Integration of Canavalia brasiliensis into the crop-livestock system of the Nicaraguan hillsides
Environmental adaptation and nitrogen dynamics

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INTEGRATION OF *CANAVALIA BRASILIENSIS* INTO
THE CROP-LIVESTOCK SYSTEM OF THE NICARAGUAN HILLSIDES:
ENVIRONMENTAL ADAPTATION AND NITROGEN DYNAMICS

A dissertation submitted to

ETH Zurich

for the degree of
Doctor of Sciences

presented by

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2010
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<tr>
<td>AR</td>
<td>site on Antonio Ruiz’ farm</td>
</tr>
<tr>
<td>ARX</td>
<td>profile n°X on AR site</td>
</tr>
<tr>
<td>BG</td>
<td>bean grain</td>
</tr>
<tr>
<td>BP</td>
<td>bean plant</td>
</tr>
<tr>
<td>C</td>
<td>carbon</td>
</tr>
<tr>
<td>CB</td>
<td>canavalia above-ground biomass</td>
</tr>
<tr>
<td>CBR</td>
<td>canavalia biomass removed</td>
</tr>
<tr>
<td>CEC</td>
<td>cation exchange capacity</td>
</tr>
<tr>
<td>CIAT</td>
<td>International Center for Tropical Agriculture</td>
</tr>
<tr>
<td>Ctot</td>
<td>soil total carbon</td>
</tr>
<tr>
<td>DAA</td>
<td>days after amendment</td>
</tr>
<tr>
<td>δ¹⁵N</td>
<td>¹⁵N natural abundance signature (‰)</td>
</tr>
<tr>
<td>DLT</td>
<td>direct labelling technique</td>
</tr>
<tr>
<td>FC</td>
<td>site on Felipe Calderón’s farm</td>
</tr>
<tr>
<td>GR</td>
<td>site on Gabriel Ruiz’ farm</td>
</tr>
<tr>
<td>ILT</td>
<td>indirect labelling technique</td>
</tr>
<tr>
<td>INTA</td>
<td>Nicaraguan Institute for Agricultural Technology</td>
</tr>
<tr>
<td>LP</td>
<td>site on Lorenzo Peralta’s farm</td>
</tr>
<tr>
<td>M/B</td>
<td>maize-bean rotation</td>
</tr>
<tr>
<td>M/C</td>
<td>maize-canavalia rotation</td>
</tr>
<tr>
<td>M/CX</td>
<td>maize-canavalia with X% of canavalia biomass removed</td>
</tr>
<tr>
<td>MG</td>
<td>maize grain</td>
</tr>
<tr>
<td>MDG</td>
<td>maize damaged grain</td>
</tr>
<tr>
<td>MC</td>
<td>maize cob</td>
</tr>
<tr>
<td>MH</td>
<td>maize husk</td>
</tr>
<tr>
<td>MP</td>
<td>site on Marcial Peralta’s farm</td>
</tr>
<tr>
<td>MRE</td>
<td>maize recycled ears</td>
</tr>
<tr>
<td>MR</td>
<td>maize residues (stalks and leaves)</td>
</tr>
<tr>
<td>N</td>
<td>nitrogen</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>$^{15}$N-X</td>
<td>$^{15}$N enrichment of the respective X pool</td>
</tr>
<tr>
<td>NCE</td>
<td>nitrogen cycling efficiency</td>
</tr>
<tr>
<td>Ndfa</td>
<td>amount of N derived from the atmosphere</td>
</tr>
<tr>
<td>Ndff</td>
<td>amount of N derived from the amendment</td>
</tr>
<tr>
<td>Ndfs</td>
<td>amount of N derived from the soil</td>
</tr>
<tr>
<td>Nfert</td>
<td>amount of nitrogen applied with mineral fertilizers (kg ha$^{-1}$)</td>
</tr>
<tr>
<td>Nfix</td>
<td>amount of nitrogen fixed (kg ha$^{-1}$)</td>
</tr>
<tr>
<td>Nmin</td>
<td>soil mineral N</td>
</tr>
<tr>
<td>Nmic</td>
<td>soil microbial N</td>
</tr>
<tr>
<td>Nseed</td>
<td>amount of nitrogen supplied through seeds (kg ha$^{-1}$) at sowing</td>
</tr>
<tr>
<td>Ntot</td>
<td>total soil N</td>
</tr>
<tr>
<td>$N_X$</td>
<td>amount of nitrogen in the plant part X (kg ha$^{-1}$)</td>
</tr>
<tr>
<td>PCA</td>
<td>principal component analysis</td>
</tr>
<tr>
<td>PT</td>
<td>site on Pedro Torres’ farm</td>
</tr>
<tr>
<td>UNA</td>
<td>National Agricultural University</td>
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ABSTRACT

Due to population growth in the rural poor areas of the Nicaraguan hillsides, land use has been intensified in a way that adversely affects soil fertility. Crop and livestock productivity have therefore declined, leading to decreased income and food insecurity. Crop production is limited to two short and successive rainy seasons, and livestock suffers forage shortage during the following five months of long dry season. Nitrogen (N) is the nutrient most limiting crop production in the area. To sustain agricultural production, the drought-tolerant cover legume Canavalia brasiliensis (canavalia) has been introduced as green manure and forage into the traditional maize-bean-livestock system. Different aspects of this introduction were studied in order to check the sustainability of the proposed technology.

The most suitable land for canavalia was identified by linking its above ground biomass production on 69 plots on-farm to the soil and topographic properties. The description of soil profiles and canavalia root system at ten contrasting sites completed the observations. Above ground biomass production for both years varied between 448 and 5357 kg ha$^{-1}$, with an average of 2117 kg ha$^{-1}$ and was significantly affected by the carbon and N content of the soil surface horizon, the amount of clay and stones in the whole profile, and the soil depth.

In order to define the net N input to the system from canavalia, and to describe how its use as forage or as green manure affects soil N stocks, N budgets were quantified on-farm over two cropping years for the traditional maize-bean rotation and the alternative maize-canavalia rotation. Canavalia derived in average 69% of its N from the atmosphere, which corresponded to a mean N input of 20 kg N ha$^{-1}$. Although canavalia increased the N balance of the rotation when used as green manure, the system N budget remained negative without mineral fertilizer application. When used as forage, it bears the risk of soil N depletion unless N would be recycled to the plot by animal manure.

To study the benefits of canavalia for the subsequent crop, microplots were installed in a six-year old field experiment. Direct and indirect $^{15}$N-labelling techniques were used to determine N recoveries in maize and soil from canavalia residues and canavalia-fed cows’ manure compared to mineral fertilizer. Most of the amendments remained in the
soil. Maize recovered 12% of N from canavalia residues. The N fertilizer value of canavalia-fed cows’ manure could not be assessed as the indirect $^{15}$N labelling technique failed due to a high N mineralization from the soil organic matter.

In conclusion, it can be stated that the integration of canavalia in the Nicaraguan hillsides is on track, but there are still knowledge gaps to be filled in order to be able to make the most of canavalia attributes. Indeed, farmers will most likely use canavalia as forage but recycling of animal manure to the plot is not yet current practice and the fertilizer value of this manure has not been determined. An option is to leave canavalia regrowth during the dry season as green manure to mitigate soil N depletion. The question of the biophysical trade-offs of using canavalia as forage or as green manure still need to be complemented with N budget studies for different rotational sequences over several years and with studies aiming at optimizing N use efficiency at farm level.
RESUME

Suite à la poussée démographique dans les pauvres zones rurales des collines du Nicaragua, la mise en valeur agricole a été intensifiée d’une manière nuisible pour la fertilité des sols. La productivité des cultures et du bétail ont donc baissé, entraînant une diminution des revenus et une insécurité alimentaire. La production agricole est limitée à deux courtes et successives saisons des pluies, et le bétail souffre une pénurie de fourrage pendant les cinq mois de saison sèche suivants. Dans la région, l’azote (N) est l’élément nutritif le plus limitant pour la production agricole. Pour soutenir celle-ci, une légumineuse tolérante à la sécheresse, *Canavalia brasiliensis* (canavalia) a été introduite comme engrais vert et fourrage dans le système traditionnel maïs-haricot-bétail. Différents aspects de cette introduction ont été étudiés afin de vérifier la durabilité de la technologie proposée.

La terre la plus appropriée pour canavalia a été identifiée en reliant sa production de biomasse aérienne aux propriétés topographiques et de sol pour 69 parcelles sur ferme. La description des profils de sol et du système racinaire de canavalia sur dix sites contrastés a complété les observations. La production de biomasse aérienne pour deux années a varié entre 448 et 5357 kg ha\(^{-1}\), avec une moyenne de 2117 kg ha\(^{-1}\) et a été significativement affectée par la teneur en carbone et en N de l’horizon de surface du sol, par les teneurs en argile et en pierre dans l’entièreté du profil, et par la profondeur du sol.

Afin de définir la contribution nette d’azote par canavalia pour le système, et de décrire comment son utilisation comme fourrage ou comme engrais vert affecte les stocks en azote du sol, des budgets de N ont été quantifiés sur ferme sur deux ans pour la rotation traditionnelle de maïs-haricot et la rotation alternative maïs-canavalia. Canavalia a dérivé en moyenne 69% de N de l'atmosphère, ce qui correspond à un apport de 20 kg N ha\(^{-1}\) en moyenne. Bien que canavalia ait augmenté le bilan azoté de la rotation lorsqu'elle est utilisée comme engrais vert, le budget de N est resté négatif en absence d’apports d'engrais minéraux. Lorsqu'elle est utilisée comme fourrage, il y a un risque d'appauvrissement des sols en N, sauf si N serait recyclé sur la parcelle par le fumier animal.

Pour étudier les avantages de canavalia pour la culture suivante, des microparcelles ont
été installés dans un champ expérimental âgé de six ans. Les techniques de marquage isotopique au $^{15}$N directes et indirectes ont été utilisées pour déterminer les recouvrements de N dans le maïs et le sol à partir de résidus de canavalia et fumier de vaches nourries par canavalia par rapport aux engrais minéraux. La plupart des amendements est restée dans le sol. Le maïs a récupéré 12% des résidus de canavalia. La valeur de l'engrais azoté du fumier de vache n'a pas pu être évaluée, car la technique de marquage indirecte a échoué en raison d'une importante minéralisation de N à partir de la matière organique du sol.

En conclusion, on peut affirmer que l'intégration de canavalia dans les collines du Nicaragua est en bonne voie, mais il y a encore des lacunes à combler pour être en mesure de tirer le meilleur parti des ses qualités. En effet en pratique, les agriculteurs utiliseront probablement canavalia comme fourrage mais le recyclage du fumier d'élevage à la parcelle n'est pas encore pratique commune et la valeur fertilisante du fumier n'a pas été déterminée. Une option est de laisser la repousse de canavalia pendant la saison sèche comme engrais vert pour atténuer l'appauvrissement des sols en N. La question des compromis biophysiques lors de l'utilisation de canavalia comme fourrage ou comme engrais vert doit encore être complétée par des études de budget de N pour les différentes séquences de rotation sur plusieurs années et par des études visant à optimiser l'efficacité d'utilisation de N au niveau de la ferme.
GENERAL INTRODUCTION
Background: the Nicaraguan hillsides

Population pressure and soil nutrient depletion

Nicaragua is the second poorest country of the LAC zone (Latin America and the Caribbean) after Haiti, with 80% of the population living with less than 2$ per day and 27% of the population undernourished (UNDP, 2008). These indicators on poverty and nutrition are stagnating since the early nineties, suggesting that it will be extremely difficult for Nicaragua to reach the Millenium Development Goals (FAO, 2009b). Moreover, global warming threatens food production particularly in tropical countries (Eakin, 2005).

Close to half (43%) of the Nicaraguan population lives in rural areas (IFAD, 2009). Population is expanding at an annual growth rate of 1.3%, increasing pressure on arable land resources (IFAD, 2009; Pfister, 2003). The expansion of cropland is only possible if fragile land is taken under plough and/or if cultivation is intensified. In the past, cropping cycles were followed by several years of bush fallows to restore soil fertility. However, as farm sizes decreased (Pfister, 2003), the fallow period has been shortened to one cropping cycle or even completely eliminated. As smallholders have no other choice than sticking to continuous staple crop production on sloping lands that are prone to erosion, and as they can hardly afford fertilizers, soil organic matter and soil nutrients are depleted, resulting in an overall soil fertility decline and a decrease in water availability (Johnson and Baltodano, 2004). As a consequence, the productivity is decreasing, resulting in further expansion of cropland, which in turn further accelerates nutrient depletion (Figure i.1). Altogether this feeds back to a decrease of income and an increase in food insecurity (Pender, 2004; Tan et al., 2005).

The major part of the country is classified as a region with severe to very severe soil degradation (FAO, 2009c), and soil erosion represents a considerable drawback in the Nicaraguan economy (Alfsen et al., 1996). In the hillsides, nitrogen (N) depletion is a major production constraint (Ayarza et al., 2007; Pfister and Baccini, 2005; Smyth et al., 2004).
Figure i.1. Relationship between poverty, production systems and soil degradation in the Nicaraguan hillsides.

The smallholders crop-livestock system

The climate of the Nicaraguan hillsides is classified as tropical savannah according to the Köppen-Geiger classification (Peel et al., 2007). Annual mean rainfall varies between 750 and 1600 mm (INETER, 2009) distributed in two peaks from June to August and from September to November. The dry season lasts from December to May with strong winds and high temperatures. More than 80% of the production systems in the hillsides of Nicaragua are crop-livestock systems (F. Holmann, personal communication). On a traditional smallholder crop-livestock farm, on average 2 ha of land are dedicated to crop production and an area shared between a few households is used for grazing on less productive pastures based on Jaragua grass (*Hyparrenia rufa*). Maize (*Zea mays*) is grown during the first rainy season, and common bean (*Phaseolus vulgaris* L.) during the
second rainy season. Cultivation is based on hand-held tools and low inputs of mineral fertilizers. Except for maize residues, no organic inputs are used. One of the main problems mentioned by farmers is that soil is “getting tired”, their way of explaining soil degradation through nutrient depletion (Pfister and Baccini, 2005; Schmidt and Orozco, 2003): “le falta vitamina al suelo” (i.e. soil is lacking vitamins), said a farmer from Santa Teresa, Nicaraguan hillsides, in May 2007.

The most important fodder resource is natural pastures. During the dry season, pasture growth ceases and the only available fodder resources are dry vegetation and crop residues of low forage quality (Bartle and Klopfenstein, 1988; Hess, 2006). This fodder shortage results each year in severe bovine malnutrition (PASOLAC, 2002) and subsequently, in a strong decrease in dairy production.

Integrated soil fertility management options have been developed to tackle the soil nutrient decline problem and the lack of forage. Since then, the traditional practice of slash and burning of crop residues to establish the next crop has been abandoned by the majority of farmers, who consider it more and more as serious environmental threat (Ravnborg, 2003). Dual purpose live barriers like elephant grass and sugar cane have also been promoted in the area (Ayarza et al., 2007). Stone barriers to control soil erosion can also be seen on some farms. Promising dry season forage options such as *Brachiaria brizantha* cv. Toledo have been identified (Peters et al., 2003). Among the various management options, cover crop legumes are attractive for the farmers as they are multipurpose, i.e. they can be used as forage or as green manure. Moreover, this technology is easy and does not demand much labour.

**Benefits of cover crop legumes in crop-livestock systems**

*Legumes used as green manures*

Legumes represent a substantial input of N in tropical agricultural system through symbiotic N\(_2\) fixation (Giller, 2001). In addition, deep rooted legumes increase N
availability in surface horizons by tapping nutrients in deep horizons and redistributing them at the soil surface in the litter (Bünemann et al., 2004b; Gathumbi et al., 2003). This N accumulated in legume biomass can become available for the succeeding crops on the short term through mineralization of the residues, and on the long term through incorporation of the decomposing residues into soil organic matter fractions (Vanlauwe et al., 1998a). This build up of soil organic N stocks is essential for the long-term sustainability of the system (Mulvaney et al., 2009). Nitrogen release patterns from residues depend on their original quality, among others in their content in total C, N, polyphenols and lignin (Palm et al., 2001). Legume residues can increase the mineral N content in the soil (Barrios et al., 1996) and the quantity of N stored in the microbial biomass (Bünemann et al., 2004b; Oberson et al., 1999) through an increase of microbial biomass due to the input of plant derived carbon substrates (Bünemann et al., 2004a). Nutrients held in the soil microbial biomass could become available to the plants when environmental conditions are conducive to the lysis of microbial cells, e.g. following a cycle of dry and wet conditions (Turner and Haygarth, 2001). In addition to the size of the biomass, microbial nutrient turnover is enhanced (Oberson et al., 2001) which can result in an overall improvement of N supply to crops, provided that N release occurs in synchrony with plant demand (Oberson et al., 2005; Turner et al., 2005). If N released by mineralization of legume residues or microbial biomass is too quick before crop roots are established to take it up, it can be lost via volatilization, denitrification or leaching (Chikowo et al., 2006; Millar et al., 2004). Symbiotic N\textsubscript{2} fixation, the N release pattern from residues, their fertilizer value to the subsequent crop and their recovery in the soil need therefore to be assessed carefully in order to determine if the legume represents a valuable N source and which are the best management practices for it.

In addition to a N fertilization effect, yield increase of the crop following a legume cover crop may also be related to a decrease in weed pressure and an increased soil cover (Schmidt et al., 2005) as well as a pest control effect (Cherr et al., 2006). Drought tolerant legumes integrated as green manure provide soil a protection against wind erosion during the dry season, and especially water erosion after heavy precipitation events at the beginning of the rainy season (Marin, 1995). The use of green manure increases therefore
**General introduction**

the agroecological resistance of the production system after natural disasters such as hurricanes (Holt-Gimenez, 2002), landslides, and earthquakes, which are frequent in Nicaragua.

From a climate change perspective, legume cover crops are part of a range of beneficial management practices helping to restore air quality by converting atmospheric carbon and N into soil organic matter (Etchevers et al., 2005). This is of particular importance for Nicaragua, as sustainable development is nowadays incompatible with unmitigated climate change (The World Bank, 2009).

**Legumes used as forage**

When legumes are used as forage, they still provide a N input to the system through N$_2$ fixation but gains are reduced as legume biomass is grazed or cut and carried for animal consumption. At farm level, this can still represent a net gain as milk and meat production increase because of the greater forage availability and quality. At plot level, N removed with harvested products can in some cases exceed the amount of fixed N, which depletes soil N (Boddey et al., 1997). Return of animal manure to the plot may compensate this depletion. Depending on their diet and performance, cattle and dairy cows can excrete 65 to 85% of the ingested N (Berry et al., 2002; Delve et al., 2001; Rufino et al., 2006). The animal manure excreted needs then to be collected, adequately stored and spread to avoid N losses (Rufino et al., 2006). How much N is released in the soil through animal manure and is effectively used by the subsequent crop depends on the same soil processes as for green manure (Bosshard et al., 2009).

**Framework: the Canavalia Project**

**State of research on canavalia**
Since 2000, CIAT in collaboration with farmers and national partners identified a number of promising cover crop legume species especially adapted to the soils and the climate of the hillsides in Nicaragua (Peters et al., 2003). Among all the legumes tested, *Canavalia brasiliensis* Mart. Ex. Benth (canavalia), also known as Brazilian jack bean, attracted most attention from farmers mainly due to its vigorous growth, good soil cover and outstanding level of adaptation to drought stress based on green forage yield (CIAT, 2004; Schloen et al., 2005; Schmidt et al., 2005). Biomass production up to 10,030 kg ha\(^{-1}\) dry matter was observed when canavalia was grown entirely during the rainy season (Carsky et al., 1990), and up to 6550 kg ha\(^{-1}\) when canavalia was planted at the end of the rainy season and grown during the dry season (Burle et al., 1999). Preliminary experiments conducted in the hillsides of San Dioniśio suggest greater grain yields in maize-canavalia than in maize-spontaneous fallow rotations (CIAT, 2004). Previous studies have indeed shown positive effects of canavalia on crop productivity when integrated in the crop rotation (Bordin et al., 2003). Maize yield was higher after a rotation with canavalia than after other cover crops, because of its high biomass production and rapid litter decomposition rate (Carvalho et al., 2008). In an on-station study over 4-years, the use of canavalia green manure in rotation with maize was equivalent to a replacement of 50 kg N ha\(^{-1}\) of mineral N fertilizer (Burle et al., 1999). When canavalia is used as intercrop, problems of competition for water between canavalia and the main crop have been reported (Daellenbach et al., 2005).

Below ground, Alvarenga (1995) observed for canavalia a deep pivoting root system with many fine roots and long lateral root extension, with good nodulation. However, symbiotic N\(_2\) fixation has not yet been quantified. Carvalho et al. (2008) reported a rapid decomposition rate of canavalia residues compared to other legumes in litter bags studies. After 20 weeks, Cobo et al. (2002) reported an N release from canavalia leaves and petiole of 116 kg ha\(^{-1}\).

Canavalia is considered resistant to adverse environmental factors (Schloen et al., 2005), well adapted to a wide range of soil pH and to low fertility conditions (Peters et al., 2002), tolerant to drought (Burle et al., 1999) and probably to salinity (Vidal et al., 2000). Except for an influence of soil compaction on root growth and biomass production
General introduction

reported by Alvarenga et al. (1977), there is no evidence of factors limiting canavalia biomass production.

The forage quality of canavalia is largely unknown (Schloen et al., 2005). Preliminary experiments showed that canavalia seems to be well accepted by goats and sheep in Nicaragua (Caballero et al., 1995). The seeds of canavalia contain anti-nutritive compounds (Schloen et al., 2005) but according to preliminary feeding experiments with sheep, the foliage seems not to be toxic (M. Peters, personal communication).

Entry point for canavalia in the crop-livestock system

The initial idea with canavalia was to take advantage of its drought tolerance and good soil cover qualities, by leaving it growing during the whole dry season and incorporate it before maize planting at the beginning of the next rainy season. Canavalia should therefore be planted during the second rainy season. It needs about six weeks of rains and can then survive the dry season (Schmidt A., personal communication). As it produces a lot of biomass, it should not be planted too early; otherwise it interferes with maize harvest. However, if planted too late, it does not have enough time to grow deep roots to tap water in the lower layers of the profile.

Besides the temporal entry point, the spatial entry point for canavalia has to be defined. To reverse nutrient depletion and enhance yields, canavalia should be planted on the 2 ha of land dedicated to crop production, where traditionally bean is grown during the second rainy season. As farmers do not plant bean on the whole cropping area, because of its high production costs (seeds and labour), the proposition is to plant canavalia on the area not occupied by beans (i.e. about 1 ha), and to improve the rotation by alternating each year the canavalia and the bean areas. On the same area, the rotational sequence would be maize-bean, maize-canavalia, maize-bean etc (Figure i.2).

The high amounts of green canavalia biomass covering the fields during the dry season are attractive for livestock. Farmers usually put their cows on maize fields to graze crop residues at the beginning of the dry season, and canavalia represent a good protein source
to combine with maize stover. Therefore, farmers face two alternatives while adopting canavalia: (a) a short-term alternative, where canavalia is grazed with crop residues to increase milk production and earn extra income during the dry season when milk prices are highest; and (b) a medium-to-long-term alternative, where canavalia is left on the soil to enhance soil fertility in order to improve crop yields in subsequent years.

**Figure i.2.** Traditional vs. alternative rotation sequence proposed for the integration of canavalia in the crop-livestock system. M/B is the maize-bean rotation; M/C is the maize-canavalia rotation.

**The project**

A multidisciplinary project, entitled “Realizing the benefits of cover crops legumes in the hillsides of Central America” was carried out from January 2007 to December 2009, aiming at assessing the biophysical and socioeconomic trade-offs of introducing canavalia either as green manure or as forage into the traditional maize-bean-livestock production system of the Nicaraguan hillsides. Farmer’s were involved through on-farm
trials, workshops and field days to enhance future legume adoption, and their perception of canavalia was studied through surveys. The project was led by ETH and realized in collaboration with CIAT (International Center for Tropical Agriculture), INTA (Nicaraguan Institute of Agricultural Technology), ILRI (International Livestock Research Institute), and the University of Zurich. It was funded by the North-South Center and the Systemwide Livestock Program of the Consultative Group on International Agricultural Research (SLP). This thesis was entirely carried out in the frame of this project.

**Objectives, structure, and study areas of the thesis**

The aim of this thesis was to study the different aspects of integrating canavalia in the crop-livestock system of the Nicaraguan hillsides from an environmental adaptation and N dynamics point of view in order to understand if the introduction of canavalia is sustainable. Three types of questions were to be answered:

- **Before** the introduction: where is the most appropriate landscape position to plant canavalia? Is there any factor limiting a good agricultural performance?
- **During** the introduction: what is its net N input to the system? How does its use as forage or as green manure affect soil N balances? How do farmers want to manage it?
- **After** the introduction: how much does it benefit to the next crop? How much legume N remains in the soil after canavalia cultivation?

These three questions correspond to the three manuscripts of this thesis, and were studied at three different scales (Figure i.3):

Chapter 1 explores the relationships between soil and topographic factors and canavalia biomass production at landscape level in farmers fields.

Chapter 2 compares the N budget of the traditional maize-bean rotation with the one of the proposed maize-canavalia rotation at plot level in farmers fields. The N inputs and
outputs on the soil surface were registered for all crops of the rotation. Symbiotic N\textsubscript{2} fixation was assessed using the sup\textsubscript{15}N natural abundance method (Shearer and Kohl, 1986). Chapter 3 investigates for maize the N fertilizer value of canavalia residues and canavalia fed cow manure and their effects on soil N dynamics at microplot level in a researcher managed field experiment, using sup\textsubscript{15}N labelling techniques (Hauck and Bremner, 1976; Hood et al., 2008).

Two typical sites of the Nicaraguan hillsides were chosen for the study (Figure i.4). On-farm trials for chapter 1 and 2 were implemented in the community of Santa Teresa, department of Estelí. The microplot study was installed in a 6-year old on-station trial in San Dionisio, department of Matagalpa. Detailed description of the sites is given in the respective chapters.
Figure 1.4. Relief map of Nicaragua. Dark grey represents higher elevation. Situation of the experimental sites is indicated by white circles: (1) Santa Teresa, (2) San Dionisio.
CHAPTER 1

Biomass production of *Canavalia brasiliensis* in the Nicaraguan hillsides
Abstract

*Canavalia brasiliensis* (canavalia), a drought tolerant legume, was introduced into the smallholder traditional crop-livestock production system of the Nicaraguan hillsides to improve soil fertility and dry season feed. The agronomic performance (dry matter production, symbiotic nitrogen (N) fixation, soil cover and N uptake) of canavalia was tested in field trials conducted on six farms located at different altitudes within the landscape of the mid-altitude hillsides agroecosystem. Canavalia was planted in rotation with maize during two successive years. Soil properties as well as topographic characteristics were defined for each plot. The soil profile and canavalia root system were described for different groups of plots with common properties. Above ground biomass production for both years varied between 448 and 5357 kg ha$^{-1}$, with an average of 2117 kg ha$^{-1}$. The variation in agronomic performance was largely determined by variation in biomass production. Soil depth, carbon and N content of the soil surface horizon and amount of clay and stones in the whole profile affected significantly canavalia biomass production. Canavalia cannot fully express its potential as drought tolerant cover legume on soils with low organic matter content as well as on shallow and stony soils that hinder deep rooting ability of the legume.

Introduction

Population growth in the rural poor areas of developing countries has led to land-use intensification and to soil degradation through soil nutrient depletion and soil erosion (Tan et al., 2005). Crop and livestock productivity in these countries is subsequently declining, causing decreased income and increased food insecurity. In the Nicaraguan hillsides, population is expanding at an annual growth rate of 1.3% (IFAD, 2009). Cropping is limited to two short and successive rainy seasons, and livestock suffers feed shortage during the five to six months long dry season. Traditional smallholder crop-livestock farmers cultivate maize and bean on about 2 ha of land, and share an area for grazing on less productive pastures based on Jaragua grass (*Hyparrhenia rufa*). Prior to
planting maize, land is usually prepared with oxen, when the accessibility to the field and the slopes allow, otherwise hoes are used. Maize is planted at the onset of the first rainy season, usually at the end of May. At maturity, plants are cut above the ears and maize ears are left drying on the stalks for two to three months. Meanwhile, beans are sown on a part of the cropped area around mid-September between the maize rows to take advantage of the second part of the bimodal rainfall pattern. Both maize and beans are harvested in December. In January, at the beginning of the dry season, feed is getting scarce and farmers let their cows enter the fields to graze crop residues.

Introduction of cover crop legumes can be beneficial to such a system due to their ability to add nitrogen (N) via symbiotic N$_{2}$ fixation (Boddey et al., 1997; Giller, 2001) and to protect the soil during the dry season or to enhance the quality of crop residues fed to livestock (Said and Tolera, 1993). Forage specialists and local extensionists, using participatory approaches, established trials with farmers to identify the most suitable legumes for the region. Among the legumes tested, *Canavalia brasiliensis* Mart. Ex. Benth (canavalia), also known as Brazilian jack bean, was selected by farmers for its high productivity, good soil cover and outstanding level of drought tolerance based on green forage yield (Peters et al., 2004). However, a high variation in canavalia biomass production was observed among farms. The major factors that are influencing this variation are not known.

Therefore, the main objective of this study was to determine the soil and topographic factors that influence canavalia above ground biomass production, in order to define the characteristics of the most suitable land for integration of canavalia for improved crop-livestock production. The biomass production of canavalia was linked to the soil properties and the topographic situation. These relations were then used to derive the landscape position where canavalia would be more productive.
Materials and methods

Sites and field experiments

The study area is located in the Rio Pire watershed (Department of Estelí, northern Nicaragua), within a 2 km radius around the community of Santa Teresa (13°18´N, 86°26´W). The altitude ranges from 600 to 900 m.a.s.l.. The climate is classified as tropical savannah according to the Köppen-Geiger classification (Peel et al., 2007). Annual mean rainfall (since 1977) is 825 mm (INETER, 2009), and has a bimodal distribution pattern. Six farmers of Santa Teresa who were interested in integrating canavalia on a part of their production area were identified. All farmers are traditional small-scale maize-bean-livestock growers. They chose themselves the site for the experiment within their farm. Crop management was done by the farmers, whereas data and samples were collected by the researchers. Cultivation was done essentially with hand-held tools. Sites were named after farmer’s initials: PT (Pedro Torres), AR (Antonio Ruiz), GR (Gabriel Ruiz), LP (Lorenzo Peralta), FC (Felipe Calderón), and MP (Marcial Peralta). Their land was distributed at different altitudes across the landscape. Three sites were located in the bottom of the valley (PT, AR and LP), two at a medium level (GR and FC) and one on the top of the hill (MP). Sites AR, GR, and MP showed high topographic within-site variability. Four 100 m$^2$ plots of maize-canavalia rotation were repeated in three completely randomized blocks on each site, for a total of 72 plots. At the end of September 2007, weeds were cut with large knives and canavalia (CIAT17009) was sown with a stick between maize rows with a row-to-row spacing of 50 cm and a plant-to-plant spacing of 20 cm. No fertilizer was applied. At the end of January 2008, four different proportions of canavalia above ground biomass were removed from each block for the purpose of the N budget experiment. In June 2008, remaining biomass of canavalia was cut before planting maize. Thereafter, the plots were managed the same way as in 2007, with canavalia sown at the end of September 2008 between the maize rows and cut four months later at the end of January 2009. Precipitation during canavalia growth (September to January) was 540 mm in 2007 and 460 mm in 2008, which is above the usual rainfall. Temperatures for both years were
similar, with a mean of 23°C, a maximum of 32°C and a minimum of 14°C (INETER, 2009).

**Agronomic performance of canavalia**

Before cutting canavalia in January 2008 and 2009, above-ground biomass production and soil cover were determined in each plot with the Comparative Yield Method (Haydock and Shaw, 1975) in which the yields of ten random 1 m$^2$-quadrats are rated with respect to a set of five reference quadrats preselected to provide a scale covering the range of biomass encountered within each plot. In each block samples of the above ground biomass were taken, dried in a wooden oven at about 40°C until constant dry weight, and ground with a rotary knife mill at CIAT-Nicaragua. All samples were then shipped to Switzerland, powdered with a ball mill (Retsch, GmbH, Germany) and analyzed for total N on a Thermo Electron FlashEA 1112 Automatic Elemental Analyzer. On four of the six sites, the rate of N derived from the atmosphere (%Ndfa) in canavalia was assessed with the $^{15}$N natural abundance method (Shearer and Kohl, 1986) using samples taken three months after planting, at the beginning of the flowering period and before the start of the dry season. Details on the method and sampling strategy and results are presented in Chapter 2.

**Soil and topographic properties**

Soil analyses

In September 2007, topsoil (0-10 cm) was collected with a soil corer in each plot, bulked together to form a composite sample, air-dried, sieved at 2 mm and brought to the CIAT laboratories at Cali, Colombia. Samples were analysed for total carbon (C) (Nelson and Sommers, 1982), total N (Krom, 1980), available phosphorus (P) using anion exchange resins (Tiessen and Moir, 1993), total P (Olsen and Sommers, 1982), pH$_{H2O}$ in a soil-water suspension, cation exchange capacity (Mackean, 1993), and mineral N (1M KCl
extraction). The same sampling was repeated in October 2008 and samples were again analysed for mineral N. A mean of the mineral N data of both years was used for the subsequent statistical analysis.

Soil physical properties of the topsoil (0-10 cm) of four contrasting sites (PT, GR, LP, MP; two plots per block) were determined in the soil physics laboratory of CIAT. An unsieved soil sample was used for the determination of aggregates stability (Yoder, 1936) with an apparatus similar to that described by Bourget and Kemp (1957). Three undisturbed soil cores of 5 cm of diameter per 5 cm length were taken per plot and analysed for water retention (Richards and Weaver, 1944), bulk density and texture (Bouyoucos, 1962).

Topography
Slope angle was measured on three representative points in each plot using an A mason level. Slope position was defined for each plot according to the five-unit model of Ruhe and Walker (1968), which include summit, upper slope (shoulder), lower slope (backslope) and bottom (footslope and toeslope) positions. As in most of the studies applying this model (Iqbal et al., 2005), the boundary lines between position types were arbitrary. The topographic description of the plots was completed for each plot by the hill form (convex, straight or concave). As the orientation of the watershed is north-south, no effect of slope orientation was expected and hence not assessed.

Soil profiles and rooting patterns
Ten groups of plots with common properties were defined based on chemical and topographic properties, i.e. on all properties measured at single plot level, using an ordination plot (Anderson, 2004). In the second year, four months after canavalia emergence, one profile was opened for each group, at a 15 cm distance parallel to plant rows, on a length of about 120 cm. Profiles were named after the site in which they were examined. Detailed profile descriptions included sketch maps, horizons identification (Brady and Weil, 2007), soil colour, structure and fractions, as well as maps of rooting pattern. Soil colour was defined following a standard colour chart (Oyama and Takehara,
Soil fractions (i.e. % of clay, silt, sand, gravel and stones) were determined visually in the field according to the diameter ranges of Kuntze et al. (1981). Stones were therefore defined in this study as soil particles with a diameter superior to 6 cm. The weight of stones, clay, silt and sand per profile was calculated from the fraction percentage of each horizon and an estimation of its bulk density following Brady and Weil (2007). The amount of each fraction per profile was the sum of the amounts in each horizon. The amount of fine earth per profile was the sum of the amounts of clay, silt and sand. A transparent plastic sheet was placed on the wall of the profile and positions of root contacts were marked with a pen (Tardieu, 1988). The resulting point patterns were then digitalized. Roots were made visible up to the plant line using small knives, and sketched. Lateral roots, which are known to be extended for canavalia (Alvarenga et al., 1995), were not included in the sketches as their excavation was not feasible in our trial.

Data analysis

Data from the profiles were assigned to all plots from the own profile group. For plots with missing parameters for soil physical properties, average values of their own group were imputed. This resulted in a single matrix with a complete set of values for all variables and plots. As usual in environmental studies, some variables were dependent from each other, especially in the profiles where variables were inevitably spatially correlated. The first step was therefore to reduce the data set to a subset of independent variables that still represent most of the variation between plots and are relevant for soil fertility. This subset (Table 1) was then subject to two types of analysis. First, the soil and topographic properties influencing canavalia biomass production were selected using stepwise multiple regression. Second, a principal component analysis was used to make the link between properties and landscape positions, and identify gradients of properties in the landscape. The profile descriptions were used to sustain the conclusions from the data subset with a representative set of concrete observations.
Statistical analysis was performed using the program R (R Development Core Team, 2007). Canavalia data were submitted to a Wilcoxon rank-sum test to check for significant differences between the two years. The significance of the effect of the cut of 2007 on the performance of 2008 was tested by an analysis of variance using the aov function in R (Chambers et al., 1992). The model contained treatment as fixed factor, site and block as random factors, block being nested within site.

In the profiles, roots aggregation index and intensity of soil exploration by roots were determined by analysing root point patterns using the package spatstat in R (Baddeley and Turner, 2005). The root aggregation index is measured based on the nearest neighbour distance, and indicates the degree of randomness in the spatial root distribution pattern. It takes values from 0 to 2, with 0 indicating the maximum degree of clustering, 1 indicating a random pattern, and 2 indicating a uniform pattern (Clark and Evans, 1954).

The linear multiple regression was done using lme in R (Pinheiro and Bates, 2000). Right-skewed variables were log-transformed before the regression. The variable site was considered as a random factor. Categorical variables were fitted by set. Model simplification was done using stepAIC in R (Venables and Ripley, 2002). Some variables showing a non significant coefficient were kept by the automated model reduction procedure as they added to a positive increase in the $R^2$ value of the model. The significance level chosen was $\alpha = 0.05$. The PCA was performed using princomp in R (Mardia et al., 1979). Before the PCA, dummy variables were created for categorical variables and Z-scores were calculated for all variables to standardize the scale of measurement.

**Results and discussion**

*Agronomic performance of canavalia*

Canavalia above ground biomass production per plot varied between 0 and 5700 kg ha$^{-1}$ in 2007 and between 290 and 6570 kg ha$^{-1}$ in 2008 (Figure 1.1). It did not significantly differ between 2007 and 2008 ($p=0.740$). The biomass removal treatments applied when
cutting canavalia at the end of the growing season 2007 had no significant effect on the production in 2008 ($p=0.407$). Therefore, for each plot, mean values of both years were used in the subsequent analysis. Compared to on-station trials in Brazil, yields were similar to the 230 to 6550 kg ha$^{-1}$ observed when canavalia was planted at the end of the rainy season and grown during the dry season (Burle et al., 1999). Soil cover by canavalia varied between 13% and 96%, with a mean value of 53%. It was positively correlated with canavalia biomass (Figure 1.2, $R^2 = 0.78$). An increase in biomass up to 3000 kg ha$^{-1}$ induced also an important increase in soil cover, whereas beyond this level this effect decreased. Cover was not included in the multiple regression analysis as it depended highly on canavalia biomass production.

![Figure 1.1. Canavalia above ground biomass production on all sites in 2007 and 2008.](image)

*Figure 1.1.* Canavalia above ground biomass production on all sites in 2007 and 2008.

![Figure 1.2. Relationship between canavalia cover and biomass](image)

*Figure 1.2.* Relationship between canavalia cover and biomass
Results from the $^{15}$N natural abundance method are detailed and discussed in Chapter 2. Average Ndfa in 2008 was significantly ($p<0.001$) higher than in 2007, with 74% and 64%, respectively. This increase in %Ndfa is likely due to an increase in nodulation during the second year. Standard deviation of Ndfa was only 10% in 2007 and 6% in 2008, which is low compared to the variation in biomass. Average N concentration in canavalia biomass was 17.5 g kg$^{-1}$ and 15.9 g kg$^{-1}$, with a standard deviation of 2.2 g kg$^{-1}$ and 2.8 g kg$^{-1}$ in 2007 and 2008, respectively.

The amount of N brought by symbiotic N$_2$ fixation into the system, defined as %Ndfa multiplied by N concentration and biomass, ranged from 0 to 63 kg N ha$^{-1}$. Since biomass production varied more than %Ndfa and N concentration, the variation in amount of N fixed was mainly due to variation in biomass.

**Soil and topographic properties**

The ranges of values taken by the soil and topographic variables of the data subset are presented in Table 1.1. In this subset, all chemical and physical properties were measured in the topsoil. The only variables integrating the information from subhorizons are the variables defined in the profiles, i.e. the amount of stones and clay. Except for water retention and pH, all quantitative variables took a broad range of values. In the plots, topsoil had no extreme pH values and available P levels indicated no P limitation for crops. In contrast, soil carbon content ranged from 38 to 3 g kg$^{-1}$, i.e. from an amount of carbon characteristic for arable soils to an amount close to the one measured on eroded soils in the Nicaraguan hillsides (Velasquez et al., 2007). About 39% of the plots had a slope angle higher than 20%. Most of the plots had a straight slope form (78%). Few plots were located on a local summit (6%) whereas 64% of the plots were in the lower part (23%) and in the bottom of the slopes (41%). In the profiles, the amount of stones ranged from 7 to 727 kg m$^{-2}$, whereas the amount of fine earth ranged from 175 to 2328 kg m$^{-2}$. The amount of fine earth per profile was highly correlated with depth ($R^2=0.89$). Therefore, from the fine earth components only the amount of clay was retained in the data subset.
### Table 1.1. Subset of soil and topographic variables used in PCA and multiple regression.

<table>
<thead>
<tr>
<th>Set</th>
<th>Abbreviation</th>
<th>Variable</th>
<th>Variable type</th>
<th>Definition</th>
<th>Range or % of total (^1) (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field</td>
<td>Alt</td>
<td>Site within the landscape</td>
<td>Quantitative</td>
<td>field</td>
<td>651 - 872 (masl)</td>
</tr>
<tr>
<td></td>
<td>oxen</td>
<td>Labour (use of oxen)</td>
<td>Categorical</td>
<td>field</td>
<td>67 (%)</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>pH</td>
<td>Quantitative</td>
<td>plot</td>
<td>5.3 - 7.1</td>
</tr>
<tr>
<td>CEC</td>
<td></td>
<td>Cation exchange capacity</td>
<td>Quantitative</td>
<td>plot</td>
<td>26.6 - 51.8 (cmol kg(^{-1}))</td>
</tr>
<tr>
<td>Ntot</td>
<td></td>
<td>Soil total nitrogen</td>
<td>Quantitative</td>
<td>plot</td>
<td>415 - 2967 (mg kg(^{-1}))</td>
</tr>
<tr>
<td>Nmin</td>
<td></td>
<td>Soil mineral nitrogen</td>
<td>Quantitative</td>
<td>plot</td>
<td>25 - 142 (mg kg(^{-1}))</td>
</tr>
<tr>
<td>Ctot</td>
<td></td>
<td>Soil total carbon</td>
<td>Quantitative</td>
<td>plot</td>
<td>3 - 38 (g kg(^{-1}))</td>
</tr>
<tr>
<td>Ptot</td>
<td></td>
<td>Soil total phosphorus</td>
<td>Quantitative</td>
<td>plot</td>
<td>122 - 730 (mg kg(^{-1}))</td>
</tr>
<tr>
<td>Presin</td>
<td></td>
<td>Soil available phosphorus</td>
<td>Quantitative</td>
<td>plot</td>
<td>6 - 86 (mg kg(^{-1}))</td>
</tr>
<tr>
<td>WSAgg</td>
<td></td>
<td>Water stable aggregates (&gt; 0.25 mm)</td>
<td>Quantitative</td>
<td>plot or profile group</td>
<td>21.3 - 73.2 (%)</td>
</tr>
<tr>
<td>UAgg</td>
<td></td>
<td>Unstable aggregates (&lt;0.125 mm)</td>
<td>Quantitative</td>
<td>plot or profile group</td>
<td>21.0 - 63.5 (%)</td>
</tr>
<tr>
<td>bulkd</td>
<td></td>
<td>Bulk density</td>
<td>Quantitative</td>
<td>plot or profile group</td>
<td>0.97 - 1.40 (g cm(^{-3}))</td>
</tr>
<tr>
<td>pF1.88</td>
<td></td>
<td>Water retention at field capacity</td>
<td>Quantitative</td>
<td>plot or profile group</td>
<td>35 - 45 (%)</td>
</tr>
<tr>
<td>pF4.18</td>
<td></td>
<td>Water retention at wilting point</td>
<td>Quantitative</td>
<td>plot or profile group</td>
<td>24 - 38 (%)</td>
</tr>
<tr>
<td>poros</td>
<td></td>
<td>Porosity</td>
<td>Quantitative</td>
<td>plot or profile group</td>
<td>47 - 62 (%)</td>
</tr>
<tr>
<td>Slope angle</td>
<td>Slope</td>
<td>Slope angle</td>
<td>Quantitative</td>
<td>plot</td>
<td>0 - 49 (%)</td>
</tr>
<tr>
<td>Slope form</td>
<td>straight</td>
<td>Straight slope</td>
<td>Categorical</td>
<td>plot</td>
<td>78 (%)</td>
</tr>
<tr>
<td></td>
<td>concav</td>
<td>Slope with concave form</td>
<td>Categorical</td>
<td>plot</td>
<td>12 (%)</td>
</tr>
<tr>
<td>Slope position</td>
<td>summit</td>
<td>Plot on the summit of local hill</td>
<td>Categorical</td>
<td>plot</td>
<td>6 (%)</td>
</tr>
<tr>
<td></td>
<td>uppersl</td>
<td>Plot on lower part of slope</td>
<td>Categorical</td>
<td>plot</td>
<td>9 (%)</td>
</tr>
<tr>
<td></td>
<td>lowersl</td>
<td>Plot on upper part of slope</td>
<td>Categorical</td>
<td>plot</td>
<td>23 (%)</td>
</tr>
<tr>
<td></td>
<td>bottom</td>
<td>Plot on the bottom of local hill</td>
<td>Categorical</td>
<td>plot</td>
<td>41 (%)</td>
</tr>
<tr>
<td>Depth</td>
<td>Depth</td>
<td>Depth of the profile</td>
<td>Quantitative</td>
<td>profile group</td>
<td>50 - 170 (cm)</td>
</tr>
<tr>
<td>Texture (^3)</td>
<td>Clay</td>
<td>Amount of clay</td>
<td>Quantitative</td>
<td>profile group</td>
<td>19 - 696 (kg m(^{-2}) profile)</td>
</tr>
<tr>
<td></td>
<td>Stone</td>
<td>Amount of stones</td>
<td>Quantitative</td>
<td>profile group</td>
<td>7 - 727 (kg m(^{-2}) profile)</td>
</tr>
</tbody>
</table>

\(^1\) range is given for quantitative variables and % of total is given for categorical variables

\(^2\) properties measured in the topsoil (0-10 cm)

\(^3\) properties measured on the whole profile, for a volume of 1 m\(^2\) x profile depth
### Table 1.2

Profiles description, including horizons identification, soil colour, structure and fractions, as well as rooting patterns. Root distribution is the number of root points per depth, in % of total. Intensity (Int., number of root points dm$^{-2}$) and aggregation index (Agg.) are given in the bottom right of each profile.

<table>
<thead>
<tr>
<th>Profile</th>
<th>Horizons</th>
<th>colour</th>
<th>structure</th>
<th>texture (%)</th>
<th>pores</th>
<th>Root system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>clay</td>
<td>sand</td>
<td>gravel</td>
<td>stones</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR1</td>
<td>A</td>
<td>brownish grey</td>
<td>granular</td>
<td>35</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>B/C</td>
<td>grey, brownish grey</td>
<td>subangular bloc</td>
<td>25</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>dull yellow orange</td>
<td>subangular bloc</td>
<td>10</td>
<td>45</td>
<td>15</td>
</tr>
<tr>
<td>AR2</td>
<td>B</td>
<td>greyish red</td>
<td>granular</td>
<td>15</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Cm</td>
<td>light reddish grey</td>
<td>-</td>
<td>1.5</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>FC1</td>
<td>A</td>
<td>reddish grey</td>
<td>granular</td>
<td>45</td>
<td>5</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>reddish grey</td>
<td>angular bloc</td>
<td>55</td>
<td>5</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>Cb</td>
<td>greyish red</td>
<td>angular bloc</td>
<td>50</td>
<td>5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>FC2</td>
<td>A</td>
<td>reddish grey</td>
<td>granular</td>
<td>30</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Bb</td>
<td>light red</td>
<td>subangular bloc</td>
<td>45</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Bb</td>
<td>light red</td>
<td>subangular bloc</td>
<td>30</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Bb</td>
<td>red brown</td>
<td>subangular bloc</td>
<td>30</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>red brown</td>
<td>subangular bloc</td>
<td>20</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>GR1</td>
<td>A</td>
<td>dull orange</td>
<td>subangular bloc</td>
<td>35</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Bt</td>
<td>dull brown</td>
<td>subangular bloc</td>
<td>50</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>dull yellow orange</td>
<td>granular</td>
<td>&lt;1</td>
<td>55</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Cm</td>
<td>light gray</td>
<td>prismatic</td>
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<td>5</td>
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<td>prismatic</td>
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### Table 1.2. Profiles description (continuing).

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<th>distribution (%)</th>
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<td>A</td>
<td>light yellow</td>
<td>subangular bloc</td>
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<td>70</td>
<td>10</td>
<td>&lt;1</td>
</tr>
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<td>prismatic</td>
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<td>60</td>
<td>&lt;1</td>
<td>&lt;1</td>
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<td>prismatic</td>
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<td>5</td>
<td>1</td>
</tr>
<tr>
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<td>C</td>
<td>dull yellow orange</td>
<td>prismatic</td>
<td>&lt;1</td>
<td>80</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Ap</td>
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<td>granular</td>
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<td>25</td>
<td>5</td>
<td>0</td>
</tr>
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<td>B</td>
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<td>subangular bloc</td>
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<td>20</td>
<td>10</td>
<td>5</td>
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<tr>
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<td>-</td>
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<td>-</td>
<td>&lt;5</td>
<td>10</td>
<td>20</td>
<td>70</td>
</tr>
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<td>granular</td>
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<td>90</td>
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<td>Ap</td>
<td>reddish grey</td>
<td>granular</td>
<td>25</td>
<td>5</td>
<td>15</td>
<td>10</td>
</tr>
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<td>subangular bloc</td>
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<td>5</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
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<td>white/light orange</td>
<td>prismatic</td>
<td>10</td>
<td>25</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>dull orange</td>
<td>columnar</td>
<td>20</td>
<td>10</td>
<td>20</td>
<td>40</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP2</td>
<td>A0</td>
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<td>granular</td>
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<td>5</td>
<td>5</td>
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</tr>
<tr>
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<td>-</td>
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<td>2</td>
<td>5</td>
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<tr>
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<td>columnar</td>
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<td>20</td>
<td>15</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT</td>
<td>Ao</td>
<td>reddish grey</td>
<td>subangular bloc</td>
<td>40</td>
<td>5</td>
<td>5</td>
<td>&lt;1</td>
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<tr>
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<td>columnar</td>
<td>45</td>
<td>5</td>
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<td>&lt;1</td>
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<tr>
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<td>Bt</td>
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<td>prismatic</td>
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</tbody>
</table>

- **GR2** and **MP1** have a reddish grey subangular bloc in the **B** horizon.
- **LP** has a light reddish grey granular layer in the **Ap** horizon.
- **MP2** shows a brownish grey granular layer in the **A0** horizon.
- **PT** features a reddish grey subangular bloc in the **Ao** horizon.

**Legend:**
- White colour: organic material slightly decomposed
- Compacted/dense material: mineral concretions
- Abrupt/clear/sharp separation: stones
- Gradual/diffuse separation: gradual/diffuse separation
Table 1.3. Soil and topographic variables for the profile groups. See Table 1.1 for the explanation of the variables. Chemical and physical characteristics were measured in the topsoil (0-10cm).

<table>
<thead>
<tr>
<th>Profile</th>
<th>Biomass</th>
<th>Field</th>
<th>pH</th>
<th>CEC (cmol kg⁻¹)</th>
<th>Ntot (mg kg⁻¹)</th>
<th>Nmin (mg kg⁻¹)</th>
<th>Cot (g kg⁻¹)</th>
<th>Pot (mg kg⁻¹)</th>
<th>Presin (mg kg⁻¹)</th>
<th>WSagg (%)</th>
<th>Uagg (g cm⁻³)</th>
<th>buildd (%)</th>
<th>pF1.88 (%)</th>
<th>pF4.18 (%)</th>
<th>poros (%)</th>
<th>Slope</th>
<th>Slope form</th>
<th>Slope position</th>
<th>Depth (cm)</th>
<th>Clay (kg m⁻²)</th>
<th>Stone (kg m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR1</td>
<td>3348</td>
<td>671</td>
<td>x</td>
<td>6.9</td>
<td>44.5</td>
<td>2967</td>
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<td>34</td>
<td>730</td>
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<td>55.5</td>
<td>1.38</td>
<td>39</td>
<td>32</td>
<td>47</td>
<td>39</td>
<td>concave</td>
<td>lowerslope</td>
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<td>460</td>
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<tr>
<td>AR2</td>
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<td>671</td>
<td>x</td>
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<td>43.8</td>
<td>1219</td>
<td>111</td>
<td>18</td>
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<td>43.4</td>
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<td>midslope</td>
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<td>696</td>
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<td>1.15</td>
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<td>12</td>
<td>straight</td>
<td>midslope</td>
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<tr>
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<td>707</td>
<td>x</td>
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<td>41.4</td>
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<td>1.15</td>
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<td>29</td>
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<td>25</td>
<td>straight</td>
<td>midslope</td>
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<td>237</td>
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<tr>
<td>GR2</td>
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<td>26.6</td>
<td>415</td>
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<td>1.08</td>
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<td>convex</td>
<td>upperslope</td>
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<td>19</td>
<td>8</td>
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<tr>
<td>LP</td>
<td>1850</td>
<td>674</td>
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<td>31.6</td>
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<td>40</td>
<td>32</td>
<td>56</td>
<td>45</td>
<td>straight</td>
<td>upperslope</td>
<td>100</td>
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<td>31.8</td>
<td>1611</td>
<td>87</td>
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<td>464</td>
<td>39.8</td>
<td>54.8</td>
<td>1.09</td>
<td>38</td>
<td>30</td>
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<td>22</td>
<td>straight</td>
<td>lowerslope</td>
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<td>727</td>
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<td>x</td>
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<td>51.9</td>
<td>1.21</td>
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<td>33</td>
<td>54</td>
<td>1</td>
<td>straight</td>
<td>bottom</td>
<td>110</td>
<td>535</td>
<td>8</td>
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</tbody>
</table>

1x means use of oxen
Soil profiles and rooting patterns

Description of soil profiles is presented in Table 1.2. Characteristics of the profiles for each variable of the data subset are presented in Table 1.3. Profiles on lower slope or bottom positions were deeper than profiles located on upper slope or summit positions. The effect of stony or compacted layers is visible on root morphology. More than 20% of roots were counted in the first 20 cm depth in the profiles with high amounts of organic matter as well as in the profiles where stony layer hindered root growth. The root aggregation index for all profiles was between 0.6 and 1. Profiles with no major obstacles hindering root growth had a relative homogeneous root distribution in depth and an aggregation index between 0.9 and 1, close to randomness (AR1, GR1, PT). Profiles with obstacles (i.e. stony layer, coarse structure, compacted layer in the upper part of the profile) had an irregular root distribution in depth and an aggregation index between 0.6 and 0.8, meaning that root patterns was slightly clustered (AR2, GR2, MP1, MP2). In rich soils, obstacles hindering root growth are less of a problem, as roots find enough nutrients where they are (MP1, MP2). In soils with coarse texture and lower nutrient content, roots have to explore a bigger area to supply plants with water and nutrients, which render obstacles more problematic (AR2, GR2).

The biomass production of canavalia associated with each profile is shown in Figure 1.3. A one-way ANOVA showed that there were significant differences between the mean canavalia biomass productions per profile group ($p<0.001$).

![Figure 1.3](image.png)

**Figure 1.3.** Canavalia above ground biomass production per profile group.
Chapter 1

Soil and topographic properties affecting canavalia biomass production

Results of the stepwise multiple regression indicated that the variables retained after the model reduction explained a significant proportion of the variation in canavalia biomass (61%, \( p<0.001 \)). Estimated parameters of the reduced model and related \( p \)-values are presented in Table 1.4. The major factors influencing canavalia biomass production were (in order of decreasing significance): soil depth, total amount of clay in the profile, slope position, total amount of stones in the profile, total N and C in the topsoil. Still, a proportion of the variation in canavalia biomass production remains unexplained by the soil and topographic properties chosen.

Table 1.4. Equation parameters of the reduced linear model assessing the relationship between canavalia biomass and soil and topographic properties, and their significance.

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>p-value</th>
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<td>Intercept</td>
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<td><strong>Soil and topographic properties</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cation exchange capacity ( ^* )(cmol kg(^{-1} ))</td>
<td>0.0006</td>
<td>0.007</td>
</tr>
<tr>
<td>Soil total nitrogen (mg kg(^{-1} ))</td>
<td>0.0006</td>
<td>0.007</td>
</tr>
<tr>
<td>Soil mineral nitrogen ( ^* )(mg kg(^{-1} ))</td>
<td>-0.0299</td>
<td>0.031</td>
</tr>
<tr>
<td>Soil total carbon (g kg(^{-1} ))</td>
<td>-0.0299</td>
<td>0.031</td>
</tr>
<tr>
<td>Soil total phosphorus (mg kg(^{-1} ))</td>
<td>-0.0299</td>
<td>0.031</td>
</tr>
<tr>
<td>Soil available phosphorus ( ^* )(mg kg(^{-1} ))</td>
<td>-0.0299</td>
<td>0.031</td>
</tr>
<tr>
<td>Water stable aggregates (( &gt;0.25 \text{ mm} )) (%)</td>
<td>-0.0046</td>
<td>0.153</td>
</tr>
<tr>
<td>Unstable aggregates (( &lt;0.125 \text{ mm} )) (%)</td>
<td>-0.0046</td>
<td>0.153</td>
</tr>
<tr>
<td>Bulk density (g cm(^{-3} ))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water retention at field capacity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water retention at wilting point (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porosity ( ^* )(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope angle (%)</td>
<td>-0.1304</td>
<td>0.137</td>
</tr>
<tr>
<td>Straight slope</td>
<td>-0.1940</td>
<td>0.341</td>
</tr>
<tr>
<td>Slope with concav form</td>
<td>0.1557</td>
<td>0.546</td>
</tr>
<tr>
<td>Plot on the summit of local hill</td>
<td>-1.0008</td>
<td>0.000</td>
</tr>
<tr>
<td>Plot on lower part of slope</td>
<td>0.0310</td>
<td>0.672</td>
</tr>
<tr>
<td>Plot on upper part of slope</td>
<td>-0.5003</td>
<td>0.001</td>
</tr>
<tr>
<td>Plot on the bottom of local hill</td>
<td>-0.0064</td>
<td>0.956</td>
</tr>
<tr>
<td>Depth of the profile (cm)</td>
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<td>0.000</td>
</tr>
<tr>
<td>Clay (kg m(^{-2} ))</td>
<td>-0.0013</td>
<td>0.000</td>
</tr>
<tr>
<td>Amount of stones (kg m(^{-2} ) profile)</td>
<td>-0.0008</td>
<td>0.002</td>
</tr>
</tbody>
</table>

\(^* \) variables log-transformed before the regression to approach a normal distribution
Gradients of properties within the landscape

The first four components of the PCA on the soil and topographic variables listed in Table 1.1 account for 67% of the variation between the plots. Loadings reported in Table 1.5 show the weight of the variables on each component. Those loadings were stable with slight changes in the data set: a decrease in the number of variables entering the PCA increased the weight of the variables but did not affect the general pattern. The components can be interpreted as gradients of properties between the plots (Olde Venterink et al., 2001; Shukla et al., 2006), reflecting soil processes that happened either at landscape level (i.e. from the upper sites of the watershed to the sites down the river), and/or at field level (i.e. from the plots on a local summit to the plot in a local depression).

Table 1.5. Loading for the first four principal components. Only loadings <-0.2 and >0.2 are shown. See Table 1.1 for the explanations of the variables.

<table>
<thead>
<tr>
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<th>Comp.1</th>
<th>Comp.2</th>
<th>Comp.3</th>
<th>Comp.4</th>
</tr>
</thead>
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<td>20</td>
<td>12</td>
<td>10</td>
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<tr>
<td>cumulated (%)</td>
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<td>57</td>
<td>67</td>
</tr>
<tr>
<td>Alt</td>
<td>0.31</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>oxen</td>
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<td>pH</td>
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<td>CEC</td>
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<td>0.27</td>
<td>0.32</td>
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<tr>
<td>Ctot</td>
<td>0.27</td>
<td>0.32</td>
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<td></td>
</tr>
<tr>
<td>Ptot</td>
<td>0.25</td>
<td>0.27</td>
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<td>-0.20</td>
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<td>-0.37</td>
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</tr>
<tr>
<td>Uagg</td>
<td>0.23</td>
<td>-0.38</td>
<td>-0.29</td>
<td></td>
</tr>
<tr>
<td>bulkd</td>
<td>0.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pF1.88</td>
<td>0.28</td>
<td>0.31</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>pF4.18</td>
<td>-0.34</td>
<td>-0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poros</td>
<td>0.28</td>
<td>0.31</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
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</tr>
<tr>
<td>straight</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>concav</td>
<td></td>
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</tr>
<tr>
<td>summit</td>
<td>0.29</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>uppersl</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>lowersl</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>bottom</td>
<td>-0.28</td>
<td>-0.23</td>
<td>0.26</td>
<td>-0.28</td>
</tr>
<tr>
<td>Depth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clay</td>
<td>-0.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stones</td>
<td>0.21</td>
<td>0.29</td>
<td>0.38</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1.4. Scores and loadings of the PCA, projected in function of the two first components. Only loadings from variables included in the reduced regression model are displayed. Variance explained by the components is given in parenthesis. Abbreviations of the variables are explained in Table 1.1. Profiles groups are drawn around their corresponding scores, and are labelled in italic.

The first component can be interpreted as a gradient of organic matter in the topsoil at watershed level. It separates the sites at the bottom of the valley where tillage is practised and which are characterized by less stable aggregates, and the sites located at higher altitude, characterized by higher C and N content. The second component represents a gradient of clay content, associated with higher water retention at wilting point and more stable aggregates in the flat and clayey areas compared to the local summits and slopes. The third component represents the accumulation of nutrients and stones in lower slopes and in concave sites. The variables with higher weight on the fourth component are depth
and amount of stones. The third and fourth gradients are both related to erosion and sedimentation processes. The interpretation of the components leads to gradients close to the variables that were retained in the reduced linear regression model (Table 1.4). Therefore, the variation in soil properties is explained by the same main factors as the variation in canavalia biomass production. The gain of information from the PCA is the link between the explaining variables and the profiles, which are associated to landscape positions. A biplot of the scores and loadings allows identifying which gradient or variable is most affecting the soil properties and therefore the biomass production of which profile (Fig. 1.4). For the sake of clarity, only the variables from the reduced linear regression model are displayed. On the positive side of the organic matter gradient (or the first component), we find MP and FC profile groups, whereas AR1 and GR1 are negatively influenced by the gradient. The clay gradient separates GR2 and AR2 vs. PT and LP profile groups.

1. Organic matter gradient, with C and N as main factor influencing canavalia growth
2. Clay gradient, with clay and slope position as main factors
3. Sedimentation gradient, with C, N and stones as main factors
4. Depth gradient, with depth, stones and slope position as main factors

Figure 1.5. Characteristics of landscape positions: example of linkage between four soil profiles and the gradients of soil properties deduced from the PCA.
By linking gradients and profiles description, we can deduce characteristics of landscape positions with different aptitude for canavalia production. Four contrasting profiles are taken as examples in Figure 1.5. The comparison between this figure and the biomass production (Figure 1.3) allows deducing the characteristics of the most suitable (AR1) and unsuitable lands (AR2 and LP) for integration of canavalia into the production system. The best soil for canavalia is deep, well drained, rich in organic matter and clay. Canavalia cannot fully express its potential as drought tolerant cover legume on soils with low organic matter content as well as on shallow and stony soils that hinder deep rooting ability of the legume. Lands with limiting characteristics can compensate with a few good characteristics (MP2: high amounts of stones but also high amounts of organic matter). Landscape position gathering most of these favourable characteristics are the lower slopes and the concave sites.

The characteristics of the best location for canavalia agronomic performance are conforming to what is commonly recognized as a good soil. Yield superiority at lower slope has been explained by increased available water, deposition of organic matter and nutrients by overland erosion and subsurface flow (Agbenin and Tiessen, 1995) and was observed in many landscape studies (Kravchenko and Bullock, 2002; Kravchenko et al., 2000; Stone et al., 1985). However, the lower slope position is not a sufficient criterion for canavalia production. If these soils are associated with bad drainage properties, they may become partially flooded during the rainy season and be less suitable. Other legumes may be more suitable to those poorly drained lands. For example, *Desmodium ovalifolium* would be suitable on periodically flooded and shallow soils (Schmidt et al., 2001), if grazed at the beginning of the dry season as it is not drought tolerant.

Except for the organic matter, the characteristics of the locations favouring high canavalia biomass production are all directly related to drought proneness, suggesting that canavalia mainly tolerates drought due to its deep rooting ability. If soil conditions do not allow tapping water from deeper soil layers, growth and biomass production could be markedly reduced. Root system observation for different types of profiles at the end of the dry season would allow confirming this hypothesis.
The adaptation of canavalia to acid and P depleted soils, as reported by Peters et al. (2002), could not be tested in this study. Indeed, the sites chosen for this study were not P depleted, and were all in a pH range of 5.3 to 7.1 (Table 1.1). The potential of canavalia to improve productivity on acid and/or low P soils would need to be confirmed by further studies.

*Perspective for integrating canavalia in the Nicaraguan hillsides*

The purpose of introducing canavalia into the Nicaraguan hillsides was twofold: (i) to restore soil fertility of degraded areas and (ii) to increase the availability of feed to livestock during the dry season. It is important to note that even when canavalia is less productive on shallow and stony soils it could still make a contribution to improving soil fertility and feed availability. However, a marked increase in agricultural production will not occur on these less productive areas in the short term without additional inputs of mineral fertilizer or animal manure. If canavalia is used on slopes, it needs to be combined with other soil conservation measures such as live barriers to restore soil fertility in the short to medium term. Biophysical and economic trade-off analysis is needed to identify the limit for the minimum biomass production at the whole farm level for farmers to adopt canavalia as a legume option. There is also need for evaluating other legume options for the less productive areas to improve the productivity and profitability of smallholder farms that are variable in their soil fertility conditions.

**Conclusions**

Topography strongly affects canavalia biomass production in farmers’ fields. Canavalia cannot fully express its potential as drought tolerant cover legume on soils with low organic matter content as well as on shallow and stony soils that hinder deep rooting ability of the legume. In these conditions, canavalia should be combined with other soil fertility management practices to be able to build up an arable layer with time. A niche-
based assessment of possibly better adapted legume species would be worthwhile for the less productive areas.
CHAPTER 2
Nitrogen balances in farmers fields under alternative uses of a cover crop legume – a case study from Nicaragua
Chapter 2

Abstract

*Canavalia brasiliensis* (canavalia), a drought tolerant legume, was introduced into the smallholder traditional crop-livestock production system of the Nicaraguan hillsides as green manure to improve soil fertility or as forage during the dry season for improving milk production. Since nitrogen (N) is considered the most limiting nutrient for agricultural production in the target area, the objective of this study was to quantify the soil surface N budgets at plot level in farmers fields over two cropping years for the traditional maize/bean rotation and the alternative maize/canavalia rotation. Mineral fertilizer N, seed N and symbiotically fixed N were summed up as N input to the system. Symbiotic N\(_2\) fixation was assessed using the \(^{15}\)N natural abundance method. Nitrogen output was quantified as N export via harvested products. Canavalia derived in average 69\% of its N from the atmosphere. The amount of N fixed per hectare varied highly according to the biomass production, which ranged from 0 to 5700 kg ha\(^{-1}\). When used as green manure, canavalia increased the N balance of the maize/canavalia rotation but had no effect on the N uptake of the following maize crop. When used as forage, it bears the risk of a soil N depletion up to 41 kg N ha\(^{-1}\) unless N would be recycled to the plot by animal manure. Without N mineral fertilizer application, the N budget remains negative even if canavalia was used as green manure. Therefore, the replenishment of soil N stocks by using canavalia may need a few years, during which the application of mineral N fertilizer needs to be maintained to sustain agricultural production.

Introduction

Population growth in the rural poor areas of developing countries has contributed to land use intensification that adversely affects soil fertility, with nutrient depletion and soil erosion being major causes of soil degradation (Tan et al. 2005). Crop and livestock productivity therefore declines, causing decreased income generation opportunities and food insecurity. In the Nicaraguan hillsides, population is expanding at an annual growth rate of 1.3\% (IFAD 2009). Cropping is limited to two short and successive rainy seasons,
and therefore livestock suffers forage shortage during the long dry season of five to six months. Smallholders are mostly affected by the declined soil fertility due to their marginalized situation and their inability to overcome production constraints (Pfister 2003). Agricultural production usually does not exceed the needs for subsistence, making the sale of products almost impossible. Sufficient amounts of mineral fertilizers are not affordable and in small-scale farms, nitrogen (N) depletion is a major production constraint (Ayarza et al. 2007; Smyth et al. 2004).

Introduction of cover crop legumes can be beneficial to such a system due to their ability to add N via symbiotic N\(_2\) fixation (Boddey et al. 1997; Ojiem et al. 2007) and to provide surface mulch during the dry season or to provide fodder to livestock (Said and Tolera 1993). In order to identify the most suitable legume for the Nicaraguan hillsides, forage specialists and local extensionists induced farmer participatory evaluation of potential legume species. Among all the legumes tested, *Canavalia brasiliensis* Mart. Ex. Benth (canavalia), also known as Brazilian jack bean, attracted most attention from farmers mainly due to its vigorous growth, good soil cover and outstanding level of adaptation to drought stress based on green forage yield. Moreover, canavalia is also adapted to a wide range of other stress factors, including low fertility soils (CIAT 2004; Schloen et al. 2005; Schmidt et al. 2005).

Previous studies have indeed shown positive effects of canavalia on crop productivity when integrated in the crop rotation (Bordin et al. 2003). Maize yield was higher after a rotation with canavalia than after other cover crops, because of its high biomass production and rapid litter decomposition rate (de Carvalho et al. 2008). In an on-station study over 4-years, the use of canavalia green manure in rotation with maize was equivalent to a replacement of 50 kg N ha\(^{-1}\) of mineral N fertilizer (Burle et al. 1999). *Canavalia brasiliensis* is known to nodulate well (Alvarenga et al. 1995) but its contribution through symbiotic N\(_2\) fixation has not been quantified. The integration of a highly productive legume crop in a cropping system could also increase mining of nutrients (Bünemann et al. 2004b), and a yield increase of the subsequent crop also means higher N export via harvested products. The contribution of a legume to a system
may also be further diminished if crop residues are used as fodder (Peoples and Craswell 1992). Before promoting the use of canavalia to smallholders, it is important to evaluate whether canavalia results in a net N input to the cropping system, i.e., whether the N input through symbiotic N\textsubscript{2} fixation exceeds N output through harvest. Such imbalances can be revealed by calculating the N budgets for the rotations of interest. Nutrient budgets are commonly used as indicators of changes in soil fertility at national or regional scale (Bindraban et al. 2000; Smaling et al. 1993), and more recently have been useful to evaluate soil fertility status and nutrient efficiency of African smallholder crop-livestock systems (Rufino et al. 2009; Zingore et al. 2007). However, there is no published information on on-farm N budgets on the alternative uses of forage legumes in Central America. We chose the soil surface budget approach where all the N entering the soil via soil surface and leaving the soil via crop uptake are recorded (Adu-Gyamfi et al. 2007; Oenema et al. 2003; Watson et al. 2002).

Canavalia was tested either as green manure to improve soil fertility or as forage to improve milk production. When used as green manure, it was left on the plot during the whole dry season and was incorporated at the onset of the next rainy season before sowing maize. As forage, it was cut and removed at the beginning of the dry season to simulate grazing. The use of the traditional maize/bean (M/B) rotation as control does not mean that canavalia should replace bean. Indeed, farmers grow bean on only half of the cultivated area. Thus there is possibility to grow canavalia on the other half, and to alternate each year between the areas under maize/canavalia (M/C) and M/B rotations.

The main objective of this study was to quantify the soil surface N budgets at plot level in farmers fields over two cropping years for the traditional M/B rotation and the alternative M/C rotation. We tested the hypothesis that the introduction of canavalia into the traditional rotation will help reversing soil N depletion by i) fixing a high proportion of N, ii) increasing the N budget of the crop rotation, and (iii) thereby increasing maize yields the year following its integration into the production system. We emphasized N output via crop harvest and N input via N\textsubscript{2} fixation of canavalia and bean. We also assessed N recycled with crop residues.
Chapter 2

Materials and methods

Study area and farmer practices

The study area is located in the hillsides of northern Nicaragua, in the Rio Pire watershed (Municipality of Condega, Department of Esteli), within a 2 km radius around the community of Santa Teresa (13°18´N, 86°26´W) (Figure 2.1). Soils are classified as Udic and Pachic Argiustolls (MAGFOR 2008). The climate is classified as tropical savannah (Aw) according to the Köppen-Geiger classification (Peel et al. 2007). Annual mean rainfall is 825 mm (INETER 2009) and has a bimodal distribution pattern (Figure 2.2).

![Figure 2.1. Location of the sites in the Rio Pire watershed (source: INETER). The map inserted at the bottom right depicts Nicaragua, the grey square being the study area.](image)

Farmers are traditional crop-livestock smallholders, cultivating maize and bean on about 2 ha of land, and sharing an area for grazing on less productive pastures based on Jaragua grass (*Hyperrenia rufa*). Cultivation is done essentially with hand-held tools. Prior to sowing maize land is usually prepared with a plough pulled by oxen if accessibility to the
field and slopes allow; otherwise it is prepared manually using a hoe. Maize is sown at the end of May, at the onset of the first rainy season. Maize is fertilized with urea and sometimes also with NPK fertilizer. At maturity, plants are cut above the ears and maize ears are left drying on the stalks for two to three months. Meanwhile, beans are sown around mid-September between the maize rows to take advantage of the part of the bimodal rainfall pattern. Both maize and beans are harvested in December. In January, at the beginning of the dry season, forage is getting scarce in the grazing area, and farmers let their cows enter the cultivated fields to graze crop residues.

Figure 2.2. Monthly rainfall distribution during the two years of the study with the historical normal value for the region (mean monthly precipitations since 1977), measured at the meteorological station of Condega (source: INETER, 2009).

System treatments and experimental design

Four farmers of Santa Teresa, who were interested in integrating canavalia in a part of their production area, were identified. They chose themselves the site for the experiment within their farm. Crop management was done by the farmers, whereas data and samples were collected by the scientists. Sites are named after farmer’s initials: FC (Felipe Calderón), GR (Gabriel Ruiz), LP (Lorenzo Peralta) and PT (Pedro Torres). General site characteristics are given in Table 2.1.
Table 2.1. Selected properties of the four study sites. Sites are named after farmer’s initials. For soil chemical properties: averages on all plots (0-10 cm depth), with standard deviation in parenthesis (n=15)

<table>
<thead>
<tr>
<th>Site</th>
<th>Altitude masl</th>
<th>Situation</th>
<th>Slope range</th>
<th>Texture</th>
<th>pH</th>
<th>total C (^1) g/kg</th>
<th>total N (^2) g/kg</th>
<th>availableP (^3) mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC</td>
<td>706</td>
<td>hill</td>
<td>3 - 17</td>
<td>Clay</td>
<td>6.4 (0.1)</td>
<td>25.2 (2.4)</td>
<td>1.90 (0.15)</td>
<td>10.1 (3.5)</td>
</tr>
<tr>
<td>GR</td>
<td>707</td>
<td>hill</td>
<td>7 - 34</td>
<td>Sandy loam</td>
<td>6.3 (0.4)</td>
<td>10.9 (4.0)</td>
<td>1.02 (0.34)</td>
<td>14.7 (6.6)</td>
</tr>
<tr>
<td>LP</td>
<td>674</td>
<td>valley</td>
<td>1 - 5</td>
<td>Clay loam</td>
<td>6.2 (0.3)</td>
<td>21.8 (0.9)</td>
<td>1.57 (0.06)</td>
<td>75.5 (7.2)</td>
</tr>
<tr>
<td>PT</td>
<td>651</td>
<td>valley</td>
<td>0 - 3</td>
<td>Sandy clay loam</td>
<td>6.7 (0.3)</td>
<td>14.8 (1.4)</td>
<td>1.13 (0.10)</td>
<td>41.9 (6.0)</td>
</tr>
</tbody>
</table>

\(^1\) measured following Nelson and Sommers (1982)
\(^2\) measured following Krom (1980)
\(^3\) measured with anion-exchange resins (Tiessen and Moir, 1993)

Figure 2.3. Treatments replicated three times on each site: the traditional maize/bean rotation and the tested maize/canavalia rotation (part 2) with different cutting intensities of canavalia during the dry season, to simulate grazing.

On each site five crop rotations were established on 70 to 100 m\(^2\)-plots, and repeated in three completely randomized blocks, for a total of 60 plots. The control treatment was the traditional M/B rotation. The four others were M/C rotations with four different cutting intensities to simulate grazing, i.e. with 0% (M/C0), 50% (M/C50), 75% (M/C75) or...
100% (M/C100) removal of canavalia biomass (Figure 2.3). Land was prepared according to the usual practice, with hoe on FC site and ploughing with oxen on the other sites. Farmers sowed maize (*Zea mays* var. Catacamas) at the end of May 2007, by hand, with a seeding rate of 23 kg per hectare, with a row-to-row spacing of 75 cm and a plant-to-plant spacing of 50 cm. Compound NPK fertilizer (12-30-10) and urea were applied 8 and 22 days after sowing respectively. The doses varied from 0 to 8 kg N ha$^{-1}$ for NPK complex and from 30 to 60 kg N ha$^{-1}$ for urea, according to each farmer’s usual practices (Table 2.2). Weed control was done before maize germination by spraying glyphosate and after germination manually with a large knife. Cypermethrin$^1$ was used for insect control. Bean (*Phaseolus vulgaris* var. INTA seda rojo) or canavalia (var. CIAT17009) were sown between maize rows at the end of September with a seeding rate of 78 and 51 kg per hectare, respectively. No fertilizer was applied to either legume crop. Maize and bean harvest occurred between November and December according to the usual practice. In January, the different percentages of canavalia above ground biomass were removed from the field. In May 2008, remaining standing maize stalks and canavalia plants were cut with large knife and left on the ground as mulch. Fields in 2008 were prepared as described for 2007, using either plough or hoe, and treatments were repeated on the same plots. Farmers did not reduce mineral fertilizer application after the first canavalia rotation.

Precipitation during crop growth (May to January), measured at the meteorological station of the nearby municipality Condega, was similar for both years (about 920 mm), which is 19% above the normal rainfall (Figure 2.2). Temperature for both years was also similar, with a mean of 24°C, a maximum of 33°C and a minimum of 16°C (INETER 2009).

*Symbiotic N$_2$ fixation*

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$^1$ (RS)-Cyano-3-phenoxybenzyl-2,2-dimethyl-3-(2,2-dichlorvinyl)-cyclopropan-1-carboxylat
The rates of N derived from the atmosphere (%Ndfa) in canavalia and bean were assessed both years using the $^{15}$N natural abundance method (Shearer and Kohl 1986), which is based on the slight natural differences between the $^{15}$N abundance of the soil and the $^{15}$N abundance of the atmosphere. As reference plants, non-fixing dicotyledonous weeds (Oberson et al. 2007) growing at the same time as the legumes have been selected and marked in the field to avoid them being cut during weed control by the farmers. Other herbaceous and shrubby weeds were excluded. Two weeds were chosen in the immediate proximity (i.e. within a radius of 50 cm) of each marked legume. Weeds were limited to four different species per site and per year, each of them being present at least five times for each legume and as well distributed as possible across the site. Species chosen were Baltimora recta, Delilia biflora, Euphorbia gramine, Euphorbia hirta, Lagascea mollis, Melanthera aspera, Mitrocarpus hirtus, Richardia scabra, Borreria suaveolens, Ageratum conyzoides, and Conyza Canadensis (Table 2.2). Sampling of canavalia occurred three months after planting, before drought, at the beginning of the flowering period. For bean, sampling occurred at the growth stage of late flowering to early pod filling. Five bean plants and five canavalia plants were harvested per block, together with their paired weeds, resulting in thirty legumes and sixty reference plants per site and per year. Table 2.2 shows slightly lower sample numbers, as in a few cases the marked plant did not develop well and was therefore not harvested. Plants were dried and analyzed for their $^{15}$N abundance (see below).

The %Ndfa for each legume plant was calculated following Shearer and Kohl (1986), using its two paired weeds as references. The final %Ndfa per legume and per site was then calculated as the average of the fifteen %Ndfa estimated from single legume plants for this site.

The $B$-value, i.e. the isotopic fractionation during $N_2$ fixation, was obtained from a pot experiment in the greenhouse at the International Centre of Tropical Agriculture (CIAT), Colombia (3° 30' N, 76° 21' W), following the procedure of Unkovich et al. (1994). Plants were grown from the end of November 2007 to the end of February 2008. Temperature in the greenhouse fluctuated from 20° to 37°C in synchrony with photoperiod, and relative humidity ranged from 40 to 90%. Eighteen 3.3 l-pots were filled with washed
white quartz, planted with canavalia or bean and watered daily with an N-free nutrient solution. The inoculum used was made from a mixture of soils from our study sites. Harvest occurred at the same development stage as in the field. Shoot $\delta^{15}N$ values were corrected for seed N effect using a mass balance (Boddey et al. 2000; Hogberg et al. 1994) accounting for the N distribution between shoots and roots.

**Evaluation and fate of crop production**

Maize
Maize production was evaluated in each of the 60 plots of the experiment. To evaluate the yield of the different maize parts, several row segments were chosen to represent the plot, excluding the border lines, and equivalent to 20 to 30 m-row length in total per plot. On these row segments, plants were counted and classified into two categories: plants with harvestable ears and plants without. Harvestable ears were considered good for human consumption by the farmers. The category “plants without harvestable ear” included plants that were without ear, with damaged ear, with already harvested ear, or with ear on the ground. Samples of ears of each category, corresponding to the number of ears for 2 m$^2$, were taken in each plot.

Harvestable ears were separated after sampling into good grain, bad grain, cobs, and husks. Samples were pooled per block, except for good grain, for which samples were kept separated per plot. Yield of the different ear parts was assessed as follow:

$$\text{Yield}_{\text{ear part}} [\text{kg ha}^{-1}] = \frac{n^0 \text{ harvestable ears per plot} \times \text{weight}_{\text{ear part}} [\text{kg ear}^{-1}]}{\text{area plot} [\text{m}^2]} \times 10000$$  \[2.1\]

where $n^0$ of harvestable ears per plot correspond to the number of plants with harvestable ears per plot, as almost no plants developed more than one ear.

Samples of not harvestable ears were not separated into plant parts because their state did not allow doing so. The amount of not harvested ears per hectare was calculated as in formula [2.1], except that the number and weight of not harvested ears were used.
Samples of stalks and leaves were taken to complete maize biomass production estimation. As traditionally farmers cut the upper part of the stalks to let the cob dry, only a few entire plants could be found and sampled on two sites. Assuming that average stalk and leaf weight was the same on all plots, yields were calculated as in formula [2.1] using the total number of plants per plot.

In order to know which plant parts are exported and which are recycled on the plot, the fate of maize harvested ears was determined by plant part for each farmer. Maize grains (MG) were exported from the plot for human consumption, maize cobs (MC) and damaged grains (MDG) (i.e. broken, discoloured, shrivelled or undersized grains) were exported to be fed to pigs or used for combustible, maize husks (MH) were either recycled on the plot or exported to be fed to cows. Maize without harvestable ears (MRE) and residues (stalks and leaves) (MR) were not removed from the plot.

Bean
Bean yields were assessed according to farmer’s current practice. On 1 m² in each plot, bean plants were removed from the soil with their roots, separated into grain (BG) and residues (shoot and root, BP), and both plant parts were sampled and weighed separately. The fate of BG is usually to be exported for consumption, while BP remains on the plots. However, during both years of this study, heavy rainfall over short periods and to larger extent diseases such as angular leaf spot killed many bean plants leading to very low yields. Farmers harvested only when expected grain value compensated labour cost for harvest.

Canavalia
At each canavalia cutting time, above-ground biomass (CB) production was assessed in each plot with the Comparative Yield Method (Haydock and Shaw 1975) in which the yields of random 1m²-quadrants are rated with respect to a set of five reference quadrants preselected to provide a scale covering the range of biomass encountered within each plot. Ten quadrants were rated per plot. Different proportions - 100%, 75%, 50% or 0% -
of the available biomass were removed according to the experimental plan, by cutting canavalia with large knives either on a plant number basis, or on a height basis when plants were undistinguishable. The M/C100 plots, where all the biomass was removed and weighed, were used as control of the biomass estimate obtained with the Comparative Yield Method. Samples of the above ground biomass were taken from each block. Removed biomass (CBR) was exported from the plot to be fed to animals.

Plant analysis
Canavalia, bean and reference plants sampled in the field were dried in a wooden oven at about 40°C until constant dry weight and ground with a rotary knife mill at CIAT-Nicaragua. Maize plant parts were dried at ambient temperature and ground with the same mill. Canavalia and bean samples from the greenhouse were dried at 70°C and ground with a rotary knife mill at CIAT-Colombia.

All samples were then shipped to Switzerland, powdered with a ball mill (Retsch, GmbH, Germany) and analyzed for total N on a Thermo Electron FlashEA 1112 Automatic Elemental Analyzer. The \( \delta^{15}N \) of legumes and reference plants were measured at the Geological Institute of the ETH Zurich on a Thermo Electron FlashEA 1112 coupled in continuous-flow with a Thermo-Fisher MAT 253 mass spectrometer. Finely ground field pea seed with an atom % \( ^{15}N \) of 0.367 was used as analytical standard.

Soil surface N budgets

Soil surface N budgets were estimated for all plots and for both years (May to January) following the equation:

\[
N \text{ budget } [\text{kg N ha}^{-1}] = N \text{ input} - N \text{ output} = (N_{\text{fix}} + N_{\text{fert}} + N_{\text{seed}}) - (N_{\text{export}}) \quad [2.2]
\]

where \( N_{\text{fix}} \) is the contribution of symbiotic \( N_2 \) fixation, \( N_{\text{fert}} \) is the mineral fertilization, \( N_{\text{seed}} \) accounts for maize, bean and canavalia seeds and \( N_{\text{export}} \) is the amount of N exported from the plot.
Nfix was calculated as the product of %Ndfa, N concentration and legume biomass. Nfert was calculated for each site based on the amount of fertilizer applied by the farmer and the N concentration in urea and NPK complex. Nseed was calculated as the product of N concentration and seed density. 

Nexport from the plot differed for each site according to the fate given by each farmer to the different plant parts of the crops and was estimated as:

$$N_{\text{export}} \ [\text{kg N ha}^{-1}] = N_{\text{MG}} + N_{\text{MDG}} + N_{\text{MC}} + N_{\text{MH}} + N_{\text{BG}} + N_{\text{CBR}} \quad [2.3]$$

where $N_X$ is the amount of N in kg ha$^{-1}$ in each of the mentioned plant part X, obtained from its N concentration multiplied by its biomass production in kg ha$^{-1}$ (dry matter basis).

$N_{\text{MH}}$ equals 0 if the farmer left the husks on the plot. $N_{\text{BG}}$ equals 0 if the farmer decided to not harvest beans, or in M/C rotations. $N_{\text{CBR}}$ equals 0 in M/B and M/C0 rotations.

**Nitrogen recycled with crop residues**

The amount of N recycled on each plot is the amount of N in crop residues and in remaining canavalia, calculated as follows:

$$N_{\text{recycled}} \ [\text{kg N ha}^{-1}] = N_{\text{MR}} + N_{\text{BP}} + N_{(\text{CB-CBR})} + N_{\text{MH}} + N_{\text{BG}} + N_{\text{MRE}} \quad [2.4]$$

where $N_X$ is the amount of N in kg ha$^{-1}$ in each of the mentioned plant material X, obtained from its N concentration multiplied by its biomass production in kg ha$^{-1}$. 

$N_{\text{MH}}$ equals 0 if the farmer exports the husks. $N_{\text{BG}}$ equals 0 if the farmer decided to harvest beans, or in M/C rotations. $N_{(\text{CB-CBR})}$ equals 0 in M/B and M/C100 rotations.

**Data analysis**
Statistical analyses were performed using the program R (R Development Core Team, 2007). Right-skewed variables were log-transformed before the analysis. Yields were submitted to a Wilcoxon’s rank-sum test to check for significant differences between the two years. The significance of the effects of site and treatment on crop production and on N balance was tested by an analysis of variance using aov and lme functions in R (Pinheiro and Bates 2000). The model was composed by treatment as fixed factor, site and bloc as random factors, bloc being nested within site.

**Results**

*N inputs: symbiotic N$_2$ fixation*

The $B$-values obtained from the greenhouse experiment were $-1.26\ %_{e}$ for canavalia and $-3.74\ %_{e}$ for bean. The $\delta^{15}$N of the reference plants ranged from $0.2\ %_{e}$ to $13.1\ %_{e}$ in 2007 and from $0.5$ and $8.4\ %_{e}$ in 2008. Table 2.2 presents the average $\delta^{15}$N per species and per site, for both years together as there was no significant difference between the two years. The $\delta^{15}$N of the legumes ranged from $-2\ %_{e}$ to $2\ %_{e}$, with extreme values up to $4.6\ %_{e}$ in 2007 and $2.6\ %_{e}$ in 2008. Each legume had significantly lower $\delta^{15}$N than its two reference plants. Figure 2.4 shows the $\delta^{15}$N of each N$_2$-fixing plant and the mean $\delta^{15}$N of its paired reference plants for all sites in 2007 and in 2008. Average %Ndfa was 55% and 58% for bean, and 64% and 74% for canavalia in 2007 and 2008, respectively. Among sites, mean %Ndfa did not vary much, with a standard deviation of 3% to 9%. For bean, average Ndfa did not differ significantly between 2007 and 2008 ($p=0.478$). For canavalia, average Ndfa in 2008 was significantly ($p=0.000$) higher than in 2007.
Figure 2.4. Delta $^{15}$N of individual legumes and mean $^{15}$N of their paired references on all sites in 2007 and 2008. The position of the vertical line between years and between legumes varies for each site according to the number of samples analyzed.
### Table 2.2. Average $\delta^{15}$N of the reference plants and the legumes for each site, year 2007 and 2008 grouped. Standard deviation is given in parenthesis.

<table>
<thead>
<tr>
<th>Species</th>
<th>FC</th>
<th>$\delta^{15}$N</th>
<th>GR</th>
<th>$\delta^{15}$N</th>
<th>LP</th>
<th>$\delta^{15}$N</th>
<th>PT</th>
<th>$\delta^{15}$N</th>
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<td>$\delta^{15}$N</td>
</tr>
<tr>
<td>Ageratum conyzoides</td>
<td>5</td>
<td>3.52 (0.63)</td>
<td></td>
<td></td>
<td>55</td>
<td>3.69 (0.81)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baltimora recta</td>
<td>30</td>
<td>3.67 (0.43)</td>
<td></td>
<td></td>
<td>5</td>
<td>3.11 (0.59)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borreria suaveolens</td>
<td>41</td>
<td>4.53 (0.87)</td>
<td></td>
<td></td>
<td>19</td>
<td>3.88 (0.65)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conyza canadensis</td>
<td></td>
<td></td>
<td>6</td>
<td>2.83 (0.54)</td>
<td></td>
<td></td>
<td>32</td>
<td>4.41 (1.43)</td>
</tr>
<tr>
<td>Delilia biflora</td>
<td></td>
<td></td>
<td>42</td>
<td>5.02 (2.26)</td>
<td></td>
<td></td>
<td>35</td>
<td>5.87 (2.06)</td>
</tr>
<tr>
<td>Euphorbia graminea</td>
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<td></td>
<td>24</td>
<td>3.64 (0.61)</td>
<td></td>
<td></td>
<td>43</td>
<td>3.68 (1.99)</td>
</tr>
<tr>
<td>Euphorbia hirta</td>
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<td></td>
<td>42</td>
<td>5.02 (2.26)</td>
<td></td>
<td></td>
<td>54</td>
<td>3.24 (0.61)</td>
</tr>
<tr>
<td>Lagascea mollis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>5.19 (0.81)</td>
</tr>
<tr>
<td>Melanthera aspera</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>6.23 (0.88)</td>
</tr>
<tr>
<td>Mitrocarpus hirtus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Richardia scabra</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bean</td>
<td>30</td>
<td>-0.12 (0.81)</td>
<td>26</td>
<td>-0.16 (1.05)</td>
<td>25</td>
<td>-0.91 (0.67)</td>
<td>30</td>
<td>0.60 (1.13)</td>
</tr>
<tr>
<td>Canavalia</td>
<td>29</td>
<td>-0.02 (0.64)</td>
<td>29</td>
<td>0.32 (0.92)</td>
<td>29</td>
<td>0.20 (0.57)</td>
<td>28</td>
<td>1.04 (1.08)</td>
</tr>
</tbody>
</table>

**N outputs: crop production**

**Maize**

Maize grain yields (Figure 2.5) conformed to the usual production of the region (personal communication from the farmers), with an average yield of 2,410 kg ha$^{-1}$ in 2007 and 2,070 kg ha$^{-1}$ in 2008. Grain yields were not significantly different between the two years ($p=0.107$). The first year of rotation made no effect on grain yields of the subsequent year ($p=0.187$). Yields were affected significantly by the site in 2008 ($p=0.025$) but not in 2007 ($p=0.135$).

**Bean**

Bean grain production (Figure 2.5) was much lower for both years compared with the farmer reported mean production value of 1300 kg ha$^{-1}$. This was mainly due to heavy rains and diseases. The grain yield ranged 13 to 320 kg ha$^{-1}$ in 2007 and from 0 to 470 kg ha$^{-1}$ in 2008. Yields were not significantly different between 2007 and 2008 ($p=0.832$).
Figure 2.5. Maize grain production (n=15), bean grain production (n=3) and canavalia biomass production (n=12). Error bars represent the standard deviation.

Canavalia

Canavalia biomass production (Figure 2.5) varied between 0 and 5,700 kg ha\(^{-1}\), with a mean value of 2,110 kg ha\(^{-1}\) in 2007. In 2008, the biomass production varied between 290 and 4,330 kg ha\(^{-1}\), with a mean value of 1,530 kg ha\(^{-1}\). Biomass did not significantly differ between both years (\(p=0.223\)) and was not influenced by the site neither in 2007
(p=0.070) nor in 2008 (p=0.999). The removal of canavalia biomass at the beginning of the dry season in 2007 had no significant effect on the production in 2008 (p=0.066). The variation in canavalia biomass production within GR and PT sites was higher than the variation between sites.

*N budgets*

The components of the soil surface N budget and the resulting balance for each treatment on each site are presented in Table 2.3. Nitrogen input from mineral fertilizers applied to maize was from 38 to 68 kg N ha\(^{-1}\). Mineral fertilizers and seeds contributed per site equally to M/B and M/C rotation. In relation to the overall N inputs, N\textsubscript{fert} represented on average for both years 88% of the total N input in the M/B rotation, and 69% in the M/C rotation. For both years, N\textsubscript{seed} represented from 3% to 6% of the total N input. The contribution of symbiotic N\textsubscript{2} fixation to the M/B rotation did not exceed 8 kg N ha\(^{-1}\) (8 and 3% of the total N input in 2007 and 2008, respectively), whereas it was on average 22 and 17 kg N ha\(^{-1}\) (or 29 and 24 % of the total N input) in the M/C rotation in 2007 and 2008, respectively. Nitrogen exported through maize harvest ranged from 16 to 67 kg N ha\(^{-1}\). Canavalia represented an export of up to 87 kg N ha\(^{-1}\) in 2007 and 39 kg N ha\(^{-1}\) in 2008 when the whole aboveground biomass was removed.

The M/C0 treatment showed in most cases the highest N balance per site, with an average surplus of 33 kg N ha\(^{-1}\) in 2007 and 26 kg N ha\(^{-1}\) in 2008 (Figure 2.6). In 2007, M/C100 treatments resulted in most cases with a negative N balance with an average depletion of 15 kg N ha\(^{-1}\). In 2008, the M/C100 balance was in average in equilibrium, with 2 kg N ha\(^{-1}\) in average. An average surplus of 14 and 17 kg N ha\(^{-1}\) in 2007 and 2008, respectively was observed with the M/B treatment. The N balance for both years was influenced by the site (p=0.015 in 2007 and p=0.003 in 2008). Treatments had a highly significant effect on the N balance in 2007 (p=0.000) and a significant effect in 2008 (p=0.006).
Table 2.3. N budget by site, in kg N ha\(^{-1}\), means for each treatment (n=3). Standard deviation is given in parenthesis. M/B is the maize-bean rotation; M/C is the maize canavalia rotation. \(N_{\text{M}}\) is N export through maize, i.e. through grains, damaged grains, cobs and husks; \(N_{\text{BG}}\) is N export through bean grains; \(N_{\text{CBR}}\) is N export through canavalia biomass removed.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N input</th>
<th>N output</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\text{Nfert})</td>
<td>(\text{Nseed})</td>
</tr>
<tr>
<td>FC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/B</td>
<td>60</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C0</td>
<td>60</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C50</td>
<td>60</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C75</td>
<td>60</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C100</td>
<td>60</td>
<td>0.4</td>
</tr>
<tr>
<td>GR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/B</td>
<td>68</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C0</td>
<td>68</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C50</td>
<td>68</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C75</td>
<td>68</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C100</td>
<td>68</td>
<td>0.4</td>
</tr>
<tr>
<td>LP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/B</td>
<td>38</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C0</td>
<td>38</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C50</td>
<td>38</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C75</td>
<td>38</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C100</td>
<td>38</td>
<td>0.4</td>
</tr>
<tr>
<td>PT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M/B</td>
<td>38</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C0</td>
<td>38</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C50</td>
<td>38</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C75</td>
<td>38</td>
<td>0.4</td>
</tr>
<tr>
<td>M/C100</td>
<td>38</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Figure 2.6. N balance for all sites in 2007 and 2008. Error bars represent the standard deviation (n=3). M/B is the maize-bean rotation; M/CX is the maize-canavalia rotation, with different percentages (X) of biomass removed during the dry season.

\[ \text{N recycled} \]

For the most contrasting treatments M/B and M/C0, the amount of N recycled and its source are presented in Figure 2.7. After maize harvest, about 18 kg N ha\(^{-1}\) were recycled on the plot with maize residues, independent of the treatment, which represents about 32\% of the overall maize N uptake. Nitrogen recycled in the M/C0 rotation is higher than in the M/B rotation. Bean residues contributed with about 3 kg N ha\(^{-1}\) to the N recycled.
When canavalia was not removed, an average value of 22 kg N ha\(^{-1}\) was recycled on the plot with canavalia biomass.

![Graph](image)

**Figure 2.7.** N recycled for the most contrasting treatments. Average of 2007 and 2008, for all sites. Error bars are standard deviation (n=24). Origin of the N recycled is indicated as CB-CBR for canavalia biomass recycled, BG for bean grain, BP for bean plants, MRE for maize ears not harvested, MP for maize plants, MH for maize husks.

**Discussion**

*Symbiotic N\(_2\) fixation estimated with the \(^{15}\)N natural abundance method*

The suggested minimum difference of 2 \(\%\) between reference plants and legumes (Unkovich et al. 1994) was reached at all sites and for both M/B and M/C treatments (Figure 2.4). Standard deviation of all reference species \(\delta^{15}\)N per site was in average 1.1 \(\%\), and was not higher than 2.2 \(\%\), which shows that soil \(\delta^{15}\)N was relatively homogeneous on each site. For canavalia, the \(B\)-value obtained was in the range reported for tropical legume species used as forage or cover crops (Unkovich et al. 2008). The
value for bean was slightly lower than -2.2 reported for common bean by Unkovitch et al. (2008).

Bean %Ndfa was higher than the average of 36% reported by Herridge et al. (2008) for common bean in farmers fields. Canavalia %Ndfa in 2007 was in the range of the 57 – 69% reported by Giller (2001) for *Canavalia ensiformis*.

Canavalia had an average %Ndfa of 64% in 2007, despite the fact that it was grown for the first time in this region and not inoculated. For the second year of cultivation of Canavalia, an average increase of about 17% was observed compared to the first year values. Results from the pot study conducted at CIAT-Colombia showed that nodulation is more rapid and abundant (30% more nodule fresh weight) when canavalia is inoculated with rhizobia from a site where it has been grown for five years (S. Douxchamps, unpublished data). Higher %Ndfa can therefore be expected after a few years of cultivation, and may reach in the third year the value of 80% as reported for many tropical green manure legumes (Giller 2001; Thomas et al. 1997).

*Parameters of the N balance and their uncertainties*

Effect of legume biomass production on Nfix

Compared to on-station trials conducted in Brazil, canavalia biomass production was similar to the values of 230 to 6,550 kg ha\(^{-1}\) observed when grown during the dry season (Burle et al. 1999) but lower than the value of 10,030 kg ha\(^{-1}\) observed when grown entirely during the rainy season (Carsky et al. 1990). Canavalia biomass production varied highly among plots. The reasons behind this variation are due to soil and topographic factors, which are discussed in Chapter 1. Because biomass production varied more than %Ndfa, the variation in Nfix was determined by variation in biomass, which has been also observed by Thomas et al. (1997) in the humid tropics for three forage legumes. Likewise, the difference of biomass production between the legumes was the main reason why Nfix by canavalia was on an average about 16 kg N ha\(^{-1}\) higher than that of bean crop.
This difference in Nfix between the two legumes was underestimated, as below-ground biomass contribution was partially taken into account for bean but not for canavalia. Canavalia is known for its deep pivoting root system with lots of fine roots and lateral root extension up to 3.5 meters (Alvarenga et al. 1995). Besides the problems encountered in trying to estimate or recover such a root system, the rapid turnover of belowground tissues and root exudation make difficult to determine below-ground N contributions (Cherr et al. 2006). Below-ground N associated with or derived from roots can represent up to 50% of the total plant N of legumes (Herridge et al. 2008). To account for below-ground N, Unkovich et al. (2008) suggested a multiplication by factor 2 for fodder legumes, which would give for canavalia in our trial an average Nfix of 44 kg N ha\(^{-1}\) in 2007 and 34 kg N ha\(^{-1}\) in 2008. For bean, only dry roots were recovered, whereas exudates and root turnover were not taken into account. By using the multiplication factor of 1.4 suggested by Unkovich et al. (2008), the maximum Nfix for bean in our trial would be of 11 kg N ha\(^{-1}\).

Effect of on-farm conditions on Nfert, Nseed, and Nexport

Nfert and Nseed were distributed by hand, by different farmers. Distribution of fertilizer and seed was not as exact as when it is done by machines or in on-station trials. As N contained in seeds remained small compared to the other factors of the budget, its potential variation had relatively small effect on the N balance estimations. Likewise, plant density was also somewhat heterogeneous between plots.

The estimation of Nexport by maize was also affected by human factors. For example, people do not enter the fields very carefully: they may drop ears on the ground, or sometimes grab an appetizing maize ear to eat on the way back home. This may be one reason why plants with empty husks were found. The amount of empty husks represented on average 6% of the good ears.

Therefore, the results from the different sites should not be combined as one single effect of canavalia when introduced on-farm, but rather be seen as a range of possible responses, taking into account farmers practices and their impacts on data variability. One may argue that those conditions render difficult to design a precise nutrient management guidelines for the region. Uncertainties are however part of budget calculations at all
scales, and there are various ways to deal with them in the subsequent decision making process (Oenema et al. 2003). As farmers cannot afford taking risks, safety margins have to be taken into account.

Interpretation of the balances
Both years and on all sites, increasing cutting intensities of canavalia reduced the N balance. On one hand, canavalia increased N input into the system compared to M/B rotation, but on the other hand it increased soil N depletion if completely removed. Under M/B rotation, balance depended much on bean yields. When beans were harvested, the balance became negative, except in the sites where high amounts of mineral fertilizer were applied (FC and GR). The positive to neutral N balance on M/B is mainly due to the low yields of common bean. Assuming yields of 800 kg N ha\(^{-1}\) (FAO 2009), N export through bean harvest would become about 30 kg N ha\(^{-1}\), which brings the balance estimate to negative in most cases, with an average value of -6 kg N ha\(^{-1}\) and a maximum value of -40 kg N ha\(^{-1}\) on LP site. A positive N balance for the M/B rotation does not mean that the system is sustainable: lower bean yields mean lower or no income. Likewise, the observation of a higher N balance for all treatments of a site is due to reduced N export by maize. For example, on FC site maize yields were much higher in 2007 than in 2008, and thus the balance resulted much lower. When export through maize grain is not compensated by mineral fertilizers, as on LP and PT sites, the N balance becomes negative. If we would include the below ground N contribution from the legumes as presented above, the deficit observed in the M/C100 rotations would be in five of eight cases compensated, with an average balance of 18 kg N ha\(^{-1}\). The impact on M/B rotations would be lower, and would not compensate the deficit observed on LP site, which would remain at about -10 kg N ha\(^{-1}\).

Effect of canavalia on maize yields

Many experiments have demonstrated the positive effect of legumes on succeeding crops (Peoples and Craswell 1992). However, in this study, the integration of canavalia as
green manure had no effect on the following maize crop, probably because (i) one year of rotation is not sufficient to observe an effect, (ii) the mineral fertilizer background is too high compared to the N input by canavalia, and (iii) other factors related to management practices may have limited a productivity increase. For example, MRE, i.e. the amount of ears not harvested, represent a potential maize yield increase if crop management is improved. On all sites and for the two years, MRE had a mean value of 350 kg ha\(^{-1}\), which corresponds to a loss of 10% of good grain yield. According to farmers, up to 50% of maize grain yield losses can occur in the region due to this problem and these losses do not include post harvest losses. Before important changes in nutrient management as the introduction of a legume in the rotation, the traditional system could be improved by a few simple efforts. There are opportunities to increase productivity with improved management, e.g., concerning plant density, timing of fertilizer application and weed control.

\textit{N recycled and rotation sequence}

According to the design of the experiment, we expected that in the green manure scenario M/C0, the N of crop residues is recycled within the plot, as no cows would enter the field to graze. However, in practice, according to participatory workshops with farmers, there will probably be only one type of M/C rotation. Farmers will allow cows to graze totally canavalia at the onset of the dry season. Regrowth, which was not expected when the experiment was designed, has been observed during the dry season when plants are not cut down to the ground level i.e., after grazing, and may be used for soil improvement. The former N recycled would in this case represent the amount of N available for grazing during the dry season. Rufino (2006) reported for African dairy studies that on average about 80% of the ingested N is returned with manure. Assuming the same proportion recycled for cows in our trial and all N excreted being returned to the grazed plot, 33 kg N ha\(^{-1}\) on average would be recycled through canavalia grazing, under the form of faeces and urine. While the urine fraction would fall on a single spot at high concentration, the faeces fraction can be uniformly distributed on the plot surface by farmers. An efficient
animal manure management would therefore be essential to maximize N recycling and compensate the N deficit observed with M/C100 rotation.

Figure 2.8. Traditional rotational sequences on the 2 ha cropping area of a smallholder farm, and proposed alternative sequence including canavalia. Bold area enlightens the rotation succeeding on the same area. M/B is the maize-bean rotation, M/C is the maize-canavalia rotation, M is maize alone.

The proposed rotation sequence would therefore be to alternate this most probable M/C rotation with the M/B rotation: canavalia would grow on the area not cultivated by beans (i.e. about 1 ha), and crops would be exchanged the following year, i.e., on the same area the sequence would be M/B-M/C-M/B-M/C etc (Figure 2.8). In the traditional sequence, the succession of M/B and M (maize alone) rotations depletes N stocks over years, moreover on sites with low mineral fertilizer applications. The alternative sequence will build up N stocks year after year. Moreover, canavalia can reduce erosion and decrease weed pressure. The time until seeing an effect on agricultural productivity depends on the biophysical limitations of each site and the management options chosen by the farmers. Canavalia yield is assumed to be maintained over years. Legume yields can decrease after a few years of cultivation due to pests and diseases, as has been reported in other trials (Bünemann et al. 2004b). However, this has not yet been observed with canavalia in a 6-
year on-station experiment where canavalia was planted on the same plots every year (A. Schmidt et al., unpublished data). Still, the proposed rotation sequence needs long term testing on-farm. The use of models, once calibrated, can also be useful in predicting the effects of rotation sequences on soil fertility (Walker et al., 2008).

Limitations of the soil surface N budget approach

The underlying assumption of a nutrient budget is that of a mass balance i.e. nutrient input to the system minus nutrient outputs from the system equals the change in storage within the system (Meissinger and Randall 1991). However, soil surface budgets consider soil as a black box, and do not provide information on the fate or origin of any budget surplus i.e. whether it is lost from the system or stored in the soil (Watson et al. 2002). Due to unaccounted N losses, like leaching and gaseous losses, N balances are overestimated unless they would be compensated by atmospheric N deposition. Accurate data being unavailable for the study region, atmospheric deposition was not included in the budget and assumed to be equal for all farms. Surface lateral nutrient flows, i.e. inputs and outputs by sedimentation, erosion and runoff were also not quantified. Despite the fact that those processes are left out, soil surface budgets based on “easy-to-measure” flows have proved their utility in providing useful information to farmers and policy makers on soil fertility and on the need for restoration (Adu-Gyamfi et al. 2007; Rego et al. 2003), even in sloping hillsides of the tropics (Briggs and Twomlow 2002). These flows are also the easiest to manipulate to influence the nutrient balances in the short term (Bekunda and Manzi 2003). However, the estimation of lateral nutrient flows and gaseous losses is essential if an extrapolation of N budgets at landscape level and for a longer time frame is envisaged (Smaling et al. 1993). Finally, to predict how much N can be expected from the use of canavalia over years, an in-depth study on soil N fluxes is needed, including a determination of the fertilizer value of manure from cows fed with canavalia, an evaluation of N losses and of the belowground contribution of the legumes, and an assessment of the N mineralization rate for the different soil types of the Nicaraguan hillsides.
Conclusions

When used as green manure, canavalia represents a net N input into the crop rotation due to symbiotic N fixation. Still, mineral fertilizers are necessary to maintain the N balance positive. Using canavalia as forage depletes soil N, and should be compensated by an effective return of animal manure on the plots. The introduction of canavalia in the Nicaraguan hillsides has the potential to improve agricultural production. However, the time needed to visualize an effect on crop productivity depends on the biophysical limitations of each site and the management done by the farmers.
CHAPTER 3

Nitrogen recoveries from organic sources in crop and soil assessed by isotope techniques under tropical field conditions
Abstract

The introduction of multipurpose legumes into low-input tropical systems is promoted because they represent a nitrogen (N) input through symbiotic fixation. The drought-tolerant cover legume canavalia (Canavalia brasiliensis) has been introduced as green manure and forage into the crop-livestock system of the Nicaraguan hillsides. To study its impact on the subsequent crop, an in-depth study on N dynamics in the soil-plant system was set up. Microplots were installed in a six-year old field experiment with maize-canavalia rotation. Direct and indirect $^{15}$N-labelling techniques were used to determine N uptake by maize from canavalia residues and canavalia-fed cows’ manure compared to mineral fertilizer. Litter bags were used to determine the N release from canavalia residues. The amendments incorporation into different soil N pools (total N, mineral N, microbial biomass) was followed during maize growth. Maize took up in average 13.3 g N m$^{-2}$, whereof 1.0 g N m$^{-2}$ from canavalia residues and 2.6 g N m$^{-2}$ from mineral fertilizer, respectively, corresponding to an amendment recovery of 12 and 32%. Most of the amendment N remained in the soil. Mineral N and microbial N were composed mainly of N derived from the soil. Combined total $^{15}$N recovery in maize and soil at harvest was highest for the residue treatment with 98%, followed by the fertilizer treatment with 83%. Despite of similar initial enrichment of soil microbial and mineral N pools, the indirect labelling technique failed in assessing the N fertilizer value of mineral and organic amendments due to a high N mineralization from the soil organic matter. A better accuracy of this technique would probably be achieved by working in soils with less potentially available soil N.

Introduction

In smallholder farming systems of the Nicaraguan hillsides, intensification of land use led to soil nutrient depletion and a decrease in crop and livestock productivity. Nitrogen (N) is the nutrient most limiting crop production in the area (Ayarza et al. 2007; Smyth et al. 2004). To sustain agricultural production, the drought-tolerant cover legume Canavalia
brasiliensis Mart. Ex. Benth (canavalia), also known as Brazilian jack bean, has been introduced as green manure and/or forage into the traditional maize-bean-livestock system (CIAT 2008; Peters et al. 2004). Maize is planted during the first rainy season, and canavalia during the second rainy season. However, when tested as green manure on farmers’ fields, canavalia showed no effect on subsequent maize yields after one year of rotation, probably because one year of rotation is not sufficient to observe an effect, the mineral fertilizer background was too high compared to the N input by canavalia, or because other factors related to management practices limited a productivity increase (Chapter 2). This absence of increase in yield does not mean that residues did not decompose and release N: their benefit to maize remains unknown. Tested as forage, canavalia increased milk yields but bears the risk of soil N depletion, except if an eventual return of animal manure to the plot would compensate N intake by cows (CIAT 2008; Chapter 2). Without knowing the fertilizer value of canavalia for maize, it may be premature to recommend it to resource-poor farmers who have limited profit margin to test new forage technologies. To determine this fertilizer value, when canavalia is used as residue or fed to animals whose manure is returned to the soil, an in-depth study on soil N fluxes was deemed necessary.

The direct $^{15}$N labelling technique (DLT), i.e. the addition of $^{15}$N labelled amendment to an unlabelled soil-plant system, has proven to be the most suitable method to trace the fate of N from amendments into different pools of the soil–plant system (Hauck and Bremner 1976; Hood et al. 2008), and was therefore applied for canavalia residues. Under tropical field conditions, applications of this method are scarce with legume residues (McDonagh et al. 1993; Toomsan et al. 1995; Vanlauwe et al. 1998b), and nonexistent with animal manure. Few field studies reported on the effects of animal manure on crop yields in the tropics (Reddy et al. 2000; Zingore et al. 2008). As it is difficult to label local cow manure, we used the indirect $^{15}$N labelling technique (ILT), where potentially available soil N is labelled instead of amendment N. Potentially available soil N includes the different soil N pools that can deliver mineral N during the growing period of the crop: mineral N, microbial N and non-living labile soil organic matter. With the ILT approach it is assumed that the potentially available soil N from the
amended plot and a non-amended control plots initially have the same $^{15}$N enrichment, so that any dilution observed in the amended plot can be attributed to the unlabelled amendment. If potentially available soil N is not labelled homogeneously, artefacts can arise due to pool substitution (Jenkinson et al. 1985), for example when labelled soil inorganic N is immobilized by a growing microbial biomass after addition of a carbon source and substituted by N of a lower enrichment. This dilution in the mineral N pool is then erroneously attributed to the unlabelled residues or manure. Labelling of the soil for a substantial time before the application of the amendments has been reported to avoid problems linked with pool substitutions (Hood 2001). This hypothesis was verified in this study by following the $^{15}$N enrichment of soil mineral and microbial N pools after amendment addition, which had not been reported by other authors for the ILT method. The accuracy of the ILT was further checked using canavalia residues, mineral fertilizer and sheep manure produced under controlled conditions.

The objectives of this study were (i) to determine for maize the N fertilizer value of canavalia residues and animal manure, (ii) to assess the recovery of the $^{15}$N in different soil N pools, (iii) to test the ILT when using animal manure.

**Materials and methods**

*Field experiment and microplot design*

The experimental work was carried out in a six-year-old field trial located in the municipality of San Dionisio, Department of Matagalpa, Nicaraguan hillside ($12^\circ 46' 47''N, 85^\circ 49' 35''W$), at 560 m.a.s.l., on a 10% slope. The climate was classified as tropical savannah according to the Köppen-Geiger classification (Peel et al. 2007). Annual mean rainfall was 1570 mm (INETER 2009) and has a bimodal pattern. Soil was a loam/clay loam classified as Ultic Tropudalf, with pH in water 6.6, total N 4.03 g kg$^{-1}$, total carbon 54.5 g kg$^{-1}$, total phosphorus 1131 mg kg$^{-1}$, available phosphorus (anion-exchange resins; Tiessen and Moir 1993) 142 mg kg$^{-1}$, cation exchange capacity 39.8 cmol kg$^{-1}$, and bulk density 0.9 g cm$^{-3}$. 
The field trial had a complete randomized block design, with six different crop rotations replicated three times on 5 x 5 m plots to test for two legumes effects on maize yields, including canavalia. At the beginning of the second rainy season in September 2007, 1.2 m²-microplots were installed down to a depth of 15 cm in the three maize-canavalia rotation plots. Some of the microplots were used for ILT and some for DLT, in a cross-labelling design (Hood 2001): two matching sets of treatments were set up, identical in all aspects except that either the available soil N or the amendment N was $^{15}$N labelled (Figure 3.1). The only treatment without mirror was the one with local cow manure. To check for the accuracy of the ILT for manure, two 0.6 m²-microplots were established with labelled and unlabelled manure obtained from a Swiss sheep (Bosshard et al. 2008). The ILT-Control treatment was used as unamended control for the ILT method, whereas the Control treatment was used as natural abundance control for all treatments of both methods (see calculations below). A timetable of the experiment is presented in Figure 3.2.
**Figure 3.2.** Rainfall distribution and work plan during the field experiment. DAA stands for days after amendments.

### Canavalia and soil N labelling

In September 2007, canavalia (cv. CIAT 17009) was sown on the whole surface of all plots at a density of 7.5 plants per m$^2$. Soil of the microplots assigned to ILT was labelled using a solution of 60 atom% $^{15}$N (NH$_4$)$_2$SO$_4$ at a rate of 50 kg N ha$^{-1}$, distributed over five applications during the first two months of canavalia development to minimize leaching by the heavy rains. The same fertilization was done on the microplots assigned to DLT with unlabelled (NH$_4$)$_2$SO$_4$, so that unlabelled canavalia was produced on DLT microplots and labelled canavalia on ILT microplots. With the last N application, sucrose was added as carbon source to give a C:N ratio of 10:1 grams in order to pre-label the soil for ILT, i.e. to allow microbial biomass to immobilize partially the label. Sucrose was added to all ILT and DLT microplots. Canavalia was harvested in February 2008 at late flowering/early pod filling. As canavalia is a climbing plant, stems grew up to 5 meters away from their origin and tightly wrapped themselves around material from other microplots. Stems were gently separated, and the small amounts of material that could not be assigned with certainty to a microplot were discarded. Yields were recorded for each single microplot, and subsamples were taken for analysis. The material from each microplot was then air dried, regularly stirred to produce hay and stored dry until application. To ensure a homogeneous soil N labelling in the ILT plots, soil was left to
equilibrate during the dry season from February to June 2008. During this time, all microplots were weeded manually and weeds were left on the surface of their microplot of origin. A composite soil (0-10 cm) sample was collected in the microplots in June 2008 to check the enrichment.

\[ N \text{ uptake by maize from different amendments} \]

At the beginning of the first rainy season in June 2008 (Figure 3.2), canavalia residues were exchanged between DLT and ILT-Residue microplots within the same replicate. Leaves and stems were applied on the surface and very slightly incorporated to prevent leaves being blown away by the wind. An N dose of 80 kg N ha\(^{-1}\), corresponding to the N yield of the least productive ILT and DLT-Residue microplots, was used as basis for all residue applications (Table 3.1). Solution of unlabelled and 10 atom% \(^{15}\)N (NH\(_4\))\(_2\)SO\(_4\) was applied with watering cans on ILT and DLT-Mineral fertilizer microplots, respectively. The total dose of 80 kg N ha\(^{-1}\) was split into two doses, one third at planting and two thirds after 25 days, according to common farmers’ practice. The two control microplots received no amendments. The fresh animal manure (feces only) for the ILT-Manure microplots was collected from a local cow fed for five days with a mixture of maize stover, grass and 8-month-old canavalia from the field experiment, and was applied at a rate of 133 kg N ha\(^{-1}\). The intended dose of 80 kg N ha\(^{-1}\) was not reached because the cow manure was more concentrated than expected due to changes during storage in San Dionisio. The manure (feces only) for the methodological control was produced by feeding a sheep with \(^{15}\)N-labelled ryegrass hay for nine days under controlled conditions in Switzerland. The unlabelled manure came from the same animal at the end of its feeding adaptation period to unlabelled ryegrass diet (Bosshard et al. 2008). Both manures were applied at an N dose of 40 kg N ha\(^{-1}\) on the small microplots. All amendments were applied with the same amount of water. No other nutrients were applied because the nutrient status of the soil of the trial was high enough to sustain maize growth without limitations, as indicated earlier. Characteristics of the amendments for each treatment are presented in Table 3.1.
Table 3.1. Amendments composition and dose of application, on a dry matter basis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amendment</th>
<th>Total N g kg(^{-1})</th>
<th>C:N ratio</th>
<th>(^{15})N abundance atom %</th>
<th>(^{15})N g kg(^{-1})</th>
<th>P g kg(^{-1})</th>
<th>K g kg(^{-1})</th>
<th>Lignin g kg(^{-1})</th>
<th>Polyphenols g kg(^{-1})</th>
<th>Dosis g N m(^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>ILT - Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ILT - Fertilizer</td>
<td>(NH(_4))(_2)SO(_4)</td>
<td>223.0</td>
<td>-</td>
<td>0.36</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>ILT - Residues</td>
<td>Canavalia</td>
<td>19.7</td>
<td>21</td>
<td>0.38</td>
<td>3.06</td>
<td>14.45</td>
<td>87.3</td>
<td>125.3</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>ILT - Manure</td>
<td>Cow manure</td>
<td>17.1</td>
<td>6</td>
<td>0.37</td>
<td>5.93</td>
<td>17.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13</td>
</tr>
<tr>
<td>DLT - Fertilizer</td>
<td>(^{15})(NH(_4))(_2)SO(_4)</td>
<td>230.0</td>
<td>-</td>
<td>10.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>DLT - Residues</td>
<td>(^{15})N-labelled canavalia</td>
<td>18.8</td>
<td>20</td>
<td>1.61</td>
<td>3.16</td>
<td>15.35</td>
<td>75.9</td>
<td>156.2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ILT - Check manure</td>
<td>Sheep manure</td>
<td>32.0</td>
<td>5</td>
<td>0.41</td>
<td>35.06</td>
<td>13.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>DLT - Check manure</td>
<td>(^{15})N-labelled sheep manure</td>
<td>35.0</td>
<td>11.23</td>
<td>39.87</td>
<td>25.85</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
</tbody>
</table>
The amended microplots were planted with *Zea mays* (cv. NB-6) two days after amendment (DAA) at a density of 6.7 plants per m$^2$. An unusually short drought hindered germination, and maize was replanted at 15 DAA. The second mineral fertilizer dose was therefore delayed until 36 DAA. Insecticide chlorpyrifos was applied around the plots to protect the seeds and young plants against ants and belowground pests such as rootworm larvae. Microplots were weeded manually and weeds were left on the surface of their microplot of origin. At maturity, maize was left to dry on the stems in the field according to usual farmers practices. Stems were cut above the ears and leaves were harvested to allow a quicker drying process. Fifteen days later, when rains had stopped and plants were dry, maize was harvested and separated into grains, damaged grains (i.e. broken, discoloured, shrivelled or undersized grains), cobs, husks, remaining stems. Maize dry matter production was evaluated as the sum of the dry weight of all plant parts, i.e. grains, damaged grains, leaves, stems, cobs and husks.

*Residue decomposition and recovery of the amendments in different soil N pools*

After amendments, remaining labelled canavalia hay from the ILT-Residue treatments was packed in 1.5 mm-mesh nylon bags of 20 x 20 cm. For all litter bags, 5 g leaves and 10 g stems were weighted, which corresponded to the ratio observed in the microplots. At 7 DAA, the five litter bags with material from the plot of the first replicate were deposited in this same plot, and the same was done for the litter bags of the other two replicates. At 14, 26, 40, 54 and 147 DAA (Figure 3.2), one litter bag was removed at random per plot.

At 1, 14, 26, 40, 54, and 147 DAA (Figure 3.2), a composite soil (0-10 cm) sample was collected in each microplot and sieved in the field at 5 mm or homogenised by hand when soil was too clumpy. Samples were analyzed for total N (Ntot), mineral N (Nmin) and microbial N (Nmic) as well as for the $^{15}$N abundance of these pools ($^{15}$N-Ntot, $^{15}$N-Nmin and $^{15}$N-Nmic, respectively).
Bulk density of the topsoil was determined by weighting a soil sample of known volume, using a metal cylinder of 5 cm of diameter and 5 cm height. Three measurements were done per plot, and their mean was used in the calculations.

Sample preparation and analysis

All plant samples were dried at about 40°C until constant dry weight and ground with a rotary knife mill at CIAT-Nicaragua. From each soil sampling point, a subsample was air-dried. All samples were then shipped to Switzerland, powdered with a ball mill (Retsch, GmbH, Germany), and analyzed for total N and $^{15}$N abundance at the Geological Institute of the ETH Zurich on a Thermo Electron FlashEA 1112 coupled in continuous-flow with a Thermo-Fisher Delta V mass spectrometer. Finely ground plant seed with an atom % $^{15}$N of 0.514 was used as an analytic standard.

At each soil sampling point, fresh samples were brought to laboratories of the Universidad Nacional Agraria in Managua, and extracted on the next day following the method of Vance et al. (1987), where two subsamples equivalent to 10 g soil dry matter were extracted with 40 ml $\text{K}_2\text{SO}_4$ (0.5 M), one of them being fumigated with chloroform prior to the extraction. Soil extracts were frozen and shipped to Switzerland. Total N was determined in all extracts on a TOC/TN Analyzer (SKALAR, Netherlands). $\text{N}_{\text{mic}}$ was obtained by subtracting for each sample the N content from non-fumigated from fumigated samples. In the extracts of non-fumigated samples, $\text{NO}_3^-$ and $\text{NH}_4^+$ contents were determined on a flow injection analyzer (SKALAR San++ System, Netherlands), and summed as $\text{N}_{\text{min}}$.

To determine $^{15}$N-$\text{N}_{\text{min}}$, extracts from non-fumigated samples were diffused on acid filters following an adaptation of the method of Goerges and Dittert (1998). Briefly, 0.02 g MgO and 0.4 g Devarda’s alloy were added to 12 ml extracts in 20 ml polyethylene vials. Quartz filters (Whatman, QM-A) of 5 mm diameter were acidified with 10 µl $\text{KHSO}_4$ 2.5 M and enclosed in polytetrafluoroethylene tape (Angst + Pfister, Dodge Fibers Nr.121) below the vial caps. Vials were shaken horizontally for 72 h at 150 rpm, before removing and drying the filters. The determination of $^{15}$N-$\text{N}_{\text{mic}}$ followed the same
principle, after an alkaline persulfate oxidation: extracts were autoclaved with K₂S₂O₈ (Cabrera and Beare 1993), then 0.4 g Devarda’s alloy, 4 ml of a saturated KCl solution and 4 ml NaOH 5 M were added to 10 ml extracts (Mayer et al. 2003) and diffusion on filters followed as described above. All filters were analyzed for ^15N abundance at the Geological Institute of the ETH Zurich as described above.

**Calculations and statistics**

For all DLT- and ILT-treatments and all compartments, the ^15N enrichments were obtained by subtracting from the ^15N abundances the mean ^15N abundance of the respective compartment from the Control microplot, which is at natural abundance (Figure 1). For the DLT, the amount of N derived from the amendments (Ndff) in a compartment was calculated as follows (Hauck and Bremner 1976):

\[
\%\text{Ndff} = \frac{\text{atom}\% \ ^15\text{N excess compartment}}{\text{atom}\% \ ^15\text{N excess amendment}} \times 100
\]  

[3.1]

where atom\% ^15N excess compartment is the ^15N enrichment of the compartment considered, i.e. either a maize plant part or a soil N pool, and atom\% ^15N excess amendment is the enrichment of the amendment applied (residues, mineral fertilizer or manure).

For each microplot, a weighted ^15N excess was used for maize, calculated from all plant parts according to Danso et al. (1993):

\[
\text{weighted } ^{15}\text{N enrichment} = \frac{\sum_{i=1}^{n} \text{atom}\% \ ^{15}\text{N excess } \times \text{total } N_i}{\sum_{i=1}^{n} \text{total } N_i}
\]  

[3.2]

where i is a particular plant part and n the total number of plant parts.

For the ILT, the Ndff was calculated as follow (Hood 2001):
where $\text{atom}\% \ N_{\text{excess control compartment}}$ is the $^{15}\text{N}$ enrichment of the compartment considered, in the ILT-Control microplot of the same replicate.

The absolute amount of N derived from the amendments in the different compartments was calculated as follows:

$$Ndff \ [g \ m^{-2}] \ or \ [mg \ kg \ soil^{-1}] = \left(\frac{\text{TN}}{100}\right) \ [g \ m^{-2}] \ or \ [mg \ kg \ soil^{-1}]$$

where TN is the total N amount in the compartment considered, in g m$^{-2}$ (for plants) or mg kg soil$^{-1}$ (for soil). TN was calculated as the product of the concentration of N in the compartment and its weight in g m$^{-2}$ (for plants) or mg kg soil$^{-1}$ (for soil). For soil, the weight of the 0-10 cm layer was calculated by multiplying its volume for a 1 m$^2$ surface by the bulk density. The amount of N derived from the soil (Ndfs) for a compartment was the difference between TN and absolute Ndff.

The amount of N recovered from the amendment was calculated as follows:

$$\text{% Recovery} = \left(\frac{Ndff}{N \text{ applied}}\right) \times 100$$

where N applied is the amount of N applied with the amendments.

The total $^{15}\text{N}$ recovery in DLT treatments was calculated as the sum of the $^{15}\text{N}$ recoveries in maize and in total soil N.

$^{15}\text{N}-\text{Nmic}$ was calculated as a mass balance according to Mayer et al. (2003):

$$^{15}\text{N}-\text{Nmic} = \frac{\text{total } N_{\text{fum}} \times \text{atom}\% \ ^{15}\text{N excess}_{\text{fum}} - \text{total } N_{\text{nonfum}} \times \text{atom}\% \ ^{15}\text{N excess}_{\text{nonfum}}}{\text{total } N_{\text{fum}} - \text{total } N_{\text{nonfum}}}$$
where fum stands for fumigated sample and nonfum for non fumigated sample.

Statistical analyses were performed using the program R (R Development Core Team, 2007). The effects of replicates and amendments were tested with a two-way analysis of variance using aov (Chambers et al. 1992). Wilcoxon’s rank-sum test was used to check for significant differences between ILT and DLT methods. The significance level chosen was $\alpha = 0.05$.

**Results**

*Canavalia and soil N labelling*

The above ground dry matter production of canavalia in the microplots was on average 820 g m$^{-2}$, with a standard deviation of 366 g m$^{-2}$. The $^{15}$N abundance of canavalia from unlabelled microplots ranged from 0.38 to 0.50 atom%, and the $^{15}$N abundance of canavalia from labelled microplots ranged from 1.23 to 2.28 atom%. Variation in canavalia $^{15}$N abundance within replicate was higher for ILT- than DLT-microplots, with a mean coefficient of variation of 15% and 5%, respectively. The recovery from labelled fertilizer in canavalia was on average 6%, with a standard deviation of 2%.

Before amendment applications in June 2008, total soil N from the ILT plots had an average abundance of 0.643 atom% $^{15}$N up to 10 cm depth, with a standard deviation of 0.076 atom% $^{15}$N. Within plot variation was on average 11% (n=5). In the 0-10 cm soil layer, the recovery from labelled fertilizer was on average 44%, with a standard deviation of 12%. Total recovery (in canavalia and in soil) from labelled fertilizer was therefore on average 50%.

*Residue decomposition*
Canavalia leaves decomposed faster than the stems (Figure 3.3). Thirty-three days after litter bags installation (i.e. 40 DAA), leaves were below the detectable weight limit. The $^{15}$N enrichment of stems and leaves decreased slightly with time, with stems being more enriched than leaves. The highest N release was observed between DAA 7 and DAA 26 with in average 202 mg N per litter bag, i.e. per 15 g residues. Knowing the amount of residues applied in the microplots per m$^2$, the 202 mg N released per litter bags corresponded to a release of 5.7 g N m$^{-2}$, whereas 72% was from the leaves.

\[ \text{Figure 3.3. Decomposition (a), } ^{15}\text{N abundance (b) and N release (c) per litter bag from canavalia stems and leaves, with days after amendments (DAA). Error bars represent the standard deviation (n=3).} \]
Figure 3.4. Changes in soil mineral N (a) and microbial N (b) pools with days after amendments (DAA) for all treatments. Averages of ILT and DLT. Error bars represent the standard deviation (n=6, except for the manure treatment where n=3).
Amendment incorporation in soil N pools

The evolution of Nmin and Nmic with time is presented on Figure 3.4, for the ILT and DLT treatments grouped as amounts of Nmin and Nmic were not significantly different between both labelling methods ($p=0.781$ and $p=0.058$, respectively). Nmin slightly decreased for all treatments after amendment addition, and then stayed stable during maize growth. The two mineral fertilizer applications were clearly reflected in the mineral pool at DAA 1 and 40 and were still observable at DAA 14. A net microbial immobilization of up to 52 mg N kg$^{-1}$ soil occurred between DAA 1 and 14 for all treatments, followed by a net N release of up to 60 mg N kg$^{-1}$ soil. In most cases the highest immobilization was observed for the residues treatment and the lowest for the mineral fertilizer treatment. Treatments had a significant effect on Nmic ($p=0.011$).

For the DLT treatments, Ndff and Ndfs were calculated for soil N pools. Ndff in Nmin (Figure 3.5) shows that the differences between treatments observed in Figure 3.4 came basically from the amendments. Except for the DLT-Mineral fertilizer treatment, most of Nmin derived from the soil. The Ndff in Nmic for the two most contrasting points regarding the size of Nmic (Figure 3.4) is presented on Figure 3.6. Most of Nmic derived from the soil. The highest Ndff in Nmic was observed with the DLT-Residues treatment just after the beginning of the rains (DAA 14) and represented 6% of Nmic. The DLT-Residue treatment has also the higher Ndff in Nmic at harvest.

For the ILT treatments, Ndff and Ndfs in soil N pools are not presented because negative estimates were often obtained. Reasons for that are discussed below. The evolution of $^{15}$N-Nmin and $^{15}$N-Nmic with time is presented on Figure 3.7. Except for the mineral fertilizer treatment, $^{15}$N-Nmin decreased with time for all treatments. The ILT-Control treatment has at most time points a higher enrichment than the other treatments. The two applications of unlabelled mineral fertilizer at DAA 1 and 40 were clearly diluting the enrichment, and were then followed by an increase of the enrichment up to a level close to the ILT-Control treatment. After the dilution by the mineral fertilizer, the strongest dilution was observed for the ILT-Residue treatment at DAA 14, and for the ILT-Manure treatment at DAA 26. For all treatments, $^{15}$N-Nmic was slightly lower than the enrichment of Nmin at DAA 14 and 147, respectively.
Figure 3.5. N derived from the amendments (Ndff) and from the soil (Ndfs) in soil mineral N for the DLT treatments at each time point. Error bars represent the standard deviation (n=3). DAA stands for days after amendments.

Figure 3.6. N derived from the amendments (Ndff) and from the soil (Ndfs) in soil microbial N for the DLT treatments for two time points. Error bars represent the standard deviation (n=3). DAA stands for days after amendments.
Figure 3.7. Changes in $^{15}$N enrichment of soil mineral N ($^{15}$N-Nmin, a) and microbial N ($^{15}$N-Nmic, b) with days after amendments (DAA) in the ILT treatments. Error bars represent the standard deviation (n=3).

Recovery in maize

Maize dry matter production was on average 1344 g m$^{-2}$, with a standard deviation of 256 g m$^{-2}$ (Table 3.2), and was not significantly different between ILT and DLT ($p=0.410$). The N uptake was on average 13.3 g N m$^{-2}$, with a standard deviation of 2.4 g N m$^{-2}$. The amendments had no significant effect on maize dry matter production ($p=0.085$) and on N uptake ($p=0.125$). Maize from the DLT-Fertilizer treatment had the highest $^{15}$N excess (Table 3.2). With the DLT, maize took up 2.6 g N m$^{-1}$ from mineral fertilizer and 1.0 g N m$^{-2}$ from canavalia residues, corresponding to an amendment recovery of 32 and 12%, respectively (Figure 3.8). Treatments had a highly significant effect on amendments recoveries determined with the DLT ($p=0.005$), and no effect on the amendments recoveries determined with the ILT ($p=0.976$). Variation within treatment with the ILT reached 204%
Table 3.2. Maize dry matter production, N uptake and enrichment for each treatment at harvest. Standard deviation is given in parenthesis (n=3).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry matter</th>
<th>N uptake</th>
<th>$^{15}$N enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total $^1$</td>
<td>Grains</td>
<td>Total $^1$</td>
</tr>
<tr>
<td></td>
<td>g m$^{-2}$</td>
<td>g m$^{-2}$</td>
<td>g m$^{-2}$</td>
</tr>
<tr>
<td>ILT - Control</td>
<td>1085 (223)</td>
<td>396 (203)</td>
<td>11.1 (2.9)</td>
</tr>
<tr>
<td>ILT - Fertilizer</td>
<td>1431 (215)</td>
<td>489 (143)</td>
<td>13.7 (2.8)</td>
</tr>
<tr>
<td>ILT - Residues</td>
<td>1461 (125)</td>
<td>583 (121)</td>
<td>15.4 (1.9)</td>
</tr>
<tr>
<td>ILT - Manure</td>
<td>1317 (115)</td>
<td>507 (137)</td>
<td>12.5 (1.2)</td>
</tr>
<tr>
<td>DLT - Fertilizer</td>
<td>1625 (147)</td>
<td>493 (107)</td>
<td>14.9 (2.4)</td>
</tr>
<tr>
<td>DLT - Residues</td>
<td>1424 (153)</td>
<td>543 (213)</td>
<td>14.5 (2.2)</td>
</tr>
<tr>
<td>Control</td>
<td>1477 (290)</td>
<td>649 (145)</td>
<td>16.7 (4.2)</td>
</tr>
<tr>
<td>ILT - Check manure</td>
<td>1244 (155)</td>
<td>477 (66)</td>
<td>11.2 (1.2)</td>
</tr>
<tr>
<td>DLT - Check manure</td>
<td>1028 (352)</td>
<td>429 (191)</td>
<td>9.5 (3.6)</td>
</tr>
</tbody>
</table>

$^1$ total for all plant parts, i.e. grains, damaged grains, leaves, stems, cobs and husks

$^2$ weighted enrichment for all plant parts

Figure 3.8 Nitrogen derived from the amendments (Ndff) and their recovery in maize, for indirect (ILT) and direct (DLT) labelling techniques. Error bars represent the standard deviation (n=3).
Amendment total recovery

Most of the amendment N was recovered in the 0-10 cm soil layer (Table 3.3). The total $^{15}$N recovery was highest for the DLT-Residue treatment with 98%, followed by the DLT-Fertilizer treatment and by the DLT-Check manure treatment. The highest recovery for the DLT-Residue treatment was due to a higher recovery in the soil. The lowest total recovery for manure was due to its low recovery in maize.

### Table 3.3. $^{15}$N recovery (%) in maize and in different soil N pools (0-10 cm) at maize harvest, for the direct labelling technique (DLT). Total recovery is the sum of recoveries in maize and total soil N. Standard deviation is given in parenthesis (n=3).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Maize</th>
<th>Soil</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ntot</td>
<td>Nmin</td>
<td>Nmic</td>
</tr>
<tr>
<td>DLT - Fertilizer</td>
<td>31.8 (1.7)</td>
<td>50.1 (11.3)</td>
<td>1.1 (0.7)</td>
</tr>
<tr>
<td>DLT - Residues</td>
<td>12.0 (4.6)</td>
<td>85.8 (7.5)</td>
<td>0.9 (0.3)</td>
</tr>
<tr>
<td>DLT - Check manure</td>
<td>2.9 (1.3)</td>
<td>73.3 (24.0)</td>
<td>1.1 (0.5)</td>
</tr>
</tbody>
</table>

Discussion

Canavalia and soil N labelling

Despite a cautious harvest, the fact that unlabelled and labelled canavalia grew climbing on each other induced a faint contamination of unlabelled canavalia biomass. Microplots were probably also to some extent influenced by the growth of lateral roots in the subsoil, which was difficult to avoid. However, this contamination did not affect the $^{15}$N abundance of soil N: as maize from the Control microplots was unlabelled (Table 3.2), we are confident that the basis for the application of DLT was fulfilled. Variation in $^{15}$N enrichment of canavalia grown on ILT plots could be due to differential mineral fertilizer leaching between microplots and differential N uptake by canavalia, which in turn could be attributed to uneven distribution of stones in the soil profile of the field. Because canavalia above ground $^{15}$N enrichment varied between microplots, $^{15}$N labelled
belowground biomass could contribute unequally to the subsequent maize. Belowground N associated with or derived from roots can represent up to 50% of the total plant N of legumes (Herridge et al. 2008) and can contribute substantially to the subsequent crop. In the calculations for both ILT and DLT, belowground N contribution from canavalia roots stand proxy for part of the soil N pool, as labelled canavalia roots remained in labelled soil and unlabelled roots in unlabelled soil. Soil $^{15}N$ enrichment before application of the amendments showed low variation between the ILT treatments, suggesting that the impact of $^{15}N$ decomposition of unevenly labelled belowground canavalia residues was minor. The low recovery of mineral fertilizer in canavalia above ground biomass of the ILT plots was due to high amounts of available soil N, to immobilisation by the microbial biomass induced by sucrose addition, and to a dilution of the label through symbiotic N$_2$ fixation. The recovery in the soil and the resulting enrichments of soil N were high enough to allow the application of the ILT. Half of the fertilizer N applied was lost, probably leached to deeper soil layers due to the heavy rains.

**Decomposition of canavalia residues**

Litter bag studies are often considered to underestimate residue decomposition through reduced litter/soil contact (Vanlauwe et al. 1997). In our trial, an overestimation of the decomposition rate is more likely, as eroded soil along the slope covered partially the litter bags with soil. The residues in litter bags were therefore slightly more mixed with soil than the residues in the microplots which were protected from soil inflow through the microplot frames. Ideally litter bags should have been applied the same day as the amendments, but due to time constraints it had to be done one week later. However, as no rain fell during this week, we assume that decomposition of the residues in the microplots hardly began before litter bags installation and that this time-lag can therefore be of a limited concern. Decomposition of canavalia litter was rapid, which is in agreement with previous studies (Carvalho et al. 2009; Carvalho et al. 2008; Cobo et al. 2002).
Nitrogen released from litter bags can be mineralized and then taken up by plants, immobilized by microorganisms or incorporated into the particulate soil organic matter fraction. In this study, most of residues N remained in the soil (Table 3.3). Indeed, the time of highest N release (between DAA 7 and 26) corresponded to the highest microbial N immobilization (Figure 3.4). At this time, maize was still at an early growth stage (with 2 or 3 leaves). From the 8 g N m\(^{-2}\) applied (Table 3.1), only 1.0 g N m\(^{-2}\) in average was recovered in maize (Figure 3.8). However, as stems were higher enriched and decomposed more slowly than leaves, the residue recovery in maize may be underestimated, as what it recovered was from less enriched leaves. If the Ndff for the DLT-Residue treatment would be calculated with the \(^{15}\)N excess of the leaves only, it would become 1.5 g N m\(^{-2}\), which corresponds to a recovery of 19%: the underestimation would be therefore around 50%.

**Soil N dynamics after amendment**

The Nmin initially decreased with the first rains, and showed later a direct relationship with N uptake by maize. During maize growth, it stayed stable on a level of 8 mg N kg\(^{-1}\) soil, and at DAA 147, when maize was not taking up N anymore as it was drying in the field for about fifteen days, it increased. Compared to Nmin, the size of the microbial pool was always at least three times larger, showing the importance of this pool in mediating soil N processes. According to the DLT, about the same amount of Nmin was derived from the soil for all treatments at each time point, the differences between treatments being rather attributable to Ndff. The Ndff in Nmic was low, and shows that this pool was mainly alimented by soil organic matter.

The steady \(^{15}\)N-Nmin decrease over time for the ILT-Control treatment (Figure 3.7) could not be due to dilution by microbial turnover as \(^{15}\)N-Nmic was close to \(^{15}\)N-Nmin at DAA 14, and was therefore attributed to mineralization of unlabeled native organic N. The five years of canavalia cultivation and application as green manure that occurred in the trial prior to our labelling have build up a big unlabelled soil organic matter pool. We can
assume that most of it entered the potentially available soil N pool, as reported before (Vanlauwe et al. 1998a).

The $^{15}$N-Nmin was in tendency lower in the amended treatments than in the control and decreased over time. The difference between treatments and control at each time point can be explained by the dilution from the unlabeled amendments. The steady decrease in $^{15}$N-Nmin over time was for all amended treatments, except for the mineral fertilizer treatment, comparable to that of the ILT-Control and can be assigned to mineralization of unlabeled native organic N. After unlabelled mineral fertilizer application, the $^{15}$N-Nmin first decreased and then increased strongly. This mineralization flush after addition of mineral fertilizers has been reported in other studies (Kuzyakov et al. 2000). As the material mineralized was of higher enrichment (labelled microbial biomass and canavalia roots) $^{15}$N-Nmin increased up to the level of the control. This flush would not have been detected by observing the evolution of Nmin only, as a net decrease in Nmin was observed at the same time (Figure 3.4).

An increase in $^{15}$N-Nmin was also observed for the residue treatment between DAA 26 and 40 and for the manure treatment between DAA 54 and 147, corresponding to microbial N release (Figure 3.4).

A decrease in $^{15}$N-Nmic was observed with time for all treatments, suggesting microbial turnover involving feeding from unlabelled N sources, in this case soil N (Figure 3.6).

**Indirect vs. direct labelling technique**

Compared to the DLT, the average Ndff ILT estimate from residues and sheep manure was overestimated, suggesting a too strong dilution of the label in the microplot treatment compared to the control. The reason for this could not be pool substitution from microorganisms as the enrichment of Nmic was only slightly lower than the enrichment of Nmin at the beginning of organic source decomposition (DAA 14).

In this study, the main problem with ILT was variation. High variation with the use of ILT has also been reported by other authors (McDonagh et al. 1993; Muñoz et al. 2003; Stevenson et al. 1998). Control and microplot treatments had about the same total soil $^{15}$N
enrichment before the application of the amendments. Then, a steady decrease in available soil N enrichment, i.e. $^{15}$N-Nmin, was observed with time (Figure 3.7). Assuming that the same basic dilution as in the control occurred in treatment plots, the dilution attributable to the amendments was very small relative to the dilution from mineralization of unlabelled organic matter. This was observed by the small difference in $^{15}$N-Nmin between control and treatment at DAA 147, relative to the differences between DAA 1 and DAA 147 for a same treatment (Figure 3.7). These observations were reflected in the differences between maize $^{15}$N enrichment from the control and the treatments in each plot. The smaller the difference between ILT-Control and treatment, the more inaccurate and variable were the Ndff estimates. Negative differences resulted in negative Ndff values.

These problems were inexistent with the DLT method, where $^{15}$N-Nmin and $^{15}$N-Nmic in the Control plot – here used as simple check – were naturally stable with time and where changes in enrichment were directly attributable to the amendments. Therefore, results from the DLT were considered as the more realistic data to define the availability of canavalia residues and manure for maize. Still, the recovery with the mineral fertilizer treatment may be underestimated due to an isotope displacement reaction, which is described by Jenkinson (1985) as the displacement of unlabelled NH$_4^+$ from clay minerals by the added labelled ammonium sulphate. Seen the rapid mineralization from canavalia residues, the recovery with the residue treatment may also be underestimated. As the trial has a clayey and N rich soil, enough native NH$_4^+$ may have been displaced to produce a measurable effect (Broadbent and Nakashima 1971; Jenkinson et al. 1985).

**Availability of canavalia residues and manure for subsequent maize**

The N recovery in maize was highest for mineral fertilizer, followed by canavalia residues and finally sheep manure. With 12%, the recovery of canavalia residues in subsequent maize was at the lower end of the range of what has been previously observed for tropical legumes in similar studies. Vanlauwe et al. (1998b) reported a recovery of 9% from Leucaena to maize, McDonagh et al. (1993) a recovery of 12 to 26% from
groundnut to maize, and Toomsan et al. (1995) a recovery of 15 to 23% from soybean to rice and 8 to 22% from groundnut to rice. The 3% recovery from sheep manure was lower than the 10% recovery in winter wheat reported for the same manure by Bosshard et al. (2009).

Maize roots and exudates were not recovered. The recovery of amendment N in maize was therefore underestimated for all treatments. We assumed that this underestimation is the same for all treatments and can therefore be omitted in the comparison of the treatments.

Most of the amended N remained in the soil. This observation is consistent with findings from a recent study that included results from thirteen tropical agroecosystems where the authors reported an average N recovery from residues of 7% in crops and 71% in soil (Dourado-Neto et al. 2010). The high total recovery for mineral fertilizer (83%), with 50% in the soil despite the heavy rains, suggest that a high amount of NH$_4^+$ has been retained on clay minerals. Since water was applied manually with watering cans, there was no significant loss of N from mineral fertilizer in gaseous form.

As N recovery in soil was higher with canavalia than with mineral fertilizer, higher residual effects can be expected from canavalia for further cropping. A part of residues N is probably retained in specific soil organic matter fractions, like particulate organic matter, and will slowly become available for crops with time (Vanlauwe et al. 1998a).

**Conclusions**

Canavalia residues represent a valuable source of N for the subsequent maize crop. Results from this study showed that despite similar enrichment of both the microbial N pool and the mineral N pool at the start of maize growth, the ILT failed in assessing the N fertilizer value of mineral and organic amendments. The reason for this was the presence of an important unlabelled mineralizable soil N pool. Pool substitution from microorganism is not the only limitation for ILT. While the labelling of the soil for a subsequent time before application of unlabelled amendment might be adequate to label potentially available soil N in poor soils, it is not sufficient in soils with high amounts of
labile soil organic matter. A better accuracy of the ILT method would possibly be achieved by working in soils with less potentially available soil N.
GENERAL DISCUSSION AND PERSPECTIVES


**Highlights**

The use of legume-based systems is nowadays the recommended way to sustainable cereal production and food security (Mulvaney et al., 2009). Particularly in low-input systems, biodiversity, crop rotation and maintenance of high levels of organic matter are key elements for sustainable food production (Spiertz, 2009). The question is therefore, which legume is most suitable for a given environment and how it is best managed.

The aim of this thesis was to contribute to knowledge about the integration of a multipurpose cover crop legume, Canavalia brasiliensis (canavalia), into the traditional crop-livestock system of the Nicaraguan hillsides. Chapter 1 declared that with an above ground biomass production up to 5357 kg ha\(^{-1}\), canavalia has the potential to improve soil fertility and feed availability. However, Chapter 1 also underlined that canavalia cannot fully express its potential as drought tolerant cover legume on soils with low organic matter content, as well as on shallow and stony soils that hinder deep rooting ability of the legume. Chapter 2 showed that canavalia makes a substantial N input to the system through symbiotic N\(_2\) fixation, with on average 20 kg N fixed ha\(^{-1}\) in the aboveground biomass. Canavalia increases the N balance of the maize-canavalia rotation when used as green manure, but bears the risk of soil N depletion if used as forage, unless N is recycled to the plot by animal manure. Further, Chapter 2 highlighted the importance of mineral N fertilizer to sustain agricultural production even in the presence of canavalia. Chapter 3 revealed that 12% of N from canavalia residues are recovered in the following maize crop, and that most of it remains in the soil. The fertilizer value of canavalia-fed cows’ manure could not be assessed as the indirect \(^{15}\)N labelling technique failed due to a high N mineralization from the soil organic matter.

Specifically, new findings of this thesis, contributing to knowledge in the field of soil fertility management in the tropics and N dynamics, can be summarized as follows:

- the soil and topographic properties limiting canavalia biomass production were determined,
- the symbiotic N\(_2\) fixation by canavalia was assessed on-farm,
General discussion and perspectives

- the isotopic fractionation during N\textsubscript{2} fixation by canavalia was determined in controlled conditions,
- insight into the trade-offs in using canavalia as green manure or as forage was provided from a N balance point of view at plot level,
- the N fertilizer value of canavalia residues was assessed for maize,
- the N recoveries into different soil N pools from legume residues, manure or fertilizer were compared in tropical field conditions,
- and finally, limitation in the use of the indirect labelling technique was put in evidence by following the \^{15}N enrichment of the mineral and microbial soil N pools over time.

Use of canavalia on-farm

From the workshops organized during the course of these studies, it is clear that farmers are motivated using canavalia as forage. “Siempre estoy pensando en los animales, yo!” (I always care about the animals) stated one of our farmers in January 2008. Due to the lack of forage during the dry season, farmers want to take advantage of this additional forage supply to feed animals and achieve higher milk production. When canavalia above ground biomass is not used as green manure, we saw in chapter 2 that the risk of N depletion is high. The return of animal manure to the soil would be the best way to mitigate soil N depletion, but this practice is yet to be developed and promoted. When canavalia is grazed, it can regrow during the dry season. The biomass production of this regrowth is, however, lower than the one of the main season. As for the main season, this biomass can be used as forage or as green manure. An alternative management option to the return of animal manure to the soil to mitigate soil N depletion would then be to leave canavalia regrowth for the soil. One may argue that belowground N from canavalia represents an input sufficient into the system (see Chapter 2) and that additional return of N to the plot, either with animal manure or with canavalia regrowth, is superfluous. However, the different options for use of canavalia above ground biomass are not equivalent in terms of N cycling efficiency (NCE) - defined as the ratio of effective or
useful output to input in a system component provided that the output can be reused within the system (Rufino et al., 2006); and this is reflected in the N availability for the subsequent maize crop. A simple attempt to compare NCE of the different management options is presented on Figure c.1. Nitrogen follows different pathways from canavalia to the next maize, going through different compartments. The NCE of each pathway can be modelled by calculating the product of the NCE of each compartment. From Figure c.1 it is clear that the use of canavalia as green manure provides a more substantial N input to the subsequent maize than the use of animal manure. The use of canavalia regrowth as green manure represents a more interesting option than the use of animal manure. What is not apparent from this approach is how much soil N stocks are built up for each option. Chapter 3 showed that the recovery in soil is higher for canavalia residues than for animal manure, which speaks in favour of the regrowth-for-soil option. Likewise, additional “losses” from the direct N pathways with the forage option does not mean that they are lost for farmers: milk and meat are produced.

A global on-farm N flow scheme for the smallholder system that was studied in this thesis is presented in Figure c.2. It highlights the changes in N flows generated by the introduction of canavalia in the system for the proposed management option: canavalia grazed, animal manure back to the plot, regrowth used as green manure. In farms located on slopes, the adoption of canavalia should be complemented by conservation works as live barriers to avoid the N gained being eroded downhill. The system would benefit from small changes in management like increase in crop planting density, timing of mineral fertilizer application and weed management (see Chapter 2). Indeed, maize productivity may be limited by agricultural management and therefore not benefit from the full N supplied by canavalia. Increased use of improved pastures like Brachiaria sp. grasses would diversify dry season feeding and allow livestock to be less dependent on canavalia amended crop residues.
Figure c.1. N pathways in maize-canavalia rotation for different uses of canavalia biomass. Dashed arrows symbolize the N pathways through various compartments according to the various management options for canavalia. Size of canavalia compartments is indicated in kg N ha\(^{-1}\), with black numbers from the on-farm study and grey numbers estimated from Herridge et al. (2008). Nutrient cycling efficiency (NCE, %) is indicated in bold above each compartments. For the soil compartment, NCE varies according to the material considered and is therefore indicated above each pathway. Overall NCE (%) is the product of the NCE of each compartment. Ndff (kg N ha\(^{-1}\)) is the amount of N in maize grain derived from the legumes. NCE not measured in this study were estimated as follows: NCE cow, Rufino et al (2006); NCE cow manure, Brouwer and Powell (1995); NCE soil below ground, downscaled from Sierra and Desfontaine (2009).
Finally, “there may be considerable opportunities for improving the efficiency with which nutrient flows are managed on-farm, by investigating losses and inefficiencies within the system, as well as economic, institutional and technical constraints. However, ultimately, it is the farmers who are the land users and decision-makers” (Scoones and Toulmin, 1998).

**Soil processes on-station vs. on-farm**

The direct $^{15}\text{N}$ labelling technique allowed to quantify the amount of N derived from canavalia residues into various soil and maize compartments. Not all processes were
considered. For example, given the size of the organic matter compartment in the trial of San Dionisio and its influence on the amount of the mineralizable soil N pool (see Chapter 3), it would have been interesting to study this compartment in more details. Figure c.3 represents the soil N processes and compartments studied in this thesis in comparison to those that were left aside, but for which an estimation of the size is provided. Belowground N from residues and soil organic matter would be two key compartments to include if one wants to determine the residual effect of a legume green manure over time.

The trial of San Dionisio does not reflect the on-farm situation. This can be seen in Figure c.3, where the size of soil and maize compartments of Santa Teresa (on-farm) is compared to that of San Dionisio (on-station). Compared to the on-station trial, total soil N is three times less in Santa Teresa and mineral N two times less. Total N in canavalia above ground biomass was in average three times less in Santa Teresa. Maize grain N yields are 2.5 times lower. The amount of N from damaged grains and from ears not harvested is proportionally higher in Santa Teresa.

It has to be pointed out that the results of San Dionisio cannot be seen as the future on-farm situation after six years of cultivation of canavalia if used as green manure. To achieve the high soil N stocks and the amount and quality of the agricultural production of San Dionisio, optimal management needs to be undertaken as discussed in Chapter 2 and in the section above, and adequate sites need to be chosen for canavalia growth (Chapter 1).

Moreover, in San Dionisio the residues amended to the soil were under the form of hay from 4.5 months-old canavalia. In Santa Teresa, the use of canavalia as green manure implies either the incorporation of 8.5 months-old fresh canavalia, or 4 months-old fresh canavalia if the regrowth is used. This difference in quality affects decomposition rates. Older material will likely decompose more slowly.

Since a study similar to the microplot study is not feasible on-farm, the real potential of canavalia in improving maize productivity on the long term needs extended on-farm validation trials.
Figure c.3. N processes in a maize-canavalia rotation and size of the compartments at maize harvest. Grey areas and arrows represent compartments and processes not measured in this study. Bold numbers are the amount of N in the respective compartment in kg ha\(^{-1}\) from the microplot study (San Dionisio), followed by the proportion of N derived from canavalia above ground residues in parenthesis. Italic numbers are the amount of N in the respective compartment, in kg ha\(^{-1}\), from the on-farm study (means of four farms; Santa Teresa). Grey numbers are estimated by assuming that total N = mineral N + microbial N + organic matter N + clay N.
The effects of amending a soil with canavalia-fed cows’ manure on soil N processes and on the subsequent maize crop remain unknown. Animal urine deposition has not been discussed in this work, and is known to increase soil mineral N and crop yields (Somda et al., 1997). If composition, rate, timing and placement are optimized, the N fertilizer value of animal manure can be enhanced and has real potential to reduce dependence on mineral fertilizers (Schröder, 2005). These aspects still need to be studied in detail in smallholder crop-livestock systems of the hillsides, to be able to provide integrated and feasible recommendations of use to farmers.

Adoption potential of canavalia by smallholder farmers

The conditions for legumes adoption by smallholder farmers have been widely debated (Shelton et al., 2005; Sumberg, 2002). Among the factors responsible for poor adoption, the lack of perceived economic benefit (Ali, 1999), lack of extension information, limited availability of seeds, shortage of labour, inappropriate land tenure and land scarcity (Elbasha et al., 1999) were mentioned. Particularly in Nicaragua, failure in taking into account local reality and perspectives has been reported as main factor for non-adoption of conservation practices (Shriar, 2007). The use of participatory approaches and the evaluation of the whole system into which legumes shall be integrated are recommended to address both the obstacles preventing farmer adoption and the complexity of legume-crop-livestock cropping systems (Cherr et al., 2006; Mugwe et al., 2009).

In this project, farmers were therefore involved since the beginning, from canavalia selection to seed production. On-farm trials and workshops allowed checking for the adequacy of the proposed technology to the cropping system locally used. Most farmers who tried canavalia want to continue planting it on their plots. First steps towards dissemination are encouraging, and local institutions follow up with seed production and validation trials. Still, there is room for improvements in the communication between legume specialists and farmers, so that the knowledge of the farmers on his own production system also increase, which would help guaranteeing sustainable adoption of canavalia (Mosimann, 2009).
**General discussion and perspectives**

**Perspectives**

The integration of canavalia in the Nicaraguan hillsides is on track, but there are still knowledge gaps to be filled in order to be able to make the most of canavalia qualities. Particularly, future studies should address the below presented points.

Regarding the best place for the integration of canavalia:
- better understand the mechanisms behind the drought tolerance of canavalia;
- establish a limit of profitability for canavalia, that delimits the level of productivity below which it will not make sense to invest in its cultivation;
- carry on a niche-based assessment of possible legume species in the region.

Regarding the best way to use canavalia:
- propose more alternatives for an efficient and sustainable management of organic resources and minimize losses using N flow and compartmental analysis;
- test the proposed rotational sequence (Figure i.2) at farm level on the long term;
- study the economic trade-offs in using canavalia as green manure or as forage.

Regarding the soil N processes following its integration in the system:
- study N fertilizer value of canavalia and animal manure in soils similar to on-farm soils;
- assess the N fertilizer value of canavalia-fed cows’ manure for the subsequent maize crop using either direct labelling technique or indirect labelling technique on soils with low amounts of potentially available soil N;
- improve knowledge on long term N fertilizer value of residues and animal manure with long term studies using $^{15}$N tracers and synchronization between N offer and N demand.
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