LABORATORY MEASUREMENTS OF SOIL MICROBIAL BIOMASS AND NITROGEN MINERALIZATION FROM TWO CHINESE SOILS AS INFLUENCED BY LONG-TERM APPLICATIONS OF MANURE AND INORGANIC FERTILIZERS

by

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ABSTRACT

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The purpose of this study was to investigate the results of two long term fertilization experiments on soil organic C, total N and mineralizable N in the Jiangsu Province of People's Republic of China. The soil samples that received manure over the years contained more soil organic C, and total N than the inorganic fertilized samples. Soil organic C was closely correlated with total N and there were correlations between crop yields and soil organic C contents and between crop yields and soil total N contents. Plant-available N was estimated using biological and chemical tests. Mineralized N formed during aerobic incubation was low except for those soil samples that received manure. Microbial biomass C and N were estimated using the chloroform fumigation-incubation method (CFIM) and fumigation-extraction procedures. Biomass measurements by CFIM were more precise and reliable then values obtained by fumigation-extraction. Treatment differences in biomass were not significant. Estimates of biomass C and N were influenced by the choice of the control soil and the period of incubation used by the CFIM. Unfumigated (10-20 d) control soils were found to be the best control for samples. Extraction of mineralized N using 0.5M $NaHCO_3$ after incubation overestimated biomass N since this extraction was found to extract non-biomass N.

RÉSUMÉ

Le but de cette étude était d'évaluer les effets à long terme de deux systemes de fertilisation, l'un à base d'engrais inorganiques et l'autre à base de fumier de cheval, sur la teneur en C organique et en N total du sol obtenus après dix années de production de blé et de mais dans la province du Jiangsu en République Populaire de Chine. Les échantillons de sol qui ont reçu du fumier au cours des années contiennent plus de C organique et de N total que les échantillons qui ont reçu des engrais minéraux. Ainsi il existe une corrélation entre le C organique et le N total du sol. Cependant, les corrélations sont plus faibles entre la production céréalière annuelle et les quantités de C organique du sol, et entre la production totale céréalière annuelle et les quantités de N total du sol. Le N du sol disponible pour la croissance des céréales a été estimé grace à deux tests: l'un biologique et l'autre chimique. Les résultats du test biologique montrent que les valeurs obtenues en anaérobiose sont faibles, excepté pour les échantillons qui ont reçu du fumier. Par contre, le test chimique s'est révélé inopérant. La méthode de fumigation-incubation au chloroforme s'est montrée plus fiable pour l'estimation de la biomasse que la technique de fumigation-extraction. Généralement, les échantillons de sol ayant reçu du fumier contiennent des teneurs en biomasse supérieures à celles des échantillons ayant reçu des fertilisants inorganiques, toutefois les différences entre les deux modes de fertilisation ne sont pas significatives. L'estimation de la taille de l'azote de la biomasse a été tentée utilisant une incubation aérobie tandis que l'utilisé d'une incubation anaérobie (sol sous une couche d'eau). L'incubation anaérobie par rapport à l'incubation aérobie présente des avantages certains de simplicité pour les laboratoires chinois. Par contre, pour l'estimation des quantités d'azote minéralisé, l'extraction utilisant $NaHCO_3$ après l'incubation aérobie s'est avérée

inefficace car cette procédure surestime les valeurs d'azote contenu dans la biomasse.

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FORWARD

This thesis presents new information on soil C and N as influenced by long-term applications of manure and inorganic fertilizers under continuous wheat and corn double-cropping systems of Xuzhou region in Jiangsu Province of the People's Republic of China. The first Chapter is a general review of literature on the methodology used to estimate plant-available N and soil microbial biomass C and N. Particular attention was given to chloroform-fumigation incubation and fumigation-extraction methods.

The second Chapter is concerned with potentially mineralized N under anaerobic conditions, soil microbial biomass C and N studies, respectively, as influenced by two different Chinese field management noted in Appendix 2. The chapter is presented with a general introduction, a description of materials and methods used, a discussion together with the results and a conclusion. Following this chapter is Appendix 1 a general survey of China's agriculture situation. Finally, Appendix 2 is a detailed discussion of the two Chinese long-term field experiments as necessary background information regarding design, management practices and data for the laboratory studies that I have carried out. The data originated from translation of three Chinese reports, supplemented by discussion of published material on other long-term trials from England, France and USA.

INTRODUCTION

In the rich agricultural Jiangsu Province of China, fertilization management using inorganic fertilizers combined with organic manures are encouraged by the government over the use of inorganic fertilizers alone. This use is for economic as well as environmental reasons.

In Appendix 2, information regarding design, management practices and results were given for two long-term field experiments in the Jiangsu Province of People's Republic of China from which soil samples were collected for subsequent laboratory measurements on soil microbial biomass and nitrogen mineralization. The most important study was conducted on long-term plots established at Xuzhou on yellow fluvoaquic soils. The objectives were as follows:

(1) to determine the influence of chemical fertilizers and organic manure on the quantity and quality of two cereal crops (winter wheat and corn),

(2) to compare short and long-term effects of continuous applications of chemical fertilizers to that of organic manure on soil fertility, nutrition balance, and fertilizer efficiency,

(3) to evaluate the effect of accumulation and exhaustion of mineral constituents in the soil on the chemical composition of wheat and corn plants, and

(4) to determine long-term trends in soil organic matter and total N under the different soil treatments.

It was clearly established that organic manure added to the soil increased amounts of nutrients provided to the soil and crop productivity. It is thought that these benefits result from increased fertility and microbial activity. It is pertinent, therefore, to quantify the N supplying capacity of the soils that have had different fertilizer treatments and the role of microbial biomass in N turnover in the soil.

In contrast to soil organic C and total N, measurement of microbial biomass C and N has been stated to provide sensitive parameters to assess changes in soil management. During the last two decades interest has developed in the quantitative determination of soil microbial biomass in the warm temperate zones. Biomass values from various areas throughout the world have been compiled in the literature, but little information has been reported on microbial biomass in Chinese soils.

Utilization of microbial biomass estimation methods for soils has produced research relating biomass to various management practices and soil processes. Consequently, for this study the hypotheses were that past fertilization management influenced soil N availability, thus manure treated soils in comparison to inorganic fertilized soils tested for 10 yr in two long-term field experiments of the Jiangsu Province should show significant differences in microbial biomass C and N and potentially mineralizable N.

The objectives of the study were:

(i) to compare N mineralized by different techniques,

(ii) to estimate microbial biomass C and N contents of Chinese soils by two methods: the chloroform fumigation-incubation (CFIM) and the fumigation-extraction (FE) methods,

(iii) to measure the effects of different extractants on biomass N estimates by CFIM,

(iv) to assess the effects of a number of soil treatments on aerobic and anaerobic incubation, and

(v) to study the relationships among various soil parameters.

CHAPTER ONE

METHODS OF MEASURING N MINERALIZATION AND MICROBIAL BIOMASS: A LITERATURE REVIEW

Soil organic matter has been studied by a variety of techniques. Net changes of organic matter occurring within the soil system have been evaluated by the combination of tracer, chemical, and biological techniques. Juma and McGill (1986) have used models simulating the dynamics of soil organic C and N. In these simulation models soil organic matter constituents were divided into four fractions (Paul 1984):

(1) the microbial biomass as determined chemically or microscopically making up 4 to 6% of soil N,

(2) the non-biomass active N determined by isotopic dilution, composed of 6 to 10% of soil N,

(3) resistant or old C associated N determined by carbon dating, forming 50% of soil N, and

(4) a stabilized N fraction providing about 36% of soil N.

Paul (1984) reported that for a Canadian Chernozemic soil the relative contribution to mineralizable N of each fraction was 30, 34, 1, and 35%, respectively. Measurements of characteristics of the small, but active fraction of soil organic matter have been used successfully for measuring changes in soil quality. In the past, it has been difficult to assess the effects of agronomic practices on the quality of soil organic matter unless widely differing management systems were used, over many years (Biederbeck et al. 1984).

In recent years, various researchers have measured such parameters as soil extractable C, mineral N flush and potentially mineralizable N to gauge qualitative soil organic matter changes. Relationships among the parameters has validated their use as possible indices to assess the nitrogen supplying-power of soil. In general, their results show that biomass and labile N in surface soils are dependent on land use.

In contrast to total organic C and N, measurements of microbial biomass and mineralizable C and N are stated to provide sensitive parameters to assess changes in agricultural practices (Carter and Rennie 1982; Paul 1984). Today's research focus on organic N pools in soil and the use of tracer techniques with new extraction techniques to aid in separating organic N into biologically meaningful fractions should unravel the mechanisms and controls of the mineralization-immobilization processes in soil as suggested by Juma and Paul (1984).

1.1 LABORATORY INDICES OF PLANT-AVAILABLE SOIL N

Considerable attention was being given to the development of a quick and reliable indice for plant-available N in soil. Stanford (1982) felt that none of the chemical or biological methods for estimating the organic N supplying capacities of soils was judged sufficiently reliable to warrant routine use in soil testing laboratories. As a rule a satisfactory method in terms of reliability and reproducibility should provide for rigorous standardization with respect to methods of sampling, drying, grinding and sieving, storing, and incubating the soils.

Still, laboratory indices of soil N availability that have been commonly proposed can be subdivide into two distinct approaches:

(1) **chemical indices** in which N mineralized as a result of chemical treatment involving acid or alkaline hydrolysis is determined (see MacLean 1964; Keeney 1982),

(2) **biological indices** in which N mineralized during a prescribed period of aerobic or anaerobic (waterlogged) incubation, (1 to 40 wk duration), is measured (see Stanford and Smith 1972; Smith et al. 1981; Keeney 1982).

1.1.1 CHEMICAL INDICES OF SOIL ORGANIC N AVAILABILITY

Stanford (1982) categorized several chemical indices on the basis of extraction intensity. They ranged from those involving relatively mild extraction, through intensive extraction to determination of total soil N. The utility of any chemical indice depends on the degree to which it correlates with reliable biological measurements of soil N availability such as N uptake, crop yield, or mineralizable N. He concluded that results obtained by more intensive procedures (such as 6N boiling HCl) were inconsistent with biological indices since they removed more N than might be mineralized during a single growing season.

Generally, the use of relatively mild extractants for estimating the potentially mineralizable N in soil (e.g., dilute solutions of $CaCl_2$, K_2SO_4 and $NaHCO_3$) has been favored by several researchers. Amongst the most interesting, MacLean (1964) proposed the use of 10mM $NaHCO_3$ on the assumption that (1) a relationship exists between solubility and availability of nitrogenous compounds to plants, and that (2) room-temperature extraction (for a short period) with that very mild alkaline solution should tend to simulate conditions produced by liming to near neutrality.

Jenkinson (1968) postulated that the soil biomass constituted the major source of plant-available N amenable to chemical dissolution or hydrolysis by relatively mild extraction procedures. He also concluded that extractants, which selectively remove microbial N, provide an indirect measure of the mineralizable N in soil.

Stanford (1982) has done an exhaustive review on the subject of mild extraction procedures relative to soil microbial biomass. However, for many investigators very little evidence supports the conclusion that mild extractants selectively remove biomass N, or that the biomass constitutes the primary source of plant-available soil N removed by extraction.

Using an Illinois soil, Kelley and Stevenson (1985) determined the relative effectiveness by which various mild extractants estimate plant-available soil N. Their results tended to support the view that less intensive extraction procedures were more selective in removing newly-immobilized N. According to Jenkinson (1968), 31 to 42% of N extracted by milder extractants of cold 10mM $NaHC_3$ and hot 5mM $NaHCO_3$ was derived from the microbial biomass. A higher percentage of the soil N was extractable with 10mM $CaCl_2$ as compared to 10mM $NaHCO_3$.

Azam et al. (1989) compared the efficiency of three mild extractants, 10mM $CaCl_2$, 10mM $NaHCO_3$ and 500mM K_2SO_4, to extract biomass N from a moist Pakistani soil after a 106 h incubation with $(15NH_4)_2SO_4$ and glucose. On one hand, they showed that the quantity of N extracted by the three extractants differed considerably. In accord with the observation of Kelley and Stevenson (1985) they confirmed that 10mM $NaHCO_3$ was somewhat more selective than 10mM $CaCl_2$ in removing the biomass N. On the other hand, they found that the three extractants solubilized almost the same proportion of applied N but of the three extractants 10mM $NaHCO_3$ extracted the largest proportion of applied 15N. This opposed the studies by Kelley and Stevenson (1985). The results of these additional studies strengthened Stanford's (1982) viewpoint on the difficulty of interpreting extractable soil N in terms of plant availability, but did not rule out the possibility of developing reliable chemical indices useful in predicting N mineralization potentials of soils.

1.1.2 BIOLOGICAL INDICES FOR MEASURING NITROGEN MINERALIZATION POTENTIALS OF SOILS

In comparison to the chemical indices, measuring plant uptake of soil N or N mineralized during incubations are laborious and time-consuming. Biological indices can be based on aerobic or anaerobic incubations.

A. Incubation under aerobic conditions

Stanford (1982) noted that biological indices based on short-term incubation were less meaningful because results were influenced by method of sample pretreatment (effects of drying, storing, freezing, or other means of handling field-moist soils) to a greater extent than with chemical extractions. With long-term incubation, transitory effects of recent crop residues or of sample handling are minimized. However, Stanford questioned the reliability of results derived from extended incubations in static, closed systems for determining the long-term N mineralization capacities of soils.

Use of short-term aerobic incubations of soil N has been motivated primarily by the need for rapid and reliable methods of assessing soil N availability. Incubation time was limited to 7 to 14 d. Most results appeared to reflect relative N-supplying capacities of soils. In contrast, Stanford and Smith (1972) argued that even with rigorous controls, results of short-term incubations do not necessarily reflect the potential, long-term N supplying capacities of soils.

Consequently, they were the first to introduce the use of long-term, aerobic incubations to determine the N-mineralization potential (No). Stanford and Smith (1972) indicated that No comprised a variable fraction of the total soil N. Since Stanford and co-workers's approach assumed there is a single capacity factor (No) for measuring soil N source for plants, this incubation technique has been widely used to determine the effect of various agricultural practices on soil fertility (Carter and Rennie 1982; El-Haris et al. 1983). In support to the approach promoted by Stanford and co-workers for estimating the nitrogen-supplying power of soils the results of Campbell et al. (1984) provided reasonably precise predictions while accounting for soil moisture and temperature differences.

Bonde and Rosswall (1987) reported that No values of soils incubated for 91 d, were a function of cropping system and sampling date. Differences in N mineralization were as large as

differences among cropping systems. Robertson et al. (1988) used the No as an estimate of the active fraction as suggested by Bonde and Rosswall (1987). Both Carter and Rennie (1982) and Robertson et al. (1988) showed that a considerable amount of C and N mineralized could be accounted for in a decrease in the microbial biomass during incubation.

Bonde et al. (1988) experiment showed that increasing additions of organic matter as farmyard manure or plant material as well as N fertilizer increased long-term aerobic mineralization and the proportion of total N present in the active fraction of soil organic N. The influence of fertilization and cropping practices on the percentages of mineralizable N was further outlined by Bonde and Rosswall (1987).

However, Bonde et al. (1988) seriously questioned whether biomass provides potentially mineralizable N in the field by means of a reduction in the size of the biomass during a growing season, rather than by exhausting other organic matter fractions. Accordingly, they concluded that likely the turnover of microbial biomass N is slower than that of biomass C but more importantly that the microbial biomass was not a major source of plant-available N during a growing season. Biomass N amounted to 3.9-6.8% of total N.

The use of short or long-term aerobic incubation techniques is complicated by problems in establishment and maintenance of satisfactory conditions of incubation, especially, control of water content during incubation (Stanford 1982).

B. Incubation under anaerobic (waterlogged) conditions

Incubation of soils under waterlogged conditions and measurements of NH_4^+ released has attracted attention because of its simplicity as compared to most aerobic incubation procedures (Waring and Bremner 1964). The main advantages of the anaerobic incubation are (Waring and Bremner 1964; Keeney 1982):

(1) it is rapid and readily applicable to both air-dried and field-moist soils,

(2) only ammonium production needs to be determined, because no nitrate (or nitrite) is produced,

(3) only a small sample of soil is needed, and

(4) it is not necessary to adjust the water-content of the soil to near optimum.

Generally, lengthy and expensive laboratory incubation studies are not feasible for many field experiments (Clay et al. 1990). Anaerobic incubation techniques seem particularly suitable for relating soil N released on incubation to yield potential under a wide range of soil and climatic conditions. Several published reports have stressed the superiority of anaerobic mineralization as an index of N availability for rice over NH_4^+ formed during aerobic incubation and also in the measurement of N turnover relative to forest productivity.

Steam distillation of the filtered extract, rather than of the soil suspension has been modified to avoid release of NH_4^+ by alkaline hydrolysis of soil organic matter. Stanford (1982) suggested incubation of soils in 2M KCl instead of water. Keeney (1982) included an anaerobic incubation procedure, which used distilled water and 4M KCl as a extractant.

For some agricultural and forest soils of Washington state, Smith et al. (1981) showed that anaerobic incubationss have smaller variation than aerobic incubation methods, since treatment conditions can be more uniformly applied. Furthermore, the authors found a significant linear relationship between the nitrogen mineralized anaerobically vs. that mineralized aerobically. They have some indication the nitrogen mineralized anaerobically was a consistent proportion of that mineralized under aerobic field conditions.

The origin of N mineralized under anaerobic conditions is not known. For Paul (1984) much of the NH_4^+ mineralized during the incubation probably was attributed to biomass N, e.g., fungal materials that decay under the anaerobic conditions.

Adams and Attiwill (1986) suggested that N was liberated from aerobic microorganisms killed by anaerobic conditions. This seems analogous to what occurs in the $CHCl_3$ fumigation-incubation method (CFIM) of Jenkinson and Powlson (1976a) for measuring microbial biomass where Myrold (1987) hypothesized that N mineralized using the 7 d anaerobic incubation method came mainly from killed microbial cells. The results obtained from soil samples representative of a wide range of forest soils in Oregon (USA), suggested that anaerobic N mineralization measured N released primarily from microbial biomass. Hence, net accumulation of NH_4^+ after 7 d of anaerobic incubation was highly correlated with NH_4^+ produced after CFIM.

Some interesting implications come from these results. On one hand, Myrold (1987) suggested that the microbial biomass contains a pool of N that is readily available to plants. Corresponding findings by Carter and Rennie (1982) have been found between microbial biomass N and other tests of N availability for agricultural soils. Perhaps through appropriate cultural practices it should be possible to manage and manipulate these easily mineralizable nutrients sequestered in the microbial biomass. The anaerobic incubation, by establishing an "anaerobic Kn value" to estimate microbial biomass N, could be used instead of the CFIM to determine microbial biomass.

1.1.3 INTERPRETING CHEMICAL AND BIOLOGICAL INDICES OF SOIL NITROGEN AVAILABILITY

Many investigations have raised serious doubts regarding the feasibility of interpreting and utilizing laboratory measurements of soil N availability under the widely varying soil and climatic conditions encountered in the field. Hence, for Stanford (1982), only with a single soil type, climatic zone, or farming system can trustworthy interpretations of mineralized N be expected. Most important, in establishing meaningful relationships between soil N measurements and

greenhouse results, researchers have a difficulty related to the variable amounts of soil mineral nitrogen present before cropping. As Stanford (1982) suggested, the problem arises because mineral N already present in the soils is readily available to plants, whereas N measured by short-term incubations or chemical methods is just an indice of the relative amounts of N that may be supplied from soil organic sources.

McCracken et al. (1989) sought to examine soil N availability indices (Keeney 1982 methods) and past N fertilization, past cover cropping, and tillage from plots of a long-term field experiment, and to evaluate the ability of these indices to predict corn response to added N. The authors found no significant correlation between the results of the anaerobic incubation indice and corn response. However, this indice was significantly affected by N-fertilizer treatment, but not in ways mirrored in crop response. The anaerobic incubation falsely predicted greater N mineralization on the no-N plots than on plots with a history of N fertilization (McCracken et al. 1989).

Normally, anaerobic incubation has been used to assess soil N lability, plant N uptake and plant productivity in studies that have attempted to deal with a range of different soils. McCracken et al. (1989) concluded that laboratory incubations or extraction procedures have a very low power to estimate long-term management effects on soil N availability. Clay et al. (1990) suggested that autoclave-extractable-N (10mM $CaCl_2$; Keeney 1982) may be sensitive to management-induced changes in soil biological activity as related to potentially mineralizable N (No) in the field.

1.2 SOIL MICROBIAL BIOMASS

Microbial biomass has been defined by Jenkinson (1981) as the living microbial component of the soil with the exclusion of plant roots and soil animals larger than 5 x $10^3 \mu m^3$. The ability to differentiate biomass from non-living soil organic matter has enabled the distribution of C, N, P and S in the soil to be measured, and the rate of turnover to be estimated by using isotopes. It is generally agreed that microbial biomass plays a crucial role in the transformation of organic matter in soil, affecting the decomposition and turnover of organic substrates, nutrient immobilization and cycling, root physiology and soil structure (see Sparling 1985).

As suggested by many authors, the organic matter entering the soil system annually should be separated into stable (~40%) and labile (~60%) fractions. Sparling (1985) reported that the microbial biomass is mainly of the labile fraction but comprises only a small proportion of the total soil organic matter.

In recent studies, Anderson and Domsch (1986, 1989, and 1990) developed a new concept on the relationship between biomass C (Cmic) and soil OM (Corg). They stated that Corg of agricultural soils in equilibrium contained 2.3 to 4.0% Cmic. They also hypothesized that soils exhibiting a ratio of biomass C-to-total organic C (Cmic-to-Corg), and deviating from these values would either be losing or accumulating OM. Constancy of the Cmic:Corg ratio is thus an indication of a system at a new equilibrium. Anderson and Domsch (1989) reported a wide spectrum of Cmic:Corg ratios ranging from 0.27 to over 7.0%, presumably, because of differences in soils, vegetative cover, management, as well to variations in sampling time and analytical methods.

Anderson and Domsch (1989) reported in detail on the ratios of microbial biomass C (Cmic) to total organic carbon (Corg) of 134 soils from 26 long-term experimental sites located in the temperate climatic zone of Central Europe. Insam

(1990), in studying Cmic:Corg ratios in long-term agricultural plots of arid to subhumid climatic zones of North America, found that precipitation-to-evaporation ratios influenced C equilibrium. This survey of Cmic:Corg ratios indicated no equilibrium constant.

Several factors have been examined and their effect on biomass C variability and/or Cmic:Corg ratio measured. Insam (1990) suggested that substrate quantity and quality as well as its distribution determined and controlled biomass C. Further, Carter and Rennie (1982), Juma and McGill (1986), Anderson and Domsch (1986), Doran (1987), Biederbeck et al. (1984), Powlson et al. (1987) and Jenkinson and Rayner (1977) reported that agricultural practices such as tillage, crop rotations, cropping sequences, manuring or residue incorporations affected the amount of OM by controlling both C input and rate of biomass turnover, therefore influencing the amount of biomass C.

Powlson et al. (1987) on long-term straw incorporation studies illustrated how microbial biomass responded more than either soil organic C or N to changes in management that altered annual inputs or decomposition of organic material to soil. Therefore, biomass measurements provided an early indication of changes in soil organic matter contents and mineralizable nitrogen, before such changes could be detected by chemical analysis. Biomass measurements have been used as sensitive indicators to detect trends in soil organic matter caused by cultivation method and for its potential use in management decisions, changes in cropping patterns (see Powlson and Jenkinson 1976), the clearing of tropical forests (see Ayanaba et al. 1976). As suggested by Paul and Voroney (1984) there is potential for improved management of soil and fertilizer nutrients through a better understanding and possible manipulation of biomass. In addition, the microbial biomass is consider a key compartment in most of the models dealing with OM and N turnover in agricultural soils (Jenkinson

and Rayner 1977; Chaussod et al. 1988).

Information has been reported on the possibility of increasing soil and fertilizer use efficiency through management of the biomass by cultivation, cropping practices, and even faunal feeding (see Paul and Voroney 1984). In analogy, many studies have been performed to elucidate both short-term and long-term effects of N fertilization on microorganisms. Schnürer et al. (1985) studied how soil microorganisms were affected by manure, crop residues and N-fertilizer additions in a 27-yr-old field experiment in Sweden, they found that treatments not receiving N contained significantly less biomass C than the corresponding treatment receiving inorganic N. In a similar manner, from Broadbalk Continuous wheat experiment, Shen et al. (1989) results showed that the plot receiving no fertilizer N contained consistently less microbial biomass C than others, presumably because the annual input of fresh plant debris is least in this plot. Nevertheless, Shen et al. (1989) felt that year-to-year variability in biomass C largely masked any differences arising from past fertilizer applications.

In his review Sparling (1985) stated that fertilizers generally increase the biomass while organic fertilizers cause a much greater increase than inorganic fertilizers. Part of the increase in the biomass caused by organic fertilizers results from a direct stimulation to the biomass, rather than from increased crop growth (Jenkinson and Powlson 1976; Rosswall and Paustian 1984).

1.2.1 ACTIVITY OF SOIL MICROBIAL BIOMASS

The term "activity" includes the many processes carried out by microbial enzymes. When interpreted in conjunction with biomass measurements, activity often refers to net nutrient mineralization and immobilization rates (Paul and Voroney 1984). In the past 20 yr, numerous empirical observations on biomass and biological activity in soils, and ecosystems, with respect to amendments, cultivation practices, and responses to

environmental and climatic factors have often produced conflicting and confusing results (Nannipieri 1984).

Nevertheless, there is general agreement, using a variety of techniques, that the bulk of the soil biomass is inactive because of low levels of available nutrients in the soil. Thus only a small proportion of the total biomass C content ranging from 2.4 to 27.2% is active (Sparling 1985).

Studies have revealed that the biomass is well adapted to survive long periods of starvation in soil and maintenance requirements are low (see Sparling 1985). Smith et al. (in Paul and Voroney 1984) have recognized three major microbial population states:

(1) the active biomass capable of growth and all metabolic functions is usually estimated to vary between 10 to 40% of the total identifiable biomass,
(2) sustained populations that are nongrowing but can dissimilate glucose and resume growth under favorable conditions, and
(3) dormant spores or other long-term resting populations.

Information has been reported on several factors that decrease biomass content. Treatments such as soil drying, freezing, thawing and waterlogging, may restrict size and activity of biomass (Ross and Tate 1984). Relationships among environment, microbial activity and rates of decomposition have been the subject of several reviews (see Sparling 1985).

1.2.2 DIFFERENT METHODS OF MEASURING MICROBIAL BIOMASS

Soil microbiologists have always hoped to obtain a general indice of total biological activity of soils, which could be used in agriculture and for describing the biological status of the environment (Nannipieri 1984). However, the need to characterize microbial cycling and the energy flow in the ecosystem has led to the consideration of the microbial biomass in soil as an undifferentiated whole, although it is known that

an enormous diversity of microbes with varying abilities to survive in extreme environmental conditions is present (Paul and Voroney 1984; Nannipieri 1984).

Tedious microscopic techniques and unreliable methods based on estimates of special cell constituents have been replaced in the past 20 yr by methods of estimating the soil biomass:

(1) $CHCl_3$ fumigation-incubation,

(2) $CHCl_3$ fumigation-extraction,

(3) substrate-induced respiration,

(4) the ATP content of biomass, and

(5) microbial biovolume by microscopy.

Relationships and differences found among biomass C from the C-flush or N-flush following fumigation, the respiration method, the ATP content of the biomass and biovolume have been discussed (Jenkinson and Ladd 1981; Sparling 1985; West et al. 1986a). None of the procedures can satisfactorily measure the biomass and activity in all soils and results will depend on aspects being investigated. Methods do not directly measure C content of the microbial biomass, but rely on conversion of a measured C value to biomass C. These conversion factors have been the subject of considerable interest and controversy as they appear to vary both within and among soils. Based on these facts, West et al. (1986a) have come to three important conclusions:

(1) in order to identify unreliable data, at least 2 and preferably 3 methods to estimate biomass should be used,

(2) the extent of correlation between the methods is partly dependent on the soils used and their condition at the time of assay, and

(3) conversion factors permitting calculation of absolute biomass C values are not applicable to all soils or situations thus probably will involve substantial error.

In spite of all this, the chloroform-fumigation technique (CFIM) by Jenkinson and Powlson (1976a,b) has permitted a more accurate determination of the content of nutrients in soil biomass and offers the possibility to quantify the relative fluxes of nutrients (Nannipieri 1984; Sparling 1985). No method, other than the CFIM offers the possibility of recovering microbial C for subsequent analysis.

1.2.3 $CHCl_3$ FUMIGATION-INCUBATION METHOD (CFIM) FOR MEASURING BIOMASS C

The method is based on the finding (Jenkinson 1966) that $CHCl_3$ vapors lyse cells of microbial biomass. This allows cytoplasmic carbon and nitrogen constituents to leak into the soil rendering them either extractable by various salt solutions, or mineralizable during a subsequent biological incubation (Chaussod et al. 1988). When a soil is fumigated with $CHCl_3$, the fumigant is removed, and the soil is inoculated with a small amount of soil having a living population (an untreated sample). The fumigation method needs no special equipment and requires only the titration of CO_2 absorbed in alkali. However, a relatively long incubation is necessary before the CO_2 can be analyzed. Some authors have used a 10 and 20 d incubation period at 25°C (Jenkinson et Powlson 1976a,b; Shen et al. 1989) or even 22°C (Anderson and Domsch 1978). Others have used a shorter period of incubation adapted to a higher temperature such as 28°C for 7 and 14 d, respectively (Nicolardot et al. 1984; Nicolardot and Chaussod 1986; Nicolardot 1988).

A. Biomass C calculation

Microbial biomass C is calculated as follows (Jenkinson and Powlson 1976a): Bc = Fc/Kc where Bc is the biomass of carbon, Fc the flush of decomposition (i.e., CO_2 from $CHCl_3$-treated soil minus soil-corrected CO_2 from untreated soil), and Kc, the percentage biomass of carbon mineralized to CO_2.

B. Assumptions of CFIM

Biomass measurements by fumigation-incubation involve a number of assumptions about extraction, shrinkage, cell density, moisture content and composition, documented by Jenkinson (1976). The most widely challenged assumption has been that microbial decomposition of native soil organic matter proceeds at the same rate in both fumigated and unfumigated soil, despite the differences in microbial population caused by fumigation. For example, the method will not give reliable results on soils that have recently received large inputs of fresh decomposable organic matter like straw or organic manures (Jenkinson and Powlson 1976b; Voroney and Paul 1984).

In analogy, West et al. (1986a) measured changes in the microbial biomass of three New Zealand soils under treatments designed to manipulate the quality and quantity of the soil microbial biomass such as: storage (i.e., starvation), air-drying (rapid decrease) and glucose amendment (rapid increase). They concluded that CFIM should not be used on soils receiving a fresh input of microbial substrate, e.g., on rewetted, previously air-dried soil or glucose-amended soil in accord with Jenkinson and Powlson (1976a,b) recommendations. This is because after such treatment the control soil can respire more than the fumigated soil resulting in no detectable flush or even a negative flush.

C. pH restrictions on the use of CFIM

Jenkinson and Powlson (1976b) have pointed out serious problems introduced through decomposition of bicarbonates in calcareous soils low in organic matter. Other experiments with soil samples at pH > 6 have shown that alkaline soils require a continuous removal of respired CO_2 from the soil air or an adsorption by an alkali in a closed system to avoid the dissolution of CO_2 in soil water.

In parallel, Powlson and Jenkinson (1976) have shown that acid soils of pH < 4.5 respond poorly to fumigation, with the

net flush sometimes being negative. As suggested by Chapman (1987) the degradation capacity of the microbial population surviving fumigation, even with inoculation, is markedly reduced in acid soils.

D. Sampling prerequisites

Comparison of biomass results of different soils are rendered very difficult because of the differences in sampling dates and conditions available throughout the scientific literature. Anderson and Domsch (1986; 1989) showed that the most appropriate sampling time would be in early spring (March-April), before any field management, fertilizer, manure application or root growth has taken place since biomass as well as its physiological state are transient events during the year.

E. Experimental variables

Ross and Tate (1984) have noted that although the principles of the CFIM have been commonly accepted, details of its use by researchers frequently differ. West et al. (1986a,b) have stressed that care should be taken in interpreting and comparing indices of microbial biomass obtained by different or modified experimental procedures.

Ross and Tate (1984) recorded at least eight different mesh sizes, from 2 to 10 mm, that have been used for sieved soil while unsieved soil (intact core) has less frequently been used. Sieving is generally considered desirable even for wet soils for subsequent measurement of microbial biomass by fumigation procedures (Jenkinson and Powlson 1976b). Other authors found sieving to reduce soil variability due to interference from plant roots, especially for grassland soils (Ross 1987).

The CFIM, commonly used to estimate soil microbial C and N, was developed for use on field moist soils adjusted to a water content between 50 and 60% of water-holding capacity (WHC) (Jenkinson and Powlson 1976a). Although 70% of WHC and no adjustment have also been employed (Ross and Tate 1984). Moist soil samples subjected to wetting-drying, and freezing-thawing showed a flush in CO_2 production and N mineralization upon incubation (Jenkinson 1966; Jenkinson and Powlson 1976a).

Shen et al. (1987) have extended the method to include rewetted air-dried soils. Jenkinson and Powlson (1976b) have strongly advised that soils should not be air-dried before fumigation since air-drying renders some non-biomass C decomposable, as well as killing an appreciable fraction of the biomass. According to the authors, the increase in extractable C is generally attributed to increased availability of OM for microbial transformations following soil treatment. In a comparable study on the effect of different soil treatments on the extractability of biomass and non-biomass N, Azam et al. (1989) suggested that enhanced metabolic activities in treated soils may not necessarily be due to the increased availability of previously inaccessible OM, but is more likely due to enhanced susceptibility of the dried OM to microbial attack.

An other important experimental variable is preincubation; direct fumigation of freshly sampled soil versus previously incubated soil at 25°C (Ross and Tate 1984). Jenkinson and Powlson (1976b) originally proposed prior incubation of samples to allow storage and handling effects to subside. Although prior incubation may be desirable in some circumstances, it was clear from Ross and Tate (1984) results that its effect on biomass C does not always follow the same pattern in all soils. They related the nature of this effect to soil temperature and moisture status at the time of sampling. In analogy, West et al. (1986b) showed that the soil properties assessed had changed appreciably during prior incubation of soil at 25°C for 7 d, a period intermediate between those used or recommended by

Jenkinson and Powlson (1976b). They concluded that measurements on fresh samples appear desirable if data on the status of a soil in the field are required.

In conclusion, many studies have confirmed that values of soil biomass are dependent upon experimental conditions. However, other factors are known to affect the reliability of the CFIM such as reinoculation of fumigated sample, different incubation periods for the control soil and calculation of the C-flush.

F. Choice of control for biomass C measurement

There is a lack of standardization in CFIM to estimate biomass C, see Jenkinson and Powlson (1976b); Paul and Voroney (1980); Voroney and Paul (1984); Ross and Tate (1984). Hence the period of mineralization and choice of an appropriate control for comparison with the fumigated samples can influence biomass C estimates.

As described by Chapman (1987) essential to the CFIM success is the development of a microbial population following fumigation that can adequately mineralizes a fixed fraction of the carbon in the killed soil biomass. This population arises either from survivors of the $CHCl_3$ treatment or from an inoculum of unfumigated soil.

Having shown that incubation without an inoculum reduced O_2 consumption of a Broadbalk soil by 7%, Jenkinson and Powlson (1976a,b) used a small inoculum of 0.4% (w/w). Anderson and Domsch (1978) used an inoculum of 2% (w/w) while others have used only a soil suspension. Jenkinson and Ladd (1981) have stated that "enough organisms survive $CHCl_3$ treatment for an inoculum not to be essential" and Chaussod and Nicolardot (1982) described a procedure for incubation without inoculation. Chapman (1987) proposed that soil biomass C determinations should be made with a 10% inoculum in fumigated soil. For some acid soils, Chapman (1987) and Vance et al. (1987) have shown that the use of a large inoculum, can provide

satisfactory measurements of CO_2-C flush, while for strongly acid soils, the use of a small inoculum has also been proposed.

A related question to the inoculum size is whether a control value should be subtracted to account for the CO_2-C derived from non-biomass C in the fumigated soil. Hence, there is no agreement in determination of microbial biomass using CFIM as to what constitutes a good control to estimate the CO_2-C attributable to mineralization of the killed population only. Chapman (1987) recognized three major sources from which CO_2 can arise in both fumigated and non-fumigated soil such as (1) biomass killed by fumigation or handling procedures, (2) basal respiration, and (3) respiration induced by the disturbance of non-biomass OM during handling.

One can find a range of recommendations for the use of an unfumigated versus a fumigated control. Hence, on the incubation of the control involving set periods of either 0-10 d (Oades and Jenkinson 1979) or 10-20 d (Jenkinson and Powlson 1976a,b) for unfumigated soil, and also no allowance for unfumigated soil (Paul and Voroney 1980), while 10-20 d for fumigated soil (Chaussod and Nicolardot 1982) have been used.

The calculation [CO_2-C fumigated (0-10 d) minus CO_2-C unfumigated (0-10 d)] was originally proposed by Jenkinson and Powlson (1976a) for soils given a prior incubation. Jenkinson (1966) suggested that basal respiration (decomposition of organic matter other than dead microorganisms) was not altered in the fumigated sample as compared with the unfumigated. However, Jenkinson and Powlson (1976a) argued that this may not always be true due to differences in the mass of the populations in incubated fumigated and unfumigated soil. Ross and Tate (1984) and Voroney and Paul (1984) have also indicated that the metabolic activity of the microorganisms present after fumigation could be different. Qualitative and quantitative differences from the original populations have been observed in organisms recolonizing soil treated with other fumigants (see McGill et al. 1986). Smith and Paul (1986) felt that native

organic C was being mineralized in fumigated samples. However, they doubted that this amount of C is similar to or even the same type of C being mineralized in non-fumigated samples. Thus it seems reasonable to subtract only a percentage of the control from the fumigated sample.

The calculation [CO_2-C **fumigated (0–10 d) minus** CO_2–C **unfumigated (10-20 d)]** was originally proposed by Jenkinson and Powlson (1976a,b) for the situation in which part of the biomass had been killed immediately before fumigation. For stored, sieved and freshly-sampled soils they recognized that over the 0-10 d period, the control soil often evolved larger amounts of CO_2-C than the fumigated sample, thus negative biomass was calculated. This suggested that the CO_2-C evolved from the control during a 10-20 d incubation be used since the CO_2-C evolution rate was more consistent with background rates.

The calculation [CO_2-C **fumigated (0–10 d) minus** CO_2–C **fumigated (10–20 d)]** was proposed by Chaussod and Nicolardot (1982). Rather than to run a parallel incubation experiment with unfumigated samples where sometimes CO_2 production does not become constant over the periods used, the incubation of the fumigated samples was carried on for ten more days, with CO_2 released from day 10 to 20 corresponding to endogenous respiration. Srivastava and Singh (1988) argued in favour of using CO_2 evolution during 10–20 d after fumigation as the control since the CO_2 comes from the same (and microbiologically comparable) sample from which the CO_2 produced during the first 10 d is measured.

In contrast, Shen et al. (1987) found that in moist soils there was a close relationship between CO_2–C evolved by fumigated soil over the 10-20 d period and CO_2-C evolved by unfumigated soil over the same period. These results lent further support to Jenkinson and Powlson (1976a) arguments that basal soil respiration was not greatly affected by fumigation.

In Shen et al. (1987) viewpoints, the weakness of using 10-20 d controls was revealed when newly-available substrate

was present. For instance, Shen et al. (1987) confirmed that substrate released by air-drying was largely decomposed during the 0-10 d period in both fumigated and unfumigated soils, so that CO_2 released from a 10-20 d fumigated soil control was too small. The use of a fumigated control appeared the most appropriate for measuring CO_2-C flush in grassland soils (Ross 1990a).

The calculation [CO_2-C fumigated (0-10 d)], which uses no control was proposed by Paul and Voroney (1980) and by Voroney and Paul (1984) for soils that have had recent amendments of available substrates. It is assumed that there is no decomposition of soil organic matter other than dead microbial biomass in fumigated soils over the measured period.

Based on the measurement of the biomass C produced during in situ growth on added 14C-glucose, Paul and Voroney (1980) suggested that it is an error to subtract a control from the total CO_2 evolved. Furthermore, since the microbial populations developing following fumigation are different from that in the soil before treatment the authors felt that an unfumigated sample cannot be a control for the fumigated sample where more than 99% of the microorganisms have been killed. In comparison, Shen et al. (1987) results stressed that a control is needed and that biomasses calculated without a control are too large, especially when fresh substrate was present.

A consensus amongst researchers is that the period of incubation used for control soils greatly influenced the estimations of biomass C. Levels of biomass C were always highest when calculated with no deduction for CO_2-C production by control soil (Voroney and Paul 1984) and generally lowest when 0-10 d values for CO_2-C production by unfumigated soils were used (Ross and Tate 1984). For many authors, the four methods of calculation produce erroneous results since the last method overestimated biomass in some instances, while the others underestimated true biomass values (Smith and Paul 1986; Bonde et al. 1988).

G. Choice of Kc value for biomass C determination

Kc is the fraction of the biomass mineralized to CO_2 used to calculate biomass from CO_2 evolution. Various values for converting the CO_2-C flush to biomass C content have been proposed in the literature ranging from 0.40 to 0.58. However, according to West et al. (1986a), these values are less variable compared to the convertion factors of other methods.

Jenkinson and Powlson (1976a,b) initially suggested a Kc factor equal to a value of 0.5 for a 10 d period at 25°C based on a small number and variety of microorganisms. Anderson and Domsch (1978) found that bacteria were decomposed more rapidly than fungi in different soils. Assuming a ratio of 1:3 for the distribution of the bacterial and fungal biomass in the soil population they suggested a value of 0.41 was more appropriate. Most researchers have employed a Kc value of 0.45 as determined by Oades and Jenkinson (1979). Ross and Tate (1984) used a Kc value of 0.45 and Jenkinson and Ladd (1981) considered that a single value might be applied to different soils without serious error. Ross (1987) used experimentally determined Kc values for each soil at each sampling time, rather than a constant Kc value. Ross (1987) results with added organisms have confirmed and extended previous observations that Kc values are dependent on the organisms and soils used for their determination (Jenkinson 1976; Nicolardot et al. 1984). Nevertheless, Ross (1987) concluded that overall the use of a single Kc value is acceptable.

West et al. (1986a) consider that CO_2-C flush can supply useful information without the complication of biomass C conversion values and thus, for comparative purposes, greater emphasis be placed on the relative differences within and among soils without converting to biomass C. Conversion of data to biomass C may result in substantial errors for both fresh and pre-incubated soil and for samples taken in different seasons (West et al. 1986a,b).

1.2.4 $CHCl_3$ FUMIGATION-INCUBATION METHOD (CFIM) FOR MEASURING BIOMASS N

Fumigation usually causes an immediate increase in the extractable NH_4^+ in soil. Following the subsequent soil incubation is an additional NH_4^+ release (Jenkinson and Powlson 1976a; Nannipieri 1984). However, determination of the mineralized N produced is difficult since it is continually transformed both in unfumigated and fumigated soils (Voroney and Paul 1984; Shen et al. 1984). Voroney and Paul (1984), Marumoto et al. (1982) and Nicolardot et al. (1986) have stated that determination of Kn coefficients related to the fraction of the biomass mineralized to NH_4^+ is relatively difficult.

Still, numerous researchers have attempted to estimate microbial biomass N from the measurements of total N or NH_4^+ liberated or free after fumigation. Aerobic incubation of sample soil performed at 25°C during 10 to 20 d has been used. Anaerobic incubation of fumigated soil has also been proposed (Nicolardot 1988).

A. Biomass N calculation

Microbial biomass N is calculated as follows: Bn = Fn/Kn where Bn the biomass of nitrogen, Fn is the flush of N mineralized and Kn is the fraction of biomass N mineralized to inorganic N during the 10 d after $CHCl_3$ fumigation. Ayanaba et al. (1976) found that there was a close correlation between biomass C and the flush of mineral N upon fumigation. They suggested that an estimate of the biomass C can be obtained from the flush of mineral N by multiplying by 8, on a wide variety of soils.

Different extractants have been used to obtain N released from biomass. Jenkinson and Powlson (1976a) noted an immediate increase in K_2SO_4-extractable NH_4^+-N following fumigation. Increases in total N (both NH_4^+- and organic-N) have also been reported (Brookes et al. 1985a). In comparison, Biederbeck et al. (1984) extracted both (NH_4 + NO_3)-N forms although others

like Carter and Rennie (1982) have used only NH_4^+. McGill et al. (1986) described an interesting way of calculating the mineral N (NH_4^+-N and NO_3^--N extracted with 2M KCl) accumulation in unfumigated controls between 0-10 d such as N10 = C10 x N20 /(C10 + C20); where N10 equals mineral N accumulated between 0 and 10 d. C10 equals CO_2 evolved between 0 and 10 d. N20 equals mineral N accumulated between 0 and 20 d, and C20 equals CO_2 evolved between 10 and 20 d. The quantity of mineral N in fumigated samples and unfumigated controls was measured after 10 d and 20 d of incubation, respectively. The flush of mineral N was calculated in the same manner as flush of CO_2, but was not converted to biomass because of variability in published methods of calculating biomass N (Voroney and Paul 1984). Like Carter and Rennie (1982) they did not convert flush of NH_4^+ released after fumigation to an estimate of biomass.

Extraction of both mineral (NH_4 + NO_3)-N forms have been used. Patra et al. (1990), for example, measured biomass N by extracting the fumigated and unfumigated soils with 0.5M K_2SO_4 after the incubations and measuring NH_4-N and NO_3-N in the extractants. The initial NH_4-N and NO_3-N contents of the soils were also measured and initial NO_3-N content used to correct for any NO_3-N denitrified when fumigated soils were incubated, since recently fumigated soils do not nitrify.

Nicolardot (1988) proposed an alternative method of measuring microbial biomass N in which N immobilization was reduced by incubating fumigated soil under anaerobic conditions for 7 and 14 d followed by an extraction with 1M K_2SO_4. Biomass N measurements are expressed as the N-flush value equal to NH_4^+ fumigated soil incubated under anaerobic conditions for 0-7 d, minus NH_4^+ produced by an identical fumigated and incubated soil over 7-14 d at 28°C.

B. CFIM assumptions for biomass N

Brookes et al. (1985a) reaffirmed that fumigation does not affect the extractability of non-biomass N. In contrast, Voroney and Paul (1984) challenged this fundamental assumption of the fumigation method for measuring biomass. Based on soil N rendered extractable due to fumigation, Azam et al. (1989) supported this suggestion since data indicated that $CHCl_3$ fumigation, besides releasing biomass N, had a significant effect on extractability of non-biomass N.

C. CFIM problems for biomass N

Many researchers like Brookes et al. (1985a) reported that problems restricted to biomass N measurements by the fumigation-incubation method arise because denitrification or (re)immobilization of N by the soil population during aerobic incubation could mask the fumigant induced release of N. Jenkinson and Powlson (1976a,b) have also indicated that there are uncertainties in making both biomass C and N measurements in soils, which have either been recently sampled or rewetted following air-drying.

D. Choice of the control for biomass N measurement

The problem of what value to use as a control seems less critical for N than for C (Paul and Voroney 1984; Smith and Paul 1986). Nevertheless, identical ways of calculating the flush-C have been used to determine the flush of N based on different controls.

Shen et al. (1984) defined the flush of N as the N mineralized by a previously fumigated soil incubated for 10 d under standard conditions, less that mineralized by a similarly incubated, but non-fumigated control soil. Voroney and Paul (1984) did not subtract a control for soil incubated with glucose and NO_3-N but their data agree with that of Ayanaba et al. (1976) who showed that the ratio of N mineralized by a fumigated soil:N mineralized by an unfumigated soil was greater

than the corresponding ratio for CO_2.

In agreement with Jenkinson (1976), Voroney and Paul (1984) reported that the net N mineralized after fumigation is directly affected by the N content of soil organisms. Estimates of biomass N are affected because the amount of mineral N that accumulates in soil is the net result of two concurrent processes, immobilization and mineralization. The Kn value for biomass N calculations will vary due to N-immobilization differences among soils. Voroney and Paul (1984) overcame this problem by correcting the Kn value used to calculate biomass based on the ratio of CO_2-C to NH_4^+-N (Cf:Nf) flushes from each soil. They found the Cf:Nf ratios to range from a low of 1.3:1 in composted wheat straw to > 300:1 in a forest floor litter.

On the other side, Chaussod et al. (1986) and Nicolardot and Chaussod (1986) argued against the use of an unfumigated control soil. They proposed a method for estimating the mineralizable microbial N fraction (NH_4^+ only) based on a fumigated control soil. This method does not overcome the difficulties linked with nitrogen immobilization in fumigated soils despite the fact that kinetic recording of the flush often improves accuracy and reliability of the measurement especially in acid soils.

E. Importance of biomass C:N ratio

As stated by Sparling (1985) the fumigation technique permits a direct estimation of the nutrient content of the biomass, yet it assumes that the flushes of nutrients from the killed biomass behave in a similar way to those from the organisms used to estimate the K values. The C:N ratio of the biomass is commonly reported to range between 5 and 15. Undoubtedly in the author's opinion there is a need to establish how the C and N content of the biomass varies under different conditions. However, Sparling (1985) showed that the biomass N comprises roughly the same proportion of total N as the biomass C does of the total C. Hence, biomass N is closely

linked to the biomass C, so that on soils with a low biomass C the biomass N is generally low.

F. Choice of Kn value for biomass N determination

The Kn has been found to cover a much wider range than Kc. N biomass estimated by the fumigation technique have to be interpreted with caution (Jenkinson and Ladd 1981; Nannipieri 1984). There is considerable divergence in the literature concerning appropriate values of Kn, some authors have decided not to present data on biomass N, but to discuss the flush of nitrogen after fumigation as a relative measure of microbial N content between soils. Jenkinson and Ladd (1981) found that relationship between mineral N (Min-N) flush and biomass C content can be relatively constant in some soils, but it can vary appreciably in others. Ross and Tate (1984) suggested that the relative insensitivity of Min-N flush to handling and changes in moisture content makes it particularly suitable for estimating microbial biomass. However, Ross (1987) found that the sampling time fluctuations in Kn values for each studied soil were generally similar to those for Kc values.

In the past ten years, Kn values ranging from 0.25 to 0.63 have been used for calculating biomass N. Carter and Rennie (1982) and Biederbeck et al. (1984) used an average value of 0.4, while Shen et al. (1984) proposed a Kn value of 0.68 derived from measurements made on microorganisms grown in vitro. This Kn of 0.68 was not recommended for acid soils (pH < 4.5), for soils that had recently received large quantities of organic matter, nor for unfumigated control soils.

Brookes et al. (1985a) felt the use of 0.68 as the Kn value was controversial since its relevancy to soil organisms in situ was not known. This Kn value is based on consideration of the measured relationship between Fc and Fn and the likely C-to-N ratio of the soil microbial biomass (Shen et al. 1984). It is much greater than the Kn values of 0.39 to 0.59 obtained by Jenkinson and Powlson (1976).

Voroney and Paul (1984) proposed to adjust the Kn value according to the ratio of CO_2-C evolved to net NH_4^+-N accumulated during the fumigation-incubation (Cf:Nf). Recent suggestions by Paul and Clark (1989) to modify the Kn value take into account the reimmobilization of nitrogen. They found the following equation: $Kn = 0.8(Cf:Nf)^{-0.43}$ where Kn was the percentage of nitrogen mineralized after fumigation, Cf the flush of CO_2-C and Nf the flush of NH_4^+-N.

The chloroform-fumigation incubation method proposed by Jenkinson and Powlson (1976a,b) has undoubtedly been the most successful in measuring the amounts of C or N held in the microbial biomass pool. A consensus among researchers is that the method satisfied the demand for a practical biomass measurement. Still some problems inherent to the methodology need to be solved. Numerous problems originate during the aerobic incubation period. For instance, uncertainties regarding the decomposition of non-biomass materials, and re-immobilization of the nitrogen compounds released by chloroform, require further examination in addition to the question of which Kc, or Kn values should be used (Merckx and Van der Linden 1988).

Recent work has shown that biomass components may be measured directly after the $CHCl_3$ fumigation without incubation. Biomass C and N measured by the fumigation-extraction methods developed by Brookes et al. (1985a,b) for biomass N and by Vance et al. (1987) for biomass C agreed closely with the measurements obtained by the classical CFIM and could be of value in avoiding drawbacks linked with the incubation.

1.2.5 INTEREST OVER THE FUMIGATION-EXTRACTION METHOD

Several reports suggested that many problems in estimating microbial C and N by the CFIM are avoided by using the fumigation-extraction (FE) method (Vance et al. 1987; Tate et al. 1988; Sparling and West 1988). FE methods use chemical oxidation or digestion to estimate the extracted organic C or N.

Three main advantages have been stressed. First, the extraction method offers the advantage of making the results immediately available, rather than after a prolonged aerobic incubation, for biomass N estimation, FE avoids the problems caused by denitrification and immobilization of N during aerobic incubation (Jenkinson and Powlson 1976a,b). Second, Merckx and Van der Linden (1988) suggested that C:N ratios of the microbial biomass were readily obtained with the FE method, thus fluctuations in the C:N ratio can effectively be studied. Third, several authors stressed that the FE method will give biomass N (and C) measurements in situations where CFIM generally does not give reliable results. Examples include freshly sampled soils, acid soils, waterlogged or dried soils, densely rooted soil samples (Merckz and Martin 1987) or soils that have recently received fresh substrates (Ocio and Brookes 1990). Minimized or neutralized flushes are measured since very often these soils are degraded faster in the non-fumigated control soil than in the fumigated soil. This problem, essentially related to the different ecological conditions that apply in fumigated versus unfumigated soil, can be avoided if the incubation is replaced by a direct extraction.

1.2.6 FUMIGATION-EXTRACTION METHOD (FE) FOR ESTIMATING BIOMASS C

Powlson and Jenkinson (1976) measured C rendered extractable by $CHCl_3$ fumigation (Ec) defined as organic C extracted by 0.5M K_2SO_4 from a fumigated soil minus organic C extracted from a non-fumigated soil. Vance et al. (1987) proposed a new method for estimating microbial biomass C in soil from the C rendered extractable by 24 h exposure to $CHCl_3$.

The possibility of estimating C and N with the same soil extract was tested by Tate et al. (1988) and their associates Sparling and West (1988a) who chose conditions for soil fumigation and extraction of soluble C that were similar to those described by Brookes et al. (1985b).

A. Relationship between biomass C and organic C rendered extractable by CHCl3

There are several reports of agreement between total extractable C (Ec) released by $CHCl_3$ and CO_2-C flush (Fc). Rather less C is rendered extractable following 24 h $CHCl_3$-fumigation compared to that mineralized during aerobic incubation. Vance et al. (1987) found that a close linear relationship existed between Fc and Ec but that Ec was 60 to 70% of Fc. Sparling and West (1988b) determined that organic C flush in 0.5M K_2SO_4 extracts could range from 48 to 84% of the CO_2-C flush. For a range of soils in New Zealand, they found the organic C flushes to be on average, only 76% of the CO_2-C flush while the Ec values and C flush: N flush ratios were reasonably consistent for all samples. Ross (1990a,b) found the measurement of C extracted to be precise and compare more than favourably with that of any other procedure for measuring biomass C for grassland soils in New Zealand. Yet, Chaussod et al. (1988) stated that the accuracy of the FE method is limited by the accuracy of organic C determinations.

B. Kec value

A basic assumption of $CHCl_3$ fumigation that applies to both FE and FI methods is that soil microorganisms alone are influenced by the fumigation, and that $CHCl_3$ has no effect on the solubility of non-biomass C (Jenkinson and Powlson 1976a,b; Vance et al. 1987). As with the CFIM, the fumigation-extraction procedure requires the use of a K value to convert extractable C to biomass C values. Microbial biomass C is equal to extractable C (Ec) divided by a Kec value.

The Kec value is a measure of the efficiency of extraction rather than mineralization, it allows for the incomplete release and extraction of the microbial C and is obtained by calibrating against alternative methods to estimate the microbial C (Sparling et al. 1990). Several reports have shown that Kec values of different soils can vary markedly (Vance et

al. 1987; Sparling et al. 1990; Ross 1990b). Tate et al. (1988) determined a mean Kec value of 0.20 based on laboratory cultured microorganisms. Sparling and West (1988a) obtained Kec values of 0.33 by 14C-labelling of indigenous soil organisms, and 0.35 by calibration of extractable-C with biomass C values estimated by the substrate-induced respiration procedure. Overall, preference was given to Sparling and West (1988a) Kec value of 0.33, which is lower than the values of 0.41 and 0.45 suggested by Anderson and Domsch (1978) and Oades and Jenkinson (1979) to convert from the CO_2-C flush to microbial C. This was felt to be a consequence of the lower recovery of microbial C in 0.5M K_2SO_4 extracts by the fumigation-extraction method.

Ross (1990b) estimated that any proposal for a common Kec value for different soils resulted in only an approximation that is subject to errors of up to 20% or more. Sparling et al. (1990) felt that a single Kec value of 0.35 applied to all soils was open to criticism. Of the 165 New Zealand soils tested only half gave Kec values within 0.28 to 0.42. Sparling et al. (1990) considered that greater precision was not needed when soil microbial C was being used to reveal differences among soils, the effects of agronomic practices or seasonal fluctuations, since the size of the biomass per se was not necessarily related to crop production and in itself did not provide a reliable indice of soil fertility.

1.2.7 FUMIGATION-EXTRACTION METHOD (FE) FOR ESTIMATING BIOMASS N

FE measures the fraction of the biomass rendered extractable to suitable reagents after lysis by $CHCl_3$. Brookes et al. (1985b) defined the amount of biomass N originally present En as total N extracted by 0.5M K_2SO_4 from soil fumigated for 24 h minus total N extracted from non-fumigated control soil.

A. Relationship between biomass N and organic N (or total N) rendered extractable by CHCl3

Brookes et al. (1985b) data are consistent with the hypothesis that Fn and one day $CHCl_3$-N (En) come from the same fraction of the soil N, probably the cytoplasmic part of the soil microbial biomass, although there is no direct evidence for this. In the same report, soil biomass N showed a close relationship with total soil N. Hence, biomass N was a constant percentage of total soil N in the 40 different soils surveyed (ranging from 2-6% with a mean of just under 4%), despite differences in their previous agricultural history.

B. Kjeldahl digestion

As noted by Brookes et al. (1985a) who compared fumigation-extraction followed by Kjeldahl-type digestion, with a 10-day incubation followed by KCl extraction of NH_4-N and NO_3-N, the amounts of N obtained by FE were less than those obtained by CFIM. They estimated that extraction in 0.5M K_2SO_4 (after 24 h $CHCl_3$ exposure) with subsequent digestion, recovered 79% of the N mineralized by the incubation method. Of the total N rendered extractable about 20% was NH_4-N. Similar observations were made by Sparling and West (1988b) who found, on average, 69% being recovered by the Kjeldahl digestion compared to CFIM.

Based on these results Chaussod et al. (1988) find the FE method in general to be quite reliable but they judged its accuracy highly dependent upon the N measurement. They argued in favour of the use of the alkaline persulphate method since they find that the Kjeldahl method, even modified to include all NO_3-N, is not very satisfactory for measuring small amounts of N in salt solutions.

C. Dichromate oxidation

Similarly, Sparling and West (1988a) studies showed that the N-flush measured as NH_4-N after fumigation-extraction and acid-dichromate-oxidation treatment of 0.5M K_2SO_4 extracts was less than that determined by N-mineralization during a 10-day incubation. Preliminary results showed N-flushes estimated by the direct extraction and oxidation to range between 23-73% of those estimated by incubation. For the authors, this lower recovery of N meant that the C:N ratios of the flushes were generally higher with the extraction and oxidation method than with the incubation method.

As suggested by Sparling and West (1988b) the dichromate oxidation method is considerably less efficient and more variable than the Kjeldahl digestion method in mineralizing organic N, with an average of 59% of the N being recovered compared to the incubation method. However, for acidic and organic soils samples direct extraction of N in 0.5M K_2SO_4 followed by Kjeldahl digestion is likely to prove the more reliable technique. Hence, they recommended to use of this last method if the extracts are also to be tested for oxidizable C. They stressed that the method was worth further research because of the potential for economies of time and reagents by using 0.5M K_2SO_4 as a single extractant for microbial C and N and a single oxidation/digestion for both elements.

D. Ken value

Brookes et al. (1985b) proposed that the Kn value of 0.68 calculated by Shen et al. (1984) for the incubation method, should be decreased by 20% to 0.54 (Ken) to convert from the flush of N to microbial N and to allow for the lower recovery when using $CHCl_3$ fumigation and extraction with 0.5M K_2SO_4 followed by Kjeldahl digestion. In a similar line of reasoning, Sparling and West (1988b) estimated that the Ken value would require to be reduced to 0.47 to allow for the even lower recovery of N when the extraction method is followed by

dichromate oxidation.

From this review of the literature it is clear that sensitive parameters such as CO_2-C evolved, extractable C, mineral flush N, and potentially mineralizable N are available to gauge qualitative soil organic matter changes due to agricultural practices in temperate regions of the world. Consequently, for this study the hypotheses were that nitrogen fertilizer history greatly influenced soil N availability, thus manure treated soils in comparison to inorganic fertilized soils tested for 10 yr in two long-term field experiments of the Jiangsu Province (China) should show significant difference upon the measurement of microbial biomass C and N and potentially mineralizable N.

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CHAPTER TWO

MINERALIZED N AND SOIL MICROBIAL BIOMASS AS INFLUENCED BY PREVIOUS FERTILIZATION

2.1 INTRODUCTION

The hypotheses of this study were that past N fertilizer management should influence the soil N fraction that is involved in mineralization and influence microbial biomass. Manure treated soils should have higher rates of N mineralization and greater microbial biomass than inorganic fertilized soils from a long-term field experiment in Jiangsu Province (China).

Objectives were:

(i) to quantify $NaHCO_3$ extractable-N as related to management induced changes,

(ii) to determine long-term effects of inorganic and manure fertilization on soil N and C mineralization,

(iii) to estimate microbial biomass C and N contents by modified fumigation-incubation (FI) and fumigation-extraction (FE) techniques.

2.2 MATERIALS AND METHODS

Soil samples from Xuzhou field plots were collected near wheat harvest in the spring of 1989. Seven to 10 cores (25 mm in diameter) of the 0-20 cm layer from each treatment plot (4 replications) were taken and similar treatments mixed to form a bulked sample of about 1 kg. Immediately after sampling, soils were stored field moist and unsieved in plastic bags at 2 to 4°C in the dark. Ideally for biological analyses most researchers recommended that experiments should be done on fresh soil (Jenkinson and Powlson 1976a; Anderson and Domsch 1989; Paul and Clark 1989). This was impossible in the present study since soil samples were collected and the subject of this study decided by the Chinese supervisor before my arrival at the Nanjing Agricultural University.

Most importantly, however, soil samples from identical field treatment replicates have been mixed and bulked together.

Thus from a statistical point of view the bulked sample contained no experimental error but only the sampling error. Therefore, all the T-test results from the comparison between different fertilizer management cannot be extrapolated to the field directly but to the bulk sample only. Further, results reported here come exclusively from the Xuzhou soils since Suzhou soil samples were statistically unreliable.

Bulked soil samples were sieved through 2 mm mesh following root and stone removal. A subsample was removed for gravimetric determination of the water content by drying for 4 h at 105°C. Part of the soil sample was air dried prior to determination of pH, organic C, and total Kjeldahl N. Samples were air dried under forced draft for 48 h, ground with a mortar and pestle to pass a 0.5 mm sieve.

Soil pH was measured in water and in 0.01M $CaCl_2$ using a glass electrode and calomel reference electrode (Beckman 61 pH meter). The water pH was measured at a 1:1 (McLean 1982) and a 1:2.5 (w/v) soil to water ratio. Soil pH in 0.01M $CaCl_2$ was obtained by adding 0.05 mL and 0.25 mL of 1M $CaCl_2$ to the 1:1 and 1:2.5 ratios, respectively, mixing and waiting 10 min prior to measurement.

Soil organic C content was measured by the modified Walkley and Black dichromate oxidation method. A mixture of 5 mL 0.8N $K_2Cr_2O_7$ and 5 mL H_2SO_4 (96%) was added to 0.5 g of air-dried soil, and digested for 5 min at 170 to 180°C using a hot plate. Back titration was performed using 0.1N $FeSO_4$ standardized solution. Soil organic matter content was calculated based on soil organic C content multiplied by a factor of 1.72.

Total soil N content was determined by the Kjeldahl method according to Bremner and Mulvaney (1982). For each gram of air-dried soil sample to be digested 1.8 g of catalyst mixture (1 g Se, 10 g $CuSO_4$-H_2O, 100 g K_2SO_4) and 5 mL of 96% concentrated H_2SO_4 was used. The digestion continued 1.30 h after the clearing of the soil solution. Determination of ammonium was performed by estimating NH_3 liberated by steam distillation of the digest with 20 mL of 10N NaOH and collected in a 5 mL 2% boric acid

indicator solution, finally, titrated with 0.1N standardized H_2SO_4 using a 5 mL burette graduated at 0.02 mL intervals.

Water holding capacity (WHC) was initially measured after soaking soil sample cores in water for 12 h followed by air pumping for 20 min in a desiccator. The wet soil cores were then oven dried thoroughly at 180°C. WHC was calculated the following way: wet soil weight (g) minus dry soil weight (g) for 100 g dry soil, and expressed as the water content in percents.

Measurement of plant-available N was estimated according to two tests. Results from the chemical test are not shown because of technical problems encountered during steam distillation to determine mineralizable N (NH_4-N + NO_3-N) following $NaHCO_3$ extraction from moist soils.

Mineralizable N was estimated by incubation. Anaerobic incubation was performed according to the method of Keeney (1982). Triplicate moist and air-dried soil samples of 5 g (oven-dry soil equivalent) were placed with 12.5 ± 1 mL of distilled water in small test tube, stoppered without shaking and kept at constant temperature (40 ± 2°C) for 7 d in the dark. Test tube contents were extracted with 12 to 15 mL of 4M KCl, steam distilled directly with 10 mL of MgO (12%), and distilled NH_3 titrated with 0.005N standardized H_2SO_4. The mineralizable N was expressed as the increase in NH_4-N concentration over the 7 d anaerobic incubation.

Microbial biomass C was estimated by fumigation-incubation and fumigation-extraction. A modified fumigation-incubation technique (Jenkinson and Powlson 1976b) used six portions of moist soil (50 g oven-dried equivalent) from each soil treatment, placed in 250 mL erlenmeyers. Three portions were used as a control (unfumigated), and three were fumigated in a desiccator (lined with wet filtered paper to maintain humidity) containing about 25 mL ethanol-free $CHCl_3$ in a small beaker with a few boiling chips. After $CHCl_3$ had boiled for 10 min, the desiccator was placed in the dark at 25 ± 2°C for 24 h. The beaker of $CHCl_3$ was then removed and the desiccator evacuated 8 times. Fumigated soils were then reinoculated with 0.2 g of

"fresh soil" while the unfumigated samples were not. Fresh inoculum and fumigated soils were mixed on an aluminium foil sheet using a spatula (Chapman 1987). Soils were remoistened to 60 to 79% of field capacity, and a small beaker with 5 mL of 1N NaOH was attached to the rubber closure and placed in the erlenmeyer flasks. Flasks were closed with a rubber stopper, sealed with paraffin wax and incubated for 10 d at 25 ± 2°C in the dark. Each day compressed O_2 was added to the flask. Blanks, in which the flask contained water and alkali but no soil, were incubated in each experiment. After the 10 d period the CO_2 in the NaOH was back-titrated with 0.1N HCl using phenolphthalein, after precipitating carbonates with 2 mL saturated 2N $BaCl_2$. Values for CO_2-C flush (Fc) were calculated as the difference between CO_2-C produced by fumigated soil minus CO_2-C produced by unfumigated soil.

In a second experiment, six portions of moist soil were compared, two portions were used as unfumigated controls and four as fumigated soil samples as previously described. Evolved CO_2 was measured after 10 d and again after 20 d.

Data obtained from both fumigation experiments were used to compare different ways of calculating biomass in analogy to Shen et al. (1987). Three ways of calculating CO_2 flush (Fc) values were examined:

(i) **Fc1 = CO_2-C fum(0-10 d) minus CO_2-C unfum(0-10 d)** (Jenkinson and Powlson 1976a),

(ii) **Fc2 = CO_2-C fum(0-10 d) minus CO_2-C unfum(10-20 d)** (Jenkinson and Powlson 1976a), and

(iii) **Fc3 = CO_2-C fum(0-10 d) minus CO_2-C fum(10-20 d)** (Chaussod and Nicolardot 1982). Values of CO_2-C (Fc) were converted to microbial biomass C (Bc) using Fc/Kc, where Kc equals 0.45 (Jenkinson and Ladd 1981; Jenkinson 1988).

Microbial biomass C determinations by the fumigation-extraction technique of Vance et al. (1987) were carried out. Five samples of moist soils (10 g oven-dry weight equivalent) were weighed into five 80 mL test tubes. Two samples served as

controls (unfumigated) and were extracted immediately with 40 mL 0.5M K_2SO_4 (1:4 w/v ratio). Three samples were fumigated in a desiccator as described in the fumigation-incubation method. After 24 h of fumigation and repeated removal of $CHCl_3$ (8 times), soil moisture was adjusted to 55% WHC. For extraction, 40 mL of 0.5M K_2SO_4 was added to the fumigated soils, suspensions shaken on an end-to-end shaker (80 rpm) for 30 min and then filtered.

The organic C in the extracts was determined by digesting 8 mL of the filtered extract with 2 mL of 0.8N (66.7mM) $K_2Cr_2O_7$, 10 mL of 95 to 98% H_2SO_4 acid and 5 mL of 85% H_3PO_4 acid (Tate et al. 1988). The mixture was boiled gently for 5 min on a hot plate at 170 to 180°C. Excess dichromate was determined by back-titration with 0.1N ferrous ammonium sulphate, using 10 drops of N-phenylantranilic acid solution as an indicator.

Extractable C flush (Ec) was calculated as the difference between the amount of organic C extracted from fumigated and unfumigated samples. Extracted biomass C was estimated two ways:

(i) based on Vance et al. (1987) where Bc = 2.64 * Ec, and

(ii) Sparling and West (1988a) procedure where Bc = Ec/Kec assuming a Kec value of 0.33.

Microbial N was estimated using four procedures:

(a) from the mineral N flush (NH_4-N) extracted by 2M KCl after a 10 d incubation of fumigated and non-fumigated soils at 25°C (Jenkinson and Powlson 1976a; Shen et al. 1984),

(b) from the (NH_4-N + NO_3-N) flush in 0.5M K_2SO_4 extracts after a 0-10 d and 10-20 d incubation of fumigated and non-fumigated soils, respectively (Brookes et al. 1985b; Azam et al. 1989),

(c) as for (b) but using 0.5M $NaHCO_3$ extractable-N (Azam et al. 1989), and finally

(d) from the NH_4-N flush in 0.5M K_2SO_4 extracts of fumigated and non-fumigated soil, using Kjeldahl digestion to convert extracted organic N to NH_4-N

(Brookes et al. 1985b; Sparling and West 1988b).

In the first experiment, soil mixtures were shaken using a 1:10 (soil: 2M KCl solution) ratio for 1 h on a rotary shaker at 250 rpm then let stand for at least 30 min before filtering. A 20 mL aliquot was steam distilled for NH_4-N determination with 10 mL MgO (12%). NH_4-N flush (Fn) values were calculated as the difference between the amounts of NH_4-N mineralized by fumigated soil in 10 d minus the amounts of NH_4-N mineralized by unfumigated soil in 10 d.

In a second experiment, microbial biomass N was determined according to Brookes et al. (1985b) and Schnürer et al. (1985). After each incubation period fumigated and unfumigated 10 g soil samples (oven-dried soil equivalent) were weighed into 80 mL test tube and shaken with 50 mL of 2M KCl (1:5 w/v) for 1 h on an end-to-end shaker (80-100 rpm) and filtered. A 20 mL aliquot was steam distilled for NH_4-N determination with 10 mL MgO (12%) and a further, 20 mL aliquot of the same soil extract was distilled for NH_4-N + NO_3-N determination with 2 g Devarda's alloy and 10 mL MgO (12%).

Similarly other soil samples of 10 g (oven-dried soil equivalent) were weighed in 80 mL test tube and shaken for 30 min on an end-to-end shaker (80-100 rpm) with 40 mL of 0.5M K_2SO_4 (1:4 w/v) and filtered. NH_4-N + NO_3-N was determined as previously. The initial NH_4-N and NO_3-N contents of the soils were measured and the initial NO_3-N content used to correct for any NO_3-N denitrified when the fumigated soils were incubated, since recently fumigated soils do not nitrify (Nicolardot et al. 1986).

Total soluble N including NO_3-N was determined using a modified method (MacLean 1964). Duplicate fumigated and unfumigated soil samples of 5 g (oven-dried soil equivalent) were extracted with 100 mL 0.5M $NaHCO_3$ adjusted to pH 8.5, shaken for 15 min on an orbital shaker (80-100 rpm) and filtered. A 20 mL aliquot was digested with 2 mL of concentrated H_2SO_4 for 30 min to remove CO_2. After cooling, 2 g of Devarda's alloy, 10 mL of 10N NaOH and 20 mL of water were added to

distillation flasks and steam distilled for 6 to 8 min. The NH_3 present was trapped into dilute 2% boric acid indicator solution. The final solution was titrated with 0.01N H_2SO_4.

Inorganic N mineralized flushes (Fn) based on different control were calculated three ways:

(i) **Fn1 = (NH_4-N + NO_3-N fum 0-10 d) minus (NH_4-N + NO_3-N unfum 0-10 d);**

(ii) **Fn2 = (NH_4-N + NO_3-N fum 0-10 d) minus (NH_4-N + NO_3-N unfum 10-20 d);** and

(iii) **Fn3 = (NH_4-N + NO_3-N fum 0-10 d) minus (NH_4-N + NO_3-N fum 10-20 d).** Inorganic N flushes (Fn) were converted to microbial biomass N values Bn = Fn/Kn, using Kn = 0.68 (Shen et al. 1984).

Ammonium-N mineralization from microbial biomass was also measured as described by Nicolardot (1988). Duplicate moist soil samples 10 g (oven-dried soil equivalent) unfumigated and fumigated for about 24 h with chloroform were anaerobically incubated with 40 mL of distilled water in 80 mL stoppered test tubes at 28 ± 2°C. After 7 and 14 d suspensions were extracted by adding 7 g of K_2SO_4 to the test tube and shaking for 30 min on an end-to-end shaker, and filtered. Extracts were kept at 2 to 4°C until analysis. Steam distillation for NH_4-N determination was performed with a 20 mL aliquot plus 10 mL MgO (12%). Mineralizable-N flushes (Fn) were calculated in two ways:

(i) **(NH_4-N fum 0-7 d) minus (NH_4-N fum 7-14 d)**, and

(ii) **(NH_4-N fum 0-7 d) minus (NH_4-N unfum 0-7 d).** The anaerobic N flushes were converted to microbial biomass N values Bn = Fn/Kn, using Kn value of 0.49 (Nicolardot et al. 1990).

Microbial biomass N determinations by the fumigation-extraction technique were performed according to Brookes et al. (1985b) from the NH_4-N flush in 0.5M K_2SO_4 extracts of fumigated and unfumigated soil, using Kjeldahl digestion to digest organic N. As proposed by Brookes et al. (1985b) a Ken value of 0.54 was used to convert the flush of N (En) to microbial biomass N.

Since all biological and chemical analyses were performed on bulked soil samples, T-tests were used to determine the effects of manure compared to inorganic fertilizers. Stepwise regression analyses were used to examine the relationships between differences in biomass and N availability indices (NAI) with differences in soil chemical properties resulting from various fertilization programs. Simple correlations were run between the different methods of measuring biomass, NAI and chemical properties using procedures of the SAS system (SAS 1976).

2.3 RESULTS AND DISCUSSION

Soils were slightly basic with no significant differences between treatments. Soils receiving manure contained more organic C and total N than the corresponding soils receiving inorganic fertilizers. The C:N ratio was between 9.5 and 10.3. Soil organic C values were correlated with total N values (r=0.96), crop yields with soil organic C contents (r=0.80) and crop yields and soil total N contents (r=0.74).

2.3.1 POTENTIALLY MINERALIZED N (PLANT-AVAILABLE N)

Biological test results based on the anaerobically mineralized N ranged from 6.9 to 26.5 mg N kg^{-1} (Table 2.1), which is in agreement with the published literature. The N mineralized anaerobically was low except from those soils that had received manure. This is in contrast to a study by McCracken et al. (1989) in which the anaerobic incubation predicted greater N mineralization on the no-N plots than on plots with a history of N fertilization. In the manured soil samples, N mineralized anaerobically was on the average, twofold higher than in the unmanured soils. Generally,"fresh" and air-dried treated soils showed similar trends between inorganic and manure fertilized samples, but more N was mineralized from dried samples. On the average, the labile N fraction of soil organic matter accounted for 2% of the total N for air-dried soil samples compared to only 1% for "fresh" soils.

The importance of soil N mineralization process in meeting plant N needs has long been recognized. However, these results supported the Stanford and Smith (1972) viewpoint that short-term incubations show little relationship to the long-term supplying capacity of the soils. Presumably NAI estimates were influenced by cropping and fertilization practices. As suggested by Shen et al. (1989) from their results on inorganic N remaining in the soil at harvest, the greater the annual input of plant residues to the field, the higher soil organic N content, which leads in turn, to more mineralizable N. It may be significant that the manured soils, although containing higher levels of total N (1.08 g kg^{-1}) compared to the unmanured samples (0.75 g kg^{-1}) showed lower fractions of mineralizable N (0.7 to 2.8% of total N).

The measurement of the N mineralized from anaerobic incubation suffered from the same imprecision as the chemical index (Purvis and Leo H_2SO_4-extractable NH_4-N) reported by Fox and Piekielek (1978). Differences among low extractable NH_4-N values were difficult to detect.

Water and $CaCl_2$ pH values were negatively correlated with N mineralizable index (NAI) (Table 2.2). Correlation analyses further showed that a somewhat better relationship existed between NAI of air-dried samples and organic C, or total N than with crop yields (Table 2.2; Appendix 3). Still, significant correlations were found between N mineralizable indices and corn yields (1989), which may suggest that NAI were sampling a biologically active N pool. This is not in agreement with McCracken et al. (1989) who found that results of the anaerobic incubation index did not correlate significantly with any corn parameter. They found that NAI was significantly affected by N-fertilizer and cover-crop treatment, but not in ways mirrored in crop response.

2.3.2 CARBON DIOXIDE EVOLUTION AND MICROBIAL BIOMASS C BY CHLOROFORM FUMIGATION-INCUBATION METHOD (CFIM)

Because of my late arrival in China, there was a delay of up to 6 months between the collection, the first and last measurement of Xuzhou soil samples, so the likelihood of anaerobic conditions in the plastic bags is not to be ignored during that time. The soils were kept throughout the study at 2 to 4°C. It is well established that storage at temperatures just above freezing does not stop microbial activity and causes ecological changes in the soil population. Thus observations on the stored samples may not represent the undisturbed field soil. Anderson and Domsch (1989) showed that for similar long-term storage conditions, loss of microbial C from unsieved soil during this period was assumed to be low, and varied between 0.5-10% with a mean of 4.5% for comparison with sieved soil.

Although the manured samples generally contained more biomass C than the mineral fertilized samples, the difference was not significant. Manure fertilization treatments showed higher soil organic C contents and higher microbial biomass C values (Table 2.3). Due to the lack of information on the specific Kc value of the Xuzhou soil, the size of microbial biomass C was estimated by dividing the flush of CO_2-C by a Kc value of 0.45 (Jenkinson and Ladd 1981). But because the method used soil samples that have been previously bulked, stored, etc., the biomass measurements may not be representative of samples from the field. The amounts of biomass C in the Xuzhou soils varied between 492 to 672 and 622 to 1011 $\mu g\ C\ g^{-1}$, in the inorganic fertilizer and manure treated samples, respectively. McGill et al. (1986) observed a similar trend in Breton plots after 50 yr of cropping, the amount of biomass C showed no statistical difference between samples of the manured and mineral fertilizer plots but organically manured plots contained significantly more soluble organic C than plots receiving NPK. Yet, literature values of biomass C tended to be lower (Bolton et al. 1985; McGill et al. 1986; Anderson and Domsch 1989; Houot et al. 1989; Insam 1990) than those reported in this study.

Biomass C levels obtained in this study ranged from 5.3 to 10.3% of the soil organic C, which is above values reported by Jenkinson and Ladd (1981) and Anderson and Domsch (1989). As a rule, when microbial biomass C is presented as a percentage of organic C in soil, the values are usually 1-5% with an average of 2-3% of the soil organic C being biomass C. However, values as high as 7.0% have also been reported presumably because of differences in soils, vegetative cover, management, as well variations in sampling time and analytical methods (Jenkinson and Ladd 1981; Anderson and Domsch 1989).

Recent studies of Anderson and Domsch (1986; 1989) based on a large survey of 129 long-term field plots selected in Europe (Denmark, F.R.G., U.K., etc.) have indicated that there is no universal Bc:Org C (or Cmic:Corg) ratio equilibrium constant. A high ratio would suggest that soil C is accumulating in the Xuzhou samples.

Table 2.3 shows that biomass C accounted on average, for 8.1 and 7.1% of soil organic C, in inorganic and manure treated samples, respectively. Differences between the two treatment managements were not significant. Anderson and Domsch (1989) also found in their study that farmyard manure (FYM) treated plots did not differ in their Cmic:Corg ratios from those of the unamended plots because the FYM amendments were applied one year prior to sampling. This suggested to the authors that there may have been differences between the various treatments in the pool of available C at the time of sampling. Regardless, McGill et al. (1986) and Anderson and Domsch (1989) have stressed that manuring practices have a direct influence on the Cmic:Corg relationship. As suggested by Insam (1990) differences between manure and inorganic fertilized samples of the same experimental site may be in part attributed to differences in crop yield and C allocation to the roots.

2.3.2.1 Effect of fumigation

Fumigated soils of both fertilization treatments evolved more CO_2 than their respective controls (Table 2.3 and 2.4), this is in agreement with Jenkinson and Powlson (1976b). Effects of fumigation persisted into the 10-20 d period while the unfumigated soils evolved much less CO_2-C during the 10-20 d period as during the 0-10 d period (Tables 2.3, 2.4). This suggests that the effects of soil sampling and handling just prior to incubation had not subsided when the first experiment was started. Also, abiotic evolution of carbon dioxide by decomposition of bicarbonate may have proceeded during the first few days of incubation (Powlson and Jenkinson 1976).

2.3.2.2 CO_2 production by fumigated soils

The total amount of CO_2 produced by fumigated soils in the first experiment was higher than in the fumigated samples of the second experiment. Soils at 70% of WHC were used in the first experiment in comparison to soils at 55% of WHC in the second experiment. As suggested by Bonde et al. (1988) growth conditions for the recolonizing bacteria were likely to be better at higher moisture contents. However, the total CO_2 production by fumigated soils was less variable in samples incubated at 55% than at 70% of WHC.

From both experiments, CO_2-C evolved from fumigated and unfumigated samples was correlated with soil organic C contents ($r=0.88$ and 0.95) ($r=0.83$ and $r=0.93$) (Table 2.2). The correlation between organic matter content and CO_2 released has been noted by several researchers. Schnürer et al. (1985) in a Swedish field experiment found that carbon mineralization upon fumigation was positively correlated with organic matter content ($r=0.83$, $P<0.001$), while in unfumigated soils the relationship was lower ($r=0.79$, $P<0.001$).

2.3.2.3 Influence of incubation conditions on CO2-C flush estimates

As predicted by the published literature, CO_2-C flush values were influenced by the choice of unfumigated or fumigated soil as the control and the incubation period selected for the control sample. Data of fumigation experiments were used to compare three ways of calculating biomass C:

C1 = [CO_2-C fum(0-10 d) - CO_2-C unfum(0-10 d)]/0.45, which is Bc1 in Table 2.3;

C2 = [CO_2-C fum(0-10 d) - CO_2-C unfum(10-20 d)]/0.45, which is Bc2 in Table 2.4; and

C3 = [CO_2-C fum(0-10 d) - CO_2-C fum(10-20 d)]/0.45, which is Bc3 in Table 2.4.

The results showed that estimates of biomass C calculated without considering CO_2-C production by a control could be twice as large as that calculated using C1 control (Voroney and Paul 1984; Schnürer et al. 1985; Shen et al. 1987). In contrast to Shen et al. (1987) who found that it mattered relatively little whether biomass C was calculated as C1, C2, C3 on soils that had been incubated moist for some time, data herein showed that calculations C1 and C2 were significantly correlated but not C3 (Table 2.5), although C2 tended to be a little greater than C1, which in turn, was larger than biomass calculated as C3, particularly in manured samples.

Unfumigated soil consistently evolved more CO_2-C during the 0-10 d period than during the 10-20 d period, thus making C2 values greater than C1. Yet, in stored samples there was a close relationship (r=0.86) between CO_2-C evolved by fumigated soil over the 10-20 d period and CO_2-C evolved by the control unfumigated soil over the same period (Table 2.6). These results were consistent with Shen et al. (1987) analyses on moist soils.

In addition, biomass C estimates were lowest when CO_2-C production by fumigated samples over 10-20 d was subtracted from the 0-10 d fumigated values, making C3 (Table 2.4) less than C1 and C2 calculations. This was consistent with observations of Paul and Voroney (1984). These results can be explained if it is

assumed that CO_2 production during 10-20 d included a contribution from the microorganisms killed by $CHCl_3$ that were still being decomposed after the 0-10 d period. But as suggested by Vance et al. (1987), this contribution is unlikely to be important in soils with pH higher than 5.0, so herein other events may have taken place in the fumigated 10-20 d samples. The recommendations of Chaussod and Nicolardot (1982) for the use of a fumigated control appeared inappropriate for measuring CO_2-C flush in these soils.

Finally, based on CO_2-C flushes used to calculate biomass C, C2 value showed the closest positive correlation with soil organic C content (r=0.91) followed by C1 (r=0.77) and C3 (r=0.76), respectively. Crop yields were positively correlated with C2 and C3 values but not significantly correlated with C1 (Table 2.2).

2.3.3 MINERALIZED NITROGEN AND BIOMASS N BY CFIM

Soil biomass N was also determined using the chloroform fumigation-incubation technique (Jenkinson and Powlson 1976b). The flushes of mineral N released (Fn1) in Table 2.7 were calculated from the expression: (NH_4-N mineralized by fumigated soil in 10 d) minus (NH_4-N mineralized by unfumigated soil over the same incubation period). Again due to the deficit of work on specific Kn value of the Xuzhou soil, the size of microbial biomass N was cautiously estimated by dividing the flush of NH_4-N by the Kn value of 0.68 (Shen et al. 1984), the latter being used in several published studies.

Based on Stanford (1982) and Nicolardot and Chaussod (1986) who have stressed the importance of measuring both NH_4^+ and NO_3^- following aerobic incubation, for the second experiment, Table 2.8 shows the flushes of N based on measurements of NH_4-N alone versus the sum of NH_4-N and NO_3-N, besides the various ways of calculating biomass N as influenced by the different controls. Amongst others, Ayanaba et al. (1976), Biederbeck et al. (1984), Shen et al. (1984), Bolton et al. (1985), McGill et al. (1986), Ritz and Robinson (1988), Shen et al. (1989) have all extracted

both NH_4^+ and NO_3^- whereas Carter and Rennie (1982) and Bonde et al. (1988) have used only NH_4^+.

2.3.3.1 Effect of fumigation

Initial NH_4-N contents of the soils were nil, and after 10 d incubation the soils contained little NH_4-N (Table 2.7). With incubation following fumigation larger values were observed, lying between 5.3-15.4 and 20.2-43.4 $\mu g\ N\ g^{-1}$ for inorganic and manure fertilized soils, respectively. Similarly, fumigation increased extractable NH_4-N on incubation (0-10 d), followed by a much smaller increase in the 10-20 d period (Table 2.8). If NO_3-N was considered, net production of (NH_4 + NO_3)-N was almost twice that of NH_4-N alone, particularly in manured soils. Further, nitrification seemed barely under way in manure fumigated samples that had been incubated for 20 d. Fumigated manure soils consistently mineralized more N than fumigated inorganic soils.

Generally Shen et al. (1984) and Nicolardot and Chaussod (1986) found that NH_4-N increased in fumigated soils while NO_3-N stayed at the same level, as nitrification seemed totally inhibited. In contrast, they showed that in unfumigated soils NH_4-N production stayed almost the same although NO_3-N levels increased following longer incubation periods. Similar trends were found in experiment 2 but with unexplained high variability between similarly treated samples. Nitrification occurred in the unfumigated soil samples as amounts of NO_3-N in unfumigated (0-20 d) soils exceeded those in fumigated (0-20 d) soils by 7.1-26.7 $\mu g\ N\ g^{-1}$ dry soil.

The (NH_4 + NO_3)-N flushes reported here were comparable to values of Bolton et al. (1985) which ranged from 2.46 to 14.50 $\mu g\ N\ g^{-1}$ for two different farm management systems in the U.S.A. Many researchers have emphasized that results on the mineral N flush and microbial biomass, obtained by different or modified experimental procedures of the CFIM, should be interpreted with caution. Woods and Schuman (1986), for example, indicated that low concentrations of mineral N do not mean lack of nutrient

cycling. Usually, however, the published values of N flush were higher. As suggested by Bolton et al. (1985), reduce N flushes could be the result of immobilization of N in fumigated samples.

2.3.3.2 Influence of incubation conditions on N flush estimates

Flush of N and biomass C had similar dynamics (Table 2.4 and 2.8). Manured samples contained almost twice as much microbial N as did the mineral fertilized soils, especially for the flush Fn2. This was consistent with McGill et al. (1986) results from Breton plots in Alberta.

Results of fumigation experiments were used to compare three ways of calculating biomass N:

N1 = [min-N fum(0-10 d) - min-N unfum(0-10 d)]/0.68 (Table 2.7);

N2 = [min-N fum(0-10 d) - min-N unfum(10-20 d)]/0.68;

N3 = [min-N fum(0-10 d) - min-N fum(10-20 d)]/0.68 (Table 2.8).

Based on NH_4-N mineralized alone, three estimates of biomass N were significantly correlated (Table 2.9). In addition, the three different flushes were closely related to soil total N content (Table 2.2).

In contrast, for five different French soils Nicolardot and Chaussod (1986) found that a precise estimate of the mineralizable microbial N fraction could only be obtained by N3 calculation: subtracting NH_4-N production from fumigated soil during the 10-20 d period to the identical fumigated soil over the 0-10 d incubation. However, the present study is in general agreement with Ross (1990a) where mineral N flush values were not consistently influenced, overall, by the use of an unfumigated or fumigated control in contrast to CO_2-flush values.

Table 2.7 and 2.8 also give the percentage of biomass N as soil total N released after fumigation and incubation according to the three calculations. Biomass N amounted to 1.1-2.2 and 2.5-5.6% of total N in inorganic fertilized and manured samples, respectively. Similar results were found in the second experi-

ment for biomass N when calculated from NH_4-N measurements only, while (NH_4-N + NO_3-N)-N biomass estimates (Bn2) showed low percentages of total N. This can be compared with estimates by Paul (1984) that microbial biomass accounted for 4-6% of total organic N and the values of biomass N reported by Bonde et al. (1988) that amounted to 3.9-6.8% of total N.

The proportion of soil N contained in the biomass was two to three times inferior to the proportion of soil C present as biomass. Biomass C:N ratios were narrowest in manured samples and widest in mineral fertilized soils. Ratios averaged 49 and 44 in inorganic fertilized samples and 20 and 28 in manured samples, when the incubation periods for CO_2-C production by unfumigated samples were 0-10 and 10-20 d, respectively. Hence, C:N ratios of the calculated biomass varied with the different soil samples, but were lowest when fumigated soil was used as the control for estimates of biomass C. The range of biomass C:N ratios was much greater than reported in the literature. Indeed, the values were high when compared to the biomass C:N ratio of 6.7 proposed by Shen et al. (1984) for arable soils cropped to grains.

In incubation of unfumigated soils, the ratio CO_2-C evolved:N mineralized was narrower where manure has been added, 192.8 compared to 391.3 (Table 2.3). Likewise, the ratios CO_2-C evolved:N mineralized for fumigated soil during the first 10 d of incubation were 51.5 and 22.5 for the inorganic and manure treated samples, respectively. This indicated that upon fumigation a larger proportion of soil N than of organic C was released during the flush of decomposition. Many authors have generally reported a three to four fold narrowing of C:N evolved due to fumigation. But according to Biederbeck et al. (1984) the narrowing of C:N ratios upon fumigation can be attributed to increased C substrate availability and microbial activity at the time of soil sampling. In contrast to most studies by Jenkinson and coauthors who have used cultivated soils sampled during the fallow period when unfumigated mineralization was low, in this experiment the soils used were sampled just shortly before

cereal crop harvest when microbial populations at the soil surface have been recognized to peak. Nevertheless, Jenkinson and Powlson (1976a) have emphasized that values for mineralized N are small compared with the total amount of mineral N in soil, and thus are subject to large errors. This implies that CO_2-C evolved:N mineralized ratios were susceptible to even larger errors.

Ratios between the flush of CO_2-C and NH_4-N mineralized (Fc/Fn) determined at the end of the 10 d incubation period following fumigation, ranged from 8.4-17.6 and 21.6-50.2, in manure and inorganic fertilized samples, respectively (Table 2.3). These values were greater than an approximate mean of 4.0 from studies summarized by Jenkinson and Ladd (1981). Still, Nannipieri (1984) also reported ratios of the flush of decomposition greater than 20.0 for a grass-legume association fertilized with labelled urea under Mediterranean climate conditions. As suggested by Nannipieri (1984), the ratio differences indicated that N immobilization occurred during biomass determination.

In both experiments, the amount of CO_2 evolved was closely correlated with the amount of N mineralized after fumigation (r=0.78-0.92) (Table 2.9). Hence, a strong relationship existed between the mineralized N and CO_2-C evolution (Fc1 and Fc2) but not with CO_2 evolution using a fumigated control. For a wide range of soils, Jenkinson and Ladd (1981) found that a rough estimate of the biomass C (Bc) could be obtained from the flush of mineral N (Fn) by multiplying by 9. Similarly, here and in the Nannipieri (1984) study this empirical relationship was invalid.

2.3.3.3 Effect of the extractant on biomass N values

In general, the three extractants showed similar trends, (NH_4 + NO_3)-N mineralized under different period of incubation, being lowest in inorganic fertilized samples and highest in manure fertilized samples (Table 2.10). However, amounts of soil N rendered extractable by the three extractants at 10 and 20 d

incubation periods preceded by $CHCl_3$ fumigation differed considerably. Extraction of organic and mineral N were always greater with $NaHCO_3$ than with KCl and K_2SO_4, respectively. The increase in $NaHCO_3$-extractable N upon fumigation was more than five times greater than with KCl or K_2SO_4 alone.

Azam et al. (1989) who studied the chemical extraction of newly immobilized 15N and native soil N as influence by substrate addition rate and soil treatments, found that 0.01M $NaHCO_3$ was the most efficient in extracting applied 15N compared to $CaCl_2$ and K_2SO_4. However, they showed that higher soil N extractability due to fumigation, also increased non-biomass N.

In this study, the $NaHCO_3$ extractable N has not correlated with any other parameters, while KCl and K_2SO_4 extractable N, corrected for unfumigated controls, were positively correlated (r=0.91) (Table 2.11). This implies that $NaHCO_3$ did not extract biomass N exclusively. Among the identical treated soil samples there was a great variability in the levels of mineralized N extracted with $NaHCO_3$. Differences between extracted N from fumigated and unfumigated soil samples did not reflect quantitatively the amounts of NH_4-N mineralized after fumigation in experiment 1. This probably was related to high standard deviations, and CFIM estimates using $NaHCO_3$ extractant may not be reliable.

2.3.3.4 Anaerobic incubation and biomass N values

Table 2.12 shows soil biomass N results that were determined using CFIM but instead of performing the standard aerobic incubation following fumigation, anaerobic incubation was used as proposed by Nicolardot (1988). The mineralized NH_4-N released were calculated from the expression: Fn1 = [NH_4 fum(0-7 d) minus NH_4 fum(7-14 d)] and Fn2 = [NH_4 fum(0-7 d) minus NH_4 unfum(0-7 d)]. The amount of microbial biomass N was calculated by dividing the flush of NH_4-N by the Kn value of 0.49 proposed by Nicolardot et al. (1990).

Fumigated soil mineralized 4 and 6 times as much N in the 10 d after fumigant removal as did the corresponding unfumigated soil, in mineral and manure fertilized samples, respectively (Table 2.12). Nitrification did not occur in the fumigated (0-14 d) samples. This is in general agreement with the results of Nicolardot et al. (1990) who found that the flush N values were systematically higher in soils incubated in anaerobiosis compared to soils incubated under aerobic conditions (Table 2.7, 2.8 and 2.12).

In parallel, a close relationship between anaerobic and aerobic mineralization of the three experiments were found here (Table 2.9), as in the Nicolardot et al. (1990) study. Based on observations obtain from 20 French soils they established the following linear relationship: anaerobic flush N = 1.29 * aerobic flush N. The greater net mineralization of N was attributed to reduced immobilization of N under anaerobic conditions. They assumed that when a soil was under a layer of water, microbial compounds liberated upon fumigation were probably more accessible to growing microorganisms compared to a soil under aerobiosis where substrate mobility was reduced.

Nicolardot et al. (1990) proposed to calculate microbial biomass N using fumigated (7-14 d) control soil incubated under anaerobiosis, based on mineralization rates of microbial materials with various C:N ratios in different soils. In contrast, results here showed that Fn1 or Fn2 flush values were significantly correlated (r=0.92) and consequently either method could be used (Table 2.5). In addition, the mineralization of N using the Fn2 calculation was also highly correlated with the flushes of N estimated using unfumigated (10-20 d) control soils incubated under aerobic conditions in experiment 2 (r=0.96)(Table 2.9).

Hence, there were significant correlations between the NH_4-N flush of the first and second experiment using aerobic incubation and the NH_4-N mineralized by anaerobic incubation measured by both procedures (Table 2.9). The anaerobic N flushes were also positively correlated with the flushes of CO_2-C

evolved of both aerobic incubation experiments (Table 2.5). In comparison, anaerobic unfumigated (0-7 d) soils were not significantly correlated with either of the aerobic unfumigated (0-10 d) nor (0-20 d) controls, whereas all fumigated soils showed a strong correlation (Table 2.9). This implies that organic matter mineralization in soils not treated with $CHCl_3$ involved differing mechanisms or substrates under waterlogged and aerobic conditions.

Notably, anaerobic biomass N flushes were not correlated with N-availability indices (NAI) of either fresh or dry soil samples. In comparison, a close relationship has been found between the amounts of NH_4-N produced by unfumigated (0-7 d) soils incubated under anaerobiosis at 28°C and the amounts of NH_4-N mineralized by soils incubated for 7 d at 40°C under waterlogged conditions (r=0.86-0.98). In contrast, it appeared that no relationship existed between the NH_4-N produced from unfumigated (0-10 d) soils incubated under aerobic conditions at 25°C and NAI values (Table 2.2). However, net accumulation of NH_4-N after 7 d of anaerobic incubation at 40°C was significantly correlated with the amount of NH_4-N produced after chloroform fumigation-incubation at 25°C. In fact, the amounts of N mineralized under waterlogged conditions from the air-dried samples were more closely correlated than the "fresh" samples with fumigated treated samples (Table 2.2). As suggested by Myrold (1987) both methods might be measuring N mineralized from the same soil N pool.

Biomass N accounted for 3.7% of the soil total N in the inorganic fertilized samples and 5.4% in samples that received manure. These values were similar to those found by others (Paul 1984; Bonde et al. 1988). As would be expected, microbial biomass N following anaerobic incubation correlated with soil total N content (r=0.69-0.75) but slightly less than biomass N based on aerobic incubation (r=0.83-0.92) (Table 2.2). This is consistent with the results of Myrold (1987) who found that microbial biomass N and C by CFIM were highly correlated with the amount of NH_4^+ released by anaerobic incubation in Oregon

soils. Crop yields showed a closer relationship with the flushes of N under anaerobic incubation than with NH_4-N mineralized upon fumigation and incubation under aerobiosis (Table 2.2).

In conclusion, these results suggest that reliable estimates of microbial biomass N can be obtained from soil incubated under waterlogged conditions upon fumigation. Standard deviations were low (< 25% of the mean) and variability generally less for the anaerobic incubation method than for the aerobic incubation method. This lower variability contributed to the increased precision in the measurements of biomass N in both mineral and manure treated soil samples. Thus CFIM followed by anaerobic incubation presents advantages over aerobic incubation, especially considering the ease with which such measurements can be obtained under laboratory conditions.

2.3.4 ORGANIC C EXTRACTED BY FUMIGATION-EXTRACTION (FE)

The fumigation-extraction (FE), which is a more rapid procedure than CFIM, was tested to obtain an approximate estimate of microbial biomass C. Since Tate et al. (1988) developed the possibility of estimating C and N flush with the same soil extract, similar experimental conditions were chosen here for soil fumigation and extraction with 0.5M K_2SO_4 of soluble C and N. Two ways of calculating microbial biomass C based on the fumigation flush of extractable C: Ec = [(organic C extracted from fumigated soil) minus (organic C extracted from unfumigated soil)].

The amounts of organic C extracted by 0.5M K_2SO_4 ranged between 0.08-0.11 and 0.10 $\mu g\ C\ g^{-1}$ for the inorganic fertilized and manure treated soil samples, respectively (Table 2.13). Fumigation with $CHCl_3$ increased organic C values. The additional C released by fumigation ranged between 0.05-0.07 and 0.07-0.09 $\mu g\ C\ g^{-1}$ for the inorganic fertilized and manure applied samples, respectively. These amounts were 500 to 1000 times lower than reported by Vance et al. (1987), Tate et al. (1988) and Sparling and West (1988a).

Similarly, estimates of biomass C based on the FE were low relative to values in the literature. However, there was a close relationship between organic C extracted by 0.5M K_2SO_4 and soil organic C. This is in general agreement with Sparling and West (1988b) results where organic C released upon fumigation was correlated (r=0.91) with total soil C content (Table 2.2).

Sparling and West (1988b) also found a correlation between the organic C flush by FE and the microbial biomass C by CFIM. Herein the data only showed positive correlations (r=0.76 and 0.59-0.71) with experiment 1 and 2 flushes of C, respectively, (Table 2.5 and 2.6). As expected by the published literature, the flushes of C determined by extraction and oxidation were less than those determined from the CO_2-C flush after a 10 d incubation. Release of C following fumigation was greater when estimated by the incubation method than with the extraction method. However, the organic C flushes were negligible, being only 0.02% of the CO_2-C flushes produced upon incubation (Table 2.4). In comparison, Vance et al. (1987) reported that the organic C extracted (Ec) was about 60-70% of the CO_2-C evolved (Fc) for 10 English soils. Sparling and West (1988a) found Ec values were 48-84% of Fc, for 26 mineral soils from pasture and arable sites in New Zealand. In addition, Sparling and West (1988b) reported that the extractable C flushes were in the range 68-98% (mean 76%) of the CO_2-C incubation flushes for 12 soils of distinctive characteristics. This emphasizes that the results reported in this study were inconclusive concerning the effectiveness of the direct extraction for estimating soil microbial biomass C probably due to organic C digestion problems.

2.3.5 N EXTRACTED BY FUMIGATION-EXTRACTION

Similarly, to the measurements of organic C extracted by 0.5M K_2SO_4, a rapid direct extraction method was used to measure NH_4-N in 0.5M K_2SO_4 extracts. The amount of N released by $CHCl_3$ after 24 h fumigation (En) was calculated from [(NH_4-N in K_2SO_4 extracts of fumigated soil after 24 h fumigation) minus (NH_4-N

in K_2SO_4 extracts of non-fumigated soil at start of fumigation)]. Soil microbial biomass N (Bn) was estimated from En/0.54 (Brookes et al. 1985b) and expressed as $\mu g\ N\ g^{-1}$ soil.

Samples treated with inorganic fertilizers contained the lowest value of extractable N, while the highest value was for samples that have been manured for 10 yr (Table 2.14). Fumigation with $CHCl_3$ did not increase the extracted and mineralized N contents of soil samples. This is in contradiction with the observations made by Brookes et al. (1985b), Sparling and West (1988a,b) and Antisari et al. (1990).

These authors have also indicated that significantly more N was measured by the CFIM after 10 d incubation period than by the FE method. This is further in conflict with the results found here where for unfumigated control samples mean values 0.5 $\mu g\ N\ g^{-1}$ NH_4-N mineralized during incubation were compared to 11.2 $\mu g\ N\ g^{-1}$ determined by Kjeldahl digestion of K_2SO_4 extracts.

Extracted N comprised 3-17% of Fn1 (Tables 2.14; 2.7), so that much less NH_4-N was rendered extractable by 24 h $CHCl_3$-fumigation than was mineralized during 10 d incubations. However, no significant correlation existed either between the flush of N released by $CHCl_3$ and extracted by K_2SO_4 (En) and the flush of N (Fn1) defined as [(NH_4-N by fumigated soil for 10 d aerobic incubation) minus (NH_4-N by non-fumigated soil under the same conditions)](Tables 2.9, 2.15) or any other parameters. In contrast, Brookes et al. (1985b) found that total N extracted (En) upon 24 h fumigation and direct extraction was closely correlated with the N mineralized from the biomass (Fn) in 37 soils during fumigation-incubation. Extracted N accounted for about 79% of the flush of N (Fn), which suggested to the authors that both methods were measuring the same pool of soil N. Soil biomass N was closely correlated with soil total N in Brookes et al. (1985b) study. The results showed that in these soils despite differences in their previous agricultural history, biomass N ranging from 2-6% was a very constant percentage of soil total N. In conclusion, the principle of using 0.5M K_2SO_4 extractant after fumigation to obtain estimates of both

microbial biomass C and N has obvious attractions but has been unreliable here.

2.4 CONCLUSIONS

The size of biomass C was quite high in both fertilizer treatments but particularly in manured samples compared to samples from fields fertilized with inorganic N. In contrast, soil biomass N was lower than reported in the published literature for arable soils.

Regarding methodology, the choice of the control and period of incubation used for the CFIM had an effect on biomass C and N estimates. For both biomass measurements, unfumigated (10-20 d) soils were found to be the best control.

Estimates of biomass N were high when $NaHCO_3$ was used as an extractant of mineralized N. Anaerobic incubation values of fumigated soil samples were closely correlated to aerobic incubation values. Biomass N estimations of soil under anaerobic conditions could be favoured since this method is simpler than the aerobic incubation method.

Nitrogen-availability indices from anaerobic incubations showed low levels of mineralizable N, and therefore these indices may be a poor guide to soil N reserves.

Measurements of biomass C and N by the fumigation-extraction procedure showed inconsistent low levels probably due to problems encountered during digestion of organic C, and Kjeldahl digestion for the content of N.

Table 2.1. Soil properties and nitrogen availability indices (NAI) from Xuzhou field plots.

Treat-ments	pH H_2O	pH $CaCl_2$	Organic C	Total N	C/N ratio	Nitrogen availability indice (NAI) (x)	NAI as % of total N
			g kg^{-1}	g kg^{-1}		mg kg^{-1}	--%--
	8.1 ±0.1 (y)	7.7 ±0.1	7.1 ±0.5	0.75 ±0.1	9.5 ±0.4	7.0 ±1.2	1.0 ±0.2
						6.9 ±1.5	0.9 ±0.1
						15.7 ±7.5	2.1 ±0.9
	8.0 ±0.2 (z)	7.6 ±0.1	11.1 ±1.0	1.08 ±0.1	10.3 ±0.7	9.5 ±1.8	0.9 ±0.2
						12.1 ±2.1	1.1 ±0.2
						26.5 ±2.9	2.5 ±0.3

(x) Nitrogen availability indice measured from two fresh-stored and one air-dried soil used in two different anaerobic incubation experiments.
(y) Mean and standard deviation; (n = 4) of Check, N, NP, and NPK plots.
(z) Mean and standard deviation; (n = 4) of Manure, MN, MNP, and MNPK treated plots.

Table 2.2. Pearson correlation coefficients among soil and crop values.

	pH w	pH s	Org C	Total N	C/N	Yields	NAI-1F	NAI-2F	NAI-2D
pH water	1.00								
pH salt	0.99	1.00							
Organic C	-0.76	-0.76	1.00						
Total N	-0.87	-0.87	0.96	1.00					
C/N ratio	0.14	0.16	0.37	0.09	1.00				
Crop yields	-0.52	-0.74	0.80	0.74	0.65	1.00			
NH_4-N mineralized under anaerobic conditions (7 d at 40°C)									
NAI 1 Fresh	-0.87	-0.87	0.76	0.85	-0.06	0.75	1.00		
NAI 2 Fresh	-0.86	-0.85	0.76	0.85	-0.07	0.62	0.99	1.00	
NAI 2 Dry	-0.95	-0.96	0.85	0.91	0.02	0.77	0.89	0.87	1.00
NH_4-N mineralized after fumigation and aerobic incubation exp. 1 (10 d at 25°C)									
Fum 0-10d	-0.71	-0.70	0.91	0.90	0.24	0.66	0.76	0.78	0.80
Unf 0-10d	0.11	0.12	-0.09	-0.05	-0.21	0.05	0.00	0.12	-0.17
Flush N	-0.71	-0.70	0.91	0.89	0.26	0.63	0.75	0.75	0.80
NH_4-N mineralized after fumigation and aerobic incubation exp. 2 (10 and 20 d at 25°C)									
Fum 0-10d	-0.06	-0.51	0.91	0.92	0.52	0.73	0.74	0.45	0.82
Fum 0-20d	-0.37	-0.65	0.90	0.87	0.62	0.73	0.58	0.57	0.80
Unf 0-20d	0.32	0.58	-0.48	-0.57	-0.08	-0.34	-0.09	-0.33	-0.37
Fum 10-20d	-0.19	-0.55	0.85	0.83	0.55	0.73	0.87	0.60	0.69
Flush N Fum	0.03	-0.41	0.81	0.83	0.43	0.61	0.54	0.29	0.78
Flush N Unf	-0.06	-0.53	0.89	0.92	0.45	0.70	0.70	0.45	0.78
NH_4-N mineralized after fumigation and anaerobic incubation (7 and 14 d at 28°C)									
Fum 0-7d	-0.73	-0.74	0.97	0.95	0.30	0.84	0.77	0.79	0.84
Fum 0-14d	-0.85	-0.87	0.94	0.97	0.12	0.82	0.89	0.89	0.95
Unf 0-7d	-0.81	-0.81	0.74	0.83	-0.07	0.74	0.98	0.98	0.86
Fum 7-14d	-0.83	-0.87	0.54	0.69	-0.32	-0.35	0.87	0.83	0.86
Flush N Fum	-0.43	-0.41	0.86	0.75	0.52	0.82	0.44	0.49	0.54
Flush N Unf	-0.38	-0.38	0.80	0.69	0.53	0.84	0.26	0.29	0.49
CO_2-C evolved during FI exp. 1									
Fum 0-10d	-0.62	-0.65	0.88	0.87	0.27	0.80	0.64	0.62	0.75
Unf 0-10d	-0.52	-0.52	0.83	0.75	0.47	0.85	0.51	0.54	0.60
Flush C	-0.59	-0.63	0.77	0.81	0.08	0.55	0.62	0.59	0.74
CO_2-C evolved during FI exp. 2									
Fum 0-10d	-0.30	-0.66	0.95	0.91	0.62	0.80	0.86	0.68	0.65
Fum 10-20d	-0.21	-0.64	0.87	0.84	0.65	0.79	0.85	0.58	0.80
Unf 10-20d	-0.43	-0.73	0.93	0.89	0.68	0.84	0.77	0.68	0.71
Flush C Fum	-0.55	-0.60	0.76	0.78	0.39	0.63	0.57	0.72	0.18
Flush C Unf	-0.26	-0.62	0.91	0.87	0.60	0.76	0.84	0.67	0.66
Organic C extracted FE									
Fum	-0.27	-0.31	0.62	0.53	0.58	0.69	0.28	0.25	0.45
Unf	0.78	0.78	-0.44	-0.55	0.48	0.46	-0.49	-0.50	-0.66
Flush C	-0.81	-0.83	0.85	0.84	0.15	0.86	0.59	0.57	0.84
N extracted after fumigation FE									
Fum	-0.15	-0.08	-0.04	0.08	-0.68	-0.26	0.11	0.14	-0.02
Unf	-0.18	-0.12	-0.04	0.09	-0.75	-0.31	0.13	0.16	0.01
Flush N	0.33	0.40	-0.36	-0.33	-0.28	-0.07	-0.39	-0.36	-0.47

Coefficients above 0.57 significant at P = 0.05, above 0.71 significant at 0.01, and finally above 0.80 at P = 0.001.

Table 2.3. Mean values of CO_2-C evolved and microbial biomass C estimated from two soil treatments by the chloroform fumigation-incubation.

Treatments		10d (0-10d)	Fc1	Bc1	Bc1 as % Org C	CO2 evol:N min	Fc:Fn	Bc:Bn
		----µg	g^{-1}----	---	-%-			
CO_2-C (x)	Fum	437 ± 62				51.5 ± 19.4		
	Unfum	177 ± 35	259 ± 34	576 ± 76	8.1 ± 0.8	391.3 ±140.9	32.8 ± 13.2	49 ± 20
CO_2-C (y)	Fum	620 ± 54				22.5 ± 6.2		
	Unfum	271 ± 53	345 ± 81	768 ±180	7.1 ± 2.3	192.8 ± 69.4	13.3 ± 4.6	20 ± 6

(x) Mean and standard deviation, (n = 4) of Check, N, NP, and NPK plots.
(y) Mean and standard deviation; (n = 4) of Manure, MN, MNP, and MNPK treated plots.
Fum - Fumigated, Unfum - Unfumigated treated soils in this and subsequent tables.
Fc1 = Flush of CO2-C evolved = Fum 10 d minus Unfum 10 d,
Bc1 = Biomass Carbon = Fc1/Kc, where Kc is 0.45 (Jenkinson and Ladd 1981),
Bc as a proportion of total organic C;
Ratio of CO2 evolved-to-N mineralized for Fum 10 d and Unfum 10 d;
Fc/Fn = Flush of Carbon-to-Flush of Nitrogen; Bc/Bn = ratio of biomass C-to-biomass N.

Table 2.4. Evolved CO_2-C and calculated microbial biomass C and N from two soil treatments in the second chloroform fumigation-incuabation experiment.

Treatments		10d (0-10d)	10d (10-20d)	Fc2	Fc3	Bc2	Bc3	Bc2 as % Org C	Bc3 as % Org C	Bc2: Bn2	Bc3: Bn3
		----	----	µg C	g^{-1}	----	----	-%-	-%-		
CO_2-C (x)	Fum	376 ±50	193 ±56	268 ±47	165 ± 3	596 ±105	366 ± 6	8.3 ±0.8	5.1 ±0.5	44¶ ±11	19¶ ± 4
	Unf	NA	98 ±24							263§ ±276	17§ ± 5
CO_2-C (ȳ)	Fum	553 ±16	324 ±36	367 ±14	219 ±51	816 ± 30	486 ±113	7.5 ±0.8	4.5 ±0.9	28¶ ± 3	16¶ ± 5
	Unf	NA	190 ±21							312§ ±455	8§ ± 2

NA = not available
(x) Mean and standard deviation, (n = 4) of Check, N, NP, and NPK plots.
(y) Mean and standard deviation, (n = 4) of Manure, MN, MNP, and MNPK treated plots.
Fc2 = Flush of CO2-C evolved calculated from Fum 10 d minus Unfum 10-20 d,
Fc3 = Flush of CO2-C evolved calculated from Fum 10 d minus Fum 10-20 d.
Bc2 = Fc2/Kc; Bc3 = Fc3/Kc, where Kc is 0.45. Bc2 and Bc3 as a proportion of soil organic C.
¶ Bc2/Bn2 and Bc3/Bn3 = ratio of biomass C-to-biomass N based on NH4-N measured, and
§ Bc2/Bn2 and Bc3/Bn3 = ratio of biomass C-to-biomass N based on (NH4 + NO3)-N measured.

Table 2.5. Pearson correlation coefficients

	NH_4-N mineralized after fumigation and anaerobic incubation						CO_2-C evolved during FI exp. 1		
	Fum 0-7d	**Fum 0-14d**	**Unf 0-7d**	**Fum 7-14d**	**Flush N-Fum**	**Flush N-Unf**	**Fum 0-10d**	**Unf 0-10d**	**Flush C**
NH_4-N mineralized after fumigation and anaerobic incubation (7 and 14 d at 28°C)									
Fum 0-7d	1.00								
Fum 0-14d	0.96	1.00							
Unf 0-7d	0.78	0.88	1.00						
Fum 7-14d	0.53	0.76	0.81	1.00					
Flush N Fum	0.90	0.73	0.49	0.10	1.00				
Flush N Unf	0.81	0.65	0.26	0.06	0.92	1.00			
CO_2-C evolved during fumigation-incubation in exp. 1									
Fum 0-10d	0.90	0.89	0.62	0.54	0.78	0.81	1.00		
Unf 0-10d	0.81	0.75	0.47	0.37	0.76	0.81	0.84	1.00	
Flush C	0.83	0.84	0.64	0.58	0.67	0.68	0.93	0.60	1.00
CO_2-C evolved during fumigation-incubation in exp. 2									
Fum 0-10d	0.96	0.90	0.68	-0.34	0.98	0.98	0.84	0.89	0.57
Fum 10-20d	0.88	0.91	0.67	-0.19	0.81	0.90	0.87	0.83	0.70
Unf 10-20d	0.90	0.86	0.71	-0.47	0.90	0.91	0.79	0.86	0.54
Flush C Fum	0.69	0.56	0.34	-0.67	0.79	0.73	0.56	0.79	0.21
Flush C Unf	0.95	0.90	0.67	-0.29	0.97	0.97	0.84	0.85	0.61
Organic C extracted after fumigation FE									
Fum	0.66	0.59	0.30	0.33	0.76	0.82	0.83	0.76	0.80
Unf	-0.38	-0.46	-0.39	-0.61	-0.20	-0.24	-0.23	-0.34	-0.17
Flush C	0.82	0.81	0.53	0.70	0.78	0.85	0.85	0.91	0.76
N extracted after fumigation FE									
Fum	-0.06	-0.05	0.13	-0.02	-0.08	-0.24	-0.15	-0.16	-0.13
Unf	-0.06	-0.04	0.14	0.03	-0.10	-0.26	-0.15	-0.18	-0.12
Flush N	-0.41	-0.46	-0.37	-0.54	-0.28	-0.31	-0.40	-0.31	-0.44

Coefficients above 0.57 significant at P = 0.05, above 0.71 significant at 0.01, and finally above 0.80 at P = 0.001.

Table 2.6. Pearson correlation coefficients

	CO_2-C evolved from FI exp. 2					Org C from FE			N extracted in FF		
	Fum 0-10d	Fum 10-20d	Unf 10-20d	Flush C-Fum	Flush C-Unf	Fum	Unf	Flush C	Fum	Unf	Flush N
CO_2-C evolved during fumigation-incubation in exp. 2											
Fum 0-10d	1.00										
Fum 10-20d	0.96	1.00									
Unf 10-20d	0.95	0.86	1.00								
Flush C Fum	0.79	0.57	0.84	1.00							
Flush C Unf	0.98	0.97	0.86	0.69	1.00						
Organic C extracted after fumigation FE											
Fum	0.82	0.80	0.76	0.48	0.80	1.00					
Unf	0.65	0.60	0.61	0.45	0.65	0.22	1.00				
Flush C	0.80	0.73	0.83	0.59	0.71	0.69	-0.55	1.00			
N extracted after fumigation FE											
Fum	-0.28	-0.39	-0.20	0.06	-0.40	-0.24	-0.16	-0.04	1.00		
Unf	-0.35	-0.44	-0.28	-0.03	-0.45	-0.28	-0.22	-0.02	0.99	1.00	
Flush N	-0.05	-0.24	0.11	0.39	-0.22	-0.17	0.30	-0.28	0.77	0.71	1.00

Coefficients above 0.57 significant at P = 0.05, above 0.71 significant at 0.01, and finally above 0.80 at P = 0.001.

Table 2.7. Nitrogen mineralized in chloroform fumigation-incubation experiment from two soil treatments.

Treatments		10d (0-10d)	Fn1	Bn1	Bn as % Total N
		------μg g^{-1}---		---	--%--
NH_4-N (x)	Fum	9.5 ±4.3	9.0 ± 4.1	14 ± 6	1.7 ± 0.7
	Unfum	0.5 ±0.4			
NH_4-N (y)	Fum	29.9 ±9.8	28.3 ±10.3	42 ±15	3.8 ± 1.3
	Unfum	1.6 ±0.8			

(x) Mean and standard deviation, (n = 4) of Check, N, NP, and NPK plots.
(y) Mean and standard deviation, (n = 4) of Manure, MN, MNP, and MNPK treated plots.
Fn1 = Flush of N mineralized = Fum 10 d minus Unfum 10 d,
Bn1 = Biomass Nitrogen = Fn1/Kn, where Kn is 0.68 (Shen et al. 1984),
Bn as a proportion of the Total N.

Table 2.8. Distribution of nitrogen after the second fumigation-incubation experiment.

Treatments		10d 0-10d	20d 0-20d	Fn2	Fn3	Bn2	Bn3	Bn2 as % Total N	Bn3 as % Total N
		-----	-----	--μg N g^{-1}		---	---		
Fum (x)	NH_4-N	13.5 ± 3.5	13.1 ± 2.9		13.9 ± 3.3		20 ± 5		2.8 ±0.5
	NH_4-N + NO_3-N	18.4 ± 4.0	36.4 ±29.0		14.9 ± 4.4		22 ± 6		3.0 ±0.7
Unf	NH_4-N	NA	3.7 ± 0.5	9.8 ± 3.9		14 ± 6		1.9 ±0.6	
	NH_4-N + NO_3-N	NA	36.2 ±39.3	1.8 ± 2.8		3 ± 4		0.4 ±0.5	
Fum (y)	NH_4-N	22.8 ± 2.0	28.9 ± 7.8		20.4 ± 1.4		30 ± 2		2.8 ±0.3
	NH_4-N + NO_3-N	41.3 ±10.4	45.3 ±10.5		41.9 ±14.6		61 ±21		5.8 ±1.9
Unf	NH_4-N	NA	2.2 ± 1.3	20.0 ±2.4		29 ± 4		2.8 ±0.4	
	NH_4-N + NO_3-N	NA	33.9 ±12.9	7.5 ±6.7		11 ±10		1.0 ±0.9	

ND = not determined, NA = not available.
(x) Mean and standard deviation, (n = 4) of Check, N, NP, and NPK plots.
(y) Mean and standard deviation, (n = 4) of Manure, MN, MNP, and MNPK treated plots.
Fn2 = Flush of N mineralized [NH4-N or (NH4 + NO3)-N]= Fum 10 d minus Unfum 20 d.
Fn3 = Flush of N mineralized = Fum 10 d minus Fum 10-20 d,
where Fum 10-20 d = Fum 20 d - Fum 10 d.
Bn2 = Fn2/Kn, Bn3 = Fn3/Kn, where Kn is 0.68.

Table 2.9. Pearson correlation coefficients among values of NH_4-N mineralized and C released as CO_2.

	NH_4-N after FI exp. 1			NH_4-N measured after FI exp. 2					
	Fum 0-10d	Unf 0-10d	Flush N	Fum 0-10d	Fum 0-20d	Unf 0-20d	Fum 10-20d	Flush N-Fum	Flush N Unf
NH_4-N mineralized after fumigation and aerobic incubation exp. 1 (10 d at 25°C)									
Fum 0-10d	1.00								
Unf 0-10d	-0.10	1.00							
Flush N	0.99	-0.22	1.00						
NH_4-N mineralized after fumigation and aerobic incubation exp. 2 (10 and 20 d at 25°C)									
Fum 0-10d	0.94	-0.11	0.92	1.00					
Fum 0-20d	0.96	-0.12	0.95	0.99	1.00				
Unf 0-20d	-0.76	-0.22	-0.69	-0.44	-0.66	1.00			
Fum 10-20d	0.79	-0.10	0.78	0.83	0.91	-0.06	1.00		
Flush N Fum	0.88	-0.10	0.86	0.94	0.87	-0.60	0.60	1.00	
Flush N Unf	0.95	-0.05	0.91	0.99	0.96	-0.55	0.78	0.96	1.00
NH_4-N mineralized after fumigation and anaerobic incubation (7 and 14 d at 28°C)									
Fum 0-7d	0.94	-0.21	0.95	0.98	0.98	-0.54	0.91	0.90	0.96
Fum 0-14d	0.89	-0.21	0.90	0.96	0.92	-0.41	0.92	0.86	0.93
Unf 0-7d	0.81	-0.04	0.80	0.79	0.88	-0.40	0.87	0.64	0.74
Fum 7-14d	0.47	-0.13	0.47	-0.13	-0.54	0.66	0.04	-0.22	-0.17
Flush N Fum	0.86	-0.18	0.87	0.97	0.98	-0.63	0.86	0.90	0.95
Flush N Unf	0.70	-0.29	0.71	0.98	0.96	-0.56	0.88	0.92	0.96
CO_2-C evolved during FI exp. 1									
Fum 0-10d	0.78	-0.09	0.78	0.92	0.78	-0.34	0.92	0.77	0.90
Unf 0-10d	0.64	0.18	0.60	0.74	0.65	-0.19	0.85	0.56	0.72
Flush C	0.75	-0.26	0.77	0.79	0.69	-0.38	0.72	0.71	0.78
CO_2-C evolved during FI exp. 2									
Fum 0-10d	0.79	0.09	0.73	0.89	0.91	-0.28	0.85	0.77	0.87
Fum 10-20d	0.69	0.04	0.66	0.93	0.78	-0.36	0.83	0.84	0.91
Unf 10-20d	0.76	0.10	0.72	0.80	0.83	-0.39	0.85	0.65	0.77
Flush C Fum	0.52	0.35	0.41	0.54	0.58	-0.16	0.67	0.40	0.52
Flush C Unf	0.78	0.10	0.72	0.89	0.90	-0.35	0.80	0.80	0.88
Organic C extracted FE									
Fum	0.49	-0.71	0.54	0.78	0.87	0.17	0.97	0.56	0.70
Unf	-0.43	-0.18	-0.41	0.55	0.65	0.46	0.80	0.33	0.44
Flush C	0.71	-0.49	0.74	0.76	0.84	0.01	0.94	0.55	0.70
N extracted after fumigation FE									
Fum	0.18	0.02	0.18	-0.22	-0.20	-0.04	-0.13	-0.24	-0.20
Unf	0.19	0.02	0.19	-0.26	-0.24	-0.08	-0.17	-0.27	-0.23
Flush N	-0.28	-0.05	-0.28	-0.12	-0.08	0.23	0.04	-0.20	-0.15

Fum - Fumigated, Unf - Unfumigated treated soils in this and subsequent tables.
0-10 d, 0-20 d, 10-20 d, 0-7 d, 0-14d, 7-14 d are all incubation period in days.
Flush N or Flush C = Fum minus Unf.
Flush C Fum = Fum 0-10 d minus Fum 10-20 d.
Flush C Unf = Fum 0-10 d minus Unf 10-20 d, where all data were measured.
Flush N Fum = Fum 0-10 d minus Fum 10-20 d, where Fum 10-20 d = Fum 0-20 d minus Fum 0-10 d
Flush N Unf = Fum 0-10 d minus Unf 0-20 d.
Coefficients above 0.57 significant at P = 0.05, above 0.71 significant at 0.01, and finally above 0.80 at P = 0.001.

Table 2.10. (NH_4 + NO_3)-N mineralized during different fumigation-incubation periods followed by extraction with three different mild chemical extractants.

Treatments		Fum (0-10d)	Fum (0-20d)	Unfum (0-20d)	Flush Fum	Flush Unfum
				$\mu g\ g^{-1}$		
2M KCl (1 5)	Ck,N,NP,NPK	18.4 ± 4.0	36.4 ±29.0	36.2 ±39.3	14.9 ± 4.4	1.8 ±1.8
	M,MN,MNP,MNPK	41.3 ±10.4	45.3 ±10.5	33.9 ±12.9	41.9 ±14.6	7.5 ±6.7
0.5M K_2SO_4 (1 4)	Ck,N,NP,NPK	28.0 ±23.3	ND	25.8 ±27.0	NA	2.2 ±4.2
	M,MN,MNP,MNPK	40.7 ±10.3	ND	26.1 ± 8.3	NA	14.6 ±8.5
0.5M $NaHCO_3$ (1 10)	Ck,N,NP,NPK (x)	156 ±77	73 ±40	147 ±34	NA	14.8 ±78.4
	M,MN,MNP,MNPK (y)	174 ±35	119 ±41	175 ±55	NA	-17.1 ±80.7

Ratio soil extractant (wt vol.), ND = not determined, NA = not available.
(x) Mean and standard deviation, (n = 4) of Check, N, NP, and NPK plots
(y) Mean and standard deviation, (n = 4) of Manure, MN, MNP, and MNPK treated plots.
Flush Fum = Flush of N mineralized = Fum 0-10 d minus Fum 10-20 d, where Fum 10-20 d = Fum 0-20 d minus Fum 0-10 d. Flush Unfum = Flush of min N = Fum 0-10 d - Unfum 0-20 d.

Table 2.11. Pearson correlation coefficients.

	$(NH_4 + NO_3)$-N measured after FI exp. 2 and extracted with 2 M KCl						$(NH_4 + NO_3)$ N measured after FI exp. 2 and extracted with 0.5 M K_2SO_4		
	Fum 0–10d	Fum 0–20d	Unf 0–20d	Fum 10–20d	Flush N-Fum	Flush N-Unf	Fum 0–10d	Unf 0–20d	Flush N
$(NH_4 + NO_3)$-N measured after FI exp. 2 and extracted with 2 M KCl (1:5)									
Fum 0–10d	1.00								
Fum 0–20d	0.96	1.00							
Unf 0–20d	0.96	0.90	1.00						
Fum 10–20d	0.66	0.51	0.66	1.00					
Flush N Fum	0.76	0.83	0.66	0.04	1.00				
Flush N Unf	−0.59	−0.57	−0.76	−0.62	−0.15	1.00			
$(NH_4 + NO_3)$-N measured after FI exp. 2 and extracted with 0.5 M K_2SO_4 (1:4)									
Fum 0–10d	0.99	0.97	0.94	0.59	0.79	−0.59	1.00		
Unf 0–20d	0.96	0.92	1.00	0.64	0.69	−0.73	0.95	1.00	
Flush N Unf	−0.46	−0.25	−0.57	−0.54	−0.01	0.91	−0.26	−0.55	1.00
$(NH_4 + NO_3)$-N measured after FI exp. 2 and extracted with 0.5 M $NaHCO_3$ (1:10)									
Fum 0–10d	0.48	0.41	0.38	0.52	0.29	−0.09	0.46	0.38	0.07
Fum 0–20d	0.79	0.71	0.77	0.26	0.81	−0.43	0.76	0.78	−0.16
Unf 0–20d	0.10	0.13	0.06	−0.45	0.56	0.13	0.09	0.05	0.07
§Flush N Fum	−0.27	−0.41	−0.37	0.20	−0.44	0.29	−0.34	−0.38	0.36
Flush N Unf	0.31	0.17	0.25	0.65	−0.10	−0.14	0.26	0.25	−0.13
$(NH_4 + NO_3)$-N extracted after fumigation but no incubation									
Fum	1.00	0.97	0.99	0.80	0.79	−0.73	0.98	1.00	−0.75
Unf	0.99	0.95	1.00	0.85	0.74	−0.78	0.98	1.00	−0.77
Flush N	0.89	0.88	0.86	0.52	0.85	−0.48	0.88	0.88	0.61

Flush N Fum = Fum 0–10d minus Fum 10–20 d, where Fum 10–20 d = Fum 0–20 d minus Fum 0–10 d.
§Flush N Fum = Fum 0–10d minus Fum 0–20 d
Flush N Unf = Fum 0–10d minus Unf 0–20 d.

Coefficients above 0.57 significant at P = 0.05, above 0.71 significant at 0.01, and finally above 0.80 at P = 0.001.

Table 2.12. N mineralized after chloroform-fumigation procedure and incubation under anaerobic conditions at 28°C for 7 and 14 d, respectively.

Treatments		7d (0-7d)	14d (0-14d)	Fn1	Fn2	Bn1	Bn2	Bn1 as % of Total N	Bn2 as % of Total N
		----	-----	µg	g^{-1}	---	---	-%-	-%-
NH_4-N (x)	Fum	17.5 ±0.5	21.5 ±0.5	13.4 ±0.6		27 ± 1		3.9 ±0.0	
	Unf	4.6 ±0.3	NA		12.9 ±0.2		26 ± 1		3.7 ±0.1
NH_4-N (y)	Fum	34.7 ±5.1	35.4 ±3.7	34.0 ±8.1		69 ±12		6.4 ±1.0	
	Unf	6.3 ±1.4	NA		28.4 ±3.9		58 ± 6		5.4 ±0.4

NA = not available.
(x) Mean and standard deviation, (n = 4) of Check, N, NP, and NPK plots.
(y) Mean and standard deviation, (n = 4) of Manure, MN, MNP, and MNPK treated plots.
Fn1 = Flush of NH4-N mineralized = Fum 7 d minus Fum 7-14 d, where Fum 7-14 d = Fum 14 d minus Fum 7 d, Fn2 = Flush of NH4-N mineralized = Fum 7 d minus Unfum 7 d.
Bn1 and Bn2 = Fn1/Kn and Fn2/Kn, where Kn is 0.49 (Nicolardot et al. 1990).

Table 2.13. Organic C measurements after fumigation with chloroform and K_2SO_4 direct extraction procedure (FE).

Treat-ments	Fum	Unfum	Flush (Ec)	Bc1	Bc2	Bc1 as % Org C	Bc2 as % Org C	Bc1:Bn	Bc2:Bn
	-----	-----	$\mu g\ g^{-1}$	-----	-----	---%--	---%--		
Ck,N	0.15 ±0.01	0.10 ±0.02	0.06 ±0.01	0.16 ±0.05	0.18 ±0.06	0.003 ±0.001	0.003 ±0.001	-0.045 ±0.134	-0.050 ±0.156
NP,NPK									
M, MN, MNP,MNPK	0.15 ±0.01	0.10 ±0.00	0.08 ±0.00	0.21 ±0.08	0.24 ±0.00	0.002 ±0.000	0.002 ±0.000	-0.185 ±0.276	-0.215 ±0.318

FE = Fumigation extraction. Mean ± std dev, (n = 4).
Ec = Flush of extracted organic C = Fumigated soil minus Unfumigated soil.
Bc1 = Biomass Carbon 1 = Ec * 2.64 (Vance et al. 1987).
Bc2 = Biomass Carbon 2 = Ec/Kec, where Kec is 0.33 (Sparling and West 1988a).

Table 2.14. Effect of two soil treatments on the N flush in 0.5 M K_2SO_4 extracts after 24 h fumigation by the FE.

Soil treatments	Fumigated	Unfumigated	Flush (En)	Biomass N (Bn)	Bn as % Total N
	----------	----$\mu g\ g^{-1}$--	----------	----------	-----%----
Ck,N,NP,NPK	11.8 ± 2 3	11.2 ±3.6	0.3 ±1.3	0.5 ± 2.1	0.06 ±0.35
M,MN,MNP,MNPK	27.0 ±10.8	21.4 ±3.6	4.7 ±7.0	8.5 ±13.4	0.81 ±1.22

Mean ± std dev, (n = 4).
En = NH4-N Flush of 0.5 M K2SO4 extracted = Fumigated soil minus Unfumigated soil.
Bn = biomass N = En/Ken, where Ken is 0.54 (Brookes et al. 1985b).

Table 2.15. Pearson correlation coefficients.

	CO_2-C evolved during FI exp. 2					NH_4-N measured after FI exp. 2					
	Fum 0-10d	Fum 10-20d	Unf 10-20d	Flush C-Fum	Flush C-Unf	Fum 0-10d	Fum 0-20d	Unf 0-20d	Fum 10-20d	Flush N-Fum	Flush N-Unf
(NH_4 + NO_3)-N measured after FI exp. 2 and extracted with 2 M KCl (1:5)											
Fum 0-10d	-0.17	-0.26	-0.10	0.05	-0.26	-0.09	-0.08	-0.09	-0.04	-0.10	-0.07
Fum 0-20d	0.06	-0.21	-0.03	0.27	-0.03	0.09	0.12	-0.16	0.18	0.02	0.11
Unf 0-20d	-0.20	-0.41	-0.27	0.09	-0.28	-0.19	-0.23	0.05	-0.06	-0.24	-0.17
Fum 10-20d	-0.70	-0.64	-0.67	-0.62	-0.71	-0.55	-0.56	0.02	-0.52	-0.48	-0.52
Flush N Fum	0.45	0.31	0.49	0.55	0.33	0.47	0.48	-0.20	0.46	0.40	0.46
Flush N Unf	0.48	0.57	0.46	0.15	0.50	0.60	0.55	-0.01	0.35	0.65	0.56
(NH_4 + NO_3)-N measured after FI exp. 2 and extracted with 0.5 M K_2SO_4 (1:4)											
Fum 0-10d	-0.06	-0.24	-0.06	0.17	-0.15	-0.01	0.07	-0.18	0.05	-0.04	0.02
Unf 0-20d	-0.17	-0.39	-0.23	0.14	-0.25	-0.17	-0.20	0.04	-0.05	-0.21	-0.14
Flush N Unf	0.46	0.56	0.56	0.02	0.50	0.68	0.80	-0.59	0.39	0.75	0.66
(NH_4 + NO_3)-N measured after FI exp. 2 and extracted with 0.5 M $NaHCO_3$ (1:10)											
Fum 0-10d	-0.19	-0.10	-0.28	-0.29	-0.14	0.14	0.02	-0.63	-0.25	0.34	0.21
Fum 0-20d	0.16	-0.05	0.17	0.48	0.03	0.18	0.07	0.00	0.35	0.04	0.19
Unf 0-20d	0.74	0.64	0.43	0.51	0.78	0.68	0.36	0.03	0.69	0.58	0.67
§Flush Fum	-0.28	-0.06	-0.45	-0.62	-0.13	-0.04	-0.18	-0.36	-0.49	0.21	0.01
Flush N Unf	-0.57	-0.50	-0.56	-0.51	-0.55	-0.29	0.39	-0.38	-0.58	-0.07	-0.22
(NH_4 + NO_3)-N extracted after fumigation but no incubation											
Fum	-0.28	-0.39	-0.20	0.06	-0.40	-0.22	-0.20	-0.04	-0.13	-0.24	-0.20
Unf	-0.35	-0.44	-0.28	-0.03	-0.45	-0.26	-0.24	-0.08	-0.17	-0.27	-0.23
Flush N	-0.05	-0.24	0.11	0.39	-0.22	-0.12	-0.08	0.23	0.04	-0.20	-0.15

Flush N Fum = Fum 0-10 d minus Fum 10-20 d, where Fum 10-20 d = Fum 0-20 d minus Fum 0-10 d.
§Flush N Fum = Fum 0-10 d minus Fum 0-20 d.
Flush N Unf = Fum 0-10 d minus Unf 0-20 d.

Coefficients above 0.57 significant at P = 0.05, above 0.71 significant at 0.01, and finally above 0.80 at P = 0.001.

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APPENDIX 1

REGRESSION OF C AND N MEASURES WITH SOIL PROPERTIES

Based on the data from different combination of manure-N and mineral-N treatments in Xuzhou soil samples: MN (0:0), (0:10), (3:7), (5:5), and (7:3), regression analyses were performed to show relationships among several soil parameters. Soil organic C content increased linearly (r^2=0.86) with increasing manure-N ratios (fIGUGRE 2.1). Total N content increased with increasing manure-N ratios (Figure 2.2). Regression of NH_4-N concentrations against increasing manure-N ratios produced a highly significant relationship (r=0.93) for dry soils (Figure 2.3).

Regression of CO_2-C evolved from fumigated samples and flush of biomass C against manure-N ratios produced highly significant relationships (r^2=0.97 and 0.93) for Xuzhou soil samples in the first experiment (Figure 2.4). Mineralizable N from fumigated samples of the first fumigation-incubation also increased with increasing manure-N ratio, although not as consistently as CO_2-C evolved by fumigated soil samples (r^2=0.87) (Fig. 2.5).

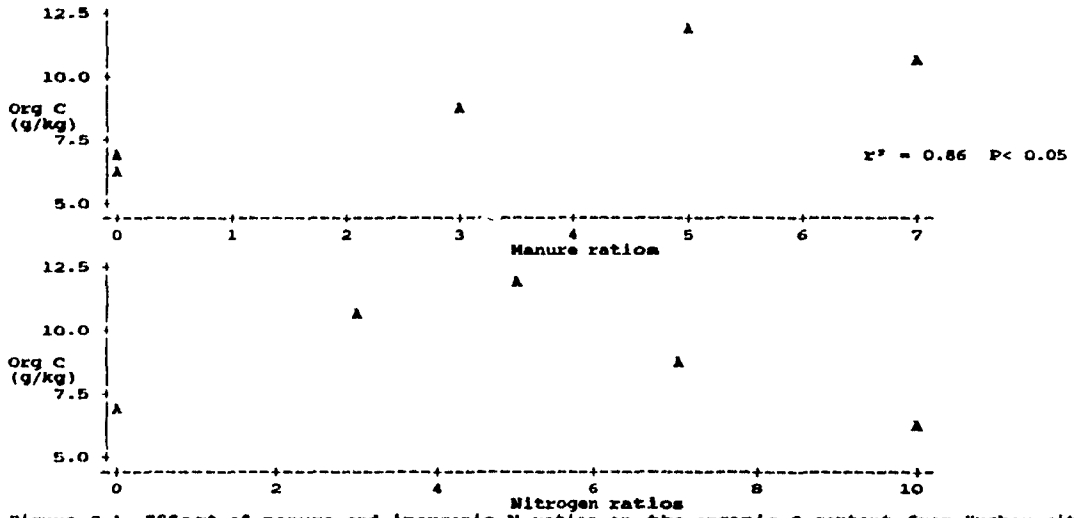

Figure 2.1. Effect of manure and inorganic N ratios on the organic C content from Xuzhou sites after 10 yr applications. Legend: A = 1 obs.

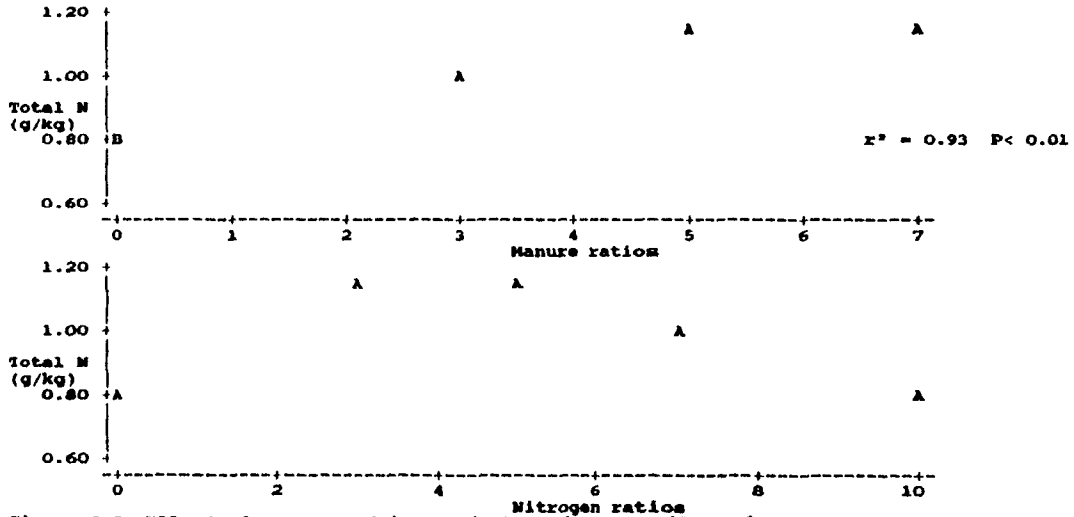

Figure 2.2. Effect of manure and inorganic N ratios on soil total N content. Legend: A = 1 obs, B = 2 obs.

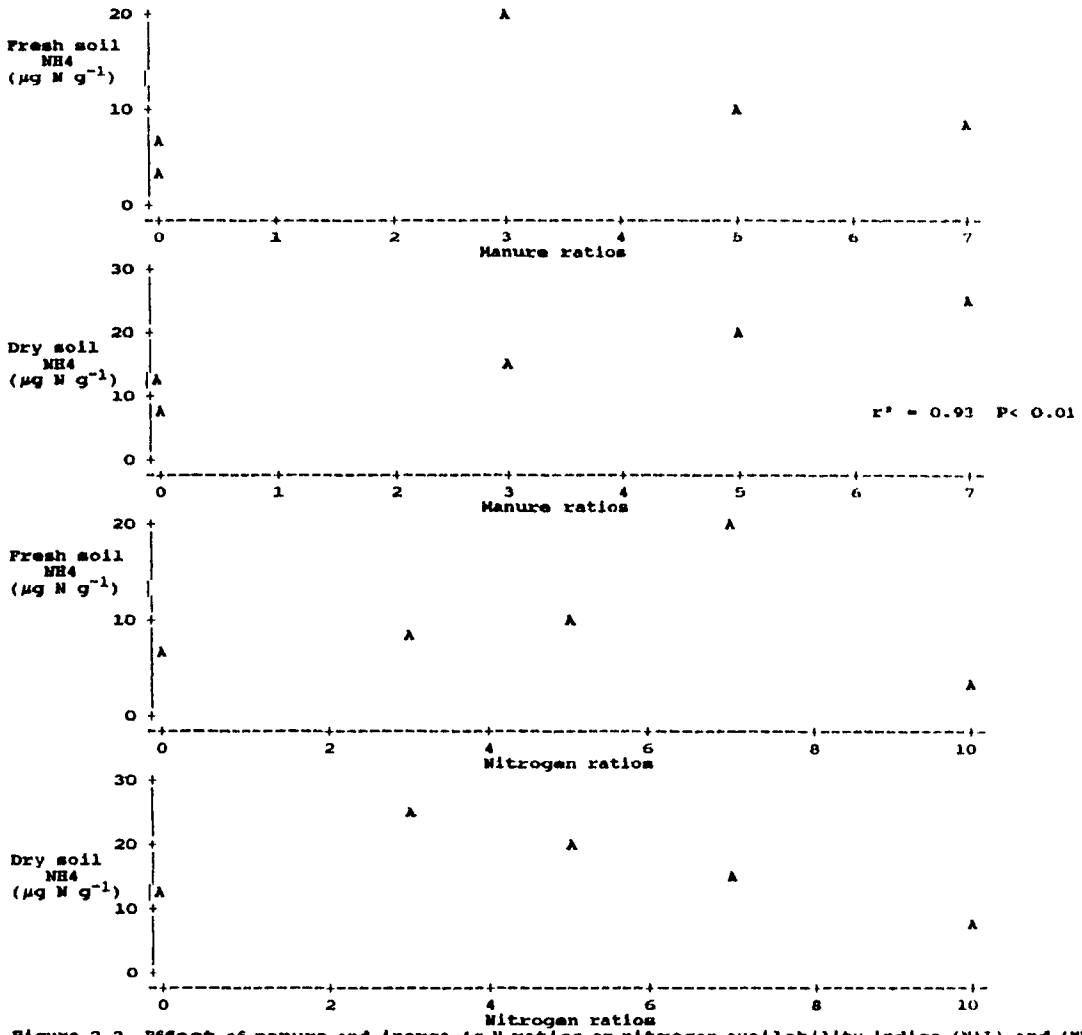

Figure 2.3. Effect of manure and inorganic N ratios on nitrogen availability indice (NAI) and (NH_4-N) measured from fresh and dry soil, from Xuzhou manure-N and mineral-N treated sites after 10 yr of applications. Legend: A = 1 obs.

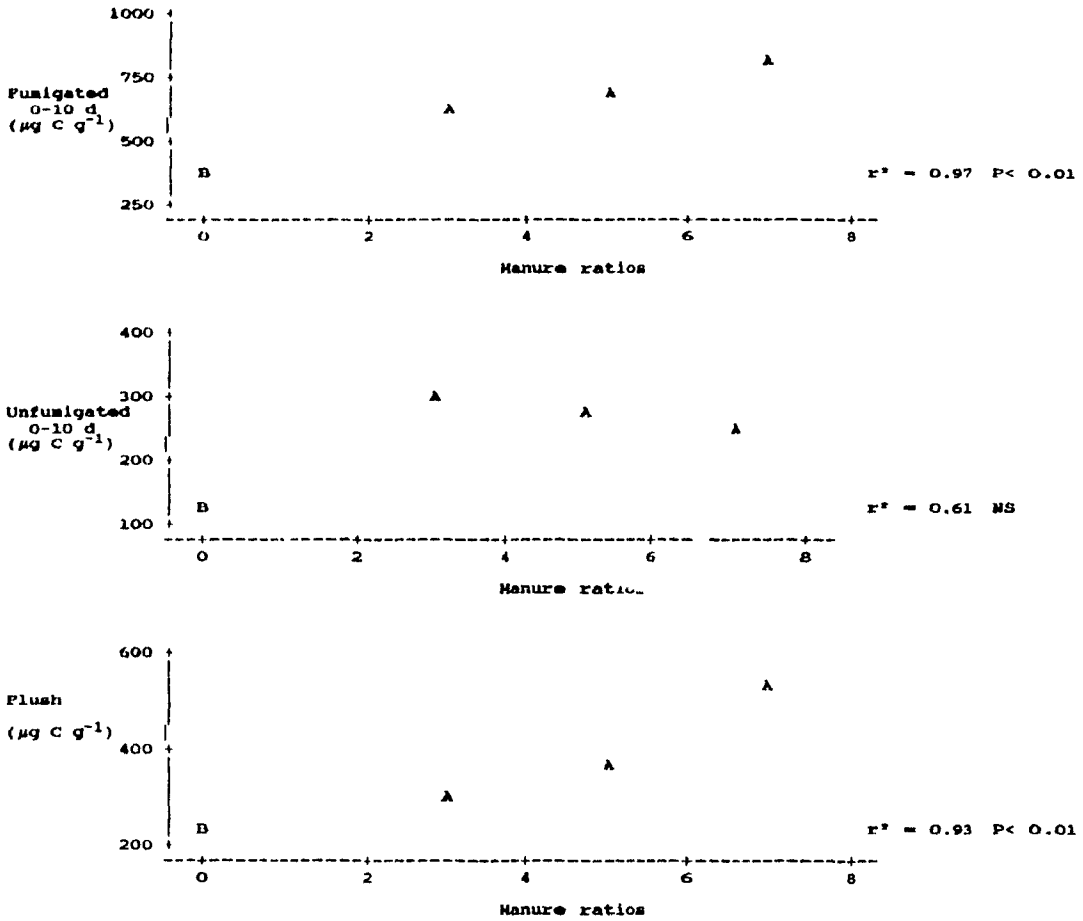

Figure 2.4. Effect of manure ratios on CO_2-C evolved from Fumigated 0-10 d, Unfumigated 0-10 d and the resulting flush C in the first fumigation-incubation experiment. Legend: A = 1 obs, B = 2 obs. NS = not significant.

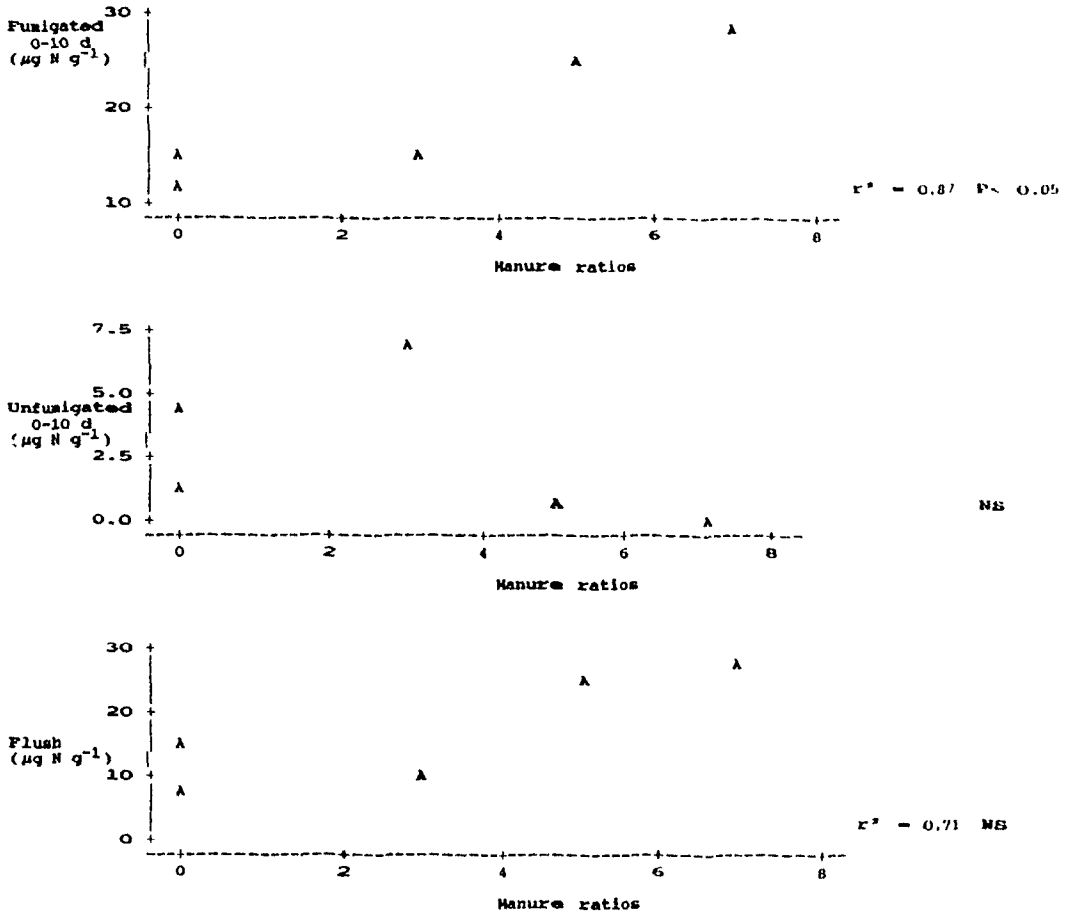

Figure 2.5. Effect of manure ratios on NH_4-N mineralized from Fumigated 0-10 d, Unfumigated 0-10 d and the resulting flush of N in the first fumigation-incubation experiment. Legend: A = 1 obs, B = 2 obs. NS = not significant.

APPENDIX 2

CHINA'S AGRICULTURE

INTRODUCTION

China's agricultural scene is extremely varied. The main axis of change is a climatic one that runs from North to South (from latitude 53° N to 18° N). Moving down this axis, differences are found in temperature and rainfall, in surface water availability, in growing season length, and in crops and cropping patterns (Howe 1978).

China is the third largest country occupying about 6.5% of the world's total land area compared to Canada with about 6.7%. Yet, cultivated land is scarce in China with only 10% of the total area being arable land. However, China supports about 23% of the world's population compared to Canada with about 0.5%. According to an official estimate an additional 5% of the land area could possibly be brought into cultivation, but most of this is in sparsely populated and inaccessible regions of North China, Xinjiang, and Tibet. The main concentrations of cultivated land are in East China, the Manchurian Plain, and in parts of the South and South-Western Provinces (Howe 1978).

For the most part, the productivity of cultivated land rises as one approaches the Eastern coast. This variation in the productivity of land is reflected in the distribution of population. Thus, crossing the North-South climatic axis, there is an East-West axis of cultivability. These two axes provide the framework of China's agricultural regions (Howe 1978). Within this framework (Figure 1) agronomists talk in terms of crops. The easiest way to analyze China's agriculture is to think of the North as a wheat (Triticum æsitivum L.) area, the South as a rice (Oryza sativa L.) area, and the Chang Jiang (Yangtze river) valley and Central China as regions that grow both. Still the real situation is much more complicated by variations in the patterns of successional planting and interplanting. These patterns that will be discussed later, are

important because changes in the extent to which it is possible to interweave several crops, or obtain multiple harvests of one crop in the agricultural year, are the keys of Chinese agricultural productivity (Howe 1978).

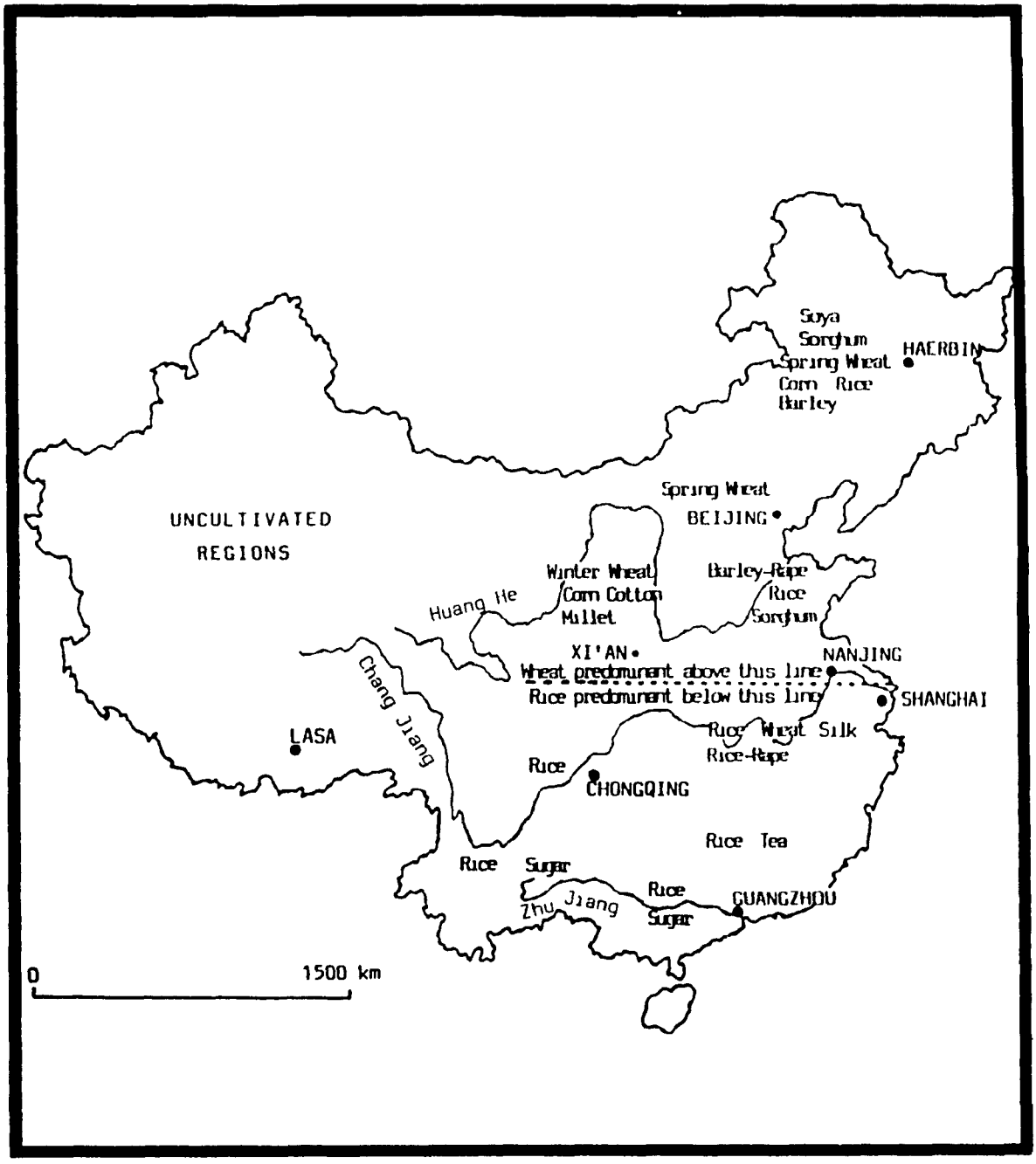

Figure 1. Major cultivated crops in China (based on Howe 1978).

Nevertheless, this impressive achievement should not hide the fact that China has not yet broken out of the range of subsistence-level production. Since the early 1960's, China has been a net importer of grains, soyabeans (Glycine max. (L.) Mess.), and raw cotton (Gossypium sp.) (Cheng 1982). In 1989, China bought from different foreign countries 60% more cereals than the previous year because it fell short of an estimated 110 millions tons of grains (Fabre and Lemoine 1990). Great inter- and intra- provincial disparities exist. Several recent Chinese statements admit that between 90 and 110 millions peoples are not still adequately fed, a proportion confirmed by the latest provincial grain availability averages (Smil 1988; Aubert 1990).

Having the world's oldest continuous civilization, agricultural tradition in China has survived uninterrupted for perhaps 7 000 to 8 000 years (Zhao 1986). When the Chinese communists took power in 1949, there was a high initial population density per hectare of cultivated land. One hectare of cultivated land nourished about 5 peoples, today one hectare of cultivated land nourishes more than 10 inhabitants (Aubert 1990).

CHINA'S AGRICULTURE CHANGES FROM 1949-1989

The development of agriculture and the balance between food and population are China's fundamental economic problems. Through a brief historical account of the major agricultural reforms since the arrival of the Popular Republic of China four decades ago, one is struck by the gradual shift of emphasis that Chinese agricultural development has undergone from institutional change and intensified use of traditional techniques to material incentives and modern techniques (Cheng 1982).

A. 1952-1956

In 1952, Mao Tse Tung abolished landlord property. This important reform was marked by the division and the redistribu-

tion of the land to poor farmers, ultimately resulting in the decrease per farmer of the average size of cultivated land. In the years following the reform there was an unavoidable decrease in quality and in quantity of agricultural products in the markets throughout China (Aubert 1990).

B. 1956-1967

In this period, the main goal of Mao Tse Tung was to increase the agricultural production and thus the industrial production. Both the National Program for agricultural development and Mao's Eight-Point Charter of 1956 stressed the intensive use of traditional techniques. The Eight-Point Charter advocated by Mao included important measures to improve agricultural production: water, fertilizer, soil conservation, seed selection, close planting, plant protection, implements, and field management (Cheng 1982). In an unprecedented reform land was collectivized by the government to increase the average cultivated farm unit. Many households were combined and the farm-owners transformed in farm-workers exploiting larger fields and sharing machine-tools (Aubert 1990). The major benefit of these reforms was that a large labour force was pooled together for big agricultural projects. The most important were irrigation works. Therefore, irrigation has been applied to an extent unequalled elsewhere. By 1978 almost half of all cultivated land was irrigated, provided with water through permanent installations such as ditches, catchment basins, dams, wells and pumps (Tang 1984).

By now two conflicting forces were affecting Chinese agriculture. On one hand, the material basis for higher productivity had been gradually formed over several years. On the other hand, Chinese agriculture was being held back by severe problems of disincentives, maladministration and poor planning, which remained unresolved after three decades (Cheng 1982).

For 2 000 years, grain has been the key factor in China's agriculture and the principal source of food. In the average

Chinese diet grain supplies 80% of the calorie intake (Howe 1978). Based on the Chinese Economic Statistics and China Agriculture yearbook of 1988, Table 1 shows that for thirty-five years the respective area devoted to grain production has been stable within the farming sector, hence more than two-thirds of the Chinese sown area is devoted to grains. In 1987, rice occupied close to 30% of grain sown area and wheat a little over 25%. However, for the total quantity of grain output since 1952 the situation has changed notably with rice still stable accounting for 43 to 45% of the total output of grain, but wheat doubling its share from 10 to 20% between 1957 and 1987 (Aubert 1990). After 1957 there was also unprecedented increase in the yield of grain crops. Wheat demonstrated the strongest rise in production, having been only 0.7 t ha^{-1} in 1952 it reached 3.0 t ha^{-1} in 1987. For rice, the yield increase was slower throughout the years nevertheless spectacular. In 1952, rice production was already high with 2.4 t ha^{-1} and peaked in 1987 with 5.4 t ha^{-1}.

Naturally, for the purpose of raising production research has been focused on rice and wheat. The rice area is being expanded to the limits of adaptation, toward the colder and drier northeast and the mountainous west, and now extends to some 34 millions hectares (Table 1). Short-stature, high-yielding cultivars have been bred well before those by the International Rice Research Institute. Besides, the Chinese strains require shorter growing periods. Early maturity comes close to being the universal objective of any plant selection programme due to the desire for greater productivity through multiple cropping. A complex cropping pattern has emerged that includes double, triple, and relay cropping also intercropping. These practices demand exact timing, whereas earliness will minimize the adverse influence of changeable weather. Even at the latitude of Beijing (Peking) i.e., 40° N where the growing season lasts only 160 d, rice preceded by wheat is a common sequence (Tang 1984).

Table 1. China's population growth and grain crop productions during the 1952-1987 period.

	1952	1957	1978	1987
China's population				
Total population (x 10^6)	575	647	963	1081
Rural population (x 10^6)	519	559	840	830
Labour force				
Total labour (x 10^6)	207	238	402	528
Rural labour (x 10^6)	182	206	306	390
Agricultural labour (x 10^6)	173	193	276	298
Agricultural/Total	84%	81%	69%	56%
Total cultivated area (x 10^6 ha)	108	112	100	96
Total irrigated area (x 10^6 ha)	21	35	45	44
Total harvested area (x 10^6 ha)	141	157	150	145
Chemical fertilizers (kg ha^{-1})	1	3	88	208
Grains				
Harvested area (x 10^6 ha)	124	134	121	111
Quantity (x 10^6 t)	161	191	316	405
Rice				
Harvested area (x 10^6 ha)	28	32	34	32
Quantity (x 10^6 t)	68	87	137	174
Yield (t ha^{-1})	2.4	2.7	4.0	5.4
Wheat				
Harvested area (x 10^6 ha)	25	28	29	29
Quantity (x 10^6 t)	18	24	54	88
Yield (t ha^{-1})	0.7	0.9	1.9	3.0
Corn				
Harvested area (x 10^6 ha)	13	15	20	20
Quantity (x 10^6 t)	17	21	56	80
Yield (t ha^{-1})	1.3	1.4	2.8	4.0

* Based on Aubert, C. 1990. Economie et société rurales. In M.C. Bergère, I. Bianco, and J. Domes (ed.) La Chine au XXe Siècle **. De 1949 a aujourd'hui. Fayard Publ. Paris, France.

For wheat, winter wheat accounts for some 85% of total output although spring wheat, traditionally cultivated in the North-west, is gaining in proportion as relay and inter-cropping patterns promote its spread southward. This trend has been possible only because of the availability of varieties with shorter life cycles like semi-dwarf high-yielding types (Tang 1984). Corn ranks third among cereals grown in China. The

main corn belt is in North China, 55% of the area in hybrids is in single cross, 40% in double cross, and 5% in three-way cross materials (Tang 1984). The progress in corn yields is comparable to wheat because often used in double cropping, wheat in winter and corn in summer, both share almost the same surface area. Before the Green Revolution there was stagnation of the grain output, from 1965 to 1978 doubling of the quantity, and finally during the last reforms a one-third increase in production. In 1987, 20 millions hectares of corn occupied close to 20% of the grain area, this corresponds to a 30% increase compared to 35 years ago (Aubert 1990).

Amongst the factors promoting agricultural advance, the most significant is the increase of chemical fertilizer production. In the early 1970's as thirteen major urea-ammonia complexes constructed with foreign technology came on stream, domestic chemical fertilizer supplies enjoyed a quantum jump (Cheng 1982). Despite a consistent rise in home production volume making the country the third largest international manufacturer of urea, China is simultaneously the biggest net importer of all nitrogenous fertilizers taken together. Although her aggregate consumption is high, China maintained a level of unit utilization of fertilizers (88 kg ha^{-1} in 1978) that exceeded only slightly the world average (Tang 1984) (Table 1).

Over the twenty-year period of 1957 to 1976, with the great incentive of growing more and more grains, in planning selt-sufficiency of grains in all provinces under the guideline of "taking grain as the key link" and in imposing grain crops in areas not suited for such crops, the result was great damage to the soil and a net decrease in the output of grain crops in many parts of China. Then agricultural production had become structurally concentrated in farming grain to the neglect of the other agricultural activities, deepening the existing imbalance between farming, forestry, and animal husbandry (Cheng 1982).

C. 1976-1989

After Mao's death in late 1976, new Chinese leaders and agro-economists reached the consensus that the traditional policy of increasing food grains as the primary goal of agricultural development must be altered. This historic shift of emphasis required a step by step restructuring of the small peasant economy, with its focus on grains, into a modern agricultural production system, with an all-around development of fishery, livestock breeding, forestry, and the cultivation and diversification of grains and cash crops in the entire China territory (Cheng 1982). The new leaders called first, for reforms in the rural incentive structures. For example, there was a general producer's price increase of 20% for within quota grain and of 50% for above quota grain, with a gradual decrease of 10 to 15% in prices of chemical fertilizers sold to the peasants. These changes raised the fertilizer-to-grain price ratio for above quota sales to a level somewhat equal to that prevailing in Taiwan (Cheng 1982). Quotas assigned to peasants were based roughly an average of the previous 3-yr crop yields and were said to be set low enough to let hard-working peasants overfill them (Cheng 1982). Second, a "responsibility system" for agriculture was instituted and the modernization process intensified. The original policy aimed at establishing a variety of organizational and remunerative techniques that linked the income of the individual peasant and their family to the quality or quantity of the agricultural goods produced. Thus, by 1983 the decollectivization had been completed and the individual household (contracting a 15-yr lease of land with the government) had again reemerged as the primary economic unit in rural China (Parish 1985). For many Chinese and foreign agriculture specialists the success of the post-1978 reforms, compared to the previous ones must be credited chiefly to the household responsibility system that has resulted in privatization of farming and in unprecedented agricultural output growth (Smil 1988).

In summary, in the view of most foreign analyses the performance of Chinese agriculture since 1949 has shown only moderate gains. Up to 1976 the strong government emphasis on grain output had slowed progress in forestry, livestock, and fishery. While the increase in the output of cash crops had been rapid especially during the last several years, supplies of cooking oil, soyabeans, and other components of the Chinese diet were chronically short. The absolute growth of food grains obtained since the end of the 1980's is very impressive, but in fact it has been modest barely exceeding population growth (Cheng 1982; Tang 1984). Because the amount of cultivated land had shrunk from 112 million hectares in 1952 to 96 million hectares in 1987 owing to urbanization, soil destruction via salinization, alkalization and desertification, increases in input of chemical products and improvements in the multiple cropping index could not increase staple crop output proportionately. Both land and labour productivities showed signs of deterioration (Cheng 1982; Tang 1984).

In contrast to other developing countries where economic growth has generally accompanied a steady decline in agricultural labour, China's industrialization didn't reduce the country's agricultural population. The nation's labour force has increased by 13 million people per annum since 1970. Consequently, the growth of the rural population has meant a steady decline in per capita output and consumption (Cheng 1982). For both Cheng (1982) and Tang (1984), the future of Chinese agriculture depends on two crucial variables: the strengthening of research efforts in agricultural science and technology to stimulate a rapid boost in per unit yield of crop harvest; and, promotion of labour efficiency by strict control of rural population growth and high peasant motivation. The progression in staple grain output is still possible in view of Aubert (1990) only within the present underestimate total cultivated land area by the Chinese government, thus overestimating the actual total grain output by 25 to 30%. The

reasons are the scarcity of new farmland, over-exploitation of soils in many regions due to intense cultivation, and increasing needs and demands of China's growing population. For these researchers, the whole situation may require again two long-standing directives, adopted in 1961 but still valid, "Agriculture is the foundation of the economy" and "Take grain as the key link."

JIANGSU PROVINCE'S AGRICULTURE

Jiangsu province is in Eastern Central China. The province with Nanjing (Nanking) the capital occupies only 1.1% of China's total area, but almost half this is arable land which makes it one of the richest regions of China. The population of Jiangsu reached over 65 million people in 1990, of these 80% live in the countryside (China Agriculture Yearbook 1989).

An important thing about Jiangsu Province is that it is one of the rice producing centers in China. Complicated rice oriented farming systems have been developed to maximize food production from the land with intensive labour input and use of all available resources. Although the major cropping system is the single rice-wheat system, there are other systems, such as the early rice-late rice-barley system, the single rice-rape system, the corn-wheat system, etc. (Tanaka et al. 1987). These multiple cropping systems are the practice of growing more than one harvested crop in sequence on the same piece of land during one year. As a rule, farming systems in China are much more complicated than in Canada. In the traditional Chinese subsistence farming system, people live on grains, animals and vegetables produced within the system; animals are fed with feeds produced within the system; the excrement of people and animals are returned to the fields. As described by Tanaka et al. (1987) the flow of plant nutrients is almost circular.

The productivity of Chinese soil has been maintained reasonably well by recycling plant nutrients efficiently with large amounts of organic manures and with tremendous labour

(Tanaka et al. 1987). Traditionally, the use of labour-intensive farming resulted in a high yield per hectare but a low yield per farmer, thus inevitably a low standard of living for the Chinese farmer. However, for the developing Jiangsu province, the farming system is changing rapidly. Since the early 1980's, labour for farming is becoming scarcer due to industrialization and a considerable amount of N and P chemical fertilizers have been introduced to increase the production of grains for a more market oriented system. When the availability of farm labour becomes more and more constrained in the future, it will become difficult to maintain combinations of cropping and animal husbandry within the farming system, and to recycle plant nutrients efficiently by using organic manures. Even under such conditions, many experts such as Tanaka et al. (1987) argue that crop production can be maintained at the present level or be increased, if chemical fertilizers are used properly.

While in the early 1980's the world wide application ratio of the three macronutrients (N-P-K) was approximately 1-0.22-0.31, in China it was a very unbalanced 1-0.11-0.03 (Smil 1988). The situation seems unchanged now. In 1987 based on the Chinese Statistics of Agricultural Economy (1989) the total chemical fertilizer consumption in Jiangsu was 8.13 millions metric tons of nitrogenous, phosphate, potash and complex fertilizers corresponding to 71.0, 22.2, 0.8, and 6.0% of the total, respectively. These numbers decrease somewhat except for potassium when the effective basis of each plant nutrient is considered. Therefore, total consumption is only 1.78 million metric tons, a decrease of 357%. On an effective basis the proportion of each type of fertilizer is as follows for N, P, K, and complex; 69.1, 19.2, 1.6, and 10.1%, respectively, showing a great disparity in utilization. It appears that the efficiency of these fertilizers for increasing the yield of crops per unit field is not necessarily very high, especially for nitrogen fertilizers (Tanaka et al. 1987; Jiang et al.

1990; Zhang et al. 1990).

Organic recycling estimated by Smil (1988) saves the Chinese government some 260 x 10^{15} Joules of mostly natural gas and electricity that would be needed to produce the replacement chemical nutrients. This comparison, of course, ignores those even more important, although not so readily measurable, agricultural benefits provided by organic recycling, above all soil organic matter accumulation, soil moisture retention, and soil conservation through protection against water and wind erosion. Another important consequence of the increasing use of chemical fertilizers and the decreasing use of organic manures is that it will become difficult to find places for disposal of residues from cropping and of human and animal wastes. A large amount of plant nutrients will be lost from the system and waterways will become plugged if mud at the bottom is not scooped out and water plants are not removed (Tanaka et al. 1987). Traditional methods for handling organic manures have played important roles in China in relation to these problems. If tradition is abandoned, new methods will be required to solve them. Furthermore, in the view of Chinese and foreign researchers, without these benefits, sustained farming is impossible, and it is thus a most unwelcome sign of modernization that the intensity of organic recycling recently has been slipping throughout China. Because of this, in 1987 at the National level, the Ministry of Agriculture urged all localities to persist in establishing a soil and fertilizer service system with the primary objective of transforming middle and low yielding fields by applying farmyard manure in combination with a balanced chemical fertilizer program.

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APPENDIX 3

TWO LONG-TERM FIELD EXPERIMENTS IN THE JIANGSU PROVINCE

INTRODUCTION

In Appendix 2, it was shown that the combination of organic-inorganic fertilizers is strongly encouraged by Chinese government officials. It is said to be good for Chinese peasants because of higher crop yields, reduced costs, and on the same time restricted environmental pollution problems. It was pertinent, therefore, to consider the effect of a combined fertilization system as to increased soil nutrients, and improved soil physical behaviour, while stabilizing crop production following increased yields.

Three Chinese reports on crop quantity, quality, soil nutrients, etc., influenced by rates of inorganic and organic fertilizers from two long-term field experiments were translated with the main objective of making these research results available, considering the importance of Chinese agriculture, and the fact that very few Chinese research papers are accessible to the western scientific community in English. Soil samples were obtained for subsequent laboratory measurements from the preceding experiments sites initiated after the Chinese culture revolution in Jiangsu Province.

Consequently, in the following Appendix after a general geographical portrait of the Province and a description of both experiment sites, long-term results originating from the translation of the reports are presented. However, often materials and methods were poorly described and statistical analyses were not specified. Hundreds of field experiments have been conducted throughout the world during the past 30 yr with the main objective of determining the nature and magnitude of crop yield responses to N fertilization (Stanford 1982). Interpretation of crop production from different experimental sites is difficult because of the broad range of soil, climatic, and management conditions (Stanford 1982). Still, an attempt has been made in Appendix 2 to summarize data from the

three oldest experiment sites in literature and to compare with the Chinese experiments.

The N supply is a general decisive factor in crop production. Nowadays, in many Chinese agricultural systems, it is customary to increase the input of N through chemical fertilization. Concern over the elevated cost and energy involved in the production of commercial fertilizer as well as increased amounts of manure that has created a disposal problem, makes imperative the utilization of manure as a fertilizer in a developing country like China.

Manure is a valuable resource, as a fertilizer and soil amendment, in crop production (Sommerfeldt et al. 1988). Experiments have shown that manures can be more effective than inorganic fertilizers in increasing crop yields. Yet, in other studies, N added in manure resulted in lower crop yields than N added in inorganic fertilizers (Xie and MacKenzie 1986). In China, a tremendous labour effort is needed to apply large amounts of manure to the intensive cropping systems. There is an urgent need for a better understanding and improved basis for managing the N factor of crop production, both by increasing the efficiency with which N is used by crops and by limiting N losses to the external environment. A goal of sound management is to adopt fertilizing practices that maximize fertilizer N use efficiency, but more significantly, that could increase soil organic matter and/or the N-supplying power (Rennie 1983).

Most fertilizer-manure applications research has been conducted in temperate areas. Therefore, the long-term effects of repeated seasonal applications of chemical fertilizers and manure under the subhumid warmed-temperate climate of eastern China are unknown. The purpose of the studies reported here was to measure changes in crop productivity and quality, also to monitor variations in soil physical, chemical, and biological properties during a 10 yr period as affected by different fertilizer-manure treatments.

MATERIALS AND METHODS

A) SITE DESCRIPTION OF XUZHOU LONG-TERM FIELD EXPERIMENT

The first site Xuzhou is 280 km northwest of Nanjing or 10 km north of the Huai He (Huai river), in a region of flat low alluvial plains (below 50 m), with good climatic conditions (Figure 1). Due to frequent southward collapse of dikes and flooding of the important Huang He (Yellow River) up to the last decades, the original river systems have been destabilized and drainage has been impeded. The outcome described by Zhao (1986) is that "small rains result in small hazards; large rains result in large floods; no rains result in drought."

The climate is essentially subhumid, warm-temperate, characterized by an average annual temperature of about 14°C, with a cold boreal winter and a warm subtropical summer. The coldest month (Jan.) has a mean temperatures of 0 to -14°, and an absolute minimum temperature below -20°C. The hottest month (July) has a mean temperatures of 24 to 29°C and an absolute maximum temperature above 40°C. The frost-free season lasts between 170 to 220 days (Institute of Soil Science 1986; Zhao 1986) (Table 1). Annual precipitation totals 500 to 900 mm. Rainfall is concentrated in summer, with a maximum daily precipitation of more than 100 to 200 mm. Winter (Dec. to Feb.) is very dry with only 3 to 7% of the annual precipitation and spring (Mar. to May) is low in humidity with only 10 to 14% of rainfall and high winds. Annual variation is as high as 25%, and some years may receive more than 10 times the precipitation of the driest year. Therefore, spring droughts and summer floods with salinization and aeolian sand often plague agricultural production in this region (Zhao 1986).

The double-cropping system of wheat in winter and corn in summer is well adapted to this area. In general, yields of both crops are reasonably high and stable. Wheat (1-20 Oct. to 10-30 May) and corn (1-20 June to 20 Sept.-10 Oct.) occupy the field for about 220 and 110 d, respectively, and the total duration of these two crops in the field is about 330 d. There are about

22 d between wheat and corn, and about 12 d between corn and wheat. These are reasonable intervals between the 2 crops for harvesting and for preparing the field properly for the next one (Tanaka et al. 1987).

Figure 1. Suzhou and Xuzhou, two long-term experiment sites in the province of Jiangsu.

Table 1. General climatic characteristics of the two long-term experimental sites.

Site	Geographical location latitude-longitude	Climate	Mean Daily Temperature (°C) Jan	July	Year	Frost-free days	Mean Total precipitation (mm)
Suzhou	31° 20' N 120° 16' E	Humid subtropical	0 to 5	28 to 43	16	200-250	1100-1400
Xuzhou	34° 15' N 117° 10' E	Subhumid-warm temperate	0 to -14	24 to 29	14	170-220	500-900

An ongoing double cropping cycle study initiated in 1979 at Xuzhou provided a unique opportunity to assess the effects of various fertilizer treatments on some physical, chemical, and biological properties of the soil over a 10 yr period. The field experiments were permanently established by the Xuzhou Agricultural Sciences Institute (Pei Xian County). The objectives of this study were:

(1) to determine the influence of chemical fertilizers and organic manure on the quantity and quality of two cereal crops (winter wheat-corn) production;

(2) to compare short and long-term effects of continuous applications of chemical fertilizers to that of organic manure on soil fertility, nutritional balance, and fertilizer efficiency;

(3) to analyze the situation of plant nutrient flow in double-cropping systems of wheat and corn in order to forecast changes that may take place when the use of chemical fertilizers increases and the use of farm wastes decreases;

(4) to provide scientific bases for rational use of nitrogenous fertilizers;

(5) to evaluate the effect of accumulation and exhaustion of mineral constituents in the soil on the chemical composition of wheat and corn plants;

(6) to determine long-time trends in soil organic

matter and total N (amounts and rates) under the different soil treatments;

(7) to develop a model to predict the accumulation of OM and total N in the soil; and finally, to determine the resulting C-to-N ratio of the soil.

Two split-plot design experiments were constructed according to a uniform plan throughout China. In the first field experiment there were two main plot treatments (134 m^2) that received:

(i) chemical fertilizers without organic manure;

(ii) chemical fertilizers with organic manure;

And four subplot treatments (33.5 m^2 replicated 4 times):

(i): Check, N, NP, NPK;

(ii): Manure alone (M), M+N, M+NP, M+NPK.

Amounts and forms of nitrogen, were 299 kg N ha^{-1} yr^{-1} (or 149 kg N ha^{-1} $season^{-1}$) as urea (46% N) were tested in the presence or absence of P, and K. The effects of phosphorus, 149 kg P_2O_5 ha^{-1} yr^{-1} (or 75 kg P_2O_5 ha^{-1} $season^{-1}$) as superphosphate (12% P_2O_5) with or without 224 kg K_2O ha^{-1} yr^{-1} (112 kg K_2O ha^{-1} $season^{-1}$) of potassium chloride (55% K_2O) in the presence of N were studied. Besides the untreated and chemically fertilized plots, there was a main group of treatments with horse manure. From 1981–1984 horse manure was applied at the total rate of 74 625 kg wet weight ha^{-1} yr^{-1} (37 313 kg ha^{-1} $season^{-1}$), but after 1985 it was reduced by half (18 656 kg ha^{-1} $season^{-1}$). Although all the horse manure came from the same feedlot, there was much variability in the manure among years. Composition of horse manure fluctuated considerably. The range was 0.516-0.577% in N, 0.356-0.474% in P_2O_5, and 0.787-0.827% in K_2O on the basis of fresh matter. The N-P_2O_5-K_2O nutrient ratio was 1.00-0.69-1.53 at the beginning of the study, and changed to 1.00-0.82-1.43 in 1989.

The fertilizers were applied according to treatment specifications and split in half so that wheat and corn received the same amount of inorganic and organic fertilizers

throughout the year. Fertilizer P, K, and one half the N were broadcast and incorporated prior to each crop sowing. The other half chemical N fertilizer remaining was applied as top dressing during both growing season. Horse manure was spread to the field and mixed with the soil before fall (for wheat) and summer (for corn) cultivation.

Based on the knowledge of Xuzhou soil properties and the findings that crop productivity in treatment MN was high without any applications of chemical P and K fertilizers, a comparative study on the effects of different ratios of manure-N-to-inorganic-N treatments was carried out. This second experiment design involved two N levels, 448 and 672 kg N ha^{-1} yr^{-1}, respectively, with a main plot dimension of 134 m^2. And five subplot treatments, 33.5 m^2 each replicated 2 to 4 times: MN = (0:0), (0:10), (3:7), (5:5), (7:3). The fertilization program was split the following way: 60% of the total N was applied to winter wheat and 40% to corn. No synthetic P and/or K fertilizers were supplied to the crops. Phosphorus and K were available through the horse manure additions with the previously described nutrient contents.

Cultural and tillage operations were performed by hand. Weed control was achieved by hand. Plots were seeded with adapted cultivars of wheat and corn. Irrigation was applied by the Chinese traditional flat-level technique using dikes. In all cases, plots were harvested at the maturity. Data were collected by cutting crops in the area without border effect. After each harvest, both grain and straw were removed from the fields, and soil samples were taken, air dried and analysed for N, P, K, and organic matter.

B) SITE DESCRIPTION OF SUZHOU LONG-TERM FIELD EXPERIMENTS

The second site, Suzhou is located 170 km southeast of Nanjing in the middle of the Chang Jiang (Yangtze River) delta plain, with a general elevation less than 50 m (Figure 1). It is characterized by rich lands and water resources (Institute

of Soil Science 1986). The region has been gradually silted up by alluvial deposits of the Chang Jiang and the Huai He as well as by extensive lacustrine and marine deposits (Zhao 1986). The 370 000 hectares of the region produce major crops of rice, wheat, cotton and oilseeds, some well-known horticultural and economic trees such as tea, orange and loquat, plus such "sideline" occupations as mulberry trees for silkworm breeding, fruits and vegetables, freshwater fish, artificial pearls, poultry and livestock (China Agriculture Yearbook 1988).

Suzhou region lies at the juncture of northern and central subtropical zones resulting in a humid subtropical climate. It is characterized by an average annual air temperature of about 16°C, with rather cold temperatures during winter owing to the east coast location and frequent invasion of cold waves, a mean January temperature of 0 to 5°C, an absolute minimum temperature below −5°C, and with high temperatures during summer, a mean July temperature of 28 to 43 °C (Table 1). According to the past ten years, the frost-free period varied between 200 and 250 d (Institute of Soil Science 1986; Zhao 1986). Mean annual total precipitation between 1 100 and 1 400 mm, with 50 to 60% concentrated in summer and fall, and 30 to 40% in spring, makes spring drought hazards practically nil. Because of heavy precipitation that comes during the long growing season agriculture is very productive.

In the very productive region of Suzhou the main double-cropping system is winter wheat and rice. Total yield average is about 11,2 t ha^{-1} yr^{-1}, rice giving the highest yield production with 7,5 t ha^{-1} yr^{-1} and wheat with barely about one half 3,7 t ha^{-1} yr^{-1} (Tanaka et al. 1987). Wheat (from 20 Oct.-1 Nov. to 10-20 May) and rice (from 20 May-1 June to 10-20 Oct.) occupied the field for about 200 and 140 d, respectively, and the total duration of these two crops in the field is about 340 d (about 10 d longer than 2-crop wheat-corn system). There are about 12 d between wheat and rice, and again about 12 d between rice and wheat. These are reasonable intervals between the two

crops for harvesting and for preparing the field properly for the next crop. Temperatures at the time of sowing and harvesting of rice are far higher than the critical low temperature. This explains why rice yield is so high compared to wheat growing under low winter temperatures (Tanaka et al. 1987). In Suzhou region, varieties grown as rice were *japonicas* while new higher productive winter wheat varieties were used.

The field experiments have been established since 1984 by Suzhou Agricultural Institute (Wu Xian County) as demonstration plots for farmers to compare the effects of the combinations of organic manure and chemical fertilizers on the typical winter wheat-rice cropping system of the region. In this long-term field experiment fewer treatments were considered suitable for the area. Only two were sampled, the untreated (check), and M+NP plots. In the check plots, neither chemical fertilizers, organic manure nor crop residues were added to the soil. In M+NP treated parcels, crops were grown for five cropping cycles beginning in the spring 1980, following farmer's standard practices.

For N, 345 kg N ha^{-1} yr^{-1} of ammonium sulphate (21% N) was split into 188 and 157 kg N ha^{-1} for wheat and rice, respectively. Chemical fertilizer-N was applied in several applications. For wheat, N was split into five portions with a ratio of 6:1:1:1:1, respectively. The first portion was added before sowing, the four others broadcast one month later, in January, in spring time, and finally, in late spring at filling stage. For the summer crop, four equal portions were applied separately one before sowing, one at tillering, one at stemming stage, and the last one, at the grain filling period. For P fertilizer, 36 kg P_2O_5 ha^{-1} yr^{-1} of superphosphate (12% P_2O_5) was broadcast before sowing winter wheat only. Therefore the application rate of synthetic P to rice was less than those to other crops.

A total of 14 925 kg fresh weight ha^{-1} of pig manure was added to the field plot each year, equivalent to 7 463 kg ha^{-1} before wheat and rice sowing seasons, respectively. Based on dry weight, pig manure content was 3.654% N, 5.027% P_2O_5, and 1.63% K_2O; the resulting N-P_2O_5-K_2O ratio was 1.00-1.38-0.45. The total amount of macronutrients supplied annually based on pig excreta dry matter content (5 970 kg ha^{-1}) was 218 kg N ha^{-1}, 300 kg P_2O_5 ha^{-1} and 97 kg K_2O ha^{-1}. Manure samples were taken and analyzed each year for moisture content and nutrient determination to permit uniform rates of application. At harvest, grain and straw yield data were collected. However, grain N was not measured regularly during this experiment so changes in relative N uptake could not be determined for Suzhou soils.

Both Xuzhou and Suzhou sites have different physical and chemical soil characteristics (Table 2 and 3). Xuzhou soil was developed from the Huang He (Yellow River) alluvial deposit, its Chinese name is "yellow fluvo-aquic" (Fluvisols-FAO system) (Jiang et al. 1987 and 1990b). The soil is sandy loam textured, lightly basic, with a medium fertility. Suzhou soil is Chinese classified as a hydromorphic paddy soil (Gleysols-FAO system) (Xu et al. 1980). It developed under the peculiar conditions of alternate wetting and drying, and ploughing and harrowing in standing water. It is an excellent example of man-made fertile soil that has long been under cultivation of rice and wheat (Xu et al. 1980). Its clay loam texture is homogeneous in the entire profile. In the ploughed horizon (0-20 cm), soils at the Suzhou site had organic matter content, organic C and total N percent, respectively, threefold higher than those of the Xuzhou site.

Table 2. Soil classification and agronomic background of the two sampling sites.

Site	Soil classification	Clay % (<0.002 mm)	Soil Texture	Double cropping system	Years under cropping	Sampling date	Field-Moisture (%)
Suzhou	Hydromorphic paddy soil	34.1	clay loam	winter wheat-rice	5	Nov 15, 1989	12-27
Xuzhou	Yellow fluvo-aquic soil	6.0	sandy loam	winter wheat-corn	10	May 21, 1989	3-15

Table 3. Physical and chemical characteristics of the soils.

Site	Depth of sampling cm	Bulk density g/cm^3	WHC (%)	pH H_2O	OM (%)	Org C (%)	Total N (%)	C:N	Total P (%)	Ava P µg/g	Ava K µg/g
Suzhou	0-20	1.15	65	6.8	3.0	1.717	0.170	10.1	0.062	27	86
Xuzhou	0-20	-	46	8.3	1.1	0.626	0.066	9.5	0.074	12	62
	20-40		-	8.4	0.5	0.265	0.033	8.0	-	3	-

WHC = water holding capacity, OM = organic matter, % Org C x 1.724 = % OM, Ava P = available P and Ava K = available K.

LONG-TERM FIELD EXPERIMENTS : RESULTS AND DISCUSSION

TOTAL CROP YIELDS

Comparison of the Chinese wheat-corn parcels with continuous winter wheat plots from Broadbalk (England) and Deherain (France) locations, and the continuous corn from Morrow plots (USA) showed that the crop yields were of the same magnitude, even under different soil conditions, duration of cultivation, and varying amounts of organic and/or mineral fertilizers employed (Table 4). Yet, in wheat, since the last 10 years the grain yield had a net tendency to increase in all Deherain treatments whereas the straw production decreased; the reverse was observed in several Xuzhou treatments. In corn, the largest difference in crop yields between similar treatments was observed in the manured plots, this may be the effect of

the large disparity in the amount of manure applied between the two sites. Prior to corn seeding, Morrow plots have received 4.5 t ha^{-1} yr^{-1} of barnyard manure compared to 18.7 t ha^{-1} of horse manure in Xuzhou organic treatments.

Table 4. Comparative results from six long-term field experiments throughout the world.

Treatments	Suzhou	Xuzhou	Jiangsu	Rothamsted	Deherain	Xuzhou	Morrow
	Wheat					Corn	
				kg ha^{-1}			
Check	3 656	1 830	2 380	2 127	2 350	3 842	3 040
N	na	4 600	na	3 039	na	6 416	na
NP	na	5 406	na	5 065	4 950	6 849	na
NPK	na	6 180	4 090	5 428	7 960	7 780	7 970
M	na	3 295	3 630	5 374	5 680	6 870	5 670
MN	na	6 257	na	na	na	7 989	na
MNP	8 060	6 321	na	na	na	8 104	na
MNPK	na	6 748	4 000	na	7 160	8 176	na

na - not available
-Suzhou double-cropping of winter wheat-rice, data of wheat production in 1989-90 season.
-Xuzhou long-term field experiment, 1986's wheat and corn yields.
-Jiangsu province rice oriented farming systems, averaged 3 yr yields (Tanaka et al. 1987).
-Rothamsted, Broadbalk continuous winter wheat experiments in England - yields of dry matter, mean of two seasons 1966-67 (Johnston 1969b).
-Deherain on Grignon experimental farm near Paris - rotation of winter wheat and sugar beets-average yields for the 1976-80 period (Morel et al. 1984).
-Morrow plots, Illinois, USA continuous corn average yields (Odell et al. 1984).

Field site			Xuzhou	Rothamsted		Deherain	
Fertilization program since			1980	1843		1875	
kg N ha^{-1}	urea		150	NH_4 salts	96	NH_4NO_3	87
kg P_2O_5 ha^{-1}	superphosphate		75		74-77		90
kg K_2O ha^{-1}	KCl		112	K_2SO_4	108	KCl	90
t ha^{-1}	manure	(1981-84)	37		31		10
		(1985-89)	18				

According to Tanaka and his co-researchers (1987) in southern Jiangsu province the double cropping of rice and wheat cycles exhibits high crop yields. For example, total production is about 12.5 t ha^{-1} yr^{-1}, whereas individual crop yield averaged range is 3.8 to 4.8, 4.8 to 6.0, and 6.4 to 7.7 t ha^{-1}, for wheat, corn, and rice, respectively (China agriculture yearbook

1988). Both Xuzhou and Suzhou adequately fertilized plots yielded above the long-term averages for the general area. Wheat production in Suzhou unfertilized parcels were about twice higher than in Xuzhou plots.

Based on Jiang et al. (1990a, b) and Zhang et al. (1990) summary reports for both Xuzhou field plots experiments, total winter wheat and corn dry matter yields were expressed on a farm production basis (t ha^{-1} yr^{-1}) after 9 and 10 yr of study, respectively (Table 5 and 6). In these reports there were neither indications on the variations of crop dry matter yield nor the nutrient uptake by wheat and corn among years with differences in weather, plant population, seeding date, and management practices. However, one can observed from these results that crop yields associated with different treatments varied greatly, depending upon the fertilization program used. The average grain yield per treatment was in the order of treatment MNPK = MNP = MN = (7:3) = (5:5) > treatment (3:7) = NPK > treatment NP > treatment M = N = (0:10) > treatment Check = (0:0). Corn yield was generally higher than wheat. This difference was especially great in both check and manure treatments. For treatment MNPK with the highest nutritious potential, the difference in yields between the two crops was small. In addition, corn dry matter yields were highest in treatments that received chemical fertilizers and horse manure, for example MN, MNP, and MNPK, while corn production was lowest in the treatment that received only 150 kg N ha^{-1}. Still, yields of corn average 125% of comparable wheat production, and were generally well above the Jiangsu provincial average of 4860 kg ha^{-1}.

Table 5. Yearly yields for the different chemical and organic fertilizers treatments in the first Xuzhou long term experiment.

Treatments	1981	1982	1983	1984	1985	1986	1987	1988	1989	9 yr Aver.
	--	----	----	----	t ha-1	----	----	----	----	----
Check	6.0 cB	5.2 dC	6.6 cC	5.8 dD	5.4 dD	5.0 dD	4.5	4.5	3.9	5.2 dC
N	12.0 bA	11.2 cB	13.6 bB	10.3 cC	9.0 cC	10.1 cC	6.5	6.6	5.9	9.4 cB
NP	11.8 bA	12.4 bB	13.5 bB	11.9 bB	10.3 bB	13.7 bB	11.0	11.0	10.9	11.8 bB
NPK	12.6 aA	14.1 aA	15.3 aA	13.4 aA	12.6 aA	15.8 aA	13.1	12.0	13.6	13.6 aA
M	8.7 bB	9.2 bB	11.8 bB	11.0 bB	10.0 cC	10.4 cC	9.2	9.5	9.6	9.9 bB
MN	12.9 aA	14.4 aA	15.8 aA	13.7 aA	12.1 bB	16.6 bB	13.9	13.1	13.9	14.0 aA
MNP	13.0 aA	14.6 aA	16.0 aA	14.1 aA	12.2 bB	16.7 bB	14.3	12.9	13.4	14.1 aA
MNPK	13.3 aA	14.4 aA	15.9 aA	14.3 aA	13.8 aA	17.8 aA	14.7	13.5	13.8	14.6 aA

a-d and A-D plots averages followed by the same letter are not significantly different at the 0.05 and 0.01 level, respectively.

Table 6. Yearly yields for various combinations of manure-N-to-inorganic-N treatments in the second Xuzhou field experiment.

Treat-ments	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	10 yr Aver.
MN	----	----	----	----	----	t ha^{-1}	----	----	----	----	----
(0 0)	5.0	6.2	5.4	6.4	5.6	5.6	4.5	4.9	5.7	5.2	5.4
(0:10)	14.7	12.2	10.3	10.6	7.7	7.9	9.0	5.1	5.4	4.9	8.8
(3:7)	15.2	14.1	13.7	14.0	12.7	12.5	16.0	13.2	13.2	12..	13.7
(5.5)	14.6	14.8	14.0	14.4	13.6	13.9	17.0	13.8	12.5	13.5	14.2
(7.3)	12.4	13.9	13.5	13.7	13.5	14.3	16.8	13.8	13.0	14.8	14.0

MN was equalled to 5 different manure-N-to-inorganic-N treatment ratios (0.0), (0.10), (3.7), (5 5) and (7 3).

The fertility status of Xuzhou soil in the experimental area was somewhat low. During the first 4 yr, total crop production varied between 5.2 and 6.6 t ha^{-1}, but thereafter, soil fertility decreased significantly. In 1989 dry matter yields reached only 65% of that of the beginning of the main

experiment (Table 5). In contrast, total crop yield was stable in the second experiment and the control treatment averaged about 5448 kg ha^{-1} yr^{-1} over 10 yr (Table 6). By taking the crop yield with N, P and K chemical fertilizer plus horse manure application (MNPK) as a base, the relative yield of crops without any nutrient application tended to decrease with the number of cropping cycles due to depletion of plant nutrients in the soil (Tanaka et al. 1987; Jiang et al. 1990a). Crop production decreased gradually with the number of cropping cycles for both wheat and corn. The calculated contribution of soil to wheat and corn yields was 27 and 47%, respectively, (Jiang et al. 1990a). Wheat crop production in Deherain long-term control fields showed similar decrease with time (Morel et al. 1984). These tendencies may suggest that:

(i) there was a significant amount of N in the soil when the experiments were started;

(ii) the soil N was depleted by growing crops with no N supply;

(iii) corn abilities to absorb nutrients from the soil was greater than wheat. Hence, corn yields without N fertilizer may provide the best index of N release by the soil (Rice et al. 1986);

(iv) the natural soil N supply was greatest during corn seasons because of a longer growth period with high temperature and good water supply. However, the soil N that became available to corn at higher temperatures was consequently depleted more rapidly than when wheat was grown at lower temperature (Tanaka et al. 1987).

With minor exceptions, both inorganic N rates, 299 and 448 kg N ha^{-1} yr^{-1} showed the same yield trends with time. In the first trial, crop production was maintained quite high with an average of 12.3 t ha^{-1} for the 1981-83 period; but after both yields of wheat and corn started to decrease gradually, to

reach in 1989 only one half the first yr production. Average total crop production in the first 5 yr compared to the last 5 yr showed a 34% decrease (9.7 to 6.4 kg ha^{-1}) in the (0:10) plots. This treatment showed the largest differences in crop production over the 10 yr period. For 1980-84, wheat crop production was equivalent to 68-73% of the manure-N/inorganic-N treatments, while it dropped to only 36-39% in 1985-89. The decrease was most remarkable, especially in the last yr, where wheat yield in the (0:10) fertilized plots showed a difference of 373-476 kg ha^{-1} compared to the untreated fields.

During the first 6 yr, except for 1985, the yearly wheat or corn production in the urea-superphosphate treated soils was higher than at the beginning of the study. But after 1987, crop production started to decrease and the soil showed signs of K deficiency. Wheat seemed to present moderate signs of K deficiency in the unbalanced fertilized plots compared to treatment NPK. Lack of K resulted in a greater decrease in production in Deherain long-term experiment compared to Xuzhou (Morel et al. 1984).

Parcels receiving manure resulted in yields significantly higher than both the control and the inorganic-N treated plots based on the average 9 yr crop production. In addition, repeated horse manure applications resulted in stable crop yields. Corn production tended to increase with time due to the residual effects of the organic compound (Jiang et al. 1990a). Amongst NPK, M, and MNPK treatments, the M plots were the least productive of this group, in each of Deherain, Jiangsu, and Xuzhou sites.

Generally, yields were almost the same whether N, P, and K were applied as chemical fertilizers or in combination with organic manure. Except, for the month of May 1985, where winter wheat was affected by heavy rainfall with lower production in all fields; treatments NPK, MN, MNP, and MNPK maintained a high and stable total crop production that averaged between 13.6 and 14.6 t ha^{-1} throughout the 9 yr period. To have some idea of the

range of yield deviation, range of total yield [(maximum yield - minimum yield)/averaged yield] was calculated for each treatment. Yields in treated N plots, and controls were less stable than those of other treatments with 28.4 and 16.2%, respectively, comparatively to about 9.9%.

As expected, there was no significant yield response to P and K fertilizers in the soils that had received yearly applications of horse manure. In MNP and MNPK treated plots, to which high rates of nutrients were applied as organic manures in combination with chemical fertilizers, the increase in crop production was considered mostly the result of inorganic N compared to the manured controls. Phosphorus and K were adequately supplied by horse manure as demonstrated by reasonably high P and K content in the straw (Jiang et al. 1990a). However, the manure-N and inorganic-N forms had a significant effect on dry matter yields. In the second experiment, treatment (3:7), (5:5), and (7:3) received a compared amount of N as the urea (0:10) parcels. The difference in crop yield between the treatments represents a difference of efficiency between fertilizer-N and manure-N (Tanaka et al. 1987). In plots (5:5), crop production was stable and the coefficient of variability between years was very small. For treatment less than (5:5) crop production showed signs of decrease, in contrast, to plots with higher ratios greater than (5:5). Major conclusions that have been drawn by Chinese researchers from the data are (Tables 5, 6):

(i) the productivity of Xuzhou "yellow fluvo-aquic" soil was reduced by the non-fertilized cropping cycles;

(ii) in comparison with the inorganic-N treated soils, manured plots produced higher yields and maintained soils in a constant state of productivity;

(iii) treatments such as manure plus inorganic-N are necessary to maintain nutrient levels in the Xuzhou soils sufficient to produce satisfactory crop yields

and to sustain high levels of productivity.

After 4 yr of high N rate of applications (672 kg ha^{-1}), total yearly production was significantly higher than the equivalent treatments of 448 N level. Crop production decreased in the subsequent order (7:3) > (5:5) > (3:7) > (0:10) (results not showed)(Zhang et al. 1990).

FERTILIZER EFFICIENCY AND PLANT NUTRIENT CONTENT

Efficiency of applied N, P_2O_5, and K_2O in the first experiment was calculated by subtracting total yields of the appropriate control plot from total yield of N, NP, and NPK applied plots within each 3 yr average divided by the rate of N, P_2O_5, and K_2O applications (Table 7).

(i) N fertilizer

During the 1981-86 period, in comparison with the unfertilized fields of the beginning the N treatment increased crop production by 5346 kg ha^{-1}. However, there was a very significant decrease in crop production marked by a decrease in N fertilizer efficiency within the last term. On average for the 9 yr period, each kg of N provided 14.1 kg of cereals. Each kg of inorganic N produced 13.7 kg of crops. For the first six yr, wheat response to inorganic-N addition was larger than with corn, especially in treatment MN. However, beyond 1984 wheat yields started to decrease significantly in the N plots only.

(ii) P fertilizer

In comparing treatment NP and N, total crop production increased due to P fertilizer application. Differences in the first 3 yr were only about 2%, but increased to 75% by the end of the 9 yr period. On average for the 9 yr period, each kg of P_2O_5 produced 16.1 kg of cereals. Wheat response to chemical P fertilizer was much greater than corn. In the last period (1987-89), one kg of P_2O_5 was produced twice as much wheat than corn, hence 43.5 compared to 19.0 kg, respectively. In

treatment MNP where superphosphate was used in combination with horse manure and N, crop response to chemical P was not significant. For the entire period treatment MNP compared to treatment MN increased production about 1%, hence one kg of P_2O_5 gave approximately one kg of cereals.

Table 7. Effects of N, P, and K chemical fertilizers on average total crop yield in Xuzhou field experiment n° 1 during the 1981-89 period.

	Treatments	Based on	81-83	84-86	87-89	9-year average
	average yields ($t\ ha^{-1}\ yr^{-1}$)	Fertilizers only ¶	12.3	9.8	6.3	9.4
		Fertilizers + Manure	14.4	14.1	13.6	14.0
N	Kg yield/Kg N applied	Fertilizers only	21.4	14.7	6.7	14.1
		Fertilizers + Manure	15.1	12.1	14.0	13.7
	% increase by N	Fertilizers only	108	81	46	81
		Fertilizers + Manure	46	34	44	41
	average yields ($t\ ha^{-1}\ yr^{-1}$)	Fertilizers only §	12.6	12.0	11.0	11.8
		Fertilizers + Manure	14.5	14.3	13.5	14.1
P	Kg yield/Kg P_2O_5 applied	Fertilizers only	2.0	14.7	31.5	16.1
		Fertilizers + Manure	1.2	1.3	0.0	0.7
	% increase by P	Fertilizers only	2	22	75	26
		Fertilizers + Manure	1	1	0	1
	average yields ($t\ ha^{-1}\ yr^{-1}$)	Fertilizers only &	14.0	13.9	12.9	13.6
		Fertilizers + Manure	14.5	15.3	14.0	14.6
K	Kg yield/Kg K_2O applied	Fertilizers only	6.3	8.5	8.5	8.0
		Fertilizers + Manure	0.0	4.5	2.2	2.2
	% increase by K	Fertilizers only	11	16	17	15
		Fertilizers + Manure	0	7	3	3

¶ average yield N treatment - Check treatment = 12.3 - 5.9 = 6.4 $t\ ha^{-1}$
RATIO (6400 kg ha^{-1}) ÷ (298.5 kg N applied ha^{-1}) = 21.4
(6.4 $t\ ha^{-1}$) ÷ (5.9 $t\ ha^{-1}$ Check treatment) x 100 = 108%

§ average yield NP treatment - N treatment = 12.6 - 12.3 = 0.3 $t\ ha^{-1}$
RATIO (300 kg ha^{-1}) ÷ (149.3 kg P2O5 applied ha^{-1}) = 2.0
(0.3 $t\ ha^{-1}$) ÷ (12.3 $t\ ha^{-1}$ N treatment) x 100 = 2%

& average yield NPK treatment - NP treatment = 14.0 - 12.6 = 1.4 $t\ ha^{-1}$
RATIO (1400 kg ha^{-1}) ÷ (223.9 kg K2O applied ha^{-1}) = 6.3
(1.4 $t\ ha^{-1}$) ÷ (12.6 $t\ ha^{-1}$ NP treatment) x 100 = 11%

(iii) K fertilizer

After 1983, differences in yields between the NP and NPK treatments increased and the effectiveness of K fertilizer was agronomically significant. Corn response to KCl applications was higher than wheat. However, on average for the 9 yr period,

the amount of cereals produced was only in a ratio of 1 kg cereal for 8 kg K_2O. In combination with organic manure, crop response to K fertilizer decreased. The average over the 9 yr was only 3%.

(iv) Organic manure

Organic manure increased plant yield in comparison with no treatment, yet the relative effect of its combination with different chemical fertilizers decreased (Table 8). The increase in total yield was 90, 49, 20, and 7%, for M in contrast to the control, MN to N, MNP to NP, and MNPK to NPK plots, respectively.

In treatment M, in which only horse manure was applied, the relative yield was rather low during the first years, especially in winter wheat, due to the expected low efficiency of manure at low temperatures in contrast to chemical fertilizers (Tanaka et al. 1987). However, since 1983 there was an apparent cumulative effect of organic manure as demonstrated by higher yields for all crops. The effect of horse manure on crop production was found to be dependent not only on varieties of crops cultivated but also on properties of the soils on which manure was applied.

Table 8. Effects of horse manure on total crop yield increase in Xuzhou first field experiment during the 1981-89 period.

Treatments	Based on	81-83	84-86	87-89	9-year average
M minus Check	t ha^{-1}	4.0	5.1	5.1	4.7
	%	68	94	119	90
MN minus N	t ha^{-1}	2.1 ¶	4.3	7.3	4.6
	%	17	44	116	49
MNP minus NP	t ha^{-1}	1.9	2.3	2.5	2.3
	%	15	19	23	20
MNPK minus NPK	t ha^{-1}	0.5	1.4	1.1	1.0
	%	4	10	8	7

¶ MN minus N = (MN treatment yield - N treatment yield) = 14.4 - 12.3 = 2.1 t ha^{-1} (2.1 t ha^{-1}) ÷ (12.3 t ha^{-1} N treatment) x 100 = 17% increase due to manure action.

Cumulative effects of manures on crop yields have been indicated by others. Xie and MacKenzie (1986) found from analyzing published data, that treatments receiving manure plus inorganic N had the highest cumulative effect on corn grain yields, inorganic N medium, and manure the lowest effect in the short term. MN, MNP, and MNPK plots received about twice as much kg N ha^{-1} than the inorganic N fertilized parcels. In the manure-inorganic-N treatment the efficiency of crop production decreased with the increase in N application rates. The finding that a twofold increase in N application rates led to about twofold decrease in the efficiency of crop production is in accord with the observation of Xie and MacKenzie (1986). Tanaka et al. (1987) considered that the efficiency of organic-N was only one half that of fertilizer-N. However, crop yield of MN fields was about 14.0 t ha^{-1} yr^{-1} from the beginning and remained constant throughout the 10 cropping cycles. Based on such tendencies it is recommended that chemical N fertilizers be used along with organic manures in the Xuzhou area of "yellow fluvo-aquic" soils (Institute of Soil Science, 1986; Tanaka et al. 1987).

The amount of organic manure used per unit field area in Jiangsu Province could be as large as 200 t ha^{-1} yr^{-1}, but in general it averaged around 80 t ha^{-1} yr^{-1} (Tanaka et al. 1987). Xuzhou manured fields received 74 t ha^{-1} annually during the first four cropping cycles. While this amount was reduced by half in the last five years it still required a tremendous labour effort.

The constitution of organic manure fluctuates considerably (Morel et al. 1984; Tanaka et al. 1987). This poses a problem if Chinese farmers intend to apply a known amount of plant nutrients to a crop, because they cannot always have analytical data. Also, if a farmer intends to apply an adequate amount of N to a crop, too much K may be applied as horse manures are not ideally balanced for crop production in N, P and K (Tanaka et al. 1987). However, such a difficulty can be easily overcome,

by using chemical N fertilizers with horse manure (Jiang et al. 1990a).

N, P, AND K CONTENTS OF WHEAT AND CORN GRAIN

Nutrient contents of wheat and corn grain production are showed in Table 9 and 10 for the first and second experiments, respectively. Amongst grains at harvest, higher percentages of N, P, and K were found in wheat than corn. Differences in mineral constituents of grains amongst treatments were small, although these tended to increase to some extent with an increase in quality and amounts of N, P, and K additions (Jiang et al. 1987; Tanaka et al. 1987; Zhang et al. 1990). From Deherain long-term wheat results, Morel et al. (1984) indicated that differences in mineral constituents due to variations in climatic conditions and wheat cultivars were greater than the effects of fertilization treatments over time. Johnston (1969b) reaffirmed, based on Lawes and Gilbert (1884) published analyses of the wheat grown on Broadbalk, that in good seasons, total uptake of N, P, and K was greater than in bad seasons; the dry matter of both grain and straw contained slightly larger percentages of K and slightly less P.

The results from both experiments showed that :

(i) Nutrient uptake and the resulting grain nutrient ratio of $N-P_2O_5-K_2O$ were in direct relation with crop yields as suggested by different studies.

(ii) Where no fertilization was applied the amount of N removed was lowest. Both crops exhibited almost the same nutrient ratio.

(iii) Total N content of the grain increased significantly with added chemical N fertilizer, yet N mineral fertilization without P induced a lower content of P_2O_5 in the grain, in agreement with Morel et al. (1984) results.

(iv) The effect of a fertilization without K was more pronounced in corn than in wheat. The nutrient

content of the grain of both crops showed that the K content was constant with time in the NPK and M treatment plots only. An exception was the MNPK treatment where N and P varied. These results were not in agreement with Broadbalk and Deherain continuous winter wheat plots where the percentage of K in the grain changed little with different treatments, confirming Lawes and Gilbert's views about the composition of ripe grain (Johnston 1969b; Morel et al. 1984).

(v) Increases in nutrient uptake with organic manure applications were noted by Xie and MacKenzie (1986) who found that pig manure applications enhanced both dry matter yields and N uptake by corn. Farmyard manure significantly increased K translocation in wheat grains from Deherain long-term experiments (Morel et al. 1984).

(vi) With increasing manure-N/inorganic-N ratios the N nutrient uptake decreased, although the removal of P and K increased.

Table 9. Effects of different fertilizers treatments on grain nutrient uptake after six years of experiment.

Treatments	WHEAT			CORN		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O
	--------	%	--------	--------	%	--------
Check	1.87	1.10	1.90	1.72	0.95	1.93
N	2.59	0.77	2.14	2.18	0.61	1.31
NP	2.52	0.95	1.94	2.03	0.74	1.24
NPK	2.36	0.92	2.43	2.12	0.71	1.91
M	2.10	1.44	2.44	1.92	1.02	2.32
MN	2.78	1.05	2.43	2.21	0.80	1.93
MNP	2.75	1.18	2.79	2.34	0.85	1.94
MNPK	2.75	1.15	3.28	2.33	0.84	2.63
Average	2.47	1.07	2.42	2.11	0.82	1.90

Table 10. Effects of various ratios of manure-N-to-inorganic-N on the crop nutrient uptake.

Treatments	WHEAT			CORN		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O
MN	--------	%	--------	--------	%	--------
(0:0)	2.08	0.91	2.20	1.64	0.74	1.75
(0:10)	3.43	0.64	2.60	2.13	0.52	1.57
(3:7)	2.76	0.84	2.10	1.95	0.66	1.53
(5:5)	2.46	0.96	2.28	1.90	0.79	1.68
(7:3)	2.25	1.03	2.34	1.78	0.80	1.81

MN was equalled to 5 different manure-N-to-inorganic-N treatment ratios (0.0), (0 10), (3.7), (5 5) and (7 3).
10 yr average for N and P, but only 6 yr for K.

PERCENTAGE RECOVERY OF N, P, AND K FERTILIZERS

The indirect method was used in Xuzhou long-term field experiments to define the simple overall effect of the different fertilizers in improving yields. The percentage recovery of applied elements within the different chemical and organic fertilizers treatments was calculated by considering the amount absorbed by the total crops in the controls as the natural supply (Johnston 1969a; Tanaka et al. 1987).

(i) Recovery of applied N

Without chemical fertilizers or organic manures, the N was low (Tables 11, 12, 13). In the control plots, the N removal in the soil was equivalent to about 20% of soil N in the period from 1981-89. Phosphorus and K also decreased significantly. Small amounts of P removal in the control plots probably limited growth and restricted N removal (Johnston 1969b)(Table 13). Added inorganic-N plots showed reduced N removal with time, but the manure plots showed increased N with time (Table 13).

Apparent recovery or use efficiency of N in whole crops grown on the different field plots were based on 299 kg N ha^{-1} yr^{-1} added (e.g. (treatment N - control)/(amount of N fertilizer

applied in the soil) x 100) (Johnston 1969a). Use efficiency of applied N was 46, 60, and 70%, for the N, NP and NPK treatments, respectively, for the 9 yr period (Table 11).

Based on the calculated additional N uptake over that on control plot with NPK, the N removed by the crops changed little with time. Crops given N without PK recovered less N by 1987-89, compared to 1981-83, probably because of diminishing available P and K in the soil limited growth (Johnston 1969a) (Table 13). In 1987-89, wheat N uptake showed a significant decrease with only 22% N recovery. In contrast to the Broadbalk continuous winter wheat experiment, Chinese wheat-corn cropping cycles showed no large increase in N removed with treatment NP, hence N and NP plots showed similar results particularly in the first year.

In proportion to the quantity applied, N removed was higher in chemical fertilized plots than in organic manured parcels, and higher at low than at high application rates (Tanaka et al. 1987)(Table 11 and 12). When inorganic N was combined with horse manure, the use efficiency of applied N increased in comparison to treatment N only, but this increase was small. The effect of time on N uptake was not important for MN, MNP, and MNPK treatments because they showed a constant N recovery throughout the 9 yr (Jiang et al. 1990a). The use efficiency of organic N treatments increased with time, particularly in M and MN plots. For example, the percentage N recovery was 26, 40, and 48% in treated M plots, in comparison to 17, 46, and 87% for MN plots in 1981-83, 84-86 and 87-89, respectively. The most apparent long-term effect of organic manure application came from its residual N effect (Jiang et al. 1990a).

In summary, when using a combination of chemical and organic fertilizers, calculated use efficiency of applied N decreased with time. For the 9 yr period, MN, MNP, and MNPK treatments averaged 43, 29, and 23%, respectively. Nitrogen use efficiency increased with manure N.

Table 11. Balance of inputs and removals of plant nutrients, over nine years in Xuzhou.

Nutrients	Input Output Difference	Check	N	NP	NPK	M	MN	MNP	MNPK
	kg ha^{-1} yr^{-1}								
N	input	0	299	299	299	310	608	608	608
	output	95	232	272	302	221	380	382	394
	diff.	-95	+67	+21	-3	+89	+228	+226	+214
P_2O_5	input	0	0	149	149	249	249	398	398
	output	53	63	99	107	145	163	173	180
	diff.	-53	-63	+50	+42	+104	+86	+225	+218
K_2O	input	0	0	0	224	443	443	443	666
	output	102	161	179	294	260	330	340	440
	diff.	-102	-161	-179	-70	+183	+113	+103	+226

Table 12. Balance of inputs and removals of plant nutrients, over ten years in Xuzhou.

Nutrients	Input Output Difference	(0:0)	(0.10)	(3:7)	(5:5)	(7:3)
	kg ha^{-1} yr^{-1}					
N	input	0	448	448	448	448
	output	110	252	333	326	293
	diff.	-110	+196	+115	+122	+155
P_2O_5	input	0	0	107	183	256
	output	54	58	109	132	133
	diff.	-54	-58	-2	+51	+123
K_2O	input	0	0	205	350	492
	output	115	183	240	284	289
	diff.	-115	-183	-35	+66	+203

MN was equalled to 5 different manure-N-to-inorganic-N treatments ratios (0 0), (0:10), (3.7), (5.5) and (7 3).

(ii) Recovery of applied P

Apparent recovery of phosphorus by grain and straw of both crops grown on Xuzhou soils from 149 kg P_2O_5 ha^{-1} added each year as superphosphate was calculated based on ((treatment NP - treatment N)/P_2O_5 added) x 100 (Johnston 1969b). With each 3 yr

period, the recovery of P significantly increased and averaged 8, 29, and 33% for 1981-83, 84-86, 87-89, respectively. The average calculated recovery of P with Xuzhou experiments showed equivalent trends as at Broadbalk (Johnston 1969b). When N was applied the apparent recovery of P increased from 24% without K to 30% with K. Johnston (1969b) suggested that this apparent increased P recovery with time was because extra fertilizer-P was removed while there was also less taken up on treatment plot N with time.

Whith NPK treatments, the percent recovery of both N and P remained much the same during the experiment. Johnston (1969b) emphasized that large residues of inorganic P accumulated but there has been no luxury uptake of P by the crops grown on NPK plots.

In parallel, removal of manure-P increased during the 1981-89 period in the manured parcels. In M treated plots, the P uptake increased from 16 to 40% showing the residual effect of repeated applications of manure. Between treatments M and MNP there was no significant difference in P recovery, while in comparison to NP the combined chemical-manure treatment showed a significantly lower inorganic-P removal. However, the uptake of inorganic P was stable and averaged about 10% in MNP plots throughout the 9 yr, thus demonstrating trends that were similar to N, although absolute values of P were lower than N (Tanaka et al. 1987).

(iii) Recovery of applied K

Apparent recovery of potassium by whole crops on the first experiment (224 kg K_2O ha^{-1} yr^{-1} added) was calculated from ((treatment NPK - treatment NP)/K added) x 100 (Johnston 1969b). The percentage recovery of K applied in NPK fertilized plots was 39, 59, and 51% for each short-term period (Table 13). Potassium recovery was consistently higher than that of N in agreement with Johnston (1969b) and Tanaka et al. (1987) results. The "yellow fluvo-aquic" soils of Xuzhou provided

large amounts of K to the crops (Jiang et al. 1990a). Crops given N recovered about 51% of the fertilizer K, but if these values were corrected for soil K uptake from O K plots, crops recovered 26% per year on the average over the 9 yr period. In contrast to N and P, the relative efficiency of fertilizer-K showed no significant differences among the fertilized treatments. With treatment MNPK, the percentage recovery of K applied was 30, 62, and 58% for 1981-83, 84-86, 87-89, respectively.

While the relative efficiency of manure-K compared to fertilizer-K added was lower, it increased significantly with time. Combined manure plus inorganic-N treatment (MN) also exhibited an important increase in manure-K recovery over the manured plots (M), especially in the last 3 yr period.

BALANCE BETWEEN THE AMOUNTS OF N, P, AND K APPLIED AND REMOVED

Soil capacity to supply plant nutrients is an important indication of its fertility status. The important correlation existing between soil capacity and crop production served as the basis to determine the amount of fertilizers to apply on untreated plots as to increase significantly crop harvest (Jiang et al. 1990a). Based on the crop output, Xuzhou sandy loam on average provided 95 kg N, 53 kg P_2O_5, and 102 kg K_2O per ha, annually. Results from N balance in both Xuzhou experiments showed that there was more N removed from only three treatment plots (control, (0:0), and NPK) than N applied as fertilizer (Table 11, 12 and 13).

When 299-149-224 kg ha^{-1} yr^{-1} of $N-P_2O_5-K_2O$ (NPK treatment) was supplied to soils, N applied and removed was almost in equilibrium, otherwise while a small excess of N was estimated to accumulate in the soil, as showed in N and NP treatments. Plots that received repeated applications of horse manure showed a N surplus that decreased with corresponding increases in crop uptake. Plots treated with MN showed the most

interesting results in relation to the amount of nutrients input and output. The highest increase in N removed was found for treatment MN of 1987-89 period.

After 10 yr, in the second experiment each treatment received a total amount of about 4480 kg N ha^{-1}. The various manure-N-to-inorganic-N treatments received from 1070 to 2561 and 2046 to 4919 kg ha^{-1} of P_2O_5 and K_2O, respectively. For N, the nutrient balance was positive for the three different treatment ratios, but the gain was greater for (7:3) > (5:5) > (3:7) treatments. In agreement with other research, N balance results noted that efficiency of N recovery was less in the plots receiving high rates of manure than those receiving low rates (Xie and MacKenzie 1986; Tanaka et al. 1987). Poor recovery of N from high manure rates was probably caused by denitrification (Meek et al. 1982).

For P balance, similar results were obtained in both Xuzhou long-term experiments. Inorganic-N treatments compared to controls and (3:7) plots showed a greater deficit in P, while all other treatments displayed a gain in P that was largest in MNP and MNPK treatments.

In contrast to N and P, K balance was negative in all inorganic fertilized treatments. The deficit was much lower for wheat and corn receiving NPK than for NP fertilizers. Still, in NPK treated plots, K was removed in greater quantity than it was added, thus inducing a deficit of about 70 kg ha^{-1} per year. An increase in K supplying power of the soil after repeated NPK applications was suggested to taken place on a long-term basis. Jiang et al. (1990a) reported that in NPK plots amounts of N and P applied were sufficient but more than 224 kg K_2O ha^{-1} was required annually for Xuzhou type of soils.

Table 13. Total nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) applied and removed by wheat and corn in each 3 yr period in Xuzhou.

Nutrients	Input Output Difference	Year	Check	N	NP	NPK	M	MN	MNP	MNPK
	kg ha^{-1} yr^{-1}									
		81-83	0	299	299	299	386	684	684	684
N	input	84-86	0	299	299	299	256	555	555	555
		87-89	0	299	299	299	286	585	585	585
		81-83	102	277	273	301	200	340	355	348
	output	84-86	99	241	279	320	202	360	373	404
		87-89	84	179	266	285	261	441	419	431
		81-83	-102	+22	+26	-3	+185	+344	+330	+337
	diff.	84-86	-99	+57	+20	-22	+54	+195	+182	+151
		87-89	-84	+120	+33	14	+26	+144	+166	+154
		81-83	0	0	149	149	215	215	364	364
P_2O_5	input	84-86	0	0	149	149	228	228	378	378
		87-89	0	0	149	149	304	304	453	453
		81-83	53	83	95	103	86	121	133	128
	output	84-86	61	67	110	121	138	139	154	166
		87-89	45	40	90	98	213	229	232	246
		81-83	-53	-83	+55	+47	+129	+94	+231	+236
	diff.	84-86	-61	-67	+40	+28	+90	+89	+224	+212
		87-89	-45	-40	+59	+51	+92	+75	+221	+207
		81-83	0	0	0	224	646	646	646	869
K_2O	input	84-86	0	0	0	224	333	333	333	557
		87-89	0	0	0	224	349	349	349	573
		81-83	112	204	196	283	231	302	348	414
	output	84-86	106	160	183	315	249	311	320	459
		87-89	88	118	158	282	299	376	351	448
		81-83	-112	-204	-196	-60	+415	+343	+297	+455
	diff.	84-86	-106	-160	-183	-91	+84	+22	+13	+98
		87-89	-88	-118	-158	-59	+50	-27	-3	+125

In summary, at first unbalanced applications of chemical N and/or P fertilizers resulted in an increase in the supplying power of the soil, but with time the depletion of essential plant nutrients resulted in crop production decreases.

Inorganic-N treatment induced the largest increase in soil K supplying power compared to the control with 82%. However after 9 yr of experiment this increase had drop to only 34%. This stressed the need to use a balanced fertilization program combined with organic manures for stable crop production, and to halt nutrient exhaustion and soil degradation as demonstrated in N plots over long-term period. In the Chinese wheat and corn system with crop dry matter production ranging from 12 686 to 14 179 kg ha^{-1} per annum, the most suitable manure-N-to-inorganic-N ratio treatment should not be lower than (5:5) (Zhang et al. 1990).

MICROBIAL COUNTS DURING WHEAT GROWTH

Many studies have been performed to elucidate both short and long-term effects of manure and N-fertilizer additions on the numbers of soil microorganisms as measured by the dilution plate counts and microbial biomass (Biederbeck et al. 1984; Rosswall and Paustian 1984; Bolton et al. 1985; Schnürer et al. 1985). During the first wheat growth season (1979-80) the relative effects of N additions as fertilizers or organic manures were compared at four sampling dates to determine differences in measurements of the biological activity between treatments were consistent and to provide extremes if any (Table 14). The soil was sieved (<2 mm) and stored field moist until used for analyses of the numbers of aerobic heterotrophic bacteria, anaerobic bacteria, fungi, and actinomycetes. Numbers of bacteria and actinomycetes were determined by the serial dilution plate count technique using soil extract agar while a different medium was used for fungi (Zhang et al. 1990).

Total counts of viable soil microorganisms and fungi showed differences among the five treatments throughout the year (Table 14). Several factors as described by Ritz and Robinson (1988) could account for the additional stimulation, including soil disturbance during sowing, fertilizer application, the increase in soil-water content and springtime rises in

soil temperature. Disturbance of the soil would disrupt soil aggregates and provide previously inaccessible soil organic matter available for microbial assimilation during sowing. Numbers of actinomycetes were unaffected by N fertilization in agreement with Biederbeck et al. (1984) results. While total microbial and actinomycete counts appeared to increase from tillering to filling and decreased significantly from filling to harvesting times, fungal numbers were about the same on the four sampling dates. Ritz and Robinson (1988) in their study of temporal variations in soil microbial biomass C and N under a spring barley crop found a similar decline from 50-80 d after sowing. However, Ritz and Robinson (1988) results and the Xuzhou experiment results do not follow the pattern of previous studies which suggest that crop growth often stimulates an increase in the size of the biomass (Carter and Rennie 1982). Elevated amounts of biomass would be expected at the harvest period because of maximal crop growth rate and presumably high root development and exudation (Ritz and Robinson 1988). Van der Werf and Verstraete (1987) also found no stimulation of total biomass by growth of a winter wheat crop, but that the active component of the biomass was stimulated considerably.

Soil plate counts from Xuzhou were similar to those of Morrow and Sanborn field plots in USA with increased numbers of bacteria when manure was added (Bolton et al. 1985). The number of aerobic bacteria in the top 7 cm of soil was lowest in the inorganic-N treatment at tillering stage and increased significantly with higher manure-N ratios. Fertilizer application would be assumed to stimulate the microbial population if it were N-limited, but in the first year of the study there was a negative effect of inorganic-N fertilizer upon microbial counts compared to the control plots.

During the growing season, in the three treatments receiving manure the aerobic bacteria population represented between 68.0 to 86.3% of the total microbial population compared to the unfertilized and inorganic-N plots with 58.0 to

73.4%, respectively. In the (5:5) and (7:3) treatments then was a corresponding increase in anaerobic bacteria populations. An exception to the augmentation of bacterial numbers with organic matter content was found in treatment (7:3) which showed slightly lower values than treatment (3:7). The first explanation for this was that the horse manure was digested before addition to the field and was not as easily decomposable and available as manure in the lower manure-N inorganic-N treatment ratio (3:7) (Schnürer et al. 1985). However, the most probable interpretation comes from the reduced crop production of the first year in treatment (7:3) versus (3:7) with 12.4 and 15.2 ton ha^{-1}, respectively (Table 6). Rosswall and Paustian (1984) described the importance of C additions to the systems, through root production, for the enhancement of microbial activity in the soil. The greater microbial numbers obtained in manure-N treatments was expected to be due to the difference in carbonaceous residues returned to the soil. As suggested by Zhang et al. (1990) soil C-to-N ratio has a great importance on soil microbial activity. Treatment (3:7) C:N ratio was lower than (5:5) and (7:3) plots when wheat was sown. This may explain why at the beginning of wheat growth in the fall of 1979, total microbial counts were highest in treatment (3:7). But as soil temperature increased, organic matter slowly decomposed, C:N ratios became adequate for boost microbial activity. Hence, during booting treatment (7:3) showed a significant increase in microbial counts. Reduced C input of roots and litter reduces energy input to soil organisms and together with an increased N input, alters the N cycle to emphasize the mineral N pool and those biological processes affecting it (Juma and McGill 1986). Nevertheless, Rosswall and Paustian (1984) pointed out that it is difficult to distinguish the effects of N additions per se from those of increased crop growth resulting in a larger input of organic matter to the soil system.

Bacterial numbers were not of the same magnitude as in other agricultural soils. Bacteria and actinomycetes counts were 100 to 1 000 times lower in Xuzhou wheat field plots compared to Biederbeck et al. (1984) and Schnürer et al. (1985) reports. For example, in the 16 yr continuous winter wheat field experiment of Swift Current (Saskatchewan) bacteria and actinomycete numbers were in the order of 10^8 and 10^6, respectively, whereas the bacteria-to-actynomycete ratio varied from 3.7-7.8 compared to 24-31 in Xuzhou soils. Biederbeck et al. (1984) insisted that poor N fertility tended to widen the bacteria-to-actinomycete ratio, indicating marked qualitative soil microbial changes in response to long-term differences in substrate availability. Yet, the Canadian soils displayed a 2.5 times higher organic C and total N contents in comparison to the Chinese "yellow fluvo-aquic" soils. Discrepancy between other long-term studies and the Xuzhou studies may come from the data that was obtained after only the first N fertilizer and manure additions (1979-1980).

In many studies on different management and agronomic practices soil dilution plate counts showed virtually no difference. Bolton et al. (1985) attributed these results to the inherent errors present in the soil dilution plate count technique that masks differences among treatments.

Table 14. Effects of different wheat growth stages and manure-N-to-inorganic-N ratios on microbial counts in the 0-7 cm soil depth layer after one season of treatment (1979-80 season).

Organisms	Crop growth	(0:0)	(0:10)	(3 7)	(5.5)	(7:3)
Total	tillering	0.714	0.512	1.354	1.306	1.258
bacteria	stemming	0.761	0.670	2.212	1.637	1.980
x 10^6 g^{-1}	filling	0.978	0.877	2.252	2.621	3.102
	harvesting	0.863	0.846	1.225	1.372	1.796
Total	tillering	2.3	2.8	7.6	5.7	4.7
fungi	stemming	2.2	1.9	7.7	6.1	5.8
x 10^3 g^{-1}	filling	2.2	2.2	2.5	5.5	5.7
	harvesting	3.3	3.5	5.0	4.8	5.6
Total	tillering	23	27	77	48	52
actinomycetes	stemming	37	74	93	81	77
x $10^3 g^{-1}$	filling	91	73	131	131	103
	harvesting	30	24	37	38	90
Total	tillering	0.739	0.542	1.439	1.360	1.315
micro-	stemming	0.800	0.746	2.313	1.724	2.063
organisms	filling	1.071	0.952	2.386	2.758	3.211
x 10^6 g^{-1}	harvesting	0.896	0.874	1.267	1.415	1.891
Aerobic	tillering	70.1	60.7	72.1	74.3	73.3
bacteria	stemming	73.4	68.4	79.3	79.4	84.0
% of total	filling	65.8	73.4	83.3	86.3	84.1
	harvesting	58.0	59.5	68.0	69.0	77.5
Anaerobic	tillering	10.0	11.4	6.5	7.8	8.2
bacteria	stemming	6.4	2.7	4.4	5.6	3.9
% of total	filling	7.1	6.4	4.7	3.7	4.1
	harvesting	12.6	11.0	9.1	9.3	7.2

MN was equalled to 5 different manure-N-to-inorganic-N treatment ratios (0 0), (0 10), (3 7), (5.5) and (7:3).

NUTRIENT CONTENT OF XUZHOU SOILS

Changes in soil organic C, total N, total P, available P, and available K content are presented in Tables 15 and 16. After only three cropping cycles, there were differences among treatments in several soil characteristics. The untreated plots

were lowest in organic C and total N. The plots where high levels of manure and chemical NPK fertilizers had been applied from 1980 to the present, had the highest soil nutrient levels. The other plots were intermediate in fertility. During the years, there were significant trends in soil tests except for available K on all treated fields.

SOIL ORGANIC MATTER AND ORGANIC CARBON

The soils were originally sampled in the 0-20 cm layer and air-dried to see how their organic matter and total nitrogen contents were affected by manuring and chemical fertilizers. The determination of organic matter was by the chromic acid titration method, using 1.17 as a correction factor for C, while total N (mostly organic) was determined with the Kjeldahl digestion method. C:N ratios were derived from the OM and total N data, i.e., (OM/1.724)/total N.

The initial C level in Xuzhou soil was 0.626% and decreased to 0.522% (17% reduction) in the untreated plots after 9 yr of experiment (Table 15). In parallel to other studies on continuous corn and continuous wheat rotations in North America where no soil treatment was applied, there was lower organic C when compared with the plots fertilized with N (Biederbeck et al. 1984; Odell et al. 1984). This perhaps was due to the low crop production where N had been withheld (Biederbeck et al. 1984). Organic C first declined rapidly and then continued downward at a slower rate. As suggested by Dalal and Mayer (1986) stubble retention reduces the rate of net OM loss by increasing inputs of organic materials through crop residues, affecting substrate accessibility and composition such as the C-to-N ratio. The C:N ratios tended to narrow between 1981 and 1989 because total N contents changed very little during this period, while there were marked decreases in C levels.

Addition of inorganic N and P fertilizers could not halt the depleting effect of the wheat-corn cropping system on the organic C concentration. Since no C had been added except for the stubble left on the soil after each growing season, on average 560 to 1 000 μg C g^{-1} dry wt had been lost from the system. Where NPK was applied, the organic matter increased but not significantly. Like Broadbalk long-term fields the Xuzhou plots receiving inorganic fertilizers accumulated a little more soil organic matter than the unmanured plots (Jenkinson and Rayner 1977). Hence, application of inorganic fertilizers reduced organic matter losses by increasing grain and straw production and presumably returning a greater amount of crop residues, especially roots (Juma and McGill 1986; Jenkinson 1988).

Jenkinson (1988) observed from field experiments strong indirect evidence that in the long run, the addition of inorganic nitrogen had little effect on the retention of carbon in soil. In this manner, the arable soil that received fertilizers for 130 yr in Rothamsted (England) contained slightly more organic matter than the soil without fertilizers additions. The C:N ratio of the fertilized soil was, if anything, slightly greater than that of the unfertilized soil. Thus there was neither accumulation of carbon-rich organic matter in the unfertilized soil, nor did fertilizer cause a decline in the amount of organic matter in the soil (Jenkinson, 1988). In comparison, large quantities of inorganic N received over the years by the Xuzhou soil in treatment N have had depleting effects on soil organic matter and nutrients except for total N, along with a drop in crop yields.

The long-term effects of winter wheat and corn double-cropping system on the amount of organic matter in soil has not often been reviewed in the literature. Organic matter contents increased for treatments that received long-term additions of manure. Similar data are available for different systems of manuring throughout the world, for example in Morrow corn

fields, Broadbalk, Deherain and Swift Current continuous wheat plots. Manure is a source of plant nutrients and improves primary productivity. Application of manure reduces the loss of organic matter compared to unamended controls. The rate of addition of organic manure affects the rate of organic matter depletion or accumulation (Meek et al. 1982; Juma and McGill 1986).

The amount of OM and total N applied to the land during the studied period varied with the following aspects:

(1) OM and total N content of the manure, (2) moisture content of the manure, and (3) amounts of manure applied among years due to annual variability in the spreading properties of manure (Sommerfeldt et al. 1988). Nearly 485 t ha^{-1} of OM (wet wt.) and 2.8 t ha^{-1} of total N were applied in Xuzhou main experiment, while to the different MN ratios experiment were added 776 to 1 164 and 4.48 to 6.72 t ha^{-1} of OM and total N, respectively, during 1980-89.

Soil C levels of the manured fields increased by 50% although treatment (7:3) plots were almost double that found in the check. Recently, in last 3 yr the rate of organic matter accumulation by the manured plots has been lower, likely because the amount of horse manure applied each season decreased by half since 1985.

Based on the short-term results the following regression equations were obtained for treatment M and MN of the first fields:

YM = 1.301 + 0.023X, r=0.729**; and,

YMN = 1.289 + 0.033X, r=0.853** (significant at the P <0.05 level). At present manure rates, organic matter content increased by 0.02%, annually.

The rate at which changes in organic C and C:N ratios are going in the subhumid region of Xuzhou appeared to be faster than in temperate climate zone. For example in Rothamsted field plots, the topsoil organic C content more than doubled, while

the C:N ratio increased only slightly, from 9.0 to 10.5 after 80 yr of management (Jenkinson 1988). In contrast, the Chinese long-term experiment exhibited a highly significant increase in organic C but a small reduction in C:N ratios after only 10 yr. The original soil had a higher C:N ratio than the various treated soils except for (5:5) and (7:3) plots. Hence, annual manure applications increased soil organic matter and nitrogen, and decreased C:N ratios (Tanaka et al. 1987; Sommerfeldt et al. 1988).

Table 15. Comparisons among organic C, total N, total P, and available P and K levels in Xuzhou experiment n°1 over different time periods.

Treat-ments	Org C	Total N	C:N	Total P	Available P	Available K
	--%--	--%--		--%--	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$
Check						
81-83	0.585	0.070	8.4	0.076	10.2	51
84-86	0.569	0.070	8.1	0.072	7.3	52
87-89	0.522	0.070	7.5	0.075	4.2	52
N						
81-83	0.679	0.076	8.9	0.074	6.8	50
84-86	0.644	0.078	8.3	0.072	3.3	50
87-89	0.579	0.080	7.2	0.074	3.2	49
NP						
81-83	0.613	0.070	8.8	0.077	14.4	52
84-86	0.602	0.073	8.3	0.079	16.6	47
87-89	0.570	0.073	7.8	0.090	13.0	47
NPK						
81-83	0.669	0.076	8.8	0.078	12.7	55
84-86	0.625	0.078	8.0	0.079	12.0	63
87-89	0.639	0.080	8.0	0.088	9.7	64
M						
81-[illegible]	0.773	0.088	8.8	0.085	33.8	71
84-[illegible]	0.968	0.103	9.4	0.086	46.0	78
87-[illegible]	0.923	0.114	8.1	0.091	52.8	72
MN						
81-83	0.756	0.087	8.7	0.079	28.5	66
84-86	0.977	0.112	8.7	0.082	37.6	68
87-89	0.996	0.121	8.2	0.090	42.4	63
MNP						
81-83	0.825	0.091	9.1	0.090	44.3	73
84-86	1.009	0.114	8.9	0.097	70.7	69
87-89	1.037	0.123	8.4	0.110	79.5	63
MNPK						
81-83	0.807	0.090	9.0	0.086	42.1	87
84-86	0.979	0.114	8.6	0.097	73.6	107
87-89	1.031	0.125	8.3	0.112	81.2	101

Table 16. Effects of various ratios of organic and inorganic nitrogen fertilizers on soil nutrient content (0-20 cm depth) after 17 seasons.

Treatments	Org C	Total N	C.N	Org P 1)	Available P	Available K
MN	--%--	--%--		$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$
Initial	0.690	0.078	8.8	-	12.0	62
(0:0) 2)	0.626	0.074	8.5	112	6.0	65
(0 10)	0.675	0.085	7.9	100	1.5	60
(3·7)	0.882	0.107	8.2	124	4.8	66
(5:5)	1.050	0.108	9.7	132	13.9	69
(7.3)	1.182	0.121	9.8	180	21.2	76

1) Organic P was measured after 11 seasons.
2) MN was equalled to 5 different manure-N-to-inorganic-N treatment ratios (0:0), (0 10), (3 7), (5 5) and (7.3).

(i) Dynamics of soil organic matter in the MN ratio experiment

The influence of manure-N applications rates on Xuzhou soil organic matter content were evident in the order of 672 less than 448 kg N per ha annually. In both levels, the effects of repeated annual additions on organic matter accumulation followed the sequence of (7:3) > (5:5) > (3:7) (Table 16) (Zhang et al. 1990). In an analogy to the Lethbridge long-term manured experiment (Canada), the amounts of organic matter increase approached linearity at lower levels of manure application (range of 30 to 180 t ha^{-1}), but as the levels of manure input increased the amounts accumulated became curvilinear with time (Sommerfeldt et al. 1988). The following curvilinear regression equations were obtained with higher manure-N treatments in Xuzhou:

448 N level (7:3)Y = 1.2377 $X^{0.24801}$, r=0.981 **;

672 N level (5:5)Y = X / (2.8577 + 0.6048 X) + 0.99, r=0.990**;

672 N level (7:3)Y = X / (3.2690 + 0.6370 X) + 0.99, r=0.844** (significant at the P <0.05 level).

The findings of Zhang et al. (1990) were in harmony with Sommerfeldt et al. (1988) results showing that organic matter increased with increasing number of years and levels of manure applications, yet an infinite amount of time will be required for maximum levels of organic matter to accumulate. Tracer studies have shown that organic materials in soil form a continuum ranging from labile to recalcitrant C that have different decomposition rates. During the initial decomposition phase of added residues, biological activity, availability of nutrients such as N, and prevailing climatic conditions controlled both the decomposition and the internal cycling of C and N for a wide variety of soils (Juma and McGill 1986). The resulting microbial materials and their metabolites, while only a small portion of the added C, undergoe stabilization in soil. Additions of manure increased turnover rate and steady-state levels, but additions of fertilizer did not (Juma and McGill 1986).

The rate of manure decomposition was greatest in the first year and decreased with time to where it approached zero in the Sommerfeldt et al.(1988) study. According to Zhang et al. (1990) after organic matter content reached 1.4-1.5% the rate of organic matter accumulation decreased with rising levels of manure applications, so that with time the rate of increase in the (7:3) plots should approach that of the (3:7) plots.

(ii) Estimated changes in organic matter concentration

Juma and McGill (1986) have evaluated trends in soil organic matter content with time by using the data on soil total N contents of various research plots existing around the world. They have estimated the annual turnover rate for soil organic matter to vary between 1 and 10%. Chinese agronomists (Zhang et al. 1990) have estimated the amount of organic matter accumulation with continued annual applications of manure plus stubble and plant roots left in the field according to the following mathematic model: $C_{t+1} = C_0 + C_0 \sum^{n}_{t=1} e^{-rt}$ where

C_0 is the amount of humus in the soil initially;

C_{t+1} is C_t the passed year accumulated humus in the soil after one year;

t is the number of years under study; and

r=0.246* is the rate of decomposition of stable organic matter to rapidly available OM (based on Wang Weimin in Zhang et al. 1990).

To maintain soil organic matter balance, the resulting estimated amount of fresh OM (m) ha^{-1} yr^{-1} must be applied to the field: $m = (P \times 22.4 \times 10^5 \times b)/(a - (M + a) \times 0.7)$ where

P is the soil organic matter % content;

22.4×10^5 is the amount of dry soil in 0-20 cm;

b is the annual soil OM mineralization rate = 5.0%;

M is the amount of organic manure added annually to the soil kg dry wt ha^{-1};

a is the amount of stubble-root crop residues left annually in the soil. According to Wang Weimin et al. (1989) research, in low wheat-corn production plots the average grain-to-straw ratio was 1:0.6. So it becomes the amount of crop production ha^{-1} yr^{-1} x 0.6;

0.3 is the average humification coefficient of organic residues in the field; and

0.7 is the coefficient of organic manure and fresh OM based on the reutilization of the roots.

The next equation come from the comparison between a MN treatment and the calculated value for the check plots (1978-80 results): $C = (aM / b) - [(aM / b) - C_0]e^{-bt}$ with unit as above.

Giving the initial soil organic matter content of 1.19% in the 0-20 cm depth, the amount of fresh organic matter that will be mineralized is equalled to: $m = (0.119 \times 22.4 \times 10^5 \times 0.05) / 0.3 = 44\ 400$ kg ha^{-1} for ten years. Since during the 10 yr period, all organic manure added (+ stubble and roots) converted in fresh OM was less than 4 440 kg ha^{-1} yr^{-1}, soil OM steady-state was not reached, OM accumulated over 4 440 kg ha^{-1}. The predicted values were quite similar with the measured

organic matter contents (Table 16). The model shows that the rate of accumulation decreases exponentially with time to where annual increase in OM becomes small after a few decades of yearly manure applications (Sommerfeldt et al. 1988). For example, at the fixed rate of 100 units of fresh OM applied annually, in 3, 6, 8, 10 and 20 yr, the model predicts that 71.8, 106.2, 118.4, 125.9 and 136.5 units of humus will be accumulated, respectively. An infinite amount of time will be required to reach maximum OM accumulation.

At the Rothamsted Experiment Station, manure was found to have residual effects for as much as 40 yr after the last application (Jenkinson 1988). A large accumulation of OM in the soil is an indication of a fertile land with a reserve of plant nutrients, including total N, to be released slowly for many years (Sommerfeldt et al. 1988). The concern in temperate region is that the amounts of OM released will exceed the growing crops capacity to utilize it as it become available. Should there be excessive amounts of total N released, mineralized, and then nitrified into the NO_3-N form, there could be potential pollution problems, depending on the NO_3-N concentration and its movement (Sommerfeldt et al. 1988). This aspect was not of great concern to Chinese soil scientists. Management practices that return residues to soil on a continual basis reduce the rate of loss of soil organic matter. Yet, the full effect of the change in management may not be known until the practice has been in effect for several years (Juma and McGill 1986).

TOTAL SOIL N

The original organic N level (0-20 cm depth) in 1980 was about 0.066% (Kjeldahl N) (not showed in Table 15). This level was lower than that observed in long-term field experiments throughout the world (Broadbalk, Morrow, Swift Current, and Deherain locations showed 0.11, 0.133, 0.18, and 0.204%, respectively). Where no soil treatment was applied in Deherain

and Morrow experiments, soil N first declined rapidly and then continued downward at a slower rate (Morel et al. 1984; Odell and al. 1984). In untreated soils of both Broadbalk and Xuzhou field trials, N content remained constant (Johnston 1969b).

In all Xuzhou fertilized treatments there was a positive increase in soil N content by the end of each 3 yr period. In 1989, the total soil N content was in the order of MNPK = MNP = MN > M > NPK = N > NP > check (Table 15). The effects of applying urea were remarkably consistent. After only nine years of study, total N contents of both N and NPK plots were 0.080% and 0.010% higher than the controls. The regression equation for the inorganic-N treatment is YN = 0.071 + 0.0009X, r=0.850** (Jiang et al. 1990a). This general increase in organic N contents was not correlated with an increase in organic C contents and reflected a decrease in C:N ratios throughout the years.

Total soil N in wheat-corn plots receiving seasonal N and P applications remained static but somewhat low, in comparison to Broadbalk continuous wheat plots (England) where NP and NPK treatments showed higher total N contents than the N treated plots. Johnston (1969b) suggested that the increase in soil N content came from the N in the larger plant residues returned each year to the soil rather than inorganic-N accumulations. The trend was different in Xuzhou N plots because even during the last years, smaller crop productions still increased the total N content.

Since the treatments started there has been an obvious gain in soil N over the check plots due to applying horse manure. Total N content in the manured treatments increased over the range of 0.048 to 0.059%, a relative augmentation of 73 to 89% compared to the original soil. The following regression equations were obtained for treatment M and MN, respectively. YM = 0.075 + 0.003X, r=0.938**; YMN = 0.078 + 0.0035X, r=0.977** (Jiang et al. 1990a). Hence, the quantities of organic matter supplied at the prevailing rate of 37.3 t ha^{-1}

of horse manure yearly, were higher than the amounts of soil organic matter decomposed since total N increased 0.002% annually (Zhang et al. 1990). Odell et al. (1984) suggested that higher soil N levels are associated with a greater return of plant residues to the field produced by stimulated vegetative growth in the manured treatments, but also from the manure itself that provides organic N and C. Fertilizer N or organic N applied undergo cycling in soil such that 30 to 50% of the N is immediately available to the crop while 30 to 40% is converted to microbial biomass and its products (Juma and McGill 1986). The N from the biomass and labile organic pools are slowly released over several years.

The manured plot response in total N was similar to that of organic matter in the topsoil surface, differing only in magnitude, like the Lethbridge long-term manured fields initiated in 1973 (Sommerfeldt et al. 1988). Organic N increased more than C, which resulted in a significant narrowing of the C:N ratio with greater N fertilization throughout the years. Rice et al. (1986) indicated that to estimate accurately the organic N accumulation or depletion in the 0-20 cm zone, requires total soil N changes over time and bulk density data. Since Xuzhou initial bulk density results are not available these changes could not be calculated.

NITROGEN STATUS IN THE MANURE-N AND INORGANIC-N EXPERIMENT

Horse manure-N and inorganic-N have been applied annually since 1979, at two designated levels of nitrogen 448 and 672 kg per ha, respectively, in the MN experiment while the main experiment plots received 608 kg N ha^{-1}. Soils from plots receiving the most manure plus inorganic-N were highest in total N. Those receiving the least manure plus inorganic-N were lowest in total N, which reflects differences in residual N from the previous manure applications. Soil C:N ratios were increased by the repeated applications of (5:5) and (7:3)

plots. In contrast, soil C:N ratios of inorganic-N, (3:7) and untreated plots decreased from 1980 to 1987 (Table 16).

Chinese mathematic model was compared with the measured changes in total N content displayed by N accumulation and/or depletion in these soils. Zhang et al. (1990) equations were based on the following hypothesis: given an annual use of manure-N named X1, inorganic-N named X2, the calculated amount of manure-N-to-inorganic-N accumulated is an independent variable, while the amount of N uptake and total soil N in the 0-20 cm layer is a dependent variable Y.

Y (uptake) = a + b1X1 + b2X2;

Y (accumulated) = a1' + b1'X1 + b2'X2; and b1(b1'), b2(b2') an intermediary model to predict the rate of manure-to-inorganic-N crop uptake (or soil N accumulation).

(i) Rate of nitrogen uptake in crops

During the 1980-87 period, the rates of manure-N uptake varied between 10.2 and 49.1%, with a mean average of 29.7%, but manure-N applications showed a positive correlation with time (r=0.966**). In contrast, N uptake rates decreased from 53.8 to 41.9%, with a mean of 47.9% in the urea fertilized plots. Hence, inorganic-N application demonstrated a negative correlation with time (r=-0.783*).

(ii) Rate of nitrogen accumulation

With manure the increase in N accumulation at Broadbalk increased according to the amount added and time (Johnston 1969b). In Xuzhou, the increase in the inorganic-N fertilized plots was only one-sixth that of (7:3) soils. In the manure-N treatment plots rates of accumulation range from 40.2 to 54.9%, and averaged 47.5%. While in the inorganic-N parcels rates were much lower 5.1 to 11.9%, with a mean average of 8.5%. All treated plots showed no significant decrease in N accumulation over the 1980-87 period.

(iii) Rate of nitrogen lost

The rates of N lost in the manure-N treated plots diminished from 41.5 to 7.0%, with a mean average of 24.2% during the 7 yr period, consequently showing an inverse correlation with time ($r=-0.881^*$). In comparison, in the inorganic N plots, rates of N lost increased from 34.2 to 51.5% and averaged about 42.9%, thus exhibiting a positive correlation with time ($r=0.765^*$).

(iv) Rate of changes in total soil N content

Where manure and urea were applied together total soil N content presented a positive correlation with time, and the r values increased with increasing manure-N ratios.
448 N (3:7) $r=0.842^{**}$, (5:5) $r=0.922^{**}$, (7:3) $r=0.943^{**}$;
672 N (3:7) $r=0.834^{**}$, (5:5) $r=0.951^{**}$, (7:3) $r=0.914^{**}$, (significant at the $P < 0.05$ level). In general, higher N rate treatments showed a more rapid increase with time in soil N than the lower N rate plots. In contrast, total soil N was slightly higher in the urea (0:10) fertilized soils, while the untreated control plots decreased 5% with regard to the original soil. Consequently, soil N balance was maintained in Xuzhou fields after 17 seasons of intense double-cropping (Zhang et al. 1990).

SOIL HUMUS CONTENT AND PHYSICAL BEHAVIOUR

To study further soil organic matter classical fractionation techniques were used to evaluate fulvic acids, humic acids, and humin (Table 17 and 18). These three humic fractions are structurally similar but differ in molecular weight, chemical analysis and functional group content (Juma and McGill 1986). The great stability of soil organic matter is due to the physical and chemical structure of these humic molecules (Jenkinson 1988).

Table 17. Effects of various fertilizers treatments on soil organic matter humic fractions and physical properties in 1986.

Treatments	Total C	Humic acid (HA)	Fluvic acid (FA)	Humin	HA/FA	Aggregate 0.25-5 mm	Bulk density	Pore space
	--%--					--%--	g cm^{-3}	--%--
Check	0.570	0.097§ 17% ¶	0.064 11%	0.409 72%	1.52	6.5	1.28	54
N	0.646	0.097 15%	0.120 19%	0.428 66%	0.81	6.2	1.32	53
NPK	-	-	-	-	-	7.9	1.13	57
M	0.983	0.073 7%	0.248 25%	0.662 67%	0.30	8.4	1.14	59
MN	1.034	0.178 17%	0.160 16%	0.697 67%	1.11	6.9	1.23	54
MNPK	-	-	-	-	-	9.4	-	-

§ % Total C = HA + FA + Humin, ¶ Percent content of total C

Table 18. Effects of different manure-N/inorganic-N fertilizers applications on the chemical and physical characteristics of organic matter in 1989.

Treatments	Humic acid (HA)	Fluvic acid (FA)	HA/FA	Paddy soil Aggregate¶	Pore space
MN				0.25 mm %	--%--
(0:0)	-	-	-	62.6	52.0
(0:10)	0.070	0.190	0.37	56.1	52.6
(3:7)	-	-	-	63.0	56.3
(5:5)	0.095	0.213	0.45	68.1	56.9
(7:3)	0.121	0.237	0.51	-	-

¶ Total content of cluster type paddy soil.
MN was equalled to 5 different manure-N-to-inorganic-N treatment ratios (0:0), (0:10), (3:7), (5:5) and (7 3).

Fertilizers treatments give different organic C contents thus varying organic matter amounts. The effects of different organic matter contents on Xuzhou soil structure were examined. Similar inorganic N treatments behaved differently. While the HA:FA ratio of (0:10) treatment plots (Table 18) was comparable to the manured parcels ratio of the first experiment (Table 17), treatment N soils showed a 2.5 times higher percent enrichment ratio. The discrepancy between similar treatments (N

and 0:10) may come from the different rates of N applied in both experiments and the three year interval between analyses. It is important to note that the unfertilized plots showed the highest HA:FA ratio and humin percent content. With repeated organic manure applications, organic C content increased significantly, fluvic acid content was much higher than in other treatments, therefore Jiang et al. (1990a) assumed that the humification process was slower.

In general, waterstable aggregate were low in Xuzhou soils. Plots that received only manure compared to NPK and control showed significant differences. The amounts of waterstable aggregates (>0.2 mm) increased with increasing organic C contents. Under the urea treatment, organic C content slightly increased, probably due to increased crop root production but the amount of waterstable aggregates was lower than in the check plots.

The formation of waterstable aggregates is not only important for good soil physical conditions but also for proper plant growth conditions - nutrient assimilation and root extension (Morel et al. 1984). Zhang et al. (1990) observed that under the present field conditions, long-term inorganic N applications can have a destructive effect on soil structure revealed by the increase in bulk density and the reduction of pore space content. Bulk density must be determined when changes in mass of organic matter over time are calculated. Bulk density increases as organic C content decreases, and conversely (Juma and McGill 1986).

In the combined manure-urea treatment, results of organic C, bulk density and percent enrichment ratio of humic-to-fluvic acids were higher than for the separate inorganic N and manure treatments. High humic acid content had a positive effect on soil structure and by consequence improved soil properties (Zhang et al. 1990). When applying a combination of inorganic-N fertilizer plus organic manure on a long-term basis, the results were significant not only at increasing soil nutrient

content but also at improving soil organic matter.

Control and inorganic N plots showed lower significant contents of big pores (>0.2 mm) and available pores (0.2-0.005 mm) in the 2-7 cm soil depth than other treatments. This condition was further accentuated in the 10-15 cm soil depth, where both treatments had a total pore content less than 50%, and big pore contents of 8.7 and 8.9%, lower the 10% critical value for good soil structure. Values of 12.8 and 18.8% were found for treatments (3:7) and (5:5), respectively (Zhang et al. 1990).

Soil particles in the size range of 2.5-500 mm diameter, could hold more water in the high ratio manure plots. For example, treatment (5:5) plots could retain more water by 54 and 42% compare to the unfertilized and inorganic N soils, respectively. Similarly, soil bulk density on average increased or decreased in relation to the manure-N-to-inorganic-N ratios. Urea treated parcels showed a 18.2% higher bulk density values than the balance fertilized (5:5) plots, compared to 16.4% for the checks in the 10-15 cm soil depth. Altogether treatment (7:3) was better than (5:5) because the increase in manure-N-to-inorganic-N ratio had a significant effect on soil structure, pore space content, etc.

CONCLUSIONS

The main conclusions based on ten years of intensive double-cropping production are that the balance combination of inorganic fertilizers with horse manure in Xuzhou treated plots resulted in higher uniform crop output, on one hand, while on the other hand, exclusive N fertilized soils showed decreasing crop yields just as soil P and K deficiencies are taken place after the third year harvest. Horse manure played an important role in maintaining plant nutrients, while increasing soil organic matter content and improving soil physical properties.

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