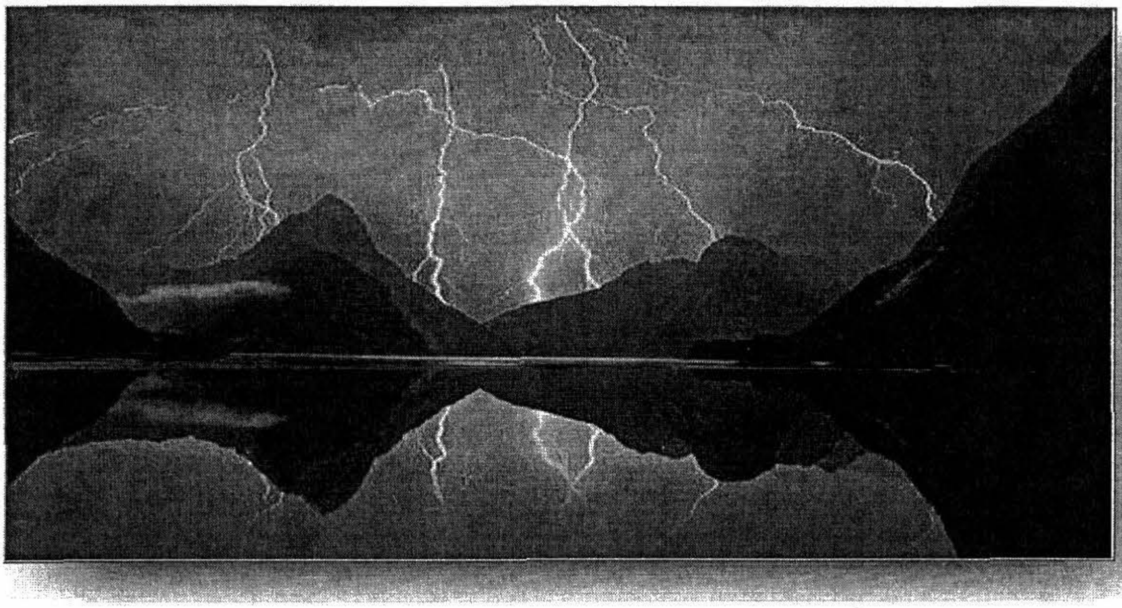
PROJECT PE-6

Confronting Global Climate Change



ANNUAL REPORT
2002

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

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Project PE-6: Confronting Global Climate Change

1. Project Description

Objective: In ecoregions within the scope of CIAT's mandate, to overcome expected reductions in productivity of some major food crops and forages as a consequence of climate change (CC), while reducing the environmental impact from agriculture.

Outputs:

1. Vulnerability and opportunity assessments of responses made by ecoregions, populations, crops and crop wild relatives 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 Greenhouse gases (GHG).
4. Impact of implemented strategies for adaptation to and mitigation of CC assessed, and institutional capacity enhanced.

Gains:

* *Plant breeders* and *agronomists* will have access to realistic and detailed definitions of the climates that will be encountered in response to CC.
* *Farmers* and *consumers* of CGIAR-mandated and other food crops will have varieties adapted to marked changes in temperature and drought conditions.
* *Farmers* benefit from informed decision capacity and sustainable systems that minimize GHG emissions (CO2, CH4, and N2O) and maximize carbon sequestration for international carbon trading.
* *Policy makers* will have information of the extent of CC on the performance of CGIAR-mandated and other food crops and possible changes required to confront it and avoid wide-spread land degradation.
* *National governments* will have more accurate information on emissions of GHG's by sources and removal by sinks for incorporation in their annual inventories under the Article 7 of the Kyoto protocol.

Milestones:

- Maps of expected changes in maize yields in response to climate change for Latin America and Africa. Alliances developed with CORPOICA, CONDESAN, MIS, FAO Lempira-Sur, CATIE, JIRCAS, Ministry of the Environment, Colombia and Honduras. Two workshops held on CC issues in Central America (CA) and Colombia. Definition of CIAT activities within the Challenge program on CC.
- - Advanced versions of FloraMap and MarkSim. Preliminary assessment of bean and cassava yields for CA and Africa. Definition of areas in urgent need to preserve wild relatives of beans. Advanced lines of drought tolerant beans available to NARS for CA. Rank of tropical grasses by their nitrification inhibition potential (greenhouse studies) Global Warming Potential (GWP) estimated for the Llanos. Preliminary estimate of GWP for the Quesungual reference site, Honduras.
- Current and expected distribution of Macrophomina in Latin America. Definition of areas to prioritize for conservation of wild relatives of cassava, release of *Briachiaria* hybrids tolerant to drought. Assessment of net reductions in N2O emissions due to *B. humidicola*: Case studies in Central America and Colombian Llanos.
- Implemented pilot project to trade C from agroforestry systems (AFS) in Colombia. Environmental Characterization of intensive high input cattle production systems: Case studies in Colombia and Central America.

Users: The immediate beneficiaries are farmers that grow CGIAR-mandated crops and the people that consume them, especially poor farmers in developing regions. Policy makers will use the information on predicted changes in climate to plan land use and to include environmental services as part of the development agenda for selected regions.

CGIAR system linkages: Enhancement and Breeding (17.5%); Crop Production Systems (20%); Livestock (20%); Protecting the Environment (35%); Training (2.5%); Information (2.5%); Networks (2.5%). Participation in the Inter-Center Working Group on Climate Change.

Collaborators: NARS and National Research Centers: Brazil (EMBRAPA, INPE, INPA); Colombia (CORPOICA, Instituto Von Humboldt, Universidad Nacional, Ministerio del Ambiente, IDEAM), Central America (Universidad de Honduras, Ministerio del Ambiente); CGIAR Centers: ILRI; CIMMYT, ICRAF. Advanced Research institutions: (Cornell University, Univeristy of Florida, USA; University of Zurich, Switzerland, Bayreuth University, Germany); Climate change modelers (e.g., Hadley Climate Center, UK; NCAR, US); international and local NGOs; farmers' and community organizations. Ongoing regional projects: Large Biosphere-Atmosphere Experiment in the Amazon (LBA).

CIAT project linkages: IP1, IP3, PE1, PE2, PE4, PE5, SN2, SN3.

Project : Confronting Global Climate Change for the Benefit of Tropical Agriculture.

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 to the 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, 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 assessment developed of the response of critical ecoregions, populations, crops and crop wild relatives to 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 GHG. 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 crop wild relatives. 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, 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 (Savannas) and no-tillage cropping systems (Hillsides). 1 B.Sc. and 2 MSc. thesis 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. Successful involvement of a suitable partners, experienced in C trading.

Introduction

The Climate Change (CC) Project was approved by the Board of Trustees to be initiated as a new CIAT project during 2002. During this initial year, the project has operated on the basis of collaborative linkages with various existing projects within CIAT (Land Use, Beans, Forages, Soils) that have being carrying CC related activities during the past few years. In this report, we present an overview of the progress made on the main research topics developed by the researchers associated with the project. Some of the activities, particularly those in the mitigation theme are new initiatives while others are continued from previous years.

During this initial year a good amount of effort was dedicated to establish links with potential partner institutions and groups, as well as to promote both within and outside CIAT the topic of Climate Change. CIAT was very active in the definition of a National Policy for Climate Change, lead by the Colombian Vicepresidency. During the month of April, the whole month seminar series was dedicated to the issue of climate change. Ample audiences were informed on various of the main themes within the scope of the CC research agenda: Assessment of the impact that changes on the climate will have of the main crops and cropping regions of the tropics; adaptation alternatives to reduce the impact of CC and mitigation options to reduce emissions of various greenhouse gases (GHG) associated with Agriculture and opportunities to enhance the CO_2 sequestration by soils and biomass. The final talk of the series was dedicated to the status of the international negotiations to implement the Kyoto protocol and the possibilities to include agriculture and agroforestry projects within the Clean Development Mechanism (CDM).

CIAT was deeply involved in the preparation of the project pre-proposal for the Climate Change Challenge Program. The final decision about the approval of the second round of challenge programs will be known towards the end of this year. We are confident that the CC-CP would be a very important source of funding for several of the proposed research activities of the project. Nevertheless, efforts will be dedicated in 2003 to seek alternative sources of funding.

In September, the Chemist Juan A. Ramírez, was appointed as research assistant to be responsible for the analytical facilities of the project, particularly on Gas Chromatography and also to help on laboratory, glasshouse and field experiments for the mitigation sub-theme.

Next year we will see the start of collaborative projects with several partners: CORPOICA, CONDESAN, Universidad Nacional, CIPAV, CONIF, CENICAFE in Colombia and Universities Such as Cornell and New Hamphire, USA and Wageningen, Netherlands.

We wish to sincerely thank many colleagues at CIAT and other partner institution for the effective collaboration received during this initial year.

Main Highlights of Research Progress in 2002

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

- Suitable habitats for wild varieties of beans will be reduced drastically in Panama (100%), Honduras (99%), Nicaragua (93%), El Salvador (87%)

- Results of the final runs of the trial modelling of maize for Latin America and Africa show that in few areas yields would increase. In many areas a mild yield decrease is expected but in others major yield decreases could be 10%, or $2,000,000,000 worth of maize crop.

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

- Nearly 600 F3-derived F5 families of common beans were evaluated for drought, of which 138 were selected for drought tolerance in combination with commercial grain type and disease resistance.

- Showed 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 greater levels of nonstructural carbohydrates in shoot tissue at mid-podfilling and efficient utilization of acquired nitrogen and phosphorus for grain production.

- Showed that the superior dry season performance of the germplasm accession of *Brachiaria* CIAT 26110 which maintained greater proportion of green leaves during moderate dry season in the Llanos of Colombia was associated with greater acquisition of nutrients from drying soil.

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

- For the first time confirmed the phenomenon of nitrification inhibition by *Brachiaria humidicola* using refined bioassay methodology and root exudates from plants grown in the hydroponics and in the field.

- Accounting all the main sources and sinks of trace gases, it was estimated that the Llanos is a net source of 0.164 Tg CH_4/y and 0.021 Tg N_2O/y. For a 20-year time horizon, the global warming potential of the Llanos under current land use distribution is 22.2 Tg CO_2 equivalents which is less than 0.005% of estimated global planetary radiative contribution.

- Tropical Secondary forest (SF) regrowing on lightly to moderately-used pasture in the Central Amazon, rapidly sequester C and rebuild total nutrient capital following pasture abandonment.

- Charcoal derived-C can account for as much as 15% of total soil C in soils of the Central Amazon and needs to be determined to allow a proper comparison of the effect of land use change on SOC.

- The slash-and-char technique is an alternative to burning of the above ground biomass. Given that the charcoal-C has very long residence times in the soil, this opens new possibilities to enhance C sequestration in soils in areas where burning is a common management practice.

- The fruit *S. saponaria* suppressed in vitro methane release by over 10% with grass-alone diets as well as from grass-legume diets. However, ciliate protozoa were only reduced by *S. saponaria* in the grass-legume diets, while the addition of legumes with high levels of tannins reduced methane release per unit of organic matter fermented

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

- Two practical training courses were held at Monteria and Florencia, Colombia on methodologies to assess C and nutrient stocks in agroforestry systems.

- Two technicians and one researcher from Corpoica and one researcher from the Universidad de Cordoba were trained on methodologies to sample gases for measurement of fluxes of GHG between the soil and the atmosphere.

- 1 Ph.D thesis ongoing on drought stress on beans, 1 BSc Thesis initiated on effect of diets to reduce methane emission by cattle and 1 BSc, thesis initiated on assessing the GWP of intensive cattle production systems in Colombia.

- One International workshop was held to advance the preparation of the Amazon Initiative proposal. It had representatives from Embrapa, CIAT, The World Bank, ICRAF, LBA and University of Quebec.

- Two meeting, were held, one in Brazil and one in Colombia to present the Amazon Initiative to potential partner institutions. It included representatives from 20 institutions in Brazil and 15 Institutions in Colombia.

Progress Report 2002

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

Activity 1.1 Definition of critical wild relatives of crops and populations requiring urgent conservation actions

Activity 1.1.1 – Wild beans and Climate Change

Contributors: Peter G. Jones (PE4), ~~Steve~~ Beebe (IP1) stephen 104975

Rationale

Because of the founder effect on domestication many genes have been left behind in wild populations of the progenitors of modern field crops. The Arcelin genes which confers resistance to grain damaging Bruchids in *Phaeolus vulgaris* is a prime example. Transfer of these genes from wild populations to modern cultivars has been achieved in a landmark collaboration between the University of Wisconsin and CIAT and is potentially worth over $1.2 Billion in prevented crop losses.

It is obviously worth preserving such potentially valuable genes and a good way of doing this is by conserving natural populations *in situ.* However the Central America wild bean populations occupy relatively small niches in the highlands. We therefore decided to look at the effect of climate change on the future stability of these populations.

Materials and methods:

A 30 arc second climate grid was created using the CIAT climate database and the digital elevation model GTOPO30. The 50 year climate change predictions were taken from the published data of the HADCM2 general circulation model, interpolated to 30 arc seconds and grafted onto the climate grid to give a predicted climate surface for the years centred on 2055.

We took 40 collected samples of wild *P.vulgaris* from the CIAT germplasm bank and four sample collection points from a collecting trip made by S. Beebe in 1996 as the calibration set and fitted a climate probability model to the present and future grids using FloraMap.

These results were overlaid with a re-classed version of the USGS land cover at 1km precision and the potential conservation areas extracted by land cover class.

Results:

Figure 1 shows the probable distribution of wild *P. vulgaris* at present and in the year 2055. We can see that there remain viable areas in southern Mexico although some are geographically removed from the present areas and intervention might be needed to assist populations to shift as the climate changes over the next 50 years.

In Guatemala there does not appear to be that much of a problem as the future areas are geographically contiguous with the present ones. However the areas in El Salvador, Honduras, Guatemala and Costa Rica are much diminished.

The USGS land cover was re-classed to 'appropriate' lands including crop and pasture lands and wild scrub land and 'inappropriate' including closed forest, urban areas and water bodies. The probable conservation areas were extracted by tese classes. The results are shown in Table 1.

Figure 1. Potential distribution of wild *P. vulgaris* in Central America

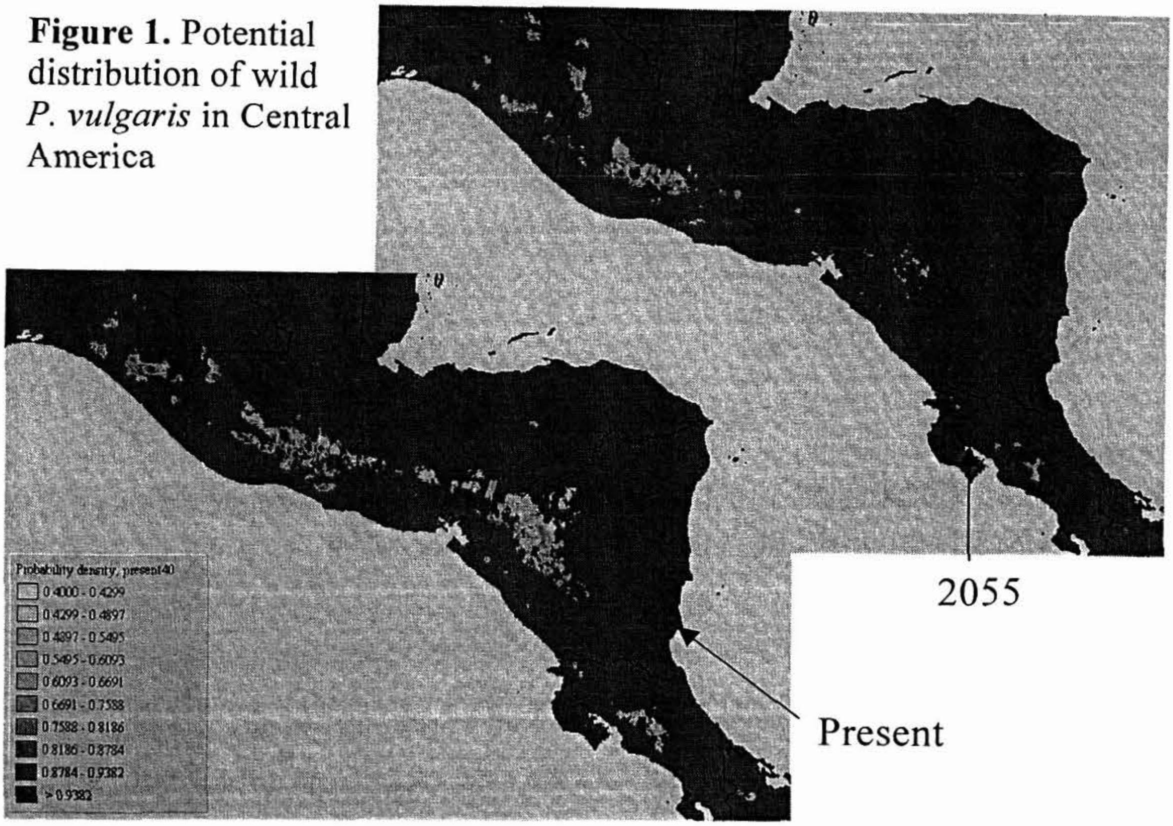

Table 1. Area of potential *in situ* conservation for *Phaseolus vulgaris* in Central America.

Country	Present		Year 2055		Percentage reduction of appropriate
	Appropriate km^2	Inappropriate km^2	Appropriate km^2	Inappropriate km^2	
Mexico[1/]	61542	84708	33696	92430	45
Guatemala	200538	149760	140634	130572	30
El Salvador	17550	42354	2340	14040	87
Honduras	92664	49608	1170	4212	99
Nicaragua	270738	106236	20124	10296	93
Costa Rica	22230	59904	8658	22698	61
Panama	4212	0	0	0	100

1/ Only that part of Mexico to 18° N 94° W.

When the area of appropriate land in each of the potential conservation areas is taken into account we can see that in El Salvador, Honduras, Nicaragua and Panama the sites suitable for *in situ* conservation almost disappear. If the genetic constitutions of these populations are highly similar to those of the Guatemalan ones then world agriculture will lose little and the problem is merely a national one for those countries.

If there are marked differences than it is a problem for everyone. The only solutions may be *ex situ* conservation in germplasm banks, or transfer to *in situ* sites in other countries. Either of these solutions may present problems under the present legal situation. Following the resolutions under Agenda 21 of Rio 1992 the native germplasm of each country is the property of that country and without the correct legal measures in place cannot be taken out of the country. To our knowledge these have not been enacted and the samples collected by S. Beebe in Honduras in 1996 are still in Honduras.

Steps should therefore be taken to check the genetic constitution of the populations and to encourage the legal steps to be taken that will enable the relevant solutions.

Outputs:
This work was presented as 'Jones PG., Beebe S. (2001) Predicting the Impact of Climate Change on the Distribution of Plant Genetic Resources in Wild Common Bean (*Phaseolus Vulgaris* L.) in Central America', at the III International Conference on Geospatial Information in Agriculture and Forestry. Denver Colorado Nov. 2001. It won the award as best paper of the conference.

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.

Activity 1.2 Development of maps of probabilities of crop yields under future climate scenarios

Activitiy 1.2.1 Farming Futures. Modelling the effects of Climate Change on agriculture.

Contributors: P.G. Jones (CIAT), P.K.Thornton (ILRI); G. Hyman (CIAT).
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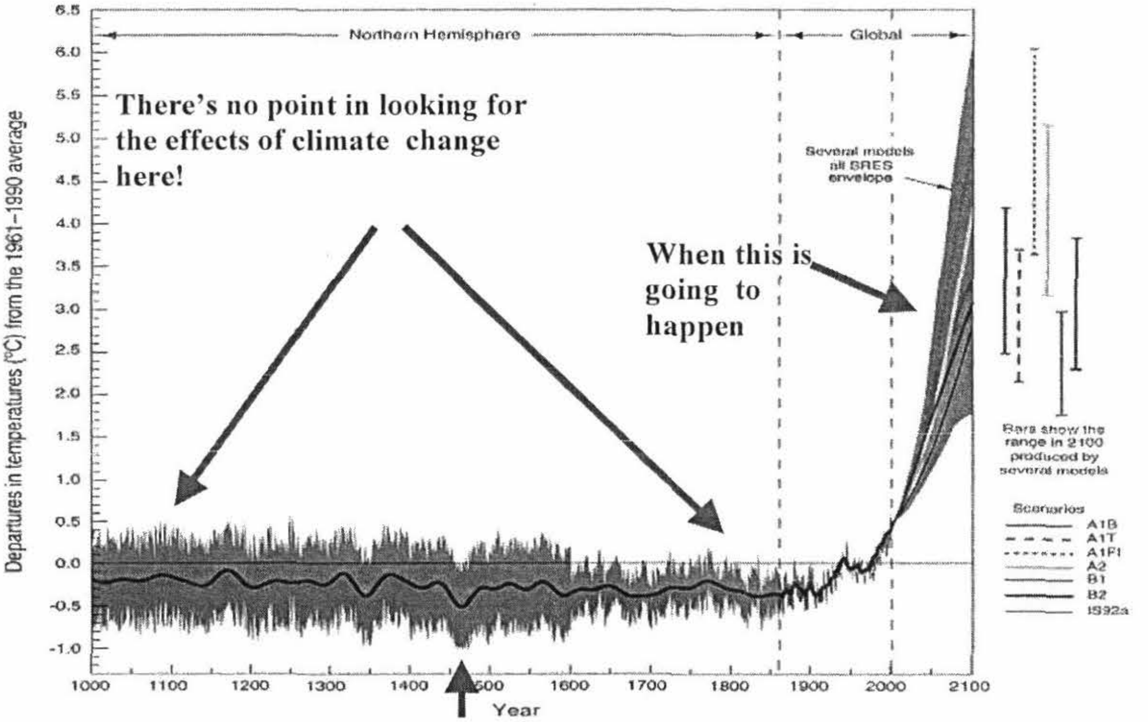
Rationale
Global warming is coming as can be seen in Figure 2; there is now no question about it. The consequences are still to be determined in detail, but agriculture will undoubtedly be changed over the next 20 years.

Table 2. Variation in the earth's surface temperature, 1000 to 1000 to 1861 N. Hemisphere, proxy data; 1861 to 2000 Global, instrumental; 2000 to 2100, SRES projections



Maunder minimum. Little Ice Age. Ice fairs on the Thames

Agricultural research has a long lead-time. A recent breakthrough in drought tolerance for beans at CIAT, although not costing more than a few million dollars, took nearly 25 years to deliver the goods. This means that scarce research resources have to be targeted with great care. This has never been easy, but with the moving goalposts of Global Climate Change upon us, the job has become much more difficult. We need to know what will be needed, and where it will be needed, **now**, to plan the next 25 years of research.

We are obviously in an area where experience is of little use beyond our knowledge of the theory of plant growth and development. We have never seen the sort of changes that are coming and so we are reduced to guessing or modelling. Guessing is never a good idea.

Methods.

We must resort to modelling the system. This is fraught with problems, but it can be done. We have shown (Jones and Thornton, 2002) that crop process models can be linked to the output from Global Circulation Models (GCMs) to estimate the effects of climate change on agriculture in the tropics. We have now extended those studies to include an assessment of potential maize yields in the year 2055 for Africa and Latin America. We used the outputs of the HADCM2 model and interpolated the changes in climate onto the CIAT climate grids. Simulated weather was then generated using MarkSim. For each of 20 simulated years the model Ceres-maize was used to estimate yield.

Results.

The results show three main scenarios with which we will be dealing:

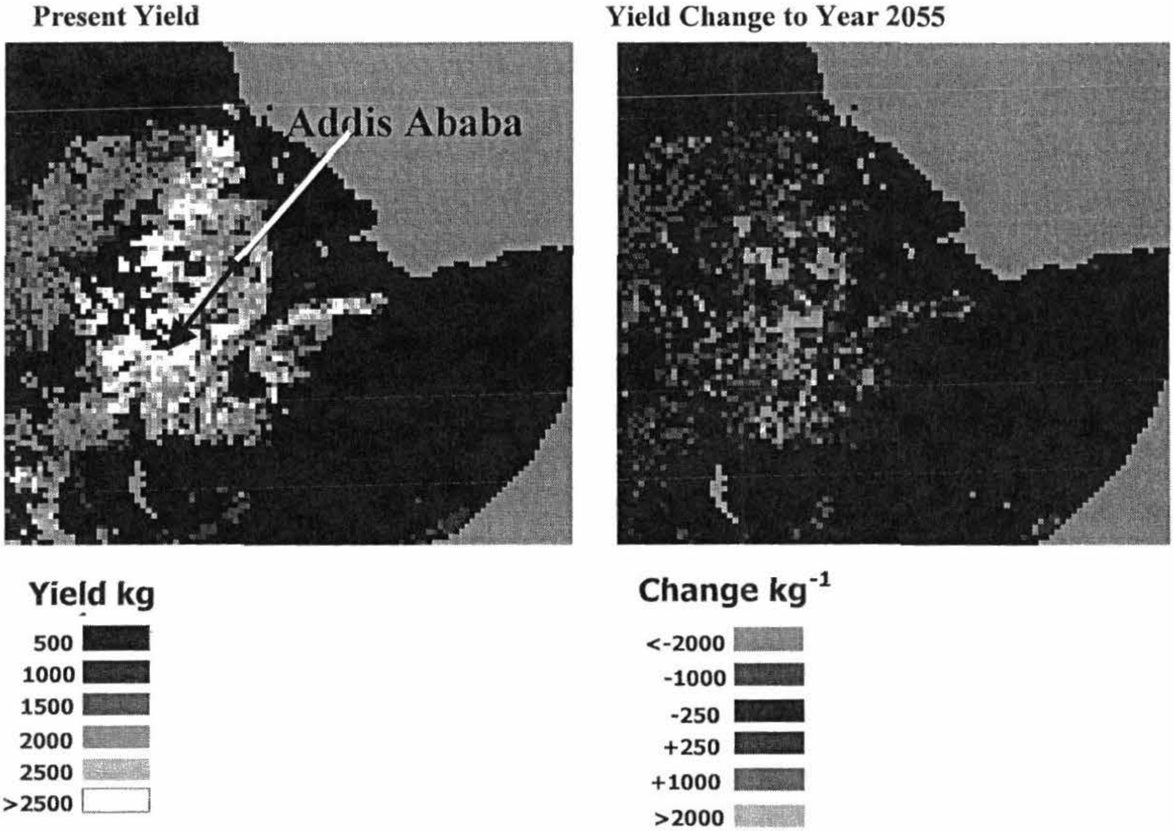
1. There will be areas (particularly in the highlands) where some crop yields may improve It is imperative that research in these areas should make the maximum use of any potential gains, because there will not be many places where this will apply.
2. Quite large areas may show minor changes in yield potential, but will require differently adapted varieties and probably some shifts in crop mixes and agricultural practices. These cases will require carefully planned research in cultivar development and farming practices.
3. A significant number of areas will require major intervention because of potential complete crop failure. Major changes in the agricultural economy of these areas will be needed. Agricultural technology may be unable to solve these problems, but farming systems research may be able to warn decision makers of the possible consequences.

Maize (the test case we decided to look at) is a C_4 plant probably better able to stand up to rising temperatures than most other crops. We chose it because we know the model used has been in use for about 30 years and has been proved in most environments. Here are some examples of what we found:

The first is some good news for Ethiopia. It is not often that happens! Ethiopia is not a major, maize-growing nation, but the indications show that cereal growth with C_4 plants such as teff could be improved in quite a few areas. You will note, however, from the maps below that local decompensations will occur. This is a typical case of the class 1 climate change problem. There are benefits to be had, but the agriculture of the area will have to be rebalanced to gain the benefits. Of course, the actual crops of the area have to be modelled under GCM conditions to assess the true situation (Figure 3).

A typical class-2 case can be found in Brazil. Here we have a mosaic of minor yield decreases associated with local increases in yield potential. This is obviously of immense importance to the farmers of the region, but could be handled with judicious application of agricultural science without major catastrophe. Knowing just what new varieties will be needed will help to capitalise on the benefits and reduce the possible losses (Figure 4).

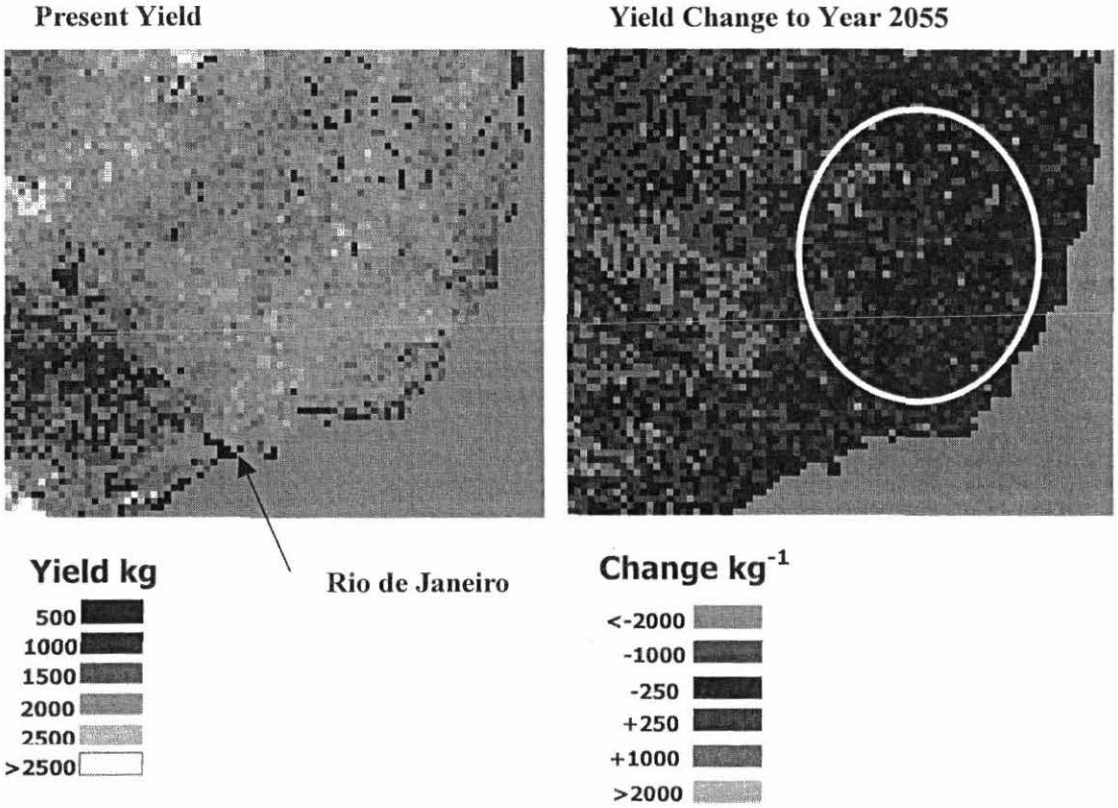
Figure 3.



Present Yield

Yield Change to Year 2055

Figure 4

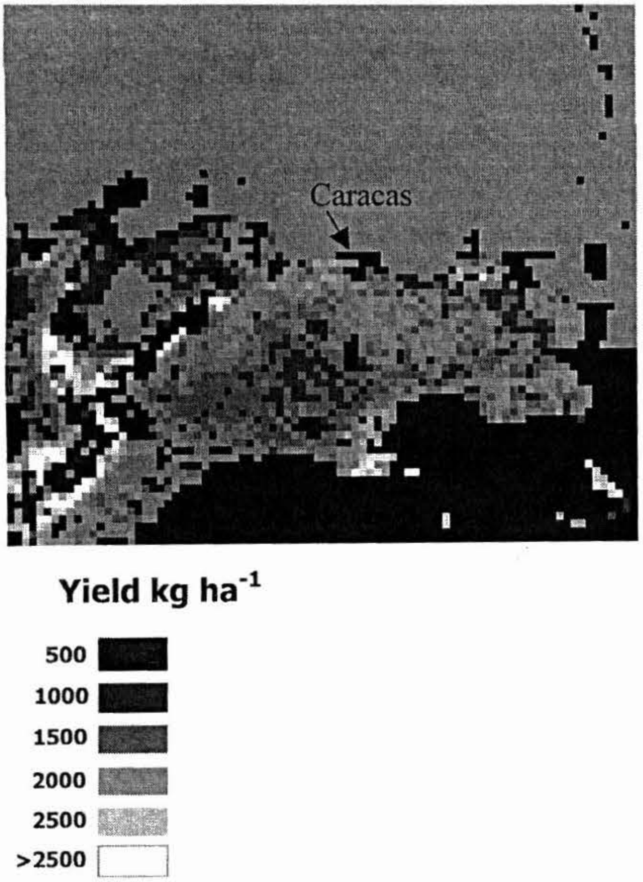


Present Yield

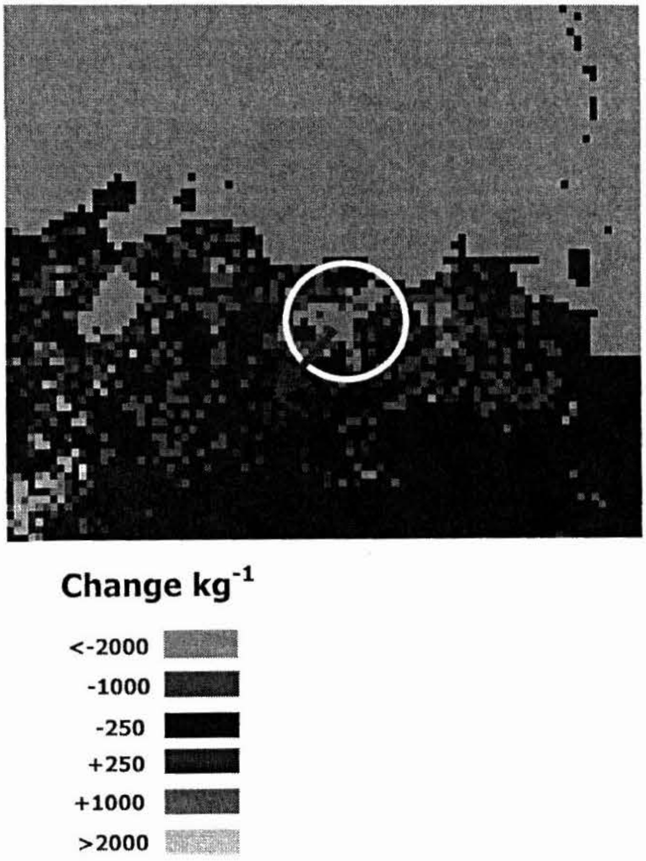
Yield Change to Year 2055

This dry area in Venezuela (see below) exemplifies the class three situations. When the markets have been right this has been a major maize producing area. It is dry, mainly scrubland, but can produce well under certain circumstances. The change in wind patterns will mean that it becomes much drier and will probably have to go back to extensive grazing. The crop production of Venezuela does not look like a happy prospect in the next 50 years, and for rainfed crops like maize it would appear that a major shift might be indicated. Venezuela, of course, has much irrigable land and so this may not be a major problem if the social implications are dealt with appropriately.

Present Yield



Yield Change to Year 2055



Outputs.

These examples show some of the complexity of the problem we are facing. Planning for agricultural research under these conditions needs pre-knowledge. We have shown that we can do this with one crop, but we need to do much more, The C_3 crops behave completely differently, and varietal adaptation is the key to successful agriculture.

These results have been used to develop project proposals. The major one entitled Farming Futures is included in the Climate Change Global Challenge Program (CCGCP) and would involve a budget of $M2.6 over 4 years. A cut down version of this to try to classify the areas showing different response types would take minimal funding and take a very quick look at the situation to assist in the planning for the CCGCP.

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

Activity 2.1 Development of new crops and forage varieties adapted to drought and heat stress

Activitiy 2.1.1 – Development and testing of segregating populations combining drought tolerance and disease resistance in small red and small black grain types of common bean

Contributors: S, Beebe, I.M. Rao, H. Terán, C.Cajiao (IP-1); C, Quintero, J. Tohme, (SB-2)

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: *F5 families:* Last year we reported on positive results with F2 populations which 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, planting successively the F3 generation in Popayán (under anthracnose inoculation and moderate fertility stress) and the F4 generation in Santander de Quilichao (under inoculation for angular leaf spot - ALS, and in moderate fertility stress). Individual plants were selected in F3, to evaluate and harvest families in F4 and perform yield trials in F5. Almost 600 families were so evaluated in five independent lattice designs of two replications each. Two trials were composed of red-seeded beans, two of black seeded beans, and a fifth of other colors. Trials were planted on-station at CIAT-Palmira in June, 2002, receiving only two irrigations, amounting to about 50 mm water. An additional 37 mm fell as rainfall during the crop cycle but the crop received no additional moisture from 30 days after planting until harvest. Common checks were included across trials: red seeded commercial cv. Tio Canela; black seeded commercial cv. DOR 390; tolerant SEA 5; and an African cowpea cultivar bred for stress conditions, Mouride.

Families also were sampled for the presence of two DNA markers for resistance to the BGYMV virus: bgm-1 and W12. With the expectation that families could be heterogeneous or segregating, four plants per family were sampled.

Additional crosses: Based upon results with F2 populations in CIAT-Palmira in 2001, superior parental materials were identified. SEA lines 15 and 21; G21212; RAB lines 608, 609, 651, and 623 performed especially well as parents. Thus, in September-2001, additional crosses were created with these parents. In 2002 some 3000 F1-derived F2 families were evaluated under drought in the same season and under the same conditions as the F5 families.

Evaluation in Central America: After the experience in 2001, about 200 F1-derived F2 families were identified as especially promising. These were increased in September, 2001 and shipped as F4 families to NARS in El Salvador and Nicaragua, as well as the Hillsides Project in Nicaragua and Honduras and the Panamerican School in Zamorano.

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 taken in the sixth week after the last irrigation (ie, in the ninth week of crop growth, or during pod-fill) registered more than 700 millibars water tension in the soil, when normally irrigations are carried out when soil water tension reaches 230 millibars.

Drought stress was slightly less severe than in 2001 due to the rainfall that occurred as the crop was approaching flowering. As a result, some materials with an intermediate reaction to drought such as Tio Canela produced an acceptable pod load. However, as the season advanced, the stress resulted in severe limitations on grain filling.

F5 families: Results of the five yield trials are presented in Tables 1a through 1e. Tio Canela produced from 173 to 1042 kg ha^{-1} in the five trials, although even when total production was acceptable, quality of grain was poor and marketable grain was much less. DOR 390 was poor yielding and produced poor quality grain in all trials (from 173 to 619 kg ha^{-1}. Thus, checks varied widely across trials, reflecting spatial variability in the field.

Selected families in the trials of red seeded beans yielded very well, as high as 1515 kg ha^{-1} and significantly more than all common bean checks. In all trials except one, families that yielded as well as the cowpea variety were identified. This is surprising since cowpea is considered to be especially tolerant to drought. However, the most striking aspect of these trials was the excellent grain quality that many families presented, in spite of the intense drought pressure during the grain fill stage. All of the 138 selected families produced a very high percentage of commercially acceptable grain, and some produced grain in the drought treatment that was superior to the quality of the commercial checks under normal conditions. This was in striking contrast to the commercial checks and represents an advantage not only in amount of marketable grain, but in labor saved in selecting grain, and also in seed quality for subsequent plantings.

The families in the black seeded trials did not show as wide an advantage over Tio Canela as did the red seeded families, but still yielded far better than the black seeded commercial check, DOR 390, and again with excellent grain quality. A few that were derived from ICTA Ligero were also early maturing.

The parents that produced the best progeny were G21212 and SEA 15, and the combination of these two parents gave especially tolerant materials with excellent grain quality. Both parents present excellent grain filling under drought stress, and may have complementary genes that combine to give superior grain filling.

Families that were selected for Central America carry either the bgm-1 or the W12 markers for BGYMV resistance. Additionally, several families presented resistance to the ALS pathogen in inoculations in Santander de Quilichao. G21212 proved to be resistant to races at that site, and was the principal source of resistance. This is another important advancement in these lines.

Additional crosses: A second set of F2 populations that was initiated a year ago presented very acceptable levels of tolerance, apparently better on average that the first set of F2 populations that were selected last year. The superior performance of the second set was undoubtedly due to the identification of the best parents in 2001 and the greater use of these in additional crosses.

Evaluation in Central America: In most sites in Central America there was no significant drought pressure, except in El Salvador where drought was severe and soil structure was unfavorable to the crop. At all sites the most promising crosses were (RAB 623 x SEA 21) x EAP 9653-16B, and (RAB 609 x SEA 21) x DOR 582, including at that site in El Salvador where these two populations were the only materials setting any young pods at all. At other sites, populations were selected for local adaptation without drought and will be advanced for selection under drought. Several farmer groups (Local Agricultural Research Committees - CIALs) will receive the populations for local selection in Honduras.

Elite lines are being crossed with each other, with sources of ALS resistance, with sources of high seed iron content, as well as with sources of BGMYV resistance to reinforce this indispensable trait.

Table 1a: Best lines in a yield trial of red seeded beans (Trial 1).

Tmt.	Family	bgm-1	W12	ALS	Yield	Mat	Pedigree
73	MR14148-54	H	+	7	1515	71	(SEA21 x RAB623) x 9653-16B
	Cowpea				1515	75	
70	MR14145-18	-	+	7	1353	74	(TAR4 x SEA18) x Tio Canela
69	MR14143-28	+	-	4	1312	70	(RAB651 x Tio Canela) x (RAB608 x SEA15)
72	MR14148-54	-	+	5	1247	73	(SEA21 x RAB623) x 9653-16B
114	MR14202-4	-	-	8	1208	73	(RAB623 x MD23-24) x (SEA15 x (RAB655 x G21212))
90	MR14152-14	-	-	5	1180	69	(SEA22 x (TLP35 x G21212)) x EAP 9504-30B
113	MR14202-2	H	-	3	1175	75	(RAB623 x MD23-24) x (SEA15 x (RAB655 x G21212))
103	MR14153-3	H	-	4	1130	72	(SEA22 x (A774 x G21212)) x (RAB619 x Tio Canela)
29	MR13937-15	-	-	1	1117	72	MD23-24 x (RAB655 x G21212)
	SEA5				1055	73	
45	MR13912-3	+	-	6	1028	70	RAB612 x (A774 x G2212)
40	MR14000-2	+	-	6	1019	74	RAB651 x (MD23-24 x G21212)
	Tio Canela				450	74	
	DOR 390				229	78	
	LSD (0.05)				456	4.2	

Table 1b: Best lines in a yield trial of red seeded beans (Trial 2).

Tmt.	Family	bgm-1	W12	ALS	Yield	Mat	Pedigree
140	MR14215-15	+	+	5	1161	74	(SEA15 x MD23-24) x (Tio Canela x G21212)
156	MR14232-27	H	+	5	1143	74	(SEA 21 x RAB 609) x DOR 582
212	MR14267-26	+	-	5	1132	73	(RAB608 x (RAB655 x TLP30)) x (Tio Canela x SEA21)
204	MR14265-58	-	-	4	1114	69	(RAB608 x DICTA17) x (SEA15 x RAB612)
	SEA5				1104	72	
143	MR14216-3	+	-	5	1101	75	(SEA15 x DOR364) x (RAB651 x (MD23-24 x G21212))
146	MR14217-12		-	2	1003	73	(SEA15 x RAB612) x (RIB66 x G21212)
227	MR14292-3	+	-	4	984	70	DICTA122 x(RAB651 x (VAX1 x RAB655))
229	MR14292-63	H	-	6	972	73	DICTA122 x(RAB651 x (VAX1 x RAB655))
144	MR14217-9	H	-	2	966	76	(SEA15 x RAB612) x (RIB66 x G21212)
200	MR14258-7	H	-	6	948	72	DICTA122 x (MD23-24 x (RAB655 x G21212))
138	MR14215-9	H	-	6	945	71	(SEA15 x MD23-24) x (Tio Canela x G21212)
	Cowpea				591	79	
	Tio Canela				173	74	
	DOR390				173	74	
	LSD (0.05)				472	3.7	

Table 1c: Best lines in a yield trial of black seeded beans (Trial 4).

Tmt.	Family	bgm-1	W12	ALS	Yield	Mat	Pedigree
457	MN14154-31	-	+	3	1353	72	(RIB68 x G21212) x ICTA Ligero
443	MR14144-31	H	-	4	1176	74	(SEA18 x (FEB192 x G21212)) x EAP9020-14
458	MN14154-31	+	+	5	1157	70	(RIB68 x G21212) x ICTA Ligero
346	MR13937-15		-	2	1142	74	MD23-24 x (RAB655 x G21212)
371	MN14059-8	+	-	7	1130	73	(FEB192 x G21212) x ICTA Ligero
402	MN13942-33	H	-	5	1107	73	(TLP35 x G21212) x ICTA Ligero
451	MN14154-10	H	-	8	1096	69	(RIB68 x G21212) x ICTA Ligero
386	MN13934-63	H	-	2	1093	78	(FEB192 x G21212) x (DOR500 x (DOR390RC)
350	MR14000-37	-	+	3	1072	74	RAB651 x (MD23-24 x G21212)
454	MN14154-22	-	+	6	1070	67	(RIB68 x G21212) x ICTA Ligero
394	MN13942-18		-	6	1067	71	(TLP35 x G21212) x ICTA Ligero
360	MR13944-32		-	2	1061	70	MD23-24 x (VAX2 x G21212)
374	MN14059-10		-	5	1045	69	(FEB192 x G21212) x ICTA Ligero
	Tio Canela				914	74	
	SEA5				767	73	
	Cowpea				654	80	
	DOR390				619	81	
	LSD (0.05)				492	5.2	

Table 1d: Best lines in a yield trial of black seeded beans (Trial 5).

Tmt.	Family	bgm-1	W12	ALS	Yield	Mat	Pedigree
	Cowpea				1503	79	
474	MR14191-26	+	-	7	1364	71	(DOR364RC x RAB609) x (SEA15 x (RAB655 x G21212))
467	MN14154-36	H	+	5	1229	68	(RIB68 x G21212) x ICTA Ligero
493	MR14212-12	+	-	7	1198	74	(SEA15 x OstuaRC) x (Tio Canela x (FEB192 x G21212))
489	MR14212-4	-	+	4	1185	74	(SEA15 x OstuaRC) x (Tio Canela x (FEB192 x G21212))
494	MN14214-11	+	-	6	1132	73	(SEA15 x (A774 x G21212)) x (NEB31 x (G21212 x OstuaRC))
495	MR14215-5	+	-	7	1131	71	(SEA15 x MD23-24) x (Tio Canela x G21212)
492	MR14212-12		-	7	1116	74	(SEA15 x OstuaRC) x (Tio Canela x (FEB192 x G21212))
465	MN14154-36		-	4	1111	67	(RIB68 x G21212) x ICTA Ligero
490	MR14212-6	+	-	3	1055	72	(SEA15 x OstuaRC) x (Tio Canela x (FEB192 x G21212))
497	MN14237-12	+	-	5	1034	71	(NEB31 x (G21212 x OstuaRC)) x (TLP36 x BAT477)
	Tio Canela				1008	77	
	SEA5				815	75	
	DOR390				366	79	
	LSD (0.05)				371	5.2	

Table 1e: Best lines in a yield trial of other colored beans (Trial 3).

Tmt	Family	bgm-1	W12	ALS	Yield	Mat	Color	
303	MR14215-6	+	-	2	1600	74	Pink	(SEA15 x MD23-24) x (Tio Canela x G21212)
	Cowpea				1598	78		
304	MR14215-6		-	1	1255	75	Red	(SEA15 x MD23-24) x (Tio Canela x G21212)
302	MR14215-5	+	-	6	1241	69	Red	(SEA15 x MD23-24) x (Tio Canela x G21212)
315	MR14217-16	H	-	3	1170	71	Purple	(SEA15 x RAB612) x (RIB66 x G21212)
325	MR14234-6	+	-	4	1128	78	Red	(SEA21 x RAB608) x (RIB66 x G21212)
312	MR14215-9	+	-	1	1127	72	Red	(SEA15 x MD23-24) x (Tio Canela x G21212)
333	MR14292-63	H	-	4	1104	74	Red	DICTA122 x (RAB651 (VAX1 x RAB655))
305	MR14215-6	+	-	3	1089	81	Red	(SEA15 x MD23-24) x (Tio Canela x G21212)
308	MR14215-6	+	-	2	1086	78	Pink	(SEA15 x MD23-24) x (Tio Canela x G21212)
298	MR14215-5	+	-	7	1073	70	Pink	(SEA15 x MD23-24) x (Tio Canela x G21212)
	Tio Canela				1042	79		
	SEA5				786	71		
	DOR390				479	78		
	LSD (0.05)				404	5.2		

Conclusions: Although drought pressure in Central America was slight, genetic advance in CIAT in Colombia was significant and an apparently high level of tolerance was obtained in families with acceptable grain type and excellent grain quality. It is expected that a certain number of these will be resistant to important diseases, especially BGYMV, based on parentage and molecular markers.

Activity 2.1.2

Interspecific progeny of beans evaluated for drought tolerance

Contributors: S, Beebe, Henry Terán (IP-1), Alvaro Mejía (SB-2) ~~104977~~

tepary Jiménez

Rationale: The center of diversity of the genus Phaseolus is found in Middle America, and largely in Mexico. Thus some species of Phaseolus evolved in dry, near-desert environments. These dry-adapted species include *P. acutifolius* (Pa) both domesticated and wild forms, and *P. parvifolius* (Pp) which is either closely related to acutifolius or is a morphotype of the same. These species are considered to pertain to the tertiary gene pool of the common bean and crosses with *P. vulgaris* (Pv) are possible through embryo rescue. Work at CIAT has advanced in obtaining intermediate types that are nearly fertile with both parental species, thus facilitating gene transfer across the species barrier. One important objective of these crosses is to transfer drought tolerance from acutifolius to common bean.

Materials and Methods: Interspecific crosses were generated in previous years through congruity backcrosses (Haghighi and Ascher 1988), to transfer resistance to common bacterial blight (Mejía Jiménez et al. 1994). Several hundred interspecific progeny were available combining accessions of Pa and Pp with common bean cultivar ICA Pijao. Subsequently, as work advanced in improving fertility and gene introgression of interspecific hybrids, more complex crosses (Mejía Jiménez et al., 2001) were made involving other accessions of common bean (ICA Pijao, MAM38, A775, A800 and Bayo Madero) and cultivated (G40001, G40020, G40065) and wild (NI576) tepary beans.

F3 seed was planted in the field under drought conditions in the June season, 2001. Promising families were harvested in bulk and in F4, individual selections were made followed by another cycle of mass selection. Thus, in 2002, F6 families were evaluated in a yield trial with RBC design and three replications under severe drought conditions, as described in point 1.1.2 above.

Results and Discussion: Highly significant differences in yield were registered among families and checks (Table 1). The tolerant check, SEA 5 yielded 715 kg ha^{-1}, and the sensitive check (ICA Pijao) which figured as the common bean parent in many of the lines produced 468 kg ha^{-1} (significantly less than SEA 5). The best line derived from ICA Pijao yielded 798 kg ha^{-1}, which was significantly different than Pijao, suggesting effective introgression from acutifolius for drought tolerance. The best families, however, were derived from the cross BKI 11 of *P. acutifolius* with the race Durango common bean variety Bayo Madero from Mexico. Race Durango typically presents some level of tolerance to drought, and the combination of Bayo with acutifolius produced lines that yielded over a ton, or about 50% over the tolerant check. This is comparable to the yield advantage obtained in the intraspecific crosses which represented far more investment of time and breeding than in these crosses with acutifolius, although the interspecific progeny are still far removed from commercial common bean type with the necessary agronomic traits and grain. The hope is that the mechanisms that operate in acutifolius might be complementary to those in vulgaris, and permit the pyrimiding of even greater tolerance. Heat tolerance is still another trait that is required in combination with drought tolerance, and these crosses may offer this trait as well.

Table 1. Yield (kg ha^{-1}) of interspecific hybrids between common bean and P. acutifolius.

Entry	Cross Code	Yield kg ha^{-1}	Maturity	Pedigree
27	BKI 11	1065	77	Bayo Madero X [CBC5(CBC3X CBC3)*]
28	BKI 11	1038	74	Bayo Madero X [CBC5(CBC3X CBC3)*]
6	4V3A1-002	798	69	(((((Pv x Pa) x Pv) x Pa) x Pv) x Pa) x Pv
54	MMNNI 14	751	73	{(CBC5X CBC5)X (CBC5X CBC3) } X {(G40065 X NI576)X [(CBC5X CBC5)X (CBC5X (CBC3X CBC3)}**
64	INB 39	737	72	(((((Pv x Pa) x Pv) x Pa) x Pv) x Pa) x Pv
30	BKI 11	731	72	Bayo Madero X [CBC5(CBC3X CBC3)*]
29	BKI 11	728	77	Bayo Madero X [CBC5(CBC3X CBC3)*]
60	SEA 5 (ch)	715	71	
	ICA Pijao (ch)	468	72	
	LSD (0.05)	184	2.3	

* Cross between congruity backcross hybrids involving the genotypes G40020 and G40001 of tepary bean, and ICA Pijao, A775, MAM38 and A800 of common bean (Mejía Jiménez et al. 1994).

** Double congruity backcross hybrids (Mejía Jiménez et al. 2001) involving the genotypes NI576, G40065, G40020 and G40001 of tepary bean, and ICA Pijao, A775, MAR1, MAM38 and A800 of common bean

Conclusions: Interspecific progeny presented significant introgression of drought tolerance from acutifolius to common bean. These represent another potential source of tolerance genes to improve common bean, and may also be a source of heat tolerance.

Haghighi, KR. and Ascher, P.D. 1998. Fertile, intermediate hybrids between *Phaseolus vulgaris* and *P. acutifolius* from congruity backcrossing. Sexual Plant Reproduction 1 : 51-58.

Mejía-Jiménez, A., Muñoz, C. Jacobsen, H.J., Roca, W.M. and Singh, S.P. 1994. Interspecific hybridization between common bean and tepary bean: Increased hybrid embryo growth, fertility, and efficiency of hybridization through recurrent and congruity backcrossing. Theor. Appl. Genet. 88: 324-331.

Mejía Jiménez, A. , Galindo L., Criollo A., Beebe S., Cardona C. and Tohme. Development of a novel backcross methodology for producing fertile common x tepary bean hybrids from otherwise incompatible genotypes. Annual report 2001. Project SB-02-CIAT

Activity 2.1.3

Identification of traits associated with drought resistance in common bean

Contributors: I. M. Rao, S. Beebe, J. Ricaurte, H. Terán and R. García

Rationale: Several bean-growing regions of endemic poverty are especially subject to drought stress, such as Central America and southern Africa. Thus, development of drought adapted bean varieties is an important strategy to minimize crop failure and improve food security in these regions. This year we report results obtained from this 36 genotypes for their adaptation to drought stress.

Last year we evaluated 36 genotypes for their adaptation to drought stress under field conditions. The results from this field study indicated that two bred lines (SEA 5 and A 801) and two landraces (Carioca and G 21212) were very well adapted to drought stress. The superior performance of these four genotypes under drought was associated with resistance to soil-borne pathogens combined with their ability to mobilize photosynthates to developing grain and to utilize the acquired N and P more efficiently for grain production. This study also provided further evidence for the usefulness of lower level of seed P as a selection method in addition to grain yield for identifying bean genotypes that are better adapted to drought.

This year we evaluated drought adaptation of 36 promising bredlines and accessions that were assembled as an international nursery. The same materials were evaluated in Haiti and Honduras (Zamorano) in addition to CIAT-Palmira (CIAT, 2001). Physiological results from CIAT-Palmira are reported here.

Materials and Methods: A field trial was conducted at Palmira in 2001 (June to September) to determine differences in tolerance to water stress conditions. The field trial included 36 bred lines and germplasm accessions. Two levels of water supply (irrigated and rainfed) were applied. A 6 x 6 partially balanced lattice design with 3 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 were measured. also

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 30.5 and 18.7 °C, respectively, while incident solar radiation ranged from 4.6 to 25.0 MJ m^{-2} d^{-1}. The total rainfall during the active crop growth was 122.7 mm with almost no-rain during flowering and podfilling time (Figure 1). The potential pan evaporation was of 432.5 mm. These data on rainfall and pan evaporation indicated that the crop suffered high level of drought stress during active growth and development. Soil water tension values in the range of 700 millibars were recorded in the ninth week of crop growth under rainfed conditions.

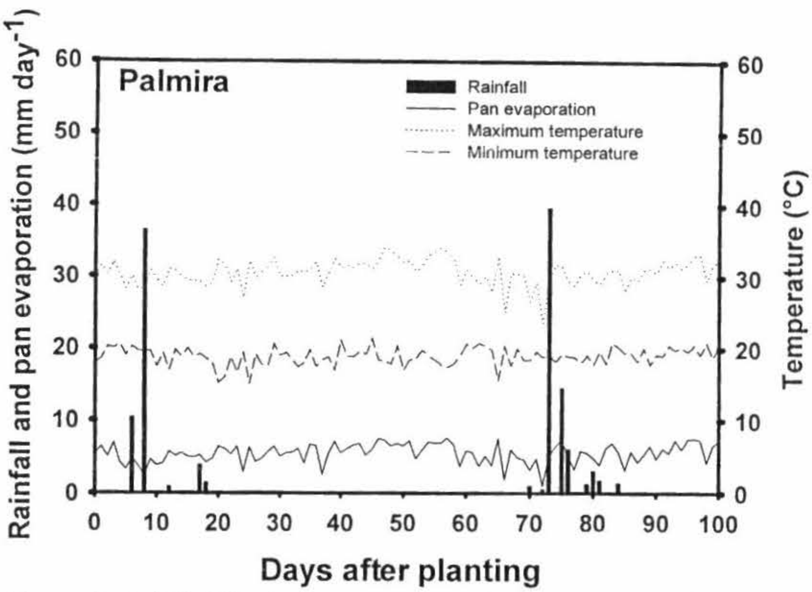


Figure 1. Rainfall distribution, pan evaporation, maximum and minimum temperatures during crop growing period at Palmira.

Grain yield and physiological traits: Under water stress conditions in the field, the seed yield of 36 genotypes ranged from 257 to 1284 kg/ha (Table 2). Among the genotypes tested, two accessions of *P. acutifolius* (G 40068 and G 40159) and two bred lines (RAB 650 and SEA 15) were outstanding in their adaptation to rainfed (water stress) conditions. There was response to irrigation in most of the genotypes but not in all genotypes as evident from the mean values (Table 1; Figure 2). This was mainly due to high incidence of a leafhopper pest (*Empoasca kraemeri*) during reproductive development in the rainfed treatment and attack of a pod-boring insect (*Epinotia opposita*) in the irrigated treatment that affected the performance of several genotypes including G 21212. The relationship between grain yield of rainfed and irrigated treatments indicated that G 40159, RAB 650, SEA 15, SEA 23 and RAB 651 were not only adapted to water stress but also responsive to irrigation (Figure 2). Among the 36 genotypes tested, INB 36 was the most poorly adapted bredline under rainfed conditions.

Under rainfed conditions, grain yield was not related to leaf area index (Table 2). This was because several genotypes (e.g., ICA Pijao, SEA 17) had greater leaf area values than the best performers such as the two accessions of *P. acutifolius* (G 40068 and G 40159) under rainfed conditions (Table 2). The superior performance of these two accessions could be due to better transport system for mobilizing photosynthates to developing grains as revealed by the greater levels of nonstructural carbohydrates in the shoot tissue at mid-podfilling stage (Figure 3). The SEA lines that were developed for improved drought adaptation showed greater levels of shoot TNC and grain yield than the mean values. Among the SEA lines, SEA 23 and SEA 15 were particularly outstanding in terms of both TNC in shoot and grain yield under rainfed conditions (Figure 3).

The two accessions of *P. acutifolius* (G 40068 and G 40159) yielded more than any line or accession of *P. vulgaris*, demonstrating the advantage that this species continues to display over *P. vulgaris*, and justifying our on-going breeding efforts in interspecific crosses. The red seeded breeding lines (sister lines RAB 650 and RAB 651) without directed selection for drought tolerance during their development performed well under rainfed conditions.

Table 1. Influence of drought stress on grain yield and leaf area index of advanced lines and germplam accessions evaluated in a Mollisol at Palmira.

	Grain yield (kg/ha)		Leaf area index (m^2/m^2)	
Line or accession	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**
G 40068 (*P. acutifolius*)	341	1284	0.83	1.45
G 40159 (*P. acutifolius*)	918	1276	0.66	1.14
RAB 650	960	1153	1.34	1.93
SEA 15	1057	1008	1.55	1.77
SEA 23	1064	1001	1.60	1.66
RAB 651	1089	963	2.08	2.66
SEA 18	897	880	1.48	2.06
SEA 5	736	820	1.58	2.17
BAT 477	1251	800	1.41	2.15
Tio Canela 75	1005	795	1.35	1.82
RAB 632	784	776	1.03	1.50
SEA 19	1081	760	1.72	2.04
RAB 619	926	751	1.85	1.86
RAB 618	733	730	1.53	1.57
Pinto Villa	1027	718	1.38	1.52
SEA 16	976	695	2.53	2.20
SEA 21	1050	656	1.83	1.67
RAB 620	832	649	1.23	1.96
RAB 636	687	620	1.24	1.34
SEA 22	1110	615	1.33	1.71
RJB 7	770	549	1.35	2.01
RAB 609	1147	533	1.64	2.26
INB 35	995	532	2.16	2.21
SEA 17	824	513	2.37	2.64
INB 39	1018	511	1.45	2.08
G 21212	344	507	1.11	2.16
SEA 20	771	499	1.30	1.71
ICA Quimbaya	500	497	1.09	1.20
INB 38	820	479	1.85	2.00
RAB 608	674	464	1.31	2.23
DOR 390	857	445	1.87	1.61
ICA Pijao	1105	435	1.99	2.68
INB 37	777	431	1.29	1.89
G 1977	397	418	1.47	2.15
Apetito	575	334	1.37	1.90
INB 36	712	257	1.53	1.74
Mean	**856**	**677**	**1.52**	**1.91**
CV (%)	**29**	**25**	**32**	**26**
LSD (0.05)	**410**	**272**	**0.80**	**0.81**

Table 2. Correlation coefficients (r) between final grain yield (kg/ha) and other plant attributes of 36 genotypes of common bean grown under rainfed and irrigated conditions in a Mollisol at Palmira.

Plant traits	Rainfed	Irrigated
Leaf area index	-0.14	0.50***
Shoot biomass	0.30**	0.62***
Shoot N uptake	0.20*	0.53***
Shoot P uptake	0.35**	0.58***
Shoot K uptake	0.14	0.49***
Shoot Ca uptake	-0.07	0.48***
Shoot Mg uptake	0.01	0.30**
Shoot TNC content	0.52***	0.30**
Shoot ash content	-0.48***	-0.34***
Seed N content	-0.42***	0.02
Seed P content	0.09	-0.10
Seed K content	-0.32***	-0.15
Seed TNC content	-0.07	0.27**
Seed ash content	-0.22*	0.04

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

It is important to note that seed TNC at harvest of the two *P. acutifolius* accessions (G 40068 and G 40159) under rainfed conditions was also greater than many genotypes (Figure 4). Among the SEA lines that were bred for improved drought adaptation, SEA 23 and SEA 15 showed greater amounts of shoot TNC and moderate levels of seed TNC (Figures 3 and 4). In general, greater yield under rainfed conditions was associated with greater shoot TNC (r = 0.52***) except for RAB 650 which combined greater yield with moderate level of shoot TNC (Table 2; Figure 3). It is possible that this advanced line (RAB 650) may maintain greater rates of photosynthate transport during grainfilling under water stress conditions.

We found that the superior performance of certain bean genotypes under rainfed conditions could be related to lower seed P content (CIAT, 2000). We tested this relationship further by measuring seed nutrient (N, P, K, Ca and Mg) content and seed ash (total mineral) content. There was significant relationship between rainfed grain yield and seed ash content (Figure 7; Table 4). The two *P. acutifolius* accessions (G 40068 and G 40159) showed lower seed ash content and lower seed P content than most of the genotypes tested under rainfed conditions (Figures 7 and 8). Several superior performers combined high grain yield with lower seed ash and seed P content (Figures 7 and 8).

The bred line SEA 23 was outstanding in combining greater yield with higher level of seed N (protein) under rainfed conditions (Figure 7). Although the two *P. acutifolius* accessions (G 40068 and G 40159) were outstanding in grain yield under rainfed conditions, these two accessions showed lower levels of seed N (Figure 7) indicating greater N use efficiency (g of grain yield per g of N uptake) (Figure 8). One of the bred lines RAB 650 was similar to the two *P. acutifolius* accessions in its outstanding ability to utilize acquired N and P for grain production.

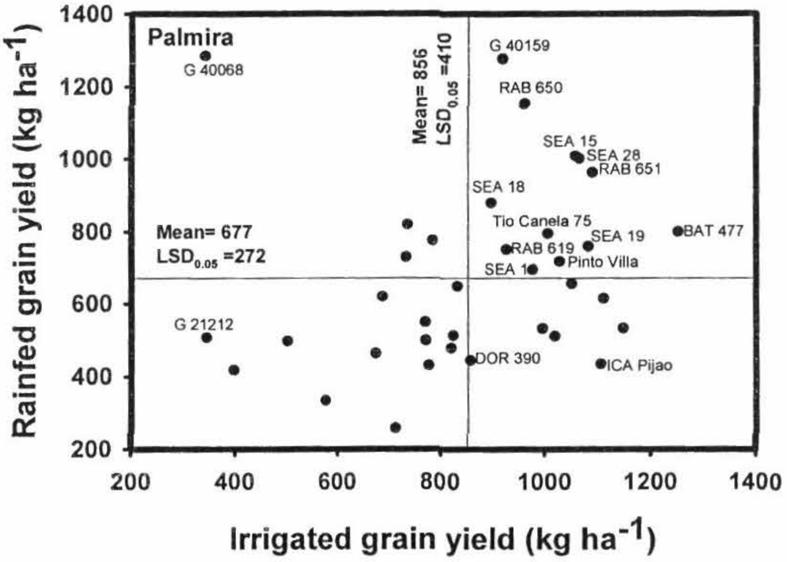


Figure 2. Identification of genotypes that are adapted to rainfed conditions and are responsive to irrigation to a Mollisol at Palmira. Genotypes that yielded superior with drought and were also responsive to irrigation were identified in the upper box of the right hand side.

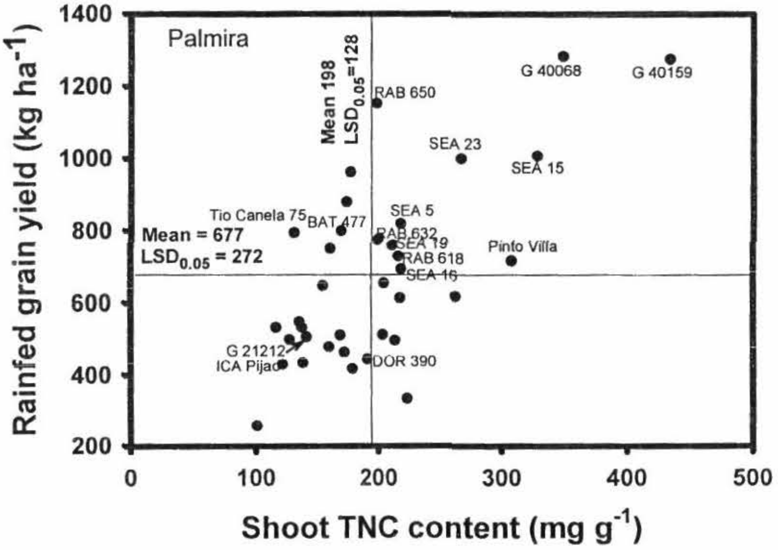


Figure 3. Identification of genotypes that combine superior seed yield with greater amount of total nonstructural carbohydrates (TNC) in the shoot when grown under rainfed conditions in a Mollisol at Palmira. Genotypes that were superior in grain yield and higher in shoot TNC were identified in the upper box of the right hand side.

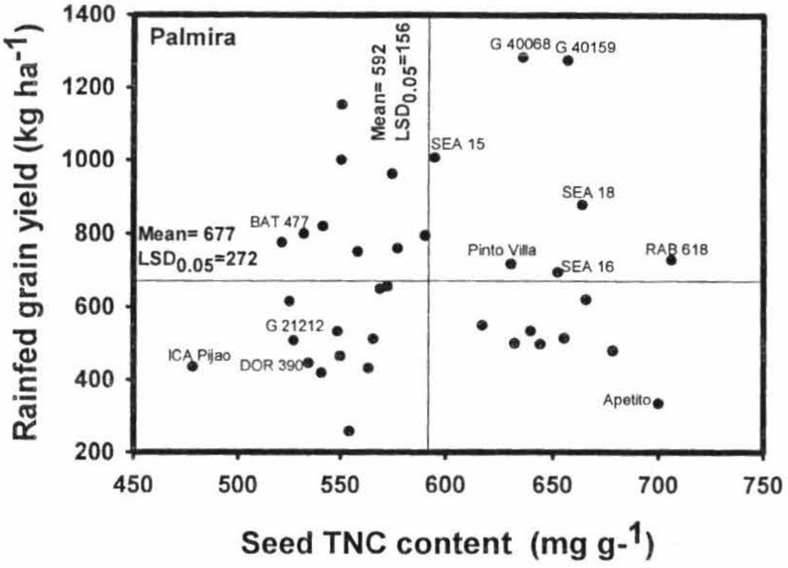


Figure 4. Identification of genotypes that combine superior seed yield with greater amount of total nonstructural carbohydrates (TNC) in the seed when grown under rainfed conditions in a Mollisol at Palmira. Genotypes that were superior in grain yield and higher in seed TNC were identified in the upper box of the right hand side.

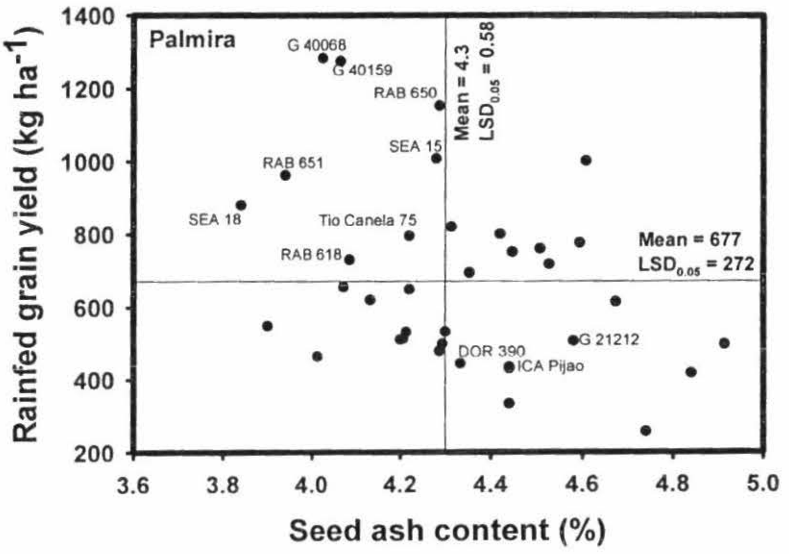


Figure 5. Identification of genotypes that combine superior seed yield with lower ash (mineral) content in seed when grown under rainfed conditions in a Mollisol at Palmira. Genotypes that were superior in grain yield and lower in seed ash were identified in the upper box of the left hand side.

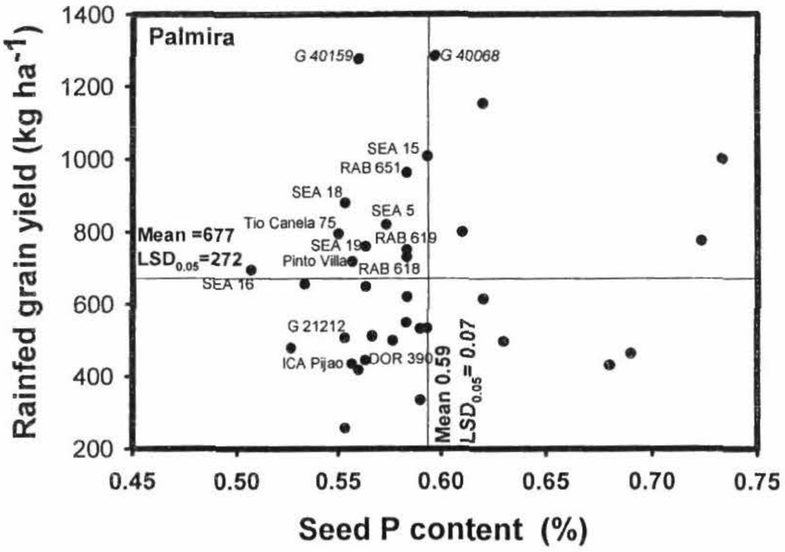


Figure 6. Identification of genotypes that combine superior seed yield with lower P content in seed when grown under rainfed conditions in a Mollisol at Palmira. Genotypes that were superior in grain yield and lower in seed P were identified in the upper box of the left hand side.

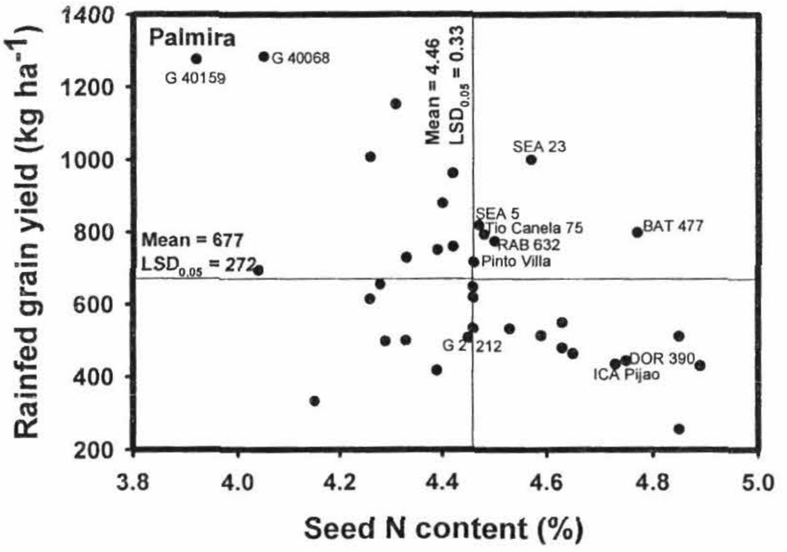


Figure 7. Identification of genotypes that combine superior seed yield with greater N content in seed when grown under rainfed conditions in a Mollisol at Palmira. Genotypes that were superior in grain yield and higher in seed N were identified in the upper box of the right hand side.

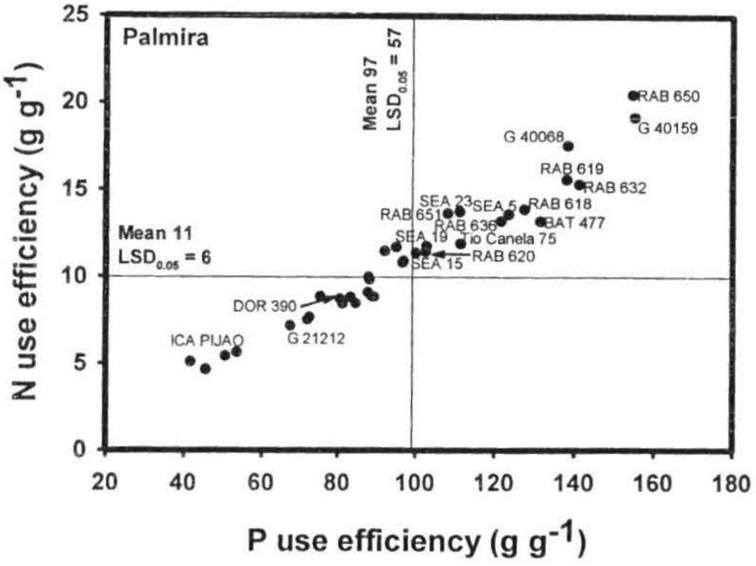


Figure 8. Identification of genotypes that combine efficient utilization of N and P to produce greater seed yield when grown under rainfed conditions in a Mollisol at Palmira. Genotypes that were superior in both N and P use efficiency were identified in the upper box of the right hand side.

Correlation coefficients between final grain yield and other shoot attributes indicated that greater seed yield under rainfed conditions was highly positively related to shoot TNC content and highly negatively related with seed ash (mineral) content, particulary seed N and K content (Table 4). Mineral accumulation in the grain primarily depends on translocation from the leaves and on minerals removed from the older parts of the plant after the onset of senescence. Under severe drought, photosynthesis is more affected than translocation of photosynthates. As a consequence, genotypes unable to maintain high rates of stomatal conductance and photosynthesis during grain filling would mainly use retranslocation of photoassimilates from preflowering reserves, and minerals from senescent tissues, for their grain filling. Thus the concentration of minerals in seed (ash content) will be greater in genotypes that are less adapted to drought. These results are consistent with the results obtained from duram wheat (Merah et al., 2001).

Seed P content was not associated with seed yield. This observation is not consistent with the results reported before (CIAT, 2001). It is possible that the interaction due to pest damage might have affected this relationship. Leaf area production was also not related to seed yield under rainfed conditions. However, shoot biomass production showed positive correlation with grain yield under both rainfed and irrigated conditions. Shoot nutrient (N, P, K, Ca and Mg) uptake was highly related to seed yield with irrigated conditions while only shoot P and N uptake was related to seed yield with rainfed conditions. Significant negative relationship was also observed between seed yield and seed N content under rainfed conditions. This observation indicates that the superior performers mobilized greater amounts of photosynthates to seed per unit amount of N in the seed.

It appears that the two accessions of *P. acutifolius* (G 40068 and G 40159) and two bred lines (SEA 23 and SEA 15) could mobilize greater amounts of photosynthates from leaves and stems to grain during water stress. Use of these genotypes in the breeding program could contribute to greater yield potential under rainfed conditions due to greater utilization of photosynthates. The superior adaptation of these genotypes to drought was found to be due to their efficient utilization of N and P for grain production (Figure 7) in addition to the mobilization of photosynthates.

Conclusions: 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.

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Activity 2.1.4
Photosynthetic adaptation to abiotic stress factors in common bean

Contributors: B. Kucera, E. Wellmann (University of Freiburg, Germany); I. M. Rao, S. Beebe, R. Garcia, J. Ricaurte and H. Terán (CIAT)

Rationale: A collaborative project funded by European commission is in its second year of progress. This project involves participation of 3 advanced research organizations from Europe (University of Freiburg, Germany: University of Sheffield, U. K.; University of Pisa, Italy) and 2 NARS partners from South America (University of Chile, Chile; CIF-UMSS, Cochabamba, Bolivia). CIAT participates in this project as a sub-contractor to the University of Chile. The project is entitled "Characterization of South American genotypes for optimal use of light under abiotic stress".

Genotypic differences in common bean for tolerance to UV-B light under abiotic stress conditions: Formation of flavonoids and cyclobutyl-pyrimidine dimers under drought and low phosphorus stress

As part of the above mentioned project work plan, a Ph. D. student, Brigit Krucera from the group of University of Freiburg, Germany headed by Prof. E. Wellmann visited CIAT during September to November of 2001 to conduct field and laboratory work on the above project. The main findings from her work are reported here.

Bean is an important crop especially in Latin American countries and it is grown virtually all over the world under very different climatic conditions. Due to its high content of proteins and essential micronutrients bean is regarded as very valuable food. Therefore, it is important to ensure bean growth even under extreme environmental conditions. During the past few years increasing UV-B (ultraviolet-blue) radiation, reaching the earth's surface especially at low latitudes, has been under discussion. UV-B radiation damages important macromolecules such as lipids, proteins and DNA (Strid et al. 1994) and also the photosynthetic machinery (Bornman, 1989). DNA damage consists mainly in the formation of dimers between adjacent pyrimidine bases (Strid et al., 1994; Taylor et al., 1997). The main type of dimers are cyclobutyl-pyrimidine dimers (CPD), which can be easily detected in the DNA isolated from irradiated plant material, using monoclonal antibodies (Mori et al., 1991). CPD may hinder replication and transcription of the DNA and therefore interfere with cell metabolism.

Two main mechanisms help the plant avoid UV-induced damage: formation of protective pigments (mainly flavonoids) that absorb UV-B radiation, and the presence of enzymes repairing DNA damage

(DNA-photolyases) (Strid et al., 1994; Taylor et al., 1997). Flavonoids are mainly located in the epidermis of the leaf and absorb radiation in the UV-B range and it is known that they reduce damage from UV-B radiation. Mutants lacking flavonoids have been shown to be more susceptible to UV-induced damage than the corresponding wild-type plants whereas other mutants overexpressing flavonoids are better protected against high UV-B levels. Flavonoid content in most plants has been shown to increase with UV-B radiation, although it was found only a slight increase in UV-B protective pigments in bean under elevated UV-B radiation. Low UV levels as present in normal solar radiation might already be saturating for flavonoid induction. Flavonoid formation is not only influenced by UV-B but also by several other internal and external factors such as developmental stage of the plant/tissue, wounding, nutrient supply, season or photoperiod. The content of phenolic compounds including flavonoids also varies between individual plants of one population.

Bean has been classified as sensitive to enhanced solar UV-B radiation, mainly because growth and plant dry weight were significantly reduced under high UV-B, although the seed yield seems to be unaffected. Several other biotic and abiotic constraints limit bean productivity in Latin American countries, e.g. soils with low P supply or high Al toxicity, drought or diseases caused by insects, fungi or bacteria (Rao, 2001). Bean is often grown under multiple stress conditions making it necessary not only to investigate each stress factor as such but also possible interactions between various factors. In our experiments we investigated bean plants grown under high solar light and UV-B fluence rates and additionaly suffering from drought stress or low P supply under field conditions. Our main objective was to determine genotypic differences in major responses to UV-B such as the formation of flavonoids and CPD under controlled environmental and field conditions.

Material and Methods: *Plant material* – Plant samples were collected from two field sites, a drought site in Palmira and a low P site in Darien managed by the bean team of CIAT, Cali, Colombia. Plants grown in Palmira suffered from severe drought stress. They were sown on July 5th, 2001, irrigated twice (during germination and on July 25th), and then maintained without further irrigation until harvest on September 4th, 2001. A total of 22 bredlines and accessions were evaluated under drought conditions. These included: SEA 5, SEA 15, MD 23-24, DICTA 122, G 21212, RAB 655, RAB 651, RAB 630, SEA 20, SEA 22, Tio Canela 75, DICTA 113, RJB 11, ICTA OSTUA, DOR 500, DOR 390, DOR 364, MUS 131, NEB 31, TLP 39, ICTA LIGERO and A 801. Three or more samples were collected for 5 genotypes (SEA 5, SEA 15, MD 23-24, DICTA 122, G 21212) while 1 or 2 samples were collected for the remaining genotypes for analysis. In Darien, the field trial was planted on October 2nd, 2001 and samples were collected on October 23rd. Plants were grown on a P-deficient soil either without additional P-fertiliser or with P-fertiliser. Each treatment was carried out in 3 repetitions. Genotypes selected for sampling included A 785, BAT 477, BAT 881, Carioca, DOR 364, G 19227A, G 21212, G 3513, ICA Quimbaya, SEA 5, Tio Canela 75 and VAX 1. Samples were collected from the same leaves for both flavonoid and dimer measurements. Leaf discs were taken from the first trifoliate leaves of 4 individual plants per repetition. All samples were immediately frozen in liquid nitrogen and kept on –80°C until extraction.

Flavonoid determination: 4 leaf discs (diameter 1 cm) were used for analysis of flavonoid content. The leaf discs were put in 200 μl extraction buffer (50% EtOH, 1% acetic acid) and heated for 30 min to 85°C. 100 μl of the extract were then placed on WHATMAN N1 paper for chromatography. Chromatography was carried out in 15% acetic acid for 2.5-3.0 h. Flavonoid spots were identified by their dark fluorescent colour under UV-A. Flavonoids spots were cut out and flavonoids extracted in 50% EtOH at 85°C for 30 min. Absorption spectra were measured from 250 nm to 400 nm and the optical density at 360 nm was used for quantification.

DNA extraction: 8 leaf discs were homogenized in 1.2 ml CTAB buffer (2% hexadecyltrimethylammonium-bromide, 1.4 M NaCl, 100 mM Tris/HCl pH 8.0, 20 mM EDTA, 0.2% (v/v) mercapoethanol) and centrifuged for 10 min at 18000 g. 1 ml of the supernatant was mixed with 1 ml chloroform/isoamylic alcohol (24:1) and centrifuged for 5 min at 7800 g. 900 µl of the supernatant were mixed with 600 µl of ice cold 2-propanol and centrifuged for 10 min at 18000 g. The pellet was washed once with washing solution (76% (v/v) EtOH, 10 mM ammonium acetate) and then dried. The pellet was resuspended in 200 µl of TE buffer (10 mM Tris/HCl pH 7.4, 1 mM EDTA), mixed with 5 µl RNAse A solution and incubated at 37°C for 1 h. After adding 120 µl of PEG/NaCl solution (20% polyethyleneglycol 6000, 2.5 M NaCl) the solution was centrifuged for 10 min at 18000 g. The pellet was washed with washing solution followed by 100% EtOH, dried and resuspended in TE buffer. DNA was quantified by measuring the absorbance at 260 nm, purity was checked by calculating the ratio of A_{260}/A_{280} which was >1.8 in all cases.

ELISA for CPD determination: The DNA was heat denatured and diluted to 4 ng/µl and 50 µl DNA used per well. DNA on the plate was allowed to dry over night at 37°C. Blocking was done with 200 µl 3% BSA solution per well. 50 µl primary antibody solution (TDM-2, 1:1000 in PBS (10 mM NaPi, 150 mM NaCl, pH 7.4)) and 50 µl secondary antibody solution (rabbit-antimouse-antibody, 1:2000 in PBS) were used. Detection of CPD was carried out with OPD (o-phenylendiamin) and quantified photometrically at 450 nm. Between each step the plate was washed with PBS-T (PBS, 0.05% TWEEN 20), and before the last step additionally with citrate-phosphate buffer (24 mM citric acid, 50 mM Na_2HPO_4).

Results and discussion: *Drought stress* - We measured flavonoid and CPD contents in leaves from 22 genotypes exposed to severe drought stress for more than one month. As expected, we found a big variation in flavonoid content among the genotypes (Figure 1; Table 1). In some genotypes such as SEA 15, SEA 5 or G 21212, flavonoid contents were much lower than in e.g. MD 23-24 or DICTA 122. Extreme values e.g. the very high flavonoid content in MUS 131 (Table 1) were obtained from only one probe and therefore have to be repeated to be cnfirmed. Measured values for flavonoid contents varied only little between individual probes as shown in Figure 1. Genotypes such as SEA 15, SEA 5 and G 21212 which showed rather low flavonoid levels are known to be well-adapted to drought stress (CIAT, 1999; 2000). On the other hand, the bred line MD23-24 that is known to be more sensitive to drought stress showed a high flavonoid level. It is known that different stress factors enhance flavonoid contents in plants. Therefore it is possible that plants that suffer more from drought accumulate more flavonoids than plants that tolerate more easily those conditions. Since we were not able to include well-watered plants as controls in this study, we cannot rule out the possibility that some bred lines such as MD 23-24 have higher flavonoid contents even under non-stress conditions and that it's simply a characteristic of the genotype. Another complication is that during the field experiment temperatures were very high (Figure 2) and that there might have been imposed heat stress on the plants at the same time as drought stress. To separate these effects, additional experiments will be carried out in more controlled (also laboratory) conditions in future.

CPD contents measured in the probes were extremely low (Figure 1 and Table 1). In most cases they were close to the detection limit and no definite statement about the existence of CPD in field-grown plants can be made from our experiment. CPD accumulation under solar radiation is not definitely proven since in most studies CPD were induced by artificial UV sources. On the other hand there is one report by (Rousseaux et al., 1999) where they were able to measure CPD in naturally grown *Gunnera magellanica* plants in Tierra Del Fuego National Park, under the influence of the ozone hole.

Nutritional stress: In another trial we determined flavonoid and CPD contents in leaves of plants grown with low P supply on a P-deficient soil in Darien compared to control plots with high P supply. Plants of 12 bean genotypes (A 785, BAT 477, BAT 881, Carioca, DOR 364, G 19227A, G 21212, G 3513, ICA Quimbaya, SEA 5, Tio Canela 75 and VAX 1) were included in the study.

Similar to the results obtained with drought stress, the levels of flavonoids varied significantly among the genotypes. In general, a small increase in flavonoid content was observed under low P conditions with the exception of two bredlines (A785 and BAT477) in which flavonoid levels decreased under low P stress. BAT 881 had the highest flavonoid level under both high and low P supply conditions. The biggest relative increase in flavonoids under stress conditions was observed in the variety ICA Quimbaya (Table 2). Among the genotypes tested, a germplasm accession G21212 showed the lowest flavonoid content under optimum (high P) growing conditions (Figure 3) and had a low flavonoid content under both drought stress (Figure 1) and low P stress. This observation indicates that this genotype which is known for its wider adaptation to different abiotic stress factors (low P, Al toxicity, drought) might be relatively less sensitive to stress conditions.

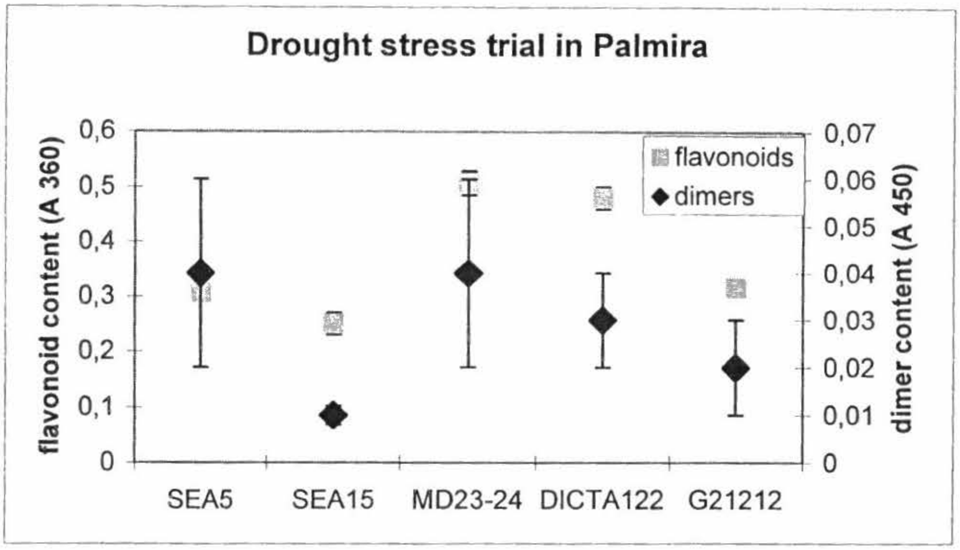

Figure 1. Flavonoid and pyrimidine dimer contents in bean genotypes grown under drought stress at Palmira field site. Mean values and standard error for at least 3 replicates. Where error bars are not visible, standard error was less than 5%.

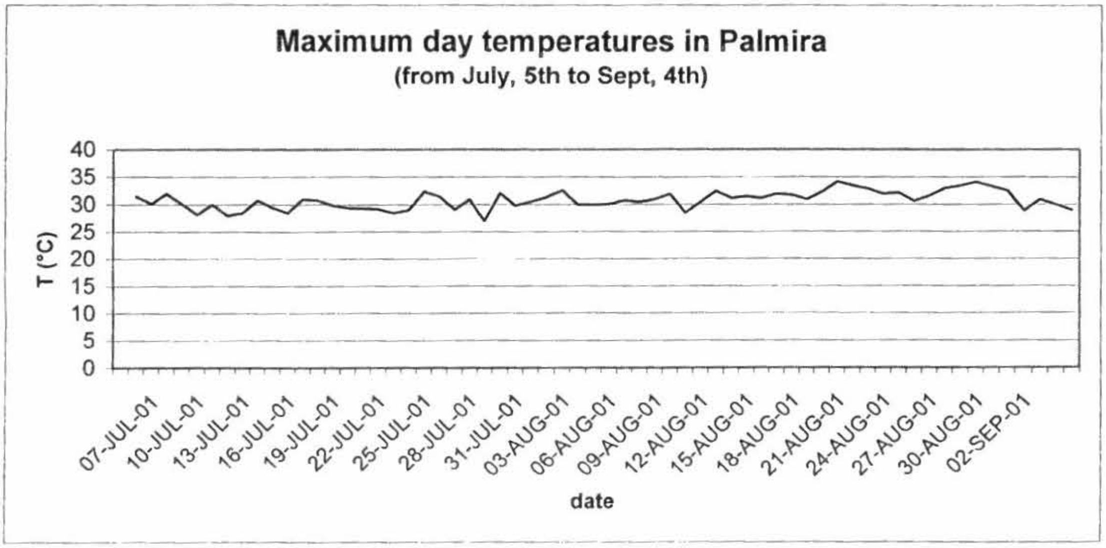

Figure 2. Variation of maximum day temperatures from July to September 2001 in Palmira.

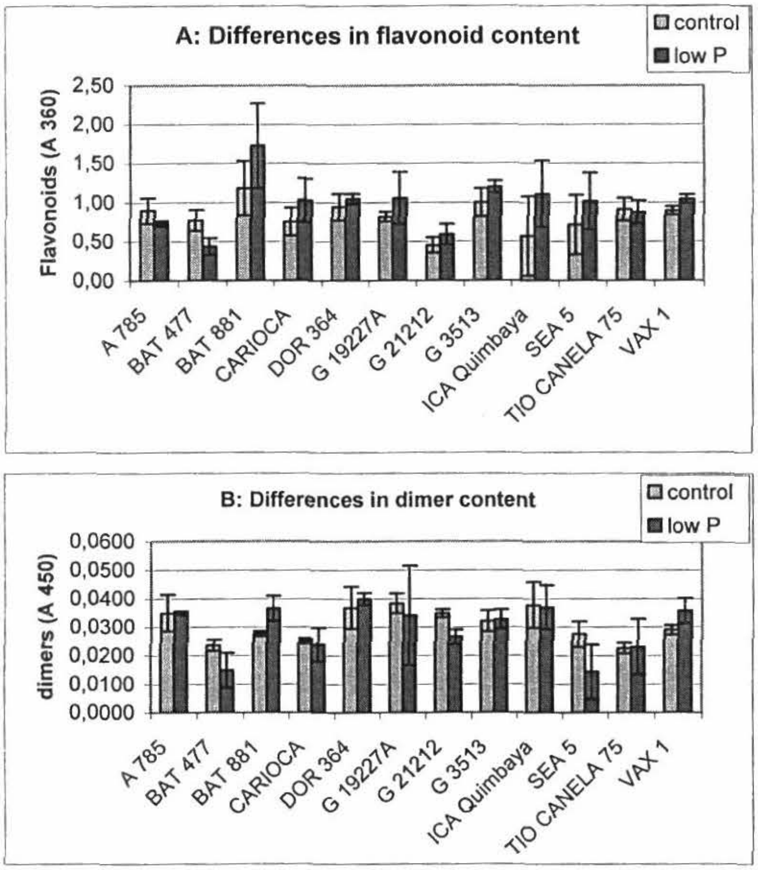

Figure 3. Flavonoid and pyrimidine dimer contents in trifoliate leaves of bean genotypes grown under low P and high P (control) conditions. Mean values with standard error for 3 replicates.

Since flavonoids fulfill various tasks in plants, among others protection from harmful solar UV radiation, some genotypes of bean probably show differences in their resistance to UV. Therefore we measured also the CPD content in the same plants. As in the drought stress experiment, again CPD contents were extremely low and close to the detection limit. There was also no clear trend in response to low P stress, making it difficult to draw conclusions from this experiment. In laboratory experiments though, differences in CPD accumulation under artificial UV have been observed among bean genotypes (unpublished results).

Conclusions: Our results support the hypothesis that flavonoids accumulate under abiotic stress conditions although the observed increases were small in most cases and even a decrease in flavonoid content was observed in two genotypes. Variation in flavonoid levels among genotypes was high. The reaction towards stress differs in different genotypes of bean. The differences in flavonoid content under stress might have an influence on several physiological processes related to plant performance. An enhancement in phenolic metabolism might for example provide better protection against abiotic stress factors such as UV-B or stress from diseases and insects. The ability of a particular genotype to accumulate UV protective flavonoids could be taken into consideration when this genotype is to be grown in a high UV-B environment. Although in our field experiments we could not prove the existence of CPD under natural sunlight, a negative effect of solar UV-B on other parameters such as photosynthesis cannot be ruled out. More work is needed under field and laboratory conditions to draw definite conclusions about the interaction between different stress factors and their impact on different bean genotypes.

Table 1: Differences in flavonoid and pyrimidine dimer contents of bean genotypes grown under drought stress (single experiment).

Genotype	**Dimer content (A_{450})**	**Flavonoid content (A_{360})**
RAB 655	0.06	0.54
RAB 651	0.10	0.50
RAB 630	0.13	0.78
SEA 20	0.07	0.30
SEA 22	0.01	0.41
Tio Canela 75	0.04	0.59
DICTA 113	0.02	0.53
RJB 11	0.01	0.75
ICTA OSTUA	0.03	0.88
DOR 500	0.04	0.58
DOR 390	0.01	0.89
DOR 364	0.12	0.43
MUS 131	0.04	1.06
NEB 31	0.04	0.89
TLP 39	0.04	0.87
ICTA LIGERO	0.02	0.45
A 801	0.04	0.48

Table 2: Differences in flavonoid content from trifoliate leaves of bean genotypes grown under P-deficient and P-optimal conditions. Relative differences from Figure 3.

Genotype	**Relative difference in flavonoid content under P-deficiency (%)**
A 785	82
BAT 477	57
BAT 881	146
Carioca	137
DOR 364	111
G 19227A	129
G 21212	131
G 3513	120
ICA QUIMBAYA	194
SEA 5	142
Tio Canela 75	96
VAX 1	117

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Activity 2.1.5

Drought tolerance of *Brachiaria:* 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. Garcia

John Camilo

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 in progress 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 2 years 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. This year, we continued our efforts to monitor the dry season performance into third 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, and shoot nutrient uptake were measured at the end of the dry season (33 months after establishment; April 2002). Maintenance fertlizer (half the levels of initial application) was applied at the beginning of the wet season of 2001 (April 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 (Table 1). At 33 months after establishment (4 months after dry sseason), live forage yield with low fertilizer application ranged from 0 to 1834 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 606 was outstanding in live forage and dead biomass production with high fertilizer application. A spittlebug resistant genetic recombinant, FM9503-S046-024 was superior among the genetic recombinants in terms of greater live shoot biomass and total shoot biomass, particularly with 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 stem ratio values of two genetic recombinanats (BR97NO-0082 and FM9301-1371) were markedly superior to other genotypes.

Table 1. Genotypic variation as influenced by fertilizer application in live shoot biomass, dead shoot biomass 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 33 months after establishment (at the end of the dry season – April 2002). 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	745	771	4.82	6.14	1506	1654
BR97NO-0383	710	972	2.88	2.84	1382	1938
BR97NO-0405	765	858	1.92	1.58	1711	1847
FM9201-1873	516	1142	2.77	2.53	1407	2256
FM9301-1371	701	848	6.79	4.01	1433	2718
FM9503-5046-024	705	1348	4.42	2.00	1762	4092
Parents:						
CIAT 606	622	2444	1.37	1.53	2086	8264
CIAT 6294	776	1077	3.24	2.51	2022	2603
BRUZ/44-02	0	0	0	0	0	0
CIAT 26646	931	1126	2.51	1.81	2173	2460
Accessions:						
CIAT 26110	1834	2069	2.17	2.22	2778	3445
CIAT 26318	974	1278	2.09	1.98	2323	2683
Mean	773	1161	2.92	2.43	1715	2830
LSD *(P=0.05)*	361	1300			843	4721

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 that differences among genetic recombinants, parents and accessions were not significant with either low or high fertilizer application (Table 2). But shoot N uptake with low fertilizer application was markedly greater for CIAT 26110 (Table 2; Figure 2). With high fertilizer application, CIAT 606 was outstanding in shoot N uptake. Shoot uptake of P, K, Ca and Mg were also greater with CIAT 26110 (Tables 3 and 4; Figure 2). Among the parents, CIAT 606 was superior in P, K, Ca and Mg acquisition from high fertilizer application.

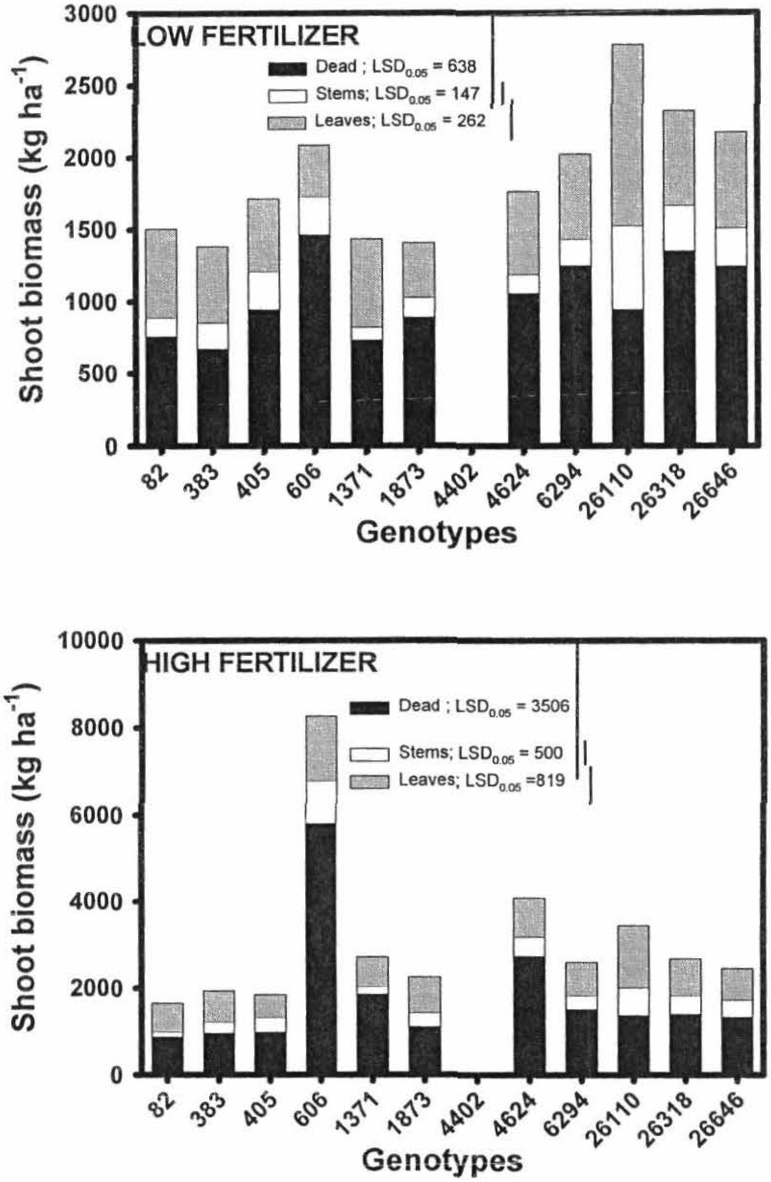


Figure 1. Genotypic variation as influenced by fertilizer application in dry matter distribution among green leaves, stems and dead biomass of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 33 months after establishment (at the end of the dry season – April 2002). LSD values are at the 0.05 probability level.

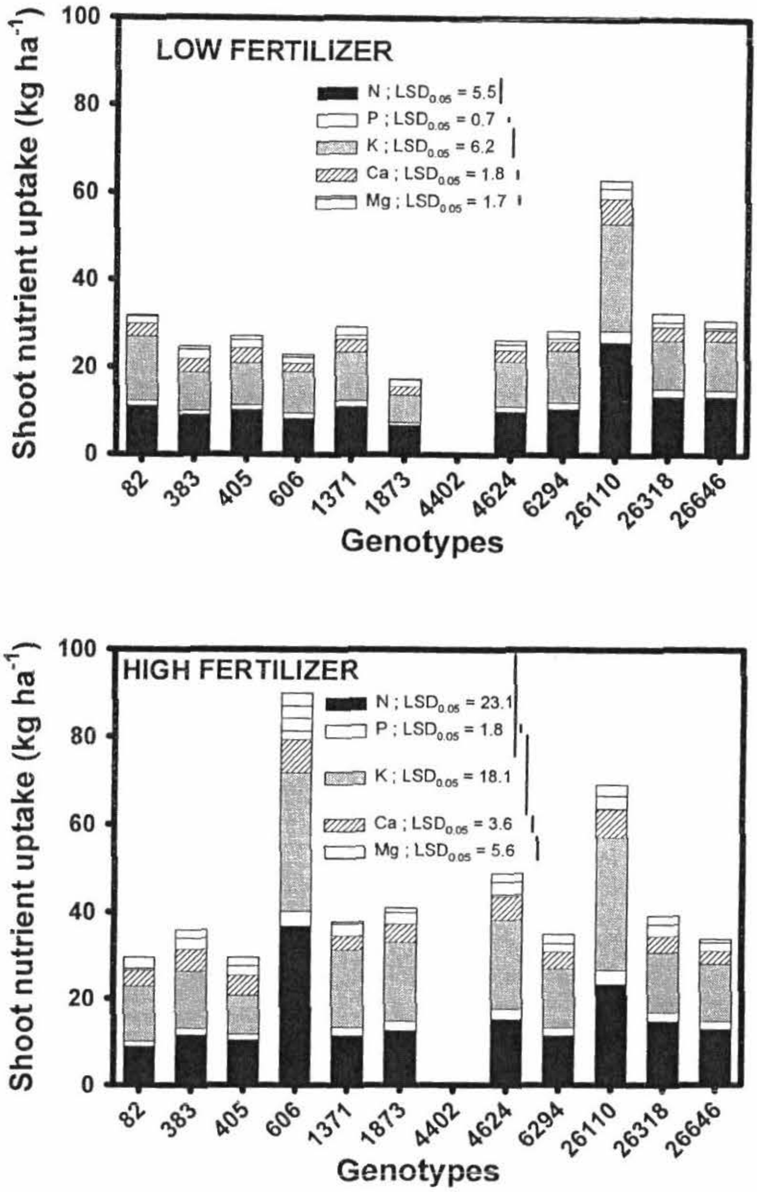


Figure 2. Genotypic variation as influenced by fertilizer application in nutrient (N, P, K, Ca, Mg) uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 33 months after establishment (at the end of the dry season – April 2002). LSD values are at the 0.05 probability level.

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 33 months after establishment (at the end of the dry season - April 2002). 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	1.51	1.26	1.34	0.88	11.07	9.02
BR97NO-0383	1.47	1.34	1.05	0.79	8.98	11.68
BR97NO-0405	1.51	1.53	1.04	0.79	1014	10.47
FM9201-1873	1.52	1.33	1.06	1.08	6.68	12.58
FM9301-1371	1.63	1.47	1.33	1.01	10.92	11.56
FM9503-5046-024	1.45	1.29	1.11	0.82	9.77	15.26
Parents:						
CIAT 606	1.72	1.41	1.07	1.01	8.25	36.88
CIAT 6294	1.47	1.12	0.92	0.89	10.48	11.47
BRUZ/44-02	ND	ND	ND	ND	ND	ND
CIAT 26646	1.64	1.31	0.89	0.9	13.38	13.0
Accessions:						
CIAT 26110	1.58	1.28	1.13	0.79	26.01	23.28
CIAT 26318	1.65	1.31	0.91	0.82	13.29	14.78
Mean	1.56	1.33	1.08	0.89	11.73	15.45
LSD *(P=0.05)*	NS	NS	0.26	NS	5.46	23.1

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

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 33 months after establishment (at the end of the dry season - April 2002). 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	0.16	0.16	0.136	0.124	1.12	1.15
BR97NO-0383	0.12	0.16	0.097	0.119	0.86	1.45
BR97NO-0405	0.15	0.17	0.096	0.098	1.07	1.30
FM9201-1873	0.13	0.17	0.103	0.135	0.65	2.12
FM9301-1371	0.18	0.23	0.153	0.188	1.34	1.83
FM9503-5046-024	0.16	0.20	0.132	0.112	1.06	2.31
Parents:						
CIAT 606	0.21	0.17	0.139	0.116	1.12	3.27
CIAT 6294	0.19	0.17	0.155	0.132	1.39	1.71
BRUZ/44-02	ND	ND	ND	ND	ND	ND
CIAT 26646	0.17	0.17	0.098	0.103	1.41	1.69
Accessions:						
CIAT 26110	0.14	0.17	0.138	0.172	2.55	3.31
CIAT 26318	0.18	0.17	0.128	0.112	1.73	1.95
Mean	0.16	0.18	0.126	0.128	1.30	2.01
LSD *(P=0.05)*	0.042	NS	0.046	0.058	0.68	1.79

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 33 months after establishment (at the end of the dry season - April 2002). 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	14.83	12.69	2.91	3.68	1.94	2.95
BR97NO-0383	8.88	13.20	3.08	5.14	2.76	4.39
BR97NO-0405	9.76	8.92	3.40	4.71	2.71	4.14
FM9201-1873	6.23	18.29	2.01	4.26	1.72	3.64
FM9301-1371	11.16	17.85	2.91	3.27	2.88	3.30
FM9503-5046-024	10.49	20.65	2.54	5.43	2.24	5.11
Parents:						
CIAT 606	9.61	31.63	1.99	7.64	1.95	10.50
CIAT 6294	12.05	13.75	2.13	3.89	2.32	4.05
BRUZ/44-02	ND	ND	ND	ND	ND	ND
CIAT 26646	11.48	13.26	2.51	2.99	2.12	2.76
Accessions:						
CIAT 26110	26.47	30.46	5.84	6.40	4.10	5.59
CIAT 26318	11.22	13.89	3.12	3.85	3.07	4.66
Mean	11.85	17.69	2.95	4.66	2.53	4.64
LSD *(P=0.05)*	6.180	18.09	1.819	3.614	1.732	5.592

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 association was observed between green leaf biomass and level of nutrients in green leaves.

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)	0.97***	0.99***
Total forage yield (live + dead) (t/ha)	0.79***	0.89***
Dead biomass (t/ha)	0.41**	0.82***
Stem biomass (t/ha)	0.77***	0.93***
Leaf N content (%)	-0.26	0.27
Leaf P content (%)	0.14	-0.10
Leaf K content (%)	0.02	-0.09
Leaf Ca content (%)	-0.13	-0.32*

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

Conclusions

Results from this field study indicated that the superior performance of the germplasm accession CIAT 26110 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.

[illegible] aumir

Activity 2.1.6

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

Contributors: I. M. Rao, J. Miles, C. Plazas and J. Ricaurte

amilo

Rationale

Previous research 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 4 *Brachiaria* hybrids (BR98NO/1251; BR99NO/4015; BR99NO/4132; FM9503-S046-024) along with 2 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 3 replications. The plot size was 5 x 2 m. A number of plant attributes including forage yield, dry matter distribution and nutrient uptake were measured at the end of dry season (April 2002; 4 months of moderate drought stress), i.e., at 11 months after establishment of the trial. The trial was managed with strong and frequent mob grazing at 2 months interval.

Results

At 11 months after establishment (4 months after dry sseason), live forage yield with low fertilizer application ranged from 900 to 1400 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 hybrid 4624 was superior to other hybrids in terms of green leaf production with both low and high initial fertilizer application. This is consistent with our previous results obtained for the past 3 years from another trial at the same location. It is important to note that one of the parents, CIAT 606, which is very well-known for its adaptation to low fertility soils, produced greater dead biomass under both low and high initial fertilizer application. This could be an important attribute for recycling of nutrients that contribute to superior persistence under low fertility conditions but may not be a desirable attribute for improved animal performance. The dry matter distribution pattern of the hybrid 4015 was in contrast with that of the parent CIAT 606. This hybrid produced very little 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.

The hybrid 4624 was also outstanding in its ability to acquire nutrients, particularly N and Ca with both low and high application of fertilizer (Figure 2). Results on nutrient uptake also showed that CIAT 6294, which is known for its moderate level of adaptation to infertile acid soils, was inferior to the 4 hybrids tested in terms of its ability to acquire nutrients, particularly N.

The results obtained on green leaf and stem N and P contents also indicate the superior nutritional quality of the hybrid 1251 under both high and low intial fertilizer application (Figures 3 and 4).

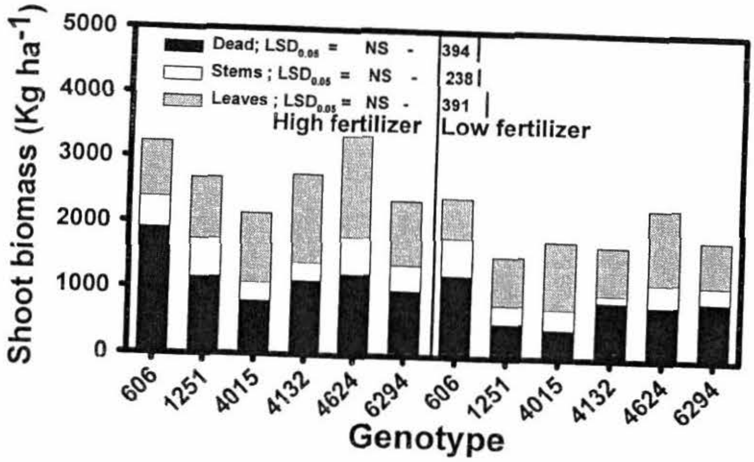


Figure 1. Genotypic variation as influenced by fertilizer application in dry matter distribution among green leaves, stems and dead biomass of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 11 months after establishment (at the end of the dry season – April 2002). LSD values are at the 0.05 probability level.

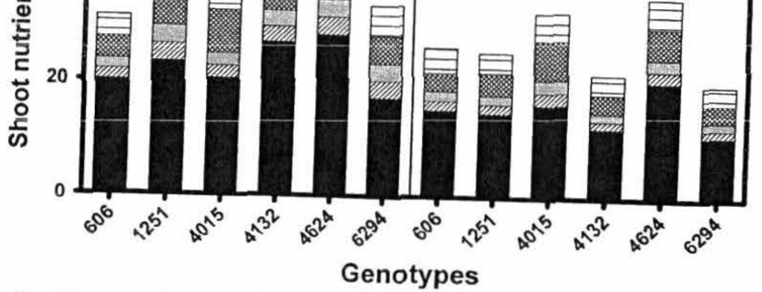


Figure 2. Genotypic variation as influenced by fertilizer application in nutrient (N, P, K, Ca, Mg) uptake of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 11 months after establishment (at the end of the dry season – April 2002). LSD values are at the 0.05 probability level. NS = not significant.

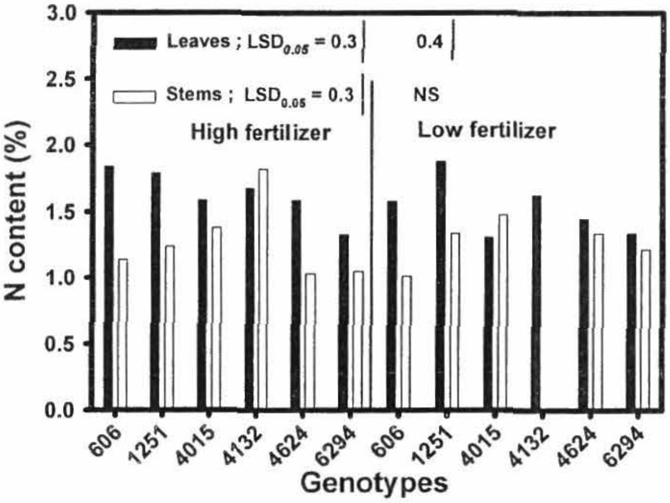


Figure 3. Genotypic variation as influenced by fertilizer application in nitrogen (N) content of leaves and stems of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 11 months after establishment (at the end of the dry season – April 2002). LSD values are at the 0.05 probability level.

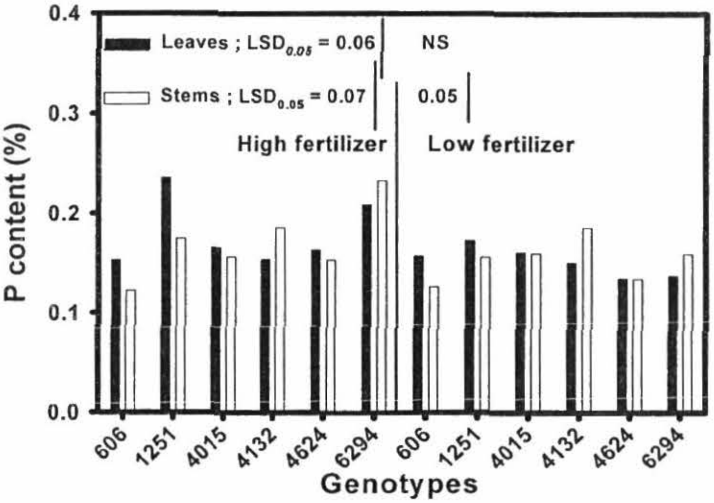


Figure 4. Genotypic variation as influenced by fertilizer application in phosphorus (P) content of leaves and stems of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 11 months after establishment (at the end of the dry season – April 2002). LSD values are at the 0.05 probability level.

Conclusions

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.

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

Activity 3.1 Study of the processes and controls involved in fluxes of GHG between the soil and the atmosphere

ensuke

Activity 3.1.1 – The biological phenomenon of nitrification inhibition in *Brachiaria humidicola*

Contributors: G.V. Subbarao, T. Ishikawa, K. Okada and O. Ito (JIRCAS) and I.M. Rao (CIAT)

104983

Rationale

Ammonium-N is transformed into nitrite-N and nitrate-N by soil microorganisms, a process known as nitrification. By controlling nitrification in soils, it will be possible in future to reduce nitrogen fertilizer inputs into agricultural production systems and also minimize nitrate pollution in aquatic systems and ground water. We have demonstrated earlier that a tropical grass, *Brachiaria humidicola* that is widely adapted to lowland agro-ecosystems (savannas) of the humid and sub-humid tropics, particularly in South America, has the ability to suppress nitrification in soil and emission of nitrous oxide to the atmosphere (Ishikawa et al., 2001; 2002 a, b, c). This year, we tested the hypothesis that root exudates of this tropical grass have the ability to suppress nitrification.

Methodology

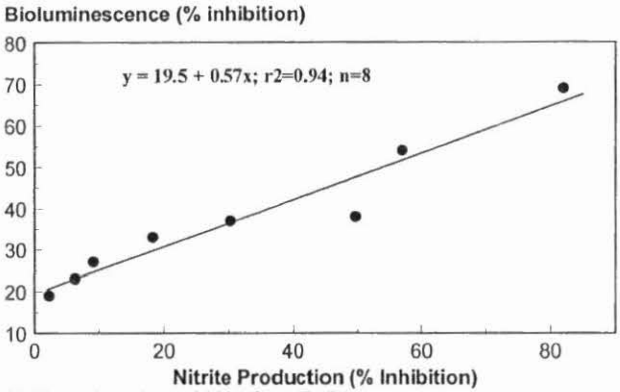
Bio-assay methodology - calibration and refinement: The basic methodology was adopted from Izumi et al. (1998), which is developed to detect nitrification inhibitors in the municipal-waste water treatment plants. This methodology was improved and calibrated to detect nitrification inhibition in the root exudates of *Brachiaria humidicola*. Different luminometer settings involving integral time of the measurement, the time to initiate the measurement after injection of the sample, the incubation time of the bacterial culture with the root exudates were tested during this phase to standardize the methodology to get reliable and repeatable data.

Root exudates collection, concentration and characterization for inhibitory activity: Using non-destructive root exudates collection techniques, root exudates were collected at weekly intervals from hydroponically grown plants. For collecting root exudates, the styroform block containing plants from each hydroponic tank is placed in 5L of distilled water for 24 h. Intact plant roots were washed initially with tap water and then with deionised water before using them for collecting root exudates. After 24 h of root exudates production in deionized water, plants were removed and returned to the hydroponic tanks, and the collected root exudates were stored at 4°C until further processing for the bioassay. This experiment was continued for about 5 months.

Protocol for bioassay using luminometer for detecting nitrification inhibition: The *Nitrosomonas* bacterial cells were concentrated by centrifuging at 10,000 g. Usually 600 ml *Nitrosomonas* broth culture of 10 d old is concentrated to 10 ml, and then suspended in fresh P buffer before the bioassay. For the bioassay, 250 μL of the concentrated *Nitrosmonas* culture is transferred to a test tube, and 20 μL of the test compound (either root exudates processed sample or rhizosphhere soil aqueous extract or phenolic test compound) is added, and incubated at the room temperature for 30 min. After incubation, then 100 μL of this *Nitrosomonas* with test compound mixture is injected into luminometer after adding 2.5 μL dicylaldehyde into luminometer to determine the bioluminescence. Every measurement is repeated four times and the average of these four measurements is used for presenting the data for each sample.

Results and Discussion

Bioassay methodology: The bioluminescence of *Nitrosomonas* showed a distinct two peak pattern during the 30 seconds time after the injection of the sample; the first peak was substantially larger than the second one and lasted about 15 seconds, thus 15 second integral time gave relatively consistent results compared with other integral times tested. Also, 2.2 seconds delay time for the initiation of the measurement after injection of the sample was found out to be the best condition. For the incubation time, 30 minutes was ideal to get consistent and stable data. With these settings in place for luminometer and incubation time, the bioassay method is found to be stable and measurements have <5% coefficient of variation. Also, the bioluminescence, which is an indicator of the metabolic status of the *Nitrosomonas* bacteria, was closely correlated with nitrite production ($r^2 = 0.94$) (Figure 1). This suggests that by measuring bioluminescence, we can quickly assess the metabolic status of the bacteria and evaluate whether the test compound (i.e. root exudates or tissue extract or synthetic chemical) has inhibitory effect on the functioning of *Nitrosomonas*, which derives its metabolic energy from the conversion of NH_3 into nitrite.



Allylthiourea is used as an inhibitor for nitrification

Figure 1. Relationship between *Nitrosomonas* activity and nitrite production in the incubation medium

Root exudates activity at various growth stages of B. humidicola: The nitrification inhibition activity increased with plant growth and reached close to maximum (i.e. >95% inhibition) at the time of heading stage of the plant growth, remained stable for the rest of the growth period (Figure 2). The specific inhibitory activity (i.e. inhibitory activity g^{-1} root dwt) decreased with plant age. The total activity per plant was maintained because of higher root mass with time. Also, root exudates collected from field grown (EMBRAPA research station experimental fields in Campo Grande, Brazil) plants of *B. humidicola*, and rhizosphere soil extracts showed inhibitory activity on nitrification (data not presented). Thus root exudates from hydroponically grown- and field -grown plants showed nitrification inhibition, thus confirming this phenomenon for the first time.

Our study is perhaps the first comprehensive study to demonstrate the biological phenomenon of nitrification inhibition using both hydroponically grown and field grown *Brachiaria humidicola*. Several studies in the past have hypothesized such a possibility based on empirical observations, but was never demonstrated through experimental evidence. Also, our research has produced a reliable technique to detect and also quantitatively determine nitrification inhibition activity in root exudates, plant tissue extracts or rhizosphere soil water extracts.

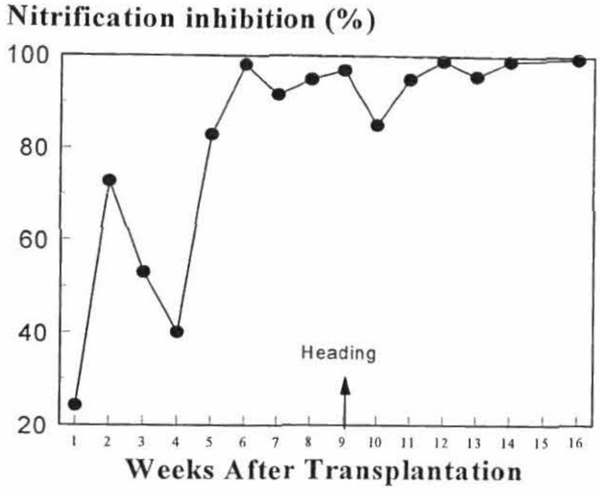


Figure 2. Total nitrification inhibition activity of the root exudates at various growth stages of *B. humidicola*

One of the most important challenges for nitrification inhibition research is the absence of a reliable technique that can detect nitrification inhibition activity rapidly and reliably. When published, our methodology to detect nitrification inhibition would become a standard procedure for researchers who wish to explore/exploit this research area in future. Also, this technique will become a powerful tool in exploiting the research potential of the biological nitrification inhibition phenomenon from a genetic improvement perspective. These technical tools are necessary and perhaps essential to develop more efficient agro-pastoral systems based on grasses such as *B. humidicola* for tropical savannas of Latin America. It appears that *B. humidicola* not only is well-adapted to infertile and poorly drained acid soils but also conserves and utilizes nitrogen more effectively because of its ability to inhibit nitrification in soils.

The nitrification inhibitory activity in the root exudates of *B. humidicola* increased with plant growth and appeared to be following in a similar fashion to normal growth curve of root or shoot. Thus, root mass in *B. humidicola* can to some extent determine the degree of nitrification inhibition activity. Nevertheless, the specific nitrification inhibitory activity (i.e. NI activity g^{-1} root dwt) decreased with the plant age (data not shown). Further studies are underway to isolate the active compound from the root exudates and determine its structure and possible metabolic pathways of synthesis.

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Activity 3.1.2

Tropical forage grasses and their influence on inhibition of nitrification and emission of nitrous oxide from acid soil

Marco Antonio Carlos Edmundo Kensuke

Contributors: M. Rondon, I. M. Rao, C. Lascano, E. Barrios (CIAT); T. Ishikawa, G. V. Subbarao, K. Okada and O. Ito (JIRCAS, Japan)

Rationale

Tropical grasses, both native and improved, account for most of pastureland in the tropics. Among the tropical grasses, *Brachiaria* species are widely adopted, particularly in Latin America where they are planted on about 70 million hectares. Their prime merits of adaptation to infertile acid soils, high yield, and ease of propagation have allowed a major economic transformation of vast areas of unproductive tropical savannas, not only by directly sustaining ruminant livestock production, but also as base of viable production systems that include annual crops. One of the most important cultivars of *Brachiaria* grasses is *B. humidicola* CIAT 679 cv. *Humidicola (koroniviagrass).*

The Soil Microbiology section of the Tropical Pastures Program (TPP) of CIAT reported inhibition of nitrification in the soil under *Brachiaria humidicola* CIAT 679 (CIAT Annual Reports of TPP – 1983, p. 211; 1985, p. 224; 1986, p. 194). Based on this research work, a paper was published showing that under pure established grasses very little nitrification occurs in Carimagua (acid soil of the Llanos of Colombia) soil where as under bare soil and under legumes high rates of nitrification are observed (Sylvester-Bradley et al., Soil Science 39: 407-416, 1988). Further research showed that: (i) the inhibitory effects on nitrate accumulation observed are a direct effect of the grass roots on nitrification and not an indirect effect of recycling of litter; (ii) rates of N mineralization under N fertilized *B. humidicola* CIAT 679 and *B. dictyoneura* CIAT 6133 were lower than those under N fertilized *B. decumbens* CIAT 606; and *B. humidicola* CIAT 679 reduces net total mineralization and specifically inhibits the conversion of ammonium-N to nitrate-N (CIAT Annual Report of TPP 1989, p. 10-23). A study conducted by soils/plant nutrition section of the TPP showed that *B. humidicola* CIAT 679 takes up nitrogen as the ammonium ion rather than as nitrate as many plants do (CIAT Annual Report of TPP 1984, p.190). The ability of *B. humidicola* CIAT 679 to take up ammonium for plant growth and inhibit nitrification in soil could contribute to its superior adaptation to very infertile acid soils of the tropics. Recent work by JIRCAS colleagues has shown that *B. humidicola* CIAT 679 inhibits nitrification of ammonium and reduces the emission of nitrous oxide into the atmosphere (IP-5 2001 Annual Report). The release of nitrous oxide from the soil comes about in the process of nitrification as plant residues decompose. A corollary of *B. humidicola*'s capacity to inhibit nitrification is to reduce nitrous oxide emissions compared with other tropical grass species.

In general, pastures of *B. humidicola* remain remarkably free of weeds as long as the pasture is reasonably vigorous. It is at least plausible that this occurs because the grass inhibits nitrification and in doing so denies nitrate to invaders. Weeding is a major cost in most agricultural systems, for cultivation and/or herbicide application in mechanized systems, and labour inputs in subsistence systems. If nitrification inhibition is the reason why *B. humidicola* CIAT 679 pastures remain weed free and if the precise mechanism can be identified, it might be possible to incorporate the characteristic into other species and make them essentially weed resistant. If the gene can be identified, it may be found or introduced in other plants so that it might be possible to 'switch on' the enabling mechanism by genomics techniques. The possibilities are mind-boggling, and potentially could affect most agricultural production with huge economic impact. This would be in addition to any potential mitigation by reducing the evolution of oxides of nitrogen, which are powerful greenhouse gases, during the nitrification process

Given these findings with one genotype of koroniviagrass, 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*, 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). 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 6 different tropical grasses were used as test plants at wo levels of ammonium sulfate application (0 and 100 kg/ha). Six tropical 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 desin with 4 replications. Plants were allowed to grow for 6 weeks and were cut to 10 cm to stimulate regrowth for 2 weeks and were cut again at 10 cm height to allow regrowth and to simulate grazing effects under field conditions. After another 10 days of regrowth ammonium sulfate was applied in solution to monitor the processes of nitrification and emission of greenhouse gases. To monitor flux measurements of gases (CH_4, CO_2 and N_2O), a closed chamber was adapted to tightly fit the pots during the 30 minute period of measurement. Gas samples were collected using glass syringes. Samples were analyzed within 36 hours of collection. Analysis of CH_4, CO_2 and N_2O were made in the same sample by combined FID-ECD gas chromatography. (Shimadzu, CG14A, Valco 14 port two-position valve). Just before application of the ammonium sulfate, soil samples were collected using a core sampler (2 cm diameter) across the soil depth. Soil was homogenized and analyzed for NO_3 and NH_4 contents. Similar procedure was used for all experimental pots at 2 weeks after application of the N fertilizer. At the end of the experiment, plants were harvested and separated into shoot and roots. Leaf area was determined using a leaf area meter. 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

The samples are being analyzed and the results will be reported next year.

Activity 3.2 Case study assessment of integrated GWP for key tropical agroecosystems

Activity 3.2.1 – Effects of Land Use Change in the Llanos of Colombia on Fluxes of Methane and Nitrous Oxide, and on Radiative Forcing of the Atmosphere

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Introduction

The Eastern plains (Llanos) of Colombia cover an area of 26 million ha and are the only land in Colombia available for agricultural expansion. The climate is sub-tropical with distinct wet and dry seasons. The natural landscape is a mosaic of grass covered, rolling savanna dissected by numerous rivers and streams that are bordered by evergreen gallery forest. Small termite mounds are abundant in the grasslands. The gallery forest accounts for nearly 10% of the total area (Rippstein and Girard, 1995). Low soil fertility and frequent natural or induced fire prevent forest from re-colonizing the land (Rippstein et al., 2001). Over the past two centuries the native grassland has been grazed by low productivity livestock and currently supports 3 million cattle (Rivas, 2000).

The soils of the Llanos are very acid (pH <4.3) Oxisols (Typic Haplustox), low in nutrients and very high in aluminum saturation (>80%). There are two contrasting textural groups; approximately 90% of the soils are clay-loams and 10% are sandy-loams. The soils are generally poorly drained and prone to compaction that can be induced by tillage or by cattle trampling (Amézquita, 1998). Despite these limitations, agricultural activities have intensified in the region during the last decade. Oil palm, rice, maize, soybeans, sorghum and improved pastures are now appearing, especially in the vicinity of the main cities and connecting roads. Intensification will be accelerated in the coming decades and various crops and management strategies have been developed to allow sustainable use of the Llanos (Friesen et al., 1997). The most promising among these are improved pastures with mixtures of grasses (e.g, *Brachiaria sp.*) and forage legumes (e.g. *Desmodium ovalifolium, Arachis pintoi, Centrosema acutifolium*), and rotations of cowpea or soybeans as green manures with varieties of upland rice or acid tolerant maize (Friesen et al., 1997b).

The Llanos is a complex ecosystem from the perspective of exchange of greenhouse gases between the land and the atmosphere, and agricultural intensification can be expected to have a large impact on these exchanges. In the natural system, fire and termites cause emissions of CO_2, CO, CH_4, NO, N_2O and volatile organic compounds to the atmosphere (Delmas, 1997;). Cattle are important sources of CH_4 and forage quality has a large impact on the amount of CH_4 emitted per unit of live weight gain or milk production. Soils are sources or sinks for carbon, N_2O and CH_4. Improved pastures have shown major increases in carbon stocks in soils of the Llanos (Fisher et al., 1994). Several studies in temperate ecosystems have found that the conversion of natural lands to agriculture may reduce oxidation rates of CH_4 and/or increase emissions of N_2O by soils (Sitaula et al., 1995; Mosier et al., 1991; Davidson et al., 1995, Willison et al., 1995). High doses of N-fertilizers stimulate N_2O emission but can reduce CH_4 oxidation (Bronson and Mosier, 1994). Less information is available for soils of the tropics; Nobre (1995) found that the conversion of native Cerrados in Brazil to high input agriculture increased N_2O emissions by a factor of ten relative to natural environments. On the other hand, Lauren et al. (1995) found little impact of land conversion to pasture or cropland on CH_4 and N_2O fluxes from a soil near Brasilia. Soils at this site were net sinks for atmospheric CH_4 throughout the year and oxidized from three to six times more CH_4 than similar land uses in temperate regions. Emission rates of N_2O were very low compared to temperate system counterparts. Venezuelan savannas are believed to be small net sources of CH_4, although only short-term measurements have been reported (Sanhueza et al., 1995).

Changing land use in the Llanos will influence more that one of the factors responsible for exchange of greenhouse gases with the atmosphere. Conversion of savannas to pastures or cropland eliminates fire and reduces the population of termites, thereby reducing emissions of CO_2, CO, CH_4, NO, N_2O and volatile organic compounds. Improved pastures will enhance carbon sequestration in soils and the higher quality forage will reduce emissions of CH_4 per unit of production. However, improved pastures also allow higher stocking rates, leading to increases in CH_4 emission by cattle per unit area of land. Conversion of land to cropland is expected to reduce the soil CH_4 sink strength and increase N_2O emissions. The objectives of this study were to assess annual fluxes of N_2O and CH_4 for the main land uses found in the Llanos, to use this and other data to make estimations of the radiative forcing potential (Global Warming Potential (GWP)) of this ecosystem under current conditions and to predict the effect of expected changes in land use over the next two decades on regional GWP. Results can be used to inform policy makers on decisions affecting the future development of the region.

Materials and Methods

Experimental Sites. Field research was conducted at the Corpoica-CIAT Carimagua station, in the middle of the Colombian Llanos (4° 37' N latitude; 71° 19' longitude). The altitude is 175 masl, and annual rainfall and mean temperature were 2498 mm and 28°C respectively for the period of the study (Nov 1997 to Dec 1998). Oxisols of two contrasting textures, a clay-loam (18% sand, 47% silt, 35% clay) and a sandy-loam (64% sand, 17% silt, 19% clay), typical of the Llanos were used in this study.

Six study sites representing native and agricultural systems were chosen. Four sites were selected from treatments in a long-term agro-pastoral/crop rotation experiment that was established on a clay loam soil in 1992 to evaluate the sustainability of several cropping and management systems in the Llanos. Details of the experiment are presented elsewhere (Friesen, 1996). The systems used were: native savanna, legume-grass pasture (B. *dictyoneura* + *A. pintoi*), upland rice as a monocrop and upland rice in a rotation with cowpea as green manure. Experimental plots were strips 20-m wide by 200-m long. A native savanna site on a sandy-loam soil (30-km southwest of the main site) and gallery forest site (9 km to the north of the main site) on a clay loam soil were also studied.

Soil properties

A detailed characterization of soil physical and chemical parameters to a depth of 30 cm was performed in May 1998. Bulk density was measured by the sand replacement method and resistance to penetration by a cone penetrometer. Both parameters were measured using procedures described by Smith and Mullins (1991). Four replicate undisturbed core samples were taken at 5-cm depth increments for determination of air permeability following the method of Koorevaar (1983). Samples were collected in metal cylinders (5-cm diameter, 5-cm height) that were capped immediately after collection. Saturated hydraulic conductivity, particle size distribution, soil porosity and pore size distribution were measured as described by Smith and Mullins (1991) and susceptibility to uniaxial soil compaction as described by Culley (1993). Chemical parameters measured were pH (water 1:1), soil organic matter (wet combustion), N content (micro Kjeldahl), and available P (Bray II). Chemical analyses followed the procedures of Hendershot et al. (1991). In April and October 1998, levels of nitrate and ammonium were determined in samples collected down to 1-m depth.

Gas Flux Measurements

The vented closed chamber technique (Conen and Smith, 1998; IAEA, 1992) was used to monitor fluxes of CH_4 and N_2O between the soil and the atmosphere. Four replicate PVC rings (10-cm high by 30-cm diameter) per treatment were permanently inserted 7-cm into the soil. White polyethylene vented chambers (10 cm high and 30 cm diameter) were attached to the rings just prior to each 1-hour measurement period. A 5 cm wide rubber band cut from a tire innertube was used to seal the joint between the chamber and the ring. The top of a chamber was fitted with a septum to facilitate extraction of gas samples, a hole for insertion of a digital thermometer, and a venting glass tube to prevent pressure differentials between the chamber and the atmosphere. The dimensions of the venting tube (0.5 cm diameter, 8 cm high) were selected as described by Hutchinson and Mosier (1981), and the tube was inserted 5 cm inside the chamber.

To reduce temperature increases within chambers, a reflecting white cover was placed over the chamber during the period of sampling. Gas samples were collected in 20 mL teflon-valved glass syringes at 0, 20, 40 and 60 minutes after installing the chamber. Immediately after collection, 15 mL of gas were transferred to high vacuum pre-evacuated glass containers (10 mL in volume). The glass containers were pre-evacuated to $2\text{-}3\times10^{-6}$ mbar, using a freeze dryer that also allowed capping of the evacuated vials with butyl rubber caps, which are impermeable to CH_4 and N_2O (IAEA, 1992). An aluminum over-cap was crimped over the rubber cap to effectively seal the flask and avoid accidental opening. Preservation of samples in these containers was found to be longer than 6 months, however analysis of samples was always completed within four weeks of collection.

At the time of gas sampling, composite soil samples (0-10 cm) were collected from around each chamber for determination of moisture content. Soil temperature was measured within the area of the chamber just before and after the sampling period. Air temperature inside the chamber was recorded at every sampling interval to later account for the change in the density of the air inside the chamber as a function of temperature. Gas flux measurements were initiated on November 1997. Sampling frequency was at three-week intervals, except for the first month when three samplings were made. The order and time of sampling was standardized; sampling was started at 8 am, 9:30 am, 11 am and 2:00 pm for the sites under clay-loam savanna, rice, rice-cowpea and pasture respectively. Samples from the sandy-loam savanna and the gallery forest were collected the next day beginning at 10 am and 3 pm respectively.

A Shimadzu model 14A gas chromatograph (GC) equipped with FID and ECD detectors was set up to simultaneously analyze CH_4, N_2O and CO_2 in the same sample, while venting N_2, O_2 and H_2O (Rondón, 2000). A 14 port, two-position valve (Valco Instruments) was used to inject samples via a 2-mL gas loop and to re-direct the stream of gas to the ECD detector just after the CH_4 peak was obtained with the FID detector. The system used a pre-column (1-m) and a main column (3-m), both were 1/8 inch stainless steel tubing filled with Porapaq Q (80-100 mesh), and a carrier gas (N_2) flow rate of 25 mL min^{-1}. An electrically operated pneumatic actuator was used to precisely time the switching of the valve. CLASS VP software (Shimadzu) was used to control the actuator as well as the GC. The GC temperatures were 24°C at injection port, 60°C for the columns and 320°C for the ECD detector. For each gas sample, a 3 mL sub-sample was withdrawn from the glass vials using a 5 mL glass syringe fitted with a Teflon valve and injected into the GC via the sample loop. The existence of positive pressure inside the vials was checked at the time of sample withdrawal. Analysis time per sample was 5.5 minutes. Gas retention times were 2.1 minutes for CH_4, 3.8 min for CO_2 and 4.8 minutes for N_2O. Class VP software was used to calculate the concentrations (ppm) of the gases relative to the standards. Compressed air and Scotty prepared mixtures containing 1 and 3 ppm CH_4 and 0.9 and 5 ppm N_2O were the most commonly used standards. For samples high in CH_4 or N_2O, other standards were used (i.e. 10, 100 ppm CH_4 and 10, 100 ppm N_2O) as appropriate.

Gas Flux Calculations and Statistical Analysis

Differences in gas concentrations over time were used to calculate gas flux to/from soil for each chamber using the condition of a linear increase for at least three points to accept a flux measurement. When the linearity condition did not occur, the four samples were reanalyzed and the chamber was not considered in the calculations of flux averages if the problem persisted. For CH_4, fluxes are shown as net oxidation rates (net CH_4 consumption by the soil) with emissions from soil shown as negative values. For N_2O, positive fluxes represent emissions from soil. Annual fluxes were estimated by integrating the net area under the curve for the plot of gas flux over time. The method was applied to each of the four chambers in every land use, in order to calculate the reported average value. Annual fluxes represent the balance between periods of net emission and net consumption.

Comparisons of annual flux averages between land-uses were made by one-way analysis of variance using SYSTAT version 8 software. Comparison of means was done using Tukey's HSD method. A level of significance of $\leq 5\%$ was used.

Results and Discussion

Environmental and Soil Parameters. Weekly rainfall and minimum, maximum and average temperatures for the period of measurement are shown in Figure 1. Annual rainfall in the study year (2323 mm) was slightly higher than the 20-year average for the station (2150 mm). March was also wetter than usual while September was drier than usual. In general, the Llanos has a dry season from November to April and a wet season from May to October. A clear alternation of dry and wet days is common in the rainy season, which helps to prevent the soil from becoming waterlogged for long periods of time. The number of days without rainfall (222) exceeded that of rainy days (142) during the year. Towards the end of the dry season, strong solar radiation and frequent winds dried grassy vegetation. Deep rooted shrubs and the gallery forest, with the ability to extract water from deep in the soil, remained green throughout the year. Temperatures are fairly constant through the year with a maximum daily variation of 10°C. For most of the day, temperature remains in a narrow band from 26-29°C. No significant correlation was observed between air temperature and gas fluxes.

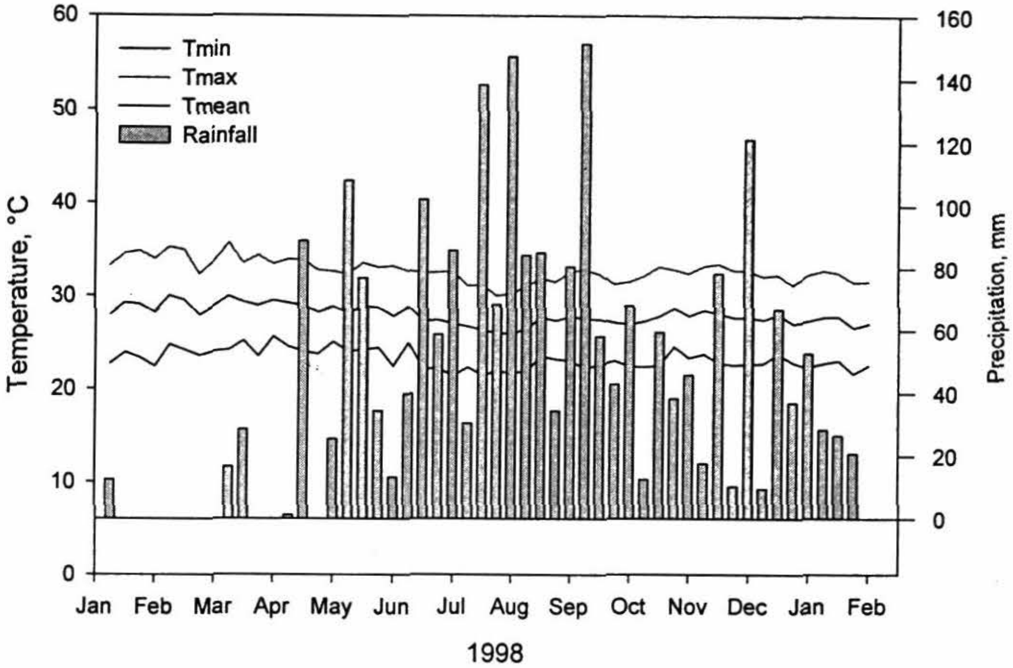

Figure 1. Weekly precipitation, maximum, minimum and average temperature at Carimagua Research Station. Tropical savannas of Colombia

The annual variation in soil water content, expressed as % water filled pore space (WFPS), in the top 10 cm of the soils is shown in Figure 2. The sandy-loam soil clearly has an intrinsically lower water retention capacity than the clay-loam soil and became extremely dry during the dry season (Figure 2A). Soil under the forest tended to be slightly drier than the clay loam savanna soil during the rainy season and slightly wetter during the dry seasonThis difference is likely a result of the combination of higher organic matter content (Figure 3G) and associated higher total porosity (Figure 3D) in the forest soil, together with reduced evaporation, as the soil is not directly exposed to sunlight. Soil under the pasture consistently retained more moisture than soils under other land uses (Figure 2B) and the difference is accentuated during the peak of the rainy season (June to August). Other studies have also found that pastures tend to retain more water than other land uses on the same soil (Roth, 1999; Keller et al., 1993). Water content in the cropped soils was very similar to that in the clay loam savanna, although laboratory measurements indicated that the amount of water that could be retained in the plow layer was reduced by cultivation (Figure 3B). Figure 3B also shows that conversion of land from savanna to pasture increased soil water retention capacity.

With the exception of the gallery forest soil, saturated hydraulic conductivity was generally low in the clay loam savanna soils, which explains the characteristic wetness of this environment. Poor drainage was exacerbated by conversion of savanna to pasture. Reduced water conductivity in the pasture (Figure 3C) is attributed to sub-surface soil compaction as can be seen from the increased resistance to penetration in the 5-10 cm depth (Figure 3J), probably as a result of cattle trampling. In fact, susceptibility to compaction (Figure 3F) is higher for the whole profile in the pasture compared to the corresponding savanna. Lower susceptibility values for the 5 to 10 cm and 20 to 25 cm layers of the pasture indicate that these layers are already compacted. Other researchers (Mosier and Delgado, 1997; Keller et al., 1993) have also reported subsurface compaction and increased water retention in pastures. As expected, hydraulic conductivity (Figure 3C) is higher in the sandy soils than in the clay-loam soils, demonstrating the influence of soil texture. The greatest hydraulic conductivity was found in the gallery forest soils, probably as a result of improved aggregation due to higher SOM contents, coupled with increased presence of root channels and faunal activity which, in turn, results in low soil bulk density values.

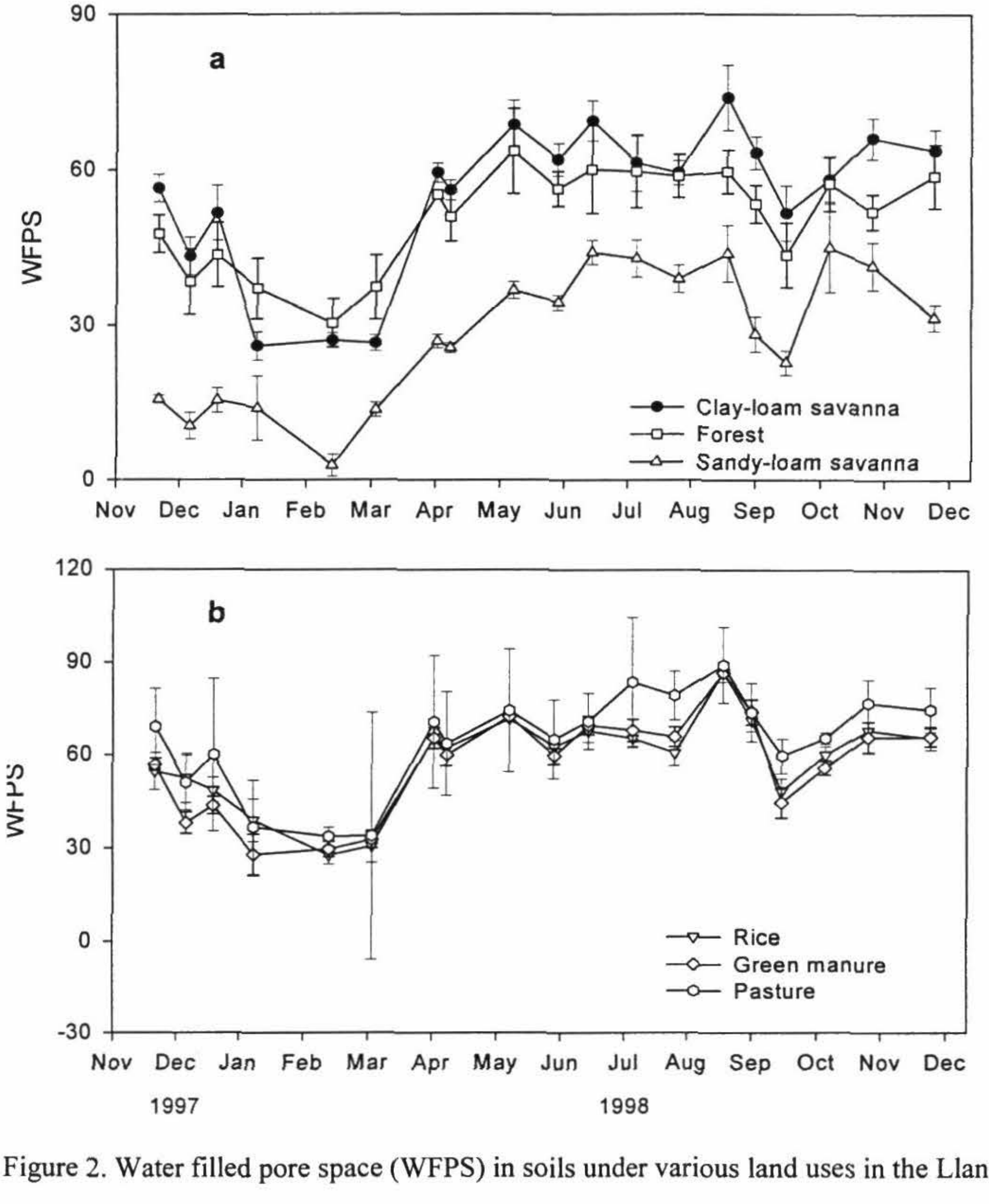

Figure 2. Water filled pore space (WFPS) in soils under various land uses in the Llanos

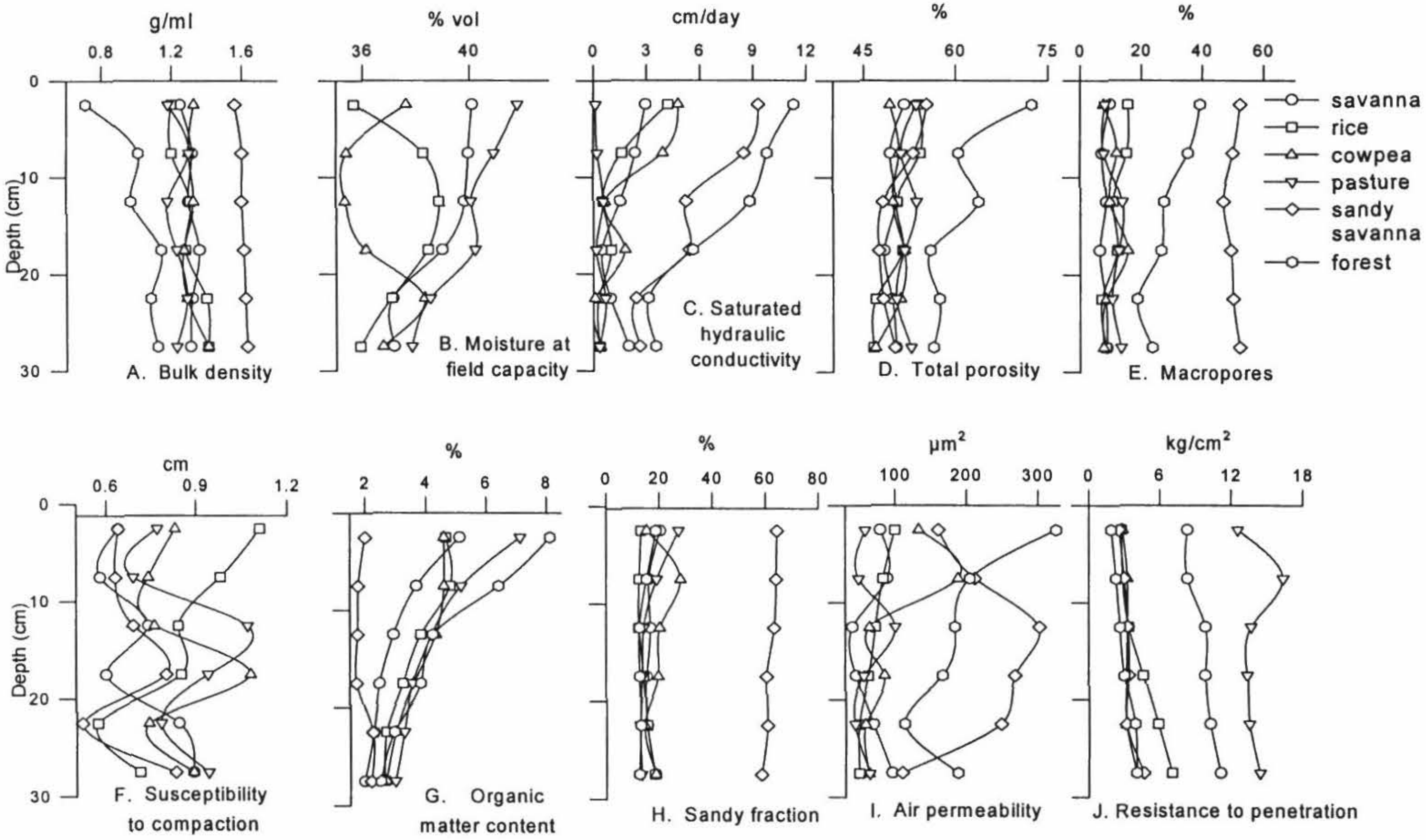

Figure 3. Selected soil properties for various land uses in Colombian savannas

Methane

Methane oxidation rates from November 1997 to December 1998 for soils under native vegetation and agricultural use are shown if Figures 4A and B, respectively. The temporal variation in CH_4 oxidation rate is high, with average values ranging from +120 µg CH_4 $m^{-2}h^{-1}$ for the forest in December to -320 µg CH_4 $m^{-2}h^{-1}$ (net emission) for pastures, in August. Spatial variability within a land use was also high, with RSD values ranging from 10% to 400%. However, for most of the sampling dates, RSD was in the range 60 to 120%, which is comparable to other studies on similar soils in the tropics (Poth et al., 1995; Cofman et al., 1998; Scharfe et al., 1990; Keller, 1994). The highest spatial variability was found with soils under rice, while forest and sandy savanna soils showed consistently lower variability.

Methane fluxes followed distinct patterns according to rainfall and soil moisture regimes. During the dry season (November to April), soils under all land uses were net sinks for atmospheric CH_4. Oxidation of CH_4 was progressively reduced as the soils became wetter in the rainy season (May to October). Soils of the clay loam savanna and the pasture eventually became a source of CH_4 during the period of peak rainfall (Figure 1) when soils were at their wettest (Figure 2).

The gallery forest soil consistently had higher CH_4 oxidation rates than the other ecosystems and none of the four sampling areas were ever a source of CH_4. Low bulk density (Figure 3A), high water infiltration rates (Figure 3C), high air permeabilities (Figure 3I) and low resistance to penetration (Figure 3J) are physical attributes of soil in the gallery forest that explain these results. In contrast, all other land uses on the clay loam soil have lower values than the gallery forest soil for air permeability and hydraulic conductivity through the 30 cm sampling depth. The clay loam savanna and pasture soils showed the most physical constraints to drainage and gas transport in the top 10cm of soil, consistent with these two systems becoming a source of CH_4 during the wet season. The pasture soil had the highest water filled pore space (Figure 2B) and the highest CH_4 emissions of all systems during the month of July. Converting the clay loam savanna into cropland, reduced CH_4 oxidation rates during the dry season (Figure 4A and B), probably as a result of reduced availability of water. On the other hand, tillage improved drainage and aeration, reducing the length of time that agricultural soils become anaerobic, which results in only small emissions from these soils in the wet season.

Soil texture was an important factor affecting CH_4 fluxes in the two savanna sites. Methane oxidation rate in the sandy savanna soil was lower than that in the clay loam savanna during the dry season, probably because low water retention (Figure 2) reduced the population and/or activity of CH_4-oxidizing bacteria (Boekx et al., 1997; Bottner 1985) relative to the clay loam savanna soil. The greater total porosity, and especially macro-porosity (Figure 3F and 3G), favored the prevalence of aerobic conditions in the sandy savanna soil, which was never a substantial source of CH_4.

Estimates of annual CH_4 fluxes are presented in Figure 5A. The gallery forest soil, with a sink strength of 3.05 kg CH_4 $ha^{-1}y^{-1}$, constituted the highest sink for CH_4. The CH_4 sink strength in the gallery forest is similar to values reported for a wet forest in Puerto Rico (Steudler et al., 1991), and other types of tropical forest in Central and South America (Keller, 1994; Keller and Reiners, 1994; Keller and Wofsy, 1986). The annual CH_4 sink strengths in the sandy and clay loam savanna soils were only about one-third and one-twelth of that of the gallery forest soil, respectively. Methane sink strength in the Llanos clay-loam savanna soil (0.26 kg CH_4 $ha^{-1}y^{-1}$) is similar to values reported for grasslands on Oxisols of Puerto Rico (Mosier and Delgado, 1997), and higher than values reported by Sanhueza et al. (1995) for comparable savannas in Venezuela, which were found to be a minor source of CH_4. However, methane sink strength on well aerated, high clay Oxisols of the Brazilian savanna (Cerrados) was 1 to 2 times higher than that of the Llanos savannas (Lauren et al., 1995).

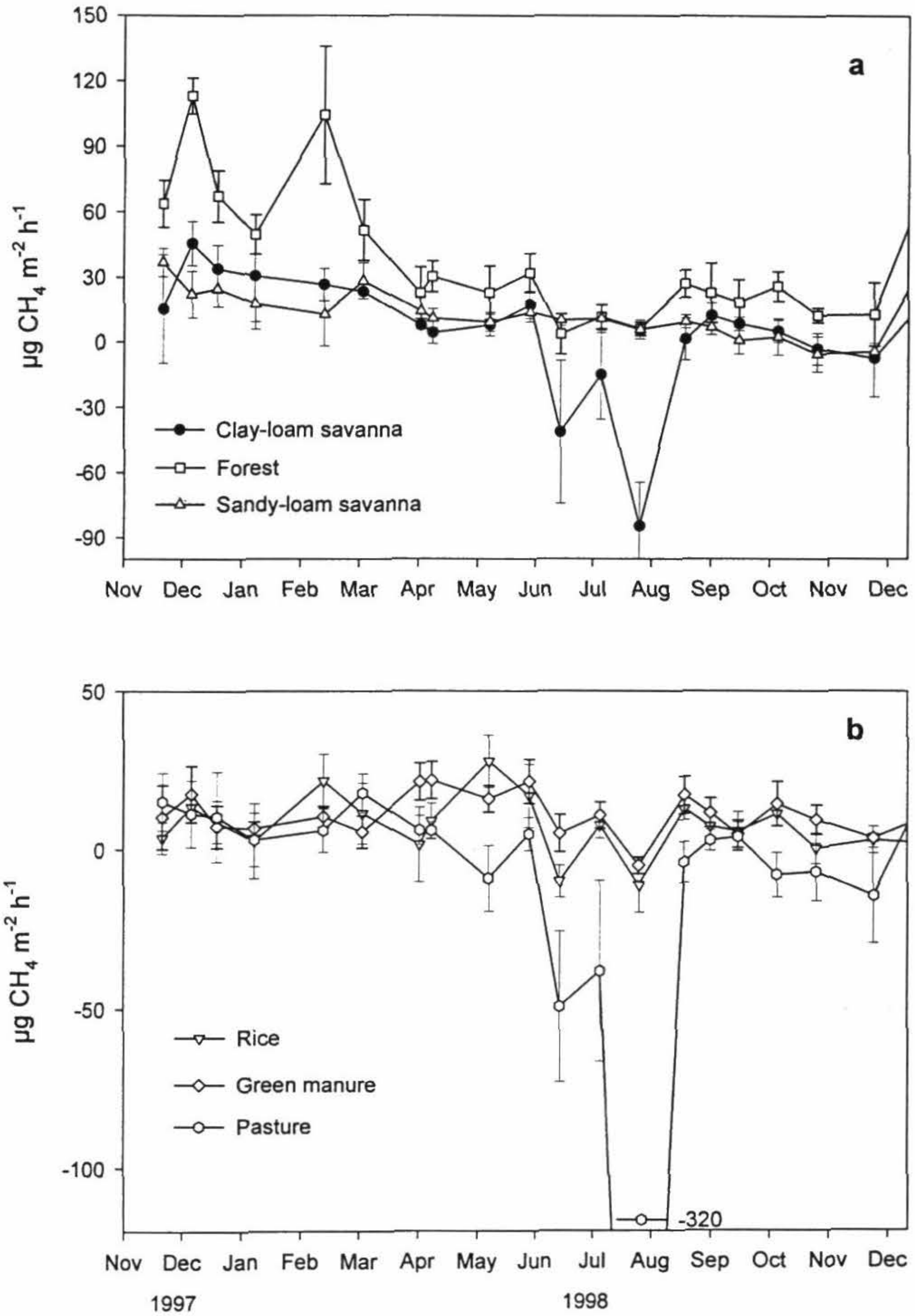

Figure 4. Land use effect on methane oxidation by soils in Colombian savannas. Positive numbers indicate net methane consumption by the soil (net sink)

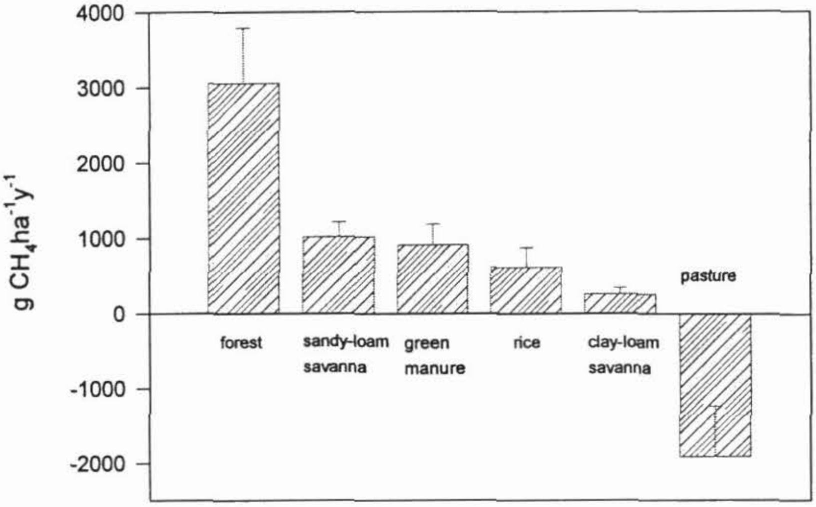

Figure 5. Annual methane oxidation by soils in Colombia savannas

Conversion of clay loam savanna to cropland increased CH_4 sink strength by 2.5x to x kg CH_4 ha^{-1}y^{-1} for rice mono-cropping and by 4x to y kg CH_4 ha^{-1}y^{-1}for the rice-cowpea rotation These results contrast with several reports (Sitaula et al., 1995; Mosier et al., 1991, Bronson and Mosier, 1994) showing a decrease in methane consumption rate when soils were fertilized with ammonium fertilizer sources. (However, information to specifically address effects of N status/source (fertilizer or green manure) on CH_4 oxidation capacity was not collected in the present study. Presumably, removal of physical constraints to gas exchange counteracted any negative effect of fertilizer on CH_4 oxidation rate.

Conversion of clay loam savanna to pasture changed the soil from a net sink to a net source (1.92 kg CH_4 ha^{-1}y^{-1}) of CH_4. A similar result was found for conversion of tropical forest in Costa Rica to pasture (Keller, 1983). Several other pastures sites in temperate (Van der Pohl, 1999) and tropical regions (Mosier and Delgado, 1997) have been found to be net sources of atmospheric CH_4. Keller and Reiners (1994) found that the condition of pastures greatly affected CH_4 emissions, with emissions from abandoned, degraded pastures being nearly five times higher than from pastures in good condition. The pasture plots used in the present study were 7-years old, originally planted with *B. dictyoneura* and *A. pintoi*, and supporting a stocking rate of 3 head/ha. Signs of pasture degradation were evident at time of sampling: low persistence of the legume, increased bulk density, subsurface compaction, reduced air permeability, occurrence of isolated termite mounds etc. Options are available to improve the management of these pastures in ways that reduce soil compaction and prevent or slow pasture degradation (Amezquita, 1998).

Nitrous Oxide

Fluxes of N_2O from soils of the native and agricultural systems over the one-year study period are shown in Figures 6a and 6b, respectively. Spatial variability in N_2O emissions was higher than that for CH_4, with RSD values mostly in the range of 100 to 200%, although values as high as 400% were sometimes found. This level of variability is similar to that found in other studies (Williams et al., 1999; Ruser et al., 1998; Veldkamp et al., 1998; Keller and Reiners, 1994).

Soils of all land-uses were net sources of N_2O for most of the year, although small sink strengths occurred in the sandy soil savanna at the end of the dry season and in the pasture soil at the end of the rains. Emission rates from natural ecosystem soils were generally low, with the gallery forest the main contributor. A peak of emissions was approximately coincident with the peak of the rainy season in the gallery forest. No consistent difference was found between the savannas on soils of contrasting texture. Observed low emission rates in native land uses may be partially the result of the small amount of nitrogen that is cycled in these nutrient-limited soils (Figure 7) and low soil pH, which reduces both nitrification and denitrification rates (Broadbent et al., 1965; Jackson, 1967).

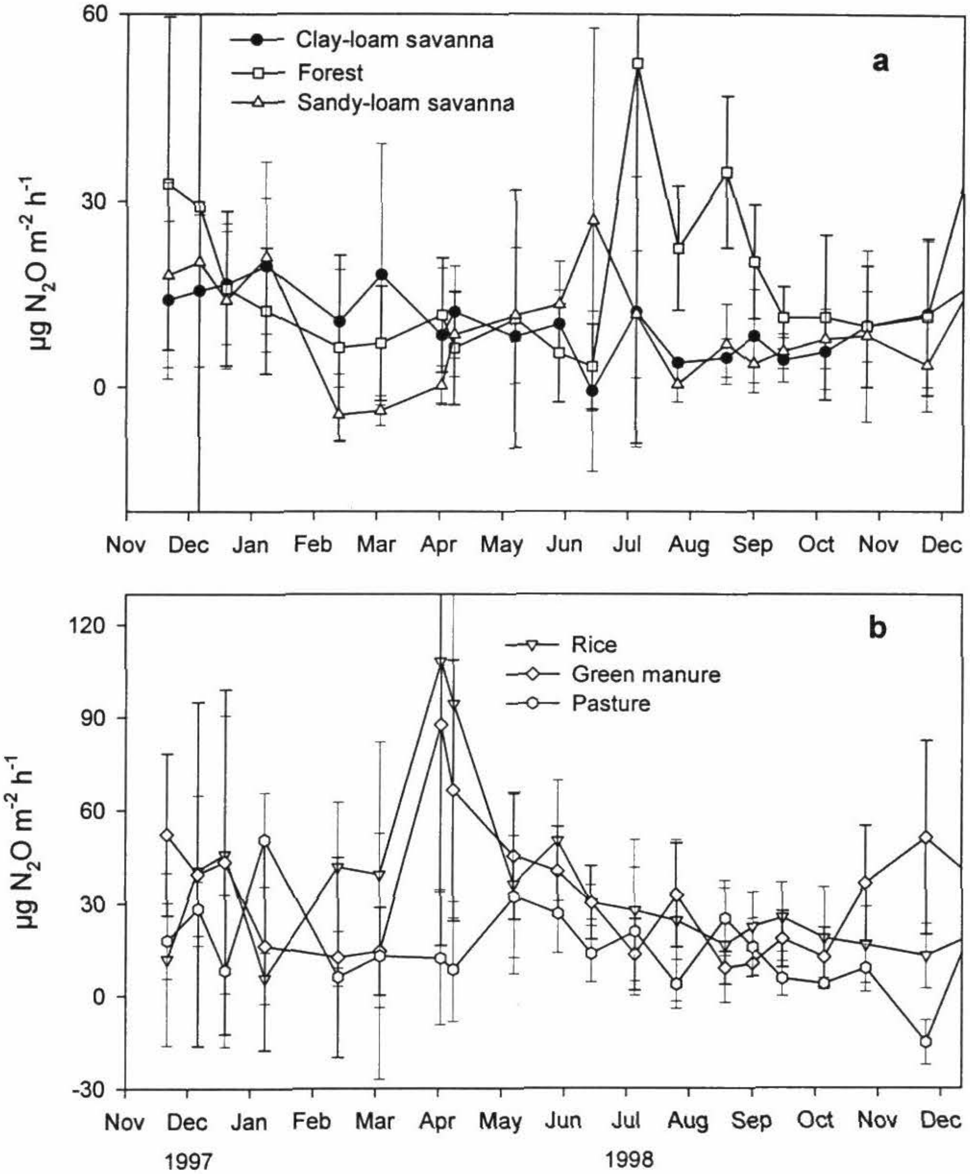

Figure 6. Land use effect on emission of nitrous oxide from soils in Colombian savannas

The increase in external nitrogen inputs and cycling increased N_2O emissions in all of the agricultural systems compared to the clay loam savanna. Biological N fixation contributes to the improved productivity of the grass-legume pasture (Thomas et al., 1997), and the cowpea green manure is estimated to supply 100-120 kg N $ha^{-1}y^{-1}$ in the rice-cowpea rotation (Friesen et al., 1997). The mono-crop rice received 80 kg N $ha^{-1}y^{-1}$. Enhanced nitrogen cycling in the pasture is due also in part to nitrogen return via urine and dung from the cattle. Yamulki et al (1998), estimated that 7% of urine-N is lost as N_2O. Both tillage and fertilization/green manuring contribute to increased nitrogen levels in cropped soils. The highest emissions of N_2O in the cropped systems were observed in April, coincident with N additions and high soil nitrate levels (Figure 7).

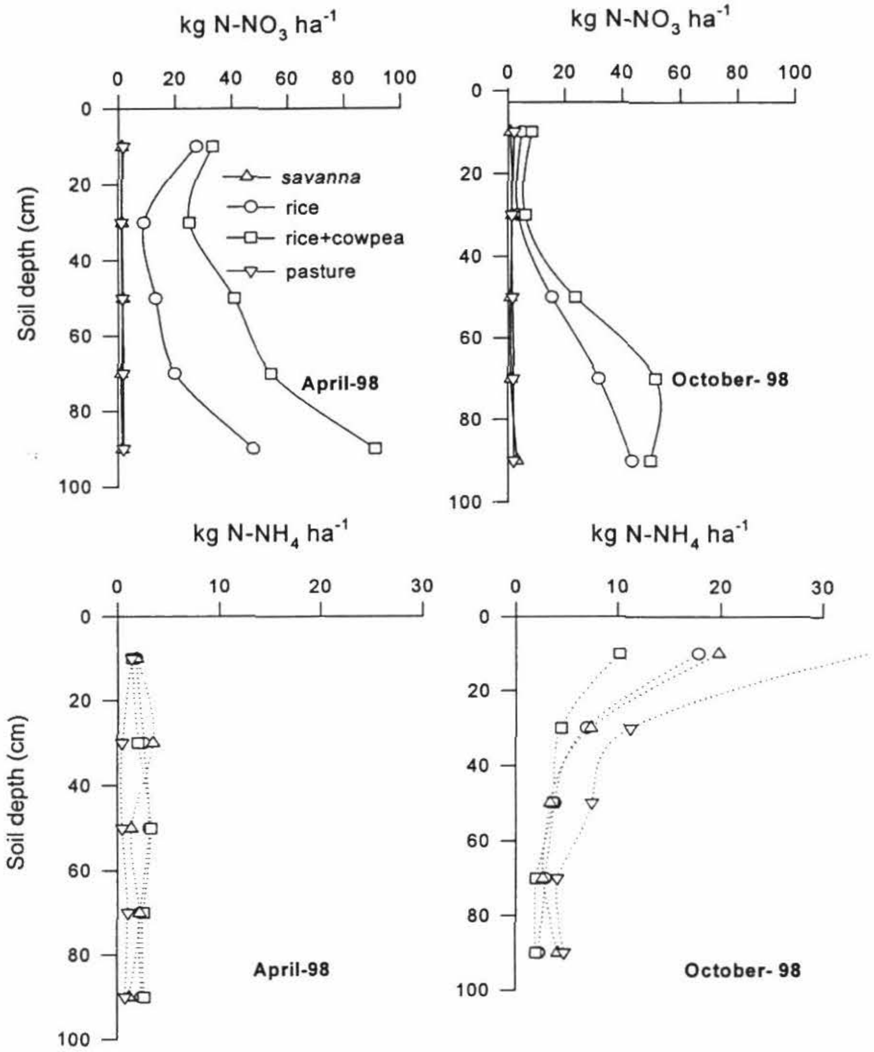

Figure 7. Nitrate and ammonium levels in the soil profile

Integrated annual net fluxes of N_2O are summarized in Figure 8. All land uses were a net source of N_2O. Within the natural ecosystems, the annual emissions from the gallery forest (1.44 kg N_2O $ha^{-1}y^{-1}$), were significantly higher than those from the clay-loam savanna (0.94 kg N_2O $ha^{-1}y^{-1}$; $p<0.01$) and the sandy savanna (0.76 kg N_2O $ha^{-1}y^{-1}$; $p<0.05$). The annual emission of N_2O from the gallery forest is low (HOW LOW??) compared to rainforest in Central America (Keller, 1994), similar to estimates for the forests in the Amazon (Culman, 1995) and eastern Venezuela (Sanhueza et al., 1990), and higher than values for semi-deciduous dry forest in Mexico (Davidson et al., 1993).

Emission rates for the savannas are similar to those reported for the Brazilian Cerrados (Nobre, 1995; Lauren et al., 1995) and for native grassland bordering the Guyana rain forest in Venezuela (Sanhueza et al., 1990), but were higher than those reported for the more comparable savannas of Venezuela (Hao et al., 1988).

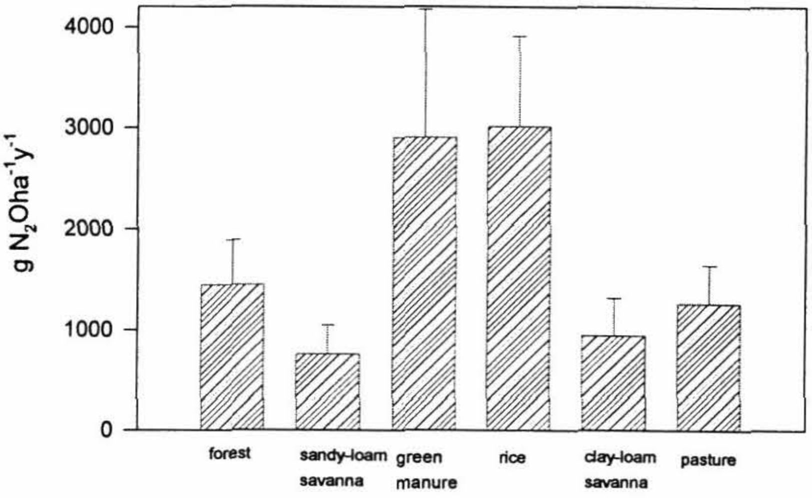

Figure 8. Annual nitrous oxide emission from soils in Colombia savannas

Conversion of clay loam savanna to a grass-legume pasture did not significantly increase N_2O emissions although legumes are known to do this (Galbally et al., 1992; Duxbury et al., 1982). A possible reason is the relatively low proportion of legume in the pasture studied. The annual emission value of 1.27 kg is lower than values for temperate grasslands (Williams, 1999; Ball et al., 1997a; Van den Pohl, 1999) or from fertilized pastures in Costa Rica (Veldkamp et al., 1997), but falls within the range found for degraded pastures in Costa Rica (Keller and Reiners, 1994), and Puerto Rico (Mosier and Delgado, 1997).

Annual emissions of N_2O were significantly increased ($p<0.01$) by slightly more than three-fold when clay loam savanna was converted to cropland. There was no difference in emissions between the two rice systems (Figure 8), where N is supplied predominantly through inorganic fertilizer in the monocrop and via organic sources in the rotation. This result does not support the suggestion (Freney, 1997), that more N_2O is emitted from organic than inorganic N sources. The observed emission rates in the rice systems of 2.8-3.0 kg N_2O ha^{-1}y^{-1} correspond to 1.8% of external nitrogen inputs after removing the contribution of the background flux from the savanna soil. This proportion is higher than the value of 1.25% x fertilizer-N which has been frequently used as an average for fertilized fields (Mosier et al., 1995), but lower than the more recently suggested proportion of 1.25% x fertilizer-N + 1 (Hopkins et al., 1997). The data is similar to other studies on tropical acid Oxisols (Mosier et al., 1998), where the conversion of the native ecosystem to agriculture, with additions of 100kg N as fertilizer, resulted in a five-fold increase in N_2O emissions from soil.

Budgets for Soil Sources of Methane and Nitrous Oxide in the Llanos

The Radiative Forcing Potential of the Llanos

The role of the soil

Table 1 shows figures for the area of the various land uses found in the Llanos, as well as their estimated contribution to annual net fluxes of methane and nitrous oxide. Data for coverage area corresponds to the nearly 14 Mha of the so called well-drained savanna which is more suitable for intensification of agriculture or pastures (Rivas, 2000).

By contributing about half of the total methane sink, soil in the gallery forest plays a key role in the net balances of this trace gas in soils of the Llanos. Given the relatively small area covered with forest, any disruption could have an important impact on regional soil methane sink strength. To illustrate this, if current forest area were reduced by 50%, the net methane sink by soils from the Llanos would be also reduced by 50%. Crops included in this study are not the only ones currently used or likely to be used in the future, and therefore a degree of uncertainty arises in regional gas budgets when it is assumed (as in Table 1) that rice as a monocrop and in rotation with green manure are representative of the effect of cropland in the region. However, given that the area under crops is still small, their potential contribution to changes in balances of methane in the Llanos is probably not too high, even under the scenario of a two fold increase in cropland expected for the next two decades (Smith at al., 1997). The annual sink strength of methane in soils from the Llanos (0.0078 Tg/y) represents around 0.02% of the estimated 40 Tg/y global soil sink strength (Minami, 1997).

Total N_2O emissions are greatly controlled by the native land uses (savannas and gallery forest), due to their high area coverage. Crops and pastures contribute currently in similar proportions to the overall budget of this gas in the Llanos. The global annual emission is low (0.1%) with respect to the estimated global planetary emissions of 13Tg/y (Bowman, 1994).

Table 1 Contribution of land uses to the budgets of CH_4 and N_2O from soils in the Llanos

Land Use	% of the area	Area x10^6 ha	CH_4 oxidation rate g $ha^{-1}y^{-1}$ (^)	Annual CH_4 oxidation Mg	N_2O emisión rate g $ha^{-1}y^{-1}$	Annual N_2O emission ton	% of total CH_4 sinks	% of total N_2O sources
Silty-Clay savanna	69	9.5	256 (90) b	2432	944 (375) ab	8968	27	61
Sandy savanna	13	1.8	1014 (199) c	1825	758 (291) a	1364	20	9
Gallery forest	10	1.4	3057 (736) a	4280	1442 (449) b	2019	48	14
Cropland (*)	4	0.5	762 (267) c	381	2961 (1079) c	1481	4	10
Pasture	5	0.6	-1915 (683) d	-1149	1266 (382) b	760	-	5
Llanos total	100	13.8	532 (201)	7769	1032 (389)	14591		

(*)Average of the values found for rice monocrop sites and cowpea - rice rotations. (^) Values in parenthesis are standard errors. In a column, values followed by the same letter indicate non-significant difference at $p<0.05$.

The contribution of termites

At least two types of termites are found in soils of the Llanos: subterranean soil-feeding termites and mound building termites (Decaëns, 1995). In a study conducted simultaneously with this, Rondón (2000) has shown that essentially all methane generated by subterranean termites is oxidized by soils before escaping into the atmosphere. The only contribution to net emissions of this gas is made by species of mound building termites of the genus Spinitermes. Annual estimated fluxes due to termites were reported as 6.7 and 7.2 g CH_4/ha for pastures of *B. humidicola* and native savanna respectively (Rondón, 2000). These values are fairly low compared to net soil sinks in the region, and consequently methane emissions by termites does not constitute an important component in the budgets of this gas in the Llanos. Soils under other land uses did not have termite mounds. To extrapolate annual fluxes of methane from termite mounds to the overall area of the Llanos covered with native savanna (in clay and sandy soils) and with pastures, it has been assumed that the density of 36 active mounds per hectare reported by Rondón (2000), for native savannas and 26 for pastures, applies throughout the Llanos. The integrated annual methane flux coming from termite mounds in the Llanos is 76 Mg CH_4/year. This value is only about 0.0004% of the total global emissions of 19.7 Tg CH_4 attributed to termites (Sanderson, 1996).

The "hot" effect of biomass burning

Towards the end of the dry season, vegetation in the savanna becomes too dry to be of value for cattle. To favor the re-growth of higher quality grasses, ranchers frequently burn their savannas. This, in addition to common natural fires results in a complete burning of the savannas at least every two years. Burning affects fluxes of GHG by two mechanisms: direct emission to the atmosphere in the form of the products of combustion, and indirect effects created by disturbances of the normal fluxes of gases from the soils. In a parallel study, Rondón (2000) measured both direct emissions of methane and nitrous oxide and long-term effects of burning on soil-atmosphere exchange. Extrapolating annual emissions to the area of the Llanos which is susceptible to burning (9.96 million hectares), direct emissions of methane due to burning were estimated to be 67,728 Mg CH_4/y, while indirect effects represent a reduction in 723 ton CH_4/y in the soil sink capacity (Rondón, 2000). The net annual release of methane by burning is then 68451 Mg per year, which is nearly 11 times higher than total methane oxidation by soils. Burning consequently has a major role in the annual budget of methane in the Llanos. The combined release of nitrous oxide in the region due to burning is 6928 Mg N_2O/year, which is about 37% of the total emissions of this gas by soils in the region. Its contribution is then also important though not dominant in the regional balance of nitrous oxide.

The "gaseous" role of cattle in the Llanos

Methane emission by cattle is a well-documented process, believed to be responsible for annual emissions on the order of 90 Tg (Johnson, 1996) or approximately half of total agricultural sources (Cole et al., 1997). Unfortunately there is a complete lack of data regarding methane emission by cattle grazing native savanna vegetation or improved pastures in the Llanos. Cattle population in the well drained Llanos is estimated to be around 2.5 million animals (Fedegan, 2000). Kurihara et al., (1999) reported methane emissions by cattle fed on tropical grasses of the order of 113g CH_4/cow-day. Though grasses are different, assuming the same methane production rate for cattle in the Llanos, the estimated production of methane by cattle in the region would be approximately 103,000 ton/year. This is about 16 times higher than the net sink by soils. This demonstrates the key role of cattle in controlling the budget of methane in the Llanos.

The total number of cattle in the Llanos is not expected to increase significantly in the near future due to market and demand constraints. What is expected with the introduction of improved pastures, is that cattle will move from native savannas frequently in remote locations, to improved pastures near to the roads and infrastructure. There are probably good opportunities to improve balances of methane in the region by offering better quality forage for the cattle. Future research in the Llanos should in consequence, try to account for the effect of improved diets on local methane budgets.

Balances of CH_4 and N_2O in the Llanos: four actors in the scene, just one landscape

In Table 2 results from the contribution of the major components of gaseous exchange are presented, and extrapolated to the respective area of influence to generate total annual fluxes for the Llanos. Data in Table 2 indicates that all together, the savanna ecosystem constitutes a net source of atmospheric methane, being largely controlled by direct emissions generated by burning and by the unfortunate 'bad breath" of cattle. The Llanos emits only about 0.03% of the total estimated global annual emissions of methane (535Tg, IPCC, 1997), and about 0.1% of total annual emissions of nitrous oxide. Covering an area of approximately 0.094% of the planetary land area, the region shows emissions of N_2O similar to planetary averages, while emission of CH_4 is only around one third of average global emissions. The Llanos can consequently be labeled as an environmentally friendly ecosystem. Despite that, there are opportunities to further reduce emissions of GHG's in the region.

Table 2 Net annual fluxes of CH_4 and N_2O in Colombian savannas

Contributing factor	CH_4 flux $g\ ha^{-1}y^{-1}$	N_2O flux $g\ ha^{-1}y^{-1}$	Total area affected Mha	Net CH_4 flux $Mg\ y^{-1}$	Net N_2O flux $Mg\ y^{-1}$
Flux from Soils (*)	-563	1,057	13.8	-7,769	14,591
Effect of burning on soil emissions	73	456	10	723	4,538
Direct emission in burning products	6,800	240	10	67,728	2,390
Emission by cattle (♣)	24,747	--	4	103,110	--
Termite mounds	7	0.008	11	76	0.1
Total in the Llanos			13.8	163,868	21,519
Total flux in the Llanos (Tg/y) (^)				0.164	0.021

(*) Value was calculated as the weighed average of fluxes and areas under various land uses in the region (Table 1).
(♣) An average stocking rate of 0.6 heads/ha was assumed for the Llanos (Rivas, 2000)
(^)Termite mounds were considered for the soils under savannas and pastures.

Mitigation strategies
Data in Table 2 indicates that mitigation strategies should be directed towards reducing the frequency of fires and reducing emissions by ruminants. Probably there is a little opportunity to favorably alter emission factors by burning at other times during the dry season, this impact has to be evaluated. Burning is however very important for maintaining the productivity and functioning of the ecosystem and also to permit the current economic exploitation of the savannas.

Therefore, unless more profitable management options are offered to farmers, there is little opportunity to reduce the scope of fires in the savannas. Pastures could play a role here, as they are economically feasible options for the development of the region (Vera, 1997). As was mentioned before, improved pastures with mixtures of high productivity grasses and forage legumes could also play a role in reducing emissions of methane by animals in the region.

In Table 3, the combined effect of all components on the balances of methane and nitrous oxide has been integrated to provide annual emissions per unit area in each land use. Though pasture soils were found to be a net source of methane, the fact that burning is eliminated in well managed pastures counteracts emissions by soil. However, given that stocking rate is increased six fold when converting a unit area of savannas into pastures, there is a 4.6 fold increase in the net release of methane to the atmosphere per unit area due to the cattle. Taking all the factors into consideration, conversion of savannas to cropland is the only alternative identified in this study, which can convert the savanna ecosystem into a net sink of methane, by eliminating the sources (burning and termites) and enhancing the soil sink. This option would however increase net emissions of nitrous oxide and consequently a "compromise solution" should be adopted when trying to include the environmental perspective within the development programs for the Llanos. It is clear however, that under the low fertilizer application rates expected to be used in the Llanos, crops will provide a good alternative to the development of the region in an "environmentally friendly way".

Clearly, regenerating forest on deforested land will provide the best alternative to mitigate emission of greenhouse gases in the Llanos. Unfortunately this is not an option easy to implement, because fire normally prevents the advance of forest into the savanna. This implies that measures to prevent the fire from reaching the borders of the forest should be reinforced, but that normally involves high cost in building roads which the farmers will not be able to afford if they are only for the sake of the environmental benefit. One possibility to cope with this problem would be to foster the use of areas near the borders of the forest as pastures or croplands. It is however reasonably to expect that governmental subsidies should be employed to make this option feasible. Perhaps there is an opportunity to recruit some funds by selling the equivalent GHG offset resulting from recovering forest (Moffat, 1997).

Table 3. Annual integrated emission of CH_4 and N_2O per hectare in various land uses in the Llanos.

	Savanna	Sandy savanna	Gallery forest	Pastures	Crops
CH_4	27.3	26.5	-3.1	125.7	-0.8
N_2O	1.6	1.5	1.4	1.3	3.0

Values are in kg $ha^{-1}y^{-1}$ (Negative values indicate a net sink)

Soil carbon, a solid component in the greenhouse gas analysis

Soils of the Llanos have been found to be able to sequester important amounts of atmospheric carbon when deep rooted grasses are introduced in these lands. Net C sequestration by pastures of *Brachiaria humidicola* in the top 1m deep soil was reported as 25.9 ton C in a ten year period, while grass-legume pastures of *B. humidicola* and the forage legume *Arachis pintoi* increased such amounts to 70.4 ton/10 years (Fisher et al., 1994). Though in this study fluxes of CO_2 were not considered, It is clear that the extent of the reported carbon accumulation in soils under pastures plays a main role in configuring the complete scenario of GHG's in the Llanos. Consequently, an analysis will be attempted here to include this component.

The radiative forcing strength of the Llanos

As a mechanism for integrating the combined effect of all greenhouse gases involved in the Llanos, the CO_2-equivalent global warming potential (E-GWP) of CO_2, CH_4 and N_2O has been calculated for two time horizon scenarios (20 and 100 years), for every land use in the Llanos. In a 20-year time scenario, CH_4 has a GWP equivalent to 62 times that of CO_2, while that of N_2O is 275 times compared to CO_2. In the 100 years time horizon, the corresponding GWP values for CH_4 and N_2O are respectively 23 and 296 times that of CO_2 (IPCC, 2001). The calculation of integrated E-GWP expressed as equivalent kg of CO_2, was done by multiplying the per hectare annual emission of CO_2, CH_4 and N_2O from each contributing factor, by the land area associated with that factor and then by the relative GWP of each gas. Adding together the values obtained for each factor gives the overall equivalent E-GWP for the Llanos, expressed as equivalent units of CO_2. For the calculation, it was assumed that burning does not make a net contribution to emissions of CO_2, because the CO2 released by fire is reabsorbed from atmosphere during vegetation regrowth. Stocking rate of cattle was assumed as 0.5 head/ha in clay and sandy savannas and 3 head/ha in pastures. The same CH_4 emission factor for cattle was used for improved pastures and for native savannas. Soil emissions of CH_4 and N_2O were assumed to be the same in grass alone and in grass-legume pastures. Figure 9 shows calculated E-GWP values (for one year total emissions of all GHG's) on a hectare basis for the various land uses and has been calculated for two time horizons, 20 and 100 years of influence. Figure 9 includes the reported (Fisher et al., 1994) values for carbon sequestration in pastures of grass-legume (0.1Mha) with a high rate of carbon sequestration (70.4 ton C/ha in a 10-year period), as well as pastures of grass alone (0.5Mha) with lower rate of carbon accumulation (25.6 ton C/ha in a 10-year period). Annual carbon sequestration by pastures was calculated assuming the same rate of accumulation for each year, and then converting it into CO_2.

All natural land uses (savannas and forests) show positive equivalent E-GWP values, indicating that they are contributing to the radiative forcing of the atmosphere. The gallery forest is clearly the best natural land use from the perspective of the heating effect on the atmosphere. Its equivalent E-GWP is very low in both time scenarios. At the 20-year horizon, the warming contribution from the gases emitted during one year by one hectare of forest is equivalent to that of the CO_2 emitted by the combustion of 35 gallons of gasoline (fuel for 8 hours trip of a small car!). Including all the sources and sinks, the radiative power of savannas is low and decreases when the 100 year time scenario is used, because most of the contribution is in the form of CH_4, which is a short lived gas in the atmosphere. Crops have integrated E-GWP lower than that of the savannas in the 20 year scenario and approximately the same as the savannas in the 100 year scenario. The conversion of savanna land into cropland does not have a detrimental effect on the E-GWP.

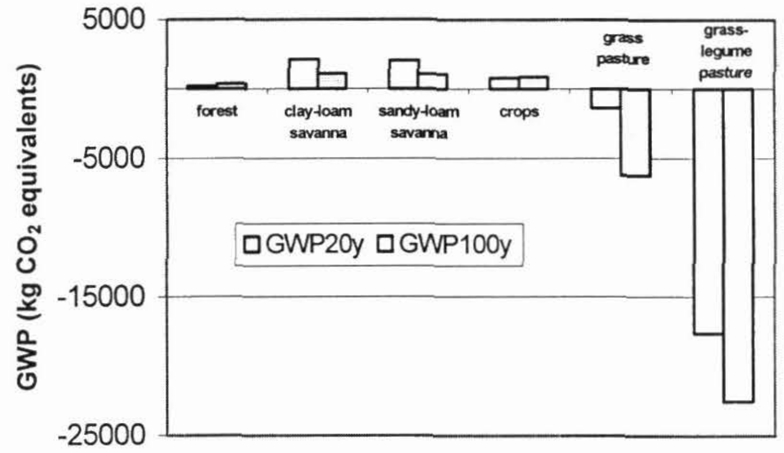

Figure 9. Global warming potential –GWP for various land uses in the llanos under two time horizons scenarios (20 and 100 years). GWP is expreed as kg of CO_2 equivalents. Values represent the contribution of annual emissions per hectare.

The inclusion of pastures in the Llanos plays a much more important role in affecting the overall E-GWP. Due to the modest emissions of methane and nitrous oxide from pastures and the very high sequestration of atmospheric CO_2 as soil organic carbon, pastures can convert the system from a modest source into an important net sink of radiatively important species. The rate and persistence of C accumulation plays a major role in the strength of the sink, especially in the 20-year horizon. Even with rates of C accumulation in soil of around half of the reported value for grass alone pastures in Carimagua, the equivalent E-GWP of annual emissions from one hectare of pastures would be zero.

The Llanos in the year 2020

Land use patterns in the Llanos are expected to change in the next two decades. Studies suggest (Smith et al., 1997; Rivas, 1999), that the area of crops could increase up to two times the current values while the area under pastures will also double in the same period. The area under grass-legume pastures is expected to grow from 0.1 to 0.3 Mha, while the area under grass alone pasture will continue to dominate and will increase from 0.5 to 0.9 Mha. This expansion will be at the expenses of the clay-loam savanna, which has slightly better levels of soil nutrients than the sandy savanna. The area under gallery forest will probably decrease by 10% in the next 20 years assuming the same rate of current intervention. Annual rate of C-sequestration by pastures in soil was assumed as reported for grass alone and grass-legume pastures at Carimagua (Fisher et al., 1994).

Table 4 shows results of a calculation of the integrated E-GWP for the Llanos at present and in the year 2020 for a 20-year time horizon. Under current land use distribution, the Llanos as a whole plays a minor role in the radiative forcing in the earth's atmosphere. Its integrated E-GWP of 9.6 Tg of CO_2 equivalents is only about 0.004% of estimated global planetary radiative contribution of about 242,000 Tg of CO_2 equivalents (IPCC, 2001).

Table 4. Integrated E-GWP for the Llanos under present and expected land use distribution in the year 2020. Values are equivalent Tg of CO_2 calculated for a 20-year time horizon.

Land use	E-GWP kg CO_2equivalents per ha	Area (Mha)		Integrated E-GWP (Tg of CO_2 equivalents)	
		present	Year 2020	present	Year 2020
Forest	207	1.4	1.26	0.29	0.26
Savanna	2140	9.5	8.54	20.33	18.28
Sandy savanna	2042	1.8	1.8	3.68	3.68
Croplands	767	0.5	1.0	0.38	0.77
Grass alone pasture	-1358	0.5	0.9	-0.68	-1.22
Grass-legume pasture	-17674	0.1	0.3	-1.76	-5.30
Total				22.24	16.46

In Figure 10, in addition to the 20-year time horizon a longer term 100-year time horizon has been used to calculate effects of present and expected land use distribution in the Llanos. The development of the Llanos will have small net benefits to the environment by reducing the radiative force of the atmosphere. This benefit will be accentuated in the longer term scenario. Once more the minor role of the Llanos in the context of warming of the planet is emphasized.

Conclusions

This study presents the first data set on fluxes of methane and nitrous oxide for the Colombian Llanos. Results indicate that gallery forest is an important sink for atmospheric methane, while savannas are a minor sink. Therefore, preservation of the gallery forest should be of priority concern as this environment also provides a home for a large biodiversity of endemic plants and animals.

Conversion of soils into cropland does not reduce their methane oxidizing capacity and some of the management practices could even increase their sink strength. This may be the result of eliminating some of the physical constraints that limit the gas exchange between soil and the atmosphere (soil compaction, surface sealing etc.). On the other hand it also increases emissions of nitrous oxide and is equally expected to increase losses of soil carbon as a result of tillage. Despite this, given that the main contributing factors (burning and cattle) are excluded in cropland, it can be anticipated that agriculture will be a better option than savanna for reducing the radiative forcing of the Llanos.

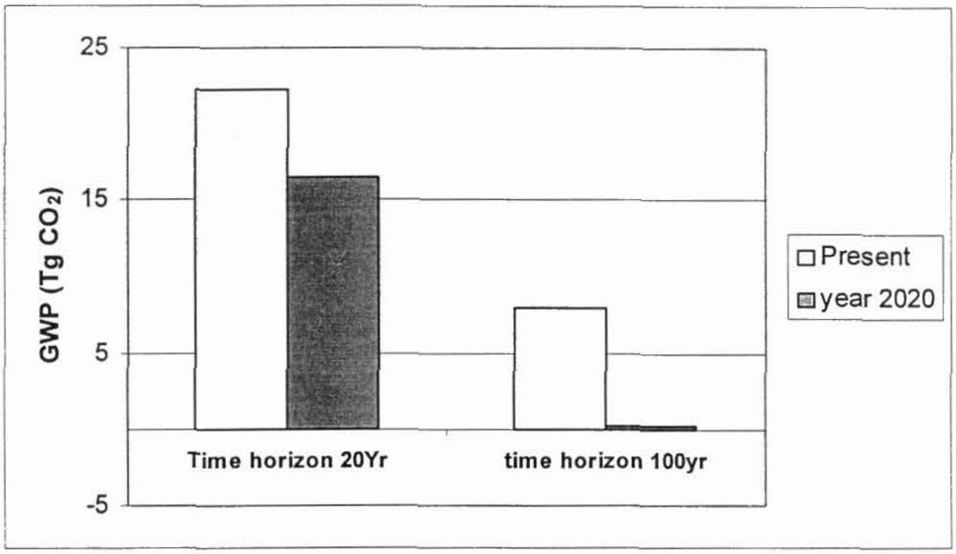

Figure 10. Integrated GWP for the Llanos under present land use and expected land distribution in the next two decades. Two time horizons (20 and 100 years) are considered.

Emission of methane by biomass burning is a key factor in the balance of this gas in the Llanos. Any action that can reduce the area submitted to burning and or the frequency of burning events, will improve the radiative balance in the region. In this respect, conversion of savanna into croplands or pastures is clearly an advantage because burning is eliminated in such land uses. Promoting the re-colonization of the land by gallery forests constitute a win-win situation as it will not only eliminate the burning, but also will increase the methane soil sink strength.

Fire plays a key role in maintaining the biodiversity in the savanna, in addition to other important though not fully understood ecological roles. Consequently, complete suppression of the burning is not a desirable option. Appropriate corridors to maintain the continuity of the savanna ecosystem should always be considered.

Cattle-associated emissions of methane dominate methane budgets in the Llanos. Improving estimates of their actual contribution as well as exploring promising opportunities to reduce their impact by offering forages of higher nutritional value, are important topics for research in the near future.

Natural ecosystems as well as converted lands constitute small net sinks for N_2O in the Llanos. Emission rates are related with the amounts of nitrogen cycled in the soil; nitrogen inputs in the form of fertilizer or green manure cause enhanced emissions. Strategies to manage these inputs in order to minimize nutrient losses and reduce environmental impact require further attention.

In general fluxes of methane and nitrous oxide from soils in the Llanos can be considered low, but fall within the range reported for similar environments in Africa (Seiler et al., 1984), Central America (Mosier et al., 1998), Brazil (Lauren et al., 1995) and Venezuela (Scharffe et al., 1980), and even for tall grass prairies in temperate regions (Tate and Striegl, 1993).

Though pastures will increase methane emissions from cattle due to the increase in the stocking rate as compared to savanna, by avoiding the fire and by sequestering atmospheric CO_2 in the form of soil organic carbon, pasture is the only land use option identified in this study, that can shift the land from a net source into a net sink of atmospheric GHG's.

Influencing the direction of change

This study has shown that the Llanos are only a very minor contributor to the warming of the atmosphere and that expected intensification of agriculture and cattle production in the coming two decades would not have negative effects on the radiative forcing potential of the region. Despite this, there are other well identified constraints for the sustainability of the natural resource base, whose impact should never be forgotten. Pasture degradation is major cause of pasture abandonment specially in the Brazilian Cerrados and the Amazon. Degradation could result not only in reduced C sequestration in soils but even turn them into net sources of Carbon (Da Silva et al., 2000). Though current pasture degradation is not too severe in the Llanos, unless appropriate management practices were adopted, this could become a critical problem in the region, whose environmental consequences are still to be evaluated.

In the jargon of optometrics, 20-20 means perfect vision. We hope that appropriate vision will be used by policy makers in the design of development plans for the Llanos which allows the region to continue being an environmentally friendly ecosystem in the year 2020.

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Activity3.3 Reducing emissions of greenhouse gases and enhancing C sequestration carbon and nutrient accumulation in secondary forests

Activity 3.3.1 – Carbon and nutrient accumulation in secondary forests regenerating from degraded pastures in central Amazônia, Brazil

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Abstract

Over the past three decades, large expanses of forest in the Amazon Basin were converted to pasture, many of which later degraded to woody fallows and were abandoned. While the majority of tropical secondary forest (SF) studies have examined post-deforestation or post-agricultural succession, we examined post-pasture forest recovery in ten forests ranging in age from 0 to 14 yrs since abandonment. We measured aboveground biomass and soil nutrients to 45 cm depth, and computed total site C and nutrient stocks to gain an understanding of the dynamics of nutrient and C buildup in regenerating SF in central Amazônia. Aboveground biomass accrual was rapid, 11.0 Mg ha^{-1} yr^{-1}, in these young SF. After 12 to 14 yrs, they accumulated up to 128.1 Mg/ha of dry aboveground biomass, equivalent to 25 to 50% of primary forest biomass in the region. Wood N and P concentrations decreased with forest age. Aboveground P and Ca stocks accumulated at a rate of 2.4 and 42.9 kg ha^{-1} yr^{-1}; extractable soil P stocks declined as forest age increased. Although soil stocks of exchangeable Ca (207.0 ± 23.7 kg/ha) and extractable P (8.3 ± 1.5 kg/ha) were low in the first 45 cm, both were rapidly translocated from soil to plant pools. Soil N stocks increased with forest age (117.8 kg ha^{-1} yr^{-1}), probably due to N fixation, atmospheric deposition, and/or subsoil mining. Total soil C storage to 45 cm depth ranged between 42 and 84 Mg/ha, with the first 15 cm storing 40 to 45% of the total. Total C accrual (7.04 Mg C ha^{-1} yr^{-1}) in both aboveground and soil pools was similar or higher than values reported in other studies. Tropical SF regrowing on lightly to moderately-used pasture rapidly sequester C and rebuild total nutrient capital following pasture abandonment. Translocation of some nutrients from deep soil (>45 cm depth) may be important to sustaining productivity and continuing biomass accumulation in these forests. The soil pool represents the greatest potential for long-term C gains; however, soil nutrient deficits may limit future productivity.

Keywords: secondary forest; abandoned pasture; carbon sequestration; plant nutrient stocks; soil nutrient stocks; nutrient loss; Oxisol; succession; Amazon.

Introduction

Primary forest conversion for subsistence agriculture, industrial logging and pasture establishment continues to be the predominant cause of tropical deforestation (Laurance, 1999). These activities have left a large portion of the tropical biome disturbed and in various states of natural regeneration (Brown and Lugo, 1990), stagnation (Fearnside and Guimaraes, 1996; Sarmiento, 1997; Silver et al., 2000), or managed recovery (Fernandes and Matos, 1995; Parrotta et al., 1997). Of the estimated 58.8 million ha of forest cleared in Brazilian Amazônia over the past three decades (INPE, 2002), approximately 24 million ha were converted to pastures (Serrão et al., 1995). Depending on management (replacement of exported or lost nutrients, stocking rates, burning frequency, etc.), region, and soil type, pasture productivity may decline after 7 to 10 years and may be recleared or abandoned to recolonizing secondary vegetation; approximately 50% of the first-cycle pastures have reached this advanced stage of degradation (Serrao et al., 1993). Based on an analysis of 1990 land use data in the Amazon, Fearnside (1996) calculated an equilibrium will be reached where ~47% of all deforested land would be regenerating forest on degraded or abandoned pastures.

Although highly altered, these lands are valuable for human use (Brown and Lugo, 1990), and provide important ecosystem services such as watershed protection, sources and havens of biodiversity, erosion prevention, soil fertility recovery by improved fallows (Szott et al., 1991), and atmospheric C sinks (Fearnside and Guimaraes, 1996, Silver et al., 2000). However, the potential of the abandoned land to recover and maintain these roles is dependant on the intensity of previous land use (Uhl et al., 1988; Nepstad et al., 1990; Aide et al., 1995; Alves et al., 1997), soil nutrient limitations (Cochrane and Sánchez, 1982; Smyth and Cravo, 1992; Laurance et al., 1999), and seed inputs and seedling establishment (Nepstad et al., 1996). These impediments to vegetation regrowth may be more extreme in abandoned pastures compared to agricultural land, resulting in lower aboveground productivity (Fearnside and Guimaraes, 1996; Silver et al., 2000), and longer regeneration times.

Pasture productivity declines rapidly with decreasing soil P availability, facilitating invasion by secondary forest (SF) species better adapted to infertile soil (Toledo and Navas, 1986); yet, soil fertility and biomass recovery is variable and dependant upon several factors. Degraded pastures are characterized by depleted soil nutrient stocks, low vegetation biomass, low primary forest seed inputs, high seed predation, depleted seed bank of forest species and low stump sprouting (Nepstad et al., 1990), as well as soil surface sealing and compaction (Eden et al., 1991). Consequently, predicting the long-term growth rate of secondary vegetation on degraded pastures and the return of primary forest characteristics becomes a complex task.

New attention has focused on fast-growing SF due to their potential to sequester large quantities of C in short time-periods. For example, worldwide tropical forests store approximately 206 Pg C in the soil (Eswaran et al., 1993), and tropical SF of less than 20 years have the potential to accrue soil C at a rate of 1.3 Mg ha^{-1} yr^{-1} (Silver et al. 2000). The growth rate of young SF is expected to increase with rising atmospheric CO_2 levels (DeLucia et al., 1999); however, high C allocation to short-lived tissues such as leaves and faster turnover of litter C may limit the potential C sink (Schlesinger and Lichter, 2001). Furthermore, soil nutrient limitations may constrain primary productivity under CO_2 enrichment (Oren et al., 2001).

Soil nutrient impediments to productivity under native vegetation are substantial in the Brazilian Amazon. Cochrane and Sánchez (1982) estimated that only 7% of the land area is free from major plant growth limitations; soil P deficiencies (<7 mg/kg) constrain productivity in 90% (436 million ha), and Al toxicity (Al saturation of $\geq$ 60%) occurs over 73% of the Brazilian Amazon. Low soil Ca (Smyth and Cravo, 1992) restrains productivity, P deficiencies (Gehring et al., 1999) limit SF growth, and the vegetation is unable to effectively capture leaching soil N (Schroth et al., 1999). Mismanagement may compound these deficiencies since pasture use-intensity appears to negatively influence regenerating vegetation biomass (Uhl et al., 1988) and nutrient stocks (Buschbacher et al., 1988). Because SF recovery is variable and dependent on previous land-use and soil fertility, the magnitude and rate of the above- and below-ground C accumulation in these regenerating SF is still relatively unknown.

Determining nutrient constraints to regrowth and the status of secondary vegetation is an important step in managing and/or enhancing abandoned site rehabilitation. We examined the dual roles of SF to rehabilitate site productivity and to increase C sinks and investigated potential soil nutrient limitations to these two processes. We examined aboveground and soil C accrual and nutrient stocks in degraded pastures that had been abandoned for a varying number of years. Our objective was to study the influence of regenerating vegetation on C and nutrient budgets following pasture abandonment. We hypothesized that C and N pools would recover with time following post-burn volatilization, while other nutrients would be redistributed from below- to above-ground pools resulting in reduced soil pools.

Methods

Study area. The study areas are located in Amazonas, Brazil, in the central Amazon Basin, north of the city of Manaus along the road BR-174. The study area spans approximately 26 km (2° 34' S, 60° 02' W and 2° 20' S, 60° 04' W). The terrain is undulating with an elevation of 50-150 m. The plateau soil is classified as dystrophic, isohyperthermic, clayey kaolinitic, Hapludox with approximately 80–85% clay (*latossolo amarelo* according to the Brazilian classification system). Slope soils are composed of Ultisols and valley bottoms by Spodosols. The plateau soils have a low cation exchange capacity and are infertile but are strongly aggregated and well drained (Van Wambeke,1992).

The regional climate is tropical humid and the mean temperature is 26.7°C. Mean annual rainfall in Manaus is 2.2 m, with March and April as the wettest months with over 300 mm of precipitation. A mild dry season occurs from August through October, with mean monthly precipitation falling below 100 mm, and in some El Niño years to as little as 50 mm (Lovejoy and Bierregaard, 1990).

The native vegetation of this region is closed-canopy, dense, evergreen *terra firme* forest (Veloso et al., 1991). Species recovery with SF development is significantly different in areas used as pasture compared to areas cut but not managed (Mesquita et al., 2001). Old growth, native vegetation remains the dominant cover in this area. The establishment of new pastures is now rare, and active pastures are a diminishing, short-lived feature of the landscape north of Manaus. However, SF are increasingly found along the primary roads where efforts to raise cattle on large ranches failed some 10 to 20 years ago.

A majority of the pastures were mechanically cleared in the early 1980's, commercial timber may or may not have been removed, the slash burned in place or mechanically piled in windrows, and the area planted with exotic African grasses such as *Brachiaria brizantha* or *B. humidicola* (Rendle). Standard pasture management for the region includes at least one application of 50 kg P/ha. The animal stocking rate and number of years that the pastures were grazed were variable. Overgrazing and annual burning to increase economic returns in the short-term accelerated pasture degradation through increased nutrient loss and soil compaction. However, even in the absence of overgrazing (1–2 animal/ha) increases in bulk density occur (0.4 g/cm^3 increase from forest values after 12 years as pasture), leading to reduced infiltration, sheetwash, and pasture decline (Eden et al., 1991). Declining pasture productivity is characterized by a reduction in the forage to weed ratio as bare ground develops and herbaceous and woody plants begin to invade. When unpalatable plants begin to dominate, livestock productivity drops, animal mortality increases and the pasture is eventually abandoned. Fire and/or labor intensive hand weeding of seedlings and roots may lengthen pasture life by reducing woody biomass while encouraging grass growth; however, species of *Vismia*, a fast-growing early successional tree, resprout rapidly after burning and dominate abandoned pastures.

Site and plot selection.

Ten SF were selected within three *fazendas* (cattle ranches) now in various stages of grazing, pasture abandonment or pasture reclamation: Fazenda Rodão (km 46), the Brazilian Agency for Agricultural Research (Embrapa Amazônia Ocidental) Agricultural District of SUFRAMA (DAS) pasture research site (km 53), and Fazenda Dimona (km 72), all along the road BR–174. Within each forest located on plateau Oxisols, we established four plots of 100 m^2 to 400 m^2, each with three subplots ranging in size from 35 to 225 m^2 depending on forest age. Forests ranged from 0 to 2 yrs to 12 to 14 yrs since pasture abandonment. Secondary forest selection was based on forest age and independence from adjacent plots within the same ranch. We selected a range of forests spanning the age of available SF in the area; however, all SF age classes do not occur at all farms. We conducted farmer interviews to determine site histories and when grazing was abandoned. The date at which the pastures were abandoned is not definitive, as cattle may infrequently graze the area until all palatable forage is replaced by woody successional vegetation. The regenerating forests within the ranches are biologically and physically distinct, each with a unique management history and vegetation cover.

Biomass and tissue analysis

Within each subplot, we measured diameter at breast height (DBH at 1.3 m above ground level; *Cecropia* were measured above prop-roots) for all live tree stems ≥1cm, tagged the stems, and recorded all species. Using two sets of allometric equations, Nelson et al. (1999) for stem >5 cm DBH and Mesquita (in preparation) for those 1 to 5 cm DBH, we calculated dry biomass for each tree and converted the estimates to Mg/ha. The two sets of equations were developed either on the EMBRAPA research site (Nelson et al., 1999) or within the same region (Mesquita, pers. comm). They provide a better estimate of SF biomass than previous equations (Saldarriaga et al., 1988; Uhl et al., 1988; Brown et al., 1989; Overman et al., 1994) developed in the Amazon Basin (Nelson et al., 1999). The Nelson equations provide valid biomass estimates from 1 to 30 cm DBH. However, since these SF have more stems in the smaller diameter range of the Mesquita equations (1 to 5 cm DBH), by using the two sets of equations rather than one, we improve biomass estimates.

We used species-specific equations for the dominant tree species *Vismia cayennensis* (Jacq.) Pers., *V. japurensis* Reich. (Clusiaceae); *Cecropia* (Moraceae; mainly *C. sciadophylla* Mart. and *C. purpurascens* C.C. Berg); *Bellucia* (Melastomataceae); *Goupia glabra* Aubl. (Celastraceae); *Laetia procera* (Poepp) Eichl. (Flacourtiaceae), and a mixed-species equation for all others. The pioneer *Cecropia*, uncommon on these sites, occurs less frequently in areas where grazing continues during secondary vegetation establishment (Mesquita et al. 2001).

To produce an aboveground forest estimate of nutrient concentrations (and nutrient stocks on a per hectare basis) within each forest, we randomly selected 15 trees ≥1 cm DBH and collected mature, upper canopy sun leaves using a telescoping tree pruner or climbing the boles. From the same trees, we drew two wood core samples (wood and bark) at 1.3 m height on opposite sides of the bole. Foliage and wood samples were pooled into three sample composites of five trees, oven dried at 70°C, ground and homogenized, and analyzed for C, N, P, K, Ca, and Mg using standard EMBRAPA laboratory operating procedures (Silva, 1999).

Vegetation stocks calculations

We developed a foliage:wood ratio (Mesquita, in prep.) for partitioning biomass into wood and foliar components. We then estimated aboveground carbon and nutrient stocks in each forest by multiplying mean nutrient concentrations for foliage and wood samples by the allometric estimates of each biomass component as partitioned by the foliage to wood ratio for individual trees. Our estimates of nutrient pools do not include aboveground biomass <1cm DBH, forest litter, or root biomass.

Soil analysis

We sampled soil to 45 cm in three depth classes (0–15, 15–30, 30–45 cm) within each of four plots per forest. The four soil samples per depth in each forest (120 soil samples) represent a composite of four to six sub-samples per sample. Soil composites were combined in the field, air dried in solar dryers, charcoal and roots removed, hand milled with a roller, sieved to 2 mm, and analyzed for C, N, P, K, Ca, and Mg. Charcoal is common in local surface soils and is present at times to 45 cm depths in both pasture and forest soils. As charcoal is heterogeneously distributed in the soil, charcoal contamination poses an important impediment to resolution in reporting soil C concentrations. We estimate that carbon concentrations in this study, as with other studies within the Amazon basin, may generally overestimate total soil carbon stocks as a result of charcoal contamination (M.A. Rondón, unpublished data). To reduce the charcoal contribution to soil C estimates, large pieces were removed while the samples were wet and again with a forceps after drying before grinding; however, the small fragment size makes total removal difficult.

Extractable soil P and K were analyzed using a double acid extraction (0.05 M hydrochloric acid and 0.0125 M sulfuric acid) and exchangeable Ca and Mg with 1 M potassium chloride. Total soil N was determined by the Kjeldahl technique and soil C (%) by wet digestion (Silva, 1999). Soil nutrient pools (kg/ha) of C, N, P, K, Mg, and Ca were calculated using mean soil bulk density data measured to 45 cm depth from abandoned pastures and SF in the same area (T.R. Feldpausch; S.A. Welch, unpublished data). Nutrient concentrations were multiplied by bulk densities for each depth class to provide soil nutrient stocks on a per hectare basis.

Statistical analysis

Statistical analyses were performed using Minitab 12.1 (Minitab Inc.). Statistical comparisons for C and nutrient concentrations and stocks were conducted separately for the different vegetation tissue types and soil depths using linear and log-linear regression and a $p<0.05$ significance level. Soil and vegetation concentrations, and soil stocks values were log transformed. Pooling the data for age classes and using regression analysis, we tested for trends in C and nutrients within aboveground and soil pools, as partitioned by depth, foliage or wood, versus time (years after pasture abandonment).

Results

A total of 1901 stems were measured in 2320 m^2, of which 138 standing dead and 177 lianas were excluded from biomass calculations due to allometric equation limitations in computing such components. Of those stems considered in biomass estimations, 68% were less than 5 cm DBH, while no stems were greater than 30 cm DBH. The two recently abandoned pastures of 0–2 years had no stems ≥1cm DBH, the minimum diameter used for the allometric equations.

Vegetation nutrient concentrations

Wood N and P concentrations declined with SF age ($r^2 = 0.85, 0.75$; $p<0.001$), with an average reduction of 50 and 60% in wood N and P from the youngest to the oldest forests. Foliage N and P concentrations tended to decline with forest age, although non-significantly. Compared to wood, foliage contained an average of 5.7 times more N and 3.7 times more P (Table 1).

Foliar and wood Ca concentrations did not show a trend with age, but the concentrations were high relative to other nutrients. Calcium concentrations in wood were comparable and at times higher than wood N values. n the foliage, Ca concentrations represented an average of 43% of N values. Foliage contained an average of 2.5 times more Ca than wood. Potassium and Mg foliar and wood concentrations showed no trends with forest age.

Vegetation nutrient stocks

Although woody biomass accumulated more quickly than foliage, nutrient stocks for all nutrients accumulated more quickly in foliage (Figure 1). Foliar N stocks (42.6 kg ha^{-1} yr^{-1}; $r^2=0.94$; $p<0.001$) increased much more rapidly than woody stocks (15.5 kg ha^{-1} yr^{-1}; $r^2=0.91$; $p<0.01$) with time after abandonment. Phosphorus stocks in foliage accrued twice as fast as wood P stocks. However, foliar Ca stocks (22.3 kg ha^{-1} yr^{-1}; $r^2=0.92$; $p<0.001$) accrued at a similar rate to wood stocks (20.6 kg ha^{-1} yr^{-1}; $r^2=0.90$; $p<0.001$) (Table 2).

Soil nutrient concentrations

Within each forest, soil carbon and nutrient concentrations generally decreased with depth (Table 3). Total soil N concentrations generally decreased with depth; however, deeper soil profile (30–45 cm depth) N concentrations increased with time after pasture abandonment ($r^2 = 0.57$; $p<0.001$), while the shallower depths showed a weaker soil N trend with time. Soil extractable P concentrations tended to decrease at all soil depths over time, with significant reductions in surface layers (0-15 cm depth) with increasing time since abandonment ($r^2 = 0.46$; $p<0.05$). Near surface Ca levels (0–15 cm depth) ranged from 0.13 to 0.33 $c.mol_{(+)}/kg$. Calcium concentrations were low below 15 cm depth, with overall means of 0.07 $c.mol_{(+)}/kg$ at 15–30 cm and 0.07 $c.mol_{(+)}/kg$ at 30–45 cm depths.

Table 1: Mean C and nutrient concentrations in foliage and wood from ten secondary forests regenerating from degraded pasture in central Amazônia, Brazil[a].

SF age (yrs)	Fazenda and forest no.	C	N	P	K	Ca	Mg
		%	g / kg				
Foliage							
0 to 2	DAS-1 and Rodão-1	46.65 (1.80)	17.11 (0.78)	0.87 (0.04)	3.71 (0.35)	7.25 (0.74)	2.31 (0.06)
2 to 4	Rodão-4	45.51 (0.33)	16.42 (0.06)	0.65 (0.03)	2.75 (0.00)	6.42 (0.17)	1.88 (0.02)
4 to 6	DAS-2 and Rodão-3	48.25 (1.26)	16.08 (0.82)	0.89 (0.04)	5.59 (1.16)	5.28 (0.14)	2.04 (0.28)
6 to 8	Dimona-1, -3 and Rodão-2	45.69 (0.59)	14.60 (0.50)	0.56 (0.03)	5.38 (0.71)	6.59 (0.41)	2.13 (0.19)
12 to 14	DAS-3 and Dimona-2	49.38 (0.64)	15.02 (0.16)	0.58 (0.02)	5.14 (0.29)	7.86 (0.12)	2.77 (0.12)
Overall Mean		47.12 (0.54)	15.66 (0.31)	0.70 (0.03)	4.78 (0.36)	6.70 (0.24)	2.25 (0.10)
Wood							
0 to 2	DAS-1 and Rodão-1	51.40 (0.49)	3.71 (0.31)	0.31 (0.01)	2.23 (0.33)	4.05 (0.58)	0.98 (0.11)
2 to 4	Rodão-4	47.64 (0.41)	2.99 (0.39)	0.19 (0.01)	1.19 (0.10)	2.76 (0.29)	0.69 (0.05)
4 to 6	DAS-2 and Rodão-3	47.45 (1.16)	2.80 (0.15)	0.21 (0.03)	1.69 (0.31)	2.01 (0.21)	0.47 (0.04)
6 to 8	Dimona-1, -3 and Rodão-2	47.68 (1.05)	2.52 (0.14)	0.16 (0.01)	1.76 (0.17)	2.33 (0.19)	0.55 (0.06)
12 to 14	DAS-3 and Dimona-2	51.05 (1.99)	1.87 (0.12)	0.09 (0.02)	1.28 (0.11)	2.51 (0.30)	0.58 (0.07)
Overall Mean		49.05 (0.63)	2.73 (0.14)	0.19 (0.01)	1.68 (0.12)	2.69 (0.19)	0.64 (0.04)

[a]Each mean nutrient concentration value represents n=3 samples of a five tree composite in each forest. Mean (standard error).

Table 2: Rate of total nutrient accumulation, vegetation nutrient immobilization (a), and soil nutrient flux to 45 cm depth (b). Results from ten secondary forests regenerating from degraded pastures in central Amazônia, Brazil.

	N	P	K	Ca	Mg
	kg ha^{-1} yr^{-1}				
Total vegetation and soil (a + b)[a]:	175.9 (r^2=0.62; $p<0.001$)	1.8 (r^2=0.75; $p<0.01$)	24.7 (r^2=0.88; $p<0.001$)	42.2 (r^2=0.84; $p<0.001$)	12.9 (r^2=0.79; $p<0.001$)
a. Total foliage and wood	58.1 (r^2=0.94; $p<0.001$)	2.4 (r^2=0.93; $p<0.001$)	25.1 (r^2=0.92; $p<0.001$)	42.9 (r^2=0.92; $p<0.001$)	12.5 (r^2=0.92; $p<0.001$)
Foliage	42.6 (r^2=0.94; $p<0.001$)	1.6 (r^2=0.95; $p<0.001$)	14.5 (r^2=0.90; $p<0.001$)	22.3 (r^2=0.92; $p<0.001$)	7.8 (r^2=0.90; $p<0.001$)
Wood	15.5 (r^2=0.91; $p<0.001$)	0.8 (r^2=0.72; $p<-.01$)	10.6 (r^2=0.89; $p<0.001$)	20.6 (r^2=0.90; $p<0.001$)	4.7 (r^2=0.93; $p=<0.001$)
b. Total soil[b]:	117.8 (r^2=0.44; $p=0.04$)	- 0.66 (r^2=0.34; $p=0.08$)	~ 0 (N.S.)	~ 0 (N.S.)	~ 0 (N.S.)

[a] Represents a new linear regression with the sum of the subtotals.

[b] Total nitrogen and extractable P, K, Ca, and Mg. N.S. indicates a non-significant change.

Table 3: Mean soil carbon and nutrient concentrations, and pH from ten secondary forests regenerating from degraded pasture in central Amazônia, Brazil[a].

SF age (yrs)	Fazenda and forest no.	C	N	P	K	Ca	Mg	Ph
		---- (g / kg) ----		---- (mg / kg) ----		---- ($cmol_{(+)}$ / kg) ----		(KCl)
0–15 cm depth								
0 to 2	DAS-1 and Rodão-1	15.39 (2.27)	1.23 (0.13)	4.83 (0.57)	20.98 (2.40)	0.13 (0.02)	0.09 (0.01)	4.1 (0.03)
2 to 4	Rodão-4	20.77 (2.64)	1.25 (0.09)	3.09 (0.17)	18.11 (0.82)	0.18 (0.01)	0.07 (<0.01)	4.0 (0.02)
4 to 6	DAS-2 and Rodão-3	20.71 (2.01)	1.42 (0.16)	6.30 (0.86)	33.20 (4.76)	0.16 (0.04)	0.14 (0.02)	4.0 (0.03)
6 to 8	Dimona-1, -3 and Rodão-2	22.91 (0.85)	1.49 (0.05)	2.46 (0.30)	23.92 (2.49)	0.33 (0.11)	0.18 (0.04)	4.0 (0.03)
12 to 14	DAS-3 and Dimona-2	19.51 (2.77)	1.75 (0.04)	1.55 (0.17)	19.45 (0.55)	0.16 (0.05)	0.11 (<0.01)	4.0 (0.01)
Overall mean		20.19 (0.94)	1.46 (0.05)	3.55 (0.36)	23.78 (1.52)	0.21 (0.04)	0.13 (0.02)	4.0 (0.01)
1 –30 cm depth								
0 to 2	DAS-1 and Rodão-1	9.17 (1.43)	0.74 (0.06)	2.03 (0.53)	8.46 (0.71)	0.08 (0.01)	0.05 (<0.01)	4.2 (0.02)
2 to 4	Rodão-4	17.14 (0.70)	0.91 (0.06)	1.87 (0.17)	10.53 (0.47)	0.11 (0.01)	0.05 (0.01)	4.2 (0.02)
4 to 6	DAS-2 and Rodão-3	10.63 (1.08)	0.86 (0.08)	1.40 (0.13)	10.86 (1.63)	0.08 (0.01)	0.05 (0.01)	4.1 (0.02)
6 to 8	Dimona-1, -3 and Rodão-2	12.09 (0.55)	0.88 (0.02)	0.86 (0.13)	11.19 (0.88)	0.05 (0.01)	0.04 (0.01)	4.1 (0.02)
12 to 14	DAS-3 and Dimona-2	11.46 (0.76)	1.01 (0.01)	0.70 (0.00)	8.56 (0.31)	0.07 (0.02)	0.05 (<0.01)	4.1 (0.02)
Overall mean		11.65 (0.52)	0.88 (0.03)	1.25 (0.13)	10.02 (0.48)	0.07 (0.01)	0.05 (<0.01)	4.1 (0.01)
30–45 cm depth								
0 to 2	DAS-1 and Rodão-1	6.57 (0.57)	0.55 (0.02)	1.15 (0.41)	5.56 (0.28)	0.06 (0.01)	0.04 (0.01)	4.2 (0.02)
2 to 4	Rodão-4	12.25 (0.86)	0.65 (0.07)	1.15 (0.16)	7.14 (0.46)	0.10 (0.02)	0.04 (<0.01)	4.2 (0.02)
4 to 6	DAS-2 and Rodão-3	10.84 (2.36)	0.65 (0.02)	0.86 (0.09)	5.84 (0.97)	0.07 (0.01)	0.04 (<0.01)	4.2 (0.02)
6 to 8	Dimona-1, -3 and Rodão-2	8.12 (0.66)	0.69 (0.01)	0.39 (0.10)	6.28 (0.58)	0.05 (0.01)	0.04 (0.01)	4.1 (0.02)
12 to 14	DAS-3 and Dimona-2	16.08 (2.49)	0.77 (0.02)	0.58 (0.09)	5.84 (0.39)	0.05 (0.01)	0.04 (<0.01)	4.1 (0.01)
Overall mean		10.46 (0.89)	0.67 (0.01)	0.74 (0.09)	6.06 (0.28)	0.06 (0.01)	0.04 (<0.01)	4.2 (0.01)

[a] Mean values are n=4 per depth in each forest from a composite of four to six sub-samples per sample and summarized by age class. Total carbon and nitrogen, extractable P and K, and exchangeable, Ca, and Mg. Mean (standard error).

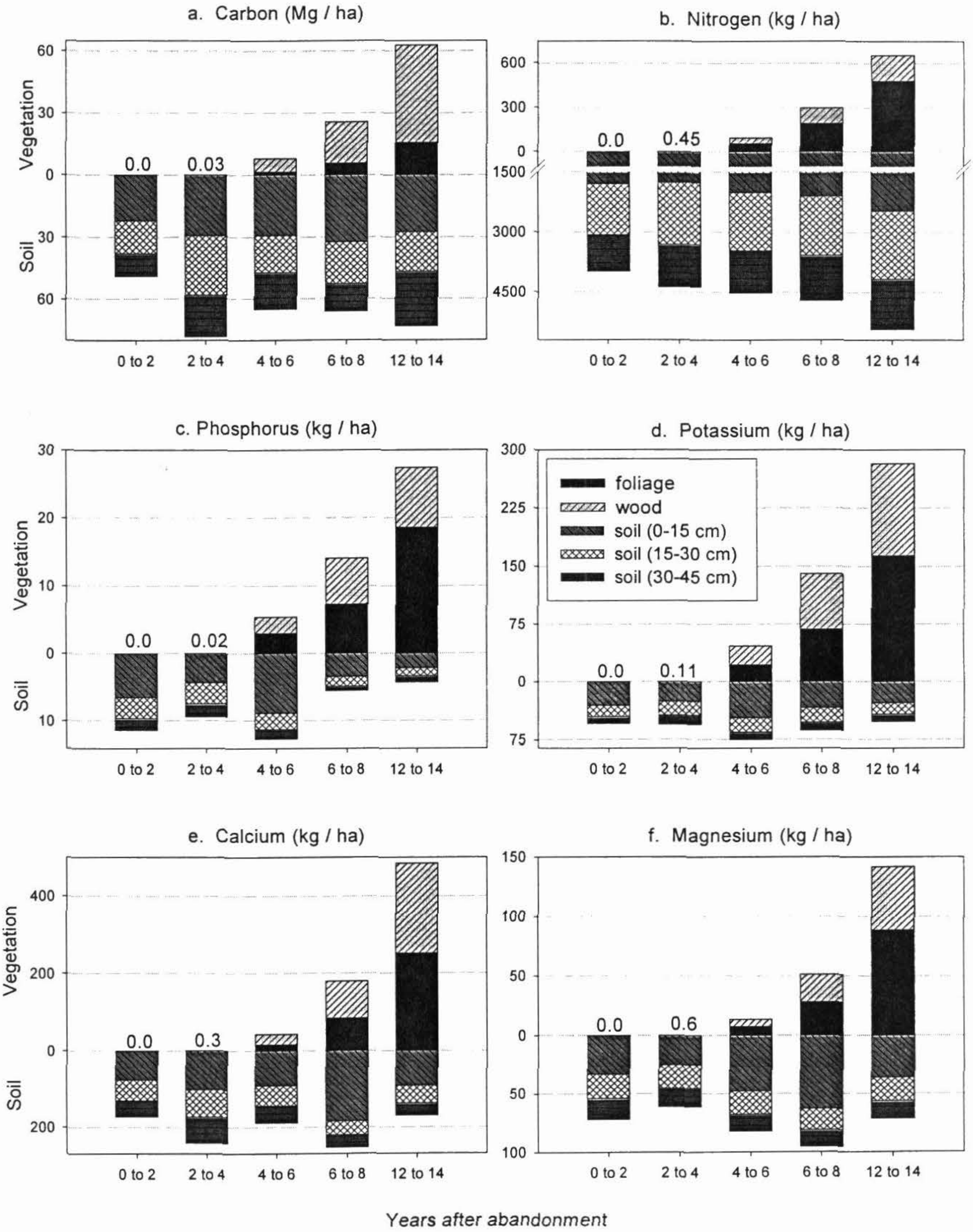

Figure 1 a – f: Total C, N, extractable P and K and exchangeable Ca and Mg in soils to 45 cm depth and live aboveground vegetation ≥1 cm DBH in ten secondary forests regenerating from degraded pastures in central Amazônia, Brazil. Mean nutrient stocks for each forest (see Table 1 for forest grouping by age-class) calculated from soil and vegetation nutrient concentrations times soil bulk density or aboveground biomass. Note the scale difference between N soil and aboveground stocks. Values above time 0 – 2 and 2 – 4 indicate aboveground quantities only.

Soil nutrient stocks

Soil extractable nutrient stocks were generally lower in deeper soil pools. Within the oldest forests, soil C, N, and Mg nutrient stocks were greater than aboveground nutrient stocks while the other nutrients resided predominantly within forest vegetation (Figure 1).

Soil N stocks, relative to aboveground stocks, were high, and increased with forest age at a rate of 117.8 kg ha^{-1} yr^{-1} ($r^2 = 0.44$; $p<0.05$). Pastures abandoned for twelve or more years stored 1.5 Mg/ha more total N to 45 cm depth than areas abandoned for two or fewer years (5.4 and 3.9 Mg N/ha). In all forests, surface nitrogen stocks (0–15 cm) represented approximately 40 to 45% of the total soil nitrogen to 45 cm depth (total 45 cm range: 3.3 to 5.5 Mg N/ha) (Figure 1).

Extractable soil P stocks to 45 cm tended to decline with increasing forest age (-0.66 kg ha^{-1} yr^{-1}), a trend most pronounced within the upper 0–15 cm. This surface layer represented 46 to 70% of total soil P stocks to 45 cm depth, with the younger areas, on average, storing 4.2 kg/ha more P in the first 15 cm than the oldest areas. Considering the entire measured soil profile (0–45 cm depth) higher extractable soil P stocks were observed in stands of 0 to 6 years (11.5 ± 4.6 kg/ha) compared to stands of 6–14 years (5.1 ± 2.1 kg/ha). Soil P in the 0–15 cm class was more variable than in deeper layers. Potassium, Ca, and Mg stocks remained constant with time after abandonment (Table 2).

Total nutrient stocks

There was a significant net gain in combined vegetation and soil nutrient stocks for all nutrients (Table 2). The total system P accumulation rate was slow and reflective of the counteracting decrease in soil P stocks with increasing forest age. Total system N stocks increased most rapidly followed by Ca. While total (biomass plus soil) nutrient stocks for all nutrients increased over time, in soils, only N increased significantly.

Carbon sequestration

Standing biomass. Foliar dry biomass in the ten forests grouped according to age after pasture abandonment (0–2, 2–4, 4–6, 6–8, 12–14 years) was 0.0, 0.02, 3.47, 13.10, 32.15 Mg/ha (Figure 1). Average biomass accrual for all SF through the first 12–14 years after pasture abandonment was 11.0 Mg ha^{-1} yr^{-1} ($r^2=0.95$, $p<0.001$), or 5.6 Mg C ha^{-1} yr^{-1} ($r^2=0.94$, $p<0.001$). As expected, carbon stocks in wood (4.2 Mg C ha^{-1} yr^{-1}) accrued more quickly than foliage C (1.4 Mg C ha^{-1} yr^{-1}) as forests matured (Table 4).

Table 4: Relationship between years after pasture abandonment (X) and the accumulation of aboveground biomass and carbon, and soil carbon (Mg ha^{-1} yr^{-1}) in ten secondary forests regenerating from degraded pastures in central Amazônia, Brazil.

Dependent Variable	Equation	(r^2)	(p)
Biomass[a]			
Foliage	$Y = 2.85\,X - 6.61$	0.94	< 0.001
Wood	$Y = 8.18\,X - 17.32$	0.95	< 0.001
Total foliage and wood	$Y = 11.0\,X - 23.92$	0.95	< 0.001
Carbon			
Foliage[a]	$Y = 1.40\,X - 3.36$	0.94	< 0.001
Wood[a]	$Y = 4.15\,X - 9.18$	0.94	< 0.001
Total foliage and wood[a]	$Y = 5.55\,X - 12.54$	0.94	< 0.001
Total soil[b]	$Y = 1.49\,X + 5.59$	0.20	= 0.20
Total foliage, wood, and soil	$Y = 7.04\,X + 43.31$	0.85	< 0.001

[a] All live trees ≥1 cm DBH with biomass converted to C based on site-specific foliage and wood C concentrations.

[b] Total soil C to 45 cm depth.

The greatest total biomass (128.1 Mg/ha) was measured in a SF with 12–14 years since abandonment; the areas abandoned 6–8 years had an average biomass of 54.4 Mg/ha, while the areas abandoned 4–6 years an average of 16.4 Mg/ha (Figure 1). Woody biomass in the ten SF by years after pasture abandonment was 0.0, 0.05, 12.92, 41.25, 92.24 Mg/ha in the 0–2, 2–4, 4–6, 6–8, and 12–14 year-old forests.

Soil Carbon. Soil carbon storage (excluding roots) tended to increase with forest age, with the oldest forests storing an average of 25 Mg/ha (65%) more total soil C to 45 cm depth than the youngest forests. Surface layers (0–15 cm) stored significantly more C (28.4 ± 2.4 Mg/ha) than deeper layers (18.3 ± 1.5 Mg/ha), from 24 to 50% of the total soil carbon to 45 cm depth in all forests ($p<0.001$). However, the oldest forests, 12–14 yr-old, stored as much carbon in the 30–45 cm layer as in the 0–15 cm surface layer. Additionally, the deeper soil profile (30–45 cm) was the only depth showing significantly increasing C stocks with time after abandonment ($r^2=0.21$; $p<0.001$). Considering all forests, total soil C to 45 cm depth increased non-significantly at a rate of 1.49 Mg ha^{-1} yr^{-1} during the first 12–14 yrs of succession (Table 4).

Total C accrual. In vegetation and soil (excluding roots), the ten SF accrued a total of 7.04 Mg C ha^{-1} yr^{-1} during the first 12–14 years after abandonment ($r^2=0.85$; $p<0.001$) (Table 4).

Discussion

Nutrient accrual

The vegetation withdraws large quantities of exchangeable Ca from low exchangeable soil reserves. After N, vegetation Ca stocks were accumulating most quickly with forest age. Wood and foliage N:Ca ratios were low, ranging from 0.7 to 1.4 for wood and 1.9 to 3.0 for foliage. In contrast, primary forest vegetation reported N:Ca ratios were 3.1 for trunks, branches and coarse roots, and 4.4 for leaves (Fernandes et al., 1997).

The high rate of Ca immobilization in vegetation but lack of reduced soil exchangeable Ca over time in our study indicates, (1) soils adequately replenish immobilized Ca from unavailable forms (Table 2); and/or (2) the vegetation is withdrawing Ca from deeper than 45 cm depth. A similar trend of a high percentage of total system Ca content in vegetation and high Ca uptake from low soil reserves of exchangeable Ca has been reported for temperate forests (Johnson and Henderson). The highly weathered Oxisols of our study provide negligible Ca from parent materials; however, atmospheric deposition may replenish depleted soil reserves by adding 0.8–12 kg ha^{-1} yr^{-1} (Vitousek and Sanford Jr., 1986; Schroth et al., 2001). For young tropical fallow vegetation, low root length density and low nutrient demand make Ca and nitrate ions susceptible to downward movement (Szott et al., 1999), which may be retrieved with increased rooting depth in later successional stages. Trees have been reported to increase soil nutrient availability over time (Sanchez et al., 1985) and net increases in total system stocks of N and Ca have been observed in older fallows, probably as a result of atmospheric deposition, N_2 fixation and uptake from subsoil (Szott et al., 1991). However, pasture soils were found to have higher exchangeable soil Ca concentrations than plantations, secondary and primary forests (McGrath et al., 2001), indicating that after the initial increase of soil Ca from cutting and burning, colonizing trees act as sinks, reducing soil Ca. Although soil Ca stocks are currently maintained in these SF, the high rate of Ca relocation from soil to vegetation, large vegetation Ca stocks, and high concentrations relative to N indicate extreme Ca demands for biomass production, which may create a soil Ca deficit and limit future vegetation growth.

The rapid total soil N stock and N concentration increase below 30 cm depth with forest age can be only partially explained by external inputs (Figure 1, Table 3). Nitrogen fixing plants may contribute 10–150 kg ha^{-1} yr^{-1} to soils (Fernandes et al., 1997; Szott et al., 1999) and atmospheric deposition may add 5.5–11.5 kg ha^{-1} yr^{-1} (Jordan et al., 1982; Vitousek and Sanford Jr., 1986; Schroth et al., 2001), explaining a fraction of the increasing total soil N. The remaining contribution to the high soil N accumulation rates could be subsoil mining of leached nitrate. Increasing extractable soil N with depth below topsoil have been measured in young SF (J. Lehmann, pers comm.); high deep soil N concentrations may be attributable to leaching from surface layers after slash-and-burning and cropping, followed by a reduced nutrient capture potential of shallow rooted colonizing secondary vegetation. Primary forest also loses nitrate to the subsoil (Schroth et al., 1999). These large N pools were deep (1-2 m) and considered at the lower limit of uptake by young SF. Leaching of surface N can be rapid in Oxisols because of the high macroporosity and hydraulic conductivity, but leaching below 0.6 m is delayed, apparently because of NO_3^- adsorption to the net positively charged subsoil (Melgar et al., 1992). Deep nutrient pools may provide a source of N as forests mature and root systems develop. Leaching of surface N (0–15 cm) to deeper layers could also explain the increase in N concentrations ($r^2 = 0.75$; $p<0.001$) we observed below 30 cm depth with forest maturation. Unless deep N mining occurs with root development, N losses to subsoil due to leaching may negatively affect surface soil fertility.

Compared to primary forest nutrient storage in soil (of the total aboveground and soil stocks), the SF stored comparable amounts of N, less P, but more Ca. Soil storage of exchangeable Ca and total N in the oldest SF accounted for an average of 26 and 89% of total nutrient storage, respectively, but just under 14% of extractable P (Figure 1). This contrasts with compartmentalization within primary vegetation, where soil storage of exchangeable Ca and total N may account for <1 and 73% of total nutrient storage, and extractable P in soil accounts for 69% of the total storage (Sanchez, 1987).

Increases in soil nutrient concentrations are followed by greater vegetation tissue concentrations in successional vegetation. Secondary forest vegetation growing on nutrient poor soil produced wood with three times less P and leaves with 50% less P than vegetation where soil P limitations were removed through P fertilizer additions (Gehring et al., 1999). The reduction in foliar P concentrations with increasing forest age observed in our study (Table 1) may indicate that this nutrient is becoming limiting as soil P levels decline (Figure 1).

Total aboveground and soil nutrient stocks increased as forests matured; yet, for P, uptake and soil P supply indicates a potential growth limitation. Concomitant decrease in extractable soil P and increase in biomass over time may be attributable to relocation from below- to above-ground pools. Plants appear to be taking up more soil P than is available (Table 2). This suggests a rapid transfer of soil P from plant unavailable to available forms or deep soil mining as the available pool is depleted with plant growth. However, subsoil P retrieval probably contributes <1 kg ha^{-1} yr^{-1} (Szott et al., 1999). The net reduction in soil P stocks from the soil (0.66 kg P ha^{-1} yr^{-1}) with increasing forest age, indicates inadequate replacement of available soil P with plant P uptake, a trend also observed elsewhere (Johnson et al., 2001). Should this trend continue, P may become limiting to growth unless other factors (1) reduce P uptake by plants, (2) increase P uptake from subsoil, (3) increase the rate at which unavailable forms of soil P shift to plant available P forms to replenish immobilized plant available soil P.

Pools of plant available (extractable) nutrients are significantly lower than the total in soils (Brown and Lugo, 1990), and the plant availability of the soil P depends on the extent of fixation or immobilization. Phosphorus fixation for Oxisols is lower in the central Amazon Basin than Oxisols in other regions of the Amazon; however, levels of plant available P in the soils are similar (Lehmann et al., 2001a). Total soil P in primary forest can be lower than under the SF replacing the vegetation (Lehmann et al., 2001b), indicating storage in biomass can significantly reduce soil extractable P stocks. Pastures grasses such as *Brachiaria* spp. may increase P availability by exuding acid phosphatase into the rhizosphere and hydrolyzing plant unavailable forms of organic phosphates (Dias-Filho et al., 2001), a benefit lost as secondary vegetation replaces the pasture grasses. Root associations with both VA-mycorrhizae and ectomycorrhizae may help the colonizing vegetation access P even at low soil P concentrations; and, in the case of ectomycorrhizae, to access P from poorly accessible pools (Boot et al., 1994). Since these colonizing species have the ability to take up P in excess of immediate growth requirements (Boot et al., 1994), P uptake by maturing trees may decline before plant levels become limiting to biomass accumulation. Fallow vegetation increased mineralizable N and available P compared to continuous cropping, probably as a result of deeper rooting (Tian et al., 2001).

Further research is needed to develop and evaluate management strategies that promote soil P acquisition, such as increasing rooting depth of regenerating vegetation.

Carbon accrual

Aboveground. Rapid biomass accrual in the SF, 11.0 Mg ha^{-1} yr^{-1}, was similar to other Amazonian findings (Uhl et al., 1988; Brown and Lugo, 1990; Alves et al., 1997), lower (Hartemink, 2001), and higher than a 20 year mean annual rate (6.17 Mg ha^{-1} yr^{-1}) in a review of tropical SF succession (Silver et al., 2000). The high C accumulation storage in the 12–14 yr-old areas represents 25–50% of equivalent primary forest biomass (230 – 500 Mg/ha) in Amazonia (Alves et al., 1997; Fujisaka et al., 1998; Laurance et al., 1999).

Aboveground carbon accrual slows with age as colonizing trees mature, die, and are replaced by slower growing species. Secondary forests in the Bragantina region of the Amazon basin were accruing biomass more rapidly in 10 yr-old (5.5 Mg ha^{-1} yr^{-1}) than in the 20–40 yr-old SF (3.3 Mg ha^{-1} yr^{-1}) (Johnson et al., 2001). And a review of 44 secondary tropical forests showed wet forests accumulating biomass significantly faster during the first 20 of 80 years of regrowth (Silver et al., 2000). Their rates through the first 10 and 20 years are still less than the rate we report through the first 12–14 years (Table 4).

Belowground. Our study indicates a trend of increasing soil C storage through the first 12–14 years (Figure 1); however, the soils are storing comparable to less C than other SF of similar or greater age (Silver et al., 2000); (Johnson et al., 2001). Although our forests only showed a weak C storage gain, other studies indicated that soil C storage (excluding roots) increases significantly with SF age, and can approach mature forests levels after 80 years regrowth (Silver et al., 2000).

Contrary to aboveground biomass accumulation rates, which proceed faster in SF following agriculture (Fearnside and Guimaraes, 1996), soils accumulate C almost twice as fast when regeneration follows pasture rather than agriculture, although this effect is only distinguishable after 20 years of recovery (Silver et al., 2000). Delays in aboveground C accrual with forest growth in early years following pasture abandonment may be offset, to a certain degree, by enhanced soil C accumulation.

Compared with soil C storage in pasture (49.5 Mg/ha; 0–30 cm depth) (Moraes et al., 1996), tropical plantation (90 Mg C/ha) and SF (61 Mg C/ha) (0–25 cm depth) (Silver et al., 2000), the SF soils in this study were storing 47.9 Mg C/ha to 30 cm and 66.1 Mg C/ha to 45 cm depth. Since aboveground C accrual appears higher than other sites, and soil C lower than other SF sites, high litter turnover and soil respiration rates at our sites may be reducing soil C residence time in this high rainfall area.

These factors pose important management implications to carbon sequestration. By choosing to maintain areas as pasture, directing SF colonization and succession after abandonment, or establishing plantations or agroforestry systems, land managers can influence the distribution of aboveground and soil C storage and the rate at which carbon accumulates within those pools. Maintaining the land cover as forest for longer time-periods rather than as degraded pasture is a more favorable practice to increase C storage.

Predictive limitations

The ≥1 cm DBH limitation imposed by the allometric equations may significantly underestimate biomass and nutrient stocks in the absence of root and biomass measurements of young SF vegetation <1cm DBH. Grasses tend to allocate a significant portion of total plant biomass within root structures (Nepstad et al., 1994) and necromass, shrubs and herbaceous vegetation dominating early pasture succession and SF understories contribute considerable quantities to C and nutrient stocks, especially P (McKerrow, 1992). Wood core measurements may overestimate nutrient concentrations in young stands since a greater portion of the sample core is nutrient rich bark. An underestimate of biomass as a result of DBH allometric limitations is inversely related to forest age. As stand dominance shifts from small- to large-stem diameter plants with understory shading and self-thinning, a greater percentage of the total stems are measured and contribute to biomass and nutrient calculations. Also, although we located our abandoned pasture study sites on plateaus, the pastures span the rolling topography. Nutrient limitations may be more severe on hillsides where erosion is more pronounced and forest recovery slower than the rates we predict.

Implications for succession, carbon sequestration, and nutrient barriers

After two and a half decades of neotropical studies of SF regeneration, we still lack the ability to make strong predictions about nutrient storage and successional shifts in forest development, and regeneration times for abandoned pastureland to attain primary forest equivalent biomass. This is largely due to an historic research focus on forest succession following agriculture rather than pasture. Since biomass recovery is significantly slower following pasture than agriculture (Fearnside and Guimaraes, 1996; Steininger, 2000), it is important to increase our understanding of pasture succession and determine potential nutrient limitations.

The paucity of forest recovery studies on abandoned pastureland and lack of detailed soil C and nutrient data make predicting forest regeneration on highly altered lands difficult. In a review of SF biomass accumulation (Silver et al., 2000), only 13% of the SF (18 of 134) were previously pastures. Additional data from SF regenerating from pasture are needed to determine long-term C accumulation rates, potential nutrient limitations to regeneration, and the time needed to attain both structural and functional properties of mature forests. This is especially relevant since primary forest biomass is positively associated with soil nutrient levels, suggesting that soil nutrient loss through pasture installation may result in lower mature regenerated forest biomass than the original forest (Laurance et al., 1999). Although studies such as ours help to fill this void, there is a need for long-term rather than chronosequential studies of forest recovery following pasture abandonment.

Summary

In this study, we show that during early successional years, biomass accumulation in light to moderately used pastures is rapid after abandonment and that soil C storage is higher in older forests. However, a slower soil C accrual rate than regenerating SF in other regions, may negatively offset total long-term C gains. The higher proportion of soil C storage compared to aboveground pools will be an important consideration of future 'carbon credit' management, as this pool is more recalcitrant to perturbations. Aboveground C re-accumulation from post-burn values is high, yet represents a finite pool which is rapidly attained in a relatively short time-period. Managing forest regeneration to maximize soil C storage, rather than aboveground pools, may prove to be more useful or meaningful when attempting to increase SF C sequestration.

Furthermore, the colonizing vegetation can extract large nutrient quantities from the soil, even when in low supply. There was not only a shift of nutrients from soil to aboveground pools, but total system nutrient stocks were increasing over time. Most of the C, N, and Mg were stored within soils, while P, K, and Ca resided within vegetation. This has important consequences to total forest nutrient stocks, in the event of removal of aboveground vegetation. In the absence of nutrient additions, removal of the vegetation a second time (pasture re-clearing or logging) could compromise the SF potential to regenerate as a result of nutrient limitations. Even after P fertilization when the areas were pasture, soil P stocks remained low. The vegetation was withdrawing more soil P than can be replenished, creating a soil P deficit which may limit system productivity. Low exchangeable soil Ca stocks seemed to be adequately replaced, apparently from atmospheric inputs and depths below 45 cm, as growing vegetation took up large nutrient quantities. Nevertheless, as vegetation Ca demands were high and soil stocks low, lack of Ca may limit future productivity.

These results demonstrate the regenerative capacity of tropical SFs to sequester C and to rebuild the nutrient capital following pasture abandonment. Aboveground carbon accrual is rapid but belowground gains represent the largest potential area for continued accumulation and management. Relocation of some nutrients from deeper soil layers may represent a substantial source of nutrients for plant growth and may be vital to sustaining long-term productivity and biomass accumulation. We recommend additional studies to explore P and Ca nutrient limitations to forest productivity and long-term measurements of soil nutrient fluxes and forest growth. Understanding nutrient limitations to resource capture will provide new options to manage forest regeneration and increase C accumulation on these globally important nutrient-limited soils.

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Activity 3.3.2

Carbon Storage in Soils from Degraded Pastures and Agroforestry Systems in Central Amazônia: The role of charcoal

ntonio arctan arian

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Introduction

The vicious cycle of deforestation: Vast areas of the Amazon rainforest have been cleared in the last decades to be converted into pastures. After few years of use, the land is degraded as a result of nutrient depletion, soil compaction and surface sealing. Productivity declines severely and pastures become abandoned, giving place for a succession to secondary forest. A new forest area is then usually cleared to start the process again. Abandoned lands are characterized by very low storage of nutrients and reduced stocks of soil organic carbon (SOC). Some alternatives do exist to recuperate degraded land including the establishment of agroforestry systems. These options are expected to help in restoring soil nutrients and allow C sequestration in both biomass and soils. This poster presents information on C storage in soils under 10 year old agroforestry systems and secondary vegetation, as well as on primary forest for an area in the Central Amazon.

The Charcoal contribution. Natural and anthropogenic fires are frequent in the Amazon forest. Combustion of plant material is never complete specially for roots, and this result in variable amounts of residual charcoal being added to the soils. Charcoal is ubiquitous in Amazonian soils and is present in a range of size particles: from coarse (>2 mm diameter) usually found mostly at the soil top layers, to very fine particles (<50 μm) distributed along the soil profile.

Charcoal is mainly carbon in an extremely inert form but until now not much effort has being devoted to define this as a separate C pool in soils. Given its inherently heterogeneous distribution, charcoal presence creates problems when trying to assess the effect of a given land management option on C sequestration by soils. Changes of SOC are normally small for short to medium terms and could be masked by the "noise signal" created by the charcoal. This noise also confounds interpretation of the dynamics of SOC when the ^{13}C technique is used (Desjardins et al., 1996). To be able to separate any difference in SOC resulting from different land use, charcoal contribution to total soil C has to be assessed. In this study we evaluated the contribution of different charcoal size classes to total soil C.

Materials and Methods

The project is being conducted at EMBRAPA-CPAA research station at km 54 north of Manaus, Brazil. In a long-term experiment, four alternatives to recover degraded land in the Central Amazon have been studied: establishment of two silvopastoral (SPS) and two agroforestry (AFS) systems: SPS included ASP1 (medium fertilizer inputs) combining *Brachiaria brizantha*, *Desmodium ovalifolium* and mahogany (*Sweithenia macrophyla*) and low input ASP2 in which *B. Brizantha* has been replaced by *B. humidicola*. AFS were: AS1 based on palm species (*Bactris gasipaes and Euterpe olearaceae*) and also includes Cupuaçu (*Theobroma grandiflorum and Colubrina acreanaea*). AS2 is based in native and exotic fruit trees (6 species) and also includes Mahogany and Brazil nut (*Bertholletia excelsa*). Secondary vegetation of similar age to the AFS and primary forest soils have been used as controls. Three repetitions of every system and control were evaluated. The area has a mean annual rainfall of 2250 mm and average temperature of 28°C. Soils are high clay Oxisols, very low in fertility.

Given the complexity of the AFS studied, to obtain a representative sample of soil from a given systems is a complex task. Soil sampling was based on a species-interaction strategy. In each plot (3000 m^2), a composite soil sample of five sites was taken from each of the main plant-plant interaction found in the plot. Samples were separated at 0-5, 5-15, 15-30, 30-60 and 60-100 cm depth. Soil was air dried and gently dissagretated to <4mm diameter. A type Jones sample divider was used to separate soil in three size classes: 2-4 mm, 0,5-2mm and less than 0,5 mm diameter. Charcoal was separated by hand in a subsample of the 2-4 mm (G) and 0.5–2mm (M) size. Then, the original sample (free from medium to large size charcoal) was reconstituted to be analyzed for total C and nutrients. In a subset of samples, the fine fraction was used to determine black carbon content using the methodology of Kuhlbusch (1995). Finely ground soil (<53 um) was oxidized with repeated doses of NaOH (four times), HCl, HNO_3, H_2O_2, H_2O and finally thermally oxidized in a Oxygen-rich environment at 350C for 3 hours. C content in the original sample and the final residue were determined in a dry combustion CHN analyzer. C content in the residual soil after the chemical and thermal oxidation is defined as black carbon, and in addition to charcoal it includes the most highly resistant components of SOM.

Results and Discussion

Charcoal. Figure 1 shows the relative contribution from charcoal in each size class to total charcoal for the case of the ASP1 system. Charcoal in medium and large sizes is more abundant in the surface and subsurface layers and decays strongly as soil gets deeper. It is worth nothing however, that medium size particles are found even at 1m depth suggesting or localized burning of deep roots or migration of charcoal from the surface probably through root or soil fauna channels. Variability in charcoal content in the M and G size classes was high indicating non-homogeneous distribution of charcoal in soils. The contribution of the fine fraction is much more homogeneously distributed through the soil profile.

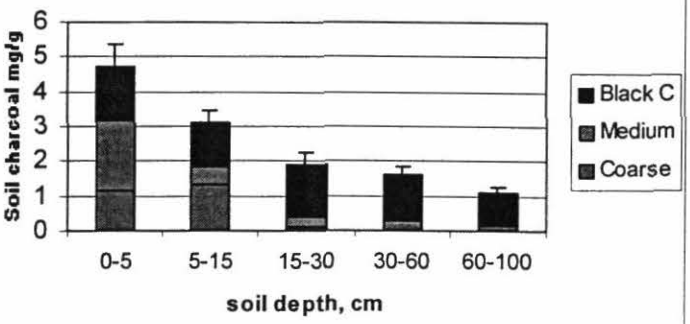


Figure 1. Charcoal distribution in various soil particle sizes. System ASP1.

Figure 2 represents the relative contribution of charcoal fractions to total soil C. It can be seen that charcoal can account for between 5 and 15% of total soil C, with higher proportions found in the top soil layers.

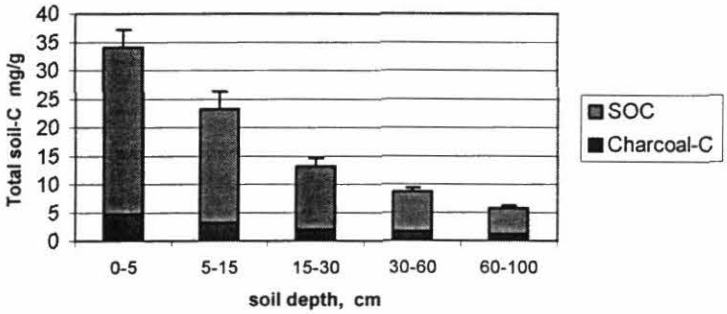
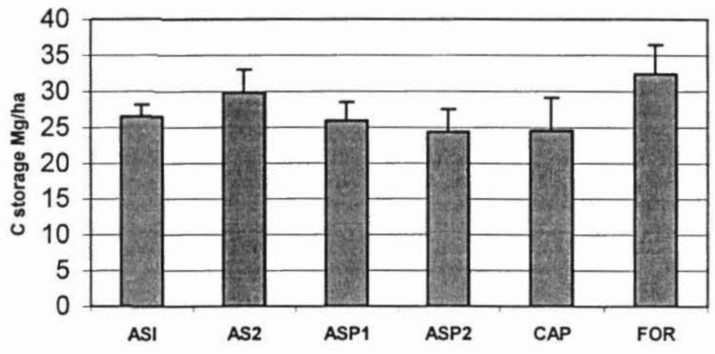


Figure 2. Charcoal contribution to total soil-C. System ASP1

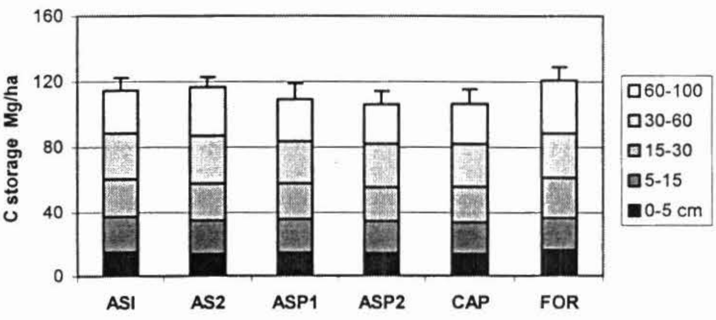
Soil C stocks. There were found significant differences among C stocks in most soil layers except in the surface layer where variability was the highest. Figure 3 shows the C stocks stored in the 60-100 cm depth soil layer. Carbon storage at that layer was higher in the primary forest soil as compared to other systems, being followed by the fruit based system (which includes some large trees), suggesting an important contribution from deep roots to C buildup. The ASP2 low input system presented the lowest C storage at such depth. This is in agreement with lower aerial biomass estimates for such system reported by McCaffery (Poster in this meeting).

Figure 3. C storage in soils at 60-100cm depth



In Figure 4, total C storage in the soil profile is presented for all systems (data correspond to C content after removing the coarse and medium size charcoal fragments). Significant differences were found between systems. When the charcoal contribution is taken into account, such differences become hidden. Forest soils store the highest amount of C (121 $Mg.ha^{-1}$), followed by the AS2 system with 116 $Mg.ha^{-1}$. On the other extreme, soils under secondary vegetation and ASP2 system presented the lowest stock (106 $Mg.ha^{-1}$). Results in this study indicate that agroforestry systems permit a moderate recovery of soil C stock relative to the control under secondary vegetation. Rates of C accrual are in the order of 1.8 $Mg.ha^{-1}.y^{-1}$. This contrasts with much higher rates reported for temperate and tropical regions (Bruce et al., 1999). It is worth mentioning however, that the initial soils were highly degraded after supporting cattle grazing for more that 12 years. Application of fertilizer was also very low to the systems. This suggests that there is space for increasing C accumulation rates through the practice of agroforestry.

Figure 4. C storage in soils under various land uses in the Central Amazon



Conclusions

Charcoal is an ubiquitous constituent of the soils in the Central Amazon and appreciable amounts can be found even at 1 m depth. Coarse fragments are located preferentially at the surface layers showing high heterogeneity in its spatial distribution. Fine particles distribute rather homogeneously through the soil profile. Charcoal derived-C can account for as much as 15% of total soil C. Separation of coarse and medium size charcoal fragments is very important to allow appropriate comparison between SOC in different land use systems in areas where fire is a factor in the natural or human influenced management of the forest. Although charcoal separation and the assessment of black carbon is a time consuming process, given that charcoal is a remarkably stable pool, once a baseline has been established for a certain site, the same information could be used in future studies.

Though various studies have shown that soils under pastures enable high rates of C sequestration (Fisher et al., 1994) in our sites, the lack of fertilizer inputs and the high initial degradation of the land prevented a significant accrual of SOC as compared to soils under secondary vegetation.

Even under unfavorable initial conditions, agroforestry systems allow net C accumulation in soils, permitting the soils to move in a 10 years time period, from 87% to 95% of the total C stocks in the primary forest.

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Activity 3.3.3

Antonio

Slash-and-char – a feasible alternative for soil fertility management in the central Amazon?

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Abstract

The application of charcoal to nutrient-poor upland soils of the central Amazon was tested in lysimeter studies in comparison to unamended control soils to evaluate the effects of charcoal on plant nutrition and nutrient leaching. Testing the application of charred organic matter was stimulated by the fact that anthropogenic soils in the Amazon (so-called "Terra Preta") with high soil organic matter contents contain large amounts of pyrogenic carbon. These soils also show high cation exchange capacity and nutrient availability. Charcoal additions significantly increased biomass production of a rice crop in comparison to a control on a Xanthic Ferralsol. This increase was largely an effect of improved P, K, and possibly Cu nutrition, whereas N and Mg uptake decreased in charcoal amended soils. In order to improve crop growth, fertilizer applications of N, S, Ca, and Mg may be necessary in addition to charcoal for optimizing rice growth. Combined application of N with charcoal resulted in a higher N uptake than what would have been expected from sole fertilizer or charcoal applications. The reason is a higher nutrient retention of applied ammonium by the charcoal amended soils. Charcoal applications therefore acted in two ways, first as a direct fertilizer and secondly as an adsorber which retained N. The amount of charcoal which can be produced from forest biomass is significant and corresponds to charcoal amounts needed for effectively improving crop growth. The slash-and-char technique is an alternative to burning of the above ground biomass and only the biomass from the same cropping area will be used for charring. Field trials need to be conducted to investigate the efficiency of charcoal production and applications under field conditions.

Keywords: Amazon; Humid Tropics; Ferralsols; Leaching; Nutrient Cycling; Slash-and-Burn

Introduction

Upland soils in the humid tropics such as in the central Amazon are highly weathered and therefore possess low plant available nutrient contents (Cravo and Smyth, 1997). This is a result of both high rainfall and low nutrient retention capacity. Applied nutrients are rapidly leached below the root zone of annual crops (Melgar et al., 1992; Cahn et al., 1993). Two basic approaches can be used to reduce nutrient leaching, first to apply slow-releasing nutrient forms such as organic fertilizers or secondly to increase adsorption sites and thereby retain applied inorganic nutrients.

Slash-and-burn is one of the main land use system in the Amazon. Secondary or primary forest is cut and burned to clear the field but also to release plant-available nutrients from slashed plant biomass. The ash from the burned biomass increases soil pH and supplies nutrients to crops which show elevated nutritient levels and yields (Sanchez et al., 1983). This effect of the ash accumulation is, however, rather short-lived. Already after a few cropping seasons the soil nutrient availability decreases and field crops have to be fertilized for optimum production (Sanchez et al., 1983) or the fields have to abandoned and new forests have to be slashed and burned. Although adequate applications of mineral fertilizers were shown to sustain yields in the Amazon (Smyth and Cassel, 1995), our efforts are intended to improve the use of biomass and nutrients contained in the plant biomass as well as that of applied fertilizer nutrients, since fertilization is expensive and crop production often has to rely on soil nutrients alone.

It is well known that about 50% of the carbon in the above ground biomass of forests can be lost upon burning (Kauffman et al., 1995). Sixty and 43% of the biomass N and S and 18, 7 and 7% of the P, Ca, and K were lost from the site (Kauffman et al., 1995). A large portion may be deposited or absorbed in surrounding ecosystems but does not contribute to the fertility of the cropped soil. The first approach should therefore aim at improving the efficiency of land clearing to preserve C and nutrients. Slash-and-mulch was successfully tested in Eastern Amazonia when fertilizer was applied (Kato et al., 1999) and has a long history in the per-humid tropics (Thurston, 1997). We are seeking an alternative technique that can be applied to the existing slash-and-burn system with minimal changes and that has the potential of being used in tree cultures as well.

Anthropogenic dark earths - evidence of sustainable soil management

Instead of burning the above ground biomass to clear the agricultural field, the biomass may be charred to produce charcoal and added to soil. Testing the application of charred organic matter was stimulated by the fact that anthropogenic dark earths in Central Amazonia (so-called "Terra Preta do Indio") with high soil organic matter contents contain large amounts of pyrogenic carbon (Glaser et al., 2001). These soils also show high cation exchange capacity, nutrient availability and organic matter (Sombroek, 1966; Kern and Kämpf, 1989). The origin of the dark earths is not entirely clear, and several conflicting theories were discussed in the past. Currently the most convincing theory states that these soils were not only used by the local population but a product of indigenous soil management as proposed by Gourou (1949).

Soil fertility increases have been observed on remnants of charcoal hearths in the Appalachian Mountains (Young et al., 1996). Tryon (1948) showed higher nutrient availability in clayey to sandy soils from the Western United States after additions of charcoal produced from conifer and hardwood. Coal from geological deposits were successfully tested for the improvement of soil physical properties (Piccolo et al., 1996). No information, however, is available about the effects of charcoal applications on nutrient availability of highly weathered soils in the humid tropics such as the central Amazon. It is also unclear whether the cation exchange capacity can be improved thereby leading to higher nutrient retention and to lower nutrient losses by leaching.

A slash-and-char technique does not advocate the destruction of existing primary forests. It should be a carbon- and nutrient-conserving alternative to existing slash-and-burn techniques. In this way, carbon will rather be retained in the system compared to slash-and-burn, since only the biomass from the same cropping area will be used for producing the charcoal.

Charcoal additions for soil fertility improvement

Experimental description

Pot experiment. A greenhouse study was carried out at the Embrapa Amazonia Ocidental near Manaus, Brazil. The mean temperature in the greenhouse was between 28-32°C. We used two different soils for our experiments: (1) a Xanthic Ferralsol taken from a secondary forest (approximately 15 years old) with high clay contents (65%), medium organic C (39 g kg^{-1}) and N contents (31.7 g kg^{-1}); (2) a Fimic Anthrosol obtained from a farmers field under fallow with low clay (5%) and high sand contents (85%), high organic C (84.7 g kg^{-1}), available P (318 mg kg^{-1}) and Ca contents (656 mg kg^{-1}), but low to medium total N (49.6 g kg^{-1}), available K (4.0 mg kg^{-1}) and Mg contents (57 mg kg^{-1}). Both soils have not been fertilized prior to the experiment.

Free-draining lysimeters were constructed with a diameter of 0.2m and a height of 0.1 m which were filled with either 3 kg of the Ferralsol or the Anthrosol. The effect of soil type, mineral fertilizer and charcoal on growth, nutrient uptake and leaching was tested using rice (*Oryza sativa* L.) as a test plant. Charcoal was applied at 20% weight which was produced by local farmers originating from secondary forests. The charcoal was ground by hand to a grain size of about 1 mm. Fertilizer was applied at 30, 21.8, and 49.8 kg ha^{-1} for N, P, and K using ammonium sulfate, TSP, and KCl, respectively. Lime was applied at 2.1 Mg ha^{-1} [all recommendations for rice from Araujo et al. (1984) Circular Técnica 18, Embrapa, unpublished].

After the soil was filled into the lysimeters, water was gently poured onto the soil at a daily rate of 6.85 mm (2500 mm y^{-1}). After four days the electrolyte content in the leachate had stabilized and fertilizer was added and rice was planted (five stands per pot with three plants per stand). Water was applied and drained daily, but only selected samples were analyzed. Nutrient contents were determined daily for the first week, twice a week for three weeks and after 5 and 10 days. The sampling was stopped when the rice was cut at 37 days after planting. The amount of leachate was determined by weight and a subsample was retained for further analyses and frozen. Cumulative leaching for the entire experimental period was calculated from the measured leachates and amounts were interpolated linearly. Plant samples were dried at 70°C for 48 hours and weighed.

In a second pot experiment, seedlings of *Inga edulis* were planted in pots with 26 cm diameter and 10 dm^3 soil (Xanthic Ferralsol) in four replicates. Charcoal was added at 0, 1, 5, 10, and 20% weight corresponding to 0, 13.3, 66.7, 133.4, and 266.7 Mg C ha^{-1} (C concentration of charcoal 70.8%). Fertilizer was applied at 100 kg N ha^{-1}, 50 kg P ha^{-1}, and 60 kg K ha^{-1} as urea, triple super phosphate and KCl, respectively. Additionally, 2 Mg ha^{-1} lime were added. Stem diameter was determined at 5-cm above soil level, and tree height was measured including the length of the uppermost leaf at 80 day after planting.

Adsorption experiment. In a laboratory experiment, we studied the adsorption of different nutrients by charcoal. The charcoal was made from the wood of black locust (*Robinia pseudoacacia*). Cubes of dried wood with 10 g were isothermically combusted in closed metal containers at 350°C for 40 minutes (65 replicates). Wood and charcoal were weighed with an accuracy of 0.1%. The charcoal was ground coarsely with mortar and pestle to pass a 2 mm sieve. One gram of charcoal was added to 10 mL of solution containing 0, 20, 50, 100, 200 mg L^{-1} using 20-mL PE bottles. In a preliminary experiment, the adsorption dynamics were determined for 10, 30 minutes, 1 and 6 hours, 1 and 3 days and 1 week using a horizontal shaker. Adsorption changed until one day, but did not differ thereafter. Therefore, adsorption experiments were done with a shaking time of one day. The effect of coating with dissolved organic matter (DOC) was tested with a manure extract. Ten grams cow manure were shaken with 20 mL deionized water and filtered. The filtrate was diluted 50 times and 10 mL of the solution was shaken with 1 g of charcoal for 24 hours. Afterwards the same adsorption experiment with different concentrations only of NH_4^+ was performed as described above with and without additions of 10% azide to inhibit microbial activity.

Chemical analyses. The aboveground biomass of rice was ground with a ball mill and analyzed for nutrients and organic carbon. C and N analyses were performed with an automatic CN analyzer (Elementar, Hanau, Germany). The K, Ca, Mg, Fe, Zn, Cu contents in the plant biomass were determined after wet digestion with sulfuric acid using atomic absorption spectrometry (AA-400, Varian Associates, Inc., Palo Alto, CA). The P contents were measured photometrically in the same extract with the molybdenum blue method.

The K, Ca, and Mg contents in the leachate and adsorption solution were measured using atomic absorption spectrometry, nitrate (NO_3^-) and ammonium (NH_4^+) concentrations were determined photometrically with a continuous flow analyzer (RFA-300, Alpkem Corp., Clackamas, OR and Scan Plus analyzer, Skalar Analytical B.V., Breda, The Netherlands) after reduction with Cd and reaction with salicylate, respectively.

Statistics. Treatment effects of the bioassay were analyzed by analysis of variance (ANOVA) with a randomized complete block design. Mean separation was done using the least significant difference test (LSD).

Charcoal as a fertilizer

Charcoal additions increased biomass production of a rice crop by 17% in comparison to a control on a Xanthic Ferralsol (Figure 1). This increase was largely an effect of improved P, K, and possibly Cu nutrition. Nitrogen and Mg uptake decreased in charcoal amended soils which resembled the uptake pattern of rice grown on an Amazonian dark earth (Fimic Anthrosol; Figure 1). Charcoal additions had no significant effects on S, Ca, Fe, Zn, and Mn uptake ($P>0.05$). In addition to charcoal, fertilization was necessary with N, S, Ca, and Mg for optimizing rice growth.

The soil fertility improvement of the dark earth was largely an effect of enhanced P, Ca, and micronutrient availability such as Mn and Cu. Crop nutrition of S and K was not better and that of N and Mg was even lower in rice grown on a dark earth in comparison to the Ferralsol. Fertilization was necessary for those elements and was effective in increasing total nutrient uptake (Figure 1)

Therefore, charcoal directly amended the soil with plant-available nutrients such as P, K, and Cu. If fertilizer was applied together with the charcoal some nutrients showed a higher uptake efficiency than the added effects of fertilization and charcoal amendment would suggest. This was the case for N, Ca, and Mg. In the following we discuss the reasons for a higher efficiency of a combined application.

Charcoal as an adsorber

Under the high leaching conditions in upland soils of the central Amazon, reduction of nutrient losses by leaching is an important aim in order to improve nutrient availability for plants. Immediately after fertilizer application, nutrient contents significantly increased as shown for ammonium (Figure 2) and leveled off to background levels only 21 days after fertilization. This was also the case with K, Ca, and Mg (data not shown).

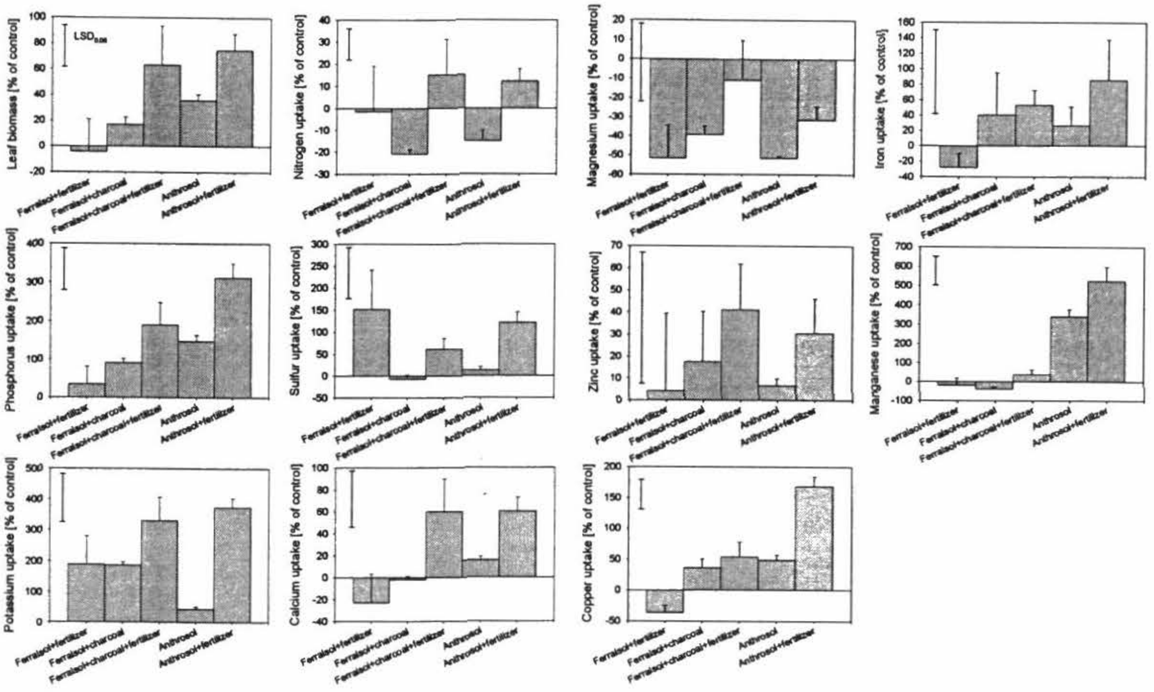

Figure 1: Biomass production and nutrient uptake by rice (*Oryza sativa*) after additions of charcoal and fertilizer to a Xanthic Ferralsol or a Fimic Anthrosol after 37 days (means and standard errors; N=4).

Leaching from the unfertilized Ferralsol was reduced when charcoal was applied and resembled the low values found in the Anthrosol (Figure 2). Ammonium concentrations in the leachate were also significantly lower in the fertilized Ferralsol after charcoal applications. These results indicate that ammonium was adsorbed by the charcoal and elevated N uptake by rice after the combined application of charcoal and fertilizer (Figure 1) was an effect of ammonium retention. This retention could not be found for other cations or anions, because K, Ca, and Mg were in higher supply with charcoal additions. After several cropping cycles, the nutrients in the charcoal may be depleted and results may differ from those shown here. Since N was applied as ammonium, nitrate contents in the leachate were controlled by biological transformation rather than physical adsorption.

In accordance with the leaching results, only ammonium was adsorbed by charcoal (Fig. 3) whereas all other nutrients (P as PO_4^{2-}, Ca, Mg, K) showed higher concentrations in the equilibrium solution than added (data not shown). The process of adsorption is largely a co-adsorption with soluble organic matter, as an addition of dissolved organic carbon (DOC) from a manure extract increased ammonium adsorption. A microbial immobilization or nitrification during shaking can be excluded, since the adsorption was similar when microbial activity was suppressed by additions of azide (Fig. 3).

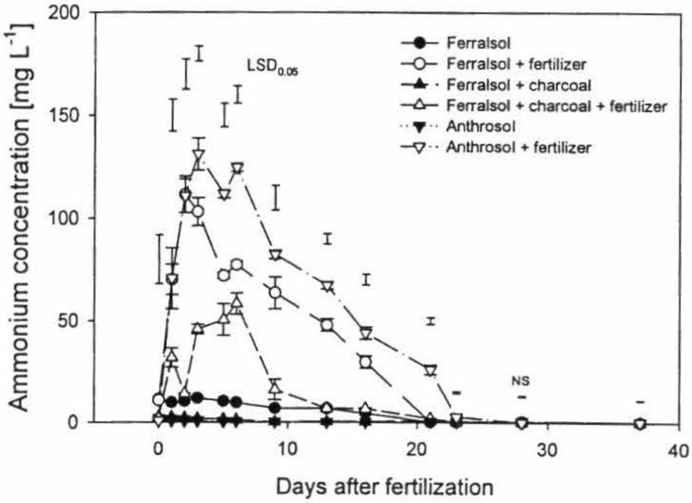

Figure 2: Ammonium concentration in the leachate of a Xanthic Ferralsol amended with charcoal and fertilizer compared to a Fimic Anthrosol; main effects significant at $P<0.001$ apart from one (NS not significant $P>0.05$) (means and standard errors; N=4).

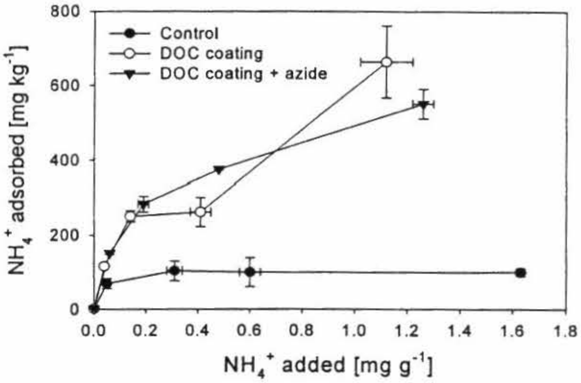

Figure 3: Ammonium adsorption by charcoal produced from black locust (*Robinia pseudoacacia*) in comparison to charcoal coated with manure or additional suppression of microbial activity using 10% azide (means and standard errors; N=2).

Slash-and-char in smallholder agriculture

If a slash-and-char technique was to be successful, (i) the quantity of applied charcoal must be produced from the same area of land that is cropped, and (ii) the periods of charcoal production must at least correspond to those of land clearing practiced so far. In other words, the slash-and-char technique must work with the same resources as conventional methods and be an alternative to slash-and-burn or slash-and-mulch. The amount of charcoal which can be produced from different forest vegetation primarily depends on the woody biomass available, and additionally on the production procedure such as charring environment (e.g., oxygen), temperature and time (e.g., Glaser et al., 2002). The average recovery of charcoal mass from woody biomass is 31% according to the published data compiled in Table 1. The effect of different charcoal production methods on its recovery in agricultural fields is not well known and the charring environment such as temperature and charring time is usually poorly documented. The carbon contents of charcoal do not vary much and lie around 63-83% with a mean of 76% (Table 1). The carbon recovery from charred woody biomass is relatively high with 54% (Table 1) due to the high carbon contents of charcoal.

Several published values of above ground biomass from secondary and primary forests in the central Amazon show a high proportion of woody biomass (Table 2). Biomass of secondary forests increase with age but depend largely on site conditions and previous land use. Larger amounts of charcoal can be produced from primary (57-66 Mg C ha^{-1}) than secondary forests calculated with the average conversion from Table 1. But also secondary forests may produce charcoal equivalents of up to 32 Mg C ha^{-1} after only 4 years (Table 2). The pot experiment shown in Figures 1 and 2 was conducted with a charcoal amount of 135 Mg C ha^{-1} (20% weight in 10 cm depth), but also 67 Mg C ha^{-1} (10%) were shown to significantly improve biomass production of cowpea (Lehmann et al., unpublished). In a pot experiment with *Inga edulis*, tree height and stem diameter significantly increased through the addition of charcoal (Figure 4; ANOVA P=0.041 and 0.007, respectively). Already at the lowest application rate (13.3 Mg C ha^{-1}), charcoal additions were equivalent to fertilizer applications. Therefore, the charcoal amounts produced from the same area of land which is used for cropping during one charring event are sufficient for improving crop performance and for reducing nutrient leaching. Lower amounts of 7.9 Mg C ha^{-1} were shown to have only minor effects on rice yield in the first cropping season under field conditions (Steiner and Nehls, unpublished data) but more information is needed from field experiments. With increasing charcoal additions, growth of *Inga* decreased when no fertilizer was applied but increased with fertilizer applications.

Table 1: Biomass conversion into charcoal

Tree species	Charring temperature [°C]	Production method	Charcoal recovery by weight [%]	n	Charcoal carbon content [%]	Carbon yield[1] [%]	Source
Acacia mangium	450	laboratory furnace	37.9	60	76.4	64.4	Lelles et al. (1996)
Eucalyptus grandis	470	laboratory furnace	33.8	60	80.7	60.6	Vital et al. (1986)
Eucalyptus camaldulensis	450	laboratory furnace	32.4	25	76.3	54.9	Vital et al. (1994)
Deciduous trees	500	laboratory furnace	30.2	8	84.7	56.8	Zhurinsh (1997)
Pinus sylvestris (sawdust)	300	laboratory furnace	21.6		62.8	30.1	Glaser et al. (1998)
Robinia pseudoacacia	350	laboratory furnace	33.2	65	71.3	52.6	this study
Leucaena leucocephala	not given	metal kiln	27.4		83.1	50.6	San Luis et al. (1984)
Coconut trunk	not given	metal kiln	25.0		77.8	43.2	San Luis et al. (1984)
Mixed tropical wood, Manaus, Brazil	not given	brick kiln	41[3]		74.8	68.2	Correa (1988)
Miombo woodland[2]	not given	earth kiln	23.3		n.d.	-	Chidumayo (1991)
Mixed tropical hardwood	not given	earth pit	nd		69.0	-	FAO (1985)
Average			**30.6**		**75.7**	**53.5**	

[1] Percentage of charcoal carbon from the carbon in wood. Assuming 45%C in wood; determined for *R. pseudoacacia* at 45.7%.

[2] Total conversion of 93% of the woody biomass from a miombo woodland, representing 97% of the total above ground biomass.

[3] Calculating a conversion of 16 m^3 to 9 m^3 with a density of 0.7 and 0.51 Mg m^{-3} for wood and charcoal, respectively.

Charred organic matter from leaves was not accounted for in the calculation and the conversion to charcoal is currently not known. The contribution of leaves to charred organic matter from secondary or primary forests may be small, however, since the proportion of leaves in these forests usually lies below 10% (Table 2). Nevertheless the contribution to total nutrient input may be significant and has to be considered in nutrient budgets

Table 2: Above ground live biomass of secondary and primary forests in the Amazon.

Region	Type	Age of forest	Total above ground biomass [Mg ha^{-1}]	Woody biomass [Mg ha^{-1}]	Wood C content[1] [%]	Charcoal yield from biomass[2] [Mg C ha^{-1}]	Source
Rondonia and Para	2nd regrowth	4	134.2	119.6	49.6	31.7	Hughes et al. (2000)
Rondonia and Para	3rd regrowth	4	90.6	72.7	49.6	19.3	Hughes et al. (2000)
San Carlos, Venezuela	Secondary forest	5	40.1	35.2	nd	8.5	Uhl and Jordan (1984)
Paragominas, Para	Secondary forest[3]	3.5	16.3	12.9	nd	3.1	Buschbacher et al. (1988)
Paragominas, Para	Secondary forest[3]	8	35.0	30.4	nd	7.3	Buschbacher et al. (1988)
Paragominas, Para	Secondary forest[4]	8	86.5	81.8	nd	19.7	Buschbacher et al. (1988)
Zona Bragantina, Para	Secondary forest	2.3	22.2	16.5	nd	4.0	Gehring et al. (1999)
Zona Bragantina, Para	Secondary forest	10	54.9	49.8	47.3	12.6	Johnson et al. (2001)
Zona Bragantina, Para	Secondary forest	20	65.5	59.2	47.9	15.2	Johnson et al. (2001)
Zona Bragantina, Para	Secondary forest	40	128.8	119.8	47.6	30.5	Johnson et al. (2001)
Manaus, Amazonas	Primary forest	-	264.6	251.2	48.9	65.7	Fearnside et al. (1993)
Altamira, Pará	Primary forest	-	262.5	222.3	49.1	58.3	Fearnside et al. (1999)
Ariquemes, Rondonia	Primary forest	-	272.2	260.0	44.4	63.5	Graça et al. (1999)
Belem, Para	Primary forest	-	256.7	247.6	48.8	59.9	Mackensen et al. (2000)
Zona Bragantina, Para	Primary forest	-	229.6	225.1	47.3	57.0	Johnson et al. (2001)

[1] Where no information was available, C contents were estimated at 45 %.

[2] Calculated using the mean conversion of wood biomass to charcoal from Table 1.

[3] With previous pasture use of moderate intensity.

[4] With previous pasture use of low intensity.

Conclusions

Charcoal applications directly increased nutrient availability such as P and K and additionally increased nutrient retention for ammonium. Whether a net nutrient retention of other cations occurs after excess nutrients have been leached or taken up by plants remains to be shown. In this respect the long-term dynamics of soil fertility with charcoal applications are very interesting in comparison to burning or mulching. It may be assumed that nutrients bound to charcoal are more persistent than those in ash or mulch but direct evidence needs to be gathered.

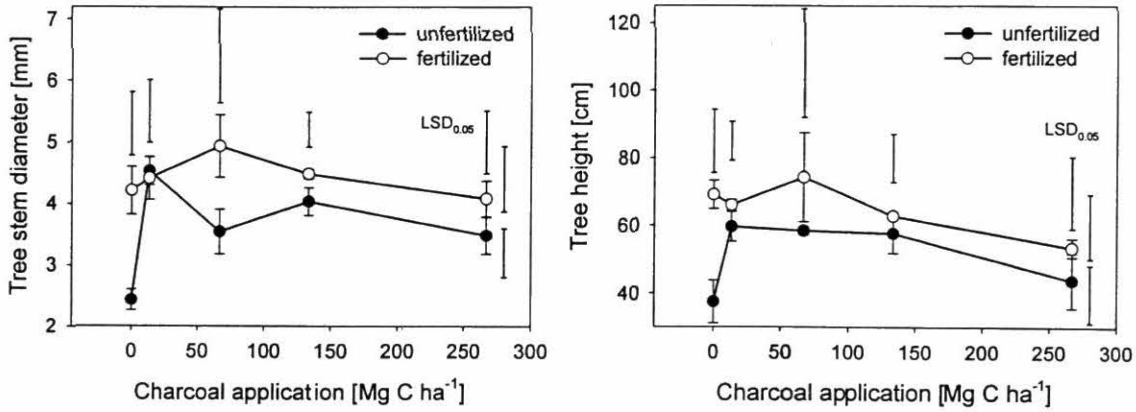

Figure 4: Tree growth of *Inga edulis* seedlings in pots amended with mineral fertilizer and increasing amounts of charcoal after 80 days (means and standard errors; N=4; Rondon et al., unpublished data).

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Activity 3.4 Delopment of suitable feeding strategies for reducing methane emissions by ruminants

Activity 3.4.1 - In vitro evaluation of the effect of Sapindus saponaria on methane release and microbial populations

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Rationale

Previous studies (See AR IP-5 2002) had shown that the supplementation with fruits of the tropical tree *Sapindus saponaria* reduced methane release from tropical diets. However, it was not clear if this methane suppressing effect was dependent on the quality of the basal diet or not. Thus an in vitro experiment was set up to test the hypothesis that the effect of *Sapindus saponaria* on methane release was independent of the quality of the diet.

Materials and Methods

A Rumen-Simulation Technique (Rusitec) experiment was performed to compare the effects of the dietary inclusion (1/3 of the basal diet DM) of the following three tropical forage legumes of contrasting quality:

Calliandra calothyrsus (low quality, high tannin content),
Cratylia argentea (medium quality, moderate tannin content) and
Arachis pintoi (high quality, negligible tannin content), to a basal diet of *Brachiaria dictyoneura*, which was also tested without legume.

All diets were evaluated with and without the addition of *Sapindus saponaria* fruits (8% of the daily DM supply).

Results and Discussion

The mean rumen fluid pH across all diets was 7.01 and the effects of legumes and *S. saponaria* on this variable were minor. *C. argentea* and *A. pintoi* increased ($P<0.05$) rumen fluid ammonia from 0.3 mmol (control) to 1.8 and 2.0 mmol, respectively, but *C. calothyrsus* had no effect ($P>0.05$) on ammonia levels and *S. saponaria* tended ($P<0.10$) to reduce rumen ammonia by 17% on average.

The legumes *A. pintoi* and *C. argentea* increased bacteria counts while *A. pintoi* increased protozoa counts ($P<0.05$). Both legumes enhanced organic matter and fiber (cellulose) degradation ($P<0.05$) (Figur 3, left). *C. calothyrsus* had no effect on microbial counts ($P>0.05$) and reduced organic matter and fiber degradation ($P<0.05$). Methane release was increased by *C. argentea* and *A. pintoi* by almost 3 to 4-fold relative to the grass-alone diet, whereas *C. calothyrsus* reduced methane release by 50% ($P<0.05$). *S. saponaria* reduced methane release by 11% on average ($P<0.05$) (Figure 3, right).

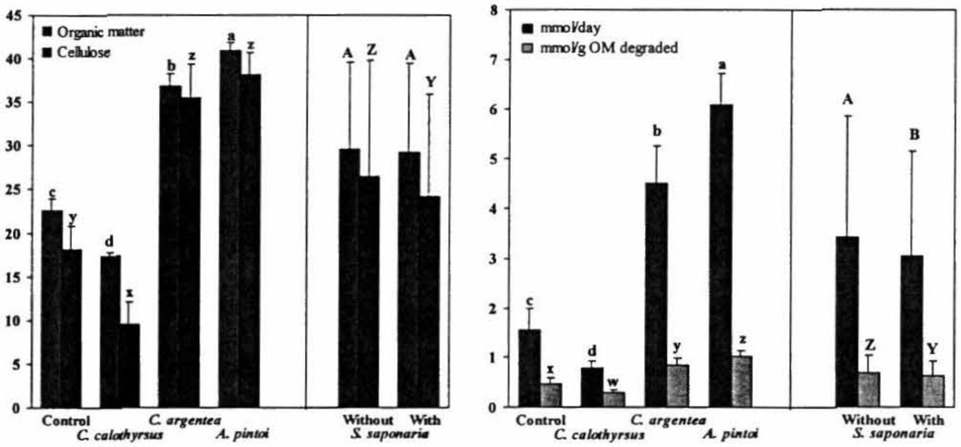

Figure 3. Apparent organic matter and cellulose degradability (%) (on the left) and methane release (on the right) of tropical diets containing no legumes (control) or 1/3 of *C. calothyrsus*, *C. argentea* or *A. pintoi*. All diets were evaluated with and without the addition of *S. saponaria* fruits (8% of daily DM supply).

Adding *S. saponaria* to the diets tended to decrease ($P<0.10$) methane release relative to organic matter degraded. Interactions between addition of legumes and *S. saponaria* were mostly insignificant, except in protozoa counts (suppressed by *S. saponaria* only in the diet containing *A. pintoi*).

In general, our results indicate that the inclusion of legumes in the diets clearly elevated N supply in all cases and this resulted in highly significant effects of legumes in all variables related to rumen fluid N turnover. Apparent N degradation rates from the grass-legume diets, compared to the grass-alone diet, were reduced by about 87% with *C. calothyrsus* but showed a 2-fold increase with *A. pintoi* and a 1.5-fold increase with *C. argentea.*

These results indicate that methane release can be reduced with *S. saponaria* when fed in combination with pure low quality grass diets or with low quality grass diets supplemented with legumes. In addition, our results suggest that the tannins present in *C. calothyrsus* could be associated with methane reduction and that methane production per unit of OM digested was reduced when high quality legumes were used as supplements of low quality grass diets. The higher organic matter and fiber degradation of low quality grass basal diets supplemented with *C. argentea* and *A. pintoi* would probably results in higher animal productivity. Therefore, the increased methane produced with these legumes will be counterbalanced by a correspondingly higher animal productivity.

Activity 3.4.2

In vitro evaluation of the effect of tannins in legumes on methane production

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Rationale

Results from the experiment reported in the previous section showed clearly that the replacement of 1/3 of low quality *Brachiaria dictyoneura* in the diet by *Calliandra calothyrsus* significantly reduced methane release per unit of organic matter digested. However, it is not known which properties of *C. calothyrsus* (*e.g.* high tannin content, low digestibility) are responsible for this effect. Thus an experiment was set to test the hypothesis that feeding legumes high in tannins could significantly reduce methane production.

Materials and Methods

A Rumen-Simulation experiment using the RUSITEC was performed to evaluate the effects of a partial (1/3) or total (1/1) replacement of *Brachiaria dictyoneura* by *Calliandra calothyrsus*. *Brachiaria dictyoneura* was also tested without the legume. All diets were evaluated with and without the addition of PEG, a tannin-binding agent that is commonly used to inactivate tannins and to evaluate tannin related effects.

Results and Discussion

These results indicate that tannins could be responsible to some extent for the suppression of methanogenesis caused by *C. calothyrsus*. The addition of PEG (i.e. inactivation of tannins) drastically increased methane release from diets containing *C. calothyrsus* and remained without any effect in the grass-alone diet (Figure 4).

It is well documented that high tannins in tropical legumes have a negative effect on digestibility and on intake by ruminants. In addition, tannins are known to bind feed protein in the rumen and make it unavailable in the small intestine. Low fiber digestibility of *C. calothyrsus* could also contribute to low methane production.

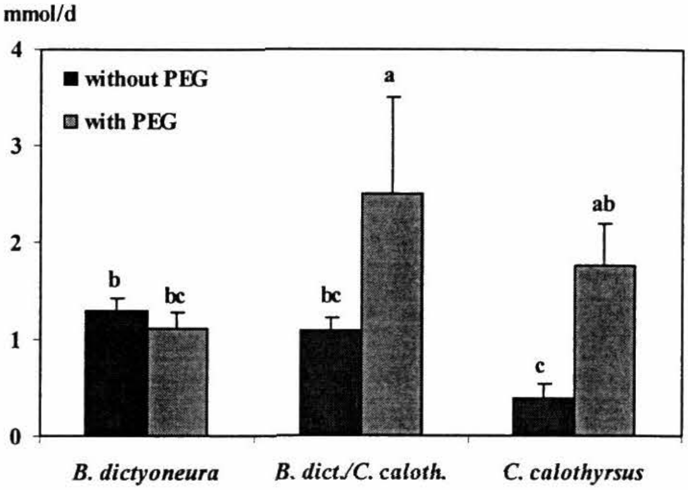

Figure 4. In vitro methane release from grass-alone (*B. dictyoneura*), grass-legume (*B. dictyoneura/C. calothyrsus*) and pure legume (*C. calothyrsus*) diets, expressed as daily release per fermenter supplied with 15 g/d of forage DM. All diets were evaluated with and without the addition of PEG (30 mg/g of forage DM).

One could speculate that by diluting tannins through the combination of legumes with and without tannins it would be possible to reach a tannin threshold that has limited effect on digestion and on intake but can still significantly reduce methane production. However, to better define feeding systems with legumes that have tannins we first need to understand the underlying mechanism on how tannins and possibly fiber in tropical legumes reduce methane.

Activity 3.4.3 or Ter

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

Patricia erarthu

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Experiment 1 - CIAT

Intake, digestion and duodenal nitrogen flow in sheep fed a low quality grass diet supplemented with fruits from *Sapindus saponaria* and legumes

Rationale

It is known that complete and partial defaunation increase the flow of microbial and dietary protein from the rumen to the duodenum and consequently can improve nitrogen utilization in ruminants. We have demonstrated through in vitro studies that the incorporation of the saponin-rich fruit *Sapindus saponaria* into forage-based diets reduces rumen protozoa population. However, little information is available on how *S. saponaria* affects nitrogen flow to the duodenum and whether these effects are dependent on the quality of the basal diet. Therefore an experiment was carried out to study the influence of a supplementation with fruits from *S. saponaria* on intake, digestion and duodenal N-flow in sheep fed tropical forage diets of contrasting quality.

Materials and Methods

Six African-type sheep were fitted with ruminal and duodenal cannulae and allotted to one of four treatments in an unbalanced simple crossover design with a 2 × 2 factorial arrangement of treatments: 2 basal diets × 2 supplementations (with and without *S. saponaria*).

The forage required for the trial was harvested, dried and stored at CIAT's research station in Quilichao (Cauca valley, Colombia) and fruits of *S. saponaria* were collected in the northern cost region of Colombia.

The traditional low–quality diet consisted of a grass hay (*Brachiaria dictyoneura* cv. Llanero, 3.7% crude protein, 72.8% neutral detergent fiber, 41.1% acid detergent fiber) and the improved diet consisted of 75% (on a DM basis) of the same hay complemented with 25% sun -dried *Cratylia argentea* leaves (18.6% CP, 60.2% NDF, 36.5% ADF).

Animals housed in individual metabolism crates were offered daily 80 g DM/kg $BW^{0.75}$ of forage in two meals (8:00 and 15:00). Both diets were fed either without supplementation or with fruits of *S. saponaria* as a supplement (8 g/kg $BW^{0.75}$ per day) dosed directly into the rumen. The fruits of *S. saponaria* were ground using a 3 mm–screen and administered via rumen cannula. Animals were offered a mineral mix *ad libitum* and water 4 times a day. Sheep used in the trial were allowed to graze for one week between experimental periods, which had a duration of 17 days, of which 7 were for adjustment and 10 for measurements.

Forage refused and feces were collected daily and on day 8 and 9 of the measurement period, samples of duodenal digesta were taken every 6 hours and on the last day rumen samples were collected every 3 hours to determine purines and bacterial nitrogen flowing to the duodenum. Cr-EDTA and indigestible acid detergent fiber were used as flow markers for the liquid and the solid phase, respectively.

Results and Discussion

Results for intake and digestibility are presented in Table 6 and as expected, voluntary dry matter intake was higher (+20%, $P<0.001$) with the grass-legume diet than with the grass-alone diet. Basal forage intake was no affected by the administration of *S. saponaria* and consequently total DM intake of animals receiving *S. saponaria* was increased. Interactions of basal diet and *S. saponaria* on intake were non significant ($P>0.05$), but significant interactions in digestibility were observed. The supplementation of *S. saponaria* reduced DM and NDF digestibility in the grass-alone diet, but no in the grass-legume diet.

Table 6. Intake and digestibility of dry matter, neutral detergent fiber and acid detergent fiber of sheep fed a grass-alone or a grass-legume diet either with or without *Sapindus saponaria*.

Variables	Grass only		Grass-Legume		SE	Significance		
	Control	Sap.	Control	Sap.		Diet	Sap.	Interact
Total intake			g/d					
Dry matter	692	774	834	918	46.9	0.009	0.097	0.989
DM/kg $BW^{0.75}$	42.3	50.0	53.5	57.8	2.57	0.003	0.035	0.534
Organic matter	633	719	769	858	42.0	0.006	0.056	0.981
NDF	515	545	575	626	36.8	0.075	0.282	0.790
ADF	282	310	341	362	21.3	0.022	0.258	0.881
Digestibility			%					
Dry matter	51.4a	47.6b	47.6b	49.4ab	1.12	0.385	0.388	0.036
Organic matter	57.5	58.5	58.5	54.7	3.38	0.682	0.679	0.498
NDF	58.0a	51.0b	51.8b	53.7ab	1.98	0.392	0.219	0.051
ADF	49.6a	42.4b	42.3b	40.2b	1.38	0.005	0.005	0.099

The main objective of carrying out this feeding trial was to test the hypothesis that the supplementation with *S. saponaria* would result in a suppression of rumen ciliate protozoa and consequently in an increased bacterial and total N flow to the duodenum. Results presented in Table 7 show that rumen ciliate protozoa were not suppressed by *S. saponaria*. Instead, counts of the group of Entodinium and of total ciliate protozoa were even increased by *S. saponaria* ($P<0.01$).

Table 7. Ciliate protozoa counts in rumen fluid of sheep fed a grass-alone or a grass-legume diet either with or without *Sapindus saponaria*.

Ciliate protozoa counts	Grass only		Grass-Legume		SE	Significance		
(10^4/ml)	Control	Sap.	Control	Sap.		Diet	Sap.	Interact.
Holotrichs	1.6	2.0	1.6	1.3	0.33	0.264	0.906	0.361
Entodinium	8.5	17.1	9.1	14.3	1.58	0.494	0.001	0.308
Total	10.4	19.2	10.7	15.6	1.80	0.421	0.002	0.290

Results on N intake, digestion and utilization are presented in Table 8. Total N intake was 128% higher ($P<0.001$) with *C. argentea* than with the grass-alone diet and the supplementation with *S. saponaria* had no effect on total N intake ($P>0.10$). The higher N intake with *C. argentea* was the result of the higher DM intake and of the higher N content in the forage offered. A higher amount of total N (+55%, $P<0.001$) and bacterial N (+30%, $P<0.05$) reached the duodenum when *C. argentea* was fed.

Table 8. Nitrogen intake, digestion and utilization and volatile fatty acid concentrations in rumen fluid of sheep fed a grass-alone or a grass-legume diet either with or without *Sapindus saponaria*.

Nitrogen	Grass only		Grass-Legume		SE	Significance		
	Control	Sap.	Control	Sap.		Diet	Sap.	Interact.
N intake (g/d)	5.54	5.74	11.86	13.91	0.800	0.001	0.179	0.29
N intake (g/kg $BW^{0.75}$/d)	0.18	0.21	0.40	0.46	0.030	0.001	0.167	0.65
Duodenal flow (g/d)	6.64	9.14	11.85	12.74	0.958	0.001	0.098	0.44
Bacterial	3.10	4.65	4.43	5.64	0.430	0.018	0.007	0.72
Duodenal flow (% of intake)	126.2	155.7	109.9	100.6	13.71	0.021	0.468	0.20
Apparent absorption (g/d)	3.67	5.41	6.68	6.74	0.925	0.035	0.344	0.40
N digestibility (%)	43.4	36.9	46.2	53.2	7.69	0.230	0.974	0.42
Ruminal escape (g/d)	3.53	4.48	7.40	7.07	0.591	0.001	0.605	0.32
Rumen ammonia N (mg/l)	30.3	17.8	89.3	79.3	8.263	0.001	0.192	0.89
Volatile fatty acids (mmol/l)	103.7	121.2	116.6	125.1	5.95	0.18	0.0047	0.48
Acetate	81.5	91.5	90.4	92.8	4.58	0.27	0.20	0.44
Propionate	14.8	19.6	18.2	22.0	1.15	0.024	0.002	0.66
n-Butyrate	7.1	9.9	7.3	9.8	0.61	0.98	0.001	0.83
iso-Butyrate	0.41	0.27	0.67	0.50	0.048	0.001	0.006	0.73
Acetate/Propionate	5.63	4.77	4.98	4.24	0.173	0.005	0.001	0.75

The supplementation with *S. saponaria* tended to increase total N flow (+18%, $P<0.10$) and significantly increase bacterial N flow (+36%, $P<0.01$) to the duodenum. This increase was considerably higher with the grass-alone diet (+50%) than with the grass-legume diet (+27%). Apparent N absorption was increased when *C. argentea* was fed (+48%, $P<0.05$) but was not affected by the supplementation with *S. saponaria* ($P>0.05$). However, it is worth mentioning that the apparent N absorption was numerically increased by over 40% with the grass-alone diet when *S. saponaria* was administered, which was not the case with the grass-legume diet, but due to the high variability this difference failed to be significant.

Nitrogen apparent digestibility was similar with all diets ($P>0.05$), whereas ruminal escape N and rumen ammonia N were increased when *C. argentea* was fed but remained unaffected by the supplementation with *S. saponaria* ($P>0.05$). The interactions between quality of the diet offered and *S. saponaria* on nitrogen intake, digestibility and duodenal N-flow were non-significant. This indicates that the potential of *S. saponaria* to increase bacterial N flow is not dependent on quality of the basal diet.

The supplementation with *C. argentea* and *S. saponaria* affected concentration of individual volatile fatty acids. Both supplements had no effect on acetate concentration but increased the concentration of propionate. While the legume did not affect n-butyrate, it was clearly increased by *S. saponaria*. This higher n-butyrate concentration could be related to the relatively high sugar concentration of *S. saponaria* (21% of DM). The legume also increased the concentration of iso-butyrate, which is one of the end products of degradation of true protein in the rumen. This finding is an indication that ruminal protein degradation was enhanced when the legume was fed and is in agreement with the higher rumen ammonia levels observed with diets containing *C. argentea*. In contrast, supplementation with *S. saponaria* reduced the iso-butyrate concentration, suggesting that this feed ingredient suppressed protein degradation. Finally, *C. argentea* and *S. saponaria* independently shifted the volatile fatty acid production towards lower acetate to propionate proportion.

Results from this study confirm previous studies that indicated that the inclusion of *Cratylia argentea* into diets based on low-quality hay increased total dry matter and nitrogen intake and improved nitrogen absorption. They also show that supplementation with the saponin-containing fruits of *Sapindus saponaria* may increase bacterial nitrogen flow to the duodenum independently of the quality of the basal diet. It is interesting to note that supplementation with *S. saponaria* may increase bacterial N flow to the duodenum even though rumen protozoa counts are not suppressed.

Finally, our results indicate that supplementations with *C. argentea* and *S. saponaria* improve the efficiency of rumen fermentation by shifting the volatile fatty acid production towards lower acetate to propionate ratio. The effects of the two supplements were independent and additive, thus the highest nutritional efficiency was obtained with the combination of *C. argentea* and *S. saponaria*.

Experiment 2 –ETH-Zurich

Methane release and energy and nitrogen utilization of sheep fed a low quality grass diet supplemented with fruits from *Sapindus saponaria* and legumes

Rationale

Previous in vitro-experiments showed that the inclusion of fruits of *Sapindus saponaria* into tropical forage-based diets could suppress methane released by over 10%. However, no information was available on its effect 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* on energy and nitrogen utilization and methane release of sheep fed tropical diets.

Materials and Methods

Three basal diets with contrasting forage quality were used. The traditional diet consisted of a low quality hay (*Brachiaria dictyoneura* cv. Llanero), and in the other two diets 1/3% and 2/3% of the grass sun-dried leaves of the shrub legume *Cratylia argentea* replaced 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 21 g/kg $BW^{0.75}$ of concentrate per day. Six growing Swiss White Hill sheep (initial body weight 30.1±2.8 kg) were allotted to one of the six treatments in a complete Latin-Square design with 3 × 2 factorial arrangement of treatments (3 basal diets × 2 concentrates) and 6 experimental periods 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 (Photo

1, right) and day 21 for blood and rumen liquid sampling. Respiratory measurements were carried out (Photo 1, left) 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 Quilichao (Cauca Valley, 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-Zurich. 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-Zurich.

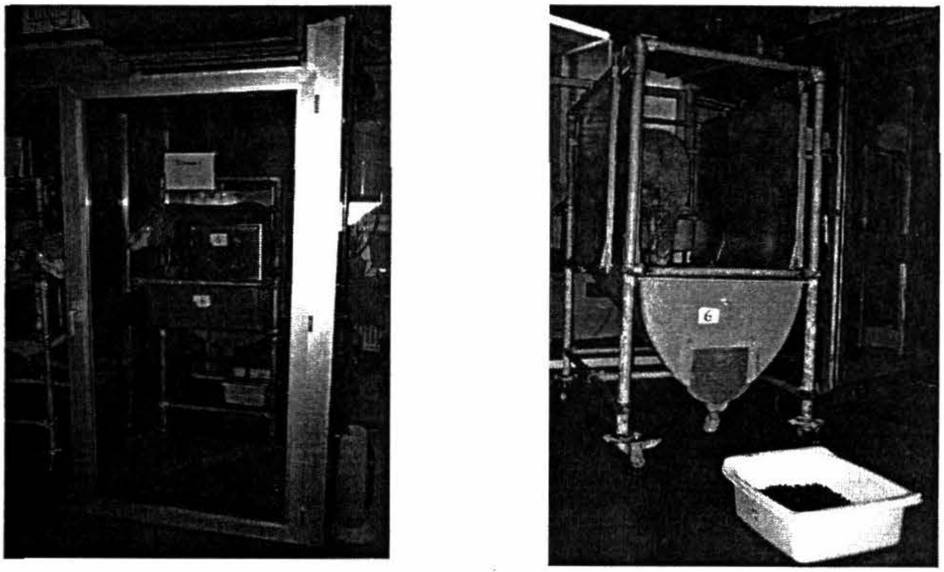

Photo 1. Sheep during measurements in respiratory chamber (on the left) and during collection period in metabolic crates (on the right).

Results and Discussion

Up to now, only data from four of the six measurement periods have been analyzed. However, some effects are already evident. Supplementing legume significantly increased:
Total daily dry matter, energy and nitrogen intake
N apparent digestibility and retention,
Rumen ammonia and blood urea levels, and
Flows of dietary and microbial nitrogen to the duodenum.

On the other hand, legume supplementation reduced apparent digestibility of OM, neutral detergent fiber and acid detergent fiber but did not affect energy retention and methane emissions. Supplementation with *S. saponaria* had no effect on dry matter and nutrient intake, but reduced the apparent digestibility of organic matter, nitrogen, fiber and energy. Additionally, *S. saponaria* shifted N excretion from urine to feces (more N in feces and less in urine), tended to reduce total N losses and to increase the flow of microbial nitrogen to the duodenum. *S. saponaria* had no significant effect on rumen ammonia but reduce the concentration of urea in blood.

Finally, the supplementation with *S. saponaria* clearly suppressed rumen ciliate protozoa by 70% and daily methane release per animal by over 10%. No interactions between the legume and the *S. saponaria* supplementation were observed.

The results from this study are in line with those from our in vitro experiments that indicated that *S. saponaria* has a high potential to suppress rumen ciliate protozoa and methane emission of ruminants fed tropical diets of contrasting quality.

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

- Two practical training courses were held at Monteria and Florencia, Colombia on methodologies to assess C and nutrient stocks in agroforestry systems.

- Two technicians and one researcher from Corpoica and one researcher from the Universidad de Cordoba were trained on methodologies to sample gases for measurement of fluxes of GHG between the soil and the atmosphere.

- One International workshop was held to advance the preparation of the Amazon Initiative proposal. It had representatives from Embrapa, CIAT, The World Bank, ICRAF, LBA and University of Quebec.

- Two meeting, were held, one in Brazil and one in Colombia to present the Amazon Initiative to potential partner institutions. It included representatives from 20 institutions in Brazil and 15 Institutions in Colombia.

- The following students are being trained

Name	Nationality	Education	Institution	Research theme
Brigit Krucera	German	Ph. D.	University of Freiburg	Characterization of bean genotypes for abiotic stress adaptation
Angela Muñoz	Colombian	BSc.	Universidad Nacional	GWP of intensive livestock production systems

Partner Institutions

- Links have being established and maintained with the following regional and international organizations:

CORPOICA. Colombia: Tito Diaz, Juan Jaramillo, Mandius Romero, Socorro Cajas, Carlos Escobar, Diego Aristizábal, Fernando Garcia, Salvador Rojas
IDEAM: Carlos Castaño, Adriana Soto
Ministry of the Environment Colombia: Juan P. Bonilla, Martha P. Castillo.
Universidad Nacional de Colombia: Alvaro Garcia

Cornell University: John Duxbury, Johannes Lehmann, Erick Fernandes, Susan Riha.
Earth Institute from the University of New Hampshire: Michael Keller, Patric Crill.
Embrapa, Brazil: Adilson Serrao, Tatiana Sa, Robert Boddey
ETH Zurich, Zwitzerland: Dieter Hess
INPA, Brazil: Flavio Luizao
JIRCAS, Japan: Kensuke Okada, T. Ishikawa.
Large Biosphere-Atmosphere Experiment in the Amazon (LBA): Carlos Nobre
University of Chile; Prof. M. Pinto
University of Freiburg: E. Wellmann
University of Wageningen: Peter Buurman.

Resource Mobilization activities:

Complementary and Special Projects

Donor/Project	Duration	Total Pledge (US$)
European Commission (EC), Brussel, Belgium Characterization of South American genotypes of bean for optimal use of light under abiotic stress (Prepared by IP1)	2001-2004	831,261 (Euros)
Approved by the MIS consortium and FAO Assessment of soil, water and environmental indicators of the performance of the Quesungual System in Honduras. Prepared by PE6 and PE2.	2002-2003	US$15,000

Full Proposals submitted:
A full proposal on "Bean genomics for improved drought tolerance in Central America" is submitted for funding to BMZ, Germany in collaboration with the University of Hannover, Germany.

Concept notes submitted:
Seeds of Hope for Central America, Phase II: A 3-Year Project to Enhance the Resilience of Agriculture in Drought-prone Areas in Nicaragua

Workshops and meetings attended:

- February: Planning Meeting of Livestock Systems research priority area organized by ZIL-SDC in ETH, Zurich, Switzerland.
- International Meeting of the MIS Consortium. Honduras, February-2002.
- International Workshop for the preparation of the Project Pre-proposal for the Climate Change CP. Nairobi, April-2002.
- Natiopnal meetings to discuss the Colombian Climate change policy. February – July 2002.
- Executive Committee Meeting of PROCITROPICOS, Santa Cruz, Bolivia. March-2002.
- Invited speak for First World Congress on "Páramos". Paipa, Colombia, April-2002.
- Invited speak to the National Congress of the Colombian Society of Soil Science. Cali, September-2002.
- Second Scientific Conference of the LBA project. Manus Amazonas, Brazil, July-2002.
- International Workshop for the Amazon Initiative (AI) with representatives of EMBRAPA, LBA, the PPG7 project and U. of Quebec. Cali, August-2002.
- International Meeting to present the AI to potential partner institutions in Brazil. Belem, September-2002.
- National Meeting to present the AI to Potential Partner institutions in Colombia, Florencia, Caquetá, October-2002.
- National meeting to discuss the national Agroforestry plan from Corpoica, Bogota, October 2002
- National meeting for preparing a pilot C trading project for the World Bank. Bogotá, September-December 2002

List of Staff

Marco Rondón, Biogeochemist, Project Manager, HQ (60%-PE6, 20%-PE2, 20%-Amazon Initiative)
Peter Jones, Climate Modeler, HW (10%-PE6, 90%-PE4)
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Steve Beebe, Breeder, Geneticist HQ (10%-PE6, 60%-IP1, 30%-SB2)
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List of Publications

Refereed journals

Feldpausch, Ted, Marco A. Rondon, Erick C.M. Fernandes, Susan J. Riha, Elisa Wandelli. 2002. Carbon and nutrient accumulation in secondary forests regenerating from degraded pastures in central Amazônia, Brazil. J. Ecol. Applic. (in press)

Jones, P. G. and P. K. Thornton. 2002. Spatial modeling of risk in natural resource management. Conservation Ecology 5(2): 27. [online] URL: http://www.consecol.org/vol5/iss2/art27

Rondón, M. , J.M. Duxbury and R.J. Thomas Effects of Land Use Change in the Llanos of Colombia on Fluxes of Methane and Nitrous Oxide, and on Radiative Forcing of the Atmosphere. Submitted to Agriculture, Ecosystems and Environment.

Refereed book chapters:

Miles, J.W., C.B. do Valle, I.M. Rao and V.P.B. Euclides. 2002. Brachiaria grasses. *In*: L.E. Sollenberger, L. Moser and B. Burson (eds.) Warm-season grasses. ASA-CSSA-SSSA, Madison, WI, USA (*in press*).

Rao, I.M. and G. Cramer 2002. 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.

Non-refereed conference presentations:

Beebe, S., H. Terán and I.M. Rao. (2002). Evaluación de poblaciones para combinar tolerancia a sequía con resistencia a BGMV en frijol de grano rojo y negro en CIAT, Cali, Colombia. Paper presented at XLVIII Annual Meeting of PCCMCA, Boca Chica, Dominican Republic.

Lehmann, Johannes, Jose Pereira da Silva Jr, Marco Rondon, Manoel da Silva Cravo, Jacqueline Greenwood, Thomas Nehls, Christoph Steiner, and Bruno Glaser. (2002). Slash-and-char – a feasible alternative for soil fertility management in the central Amazon?. Proceedings of the World Congress of Soil Science. Bangkok, Thailand, 2002.

Rao, I.M., S. Beebe, J. Ricaurte, H. Terán and G. Mahuku. (2002). Identificación de los caracteres asociados con la resistencia a la sequía en frijol común (*Phaseolus vulgaris* L.). Paper presented at XLVIII Annual Meeting of PCCMCA, Boca Chica, Dominican Republic.

Rondón, Marco A. (2002). Efecto de los Cambios en el uso del suelo sobre los almacenamientos de carbono y flujos de gases de efecto Invernadero en areas de paramo de las Animas, Cauca. Paper presented to the World Congress on "Páramos". Paipa, Colombia. July 2002.

Rondón, Marco A. (2002). Cambio Climatico y Agricultura: desafios y oportunidades. Paper presented to the Meeting of the Colombian Society of Soil Science. Cali, Colombia, September 2002.

Rondón, Marco A. , Erick C.M. Fernandes, Rubenildo Lima, Elisa Wandelli. (2002). Carbon Storage in Soils from Degraded Pastures and Agroforestry Systems in Central Amazônia: The role of charcoal. Proceedings of the Second International Conference of the Large Biosphere – Atmosphere Experiment in the Amzon- (LBA project). Manaus, Brazil, July 2002.

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