Research Article

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Estimates of nitrogen fixation by legume cover crops grown in young rubber plantations using ^{15}N isotope dilution technique

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Abstract

^{15}N Isotope dilution technique has been used to estimate the amounts of N_2 fixed by leguminous cover crops, *Pueraria phaseoloides* and *Mucuna bracteata* grown in the inter-rows of immature rubber plantation, using *Ipomoea batatas* as the reference plant. The field experiment was conducted in the RRII farm during 2009, in an area planted with *Hevea brasiliensis*, clone RRII 105. The cover crops and the reference plant were established in separate blocks. For labeled urea application, micro-plots (1m x 1m) were established and ^{15}N labeled urea, 5 atom % ^{15}N excess, was applied @ 5 kg ha^{-1}. After 45 days, the above ground biomass from the micro-plots was harvested, weighed and sub-samples were collected for determination of dry matter accumulation, N content and ^{15}N enrichment. Micro-plot experiments were conducted thrice during the active growth period of the legumes and the reference plant, *i.e.*, during June-July, August-September and October-November. Estimates of N_2-fixation for *P. phaseoloides* ranged from 79.82 kg N ha^{-1} during June-July to 39.32 kg N ha^{-1} during October-November and averaged 58.1 kg N ha^{-1}. Corresponding estimates for *M. bracteata* ranged from 83.95 kg N ha^{-1} during June-July to 57.03 kg N ha^{-1} during October-Nov and averaged 69.7 kg N ha^{-1}. The study showed that both the cover crops fixed considerable quantities of atmospheric nitrogen and that N_2 fixation by *M. bracteata* was higher than that of *P. phaseoloides.*

Keywords: N_2 fixation, ^{15}N isotope dilution technique, *P. phaseoloides, M. bracteata*

Introduction

Establishment of leguminous cover crops in young rubber plantations is a common agronomic practice and its beneficial effects on growth and yield of rubber are well documented. Leguminous cover crops fix atmospheric nitrogen, enrich soil with organic matter, improve soil fertility, reduce soil erosion and suppress weed growth.

Major advantage of growing legume cover crops is their ability to fix atmospheric nitrogen in the root nodules in association with a group of bacteria belonging to the genus *Bradyrhizobium.* Legume cover crops fix substantial quantity of atmospheric nitrogen and thus help in reducing the use of costly nitrogenous fertilizers (Broughton, 1977, Punnoose *et al*., 1994). By reducing the inputs of fertilizer N, legumes reduce the cost of production and the potential for N contamination of water resources. Leguminous plants provide the major N input into the biosphere as a result of their ability to convert atmospheric N (N_2) to a form that can be assimilated by plants (Hardarson and Atkins, 2003). Legume covers help in enhancing the growth of rubber during immature phase and reduce the immaturity period (Watson, 1961; Broughton, 1977; Mathew *et al*., 1989; Punnoose *et al*., 1994). *Pueraria phaseoloies* and *Mucuna bracteata* are the two common cover crops grown in rubber plantations in India.

Large scale production of nitrogenous fertilizers is no longer economically feasible, and this commodity will soon become too costly for wide usage in agriculture. Improved use of symbiotic N fixation in legumes could be the key to sustaining agricultural productivity with reduced dependence on the non-renewable resources (RRIM, 1983).

The quantity of nitrogen fixed by *P. phaseoloides* and *M. bracteata* grown in association with

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immature rubber in the traditional rubber growing tracts of India has not been estimated by the isotope method. In the present work, ^{15}N isotope dilution method has been used to estimate the quantity of N_2 fixed by these legumes grown in the inter-rows of immature rubber plantation, using *Ipomoea batatas* as the reference plant.

The ^{15}N isotope dilution method involving the application of small quantity of ^{15}N enriched fertilizer to the soil, has been widely used for quantitative assessment of symbiotic nitrogen fixation under field conditions (Fried and Middelboe, 1977; Witty, 1983; Carlsson and HussDanel, 2003). N_2 fixing plants will absorb atmospheric N supplied by the root nodule symbionts in addition to the isotope-enriched N from the soil. Since the non-fixing plant (reference plant) do not have access directly to atmospheric N_2, the resulting $^{15}N/^{14}N$ ratio of tissue N in the two species will differ in proportion with the amount of N_2 fixed (Hardarson and Atkins, 2003: Danso, 1992). Measurements of the extent to which the N_2 fixing plant dilutes the ^{15}N enriched N taken up from the soil allows quantification of the fixed N_2.

Materials and methods

A field experiment was conducted in the Rubber Research Institute of India (RRII) farm during the year 2009, in an area planted with *Hevea brasiliensis* clone RRII 105 in the second year of planting. The soil of the experimental area was sandy clay loam, with a pH of 4.4, organic carbon 1.86 per cent, available P 3.5 mg kg^{-1} and available K 26.6 mg kg^{-1}. *Pueraria phaseoloides, Mucuna bracteata* and *Ipomoea batatas* were established in the inter-rows of rubber plants in separate blocks, after planting rubber. Seed rate of legumes was about 4 kg ha^{-1} for *P. phaseoloides* and 200 g ha^{-1} for *M. bracteata.* Legume seeds were not inoculated with *Bradyrhizobium* prior to sowing. Uniform cuttings of *I. batatas,* (10 cm length), collected from Central Tuber Crops Research Institute, Trivandrum, were planted, at the rate of three cuttings per planting point, spaced 15 cm apart. Recommended doses of NPK fertilizers were applied to both cover crops and reference plant. *I. batatas* was established again during the second year by planting fresh cuttings.

For labeled urea application, micro-plots (1m x 1m) were established by erecting GI sheets on the boundaries, inserted 6 cm into the soil in between two rows of rubber plants. There were seven replicate micro-plots for each legume and the reference plant. ^{15}N labeled urea, 5 atom per cent ^{15}N excess, @ 5 kg ha^{-1} was applied in each micro-plot. Labeled urea was dissolved in 1 liter water and then poured with a watering can (Ikram *et al.*, 1995). The area outside the micro-plot was fertilized with equivalent amounts of non-labeled urea. The above ground biomass from the ^{15}N applied micro-plots was harvested 45 days after the application. Fresh biomass was weighed at the site and sub samples were collected for estimating dry matter accumulation and N content. The plant samples were dried at 70 °C in an air draught oven, weighed for dry matter determination, powdered and analyzed for tissue N and atom per cent ^{15}N. Total N in plant tissue was determined by micro-Kjeldahl method and N isotope ratio by Mass Spectrometry (Jensen, 1991).

The micro-plot experiment with labeled urea was carried out thrice during the active growth period of the cover crops and the reference plant, *i.e.,* during June-July, August-September and October-November.

The percentages of plant N derived from the atmosphere *(%Ndfa),* from labeled N *(%Ndfl),* and the amount of nitrogen fixed (kg ha^{-1}) were calculated from ^{15}N derived data using equations 1, 2 and 3, respectively (Fried and Middelboe, 1977). The data were subjected to analysis of variance.

Calculations

$$\%\,Ndfa = 1 - \left[\frac{atom\,\%\ ^{15}N\ excess\ in\ fixing\ plants}{atom\,\%\ ^{15}N\ excess\ in\,non-fixing\ plants}\right] \mathrm{x}100 \quad \ldots 1$$

where % *Ndfa* = %N derived from atmosphere

$$\%\,Ndfl = \left[\frac{atom\,\%\ ^{15}N\ excess\ (plant)}{atom\,\%\ ^{15}N\ excess\ (labelled\ N)}\right] \mathrm{x}100 \quad \ldots 2$$

where *%Ndfl* = %N derived from labeled N

$$\text{Nitrogen fixed (kg ha}^{-1}\text{)} = \frac{\%Ndfa}{100}\ X\,N\,yield \quad \ldots 3$$

Results and discussion

Above-ground biomass (dry matter), N content, and N yield in biomass of legumes and reference

Table 1. Above ground biomass (dry matter), nitrogen content and N yield in biomass of legumes and reference plant

Crop	Above ground biomass (kg ha^{-1})	N content of biomass (%)	N yield in biomass (kg ha^{-1})
June - July			
Pueraria phaseoloides	3601.7	3.11	112.0
Mucuna bracteata	3490.0	3.26	113.9
Ipomoea batatas	2018.5	2.66	53.5
SE	234.0	0.09	7.71
CD	1006.5	0.39	33.3
August - September			
Pueraria phaseoloides	2951.3	3.10	91.2
Mucuna bracteata	3178.2	3.22	102.4
Ipomoea batatas	1777.2	2.64	47.4
SE	167.5	0.07	5.49
CD	723.7	0.31	23.7
October -November			
Pueraria phaseoloides	2872.8	3.20	91.9
Mucuna bracteata	2869.4	3.28	94.5
Ipomoea batatas	992.0	2.56	25.6
SE	110.5	0.10	4.44
CD	477.5	0.42	19.18

plant are shown in Table 1. Above-ground biomass was comparable for the two legumes, *P. phaseoloides* and *M. bracteata,* during all the three harvests while it was significantly lower for the reference plant, *I. batatas*. N content and N yield of above ground biomass were also comparable for the two legumes, and significantly lower for the reference plant.

^{15}N enrichment in plant tissue (Table 2) was significantly lower for the legumes than the enrichment in the reference plant, irrespective of time of harvest, indicating a dilution of the legume plant N by non-labeled N from the atmosphere, which is a clear indication of nitrogen fixation by the legumes. The proportion and amount of total N derived from labeled N *(%Ndfl)* was significantly lower for the legumes compared to the reference plant, during all the three harvests and consequently, the uptake of non-labeled N was significantly higher for the legumes. During the third harvest, *%Ndfl* and uptake of labeled N were significantly higher for *P. phaseoloides* compared to *M. bracteata* and the reference plant *I.batatas*, indicating a reduction in isotope dilution by fixed N_2.

Table 2. ^{15}N enrichment in plant, N uptake from labeled urea and from soil, and non- labeled N in the plant

Crop	Atom % ^{15}N excess in plant	% N derived from labeled N *(%Ndfl)*	Labeled N uptake (kg ha^{-1})	Uptake of non-labeled N (kg ha^{-1})
June-July				
Pueraria phaseoloides	0.0745	1.49	1.63	110.40
Mucuna bracteata	0.0712	1.42	1.66	112.27
Ipomoea batatas	0.2872	5.75	3.07	50.46
SE	0.14	0.51	0.31	7.63
CD	0.11	1.57	0.94	23.52
August-September				
Pueraria phaseoloides	0.0793	1.59	1.46	89.73
Mucuna bracteata	0.0672	1.34	1.39	101.00
Ipomoea batatas	0.2034	4.06	1.92	45.50
SE	0.006	0.12	0.21	5.31
CD	0.03	0.39	NS	16.37
October -November				
Pueraria phaseoloides	0.1034	2.06	1.87	90.03
Mucuna bracteata	0.0704	1.41	1.33	93.15
Ipomoea batatas	0.1804	3.61	0.92	24.63
SE	0.006	0.13	0.08	4.41
CD	0.03	0.39	0.24	13.58

Table 3. Per cent N derived from atmosphere (*%Ndfa*) and quantity of N_2 fixed by legumes

	% Ndfa	N_2 fixed (kg ha^{-1})
June-July		
Pueraria	71.07	79.82
Mucuna	73.27	83.95
t stat	NS	NS
August –Sept.		
Pueraria	60.77	55.36
Mucuna	66.99	68.34
t stat	NS	NS
Oct-Nov.		
Pueraria	42.96	39.32
Mucuna	60.13	57.03
t stat	4.01 **	2.90 **

** Significant at $P<0.01$

Per cent N derived from atmosphere (*%Ndfa)* and estimated N_2-fixation for the two legumes studied are shown in Table 3. *%Ndfa* for *P. phaseoloides* ranged from 71.1 per cent of total plant N during June-July, to 42.96 per cent during October-November period. Corresponding estimates of N_2-fixation for *P. phaseoloides* ranged from 79.82 kg N ha^{-1} during June-July to 39.32 kg N ha^{-1} during October-November and averaged 58.1 kg N ha^{-1} year^{-1} (Table 4). *%Ndfa* for *M. bracteata* ranged from 73.27 per cent of total plant N during June-July to 60.13 per cent during October-November and corresponding estimates of N_2 fixation ranged from 83.95 kg N ha^{-1} during June-July to 57.03 kg N ha^{-1} during October-November and averaged 69.7 kg N ha^{-1} year^{-1}.

Table 4. Estimate of N_2 fixation by legumes (kg ha^{-1})

	June-July	Aug-Sept.	Oct-Nov	Mean
Pueraria	79.8	55.4	39.3	58.1
Mucuna	84.0	68.3	57.0	69.7

The amount of N_2 fixed in the above-ground biomass showed a decrease from the first to the third harvest for both the legumes. This is more pronounced in the case of *P. phaseoloides*, which may probably be due to the higher litter production of this legume during this period. In this study, the amount of N_2 fixed in litter has not been estimated because the litter is decomposing with constantly changing amounts of labeled and non-labeled N (Ikram *et al*., 1995).

In our study, N_2 fixation by *M. bracteata*, was higher than that of *P. phaseoloides,* and the difference was significant during the October-November period. Kothandaraman *et al*. (1993) observed that the population of *Bradyrhizobium* which could nodulate *P. phaseoloides* in acid soils was low, and the effectiveness of nodules produced was also found to be low. Nitrogen fixation by *P. phaseoloides* observed in this study, *i.e.,* 58.1 kg N ha^{-1} is comparable with the estimate of 49.0 kg N ha^{-1} given by Ikram *et al*. (1995), estimated by ^{15}N isotope dilution method using *Ipomea batatas* as reference plant, in a Malaysian oxisol. Isotopic estimates of nitrogen fixation by *M. bracteata* are not reported, but significantly higher N-accumulation in biomass compared to *P. phaseoloides* of the same age has been reported (Philip *et al.*, 2005).

The study showed that both the cover crops fixed considerable quantities of atmospheric nitrogen and that N_2 fixation by *M. bracteata* was higher compared to that of *P. phaseoloides.*

Although cover crop has long been a component of *Hevea* cultivation, its importance has diminished in recent years due to a shift towards intercropping in immature phase. However, for rubber agro-ecosystem to remain productive and sustainable in the long term, it will be necessary to replenish the resources of organic matter and nutrients which are removed or lost from the soil, through renewable resources.

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