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

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Abstract

The integration of multipurpose legumes into low-input tropical agricultural systems is needed because they are a nitrogen (N) input through symbiotic fixation. The drought-tolerant cover legume canavalia (*Canavalia brasiliensis*) has been introduced for use either as forage or as a green manure into the crop-livestock system of the Nicaraguan hillsides. To evaluate its impact on the subsequent maize crop, an in-depth study on N dynamics in the soil-plant system was conducted. 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 incorporation of N from the amendment into different soil N pools (total N, mineral N, microbial biomass) was followed during the maize cropping season. Maize took up an average of 13.3 g N m^{-2}, within which 1.0 g N m^{-2} was from canavalia residues and 2.6 g N m^{-2} was from mineral fertilizer, corresponding to an amendment N recovery of 12% and 32%, respectively. Recoveries in maize would probably be higher at a site with lower soil available N content. 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 canavalia residue treatment with 98% recovery, followed by the mineral fertilizer treatment with 83% recovery. Despite similar initial enrichment of soil microbial and mineral N pools, the indirect labelling technique failed

to assess the N fertilizer value of mineral and organic amendments due to a high N mineralization from the soil organic matter.

Key words

Canavalia brasiliensis; ^{15}N; indirect and direct labelling techniques; microplot study; organic amendments.

List of abbreviations

DAA, days after amendment; DLT, direct labelling technique; ILT, indirect labelling technique; N, nitrogen; Ndff, amount of N derived from the amendment; Ndfs, amount of N derived from the soil; Nmin, soil mineral N; Ntot, total soil N; Nmic, soil microbial N; ^{15}N-X, ^{15}N enrichment of the respective X pool.

Introduction

The integration of multipurpose legumes into low-input tropical agricultural systems is needed because they represent a nitrogen (N) input through symbiotic fixation. This can benefit the subsequent crop and build up soil organic matter stocks over time, either when their biomass is used as green manure or when fed to animals whose manure is recycled into the soil. To adequately manage legumes in crop rotations, their N fertilizer value (i.e. the legume N uptake by the succeeding crop and the amount and form of legume N

66 remaining in the soil) must be known. The drought-tolerant cover legume *Canavalia*
67 *brasiliensis* Mart. Ex. Benth (canavalia), also known as Brazilian jack bean, has recently
68 been introduced as a green manure and/or forage into the traditional maize-bean-livestock
69 system of the Nicaraguan hillsides (CIAT 2008; Peters et al. 2004). Canavalia is well
70 accepted by farmers, but its fertilizer value remains unknown (Douxchamps et al. 2010).
71
72 The direct ^{15}N labelling technique (DLT), i.e. the addition of ^{15}N labelled amendment to
73 an unlabelled soil-plant system, has proven to be the most suitable method to trace the
74 fate of N from amendments into different pools of the soil–plant system (Hauck and
75 Bremner 1976; Hood et al. 2008), and was therefore applied to canavalia residues. Under
76 tropical field conditions, previous use of this method with legume residues are scarce
77 (McDonagh et al. 1993; Toomsan et al. 1995; Vanlauwe et al. 1998a), and, to our
78 knowledge nonexistent with animal manure. As it is difficult to label local cow manure,
79 we used the indirect ^{15}N labelling technique (ILT), where potentially available soil N is
80 labelled instead of amendment N. Potentially available soil N includes the different soil N
81 pools that can deliver mineral N during the growing period of the crop: mineral N,
82 microbial N and non-living labile soil organic matter. With the ILT approach it is
83 assumed that the potentially available soil N from the amended plot and a non-amended
84 control plot initially have the same ^{15}N enrichment, so that any dilution observed in the
85 amended plot results from the unlabelled amendment. If potentially available soil N is not
86 labelled homogeneously, artefacts can arise due to pool substitution (Jenkinson et al.
87 1985), for example when labelled soil inorganic N is immobilized by growing microbial
88 cells 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 legume residues or manure. Labelling of the soil for a substantial time before the application of the amendments has been reported to prevent 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 with DLT using canavalia residues, mineral fertilizer and sheep manure produced under controlled conditions.

The objectives of this study were (i) to determine the N fertilizer value of canavalia for maize, when canavalia biomass is used as green manure or fed to animals whose manure is returned to the soil, (ii) to compare the ILT and DLT methods under tropical field conditions for amendments N uptake by maize and (iii) to explain any discrepancies between ILT and DLT by the evolution of the ^{15}N excess in different soil N pools.

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°46’47’’N, 85°49’35’’W), at 560 meter above sea level, 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 had a bimodal pattern (Figure 1). 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 the effect on maize yields of two different legumes, which included canavalia. At the beginning of the second rainy season in September 2007, 1.2 m^2-microplots made from tin sheets 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 1). The only treatment without a mirror was the plot with local cow manure. To check for the accuracy of the ILT for manure, two 0.6 m^2-microplots were established with labelled and unlabelled manure obtained from a Swiss sheep (Bosshard et al. 2008). The ILT-Control treatment was used as an 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).

Labelling of canavalia and soil N

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 $(NH_4)_2SO_4$ at a rate of 50 kg N ha^{-1}. To minimize leaching by the heavy rains, the dose was distributed over five applications during the first two months of canavalia development. The solution was applied to the soil surface between the canavalia plants using a watering can. Likewise, unlabelled $(NH_4)_2SO_4$ was applied using the same procedure to the microplots assigned to DLT. Thus, 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 in order to promote homogenous soil N labelling for ILT through microbial immobilization of a part of the ^{15}N. Sucrose was added to all ILT and DLT microplots. Canavalia was harvested in February 2008 in the late flowering/early pod filling development stage. 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 (i.e. leaves detached from the stems) were discarded. Yields were recorded for each single microplot, and subsamples were taken for analysis. The material from each microplot was then air dried, stirred regularly 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 the microplots were weeded manually and weeds were left on the

surface of their microplot of origin. A composite soil (0-10 cm and 10-20 cm) sample was collected in the microplots in June 2008 to check the enrichment.

N uptake by maize from different amendments

At the beginning of the first rainy season in June 2008 (Figure 2), canavalia residues were exchanged between DLT and ILT-Residue microplots within the same replicate. Leaves and stems were applied on the surface and slightly incorporated to prevent wind dispersal. A 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 1). Solution of unlabelled and 10 atom% ^{15}N $(NH_4)_2SO_4$ 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 (faeces 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 exceeded because the cow manure was more concentrated than expected due to water loss during storage in San Dionisio. The manure 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

180 unlabelled ryegrass diet (Bosshard et al. 2008). Both manures were applied at a dose of
181 40 kg N ha^{-1} on the small microplots. All amendments were applied with the same
182 amount of water. No other nutrients were applied because the nutrient status of the trial
183 soil was high enough to sustain maize growth without limitations. Characteristics of the
184 amendments for each treatment are presented in Table 1.
185 The amended microplots were planted with *Zea mays* (cv. NB-6) two days after
186 amendment (DAA) at a density of 8 plants per 1.2 m^2 (microplot surface). Per microplot,
187 there were 4 planting points with 2 seeds each, with 0.8 m distance between rows and 0.6
188 m distance between the planting points within the rows. The distance between the plants
189 and the border of the microplots was 0.2 m. An unusual, short drought hindered
190 germination, and maize was replanted at 15 DAA. The second mineral fertilizer dose was
191 therefore delayed until 36 DAA. Insecticide chlorpyrifos was applied around the plots to
192 protect the seeds and young plants against ants. Microplots were weeded manually and
193 weeds were left on the surface of their microplot of origin. At maturity, maize was left to
194 dry on the stems in the field according to usual farmer practices. Stems were cut above
195 the ears and leaves were harvested to allow a quicker drying process. Fifteen days later,
196 when rains had stopped and plants were dry, maize was harvested and separated into
197 grains, damaged grains (i.e. broken, discoloured, shrivelled or undersized grains), cobs,
198 husks, and remaining stems. Maize dry matter production was evaluated as the sum of the
199 dry weight of all plant parts, i.e. grains, damaged grains, leaves, stems, cobs and husks.
200
201
202 *Residue decomposition and recovery of the amendments in different soil N pools*

After amendments, litter bags were made by packing remaining labelled canavalia hay from the ILT-Residue treatments 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, one litter bag was removed at random per plot.

At 1, 14, 26, 40, 54, and 147 DAA, 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 agglomerated. 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).

Three measurements of the bulk density of the topsoil were done per plot, and their mean was used in subsequent calculations.

Sample preparation and analysis

All plant samples were dried at about 40°C until a constant dry weight was reached, weighed and ground with a rotary knife mill at CIAT-Nicaragua. From each soil sample, a subsample was air-dried. All plant and soil samples were shipped to Switzerland where they were powdered with a ball mill (Retsch, GmbH, Germany) and analyzed for total N

226 and ^{15}N abundance at the Geological Institute of the ETH Zurich on a Thermo Electron
227 FlashEA 1112 coupled in continuous-flow with a Thermo-Fisher Delta V mass
228 spectrometer. Finely ground plant seed with an atom % ^{15}N of 0.514 was used as an
229 analytic standard.
230 The fresh samples were brought to laboratories of the Universidad Nacional Agraria in
231 Managua, and extracted on the next day following the method of Vance et al. (1987),
232 where two subsamples equivalent to 10 g soil dry matter were weighed and one was
233 fumigated with chloroform. Both subsamples were then extracted with 40 ml K_2SO_4 (0.5
234 M), and soil extracts were frozen and shipped to Switzerland. Total N was determined in
235 all extracts on a TOC/TN Analyzer (SKALAR, Netherlands). Nmic for each sample was
236 obtained by subtracting the N content of non-fumigated subsamples from fumigated
237 subsamples. In the extracts of the non-fumigated subsamples, NO_3^- and NH_4^+ contents
238 were determined on a flow injection analyzer (SKALAR San++ System, Netherlands),
239 and summed to obtain Nmin.
240 To determine ^{15}N-Nmin, extracts from non-fumigated samples were diffused on acid
241 filters following an adaptation of the method of Goerges and Dittert (1998). Briefly, 0.02
242 g MgO and 0.4 g Devarda's alloy were added to 12 ml extracts in 20 ml polyethylene
243 vials. Quartz filters (Whatman, QM-A) of 5 mm diameter were acidified with 10 µl
244 $KHSO_4$ 2.5 M and enclosed in polytetrafluoroethylene tape (Angst + Pfister, Dodge
245 Fibers Nr.121) below the vial caps. Vials were shaken horizontally for 72 h at 150 rpm,
246 before removing and drying the filters. The determination of ^{15}N-Nmic followed the same
247 principle. Extracts were autoclaved with $K_2S_2O_8$ (Cabrera and Beare 1993). Then 0.4 g
248 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 ^{15}N 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 ^{15}N enrichments were obtained by subtracting from the ^{15}N abundances the mean ^{15}N 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):

$$\%Ndff = \frac{\text{atom\% } ^{15}N\text{excess compartment}}{\text{atom\% } ^{15}N\text{excess amendment}} \times 100 \quad [1]$$

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

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

$$\text{weighted } ^{15}N \text{ enrichment} = \frac{\sum_{i=1}^{n} \text{atom\% } ^{15}N \text{ excess}_i \times \text{total } N_i}{\sum_{i=1}^{n} \text{total } N_i} \quad [2]$$

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):

$$\%Ndff = \left(1 - \frac{\text{atom\% } ^{15}\text{Nexcess compartment}}{\text{atom\% } ^{15}\text{Nexcess control compartment}} \right) \text{x } 100 \quad [3]$$

where atom% ^{15}Nexcess control compartment is the ^{15}N enrichment of the compartment considered, in the ILT-Control microplot of the same replicate (Figure 1).

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

$$\text{Ndff } [\text{g m}^{-2}] \text{ or } [\text{mg kg soil}^{-1}] = (\%\text{Ndff x TN}) / 100 \quad [4]$$

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:

$$\% \text{ Recovery} = \frac{\text{Ndff}}{\text{N applied}} \times 100 \quad [5]$$

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

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

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

$$^{15}\text{N-Nmic} = \frac{\text{total N}_{\text{fum}} \times \text{atom\% } ^{15}\text{N excess}_{\text{fum}} - \text{total N}_{\text{nonfum}} \times \text{atom\% } ^{15}\text{N excess}_{\text{nonfum}}}{\text{total N}_{\text{fum}} - \text{total N}_{\text{nonfum}}} \quad [6]$$

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

Labelling of canavalia and soil N

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 replicates 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%. In the 10-20 cm layer, total soil N had an average abundance of 0.626 atom% ^{15}N with a standard deviation of 0.067 atom% ^{15}N. In the 10-20 cm soil layer, the recovery from labelled fertilizer was on average 48%, with a standard deviation of 16%. Total recovery (in canavalia and in soil) from labelled fertilizer was therefore on average 98%.

Residue decomposition

340 The canavalia leaves decomposed faster than the stems (Figure 3). Thirty-three days after
341 the litter bag installation (i.e. 40 DAA), leaves were below the detectable weight limit.
342 The ^{15}N enrichment of stems and leaves decreased slightly with time, with stems more
343 enriched than leaves. The highest N release was observed between DAA 7 and DAA 26
344 with on average 202 mg N per litter bag, i.e. per 15 g residues. Knowing the amount of
345 residues applied in the microplots per m^2, the 202 mg N released per litter bags
346 corresponded to a release of 5.7 g N m^{-2}, of which 72% was from the leaves.
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349 *Incorporation of amendment N into soil N pools*
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351 The evolution of Nmin and Nmic with time is presented on Figure 4. The ILT and DLT
352 treatments are merged as amounts of Nmin and Nmic were not significantly different
353 between labelling methods (p=0.781 and p=0.058, respectively). After amendment
354 addition, Nmin slightly decreased for all treatments and then stayed stable during maize
355 growth. The two mineral fertilizer applications clearly affected the mineral soil N pool at
356 DAA 1 and 40 and were still observable at DAA 14 and 54. A net microbial
357 immobilization of up to 52 mg N kg^{-1} soil occurred between DAA 1 and 14 for all
358 treatments, followed by a net N release of up to 60 mg N kg^{-1} soil. The highest
359 immobilization was observed for the residues treatment and the lowest for the mineral
360 fertilizer treatment. Treatments had a significant effect on Nmic (p=0.011).
361 For the DLT treatments, Ndff and Ndfs were calculated for soil N pools. Ndff in Nmin
362 (Figure 5) shows that the differences between treatments observed in Figure 4 came from

363 the amendments. Except for the DLT-Mineral fertilizer treatment, most of Nmin was
364 derived from the soil. The Ndff in Nmic for the two most contrasting points regarding the
365 size of Nmic (Figure 4) is presented on Figure 6. Most of Nmic was derived from the soil.
366 The highest Ndff in Nmic was observed with the DLT-Residues treatment just after the
367 beginning of the rains (DAA 14) and represented 6% of Nmic. The DLT-Residue
368 treatment had also a higher Ndff in Nmic at harvest than the other treatments.
369 For the ILT treatments, Ndff and Ndfs in soil N pools are not presented because negative
370 estimates were often obtained; this is considered further in the discussion section. The
371 evolution of ^{15}N-Nmin and ^{15}N-Nmic with time is presented on Figure 7. Except for the
372 mineral fertilizer treatment, ^{15}N-Nmin decreased with time for all treatments. The ILT-
373 Control treatment had, at most time points, a higher enrichment than the other treatments.
374 The two applications of unlabelled mineral fertilizer at DAA 1 and 40 were clearly
375 diluting the enrichment, and were then followed by an increase of the enrichment up to a
376 level close to the ILT-Control treatment. After the dilution by the mineral fertilizer, the
377 strongest dilution was observed for the ILT-Residue treatment at DAA 14, and for the
378 ILT-Manure treatment at DAA 26.
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380
381 *Amendment N recovery in maize*
382
383 Maize dry matter production was on average 1344 g m^{-2}, with a standard deviation of 256
384 g m^{-2} (Table 2), and was not significantly different between ILT and DLT (p=0.410). The
385 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) or on N uptake (p=0.125). Maize from the DLT-Fertilizer treatment had the highest ^{15}N excess (Table 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 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%.

Total recovery of amendment N

Most of the amendment N was recovered in the 0-10 cm soil layer (Table 3). The total ^{15}N recovery was highest for the DLT-Residue treatment with 98% recovery, 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.

Discussion

Labelling of canavalia and soil N

409 Despite a cautious harvest, the nature of canavalia growth and the proximity of labelled
410 and unlabelled microplots introduced a small contamination of unlabelled canavalia
411 biomass. However, this contamination did not affect the ^{15}N abundance of soil N because
412 maize from the Control microplots was unlabelled (Table 2) and because soil N of the
413 control plots was close to the basic natural abundance (0.372 atom% ^{15}N, after harvest in
414 November 08).
415 Variation in ^{15}N enrichment of canavalia grown on ILT plots could be due to differential
416 mineral fertilizer leaching between microplots and varying N uptake by canavalia from
417 different soil layers, which in turn could be attributed to uneven distribution of stones in
418 the soil profile of the field. Particularly in the layer below 20 cm, total soil N was less ^{15}N
419 enriched than in the 0-20 cm layers (data not shown).
420 Because canavalia above ground ^{15}N enrichment varied between microplots, ^{15}N labelled
421 belowground biomass could contribute unequally to the N uptake of the subsequent
422 maize. Belowground N associated with or derived from roots can represent up to 50% of
423 the total plant N of legumes (Herridge et al. 2008) and can contribute substantially to the
424 subsequent crop. In both methods, ILT and DLT, belowground N contribution from
425 canavalia roots stood proxy for part of the soil N pool because labelled canavalia roots
426 remained in labelled soil and unlabelled roots in unlabelled soil. Soil ^{15}N enrichment
427 before application of the amendments showed low variation between the ILT treatments
428 (12% and 16% at 0-10 cm depth and 10-20 cm depth, respectively), suggesting that the
429 impact of ^{15}N decomposition of unevenly labelled belowground canavalia residues was
430 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 immobilization 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. Also, the 0-10 and 10-20 cm layer had similar enrichments.

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 partially covered the litter bags with soil. The residues in the 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, the litter bags should have been applied on the same day as the amendments, but due to time constraints it was done one week later. However, as no rain fell during this week, we assume that decomposition of the residues in the microplots was minimal before litter bags installation. 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 the litter bags by mineralization can be taken up by plants, get immobilized by microorganisms, be sorbed onto soil particles or be transformed into

forms prone to losses. The residues can also be incorporated into the particulate soil organic matter fraction. In the microplots, most residue N remained in the soil (Table 3). The time of highest N release (between DAA 7 and 26) corresponded to the highest microbial N immobilization (Figure 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 1), only 1.0 g N m^{-2} in average was recovered in maize (Figure 8). However, as stems were more enriched and decomposed more slowly than leaves, the residue recovery in maize may be underestimated because the maize took up N from the less enriched leaves. The Ndff for the DLT-Residue treatment calculated with the ^{15}N excess of the leaves was 1.5 g N m^{-2}, which corresponds to a recovery of 19%. The underestimation would be therefore around 50%.

Soil N dynamics after amendments

The Nmin initially decreased with the first rains. During the following period of maize growth, it stayed stable on a level of 8 mg N kg^{-1} soil. At DAA 147, after maize had been drying in the field for 15 days and was, therefore, no longer taking up N, it increased. 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 attributable to Ndff. The Ndff in Nmic was low and shows that this pool was mainly alimented by soil organic matter N.

476 The steady ^{15}N-Nmin decrease over time for all ILT treatments except the mineral
477 fertilizer treatment (Figure 7) could not be due to dilution by microbial turnover as ^{15}N-
478 Nmic was close to ^{15}N-Nmin at DAA 14 and was therefore attributed to mineralization of
479 unlabeled native organic N. The five years of canavalia cultivation and application as
480 green manure that occurred in the trial prior to our labelling resulted in the build up a
481 large unlabelled soil organic matter pool. We can assume that most of it entered the
482 potentially available soil N pool (Vanlauwe et al. 1998b).
483 The ^{15}N-Nmin was in general lower in the amended treatments than in the control which
484 can be explained by the dilution from the unlabeled amendments. After unlabelled
485 mineral fertilizer application, the ^{15}N-Nmin first decreased and then increased strongly.
486 This mineralization flush after addition of mineral fertilizers has been reported in other
487 studies (Kuzyakov et al. 2000). As the material mineralized was of higher enrichment
488 (labelled microbial biomass and canavalia roots) ^{15}N-Nmin increased up to the level of
489 the control. This flush can not be detected by observing the evolution of Nmin only, as a
490 net decrease in Nmin was observed at the same time (Figure 4).
491
492
493 *Indirect vs. direct labelling technique*
494
495 Compared to the DLT, the average Ndff ILT estimate from residues and sheep manure
496 was overestimated, suggesting a greater dilution of the label in the microplot treatment
497 compared to the control. The reason for this is not likely to be as a result of pool
498 substitution from microorganisms as the enrichment of Nmic was only slightly lower than

499 the enrichment of Nmin at the beginning of organic amendments decomposition (DAA
500 14). If pool substitution occurred, then it must result from soil N pools other than Nmin
501 and Nmic.
502 In this study, the main problem of ILT was high variation of the results caused by small
503 dilutions of the ^{15}N enrichments of the relevant pools. High variation with the use of ILT
504 has also been reported by other authors (McDonagh et al. 1993; Muñoz et al. 2003;
505 Stevenson et al. 1998). The dilution of ^{15}N-Nmin attributable to the amendments was
506 very small relative to the dilution from mineralization of unlabelled organic matter
507 (Figure 7). This was reflected in the differences between maize ^{15}N enrichment from the
508 control and the treatments in each plot. The smaller the difference between ILT-Control
509 and treatment, the more inaccurate and variable the Ndff estimates were. Negative
510 differences resulted in negative Ndff values.
511 These problems did not occur with the DLT method, where ^{15}N-Nmin and ^{15}N-Nmic
512 were directly attributable to the amendments. Therefore, results from the DLT are
513 considered more relevant to define the availability of canavalia residues and manure for
514 maize. Still, the recovery with the mineral fertilizer treatment may be underestimated due
515 to an isotope displacement reaction, described by Jenkinson et al. (1985) as the
516 displacement of unlabelled NH_4^+ from clay minerals by the added labelled ammonium
517 sulphate, or through the priming of soil organic N mineralization seen from the evolution
518 of ^{15}N-Nmin in the ILT (as noted earlier). Seen the rapid mineralization from canavalia
519 residues, the recovery with the residue treatment may also be underestimated.
520
521

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. At a recovery of 12% of applied N, the recovery of canavalia residue N 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. (1998a) reported 9% Leucaena N recovery in maize, McDonagh et al. (1993) 12 to 26% groundnut N recovery in maize, and Toomsan et al. (1995) 15 to 23% soybean N recovery in rice and 8 to 22% groundnut N recovery in rice. The 3% recovery of sheep manure N was lower than the 10% recovery in winter wheat reported for the same manure by Bosshard et al. (2009). These rather lower recoveries are most probably due to the high soil N availability at the research site. Furthermore, lateral root growth of maize growing inside the microplots at a soil depth of more than 15 cm (i.e., underneath the 15 cm deep microplot borders) might have given access to additional unlabelled soil N.

Most of the amended N remained in the soil. This observation is consistent with 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, suggests that a high amount of NH_4^+ has been retained on clay minerals. Since the mineral fertilizer was applied as solution which rapidly infiltrated into the soil, there was no significant loss of N from mineral fertilizer in gaseous form. As N recovery in soil was higher for canavalia residues than for mineral

fertilizer, higher residual effects can be expected from canavalia for further cropping (Vanlauwe et al. 1998b).

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 to assess the N fertilizer value of mineral and organic amendments. This is due to the presence of an important unlabelled mineralizable soil N pool. Pool substitution from microorganisms 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 less fertile soils, it is not sufficient in soils with high amounts of labile soil organic matter. With DLT amendment recoveries in maize would probably be higher at a site with lower soil available N content.

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677 **Figure captions**

678

679 **Fig 1.** Microplot design for one of the three replicates of the trial. ILT and DLT stand for
680 indirect and direct labelling technique, respectively. Grey colour indicates microplots
681 with labelled available soil N. Dark grey squares represent the litter bags. Dashed line is
682 the border of the plot.

683

684 **Fig 2.** Rainfall distribution and crops during the field experiment.

685

686 **Fig 3.** Decomposition (a), ^{15}N abundance (b) and N release (c) per litter bag from
687 canavalia stems and leaves, with days after amendments (DAA). Error bars represent the
688 least significant difference (LSD).

689

690 **Fig 4.** Changes in soil mineral N (a) and microbial N (b) pools with days after
691 amendments (DAA) for all treatments. Averages of ILT and DLT. Error bars represent
692 the least significant difference (LSD).

693

694 **Fig 5.** N derived from the amendments (Ndff) and from the soil (Ndfs) in soil mineral N
695 for the DLT treatments at each time point. DAA stands for days after amendments. Error
696 bars represent the least significant difference (LSD): the LSD above is for Ndff and the
697 LSD below is for Ndfs.

698

Fig 6. N derived from the amendments (Ndff) and from the soil (Ndfs) in soil microbial N for the DLT treatments for two time points. DAA stands for days after amendments. Error bars represent the least significant difference (LSD): the LSD above is for Ndff and the LSD below is for Ndfs.

Fig 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 least significant difference (LSD).

Fig 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). Least significant difference is 6.1 g N m^{-2} for the ILT Ndff and 0.6 g N m^{-2} for the DLT Ndff. Least significant difference is 86.7% for the ILT recovery and 8.8% for the DLT recovery.

Table 1. Amendments composition and dose of application, on a dry matter basis.

Table 1. Amendments composition and dose of application, on a dry matter basis.

Treatment	Amendment	Total N $g\ kg^{-1}$	C:N ratio	^{15}N abundance atom % ^{15}N	P $g\ kg^{-1}$	K $g\ kg^{-1}$	Lignin $g\ kg^{-1}$	Polyphenols $g\ kg^{-1}$	Dosis $g\ N\ m^{-2}$
ILT - Control	-	-	-	-	-	-	-	-	-
ILT - Fertilizer	$(NH_4)_2SO_4$	223.0	-	0.36	-	-	-	-	8
ILT - Residues	Canavalia	19.7	21	0.38	3.1	14.4	87.3	125.3	8
ILT - Manure	Cow manure	17.1	6	0.37	5.9	17.0	-	-	13
DLT - Fertilizer	$^{15}(NH4)_2SO_4$	230.0	-	10.00	-	-	-	-	8
DLT - Residues	^{15}N-labelled canavalia	18.8	20	1.61	3.2	15.3	75.9	156.2	8
Control	-	-	-	-	-	-	-	-	-
ILT - Check manure	Sheep manure	32.0	5	0.41	35.1	13.3	-	-	4
DLT - Check manure	^{15}N-labelled sheep manure	35.0		11.23	39.9	25.9	-	-	4

Table 2. Maize dry matter production, nitrogen uptake and enrichment for each treatment at harvest.

Treatment	Dry matter		N uptake		^{15}N enrichment [2]
	Total [1]	Grains	Total [1]	Grains	
	g m^{-2}	g m^{-2}	g m^{-2}	g m^{-2}	atom% ^{15}N excess
ILT - Control	1085	396	11.1	5.4	0.466
ILT - Fertilizer	1431	489	13.7	7.0	0.404
ILT - Residues	1461	583	15.4	9.1	0.383
ILT - Manure	1317	507	12.5	6.9	0.342
DLT - Fertilizer	1625	493	14.9	6.7	1.680
DLT - Residues	1424	543	14.5	7.7	0.075
Control	1477	649	16.7	10.8	0.000
ILT - Check manure	1244	477	11.2	6.6	0.410
DLT - Check manure	1028	429	9.5	5.6	0.143
LSD	535	326	6.9	6.0	0.101 / 0.383 [3]

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

[2] weighted enrichment for all plant parts

[3] ILT / DLT

Table 3. ^{15}N recovery (%) in maize and in different soil N pools (0 - 10 cm) at maize harvest, for the direct labelling technique. Total recovery is the sum of recoveries in maize and total soil N.

Treatment	Maize	Soil			Total
		Ntot	Nmin	Nmic	
DLT - Fertilizer	31.8	50.1	1.1	0.82	82
DLT - Residues	12.0	85.8	0.9	2.94	98
DLT - Check manure	2.9	73.3	1.1	~ 0	76
LSD	8.8	31.1	1.3	8.8	