Nitrogen Use in a Maize-Bean rotation in Nicaragua

Effects of organic and mineral fertilisers

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Cover: An overview of the maize field experiment of this study
(photo: Maize)

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Abstract

Nitrogen (N) is recognised as a major constraint to crop production worldwide. This thesis evaluated the effects of organic and mineral fertilisers on N use in the field through studying the main N fluxes in soil and plants on a 30-day basis over four consecutive growing seasons in a maize-bean rotation in southern Nicaragua. The soil net N mineralisation response to the application of organic (chicken and cow manure applied at recommended dose and double dose) or mineral N sources was evaluated in an *in situ* experiment. To determine the N available for plant use, total plant N uptake was estimated by calculating the mass balance of soil mineral N pool. The measured aboveground N was related to the total plant N uptake within and between the growing seasons. Net mineralisation was found to increase significantly at the higher N rate of chicken manure compared with the other treatments. The soil mineral N increased significantly in the middle of the season in both maize and beans. Total N uptake was mainly determined by mineralisation rate and less by changes in the soil mineral N pool and very little by N leaching. For beans, the estimated partitioning of mineral N to aboveground parts was practically linearly related to the estimated total mineral N uptake. For maize, the fraction of N allocated to above ground decreased during the middle of the growing season. Grain yield increased at the higher N rates of chicken manure and mineral fertiliser. The grain yield response to added fertiliser N was similar to the corresponding response of net N mineralisation. This was explained by the aboveground N being almost proportional to the N mineralisation, the aboveground biomass being related to above ground N, and that grain yield was basically proportional to the aboveground biomass. It was concluded that the effect of fertilisation type on crop yield was mainly through its effect on net mineralisation, and that crop growth was always N-limited. Hence, the study suggests increased recommended fertiliser dose to increase yields in Nicaragua, and that quantification of soil N mineralisation is a main factor to control the effects of manure applications.

Keywords: N mineralisation, N uptake, N_2 fixation, grain DW, N yield, maize, beans, tropics.

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Dedication

To my wonderful family

Contents

List of Publications

This thesis is based on the work contained in the following papers, which are referred to in the text by their Roman numerals:

I Salmerón-Miranda, F., Eckersten, H. & Wivstad, M. (2008). Net N mineralization of an andosol influenced by chicken and cow manure applications in a maize-bean rotation in Nicaragua. *Scientific Research and Essay* 3, 280-286.

II Salmerón-Miranda, F., Båth, B., Eckersten, H., Forkman, J. & Wivstad, M. (2007). Aboveground nitrogen in relation to estimated total plant uptake in maize and beans. *Nutr. Cycl. Agroecosystem* 79, 125-139.

III Salmerón-Miranda, F., Båth, B., Eckersten, H., Forkman, J. & Wivstad, M. N utilisation in a maize-bean rotation in Nicaragua in relation to organic and mineral fertilisers

IV Salmerón-Miranda, F., Eckersten, H. & Wivstad, M. Effects of Manure Amendment on Dinitrogen Fixation of Common Beans (*Submitted to Journal Plant Soil Environment*)

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1 Introduction

Maize (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.) have been dominant staple crops in the low to middle Americas for millennia (CIAT, 2002; Morris & Paulsen, 2002; Broughton *et al.*, 2003). In Nicaragua, these crops are strongly related to the food security of the population, but are also important commodities. The maize is consumed almost every day in the form of *tortilla*, while beans are eaten either in soup or mixed with rice as *gallo pinto*. Exports of Nicaragua beans have increased by 50% over the past five years. Despite their importance, the grain yield of these crops has been reported to be low. During recent years (2000-2005), the average Nicaraguan yield (14% moisture) of maize has been about 1400 kg ha^{-1} and of beans 700 kg ha^{-1} (BCN, 2007). This level of maize yield is about 20% of that obtained on a rain-fed sandy soil in the USA (Mason *et al.*, 2008), while the yield of beans is about 40% of that reported in tropical lowlands of Mexico (Rosales-Serna et al., 2004).

An improved understanding of nitrogen (N) use in the plant-soil system is needed to improve crop production in Nicaragua. The term 'N use' refers here to the study of N from fertilisation through soil N mineralisation to plant N uptake and the allocation to crop parts. In the tropics, as in many regions of the world, N is often the most limiting nutrient for crop yield (Sanchez, 2002; Giller, 2004). In the Carazo region of Nicaragua, where this research was conducted, climatic conditions for maize and bean cultivation are reported to be good (Alemán, 2000; Gómez *et al.*, 2004), but field yields have remained small. Numerous studies have reported that N limits crop yields in this region (Quintana, 1983; Quintana *et al.*, 1992; Talavera, 1991; Izquierdo, 1992; Larios & Garcia, 1999). However, application of N, organic or mineral, does not necessarily mean that all N is available, taken up by the crop and used to increase yield. In the Carazo region, with predominantly volcanic soil, small-scale farmers cultivate maize and beans following the bimodal pattern of rainfall that allows two growing seasons per year. Maize is grown in the first season (*primera*), and after the maize is harvested beans are grown during the second season of the year (*postrera*). This rotation is repeated year after year. In maize, fertilisers are usually applied at sowing with a top-dressing at 30 or 40 days after sowing, while beans are fertilised only at sowing. The fertilisation rates for these crops are based on recommendations for mineral fertilisers by the national extension services (INTA, 2002), and not for organic fertilisers. In Nicaragua, however, the use of manure as an organic fertiliser has been promoted by organic grower organisations as an alternative to commercial mineral fertilisers. In this context, studies of animal manure as N fertiliser are needed to clarify its contribution to crop N uptake and yield. Chicken and cow manure are accessible in the Carazo region. Chicken manure is available from large Nicaraguan poultry enterprises (e.g. Pollo Estrella, Tip-top and Pollo Rico), which sell it at a reasonable price. Cow manure can be collected from corrals on farms with mixed cropping systems. The costs of manure application depend mainly on manure handling, storage and labour, but these aspects were beyond the scope of this study.

The use of mineral or organic fertilisers has been shown to have a positive effect on crop yield through changes in soil N status (Soumaré *et al.*, 2003; Muñoz *et al.*, 2008). However, the degree of yield increase and the effects on N cycling within the soil-plant system in relation to fertiliser type are unclear. In addition, cumulative effects from the repeated manure applications over consecutive years are of critical importance. To provide guidelines for optimal use of mineral or organic N sources, it must be possible to predict the effects on crop yield. This in turn requires understanding of the reasons for the variation between seasons, crops, fertilisation rates and fertiliser types.

Many processes are involved in determining fertilisation effects, e.g. soil N mineralisation, crop N uptake and biomass allocation to grain, and N yield. The flux of N through mineralisation is the major determining factor for soil mineral N (Accoe et al., 2005) especially when organic fertilisers are applied. Nitrogen availability is defined here as the cumulative fluxes of the soil mineral N pool in the root zone. The N mineralisation process is affected by site-specific factors, e.g. climate, soil properties and soil management, but the quality of the added organic N is perhaps one of the most determining factors. Quality is to some extent expressed by the C/N ratio of the applied materials, as it influences the N mineralisation process (Paul & Beauchamp, 1993; Giller, 2004) and can lead to immobilisation of N if the ratio is high. Both N mineralisation and immobilisation occur simultaneously and cannot be quantified separately under field conditions (Amlinger et al., 2003). In this study, net N mineralisation is used in the meaning of the net result of mineralisation and immobilisation. The net N mineralisation can be enhanced by the application of organic sources with high quality, i.e. low C/N ratio. The use of soil total N, rather than soil total C/N, as an indicator of the N mineralisation process could be relevant for some volcanic soils, e.g. andosols, which contain high quantities of elementary soil carbon highly resistant to decomposition (Barrios & Trejo, 2003).

Variation in net N mineralisation can also affect the N availability, which can increase depending on the timing of mineralisation (Ma et al., 1999). For instance, under tropical conditions net N mineralisation shows marked seasonal variations, with significant lower rates during the dry season (Babbar & Zak, 1994). In contrast, a high initial rate of net N mineralisation is expected when the dry soil is moistened at the beginning of the rainy season, but it decreases considerably within a few days thereafter (Birch, 1959, 1960; Ohlander, 1980). Fertilisation can also influence the variability of net N mineralisation and the available N in soil from repeated manure applications (Muñoz et al., 2008). Furthermore, crop rotation can affect net N mineralisation. An increase in the mineral N pool has been reported under a legume-based rotation system, possibly due to increased degradability of the organic matter due to an increase in root exudates (Neider et al., 1996). A rapid turnover of legume roots and nodules together with rhizodeposition can also increase soil mineral N (Unkovich et al., 1997).

Nitrogen availability can be significantly reduced by N losses, mainly though leaching, volatilisation and denitrification. The magnitude of the N losses is determined by e.g. weather conditions, soil properties, management and fertilisation practices. Chang & Janzen (1996) found that losses of N through leaching and volatilisation were lower for rain-fed crops than irrigated crops in a clay loam soil supplied with up to 180 Mg $ha^{-1}y^{-1}$ of cow manure over 20 years, while Powell et al. (2005) found that most of the mineralised N was in the upper 30 cm of the soil, indicating little downward movement of the applied manure. Ma et al. (1999) reported that slow release of soil mineral N in manured soil during the growing season can reduce N losses compared with mineral fertilisers. However, other studies have reported large N leaching due to a mismatch between manure application and crop demand (Yuan et al., 2000). Nitrogen leaching can also increase due to reduced N demand through lack of a well-developed root system in early stages of crop growth or when plants are reaching senescence.

Plant N uptake has been widely defined, but in this thesis it means total plant uptake from the soil mineral N pool. Across a range of crops, plant uptake of soil mineral N has been reported to be regulated by both N availability and plant N demand (Gastal & Lemaire, 2002; Lemaire et al., 2007). Under ample N availability, plant N uptake is mainly regulated by biomass accumulation and increased N demand (Greenwood, 1990). Under limited N conditions, N availability mainly regulates plant uptake. Many other factors have also been reported to affect crop N demand, e.g. crop developmental stage (Uhart & Andrade, 1995), growing season (Soon et al., 2001), crop type (Lemaire et al., 2005) and crop rotation (Weston et al., 2002). However, crop plants take up only a fraction of the mineral N per day and this could be considered an indicator of the efficiency with which the crop can use the available N (Eckersten, 2007).

Estimates of total plant N uptake based on available N can be influenced by root turnover, which can enhance mineralisation and enrich soil mineral N (Rochester et al., 1998). The contribution of root exudates and litter to N availability and plant N uptake have been estimated to account for 7-20% of the total plant N (Jensen, 1996; McNeill et al., 1997). Extra N input from air fixation is important when a leguminous crop is part of the crop rotation (Oliveira et al., 2004).

Grain biomass and N yield are the result of allocation of plant N uptake and accumulated biomass to the grain. Farmers try to maximise yield production by application of N fertilisers. Increased crop yield should be one of the main goals when any source of N is used, especially in the tropics where poor yields are commonly reported (Sanchez, 2002). There is a large body of literature to show that increased crop yields over recent decades have been related to the amount of fertiliser N applied, as shown in a review by Sinclair (1998). However, there is growing interest in not only increasing yields, but also improving the N use efficiency (Dawson et al., 2008). Despite its importance, the major mechanisms of N use efficiency have still not been fully quantified (Basra & Goyal, 2002). To rectify this, an understanding is needed of the N plant and soil components that can influence crop yield. To explain the effect of fertiliser N on crop yield, some studies have examined the relationship to plant biomass production (Saxena et al., 1990; Tollenaar & Aguilera, 1992; Andrade et al., 1999; Rosales-Serna et al., 2004). These studies suggest that yield response to fertiliser N is influenced by the physiological ability of the crop to transform the accumulated plant biomass into grain, an ability that varies greatly between crops (Bruce et al., 2002). Plant biomass accumulation has been reported to be coupled with N in the plant, complicating the simple relationship between biomass and crop yield. Several other studies have tried to predict grain yield based on plant N content (Sinclair, 1998; Cassman et al., 2002; Sinclair et al., 2002). The N in plants not only supports the grain biomass but also determines the N content of the grain. The N in grain is regulated by the physiological activity of grain formation and has been reported to be influenced by crop management (Weiland & Ta, 1992; Uribelarrea et al., 2004). The response of crop yield to N fertilisation varies greatly depending on different fertilisation strategies (Sanchez, 2002), and can also be influenced by crop rotation (Varvel & Wilhelm, 2003; Stanger & Lauer, 2008). The use of organic or mineral sources of N can have different impacts on the grain yield. Since fertiliser type and regime are highly influenced by local conditions (Muñoz et al., 2008), it is of interest to quantify the N fraction of applied N that plants can use for grain and N yield in a crop rotation.

2 Objectives

The overall aim of this study was to evaluate how organic and mineral fertilisers influence N use in a maize-bean crop rotation. The primary aim was to determine the extent to which the N sources applied altered net N mineralisation and soil mineral N content. A secondary aim was to estimate the amount of soil mineral N taken up by the crops and how this was influenced by inputs from N fixation. The final aim was to evaluate how grain yield was influenced by soil and plant N and biomass allocation to grain and to relate these changes to the organic or mineral fertilisers added.

To achieve these aims, the specific objectives of the four studies on which this thesis is based were:

1) To examine how net soil N mineralisation was affected by repeated applications of organic N sources and to express the differences in terms of the efficiency with which N was mineralised per unit of soil organic N (Paper I).

2) To estimate total plant uptake of N from soil mineral N and to determine the partitioning to aboveground parts as a function of growing period, season and crop (Paper II).

3) To evaluate variations in crop yield response in the maize-bean rotation in relation to N fertiliser application, and to explore reasons for these variations in terms of soil net N mineralisation, aboveground N and aboveground biomass over two years of crop rotation (Paper III).

4) To estimate N_2 fixation as a function of aboveground biomass of beans over three consecutive growing seasons (Paper IV), and to apply the functions obtained in the main fertiliser experiment to estimate the contribution of N_2 fixation to aboveground N in beans (Paper II).

3 Materials and Methods

A brief summary of material and methods is given below. For further details the reader is referred to Papers I–IV.

3.1 Thesis framework

In order to achieve a holistic picture of N use, the main N fluxes in the soil and in the plant were studied. An overview of the components considered in the N use approach is given in Figure 1.

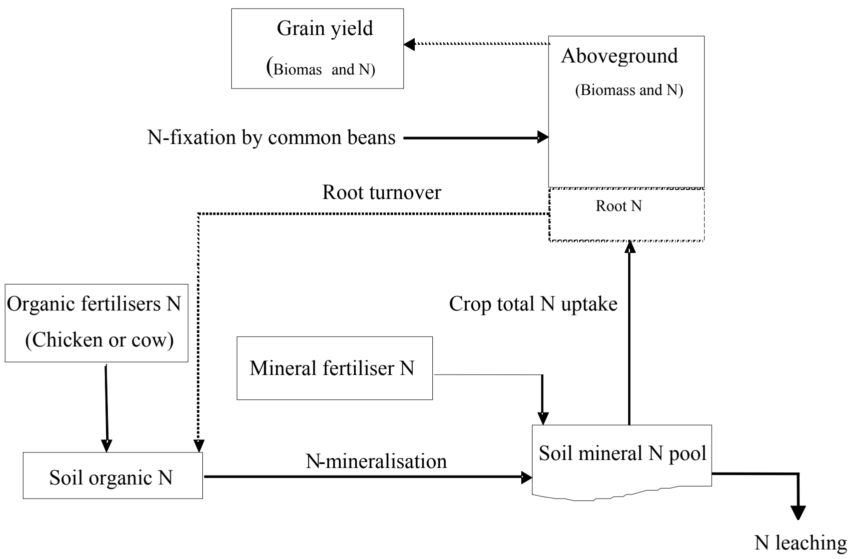

Figure 1. Flow (arrows) and states (boxes) considered in the estimates of N use in the maize-bean rotation. Denitrification, volatilisation and deposition were regarded as unknowns. Solid lines represent observed or estimated values. Root N and root turnover were not explicitly considered.

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(N-NO_3)

3.2 Study site

This study was carried out on the Carazo plateau in the Pacific region of Nicaragua (Figure 2), at the *La Compañía* research station, which is managed by the Universidad Nacional Agraria (UNA). The station is one of the reference sites where the national extension service carries out research on bean cropping.

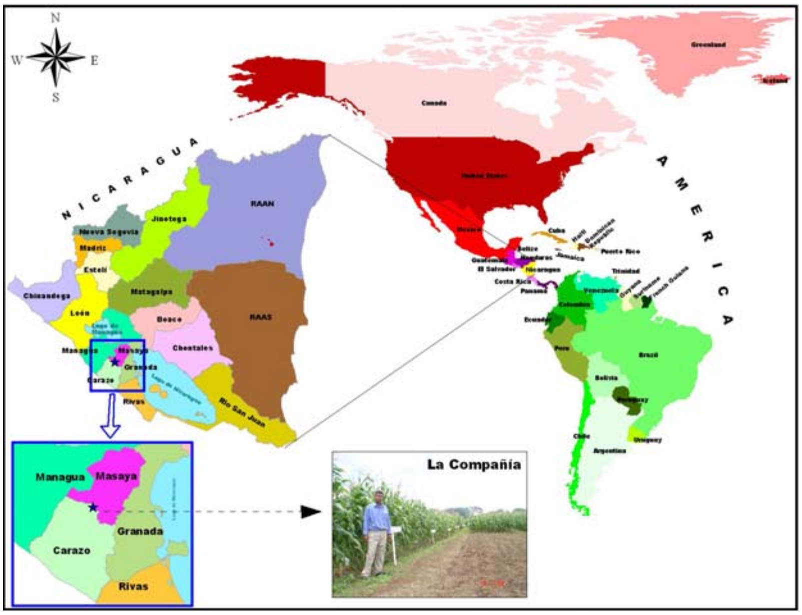


Figure 2. Research area in the Carazo region of Nicaragua. Photo: Jannette Gutiérrez Barrera, MSc.

3.3 Rainfall and crops

The mean annual rainfall at the experimental area is about 1 500 mm, with a bimodal pattern that gives two growing seasons per year. The mean annual temperature in the area is a stable 27 °C. Maize and beans were chosen due to their importance as food crops and were sown in a maize-bean within-year sequence for two consecutive years, 2002-2003 (Figure 3). Cultivar NB-6 was selected for maize and cultivar DOR-364 for beans because they are commonly used by Nicaraguan farmers. Certified seeds of these cultivars were obtained from the national extension service (INTA, 2002). The crops were managed following normal agronomic practices in the area as regards soil tillage, sowing, weeding and harvest.

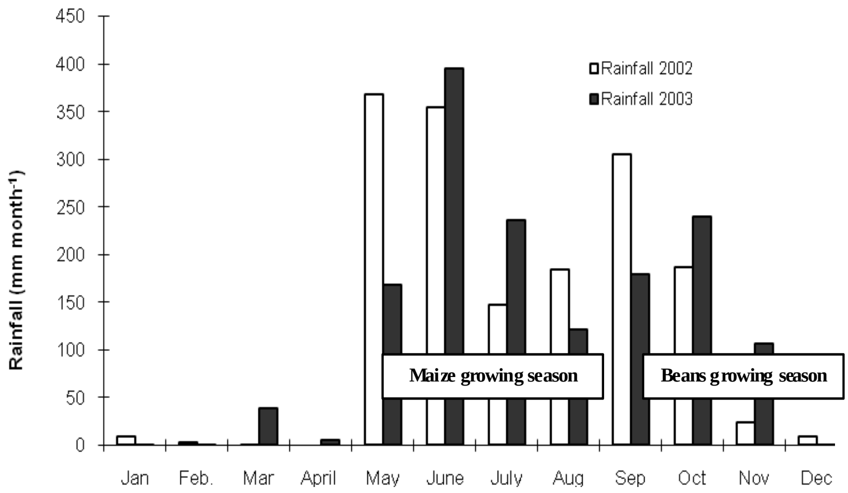


Figure 3. Rainfall 2002-2003 in relation to the growing seasons of maize and beans at the La Companía research station, Carazo, Nicaragua (INETER, 2004).

3.4 Fertilisers

Mineral and organic N fertilisers were used in this study. The mineral N fertiliser consisted of urea and N-P-K formula and the organic fertiliser of chicken and cow manure. A detailed description of the fertilisers applied is given in Table 1. The N fertilisers were applied at two doses, referred to here as lower and higher. The lower dose of mineral fertiliser was that recommended by the extension services (INTA, 2002) for maize or beans, while the higher dose was double the recommended dose. The lower dose of chicken and cow manure was assumed to be 5 Mg DW ha^{-1}, based on previous research carried out in the same experimental area (Larios & Garcia, 1999), while the higher dose was 10 Mg DW ha^{-1}. Before application, the chicken and cow manure was aerobically composted for about 12 weeks following procedures taken from the Indore method (FAO, 1980). In brief, the manure and urine, together with bedding materials, were placed in a heap that was turned and watered once a week. The manure was regarded as ready for application when the temperature in the heap was similar to the air temperature and the manure had turned a dark colour and become odourless. The composted manure was stored in sacks under cover at the experimental station. The manure composted during a previous year was used to fertiliser the soil plots of the current year.

Table 1. *Data on the fertiliser doses (g m^{-2}) applied to maize and bean crops in each growing season in 2002 and 2003.*

Treatment	FW[a]	DW[b]	Tot-C[c]	Tot-N[d]	C/N[e]	Min-N[f]	P[g]	K[h]
Chicken manure								
Higher (CHH)	1083	1000	306	33	9	2.9	3.6	17.2
Lower (CHL)	541	500	153	16	9	1.4	1.8	8.6
Cow manure								
Higher (COH)	1044	1000	173	11	15	0.5	3.1	15.6
Lower (COL)	522	500	86	6	15	0.2	1.6	7.8
Mineral fertiliser								
Maize[i] (NPK12-30-10 + Urea N 46)								
Higher (MFH)	33 + 39	-	-	22.0	-	22.0	4.4	2.7
Lower (MFL)	17 + 20	-	-	11.0	-	11.0	2.2	1.4
***Bean* (NPK18-46-0 + Urea N 46)**								
Higher (MFH)	26+7	-	-	8.0	-	8.0	5.3	-
Lower (MFL)	13+4	-	-	4.0	-	4.0	2.7	-
Unfertilised (UF)	-	-	-	-	-	-	-	-

[a]Fresh weight, [b]Dry weight [c]Total amount of carbon [d]Total nitrogen [e]Ratio of tot-C to tot-N [f]Mineral N content of the fertiliser applied [g]Phosphorus [h]Potassium [i]For maize, the mineral fertiliser was split into initial doses of 12 and 6 g m^{-2} of N, and a second application of 10 and 5 g N m^{-2} 40 days after sowing, for the higher and lower rates respectively.

3.5 Experiment trial

Two field experiments were carried out. The first and main experiment was performed in two successive years in a maize-bean rotation to achieve the objectives described for Papers I, II and III.

Figure 4. Experimental plot of the main field experiment showing the *in situ* N mineralisation experiment (inserted soil tubes) and the maize crop cultivated in rotation with beans. Photo: Jannette Gutiérrez Barrera, MSc.

The soil mineral N was studied through an *in situ* N mineralisation experiment (Figure 4), in which the response of soil mineral N to the application of chicken and cow manure was examined (Paper I). Net N mineralisation at 0-0.3 m depth was estimated as the difference in soil mineral N over time periods of 30 days in inserted plastic tubes isolated from crop roots. Soil mineral N was sampled every 30 days for three consecutive periods, from 0 to 90 days after sowing (DAS). As an indicator of the mineralisation efficiency, the specific mineralisation was expressed as the ratio between the estimated net N mineralisation and the soil total organic N content.

The total N uptake of maize and bean plants was estimated as the residual in a mass balance calculation of the soil mineral N (Paper II). The N input flows to the balance were net N mineralisation from the tubes and the differences in soil mineral N (outside the tubes) between 30-day periods. The N output flow was the estimated N leaching during the corresponding period. Other N fluxes, e.g. N deposition, denitrification and volatilisation, were assumed to be either the same for all the treatments or negligible and were therefore ignored. Nitrogen uptake below 0.30 m was also ignored in the calculations. The efficiency of plant N uptake was evaluated as the fraction of soil mineral N taken up daily. The estimated total N uptake was also evaluated to explore partitioning of N to aboveground biomass. The N in aboveground biomass was sampled at every 30 days, from 0-30, 30-60 and 60-90 days for maize, but 0-30 and 30-60 days for beans due to senescence of the crop. All relationships evaluated between estimated total N uptake and N in biomass considered only soil mineral N. Therefore the input from N_2 fixation was estimated to evaluate its relative contribution to the N in the aboveground biomass of beans. Calculation of N fixation was based on linear functions obtained in Paper IV.

The crop yield response to the application rates and types of N fertilisers was examined by quantifying different utilisation and use relationships (Paper III). The ratios of grain dry weight to added N (N utilisation), soil N mineralisation to added N, aboveground N to soil N mineralisation (N uptake efficiency), dry weight biomass to aboveground N (N productivity), dry weight biomass to grain dry weight, grain N to aboveground N (N harvest index) were evaluated. Most ratios were expressed as cumulative values in order to explore the trends over the two years of the crop rotation period. The grain yield values refer to date of harvest, at 115 and 80 days after sowing for maize and beans respectively. The N mineralisation data used to calculate the ratios were those estimated from soil tubes (Paper I). The data used for aboveground biomass and aboveground N were those sampled at 90 and 60 days after sowing for maize and beans respectively (Paper II).

The second experiment was established near the main experiment to estimate the input of N fixation derived as a function of aboveground biomass of beans over three consecutive growing seasons (Paper IV). These seasonal functions were applied to data from the first experiment to evaluate the specific contribution of N fixation to measured bean aboveground N (Paper II).

3.6 Data analyses

The relationship between the organic N fertilisers applied and soil N mineralisation ($N_{Mineralisation}$) was analysed with an ANOVA procedure to ascertain statistical significance between 30-day periods and between growing seasons through applying the GLM statistical model (Paper I). The components of the estimated total N uptake were analysed with a linear model to test the hypothesis of no differences between the 30-day periods.

The relationship between estimated total N uptake ($N_{TotUptake}$) and measured change in aboveground biomass ($\Delta N_{AboveGr}$) was investigated for differences between sampling periods using a linear model in which maize and beans was analysed separately. The LSD between means of the sampling periods were calculated and pairwise comparisons were made (Paper II). Different N utilisation and use ratios were analysed with a linear model to test the hypothesis of no difference between crops, growing seasons, and fertiliser type and rates (Paper III). To explore differences for the crop rotation period, the hypothesis of specific growing season slope to the treatments was tested by a proc-mixed model. The response of bean N_2 fixation was analysed following the ANOVA procedure to ascertain the statistical significance of manure effects and interactions with crop type and season by applying the GLM procedure (Paper IV). The relationship between aboveground dry matter and N_2 fixation from each season was examined using the regression procedure. Transformation of data was performed when necessary to fulfil the assumptions on normality and equal variances. All the analyses were made by applying the SAS statistical package SAS institute Inc. NC, USA, 2002-2003 version 9.

4 Results

4.1 N mineralisation, plant N uptake and allocation

The net N mineralisation significantly increased in plots where chicken manure was applied at the higher rate of 10 Mg DW ha^{-1} (CHH treatment; Paper I). In the third season (*primera* 2003) in particular, the net N mineralisation showed an increased trend of up to 12 g N m^{-2} 30 $days^{-1}$ (Figure 5). From the second to the fourth season, the N mineralisation in both cow manure treatments (COH and COL) was low and similar to that for the lower rate of chicken manure (CHL). Variation in net N mineralisation was larger in maize than in beans and larger for the period 30-60 days than for the other periods. The net N mineralisation decreased in unfertilised plots from 4.7 to 2.6 g N m^{-2} 30 $days^{-1}$ from the first to the fourth season. When net N mineralisation was expressed as a ratio of the total soil organic N (a-value) it followed a similar trend to N mineralisation (Paper I).

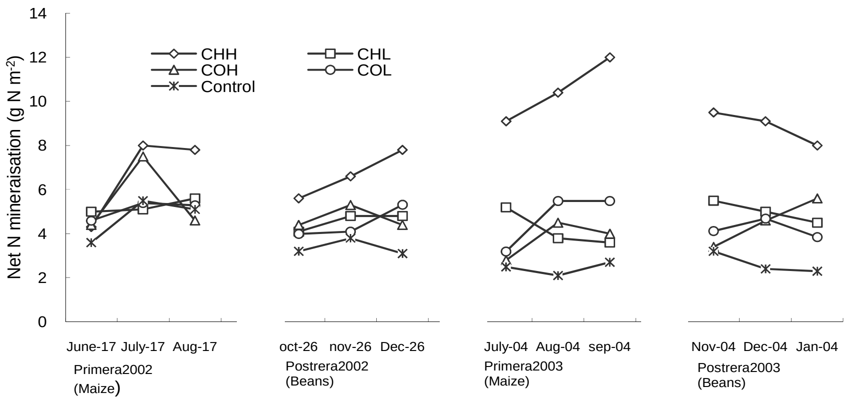


Figure 5. Estimated net N mineralisation of the 0-30 cm soil layer (g N m^{-2} $30d^{-1}$) between sampling occasions in the four manure treatments and the control. CHH= higher rate chicken manure, CHL=lower rate of chicken manure, COH= higher rate of cow manure COL= lower rate of cow manure Control=unfertilised (from Paper I).

Estimated plant N uptake of soil mineral N ($N_{TotUptake}$) was highly dependent on the net N mineralisation (Paper II). Net mineralisation values, averaged over applied N fertilisers and crops, ranged between 5-7 g N m^{-2} 30 day^{-1} while the change in mineral N was about 1 g N m^{-2} 30 day^{-1}. Nitrogen leaching had a minor influence and values from single plots ranged from 0 to 0.8 g N m^{-2} 30 $days^{-1}$, with the highest values recorded between 0 and 30 days after sowing (DAS).

The $N_{TotUptake}$ varied considerably between crops, sampling periods and N fertilisers. In maize, it ranged from 2.5 to 13.0 g N m^{-2} 30 $days^{-1}$ and in beans from 3.0 to 14.0 g N m^{-2} 30 $days^{-1}$. It was generally larger between 30 and 60 DAS than in the earlier period (0-30 days) for most of the plots. The highest plant N uptake was estimated for the higher doses of chicken manure (CHH) and mineral fertiliser (MFH), but an increasing trend over years was only observed for the chicken manure.

The monthly trend in aboveground crop N ($\Delta N_{AboveGr}$) expressed in relation to $N_{TotUptake}$ was linear for beans, but not for maize. For maize the relationship was significantly different between 30-day periods of the growing season. The aboveground N to total N uptake ratio approached 75-80% for the periods 0-30 and 60-90 DAS, and 55% for the period in between (Figure 6).

For beans, the aboveground N to total N uptake ratio ranged from 20 to 40% and there were no significant differences between periods (Figure 7).

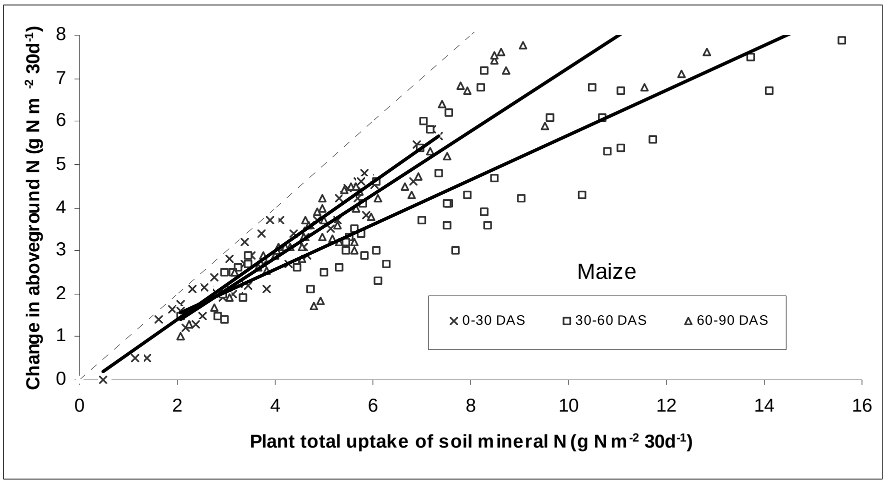


Figure 6. Change in maize aboveground N ($\Delta N_{AboveGr}$; g N m^{-2} $30d^{-1}$) in relation to estimated total N uptake ($N_{TotUptake}$; g N m^{-2} $30d^{-1}$) for 30-day periods in 2002 and 2003. The solid lines were derived by linear regression (y=*a*x+*b*) for sampling periods (the two years combined). The dotted line denotes the 1:1 relationship (from Paper II)

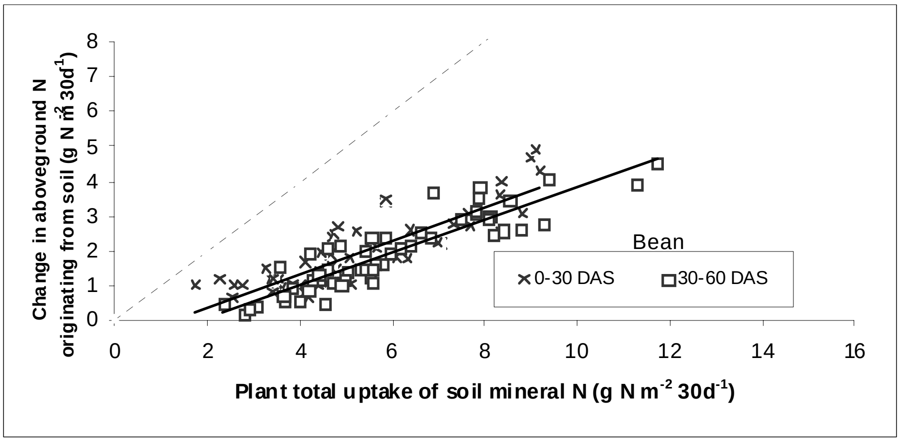


Figure 7. Change in bean aboveground N originating from soil mineral N ($\Delta N_{AboveGrNoFix}$; g N m^{-2} $30d^{-1}$) in relation to estimated total N uptake ($N_{TotUptake}$; g N m^{-2} $30d^{-1}$) for 30-day periods in 2002 and 2003. The solid lines were derived by linear regression (y=ax+b) for sampling periods (the two years combined). The dotted line denotes the 1:1 relationship (from Paper II).

Both the relative and absolute contribution of N from N_2 fixation to aboveground N in beans was higher in the second period (30-60 DAS) than in the first period (0-30 DAS), as shown in Figure 8. Nitrogen fixation showed an increased trend by the second year. In beans, changes in measured aboveground N, including the input from N fixation, ranged from 0.8 to 7.0 g N m^{-2} 30 $days^{-1}$ and increased within the growing seasons (Paper II).

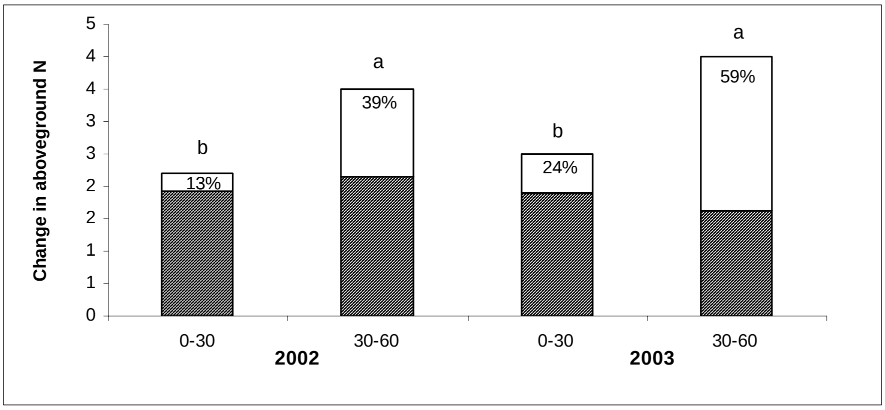


Figure 8. Contribution of the estimated N_2 fixation ($\Delta N_{AboveGrFix}$) to the change in measured bean aboveground N during 30-day periods within seasons ($\Delta N_{AboveGr}$). The contribution of N_2 fixation ($\Delta N_{AboveGrFix}$) is denoted with white and given as a percentage. The values are averaged over all fertiliser treatments. Different letters denote statistical differences between 30-day periods ($p<0.05$) (from Paper II).

4.2 Crop yield

The highest grain yield was achieved in the mineral fertiliser treatments (MFH and MFL) and with the higher N dose of chicken manure (CHH) over the four seasons of the maize-bean rotation (Paper III). For mineral fertiliser (single season average), the maize grain yield ranged from 386 to 391 g DW m^{-2}, while for beans the range was 203-220 g DW m^{-2}. For chicken manure, the maize grain yield range was 300-371 g DW m^{-2} and for beans 187- 224 g DW m^{-2} .

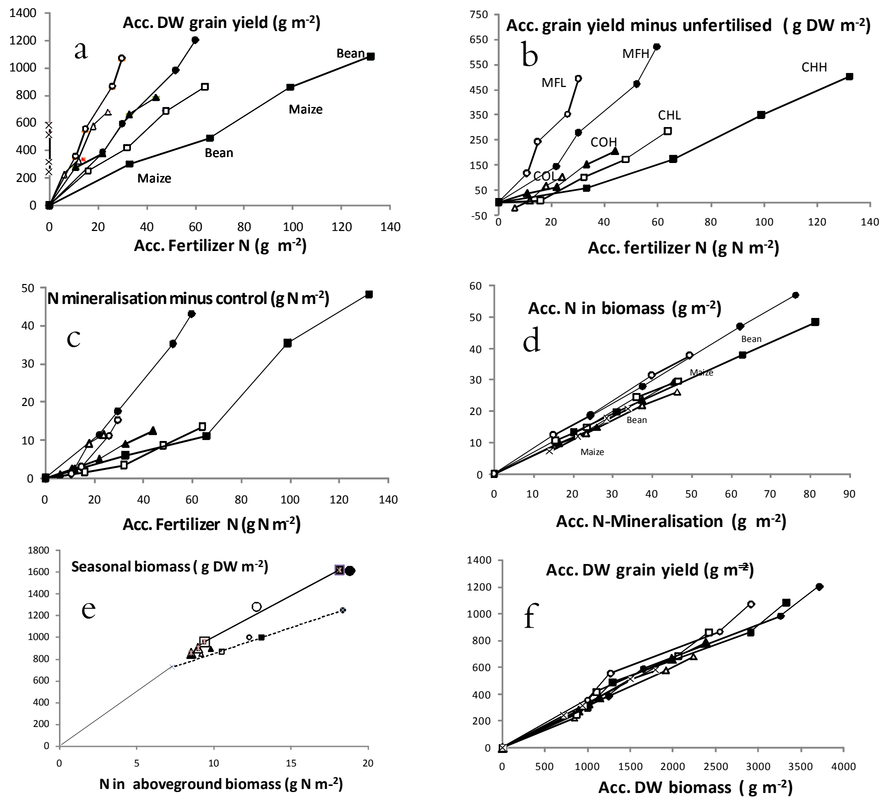
Table 2. *Grain yield of maize and beans for different fertiliser N treatments and the control plot in four seasons in 2002 and 2003*

Treatment		Grain yield (g DW m^{-2})			
		2002		2003	
Fertiliser type	Fertiliser N Doses (g m^{-2})	Maize Season1	Bean Season 2	Maize Season 3	Bean Season 4
COL	6	222d*	100d	254c	103c
COH	11	277cb	98d	287cb	122c
CHL	16	249cd	168c	268c	176b
COH	33	300b	187b	371a	224a
MFL *maize*	11	256a		311b	
MFL *maize*	22	386a		391a	
MFL *bean*	4		197a		206a
MFL *bean*	8		203a		220a
UF	0	241cd	72e	197d	68d

For description of the treatments see Table 1.

*: Different letters in the same column indicate significant differences between fertiliser N treatments for the same growing season ($p<0.05$).

The utilisation efficiency of fertiliser N for grain yield was related to fertiliser dose and type and crop type (the slope in Fig. 9a). The N utilisation was higher for the lower application rates and higher for mineral fertiliser and cow manure than for chicken manure. The highest N utilisation was observed for the lower rates of the MFL treatment (36 g DW grain per g of added N) and the COL treatment (28 g DW grain per g of added N). It was only for maize in the CHH treatment that the utilisation differed significantly between years. When the N utilisation ratio was related only to the yield increase caused by fertilisation, there was only an influence of fertiliser type (Fig. 9b). The mineral fertiliser was used more efficiently than the organic fertilisers.



Figures 9 a-f. (a-b): Crop yield response vs. added N, (c): N mineralisation minus the control vs. added N, (d): Aboveground N vs. N mineralisation, (e): Aboveground biomass vs. aboveground N on a seasonal basis (lines are arbitrarily drawn), and (f): Grain DW vs aboveground biomass. All values are cumulative for the four-season crop rotation, except for the aboveground biomass vs. aboveground N, which are seasonal values. Units are g dry weight m^{-2} for biomass and g N m^{-2} for nitrogen. Triangles = cow, squares = chicken, circles = mineral, cross = unfertilised, filled symbols = high rate and the others low rate. Small dots in (e) represent first year. (a, d, f are from Paper III).

Furthermore, the grain yield of some fertilisation treatments was lower than that of the control in the first growing season, but from the second season all were higher. The net N mineralisation minus the control was related to the added fertiliser N in a similar way as was the grain yield (Fig. 9c). The highest value of N mineralisation was observed for the higher rate of chicken manure (CHH), but the highest N mineralisation per unit of added N was observed for the higher mineral fertiliser treatment (Paper III).

The aboveground N (g N m^{-2}) to net N mineralisation (g N m^{-2}) ratio showed a linear trend, with almost constant values over the four seasons (Fig. 9d). This ratio was independent of crop and fertiliser rates, was slightly higher for mineral fertiliser (0.7) than for organic fertilisers (0.6) and was independent of crop type (Paper III).

The seasonal N productivity, aboveground biomass (g DW m^{-2}) to aboveground N (g N m^{-2}) ratio, showed the highest values in the treatments with lower fertilisation rates independent of fertiliser type (Fig. 9e). For the higher fertilisation treatments the N productivity decreased overall, but increased from the first to the second year (Paper III).

The grain dry weight to aboveground biomass to ratio showed no trend over the crop rotation and was similar for all treatments, but was higher for beans than for maize (Fig. 9f). The ratio, averaged over treatments and seasons, was 0.3 for maize and 0.4 for beans (Paper III).

5 Discussion

5.1 N mineralisation, plant N uptake and allocation

5.1.1 N mineralisation estimates

In this study, soil net N mineralisation was estimated as the measured change in soil mineral N in tubes inserted into the soil *in situ*. A number of factors could have influenced the net N mineralisation estimates. The soil inside the tubes was isolated from plant roots. Several studies have reported a positive priming effect, meaning that N mineralisation is enhanced by litter and substances in root exudate (Phillips & Fahey, 2008) probably due to an increased degradability of soil organic matter. If that were the case, the absence of roots in the tubes may have led to underestimation of the N mineralisation. Other studies, although less common, have reported a negative root priming effect on N mineralisation due to changes in the soil C/N from added root litter leading to temporal N immobilisation (Hamer & Marschner, 2005). The contribution from nodules and litter under legumes has been reported to be between 7 and 20% of the N available for plant uptake (McNeill *et al.*, 1997). However, this contribution depends on the growing conditions determining belowground N in the leguminous crop (Rochester *et al.*, 1998). In this study, when the influx of belowground N was large in the maize-bean rotation, the mineralisation was most probably underestimated. On the other hand, N losses by leaching were neglected in mineralisation estimations, which might have caused an overestimation of N mineralisation. However, the maximum N leaching outside the tubes was estimated to have been less than 10% of that mineralised (Salmerón-Miranda *et al.*, 2007). The soil moisture may also have been lower in the tubes than in the cropped soil, since the tubes were covered with a cup and not directly influenced by rainfall. Although the tubes were watered weekly, short periods of dryness might have depressed the mineralisation process, leading to an underestimation of N mineralisation.

5.1.2 N mineralisation and fertiliser type

The net N mineralisation was significantly increased in the chicken manure treatment at the higher rate. This was particularly true in the third season, in maize grown after the bean crop (Paper I; Figure 5). The explanation may be related to the large amount of N applied every season with the higher rate of chicken manure (330 kg organic N ha^{-1} $season^{-1}$), but also to the lower C/N ratio of chicken manure (9) compared with cow manure (15). Previous studies have reported that with applications of organic materials with a C/N ratio less than 15, net N mineralisation occurs almost immediately (Jenkinson, 1990; Marstorp & Kirchman, 1991). The increased N mineralisation in the third season might also have been due to an N effect of the bean crop grown in the previous season. Numerous studies have shown an increased mineralisation of N with a leguminous crop as the preceding crop (e.g. Andersson & Domsch, 1989; Varvel & Wilhelm, 2003). In addition, the much larger precipitation in the third season than in the other seasons might have had a positive effect on N mineralisation (Figure 3). However, the most important reason for the systematic increase in N mineralisation was probably the continued supply of chicken manure at a high N rate.

5.1.3 Estimated plant uptake of mineral N

The total plant N uptake, estimated as the residual of the soil mineral N balance, would have been underestimated in the event of underestimation of the N mineralisation. In addition, the fact that only a soil depth of 0.3 m was considered in the estimates might have contributed to an underestimate of the total N uptake. Roots are expected to extract N from deeper soil layers during a whole season (Bolaños & Edmeades 1993, 1996). Nitrogen leaching was also part of the estimated soil mineral N balance and could have been underestimated by not considering soil layers below 0.3 m.

As regards other N fluxes affecting the estimated soil mineral N balance, denitrification of N was assumed to be low because of prevailing non-water saturated soil conditions at the experimental site. Volcanic soils such as andosols usually have good drainage conditions due to a well-structured soil texture and high soil organic matter content (Sanchez, 2002). In the present study, ammonia losses were assumed to have had a minor effect on the soil mineral N balance since the fertilisers were incorporated into the soil just before the first measurement of soil mineral N. Atmospheric N deposition could have been an important input of N to the estimated total uptake. This input has been reported to be about 5 g N m^{-2} in the tropics (Zougmoré *et al.*, 2004), and represents less than 5% of the estimated total N uptake. However, deposition was neglected here because it was assumed to be the same for all the experimental plots. In summary, the estimated plant N uptake of mineral N was probably underestimated due to possible underestimation of net N mineralisation and due to root uptake deeper than the sampled 0.3 m being neglected, although this was possibly counterbalanced by underestimation of N leaching.

5.1.4 Partitioning of N in the plant

The measured N in aboveground biomass was linearly related to estimated total N uptake. This suggests that the shoot to total plant ratio tends to be constant as the total N uptake increases. However, this ratio was crop-sensitive, showing higher variation in maize than in beans. For beans the ratio (N fixation excluded) remained fairly stable over the two growing periods evaluated, but for maize the ratio showed larger variation between periods. The lower ratio between 30 and 60 DAS of maize indicated an increased root to shoot ratio. On the other hand, higher shoot to total N uptake ratio for maize in the 60-90 DAS period, related to reproductive stages, indicated an increased N translocation from root to aboveground parts. This is in line with other studies reporting that root death in annual species is often considered a predetermined response that occurs when plant resources, such as N, are diverted from root growth and maintenance to flowering and yield (Ma & Subedi, 2005). However, the high shoot N content to estimated total N uptake ratio for maize during the early 0-30 DAS period was more difficult to explain. One explanation might be a possible underestimation of the total N uptake. The lower ratio for beans compared with maize seems reasonable, since a higher root N proportion is expected in the legume than in the cereal crop (Rochester et al., 1998). This is also supported by the finding that common bean does not exhibit root senescence during the pod filling stage (Fichtner & Schulze, 1992), which occurred during the 30-60 DAS period in this study.

5.2 Crop yield

Grain yields were significantly increased in the treatments receiving higher N doses of mineral fertiliser or chicken manure over the two-year crop rotation compared with the lower N treatments (Figure 9). The observed variation in grain yield was strongly influenced by the net N mineralisation rather than the plant N demand.

The utilisation of N (dry weight grain per unit of added N; Fig. 9a) decreased with fertilisation rate and was higher for mineral N and cow manure. A possible explanation for the higher utilisation of mineral fertiliser could be that the applied rate was split into an initial application at sowing and a second 35 days later. Plénet & Leamaire (2000) reported that utilisation of added N can be increased by splitting the N dose over the growing period. One explanation for the higher N utilisation in the lower rate cow manure treatment could be a higher uptake of native soil N in relation to cow manure N compared with the higher N rate treatments, as showed by the grain yield less the control value (Fig. 9b) and the net N mineralisation less the control vs. added N (Fig. 9c).

The response to fertilisation was similar for beans and maize, despite N fixation in beans. Utilisation of fertiliser N appeared to be independent of the N fixation rate. One possible explanation is that N fixation was depressed by the N fertilisation (Oliveira et al., 2004). The N fixation rate of beans decreased over three growing seasons, possibly due to continuous addition of N, as reported in the neighbouring experiment examining N fixation response to organic fertiliser applications (Paper IV).

The cumulative net N mineralisation per unit of added fertiliser (Fig. 9c) showed an almost similar pattern to the N utilisation (grain yield/added N) over the crop rotation. This may indicate that crop yield increased mainly through the effects of applied fertiliser N on N mineralisation. This is in line with other studies reporting that grain yield is determined by N availability (Jokela, 1992; Liang et al., 1996; D•Andrea et al., 2008).

The aboveground N to net N mineralisation ratio (N use efficiency) was in most cases independent of the crop and of the type and rate of N fertiliser applied, but the efficiency was slightly higher for the higher rate of mineral fertiliser than for the chicken manure (Fig. 9d). The N use efficiency was constant over the whole crop rotation. One interpretation could be that the aboveground plant N was determined by soil N mineralisation and that the N uptake was N-limited even with the highest N fertilisation rate in the experiment, the higher rate chicken manure treatment. The ratio between aboveground biomass and aboveground N (N productivity) was not sensitive to fertilisation treatment or crop type, but tended to increase during the crop rotation (Fig. 9e). This may be a confirmation that plant growth was N-limited even for the high fertiliser application rates. This relationship seemed never to reach a plateau that would indicate luxury consumption of N by plants, as described in other studies (Greenwood et al., 1990). The N productivity was slightly crop-sensitive, being higher for maize than for beans. The higher relationship in maize may have been related to the higher shoot dry weights compared with legumes during all growth stages (Fageria & Baligar, 2005).

The aboveground biomass to grain yield ratio, a sort of harvest index, was not influenced by the N sources applied and was rather constant over the crop rotation (Fig. 9f). This is in line with studies reporting that the harvest index is genetically determined and not influenced by fertilisation rate (Sinclair, 1998; Khan et al., 2007). The harvest index is usually measured at the time of crop harvest for both grain and biomass. In this study, however, for practical reasons the aboveground biomass for maize was sampled at 90 days after sowing (when it was at its maximum), while grain was harvested at 115 days, and this might have caused an underestimation of the harvest index. This is probably the reason why our ratios are lower than those reported in the literature as the harvest index for maize. For beans the observed ratio was 0.37, which is in the lower range of reported harvest index values of 0.36-0.67 for Mexican cultivars (Rosales-Serna et al., 2004). For maize, the observed biomass to grain ratio of 0.33 was considerably lower than the 0.5 reported for maize in Argentine (Tollenar et al., 1994). The possible low harvest index of the locally cultivated maize variety used might have contributed to the low index.

5.3 Other nutrients

Other nutrients were added with the fertilisers applied in the experiment in addition to N, of which phosphorus (P) probably deserves most attention because of the potential P-fixing problem in the volcanic experimental soil (Izquierdo, 1991; Talavera, 1991). The P added with the different fertilisers ranged from 1.6 to 5.3 g P m^{-2} $season^{-1}$ (Table 1). Plotting the accumulated increase in grain yield, compared with the control, against the cumulative added P in the grain (data not shown) revealed a similar response to the corresponding graph for N (Fig. 9b) except that cow manure always had the lowest P utilisation efficiency. This suggests that P might also have been an important factor regulating yield. However, the current experimental data could not be used to evaluate the interactive effects of P and N on yield (cf. Knecht-Billberger, 2006).

6 Conclusions

Of the organic fertiliser treatments, N mineralisation increased significantly only with the higher rate of chicken manure, when it resulted in a higher mineralisation rate per unit total organic N. Nitrogen mineralisation was the main source of N for plant N uptake, which significantly increased in both maize and beans in the middle of the season. The fraction of total N uptake partitioned to aboveground parts was almost unrelated to the magnitude of N uptake for beans, but for maize it decreased during the middle of the season, indicating increased allocation to root growth during this period. The relative contribution of N_2 fixation to bean aboveground N was substantial in both fertilised and unfertilised bean, and increased in the second year. The utilisation of fertiliser N for grain yield could be attributed mainly to the effect of the fertiliser on N mineralisation. This was examined by estimating the effect of N mineralisation on aboveground N, which was almost linear, N productivity (aboveground biomass to N ratio), which was the same for all types of fertilisers but decreased slightly for higher rates, and harvest index, which was independent of N treatment. In conclusion, the effect of fertiliser type and rate on crop yield was mainly through its effect on mineralisation, and after four seasons of high application rates plant growth was still N limited. Hence, improving and controlling soil net N mineralisation is an important approach for increasing crop yields in the tropics.

7 Practical implications

This thesis adopted a methodological approach to quantify central relationships between N dynamics and crop yield of maize and beans. The results suggest the relationship between the type and amount of fertiliser N and N mineralisation to be critical. If the soil N mineralisation rates were known, crop yield would be more predictable and it would be possible to estimate the appropriate application rates of chicken and cow manure to achieve increased yields. The methodological approach estimates the use efficiency of the applied N, allowing the increased yield to be related to increased soil N and N losses. One important feature of this approach is that it would allow the impact of long-term fertiliser application on the N use and utilisation efficiencies to be monitored under different fertilisation regimes and scenarios in Nicaraguan conditions. This approach could contribute to the defining the productivity limits of the farming systems and to relating fertilisation treatments to the risk of environmental impacts. From a practical perspective the results indicated that grain yield of the current cropping system was limited by fertilisation rates. This suggest a considered increase of recommended rates from a production point of view, however, this study is not a base for evaluating the environmental consequences of such increase.

Finally, an issue of practical importance is the estimation of N_2 fixation based on the aboveground biomass, which could help farmers to quantify the N contribution in legume-based crop systems and thus decrease fertiliser applications.

8 Future research

Reducing the sources of uncertainty in N mineralisation estimations is an important goal for future research. For example, an improvement in root N measurements is needed to estimate the contribution from root turnover. Although the current study did not find N losses of significant importance for crop yield, it is important to increase the reliability of estimated N losses, e.g. through leaching, denitrification and volatilisation, especially considering the advice on increasing fertilisation rates, which might increase the risk of such losses. The use of physical methods, e.g. lysimeter techniques, might prove useful in determining N losses.

The influence of climate on N inputs and outputs was not specifically examined in the current study, but potential changes in soil water conditions due to climatic variability and/or climate change could influence the results. Future studies should examine how the relationships found between fertiliser N, mineralisation, uptake, allocation growth and yield are affected by short-term and long-term changes in climatic conditions. In addition, this study was performed only for four seasons and longer trials would be needed to evaluate the long-term effects of high application rates on N mineralisation, plant use and N losses.

Other questions not considered in this thesis, but that might have influenced the results, relate to the effects of organic fertilisers on soil P and the effects of N and P interactions on crop yield.

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