MATERIALS AND METHODS

Treatments and design

The experiment was carried out on San sai soil (sandy loam, PH 5.7-5.9, 0.05-0.06% total nitrogen) at the Multiple Cropping Center Experiment Station of Chiang Mai University (19 ^{o}N, 99 ^{o}E) from August 1988 to April 1989, The climate of Chiang Mai is characterized by definite wet and dry seasons. Precipitation during the wet season (May to October) totals 1100 mm on average; temperatures during this time are high (26 to 28 ^{o}C, daily means). The dry season (November to April) is, for the most part, cooler; rainfall totals 150 mm.

The nine treatments included a factorial combinations of nitrogen fertilizer applied to the rice (*Oryza sativa* L. cv RD21) at levels of 0 (R0), 100 (R100), 300 (R300) kg N/ha and to the following soybean crop (*Glycine max* L. Merrill cv SJ5) at 0 (S0), 25 (S25), 50 (S50) kg N/ha. The experimental design was a randomized complete block with six replications. Rice was transplanted on 19 August 1988. Hills were arranged on a 25 x 25 cm lattice; there were 2 to 3 seedlings/hill. Each plot was 4 x 9 m with small earthen bunds separating the plots. Nitrogen was applied to the rice as urea in two applications, three

weeks after transplanting (70%) and just prior to panicle initiation (30%). A basal fertilizer, containing 50 kg K_2O /ha (as potassium sulphate) and 50 kg P_2O_5 /ha (as triple superphosphate) was applied to the rice three weeks after transplanting.

After the rice was harvested and threshed on 17 November 1988, the rice straw was spread over the field and burnt, according to local farmer practice. The various levels of `starter´ nitrogen were broadcasted into the plots. The field was then flood irrigated. Soybean seed was inoculated with *Bradyrhizobium japonicum* (Commercial peat inoculum) and sown by hand into moist soil on 20 December 1988. The seeds as sown at the rate of five per hill; hills were arranged in the same 25 x 25 cm lattice as for the rice. Once sowing had been completed, the field was sprayed with a pre-emergent herbicide, Alachor. Two weeks after emergence, plants were thinned to three per hill. The crop was irrigated as required.

Sampling

Soil

From each plot a composition sample was collected (to 30 cm) and analyzed (2 mM Potassium choloride) for available nitrogen at soybean sowing and when the crop had reached early seed development stage (R5).

Rice

Rice was sampled for assessments of growth and grain production. Total dry matter and grain yield were measured from harvesting area 2 m^2. Plant samples were dried under sunshine, then weighed and threshed to obtain the grain yield. Grain and straw samples were dried, chopped and ground, subsampled for analysis total (Kjeldahl) nitrogen. Plant nitrogen content was estimated from the total nitrogen (grain nitrogen plus straw nitrogen) over the total dry matter weight.

Soybean

On 6 occasions during growth a 1 m^2 quadrat was sampled from each plot for assessments of nodulation, crop growth and nitrogen fixation, the latter based on analysis of xylem sap for nitrogen solutes (Peoples *et al.*, 1989). Sampling commenced in vegetative growth (V6) with final sampling at physiological maturity (R7) (Fehr *et al.*, 1971). Sets of plants were sampled by first removing the shoots and leaving the root stumps Xylem sap and nodulation data were collected from at least 10 plants. Xylem sap was collected as root-bleeding sap from the root stumps (Peoples *et al.*, 1989), and placed immediately on ice then frozen at -10 ^{o}C for later analysis. The roots were dug from within

each sampling area, nodules were removed from the roots, dried at 80 ^{o}C for 48 hours and weighed. The sample of shoots were dried to constant weight at 80 ^{o}C, weighed and analyzed for total (Kjeldahl) nitrogen. At maturity (R8), seed yields were estimated from harvest areas of 2 m^2, subsamples of seed and straw were analyzed for total nitrogen. Fallen leaves were collected through the growing period and dried at 80 ^{o}C for 48 hours, weighed and analyzed total nitrogen.

Chemical analysis, determinations of plant nitrogen derived from nitrogen fixation (Pfix)

Concentrations of ureides (allantoin and allantoic acid) in root-bleeding sap were estimated colorimetrically as the phenylhydrazone of glyoxalate. Nitrate was measured by the salicylic acid technique. The amino-nitrogen content of sap was determined colorimetrically with ninhydrin, using a 1:1, asparagine:glutamine standard (Peoples *et al.*, 1989)

The relative abundance of ureide-N in sap was calculated as:

$$\text{Relative ureide-N (RU, \%)} = [4a/(4a+b+c)]x100 \quad [1]$$

where a, b and c are respectively the molar

concentrations of ureides (ureides contain 4 nitrogen atoms per molecule), nitrate and amino-acid-N (Herridge, 1984). Calculation of the proportion of plant nitrogen derived from nitrogen fixation was based on regressions established from glasshouse calibrations (Peoples *et al.*,1989) as follows:

$$P\ fix\ (\%) = 1.21 \times (RU - 4.8) \qquad [\ 2\ ]$$

for plants in vegetative and flowering stages (up to R2)

$$P\ fix\ (\%) = 1.49 \times (RU - 21.3) \qquad [\ 3\ ]$$

for plants during pod-fill (reproductive stages of development after R2)

where RU is the % relative abundance of ureide-N in root-bleeding sap (Peoples *et al.*, 1989).