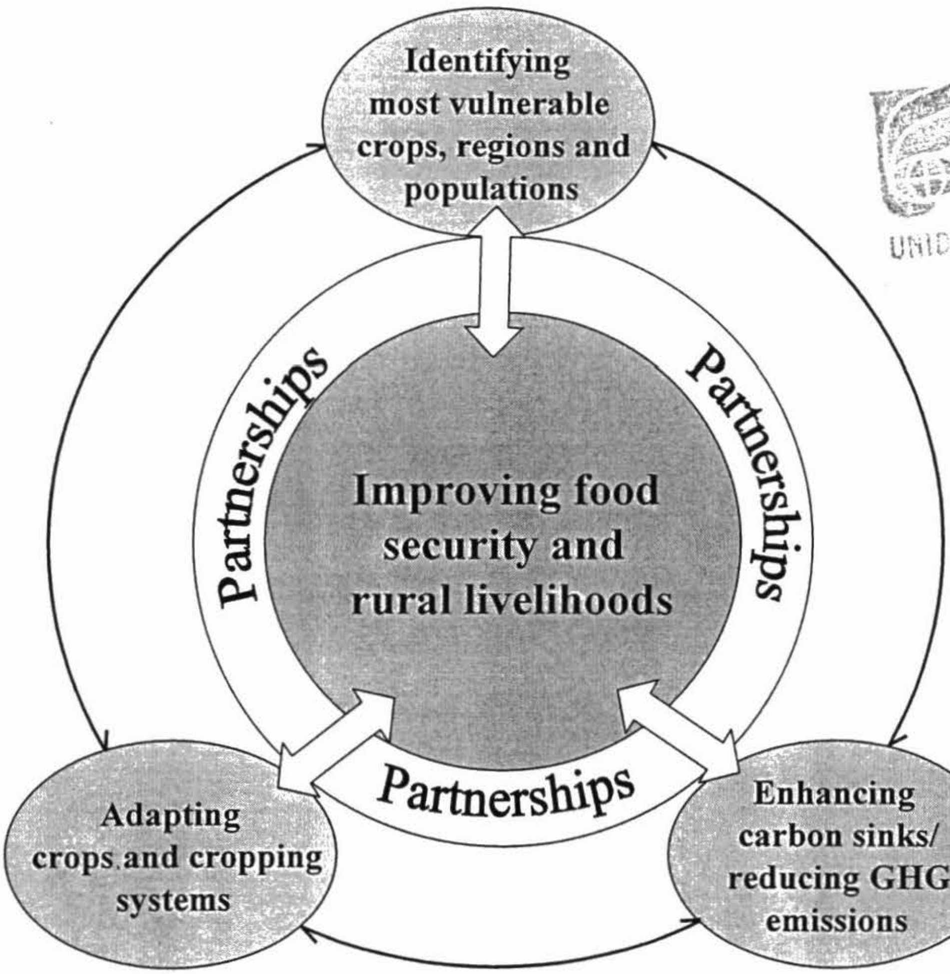
ANNUAL REPORT 2003

PROJECT PE-6

Confronting Global Climate Change



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CIAT: PE-6 Project Log Frame (2003-2005)

Project: Confronting Global Climate Change
Project Manager: Marco Rondón

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Goal To contribute to long-term increases in agricultural productivity, poverty reduction, and conservation of the global environment.	Agricultural production increased. Farmers' income increased. Agriculture-related emissions of greenhouse gases (GHG) reduced. Water production levels maintained or increased.	National statistics of agricultural production and rural income. National and international inventories of GHG. National and regional inventories of water resources.	
Purpose To overcome expected reductions in productivity of some major food crops and forages as a consequence of global climate change, while reducing the environmental impact from agriculture in ecoregions within the scope of CIAT's mandate.	Net increase in agricultural productivity resulting from adoption of climate change (CC)-adapted crops. Net reduction in the global warming potential (GWP) of key ecoregions: tropical lowlands, hillsides, and Andes.	National and regional statistics of food and forage production. Regional and national inventories of GHG compared over time.	NARS partners show interest in collaborative research. Adequate funds from global challenge programs (climate change, water) allocated. Favorable policies for the release and adoption of new crop and forage varieties. Timely implementation of policy and trading incentives to favor adoption of environmentally safe management practices.
Outputs 1. Vulnerability and opportunity assessments of responses of ecoregions, populations, crops, and wild relatives of crops in crisis from changing climates. 2. Germplasm and management systems adapted to changing climatic conditions and exacerbated incidence of pests and diseases. 3. Crop, forage, water, and soil management strategies developed to minimize sources and/or increase sinks of GHGs. 4. Impact of implemented strategies for adaptation to and mitigation of GCC assessed, and institutional capacity enhanced.	Maps of risk of yield decline (maize, beans, cassava) for Africa and Latin America. Maps of risks of loss of habitat for wild relatives of crops. Adoption of drought-adapted crop and forage varieties as key components of production systems that minimize crop failures. Pilot testing of developed methodologies in at least three benchmark ecoregions: tropical lowlands, hillsides, and Andes. Implementation of a pilot project for trading C sequestered in soils and/or biomass. Studies to assess economic benefits of adopting drought-tolerant beans and pastures in LA. Study prepared on scenarios for potential C trading in improved pastures and no-tillage cropping systems. One BSc and two MSc theses submitted.	Maps available. Information transferred to policy makers. Field verification. Project reports. National average of yields in dry seasons. National GHG inventories. Pilot contract for C trading. Studies transferred to policy makers.	Active participation of germplasm development projects. Access to benchmark sites continued. Continued commitment of local partners to project activities. Successful involvement NARS partners for release of new varieties. Approval of the CDM Successful involvement of suitable partners experienced in C trading.

Main Highlights of Research Progress in 2003

Output 1.
Vulnerability and opportunity assessment developed of the response of critical ecoregions, populations, crops and crop wild relatives to changing climates.

- Found that maize yields in Colombia could be expected to decrease as a consequence of climate change in the coming decades.

Output 2.
Germplasm and management systems adapted to changing climatic conditions and exacerbated incidence of pests and diseases.

Developing bean germplasm tolerant to drought

- Breeding lines with commercial grain type and selected for tolerance to terminal drought in previous years also expressed tolerance to intermittent drought, exceeding the commercial checks by 200% or more in yield. New drought tolerant lines show up to 42% yield improvement over local commercial cultivars under both stress and non-stress conditions in Eastern Africa.
- Field evaluation of 16 elite lines showed that a bred line (SEA 15) was outstanding in its adaptation to drought stress conditions. The superior performance of this bred line under drought stress in comparison with 15 other elite parents of recombinant inbred lines (RILs) was associated with lower seed ash (mineral) content indicating efficient utilization of acquired nutrients for grain production.

- Among the 95 advanced lines of the cross BAT 881 × G 21212, three lines (BH 21134-9-1-1-M-M-M-M; BH 21134-154-1-1-M-M-M-M-M; BH 21134-97-1-1-M-M-M-M-M) were superior in their adaptation to drought stress conditions. The superior performance of these three lines was related to lower levels of seed ash (mineral) and seed P indicating the usefulness of these traits for selection for drought adaptation in common bean.

2.2 Identify genotypes of grasses and legumes with dry season tolerance

- Showed that the superior performance of one germplasm accession (CIAT 26110) and one hybrid (FM9503-S046-024) of *Brachiaria* which maintained greater proportion of green leaves during dry season in the Llanos of Colombia, was associated with greater acquisition of nutrients under water deficit conditions.

Output 3.
Crop, forage, water and soil management strategies developed to minimize sources and/or increase sinks of GHG.

3.1 The biological phenomenon of nitrification inhibition in *Brachiaria humidicola* and other tropical grasses

- Found substantial differences in total and specific NI (nitrification inhibition) activity among tropical grasses.

- Developed and tested a simple protocol to quantify the impact of addition of root exudates from plants on nitrification inhibition of incubated soils.

3.2 Silvopastoral multistrata systems to improve cattle productivity and reduce GHG emissions.

- Found that the conversion of traditional grass only pastures in the seasonally dry savannas of the Caribbean region in Colombia, to grass- forage shrubs or grass-trees silvopastoral systems results not only in increases in cattle productivity, but also in net reductions in emissions of greenhouse gases into the atmosphere.

3.3 Charcoal interaction with soils

- Found that modest additions of charcoal to soils can increase plant growth and yields and enhance availability of various soil nutrients. Additionally net decreases in emissions of nitrous oxide were observed after additions of charcoal.

3.4 Nitrogen dynamics from mixed species litter in the Amazon.

- Found that In the field, the release of nutrients and available soil N from litter of mixed species depends more on soil fauna, roots, and microclimatic conditions than on residue quality.

3.5 Agroforestry trees increase phosphorous availability in Amazonian Oxisols

- Found that in soils from Central Amazon, tree species such as Peach palm and Brazil nut grown in in agroforestry systems with moderate fertilization, better maintain available phosphorous in soils than other tree species.

3.6 Assessment of the potential of tannins in legumes and saponins in tropical fruits to reduce methane in ruminants.

- The supplementation with *S. saponaria* reduced daily methane release from sheep by over 10%.

- The addition of legumes with high levels of tannins reduced methane release per unit of organic matter fermented

- Supplementation with molasses reduced the negative nutritional effects high concentrations of condensed tannins in legume by enhancing N turnover.

Advances in Research 2003

Output 1: 107652

Vulnerability and opportunity assessment developed of the response of critical ecoregions, populations, crops and crop wild relatives to changing climates.

1.1 Climate change and maize yields in Colombia.

Contributor: Peter Jones, CIAT (PE4)

Highlights:

- Found that maize yields in Colombia could be expected to decrease as a consequence of climate change in the coming decades.

A study of the potential scenarios for the effects of climate change on maize yields in Latin America and Africa was reported last year. In general major decreases could be expected on a regional basis for both large regions. The journal article was published this year and received great attention in the mass media worldwide. This year using the same methodologies (MarkSim coupled to the Ceres maize model), the study has being done specifically for Colombia. Results have shown the typical trends (Figure 1) with a threefold potential outcome.

1. There will be areas (particularly in the highlands) where some crop yields may improve
2. Quite large areas may show minor changes in yield potential, but will require differently adapted varieties and probably some shifts in crop mixes and agricultural practices.
3. A significant number of areas will require major intervention because of potential complete crop failure..

It is especially important to note the scale of the variations. Pixel size in this output is approximately 18 km. It is possible that areas with disastrous yield losses will be within 20 km of where yields may actually increase. This will cause disruption in the local economy and recommendations for coping with the change will vary extremely locally.

Reference:

Jones, P. G. and P. K. Thornton (2003) The potential impacts of climate change on maize production in Africa and Latin America in 2055. Global Environmental Change 13: 51–59.

1.2 Bean production at risk in Central America.

Contributors: Peter G. Jones (PE4), Steve Beebe (IP1)

Last year, it was shown that climate change in Central America will be characterized by a more frequent and intense droughts. Niches for wild species of cultivated common beans will shrink considerably (Figure 2). The results have drawn attention to the need for conservation of the germplasm of Honduras, Nicaragua, El Salvador and Costa Rica. It is hoped that they will stimulate conservation efforts.

It is highly likely that droughts will negatively affect bean production in the region and that a net decline in expected production of this basic staple food for at least 10 million people in Central America. This year, research has being initiated and will be completed next year, to couple daily weather forecast from MarkSim with common bean crop models to predict the expected performance of the bean production in the region.

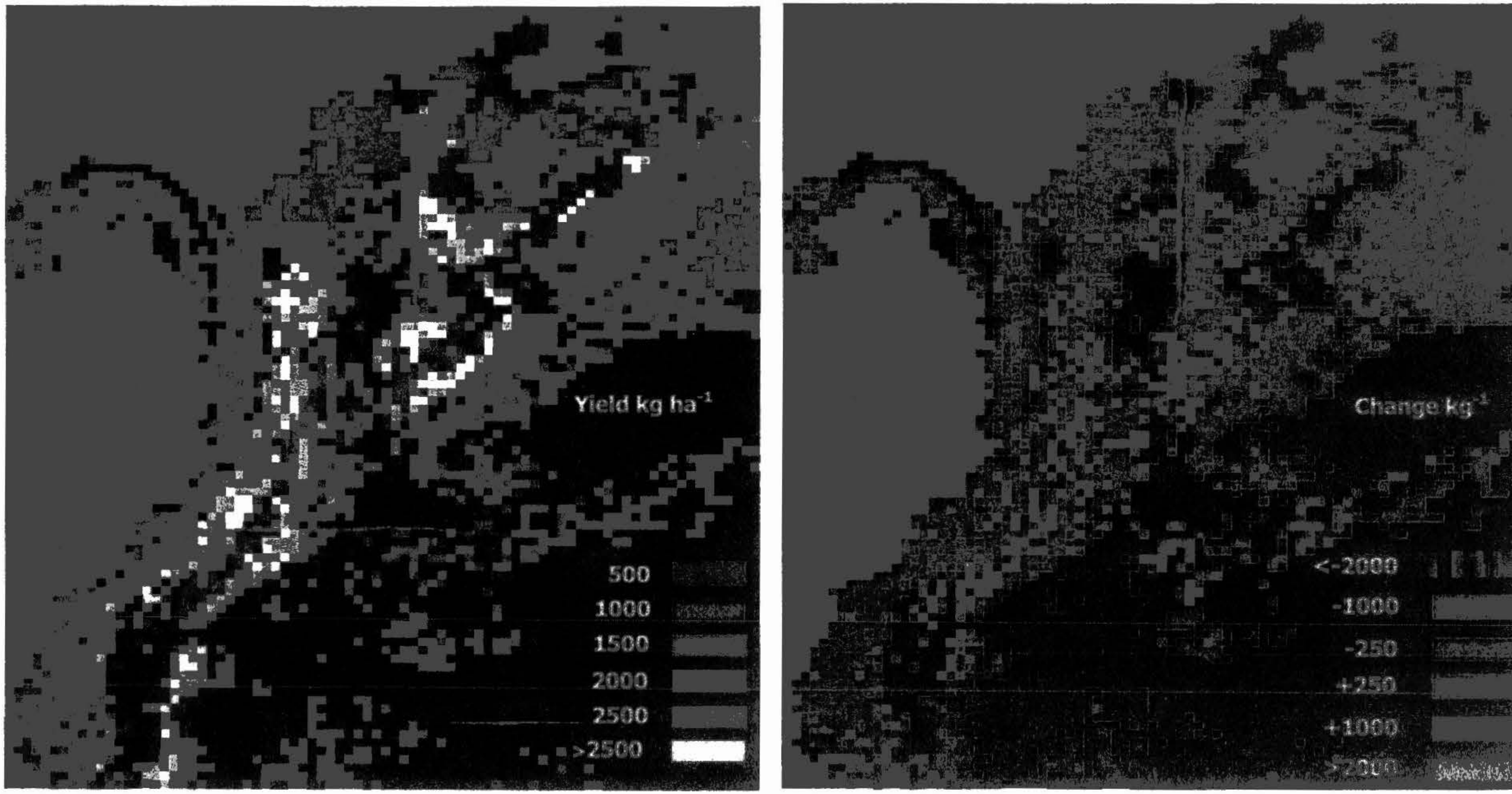

Figure 1. Maize yield potential at present and thee change in 50 years for Colombia

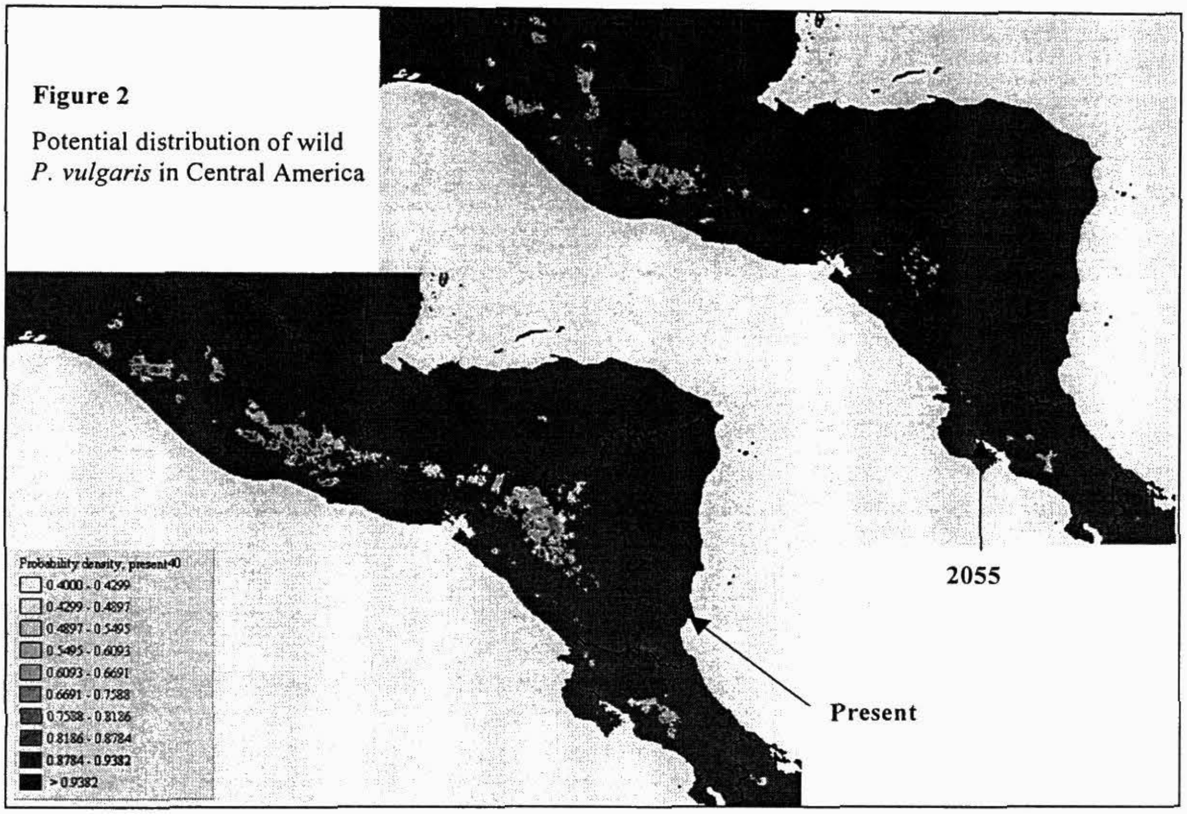

Figure 2

Potential distribution of wild *P. vulgaris* in Central America

107653

Output 2.

Germplasm, and management systems adapted to changing climatic conditions and exacerbated incidence of pests and diseases.

2.1 Developing bean germplasm tolerant to drought

Highlights:

- Breeding lines with commercial grain type and selected for tolerance to terminal drought in previous years also expressed tolerance to intermittent drought, exceeding the commercial checks by 200% or more in yield. New drought tolerant lines show up to 42% yield improvement over local commercial cultivars under both stress and non-stress conditions in Eastern Africa.
- Field evaluation of 16 elite lines showed that a bred line (SEA 15) was outstanding in its adaptation to drought stress conditions. The superior performance of this bred line under drought stress in comparison with 15 other elite parents of recombinant inbred lines (RILs) was associated with lower seed ash (mineral) content indicating efficient utilization of acquired nutrients for grain production.

- Among the 95 advanced lines of the cross BAT 881 × G 21212, three lines (BH 21134-9-1-1-M-M-M-M; BH 21134-154-1-1-M-M-M-M-M; BH 21134-97-1-1-M-M-M-M-M) were superior in their adaptation to drought stress conditions. The superior performance of these three lines was related to lower levels of seed ash (mineral) and seed P indicating the usefulness of these traits for selection for drought adaptation in common bean.

2.1.1 Development and testing of lines and segregating populations combining drought tolerance and disease resistance in small red and small black grain types

Contributors: S. Beebe, I.M. Rao, H. Terán, C. Cajiao, Miguel Grajales (IP1); C. Quintero, J. Tohme (SB2)

Rationale. Drought tolerance must be combined with other traits to be employed in commercial varieties. In most regions where drought is a problem in the Americas, Bean Golden Yellow Mosaic Virus (BGYMV) is also a serious limitation. For the Central American region, small red and small black seeded grain type is required. In Africa a more diverse range of grain types are acceptable, although BGYMV is not yet a problem. However, recessive resistance to BCMV is highly desirable.

Materials and Methods. *F_8 lines:* Last year we reported on positive results with $F_{3\text{-}5}$ families that represented our first experience with combining drought tolerance with other traits. In the course of the past year we have advanced these populations through three more generations, to $F_{6\text{-}8}$ families that were again yield tested under drought conditions. Three yield trials were composed of red-seeded beans (each in 7×7 lattice design), two of black seeded beans (also 7×7 lattice design), and a sixth of other colors (in a 6×6 lattice design). Trials were planted on-station at CIAT-Palmira in July, 2003, receiving only two irrigations, amounting to about 60 mm water. An additional 117 mm of rain fell during the crop cycle. Common checks were included across trials: red seeded commercial cv. Tio Canela; black seeded commercial cv. DOR 390; tolerant lines SEA 5 and SEA 15; and an African cowpea cultivar bred for stress conditions, Mouride. Families also were sampled in F_7 for the presence of two DNA markers for resistance to the BGYMV virus: bgm-1 and W12.

Additional crosses. Based upon results with F_2 populations in CIAT-Palmira in 2001, superior parental materials were identified and additional crosses were created with these parents. These were evaluated in F_2 for drought, in F_3 for anthracnose, and then as F_4 families in Santander de Quilichao under moderate fertility stress and ALS pressure. In 2003 they were returned to Palmira for a second drought evaluation. Additionally, crosses for carioca grain type were evaluated in F_4 generation for drought tolerance.

New populations were created to combine high mineral content with drought tolerance, low fertility tolerance, and disease resistance. F_2 populations were evaluated for the first time in the 2003 summer planting season.

Results and Discussion. During the crop cycle, day-time temperatures ranged from moderate (28°C) on cloudy days, to quite high (35°C) on clear days. Tensiometer readings were more moderate than in past years, and did not drop below –500 millibars, when in 2002 readings of -700 millibars were registered. Rainfall occurred sporadically throughout the crop cycle resulting in an intermittent stress, unlike the severe terminal stress in the previous two years. This more moderate stress was evidenced in the higher yields of checks, for example, Tio Canela yielded around a ton per hectare in 2003, while in 2002 its yield ranged from 500-800 kg/ha.

A range of plant response was evident among the F_8 families and the checks. Some materials like DOR 390 and BAT 881 were severely stunted by early drought after germination and recovered very slowly if at all. Tio Canela recovered steadily but its flowering and pod development were delayed. Its development suggested a conservative survival mode, whereby its resources were deployed gradually and steadily toward the formation of pod and seed. EAP 9510-77 set pods more readily than Tio Canela but aborted much seed. Among the lines bred for tolerance, a few presented an almost normal development pattern, with little delay in flowering and podding. This was the case with the progeny of the cross (RAB 651 × Tio Canela) × (RAB 608 × SEA 15). Others expressed a delayed flowering or early abortion of buds, but set pods quickly and uniformly after a long quiescent period. This mimicked the pattern of G21212 and was observed in its progeny from the cross RAB 651 × (MD23-24 × G21212). Most families had been selected intensively for good grain filling and this trait was maintained in these trials.

Out of nearly 250 F_8 lines, 33 red seeded, 33 black seeded, and 17 lines of other colors were selected, for a total of 83 lines (Tables 1 to 3). These presented a yield advantage of as much as 200% over the average of the commercial checks, Tio Canela and DOR 390. As in the past, some lines yielded as well as the cowpea check, in a growth cycle that was 8-10 days shorter than the cowpea. It is reassuring that the tolerance is expressed under intermittent drought as well as in terminal drought under which the materials were originally selected. This gives us greater confidence that the tolerance will be expressed over a wider range of environments. One set of sister lines derived from the cross (RIB 68 × G21212) × ICTA Ligero [MN14154-31-MC-9P-MQ-MC-...] yields very well under drought and also carries the *bc-3* gene for BCMV resistance.

F_5 families and F_2 populations: Some 17 F_5 families were selected out of nearly 100 families for further evaluation and selection. Their principal advantage in relation to the more advanced materials is the inclusion in most of these crosses of the *bc-3* gene for recessive non-necrotic resistance to necrotic strains of BCMV. Promising F_4 families in carioca type were also identified and selected. These will serve for a drought breeding project that we hope to initiate under the Water Challenge Program with EMBRAPA-Brazil.

Among nearly 1500 F_1-derived F_2 families made to combine drought tolerance with high mineral traits, 250 were selected for additional study and are being analyzed for iron and zinc content.

Conclusions. Progress in drought tolerance was confirmed in F_8 families, under intermittent drought. The fact that these materials resist both terminal and intermittent drought gives us more confidence in the tolerance of these lines, and their potential performance over environments.

2.1.2 Screening drought tolerant bean lines in Eastern Africa

Contributors: P. Kimani and S. Beebe (IP1)

Rationale. Drought is one of the most important constraints to bean production in East, Central and Southern Africa. Over 396,000 t of grain are lost annually in Africa due to drought. Drought may occur early in the season, mid-season or late in the cropping season. Drought is ranked as major constraint to bean production in Kenya, Ethiopia, parts of south western Uganda, northern and central Tanzania, South Africa, southern Rwanda, Sudan, Angola, central plateau of Madagascar and southeast DR Congo. Although the adverse effects of drought can be alleviated through irrigation, few smallholder bean growers in East and Central Africa (except in Sudan) have access to irrigation water. Bean production in this region is predominantly rain fed. Crop failures are frequent. Growing drought tolerant bean cultivars is probably the most cost-effective strategy for smallholder, resource-poor farmers in drought prone environments. However, few drought tolerant cultivars are available in sub-Saharan Africa. CIAT has been screening bean cultivars for drought since 1983 as part of integrated genetic improvement of the common bean. Recently, an international drought nursery was constituted which included the most promising drought tolerant lines. This report highly the performance of this nursery in trials conducted in Eastern Africa.

Table 1. Elite red seeded lines tolerant to drought identified in three separate yield trials.

Cross / Line	Yield as % checks[1]	Yield checks
(RAB 651 × Tio Canela) × (RAB 608 × SEA 15)		
MR14143-28-MC-2P-MQ-MQ-27C	245	856
MR14143-28-MC-2P-MQ-MQ-3C	245	856
MR14143-28-MC-2P-MQ-MQ-15C	242	856
MR14143-28-MC-2P-MQ-MQ-17C	236	856
MR14143-28-MC-2P-MQ-MQ-20C	233	856
MR14143-28-MC-2P-MQ-MQ-4C	231	856
MR14143-28-MC-2P-MQ-MQ-25C	229	856
MR14143-28-MC-2P-MQ-MQ-6C	221	856
MR14143-28-MC-2P-MQ-MQ-8C	211	856
RAB 651 × (MD23-24 × G21212)		
MR 14000-2-MC-1P-MQ-MC-15	213	856
MR 14000-2-MC-1P-MQ-MC-21	206	856
MR 14000-2-MC-10P-MQ-MC-1	223	856
MR 14000-2-MC-10P-MQ-MC-21	223	856
MR 14000-2-MC-10P-MQ-MC-13	203	856
MD23-24 × (RAB 655 × G21212)		
MR13937-15- MC-3P-MQ-MC-2C	175	856
MR13937-21- MC-7P-MQ-MC-9C	183	856
(SEA 22 × (TLP 35 × G21212) ×EAP9504-30B		
MR14152-14-MC-3P-MQ-MC-1C	272	896
MR14152-14-MC-3P-MQ-MC-6C	234	896
(SEA 21 × RAB 623) × 9653-16B		
MR14148-80-MC-2P-MQ-MC-3C	255	896
MR14148-72-MC-3P-MQ-MC-3C	251	896
MR14148-80-MC-2P-MQ-MC-2C	238	896
MR14148-54-MC-3P-MQ-MC-13C	221	896
[SEA22 × (A774 x G21212)] × (RAB 619 × Tio Canela)		
MR14153-3-MC-3P-MQ-MC-21C	235	896
MR14153-3-MC-3P-MQ-MC-17C	230	896
MR14153-3-MC-2P-MQ-MC-4C	203	896
(RAB618 × (DOR 364 RC) × [(RAB 655 × G21212) × SEA 21]		
MR14198-13-MC-1P-MQ-MC-11C	228	896
MR14198-13-MC-1P-MQ-MC-23C	210	896
DICTA 122 RC × [RAB 651 × (VAX1 × RAB 655)]		
MR14292-63-MC-2P-MQ-MC-11C	364	570
MR14292-63-MC-2P-MQ-MC-1C	336	570
MR14292-63-MC-2P-MQ-MC-10C	310	570
DICTA 122 RC × [MD23-24 × (RAB 655 × G21212)]		
MR14258-7-MC-9P-MQ-MC-8C	317	570
MR14258-7-MC-9P-MQ-MC-4C	303	570
(RAB 623 × DICTA 17) × (RAB 630 × SEA 21)		
MR14273-4-MC-3P-MQ-MC-6C	302	570
MR14273-4-MC-3P-MQ-MC-11C	297	570
(SEA 15 × MD23-24) × (Tio Canela × G21212)		
MR14215-9-MC-6P-MQ-MC-11C	311	570

[1] *Yields of lines were calculated as a per cent of the average of commercial checks Tio Canela and DOR 390.*

Table 2. Elite black seeded lines tolerant to drought identified in two separate yield trials.

Cross / Line	Yield as % checks[1]	Yield checks	Cross / Line	Yield as % checks	Yield checks
(RJB 10 × Tio Canela) × [SEA 18 × (FEB 192 × G21212)]			**(RIB 68 ×G21212) × ICTA Ligero**		
MN14142-27-MC-3P-MQ-MC-1C	355	741	MN14154-31-MC-9P-MQ-MC-5C	301	761
MN14142-27-MC-3P-MQ-MC-11C	325	741	MN14154-31-MC-9P-MQ-MC-9C	264	761
MN14142-27-MC-3P-MQ-MC-4C	294	741	MN14154-31-MC-9P-MQ-MC-14C	250	761
MN14142-27-MC-3P-MQ-MC-5C	280	741	MN14154-31-MC-9P-MQ-MC-4C	243	761
MN14142-51-MC-1P-MQ-MC-3C	240	741	MN14154-36-MC-12P-MQ-MC-2C	221	761
MN14142-51-MC-1P-MQ-MC-5C	165	741	MN14154-10-MC-4P-MQ-MC-11C	198	761
(TLP 35 × G21212) × ICTA Ligero			**(SEA 15 × Ostua RC) × (Tio Canela × (FEB 192 × G21212)**		
MN13942-22-MC-MC-1P-MQ-MC-1C	279	741	MN14212-12-MC-5P-MQ-MC-4C	280	761
MN13942-22-MC-MC-1P-MQ-MC-11C	252	741	MN14212-12-MC-5P-MQ-MC-7C	276	761
MN13942-22-MC-MC-1P-MQ-MC-3C	247	741	MN14212-12-MC-5P-MQ-MC-3C	263	761
MN13942-22-MC-MC-1P-MQ-MC-5C	207	741	MN14212-4-MC-2P-MQ-MC-4C	239	761
MN13942-33-MC-MC-7P-MQ-MC-6C	233	741	(SEA 15 x MD 23-24) x (Tio Canela x G21212)		
MN13942-31-MC-MC-1P-MQ-MC-4C	165	741	MR14215-5-MC-9P-MQ-MC-4C	245	761
(FEB192 × G21212) × DOR 500 RC			**LORE 87 × [(VAX 1 × IPA 7) × Ostua RC]**		
MN13934-63-MC-2P-MQ-MC-15C	270	741	MN13862-44-MQ-1P-MQ-MC-2C	221	761
MN13934-63-MC-2P-MQ-MC-9C	221	741	**[(A774 × G21212) × RAB 609) × (DOR 500 RC × SEA 18)**		
[SEA 18 × (FEB 192 × G21212) × EAP9020-14			MR14194-19-MC-3P-MQ-MC-6C	219	761
MR14144-11-MC-1P-MQ-MC-8C	262	741	**LORE 24 × [(VAX 1 × A774) × Ostua RC]**		
MR14144-11-MC-1P-MQ-MC-2C	251	741	MN13856-6-MQ-3P-MQ-MC-2C	179	761
MR14144-15-MC-1P-MQ-MC-1C	195	741	MN13856-35-MQ-3P-MQ-MC-3C	171	761
(FEB 192 × G21212) × ICTA Ligero					
MN14059-8-MC-4P-MQ-MC-4C	221	741			

[1] *Yields of lines were calculated as a per cent of the average of commercial checks Tio Canela and DOR 390.*

Table 3. Elite lines with pink, purple, yellow or cream seed color, tolerant to drought.

Cross / Line	Yield as % checks[1]	Yield checks	Color[2]	Cross / Line	Yield as % checks	Yield checks	Color
(SEA 15 × MD 23-24) × (Tio Canela × G21212)				**(RAB 623 × MD23-24) × [SEA 15 × (RAB 655 × G21212)]**			
MR14215-6-MC-2P-MQ-MC-12C	199	1101	Pk	MR14202-4-MC-3P-MQ-MC-12C	178	1101	Pur
MR14215-5-MC-5P-MQ-MC-1C	199	1101	Pk	MR14202-4-MC-3P-MQ-MC-9C	178	1101	Pur
MR14215-6-MC-2P-MQ-MC-4C	194	1101	Pk	(FEB 192 x G21212) x ICTA Ligero			
MR14215-6-MC-2P-MQ-MC-5C	191	1101	Pk	MN14059-16-MC-2P-MQ-MC-4C	168	1101	Cr
MR14215-6-MC-4P-MQ-MC-12C	181	1101	Pur	**RAB 651 × (MD23-24 × G21212)**			
MR14215-5-MC-4P-MQ-MC-8C	174	1101	Red	MR14000-20-MC-6P-MQ-MC-10C	168	1101	Ye
MR14215-5-MC-5P-MQ-MC-10C	160	1101	Red	MR14000-20-MC-6P-MQ-MC-1C	161	1101	Ye
MR14215-9-MC-8P-MQ-MC-1C	159	1101	Pk	MR14000-20-MC-6P-MQ-MC-4C	148	1101	Ye
MR14215-9-MC-8P-MQ-MC-4C	157	1101	Pk				
(SEA 15 × DOR 364 RC) × [RAB 651 × (MD23-24 × G21212)]							
MR14216-3-MC-7P-MQ-MC-9C	194	1101	Ye				
MR14216-3-MC-7P-MQ-MC-1C	169	1101	Ye				

[1] Yields of lines were calculated as a percent of the average of commercial checks Tio Canela and DOR 390.
[2] Color code: Cr = cream; Pk = pink; Pur = purple; Ye = yellow

Materials and Methods. Thirty-six drought tolerant bean lines including two susceptible checks were evaluated at Thika, Kenya in 2001, 2002 and 2003. Each year, the trial was laid out in 6 × 6 lattice design with three replicates. The 36 genotypes were evaluated in drought stressed and non-stressed environments for the three cropping seasons. Each entry was sown on four, 5 m rows. Data was recorded from the two inner rows. Entries in non-stressed plots were provided with 1 to 2 supplemental irrigations. In stressed plots, the entries were grown under natural rainfed conditions. For data analysis, the cropping seasons (environments) and replications were considered as random effects, whereas irrigation treatments (stress levels) and genotypes were fixed effects. All data was analyzed using Genstat (6ed, 2002) statistical package. Two local cultivars, GLP × 92 and GLP 585 were included as checks.

Results and Discussion. There were significant grain yield differences due to environments, stress levels and genotypes (Table 4). Significant environment × stress levels, genotype × stress level, genotype × environment interactions were detected. This indicated that performance of the genotypes varied with stress level and with environments. Yield reduction due to drought was highest in 2001 (58%) but remained at 40% in 2002 and 2003. The ten most promising lines under both stress and nonstress conditions are shown in Table 4. SEA 16 and SEA 20 consistently ranked among the top five best yielding lines under stress conditions for the three seasons. RAB 608, RAB 636 and INIB 35 ranked among the top five for two seasons under stress conditions. However, SEA 23, RAB 608, SEA 16 and RAB 618 were the best yielding lines under stress and nonstress environments. These four lines out yielded all the checks. These results indicate new possibilities of stabilizing bean yields in drought prone environments in Eastern Africa. These lines can be utilized as sources for drought tolerance in breeding drought tolerant marketable bean cultivars.

Table 4. Grain yield (kg ha^{-1}) of drought tolerant lines grown under stress and nonstress conditions over three seasons at Thika, Kenya, 2001-2003.

Genotype	2001		2002		2003		
	Nonstress	Stress	Nonstress	Stress	Nonstress	Stress	Mean
RAB 608	1435	986	935	513	3031	739	1273
SEA 23	2780	562	1048	585	2405	1032	1402
RAB 636	1196	621	1075	443	2551	983	1145
SEA 16	1885	792	1236	972	1467	1105	1243
RAB 618	1551	564	861	896	1956	861	1115
Pinto Villa	1437	279	1188	407	2119	836	1044
SEA 20	1298	806	1704	681	1139	862	1082
INB 38	1370	528	1269	1091	1149	841	1041
INB 35	1432	881	1207	992	1287	697	1083
INB 39	1468	388	1162	828	1180	823	948
Checks							
Tio Canela	1253	300	1264	664	1591	1059	1022
SEA 5	1934	570	1316	546	1040	546	992
GLP × 92	698	676	1124	265	1288	757	801
GLP 585	1154	685	1267	512	1113	523	876
Trial mean	1561	654	1072	652	1340	801	1037
Reps/Environments							
Environments (E)		**					
Stress levels (S)		**					
Genotypes (G)		*					
E × S		NS					
G × E		**					
G × S		**					
G × E × S		**					
Residual							

*, **: *Significant at 5 and 1% probability levels, respectively; NS= not significant*

2.1.3 Identification of traits associated with drought resistance

Contributors: I. M. Rao, S. Beebe, J. Ricaurte, H. Terán and R. García (IP-1)

Rationale. Development of drought adapted bean varieties is an important strategy to minimize crop failure and improve food security in bean growing regions. Last year we evaluated 36 promising bred lines for their adaptation to drought stress under field conditions. The results from this field study indicated that two accessions of *P. acutifolius* (G 40068 and G 40159) and one bred line (RAB 650) were outstanding in their adaptation to water stress conditions. The superior performance of these three genotypes under drought was associated with their ability to mobilize photosynthates to developing grain and to utilize the acquired N and P more efficiently for grain production. This year, using two separate field trials, we evaluated drought adaptation of 16 elite parents of RILs and 95 RILs of the cross BAT 881 × G 21212.

Materials and Methods. Two field trials were conducted at Palmira in 2002 (June to September) to determine differences in tolerance to water stress conditions. The *field trial 1* included 16 elite parents of RILs. Two levels of water supply (irrigated and rainfed) were applied. A 4 × 4 partially balanced lattice design with three replications was used. Details on planting and management of the trial were similar to those reported before (CIAT, 1998). Experimental units consisted of 4 rows, 5 m long by 0.6 m wide. A number of plant attributes were measured at mid-podfilling in order to determine genotypic variation in drought resistance. These plant traits included leaf area index; canopy dry weight per plant; shoot nutrient (N, P, K, Ca and Mg) uptake; shoot and seed ash content; and shoot and seed TNC (total nonstructural carbohydrates). At the time of harvest, grain yield and yield components (number of pods per plant, number of seeds per pod, 100 seed weight) were determined. Seed N, P, ash content and TNC (total nonstructural carbohydrates) were also measured. The *field trial 2* included 95 RILs of BAT 881 × G 21212 along with five checks including the two parents. A 10 × 10 partially balanced lattice design with three replicates was used. Details on treatments and measurements are same as for trial 1.

Results and Discussion

Palmira – Soil, temperature, rainfall and evaporation. The soil is a Mollisol (Aquic Hapludoll) with no major fertility problems (pH = 7.7), and is estimated to permit storage of 130 mm of available water (assuming 1.0 m of effective root growth with –0.03 MPa and –1.5 MPa upper and lower limits for soil matric potential). During the crop-growing season, maximum and minimum air temperatures were 34 and 15.8°C, respectively, while incident solar radiation ranged from 11.2 to 24.7 MJ m^{-2} d^{-1} (Figure 1). The total rainfall during the active crop growth was 43.2 mm. The potential pan evaporation was of 337 mm. These data on rainfall and pan evaporation indicated that the crop suffered high level of drought stress during active growth and development.

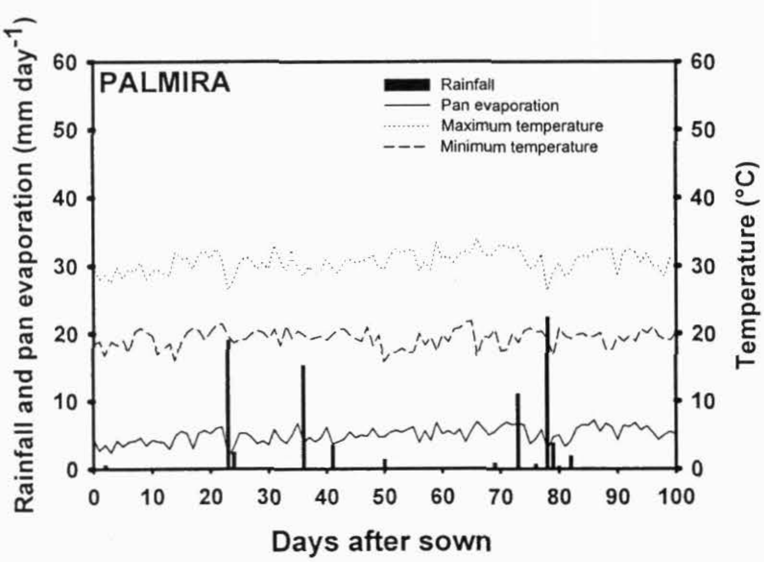

Figure 1. Rainfall distribution, pan evaporation, maximum and minimum temperatures during crop growing period at Palmira.

Trial 1 - Under water stress (rainfed) conditions in the field, the seed yield of 16 elite bred lines ranged from 439 to 1071 kg/ha (Table 5). Among the lines tested, SEA 15 was outstanding in its adaptation to rainfed (water stress) conditions. There was response to irrigation by all lines and the highest value of seed yield under both irrigated and rainfed conditions was recorded with SEA 15 (Table 5). The relationship between grain yield of rainfed and irrigated treatments indicated that SEA 15 was not only adapted to water stress but also very responsive to irrigation. Among the 16 lines tested, BAT 881 was the most poorly adapted bredline under rainfed conditions. The mean seed P content decreased under rainfed conditions (Table 5) while the mean seed TNC content was not affected by water stress conditions. The lowest value of seed P under rainfed conditions was observed with SEA 21. Seed ash content that reflects the mineral status of the grain was also lower in SEA lines compared with other lines under rainfed conditions (Table 5). Greater amount of seed N was observed with MCD 2004 under both rainfed and irrigated conditions.

Table 5. Influence of drought stress on grain yield, seed P content and seed ash content of 16 elite bred lines evaluated in a Mollisol at Palmira.

	Grain yield (kg/ha)		Seed P content (%)		Seed ash content (%)	
Elite bred line	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**
SEA 15	1840	1071	0.597	0.502	4.45	4.23
A 774	1397	751	0.543	0.476	4.47	4.32
BAT 477	1315	743	0.600	0.580	4.99	4.86
DOR 364	1353	695	0.577	0.540	4.76	4.33
EAP 9510-77	1661	705	0.513	0.477	4.40	4.29
G 21212	1390	777	0.587	0.540	5.05	4.77
MCD 2004	1434	689	0.583	0.510	4.78	4.39
RAB 609	1372	715	0.537	0.530	4.54	4.24
RAB 612	1134	473	0.590	0.489	4.71	4.33
RAB 651	1608	848	0.503	0.517	4.29	4.45
RAB 655	1123	640	0.603	0.501	4.79	4.45
SEA 5	1567	950	0.587	0.577	4.79	4.56
SEA 21	1422	733	0.493	0.473	4.37	4.13
SEA 23	1440	838	0.643	0.590	4.39	4.07
Tio Canela 75	1523	767	0.623	0.479	4.70	4.49
BAT 881	1131	439	0.633	0.523	5.01	4.41
Mean	**1419**	**740**	**0.576**	**0.519**	**4.65**	**4.39**
CV (%)	**15**	**23**	**33**	**33**	**8**	**8**

Grain filling index (100 seed weight of rainfed/100 seed weight of irrigated × 100), which is a measure of the ability of the genotype to fill the seed with photoassimilates under rainfed conditions relative to irrigated conditions, was markedly superior with G 21212 (Figure 2). This landrace is also found to be well adapted to other abiotic stress factors such as low soil fertility and acid soil stress. Thus the superior ability to fill the grain under stressful conditions may be a common physiological mechanism for stress adaptation.

Conclusions. Results from this study indicated that a bred line (SEA 15) was outstanding in its adaptation to drought stress conditions. The superior performance of this bred line under drought stress in comparison with 15 other elite parents of recombinant inbred lines (RILs) was associated with lower seed ash (mineral) content indicating efficient utilization of acquired nutrients for grain production.

Trial 2 - Under water stress conditions in the field, the seed yield of 95 RILs ranged from 188 to 974 kg/ha (Figure 3). Among the lines tested, line BH9 (BH 21134-9-1-1-M-M-M-M) was outstanding in its adaptation to rainfed (water stress) conditions. There was response to irrigation by most of the lines. The relationship between grain yield of rainfed and irrigated treatments indicated that lines BH97, BH154 and BH47 were not only adapted to water stress but also responsive to irrigation. Among the 95 lines tested, BH36 was the most poorly adapted bredline under rainfed conditions.

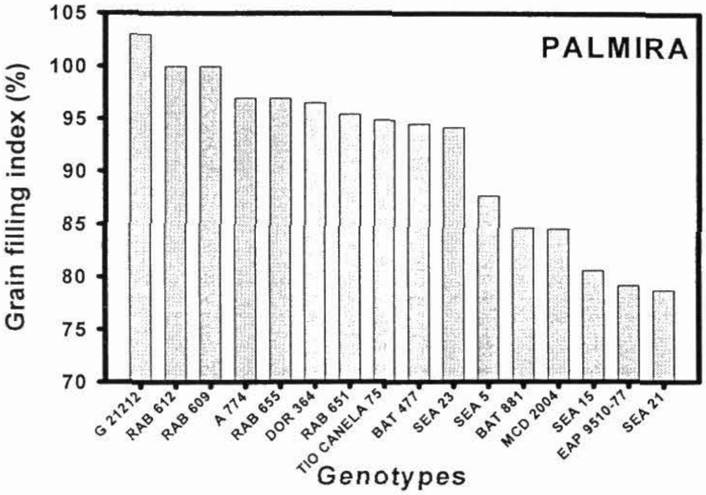

Figure 2. Differences in grain filling index (100 seed weight of rainfed/100 seed weight of irrigated × 100) among 16 elite parents of RILs.

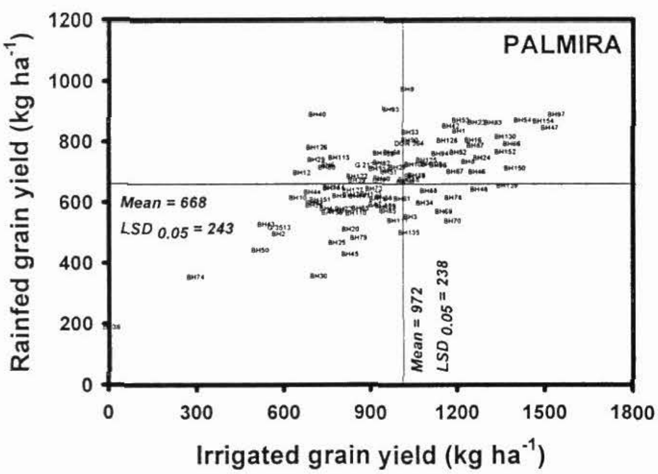

Figure 3. Identification of genotypes that are adapted to rainfed conditions and are responsive to irrigation to a Mollisol at Palmira. Genotypes that yielded superior with drought and were also responsive to irrigation were identified in the upper box of the right hand side.

The relation ship between rainfed seed yield and other plant attributes indicated that the superior performance of lines BH9, BH154 and BH97 was associated with lower levels of seed P and seed ash content (Figures 4 and 5). Line BH9 also showed greater level of seed TNC under rainfed conditions. It is important to note that seed N content of BH9 was also markedly greater than most of the lines. Relationship between N use efficiency and P use efficiency indicated that the lines BH47 and BH130 were outstanding in utilizing N and P for grain production.

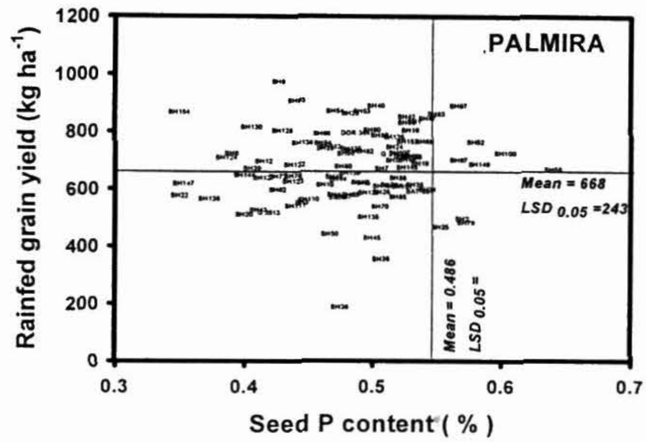

Figure 4. Identification of genotypes that combine superior seed yield with lower P content in seed when grown under rainfed conditions in a Mollisol at Palmira. Genotypes that were superior in grain yield and lower in seed P were identified in the upper box of the left hand side.

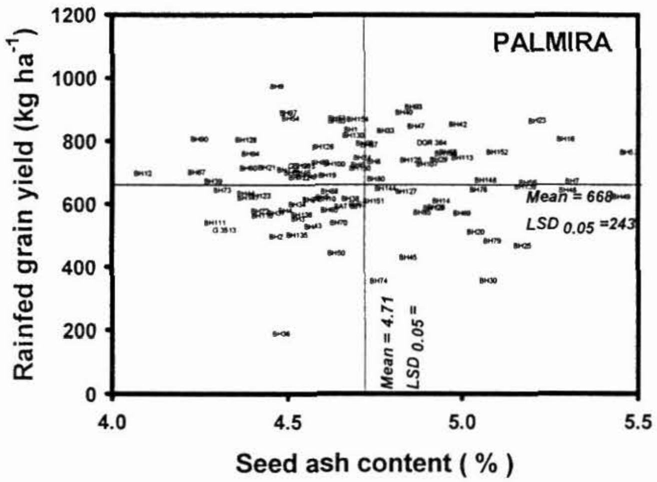

Figure 5. Identification of genotypes that combine superior seed yield with lower ash (mineral) content in seed when grown under rainfed conditions in a Mollisol at Palmira. Genotypes that were superior in grain yield and lower in seed ash were identified in the upper box of the left hand side.

Conclusions. Results from this field study indicated that among the 95 advanced lines of the cross BAT 881 × G 21212, three lines (BH 21134-9-1-1-M-M-M-M; BH 21134-154-1-1-M-M-M-M-M; BH 21134-97-1-1-M-M-M-M-M) were superior in their adaptation to drought stress conditions. The superior performance of these three lines was related to lower levels of seed ash (mineral) and seed P indicating the usefulness of these traits for selection for drought adaptation in common bean.

References:

CIAT. 1998. Bean Project Annual Report 1997. CIAT, Cali, Colombia. 197 p. Working Document No. 177.

2.2. Identify genotypes of grasses and legumes with dry season tolerance

Highlights

- Showed that the superior performance of one germplasm accession (CIAT 26110) and one hybrid (FM9503-S046-024) of *Brachiaria* which maintained greater proportion of green leaves during dry season in the Llanos of Colombia, was associated with greater acquisition of nutrients under water deficit conditions.

2.2.1 Determination of the genotypic variation in dry season tolerance in *Brachiaria* accessions and genetic recombinants in the Llanos of Colombia

Contributors: I. M. Rao, J. W. Miles, C. Plazas, J. Ricaurte and R. García (IP5)

Rationale. A major limitation to livestock productivity in subhumid regions of tropical America is quantity and quality of dry season feed. A field study is completed this year at Matazul Farm in the Llanos of Colombia. The main objective was to evaluate genotypic differences in dry season (4 months of moderate drought stress) tolerance of most promising genetic recombinants of *Brachiaria*. Results from this field study for the past two years indicated that the superior performance of the germplasm accession CIAT 26110 and the *Brachiaria* hybrid, FM9503-S046-024, which maintained greater proportion of green leaves during moderate dry season in the llanos of Colombia, was associated with greater acquisition of nutrients under water deficit conditions. This year, we report results from the dry season performance into fourth year after establishment.

Materials and Methods. A field trial was established on a sandy loam Oxisol at Matazul farm in the Llanos of Colombia in July, 1999. The trial comprises 12 entries, including six natural accessions (four parents) and six genetic recombinants of *Brachiaria*. Among the germplasm accessions, CIAT 26110 was identified from previous work in Atenas, Costa Rica as an outstanding genotype for tolerance to long dry season (up to 6 months). The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20 P, 20 K, 33 Ca, 14 Mg, 10 S; and high: 80 N, 50 P, 100 K, 66 Ca, 28 Mg, 20 S and micronutrients) as main plots and genotypes as sub-plots. Live and dead forage yield, shoot nutrient composition, shoot nutrient uptake and leaf and stem TNC (total nonstructural carbohydrates) were measured at the end of the dry season (44 months after establishment; March 3, 2003). Maintenance fertlizer (half the levels of initial application) was applied at the beginning of the wet season of 2001 (July, 2001).

Results and Discussion. Because of the application of maintenance fertilizer, forage yields with high fertilizer treatment were greater than those with low fertilizer treatment (Figure 1). At 44 months after establishment (4 months after dry sseason – March 3, 2003), live forage yield with low fertilizer application ranged from 0 to 329 kg/ha and the highest value of forage yield was observed with a germplasm accession CIAT 26110. This accession was released in Costa Rica as cultivar Toledo and is known for its dry season tolerance. Among the 4 parents, CIAT 6294 was outstanding in live forage and dead biomass production with low fertilizer application. A spittlebug resistant genetic recombinant, FM9503-S046-024 was superior among the genetic recombinants in terms of greater live shoot biomass, both with low and high fertilizer application. As expected, the performance of one of the parents, BRUZ/44-02 was very poor compared with other parents and genetic recombinants as it produced almost no live forage after dry season. The leaf to stem ratio values of one of the genetic recombinants (BR97NO-0082) were markedly superior to other genotypes under low and high levels of initial fertilizer application (Table 1).

The superior performance of the accession CIAT 26110 with low fertilizer application was mainly attributed to its ability to produce green leaf biomass during dry season (Figure 1). Results on leaf and stem N content indicated significant differences among genetic recombinants, parents and accessions with both levels of fertilizer application (Table 2). But shoot N uptake with low fertilizer application was markedly greater for the hybrid, FM9503-5046-024 (Table 2; Figure 2). With high fertilizer application, the hybrid FM9503-5046-024 and CIAT 26110 were outstanding in shoot N uptake. Shoot uptake of P, K, Ca and Mg were also greater with the hybrid FM9503-5046-024 and CIAT 26110 (Tables 3 and 4; Figure 2). Among the parents, CIAT 6294 was superior in P, K, Ca and Mg acquisition from both low and high fertilizer application.

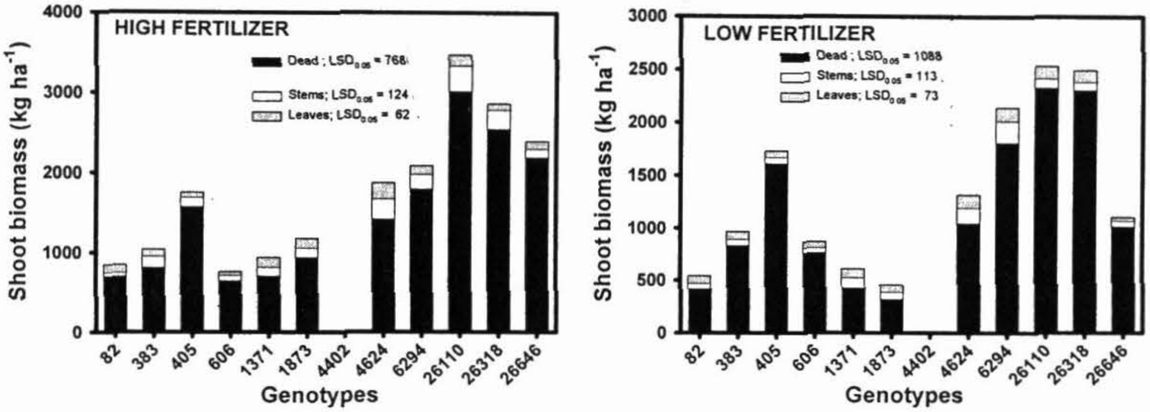

Figure 1. Genotypic variation as influenced by fertilizer application in dry matter distribution among green leaves, stems and dead biomass of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam Oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level.

Table 1. Genotypic variation as influenced by fertilizer application in live shoot biomass, leaf to stem ratio and total forage yield of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam Oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level.

Genotype	Live shoot biomass		Leaf to stem ratio		Total forage yield	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	---------- (kg/ha) ----------					
Recombinants:						
BR97NO-0082	121	145	1.5	2.3	544	848
BR97NO-0383	130	227	1.5	0.7	969	1040
BR97NO-0405	116	181	1.1	0.5	1734	1759
FM9201-1873	136	238	1.1	1.1	460	1181
FM9301-1371	180	226	0.9	1.2	612	938
FM9503-5046-024	269	386	0.9	0.8	1323	1814
Parents:						
CIAT 606	100	108	1.4	0.7	870	758
CIAT 6294	326	288	0.6	0.6	2145	2093
BRUZ/44-02	0	0	.	.	0	0
CIAT 26646	88	198	0.8	1.0	1115	2397
Accessions:						
CIAT 26110	203	454	1.4	0.4	2536	3474
CIAT 26318	185	314	1.6	0.3	2498	2865
Mean	160	231			1309	1615
LSD *(P=0.05)*	156	171			1146	808

Table 2. Genotypic variation as influenced by fertilizer application in leaf N content, stem N content and shoot N uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam Oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season - March 2003). LSD values are at the 0.05 probability level.

Genotype	Leaf N content		Stem N content		Shoot N uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	---------- (%) ---------		---------- (%) -----------		---------- (kg/ha) -----	
Recombinants:						
BR97NO-0082	2.02	2.21	0.84	0.91	1.75	2.64
BR97NO-0383	2.32	1.94	0.87	0.59	2.15	2.49
BR97NO-0405	1.98	2	1.03	0.63	1.79	1.89
FM9201-1873	1.79	2.02	0.97	0.55	1.91	3.06
FM9301-1371	2.42	2.16	1.15	0.98	3.07	3.63
FM9503-5046-024	2.26	2.02	0.85	0.5	3.63	4.77
Parents:						
CIAT 606	2.1	2.16	0.72	0.65	1.51	1.46
CIAT 6294	2.1	1.97	0.66	0.54	3.94	3.03
BRUZ/44-02	2.03	.	.	.	.	.
CIAT 26646	1.53	1.64	0.69	0.44	0.78	2.05
Accessions:						
CIAT 26110	2.32	2.14	0.68	0.51	3.18	4.36
CIAT 26318	2.18	2.27	0.45	0.4	2.80	2.65
Mean	2.08	2.05	0.78	0.61	2.41	2.98
LSD *(P=0.05)*	0.48	0.33	0.44	0.26	1.99	1.97

ND = not determined due to small size of the sample; NS = not significant.

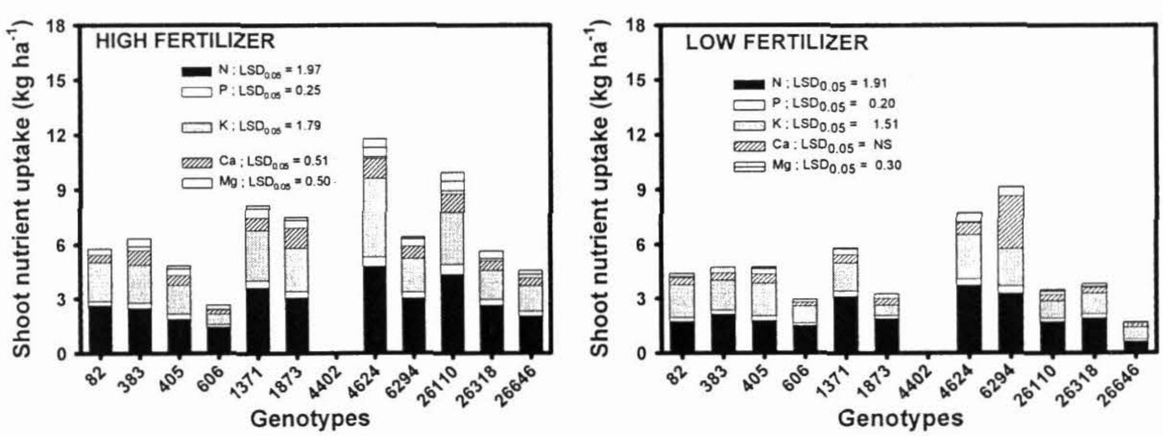

Figure 2. Genotypic variation as influenced by fertilizer application in nutrient (N, P, K, Ca, Mg) uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam Oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level.

Table 3. Genotypic variation as influenced by fertilizer application in leaf P content, stem P content and shoot P uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam Oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season - March 2003). LSD values are at the 0.05 probability level.

Genotype	Leaf P content		Stem P content		Shoot P uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(%)		(%)		(kg/ha)	
Recombinants:						
BR97NO-0082	0.19	0.18	0.13	0.09	0.20	0.22
BR97NO-0383	0.18	0.17	0.09	0.08	0.19	0.26
BR97NO-0405	0.30	0.24	0.10	0.10	0.24	0.26
FM9201-1873	0.14	0.18	0.08	0.07	0.15	0.33
FM9301-1371	0.21	0.20	0.11	0.11	0.28	0.36
FM9503-5046-024	0.19	0.20	0.08	0.06	0.34	0.50
Parents:						
CIAT 606	0.15	0.17	0.08	0.05	0.12	0.10
CIAT 6294	0.24	0.20	0.09	0.05	0.47	0.31
BRUZ/44-02	0.20	.	.	.	.	.
CIAT 26646	0.18	0.17	0.09	0.06	0.12	0.23
Accessions:						
CIAT 26110	0.23	0.23	0.07	0.06	0.32	0.53
CIAT 26318	0.20	0.20	0.06	0.06	0.27	0.29
Mean	0.20	0.19	0.09	0.07	0.25	0.31
LSD *(P=0.05)*	0.07	0.05	0.03	0.04	0.20	0.24

ND = not determined due to small size of the sample; NS = not significant.

Table 4. Genotypic variation as influenced by fertilizer application in shoot K uptake, shoot Ca uptake and shoot Mg uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam Oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season - March 2003). LSD values are at the 0.05 probability level.

Genotype	Shoot K uptake		Shoot Ca uptake		Shoot Mg uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(kg/ha)					
Recombinants:						
BR97NO-0082	1.82	2.14	0.35	0.41	0.25	0.34
BR97NO-0383	1.67	2.10	0.39	0.82	0.30	0.63
BR97NO-0405	1.81	1.59	0.52	0.54	0.39	0.50
FM9201-1873	0.59	2.41	0.36	1.11	0.24	0.57
FM9301-1371	1.85	2.79	0.49	0.69	0.43	0.64
FM9503-5046-024	2.56	4.34	0.70	1.08	0.55	1.08
Parents:						
CIAT 606	0.93	0.58	0.21	0.24	0.16	0.24
CIAT 6294	2.50	1.85	0.69	0.68	0.54	0.50
BRUZ/44-02	-	-	-	-	-	-
CIAT 26646	0.74	1.42	0.20	0.41	0.14	0.40
Accessions:						
CIAT 26110	1.70	2.88	0.52	0.98	0.42	1.18
CIAT 26318	1.71	1.60	0.35	0.53	0.28	0.53
Mean	1.63	2.16	0.44	0.67	0.34	0.60
LSD *(P=0.05)*	1.48	1.79	0.33	0.51	0.27	0.50

ND = not determined due to small size of the sample.

Correlation analysis between green leaf biomass produced in the dry season and other shoot attributes indicated that superior performance with low and high fertilizer application was associated with greater stem biomass indicating the importance of stem reserves for production of green leaf biomass (Table 5). No significant negative association was observed between green leaf biomass and level of nutrients in green leaves. But significant negative association was observed between green leaf biomass production and stem P and Ca content. The usefulness of this trait for evaluating dry season tolerance needs further research work. Stem ash (mineral) content was also negatively associated with green leaf biomass.

Results from this field study indicated that the superior performance of one germplasm accession (CIAT 26110) and one genetic recombinant (FM9503-S046-024) which maintained greater proportion of green leaves during dry season in the Llanos of Colombia, was associated with greater acquisition of nutrients under water deficit conditions.

Table 5. Correlation coefficients (r) between green leaf biomass (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low or high fertilizer application in a sandy loam Oxisol in Matazul, Colombia.

Shoot traits	Low fertilizer	High fertilizer
Live forage yield (t/ha)	0.85***	0.77***
Total forage yield (live + dead) (t/ha)	0.57***	0.66***
Dead biomass (t/ha)	0.48***	0.57***
Stem biomass (t/ha)	0.93***	0.94***
Leaf TNC content (mg g^{-1})	0.10	-0.08
Leaf ash content (%)	-0.04	0.04
Stem N content (%)	-0.32	0.08
Stem P content (%)	-0.41*	0.04
Stem K content (%)	-0.27	0.21
Stem Ca content (%)	-0.42**	0.22
Stem Mg content (%)	-0.25	0.11
Stem TNC content (mg g^{-1})	-0.13	0.23
Stem ash content (%)	-0.24	0.14

*,**,*** *Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.*

2.2.2 Dry season tolerance of most promising hybrids of *Brachiaria* in the Llanos of Colombia

Contributors: I. M. Rao, J. Miles, C. Plazas, J. Ricaurte and R. García (IP-5)

Rationale. Previous research on evaluation for dry season tolerance in *Brachiaria* grasses indicated that the superior performance of the *Brachiaria* hybrid, FM9503-S046-024 which maintained greater proportion of green leaves during moderate dry season in the Llanos of Colombia, was associated with lower levels of K and N content in green leaves. The main objective of this field study was to evaluate dry season tolerance of the more recent hybrids of *Brachiaria* in comparison with their parents when grown with low nutrient supply in soil at Matazul farm of the Altillanura.

Materials and Methods. A field trial was established at Matazul farm on 31 May of 2001. The trial included four *Brachiaria* hybrids (BR98NO/1251; BR99NO/4015; BR99NO/4132; FM9503-S046-024) along with two parents (*B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294). The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots with three replications. The plot size was 5 × 2 m. A number of plant attributes including forage yield, dry matter distribution and nutrient uptake were measured at the end of dry season (march 2003; 4 months of drought stress), i.e., at 22 months after establishment of the trial. The trial was managed with strong and frequent mob grazing at two months interval.

Results. At 22 months after establishment (4 months after dry season), live forage yield with low fertilizer application ranged from 65 to 235 kg/ha and the highest value of forage yield was observed with the hybrid 4624 (Figure 1). Differences in dry matter distribution among the hybrids and parents indicated that the parent CIAT 6294 was superior to other genotypes in terms of shoot biomass production with both low and high initial fertilizer application. This parent produced greater dead biomass under both low and high initial fertilizer application. The dry matter distribution pattern of the some hybrids such as 4015 was in contrast with that of the parent CIAT 6294. These hybrids produced lower amounts of dead biomass and a greater proportion of aboveground biomass was in green leaves. Another hybrid, 4132 had markedly lower stem biomass compared with green leaf biomass.

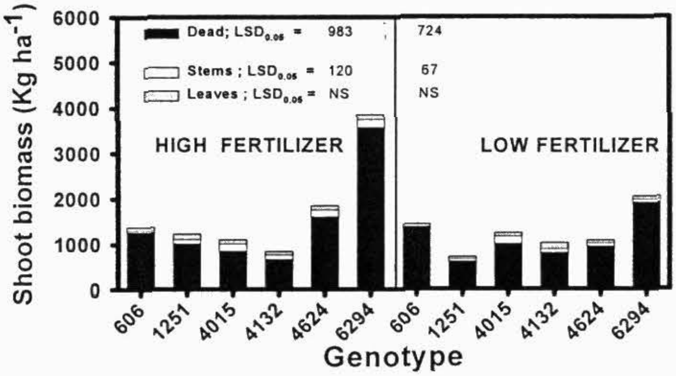

Figure 1. Genotypic variation as influenced by fertilizer application in dry matter distribution among green leaves, stems and dead biomass of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam Oxisol at Matazul, Colombia. Plant attributes were measured at 22 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level.

The hybrid 4132 was also outstanding in its ability to acquire nutrients, particularly from low application of fertilizer (Figure 2). Results on nutrient uptake also showed that two other hybrids 1251 and 4624 were also superior in their ability to acquire nutrients.

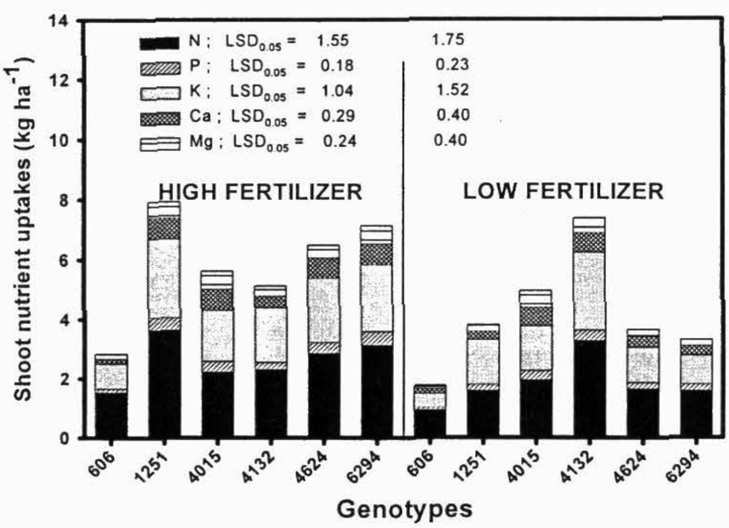

Figure 2. Genotypic variation as influenced by fertilizer application in nutrient (N, P, K, Ca, Mg) uptake of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam Oxisol at Matazul, Colombia. Plant attributes were measured at 22 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level. NS = not significant.

The results obtained on green leaf and stem N and P contents also indicated the superior nutritional quality of the hybrid 1251 under both high and low initial fertilizer application (Figure 3).

The dry season performance of the four hybrids will be monitored for the next two years in comparison with the two parents in terms of forage yield and nutrient acquisition.

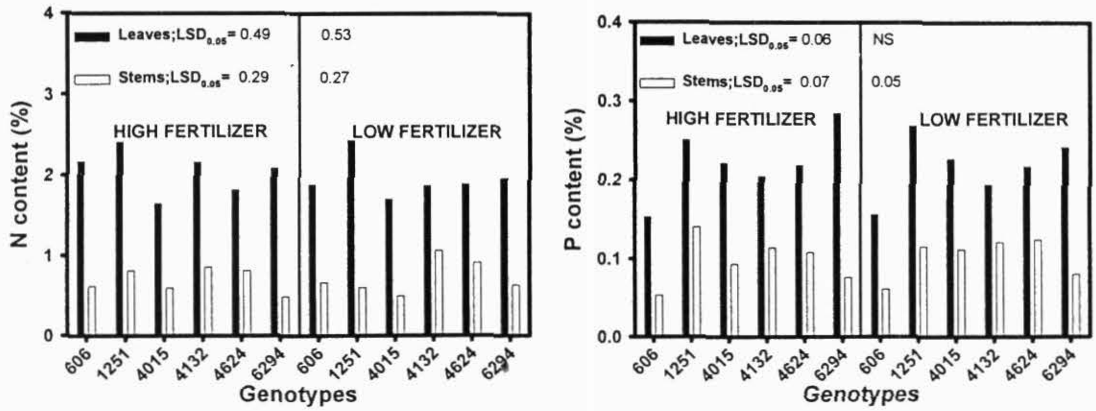

Figure 3. Genotypic variation as influenced by fertilizer application in nitrogen (N) and phosphorus (P) content of leaves and stems of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam Oxisol at Matazul, Colombia. Plant attributes were measured at 22 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level.

Output 3.

Crop, forage, water and soil management strategies developed to minimize sources and/or increase sinks of GHG.

3.1 The biological phenomenon of nitrification inhibition in *Brachiaria humidicola* and other tropical grasses

Highlights

- Found substantial differences in total and specific NI (nitrification inhibition) activity among tropical grasses.
- Developed and tested a simple protocol to quantify the impact of addition of root exudates from plants on nitrification inhibition of incubated soils.

Last year, we showed the feasibility of using a bioassay (with recombinant *Nitrosomonas europaea*) that detects nitrification inhibitory activity in plant (such as root exudates or tissue extracts) or soil samples (such as soil water extracts). Using this bioassay we have shown that root exudates from *B. humidicola* inhibit nitrification. The inhibitory activity of the root exudates increased with the plant age (mostly because of the increase in root mass) and showed a sigmoid pattern; subsequently, NI activity in root exudates declined as the plants reach the maturity stage.

This year we report our ongoing research activities on methodology development, and comparative evaluation of other tropical grasses for the ability to inhibit nitrification. The other ongoing research activities in this collaborative project include: isolation of the active compound responsible for NI activity in *B. humidicola*, mechanisms underlying the inhibition of nitrification in root exudates, and factors that regulate the expression of NI activity. Results from these activities will be reported next year.

3.1.1 Bioassay – Further improvements and refinements in the methodology

Contributors: G.V. Subbarao, K. Nakahara, T. Ishikawa, K. Okada and O. Ito (JIRCAS, Japan)

The bioassay methodology was adopted from Izumi *et al.* (1998), which was initially developed to detect nitrification inhibitors in the municipal-waste water treatment plants. This methodology has gone through improvements to get reliable and stable measurements in detecting inhibitory effect on nitrification from root exudates, tissue extracts and soil-water extracts. Of the various factors, one of the most important is the incubation temperature of the bacterial culture with the test compound (i.e. root exudates) for the bioassay measurements. The bioassay appears to be at its best in detecting the inhibitory activity at 15°C; the bioassay's ability to detect inhibitory effect from a known inhibitor (allylthiourea) decreased with an increase in incubation temperature. Also, the room temperature where the luminometer is located should be maintained close to 20 to 22°C to obtain stable measurements.

The *Nitrosomonas* culture age of 6 to 7 days is found to be the optimum stage for the bioassay measurements; beyond this, the response to known inhibitor (Allyl thiourea) decreased with the culture age. These modifications are now part of the bioassay methodology (for the other details on the methodology please see CIAT, 2002 – PE-6 Annual Report) to evaluate nitrification inhibitory activity of the plant samples and will improve our ability to generate reliable and stable measurements for this research project.

References

Izumi, T., Mizumoto, M., and Nakamura, K. 1998. A Bioluminescence assay using *Nitrosomonas europaea* for rapid and sensitive detection of nitrification inhibitors. Applied and Environmental Microbiology, 64:3656-3662.

3.1.2 Root exudates – Development of sample processing and preparation protocols for the determination of NI (nitrification inhibitory) activity using bioassay

Contributors: G.V. Subbarao, K. Nakahara, T. Ishikawa, K. Okada and O. Ito (JIRCAS, Japan)

In most cases root exudates when collected from the plant roots (by keeping intact plant roots in deionised/distilled water for 24 hours) needs to be condensed several fold (about 50 to 100) before they can be used for the determination of NI activity. We have noticed several times that the contamination of chloride (either from water that is used for collecting root exudates or from the soil-water extracts) interferes with the inhibitory activity measurements. To avoid chloride contamination problems in the root exudates and soil-water extracts, we have developed a sample preparation protocol where the root exudates sample is evaporated to dryness using a rotari-evaporator at 45°C, and then extracted with methanol; the methanol extract is further evaporated to dryness and then re-dissolved in dimethyl sulfoxide. Using this sample preparation protocol, we could eliminate completely the problems of chloride interference, as chloride does not dissolve in methanol.

Using several root exudates samples from *B. humidicola*, we have shown that the NI activity from the root exudates or plant tissue extracts can be recovered into the methanol extract. The remaining portion of the root exudates did not show inhibitory activity (data not shown). This sample preparation protocol has now become a standard procedure for processing the root exudates samples for the determination of NI activity.

3.1.3 Comparative evaluation of six species of tropical grasses for the ability to inhibit nitrification from acid soil

Contributors: M. Rondón, Juan Andrés Ramírez, I.M. Rao and C.E. Lascano (CIAT); G.V. Subbarao, K. Nakahara, T. Ishikawa, K. Okada and O. Ito (JIRCAS, Japan)

Rationale. Collaborative research with JIRCAS colleagues has shown that *B. humidicola* CIAT 679 inhibits nitrification of ammonium and reduces the emission of nitrous oxide into the atmosphere (PE-6 2002 Annual Report). Given these findings with one genotype of *B. humidicola*, there is a need to determine the extent of genetic variation among tropical grasses in their ability to inhibit nitrification and reduce emissions of N_2O. This information will be extremely useful to develop screening methods to select genetic recombinants of *Brachiaria* grasses that not only are resistant to major biotic and abiotic constraints but also can protect the environment. Given the vast areas under *B. humidicola* in the tropics, reductions in net emissions of N_2O could have important environmental implications. The main objective was to quantify differences among several tropical grasses to inhibit nitrification and associated reductions in N_2O emission under greenhouse conditions using infertile acid soil. Also we intend to correlate nitrification inhibition with root biomass and length, and to monitor nitrate and ammonium levels in the soil after addition of ammonium –N as fertilizer.

Materials and Methods. A sandy loam Oxisol from the Llanos (Matazul) of Colombia was used to grow the plants (4 kg of soil/pot). A basal level of nutrients were applied before planting (kg/ha): 40 N, 50 P, 100 K, 66 Ca, 28.5 Mg, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo. A total of six different tropical grasses were used as test plants at two levels of ammonium sulfate application (0 and 100 kg/ha). The grasses included: *B. humidicola* cv. Humidicola; *B. decumbens* cv. Basilisk; *B. dictyoneura* cv. Llanero; *B.* hybrid cv. Mulato; *B. brizantha* cv Marandu; *P. maximum* cv. Common. Two control treatments were included: soil without plants that had no application of ammonium sulfate or had application of ammonium sulfate. The experiment was arranged as a completely randomized block design with four replications. Plants were allowed to grow for seven weeks and were cut to 10 cm to stimulate regrowth for three weeks and were cut again at 10 cm height to allow regrowth and to simulate grazing effects under field conditions. After another week (at 11 weeks) of regrowth, ammonium sulfate was applied in solution at a rate equivalent to 100 kg $N\text{-}NH_4$ per hectare. To favor a greater development of plant roots in the pots, which could increase the NI effects, the plants were allowed to grow for 13 weeks more (at 24 weeks) before making a second application of ammonium sulphate at the same dose. Plants grew for four weeks more before final harvest (at 28 weeks). Thus the total length of the experiment was seven months. At the end of the experiment, plants were harvested and separated into shoot and roots. Root length was measured using a root length scanner. Dry matter content and N status of both shoot and roots was determined.

Results and Discussion. Results from the comparative evaluation of six tropical grasses (that are predominantly grown in South America) indicate that substantial levels of NI (Nitrification inhibitory) activity is present in the root exudates of other *Brachiaria* grasses in addition to *B. humidicola* (Table 1). However, among *Brachiaria* grasses tested in this study, NI activity is substantially lower in *B. brizantha* cv. Marandú. It is interesting to note that a complete absence (below the detectable limit to our bioassay) of NI activity from the root exudates of *P. maximum*.

The total NI activity (i.e. NI activity from four plants pot^{-1}) of *B. humidicola, B. decumbens, B. dictyoneura and Brachiaria* hybrid cv. Mulato were similar based on our bioassay estimations (Table 1). Nevertheless, the NI activity of the root exudates needs to be confirmed further using soil incubation studies.

Table 1. Nitrification inhibitory activity (total NI activity pot^{-1}) of the root exudates from 6 tropical grasses grown under two levels of N fertilization. Plants were grown for seven months before being used for collecting root exudates.

Pasture species	N Fertilizer Treatment	NI activity (in AT units pot^{-1})	SD
B. humidicola	With ammonium supply	81.95	7.35
B. decumbens	With ammonium supply	77.39	5.33
B. dictyoneura	With ammonium supply	70.54	11.30
B. hybrid Mulato	With ammonium supply	84.52	11.20
B. brizantha	With ammonium supply	49.62	13.33
P. maximum	With ammonium supply	-9.43*	8.80
B. humidicola	Without ammonium supply	13.04	6.46
B. decumbens	Without ammonium supply	21.43	1.28
B. dictyoneura	Without ammonium supply	19.22	1.90
B. hybrid cv. Mulato	Without ammonium supply	23.19	1.45
B. brizantha	Without ammonium supply	23.91	0.36
P. maximum	Without ammonium supply	1.81	2.39

Note: Ammonium was supplied as ammonium sulfate at 100 kg ha^{-1} rate. NI activity is expressed as AT units; One AT unit is defined as the inhibitory activity caused by the addition of 0.44 μM of allylthiourea in the bioassay medium. Thus, the inhibitory activity of the test samples of root exudates is converted into AT units for the ease of expression in numerical form.

**Negative activity - nitrification was stimulated by the root exudates. SD = standard deviation.*

The presence of substantial levels of NI activity in the root exudates of *Brachiaria* hybrid cv. Mulato has immediate practical implications as this hybrid has a huge potential for being planted in large areas of South America. Also, the implications of such high levels of nitrification inhibition (if confirmed further from the soil incubation studies and field studies) in *Brachiaria* hybrid cv. Mulato opens the possibility to screen for this trait in the *Brachiaria* improvement program. The lack of nitrification inhibition ability in *P. maximum* is an interesting issue as this is the most commonly grown pasture species with high levels of N inputs.

Large differences in specific NI activity among the tropical grasses were found in this study, with *B. humidicola* exhibiting the highest level of inhibition (Table 2). Furthermore, the total and specific NI activities were substantially lower in the treatment without ammonium in all the *Brachiaria* grasses tested. Similar trends (data not shown) were found when the specific activity was calculated using total root length instead of root dry weight that is shown in Table 2.

The immediate task is to characterize and quantify this phenomenon further contrasting grasses (e.g., *B. humidicola* vs. *Panicum maximum*) both under glasshouse and field conditions. This would help us understand the potential impact of this relatively new phenomenon in nitrogen dynamics in pasture systems. We also need to determine the relative importance of total NI activity vs. specific NI activity in influencing the nitrification process (i.e. inhibition) in a soil environment. Whether the presence of ammonium nitrogen (or any form of nitrogen) near the root zone of the soil acts as a triggering factor for the release of inhibitory compounds or if the plant nitrogen status regulates the NI activity in the root exudates are other issues that need to be resolved through further research work.

Table 2. Specific NI (Nitrification inhibitory) activity (NI activity g^{-1} root dry wt) of the root exudates from six tropical grasses grown under two levels of N fertilization.

Pasture species	N Fertilizer Treatment	Specific NI activity	SD
B. humidicola	With ammonium supply	17.30	0.34
B. decumbens	With ammonium supply	12.31	0.27
B. dictyoneura	With ammonium supply	5.93	0.68
B. hybrid *Mulato*	With ammonium supply	10.16	2.26
B. brizantha	With ammonium supply	8.35	2.20
P. maximum	With ammonium supply	-1.67	1.34
B. humidicola	Without ammonium supply	2.87	1.17
B. decumbens	Without ammonium supply	7.22	2.19
B. dictyoneura	Without ammonium supply	2.90	0.31
B. hybrid Mulato	Without ammonium supply	6.46	0.45
B. brizantha	Without ammonium supply	6.75	0.40
P. maximum	Without ammonium supply	0.69	0.75

3.1.4. Development of an incubation protocol to assess *nitrification inhibition* by addition of root exudates to soils.

Contributors: M. Rondón, Juan Andrés Ramírez (PE-6), I.M. Rao and C. E. Lascano (IP5) (CIAT); G.V. Subbarao, K. Nakahara, T. Ishikawa, K. Okada and O. Ito (JIRCAS, Japan)

Rationale. According to the results presented in the preceding section, root exudates from various tropical grasses have differential ability to reduce nitrification activity as demonstrated by the bioassay. As indicated before, the next sequential step is to assess the effect of application of root exudates directly to soil in relation with Nitrification Inhibition. Given that the process of collecting and concentrating root exudates is time and labor intensive, and that it is difficult to generate large amounts of root exudates, it is desirable to develop a methodology to apply root exudates to small amounts of soil in conditions that allow a proper monitoring of inorganic nitrogen in the soil as well as reliable monitoring of fluxes of nitrous oxide. This later process is especially difficult to follow, given the inherent variability in gas fluxes from soil even under controlled incubation conditions.

The purpose of a recent scientific internship of Marco Rondón at JIRCAS laboratories was to develop and test a simple incubation methodology to quantify the effect of application of root exudates to soils on nitrate and ammonium levels and on fluxes of nitrous oxide. As nitrous oxide fluxes are highly dependent on the moisture content of the soil, the method needs to maintain moisture content at levels of 50-60% of water filled pore space (WFPS), which is considered optimum for nitrification in most soils (Del Grosse et al., 2002) After trying various alternatives, a suitable method was developed and tested. The final protocol is described here, and some results using it are presented.

Description of the method. Plastic syringes (20 ml) are used both as incubation containers and as gas collection chambers for monitoring gas fluxes. 10 grams of air-dried soil are loaded inside the syringe on top of an inert fine nylon (100 µm mesh), which allows gas exchange but prevents soil to move out of the syringe. On top of the soil surface, another two discs of nylon mesh are placed to hold the soil in place and serve as barriers to reduce moisture evaporation from the soil surface. The nylon barrier also serves to isolate the soil from the syringe piston during the gas collection process. Once the soil and nylon discs are placed inside the syringe, the syringe is tapped several times until the soil redistributes itself into a minimum volume. Water, fertilizer solution or the root exudates to be tested are then added to the soil to reach 60% WFPS. Water is sprinkled slowly and uniformly on top of the nylon mesh with a fine syringe needle. The liquid progressively moistens the soil by capillary movement and after about one hour, the soil is well mixed with the liquid without a need for further disturbances. This procedure allows a very effective control of the moisture content in the syringes, which otherwise, is a very time consuming and error causing procedure. Temperature is maintained stable during the incubation time by placing the syringes in an incubation chamber. During the incubation time, the soil can freely exchange gases with the surrounding air. The combination of controlled temperature and moisture content permits reliable and reproducible monitoring of gas fluxes.

At the time of collecting gas samples, syringes are removed from the incubation chamber. A Teflon valve is attached to the lower end of the syringe. The syringe piston is inserted into the barrel and the piston is depressed fully (without compressing the soil) to force the air inside the soil pore space to move outside the syringe. The soil air is replenished with fresh air by moving backwards the syringe piston. This procedure is repeated four times to warrant a complete exchange of the original air inside the soil pores. Once this is done, the gas sample corresponding to time zero could be collected. Then, the piston is raised at a predetermined height inside the syringe, to provide a preset soil to air volume ratio. The needle valve is closed creating a tight sealed chamber. The chamber is allowed to exchange gases during a determined gas collection period, typically one hour. After this time, the air inside the chamber could be directly analyzed by gas chromatography or transferred into pre-evacuated glass vials to be analyzed at a later time. Once the sample is collected, the syringe piston is removed to allow the soil to exchange again gases freely with the surrounding air. he procedure permits an easy way to adjust the air to soil volume ratio, so very low fluxes of gases could be detected. The procedure was tested repeatedly and good reproducibility in gas measurements was obtained.

References

Del Grosse, S., Jim, D. Patron, W. Mosier, A. Peterson G., Scheme D. 2002. Simulated effects of dry land cropping intensification on soil organic matter and greenhouse gas exchanges using DAYCENT ecosystem model. *Environmental Pollution* 116, S75-S83.

3.1.5 Effect of application of root exudates on inorganic nitrogen and fluxes of nitrous oxides from incubated soils.

Contributors: M. Rondón, Juan Andrés Ramírez (PE-6), I.M. Rao and C. E. Lascano (IP-5) (CIAT); G.V. Subbarao, K. Nakahara, T. Ishikawa, K. Okada and O. Ito (JIRCAS, Japan)

Rationale. Once the nitrification inhibition activity in tropical grasses was confirmed in the bioassay medium, the next logical step was to validate whether or not the same phenomenon was evident in soils.

Materials and methods. The procedure described in activity 3.1.4 was used to study the effect of application of root exudates on fluxes of N_2O from incubated soils. Root exudates were obtained from intact plants grown in solution media, which were transferred from the nutrient media into de-ionized water. Plants were allowed to exudate during 24 hours and the resulting root exudates were concentrated using a rotovapor at 45°C.

Experimental treatments included:

1. Blank (W): Application of water
2. Control (AS): application of aqueous solution of ammonium sulfate at a rate of 91 ug N-NH_4/ g soil

3. Root exudates from *B. humidicola* at low concentration (BL). Exudates were concentrated from 30 liters into 900 ml with a rotovapor.
4. Root exudates from *B. humidicola* at high concentration (BH). Exudates were concentrated from 30 liters into 200 ml with a rotovapor.
5. Root Exudates from soybean at low concentration (SNB). Exudates were concentrated from 30 liters into 900 ml.

Treatments with root exudates received the same dose of ammonium sulfate as the Control AS. The ammonium was dissolved in the root exudates prior to adding the solution to soils.

A fertile Andisoil from Tsukuba was used. Soil was collected from the top 20 cm in the field, air dried and then sieved with a 2 mm mesh. Soil was well mixed and 10 g sub samples were loaded into plastic syringes. The corresponding treatment liquid (Water, ammonium sulfate, root exudates + AS) was then applied to the syringes. 3.6 ml of aqueous solution was used in each syringe, to raise the soil moisture content to 60% WFPS. Once wetted, the syringes were kept at 21°C in an incubation chamber for 24 days. These syringes were subsequently used to monitor fluxes of N_2O over time, according to the procedure described above (activity 3.1.4). Gas samples were collected at 5, 9, 14, 19 and 24 days after ammonium sulfate application. 8ml of the gas sample were transferred into a 4ml preevacuated glass vial. Analysis of N_2O was performed typically within two days after collection using a Shimadzu-14B GC equipped with an ECD detector, and a stainless steel column packed with Porapaq Q80 mesh.

Gas fluxes were calculated by linear regression of chamber headspace concentration vs. time. The results are presented as arithmetic means of three replicates.

For monitoring of nitrate and ammonium levels, parallel samples of soil were incubated. Due to limited availability of root exudates, only 2 grams of air-dried soil was used for monitoring of inorganic N. The vials received the same treatments as the syringes and incubation was started at the same date. KCl extracts were made of the incubated soil in the vials at the same dates of gas collection. 20 ml of 2M KCl were used to extract the 2 g of soil in each vial. Shaking time was 1 hour. The KCl extract was filtered immediately after shaking using Whatman 5C filter paper that was prewashed with KCL. Extracts were maintained refrigerated until the time of analysis. Analysis was done colorimetrically using an Autoanalyzer.

Results and Discussion. As expected (Figure 1), addition of water or aqueous solutions to air dried soil resulted in a temporary increase in fluxes of N_2O. The addition of root exudates from soybean strongly promoted the fluxes of the gas from the soil during the initial week of incubation compared to the control treatment. Recent research (G.V. Subbarao, personal communication) has shown that root exudates from soybean also promote nitrification in the bioassay medium. This may be the result of addition of readily available carbon sources in the root exudates. The large peak of N_2O could be caused by rapid denitrification. Reports from the literature (Azam et al., 2002), indicate that when nitrogen is added to the soil, in addition to nitrogen sources, microbial respiratory activity is greatly enhanced and this may result in a rapid depletion of oxygen in the soil micropores which favors the formation of anaerobic microsites where denitrification may take place. After one week, the initial peak of nitrous oxide decays and soils that received root exudates from B. *humidicola* at high concentration, show subsequently lower emissions of nitrous oxide.

In Figure 2, accumulated net fluxes of nitrous oxide for a period of 24 days are presented. Exudates from soybean also result in appreciable higher net accumulated fluxes of N_2O during the incubation period. Whether these fluxes are resulting from enhanced nitrification or denitrification needs to be clarified in further studies. No appreciable reduction in fluxes of the gas appear to occur as a consequence of the addition of root exudates from *B. humidicola* at low concentration, suggesting that the added dose of the active compound was probably not high enough to interact with all the population of nitrifying bacteria in the soil. In contrast to this, the addition of exudates of *B. humidicola* at high concentration resulted in a net decrease of around 45% of total emissions of N_2O relative to the control treatment.

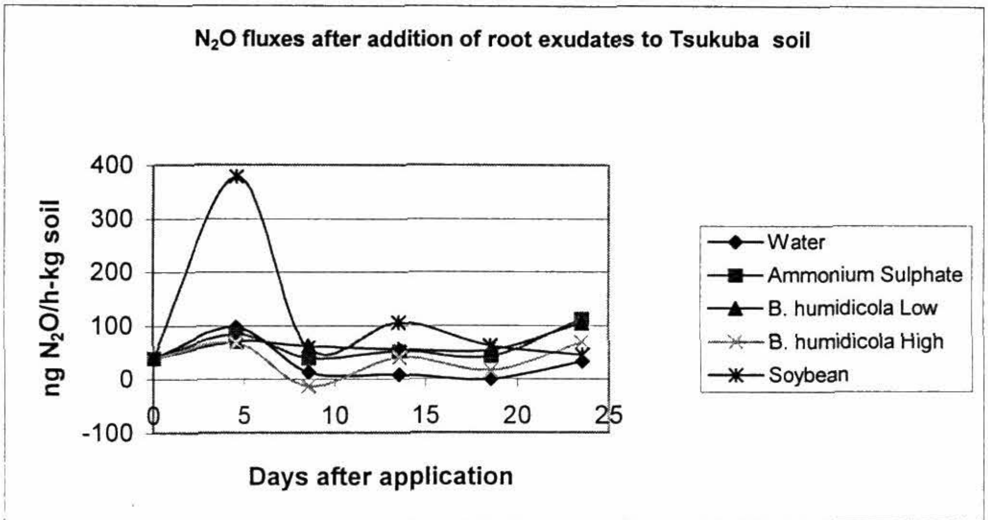

Figure 1. Fluxes of nitrous oxide following addition of root exudates from different species to incubated Andisol.

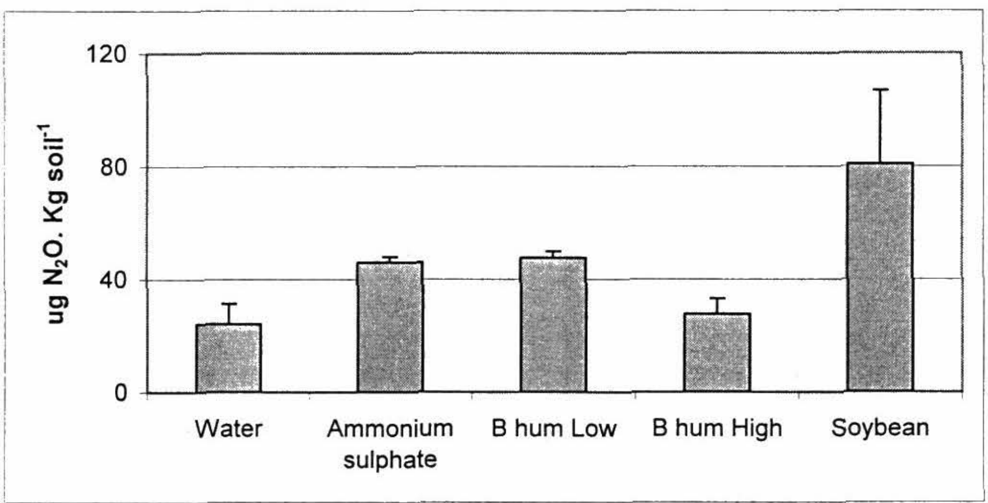

Figure 2. Accumulated fluxes of nitrous oxide over a 24-day incubation period.

Similar trends were observed in levels of ammonium in the soils, (Figure 3). Ammonium levels were consistently higher in the *B. humidicola* treatment indicating reduced nitrification rate, while consistently lower levels were found in the soybean exudates treatment confirming promoted nitrification.

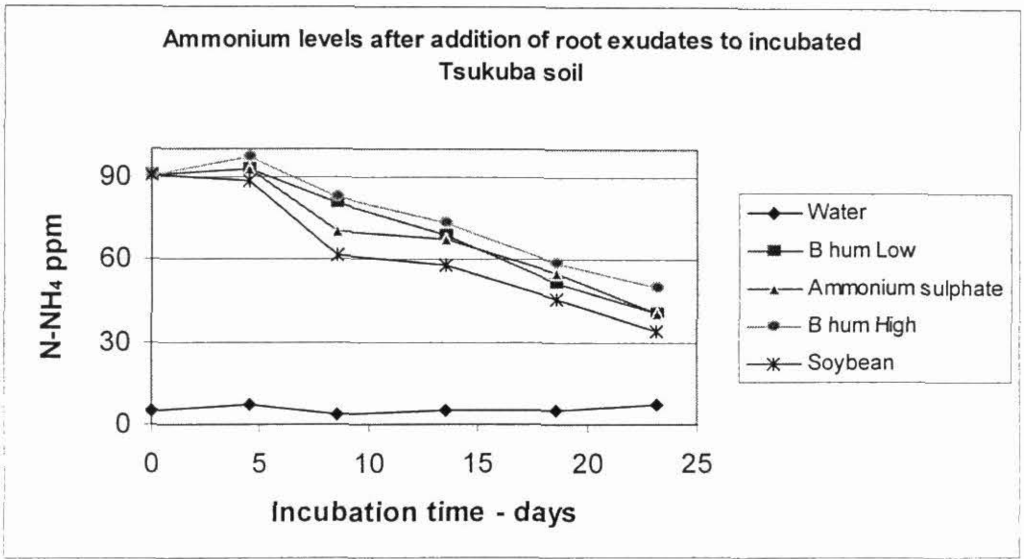

Figure 3. Ammonium evolution on incubated soils after receiving root exudates.

The nitrification inhibition seems to be more effective between 5 and 9 days as indicated by the slope of the graphs in Figure 3. After two weeks of incubation, the curves show similar slopes suggesting that the residual effect of the root exudates could be of around two weeks. This is shorter residual time than the three weeks suggested by previous studies in pots (Ishikawa *et al.*, in press), and could be an indication of low additions of the active compound with the root exudates as compared with concentrations generated by the root system in pot experiments or in the field.

Results from this study suggest that there is a minimum threshold concentration of root exudates (active compound) required to effectively reduce nitrification in soils. Once the active compounds in root exudates that are responsible for NI activity are identified, it would be possible to conduct more controlled experiments to add known amounts of the active compound to the soils.

The procedures developed and tested here, have proven indeed to be easy, and reliable for monitoring fluxes of nitrous oxide and appropriate to follow the levels of inorganic nitrogen in the soil. These methods could be applied to test the effect of additions of root exudates from different plant species to soils on Nitrification Inhibition and associated fluxes of N_2O and would contribute to rapid advances in this field of research.

The method is being currently used to assess the effect of application of various root exudates into soils of contrasting chemical and physical characteristics including Oxisols from Colombian savannas and inceptisols from Andean hillsides. Results will be reported next year.

References

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Ishikawa, T., G.V. Subbarao, O. Ito, and K. Okada. 2003. Suppression of nitrification and nitrous oxide emission from soil by a tropical grass, *Brachiaria humidicola. Plant and Soil* (in press).

3.2 Silvopastoral multistrata systems to improve cattle productivity and reduce GHG emissions.

Contributors: Socorro Cajas (CORPOICA), Marco Rondón, Juan Andrés Ramírez, CIAT (PE-6). Edgar Amézquita, Mariela Rivera, CIAT (PE-2).

Highlights

- Found that the conversion of traditional grass only pastures in the seasonally dry savannas of the Caribbean region in Colombia, to grass-forage shrubs or grass-trees silvopastoral systems results not only in increases in cattle productivity, but also in net reductions in emissions of greenhouse gases into the atmosphere.

Rationale. The savannas in the Caribbean region of Northern Colombia have been traditionally used for cattle ranching, being the first cattle producing area in the country. Productivity in these savannas is limited by lack of forage during the four months of severe dry season. As an attempt to improve availability of forage during the dry season, a 30 hectares multistrata silvopastoral experimental system, with varying proportion of tree components, was established by CORPOICA in Macagual, Córdoba in mid-1998. The systems have been continuously evaluated and resulted in net increases in the cattle carrying capacity of the land. Economic analysis indicate that the cost of establishing and maintaining the systems could be recovered in three years. This year we started research to establish the baseline of carbon stocks and emissions of GHG for some of the plots in the multistrata systems.

Materials and Methods

Field site, location and general description. The field site was situated within the Turipaná Research Centre, Cereté-Córdoba, in the Valle del Sinú, microregion of Colombia (8° 51'N, 75° 49'W, 18 m above sea level). The experiment was established on 30 ha of cattle-grazed pasture that was predominantly covered by *Dichanthium aristatum* grass with *Brachiaria mutica* found as a major componente in some wetter parts. The most frequent herbaceous legumes found at the site were *Centrosema pubescens, Desmodium uncinatum, Rhynchosis minima, Teramnus uncinatus* and *Vigna sp.*

Layout and experimental design. A randomized complete block design with three replications was used. The experiment was established using four multistrata treatments of different structural complexity plus a control with pasture only. The treatments comprised different combinations of four distinct strata: pasture, shrub layer, arboreal (fruit producing) tree layer and timber tree layer. The control treatment contained only pasture, treatments 2 and 3 involved only shrub and arboreal layers respectively in addition to pasture, and treatment four combined pasture, shrubs and arboreal strata while treatment 5 had all four strata. The target density of plants was higher for lower strata species (625, 156 and 39 trees ha^{-1} for shrubs, arboreal and timber trees respectively) and a substitutive design was used where multiple strata were combined. This meant that treatments 2, 4 and 5 formed a substitutive sequence but treatments 3 and 4 were an additive comparison and treatments 2 and 3 were alternatives. Each of the woody perennial strata contained three species. The shrub layer comprised *Crescentia cujete, Gliricidia sepium* and *Leucaena leucocephala* planted in a repeating alternate sequence. For the arboreal tree layer, *Albizia saman, Cassia grandis* and *Guazuma ulmifolia* were planted at a lower density but also in a repeating alternate pattern. In treatment 4, in every second row shrubs were replaced in alternate positions by the three arboreal species. This meant that over the plot as a whole, 23% of the total shrubs were replaced by fruiting trees. For treatment 5, in every fourth row arboreal trees were substituted in alternate positions by the three timber species *Pachira quinata, Swietenia macrophylla* and *Tabebuia rosea*, which meant that over the plot as a whole, 25% of arboreal trees were replaced by timber trees. Table 1 illustrate the differences amongst the five treatments.

Table 1. Description of treatments in the multistrata silvopastoral systems experiment at Turipaná, Colombia, showing the different levels of tree and shrub diversity.

Treatment	Label	Number of strata	Species
1	Control	1	*Dichanthium aristatum* + herbaceous legumes
2	Shrub	2	*Dichanthium aristatum* + herbaceous legumes Shrub species = *Leucaena leucocephala, Gliricidia sepium, Crescentia cujete* Tree density = 625 trees ha^{-1} 4 m × 4 m planting distance
3	Arboreal	2	*Dichanthium aristatum* + herbaceous legumes Arboreal species = *Albizia saman, Guazuma ulmifolia, Cassia grandis* Tree density = 156 trees ha^{-1} 8 × 8 m planting distance
4	Shrub –Arboreal	3	*Dichanthium aristatum* + herbaceous legumes Shrub species = *Leucaena leucocephala, Gliricidia sepium, Crescentia cujete* Arboreal species = *Albizia saman, Guazuma ulmifolia, Cassia grandis* Shrub density = 469 trees ha^{-1} 4 m × 4 m planting distance Arboreal tree density = 156 trees ha^{-1} Arboreal trees substitute for shrubs at 8 m × 8 m spacing
5	Timber	4	*Dichanthium aristatum* + herbaceous legumes Shrub species = *Leucaena leucocephala, Gliricidia sepium, Crescentia cujete* Arboreal species = *Albizia saman, Guazuma ulmifolia, Cassia grandis* Timber species = *Pachira quinata, Swietenia, macrophylla, Tabebuia rosea* Shrub density = 469 trees ha^{-1} Arboreal tree density = 117 trees ha^{-1} Timber tree density = 39 trees ha^{-1} Timber trees substitute for arboreal trees at 16 m × 16 m spacing

Planting conditions. Seeds were sown at the beginning of February and during March 1998, in a nursery at Turipaná research station. *Gliricidia sepium* and *Leucaena leucocephala* were sown in beds, and the remaining species were sown in 15 cm deep black polythene bags. *Guazuma ulmifolia* was first germinated in beds and transferred to polythene bags after germination. *Leucaena leucocephala* and *Guazuma ulmifolia* seeds were sown after inmersion for 1 minute in boiling (100°C) water.

The soil mixture for beds and bags consisted of loam and dry cattle manure, at a ratio of 3:1. All seedlings were kept under dry leaf palm shade and were watered daily in the morning and afternoon. All seedlings were maintained weed-free and those sown in polythene bags were root pruned if they penetrated into the nursery soil.

Once the rainy season was advanced, the seedlings were transferred from the nursery to the experimental plots. Before seedlings were transferred to the field, the herbaceous vegetation of the entire area was cut to ground level using a tractor-drawn grass cutter. All seedlings were hand planted between 24^{th} May and the 15^{th} June 1998. Replacement of dead seedlings was done between 16^{th} June and 16^{th} July in the same year and then again in September after heavy rains. Treatments 2, 3 and 5 of block 3 were seriously flooded at this time and, therefore, extensive replacement planting was necessary in these plots.

Seedlings were planted in plots of 2 ha (98 m × 204 m). Each plot consisted of 25 rows, and each row was demarcated with 50 planting sites at 4 m × 4 m distance, constituting 1250 sites per plot for treatments 2, 4 and 5. Treatment 3 was demarcated with 12 rows and 24 sites per row at 8 m × 8 m distance constituting 288 sites per plot. The shrub species *Gliricidia sepium* and *Leucaena leucocephala* were planted in clumps of four seedlings per site. The remaining species were planted with a single seedling per site.

Manual weeding within a radius of 1 m around the seedlings was done during the first month and then chemical weed control by spraying with a direct application of glyphosate was used to reduce competition during establishment (all seedlings were protected against the glyphosate). During the first 10 months, manual weeding around each seedling was carried out every three months, and subsequently only weeding of climbing weeds was done as necessary (about twice per year). The herbaceous vegetation between rows was kept cut using a grass cutter until grazing started in June 01-1999. Before cattle were introduced to plots, the timber trees were protected with a simple tree guard comprised of blue nylon netting supported by three wooden stakes (2.2 m high × 65 mm diameter) in a triangular arrangement 1 m × 1 m apart, costing approximately 2 USD for materials and 0.3 USD in labor for construction. Cattle were grazed on a rotational basis on the plots from June to September 1999, and from January to May 2000.

Soil Analysis. Four years after experiment establishment, a detailed characterization of soil physical and chemical properties was performed. Each plot was divided in 3-transepts (67 × 99 m). The samples were collected only in treatments 1 (Control), 2 (Shrub), and 3 (Timber). Undisturbed core samples were taken for depths between 0 and 60 cm. The following parameters were measured: Saturated hydraulic conductivity, susceptibility o compaction, bulk density, total porosity, macroporosity, air permeability, moisture at field capacity, texture and organic carbon content.

Analysis of CH_4 and N_2O. Air samples were collected using the vented close chamber method. The chambers were installed at the Control, Shrub, and Timber treatments inside the block 1. Four chambers were used by treatment. Air samples were collected at 0, 10, 20, and 30 minutes after chamber installation. Gas sampling was started in November 2002 and continues so far. CH_4 and N_2O are analyzed using a gas chromatograph (Shimadzu GC 14A) equipped with ECD and FID detectors. Compressed Air and Scotty prepared standard containing 1 and 3 ppm CH_4 and 0 and 1 ppm N_2O were the most commonly used standard.

Biomass Determination. Grass biomass was measured using 10-one square meter quadrats in each plot. For the shrub and arboreal components, allometric equations were developed by destructively harvesting and measuring at least 10 trees/ shrubs of each species. Diameter at breast height (DBH) and total height were used to correlate with total dry weight and total carbon. All trees and shrubs in the plots were measured for DBH and the total biomass per unit area calculated using the specific *allometric equations.*

Results and Discussion. Soils are in general very heavy textured with bulk densities in the range of 1.35 or more below 5 cm depth. Bulk density is generally lower only at the surface layer. Saturated hydraulic conductivity is low which favor low infiltration rates and results in frequent saturation of the top layers during the rainy season.

Carbon content in the soil is high on the surface layers but decreases very rapidly with depth (Figure 1). Plots with shrubs-grass have significantly higher carbon content in the surface layer, but not appreciable differences were found in the rest of the soil profile. Table 2 shows total carbon stocks in the soil profile to 60 cm depth. Though the shrub plots show slightly higher C stocks, differences were not significant (P>0.05%) between treatments. Carbon stock in these soils is high compared to other tropical environments.

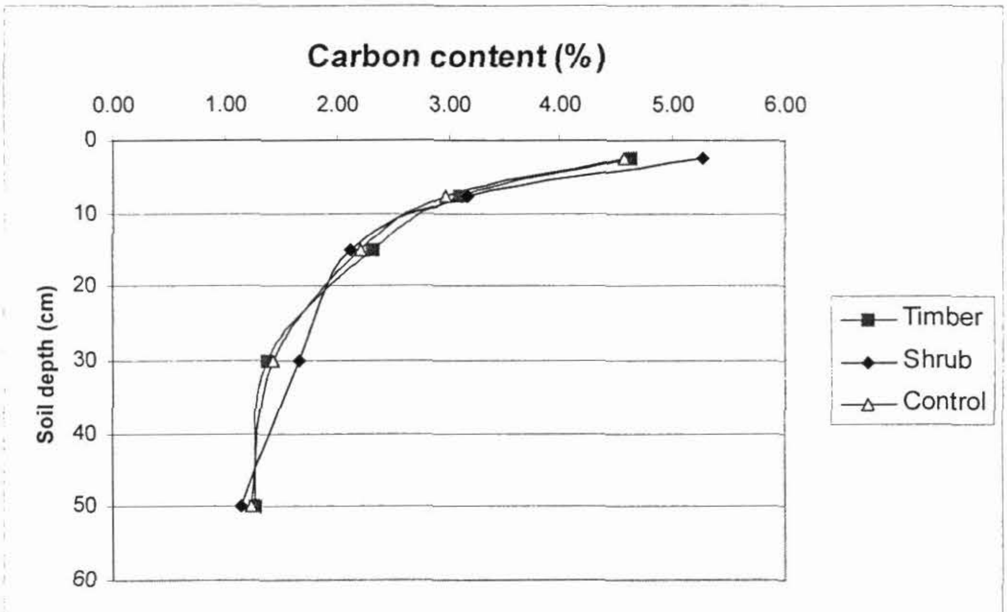

Figure 1. Carbon content in soils under different silvopastoral systems. Turipaná, Colombia.

Table 2. Total carbon stocks (ton C/ha) in the soil profile of 4 years old silvopastoral systems. Montería, Colombia. Numbers in parenthesis indicate standard deviations.

Depth	Control		Shrub		Timber	
0-5	28.27	(4.32)	34.50	(6.65)	29.88	(3.81)
5-10	20.04	(1.98)	21.08	(2.59)	20.83	(3.34)
10-20	29.52	(4.73)	28.47	(6.44)	31.74	(4.78)
20-40	37.09	(3.57)	43.06	(2.82)	37.19	(2.09)
40-60	32.92	(5.63)	29.97	(5.92)	33.31	(4.27)
TOTAL	147.84	(20.23)	157.08	(24.42)	152.93	(18.29)

Fluxes of CH_4 and N_2O. As usually occurs in soils, temporal variation of CH_4 emissions is high, with values ($\mu g\ CH_4\ m^{-2}h^{-1}$) ranging between 31.85 and –29.78, for Shrub and Control, respectively. Spatial variability is also high, with RSD values ranging between 10 and 600% among chambers. Nevertheless, RSD for the majority of samples is between 40 and 120%. Integrated methane fluxes from the Shrub plots were consistently higher than fluxes from the control plots as indicated in Figure 2.

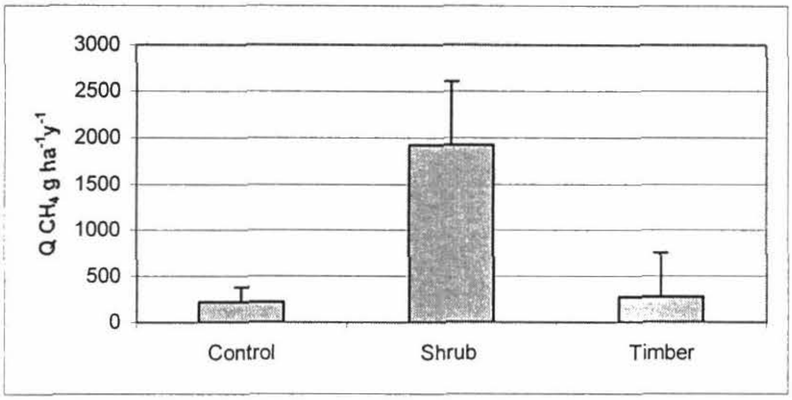

Figure 2. Integrated annual methane emissions from silvopastoral systems at Turipaná, Colombia. Flux measurements were made from December 2002 to June 2003. Data has being extrapolated to an annual estimate.

On an annual basis, all systems are net methane sources. No significant difference is observed between control and timber plots, but the shrub plots showed significantly ($P>0.01\%$) higher emissions which could be attributable to the lower water infiltration rates in these plots which may result in longer prevalence of anaerobic conditions in the soils, which is known to promote methanogenesis.

Fluxes of N_2O for the period December 2002 to June 2003 are illustrated in Figure 3. As for methane, nitrous oxide emissions present high temporal and spatial variability. Major flux peaks follow periods of intense rainfall. Temporal trends were very similar between treatments but integrated net N_2O emissions show that with an annual release of 27.3 kg N_2O/ha-y, Shrub emits significantly higher ($P<0.05\%$) amounts of nitrous oxide than the control (16.5 kg/ha-y) and similar to the timber treatment (23,2 kg/ha-y)

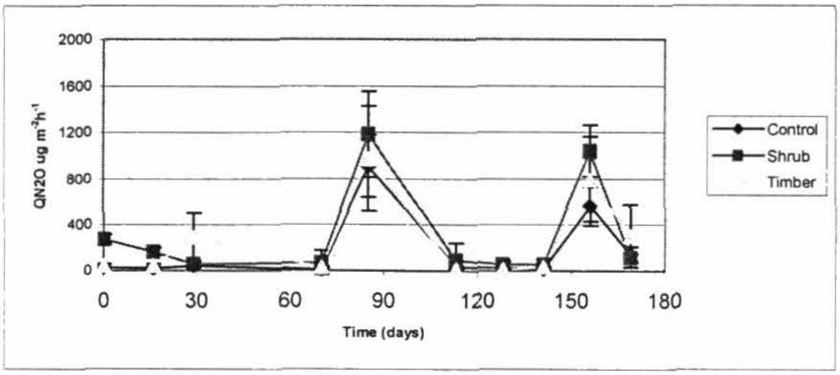

Figure 3. Temporal evolution of fluxes of N_2O from soils under silvopastoral systems. Turipaná, Colombia.

Higher gas emissions in the shrub-grass plots may be associated to the higher organic matter content of the surface layer in these plots (Figure 1) and to the higher nitrogen turnover that should result from higher density of legume species in these plots. Nitrogen turnover however still needs to be monitored. Similar trends have been reported by Palm et al. (2002).

C accumulation in biomass. Figure 4 shows carbon accumulation in the biomass of all the systems implemented in the study. Data includes both aerial and root biomass. Root biomass was estimated as 30% of the aerial biomass (Brown et al., 1989). There are no differences between the grass alone control pasture and the grass-shrub system. However, as the arboreal component increases in the system, the accumulated biomass increases accordingly, being maximum at the timber plots where number of large trees is highest. As the systems were four years old at the time of measurement, carbon accumulation rates reach 6 ton C/ha-y which is similar to what secondary forest regeneration could accumulate in the Central Amazon (Feldpausch et al., in press).

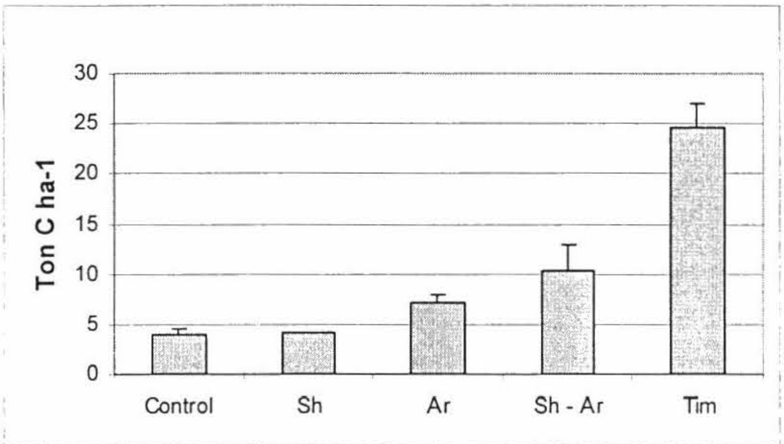

Figure 4. Total carbon accumulation in aerial biomass of silvopastoral systems. Turipaná, Colombia. Evaluated plots included: grass alone pasture (Control), grass-forage shrubs (Sh), grass – arboreal components (Ar), grass-shrubs and arboreal components (Sh-Ar) and complex system include all the species plus timber trees (Tim) - Specifically developed allometric equations were used to estimate carbon stocks.

Methane emissions by cattle. Direct emissions of methane associated to cattle were not directly measured due to lack of appropriate equipment. Instead, emission factors from the literature were used to estimate total annual emissions (Flessa et al., 2002). Table 3 presents the actual animal carrying capacity of the plots and their estimated annual emissions of methane. The same factor was used for all the treatments. It is expected however that different diets will result in different emission factors for each plot.

Table 3. Pasture carrying capacity of silvopastoral systems and estimated annual methane emissions by cattle units are based on a 450 kg beef

System	Cattle units/ha	Annual methane emission kg CH_4/ha-y
Control	1.54	83.4
Shrub	2.01	104.9
Timber	1.52	79.3

Integrated Global Warming Potential (GWP). In order to generate an integrated parameter to include all factors that contribute to balances of greenhouse gases in the systems, the GWP is evaluated. GWP is expressed as units of CO_2 equivalents. Relative radiative forcing potential of the diverse greenhouse gases reported by IPCC (2001) have been used in this assessment. The following parameters have been included in our estimation: total carbon stocks in soils (to 60 cm depth) and biomass, methane emissions by cattle, fluxes of methane and nitrous oxide from the soil. All factors are converted into units of equivalent CO_2 fluxes (kg y^{-1}), before adding them to calculate net GWP. Values for GWP are presented in Figure 4.

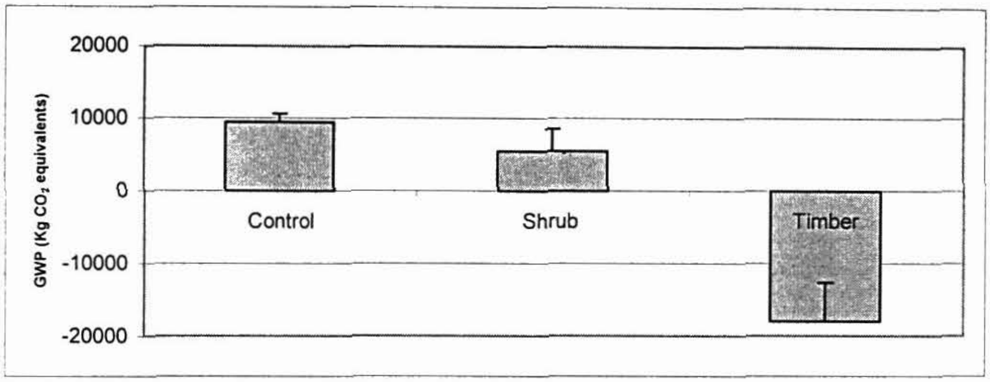

Figure 4. Integrated Global Warming Potential of silvopastoral systems. Turipaná, Colombia.

Values correspond to the radiative forcing resulting from net annual fluxes of greenhouse gases per hectare in the systems

Conventional grass alone pastures are net sources of greenhouse gases to the atmosphere and the main contribution comes from methane emissions from the cattle followed by nitrous oxide emission from the soils. Introducing forage shrubs in the pasture increase contribution from both soils and cattle, but results in net carbon accumulation in the top layers of the soils, with a net small decrease in total emissions. The introduction of trees and timber species in the pastures drastically modifies net fluxes and the high carbon accumulation rate found in the trees turns the system to be an important net sink for GHG.

Conclusions. The conversion of traditional grass only pastures which frequently are in progressing stages of degradation, into silvopastoral systems has proven to improve considerably the productivity of the pastures, mainly through an increase in food supply during the more than four months dry season (Cajas et al., 2002). Economic analysis indicate that the costs of establishing and maintaining the systems could be recovered in three years (Cajas et al., 2001). There are thus economic incentives to farmers to introduce shrubs and trees into their pastures and effectively this research has generated great interest from the cattle ranchers in the region who have been exposed to the system. Results from this study indicate that the introduction of silvopastoral systems also result in a net beneficial environmental impact, evidenced by a reduction in net emissions of greenhouse gases to the atmosphere. Potential implications for the region are important given that the Caribbean savannas account for nearly 40% of the beef and milk produced in Colombia. The difference in GWP between the control and the silvopastoral system could in principle be legible for trading carbon quotas in the emerging market for GH as part of the Clean Development Mechanisms of the Kyoto Protocol when this would be fully ratified. Effort will be dedicated jointly with CORPOICA and the Ministry of environment, to promote implementation of these prototype systems for a national project for the CDM.

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3.3 The use of charcoal in soils: Agronomic and environmental implications

Charcoal is an ubiquitous material that has being used in agriculture by almost every culture in the planet (Coomes and Burt, 2001; Goldberg, 1985). Increasing evidence indicates that in very low fertility soils like in savannas from South America and the Amazon, additions of charcoal could increase plant yield and improve several soil quality indicators (Iswaran et al., 1980). Charcoal is a very stable material in soils, with residence times in the order of thousands of years contrasting with mean residence times of decades to centuries for most SOM pools. This provides the possibility to use charcoal as a suitable option for long-term storage of C in soils. This year we advanced research to assess the effect of additions of charcoal to low fertility tropical soils and explore the use of charcoal as a tool for long term carbon storage in soils.

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3.3.1 Effect of charcoal application on crop/forage yields and on fluxes of greenhouse gases under glasshouse conditions.

Contributors: M. Rondón, J.A. Ramírez CIAT (PE-6)

Rationale. There is evidence indicating that additions of charcoal to soils could increase yields of legume plants (Iswaran et al., 1980) and other plant species (Kishimoto and Sugiura, 1985) but not information seems to be available on the effects on grasses. So far there is not any information related to the effects of charcoal additions to soils on fluxes of CO_2, methane and nitroux oside, but these fluxes are likely to be influenced, given that charcoal can affect microhabitats fro microbial populations. To advance on the study of effects of charcoal on soils, a glasshouse pot experiment was conducted to assess the effect of increasing doses of charcoal addition to soils as reflected on plant yield, soil nutrients and fluxes of methane and nitrous oxide.

Materials and Methods. A low fertility Oxisol from Matazul in Colombia was used. Before filling the pots, the soil received a basal dose of fertilization including the equivalent to 300 kg of lime, 20 kg P, and 30 kg N per hectare to enable proper plant growth. Four replicated pots per treatment were filled with 2 kilograms of air dried soil and charcoal was added to pots in four doses: 0, 7.5, 18.8 and 30 g charcoal/kg soil. Charcoal was very well mixed with the soil during the process of filling the pots. Water was then applied to the pots to reach 60 to 70% field capacity and allow to stabilize during three weeks before planting. *B. humidicola* and soybean were transplanted at four plants per pot for the grass and two plants per pot for the legume. Plants were allowed to grow for 50 days until soybean complete pod filling. Moisture was maintained in the pots at 50-60% of field capacity by periodical additions of water. Gas samples were taken at 15 days after sowing and at harvest, using the vented close chamber method. Gas samples were analyzed for methane, and nitrous oxide within one week of collection, by using a Gas chromatograph (Shimadzu-14A) equipped with FID and ECD detectors.

Results and Discussion. Table 1 presents values of soil pH at the time of harvest. Increasing additions of charcoal to soil clearly influences soil pH in a fairly linear progression. Nearly one unit of pH was increased with the high charcoal dose application. Potential implications to reduce aluminum saturation and correct some low pH induced nutrient deficiencies are clear.

Table 1. Effects of charcoal additions on soil pH at harvest time.

Charcoal doses ($g\ kg^{-1}$)	*B. humidicola*	SD	Soy Bean	SD
0.0	4.87	0.12	5.11	0.11
7.5	5.20	0.07	5.34	0.02
18.8	6.15	0.28	5.82	0.28
30.0	6.09	0.04	6.06	0.06

Figure 1 presents the effects of increasing additions of charcoal on total dry biomass of *B. humidicola* and soybean. Biomass production of *B. humidicola* was not affected by additions of charcoal, but dry biomass and grain yields of soybean were positively affected by the additions of charcoal reaching maximum values (net increase of 15% in dry biomass and 20% increase in grain yield compared to the zero charcoal control), at doses of 18.8 g of charcoal/kg soil. Beyond this level, no additional yield increases were observed. This could be probably the result of differential nutrient demands among plants; *B. humidicola* is a relatively low nutrient demanding plant, while soybeans responds very effectively to fertilization.

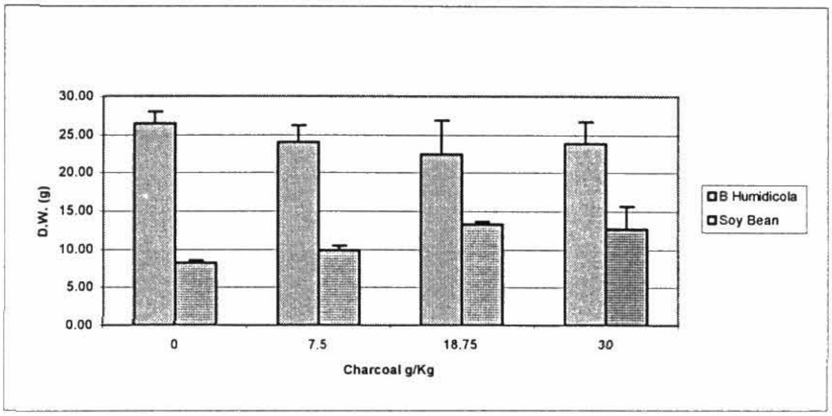

Figure 1. Total dry weight of *B. humidicola* and soybean; effect of increasing additions of charcoal to an Oxisol

Influence of charcoal was also noted in the levels of various soil nutrients after harvest: Figure 2 shows how available phosphorous increased 50% in the high dose application (30 g charcoal/kg soil) relative to the non charcoal control, while for both available K and available Ca, a linear increase with increasing doses of charcoal was observed, reaching a five fold increase with the high charcoal dose. Increases in available Mg were also found but to a lesser degree. Some of these changes may be attributable to increases in soil pH found with even modest additions of charcoal to acid soils (Table 1). In environments severely limited by nutrient deficiencies such as the soils from the Amazon or South American Savannas, even the small amounts of nutrient that could be directly released by charcoal additions or being made available from the soil pools through changes in soil pH, could make a difference in plant growth and performance.

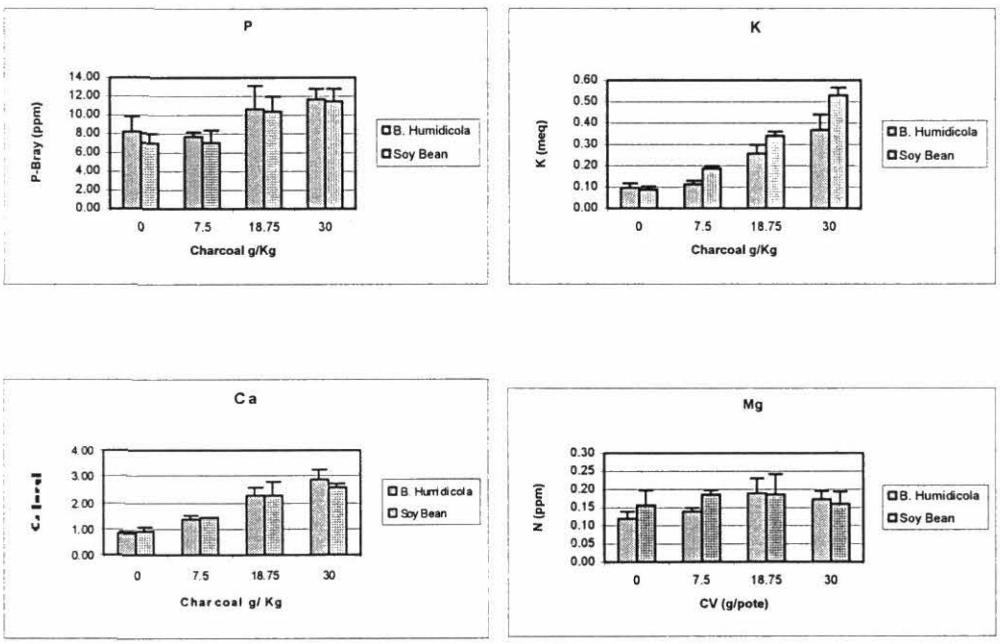

Figure 2. Effects of charcoal additions on the status of various soil nutrients at harvesting time.

Greenhouse gas emissions. Fluxes of methane and nitrous oxide were significantly reduced by increased additions of charcoal. Reductions were more accentuated in the pots with *B. humidicola* (Figures 3 and 4) Control pots were net sources of methane to the atmosphere (Figure 3) and the addition of charcoal reduces progressively net emissions and at the high dose turns the soil to be a small net sink. Nitrous oxide fluxes are reduced very effectively (Figure 4), indicating that charcoal is interacting with nitrogen dynamics in soils. Mechanisms responsible for decreases in nitrous oxide fluxes are not clear yet and will require further research.

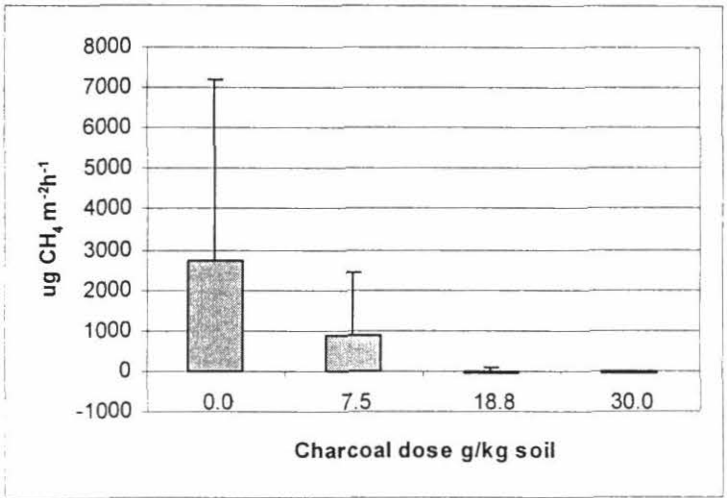

Figure 3. Fluxes of methane from soils with varying doses of charcoal addition. Pots with *B. humidicola* sampled one week after planting.

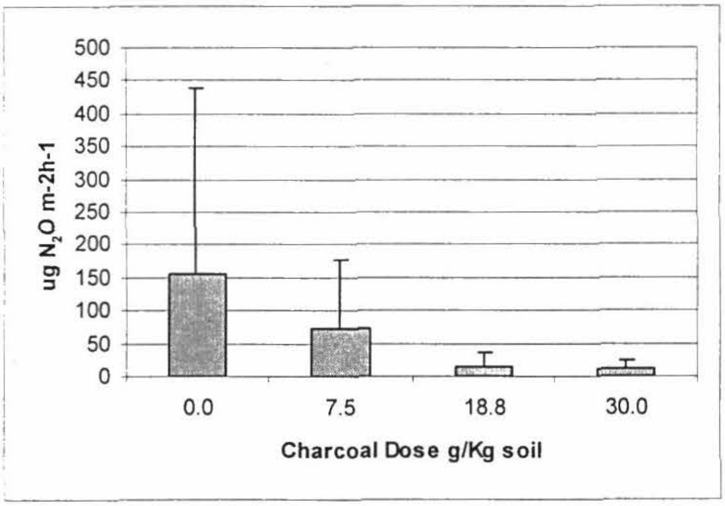

Figure 4. Fluxes of nitrous oxide from soils with varying doses of charcoal addition. Pots with *B. humidicola* sampled one week after planting

Conclusions. Charcoal additions to soils did not have an effect on accumulation of dry biomass of *B. humidicola*. The effect was positive to increase dry matter and grain yields of soybean. Since the amounts of nutrients directly released by the added charcoal are small, most of the positive effects could likely come from indirect effects on enhancing the availability of soil nutrients probably as a result of clear increases on soil pH. Effects were beneficial in terms of reductions of net emissions of both methane and nitrous oxide. Mechanisms responsible for these reductions needs further research.

Qualitative observations at harvesting time indicate that the number of nodules on the roots of soybean were increased by additions of charcoal. Another experiment is in progress using ^{15}N methodology, to specifically address the effect of charcoal additions on biological nitrogen fixation by common beans.

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3.3.2 Charcoal as amendment for high fertility trenches.

Contributors: M. Rondón, J.A. Ramírez CIAT (PE-6) and E. Amézquita CIAT (PE-2); J. Lehmann, Cornell University.

Rationale. Soil fertility is perhaps the most prevalent constraint preventing small scale farmers to improve their agricultural productivity and livelihoods. Worldwide, farmers try to improve pockets of soils to grow their most profitable cash crops: vegetables, fruits medicinal plants, etc. Improving all the land of a farm is often inaccessible to farmers and requires major investments and long-term commitments. There is however a possibility of progressively improve patches of the land which could be expanded as more resources become available. One alternative for this is to create high fertility trenches where the soil quality could be improved in belts of the minimum width and depth to allow good development of the crops of interest to the farmer, typically vegetable or fruit crops. Charcoal is frequently available at the farms in the form of residues of incomplete combustion of firewood etc. Charcoal could be used as an additional mechanism to improve some soil quality characteristics (reduce bulk density, enhance porosity, water retention and improve soil drainage) in such high fertility trenches.

Materials and Methods. A field experiment was initiated this year to establish high fertility trenches on a steep slope farm under a low fertility Andisol in Pescador, Cauca Colombia. The effects of charcoal additions on plant yield (vegetable crops) and soil quality are being studied. Additionally, the effects of charcoal on C dynamics and fluxes of GHG are being evaluated. Trenches 40 cm wide and 20 cm deep were excavated in contour lines on a very steep slope (45% slope). Trenches are separated 1-m from each other. The soil taken out of the trenches was mixed with lime (600 kg/ha) and received the following doses of fertilizers: 45 kg N/ha, 45 kg P/ha, 45 kg K/ha, 30 kg Mg/ha and additionally 3000 kg of chicken manure per hectare. Chicken manure and fertilizers were homogeneously mixed with the soil, which was then repacked into the open trenches. This level of fertilization is considered appropriate to obtain high yields of vegetable crops in these soils. The trenches were irrigated and allowed to stabilize for several weeks before transplanting the seedlings. Trenches were prepared and fertilized on 25-July-2003. Due to lack of rainfall in the area, transplanting had to be postponed until 1-October-2003. Crops are expected to be harvested toward the middle of December 2003. Two vegetable crops are used: carrots and red beans planted in rows at 20 cm between plants in the center of the trenches.

Experimental treatments included carrot without charcoal (CNC), carrot with charcoal (CC), Bean without charcoal (BNC), Bean with charcoal (BC), and original soil from between trenches (control). The Charcoal dose applied was 1.5 kg per lineal meter of trench which approximately equivalent to the amount of soil carbon originally present in the soil to a depth of 20cm. Charcoal was applied simultaneously with other amendments and uniformly mixed over the whole trench depth. Each treatment had four replicates in a complete randomized block design. Position on the slope was the blocking factor. Trenches were distributed along a hill, and each experimental unit plot had the following dimensions: 3 rows, 5 m long × 0.4 m width × 0.2 m deep. After transplanting, the plants have being irrigated when necessary to maintain appropriate moisture for the crops. Required phytosanitary controls have being applied to maintain the crops in good health condition.

Soil Analysis. Before fertilization, soil physical and chemical properties were measured. Undisturbed core samples were taken for depths between 0 and 60 cm, to establish initial baseline conditions for all treatments. Saturated hydraulic conductivity, susceptibility to compaction, bulk density, total porosity, macropores, air permeability, moisture at field capacity, C, N, NO_3, NH_4, P, K, Ca, Mg, and pH were evaluated. Similar soil analysis will be done it during crop cycle and at final harvest.

Analysis of CH_4 and N_2O. Air samples were collected using the vented close chamber method. One chamber was installed by plot, air sampling was made at times 0, 10, 20, and 30 minutes after chamber installation. First gas sampling was collected 15 days after carbon application and subsequent gas collection have been taken at 10 days interval. CH_4 and N_2O analysis is done using a gas chromatograph (Shimadzu GC 14A) with ECD and FID detectors. Compressed Air and Scotty prepared standard containing 1 and 3 ppm CH_4 and 0 and 1 ppm N_2O being the most commonly used standards.

Results and Discussion. Soil determinations are still under analysis. Preliminary results are showed for N_2O and CH_4 flux measurements. N_2O rate of emissions for treatments CNC, CC, and control; are showed in figure 1. Integrated N_2O and CH_4 emissions are presented in Table 1.

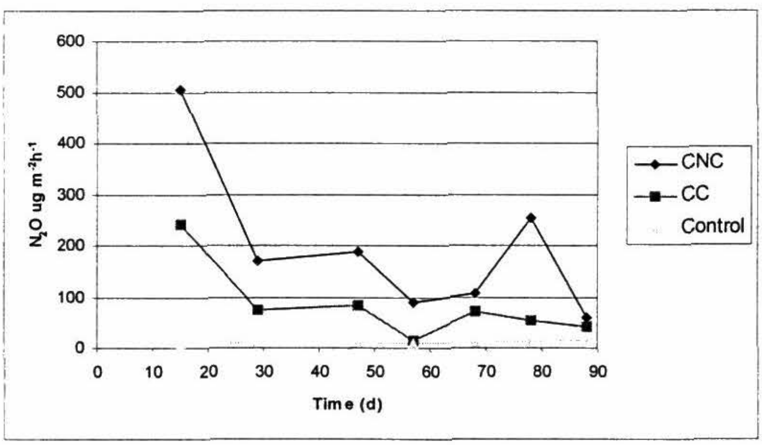

Figure 1. Evolution of fluxes of nitrous oxide on high fertility trenches on an Andisol. Pescador-Cauca, Colombia.

CNC (carrots non charcoal added),
CC (carrots, charcoal added)
Control is original soil without fertilizer application. Transplanting of seedlings was done at day 67.

Fluxes of N_2O from the original soil are very low over time indicating a low nutrient turnover. As expected, fertilization increases considerably net fluxes of nitrous oxide in the high fertility trenches. Charcoal addition clearly reduces net emissions of N_2O and the effect is persistent for at least 90 days after charcoal application. On the other hand the original soil behaves as a net sink for atmospheric methane. The application of fertilizer significantly reduces methane oxidation by the soils and the addition of charcoal reduces that even more (Table 1). At this point, the crop type is not introducing significant differences between treatments.

In Table 1, integrated values for fluxes of methane and nitrous oxide are presented. Values correspond to accumulated fluxes during the measurement period expressed per unit length of trench.

Table 1. Integrated Greenhouse Gases Emissions

Treatment		**N_2O mg m^{-2}**		**CH_4 mg m^{-2}**	
CNC	a	292.8	(61.4)	-74.73	(18.92)
CC	b	125.2	(65.0)	-44.80	(22.73)
BNC	a	273.4	(5.0)	-76.76	(7.89)
BC	b	128.2	(8.2)	-42.81	(21.24)
Control	c	28.4	(8.3)	-96.03	(5.02)

Treatments with the same letter are not significantly different ($P<0.05$).
Values in parentheses indicate standard deviations

Integrated N_2O emissions are reduced on average 55% in the treatments with charcoal. The most appreciable difference for N_2O fluxes, between charcoal and no charcoal treatments, is founded in the first gas sampling. Glaser et al. (2002) and Lehmann et al. (2003) report that charcoal amendments can improve nutrient retention, specifically NH_4^+. Reduction of N_2O emissions could be partially explained by NH_4^+ retention which could result in a net decrease in the availability of nitrogen for nitrification and or denitrification.

Lower CH_4 oxidation in charcoal treatments could be probably associated to enhanced water retention expected in the plots receiving charcoal. This could favor a net increase in anaerobic microsites where methane is generated Piccolo et al. (1996) showed that charcoal may increase water retention and soil aggregation.

Conclusions. So far, charcoal addition to high fertility trenches does not seem to be negatively affecting the growth of vegetable crops. Fertilization has in general negative impact on net fluxes of greenhouse gases, but the addition of charcoal is showing to be very effective in minimizing that impact. Taking into account that the persistence of charcoal in soils is in the orders of thousands of years (Lehmann et al., 2002), small amount of added charcoal could easily revert the negative climate forcing impacts of applying high fertility doses to trenches, and even could result in a net sink of greenhouse gas emissions of the atmosphere. The work is still in progress and full results of this experiment including effect of charcoal on plant yields will be presented next year.

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3.4 Soil mineral N dynamics beneath mixtures of leaves from legume and fruit trees in Central Amazonian multi-strata agroforests

Contributors: Carol M. Schwendener, Johannes Lehmann (Cornell University), Marco Rondón (CIAT), Elisa V. Wandelli (Embrapa), and Erick C. M. Fernandes (Cornell University).

Abstract. Long-term applications of leguminous green mulch could increase mineralizable nitrogen (N) beneath cupuaçu trees produced on the infertile acidic Ultisols and Oxisols of the Amazon Basin. However, low quality standing cupuaçu litter could interfere with green mulch N release and soil N mineralization. This study compared mineral N, total N, and microbial biomass N beneath cupuaçu trees grown in two different agroforestry systems, north of Manaus, Brazil, following seven years of different green mulch application rates. To test for net interactions between green mulch and cupuaçu litter, dried gliricidia and inga leaves were mixed with senescent cupuaçu leaves, surface applied to an Oxisol soil, and incubated in a greenhouse for 162 days. Leaf decomposition, N release and soil N mineralization were periodically measured in the mixed species litter treatments and compared to single species applications. The effect of legume biomass and cupuaçu litter on soil mineral N was additive implying that recommendations for green mulch applications to cupuaçu trees can be based on N dynamics of individual green mulch species. Results demonstrated that residue quality, not quantity, was the dominant factor affecting the rate of N release from leaves and soil N mineralization in a controlled environment. In the field, complex N cycling and other factors, including soil fauna, roots, and microclimatic effects, had a stronger influence on available soil N than residue quality.

Key words: *Theobroma grandiflorum, Gliricidia sepium,* agroforestry, mineral N, green mulch

Introduction. Prunings from leguminous trees provide an accessable organic nitrogen (N) source for small-scale agroforestry farmers on the acid infertile Oxisol and Ultisol soils that dominate the Amazon Basin (Sanchez et al., 1982). In alley cropping and shade agroforestry systems N applied with prunings can amount to 136-240 kg N ha^{-1} y^{-1} on infertile soils (Kang et al., 1981; Russo and Budowski, 1986). 37-92% of the N in gliricidia (*Gliricidia sepium*) prunings can be fixed from atmospheric N_2 (Giller, 2001). Although applications of prunings from leguminous species can increase crop yields (Haggar et al., 1993; Kang et al., 1981; Mulongoy et al., 1993) and organic soil N and N mineralization (Isaac et al., 2003), often only 10-20% or less of the N added in the prunings is recovered by the first subsequent annual crops (Haggar et al., 1993; Palm, 1995). Much of the N not used by crops remains in undecomposed material or moves to the readily mineralizable fraction of soil organic matter (Haggar et al., 1993), while significant amounts can be leached to the subsoil (Schroth et al., 1999), or lost through volatilization (Palm, 1995). Agroforestry tree species have the potential to benefit from residual N or priming effects of long term green mulch applications (Cadisch et al., 1998) due to their longer growing periods, more established root systems and a lower need for nutrient synchrony than annual crops.

Extensive research has shown that applications of legume tree prunings increase available N in agroforestry systems, but no studies have focused specifically on application in tree cropping systems. Cupuaçu (*Theobroma grandiflorum* (Wild. ex Spring) Schumann) is a common fruit tree species in agroforestry systems in the Amazon Basin that produces a low quality litter (high C:N ratio and high lignin contents) (McGrath et al., 2000; Uguen, 2001). Applications of high quality prunings, such as gliricidia, could increase the nutrient release from cupuaçu litter. It has been shown that cupuaçu litter can immobilize P from higher quality litter and decrease available P in the soil (McGrath et al., 2000), but little is known about whether low quality cupuaçu litter decreases soil N mineralization, green mulch decomposition and N release.

Green mulch and litter decomposition and N mineralization rates have been related to various intrinsic chemical properties of residues and ratios of the parameters, including initial N, lignin, and polyphenol contents, C/N ratio (Constantinides and Fownes, 1994; Handayanto et al., 1997; Lehmann et al., 1995; Mafongoya et al., 1998a; Palm and Sanchez, 1991; Tian et al., 1992). These predictors of residue decomposition and N mineralization rates can be altered by mixing different quality leaves to regulate N release (Handayanto et al., 1997). Although much work has been done on the residue quality parameters to predict N release and decomposition, only a few studies have looked at interactions of multi-species mulch or litter mixtures (Handayanto et al., 1997; Mafongoya et al., 1998b; McGrath et al., 2000) and none specifically at low-quality litter from tree crops such as cupuaçu mixed with high-quality legume prunings such as gliricidia and inga (*Inga edulis*).

The objectives of this research were 1) to determine whether green mulch quality and quantity increase soil mineral N beneath cupuaçu in a controlled environment and in an agroforestry system; 2) to assess whether cupuaçu litter decreases soil N mineralization and N release by green mulch.

Methods

Site Description. The project site is located in Central Amazonia at the Empresa Brasileira de Pesquisa Agropecuaria – Centro da Pesquisa Agroflorestal (EMBRAPA-CPAA) research station 53 km north of Manaus, Brazil along the highway BR-174. The soil is a fine, isohyperthermic, Xanthic Hapludox (Staff, 1998) or Ferrasol (FAO-UNESCO, 1990). The annual average rainfall is 2500 mm per year with a 2-3 month dry season, and the mean annual temp is 26°C. Between 1991 and 1992, three 50 m × 60 m plots each of a palm-based agroforestry system (Agrosilvicultural I - ASI) and a fruit-tree based home garden system (Agrosilvicultural II - ASII) were established on abandoned, degraded pastureland. Details on site preparation and establishment can be found in Fernandes et al. (submitted). Species composition of each system is shown in Figure 1.

Both systems were bordered with around 90 gliricidia trees spaced two meters apart and intercropped with cupuaçu trees. In ASII, the cupuaçu trees were planted in two 13-tree rows in six by two meter spacing, for a total of 26 cupuaçu trees with 25 intercropped inga trees, also spaced six by two meters apart. Agrosilvicultural system I had 99 cupuaçu trees spaced six by two meters apart and intercropped with açaí (*Euterpe oleracea)* trees only or with açai and capoeirão (*Colubrina glandulosa)* trees (Fig. 1). Gliricidia prunings were applied two to three times annually to cupuaçu trees in both systems for seven years. In ASII, inga prunings were applied to cupuaçu trees in addition to the gliricidia prunings two to three times annually for seven years. The lower density of cupuaçu trees in ASII and similar total gliricidia pruning biomass resulted in individual trees in ASII receiving higher rates of gliricidia prunings than trees in ASI. The addition of inga prunings to trees in ASII further increased the difference in green mulch applications between the two systems. The nitrogen input per hectare was 1.46 times greater in ASII than in ASI (16.8 and 24.5 kg N ha^{-1} y^{-1} for ASI and ASII, respectively) (EMBRAPA-CPAA, 1997), or 5.6 times greater per cupuaçu tree (approximately 0.17 and 0.94 kg $tree^{-1}$ for ASI and ASII, respectively). At the time of sampling, the cupuaçu trees were 10 years of age and green mulch applications had been occurring for seven years.

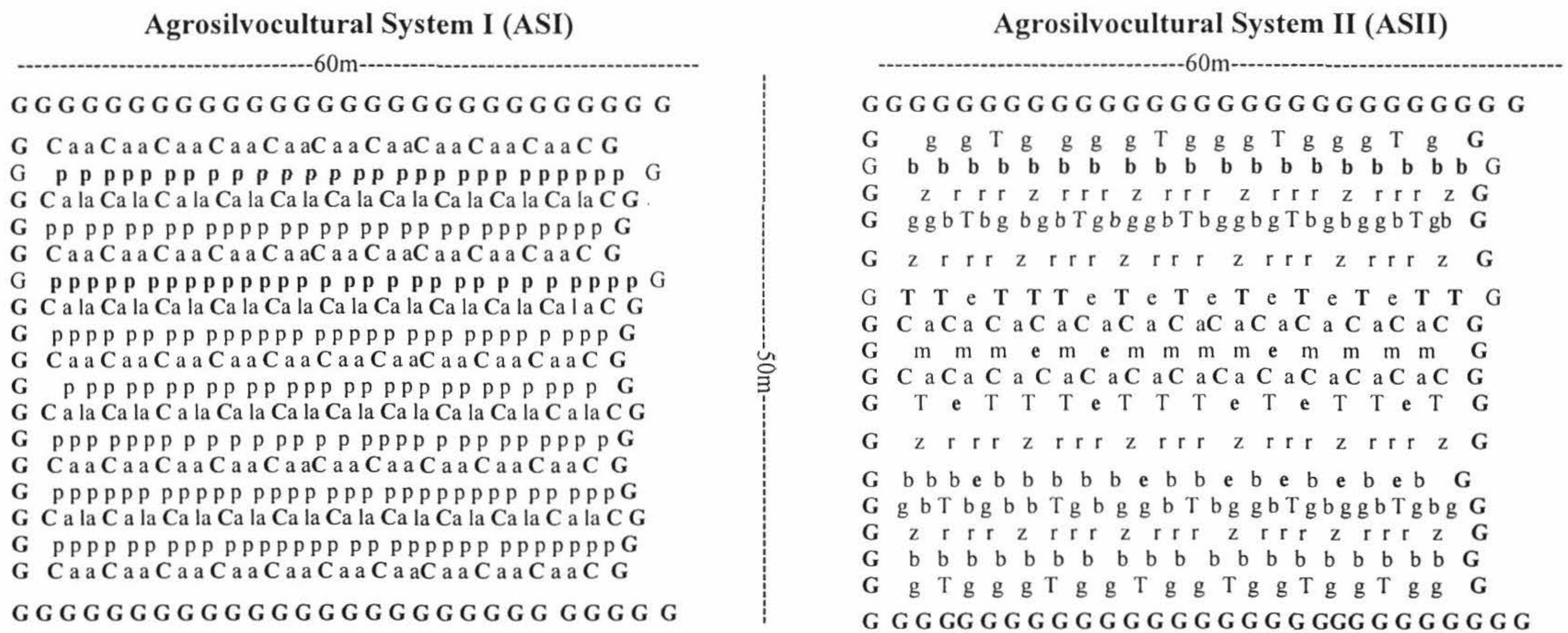
Sampling. Fresh gliricidia prunings were applied to four randomly selected cupuaçu trees (excluding edge trees) within each block in ASI and ASII. Prunings were applied at a rate of 10 kg fresh weight per tree (3 kg leaves, 7 kg branches), branches placed on top to hold the leaves in place, within a 0.5 m radius of the tree trunks. The application rate and method was similar to past applications in the agroforestry systems. One week after application, during which at least one rainfall occurred, the top 0-10 cm of soil was sampled at 0.2 m and 1 m from the base of the tree. The 0.5m distance represented the area that always received inga and/or gliricidia prunings, while the 1 m location was primarily covered with cupuaçu litter and some litter from adjacent species. The soil was sampled at two locations for each distance from each tree and composited for each distance for each treatment and replicate, resulting in a total of 12 samples (two treatments, two distances, three replicates). Plots were sampled in July 2001 at the end of the rainy season and again in November 2001 at the end of the dry season.

Greenhouse Incubation. The substrate soil was the same Xanthic Hapludox (Ferrasol) on which the agroforestry systems were established, but it was collected beneath an 8-year old secondary forest adjacent to the ASI and ASII plots. Soil was collected from 7.5 cm to 15 cm depth and the large roots and charcoal pieces were excluded to minimize initial organic matter content and facilitate detection of treatment effects. Aggregates greater than 2 cm were broken apart to increase homogeneity of the soil without complete destruction of macro-aggregation in the soil. The soil had the following characteristics: pH (in water) 4.2; 1.6 g kg^{-1} N (Kjeldahl), 21.2 g kg^{-1} C (Walkley-Black), 1.8 g kg^{-1} available P (Mehlich-1), 18.3 g kg^{-1} available K (1M KCl), 22.7 g kg^{-1} Ca (1M KCl), and 11.2 g kg^{-1} Mg (1M KCl).

Senescent cupuaçu leaves, collected during the four weeks prior to application, and fresh gliricidia, and inga leaflets were cut into 1 cm^2 pieces, the internodes of gliricidia and inga to 2 cm, dried to a constant weight at 55-65 °C, and stored in dry conditions. The application rates included internodes and leaves at the same ratio found in leaves of the plants. Initial C and N contents of the leaves for gliricidia were 33.6 mg g^{-1} N, 42.1 mg g^{-1} C, for inga 28.4 mg g^{-1} N and 436 mg g^{-1} C and for cupuaçu 10.1 mg g^{-1} N and 361 mg g^{-1} C.

Exactly 150 g of fresh soil were weighed into 500 mL plastic cups, resulting in a surface area of 50 cm^3. Care was taken to prevent drying of the soil. The samples were placed in a randomized block design in a greenhouse protected from rain and direct sun. The soils were left undisturbed for three weeks prior to application of litter treatments to allow microbial activity to stabilize. Daytime soil temperatures averaged 32-36 °C. Soil moisture was maintained at 66% field capacity, except at 12 days prior to each collection when a rainfall was simulated and the moisture content was raised to 80% field capacity. This flush of water was intended to facilitate movement of N through the litter layer to the soil.

Treatments consisted of single and mixed species litter applications and a bare soil control. Gliricidia, inga, and cupuaçu were applied separately, as single species. Multiple species treatments were composed of the following combinations: gliricidia + inga, cupuaçu + gliricidia, cupuaçu + gliricidia (high rate), cupuaçu + gliricidia + inga and cupuaçu + gliricidia + inga (high rate). Gliricidia and inga leaves were applied at a low rate in all treatments, except those indicated as high rate. The high and low application rates of gliricidia and inga, 2 Mg ha^{-1} (1.0 g cup^{-1}), 10 Mg ha^{-1} (5.0 g cup^{-1}) and 2.6 Mg ha^{-1} (1.3 g cup^{-1}), 13 Mg ha^{-1} (6.5 g cup^{-1}), respectively, were selected to extend the range of application rates in the field. The application rate for cupuaçu litter of 4 Mg ha^{-1} (2.0 g cup^{-1}) was based on standing cupuaçu litter and litter production in agroforestry systems similar to ASI (Uguen, 2001).



Symbol	Scientific Name	ommon Name
C	*Theobroma grandiflorum* (Wild. ex Spring) Schumann	cupuaçu
G	*Gliricidia sepium*	gliricídia (gliricidia)
a	*Euterpe oleracea*	açaí
p	*Bactris gassipaes*	pupunha (peach palm)
I	*Columbrina glandulosa*	capoeirão

Symbol	Scientific Name	Common Name
C	*Theobroma grandiflorum* (Wild. ex Spring) Schumann	cupuaçu
G	*Gliricidia sepium*	gliricídia (gliricidia)
e	*Inga edulis*	ingá (inga)
r	*Malphigia glabra*	acerola (barbados cherry)
a	*Eugenia stipitata*	araça-boi
b	*Musa paradisiaca*	banana
z	*Bertholletia excelsa*	castanha (Brazil nut)
g	*Genipa americana*	genipapo
m	*Swietenia macrophylla*	mogno (mahogany)
T	*Tectona grandis*	teca (teak)

Figure 1. Location of cupuaçu (*Theobroma grandiflorum*), gliricidia (*Gliricidia sepium*), and inga *(Inga edulis)* in the two agroforestry systems established on abandoned pastures near Manaus, Brazil.

The total N applied per cup as litter differed for each treatment as follows: 20.2 mg (cupuaçu), 33.6 mg (gliricidia), 36.9 mg (inga), 70.5 mg (gliricidia + inga), 53.8 mg (cupuaçu + gliricidia, low rate), 188.2 mg (cupuaçu + gliricidia, high rate), 90.8 mg (cupuaçu + gliricidia + inga, low rate), and 372.8 mg (cupuaçu + gliricidia + inga, high rate). All leaf combinations were mixed and surface applied to the soil with an amount of water equivalent to the water weight lost during drying. There were five replicates of each treatment and the control. Soil and litter were destructively sampled at 7, 29, 50, 98, and 162 days after litter application.

Laboratory Analyses. Soils from the field and greenhouse incubation were analyzed for mineral N, total N and C, and field soil samples were also analyzed for microbial biomass C and N. Mineral N was extracted from 25 g fresh soil within two hours after collection with 75 mL of 1N KCl for 0.5 hour on a horizontal shaker at the highest speed. After decanting, the supernatant was pipetted and stored below 0°C until it was analyzed for ammonium and nitrate on a Skalar continuous flow analyzer. Results are reported per dry weight soil, determined by drying at 105°C for 24 hours. Net interactive effects on decomposition, litter N release, and soil mineral N were estimated by summing values for single species treatments and statistically comparing them to the equivalent low rate mixed species treatment values. The control soil mineral N value was subtracted from all treatments to discount the mineralized N in the soil that was not a direct result of litter application. Microbial biomass N was measured by chloroform fumigation-extraction (Brookes et al., 1985) adapted for acid soils. The remaining soil was dried and ground to 2 mm prior to N and C analysis. Remaining plant material was cleaned by gentle brushing, dried for 48 hours at 65-70 °C, weighed, ground, and analyzed as a mixture for N and C. Plant and soil N were analyzed by Kjeldahl digestion, and carbon was analyzed by the Walkley-Black method (Silva, 1999).

Statistical Analyses. Statistical analysis of the data was performed with regressions and 1-way and 2-way ANOVAs in Minitab 13 (Minitab, 2000). Where $p<0.05$, means were compared using Fisher's least significant difference test.

Results

Greenhouse incubation - Litter decomposition. The gliricidia leaves applied in isolation decomposed more rapidly and completely of all single and mixed species litters as a percent of the dry weight applied. The cupuaçu litter applied alone decomposed the slowest during the 162-day experimental period (Fig. 2). Among the multiple species leaf mixtures, the cupuaçu + gliricidia leaves applied at a high rate decomposed significantly faster and to a greater extent than other mixed species litters. The leaf mixtures with high rates of green mulch decomposed significantly faster than equivalent mixtures with low green mulch application rates; however, only the high and low rate cupuaçu + gliricidia leaf mixtures differed significantly in total decomposition with the high rate decomposing to a greater extent during the experimental period. Total leaf decomposition of species applied together was not significantly different from the total decomposition of the species when applied singly.

Greenhouse incubation - N release from litter. The gliricidia leaves released the highest percent N of all single and mixed species litters, while the cupuaçu and inga leaves released the least percent N (Fig. 3a). The percent N release by inga was only significantly greater than the percent N released by cupuaçu at 50 days after application. The cupuaçu + gliricidia (high rate) leaf mixture initially immobilized less than 1% of the nitrogen before quickly releasing more than 60% and then immobilizing about 10% N through to 162 days after application (Fig. 3b). This release pattern was different from that of the other cupuaçu and green mulch litter mixtures, which had an initial rapid N release leveling at or prior to 162 days. Both treatments with cupuaçu + gliricidia + inga leaves released N more slowly than the treatments with only cupuaçu + gliricidia leaves. Despite different release patterns, there was no significant difference among the mixtures in total N released 162 days after litter application.

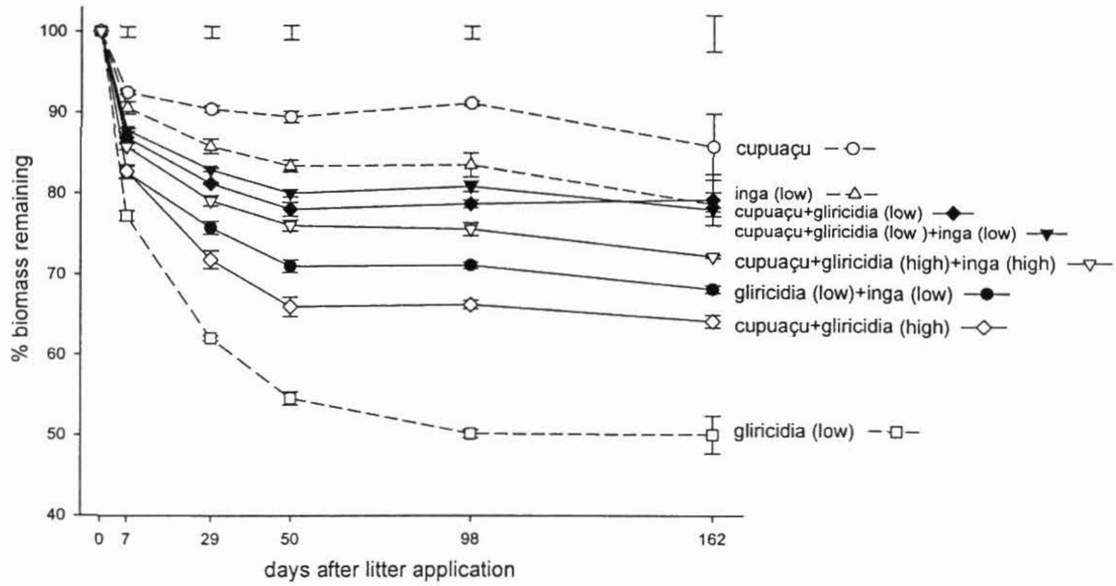


Figure 2. Decomposition of cupuaçu (*Theobroma grandiflorum*), gliricidia (*Gliricidia sepium*), and inga (*Inga edulis*) leaves after surface application on an Oxisol near Manaus, Brazil. Single species litter applications are indicated by dashed lines. High and low refer to gliricidia and inga application rates (2 and 10 Mg ha^{-1} for gliricidia and 2.6 and 13 Mg ha^{-1} for inga). Cupuaçu was applied at 4 Mg ha^{-1} in all treatments. Bars represent standard error and Fisher's LSDs ($p<0.05$, $n = 5$).

Greenhouse incubation - Soil N. Net mineral N accumulation in the soil was better correlated with the amount of gliricidia N applied ($r = 0.498$; $n = 219$; $p<0.001$) than total plant N applied ($r = 0.204$; $n = 219$; $p<0.001$). Treatments with the higher gliricidia application rate had significantly greater soil mineral N levels than the treatments with less gliricidia applied (Fig. 4). Cumulative N mineralized as a percent of the N applied in the litter mixture was lower in the cupuaçu +gliricidia + inga treatments compared to the cupuaçu +gliricidia treatments when high and low application rates were averaged (Fig. 4, inset). Calculations to determine net interactions showed no significant differences in soil mineral N when the leaves were applied separately or in combination. The presence of cupuaçu leaves with inga and/or gliricidia leaves did not reduce soil mineral N compared to identical treatments without cupuaçu. The mineral N in the gliricidia + inga treatment was not significantly different from the treatment with only gliricidia leaves. There was no treatment effect on total soil N during the 162-day experimental period.

Mineral N, total N, and microbial biomass C and N did not differ between ASI and ASII at either distance from cupuaçu trees, however, nitrate was higher in ASII, due to higher nitrate levels 0.20 m from the base of the tree. In both systems, soil mineral N was greater at 0.2 m from the tree than at 1 m, but distance had no effect on total N or microbial biomass C or N.

Discussion. As expected, residue quality, defined by the C:N ratio, was the dominant factor affecting the rate of N release from leaves and soil N mineralization in the greenhouse incubations. This was shown by the stronger positive correlation between gliricidia N applied and soil mineral N than between total mulch N applied and soil mineral N. Further evidence was the more rapid, but not greater, N release from litter mixtures with gliricidia leaves. Nitrogen mineralization in the soil as a percent of the N applied in the mulch decreased significantly when lower quality litter (inga) was included in the green mulch added to cupuaçu. Also, inga and cupuaçu leaves applied separately did not increase soil mineral N above bare soil values, and leaf mixtures with inga did not increase soil mineral N above that of similar mixtures without inga. This suggests that during the 162-day experimental period, inga and cupuaçu did not increase or decrease soil mineral N as is expected for litter species with decomposition half-lives greater than the study period (over 1 year for cupuaçu (McGrath et al., 2000) and 39 weeks for air-dried inga (Palm and Sanchez, 1990)).

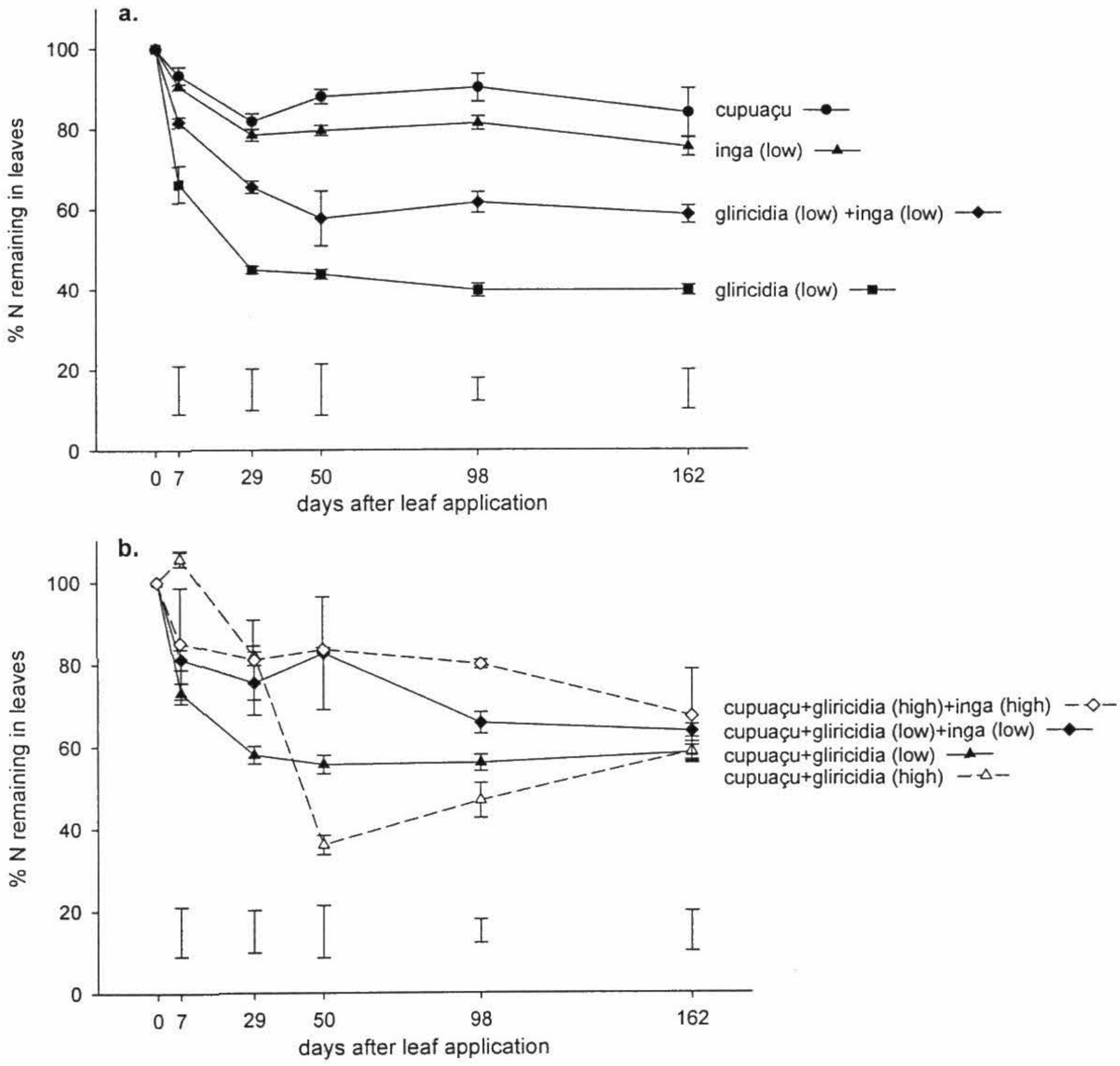


Figure 3. Percent N remaining in leaves following single or mixed species surface applications of cupuaçu (*Theobroma grandiflorum*), gliricidia (*Gliricidia sepium*), and inga (*Inga edulis*) on an Oxisol near Manaus, Brazil. Mixed species treatments with cupuaçu are shown in Figure 3b and single species and green mulch only treatments in Figure 3a. High and low refer to gliricidia and inga application rates (2 and 10 Mg ha^{-1} for gliricidia and 2.6 and 13 Mg ha^{-1} for inga). Cupuaçu was applied at 4 Mg ha^{-1} in all treatments. Dashed lines represent treatments with high green mulch application rates. Bars indicate standard errors and Fisher's LSDs ($p<0.05$, n = 5).

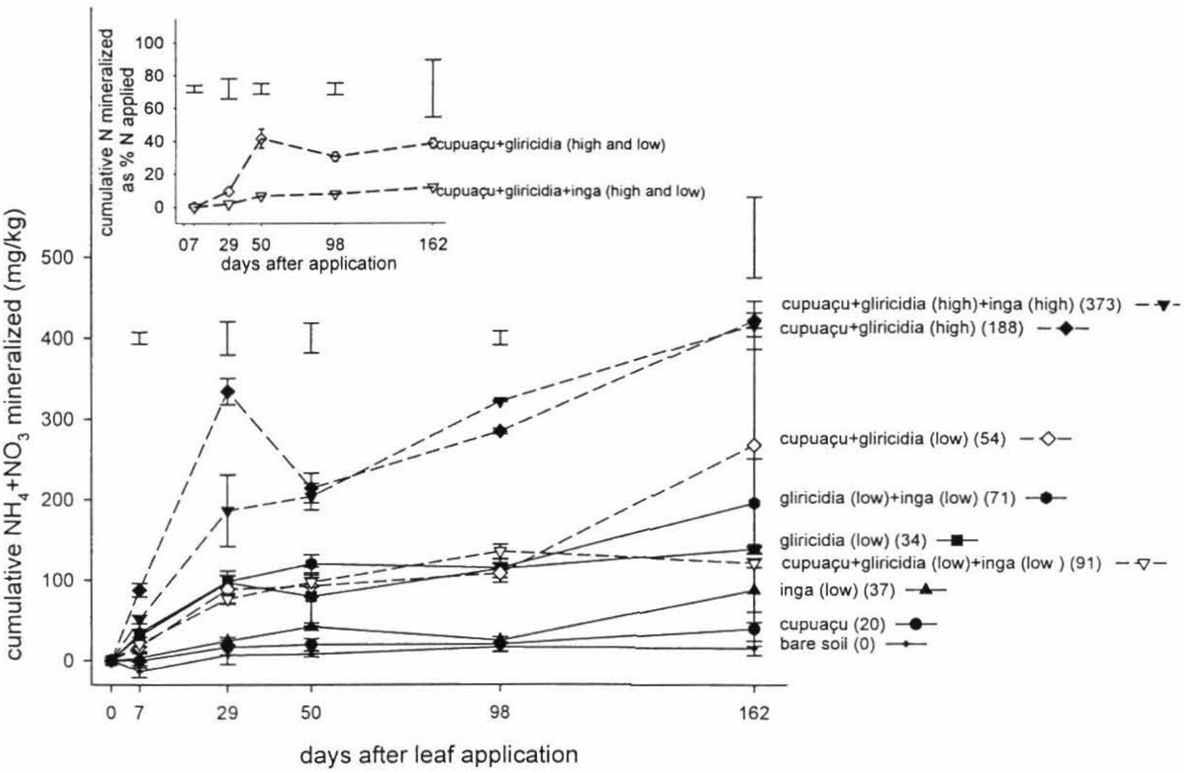


Figure 4. Cumulative N mineralization in the soil following single or mixed species surface applications of cupuaçu (*Theobroma grandiflorum*), gliricidia (*Gliridicia sepium*), and inga (*Inga edulis*) on an Oxisol near Manaus, Brazil and a bare soil control. Dashed lines indicated mixed species treatments. The amount of N applied with leaves is shown in brackets (in mg per pot). The inset shows cumulative N mineralizated as a percent of the N applied in the leaves; the high and low green mulch application rates were averaged for the cupuassu + gliridia and cupuassu + gliricidia + inga mixtures. Bars indicate standard errors and Fisher's LSDs ($p<0.05$, n=5, n=10 for the inset).

Soil mineral N levels were additive in the lower rate mixtures, and, contrary to expectations, no net interactions between the cupuaçu, gliricidia, and inga litters occurred. The presence of cupuaçu did not decrease net N mineralization or N release in the lower rate green mulch applications. The additive effect of legume biomass and cupuaçu litter on soil mineral N levels implies that recommendations for green mulch applications to cupuaçu trees can be based on N dynamics of individual green mulch species and crop N requirements. Soil mineral N quantities, however, cannot be extrapolated from lower application rates because the increase in soil mineral N was not proportional to the increase in applied N. This may be due to a larger litter layer with higher application rates, which decreased soil contact with the leaves and consequently slower decomposition (Henriksen and Breland, 2002; Wilson et al., 1986). The absence of net interactions does not preclude gross interactions between cupuaçu litter N and green mulch N, which were not studied here. Schwendener et al. (unpublished data) also found no net interactions between cupuaçu and gliricidia leaves, but with the use of ^{15}N were able to confirm that gross interactions, including temporary immobilization by cupuaçu of N released by gliricidia, were occurring. An understanding of gross interactions among cupuaçu litter, green mulch and soil could facilitate the management of litter and green mulch N dynamics farmer field conditions.

In a homogenous soil and controlled environment, higher quantities of high quality litter (i.e. gliricidia) increased soil mineral N; under field conditions in the agroforests, however, other factors had a greater effect on soil mineral N than the quality of the green mulch. Soil mineral N and total soil N were expected to be higher in ASII due to accumulation of readily mineralizable N fraction in soil organic matter during the seven years of higher green mulch applications, but soil mineral N, microbial biomass N, and total N did not differ between the two agroforestry systems. Temporal variability of mineral N and the short sampling period of this study made it difficult to examine N mineralization rates which may have better reflected the greater accumulation of a mineralizable N fraction as a result of long term green mulch applications (Haggar et al., 1993). Other factors present in the field and absent in a controlled experiment, such as soil fauna (Hofer et al., 2001; Tian et al., 1992; Vohland and Schroth, 1999), roots (Lehmann et al., 2001; Schroth et al., 1999) and microclimatic effects such as increased shade and moisture (Giller, 2001; Rao et al., 1997), may have had larger effects on soil mineral N than the application of green mulch and may have affected soil mineral N differently in ASI than in ASII. For instance, macrofauna may have been more abundant in ASI due to the close proximity of cupuaçu to peach palm and consequently increased decomposition of the higher quality litter (Vohland and Schroth, 1999). Alternatively, the additional gliricidia N added to ASII could have been removed from the system through increased export of fruit (which was higher in ASII (Wandelli, pers. com.)) or lost via leaching of nitrate to the subsoil (Schroth et al., 1999). Higher mineral N closer to the trunk in both systems suggests that in addition to higher rates of gliricidia prunings at that location, increased shade and thicker litter layer contribute to a favorable microclimate and increased N mineralization (Babbar and Zak, 1994).

The strong influence of gliricidia on soil N mineralization in the greenhouse study demonstrates the importance of the quality of N applied, rather the quantity of N applied as mulch for short term soil N availability. When referring only to higher quality mulch, quantity is also significant for increasing N mineralization. The lower quality inga mulch and cupuaçu litter did not interfere with net decomposition of and N release of gliricidia mulch and soil mineral N accumulation in the soil mulched with gliricidia. Although the low-quality and slow-decomposing inga and cupuaçu leaves did not contribute to soil mineral N in the short term, they could benefit soil fertility in ways not studied in this paper, by providing a litter layer for soil fauna, reducing erosion, increasing soil moisture availability, and controlling weeds. Laboratory studies on soil N availability are limited to green mulch management recommendations with respect to soil N availability. Final recommendations for green mulch applications should be based on field trials that include the N sources and sinks which contribute to the complex N cycling in agroforestry systems.

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3.5 Agroforestry trees increase phosphorous availability in an Oxisol of the Brazilian humid tropics

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Summary
We investigated the effect of land-use, i.e., agroforestry systems (AGR), pasture (PAS), and secondary forest (SEC), and specific agroforestry tree species, i.e., áraça-boi (*Eugenia stipitata*), Brazil nut (*Bertholletia excelsa*), cupuaçu (*Theobroma grandiflorum*), and pupunha (*Bactris gasipaes*), on P availability of acid upland soils of the central Amazon basin. The land-use systems were established in 1991 and underwent different management regimes, with low-input fertilization in AGR and PAS, and no fertilization in SEC. A modified sequential P extraction was used to determine P availability, and total N and other nutrients were also measured.

Pupunha increased resin P and Brazil nut increased bicarbonate organic P. Fertilization increased the hydroxide organic P. Áraça-boi increased hydroxide organic P. Pupunha and Brazil nut increased soil available P (sum of available Hedley fractions – AP) and fertilization increased moderately available P (sum of moderately available Hedley fractions – MAP). This suggests the use of pupunha and Brazil nut in agroforestry systems with moderate fertilization better maintain

AP and MAP in soils of the central Brazilian Amazon than other tree species and land-use systems studied.

Keywords: Amazon, *Bactris gasipaes, Bertholletia excelsa,* Phosphorus fractions, Oxisol

Introduction. Phosphorus is extremely limiting to agricultural production throughout many parts of the tropics, especially the upland soils of the Brazilian Amazon (Dematê and Dematê, 1997). The highly weathered Oxisols of the Central Brazilian Amazon have considerably less available P and total P than Oxisols in other regions of South America, which is caused by low total P contents in the parent material, and not primarily by a high P fixation (Lehmann et al., 2001b). However, it is thought that effective agricultural management can improve soil P availability (Lehmann et al., 2001b; Solomon et al., 2002; Tiessen and Moir, 1993), and possibly decrease the need for fertilizers that are often expensive and difficult to obtain.

Agroforestry systems have been suggested as an alternative to slash-and-burn agriculture and as a strategy to improve soil chemical and physical characteristics of degraded lands, especially degraded pastures, in the Amazon basin (Fernandes et al., 1995). The use of perennial tree crop systems may also lower economic risk and increase diversity of the agricultural system, improve standard of living, and decrease deforestation pressures (Fernandes and Matos, 1995; Lal, 1991; McGrath et al., 2000b; Sanchez et al., 1985).

To date, most agroforestry studies in the Amazon have focused on a select and limited number of species, due to the nature of the research, and few have focused on nutrient cycling (McGrath et al., 2001b; Schroth et al., 2001; 2000), with equally few focusing on P fractionation (Lehmann et al., 2001b; McGrath et al., 2001a; 2000b; Solomon and Lehmann, 2000). Sequential P fractionation is a method used to quantify pools/fractions of P according to their bioavailability (Hedley et al., 1982; Tiessen and Moir, 1993).

The objectives of this study were: 1) to quantify the effects of 10-year-old Central Amazonian agroforestry, pasture, and secondary forest systems in addition to agroforestry tree species on available and total soil P pools, and 2) to determine the effect of these systems and tree species on soil nutrients.

Methods and Measurements

Site description. The study area is located in central Amazônia on an upland soil at the EMBRAPA-CPAA field station located along highway BR-174, 53 km north of Manaus (2°30'36" S and 2°30'42" S and 60°01'29" W and 60°01'46" W (Coolman, 1994)), in the State of Amazonas, Brazil. The natural vegetation is evergreen tropical rainforest and the soil is a fine, isohyperthermic, Xanthic Hapludox (US Soil Taxonomy) with a pH of 4.3, available P of 2.5 mg P kg^{-1} soil (Mehlich-1), organic C of 2.6 mg g^{-1}, total N of 2 mg g^{-1}, and 356.7, 77.8, and 35.2 mg of Ca, Mg and K, respectively, kg^{-1} soil in the top 15 cm (McKerrow, 1992). Soil bulk density is 0.96 g cm^{-3} (Coolman, 1994) and was used in calculations throughout this study. The climate is humid tropical and the mean annual rainfall is 2500 mm, and mean annual temperature is 26.2°C. The mean relative air humidity is 83.9% (Tapia-Coral et al., 1999).

The site is a former *Brachiaria decumbens* pasture that was grazed for four to eight years prior to being abandoned. The scrub forest that regenerated on the abandoned pasture was slashed and burned in 1991, three to five years post-abandonment, and four agroforestry prototype systems were established (Figure 1) (Fernandes et al., 1995). Three of the four existing systems were studied, with sampling focused on soils beneath specific trees and grasses, representing useful and profitable species for local economies, within those systems.

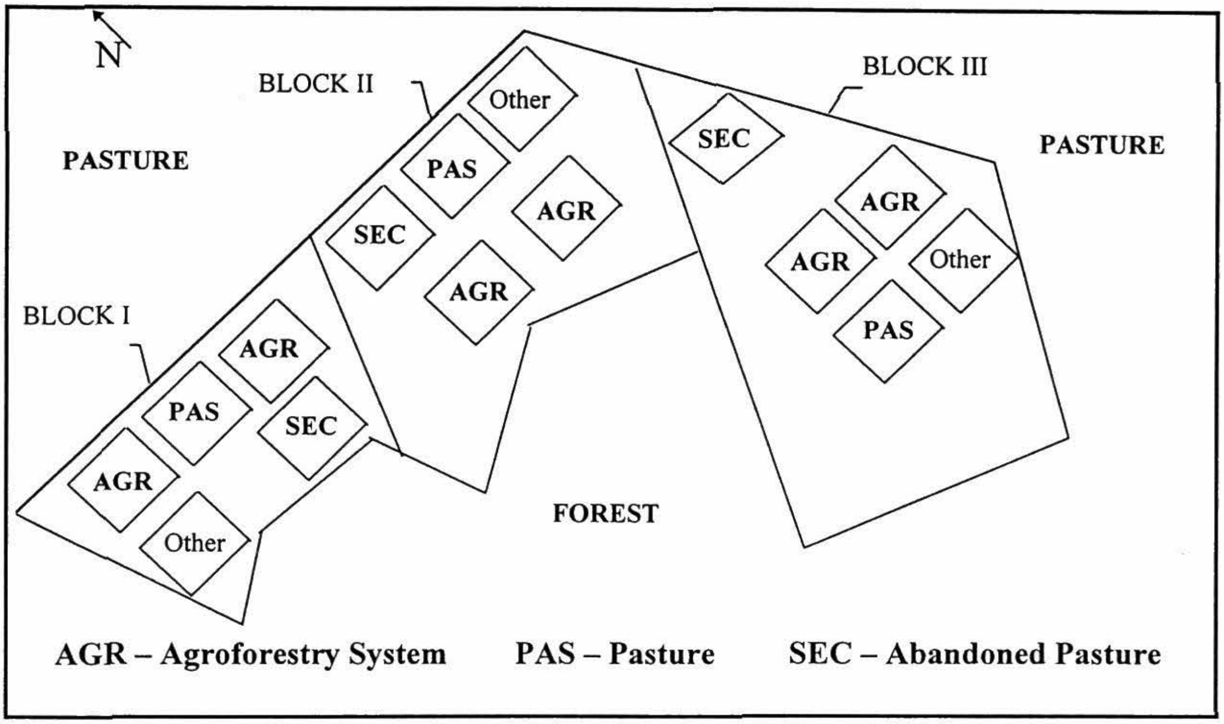

Figure 1. Layout of AGR, PAS, and SEC plots with three blocks. Each plot was 50 × 60 m. The total experiment area occupies approximately 5 ha

The agroforestry system (AGR) was fertilized with Triple Super Phosphate (20.1% P), KCl (49.8% K), lime (40.0% Ca), and urea (45% N) to provide 15.8 kg N ha^{-1} yr^{-1}, 23.7 kg P ha^{-1} yr^{-1}, 12.3 kg K ha^{-1} yr^{-1}, and 4.7 kg Ca ha^{-1} yr^{-1} for the period of 1991, when the systems were established, through 2001, when sampling was conducted (Table 1). The pasture (PAS) received fertilization over the same time period with the same products, in addition to ammonium sulfate (21% N; 23% S), at the rate of 4.6 kg N ha^{-1} yr^{-1}, 5.7 kg P ha^{-1} yr^{-1}, 0.5 kg K ha^{-1} yr^{-1}, 1.6 kg S ha^{-1} yr^{-1}, and 80.0 kg Ca ha^{-1} yr^{-1} (Table 1). PAS was originally grazed for 7 days with a 21-day rest period. Stocking rates and grazing periods were adjusted with varying plant-growing conditions. The secondary forest (SEC) was left to grow post-burn since 1991, without chemical amendments.

Table 1. Mean annual fertilization of the land-use systems and AGR tree species from 1991 to 2001

LAND-USE SYSTEM / **Tree species**	**N**	**P**	**K** (kg ha^{-1})	**S**	**Ca**
AGR (total of species)	15.8	23.7	12.3	0.0	4.7
Araca-boi	5.0	3.1	2.2	0.0	0.3
Brazil nut	0.4	3.0	0.4	0.0	0.0
Cupuacu	3.8	6.1	2.8	0.0	1.3
Pupunha	6.6	11.5	7.0	0.0	3.1
PAS	4.6	5.7	0.5	1.6	80.0
SEC	0.0	0.0	0.0	0.0	0.0

AGR = Agroforestry System; PAS = Pasture; SEC = Secondary Forest

Sample collection. Soil samples were taken within a 1 m radius from the base of tree and grass species. Each soil sample was a composite of five soil samples. Beneath each of the tree species in AGR and SEC, nine soil samples were taken from the entire sampling area, with three soil samples per block (3 samples × 3 blocks = 9 samples total per tree). In PAS, 18 soil samples were taken from the entire sampling area, with six soil samples per block (6 samples × 3 blocks = 18 samples total). The species sampled in AGR were *Theobroma grandiflorum* Schumann, *Bactris gasipaes* Kunth, *Bertholletia excelsa* Humb. & Bonpl., and *Eugenia stipitata* McVaugh. PAS was sampled beneath *Brachiaria humidicola* Rendle, and SEC was sampled around the base of *Vismia* and *Cecropia* trees. Species were chosen because of their utility and profitability.

Sampling was random by tree species within each block. Soils were sampled at 0-15 cm depth, air-dried and sieved through a 2 mm sieve. Soils were contaminated with a high frequency of small charcoal pieces, which were removed to the greatest extent possible.

Analyses. Samples were sequentially extracted and analyzed according to a modified sequential phosphorus extraction (Hedley et al., 1982; Tiessen and Moir, 1993) (Table 2), that closely followed the extraction procedure detailed by Tiessen and Moir (1993). However, residual P was extracted using concentrated HNO_3 and $HClO_4$ at 200°C in a sand bath until dry, because of P impurities in locally available H_2SO_4.

Because P fraction differences between soil of the land-use systems studied and soil beneath individual AGR tree species were significant, but not numerous, we grouped P fractions into inorganic P (Pi) and organic P (Po) and availability groups to determine if different or additional trends existed.

Hedley P fractions were summed as total Pi and total Po by adding mean P in inorganic and organic fractions, respectively, by species and land-use system. Phosphorus fractions were also grouped by availability into available P (AP), moderately available P (MAP), and resistant P (RP). Phosphorus in each availability group was determined through summation of mean P by individual AGR species and land-use systems. AP is the sum of P in the resin fraction and the bicarbonate inorganic and organic fractions. MAP is the sum of P in the hydroxide inorganic and organic fractions and the dilute acid fraction, and RP is the sum of P in the concentrated acid inorganic and organic fractions and the residual fraction.

Nutrient analysis. Exchangeable K was analyzed using a double acid extraction (0.05 M HCl and 0.0125 M H_2SO_4) and exchangeable Ca, Mg, and Al with 1 M KCl (EMBRAPA, 1999). Total soil N was determined by the Kjeldahl technique (EMBRAPA, 1999).

Statistical analysis. The results were compared by analysis of variance (repeated measures, general linear model) using a completely randomized design. The SAS System and Minitab computer software were used to determine statistical differences (SAS Institute, Inc., Cary, NC). The repeated measures tool within the SAS System was used to account for repeated measures (nine extracts per sample for Hedley fractions) of the dependent variable for each sample. Where the variances were not homogeneous, a logarithmic transformation was applied. Differences were considered significant at $P<0.05$.

Table 2. Phosphorus pools, extractions procedures, and P pool properties

Pool	Extraction procedure or fractions represented *	Pool properties **
Resin - Pi	Resin strips, distilled water, 16h	Freely exchangeable Pi
Bicarbonate - P	0.5 M $NaHCO_3$, 16h	Immediately plant available P; bound to mineral surfaces
Hydroxide - P	0.1 M NaOH, 16h	Successively available P; bound to oxides; amorphous phosphates
Dil. Acid - Pi	1 M HCl, 16h	Successively available Pi; Ca-bound
Acid – P	Concentrated HCl, 10 min., 80°C	Long-term available P; Ca-bound and occluded P
Residual - Pi	Concentrated HNO_3 and concentrated $HClO_4$, 200°C until dry	Highly resistant and occluded P
Available P (AP)	Sum of P in resin and bicarbonate fractions	Plant available P, immediately available and/or bound to mineral surfaces
Moderately Available P (MAP)	Sum of P in hydroxide and dil. acid fractions	Successively available P; bound to oxides, amorphous phosphates, and Ca-bound
Resistant P (RP)	Sum of P in acid and residual fractions	Highly resistant and occluded P
Inorganic P (Pi)	Sum of P in resin, bicarbonate Pi, hydroxide Pi, dil. Acid, acid Pi, and residual fractions	
Organic P (Po)	Sum of P in bicarbonate Po, hydroxide Po, and acid Po fractions	
Total P	Sum of P in all Hedley fractions	

* *Based on Lehmann et al. (2001) and Tiessen and Moir (1993)*
** *Based on Lehmann et al. (2001), Cross and Schlesinger (1995), and Tiessen and Moir (1993)*

Results

Phosphorus pools. Soil beneath pupunha contained four and six times more resin P than PAS and SEC soil, respectively (Table 3). Soil beneath Brazil nut contained six times more bicarbonate organic P than SEC soil. AGR soil, including soil beneath all species studied, contained about two times more hydroxide organic P than SEC soil. Soil beneath áraça-boi contained more hydroxide organic P than PAS and SEC soils, while PAS soil contained more hydroxide organic P than SEC soil. There was more total P (sum of Hedley fractions) in soils beneath áraça-boi, Brazil nut, and cupuaçu than in SEC soils.

Po was greater in AGR soil than in SEC soil (Table 3), and soils beneath áraça-boi, Brazil nut, and pupunha contained more Po than SEC soils also. AP was greater in AGR soil than PAS and SEC soils (Table 4), and soils beneath Brazil nut and pupunha contained more AP than SEC soil. Although AGR soil did not contain more MAP than SEC soil, individually, soil beneath all species studied in AGR and PAS soil contained more MAP than SEC soil. For all land-use systems, MAP was greater than either AP or RP.

Soil nutrient concentrations in land-use systems and agroforestry species: Calcium was significantly greater in AGR and PAS soils than in SEC soils (Table 5), and soils beneath Brazil nut and pupunha contained more exchangeable Ca than SEC soils. SEC soils contained five and six times less exchangeable Ca than soils beneath pupunha and Brazil nut, respectively, and eight times less exchangeable Ca than PAS soil. PAS soils also contained more exchangeable Ca than soil beneath áraça-boi, close to three times more.

There was more K in AGR soil and soils beneath individual AGR species than in SEC soil. PAS soil contained more Mg than SEC soil, and soils beneath Brazil nut and pupunha contained more Mg than SEC soil. Comparing soils beneath individual AGR species, soil beneath pupunha contained more Mg than soil beneath cupuaçu. PAS soil had significantly less Al than SEC soils and soil beneath áraça-boi and Brazil nut. Additionally, soil beneath cupuaçu and pupunha contained less Al than soil beneath áraça-boi and SEC soil. Soil acidity was less beneath pupunha than beneath áraça-boi and Brazil nut.

Discussion

Phosphorus pools. Resin P was increased by tree species and fertilization, i.e., pupunha increased resin P where fertilization minimally increased resin P (Table 3). It may be that pupunha, being adapted to acidic and chemically poor soil (Sanchez and Salinas 1981; FAO 1986), affects surrounding soil through efficient nutrient cycling. Fernandes and Sanford (1995) suggested that pupunha may be able to access less soluble forms of soil P after observing significantly lower hydroxide organic P in soil beneath a 30 year old pupunha orchard than in soil beneath adjacent Costa Rica forests. Additionally, soil P pools were significantly increased by the transformation of P by litterfall, throughfall, and stemflow of pupunha in a Central Amazonian agroforestry system, due to relatively high foliar concentrations of pupunha (Lehmann et al., 2001b; Schroth et al., 2001).

Additionally, pupunha has surface roots that extend approximately 5 to 25 cm above the soil surface (personal observation). They are notable because they have a gelatinous covering, which is seasonal in its existence. Where these roots make contact with the soil surface, there is a change in the physical appearance of the soil, making it richer in color, with a seemingly 'worked' texture, influenced possibly by soil macrofauna or microbial biomass. It has being suggested that the gelatinous root covering might be a labile and easily assimilated source of C, which draws soil fauna to feed upon it. However, no studies have investigated the gelatinous covering on pupunha surface roots.

Table 3 Phosphorus fractions in soils by land-use system and agroforestry tree species

Phosphorus Fractions / LAND-USE SYSTEM / Tree Species	Resin Pi	Bicarb. Pi	Bicarb. Po	Hydrox. Pi	Hydrox. Po	Dil. Acid Pi ($g\ m^{-3}$)	Acid Pi	Acid Po	Residual Pi	Total Pi	Total Po	Total P
AGR	11.3 ab	2.3	6.5 ab	15.2	28.8 ab	1.7	4.1	5.4	5.2	39.8	40.7 a	80.5 ab
Áraça-boi	11.2 ab	4.0	4.8 ab	17.8	31.6 a	1.5	4.1	4.4	5.0	43.7	40.8 a	84.4 a
Brazil nut	9.6 ab	2.2	11.6 a	16.4	29.7 ab	1.9	4.2	5.9	6.8	41.1	47.2 a	88.4 a
Cupuaçu	9.3 ab	1.2	5.1 ab	13.5	25.8 ab	1.7	3.1	4.3	3.0	31.7	35.1 ab	66.8 a
Pupunha	15.1 a	1.8	4.7 ab	13.2	28.1 ab	1.8	5.0	6.8	6.1	42.9	39.7 a	82.6 ab
PAS	3.7 b	2.9	3.3 ab	17.0	21.6 b	1.8	2.9	2.7	9.9	38.1	27.6 ab	65.7 ab
SEC	2.2 b	2.3	1.9 b	10.2	13.0 c	0.6	3.4	1.3	6.1	24.8	16.2 b	40.9 b

AGR = Agroforestry System; PAS = Pasture; SEC = Secondary Forest
Bicarb. = Bicarbonate; Hydrox. = Hydroxide; Dil. Acid = Dilute Acid
Pi = Inorganic P; Po = Organic P
Pi Total = Sum of inorganic fractions; Po Total = Sum of organic fractions; Total P = Sum of all fractions
Letters indicate significant differences within each column ($P < 0.05$)

Table 4 Phosphorus pools in soils by land-use system and agroforestry tree species

LAND-USE SYSTEM Tree Species	AP	MAP ($g\ m^{-3}$)	RP
AGR	20.1 a	45.8 ab	14.7
Araça-boi	20.0 abc	51.0 a	13.5
Brazil nut	23.4 ab	48.0 a	17.0
Cupuaçu	15.5 abc	41.0 a	10.3
Pupunha	21.6 ab	43.1 a	17.9
PAS	9.9 bc	40.7 a	15.8
SEC	6.4 c	23.8 b	10.7

AP = Available P, Sum of resin and bicarbonate fractions
MAP = Moderately Available P, Sum of hydroxide and dilute acid fractions
RP = Resistant P, Sum of acid and residual fractions
AGR = Agroforestry System; PAS = Pasture; SEC = Secondary Forest
Letters indicate statistical differences ($P < 0.05$) within P pools

Table 5 Nutrients in soils by land-use system and agroforestry tree species

LAND-USE SYSTEM Tree Species	Total N ($g\ kg^{-1}$)	Exch. Ca ($ug\ g^{-1}$)	Exch. K ($g\ m^{-3}$)	Exch. Mg ($ug\ g^{-1}$)	Exch. Al ($ug\ g^{-1}$)	pH H_2O
AGR	1.8	143.0 b	30.7 a	45.6 abc	323.9 abc	4.8 ab
Áraça-boi	1.7	84.6 bc	30.8 a	40.4 abc	431.7 a	4.6 b
Brazil nut	1.8	159.9 ab	32.2 a	48.9 ab	328.0 ab	4.5 b
Cupuaçu	1.7	135.7 abc	28.9 a	32.0 bc	285.0 bc	4.8 ab
Pupunha	1.8	192.6 ab	31.1 a	63.1 a	263.2 bc	5.1 a
PAS	1.7	246.9 a	26.5 ab	50.2 ab	194.8 c	4.9 ab
SEC	1.7	28.4 c	17.8 b	17.9 c	414.0 a	4.7 ab

Exch. = Exchangable
AGR = Agroforestry System; PAS = Pasture; SEC = Secondary Forest
Letters indicate significant difference ($P < 0.05$) within column

Resin P in soil beneath all AGR species and SEC soil was greater than a representative mean for Amazonian soils (McGrath et al., 2001b). Additionally, resin P, bicarbonate inorganic P, and bicarbonate organic P values were consistently greater than those of 6-year-old pupunha and cupuaçu plantation agroforestry systems in the Brazilian states of Acré and Rondônia (McGrath et al., 2001a). However, in a study of single agroforestry tree effects on P fractions north of Manaus, Brazil, each of our P fraction values were consistently less in SEC soil than soil beneath *Vismia* in their study. Additionally, each of our P fraction values were consistently less in soil beneath AGR species the study sites had in common (Lehmann et al., 2001c). Fertilization in these other studies was greater than fertilization in this study. Greater time after establishment, the use of more agroforestry tree species, greater fertilization, and the possibility of some of the soils having been Ultisols are likely reasons that the aforementioned P fractions were greater in our systems compared to those of McGrath et al. (2001b).

Bicarbonate organic P was affected by tree species, i.e., Brazil nut increased bicarbonate organic P (Table 3). Brazil nut had considerably more above ground biomass (372.4 ± 22.0 kg plant dry matter) than any of the other species studied (McCaffery, 2002). This may be indicative of considerably greater below ground biomass, which could be more efficient in taking up and recycling this P fraction.

AP was greater in soils beneath Brazil nut and pupunha than SEC soil, and was greater in AGR soil than PAS soil and SEC soil (Table 4). This suggests that P additions and the use of AGR species and management was beneficial to maintaining AP. Additionally, it suggests that Brazil nut and pupunha may have a mechanism to 'mine' P better than the other AGR species.

Air drying soils from England and Wales increased bicarbonate inorganic and organic P, compared to field-moist soils (Turner and Haygarth, 2003). Although errors in this study may have been caused by air-drying, we suspect error will likely be consistent, making values comparable across species within this study. Additionally, the soils in the study in England and Wales were of a different mineralogy in a temperate climate, which may influence the transferability of air drying errors of soil bicarbonate inorganic and organic P (Turner and Haygarth, 2003).

Fertilization had the greatest impact on producing differences in soil P in the hydroxide organic P fraction (Table 3), which more or less mirror P fertilization differences. Tiessen *et al.* (1992) also found that fertilizer applied P to an Oxisol in Northeastern Brazil was incorporated into hydroxide organic P. Tree species effects were limited only to áraça-boi, which is inexplicable.

MAP was greater in soil beneath all individual AGR species and PAS soil than in SEC soil (Table 4). This suggests that moderate fertilization is key in maintaining MAP. MAP is likely in equilibrium with AP and RP, contributing to plant nutrition and P cycling. We expected that all species would have had more total soil P (Table 3) beneath AGR species than in SEC soil due to fertilization of AGR species. However, soil P concentrations in soil underneath pupunha did not increase. It is likely that more of the fertilizer P applied to pupunha was removed through harvest of heart of palm and pupunha fruits than was replenished by fertilization. Fertilizer P applied to the other species was also removed through fruits, nuts, wood, etc., but may not have exceeded P additions.

The total P values for soils of AGR, PAS, and SEC of 83.6, 68.2, and 42.5 mg P kg^{-1} soil, respectively were comparable with total soil P values of other studies in Central Amazônia (Lehmann et al., 2001b; 2001c), and were considerably lower than soils of other regions in Brazil (141 to 388 mg P kg^{-1} soil) (Lehmann et al., 2001b), which were considerably less than other worldwide soils (two soils from Africa contained 874 to 1426 mg P kg^{-1} soil) (Solomon et al., 2002).

Organic P was greater in AGR soil, except cupuaçu, than in SEC soil which can be explained by fertilization. Cupuaçu did not follow this pattern due to its capability to immobilize P. McGrath *et al.* (2000a) found cupuaçu litter to immobilize P for a period of one year in a litterbag experiment.

Soil nutrient concentrations in land-use systems and agroforestry species. It is interesting that soil beneath Brazil nut and pupunha had more Ca than SEC soil, considering Brazil nut received no lime and pupunha received very little (Table 5). This may be indicative of a tree specific effect that increased soil AP beneath pupunha and Brazil nut. PAS soil did not contain more Ca than soil beneath the other AGR species probably because of Ca uptake by the pasture grass or litter cycling of Ca in the other AGR species. Other differences in soil acidity, Al, and Ca were most likely due to differences in lime additions to different plants and land-use systems.

Mg was greater in soil beneath Brazil nut and pupunha and PAS soil than SEC soil, which is likely an additional result of lime amendments (Table 5). Soil beneath pupunha additionally contained more Mg than soil beneath cupuaçu. This may be related to the foliar C:N ratio, which is higher for cupuaçu than pupunha (Lehmann et al., 2001a), which could reduce decomposition and Mg release from litter. Fertilization affected soil K. All species that received some fertilization contained more than SEC, where there was no fertilization

Conclusions. Low-input fertilization and agricultural management through the use of native agroforestry tree species increased available soil P and other soil nutrients. Pupunha and Brazil nut increased soil AP, whereas áraça-boi, cupuaçu, PAS, and SEC did not. Pupunha and Brazil nut are suggested for use in designing perennial tree crop systems in Central Amazônia where sufficient soil P availability is of concern. We also suggest further research of the physiology of these species in order to better understand how they increase AP, so other species with similar physiology may be tested for use in maintaining AP in soils of central Amazônia.

Fertilization increased MAP, which is important in maintaining long-term P availability. Therefore, in addition to establishing agroforestry systems in central Amazônia with pupunha and Brazil nut tree species, among other native perennial tree crops, it is important to apply or maintain low-level fertilization to at least account for nutrients removed in harvests.

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3.6 Assessment of the potential of tannins in legumes and saponins in tropical fruits to reduce methane in ruminants

Highlights:

- The supplementation with *S. saponaria* reduced daily methane release from sheep by over 10%.
- The addition of legumes with high levels of tannins reduced methane release per unit of organic matter fermented
- Supplementation with molasses reduced the negative nutritional effects high concentrations of condensed tannins in legume by enhancing N turnover.

We have made considerable progress in defining the potential of saponin-rich fruits to reduce methane emission from rumen fermentation and enhance N utilization by sheep. The results of experiments carried out during the last two years showed that the fruits of *Sapindus saponaria* are valuable in supplementing tropical forage-based diets since they are effective in improving duodenal microbial protein flow and efficiency of rumen fermentation, and in suppressing ruminal methane release.

This year we confirmed that the inclusion of tannin-rich legumes such as *Calliandra calothyrsus* and *Flemingia macrophylla* in forage-based diets significantly reduces methane release but also inhibits nutrient degradation and N turnover. It was hypothesized that to take advantage of the methane suppressing effect of tannin-rich legumes without affecting nutrient degradation and N turnover, it was necessary to combine them with legumes free of tannin. This hypothesis was partially confirmed this year. However, the effects of legume mixtures were dependent on the type and proportion of tannin rich legumes included in the diet. Although *C. calothyrsus* and *F. macrophylla* presented similar tannin contents, the inclusion of these legumes in mixtures resulted in considerably different effects on rumen fermentation, and *F. macrophylla* was less effective in suppressing methanogenesis than *C. calothyrsus*. Thus, we still need to define the optimal type and proportion of tannin rich legumes in mixtures to take advantage of their methane suppressing potential without affecting nutrient degradation.

3.6.1 In vivo evaluation of *Sapindus saponaria* and legumes as supplement on rumen fermentation and N utilization by sheep fed a low quality grass

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Rationale. Previous *in vitro*-experiments showed that the inclusion of fruits of *Sapindus saponaria* into tropical diets may suppress methane release by over 10% and that supplementation with leaves from the shrub legume *Cratylia argentea* increased methane release by 300%. However, no information was available on the effect of these tropical forage resources on methane release *in vivo*. Thus a respiratory chamber experiment was carried out to study the influence of a supplementation with fruits of *S. saponaria* and leaves of *C. argentea* on energy utilization and methane release of sheep fed a basal diet of *Brachiaria dictyoneura*.

Materials and Methods. Three basal diets with contrasting forage quality were included. The traditional diet consisted of a low quality hay (*Brachiaria dictyoneura* cv. Llanero), in the other two diets 33.3%, respectively sun-dried leaves of the shrub legume *Cratylia argentea* replaced 66.7% of the grass hay. All three diets were fed either with a concentrate containing 25% *S. saponaria* or with a control concentrate without *S. saponaria*. Animals were offered 60 g/kg $BW^{0.75}$ of forage and 20 g/kg $BW^{0.75}$ of concentrate per day. Six castrated growing lambs of the Swiss White Hill breed with a initial body weight of 30.1±2.8 kg were allotted to one of six treatments in a complete Latin Square design with 3 × 2 factorial arrangement of treatments (3 basal diets × 2 concentrates) and the six experimental periods were of 21 days each. The first 12 days of each experimental period were used for adaptation, days 13 to 20 for measurement of forage intake and total collection of feces and urine and day 21 for blood and rumen liquid sampling. Respiratory measurements were carried out during two 22.5 h-periods on days 19 and 20.

The forage required for the trial was harvested and dried at CIAT's Research Station in Santander de Quilichao (Cauca, Colombia) and fruits of *S. saponaria* were collected in the rural area near Cali (Cauca Valley, Colombia). Forage and fruits were then shipped to Switzerland and stored at the ETH. The two concentrates were formulated to contain similar amounts of protein, fiber and energy. Approximately 180 kg of each concentrate were mixed and pelleted at the Institute of Animal Sciences at the ETH.

Results and Discussion. The two concentrates offered were consumed completely by all animals throughout the experiment and total DM and OM intakes per kg of metabolic body weight ($BW^{0.75}$) were not affected ($P>0.05$) by type of concentrate. Total DM and OM intakes responded linearly ($P<0.001$) as legume proportion increased, with the highest DM and OM intakes occurring in the treatments with 2/3 of legume in the basal diet. Intake of CP did not vary with type of concentrate ($P>0.05$) but responded linearly ($P<0.001$) to dietary legume proportion and increased from 5.98 g/kg $BW^{0.75}$ in the grass-alone treatments to 13.91 g/kg $BW^{0.75}$ in the 2/3-legume treatments.

Apparent total tract digestibilities of OM, CP, NDF and ADF were reduced ($P<0.01$) by supplementation with *S. saponaria* and digestibilities of OM, NDF, ADF were linearly reduced with increasing legume proportion. Apparent digestibility of CP, however, increased linearly and quadratically ($P<0.001$) as legume proportion increased.

Rumen fluid pH did not vary ($P > 0.05$) with dietary treatment and averaged 6.76. Rumen fluid ammonia concentration was not affected ($P > 0.05$) by type of concentrate but responded linearly ($P<0.001$) as dietary legume proportion increased. In the grass-alone treatments rumen ammonia concentration was as low as 2.45 mmol/l and increased to a level of 13.61in the 2/3-legume treatments.

Total VFA concentration was higher ($P<0.01$) when the concentrate containing *S. saponaria* was supplied than with the control concentrate but did not vary ($P>0.05$) with dietary legume proportion. Supplementation with *S. saponaria* reduced ($P<0.001$) the molar proportions of iso-Butyrate and iso-Valerate but had no effect ($P>0.05$) on the other VFA. The molar proportion of acetate responded linearly to increasing legume proportion and decreased from 0.73 in the grass-alone treatments to 0.69 in the 2/3-legume treatments. The molar proportions of propionate, iso-Butyrate, n-Valerate and iso-Valerate increased linearly ($P<0.001$) as dietary legume proportion was raised. The acetate-to-propionate ratio tended to be lower ($P=0.078$) in the treatments with *S. saponaria* supplementation and decreased linearly ($P<0.001$) as legume proportion increased.

Total bacteria count was increased ($P<0.01$) by *S. saponaria* supplementation but showed no clear trend due to dietary legume proportion ($P>0.05$). Total ciliate protozoa count was reduced by over 50% in the treatments with *S. saponaria* but did not vary ($P>0.05$) with legume proportion. No significant interactions of dietary legume proportion and *S. saponaria* supplementation on rumen fluid characteristics were found ($P>0.05$).

Energy intake did not vary ($P>0.05$) with type of concentrate but responded to dietary legume proportion. Gross energy (GE), digestible energy (DE) and metabolizable energy (ME) intakes increased linearly ($P<0.001$) as legume proportion increased. Feeding the *S. saponaria* containing concentrate increased ($P<0.001$) energy losses through faeces, reduced energy losses through urine ($P<0.05$) and methane ($P<0.01$), had no effect ($P>0.05$) on energy expenditure (heat energy) and tended to increase ($P=0.080$) total energy losses. Energy losses through faeces, urine and heat, as well as total energy losses increased linearly ($P<0.001$) with increasing legume proportion, whereas energy losses through methane tended to decrease ($P=0.075$) with increasing legume proportion. Energy retention did not vary ($P>0.05$) with dietary treatments. No significant interactions of dietary legume proportion and *S. saponaria* supplementation on energy balance were found ($P>0.05$).

Daily methane release per kg of body weight was reduced ($P<0.001$) by 9% on average by *S. saponaria* supplementation and was not affected ($P>0.05$) by dietary legume proportion. Compared to the control concentrate, the *S. saponaria* containing concentrate reduced ($P<0.05$) methane release relative to CO_2 production and tended to reduce ($P=0.063$) methane release relative to OM digested but had no effect ($P>0.05$) on methane release relative to NDF digested. With increasing legume proportion, methane release relative to CO_2 produced and OM digested decreased ($P<0.01$), and methane release relative to NDF digested increased linearly ($P<0.001$). Methane release relative to energy retained was reduced by 30% ($P<0.05$) when *S. saponaria* was supplemented bud did not vary ($P>0.05$) with legume proportion. In contrast, methane release relative to N retained was not affected ($P>0.05$) by *S. saponaria* and decreased linearly ($P<0.05$) with increasing legume proportion. On average, methane release per gram of N retained was reduced by 60% due to legume supplementation.

Haematocrite values and activity of liver enzymes (ASAT and GLDH) were not affected by dietary treatments and averaged 34.9%, 80.2 U/l and 10.3 U/l, respectively. This indicates that the dietary proportion of *S. saponaria* tested in this experiment did not affect the health status of sheep.

The fact that interactions were mostly insignificant indicates that supplementation of *S. saponaria* fruits is a useful means to reduce methane emission from sheep fed tropical grass-alone and legume-supplemented diets. Legume supplementation represents an environmentally friendly way to increase animal performance of tropical livestock, since it was shown to improve N retention and to reduce methane release relative to body protein retention.

3.6.2 Effect of legumes with contrasting tannin content and their mixtures on *in vitro* rumen fermentation parameters and methane emission

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Rationale. A previous *in vitro* experiment had shown that the supplementation of a low-quality grass diet (*Brachiaria dictyoneura*) with *Cratylia argentea* drastically increased organic matter degradation, N turnover and methane release per unit of organic matter degraded, whereas the partial replacement of *B. dictyoneura* by *Calliandra calothyrsus* significantly reduced organic matter degradation, N turnover and methane release. Thus it was hypothesised that, to take advantage of the methane suppressing effect of *C. calothyrsus* without affecting nutrient degradation and N turnover, it was necessary to combine tannin-rich legumes with legumes free of or low in tannins. To test this hypothesis three Rusitec-experiments were performed (see also Activity 3.3 in this section) to evaluate the effect of supplementing legumes with contrasting contents of condensed tannins on methane production and N turnover *in vitro*.

Materials and Methods. In each experiment four basal diets were evaluated in four replicates. In experiment 1 the effect of the inclusion of *C. argentea* (0, 25, 50 and 100% of diet DM) in a diet of *C. calothyrsus* was investigated. In experiment 2 the effect of a partial replacement (50% of diet

DM) of *F. macrophylla* by *C. argentea*, *C. calothyrsus* or by a mixture of both legumes was evaluated (Table 1). The daily dry matter supply was maintained constant at 15 g DM.

Table 1 Composition (% of DM) of the experimental diets

	Experiment 1			
Diet	1	2	3	4
Calliandra calothyrsus	100	75	50	-
Cratylia argentea	-	25	50	100
	Experiment 2			
Diet	1	2	3	4
Flemingia macrophylla	100	50	50	50
Calliandra calothyrsus	-	50	25	-
Cratylia argentea	-	-	25	50
	Experiment 3 (Activity 3.3)			
Diet	1	2	3	4
Brachiaria dictyoneura	100	50	50	50
Calliandra calothyrsus	-	50	25	-
Cratylia argentea	-	-	25	50

Results and discussion. In experiment 1, ammonia concentration in the fermenter fluid increased ($P<0.001$) from 0.81 mmol/l to 13.02 mmol/l when the proportion of *C. argentea* in the diet was increased from 0 to 100%. Counts of ciliate protozoa and bacteria also varied with the composition of the diet and were highest ($P<0.05$) with 50 and 100% *C. argentea*, intermediate with 25% *C. argentea* and lowest with 100% *C. calothyrsus*. Similarly, total bacteria counts were lowest with 100% *C. calothyrsus* and increased linearly ($P<0.01$) with increasing dietary proportions of *C. argentea*.

Apparent degradation of nutrients was also related to the level of *C. argentea* in the diet and was lowest in the diet with *C. calothyrsus* alone and increased linearly ($P<0.001$) with the proportion of *C. argentea*. Apparent organic matter degradation was twice as high in the diet of *C. argentea* alone (35.5%) as in the diet with *C. calothyrsus* alone (17.4%).

Daily methane release increased linearly ($P<0.001$) from 0.16 to 3.53 mmol/d when the proportion of *C. argentea* increased from 0 to 100%. When 25 or 50% of *C. calothyrsus* were replaced by *C. argentea* no changes occurred ($P>0.05$) in apparent crude protein degradation and only minor changes were observed in ruminal N turnover. Only the complete replacement of *C. calothyrsus* clearly increased apparent crude protein degradation and enhanced ruminal N turnover. These results suggest that the use of mixtures of *C. calothyrsus* and *C. argentea* is no means to improve ruminal N turnover without increasing methane release. This contrasts with the observations made in the third experiment (Activity 3.3) where the supplementation of low-quality *B. dictyoneura* with a mixture of *C. calothyrsus* and *C. argentea* (50:50) improved the nutritional quality of the diet and increase the ammonia concentration in the fermenter fluid without enhancing methane emission relative to organic matter degraded.

Therefore we suggest that the effects of legume mixtures on rumen fermentation not only depend on the quality and proportion of the different legumes, but also on the remaining components of the diet. Consequently, the evaluation of legume mixtures has to be done in combination with grasses, which represent the most important diet ingredient for ruminants in tropical smallholder livestock systems.

In experiment 2, ammonia concentration in the fermenter fluid was not affected (P>0.05) when 1/2 of *F. macrophylla* was replaced by *C. calothyrsus*, but increased (P<0.05) by 50% when 1/4 of the tannin rich legumes was replaced by *C. argentea*. With 50% of *C. argentea* in the diet, the ammonia concentration was 280% higher (4.6 mmol/l) than with *C. calothyrsus* alone (1.2 mmol/l).

Count of rumen ciliate protozoa was not affected (P>0.05) by the composition of the diet, but total bacteria count was increased (P<0.05) by 45% due to the inclusion of *C. argentea*. A small (-7%) but significant reduction in apparent organic matter degradation was recorded when 1/2 of *F. macrophylla* was replaced by *C. calothyrsus*.

The inclusion of 1/4 of *C. argentea* increased (P<0.05) apparent organic matter degradation by 13%, and with 1/2 of *C. argentea* in the diet, organic matter degradation increased (P<0.05) by 38% relative to the control (*F. macrophylla* alone). Methane release per gram of organic matter apparently degraded, decreased by 24% when 1/2 of *F. macrophylla* was replaced by *C. calothyrsus* and increased (P<0.05) by 27% and 57%, respectively, when 1/4 and 1/2 of *C. argentea* were included in the diet.

When 1/2 of *F. macrophylla* was replaced by *C. calothyrsus* no changes occurred (P>0.05) in apparent crude protein degradation and ruminal N turnover. In contrast, the inclusion of *C. argentea* in the diet resulted in a clear improvement of crude protein degradation and N turnover.

Overall, the replacement of 1/2 of *F. macrophylla* by *C. calothyrsus* resulted in only minor changes in rumen fermentation. Apparent organic matter degradation and methane emission were slightly reduced due to *C. calothyrsus* but the other major fermentation parameters remained unchanged. This suggests that both legumes had similar (negative) effects on rumen fermentation.

In contrast, the inclusion of *C. argentea* in the mixtures with the tannin-rich legumes enhanced rumen fermentation, as indicated by higher ammonia concentration and total bacteria count in the fermenter fluid and by improved apparent nutrient degradation and increased methane emission, both in absolute terms (mmol/d) and relative to organic matter degraded. It's worth mentioning, that apparent crude protein degradation in the mixture of *F. macrophylla* with *C. argentea* (50:50) was twice as high as in the mixture of *C. calothyrsus* with *C. argentea* in experiment 1 (27% vs. 14%). This indicates that *C. calothyrsus* and *F. macrophylla* have similar effects on rumen fermentation when used alone or in combination with each other, but contrasting effects are observed, when these tannin-rich species are mixed with a legume free of or low in tannins.

In general our results suggest that even though *C. calothyrsus* and *F. macrophylla* have similar chemical compositions and tannin contents, the nutritional value of *F. macrophylla* is higher than that of *C. calothyrsus* when these species are used in combination with a good-quality legume, but that is less effective in suppressing methane emission than *C. calothyrsus*.

3.6.3 Effect of supplementing low quality grasses with legumes and soluble carbohydrates on *in vitro* rumen fermentation parameters and methane production

Contributors: F.L. Valencia (National University of Palmira), L.M. Monsalve (University of Pereira), H.D. Hess (ETH Zurich), C.E. Lascano (CIAT), M. Kreuzer (ETH Zurich)

Material and Methods. In this in vitro experiment a grass-alone and three legume supplemented (50% of DM) diets were evaluated. The legume supplements consisted of *C. calothyrsus* (100%), *C.*

argentea (100%) or a mixture of both legumes (1:1). All four basal diets were evaluated with and without the addition of sugarcane molasses (10% of DM). The eight treatments were tested during 4 × 10 day periods (n=4).

Results and Discussion. The pH of fermenter fluid averaged 6.97 and showed only minor variations between basal diets and due to the addition of molasses. The replacement of 50% of the basal diet by *C. calothyrsus* reduced (P<0.05) apparent degradability of N from 350 mg/g (grass-alone diet) to 80 mg/g without affecting fermenter fluid ammonia (P>0.05) (Fig. 1). Compared to the grass-alone diet, the inclusion of *C. argentea* did not affect (P>0.05) apparent degradability of N but drastically increased fermenter fluid ammonia (P<0.05) from 1.0 mmol/l (grass-alone diet) to 4.2 mmol/l. When the legume mixture was included, apparent degradability of N was reduced (P<0.05) but fermenter fluid ammonia was increased (P<0.05).

As expected, the addition of molasses reduced (P<0.001) ammonia concentration with all diets. The effect of molasses on apparent N degradability was dependent on the kind of legume supplementation. While no effect was observed (P>0.05) when molasses was added to the grass-alone diet and the diets containing *C. argentea*, the addition of molasses to the diet supplemented with *C. calothyrsus* alone, drastically increased apparent N degradability from -45 to 203 mg/g (interaction between diet and molasses, P<0.001). The reasons for this unexpected increase are not well understood but could be related to the higher availability of fermentable energy and hence increased microbial activity when molasses was added, or to the inactivation of condensed tannins due to the formation of complexes between tannins and soluble carbohydrates. Even though we do not completely understand this phenomenon, it is highly interesting, because it indicates that supplementation with molasses could be an alternative to partially reduce the negative nutritional effects of feeding legumes with high concentrations of condensed tannins.

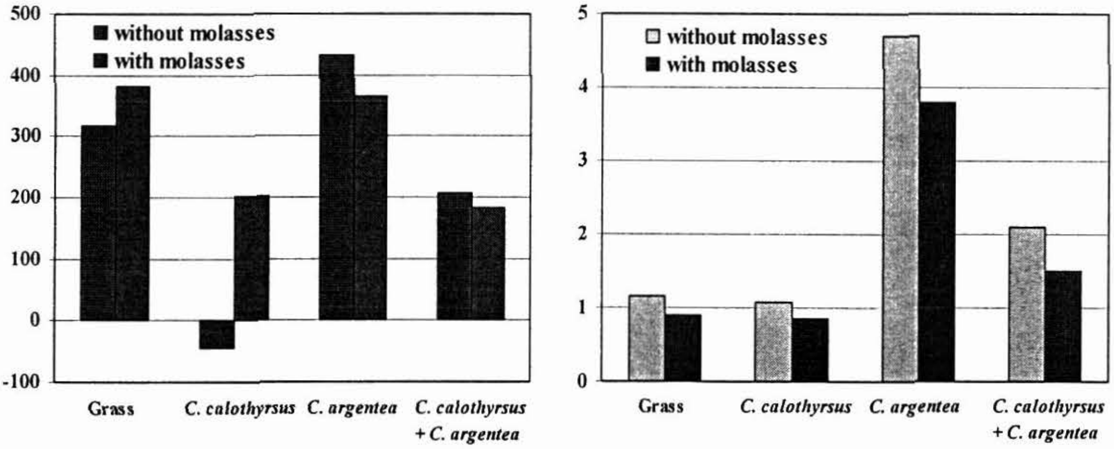

Figure 1: Apparent rumen degradability of nitrogen (mg/g) (on the left) and ammonia concentration (mmol/l) (on the right) observed in Rusitec-fermenters supplied with a grass-alone and with legume supplemented (50% of DM) diets. The legume supplements consisted of *C. calothyrsus* (100%), *C. argentea* (100%) or a mixture of both legumes (50:50). All diets were evaluated with and without the addition of sugarcane molasses (10% of DM).

The supplementation with *C. calothyrsus* alone reduced the apparent degradability of organic matter and neutral detergent fiber (P<0.05) (Fig. 2, left). The supplementation with *C. argentea*, in contrast, increased organic matter and fiber degradation (P<0.05). Supplementation with the legume mixture increased organic matter degradation (P<0.05) and had no effect on fiber degradation (P>0.05), indicating that the negative effects of supplementing *C. calothyrsus* on fiber degradation can be avoided when this legume is supplied in combination with a legume low in tannins.

Daily methane release (-50%) and methane release relative to organic matter degraded (-40%) were reduced (P<0.05) by supplementation with *C. calothyrsus* alone (Fig. 2 +1), which agrees with the results from a previous experiment. Supplementation with *C. argentea* alone increased (P<0.05) daily methane release (+100%) and methane release relative to organic matter degraded (+40%), which is also in agreement with the results of the previous experiment.

When the low-quality grass diet was supplemented with the mixture of *C. calothyrsus* and *C. argentea*, daily methane release was increased (P<0.05) by 30% but methane release relative to organic matter degraded remained unaffected (P>0.05). Interactions between basal diet and molasses addition were mostly insignificant, except for apparent N degradation (see above). On average among all diets, molasses addition increased (P<0.05) organic matter degradation and methane release and reduced (P<0.05) fiber degradation.

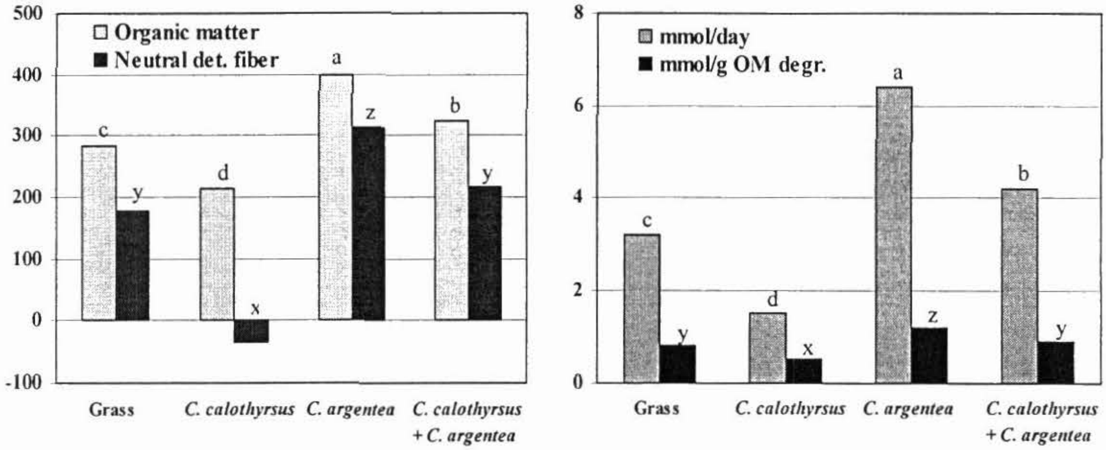

Figure 2: Apparent degradability of organic matter and neutral detergent fiber (mg/g) (on the left) and methane release (on the right) observed in Rusitec-fermenters supplied with a grass-alone and with legume supplemented (50% of dry matter) diets. The legume supplements consisted of *C. calothyrsus* (100%), *C. argentea* (100%) or a mixture of both legumes (50:50).

Results of this experiment confirmed the methane suppressing potential of the tannin-rich *Calliandra calothyrsus* and suggest that supplementing mixtures of legumes with high and low contents of condensed tannins could be a useful alternative to improve nutrient supply and ruminal organic matter degradation avoiding the dramatic increase in methane release typically observed when low-tannin legumes are supplemented alone. Additionally, results indicate that supplementation with molasses could be an alternative to partially reduce the negative nutritional effects of feeding legumes with high concentrations of condensed tannins as it enhances N turnover.

Output 4.
Impact of implemented strategies for adaptation to and mitigation of GHG assessed, and institutional capacity enhanced

Most of the efforts in this output were dedicated this year to enhance institutional capacity of our partners in national programs and educational institutions. Staff from the project participated actively in several training courses and workshops. Great effort was made to involve students from local universities. Staff from the project has being very active in collaborating with the Ministry of Agriculture of Colombia to discuss national policies related to the mechanisms for the implementation of projects for the CDM.

Strengthening NARs

Training Courses:
Curso Internacional de Agroclimatología Tropical. CORPOICA, July 5-10, 2003, Bogotá, Colombia. Attended by 35 participants from CORPOICA, CENICAFE, CENICAÑA and Universities in Colombia.

Encuentro Regional de ONG Ambientalistas del Valle del Cauca. Cali, Colombia, August 22, 2003. Attended by 50 participants from a wide range of NGOs from Valle, Cauca and Nariño Departments in Colombia.

Taller Nacional de la Sociedad Colombiana de Ingeniería (ACIEM) sobre el mecanismo de desarrollo limpio (DML) del protocolo de Kyoto. Cali, May 10, 2003. Atended by 100 participants from the private sector and Universities from Colombia.

Practical training course for Corpoica staff from the Regional-8 to monitor Carbon stocks in soils and biomass on forestry and agroforestry systems in the Colombian Llanos. Bogotá, June, 2003. Two staff trained.

Thesis

Undergraduate Thesis.
Angela Muñoz. Balances de Carbono y gases de efecto invernadero en un sistema intensivo de producción de ganado de leche en la Hacienda Pasoancho (Valle, Colombia). Universidad Nacional de Colombia, Palmira. In progress.

Enna Díaz and Liliana Patricia Paz. Evaluación del regimen de humedad del suelo bajo diferentes usos en los Páramos "Las Animas y Piedra de León", Departamento del Cauca. Universidad del Cauca., Popayán, Completed.

Mera Monica Lorena. Efecto de reducción de taninos en leguminosas forrajeras tropicales en producción de metano en un sistema de fermentación *in vitro*. Universidad Nacional de Colombia, Palmira. In progress.

Monsalve, Lina M. Suplementación de una gramínea tropical con leguminosas y *Sapindus saponaria*: efecto sobre fermentación ruminal y metanogénesis *in vitro*. Universidad de Santa Rosa de Cabal. Pereira, Colombia. In progress.

MS thesis.

Abreu S. Andrés. 2003. Utilización del fruto de *Sapindus saponaria* como fuente de saponinas para reducir la metanogénesis y mejorar la utilización del alimento en rumiantes con dietas tropicales. Universidad Nacional de Colombia. Bogotá. Completed.

Valencia Francis Liliana. Determinación del efecto de la calidad de la dieta en relación con la presencia de taninos y emisiones de metano en un sistema *in vitro*. Universidad Nacional de Colombia. In preparation.

Partnerships with NARS, Universities, NGO's and Producer associations

- Environmental services of silvopastoral systems in the Carribean Region, Colombia. Corpoica, Universidad de Córdoba, Asociación Nacional de Ganaderos.

- The role of a prototype intensive cattle production systems in net balances of greenhouse gases in Valle, Colombia. Universidad Nacional, Palmira, Hacienda Pasoancho.

- Production and chemical characterization of charcoal generated from selected tree species. Universidad Nacional de Colombia, Bogotá.

- Carbon storage on pastures in different agroecosystems. CIPAV, CATIE, Universidad del Amazonas.

- Quesungual slash and mulch agroforestry system. MIS consortium, Universidad Nacional de Honduras, ESNACIFOR Honduras, FAO.

- Environmental externalities to promote sustainable development of rural communities in the Andes. CONDESAN, Universidad de Caldas, CENICAFE, Universidad Nacional, Bogotá, GTZ.

Partnerships with ARO's

- Cornell University: Interaction of charcoal with soils

- ETH, Zurich, Switzerland: Effect of saponin-rich fruits on methane emissions by ruminants.

- GTZ, Germany: Environmental externalities to promote sustainable development of rural communities in the Andes.

- ILRI, Nairobi, Kenya: Modelling of expected effects of climate change on yields of main food crops.

- JIRCAS, Japan: Nitrification Inhibition by tropical grasses

- Wageningen University, The Netherlands and CATIE, Costa Rica: Carbon storage on pastures in different agroecosystems.

Workshops/ Conferences/Meetings (attended by at least one staff of the project):

- Annual Meeting of the Japanese Soil Science and Plant Nutrition Societies, August 21-24, 2003 at Yokohama, Japan.

- Eighth Conference of the Parties to the UN Framework Convention on Climate Change in New Delhi, India, October, 2002. Made a poster presentation in conjunction with UNEP, explaining the work of the CGIAR on Climate Change, including distribution of a two-page summary of the Challenge Program Pre-proposal. Made a presentation of Climate Change in the CGIAR at a side-event meeting chaired by Dr Klaus Topfer, UNEP Executive Director.

- Third International Coordination Meeting of the Netherlands Cooperation Activity CO-010402, *Research Network for the Evaluation of Carbon Sequestration Capacity of Pasture, Agropastoral and Silvopastoral Systems in the American Tropical Forest Ecosystem,* at CATIE, Costa Rica, December, 2002. Presented a summary of activities on Climate Change in the CGIAR.

- Fourth International Coordination Meeting of the Netherlands Cooperation Activity CO-010402, *Research Network for the Evaluation of Carbon Sequestration Capacity of Pasture, Agropastoral and Silvopastoral Systems in the American Tropical Forest Ecosystem,* at CIAT.

- Curso Internacional de Agroclimatología Tropical. Corpoica, June 5-10, 2003, Bogotá, Colombia.

- International workshop on no-tillage agriculture on tropical lowlands. Bogotá, Procitropicos, June 10, 2003.

- Taller Nacional de la Sociedad Colombiana de Ingeniería (ACIEM) sobre el mecanismo de desarrollo limpio (DML) del protocolo de Kyoto. Cali, May 10, 2003.

- Encuentro Regional de ONG Ambientalistas del Valle del Cauca. Cali, Colombia, August 22, 2003.

Resource Mobilization:

Proposals being funded or approved:
Unravelling the mysteries of the Quesungual slash and much agroforestry system. 3-year project (2004-2006) grant from the CGIAR Water Challenge Program. Led by PE2 in collaboration with PE6, MIS Consortium, Universidad Nacional de Honduras, ESNACIFOR-Honduras, FAO.
Total pledge: US$ 650.000

Bean genomics for improved drought tolerance in Latin America. 3-year project (2003-2006) approved to CIAT bean project, funded by BMZ-GTZ, Bonn, Germany.
Total pledge: 740,000 (Euros)

Environmental externalities to promote sustainable development of rural communities in the Andes. 5-year project funded by GTZ Germany to CONDESAN in collaboration with a large number of partner Institutions in Colombia, Ecuador, Peru, Venezuela and Bolivia.

The forage potential of tanniniferous legumes: 3-year project funded by SDC – ZIL Switzerland to IP5 in collaboration with ETH, ILRI, and Universidad Nacional de Colombia.
Total pledge: US$ 221,000.

Proposals Submitted
Nitrification Inhibition by tropical grasses as tools to improve nitrogen use efficiency and reduce nitrous oxide emissions. Submitted to the Water Challenge program and to the New energy and Industrial technology development Organization (NEDO, Japan). Proposal were not approved.

Farming Futures: Submitted to the Water Challenge program. Proposal was not approved.

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University of Quebec-Canada: Marc Luccotte
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Wageningen University-Netherlands: Peter Buurman

List of Publications

Refereed journal articles:

Carol M. Schwendener, Johannes Lehmann, Marco Rondón, Elisa V. Wandelli and Erick C.M. Fernandes. Soil mineral N dynamics beneath mixtures of leaves from legume and fruit trees in Central Amazonian multi-strata agroforests. *Acta Amazonica*. Special LBA Issue (in press).

Christienne N. Pereira, Erick C.M. Fernandes, Johannes Lehmann, Marco Rondón, Flavio J. Luizão. Agroforestry trees increase phosphorus availability in an Oxisol of the Brazilian Humid Tropics. *Acta Amazonica*. Special LBA Issue (in press).

Cajas-Girón YS and Sinclair FL (2001) Characterisation of multistrata silvopastoral systems on seasonally dry pasture in the Caribbean region of Colombia. *Agroforestry Systems* 53:215-225.

Feldpausch, Ted, M. Rondón, E. Fernández, S. Riha, E. Wandelli, 2003. Carbon and nutrient accumulation in secondary forest regenerating from degraded pastures in Central Amazonia. *J. Ecol. Applic.*

Fisher, M.J. and Thomas, R.J. (2003). Case studies of land use changes in the central lowlands of tropical South America. *Environment, Development and Sustainability* (in press).

Hess D., Kreuser M., Diat T., Lascano C., Carulla J., Soliva C. Machmuller A. (2003) Saponin rich tropical fruits affect fermentation and methanogenesis in faunated and defaunated rumen fluid. *Animal Feed Science and technology* 109:79-94.

Hess D., Monsalve L., Lascano C., Carulla J., Diaz T., Kreuzer M. (2003) Supplementation of a tropical grass diet with forage legumes and *Sapindus saponaria* fruits: efeccts of *in vitro* ruminal nitrogen turnover and methanogenesis. *Australian Journal of Agricultural Research* 54: 703-713.

Ishikawa T., G.V. Subbarao, O. Ito, and K. Okada. 2003. Suppression of nitrification and nitrous oxide emission from soil by a tropical grass, *Brachiaria humidicola*. *Plant and Soil* (in press).

Jarvis A., A. Ferguson. D. Williams, L. Guarino, P. Jones, T. Stalker, J. Valls, T. Pittman, C. Simpson, P. Bramel. (2003). Biogeography of wild *Arachi:* Assessing conservation status and setting future priorities. *Crop Sci.* 43:1100-1108.

Jones, P.G. and P.K. Thornton (2003). The potential impacts of climate change on maize production in Africa and Latin America in 2055. *Global Environmental Change* 13: 51-59.

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Rao, I. and G. Cramer. 2003. Plant nutrition and crop improvement in adverse soil conditions. In: M. Chrispeels and D. Sadava (eds). *Plants, Genes, and Crop Biotechnology*. Published in partnership with the American Society of Plant Biologists and ASPB Education Foundation. Jones and Bartlett Publishers, Sudbury, Massachusetts, USA, pp 270-303.

Rychter, A.M. and I.M. Rao. 2003. Role of phosphorus in photosynthetic carbon metabolism. In: M. Pessarakli (ed). Handbook of Photosynthesis. 2nd Edition. Marcel Dekker, Inc., New York (in press).

R.J. Thomas, M. Rondón, E. Amézquita, D.K. Friessen. Overcoming Soil Constraints in Latin American Savannas: New Approaches and Potential Trade-offs. In: Agropastoral Systems for Tropical Savannas in Latin America: E. Guimaraes; J.I. Sanz; I.M. Rao; M.C. Amézquita, E. Amézquita (Editors). CIAT, in press.

Conference proceedings:

Amede, T., E. Amézquita, J. Ashby, M. Ayarza, E. Barrios, A. Bationo, S. Beebe, A. Bellotti, M.Blair, R. Delve, S. Fujisaka, R. Howeler, N. Johnson, S. Kaaria, S. Kelemu, P. Kerridge, R. Kirkby, C. Lascano, R. Lefroy, G. Mahuku, H. Murwira, T. Obertur, D. Pachico, M. Peters, J. Ramisch, I.M. Rao, M. Rondón, P. Sanginga, M. Swift and B. Vanlauwe. 2002. Biological nitrogen fixation: A key input to integrated soil fertility management in the tropics. Position paper by CIAT-TSBF Working Group on BNF-CP for " International Workshop on Biological Nitrogen Fixation for Increased Crop Productivity, Enhanced Human Health and Sustained Soil Fertility". ENSA-INRA, Montpellier, France (10-14 June, 2002).

Hess D., Monsalve L., Lascano C., Carulla J., Diaz T., Kreuzer M. (2003) Potential of forage legumes and of saponin containing fruits as tropical feed resources to manipulate rumen fermentation and to improve ruminant nutrition. The Sixth International Symposium on the Nutrition of Hervibores, October 17–24, 2003. Yucatán, México.

Subbarao G.V., Nakahara K., Ishikawa T., Ito O., and Okada K. 2003. The Biological Phenomenon of Nitrification Inhibition in *Brachiaria humidicola* – Possible Mechanisms and Active Compounds. Soil Science and Plant Nutrition Annual Meeting, Vol. 59, Abstract No.9-58; August, 2003 at Yokohama, Japan.

Ishikawa T., Subbarao G.V., Okada K, Ito O. 2003. Nitrogen status and plant growth stage influence nitrification inhibitory activity of root exudates in *Brachiaria humidicola*. Soil Science and Plant Nutrition Annual Meeting, Vol. 59, Abstract No. 9-57; August 2003 at Yokohama, Japan.

Non-refereed conference presentations:

Beebe S., I.M. Rao, H. Terán and C. Cajiao. 2003. Breeding concepts and approaches in food legumes: The example of common bean. Invited paper presented at the "Second National Workshop on Food and Forage Legumes". Addis Ababa, Ethiopia. 22-27 September, 2003 (in press).

Beebe S., Rao I.M., Terán H., Cajiao C., Ricaurte J., and Beltrán J. 2003. Progreso en Aumentar Tolerancia a Estrés Abiótico en Frijol Común. Paper presented in the XLIX Meeting of the PCCMCA, Programa Cooperativo Centroamericano de Mejoramiento de Cultivos y Animales. La Ceiba, Honduras.April 27-May 3, 2003.

Fisher, M. Case studies of land use changes in the central lowlands of tropical South America. Internal CIAT Seminar, October 2003.

Ramírez, J.A. Contribución de las actividades antropogénicas a las emisiones globales de gases de efecto invernadero. Seminar presented at the Encuentro Regional de ONG Ambientalistas del Valle del Cauca. Cali, Colombia, August 22, 2003.

Rondón, M. Ramírez, J.A. The challenge of climate change to small scale farmers in Colombia. Paper presented at the Curso Internacional de Agroclimatología Tropical. Corpoica, July 5-10, 2003, Bogotá, Colombia.

Rondón, M. Oportunidades para mitigar la acumulación de gases de efecto invernadero en la atmósfera mediante intensificación agrícola y ganadera en las sabanas de Latinoamérica. Paper presented at the International workshop on No tillage agriculture on tropical lowlands. Bogotá, Procitrópicos, June 10, 2003.

Rondón, M. Oportunidades en los sectores agrícola y forestal para contribuir a la reducción de emisiones de gases de efecto invernadero mediante proyectos MDL. Taller Nacional de la Sociedad Colombiana de Ingenieria (ACIEM) sobre el mecanismo de desarrollo limpio (DML) del protocolo de Kyoto. Cali, May 10, 2003.

Rondón, M. Nitrification inhibition by root exudates of *B. humidicola* in soils. Seminars presented on September 28th and October 1st. at The Japan National Institute for Agroenvironmental Studies (NIAES) and at JIRCAS. Tsukuba, Japan.

List of Acronims

CATIE:	Centro Agronómico de Investigación y Enseñanza, Turrialba, Costa Rica
CDM:	Clean Development Mechanism
CIPAV:	Centro para la Investigación en Sistemas Sostenibles, Colombia
CONDENSAN:	Consorcio para el Desarrollo Sostenible de la Ecoregión Andina, Colombia
CORPOICA:	Corporación Colombiana de Investigaciones Agrícolas
EMBRAPA:	Empresa Brasilera de Pesquisa Agropecuaria, Brazil
ETH:	Institut for Plant Scince, Zurich
GHG:	Greenhouse Gases
GWP:	Global Warming Potential
GWP:	Global Warming Potential
IDEAM:	Instituto de Hidrología, Meteorología y Estudios Ambientales, Bogotá, Colombia
INPA:	Instituto Nacional de Pesquisa na Amazonia. Manaus, Brazil
LBA:	Large Scale Biosphere-Atmosphere Experiment in the Amazon, Brazil
MIS:	Integrated Soil Management. CIAT, Honduras