PRELIMINARY STUDIES ON NITRIFICATION UNDER COCONUT-PASTURE ASSOCIATION

BY
NADARAJA SELVARAJAH

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ABSTRACT

The number of nitrifiers (NH_4 - oxidizers and NO_2 -oxidizers), total heterotrophs, amount of ammonium nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) were determined in a Red Yellow Podzolic soil with gravels (pH 4.2), under a coconut-pasture association to understand the distribution of inorganic nitrogen in coconut-growing soils and to study the effect of pasture on nitrification.

It has been found that the content of NH_4-N was higher than NO_3-N in all treatments. Under pasture, NH_4-N was higher and NO_3-N was lower than under no cover. Although nitrifying population and amounts of NH_4-N were abundant under pasture, less amount of NO_3-N was detected probably due to the favourable effects of rhizosphere on NO_3-N losses. The dominancy of NH_4-N over NO_3-N in the soil profile may be due to leaching-reduction-denitrification losses of NO_3-N, NO_2-N losses due to some chemical reactions and the higher number of total heterotrophs (about 10,000 fold). Both NH_4-N and NO_3-N were higher in the surface soil (0-15 cm) than sub soil (15-30 cm) due to higher availability of substrate and aeration.

1. INTRODUCTION

Soil nitrogen is considered to be the most prominent essential nutrient for crop growth. Nitrogen occupies a unique position among the major nutrients because plants require nitrogen in relatively large amounts whereas it occurs only in trace amounts in soil parent materials.

Manuring coconut, particularly with nitrogen fertilizers has been a very expensive operation in coconut plantations. Although nitrogen fertilizers show high N x P and N x K interactions, the response due to nitrogen alone is negligible after the first few years of nitrogen fertilizer application (18). Unlike potassium phosphorus and magnesium, nitrogen availability and transformations in coconut soils have not received sufficient attention.

Coconut is generally planted at distances of 26' x 26' (7.9 m x 7.9 m) on a square and hence, there is ample space between the palms for the cultivation of other crops. Pasture is commonly grown in this space for the support of livestock. Although there is evidence to show that growing pasture improves coconut production the reasons for this are not clear.

Early work carried out on nitrogen transformations under coconut-pasture associations had led to conflicting results. Salgado and Nethsinghe (102) studied nitrogen transformation in coconut growing soils under a temporary weed cover (biennially ploughed), and reported that the content of ammonium-nitrogen (NH_4-N) was very much higher (over 5% of total nitrogen) than nitrate nitrogen (NO_3-N). They also reported that the content of NH_4-N was higher in the illuvial horizon than the surface. In another experiment they were not able to detect any NO_3-N in soils under a grazed pasture cover whereas NH_4-N was detected in large amounts (15 to 20 ppm) (19). Ferdinandez, however, reported that there were higher amounts of NO_3-N than NH_4-N in soils under pasture (51).

Although number of explanations for this uneven distribution of soil nitrogen have been suggested in the literature (20, 21, 23, 24) no conclusive evidence is yet available. One of the explanations given for higher NH_4-N content under pasture is that nitrifiers are inhibited by toxic substances secreted in the rhizosphere of grasses (119). Present study has been designed to investigate the distribution of NH_4-N and NO_3-N contents in a soil at two depths under a coconut-pasture association. The microbes responsible for nitrification were counted in order to explain the distribution of NH_4-N and NO_3-N in the soil.

2. LITERATURE REVIEW

2.1. The Nitrogen Cycle

Nitrogen is the key building block of the protein molecule in which, all life is based and it is thus an indispensable component of the protoplasm of all plants, animals, and micro-organisms. Although nitrogen is 79% of the earth's atmosphere, it cannot be directly utilized by majority of living organisms. Nitrogen is an inert gas and only a few micro-organisms have the ability to utilize it by converting the element to a combined form. By combined nitrogen it is meant the nitrogen incorporated in a chemical compound that can be utilized by plants

and animals. Thus availability of combined nitrogen limits supply of food for man and animals than any other plant nutrient.

Nitrogen undergoes a number of transformations, which involve organic and inorganic compounds, some of which are volatile. These transformations occur simultaneously and the reactions involved may be viewed in the form of a cycle, in which nitrogen is transformed as a result of microbial activity. A diagrammatic representation of the biological nitrogen cycle is given in Figure.1. As the present study is largely concerned with nitrification in coconut soils, it is hoped to review the literature only on this process.

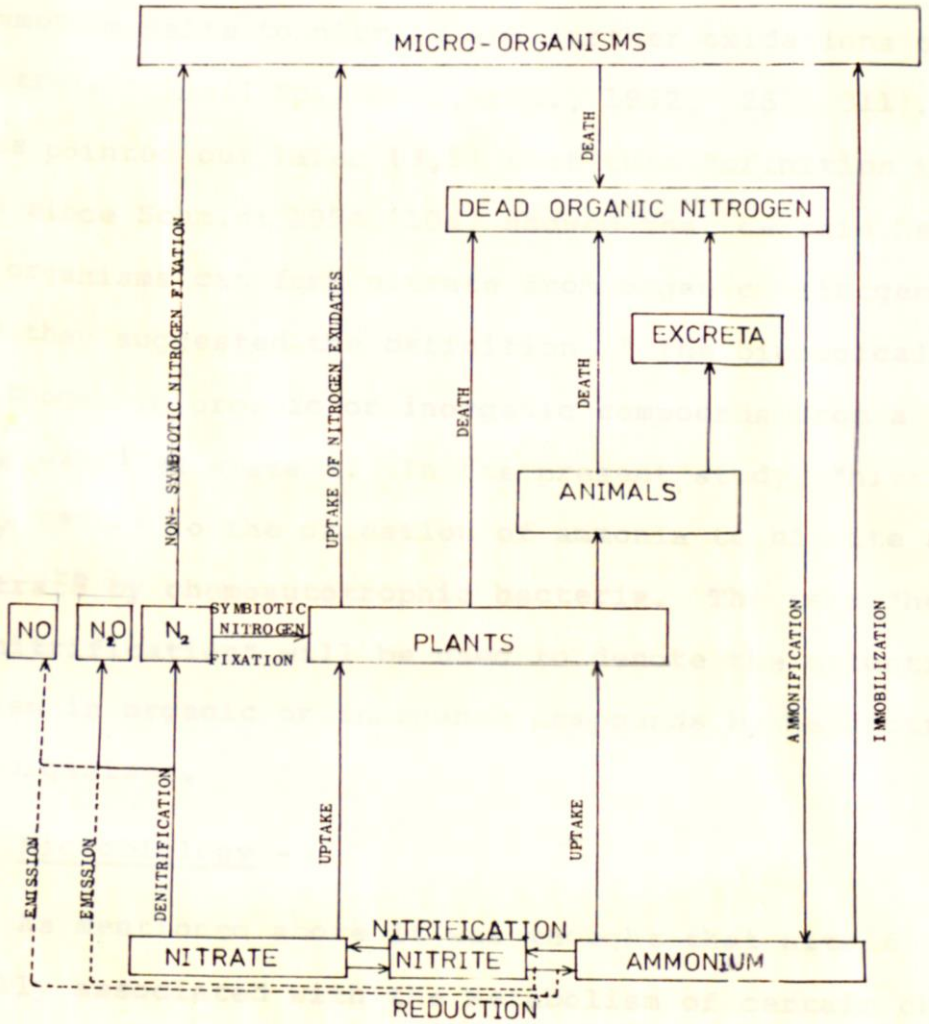
2.2. Nitrification

Nitrification is defined as "the biological oxidation of ammonium salts to nitrite and further oxidations of nitrite to nitrate" (Soil Sci. Amer. Proc., 1962, 26 - 311). However, it was pointed out later (3, 5) that this definition is inadequate since Schmidt 1954 (104) showed that certain heterotrophic organisms can form nitrate from organic nitrogen compounds. Hence they suggested the definition, "the biological conversion of nitrogen in organic or inorganic compounds from a reduced to a more oxidized state ". In the present study, "nitrification" simply refers to the oxidation of ammonium to nitrite and then to nitrate by chemoautotrophic bacteria. The term "heterotrophic nitrification" will be used to denote the oxidation of nitrogen in organic or inorganic compounds by heterotrophic micro-organisms.

2.2.1. Microbiology

As mentioned above it is thought that nitrification is typically associated with the metabolism of certain chemoautotrophic bacteria. Winogradsky (cited by Walker 1975 (133)) identified two groups of these bacteria, one deriving its energy for cell synthesis by the oxidation of ammonium (*Nitrosomonas*) and the other by the oxidation of nitrite (*Nitrobacter*). Since then several other genera capable of either converting ammonium to nitrite or nitrate have been isolated. To date, four genera of ammonium oxidizing bacteria, *Nitrosomonas, Nitrosospira, Nitrosoccus* and *Nitrosolobus,* and three genera of nitrite oxidizing bacteria, *Nitrobacter, Nitrospina* and *Nitrococcus* (135).

These bacteria are classified under the family *Nitrobacteraceae* and order *Pseudomonadales*. Out of these species *Nitrosomonas* and *Nitrobacter* are considered to be frequent in soils and are the major nitrifying chemoautotrophs (6), probably because of their ability to tolerate more difficult environmental conditions than the other genera (52). However, it has been recently found that *Nitrosomonas* species are common in soils receiving organic manure and the common organisms oxidizing ammonium in fields receiving mineral fertilizers are *Nitrosolobus, Nitroso-cystis* and *Nitrosospira* (31, 115). The ammonium oxidizers and nitrite oxidizers may be considered fairly similar since they compete for a highly specialized niche (108, 13). In most soils ammonium oxidizers and nitrite oxidizers are found together and their numbers are some thousands per gram of soil (11, 133). The population of ammonium oxidizers to nitrite oxidizers in soils varies markedly (26) depending on the environmental conditions such as pH (86) but it is usually known that the number of *Nitrasomonas* id slightly greater than that of *Nitrobacter*.



THE BIOLOGICAL NITROGEN CYCLE

Figure - 1

Growth of nitrifiers is a slow process as compared to that of most heterotrophic bacteria. Although the generation times are as short as 8 hours for the growth of *Nitrosomonas* (16) and *Nitrobacter* (86) in pure culture are known, this time in nature are larger; between 20 - 40 hours for both organisms (38, 75). The amount of nitrogen oxidized and carbon-dioxide fixed per unit biomass formed can be narrowly determined. *Nitrosomonas* and *Nitrobacter* have been found to oxidize about 35 and 100 atoms of nitrogen respectively for fixation of a molecule of (31). Cell yields calculated from the relationship of CO_2 fixation or empirically

determined from bacterial counts and substrate oxidation have given similar results. About 1 to 4 x 10^4 cells of *Nitrobacter* per μg N are produced from the oxidation of nitrite (27). Three times this number of cells are produced from a of ammonium nitrogen oxidized by *Nitrosomonas* (11, 131). *Nitrobacter* require three times more nitrogenous substrate than *Nitrosomonas* because the resulting free energy change is about - 65 Vs - 20 kcal/mol for the oxidation of ammonium and nitrite respectively.

2.2.2. Heterotrophic nitrifiers

In 1926, Mishustin discovered the formation of nitrite from organic nitrogen by a *Bacillus* strain and thereafter several bacteria, actinomycetes and fungi have been found to oxidize nitrogen heterotrophically (cited by Focht and Verstraete, 1977) (52).

Unlike in the case of autotrophic nitrifiers, nitrogenous substrates that are oxidized by heterotrophic nitrifiers are restricted to ammonium or nitrite or even to inorganic compounds. Several reduced forms of organic nitrogen compounds may serve as substrate for nitrite formation by various individual organisms (46). Nitrite is formed by a wide variety of bacteria and actinomycetes but only few bacteria (e.g. *Arthrobacter*) and fungi (e.g. *Aspergillus flavus*) (16) have the ability to oxidize ammonium to nitrate.

Heterotrophic nitrification, unlike the autotrophic nitrification is not obligately associated with the formation of heterotrophs, so that the presence of these micro-organisms merely indicate a potential for activity rather than an occurrence of actual transformation, for example, it has been found that nitrate is formed even if the soil contained only few autotrophs; sometimes nitrate is formed at temperatures too high for significant growth of known autotrophs (95). It has also been suggested that heterotrophic nitrification may be important in acidic and alkaline soils where autotrophic nitrification is not observed to occur (70).

Focht and Verstraete (52) comparing the rate of nitrification and maximum concentration, of products formed between heterotrophic and autotrophic nitrification reported that the nitrification rate of heterotrophs in 10^3 to 10^4 times smaller than that of autotrophs. Furthermore, the conversion capacities of nitrification substrates by the heterotrophic nitrifiers is 10^2 to 10^3 times smaller on a cell number basis than those of that autotrophic counterparts.

2.2.3. Biochemistry

Since the present study is mainly concerned with the autotrophic rather than the heretotrophic nitrifiers, only the biochemistry of autotrophic nitrification will be discussed here. Autotrophic bacteria are, micro-organisms that oxidize mineral substances for their metabolism, gaining carbon from carbon-dioxide and nitrogen from inorganic compounds (37). Nitrifiers are chemoautotrophs which obtain carbon from carbon-dioxide, and energy from oxidation of ammonium or nitrite.

Organisms using energy from photochemical reactions are called photoautotrophs. On the other hand, heterotrophic bacteria obtain energy solely from organic substances. Although

main source of energy for autotrophic nitrifiers is obtained from inorganic oxidation, they have a metabolism comparable with that of heterotroph (65).

Hydroxylamine is found to be the first intermediate in the pathway of ammonium oxidation (141). Nitrous oxide is formed during the conversion of ammonium to nitrite from an actual intermediate or by reduction of nitrite (142, 143) but nitrous oxide cannot be considered as an intermediate because, it is not converted to nitrite. Anderson, 1964 (16) reported that small amount of nitric oxide also evolved during this transformation. He showed the ability of enzymes derived from ammonium oxidizers in oxidizing hydroxylamine to nitrous oxide, nitric oxide and nitrite (17).

Little is known about nitrite oxidizing systems but, Aleem and Alexander, 1958 (1) showed that enzymes prepared from cells of *Nitrobacter* converted nitrite to nitrate and consumed oxygen in the process. It was also shown that cells provided with nitrite and $^{18}O_2$ made nitrate containing no $^{18}O_2$ (2).

Nitrosomonas oxidizes ammonium to nitrite and *Nitrobacter* converts nitrite to nitrate. These reactions yield energy and a source of reducing power for the on-corporation of carbon-dioxide into cell (59).

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O + 66\ kcal$$

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- + 17.5\ kcal$$

It is evident that many autotrophs, even those previously regarded as strict autotrophs can use some form or organic matter either as energy source or as a carbon source and sometime as both (59). In addition, many organisms can live both autotrophically and heterotrophically.

2.2.4. Environmental Factors affecting Nitrification

The autotrophic nitrifying bacteria are more sensitive to unfavourable conditions than are most other common microorganisms. The effects of nutrient supply, temperature, PH, aeration, organic matter, depth, water-logging, plant growth, cultural practices, and seasonally related influences have been discussed in detail (3, 52, 59). Only a few of these will be considered here.

2.2.4.1. Soil Moisture

The nitritifation process can be readily affected by the alteration of soil moisture status in soil (32, 50, 137). The optimum moisture level varies considerably with different soils (100). Nitrate generally appears more readily at half to two thirds the moisture holding capacity - cited by Amarasinghe, 1979 (11). It has been reported that active nitrification of soil nitrogen ceases at a soil moisture level slightly below permanent wilting point (96). However viable cells of nitrifiers have been isolated from desert soils (109) and when ammonia was added to dry soil samples in bottles of more than 15 years old, nitrification has been observed (53).

2.2.4.2. pH

Several reports (3, 59, 103, 136) are available on the relationship between the pH and the abundance of nitrifiers in soil. Nitrification is closely and directly related to pH and affected significantly at low pH values (43). Although the optimum pH value is considered between 7 and 9 for both *Nitrosomonas* and *Nitrobacter*, nitrification has also been found in soils having pH values of 4.0 (136) and 4.5 (76).

The growth of nitrifiers is also pH dependant. Generation times varied from 100 hours at pH 6.2 to 38 hours at pH 7.6 for *Nitrosomonas* and from 58 hours at pH 6.2 to 21 hours at pH 6.6 for *Nitrobacter* (59).

2.2.4.3. Aeration

Nitrifying bacteria are obligate aerobes and thus they are sensitive to oxygen deficiency. The optimum percentage of oxygen for rapid nitrate production in soil is similar to that found in air and low or unnaturally high partial pressures of oxygen suppress the organisms (15, 61). Recent evidence (62) suggests that they can still grow in very low concentrations of oxygen, (e.g. up to 5 x 10^{-6} M) and possession of cytochromes (which have a very high affinity for oxygen) by nitrifying bacteria has been put forward as a possible reason.

2.2.4.4. Nutrient Supply

In a natural environment the only source of mineral ammonium is ammonifiers. Therefore, at this condition nitrifiers have to depend on ammonifiers for substrate. If the rate of nitrification exceeds the rate of ammonification the soil would become devoid of ammonium except the fixed ammonium. A direct relationship between the amounts of ammoniacal nitrogen applied and nitrate nitrogen accumulated has been observed (66).

Fixed ammonium is sometimes oxidized. For instance, Raju and Nukhopadyay, 1974 (94) found that nitrification of fixed ammonium increased with incubation period and when NH_4^+ fixing power of soil was increased, the availability of fixed ammonium to nitrifying bacteria decreased. Allison *et al*, 1953 (9) reported that soils containing montmorrilonite exhibited the greatest oxidation of fixed ammonium and nitrification of soils containing illite and vermiculite was slow.

It has been found that the growth of nitrite oxidizers is enhanced by the additions of nitrite to the soils (14). On the other hand the growth of *Nitrobacter* has been found to be greatly suppressed when nitrite level in soil increased due to application of large quantities of urea, anhydrous ammonia or ammonium salts to soils of high pH (3). Nitrite oxidizers are also known to be more sensitive than ammonium oxidizers to other nutrient such as phosphorus (92). Possible effects of metals on nitrification have also been studied for example adverse effect of manganese (> 1000 ppm) (139) and same effects of Zn, Cu, Cr, Cd, Hg and Pb (91, 121, 124) on nitrification have been considered in detail.

2.2.4.5. Depth

Effect of soil depth on nitrification has not been considered sufficiently in the past. Martin and Cox, 1956 (82) suggested that the pattern of nitrification is not same in all horizons. Lack of nitrate formation with absence of nitrifying bacteria has been reported in the horizon than the A horizon.

2.2.4.6. Temperature

Contradictory results appear in the literature on the effects of temperature on nitrification (54, 54b, 122a, 123). For example, while oxidation of ammonium was greater at 40°C than at 5, 15 or at 25°C (123), a slow but significant ammonium and nitrite oxidation has been observed at 2°C (54, 56). However, the optimum temperature both in soil and culture is considered to fall between 30° and 35°C.

2.2.4.7. Organic Matter

The results of studies on the effect of organic matter on nitrification also have been contradictory. (3, 55, 59, 113, 126, 127, 130). Amarasinghe, 1979 (12) found that the addition of dried sub-clover tops of soil did not affect the population of nitrifiers but increased the population of heterotrophs. Similar observations made by Van Schreven, 1964 (127), and he found that nitrogen mineralization was retarded when dried organic matter was applied in mechanically broken pieces.

Envezor, 1967 (50) reported that addition of decomposable organic materials caused nitrate immobilization and retarded the nitrification of ammonia produced. It was also reported (71) that when C/N ratio was high nitrification was poor, and when organic matter rich in nitrogen was applied nitrification increased significantly.

2.2.4.8. Plant Effects

Effects of rhizosphere and rhizoplane on nitrification have been studied widely. Vlassak, 1970 (130) found relatively rapid nitrification with soils from cultivated land and pastures but soils under natural vegetative covers of conifers and hardwoods were mostly ammonifying. Inhibition of nitrification caused in the rhizosphere has been reported by many workers (48, 74, 85, 88, 116, 119). Theron, 1951 (119) stated that inhibition of nitrification in grasslands was due to toxic substances secreted by the grass roots. Similarly, Purchase, 1974 (93) and Robinson, 1963 (97) reported that nitrifying microbes in the root zones are restricted by ammonium deficiency which has been confirmed with the addition of urea or ammonium salts to the soil. On the other hand no evidence for rhizosphere inhibition was shown by Starkey, 1929 (118), Katznelson, 1946 (73), Purchase, 1974 (93), Huntigens, 1971 (60), Walker et al, 1954 (134), Robinson, 1963 (97) and Millbank, 1959 (83). Millbank, 1959 (83), Vasantharajan and Bhat, 1968 (128) found methionine which is stimulated in rhizosphere has an inhibitory effect on soil nitrification. They found beneficial heterotrophs such as *Pseudomonas, Achromobacter* and *Bacillus* were capable in degrading methionine to greater extent. Walker et al, 1954 (134), Leshem and Levin, 1978 (78) have shown that the continuous low level of nitrate nitrogen under grass was due to rapid removal of nitrate from soil by grass.

2.2.4.9. Manuring

Fertilizers control nitrification directly by altering the source of energy available for nitrifiers (66, 71, 76) and indirectly by influencing the pH of the soil (3, 43). Although nitrogen fertilizers are source of energy, *Nitrobacter* has been found to be sensitive to the presence of ammonia even at comparatively low concentrations (122b). Nitrification in acid soils, has been found to be slow when ammonium sulphate was added (49, 122 a). A calculation using the most recent information available showed that the added amounts (150 ppm) of $(NH_4)_2SO_4$ could theoretically produce about 1.6 x 10 cells of *Nitrosomonas europaca* g ^{1} soil, which is much higher than the actual cells produced (14). The absence of significant changes in ammonium oxidizing and nitrite oxidizing populations to added ammonium or ammonium formed from organic material was mainly ascribed to their capacity to oxidize relatively large quantities of energy yielding substrates.

Purchase, 1974 (92) and Sinha, 1957 (110) reported that phosphorus is very important for the nitrification process. However, for the oxidation of 1000 lbs ammonium nitrogen, only one pound of phosphorus is needed by nitrifiers which can be easily satisfied without seeking for additional sources of supply (3). Potassium tends to have a depressing action on nitrification (110).

2.2.4.10. Pesticides

Effectiveness of various pesticides on nitrification varies with soil types and dosage (120). Nitrifiers are used as indicators of pesticide damage is being investigated because they are known to be very sensitive to certain chemicals including pesticides. But recent findings (99), reveal that *Nitroso-cystis* is the most dominate in resisting toxic substances, and none of the pesticide in any condition depress growth of this organism. Although several studies have been made on the effect of pesticide on nitrification (11, 47, 63, 77, 106, 120, 129) it has been briefly dealt because, pesticide application did not take place in our experimental site.

Likewise studies made on the effect of burning (45, 57, 112), crop establishment (12), inhibitors (13, 72, 76, 89, 98), amendments (81, 101, 111) and gamma irradiation (69) on nitrification also have been ignored in our present studies.

2.3. Nitrogen Status of Coconut growing Soils

As our present study has been designed to investigate nitrification in coconut growing soils, it is very useful to focus the review on a general topic such as nitrogen status of these soils with a historical background.

Most of the experiments carried out by the Coconut Research Institute of Sri Lanka have shown that coconut respond to nitrogen application only during the first few years. Thereafter there has been either very small response or no response at all (Appendix 1a, & 1b). Although application of nitrogen fertilizer did not give any significant returns, it has been found that nitrogen fertilizer had significant interaction with K and P fertilizers (18).

Statistically non-significant responses to nitrogen fertilizers with occasional depressions in yield in the 3^3- Factorial NPK fertilizer trial at Bandiruppuwa Estate had been one of the

primary reasons to commence experiments on nitrogen status of coconut growing soils. The absence of response to nitrogen was suspected to be due to the accumulation of nitrogen under a cover (22 a). It was felt that season x nitrogen interaction could have been the reason for the absence of response and an analysis of variance of 14 years integrated data was done, but there was no nitrogen x season interaction (18).

In 1947 preliminary studies on soil nitrogen under coconut were commenced. The main results are shown in Appendix 1 a. Subsequently in 1952 (19) three series of samples were collected at 16 and 8 days interval from a deep loamy, well-drained soil overlying a subsoil of lateritic gravel. Some important features in this experiments were -

1. a high amount of NH_4-N (about 5% of total-N-outside the manure circle) in the soil profile (Appendix 2),
2. NH_4-N concentration in subsoil (22.5 to 45 cm) was higher than in top (0 - 22.5 cm) and appreciable amounts of NH_4-N and NO_3-N were even noticed at 76 cm to 107 cm depth, and
3. the consistent nature of results in all series of samples.

Reasons suggested on these observations were -

a) ammonium may have leached or hydrolysed to the lower layers as NH_4-N - clay complex; and
b) in coconut cultivation a two year ley of grass was maintained and whether the accumulation of ammoniacal nitrogen might be due to the selective absorption of NH_4-N by coconut roots but not NO_3-N.

In 1954, experiments on transformation of soil nitrogen under coconut was carried out in five different plots at Bandirippuwa Estate. Same results were obtained, except in three plots concentration of NH_4-N in top soil was higher than sub (Appendix - 3a and 3b). Soil nitrogen studies carried out at Ratmalagara Estate, Manurial Cultivation Experiment plots in 1955 also showed similar results (Appendix - 3c). The results of Bandiruppuwa Estate also showed that except in a sandy plot where NH_4-N was (2.2 ppm) nearly the same as NO_3-N (2.3 ppm), the other plots had very much higher NH_4-N (10 to 15 ppm) (Appendix 3b) studies done in 1956 and 1959 followed the same pattern of NH_4-N and NO_3-N occurrence in soils under coconut (22c, 24, 25) - higher NH_4-N in the top soil and lower NO_3-N in top and subsoil.

In 1956, soil nitrogen studies were initiated on nitrogen rich soils from virgin jungles of Wellawaya, Puttalam and Mannar. It was reported that concentration of NH_4-N was higher than NO_3-N in all jungle soils (Appendix 7a). However, some contradictory results were obtained by the Chemistry Division, C.R.I, in 1957, when they were trying to trace the reasons for ‘yellowing’ of palms in Galle District. An unusual occurrence of high NO_3-N (32.5 ppm to 177.5 ppm) in the soil profile was one of the significant features in their results (Appendix 8). Unlike all the previous results reported earlier, concentration of NO_3-N was significantly higher than the concentration of NH_4-N (20 ppm to 40 ppm). As their interest was mainly focused to the yellowing of palms, they did not attempt to reason out the 'shifted' dominancy of NO_3-N over NH_4-N in coconut growing soils.

When yellowing of palms in Waligama Estate was reported in 1959, the Division of Soils, C.R.I, collected soils samples from manure circle and centre of square at 0 to 22.5 cm and 22.5 cm to 45 cm depths and analysed for NO_3-N and NH_4-N. None of the results obtained from this experiment showed higher amount of NH_4-N in illuvial horizon than eluvial (Appendix 9). Also concentration of NO_3-N was lower than concentration of NH_4-N in soils under affected and healthy palms from both manurial circle and centre of squares at both depths - except one sample from the manurial circle of affected palms at 22.5 cm - 45 cm depth where NO_3-N was slightly higher than NH_4-N.

According to soil nitrogen studies made on Iraneville soils (which were suspected as deficient in nitrogen) it can be noted that concentration of NH_4-N and NO_3-N were significantly low after rainy season (October to December) (40). The conclusion drawn out from this experiment was treatments did not favour either ammonification or nitrification (Appendix 5a and 5b).

2.4. Preliminary Studies on Nitrification under Coconut

In 1965 it was again decided to investigate the cause for 'yellowing' of palms at Iraneville Estate (22b). According to the report of the Superintendent, affected palms treated with cattle manure showed some improvements. Chemical analysis of 14^{th} leaf indicated a marked deficiency of nitrogen. It was suspected that in sandy soils of Iraneville, little or no nitrification occurred due to sterile soil condition, absence of vegetation, high temperature and desiccation. Therefore it had been decided to have a preliminary study on nitrification of soils under yellowed palms, both from Iraneville and Bandirippuwa. Soil samples were brought to the laboratory and kept under incubation. At the end of 10 days NH_4-N and NO_3-N were analysed and nitrification rate was measured (Appendix 6a and 6b). It was concluded that nitrification was less in Iraneville soils. The method of incubation of soil samples was not given in the literature (40). Therefore the results of this experiment are ambiguous to draw any conclusions.

Except in one or two cases the studies reviewed above indicate that NH_4-N dominates over NO_3-N in coconut growing soils at various depths. Nitrate nitrogen is detected in trace amounts in most of the cases.

2.5. Nitrogen status of coconut growing Soils in association with pasture

Number of beneficial effects of cover crops on coconut and enthusiasm shown by many planters on cover crops initiated systematic studies on the beneficial and harmful effects of grasses; legumes and weeds on coconut in 1934 (114). It is well known that when cover crops are not established under coconut, there is a tendency for the weeds to cover the soil. In this case weeds act as a cover. Therefore the association of grass is not artificial, it is a natural phenomenon in which coconut may get some benefits. But precise ecological nature of this beneficial association had not been clearly understood. In addition, the mysterious fate of nitrogen fertilizers applied to the coconut also had been unknown. This was sufficient enough to commence studies on soil nitrogen under a lay of grass in coconut plantations.

The first experiment carried out at Kirimetiyana Estate, Lunuwila, in 1947 showed a high concentration of NH_4-N under grazed pasture (79). Amount of NH_4-N also higher in illuvial horizon than eluvial. Surprisingly, amount of NO_3-N under the same condition was zero. Considering this, Salgado and Nethsinghe, 1952 (102) started another experiment to investigate the transformation of nitrogen under coconut with a cover of weed. Same results were obtained. Nitrate nitrogen was detected but comparatively in trace amounts (1 to 3 ppm). It was thought that some substances secreted by grass roots would have caused toxic effect on micro-organisms which convert $NH_4^+ \rightarrow NO_3^-$. However, an invaluable comparison of nutrient status of soils cropped continuously with different pasture species was made in 1971 with two pasture species and fodder (51) (Appendix 6c). According to this, concentration of NO_3-N was higher than NH_4-N in soils under *Brachiaria mililformis*, *Brachiaria brizantha* and *Panicum maximum*. But in the soil under weed cover (control) this was reversed.

It has been found that cultivation of pasture under coconut with optimum condition increases coconut yield (51). Microbiological studies conducted in the rhizosphere of coconut and cocoa under mixed cropping by Nair and Rao, 1970 (87) in Kerala may be useful in understanding the microbial effects on nitrogen transformation under pasture with the association of coconut. A 95% and 65% increase in yield was observed in coconut with double and single hedged cocoa respectively. The reasons suggested were -

a. increase in organic carbon context in the soils,
b. population of *Beijerinekia* (micro-organism involved-in N - fixation) was high in coconut rhizosphere with double hedged cocoa and
c. phosphate solubilisation was very high due to the presence of *Pseudomonas sp* and *Aspergillus nigor*.

3. MATERIALS AND METHODS

The experimental site for the study was selected at Ratmalgara Research Station, Madampe (Puttalam District) where a pasture cover (*Brachiaria miliiferais*) has been maintained since 1975 for a pasture experiment (80).

3.1. Sampling

There were four plots, two pasture (replicates) and two control plots (bare) in the experimental site. Sampling was done at the centre of the square. (Appendix 10). Sites where tensio-meter or neutron probe tubes installed had been deliberately avoided. Likewise sites which had termite mounds, pits, decaying stems of coconut and sites near to the gravel road were also avoided. Ten to twelve sampling sites were selected randomly. Sampling was carried out fortnightly using a 3.75 cm diameter, 45 cm long galvanised pipe auger, specially made for this purpose. Each sample consisted of 10 - 12 cores taken with the auger to the depth of 0 - 15 cm and 15 - 30 cm. Care was taken to avoid any litter of pasture mixing with sample. The samples were placed in clean polythene bags, and transported immediately to the laboratory where they were stored overnight at room temperature. Notes were made at

each sampling visit of the weather conditions, state of the soil and pasture. Sampling was done for two months.

Date of Samplinq	Weather	Soil Condition	Pasture
17.07.1980	Sunny	Not dry	Good cover
31.07.1980	Sunny	Slightly dry especially top soil was dry and loose	Cover showed symptoms of water stress slightly
14.08.1980	Sunny (previous days drizzling[1])	Dry	Water stress was more pronounced
28.10.1980	Sunny (rained a day back)	Not dry	Water stress was more pronounced

[1] Rainfall data in Appendix 11

Physical soil property of the experimental site – Ratmalagara Research Station

Depth (cm)	% Coarse sand	%Fine sand	% Silt	% Clay	Texture[1]	Soil pH[2]
0-15	69.00	7.50	3.40	19.40	Sandy loam	4.2
15-30	63.00	6.50	1.62	28.18	Sandy clay loam	4.1

[1] Texture was determined by pipette method (44)

[2] Soil pH was measured in 0.01 M $CaCl_2$ (1:2 soil:$CaCl_2$) (9)

3.2. Treatments

One day after sampling, each sample was sieved through 2 mm mesh; thoroughly mixed several times and divided into two sub samples. One sub sample was used for moisture and chemical analysis and the other for microbiological analysis.

3.3. Microbiological Analysis

The number of ammonium oxidizers and nitrite oxidizers were estimated by the most probable number method (4), as given below:

Each soil sample was mixed as thoroughly as possible in the bag and a representative 2 g soil was suspended in 18 mL sterile water; shaken for 15 minutes in a horizontal reciprocating shaker (150 RPM) and later for one minute by hand. Tenfold dilution series was prepared by transferring 2 mL of soil suspension to 18 mL water blank with 1 mL sterile blow-out pipettes.

Dilution series were prepared up to 10^{-5} dilution. One mL aliquots from each dilution was transferred to Mc Cartney bottles, each containing 3 ml sterile culture medium. The inoculated tubes were incubated for 3 weeks at room temperature (30 - 31°C). At the end of the inoculation period, tubes were scored either positive or negative for microbial growth using suitable tests. The number of viable cells g^{-1} dry soil was calculated using the MPN table (P 1470 (7)) and correcting for moisture content of the soil.

Heterotrophic bacteria were counted by surface dilution plate count method using 1/5 M_{32} (Dr. K. Sivasithamparam, Dept. of Agriculture, Perth, Australia) agar medium - the detail of the method is given elsewhere in this section.

3.3.1. Ammonium Oxidizers

The following culture medium was used in counting the ammonium oxidizers. P 1479 (4))

$(NH_4)_2SO_4$	0.50 g
K_2HPO_4	1.00 g
$FeSO_4.7H_2O$	0.03 g
NaCl	0.30 g
$MgS0_4.7H_2O$	0.30 g
$CaCO_3$	7.50 g
Water distilled	1000 mL

Ilosvay reagent (P 1479 (4)) and the Improved Griess Ilosvay reagent (28). Tubes that gave pink, dark pinkish purple, orange red, orange yellow and brown colour with the reagents were marked positive. All tubes that yielded a negative test for nitrite were tested for nitrate by adding approximately 0.01 g of the Zn-Cu-MnO_2 mixture (1.0 g Zn, 0.1 g Cu and 1.0 g MnO_2) or Zn powder (Analar) alone. The tubes which gave red colour were marked positive for ammonium oxidizers on the basis that the initially negative reading for nitrite meant only that nitrite formed by ammonium oxidizers would have been oxidized to nitrate by nitrite oxidizers.

3.3.2. Nitrite Oxidizers

Formula for culture medium (P 1480 (4)) and weight of chemicals in grams.

KNO_2	0.006
K_2HPO_4	1.0
NaCl	0.3
$MgS0_4.7\ H_2O$	0.1
$FeSO_4.7\ H_2O$	0.3
$CaCO_3$	1.0
Water	1000 ML

Tubes were tested for nitrite, using Griess Ilosvay or Improved Griess Ilosvay reagent. If the medium failed to show reddish colour characteristic of nitrite, those tubes were recorded as positive. If the red colour developed they were marked as negative.

3.3.3. Total Heterotrophs

Formula of agar media 1/5 M_{32}

K_2HP0_4	0.50 g
Ferric tartarate	1 mL (0.5% solution)
$MgS0_4$	0.50 g
Glucose	5.09 g
Peptone	2.50 g
Yeast Extract	0.50 g
Agar 38 g	(50 g for *2%* agar)
Trace elements	5 mL ($MnS0_4$ – 2.00 g, $NaMn0_4$ – 2.00 g, $ZnS0_4$ - 0.20 g, and $CuSO_4$ -0.10 g in 1000 mL distilled water)

Agar media + trace element Solution + 2494 mL distilled water — 2.5 1 media

Agar (Difco) was melted in a portable autoclave at 0.36 kg/cm^2 for 10 min; added to the media and stirred thoroughly with magnetic stirrer. Media was poured into five 1000 mL conical flasks; closed with cotton plugs and sterilized in an autoclave at 0.73 kg/cm^2 for 15 min. Un-used media was stored at room temperature and used in subsequent analysis after melting at 0.36 kg/cm^2 for 5 min.

About 10 mL of media was poured into each sterile petri-dish. Dishes were then kept upright until agar had solidified. Then petri-dishes were inverted and kept aside until inoculation. From each dilution series (10^{-3}, 10^{-4} and 10^{-5}) soil suspension was drawn out by sterile pipettes (1 mL) and 0.1 mL of suspension was transferred to each plate (each dilution had two plates). Then using a sterile L shape glass rod, the suspension on the surface was spread thoroughly (for each spreading, rod was dipped into 100% alcohol and burnt). Plates were inverted one hour after inoculation and kept for incubation at room temperature (30 - 31°C) for 7 days. Plates which were selected from the dilution at which 30 - 300 colonies had developed per plate were counted for number of colonies.

3.4. Determination of Ammonium and Nitrate in soil

Ten g of moist sub samples (< 2 mm) were extracted with 2 N KCl (100 mL) by shaking for one hour in a reciprocal shaker at 150 RPM. The soil suspension was left overnight and filtered on

the following day. Ammonium nitrogen and NO_3-N in the filtrate were determined by Kjeldahl distillation using magnesium oxide - Devarda alloy (pp. 1195 - 1198) (35).

3.5. Determination of Organic Carbon in soil

Sub samples were air dried; ground well and sieved through a 100 mesh. One g soil was analysed by the Walkley & Black method (pp 1374 - 1375) (10).

3.6. Determination of Total Nitrogen in soil

Sub samples were air dried; passed through 100 mesh and 10 g samples were digested and nitrogen was analysed by the Macro-Kjeldahl method (pp 1162 - 11643 (36).

4. RESULTS

4.1. Ammonium and nitrate nitrogen

There was no statistically significant difference in the NH_4-N contents under pasture and no pasture, although, the data showed a slightly higher amount of NH_4-N in the soil under pasture. In contrast, the effect of pasture on the NO_3-N content was statistically significant - soils under no pasture had more NO_3-N than those under pasture. The concentration of NH_4-N in the surface horizon (0-15 cm) was significantly higher than the sub surface horizon (15 - 30 cm). Nitrate nitrogen in the surface soil was also higher than that in the sub surface but relation was not significant. The variation of the amount of NO_3-N and NH_4-N with time of sampling at both depths and with and without pasture generally followed the same pattern (Figure 24 & 26). The variation of the two forms of inorganic nitrogen with time of sampling appears to be related to the moisture content of the soil (Figure 4a) - higher the moisture content lower the amount of NH_4-N and NO_3-N contents.

4.2. Population of nitrifiers

The population of NH_4-oxidizers was very high under pasture and higher number of viable cells were detected in the 0-15 cm horizon (Figure 3a). The number of NH_4-oxidizers increased with the amount of soil moisture and decreased when the soil was dry (Figure 4a) Nitrite oxidizers were not much affected by moisture content.

Also pasture cover did not have any influence on NO_2-oxidizers. Furthermore, there was a significant number of nitrite oxidizers in the top soil. Although, NH_4-oxidizers were found to dominate NO_2-oxidizers under pasture, this condition was reversed under cover i.e. NO_2-oxidizers were higher than the amount of NH_4-oxidizers under no cover (Figure 3b).

4.3. Population of total heterotrophs

Due to some laboratory mishaps the total counts of heterotrophs could not be detected in the first sampling and therefore only the results of subsequent three samplings are presented in this report. The population of heterotrophs was highly influenced by both

Figure 2a

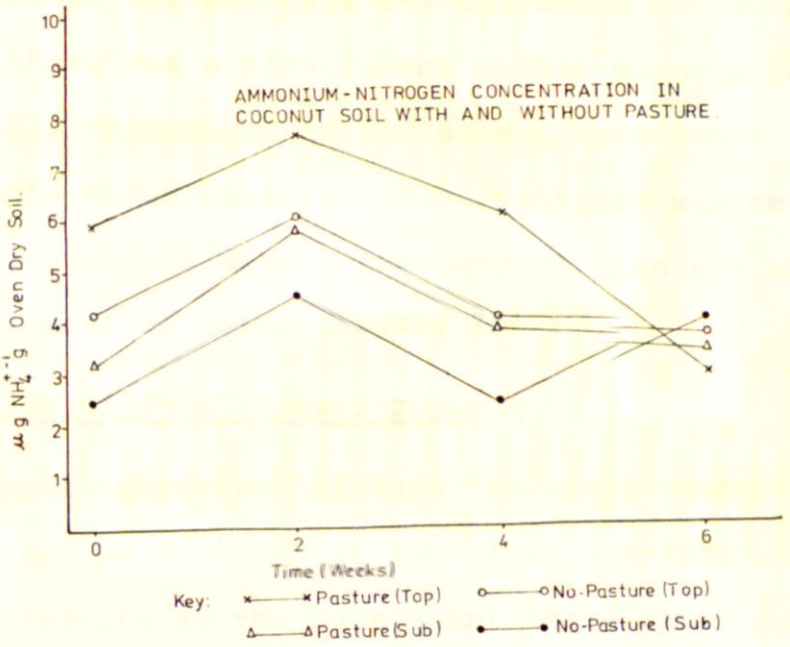


Figure 2b

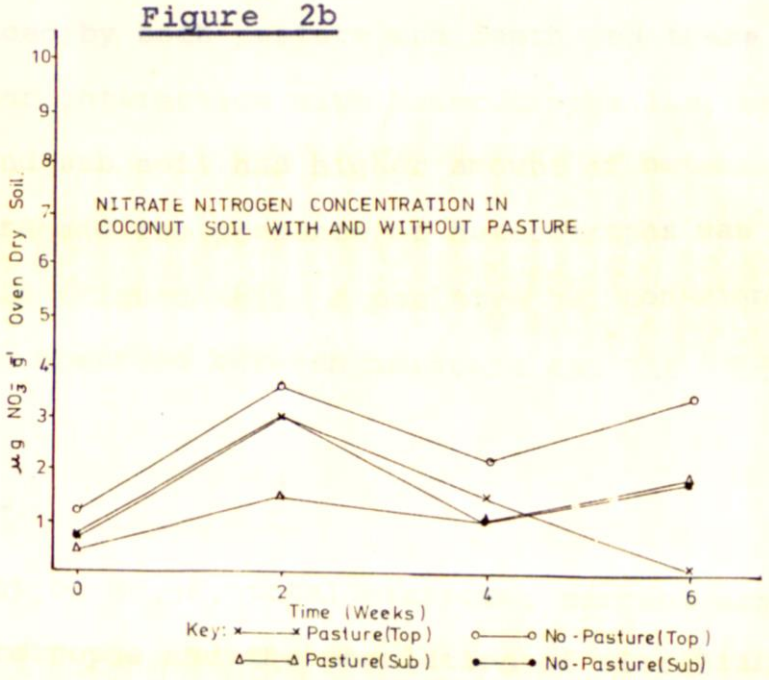


Figure - 3a

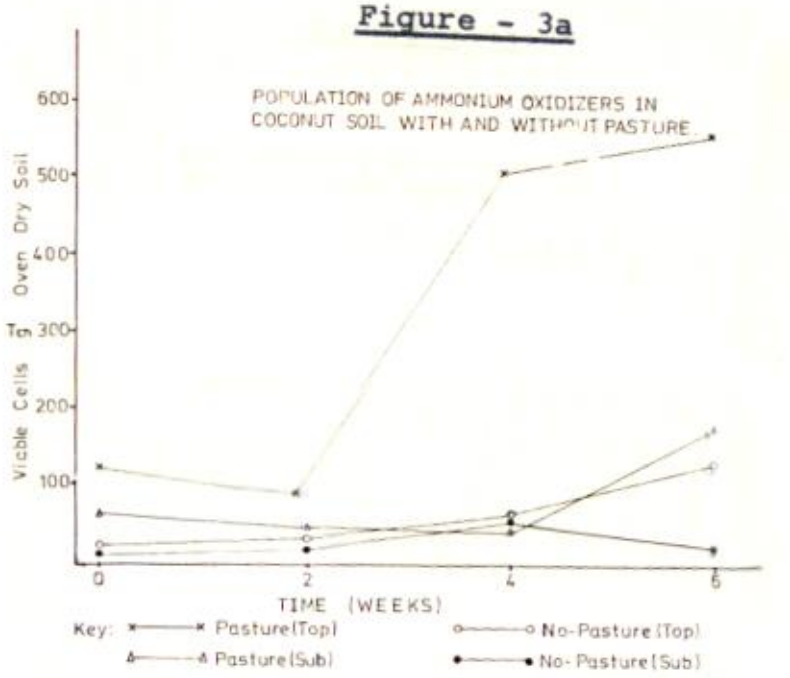
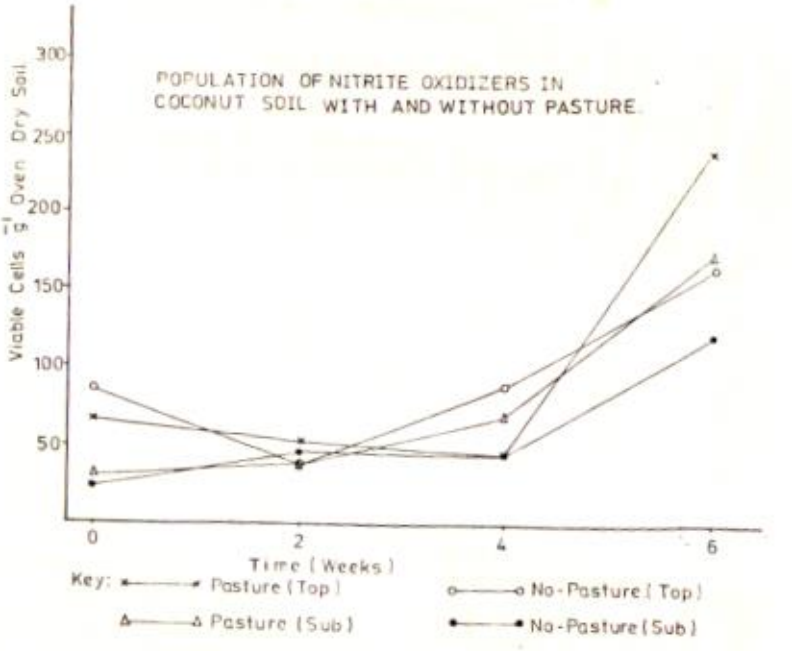


Figure - 3b



pasture and depth and these two factors had a higher interaction with heterotrophs i.e. under pasture both top and sub soil had higher amount of heterotrophs than under no pasture and the presence of heterotrophs was prominent in the top soils (Figure 4b). A positive but non-significant correlation was observed between moisture and the number of heterotrophs.

4.4. General

The amount of NH_4-N, total nitrogen, percent organic carbon, total heterotrophs and the population of nitrifiers were higher in the 0 - 15 cm horizon under pasture. Amount of organic carbon did not vary with time but a significant in the amount of total nitrogen was found hence a highly significant change in C/N ratio was also observed.

5. DISCUSSION

5.1. NH_4-N versus NO_3-N

The data presented here showed that the amount of ammoniacal nitrogen (NH_4-N) is higher than the nitrate nitrogen (NO_3-N) in the soils at both depths (0 - 15 cm and 15 - 30 cm) with and without pasture. This is in general agreement with the observation made by other workers (19, 20, 21).

Various reasons can be suggested for the dominancy of NH_4-N over NO_3-N. The leaching losses of the soil nitrogen mainly occur through NO_3-N because NH4-N can be strongly adsorbed on soil colloids. At the beginning, sampling was done soon after a heavy rainfall and hence a very trace amount of NO_3-N was detected (Figure 2b). Ammonium nitrogen content however was not low (Figure 2a). The results of Nethsinghe (40) on the distribution of NH_4-N and NO_3-N under coconut in Iranaville soils supports the above explanation (Appendix 5b).

A tendency for the NO_3-N to be lost as and N_20 in the process of denitrification under aerobic as well as anaerobic conditions in the soil profile may also be a cause for the lower content of NO_3-N than NH_4-N (64). Besides nitrite nitrogen is one of the main intermediate products in the process of nitrification (3, 59). This NO_2-N may have been lost in gaseous forms of nitrogen in acidic soils by chemical process (8, 33, 42, 122b, 138) which would have been otherwise converted to NO_3-N by nitrite oxidizers.

The total counts of the number of heterotrophs include the ammonifiers and denitrifiers. Under upland conditions the number of ammonifiers are very much higher than the denitrifiers (60) and therefore the total number of heterotrophs in our study could represent mainly the number of ammonifiers. The number of heterotrophs in our study is about 10,000 times higher than the number of nitrifiers. This may also be a reason for the higher levels of NH_4-N than NO_3-N in the present study. Furthermore, ammonification is always higher than nitrification process due to the physiological heterogeneity of ammonifiers and therefore even in adverse conditions one segment of this population carries out ammonification satisfactorily (14, 43, 84, 92). The NH_4-N formed do not get lost by volatilisation as one would expect because the soil is acidic (pH 4.2)

Figure - 4a

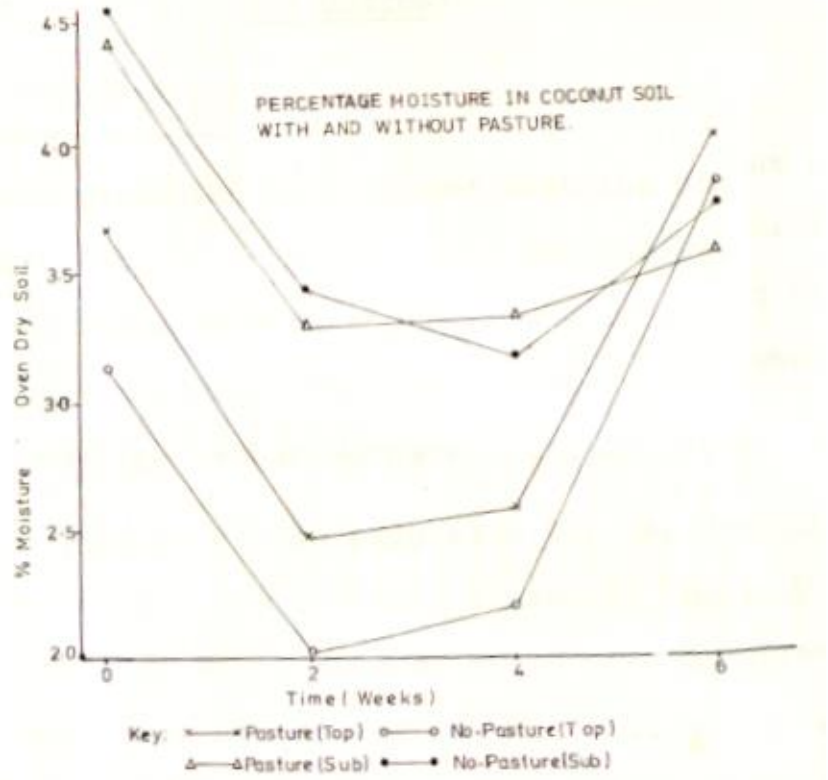
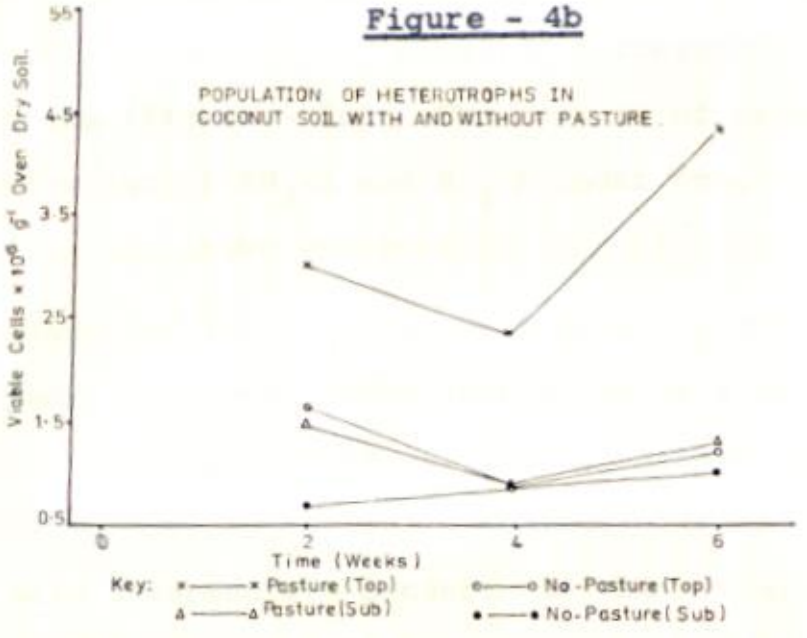


Figure - 4b



With all these, reduction of NO_3-N to NH_4-N also adds appreciable amount of NH_4-N which gives rise to further increase in NH_4-N in the soil (39, 117).

5.2. Effect of Pasture on NH_4-N -

It has been much exaggerated of the significant accumulation of NH_4-N under the cover (85,102,119). Although statistically non-significant, our result also showed that NH_4-N is higher under pasture (Figure 2a). Data presented here are sufficient to explain the higher accumulation of NH_4-N under pasture.

Under pasture total nitrogen, percent organic carbon and total heterotrophs (mainly ammonifiers) were detected in significantly higher amounts than under no pasture. Others also reported that the presence of ammonifiers under cover is high (about 100 times) (60). Thus ammonification process in the rhizosphere is resulting in higher NH_4-N. The data for the 6^{th} week sample is an exception. This sample was collected following some rainy days and the soil moisture was also high (Appendix 11) (Figure 4a). Under this condition the nitrification rate was high (Figure 3a and 3b) and there was reduction in the NH_4-N content. Though the ammonification rate is also high, the removal of NH_4-N and NO_3-N by plants and the loss of NO_3-N by leaching may not have allowed the NH_4-N in the soil to accumulate.

5.3. Effect of Pasture on NO_3-N

Various explanations have been given for the higher accumulations of NO_3-N in fallow soil than under cover (67, 93, 97).

Earlier it was believed that nitrification was inhibited by some toxic substances exuded in the rhizosphere (119) and later on many workers suggested that a depression in nitrification was due to high amount of carbonaceous materials available in the rhizosphere which would have resulted in multiplication of heterotrophs at the expense of NH_4-N. Hence there was shortage of substrate which caused NH_4-N oxidizers to starve indefinitely (58, 68). The results obtained in the present study also showed that NO_3-N under cover is less than under no cover. Immobilization cannot be the reason for the losses in NO_3-N as suggested by some workers (71, 83) because the C/N ratio in the soil in all cases was less than 10 (mean 9.2).

It was discussed earlier that the most favourable conditions under the cover gave rise to higher accumulation of NH_4-N due to the abundance of ammonifiers. This may be one of the reasons for the detection of higher number of nitrifiers -the number of NH_4-oxidizers being higher than the NO_3-oxidizers. In contrast, in soils under no cover the presence of NO_2-oxidizers were always higher than NH_4-oxidizers. This is probably due to rapid loss of NO_2-N in the rhizosphere which is the substrate for NO_2-oxidizers. (33,138). Though the population of NH_4-oxidizers under no cover is very low it may be sufficient to supply the required amounts of substrate (NO_2-N) for the NO_2-oxidizers as the losses of NO_2-N is very low in the absence of rhizosphere. This may be one of the reasons for the abundance of the NO_2-oxidizers under no cover hence more production of NO_3-N in this soil condition.

Figure 5a

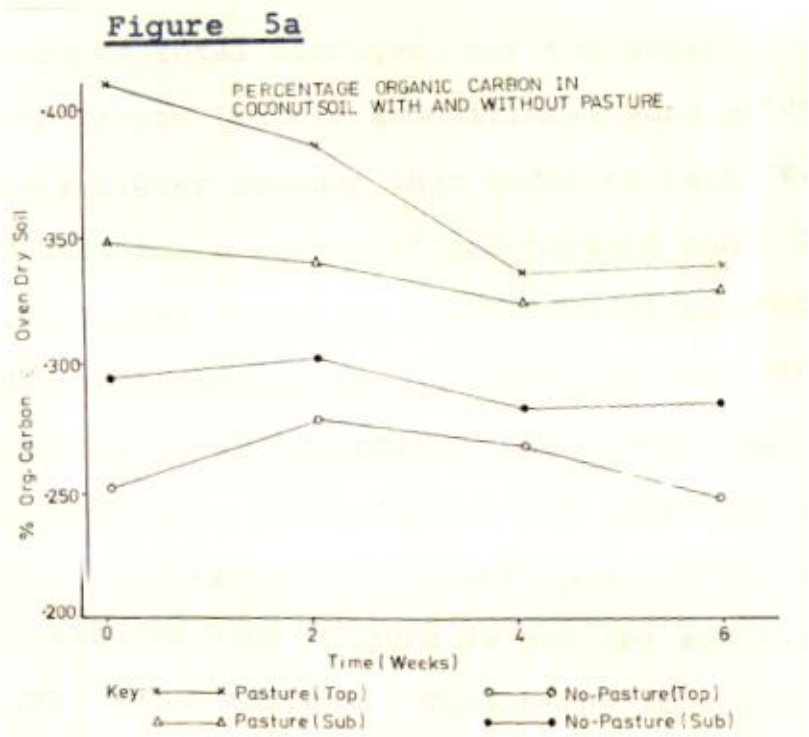
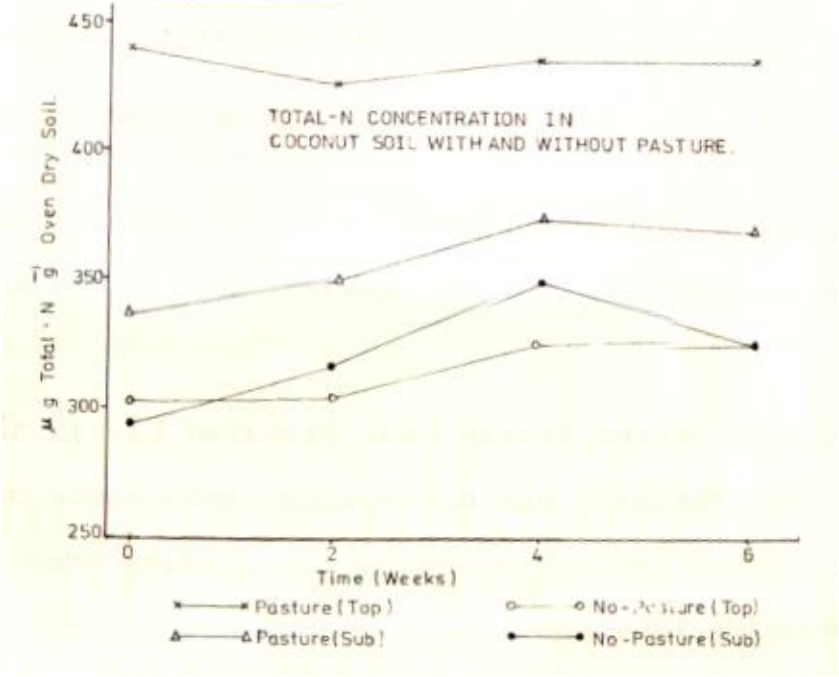


Figure 5b



There is sufficient information in the literature available to show a fair amount of NO_3-N is lost by denitrification process in the rhizosphere (34, 105, 140). Presence of higher amount of organic material, anaerobic conditions and higher nitrification are some of the possible reasons given for losses of NO_3-N under pasture. This may be one of the reasons for the low

content of NO_3-N under pasture at 0 - 15 cm depth at the 6th week sampling (Figure 2b) - moisture at this time was high (Figure 4a) suggesting localized anaerobic conditions.

In addition to these NO_3-N also may be lost from the rhizosphere by plant uptake as explained earlier and reduction process of NO_3-N to NH_4-N in the rhizosphere 30, 39, 129). The above discussion clearly shows that a fair amount of NO_3-N is lost by various process in the presence of plants.

5.4. NH_4-N and NO_3-N distribution in the surface and sub-surface horizon

The higher concentration of NH_4-N in the surface horizons (0 - 15 cm) than the sub-surface (14 - 30 cm) observed in the present study is not in agreement with the results obtained by Salgado and Nethsinghe (102). They found larger amounts of NH_4-N in the illuvial horizons (22.5 - 45 cm) than in the eluvial (0 - 22.5 cm). It is not a surprise because biennial ploughing and nitrogen fertilizer (ammonium sulphate) applications would have caused excess NH_4-N to get hydrolysed and leached to the lower layers under their conditions (19) whereas, the present study was conducted in a plot where both ploughing and fertilizer application had been stopped since last 5 years. (80). In the present study it is very prominent that population of nitrifiers and total heterotrophs were higher in the 0 - 15 cm horizons. This may be due to more aeration and higher substrate availability at the surface. This is sufficient to maintain the levels of NH_4-N and NO_3-N higher in the surface horizons than in the subsurface horizons.

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Appendix 1a

The 3 x 3 x 3 - N.P.K .Factorial at Bandiruppuwa Estate. Commenced in 1935 (66 palms/acre)

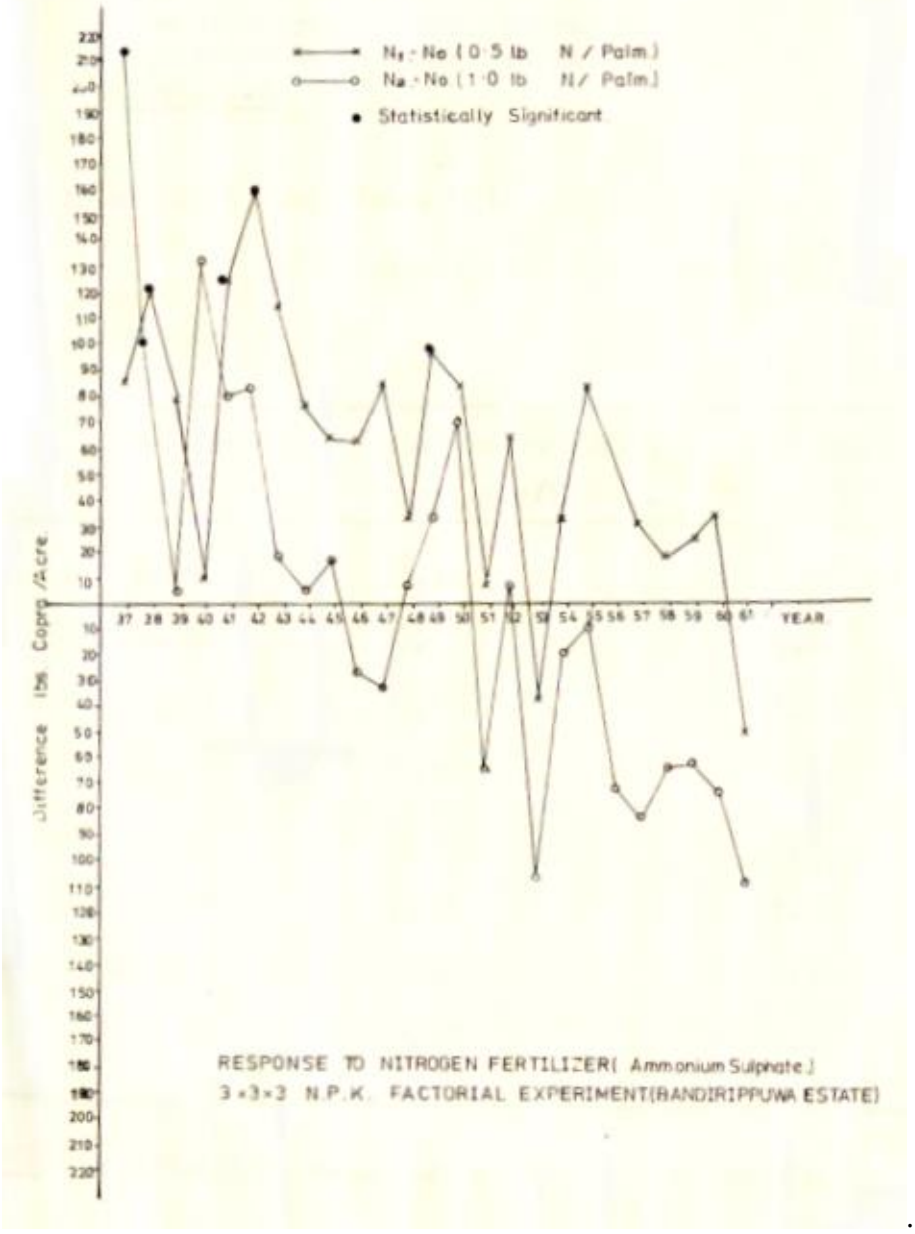
Year	N1 No	N2 No.
1937	86	212
1938	121	100
1939	80	4
1940	11	132
1941	126	80
1942	159	84
1943	113	19
1944	76	5
1945	64	19
1946	63	-27
1947	87	-31
1948	32	8
1949	97	8
1950	85	71
1951	6	-68
1952	65	8
1953	- 35	-109 (wet)
1954	31	-20
1955	81	-9
1956	-	-71
1957	30	-82
1958	19	-65
1959	24	-61
I960	33	-71
1961	51	-109
1962		
1963	38	-45
1964	48	-44
1965	(experiment was terminated)	

Ref. C.R.I. Annual Reports 1947-196.

Transformation of soil nitrogen under coconuts. Date of sampling- August 4,1947

Number of plots	% Carbon	%Nitrogen	C/N ratio	NH_4-N (ppm)
1	0.65	0.053	12.26	16
2	0.49	0.042	11.67	22
3	0.56	0.060	9.33	11

Appendix 1 b



RESPONSE TO NITROGEN FERTILIZER(Ammonium Sulphate)
3×3×3 N.P.K. FACTORIAL EXPERIMENT(RANDIRIPPUWA ESTATE)

Appendix 2

Transformation of soil nitrogen under coconuts at Bandiruppuwa Estate 1952 Plot No. 42.

Description of soil: Deep loam, well drained overlying a sub-soil of laterite gravel much below the sampled depth.

Exchangeable Bases

Sample No	Horizon depth	Total exchangeable bases m.e/100 g	Exchangeable Ca m.e./100 g	Exchangeable K m.e./100 g
RT	0”-9”	3.46	1.86	0.095
RSI	9”-18”	2.83	1.66	0.026
RS2	1.5’-2.5’	2.75	1.82	0.056
RS3	2.5’-3.5’	3.73	2.31	0.030
ST	0”-9”	3.38	1.96	0.095
SSI	9”-18”	3.91	1.62	0.088
SS2	1.5’-2.5’	4.26	2.35	0.015
SS3	2.5’-3.5’	3.54	1.86	0.330

Rainfall data proceeding manuring up to last soil sampling:

Month	Year	Inches	Rainy days
December	1951	1.84	10
January	1952	4.10	07
February	1952	4.70	06
March	1952	0.23	01
April	1952	3.71	12
May	1952	11.54	19
June	1952	7.40	16

Appendix 3

Bandiruppuuwa Estate Plot No.42

	Series I 3 April 1952				Series II 19 April 1952				Series III 27 July 1952		
	NH_4-N	NO_3-N	NO_2-N	Total-N	NH_4-N	NO_3-N	NO_2-N	Total-N	NH_4-N	NO_3-N	NO_2-N
R1	19.5	8.0	0.4	280	18.1	2.0	0.3	338	16.3	2.0	0.02
RS1	30.8	1.0	0.1	307	23.3	1.5	0.04	376	18.4	1.0	0.10
RS2	17.8	3.0	0.5	224	17.1	1.0	0.01	266	14.6	1.0	0.08
RS3				270	6.9	1.0	0.04	231	9.3	2.0	0.05
S1	12.0	10.3	0.3	400	16.3	3.0	0.01	369	22.6	3.0	0.10
SS1	19.5	0.10	0.1	280	11.3	1.5	0.02	307	12.0	1.0	0.04
SS2	14.5	0.20	0.2	250	6.9	1.0	0.01	320	5.6	3.0	0.04
SS3				210	2.6	1.0	0.06	310	9.9	1.0	0.04

Appendix 3a

Transformation of soil nitrogen under coconuts (Location- Bandiruppuwa Estate, Sampling date: 6 August 1954 preceded by a period of wet weather)

Sample	Depth	Bases m.e./100 g	Fresh soil		Toluene treated		Clay %	Total N (ppm)
			NH_4-N ppm	NO_3-N ppm	NH_4-N ppm	NO_3-N ppm		
Lower end of Botanist's replanting block	0-9"	1.28	14.5	6.0	8.5	2.2	5.2	34
	9-18"	1.18	15.8	5.5	8.4	2.0	1.3	224
	18-30"	0.93	15.3	5.5	11.5	1.6	1.6	207
Upper end of Botanist's replanting block	0-9"	0.00	16.1	6.5	14.5	2.0	1.8	347
	9-18"	0.98	11.7	5.8	11.6	1.4	2.6	185
	18-30"	1.38	14.9	5.5	14.0	2.0	2.7	180
NPK plot 18	0-9"	1.53	15.9	5.9	11.0	2.2	6.6	403
	9-18"	2.08	15.5	6.5	11.6	2.4	1.5	286
	18-30"	2.15	15.5	6.0	7.2	1.6	9.7	347
Cover crop demonstration plot	0-9"	2.25	14.8	5.2	11.1	2.4	6.3	380
	9-18"	1.90	11.9	5.2	13.0	2.4	11.4	46O
	18-30"	1.38	14.3	5.2	11.1	1.2	1.1	341

Appendix 3b

Soil N studies Bandirippuwa Estate 1955 (A Period of dry weather),

Plot	Sample	NH_4-N (ppm)	NO_3-N (ppm)	Total-N (ppm)
18	Square Top. (0-9”)	12.4	0.6	427
	Square Sub. (9-18”)	10.1	0.8	390
	Square Sub. (18-30”)	12.7	0.6	336
2	Square Top (0-9”)	12.5	0.4	534
	Square Sub (9-18”)	10.2	0.8	420
	Square Sub (18-30”)	10.3	0.6	326
Bot.block	Square Top (0-9”)	2.2	2.3	281
	Square Sub (9-18”)	2.2	0.5	205
	Square Sub (18-30”)	0.0	0.0	124

Appendix 3c

Ratmalgara Estate, 1955

Plot	Sample	NH_4-N ppm	NO_3-N ppm	Total-N ppm	% Clay	Total exchangeable bases m.e./100 g
Plot 3	S.T. 0-9”	NA	NA	677	11.1	4.01
	S.S. 9-18”	14.21	2.5	721	8.5	3.76
Plot 8	S.T. 0-9”	14.30	1.4	607	4.8	1.71
	S.S. 9-18”	11.70	1.8	770	5.7	0.98
Plot 14	S.T. 0-9”	12.90	1.7	670	10.6	0.57
	S.S. 9-18”	15.50	1.6	457	7.1	0.81
Plot 23	S.T. 0-9”	13.30	1.9	634	10.1	0.71
	S.S. 9-18”	9.60	2.6	541	2.4	O.51
Plot 27	S.T. 0-9”	14.42	1.5	500	4.2	0.81
	S.S. 9-18”	10.80	1.7	392	7.0	0.69
Plot 35	S.T. 0-9”	12.60	1.9	429	10.3	2.09
	S.S. 9-18”	NA	1.5	NA	NA	NA

Reference C.R.I. Annual Reports, 1955 pp.22-23

Appendix 3d

Series	Soil depth	NH_4-N ppm	NO_3-N ppm
I	Top 0-9”	18.1	9.1
	Sub1 9-18”	8.6	7.9
	Sub2 18-27”	12.4	9.1
II	Top 0-9”	14.3	7.4
	Sub1 9-18”	15.6	6.9
	Sub2 18-27”	10.1	7.5
III	Top 0-9”	14.3	8.4
	Sub1 9-18”	9.7	7.6
	Sub2 18-27”	10.4	8.2

Ref. C.R.I. Annual Report 1956- p.20.

Appendix 4a

Soil N studies (1959) *Bracharia brizantha* Plot Agrostologist’s Division

Depth	Moisture %	NO_3-N ppm	NH_4-N ppm	pH	Total exchangeable bases m.e./100 g
0-9"	11.6	9.7	17.6	5.8	0.91
9-18"	4.2	8.0	15.4	5.4	1.04
18-27"	13.1	6.0	8.8	5.2	0.71
27-36"	6.2	5.5	9.9	4.9	NA
36-45"	9.2	5.5	7.7	4.9	1.45
45-54"	10.3	7.0	6.6	4.6	1.15
54-63"	11.9	5.5	9.9	4.8	1.04
63-72"	9.4	6.2	11.5	4.7	0.86
72-81"	9.6	6.2	11.5	4.7	0.89
81-90"	10.5	11.0	8.8	4.9	0.97
90-96"	10.9	12.0	4.9	4.6	1.02

Ref. C.R. I. Annual Report 1961 - p. 24.

Appendix 4b

Bandirippuwa Estate Opposite the Chemist's Laboratory

	Series 1		Series 2		Series 3	
Depth of Soil Profile	NO_3-N ppm	NH_4-N ppm	NO_3-N ppm	NH_4-N ppm	NO_3-N ppm	NH_4-N ppm
0-9"	7.0	16.6	5.5	22.4	6.25	12.8
9-18"	6.0	13.9	2.5	16.0	19.9	11.8
18-27"	1.3	16.0	2.5	16.0	5-5	10.1
27-36"	4.0	18.0	6.2	16.0	4.0	10.7
36-45"	2.5	17.1	2.7	14.4	9.0	26.2
45-54"	2.0	16.6	6.2	14.4	5.5	26.7
54-63"	2.7	16.0	2.5	19.3	5.5	19.8
63-72"	2.5	18.0	7.0	10.7	6.0	23.5
72-84"	4.0	17.1	2.0	18.0	2.5	28.9
84-96"	6.2	21.4	5.5	17.1	5.6	28.3
96-108"	8.8	14.9	NA	NA	NA	NA
106-120"	5.5	17.1	NA	NA	NA	NA
120-132"	6.2	22.4	NA	NA	NA	NA

Appendix 4

Chemical analysis of Pothykuluma Soils (0-9”)

Blocks	Total exchangeable bases m.e./100 g	Total-N ppm	NO_3-N ppm	NH_4-N ppm	Total-C g/kg	C:N
1	3.12	336	3.6	7.6	0.680	20.2
2	3.81	389	2.2	7.2	0.522	13.4
3	2.70	392	2.5	7.2	0.58I	14.8
4	5.03	480	3.0	8.6	0.757	15.8

Ref. C.R.I. Annual Report 1961- p.24.

Appendix 5a

Soil Nitrogen Studies on Iranaville Soils 1966 (Problem of "Yellowing")

Total, ammoniacal and nitrate nitrogen in Iranaville Soils.

Date of sampling	Treatment	Total-N ppm		NH_4-N ppm		NO_3-N ppm	
		Surface	Basal	Surface	Basal	Surface	Basal
16.9.1966	1	308.50	260.16	4.22	35.27	1.50	0.90
	2	291.60	234.90	0.90	8.96	29.60	2.25
	3	342.93	181.35	2.97	27.79	29.34	19.74
	4	411.12	1S2.09	4.90	24.20	0.75	12.83
	5	252.45	208.18	6.40	5.97	3.51	25.15
	6	228.22	165.17	8.68	5.48	0.50	0.50
	7	279.19	181.44	9.57	14.35	22.06	1.20
	8	268.52	154.51	15.54	5.08	1.50	14.83
	9	201.64	255.29	13.69	48.88	1.50	1.90
	10	311.35	134.31	27.2C	11.95	12.35	2.30
15.12.1966	1	166.35	146.79	5.43	5.11	0.401	1.66
	2	126.89	186.54	5.43	6.59	0.705	0.75
	3	159.47	189.81	7.49	3.49	3.000	0.61
	4	139.19	216.47	6.71	3.91	0.850	0.45
	5	146.39	208.92	5.62	2.39	1.054	1.50
	6	207.27	209.76	6.62	3.59	3.633	0.91
	7	153.44	194.70	4.20	4.32	1.661	1.75
	8	233.38	195.81	6.90	10.19	2.87	0.75
	9	160.10	209.32	9.01	2.69	2,266	1.36
	10	219.47	183.13	4.79	5.12	2,258	0.45

Samples of soils were collected before each quarterly application of fertilisers. Five individual samples were taken from each plot at random and bulked to form a composite sample.

Appendix 5b

No.1. Ammonium sulphate 5 lbs
Muriate of Potash 2.5 lbs
Saphos phosphate 2.5 lbs

No.2. Spdium nitrate 7 lbs
Muriate of Potash 2.5 lbs
Saphos phosphate 2.5 lbs

No.3. Calcium ammonium nitrate 5 lbs
Muriate of Potash 2.5 lbs
Saphos phosphate 1.5 lbs

No.4. Calcium ammonium nitrate 5 lbs
Muriate of Potash 2.5 lbs
Conc. Super phosphate 1.5 lbs

No.5. Sodium nitrate 7 lbs
Saphos phosphate 2.5 lbs
Muriate of potash 2.5 lbs

No.6. Cattle manure+ Muriate Potash 2.5 lbs
Conc. Super phosphate 1.5 lbs

No.7. Animal meal 5 lbs
Crushed fish 4 lbs
Crushed oil cake 15 lbs
Muriate of potash 2.5 lbs

No.8. Blood meal 6 lbs
Crushed fish 8 lbs
Bone meal 1.5 lbs
Muriate of potash 2.5 lbs

No.9. Calcium ammonium nitrate 5 lbs
Muriate of potash 2.5 lbs
Conc super phosphate 1.5 lbs
1 lb
1 lb
2 lbs

No.10. Foliar sodium nitrate 2 p
Saphos phosphate 2.5 lbs
Muriate of potash 2.5 lbs Borate
Copper sulphate
Kieserite

+ Cattle manure treatment: A pair of cattle tethered to a palm for 15 nights once in 6 months.

Appendix 6a

Nitrification studies- Inorganic nitrogen in Bandirippuwa Soil 1966

Date	NH_4-N ppm				NO_3-N ppm			
	Without Ca		With Ca		Without Ca		With Ca	
29.04.1966	16.88	14.38	12.52	12.0	7.12	7.88	4.56	5.37
	8.93		13.8		6.14		1.84	
	17.86		9.7		10.38		9.70	
5.5.1966	9.24	9.25	10.47	8.07	6.65	6.08	2.10	6.49
	8.64		6.37		4.37		6.15	
	9.86		7.36		7.23		8.23	
15.5.1966	8.24	8.15	3.59	4.67	5.37	5.32	4.34	6.83
	7.78		7.43		4.8		7.27	
	8.44		2.99		5.79		8.9	

Ref. Ceylon Coconut Quarterly- Vol 18 pp. 52-53.

Appendix 6b

Inorganic nitrogen in Iranaville Soil

Position	Date	NH_4-N ppm		NO_3-N ppm	
		Cattle manure	Inorganic fertiliser	Cattle manure	Inorganic fertiliser
Manure circle	14.6.19966	14.34	15.34	2.15	1.01
	23.6.1966	6.11	14.64	2.60	0.75
	4.7.1966	6.77	10.29	2.87	6.80
Centre of square	14.6.1966	8.13	3.30	0.78	1.05
	23.6.1966	5.73	5.23	0.44	0.50
	4.7.1966	8.54	9.92	1.23	0.69

Ref. Ceylon Coconut Quarterly Volume 18 pp.52-53.

Appendix 6c

Comparison of the nutrient status of soils cropped continuously with different pasture spp. 1971.

Grasses	Total-N ppm	NO_3-N ppm	NH_4-N ppm	Exch K m.e./100 g	CEC m.e./100 g
Weed	1053	6.27	27.26	0.26	2.66
Brichiaria brizantha	1395	15.99	8.71	0.31	2.94
Brichiaria miliformis	1336	12.5	9.2	0.35	3.48
Panicum maximum	1289	10.85	10.51	0.32	3.77

Ref: Ceylon Coconut Quarterly 23. P. 51.

Appendix 7

Soil nitrogen studies (Virgin Jungles) 1956

Locality	Layer	Total-N %	NO_3-N ppm	NH_4-N
Veherayaya	Top	0.0624	12.5	24.9
Veherayaya	Sub	0.0123	7.5	22.4
Dambakotayaya (Pit 2)	Top	0.1101	12.5	30.7
Anapallarna (4 M.P left hand side)	Top	0.1204	13.8	38.6
Harathgamuwa	Top	0.0782	22.0	34.4
Kataithivu	Top	0.1289	3.0	21.7
Marichchukkaddi	Top	NA	2.5	NA
Marichchukkaddi	Sub	NA	5.0	NA

Ref. C.R.I. Annual Report 1956. P.20

Soil nitrogen studies (Virgin Jungles) 1957

Locality	Layer	Total-N ppm	NO_3-N ppm	NH_4-N ppm
Marichchukkaddi (Mannar)	Top	270.1	0.75	5.88
Marichchukkaddi	Sub	267.4	0.60	7.06
Dambakotarayaya (Wellawaya)	Top	1128.4	2.20	11.77

Ref. C.R.I. Annual Report 1957. p.10.

Appendix 8

Soil nitrogen studies 1957- Galle District (yellowing problem)

Depth	pH	Total-N %	NH_4-N ppm	NO_3-N ppm
0-9”	5.0-5.5	0.115	40.0	59.5
9-18”	4.5-5.0	0.310	30.0	43.5
18-27”	5.5-6.0	0.392	20.0	177.5
27-36”	5.0-5.5	0.079	20.0	32.5
36-45”	5.5	0.067	40.0	61.3

Ref. C.R.I. Annual Report 1957. p.36.

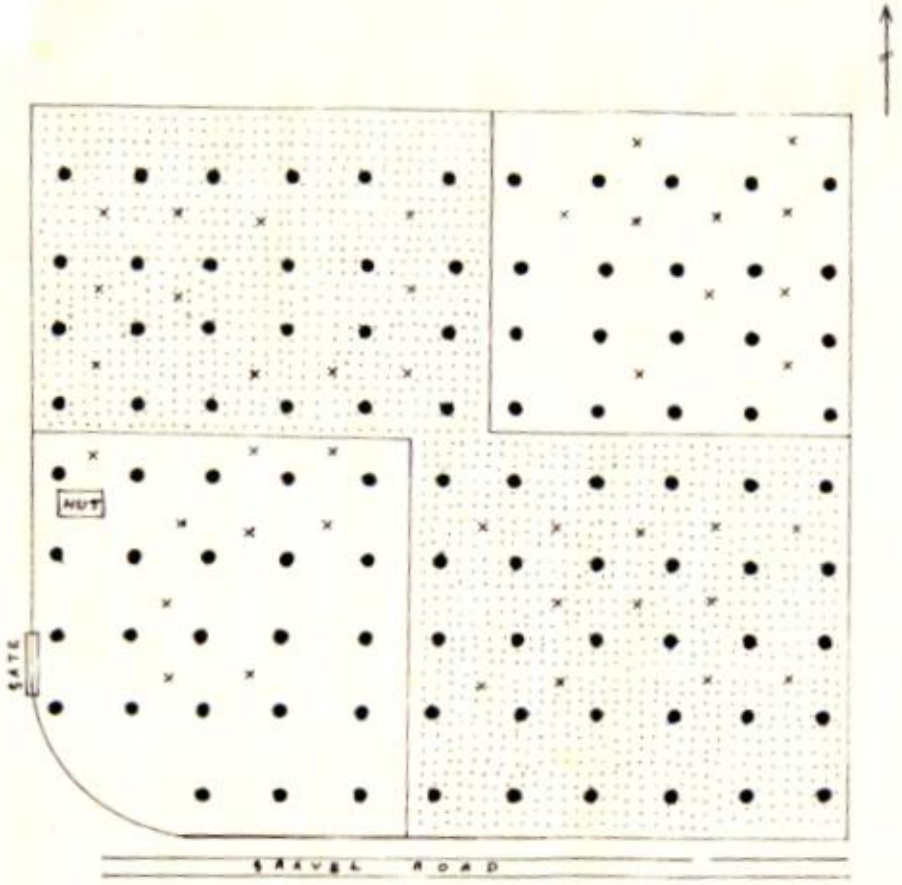
Appendix 9

Nitrogen Analysis of Soils from Walgama Estate (Series II Sampled on 12.05.1959).

Under healthy palms					
	Layer	Moisture %	Total-N ppm	NO_3-N ppm	NH_4-N ppm
Manure Circle					
8	0-9"	12.4	980	7.1	40.1
	9-18"	10.9	650	9.0	26.4
10	0-9"	12.8	1040	8.0	32.0
12	0-9"	14.9	1020	9.4	36.0
Square centre					
7	0-9"	13.8	1320	16.0	31.8
	9-18"	12.4	430	3.1	25.0
9	0-9"	15.2	1220	6.5	39.5
11	0-9"	15.6	1290	9.5	42.9
Under Affected Palms (Yellow palm)					
Manure circle					
Sample No					
2	0-9"	14.5	1230	7.3	24.3
	9-18"	10.4	330	7.0	6.7
4	0-9”	15.9	1150	13.4	24.8
	9-18”	13.9	870	10.2	8.2
6	0-9”	15.0	1020	11.8	23.2
	9-18”	12.1	660	11.4	6.9
Square centre					
1	0-9”	14.7	1290	9.4	28.3
	9-18”	12.7	620	10.0	21.6
3	0-9”	16.7	920	12.0	25.0
	9-18”	13.2	480	9.2	13.3
5	0-9”	16.2	1110	9.5	29.5
	9-18”	15.2	640	9.5	26.0

Ref. C.R.I Annual Report 1959. p.25.

Appendix 10



PASTURE (Brachiaria miliiformis)

COCONUT PALM

SITE OF SAMPLING

Appendix 11

Rainfall data (mm) for the experimental site during the nitrification studies at Ratmalagara Research Station 1980

Dates	July	August
1	2.0	0.0
2	7.1	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
6	0.0	0.0
7	0.0	0.0
8	0.0	0.0
9	0.0	3.6
10	0.0	0.4
11	0.3	0.6
12	2.4	0.0
13	1.2	0.5
14	1.3	0.7+
15	0.0	3.2
16	0.0	0.0
17	0.0+	5.9
18	0.0	5.2
19	0.0	1.9
20	0.0	0.0
21	0.0	0.0
22	0.0	0.0
23	0.0	0.0
24	0.0	0.0
25	0.0	2.4
26	0.0	2.6
27	0.0	0.0
28	0.0	0.0+
29	0.0	0.0
30	0.0	0.0
31	0.0+	0.0
Total rainy days	6	11
Total rainfall	14.3	31.2

+ Date of soil sampling