5. SUMMARY

This study on heterotrophic N\textsubscript{2} fixation in tropical Indian rice soils employing \textsuperscript{15}N-tracer and acetylene reduction assays is concerned with the following aspects:

- Influence of carbon substrates, combined N and water regimes on N\textsubscript{2} fixation in several rice soils and rice rhizosphere
- Influence of pesticides on bacterial N\textsubscript{2} fixation
- The transfer of biologically fixed N from the soil to the rice plant.

Studies involving labelled nitrogen gas (\textsuperscript{15}N\textsubscript{2}) showed that indigenous N\textsubscript{2} fixation was low, but detectable in several paddy soils especially in submerged laterite, alluvial and acid sulphate saline (Pokkali) soils. In all subsequent experiments, soils were supplemented with cellulose to accelerate N\textsubscript{2} fixation in the soils.

Of the organic sources, cellulose, in particular, and rice straw accelerated N\textsubscript{2} fixation in all soils under submerged conditions. Likewise, N\textsubscript{2} fixation under submerged conditions was stimulated by sucrose, glucose and malate in that order in alluvial soil, by sucrose alone in laterite soil and by none of these sources in more acidic soils. Microbiological analysis
indicated that the population of $\text{N}_2$-fixing microorganisms was low in more acidic soils compared to other submerged soils. Under nonflooded conditions, $\text{N}_2$ fixation was stimulated by cellulose, sucrose and glucose in alluvial and acid saline (Karapadam) soils, by cellulose in laterite soil and by rice straw in acid sulphate saline (Pokkali) soil. The stimulatory effect of sucrose on $\text{N}_2$ fixation in alluvial soil especially under nonflooded conditions could perhaps be due to the greater invertase activity in nonflooded alluvial soil leading to increased glucose availability.

$\text{N}_2$ fixation in two of the three soils under submerged and nonflooded conditions increased progressively with increasing levels of cellulose upto 2 per cent and declined thereafter. But, in an acid saline (Karapadam) soil under nonflooded conditions, $\text{N}_2$ fixation increased only upto 1 per cent of cellulose.

Added $\text{N}$ to paddy soils significantly stimulated the $\text{N}_2$-ase activity until 10 ppm level and inhibited it above 10 ppm under both submerged and nonflooded conditions. $^{15}\text{N}$-tracer technique studies showed that $\text{N}_2$ fixation decreased with a progressive increase in the concentration of added $\text{NH}_4^+$-N in paddy soils under both water regimes. This inhibition was more pronounced
under nonflooded conditions. In contrast, in acid sulphate (Pokkali) and acid saline (Karapadum) soils, $N_2$ fixation was completely suppressed even by the low concentrations of added $N$ irrespective of water regimes. The decrease in $N_2$-fixing activity in soils was correlated with a gradual increase in soil solution $NH_4^+$-N.

$^{15}N_2$ incorporation was higher in soil samples from rice rhizosphere than in non-rhizosphere soil. Rhizosphere samples from rice straw amended (3 and 6 t/ha) soil exhibited more pronounced $N_2$-fixing activity than the samples from unamended soil. The $N_2$-fixing activity of rhizosphere samples from soils receiving combined $N$ (40 and 80 kg $N$/ha) was relatively low. However, the inhibitory effect of combined $N$ on $N_2$ fixation was alleviated in the presence of rice straw (6 t/ha).

Rice straw amendment to alluvial and laterite soils enhanced the populations of *Azotobacter* and anaerobic $N_2$-fixing microorganisms under both water regimes, especially under submerged conditions. In contrast, in an acid sulphate saline (Pokkali) soil, rice straw application decreased their populations under both water regimes.
Rice straw amendment increased the population of facultative symbiotrophic $N_2$-fixing microorganisms (which seldom fix $N_2$ in pure cultures but readily do so in mixed cultures) and their $N_2$-fixing activity in submerged and nonflooded soils. *Pseudomonas* sp., *Mycobacterium* sp., *Arthrobacter* sp., *Bacillus* sp., and *Azospirillum* sp. were predominant in these associations.

Almost all rice soils screened harboured *Azospirillum* sp. The occurrence of *Azospirillum* sp., though in low numbers, in an extremely acid sulphate saline, Kari (pH 3.2) soil indicated their tolerance to extremes of acidity and salinity. Submerged soils harboured higher population of *Azospirillum* than non-flooded soils. Rice straw amendment considerably enhanced the population of *Azospirillum* sp. in submerged alluvial, laterite and acid sulphate saline (Pokkali) soils but not in Kari soil. Moreover, excepting from Kari soil, *Azospirillum* cultures isolated from all other rice straw amended flooded and nonflooded soils exhibited higher $N_2$-fixing activity.

A relationship existed between soil pH, Eh and the $N_2$-fixing activity of *Azospirillum* cultures isolated from different soils. Thus, several enrichment cultures isolated from soils with a low pH ($< 5.0$) possessed lower
$N_2$-fixing activity than cultures from soils of higher pH. Similarly, enrichment cultures originating from flooded soils with relatively low redox potentials (-50 to -150 mV) showed higher $N_2$-fixing ability in vitro than enrichment cultures from soils with higher potentials. Moreover, the increased activity of the cultures from rice straw amended alluvial, laterite and acid sulphate saline (Pokkali) soils over the cultures from respective unamended soils was apparently related to lower potentials of amended soils.

Benomyl, a fungicide, increased the population of *Azospirillum* under flooded conditions. *Azospirillum* cultures isolated from benomyl amended soil, in general, possessed higher $N_2$-fixing activity than the cultures from unamended soils. Likewise, 2-AB, a degradation product of benomyl, increased the population of *Azospirillum* in contrast to a decrease in the symbiotrophic $N_2$ fixers. Interestingly, *Azospirillum* cultures from 2-AB amended soil possessed lower $N_2$-ase activity vis-a-vis the higher $N_2$-ase activity of symbiotrophic $N_2$-fixing microorganisms from 2-AB amended soil as compared to respective cultures from unamended soil.

$^{15}N$ studies showed that the grain derived a major portion of the biologically fixed $N$ in the soil followed
by the roots and the leaves. A substantial portion of the biologically fixed N remained in the soil after harvest while about 40 per cent of the fixed N was not accounted for.

The important conclusions of the present study are:

- Soil submergence increased populations of $N_2$ fixers and their $N_2$-fixing activity.
- Carbon substrates like cellulose and rice straw stimulated the population of $N_2$ fixers and their $N_2$-fixing activity in paddy soils.
- High levels of combined N inhibited and low levels stimulated $N_2$ fixation in paddy soils.
- Rice rhizosphere soil amended with combined N exhibited low activity while this inhibition was alleviated by the addition of high levels of organic matter.
- Application of pesticides to a paddy soil exerted differential response on the $N_2$-fixing population and $N_2$-ase activity.
- Biologically fixed N was transferred from the soil and distributed in different parts of rice plant.