above, the high NH_4^+ concentration in the saturated soil sample could have been due to a low rate of immobilization, with the result that net mineralization appeared to be more efficient than in the other treatments. This effect is well known and together with the fact that autotrophic nitrification effectively ceases under anaerobic conditions, forms the basis of a technique for estimating the nitrogen availability in soils (Bremner, 1965), in which the amount of N mineralized under anaerobic conditions is measured. When the extreme values of the air-dry and saturated samples were omitted from the statistical analysis, no statistically significant relationship was found between any pair of properties.

iii. Discussion

The finding that the soil sample which had been stored in the field-moist state for 124 days had a significantly lower SNA value than the fresh sample (0.015 compared to 0.019 μmol N g^{-1} h^{-1}), together with fact that there was more Ex-NH_4^+ in the stored sample, a similar result to that of Gasser (1961), suggests that the effects of storage may cause complications in the interpretation of the experimental results. This was especially likely in the case of the drier samples (Gasser, 1961; Bartlett and James, 1980). Bartlett and James (1980) warned against studying dried stored soil noting that the behaviour of a dry soil immediately after adding water is different to that of continuously moist soil. There is little to suggest that the results reported here show evidence of a microbial explosion, or *flush* (Birch, 1958; 1960), since the air-dry sample had a significantly lower SNA value than all other treatments except treatment 2, the next driest, and the saturated sample which had a lower nitrifier activity. One could argue that the SNA value of the air-dry sample would have been expected to be even lower in

relation to the other samples, especially as Harris (1980) quoted 40 bars (pF 4.61) as the maximum stress tolerated by nitrifiers. However, the results of Chapter 7 suggested that the initial nitrifier activity of soil sampled in July is expected to be relatively low (i.e. the population was small), probably due to the cold wet winter conditions. Since the only factors which were altered under the conditions of the experiment were the soil moisture status, and the soil temperature which changed according to season and was assumed to be the same in all treatments, any difference in the nitrifier activity between treatments was assumed to be due to differences in the soil moisture status. If the population were initially small, its characteristics might not be expected to change very noticeably, even if it were living under conditions of near-optimum pF. This would especially be the case if some other factor (e.g. substrate) was limiting. It would therefore be of interest to repeat this experiment with soil sampled at a time of high nitrifier activity, such as April, when one presumes that substrate levels are relatively high and that the population is both large and active, to see whether the difference between the SNA value of air-dry soil following 124 days of storage and that of soil kept at a near optimal moisture status was of a similar order of magnitude to that observed here.

Bartlett and James (1980) commented that air-drying leads to an increase in the amount of water-soluble organic matter that may be extracted upon rewetting. Thus, the amount of substrate available to ammonifiers and nitrifiers on remoistening would be expected to be higher following drying. However, since an excess of NH_4^+ substrate is supplied in the SNA technique, it is unlikely that a flush of nitrifier activity would be observed under the conditions of the incubation. Even if a blank incubation had been carried out, a microbial flush would still probably not have been observed, as the pre-leaching would have removed most of this new substrate, and since nitrifiers are slow to regenerate (Aleem & Alexander, 1960, Sarathchandra, 1978), SNA values measured over an eight hour incubation such as used here would not be expected to reflect high activity immediately after rewetting. Thus, the SNA technique can be considered to be entirely satisfactory for use in this kind of investigation.

Miller and Johnson (1964) incubated soils at 30 °C for 14 days under moisture tensions ranging from zero to air-dryness, and found maximum nitrification to occur between 0.5 and 0.15 bars (pF 2.70-2.18). They also measured nitrification at tensions greater than 15 bars (pF 4.2) but at very low rates, and noted that ammonification occurred at higher rates than nitrification at both high and low tensions; NH_4-N built up in both air-dry and saturated samples. Sabey (1969) found the optimum moisture tension for nitrification in a limed loessial silt loam to be of the order of 0.1 bar (pF 2.01), whilst Dubey (1968) studied two sandy loams and found nitrification to be greatest at 2 bars (pF 3.31). He also noted marked nitrification under flooded (saturated) conditions. Whilst the saturated sample tested here had the lowest SNA value, it was interesting to note that the anaerobic conditions did not kill off the nitrifier population - a possible reflection of some heterotrophic nitrifier activity in this soil (Focht & Verstraete, 1977). Mahli and M^{c}Gill (1982) found that appreciable nitrification occurred at the permanent wilting point in a loam, a silt loam, and a silty clay loam from Alberta, which is in agreement with the results of this experiment (Figure 8.3), and found nitrification to be at a maximum at -33 kPa (pF 3.53) for all soils. A summary of published results is given in Table 8.2.

The difference between the SNA values reported here and those calculated from the results of Yadvinder-Singh and Beauchamp (1988) and Mahli and M^{c}Gill (1982) at comparable moisture tensions (Table 8.2) is further evidence of the marked difference in nitrifier activities between different soils. The range in apparent pF optima, pF_{opt}, for nitrifier activities in soils of differing texture (Table 8.2) suggests that some factor related to the particle size distribution is important in controlling the moisture relations of nitrifier activity. However, the results of Mahli and M^{c}Gill (1982) for sandy loam and silty loam soils (pF_{opt} = 3.53) contrast markedly with the result for the sandy loam of Reichmann *et al.* (1966) and the silty loam studied by Sabey (1969) which had values of pF_{opt} of 2.31 and 2.01 respectively. It may well be therefore that it is soil structure which is of greatest importance in controlling the nitrifier response to changes in the soil moisture status. Brandt *et al.* (1964) found that rates of NH_4^+ oxidation were

Table 8.2 Summary of results for the suggested optimum moisture tension for nitrification

Reference	Soil texture	Suggested Optimum pF	Nitrification rate* (μmol N g^{-1} h^{-1})
Miller & Johnson (1964)	Silt loam Clay loam	range of 2.18-2.70	
Reichmann *et al.* (1966)	Sandy loam Loam	2.31	
Dubey (1968)	Sandy loam Loamy sand	3.30	
Sabey (1969)	Silt loam	2.01	
Mahli & McGill (1982)	Sandy loam Loam Silty clay loam	3.53	0.010[a]
Yadvinder-Singh & Beauchamp (1988)	Clay Silt loam	3.55	0.326[b]
This Chapter (Equation 8.5)	Silt loam	3.39	0.023[c]

*Nitrification rates estimated from the results of incubations of [a]6 days, [b]16 hours following 35 days at 15 °C and [c]8 hours standard SNA

directly related to the level of oxygen supply as determined by the oxygen diffusion rate, although he also found that the independent effects of aggregate size and moisture content on N transformations could not be explained by measured differences in the oxygen diffusion rate. The sieved soil samples used in this study could not be said to have structure *sensu stricto*, although one might argue that the observed difference between the saturated sample, which resembled a mud, and the soil in the other treatments which retained its sieved appearance, was a structural difference. However, since all the treatments other than the saturated one maintained their sieved *structure*, the differences in SNA values between them could not be attributed to structural differences, and accordingly, the low SNA value of the saturated treatment was attributed to the anaerobic conditions caused by the high moisture content rather than any loss of structure. It would therefore be of interest to repeat this experiment with dispersed or ground soil samples, and see if any differences between measured nitrifier activities were similar to those observed here.

Macduff and White (1985) concluded that their model for predicting nitrification and mineralization rates on the basis of soil temperature and moisture content was biased in terms of the weight attached to soil moisture. Kowalenko and Cameron (1976) did not account for the soil moisture characteristics of the soil they studied, but found that it was not possible to distinguish between the effects of temperature and moisture on nitrification rates on account of the strong interaction between these properties. In view of these findings, together with the small range in mean SNA values between field capacity and the wilting point (Figure 8.3) and the resultant weakly defined pF optimum for soil T (Figure 8.3), it appears that Macduff and White (1985) were probably correct in stating that the form of the relationship between soil moisture and nitrification rate (and by implication, nitrifier activty) is dependent on the moisture holding characteristics, porosity, organic matter content and pH, and that as a consequence, it varies considerably between soils.

CHAPTER 9

HETEROTROPHIC NITRIFICATION - EXACTLY WHAT HAS BEEN MEASURED BY THE SNA ?

As was stated in Chapter 1, one of the attractions of the SNA technique to a project such as this, was that because SNA values are determined on the basis of the *net* rate of nitrate production, the possibility that the SNA value represents the activity of a range of species, rather than that of a single one, can be ignored. In terms of the difficulties of modelling nitrate leaching caused by the spatial variability of the concentration of soil nitrate, accounting for differences in the activity of different species of nitrifiers is of little importance, providing that the species present contribute towards the measured net activity in similar proportions to one another under the conditions of the SNA as they do in the field. This is because it is the amount of nitrate which is present and leachable that is crucial to the nitrate leaching model, and not necessarily the means by which the nitrate is produced. The biochemistry and microbiology of nitrification in the field have not been a main concern at any stage of this project. However, the broad assumption throughout has been that the activity measured by the SNA has been that of *autotrophic* nitrifiers; *heterotrophic* organisms have been ignored. It follows from the preceding discussion that whether or not heterotrophic nitrification is significant is of little consequence to the spatial variability studies (Chapters 5 and 6) providing it is measured under the conditions of the SNA. Nevertheless, it may have considerable implications in the study of the pH and moisture relations of nitrifiers because the degree to which soil properties affect the activity of nitrifiers may not be the same for heterotrophs as for autotrophs. As a consequence, one mode of nitrification may mask the effects of unfavourable conditions on the other. For example, nitrification in acid forest soils is predominantly heterotrophic (Schimel *et al.*, 1984; Adams, 1986a; 1986b).

Heterotrophic soil micro-organisms such as *Arthrobacter spp.* and *Aspergillus spp.* (Focht & Verstraete, 1977) will produce NO_3^- from organic substrates if the N content of these substrates is sufficiently high (Wild, 1988), presumably such that it is in excess of the amount required for protein synthesis. In addition, heterotrophic micro-organisms will produce NO_2^- from NH_4^+ if the latter is at a high enough concentration and providing that an appropriate carbon source is also present (Focht & Verstraete, 1977, Wild, 1988). Assuming that the concentration of the added ammonium in the SNA is at least as great as it is in the field, and/or that the N content of the soil organic matter is high enough, the activity of heterotrophic nitrifiers (if present) will be accounted for under the conditions of the SNA. However, given that the amount of Ex-NH_4^+ in the Tokomaru silt loam is low (Chapter 5), it seems unlikely that the heterotrophic production of NO_2^- from NH_4^+ will be significant. Furthermore, if the N content of the soil organic matter is not high enough for NO_3^- production, then this form of heterotrophic nitrification will also be insignificant in the field, and will therefore not be measured in the SNA.

Ishaque and Cornfield (1972) studied nitrification in two "tea" soils in Pakistan with pH values of 4.1 and 4.2. They observed an increase in the nitrification rate in the pH range 4-5 but a decline with further increases in soil pH, which they concluded was evidence of either an adaption of the nitrifiers to the acid nature of the soils (*c.f.* Chapter 7), or alternatively, was evidence of heterotrophic nitrifier activity. Examination of the micro-organisms present in these soils indicated a complete absence of autotrophic nitrifiers leading to the conclusion that heterotrophs were entirely responsible for the measured nitrification. The plots of SNA *vs.* pH shown in Figures 7.3 and 7.4 suggest that significant nitrification occurs in the Tokomaru silt loam at pH values below that regarded in the literature as the likely lower limit for nitrification (Chapter 2). Indeed, Schmidt (1982) noted that most observations reported on the literature indicated a lower limit for nitrification of pH 4. The cessation of nitrification in the Tokomaru silt loam is predicted by equation (8.4) at a pH of approximately 4.1 (Figure 9.1a). Although this is in agreement with the summary conclusions of Schmidt (1982), it is not inconsistent with the results of Ishaque and Cornfield (1972). Thus, there was a suspicion that some of the nitrifier activity measured in the Tokomaru silt loam may have been heterotrophic.

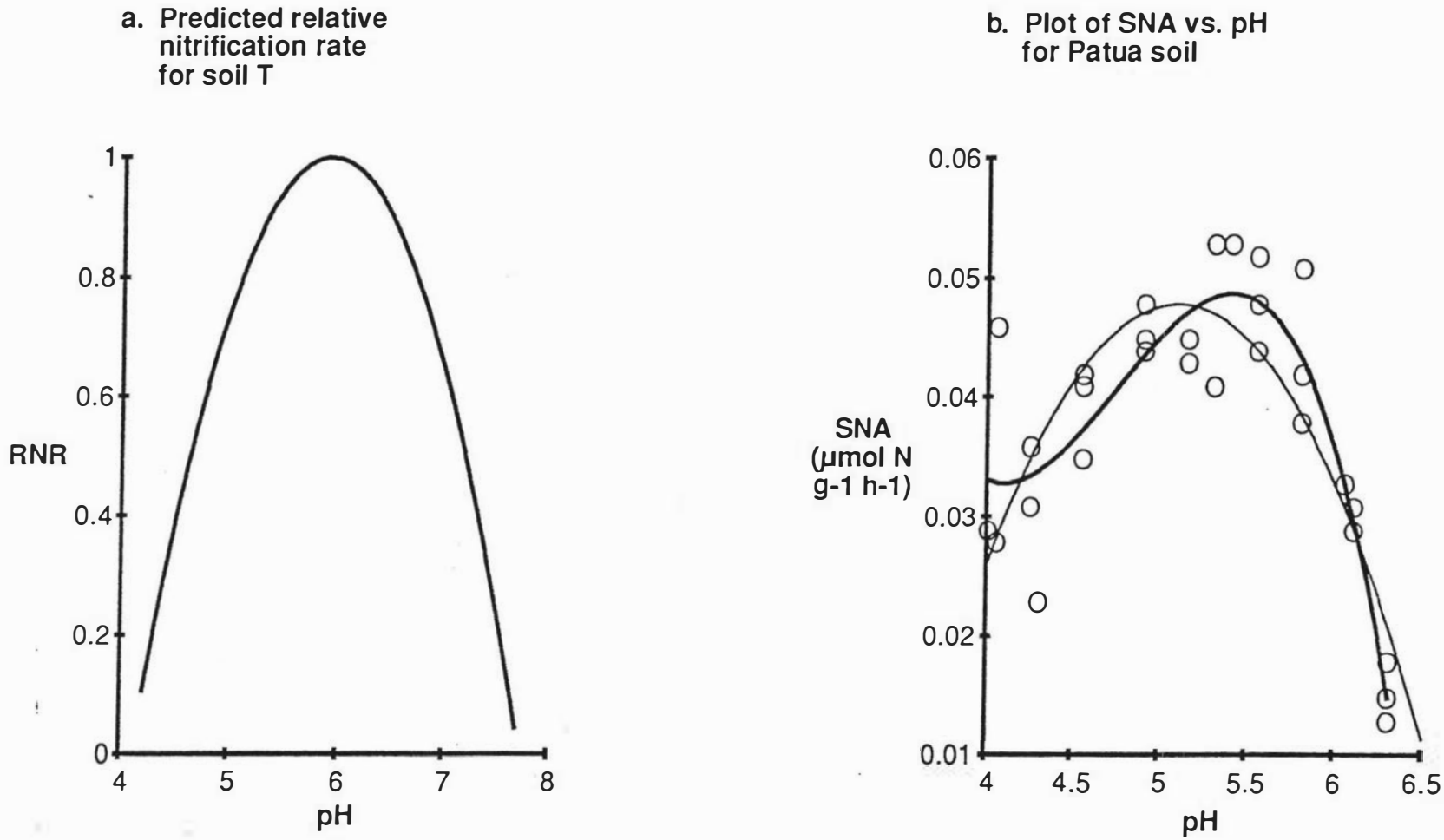

Figure 9.1 (a) Realtive nitrification rate in the Tokomaru silt loam at different soil pH and (b) a pH optimum curve for nitrifier activity in the Patua soil

When the pH work was started, the pH relations of nitrifier activity in the Patua soil were also investigated. This strongly leached yellow brown loam (N.Z. Soil Bureau, 1968), which was sampled from the lower slopes of Mt Egmont, had a pH of 4.9, and on the basis of the fitted quadratic equation (Figure 9.1b, light line; R^2 = 0.65, $p<0.1\%$) had a pH_{opt} of 5.09. However, as can be seen from Figure 9.1b, this soil exhibited considerable nitrifier activity at low pH, so much so that the relationship between SNA and pH was better described by a cubic equation (Figure 9.1b, heavy line; R^2 = 0.79, $p<0.1\%$). Whilst this model may be of dubious theoretical standing due to the prediction of increasing SNA with increased acidity below a pH of about 4, it does nevertheless suggest that at a certain critical pH, a minimum SNA value may be reached, and that further increases in the level of acidity will have negligible effect on the nitrifier activity. This was taken to be an indication of heterotrophic nitrifier activity. Unfortunately the cost of obtaining samples of the Patua soil at regular intervals for a study similar to that described in Chapter 7 meant that no further experiments were done using this soil. However, in view of the possibility of heterotrophic nitrifier activity as indicated by both Figure 9.1a and 9.1b, it was considered important to try to establish the extent of heterotrophic nitrification (if any) in the Tokomaru silt loam. It was decided that a suitable means of doing this would be to carry out a series of SNA incubations using a range of substrates including peptone (Van de Dijk & Troelstra, 1980; Schimel *et al.*, 1984; De Boer *et al.*, 1988). The assumption was made that any nitrifier activity measured in a medium with peptone as the only substrate, would be an indication of heterotrophic nitrification.

i. Methods and materials

On 11 June, 1988, two bulk soil samples (approx. 5 kg each) were taken from field No. 6; one between 0 and 3 cm depth and the other from the 12-15 cm depth range. The samples were sieved and stored as before. 6 g subsamples (oven-dry equivalent) were taken from each sample (5 replicates each) and extracted with 2 M KCl for analysis of exchangeable ammonium (Chapter 4).

A 200 g subsample of soil from each depth was prepared for SNA incubation by overnight leaching with 0.005 M KCl as before (Chapter 4). Following removal of the excess moisture by suction filtration for 90 minutes, 5 g subsamples (oven-dry equivalent) were weighed into 64 incubation tubes (32 tubes per sample depth). To each tube, 10 cm^3 of the following treatments were added (8 replicates per treatment per sample depth); (a) 0.01 M $(NH_4)_2SO_4$, the *standard* SNA substrate; (b) 0.01 M K_2SO_4, a *blank* substrate; (c) peptone (Gibco Laboratories; Gelatin hydrolysate, peptone No. 190); and (d) a solution containing both 0.01 M $(NH_4)_2SO_4$ and peptone. All solutions were made up in 0.005 M KCl as before. The total N content of the peptone according to the manufacturers specification was 16 %. The amount of peptone dissolved in 0.005 M KCl (1.73 g dm^{-3}) to make up treatments (c) and (d) was calculated so as to give the same N content in the peptone solution as was in the standard treatment (a). In the case of treatment (d), it was assumed that the NH_4^+ was effectively unavailable to the heterotrophs and the substrate for this treatment was made up by dissolving 1.73 g peptone in 1 dm^{-3} 0.01 M $(NH_4)_2SO_4$. Thus, the concentration of N as peptone and as NH_4^+ was the same in treatment (d) as it was in (a) and (c). Following addition of the substrate, the incubations were carried out as before, and were sampled after 1 and 8 hours. The pH of the incubating media was recorded following the 8 hour sampling.

A complicating factor in this experiment was that excess peptone remaining in the samples taken after 1 and 8 hours incubation was found to upset the chemistry of the analysis of NO_3-N. Accordingly, to a 2 cm^3 subsample of each supernatant, 0.2 cm^3 2 % hydrogen peroxide (H_2O_2) were added. The solutions were mixed and then dried overnight (105 °C) to complete dryness (S.K. Saggar, Dept Soil Science, Massey University - personal communication). Following drying, 2 cm^3 deionized double-distilled water were added to each dry tube. The tubes were thoroughly shaken using a vortex shaker, and the solutions analysed for NO_3-N in the usual way (Downes, 1978). A series of blank (no peptone) treatments with H_2O_2 and standard NO_3-N solutions demonstrated that this procedure had no effect on the measured NO_3-N concentration of solutions containing no peptone.

On 27 October, 1988, further bulk samples were taken from field No. 6 from both the 0-3 and 12-15 cm depth ranges, and the experiment was repeated.

ii. Results

As was the case in the moisture stress experiment (Chapter 8), the incubation pH was found to vary between treatments, the pH of the standard and peptone incubations being approximately 5.0 and 5.9 respectively. Accordingly, the SNA data were adjusted to account for this variation using equations (8.3) and (8.4). The mean pH-adjusted values of SNA in each treatment are shown in Figure 9.2. Generally, the nitrifier activity between 0 and 3 cm was higher than in soil sampled between 12 and 15 cm depth, which is consistent with the results reported in Chapter 5. The difference between the 0-3 and 12-15 cm samples in treatment (b) was not significant ($p<5\%$), and in the light of the results for the other treatments, and those of the depth experiment (Chapter 5), this result was regarded as an anomaly.

Treatment (b) showed significantly lower ($p<0.1\%$) nitrifier activity in the 0-3 cm sample than treatments (a), (c) and (d) whose SNA values were not significantly different from each other at the $p<5\%$ level of significance. The same applied for the 12-15 cm samples. As might have been expected, the lowest SNA values were measured in the blank treatment (b) (0.006 ± 0.0004 and 0.008 ± 0.0008 µmol N g-1 h-1 for the 0-3 and 12-15 cm samples respectively). The fact that the SNA values measured for treatments (c) and (d) at 0-3 cm (0.021 ± 0.004 and 0.022 ± 0.002 µmol N g^{-1} h^{-1}) were not significantly different from the standard incubation (0.021 ± 0.003 µmol N g^{-1} h^{-1}) makes the interpretation of the results difficult. Nevertheless, the result for treatment (c) suggests that peptone has stimulated heterotrophic nitrifier activity. In view of the low concentration of Ex-NH_4^+ in the field (0.32 ± 0.035 and 0.05 ± 0.007 µmol N g^{-1} at 0-3 and 12-15 cm respectively) and the low content of total N in the soil (Chapter 5), it was assumed that there was insufficient substrate for measurable heterotrophic activity under field conditions.

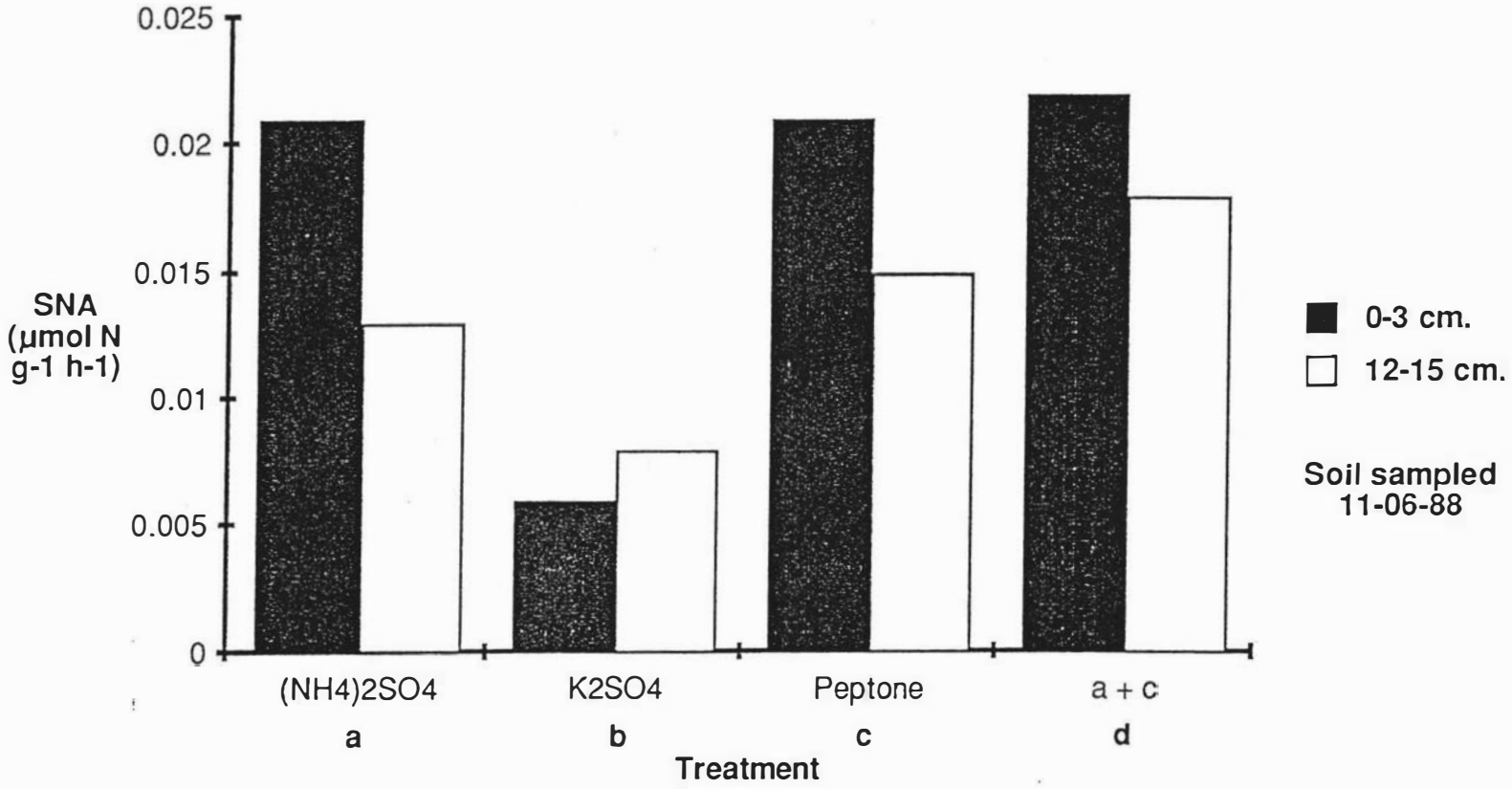

Figure 9.2 Nitrifier activities in soil sampled in June incubated with a range of N-substrates

The SNA value for treatment (b) was therefore considered to represent the activity of an autotrophic population which was surviving on the small amount of substrate available in the field. In treatment (a) the nitrifiers were stimulated by the addition of $(NH_4)_2SO_4$, but as the work reported in Chapter 4 indicated, the nitrifiers did not increase in number during the time of the incubation. Assuming that there was no growth in the nitrifier population in treatments (a) and (b) and that the concentration of enyzme in the ammonium oxidase system was the same, the idea of stimulated activity following addition of NH_4^+ is consistent with the theory of Michaelis-Menten kinetics. However, it is not certain whether the activity measured in treatment (a) was entirely autotrophic, especially since treatment (d) had a mean SNA value which was not significantly different to that of treatment (c).

The latter result indicated that there was no increase in activity in treatment (d) following addition of $(NH_4)_2SO_4$ in addition to peptone, over and above that measured in treatment (c) where the measured activity was assumed to be predominantly heterotrophic. Assuming that the activity measured in treatment (b) effectively occurs in all treatments, it follows that the difference between (b) and (c) (i.e. 0.015 μmol N g^{-1} h^{-1}) represents the *actual* amount of heterotrophic activity in (c). If the measured SNA value in treatment (a) was entirely due to autotrophic activity, then the SNA value of treatment (d) would be expected to be of the order of that measured for (a) plus the difference between (b) and (c), that is, 0.036 μmol N g^{-1} h^{-1}. The fact that this was not the case suggests that there may be some heterotrophic activity in treatment (a). The difference between treatment (c) and the expected value of (d) is equivalent to 0.015 μmol N g^{-1} h^{-1}. Therefore in treatment (a), activity equivalent to 0.006 μmol N g^{-1} h^{-1} (i.e. the difference between 0.021 and 0.015) may be due to heterotrophic nitrifiers; that is, approximately $^1/_3$ of the nitrifier activity measured in the standard incubation may have been heterotrophic. This seems a reasonable interpretation of the experimental results because when 10 cm^3 0.01 M $(NH_4)_2SO_4$ were added to media containing the equivalent of 5 g oven-dry soil, the effective concentration of NH_4^+ substrate increased, in the case of the 0-3 cm sample, from 0.32 μmol N g^{-1}, the field concentration, to 40 μmol N g^{-1} in treatment (a). Thus, the concentration of NH_4^+ may have been great

enough to stimulate the heterotrophic production of NO_2^- from NH_4^+. However, the anomalous result for treatment (b) at 12-15 cm, the unexpected lack of any significant difference between treatments (a), (c) and (d), and the fact that the interpretation of these results is speculative and cannot be regarded as conclusive on the basis of a single experiment, led to the conclusion that it was necessary to repeat the experiment. This was done in late October when, in contrast to June when the first experiment was carried out, nitrifier activity was expected to be high (Chapter 7).

The mean SNA value for treatment (a) in the second experiment (Figure 9.3) reflects the higher nitrifier activity in spring (0.036 ± 0.002 and 0.017 ± 0.001 μmol N g^{-1} h^{-1} for the 0-3 and 12-15 cm depth ranges). This increased activity was most likely brought about by the greater concentration of Ex-NH_4^+ in the soil in October (1.588 ± 0.056 and 0.415 ± 0.064 for the 0-3 and 12-15 cm samples respectively) compared to June. The higher SNA values for the 0-3 cm sample compared with the 12-15 cm sample are again consistent with the findings of Chapter 5. However, as can be seen from Figures 9.2 and 9.3, the pattern of differences between the mean SNA values of each treatment at 0-3 cm is different in the two experiments. In the first experiment, the trend in mean SNA value was (d) ≈ (a) = (c) > (b). In the second experiment the trend is (a) > (c) > (d) = (b) which, ignoring treatment (d), may simply be a reflection of differing seasonal effects on the various species which make up the total soil nitrifier population. If this is not the case, these results cast serious doubt on the interpretation of the results of the first experiment, especially with respect to the relative magnitude of the activity of autotrophs and heterotrophs.

In contrast to the first experiment, the mean adjusted SNA value of the standard incubation for 0-3 cm was significantly different from treatments (b) and (d) at the $p<0.1\%$ level of significance and from treatment (c) at $p<1.0\%$. Treatments (b) and (c) were significantly different ($p<0.1\%$) from each other as were treatments (c) and (d). In the case of the 12-15 cm sample, all treatments were significantly different ($p<0.1\%$) except treatments (b) and (c) which were not significantly different, and treatments (c) and (d) which were significantly different

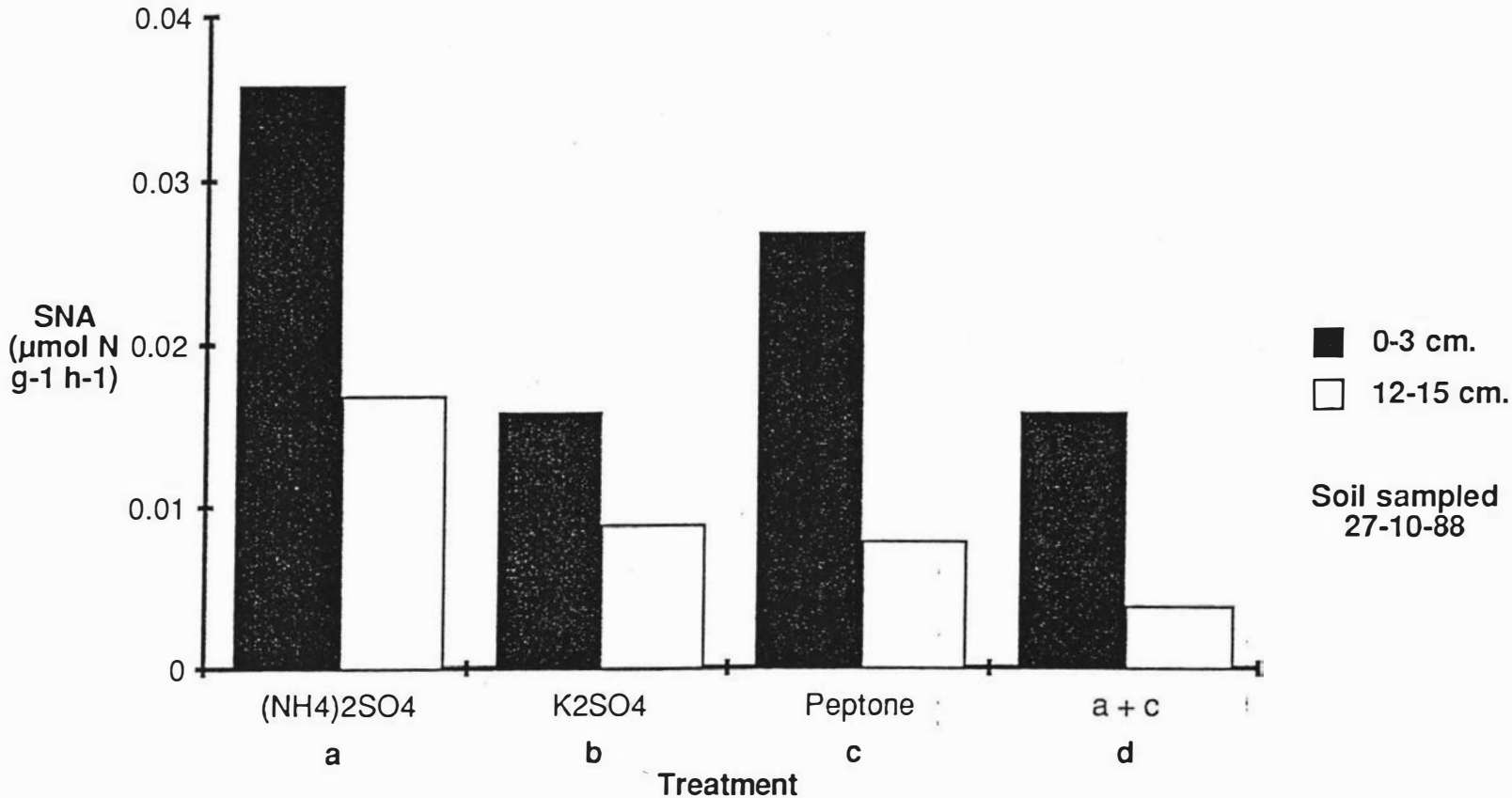

Figure 9.3 Nitrifier activities in soil sampled in October incubated with a range of N-substrates

at the $p<1\%$ level of significance. The fact that the peptone treatment had a lower mean SNA value than the standard incubation (0.027 ± 0.001 compared to 0.036 ± 0.002 for the 0-3 cm depth range, and 0.008 ± 0.0006 compared to 0.017 ± 0.001 μmol N g^{-1} h^{-1} at 12-15 cm) suggests that at this time of year, the heterotrophic population may not be as active relative to the autotrophic population as was the case in winter, assuming that the activity measured in the standard incubation was predominantly autotrophic. Alternatively, one could say that the autotrophic population was more active at this time of the year than the heterotrophic population. This would certainly be the expected result if the soil had become much drier, since many soil heterotrophic organisms are not obligate aerobes (Focht & Verstraete, 1977) and therefore drier, or more aerobic, conditions may not have a significant beneficial effect on their activity. In June the soil was wet, with moisture contents for the 0-3 and 12-15 cm samples of 0.495 and 0.290 g g^{-1} respectively, equivalent to pF values of 1.17 and 2.54. In October, the soil was wetter in the 0-3 cm sample (0.546 g g^{-1}, pF 0.91) following rain the day before sampling, and had approximately the same moisture content at 12-15 cm as it was in June (0.287 g g^{-1}, pF 2.56). Thus, on the basis of the results of the moisture stress experiments (Chapter 8; Figure 8.3), the differences in activities between the two sampling times cannot be attributed to differences in the soil moisture content because the 0-3 cm sample in October would be expected to show a lower nitrifier activity than in June. The fact that the SNA value at 12-15 cm in October was higher than in June despite the soil moisture status being similar at both sampling times, indicates that moisture was not a factor in accounting for the difference in nitrifier activity between the two sampling dates. It is also unlikely that the increased temperature would have caused an increase in autotrophic relative to heterotrophic activity, since warmer temperatures might be expected to favour autotrophs and heterotrophs equally. The increased activity may therefore simply be a reflection of the increase in Ex-NH_4^+ in the soil, caused by the relative inactivity of the nitrifier population during the winter which allowed the concentration of NH_4^+ to build up. On the evidence of these results, the build-up of Ex-NH_4^+ was not sufficient to promote the heterotrophic production of NO_2^- from NH_4^+.

The explanation of seasonal differences between the two sets of results does not contribute to an explanation of the differences between the measured SNA values for treatments (a), (c) and (d). Indeed, following the argument used for the first experiment, the 0-3 cm sample with treatment (d) would be expected to have an SNA value of 0.047 μmol N g^{-1} h^{-1} (a+(c-b)), that is, nearly three times as large as the measured value of 0.016 ± 0.002 μmol N g^{-1} h^{-1}. In fact the SNA value of treatment (d) was identical to that for treatment (b). A possible explanation for this result is given below.

iii. Discussion

A number of authors have attempted to identify and quantify heterotrophic nitrification in forest and heath soils (e.g. Van de Dijk & Troelstra, 1980; Hynes & Knowles, 1982; Schimel *et al.*, 1984), but none have investigated heterotrophic activity in agricultural soils. No worker has succeeded in obtaining values for the actual amounts of heterotrophic nitrification *in situ*, and most have merely confirmed that it is occurring in their soils. Van de Dijk and Troelstra (1980) measured nitrate reductase activity (NRA) in the leaves of two sub-species of *Hypochaeris radicata* as an indicator of *in situ* nitrate production in two heath soils. In one of these (pH 4.3), addition of ammonium did not affect NRA in the leaves, and analysis of the microbial population failed to identify any autotrophic nitrifiers. However, the addition of peptone led to an increase in NRA indicating the presence of heterotrophic nitrifying organisms. In a similar experiment in the laboratory, the addition of NH_4^+ led to a decrease in the production of NO_3^- to zero, whereas the addition of peptone led to a doubling of the amount of NO_3^- produced. In the other soil (pH 6.3), addition of both NH_4^+ and peptone increased the amount of NO_3^- produced. These results are of interest with respect to the results for treatments (c) and (d) in the second experiment. Since treatment (c) showed an increase in nitrifier activity over treatment (b), it appeared that when peptone was added, heterotrophic activity increased, but when NH_4^+ was also added, nitrifier activity was suppressed. However, this type of explanation is at odds

with the results of the first experiment when treatment (d) showed equal highest activity, and also the other results in the second experiment, since treatment (a) showed autotrophic activity to be greater than heterotrophic activity. One might have therefore expected treatment (d) to show nitrifier activity of a similar magnitude, assuming that peptone does not suppress autotrophic activity, because the same concentration of NH_4-N was present in treatment (d) as was present in treatment (a). It should be noted however, that this kind of argument is based on the assumption that the concentration of peptone used in these experiments was as optimal for heterotrophs as 0.01 M $(NH_4)_2SO_4$ was shown to be for autotrophs (Chapter 4). Whether or not this assumption is correct is not known, and therefore both the results and their interpretation must be regarded as speculative.

Adams (1986a,b) also noted suppression of heterotrophic activity following addition of $(NH_4)_2SO_4$. He studied "strongly acid" forest litter (pH 4.50) and humus (pH 3.88-4.23) beneath sitka spruce, heather, Scots pine and larch in Scotland, and found that net NO_3-N production during aerobic incubation was generally greater in litter than in humus, and that it was correlated ($p<0.1\%$) with the initial concentration of organic N soluble in 1 M KCl, thus suggesting heterotrophic activity. However, Adams (1986a) argued that the very low amount of NO_3-N produced in most of the samples suggested that the factor determining whether or not nitrification occured in a particular forest floor sample was the amount of readily mineralizable carbon available for heterotrophic utilization and not the supply of a source of organic N. He supported this idea in the case of sites where the trees had received fertilizer, by suggesting that leaf litter falling from fertilized spruce contained less lignin and more metabolizable carbon following fertilization of the trees. Not all the samples tested exhibited nitrification, but of those that did, the rate of NO_3-N production was increased by the addition of peptone, whilst addition of $(NH_4)_2SO_4$ generally had no effect. In larch humus, however, addition of $(NH_4)_2SO_4$ significantly reduced NO_3-N production. Adams (1986b) found that whilst the reason for this apparent suppression of heterotrophic nitrification by NH_4-N was unclear, it could not be attributed to any direct toxic effect of added NH_4^+.

Meiklejohn (1953) stated that peptone was very poisonous to autotrophic nitrifiers, especially if it contained free amino acids. De Boer *et al.* 1988) also found peptone to be inhibitory to the nitrifier population in a fertilized acid heath soil when added weekly at a rate of 4 mg peptone-N dm^{-3} soil suspension. This kind of result might go some way to explain the results of the second experiment, but seems an unlikely reason for the depressed SNA value in treatment (d) in the second relative to the first experiment since the same peptone was used in both. However, if the winter conditions were sufficiently unfavourable to autotrophs that the nitrifier activity measured in the first experiment was predominantly heterotrophic, then the relative magnitude of the observed nitrifier activities in the two experiments makes sense. In the first experiment, the SNA value was greatest in treatments (a), (c) and (d), and the lack of a significant increase in treatment (d) compared to (c) or (a) might have been due to inhibition of autotrophic nitrification (which was low anyway) by peptone. In the second experiment the field conditions favoured a larger autotrophic population so that treatment (a) exhibited greater nitrifier activity than treatment (c), but when both peptone and NH_4^+ were supplied as substrates as in treatment (d), the activity of the autotrophs was inhibited by the peptone. This explanation would appear reasonable but would be more convincing if treatments (c) and (d) in the second experiment had recorded similar SNA values, just as they did in the first experiment.

Schimel *et al.* (1984) used a variety of agents including acetylene and chlorate to block the oxidation of NH_4^+ and NO_2^- in an attempt to identify the presence of heterotrophic nitrification in a Sierran forest soil in California. They found that NO_3^- production was not inhibited by either acetylene or chlorate which suggested that the NO_3^- had been produced by heterotrophs. However, neither peptone nor NH_4^+ stimulated the production of NO_3^-, suggesting that either these were not suitable substrates, or that the rate of nitrification was not substrate limited in this soil. In fact, the rate of NO_3^- production decreased during the assay period, which Schimel *et al.* (1984) considered sufficient evidence to conclude that neither peptone nor NH_4^+ were suitable substrates. This seems a curious result, but along with those of Adams (1986a,b), Van de

Dijk and Troelstra (1980) and those reported here, reinforces the conclusion of Chapters 7 and 8, that the nitrifier population is dynamic and will change to suit particular soil conditions. On the basis of the results of the work reported in this chapter, this adaption to specific environments must include the possibility that the make up of the population, in terms of the relative numbers of different species present, is also dynamic.

iv. Conclusions

Like those of other workers, this experiment has failed to provide conclusive evidence as to the relative importance of heterotrophic nitrification; indeed much of the interpretation of results is speculative. Nevertheless, it seems likely that there was a potential for heterotrophic activity in the Tokomaru silt loam at both sampling times, and that the activity of the autotrophic population (and therefore the population size) was approximately twice as high in October as it was in June. The effect of adding both peptone and $(NH_4)_2SO_4$ together is complex and the results for treatment (d) were ambiguous; there was a suggestion that the concentration of peptone used inhibited autotrophic activity, but only at the October sampling. To further investigate the importance of heterotrophic activity in this soil, one would have to do much more detailed experiments with different concentrations of peptone and $(NH_4)_2SO_4$, and examine any interactions between these substrates. Looking at the Patua soil (Figure 9.1b), one might think that about half the nitrifier activity at pH 4-4.5 was heterotrophic judging by the lack of a steep decline in SNA value below pH 5, such as that seen for the Tokomaru silt loam (Figure 9.1a). However, in the absence of extensive analysis of the soil microbial population, and identification of all the soil microbes present that might be capable of nitrification, it seems that the SNA will have to be assumed to be a good measure of *in situ* nitrifier activity. The fact that the very low pH values at which heterotrophic activity seemed likely to occur have not been measured in soil samples taken from either Field No. 2 or No. 6, suggests that it is reasonable to assume that autotrophs either predominate or are at least as important as heterotrophs in the Tokomaru silt loam as sampled. If this is the case, the SNA may be considered to be an entirely satisfactory means of measuring *in situ* nitrifier activities.

SECTION IV

CHAPTER 10

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER WORK

A. A CRITIQUE OF THE GEOSTATISTICAL TECHNIQUES USED FOR THE ASSESSMENT OF SPATIAL VARIABILITY IN NITRIFIER ACTIVITY

Geostatistics were first introduced to soil scientists at the beginning of the decade in a series of papers by Burgess and Webster (1980a,b), Webster and Burgess (1980) and Burgess *et al.* (1981). Since then, the literature has contained reports of the spatial analysis of a range of soil properties including soil temperature (Davidoff *et al.*, 1986, Davidoff & Selim, 1988), soil moisture content (Davidoff & Selim, 1988), soil particle size (Oliver and Webster, 1987), topsoil properties (Xu & Webster, 1984), crop yield-soil water relationships (Russo, 1984b), soil surface roughness (Lehrsch *et al.*, 1988), soil fertility (Webster & M^{c}Bratney, 1987), soil nitrate concentration (Tabor *et al.*, 1985, White *et al.*, 1987) and the heavy metal content of soils (Wopereis *et al.*, 1988). Some authors have proceeded from the experimental variogram and used kriging to produce maps of the properties under investigation (e.g. Xu & Webster, 1984, Webster & M^{c}Bratney, 1987), whilst others have used the variogram to design so-called *optimal sampling schemes* (e.g. Burgess *et al.*, 1981, M^{c}Bratney & Webster, 1981, M^{c}Bratney & Webster, 1983, M^{c}Bratney *et al.*, 1981). However, because soil scientists are not necessarily also mathematicians, few have given theoretical consideration to the problems that may be encountered in the practical use of geostatistics for soil science problems. In many of the instances listed above, this is probably because workers have produced a single variogram based on a single sampling occasion and therefore have been unaware of the possibility of changes in the variogram model with time and sampling design (Chapters 5 and 6). Indeed, the selection of models for fitting to experimental variograms has been the only aspect of geostatistical theory to receive much attention in the soil science literature (M^{c}Bratney & Webster, 1986).

Some soil scientists might draw a "perverse comfort" from the notion that geostatistical theory may have outpaced its application to soil science (M^{c}Bratney & Webster, 1986) since by and large, they have been left behind in the development of the subject by mathematicians. Nevertheless, if better practical use is to be made of these techniques, which are seen by many (e.g. Dudal, 1986, Nielsen, 1987) as a potentially major avenue of progress in soil science, the problems of estimation and interpretation of the variogram must be resolved. In the following discussion, an attempt is made at addressing some of these problems, particularly in relation to the work reported in Chapters 5 and 6.

i. Problems caused by anisotropy

With the exception of the variogram for moisture content in the nested experiment (Figure 6.3e), spatial variation in the data was assumed to be isotropic because as explained in Chapter 5, anisotropy is difficult to identify given the tendency of values of $\hat{\gamma}(h)$ to be more variable at large lags. However, inspection of many of the variogram models, such as those for incubation pH (Figure 5.5c) and moisture content (Figure 5.5d) in the first experiment over 625 m^2, and SNA over 9 m^2 (Figure 5.7a), suggested that isotropy may not have been a good assumption.

When fitting models to the experimental variograms shown in Figure 6.3, it was apparent that by considering the north-south and east-west directions separately, direction-dependent values of the range, nugget variance, C_o, and the spatially dependent variance, C, would be obtained. For example, in the case of incubation pH (Figure 6.3d), in the north-south direction the best fit model was exponential with values of C_o, C and r equal to 0.0044305, 0.020437 and 1.774 respectively (Figure 10.1). The range (equal to 3r) was estimated as 5.3 m. In the east-west direction, in contrast, the best fit model was spherical, with values for C_o, C and the range of 0.008814, 0.011469, and 4.1 m (Figure 10.1). The difference between the sill variances of the two models (0.024872 in the north-south direction compared to 0.020283 in the east-west direction) might be taken to indicate *zonal anisotropy* (Webster, 1985). However, it

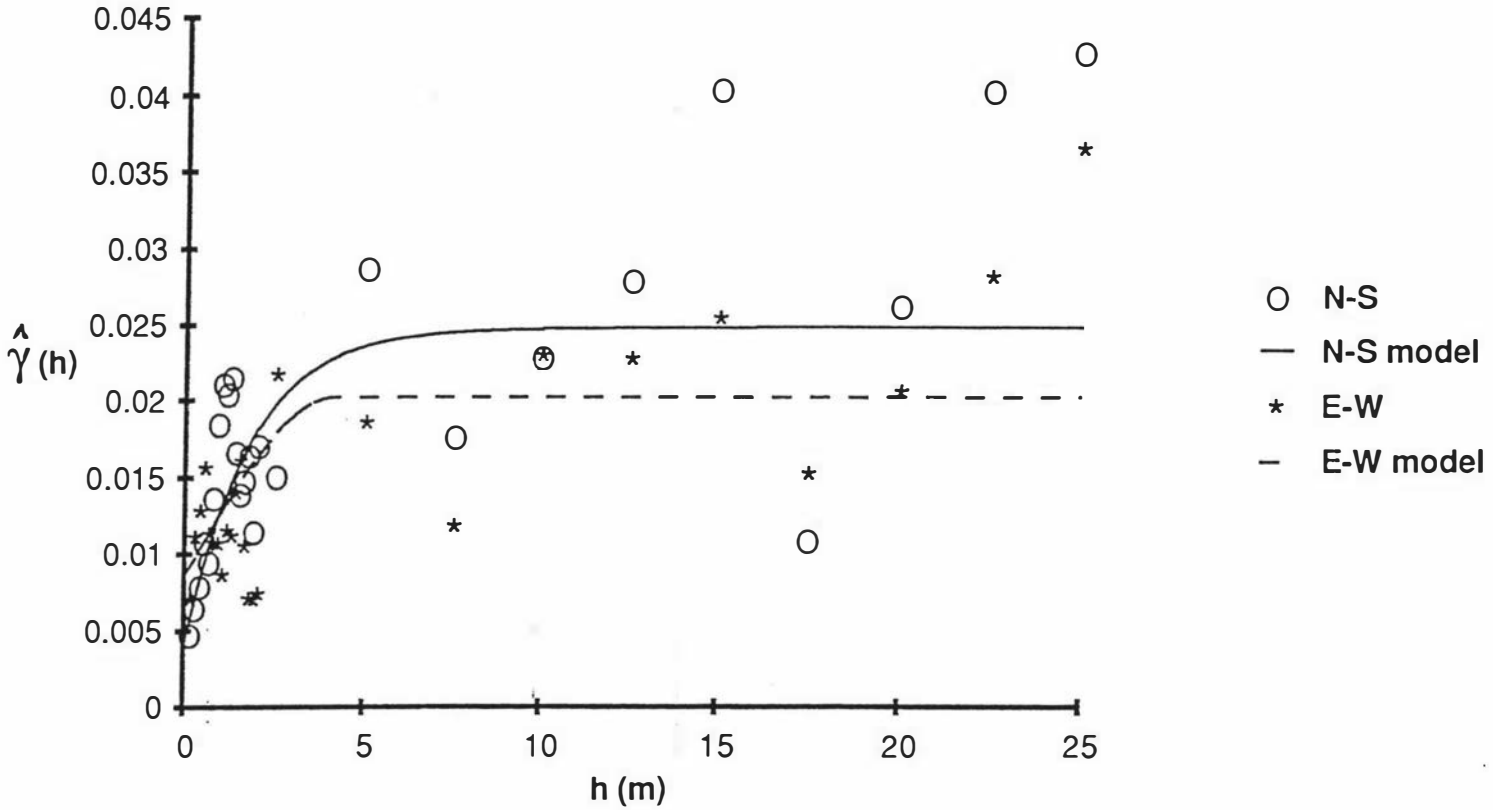

Figure 10.1 Experimental variogram of incubation pH of soil sampled between 3-12 cm over 625 m^2 using a nested sampling design (Figure 6.1) with separate models fitted by weighted least squares optimization for the north-south and east-west directions

seems unlikely that these differences are significant in view of the large error associated with the estimate of the sill variance as indicated by the spread of values of $\hat{\gamma}(h)$ about the sill at values of h greater than the range. Thus, on the basis of differences between the sill variance of the two models, the assumption of isotropy seemed satisfactory. However, by expressing the nugget variance as a percentage of the sill variance (17.8% in the north-south direction compared to 43.5% in the east-west direction), the differences between the models for the two directions seem more significant and might therefore suggest that the assumption of isotropic variation in incubation pH was incorrect.

Many authors (e.g. Burgess & Webster, 1980a, Webster & Burgess, 1980) have calculated anisotropy ratios over the first few lags where the variogram is approximately linear using the equation (Trangmar *et al.*, 1985):

$$\hat{\gamma}(h,\theta) = C_o + [A\cos^2(\theta-\Omega) + B\sin^2(\theta-\Omega)]h \qquad (10.1)$$

where $\hat{\gamma}(h,\theta)$ is the semivariance estimated for lag h in direction θ, C_o is the nugget variance, Ω is the direction of maximum slope *A*, and *B* is the slope of the variogram at 90° to Ω. The anisotropy ratio is given by A/B. In the first instance, equation (10.1) appears to have much potential as a descriptor of the difference in spatial dependence between two directions. However, deciding whether or not it is valid to use equation (10.1) presents a considerable problem. Firstly, equation (10.1) assumes that the value of C_o is common to the variogram model in both directions which, from the above discussion, is clearly not the case. Secondly, a significant difference between linear models fitted over the first few lags may not necessarily indicate a significant difference between the variogram models fitted over all values of h. The relatively small difference between the sills of the models fitted to the north-south and east-west directions (Figure 10.1), in spite of an apparent difference between the models over the first few lags, might indicate this. In addition, the situation could arise where the slope of the variogram over the first few lags was the same in two (or more) directions, but values of C_o and C, and therefore the value of the sill variance and range, were quite different. In this event, equation (10.1)

would indicate isotropy despite the variogram models showing large differences in the variation in different directions. Thus, the use of equation (10.1) seems limited; what is needed is a test for anisotropy which applies over all values of h.

Laslett *et al.* (1987) pointed out that there is no statistical test for anisotropy, so whether or not the differences between the north-south and east-west models (Figure 10.1) are significant cannot be discerned. One might expect differences in the variogram models fitted in different directions in any case, simply on account of experimental error and the error associated with the model fitting process. The use of weighted least squares prevents the use of a test for significant differences between regressions (Freund & Minton, 1979) to differentiate between variogram models, due to the complication caused by the weighting factors. In light of the generally held view that models must be fitted by weighted least squares, it therefore appears that finding an objective test for anisotropy will prove very difficult. Nevertheless, this should be an essential aspect of future geostatistical research.

With regard to the aims of the work described in this thesis, it is suggested that whether or not anisotropy occurs may be of little consequence. The intention here was to identify the distance over which soil mineral nitrogen and the factors affecting it might be spatially dependent, with a view to improving the estimation of a field mean nitrate concentration. Continuing with the example of incubation pH, whether the range is taken to be 4.1 or 5.3 m is probably only of academic interest, and therefore irrelevant to further field-scale sampling. By taking samples at separations greater than 5.3 m, the effect of spatial dependence on measurements made in either direction will be avoided since there is no increase in the fitted value of $\hat{\gamma}(h)$ with h for values of $h>a$, where a denotes the range of spatial dependence. Thus, for studies such this, it seems reasonable to ignore anisotropy unless it is obvious, as was the case for moisture content in the nested experiment. It should be noted, however, that this approach may not be acceptable if interpolation of unsampled sites by kriging is to be used because the presence of anisotropy will necessitate consideration of the direction of separation between samples, as well as the distance of separation, and as

a result will affect the weights given to near neighbours in the kriging procedure. It is therefore interesting to note that Laslett *et al.* (1987) found isotropic kriging to be better than anisotropic kriging despite clear evidence of anisotropy in their data. A similar result was found in the case of incubation pH (Figure 6.3). When the number of sampling points separated by each lag is the same in both directions, as is the case in a symmetrical sampling design, $\hat{\gamma}(h)$ in both directions combined is equal to the average of $\hat{\gamma}(h)$ in the two directions separately; that is, the number of points fitted to the model is the same and so comparison of AIC values (equation (3.22)) is valid. Comparison of the AIC values for the pH variograms (Figures 6.3d and 10.1) indicated that the model describing both directions (AIC = -451.59) described the spatial variation better than either the north-south (AIC = -432.06) or east-west (AIC = -445.17) directions separately. It is therefore concluded that the assumption of isotropy in this work was acceptable.

ii. Problems caused by changes in the sample variance and variation in the variogram model with time and scale of sampling

In Chapter 5 it was observed that the form of the model fitted to the experimental variogram appeared to be a function of the scale of sampling. It also appeared that the variogram model was affected by the time of sampling, although in view of the fact that sampling was only carried out on two occasions, this effect was less conclusive. The observation that the scale of sampling affected the form of the variogram was supported by the finding in Chapter 6 that the variograms produced from data sampled on a nested grid were more meaningful than those produced from regular grided data, in that the nugget variance was reduced, and the range of spatial dependence was better defined as a result. On this basis, it was concluded that the efficacy of a sampling scheme was a function of the ratio of the smallest:largest lag. However, the observation in Chapter 5 that both the sample variance and the form of the variogram model changed with time and size of the sampling area, and the similar finding in Chapter 6 that the sample variance was also dependent on the size of the sampling area, raises serious questions as to the reliability of the variogram irrespective of the sampling design.

It also suggests that a given variogram model may only give information that is of use in considering the spatial distribution of a soil property measured at the time and scale of sampling used for production of that variogram. In the case of soil physical characteristics, the error due to time is probably insignificant and so the reproducibility of the variogram may be satisfactory. However, in the case of biological soil properties such as those measured here, variation of the variogram model and its parameters with time may reflect either a serious shortcoming in geostatistical techniques, or may simply indicate that these techniques are not appropriate for the study of temporally variable biological properties.

The variation in sample variance with the scale of sampling, as identified in Chapter 6, may reflect a shortcoming of nested sampling designs. The sample variance of data in the nested grid (0.012 in the case of incubation pH) was less than the sample variance for the whole data set (0.0165), and much less than the sample variance of the main grid alone (0.022). This suggests that the estimate of σ^2 by s^2 was not an unbiased estimate. s^2 calculated for the whole data set underestimated σ^2 for the sampling area due to bias caused by the spatial dependence of sample values measured at sites within the nested grid on one another - a reasonable explanation given that the nested data points were confined to an area of 2.5 m $\times$ 2.5 m, and the range of spatial dependence (for incubation pH) was 6.1 m. In view of the tendency for estimates of the variance to vary without limit as the size of the sampling area increases (Oliver, 1987), the degree of this bias will be a function of the ratio of the area covered by the nested grid to that covered by the whole grid. There may be further bias in the nested design because the location of the nested grid within the main grid was arbitrarily decided. Overall, it appears that the sampling design used for the experiment described in Chapter 6 was not as good as previously suggested. Indeed, in light of the above discussion, it seems that the problem of s^2 varying with changing sampling design may be avoided by positioning the nested sampling points over the whole sampling area adjacent to points on a regular grid, in a similar way to that used by Laslett *et al.* (1987).

Changes in the sample variance with time and scale of sampling, as opposed to changes in the actual sampling design, present more difficult problems, especially since changes in the value of s^2 will probably be mirrored by changes in the sill variance (C_o+C), given that both are a measure of the variation of the measured property over the area in which it was sampled. Analysis of the differences in sample variance for the properties measured in the three experiments described in Chapters 5 and 6 is made difficult in the case of the first experiment over 625 m^2, and the 9 m^2 experiment, by the different times of sampling; and in the case of the two experiments over 625 m^2 by the difference in the depth of sampling.

In the case of incubation pH, which was not expected to vary much with season, the values of the mean and sample variance in the first 625 m^2 experiment were 4.92 and 0.0335 respectively, compared to 4.82 and 0.0240 in the 9 m^2 experiment. In light of the discussion in Chapter 6, this difference is probably simply a result of the reduction in the size of the sampling area. In the case of SNA, however, the mean value was 0.028 μmol N g^{-1} h^{-1} in both the first 625 m^2 experiment and the 9 m^2 experiment, yet the value of s^2 (ln transformed data in ng N g^{-1} h^{-1}) changed from 0.2298 over 625 m^2 to 0.3415 over 9 m^2. A similar increase in the sample variance was observed for soil NO_3^- (ln transformed values; 0.3976 and 0.9225 for 625 m^2 and 9 m^2 respectively). Both these differences were probably a reflection of the different time of sampling.

Thus, explanation of the differences in the value of s^2 between the three experiments is complex, and is a problem that will not be dealt with further here. However, the fact that the variance of the sampled properties can change with the design and scale of sampling (in spite of the log transformation which is expected to stabilize the variance) indicates that both of these factors can markedly affect the results of the data analysis. As indicated above, in the case of biological soil properties, temporal variation may also have to be accounted for. Other than by carrying out a series of identical spatial analyses over a period of at least a year - which would require a massive sampling effort overall - how temporal variation in the variogram should be accounted for is unclear.

With regard to geostatistical data analysis, the change in the variogram with time and scale of sampling is somewhat disturbing. Which variogram is the correct one ? In Chapter 6, it was assumed that since the sampling design appeared good, the models fitted to the experimental variograms defined the spatial variation of the properties measured for $^1/_{16}$ ha (the area of field No. 6) in the Tokomaru silt loam. However, differences between the variogram models, especially those for the first two experiments where the only difference in sampling was in terms of the area sampled, require some examination.

Table 10.1 gives a summary of the results of the three spatial analyses of SNA and incubation pH. The variogram for incubation pH over 625 m^2 (Figure 5.5c) was best fitted by a spherical model predicting a range of 9.9 m, yet over 9 m^2 the variation was pure nugget (Figure 5.7c). Since the largest lag over 9 m^2 was less than the range as defined over 625 m^2 (in both the regular and nested experiments) one would not expect to identify a range when sampling pH over 9 m^2. However, it would be expected that the variogram model should be linear upwards, because as the variogram over 625 m^2 shows (Figure 5.5c), $\hat{\gamma}(h)$ increases with increasing h between 2.5 and 9.9 m. Since by definition $\hat{\gamma}(h)$ at h=0 is zero, one can say that $\hat{\gamma}(h)$ in this variogram should increase between h=0 and h=9.9 even though no measurements were made at values of $h<2.5$ m. Indeed, convention was followed and the model was plotted over this range of values of h. Thus, there is an inconsistency between these results which could perhaps reflect either seasonal variation, or more likely, an inadequacy of the 9 m^2 sampling design.

In the case of SNA, in the first experiment over 625 m^2, the variance was pure nugget (Figure 5.5a). In the 9 m^2 experiment, SNA showed spatial dependence within 0.6 m (Figure 5.7a). In contrast to incubation pH (see above), these results are not inconsistent with one another because the shortest lag in the first experiment was 2.5 m, and the other two experiments indicated that the range of spatial dependence was less than 2.5 m. However, the result for the 9 m^2 experiment is at odds with that for the nested experiment (Figure 6.3a). Given that the sampling design in the nested experiment was good, and that sample separations less than

Table 10.1 Summary of results for the three spatial analyses of SNA and incubation pH

	pH		SNA	
	Variogram Model	Range (m)	Variogram Model	Range (m)
625 m² (regular grid) (Figure 5.5)	Spherical	9.9	Nugget	-
9 m² (regular grid) (Figure 5.7)	Nugget	-	Spherical	0.6
625 m² (Nested grid) (Figure 6.3)	Exponential	6.1	Exponential	2.4

0.6 m were covered under the nested design, one wonders whether the difference in the range as defined under the nested and 9 m^2 experiments might not be a function of seasonal variation. If this is so, and assuming that only two values of $\hat{\gamma}(h)$, those at h=30 and 60 cm, can provide sufficient evidence of spatial dependence within 0.6 m, the question arises as to why the spatial dependence of nitrifier activity in June should be so much more intense than it is in October, given that the sill is greater, and the range shorter, in the 9 m^2 (June) experiment compared to either the nested experiment or indeed the regular grid experiment over 625 m^2 where the variance was pure nugget ? Finding answers to these questions is perhaps beyond the scope of this work and in the absence of an understanding of why SNA should be spatially dependent within 2.4 m, may not be possible. However, the evidence from these experiments is that SNA variability is predominantly short-range. With respect to estimating field mean values of such properties as SNA, NO_3^- and NH_4^+, which may be referred to as the parameters of soil mineral N, this is valuable information.

Oliver and Webster (1986) and Russo and Jury (1987a) noted that the variability of a soil property may occur over a range of different scales which may change by several orders of magnitude, such that each scale of observation will integrate the variabilities apparent at smaller scales. It is tempting to explain the differences between the variograms for the experiments done in Chapters 5 and 6 in these terms. However, on the basis of the results for these three experiments, it seems more likely that the variograms are unreliable, certainly in the case of the regular grid experiments. This conclusion presumes that variograms should have a high degree of accuracy. In view of the wide scatter of the values of $\hat{\gamma}(h)$, it may be that in the case of some soil properties, this degree of accuracy cannot be expected. Nevertheless, it would be of great interest, if time were available, to investigate the (temporal) reproducibility of the variogram. Obviously, if it cannot be accurately reproduced, particularly with regard to the value of the range, its value may be limited, especially if kriging, which depends on an accurate variogram, is to be used. Consideration of this problem is seen as an essential element of future research.

A further question of importance is whether there is any value to the *non-kriger* in fitting variogram models at all. In investigations such as this, where the aim was to define a minimum acceptable sample separation for future sampling, the increased precision of a range defined by a fitted model over that defined by simple inspection of the experimental variogram is almost certainly not significant in terms of future sampling at the field scale. Furthermore, the fitted model is only a *best fit* model. It is not necessarily the *perfect fit*, nor the only *acceptable* best fit (A. Swift, Dept. Mathematics and Statistics, Massey University - personal communication); and as Burrough (1983) pointed out, just because variation in a soil property can be defined in terms of a variogram model, it does not mean that the variation can be explained in physical terms. For example, nitrifier activity has been found to be spatially dependent within 2.4 m, but in view of the microscopic size of microbial clusters, the physical significance of a range of spatially dependent variability in nitrifier activity of this magnitude is difficult to discern. The lack of any spatial dependence in the variability of exchangeable ammonium suggests that variability in the substrate supply was not responsible for the observed variability in nitrifier activity, and that variability in some other factor(s) causes the variability in nitrifier activity. On the basis of the results of Chapters 5 and 6, and without further extensive investigation into the variability of nitrifier activity, one is inclined to think that Burrough's observation was not only correct, but also points to a serious shortcoming in the use of geostatistical techniques. The fact that very little attempt has been made in the literature to account for the spatially dependent variability that has been defined for various soil properties, further supports this conclusion.

iii. The relationship between the sill and the sample variance

According to Webster (1985) the population variance of a finite region, known to geostatisticians as the *dispersion variance*, is the average semivariance within that region. It therefore follows that the sample variance must be less than the sill unless the variogram is pure nugget. In Figures 5.5a-c, 5.7b-d and 6.3c,d the position of the sample variance in relation to the values of $\hat{\gamma}(h)$ is consistent with the above. In contrast, values of $\hat{\gamma}(h)$ in the variograms for moisture content in both experiments over 625 m^2 (Figures 5.5d and 6.3e), SNA over 9 m^2 (Figure 5.7a) and 625 m^2 under the nested sampling design (Figure 6.3a), and initial NO_3^- in the nested experiment (Figure 6.3b) are not consistent with the value of the sample variance. Indeed, in all these cases, the values of $\hat{\gamma}(h)$ appear too low. In the other variograms, the values of $\hat{\gamma}(h)$ and s^2, which were calculated in the same way as for the variograms listed above (i.e. using GAMMAH), were consistent with one another. Thus, the inconsistencies between values of $\hat{\gamma}(h)$ and s^2 cannot be ascribed to mathematical error, and therefore require some explanation.

In section (ii) above, a problem was identified with the nested sampling design with respect to bias in the estimation of the sample variance. Since the effect of the nesting was to reduce the value of s^2 as estimated for the whole sampling area (i.e. the whole data set), the nesting cannot be blamed for the apparently high value of s^2 for SNA and NO_3^- in relation to the values of $\hat{\gamma}(h)$. Indeed, since the sample variance is calculated independently of the position of the data points in the sampling area, one might conclude that it is the values of $\hat{\gamma}(h)$ which are too low. A similar bias to that observed in estimating s^2 in the nested grid may occur in the estimation of values of $\hat{\gamma}(h)$. It follows from equation (3.14) that the semivariance at any given lag represents the variation associated with property values separated by that lag. Under the regular grid sampling design (Chapter 5), data from every sampling point is considered at least once in the calculation of $\hat{\gamma}(h)$ for any value of h; at shorter lags a point may be considered twice as it may have neighbours separated by a given lag either side of it along the same plane. For example, every point in the regular grid over 625 m^2 except those on the outside of the grid had two neighbours 2.5 m away in both

the north-south and east-west directions. Thus, every sampling point is included in the calculation of $\hat{\gamma}(h)$ at any value of h in any direction either once or twice. Under the nested design in contrast, this is not the case because some points do not have neighbours at all lags.

Four points were randomly selected from the main grid of the nested sampling design (Figure 10.2), and the grid was examined to investigate how many times these points were considered in the calculation of $\hat{\gamma}(h)$ at each value of h, and thus in the spatial analysis as a whole. The four points selected (henceforth denoted by A, B, C and D) were those occurring at 5x 7.5y, 10x 15y, 12.5x 2.5y, and 17.5x 20y where x denotes east-west, and y denotes the north-south direction (Figure 10.2). Point A has one neighbour at lags of 17.5, 10, 7.5 and 5 m in the north-south direction, and in the east-west direction has a neighbour at 20, 17.5, 15 and 2.5 m. Thus, it is considered four times in the variogram for any one direction and eight times assuming isotropy. Similarly, point B has a neighbour in *both* the north-south and east-west directions at distances of 15, 12.5, 10, 7.5, 5 and 2.5 m and so is referenced six times in either direction, or twelve times assuming isotropy. Like point A, point D has a neighbour at four different lags; at 20, 17.5, 15 and 2.5 m in the north-south direction, and at separations of 17.5, 10, 7.5 and 5 m in the east-west direction. Thus, assuming isotropy it is referenced eight times. In marked contrast to the others, point C has a neighbour in the north-south direction at 22.5, 20, 17.5, 12.5, 10 and 7.5 m, but has two neighbours at a lag of 2.5 m, that is, it is referenced eight times in the estimation of the north-south variogram. In the east-west direction, point C has two neighbours at lags of 12.5, 5 and 2.5 m and thus is referenced six times. Assuming isotropy however, point C is referenced fourteen times, that is, two times more than point B, and six times more than points A and D. Thus, under the nested design, the estimation of the experimental variogram can be unequally influenced by some sampling sites which, if they represent extreme values of the soil property, could lead to bias in the values of $\hat{\gamma}(h)$ in relation to s^2. In the case where the data are anisotropic, values of $\hat{\gamma}(h)$ which involve reference to point C might be expected to differ in the north-south compared to the east-west direction.

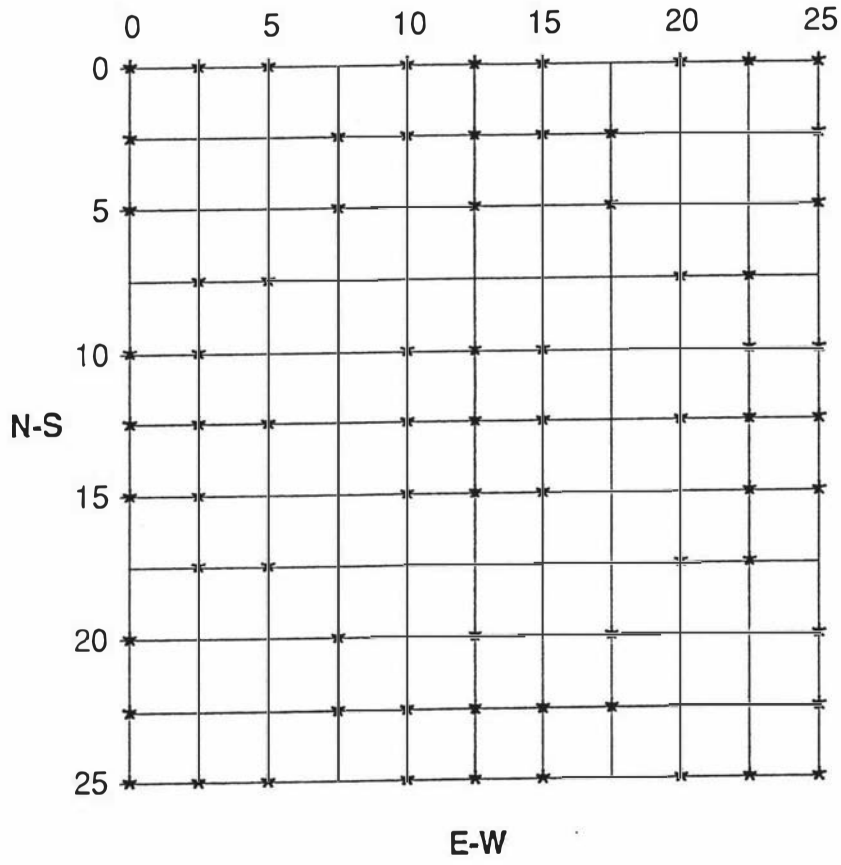

Figure 10.2 The main grid of the nested sampling design

A further complication to the above is that in the case of lognormally distributed data such as SNA and initial NO_3^- which are highly skewed, most of the measured values occur in the low end of the range. However, extreme (high) values, which may still be outliers after the log transformation, are included in the calculation of s^2 but may not carry the same weight as intermediate and low values in the calculation of values of $\hat{\gamma}(h)$, thus making the values of $\hat{\gamma}(h)$ appear low in relation to s^2. The fact that the values of $\hat{\gamma}(h)$ in the case of incubation pH are not inconsistent with the values of s^2 may be a reflection of the lack of extreme values in the data. Indeed, incubation pH ranged between 4.4 and 5.3 yet the standard error was only 0.01 pH units which is equivalent to only 0.2% of the mean value value of 5.04. In contrast, the standard error for SNA values calculated from the ln transformed data using equations (5.5) and (5.6), was equal to 0.0004 μmol N g^{-1} h^{-1}, equivalent to 5% of the mean value of 0.008 μmol N g^{-1} h^{-1}. In the case of initial NO_3^- values, the standard error (0.010 μmol N g^{-1}) was equivalent to 8.1% of the mean value (0.124 μmol N g^{-1}). Thus, SNA and NO_3^- had more extreme values than incubation pH, and this was reflected by the relative position of values of $\hat{\gamma}(h)$ and s^2 in Figures 6.3a,b, and d. Indeed, NO_3^- which had the greatest number of extremes also had the greatest discrepancy between s^2 and values of $\hat{\gamma}(h)$ (Figure 6.3b). Figure 10.3 shows the distribution of the ln transformed values of initial NO_3^-, fitted by ordinary least squares optimization with a normal distribution (R^2 = 0.68, $p<1\%$). Comparison of Figure 10.3 with Figures 5.4c,d; 5.6c,d and 6.2d,e suggests that the fit of the normal distribution to these log transformed data is not as good as the fit of the normal distribution to the data for incubation pH and moisture content, which did not require transformation. One might therefore conclude that the normalization of the lognormally distributed SNA and NO_3^- data has not been entirely satisfactory. The unexpected large number of ln NO_3^- values in the range 7.8-8.2 (Figure 10.3) in relation to the expected small number of values in the range 5.8-6.2 suggests this to be the case.

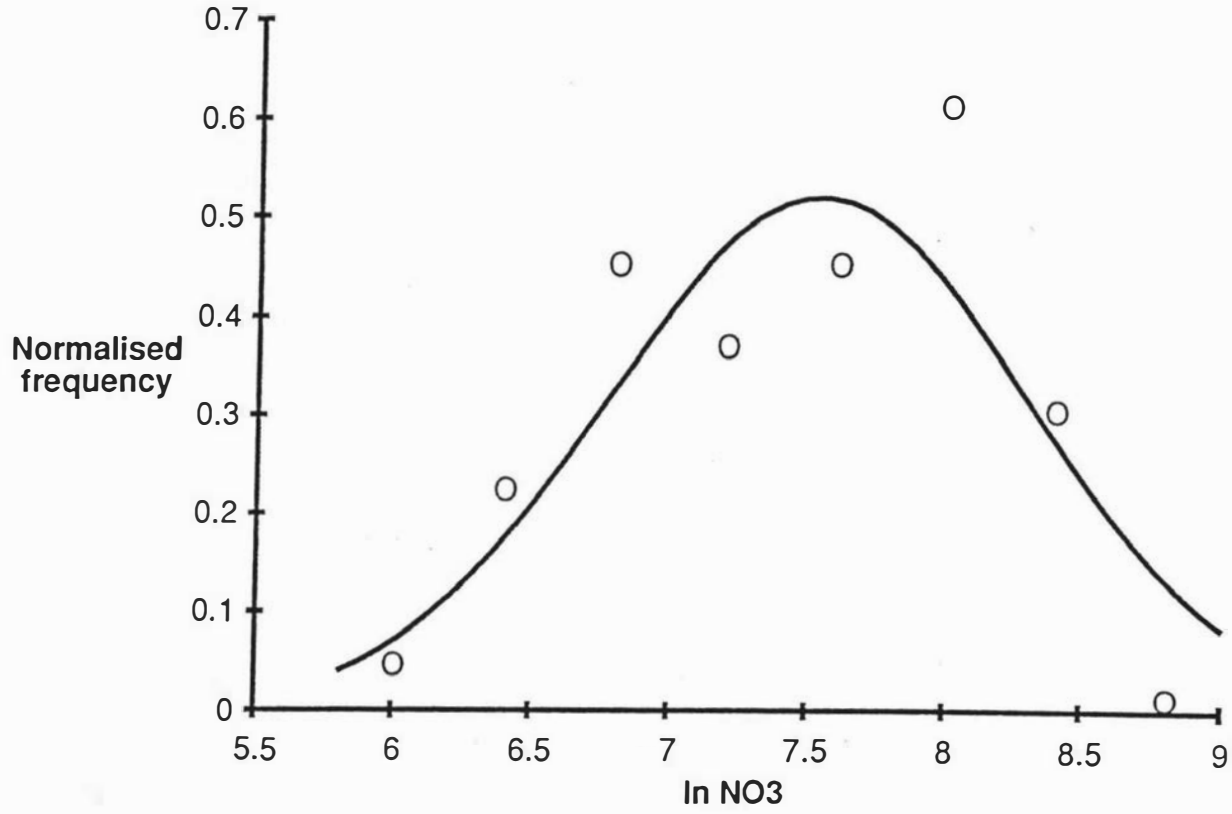

Figure 10.3 Distribution of the ln transformed values of initial NO_3^- sampled between 3-12 cm depth over 625 m^2 using a nested sampling design (Figure 6.1)

The fact that the above argument does not hold for the Ex-NH_4^+ variogram (Figure 6.3c), where the values of $\hat{\gamma}(h)$ were consistent with s^2, is no doubt due to the fact that values of Ex-NH_4^+ were highly variable and showed no spatial dependence. Thus, variation in Ex-NH_4^+ was pure nugget and so the value of s^2 and the sill, in so far as one could be defined by the linear model, were in close agreement.

In the light of the above discussion, it appears that the nested sampling design used was not in fact as good as was thought earlier. It is therefore suggested that designs of this type, where the nested sampling points are all closely grouped with each other, should be avoided. An alternative means of achieving a small ratio of smallest:largest lag is therefore required. In conclusion, it appears that in order to produce a variogram of good reliability and reproducibility, not only must the distribution of the number of pairs at each lag be as even as possible (Russo, 1984a, Cressie, 1985), but the number of times the sampling points are referenced in the estimation of the variogram must also be approximately equal. The design of such a sampling scheme is another avenue of important future research.

iv. Crossvariogram analysis

In Chapter 6, the correlation of SNA with NO_3^- and incubation pH over space was investigated using crossvariogram analysis, and on the basis of the crossvariograms produced (Figure 6.4a,b), it was concluded that SNA was not closely correlated with either NO_3^- or pH over space. However, it was not clear what information crossvariograms could provide over and above ordinary correlation. Given that the SNA and NO_3^- data, for example, were sampled at the same points in the field, it follows that any correlation between them must occur over space. A standard correlation analysis was therefore carried out between the SNA, NO_3^- and incubation pH data from the nested 625 m^2 experiment. It was found that SNA was not correlated ($p<5\%$) with either pH or NO_3^-. This may explain why the models fitted to the crossvariograms for these properties were horizontal linear, or pure nugget; that is, there was no spatial dependence between them. However, the fact that values of $\hat{\gamma}_{zv}(h)$ were

generally positive was taken to mean that these properties were generally correlated over space (Davidoff & Selim, 1988), although no indication was given as to the level of significance of the correlations (- a shortcoming of the analysis perhaps ?). That the results of these two analyses were at odds was further indicated by the fact that the correlation coefficient for SNA and NO_3^- was negative, and although insignificant at the 5% level, this would suggest that the interpretation of the crossvariogram was incorrect.

A separate correlation analysis was carried out on the values of $\hat{\gamma}(h)$ for SNA, NO_3^- and incubation pH, and it was found that these were significantly correlated. In the case of SNA and pH, the correlation was significant at the $p<0.1\%$ confidence level, whilst that for SNA and NO_3^- was significant at $p<1\%$. Since SNA, NO_3^- and incubation pH are all spatially dependent, that is, the variability in their values depends on the distance of separation of sampling sites, it follows from first principles that at lags greater than the range, their values will fluctuate more than at lags shorter than the range of spatially dependent variability. Their values may therefore not be expected to be closely correlated. By the same token, since the variability in all these properties is spatially dependent within ranges of a similar order of magnitude (2.4, 5.4 and 6.1 m respectively for SNA, NO_3^- and incubation pH), close correlations between their values of $\hat{\gamma}(h)$ over a range of values of h would be expected. Thus, it is concluded that the crossvariogram will only fit an exponential or spherical model, that is, will only show spatial dependence of one property on another, when the correlation between values of $Z(x_i)$ and $Y(x_i)$, in addition to that between $\hat{\gamma}_z(h)$ and $\hat{\gamma}_y(h)$, is significant. Alternatively one can say that the crossvariogram is a true test of *spatial co-dependence*. It therefore seems reasonable to conclude that crossvariogram analysis is potentially useful, but that time and effort will be saved in the case of *spatially independent covariables* if estimation of the crossvariogram is preceded by a classical test of correlation because this will indicate whether estimation of the crossvariogram is likely to be worthwhile.

In view of the conclusion that nitrifiers are sufficiently adapted to the prevailing soil pH that their activity in the field is close to that when operating at the pH optimum for nitrification, small fluctuations in pH over space might not be expected to cause spatial variation in nitrifier activity. Hence, the lack of any close correlation over space between SNA and pH in the nested spatial variability experiment as described in section A(iv) above, should not be regarded as an anomalous result. Indeed, it is concluded that for the Tokomaru silt loam, in any given area, a pH value within the range 5-6.5 would not be a good predictor of nitrifier activity.

The indications from the literature are that the degree to which nitrifier activity is affected by various soil properties is soil-specific. In the case of soil pH and moisture effects, this conclusion is supported by the differences between values of pH_{opt} in soils **T**, **TL**, **TX**, **TLX** and the Patua soil, and also between these values and those for other soils (Chapter 7), and by the similar differences between pF_{opt} for the Tokomaru silt loam and published results for other soils. It might therefore be concluded that the spatial variability of nitrifier activity will also be soil-specific. Thus, different soils will have different ranges of spatial dependence for the parameters of mineral N. Furthermore, the fact that SNA is not the only factor governing the soil NO_3^- concentration, and that other factors such as plant uptake and leaching are also important, indicates that SNA variability is probably not a good estimator of soil NO_3^- variability. This conclusion is certainly supported by the geostatistical aspects of the work reported in this thesis. Thus, with respect to field scale estimates of the areal mean soil nitrate concentration, it is concluded that soils should be sampled in the manner described at the end of Chapter 6, that is, using clusters of samples separated by a minimum distance of 5 m, and analysed primarily for NO_3^- concentration.

Whether further spatial analysis of the parameters of mineral N in other regions and in other soils is warranted, so as to define an optimum distance for the separation between sampling clusters, will probably be determined by the cost of such analysis. Nevertheless, in spite of the apparent shortcomings of geostatistical techniques, they obviously have

the potential to allow for the precise definition of spatial variability. Assuming that the range of spatial dependence (if any) of the other input parameters to the nitrate leaching model can be determined, in addition to that for the soil nitrate concentration, it should be possible to map soil nitrate *leachability* over large areas using kriging. However, the inferred soil-specificity of N variability, together with the possibility that in addition to the soil nitrate concentration, the other input parameters to the nitrate leaching model may also be soil-specific, might necessitate sampling and measurement of a range of properties over a wide range of soils. Furthermore, the fact that the variability of mineral N is predominantly short-range will make it necessary for sampling to be very intensive for nitrate leachability maps to be of real value. The costs involved may be therefore be prohibitive. However, in view of the public concern about the environmental consequences of nitrate leaching, this is an avenue of future research that should be pursued.

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APPENDIX I

COMPUTER PROGRAMMES - GAMMAH

COVGM

```
c
c   ****************************** GAMMAH ************************
c
c   Robert G.V. Bramley, Dept. Soil Science, Massey University
c                        Palmerston North, New Zealand.
c
c   This programme performs all the necessary calculations for the
c   spatial analysis of grided data in two perpendicular directions.
c
c   The programme assumes a nested design whereby a 21 * 21 grid
c   with a grid spacing of 12.5 cm is nested at the centre of an 11
c   * 11 grid with a 2.5 m grid spacing; samples need not be taken
c   at every node, but where there are gaps, a value of zero should
c   be entered in the data file. The data are read in from a data
c   file in which the data are arranged in two matrices - an 11 *
c   11 matrix containing the main grid data, and a 21 * 21 grid
c   immediately below it containing the nested grid data.
c
c   (N.B. The size of the grid can be altered by changing the i,j,
c   lagA and lagB parameters, and the grid spacing may be changed by
c   altering the initial values of gapA and gapB.)
c
c   As well as calculating the semivariance at each lag, the
c   programme output also contains a check on the number of data
c   points used in the calculations, separated by each lag.
c   The mean, sample variance and total sum of squares are also
c   calculated.
c
c   (N.B. The "call openfile" command must be replaced by the
c   longhand FORTRAN alternatives, Read and Write, if the programme is
c   to be run outside Massey University. Access to this routine may be
c   obtained from H. Murphy, Dept. Soil Science.)
c
c
c
c   Declare Parameters
c
      integer i, j, n, q
      integer lagA, lagB, mnsA, mnsB, mewA, mewB, mA, mB
c
      Real ZA (11, 11)
      Real ZB (21, 21)
      Real mean, sum, var, zsqs, sq, squm, p, sumsqs
      Real gapA, totnsA, totewA, seminsA, semiewA, semiA, diff
      Real gapB, totnsB, totewB, seminsB, semiewB, semiB
c
c   Read in the data
c
      call openfile (5,'  ','read')
      call openfile (6,'  ','write')

c   i corresponds to rows; j to columns.
c
      do 10 i = 1, 11
         read (5, *) (ZA(i,j), j = 1, 11)
10    continue
```

```
c
      do 20 i = 1, 21
         read (5, *) (ZB(i,j), j = 1, 21)
20    continue
c
c
c   Calculate sample mean and variance
c
      n = 0
      sum = 0.0
      zsqs = 0.0
      do 30 i = 1, 11
         do 40 j = 1, 11
            if (i.eq.5.and.j.eq.6) goto 40
            if (i.eq.6.and.j.eq.6) goto 40
            if (i.eq.5.and.j.eq.7) goto 40
            if (i.eq.6.and.j.eq.7) goto 40
            if ((ZA(i,j).lt.0).or.(ZA(i,j).gt.0)) then
            sum = sum + ZA(i,j)
            n = n + 1
            sq = (ZA(i,j)) * (ZA(i,j))
            zsqs = zsqs + sq
            endif
40       continue
30     continue
c
       do 50 i = 1, 21
          do 60 j = 1, 21
             if ((ZB(i,j).lt.0).or.(ZB(i,j).gt.0)) then
             sum = sum + ZB(i,j)
             n = n + 1
             sq = (ZB(i,j)) * (ZB(i,j))
             zsqs = zsqs + sq
             endif
60        continue
50     continue

      mean = sum / n
c
      write (6,70) n, mean, sum
70    format (//,'Mean of',i4,' data points is',f8.4,' ; sum of Z is',f1
     *0.4/)
c
      squm = sum * sum
      p = squm / n
      sumsqs = zsqs - p
      q = n - 1
c
      var = sumsqs / q
c
      write (6,80) var, sumsqs
80    format ('Sample variance is',f12.6,' ; Sum of squares is',f15.6//
     */)
c
c
```

```
c     Calculate semivariances
c
c     a) Nested Grid
c
      mnsB = 0
      mewB = 0
      do 180 lagB = 1, 20
         if (lagB.eq.1) then
         gapB = 0.125
         endif
         if (lagB.ne.1) then
         gapB = gapB + 0.125
         endif
         if (gapB.gt.2.0) goto 180
c
         totnsB = 0.0
         totewB = 0.0
c
         do 190 i = 1, 21 - lagB
            do 200 j = 1, 21
               if (((ZB(i,j).lt.0).or.(ZB(i,j).gt.0)).and.((ZB(i + lagB,
     *j).lt.0).or.(ZB(i + lagB,j).gt.0))) then
               diff = ZB(i,j) - ZB(i + lagB,j)
               mnsB = mnsB + 1
               totnsB = totnsB + (diff * diff)
               endif
200         continue
190      continue
c
         do 210 j = 1, 21 - lagB
            do 220 i = 1, 21
               if (((ZB(i,j).lt.0).or.(ZB(i,j).gt.0)).and.((ZB(i,j + lag
     *B).lt.0).or.(ZB(i,j + lagB).gt.0))) then
               diff = ZB(i,j) - ZB(i,j + lagB)
               mewB = mewB + 1
               totewB = totewB + (diff * diff)
               endif
220         continue
210      continue
c
c
         seminsB = totnsB / (2.0 * mnsB)
         semiewB = totewB / (2.0 * mewB)
         mB = mnsB + mewB
         semiB = (totnsB + totewB) / (2.0 * mB)
c
         write (6,230) gapB
         write (6,240) mnsB, seminsB
         write (6,250) mewB, semiewB
         write (6,260) mB, semiB
c
230      format ('With a lag of ',f6.3,' m :'//)
240      format ('In a "north-south" direction with',i3,' pairs of point
     *s, gamma = ',f10.8/)
250      format ('In an "east-west" direction with',i3,' pairs of points
     *, gamma = ',f10.8//)
```

```
260      format ('If data are isotropic, there are a total of',i5,' pair
     *s of points,',/,'with gamma = ',f10.8/////)
cc
         mnsB = 0
         mewB = 0
180      continue
c
c
c     b) Main Grid
c
      mnsA = 0
      mewA = 0
      do 90 lagA = 1, 10
         if (lagA.eq.1) then
         gapA = 2.5
         endif
         if (lagA.ne.1) then
         gapA = gapA + 2.5
         endif
c
         totnsA = 0.0
         totewA = 0.0
c
         do 100 i=1, 11 - lagA
            do 110 j=1, 11
               if (((ZA(i,j).lt.0).or.(ZA(i,j).gt.0)).and.((ZA(i + lagA,
     *j).lt.0).or.(ZA(i + lagA,j).gt.0))) then
               diff = ZA(i,j) - ZA(i + lagA,j)
               mnsA = mnsA + 1
               totnsA = totnsA + (diff * diff)
               endif
110         continue
100      continue
c
c
         do 120 j = 1, 11 - lagA
            do 130 i = 1, 11
               if (((ZA(i,j).lt.0).or.(ZA(i,j).gt.0)).and.((ZA(i,j + lag
     *A).lt.0).or.(ZA(i,j + lagA).gt.0))) then
               diff = ZA(i,j) - ZA(i,j + lagA)
               mewA = mewA + 1
               totewA = totewA + (diff * diff)
               endif
130         continue
120      continue
c
c
         seminsA = totnsA / (2.0 * mnsA)
         semiewA = totewA / (2.0 * mewA)
         mA = mnsA + mewA
         semiA = (totnsA + totewA) / (2.0 * mA)
c
         write (6,140) gapA
         write (6,150) mnsA, seminsA
         write (6,160) mewA, semiewA
         write (6,170) mA, semiA
```

```
c
140       format ('With a lag of ',f4.1,' m :'//)
150       format ('In a "north-south" direction with',i3,' pairs of point
     *s, gamma = ',f10.8/)
160       format ('In an "east-west" direction with',i3,' pairs of points
     *, gamma = ',f10.8//)
170       format ('If data are isotropic, there are a total of',i5,' pair
     *s of points,',/,'with gamma = ',f10.8///)
c
          mnsA = 0
          mewA = 0
90        continue
c
c
      write (6,270)
270   format (//,'N.B. Formulae used in this programme are as follows:'
     *,//,'Mean = Sum Z(i,j) / n',//,'Sum of squares = Sum Z(i,j)^2 - {[
     *Sum Z(i,j)]^2 / n} / {n - 1}',//,'Variance = Sum of squares / {n -
     * 1}'//'Gamma(h) = {1 / 2m(h)} * Sum {[Z(x) - Z(x + h)]^2}')
c
      stop
      end
```

```
c
c   ****************************** COVGM ************************
c
c   Robert G.V. Bramley, Dept. Soil Science, Massey University
c                        Palmerston North, New Zealand.
c
c   This programme is an adapted version of GAMMAH and calculates
c   cross-semivariances for two properties arranged in a nested
c   grid as per GAMMAH.
c
c
c   Declare Parameters
c
      integer i, j
      integer lagA, lagB, mnsA, mnsB, mewA, mewB, mA, mB
c
      Real ZA (11, 11)
      Real ZB (21, 21)
      Real QA (11, 11)
      Real QB (21, 21)
      Real diffZ, diffQ
      Real gapA, totnsA, totewA, seminsA, semiewA, semiA
      Real gapB, totnsB, totewB, seminsB, semiewB, semiB
c
      call openfile (6,'  ','write')
c
c   Read in the data
c
c   i corresponds to rows; j to columns.
c
      call openfile (5,'  ','read')
c
      do 10 i = 1, 11
         read (5, *) (ZA(i,j), j = 1, 11)
10    continue
c
      do 20 i = 1, 21
         read (5, *) (ZB(i,j), j = 1, 21)
20    continue
c
      call openfile (5,'  ','read')
c
      do 11 i = 1, 11
         read (5, *) (QA(i,j), j = 1, 11)
11    continue
c
      do 21 i = 1, 21
         read (5, *) (QB(i,j), j = 1, 21)
21    continue
c
c
c
```

```
c     Calculate cross semi-variances
c
c     a) Nested Grid
c
      mnsB = 0
      mewB = 0
      do 180 lagB = 1, 20
         if (lagB.eq.1) then
         gapB = 0.125
         endif
         if (lagB.ne.1) then
         gapB = gapB + 0.125
         endif
         if (gapB.gt.2.0) goto 180
c
         totnsB = 0.0
         totewB = 0.0
c
         do 190 i = 1, 21 - lagB
            do 200 j = 1, 21
               if (((ZB(i,j).lt.0).or.(ZB(i,j).gt.0)).and.((ZB(i + lagB,
     *j).lt.0).or.(ZB(i + lagB,j).gt.0))) then
               diffZ = ZB(i,j) - ZB(i + lagB,j)
               diffQ = QB(i,j) - QB(i + lagB,j)
               mnsB = mnsB + 1
               totnsB = totnsB + (diffZ * diffQ)
               endif
200         continue
190      continue
c
         do 210 j = 1, 21 - lagB
            do 220 i = 1, 21
               if (((ZB(i,j).lt.0).or.(ZB(i,j).gt.0)).and.((ZB(i,j + lag
     *B).lt.0).or.(ZB(i,j + lagB).gt.0))) then
               diffZ = ZB(i,j) - ZB(i,j + lagB)
               diffQ = QB(i,j) - QB(i,j + lagB)
               mewB = mewB + 1
               totewB = totewB + (diffZ * diffQ)
               endif
220         continue
210      continue
c
c
         seminsB = totnsB / (2.0 * mnsB)
         semiewB = totewB / (2.0 * mewB)
         mB = mnsB + mewB
         semiB = (totnsB + totewB) / (2.0 * mB)
c
         write (6,230) gapB
         write (6,240) mnsB, seminsB
         write (6,250) mewB, semiewB
         write (6,260) mB, semiB
c
230      format ('With a lag of ',f6.3,' m :'//)
240      format ('In a "north-south" direction with',i3,' pairs of point
     *s, gamma = ',f10.8/)
```

```
250      format ('In an "east-west" direction with',i3,' pairs of points
     *, gamma = ',f10.8//)
260      format ('If data are isotropic, there are a total of',i5,' pair
     *s of points,',/,'with gamma = ',f10.8/////)
cc
         mnsB = 0
         mewB = 0
180      continue
c
c
c     b) Main Grid
c
      mnsA = 0
      mewA = 0
      do 90 lagA = 1, 10
         if (lagA.eq.1) then
         gapA = 2.5
         endif
         if (lagA.ne.1) then
         gapA = gapA + 2.5
         endif
c
         totnsA = 0.0
         totewA = 0.0
c
         do 100 i=1, 11 - lagA
            do 110 j=1, 11
               if (((ZA(i,j).lt.0).or.(ZA(i,j).gt.0)).and.((ZA(i + lagA,
     *j).lt.0).or.(ZA(i + lagA,j).gt.0))) then
               diffZ = ZA(i,j) - ZA(i + lagA,j)
               diffQ = QA(i,j) - QA(i + lagA,j)
               mnsA = mnsA + 1
               totnsA = totnsA + (diffZ * diffQ)
               endif
110         continue
100      continue
c
c
         do 120 j = 1, 11 - lagA
            do 130 i = 1, 11
               if (((ZA(i,j).lt.0).or.(ZA(i,j).gt.0)).and.((ZA(i,j + lag
     *A).lt.0).or.(ZA(i,j + lagA).gt.0))) then
               diffZ = ZA(i,j) - ZA(i,j + lagA)
               diffQ = QA(i,j) - QA(i,j + lagA)
               mewA = mewA + 1
               totewA = totewA + (diffZ * diffQ)
               endif
130         continue
120      continue
c
c
         seminsA = totnsA / (2.0 * mnsA)
         semiewA = totewA / (2.0 * mewA)
         mA = mnsA + mewA
         semiA = (totnsA + totewA) / (2.0 * mA)
```

```
c
        write (6,140) gapA
        write (6,150) mnsA, seminsA
        write (6,160) mewA, semiewA
        write (6,170) mA, semiA
c
140     format ('With a lag of ',f4.1,' m :'//)
150     format ('In a "north-south" direction with',i3,' pairs of point
     *s, gamma = ',f10.8/)
160     format ('In an "east-west" direction with',i3,' pairs of points
     *, gamma = ',f10.8//)
170     format ('If data are isotropic, there are a total of',i5,' pair
     *s of points,',/,'with gamma = ',f10.8///)
c
        mnsA = 0
        mewA = 0
90      continue
c
c
      write (6,270)
270   format (//,'N.B. Formulae used in this programme are as follows:'
     *,//'Gamma(h) = {1/2m(h)} * Sum {[Z(x)-Z(x+h)][Q(x)-Q(x+h)]}')
c
      stop
      end
```