Université des Sciences et Techniques du Languedoc

Master « Sciences et technologies »

Mention " Biodiversité Ecologie Evolution B2E"

Parcours « Ecosystèmes »

Projet de Recherche:

OPTIMIZING SAMPLING PROCEDURES IN SUGARCANE PLANTATIONS TO STUDY

NITROGEN CYCLING WITH ^{15}N LABELLING METHOD

Par
POULTNEY Daniel

Stage de M2

Réalisé sous la direction de
Versini Antoine
Chercheur encadrant

UPR Recyclage et risque - CIRAD

1. Introduction

1.1. Global nitrogen cycles in terrestrial ecosystems

Nitrogen (N) is fundamental to life as a major component of the nucleic acids, that determine the genetic character of all living organisms, and of enzyme proteins that drive the metabolic machinery of every living cell (Galloway & Cowling, 2002). N_2 is highly abundant in the atmosphere, but needs to be broken down into a form biologically available to organisms, by bonding chemically to other essential elements (Cowling & Galloway, 2002). Non-biological fixation occurs in the air by means of lightning but most natural fixation is done biologically by free-living, symbiotic or associative bacteria and blue-green algae. In the pre-human world, biological N fixation was thus the dominant means by which new reactive nitrogen (Nr) was made available to living organisms (Galloway & Cowling, 2002). The term reactive nitrogen is defined as all biologically, photochemically, and/or radiatively active forms of N. This has changed drastically with population growth and increasing consumption. Between 1890 and 1990, the human population increased by a factor of approximately 3.5, global food and energy production increased approximately 7-fold and 90-fold respectively, leading to a 9-fold increase of anthropogenic Nr (Galloway & Cowling, 2002). This trend is the result of increased NOx emissions due to fossil fuel energy production (particularly for transport and industrial plants), increased biological fixation due to extensive cultivation of legumes and, above all, the growing use of N fertilisation with the invention of artificial N fixation (the industrial Haber-Bosh process). Nitrogenous fertilisers now account for 33% of the total annual creation of Nr, and for 63% of all anthropogenic sources of Nr (Doberman, 2005). Nitrogenous fertilisers used for food production have played a crucial role in contributing to this substantial increase (Smil, 2002). Although fertilizers have had, in part, a largely positive impact on society by contributing to food security and adequate nutrition (Doberman, 2005), the consequence has been that when accumulated in excessive amounts in terrestrial and aquatic ecosystems, and in the atmosphere, Nr leads to a significant impact on environmental quality, ecosystems, biodiversity and human health (Doberman, 2005). The greatest overall challenge, therefore, as posed by Galloway *et al*. (2008), would be to find the means to maximise the benefits of anthropogenic Nr while minimising its unwanted consequences.

1.2. Nitrogen biogeochemical cycles in agroecosystems

Nitrogen is essential for plant growth and often the primary nutrient limiting agricultural productivity (Barrios, 2007). In agroecosystems, the crop N requirement can be supplied by several sources: microbial-mediated mineralization of soil organic matter, mulch and root harvest residues, application of organo-mineral fertilisers, biological N fixation and, to a lesser extent, atmospheric deposition. With the development of intensive agriculture since the Green Revolution, fertilisation has been used to meet the crop demand for N with the application of various forms of organic and inorganic N. Fertiliser N can be lost to the atmosphere via NH_3 volatilization, which can be harmful to plants and reduces air and water quality. In the troposphere, NH_3 gas reacts with nitric and sulfuric acids to form nitrate-containing particles that contribute to aerosol pollution that is damaging to human health. Ammonia gas can also fall back to Earth and enter the hydrosphere, contributing to acid rain events and causing eutrophication. This process leads to high algal population and growth, which reduces dissolved oxygen in the water and at high enough levels would lead to dead zones. At a local scale, fertiliser N can alternatively be converted into nitrate (NO_3) during the process of nitrification and be lost to the hydrosphere via deep drainage. NO_3 produced by this oxidation process can enter groundwater, which can be hazardous in drinking water.

When groundwater recharges stream flow, nitrate-enriched groundwater can also contribute to eutrophication. At a global scale, agricultural activities play a major role in the global fluxes of the greenhouse gases CO_2, CH_4 and N_2O. Together, agriculture, forest and land use change are responsible for 24% of anthropogenic greenhouse gases emissions expressed in CO_2 equivalent (IPCC, 2014). The contribution of agriculture alone was 10-12% in 2007 and is continually increasing. The agricultural sector produces approximately 50% of the CH_4 emissions and 85% of the anthropogenic emissions of N_2O; a gas with a global warming potential approximately 300 times higher to that of the CO_2 (IPCC, 2007). Consequently, crop nutrition, and in particular N fertilization in agroecosystems, should therefore be optimized to sustain crop productivity while limiting N contaminations at local and global scales.

1.3. Nitrogen use efficiency improvement through fine-tuning of N fertilisation timing

One important means to optimize N cycling and to mitigate Nr creation in agroecosystems is through improvements in fertiliser nitrogen use efficiency (NUE), where less N fertiliser is used per unit food produced. This has been a concern for decades and it is anticipated that fertiliser management will be at the forefront of measures to improve the global N balance over the short- and long-term (Dobermann, 2005). In particular, achieving synchrony between N supply and crop N demand without excess or deficiency is the key to optimizing trade-offs between yield, profit and environmental protection (Cassman *et al.*, 2002). Two main indices are used in agronomic research to assess the efficiency of crops to use nitrogen applied to soils through fertiliser (both indices are known as Recovery Efficiency of N or RE_N). The first approach, the "difference method", corresponds to the broadest measures of NUE. It is based on crop yield variations observed in the relationship between applied N and aboveground biomass (in French the term is *coefficient apparent d'utilisation* or CAU). The second approach to studying NUE uses ^{15}N-labelled fertilisers to estimate the crop recovery of applied N (*coefficient reel d'utilisation* or CRU in French). In addition, the ^{15}N tracer can be used to determine the fate of fertiliser N in distinct compartments of an ecosystem (plant, soil and soil solutions), and to estimate the contribution of an N source to the N stocks of a given compartment (Versini *et al.* 2014). Whichever approach is used, the calculation of the RE_N indices requires a precise estimation of the amount of N contained in the aboveground biomass of the sugarcane. It is for this reason that it remains challenging to study NUE at different phases of crop growth as the biomass can normally only be determined at the end of the crop cycle when the crop is harvested.

1.4. Studying NUE in sugarcane agroecosystems with ^{15}N labelling method

Due to the high cost of ^{15}N labelled compounds, the size of the field plots is a major constraint in the use of the ^{15}N labelling method. In most studies involving annual crops, ^{15}N microplots have a minimum of 3 row segments, 2 to 3 meters long, placed inside larger plots fertilised at the same rate with non-labelled fertiliser, that are used to obtain yield results (Trivelin *et al.*, 1994). Trivelin *et al.* (1994) established that 2 m long single row microplots of ratoon cane[1] are sufficient to determine fertiliser N recovery by the crop using ^{15}N fertiliser, therefore saving one third of the labelled isotope used in conventional designs.

Most studies using the ^{15}N method to estimate RE_N in sugarcane agroecosystems have focused on fertiliser NUE at the end of the crop cycle (Chapman *et al.*, 1994, Basanta *et al.*, 2003, Isa *et al.*, 2006, Fortes *et al.*, 2010, Ambrosano *et al.*, 2011). Although there are a select few studies which have investigated NUE during the crop cycle and crop development (Courtaillac *et al.*, 1988, Kee Kwong *et al.*, 1994), and certain others the plant utilisation of

---

[1] "Ratoon" cycles are each successive cycle of plant growth after harvesting.

the N derived from fertilizer (Ndff) at different phases of the crop cycle (Franco *et al.*, 2011; Vieira-Megda *et al.,* 2015). Ndff is not directly related to NUE but provides information on the relative importance of an N source such as fertiliser on plant nutrition. One study shows that the Ndff was 10% of total N at the harvest of a planted cane and 30% at the harvest of the first ratoon in Brazil (Franco *et al.*, 2011). However, the same study showed that at initial stages of crop development, the Ndff was between 40 and 70%, highlighting the crucial role of fertilisation for sugarcane nutrition at early stages of development. These results point to the relevance of considering the whole crop cycle when studying fertiliser NUE in sugarcane agroecosystems, and for this purpose, to develop non-destructive methods of biomass and N content estimations. Destructive sampling of biomass during the crop development is indeed not possible in agronomic trials. Alternatively, sugarcane biomass can be simulated with sugarcane growth models or estimated non-destructively using allometric relationships (Jean-François Martine, pers. comm.), while their N content could be estimated with representative leaves (Trivelin *et al.*, 1994) or from dilution curves (Jean-François Martine, pers. comm.).

In most of the studies cited, the root compartment (i.e. belowground biomass) is not accounted for, where belowground biomass amounts to approximately 20% of the aboveground biomass (Smith *et al.*, 2005) and should therefore be considered in ^{15}N recovery calculations. To conclude, most of the studies have been done in a Brazilian agricultural context. It would therefore be important to test whether similar findings would apply to other sugarcane varieties and pedoclimatic conditions, which is especially relevant as efficient N fertilisation depends a number of factors, including soil properties, crop variety, the source of N, and climate.

1.5. Evaluating organic fertilisation in sugarcane agroecosystems in Reunion Island

The volcanic island of Réunion is situated in the Indian Ocean, and has a growing population of about 850,000 (growing by over 10,000 a year) and a small portion of arable land (17% of the 2,5 000 km^2 area). There is a strong dependence on import, for example inputs for agricultural production or food consumption, which is increasing. This means, on the one hand, that smallholders depend on increasingly expensive inputs (e.g. fertilizers) from the global market, thus threatening the competitiveness of their produce. On the other hand, this situation, exacerbated by stringent EU regulation, leads to a pressing need for solutions to rapidly increasing organic waste management problems. The recycling of organic residues in agricultural land, therefore, appeared to be a potentially promising alternative, and a means to promote circular economic and agricultural sustainability in the framework of this agroecological transition. Rehabilitating disrupted nutrient cycles through organic residue recycling in agriculture may carry the promise of enhancing the eco-efficiency and resilience of agriculture while reducing environmental pressure (Wassenaar *et al.*, 2014). In this context, it is important to investigate the efficiency of organic fertilizer in order to assess its potential effect both on crop nutrition and environmental impact, compared to conventional fertilization practices. However, the production of ^{15}N labelled organic fertilizers, such as liquid pig manure or sewage sludge, is particularly costly and time consuming. The experimental design and the sampling procedure that make possible the study of fertilizer NUE in sugarcane agroecosystems with the ^{15}N labelling method should therefore first be optimized with less costly ^{15}N-labelled urea.

1.6. Study hypotheses

In this methodological study, we hypothesised that: 1/ leaf collection in a 1.5 x 1.5 m ^{15}N microplot can be used to determine Ndff; 2/ non-destructive methods such as allometric relationships can be developed in order to estimate aboveground biomass during the

sugarcane crop development; 3/ non-destructive methods involving leaf representativity or dilution curves can be developed in order to estimate the N content of the biomass during the sugarcane crop development; and 4/ the root biomass is not an insignificant compartment, and can be taken into account through soil core sampling in ^{15}N recovery budgets.

2. Objectives

The overall objective of this project was to experiment sampling procedures that make possible the determination of fertiliser NUE during the various phases of sugarcane development. More specifically, non-destructive methods of estimating biomass, N and ^{15}N content were tested to allow RE_N to be calculated from Ndff. Firstly, the ^{15}N microplot methodology, which is commonly used to assess Ndff in sugarcane agroecosystems, was tested and adapted to the present context. Secondly, three methods of estimating sugarcane aboveground biomass were studied: 1/Localised harvesting of 3 m linear sugarcane plots; 2/ Application of allometric relationships to cane height inventories; and 3/ Simulation with the sugarcane growth model MOSIWEB. The third objective was to investigate how to determine the N content of the sugarcane biomass where two methods were tested: 1/ The use of a single leaf or combination of leaves which were representative of the N content of the entire plant; and 2/ The use of a dilution curve of sugarcane N content in response to biomass, as a reference to predict the N content of the sugarcane at a given time. The investigated procedures were applied to a case study dealing with the fate of urea-derived N in the different biomass compartments of a sugarcane agroecosystem of Reunion Island. Finally, the relative importance of the root compartment in NUE estimations were considered, and the ^{15}N recovery in the soil compartment computed to obtain further information that may be of relevance to this case study.

3. Methodological strategy

3.1. Study site and experimental design

The study site is the experimental station of La Mare, which is closely situated to Saint-Denis, Reunion Island (Lat 20°54'12.2"S, 55°31'46.6"E). The experimental trial takes place at a highly monitored site of the SOERE-PRO network (Système d'Observatoires, d'Expérimentations et de Recherche en Environnement sur les Produits Résiduaires Organiques) designed to investigate the long-term impact of organic fertilization on the different compartments of the sugarcane agroecosystem (Figure 1). The site is characterized by a tropical climate with an average annual temperature of 25°C and annual precipitation of 1650 mm. The soil is a silt-clay nitisol (FAO, 1998) with a CEC of 108.6 mmol/kg and a soil organic carbon content of 2%. The trial was planted on March 2014 from viable buds of the R579 sugarcane variety placed with 1.5 m spacing between rows. The trial consists of 6 plots, each with a different fertilizer treatment, which is repeated in 5 blocks, with each plot made up of 6 sugarcane rows of 28 m, constituting a total plot area of 250 m^2.

This experiment was conducted during the third ratoon of sugarcane in a control plot fertilized with urea (plot TA1 in Figure 1). In this plot, 97 kg N ha^{-1} was applied following the annual harvest in December 2016. Inside the experimental plot of sugarcane ratoon, three sugarcane microplots of 2.25 m^2 (1.5 m x 1.5 m), as well as nine soil microplots of 22.5 cm^2 (15 cm x 15 cm) received labeled urea (3 atom% ^{15}N) in the same quantity as the conventional non-labeled urea (Figure 1). The soil microplots were clustered into three groups, with each group at a different position in the sugarcane plot, and the microplots spaced at equal distances to the row of sugarcane. ^{15}N urea fertilizer was applied homogeneously across the microplots on December 7, 2016.
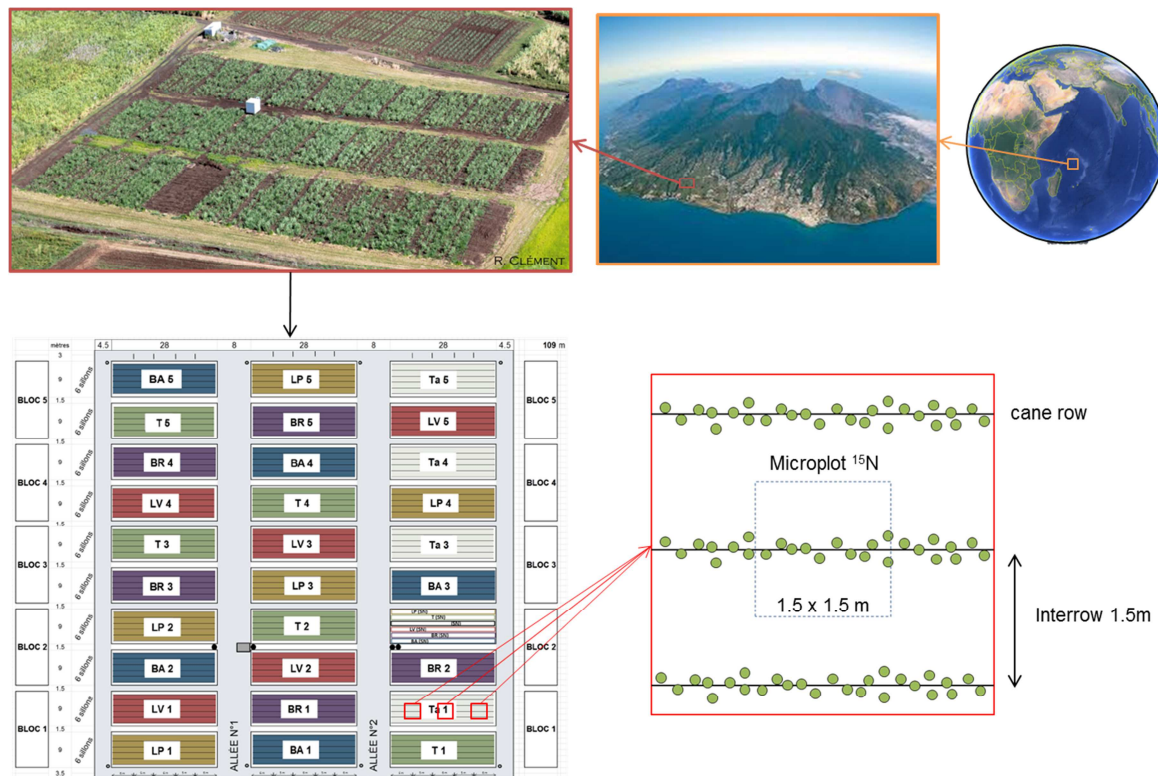
Figure 1. Study site localization (top) and experimental design schemes (bottom). Experimental design caption: T control with annual urea fertilization, BA annual organic fertilization with sewage sludge, BR organic fertilization with sewage sludge at planting, LP annual organic fertilization with pig slurry, LV organic fertilization with poultry manure at planting, Ta control without urea fertilization once in every four years, SN bare soil.

There were two main sampling dates, 1 February 2017 (t1) and 1 April 2017 (t3), respectively 2 and 4 months after the urea application, and 3 and 5 months after the beginning of the 3rd ratoon. Two additional sampling dates were scheduled for the biomass estimation components of the experiment, on 2 March 2017 (t2) and 4 May 2017 (t4).

3.2. Estimation of the Ndff

3.2.1. Leaf+3 collection at various distances relative to the ^{15}N microplot centre

Lateral ^{15}N movement was tested along a horizontal gradient to validate Trivelin *et al.*'s (1994) use of 2m X1.5 m ^{15}N enriched microplots to determine Ndff, and to test the underlying assumptions of the lateral transfer of ^{15}N between the microplot and its surroundings. The third leaf below the top visible dewlap (Leaf+3) was sampled within the microplot (Figure 2; zones A and B) and at 50 cm distance intervals relative to the microplot enriched in ^{15}N, along the row of sugarcane (zones C, D and E). Samples were also taken at the sugarcane row adjacent, in line with the microplot (zones F and G).

3.2.2. Leaf representativity of ^{15}N content

Individual sugarcane were harvested at the centre of each of the three microplots. The ^{15}N enrichment of different sugarcane leaves were analyzed and compared to the ^{15}N enrichment of the total aboveground biomass in order to test their reliability to represent the aboveground biomass ^{15}N in Ndff calculations as proposed by Trivelin *et al.* (1994). The "Leaf+3" is typically used to represent the aboveground (Trivelin *et al.* 1994), and was further divided into three segments: the base of the leaf (notated as Leaf + 3 1/3); the middle segment (Leaf + 3 2/3) and the tip of the leaf (Leaf + 3 3/3). An independent sample t-test was used to test whether the N and ^{15}N values from the plant compartments were significantly different from the aboveground biomass at a 95% confidence interval.

3.3. Estimation of the sugarcane biomass

3.3.1. 3 m linear biomass collection

A 3 m linear plot of sugarcane was harvested at different locations in the sugarcane plot at each of the four sampling dates to give an actual measure of sugarcane biomass to which the non-destructive estimations of biomass could be compared.

3.3.2. Cane height inventory and cane collection for allometric relationships

Despite few research papers dealing with the use of allometric relationships in estimating biomass, sugarcane biomass can theoretically be estimated non-destructively using allometric relationships (Jean-François Martine, pers. comm.), i.e. the relationship between the mass of a sugarcane plant and other measurable traits. From a preliminary study, the measurable traits selected were sugarcane height (length from the base to the top visible dewlap (TVD) and to the "Leaf+3"), as well as the diameter of the plant, measured at the base of the Leaf+3. The biomass of the different plant compartments were measured separately, being the leaf blade, sheath, useable stem and straw. 3 m linear plots of sugarcane were harvested at the beginning of each month, for four consecutive months (t1, t2, t3 and t4). Before harvesting, height measurements were taken for all the sugarcane at four 3 m linear plots. Height classes were established as the mean of each quintile of sugarcane heights. Twenty individual sugarcanes were then harvested at each date, comprising four individuals from each of the five height classes. These samples were then oven dried at 60°C for 72 hours, and the dry mass of the different compartments measured. For each sampling date, four functions were tested in their ability to represent sugarcane biomass as a function of sugarcane height: linear, exponential, power and polynomial functions. The function was fitted to the data points and the parameters of the model determined, using the *nls* command of the software R. In the preliminary study, cane height

Figure 2. Isotopic enrichment of leaves+3 (^{15}N atom%) in 50 cm long zones along and adjacent to sugarcane row for sampling dates t1 and t3. Colour-coded according to the level of ^{15}N enrichment, with green being the highest, yellow intermediate and red indicating zero enrichment. Excess ^{15}N atom% was calculated by deducting the atom% of a control leaf from the atom% of the Leaf+3 samples. Standard deviation is given (n=3).

(TVD) was found to be the most suitable measurable trait for comparison to cane biomass, as it provided both the best fit for functions, and is the most practical plant trait to measure in the field. For each date, the most suitable model was selected which best fit the data, according to its R^2 value and its AIC rank with an Aikaikee test. The allometric relationships were applied to four 3 m linear sugarcane height inventories in the plot. This allowed biomass to be estimated at the plot scale. The difference between measured and estimated biomass (Δ Biomass %) was also used to select the best functions to estimate sugarcane biomass. For this calculation, the allometric relationships were applied to the height inventory of the same 3 m linear plots used for the measured biomass values (cf. 3.3.1).

3.3.3. MOSICAS sugarcane growth model

The potential of the sugarcane growth model MOSICAS to estimate aboveground biomass at the plot scale was also tested. MOSICAS is a semi-mechanistic and climate-dependent model of sugarcane growth developed by Jean-François Martine for different varieties in La Reunion (Martine, 2003). The simulated growth depends on climate variables such as water supply (precipitation and irrigation), potential evapotranspiration, temperature and solar radiation, as well as soil and plant parameters. The version of the MOSICAS model used is available on the Margouill@ platform (*Modélisation de l'Agriculture Réunionnaise par Géolocalisation et OUtils Internet et Libres*) under the name "MOSIWEB".

The three methods of estimating sugarcane biomass were then compared. The values of the biomass weighed (averaged as $g.m^{-2}$); the sugarcane biomass estimated by allometric relationships and applied as an average at the plot scale using the 4 height inventories, and the biomass estimation by the MOSIWEB growth model, also at the plot scale.

3.4. Estimation of the sugarcane nitrogen content

3.4.1. Leaf representativity of N content

The reliability of leaves to represent the aboveground biomass N content was tested in the same way as for ^{15}N content (cf. 3.2.2).

3.4.2. Dilution curve

The concept of the N dilution curve based on plant N concentration was developed by Lemaire and Salette (1984) for tall fescue, and it is a curve representing the quantity of N as a function of plant biomass. It is primarily used to monitor and fine-tune fertilizer input in sugarcane agroecosystems, but can also be used as a predictive reference from which N content can be determined from plant biomass at a plot scale. N dilution curves have been determined for various crops and were established for several varieties of sugarcane at various sites in Reunion by Pouzet *et al.* (1999). We investigated the use of this curve for our sugarcane variety (R579) and site, by analysing the N content in 3 m linear plots harvested at each date (cf. 3.3.1). In addition, the mean value of three individual cane at the two sampling times were extrapolated to a metre squared, by multiplying the biomass by the average number of cane individuals over a metre squared.

3.5. 15N recovery budget

3.5.1. ^{15}N recovery calculations

All samples requiring N and ^{15}N analysis were ground to 500 μm using a Cyclotec grinder (CT Tecator Cyclotec Sample Mill, Foss), sent to the PTEF laboratory in Nancy (Plateforme Technique d'Ecologie Fonctionnelle, INRA), where samples were further ground to 100 μm using a mixer mill (MM400, Retsch) and analysed for N and ^{15}N concentrations with an Elemental analyzer (vario ISOTOPE cube, Elementar, Hanau, Germany) interfaced in line with a gas isotope ratio mass spectrometer (IsoPrime 100, Isoprime Ltd, Cheadle, UK).

Nitrogen derived from fertilizer (Ndff) was determined by the following formula :

$$Ndff = \left[\frac{a-b}{c-d}\right].100 \qquad (1)$$

where Ndff is the proportion of N in the plant derived from fertilizer (%), *a* is the abundance of ^{15}N atoms in the plant (%), *b* is the natural abundance of ^{15}N atoms in a control plant sample (%), *c* is the abundance of ^{15}N atoms in the fertilizer (%) and d is the natural abundance of ^{15}N atoms of a standard (0.366%).

Due to sugarcane's ability to uptake N via roots up at considerable distances from the stem of the plant (Smith *et al.*, 2005), the Ndff of sugarcane outside of the microplot should be considered as follows:

$$Ndff_T = Ndff_M + 2 \times Ndff_{AR} \qquad (2)$$

where *Ndff_T* is the sum of the Ndff determined from the centre of the ^{15}N microplot (*Ndff_M*); and the Ndff from the two adjacent cane rows in line with the microplot (*Ndff_AR*).

The assumption is that sugarcane in the row adjacent to the microplot take up the same amount of N inside the microplot as the cane inside the microplot take from the row adjacent (Trivelin *et al.*, 1994). Ndff is therefore diluted within the microplot and the Ndff in the row adjacent should be added. Ndff of cane in the same row but outside the microplot is also considered.

Nitrogen recovery in the plant biomass, was calculated by this formula:

$$RE_N = \frac{Ndff*N_{plant}}{N_{fertilizer}} \qquad (3)$$

where *RE_N* is the recovery efficiency of fertiliser N in the plant (%), *N_plant* is the quantity of N in the plant (g.m^-2) and *N_fertilizer* is the quantity of N applied with the fertilizer (g.m^-2).

3.5.2. Root sampling

The relative importance of the root compartment on Ndff and *RE_N* estimations was investigated in addition to the aboveground plant biomass. Soil cores were sampled with a 1 m gouge coupled with a percussion hammer (Cobra TT, SDEC). Three soil cores were taken at t1 and t3 at three distances relative to the sugarcane row: 0-25 cm; 25-50 cm and 50-75 cm (cf. Annex).

3.5.3. Soil sampling

Soil samples were taken at the 15 cm x 15 cm microplots at t1 and t3, sampled to a depth of 50cm (cf. Annex). N recovery in the soil was calculated by this formula:

$$RS_N = (A^{15}N - A^{15}N_{CTL}) * \frac{N_{soil}}{N_{fertilizer}} \qquad (4)$$

where *RS_N* is the recovery of fertiliser N in the soil (%), *A^{15}N* is the abundance of the soil sample (%), *A^{15}N_{CTL}* is the natural abundance of a control sample (%), *N_soil* is the quantity of N in the soil layer (g.m^-2) and *N_fertilizer* is the quantity of N applied with the fertilizer (g.m^-2).

4. Results

4.1. Estimation of the Ndff

4.1.1. 15N horizontal gradient from the microplot centre

The ^{15}N enrichment is more than twice as high at the first sampling date (t1) than the second (t3) in the microplot and zone adjacent (zones A, B and C 2.4, 2.4 and 2.2 times higher in t1 than in t3 Figure 2, respectively). However, the lateral ^{15}N movement follows a similar trend over the two dates. The ^{15}N enrichment is highest in the centre of the microplot and decreases steadily, moving laterally away from the centre.

[15]N excess atom percentages were respectively 1.39, 0.77, 0.21, 0.07, 0.00 at t1 and 0.57, 0.32, 0.09, 0.01, 0.00 at t3, in zones A, B, C, D and E. At a distance of 1 m from the border of the microplot (zone E), there is no longer ^{15}N enrichment. The sugarcane row opposite the microplot is also enriched in [15]N (zone F with a ^{15}N excess atom% of 0.05 at t1 and 0.01 t3; and in addition zone G with a ^{15}N excess atom% at t3 and 0 at t1). The red zones with approximately zero enrichment indicate the spatial limits of ^{15}N lateral transfer in the experiment.

4.1.2. Aboveground biomass 15N representativity of leaves

There was no significant difference ($p < 0.05$) between [15]N% of the aboveground biomass and each of the cane compartments at both t1 and t3 (Table 1). The order of the most to least representative leaves at t1 relative to the aboveground biomass ^{15}N was : Leaf+3 1/3 > Leaf+5 > Leaf+1 ≥ Leaf+2 > Leaf+3 ≥ Leaf+4 > Leaf+3 2/3 > Leaf+3 3/3; and for t3: Leaf+3 2/3 > Leaf+3 > Leaf+3 1/3 ≥ Leaf+3 3/3 > Leaf+2 > Leaf+4 > Leaf+1 > Leaf+5.

4.2. Estimation of sugarcane biomass

4.2.1. Destructive measurement of sugarcane biomass

The biomass collected showed a steady increase over the first three sampling dates (546 g.m^{-2},1271 g.m^{-2}, 2233 g.m^{-2} at t1, t2 and t3). The biomass collected at t4 (2069 g.m^{-2}) is lower than at t3.

4.2.2. Construction of allometric relationships

For t1, the power function has the highest R^2 value and lowest AIC weight, as well as the lowest Δ biomass % (Table 2). For t2, the exponential and polynomial functions have the highest R^2 values and lowest AIC weights, followed closely by the power function. However, the power function has by far the lowest Δ biomass % value, and is selected as the most suitable function across these criteria. For t3, the power function is the most suitable function across selection criteria, having the highest R^2 value, lowest AIC weight and lowest Δ biomass % value. For t4, the exponential, power and polynomial models are closely ranked, having the highest R^2 values, the lowest AIC weights, and lowest Δ biomass % values, with very similar values for these three selection criteria. Although each of these functions could feasibly be used, the power model is selected in order to keep a consistent model between dates. The allometric relationships between cane height and biomass are displayed graphically using the power function for each sampling time (Figure 3). When the power functions at each respective date are applied to the four height inventories of the cane to predict their corresponding biomass (Figure 4), a steady increase in biomass estimated is observed with time (509 ± 31 g.m^{-2}, 1252 ± 84 g.m^{-2}, 2203 ± 68 g.m^{-2}, 2726 ± 144 g.m^{-2} at t1, t2, t3 and t4, respectively). A very similar trend is observed for the biomass collected and estimated with allometric relationships for the first three dates (Figure 3). However, at t4, there is a divergence between these values.

4.2.3. Simulation with MOSIWEB

The MOSIWEB simulated biomass values also show a steady increase between sampling dates (1870 g.m^{-2}, 2425 g.m^{-2}, 2872 g.m^{-2}, 3381 g.m^{-2} at t1, t2, t3, t4, respectively; Figure 4). The biomass values estimated by the MOSIWEB growth model were higher than the measured values at each date (by a difference of 1324 g.m^{-2}, 1154 g.m^{-2}, 639 g.m^{-2}, 1365 g.m^{-2} at t1, t2, t3, t4, respectively).

Table 1. Nitrogen concentration (%) and ^{15}N abundance (%) of the different plant components at sampling times t1 and t3 (*n*=3).

Sugarcane compartment	t1		t3	
	N%	^{15}N%	N%	^{15}N%
Leaf+1	1.06 ± 0.13*	1.36 ± 0.42	0.91 ± 0.05*	0.89 ± 0.12
Leaf+2	1.06 ± 0.06*	1.48 ± 0.49	0.95 ± 0.11*	0.92 ± 0.12
Leaf+3 1/3	0.79 ± 0.06	1.66 ± 0.63	0.61 ± 0.04*	0.93 ± 0.13
Leaf+3 2/3	1.39 ± 0.17*	1.78 ± 0.71	1.20 ± 0.08*	0.96 ± 0.14
Leaf+3 3/3	1.59 ± 0.16*	1.91 ± 0.91	1.45 ± 0.08*	0.97 ± 0.15
Leaf+3	1.11 ± 0.10*	1.76 ± 0.72	0.76 ± 0.17*	0.93 ± 0.13
Leaf+4	1.11 ± 0.10 *	1.70 ± 0.47	0.96 ± 0.07*	1.01 ± 0.15
Leaf+5	1.04 ± 0.15	1.67 ± 0.41	0.89 ± 0.05*	1.07 ± 0.20
Leaves	1.05 ± 0.08 *	1.68 ± 0.48	0.90 ± 0.09*	0.99 ± 0.12
Sheath	1.07 ± 0.10	1.57 ± 0.49	0.61 ± 0.10*	0.91 ± 0.08
Straw	0.47 ± 0.09	1.24 ± 0.44	0.30 ± 0.04	1.24 ± 0.23
Stem	0.41 ± 0.11	1.21 ± 0.37	0.16 ± 0.02*	0.91 ± 0.11
Aboveground biomass	0.80 ± 0.05	1.42 ± 0.39	0.34 ± 0.04	0.96 ± 0.11

Plant compartment N and ^{15}N% values which are significantly different from aboveground biomass are followed by *.

Table 2. Selection of the most suitable models for allometric relationships

Sampling time	Function	Model	R^2	AIC	Δ Biomass % *
t1	Linear	y = 1.32x - 25.72	0.94	185.0	12.0
	Exponential	y = 2.79e$^{0.05x}$	0.93	185.0	-8.8
	Power	y = 1.39*10^{-2} x$^{2.01}$	0.97	173.8	4.5
	Polynomial	y = 0.01x^2 + 0.18x - 3.21	0.96	175.6	4.6
t2	Linear	y = 2.06x - 92.45	0.87	182.7	44.5
	Exponential	y = 7.58e$^{0.03x}$	0.97	157.1	-15.3
	Power	y = 8.71*10^{-3}x$^{2.04}$	0.94	163.3	-0.6
	Polynomial	y = 0.03x^2 - 2.93x + 103.87	0.96	159.7	-50.3
t3	Linear	y = 2.29x - 129.99	0.85	261.2	11.0
	Exponential	y = 9.75e$^{0.02x}$	0.93	255.8	-12.2
	Power	y = 3.49*10^{-3}x$^{2.19}$	0.95	255.5	-7.6
	Polynomial	y = 0.01x^2 - 1.07x + 43.68	0.88	257.4	-14.6
t4	Linear	y = 3.68x - 419.29	0,89	196.0	61.5
	Exponential	y = 7.15e$^{0.012x}$	0,92	189.3	6.3
	Power	y = 1.79*10^{-5}x$^{3.15}$	0,92	189.3	7.6
	Polynomial	y = 0.03x^2 - 7.41x + 498.80	0,92	190.8	12.0

* Δ Biomass % values are calculated with the formula $\left(\frac{\text{measured biomass} - \text{estimated biomass}}{\text{measured biomass}}\right)$

4.3. Estimation of N content

4.3.1. Nitrogen representativity

At sampling time t1, the only leaves which have an N percentage not significantly different from the aboveground biomass are the Leaf+5 and the base of the Leaf+3 (Table 1). At t2, all of the leaves have significantly different N percentages from the aboveground biomass. The only plant compartment which is not significantly different is the plant straw.

4.3.2. Dilution Curve

With the exception of an outlier above the theoretical dilution curve, the plotted points are consistently lower than the theoretical dilution curve established by Pouzet et al., (1999) (Figure 5). However, when a new curve is fitted to the plotted points, there is a similar trend to that of the dilution curve.

4.4. ^{15}N recovery in other sugarcane compartments

4.4.1. ^{15}N recovery in belowground biomass

Fine roots constitute the majority of root biomass (87% of living roots at t1 and 79% at t3), and have higher N concentration than the other root types, at both dates (Table 3).

4.4.2 ^{15}N recovery in the soil compartment

The ^{15}N soil recovery decreases with soil depth at both sampling dates (at soil horizons 0-5cm, 5-10cm, 10-30cm, 30-50cm; soil recovery was 9.5 %, 1.9 %, 2.7 %, 1.4 % at t1 and 8.2 %, 2.2 %, 3.6 %, 1.6 % at t3).

5. Discussion

5.1. Estimating Nitrogen derived from fertiliser

The 2.25 m^2 ^{15}N microplots were sufficiently large to study the passage of N from fertiliser to the crop biomass. However, given that a substantial amount of ^{15}N was transferred outside the microplot, cane collected at the centre of the microplot was clearly capable of ^{15}N uptake from outside the microplot, which would dilute its ^{15}N content. As a consequence, Ndff and RE_N calculations would underestimate N recovery if lateral ^{15}N transfer was not taken into consideration. The Ndff of cane opposite the microplot in the adjacent rows were 5 % two months after the ^{15}N enriched urea application (t1), and 1 % four months after the urea application (t3), which confirms the need to consider lateral transfers when computing Ndff estimations (Trivelin *et al.*, 1994). By reducing the length of the microplot from 2 m (as proposed by Trivelin *et al.*, 1994) to 1.5 m, an additional challenge was encountered. The ^{15}N of cane at the centre of the plot was not only affected by lateral ^{15}N transfer between the microplot and the adjacent cane row, but also by lateral ^{15}N transfer between the microplot and the zones outside, in the same sugarcane row. The Ndff in the current row adjacent to the microplot amounted to 4 % at t1 and 1 % at t3, and should therefore be considered as follows:

$$Ndff_T = Ndff_M + 2 \times Ndff_{AR} + 2 \times Ndff_{CR}$$

where *Ndff$_T$* is the sum of the Ndff determined from the centre of the ^{15}N microplot (*Ndff$_M$*); the Ndff from the two adjacent cane rows in line with the microplot (*Ndff$_{AR}$*) and the Ndff from the same row but outside the microplot (*Ndff$_{CR}$*).
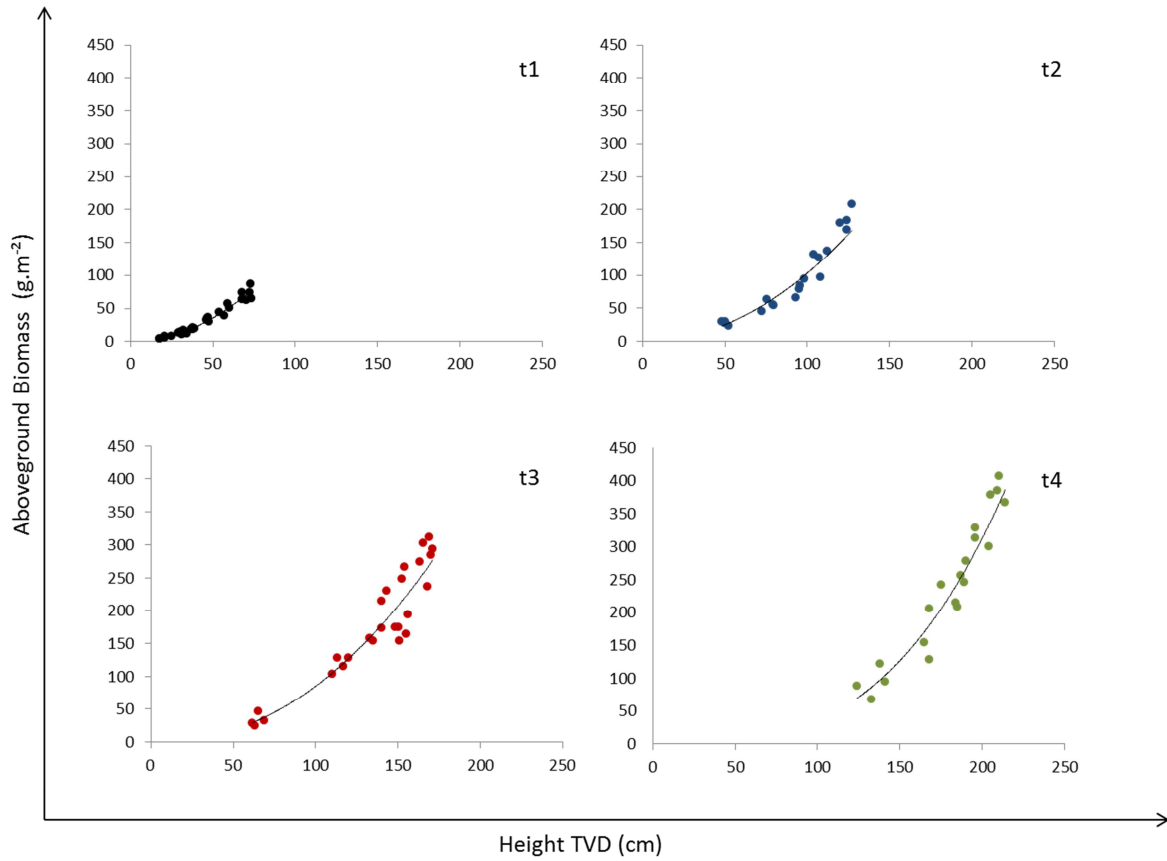
Figure 3. Allometric relationships between cane height to the top visible dewlap (TVD) and aboveground biomass at sampling time t1, t2, t3, t4.
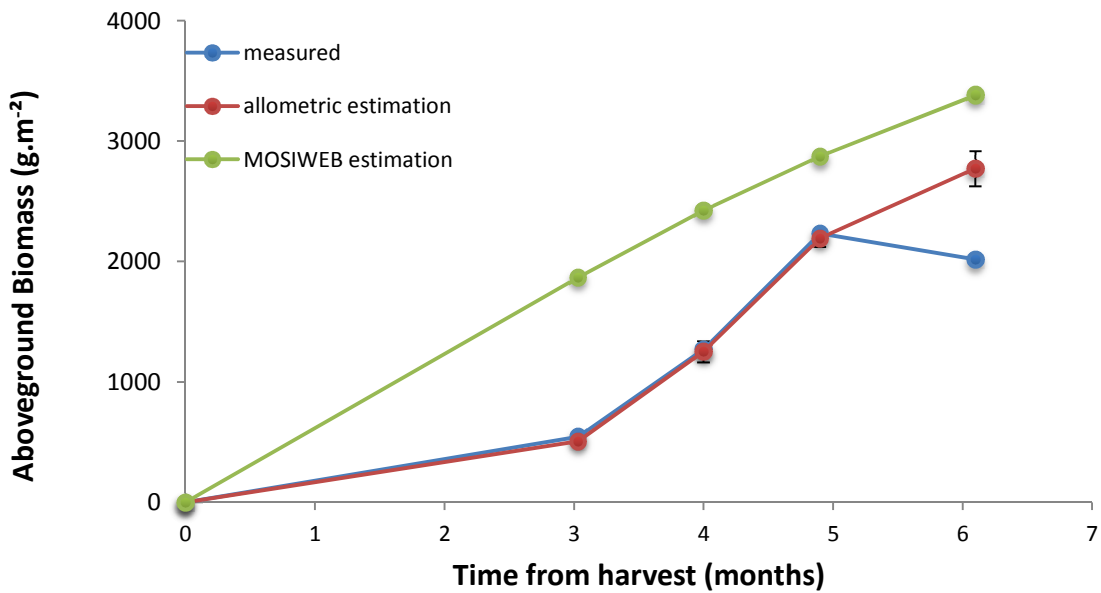

Figure 4. Sugarcane aboveground biomass obtained at the plot scale at the four sampling dates from three different methods: destructive measurements, estimation with allometric relationships and simulation with MOSIWEB. Standard deviation is given for allometric estimations (*n*=4).

In this study, none of the leaves tested had an ^{15}N abundance significantly different from the aboveground biomass. This is consistent with other studies, where the leaf tested was almost always not significantly different from the entire aboveground biomass (Takahashi, 1967, Sampaio et al., 1988, Franco *et al.*, 2011). Some have argued that any green leaf could therefore be used to represent the average ^{15}N abundance of the aboveground biomass (Carnauba, 1989 in Franco *et al.*, 2011). However, the non-significant difference between the different leaves of the cane and the aboveground biomass is most probably due to high variability among repetitions rather than the effective proximity of ^{15}N between each leaf and the total biomass.

The partitioning of ^{15}N between different compartments of sugarcane is strongly dependent on its growth dynamic. Given that the growth and development of different sugarcane individuals is variable, the response of different cane individuals to the "pulse" of ^{15}N abundance generated by ^{15}N enriched fertiliser applied to the soil will also be variable. However, there can be general tendencies across the sugarcane plot. In this study, there was the overall trend that leaves +1 and +2 have closer ^{15}N abundance values to the aboveground biomass at the first sampling date, and that the ^{15}N abundance of the Leaf + 3 was closer to that of the aboveground biomass at the second sampling date. This lag effect makes sense in relation to the timing of ^{15}N enriched fertiliser. The more time passes after fertiliser application, the more ^{15}N is found in the older leaves which developed shortly after the ^{15}N fertiliser application, and higher ^{15}N abundance is therefore found in the leaves positioned closer to the base of the plant. These results are consistent with the studies of Sampaio *et al.* (1988) and Takahashi (1967), which analysed ^{15}N abundance of different plant compartments over time, and chose representative leaves accordingly. Since the Leaf+3 is the standard leaf used to represent aboveground biomass ^{15}N content (Sampaio *et al.*, 1988, Trivelin *et al.*, 1994; Franco *et al.*, 2011), and in this study it was not significantly different from the aboveground biomass ^{15}N content, the Leaf+3 will continue to be used as the plant compartment to represent ^{15}N abundance of aboveground biomass non-destructively in Ndff and RE$_N$ calculations.

5.2. Estimating sugarcane aboveground biomass

The use of allometric relationships appears to be an effective method of estimating sugarcane biomass. The most suitable function to predict biomass from its height is the power function over the four dates. Although the function remains the same, its parameters change between dates. At the first three sampling dates, the biomass estimated by the allometric relationships had very similar values to the biomass harvested for these dates. Between the third and final sampling date, the expectation was that the plot biomass would increase, and this expectation was supported by the biomass estimation of the allometric calculations. However, the biomass harvested at the t4 showed a decrease from t3, and was therefore lower than the estimation. It is likely that the 3m linear patch selected for harvest was a particularly underproductive patch in terms of cane growth relative to the rest of the plot. While this would account for why there was a lower biomass of cane harvested, it does not refute the potential use of the allometric relationships to estimate plant biomass.

The measured biomasses were, however, very different from the biomasses predicted by the MOSIWEB sugarcane growth model. The MOSIWEB model may be an effective means of predicting sugarcane biomass over larger terrain, where there is greater variability and more productive sugarcane plots, but it clearly does not work effectively for such a precisely defined plot. For further studies, allometric relationships will be used as the non-destructive method to determine cane biomass of the sugarcane plot.

Table 3. Dry Mass, N concentration (%) and ^{15}N abundance (%) for fine roots (Ø <1mm), thick roots (Ø>1mm) and dead roots at sampling times t1 and t3. Standard deviation is given (*n*=3).

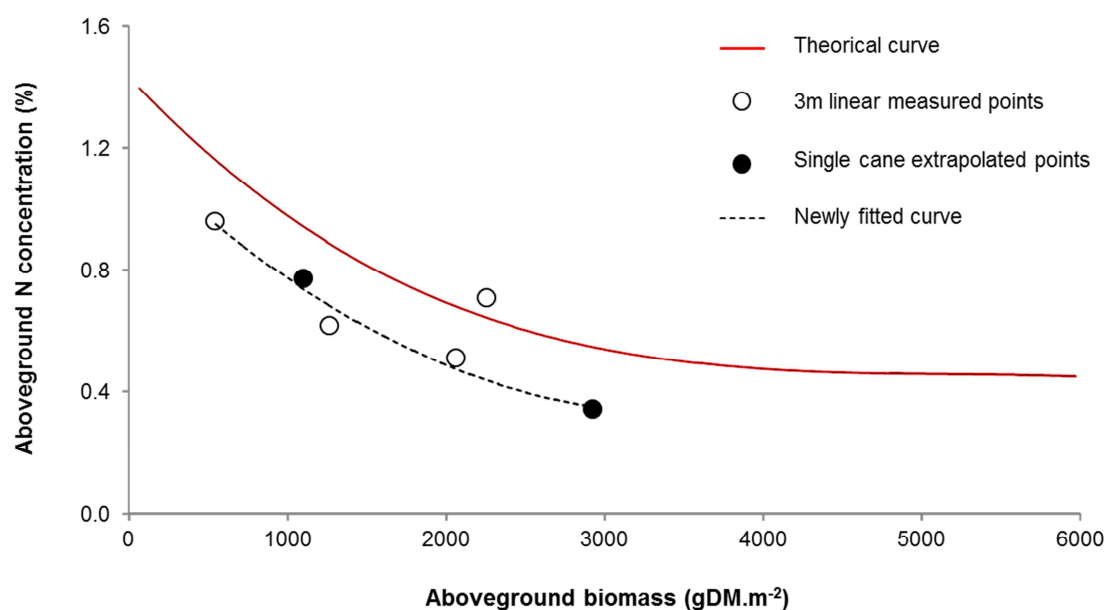| Sampling time	Root class	Biomass (g DM.m^{-2})	N concentration (%)	^{15}N atom% (%)
t1	Fine roots	561.13	0.55 ± 0.05	0.69 ± 0.15
	Thick roots	87.29	0.41 ± 0.06	0.73 ± 0.20
	Dead roots	83.61	nd	nd
t2	Fine roots	808.04	0.63 ± 0.05	0.55 ± 0.07
	Thick roots	220.51	0.40 ± 0.07	0.59 ± 0.08
	Dead roots	236.06	0.46 ± 0.05	0.64 ± 0.15

nd non-determined

Figure 5. Nitrogen dilution curves for sugarcane R579 aboveground biomass by Pouzet *et al.,* (1999, red line) and from the values of the present study (dotted black line). Values for the newly fitted curve are plotted from the 3m linear plot harvested (hollow points), and from values of single cane individuals extrapolated to a metre squared scale (solid points).

5.3. Estimating the N content of sugarcane biomass

The sugarcane leaves had significantly different N concentration values from the aboveground biomass over the two sampling dates, and therefore cannot be used to represent the aboveground biomass in Ndff and RE_N calculations. This finding was coherent with results reported on in the literature, where representative leaves are only used to represent aboveground biomass for ^{15}N abundance, and N abundance is obtained from the entire aboveground biomass of sugarcane individuals (Sampaio *et al.*, 1988, Trivelin *et al.*, 1994; Franco *et al.*, 2011). The aboveground biomass of cane can also be harvested from outside the microplot, under the same fertiliser treatment (Franco *et al.* 2011). The dilution curve appeared to be a feasible means of determining N content of the sugarcane, during its development. This would mean constructing a dilution curve fitted to values of N in relation to biomass for our specific sugarcane plot. The compromise with this approach is that it would entail sugarcane harvesting at each date to determine the N content of the plot which is not a non-destructive approach.

A promising alternative could be to use allometric relationships directly to predict the aboveground N amount from cane height TVD (J.P. Laclau, pers. comm). Since it appears that sugarcane biomass can feasibly be determined from corresponding height with the use of allometric relationships, and that cane N concentration can be determined by corresponding cane biomass with the use of a dilution curve, in theory it should be possible to establish a relationship between cane height and its corresponding N concentration. This approach will be tested in one of the next phases of the current project.

5.4. Fate of urea-derived N in a sugarcane agroecosystem

The application of the methods developed is the determination of the fate of N applied as urea fertiliser, i.e. where N ends up in the sugarcane agroecosystem. This is determined by the N recovery of the various components of the agroecosystem and gives an indication of the NUE, which is the amount of N effectively used from what was applied as fertiliser, versus what is lost in the agroecosystem. The aboveground biomass had an N recovery of 14.9% two months after urea application (t1), and 15.1 % four months after (t3). ^{15}N recovery was higher in the leaf compartments at the first sampling date than the second (5.9 % for leaf blade and 7.9 % for leaf sheath at t1; reduced to 4.1 % and 5.5 %, respectively, at t3). By contrast, for the straw and stem compartments, ^{15}N recovery was higher at the second sampling date than the first (0.4 % and 0.7 % at t1; and 1.6 % and 3.9 % at t3, respectively). This is coherent with ^{15}N crop recovery determined in sugarcane agroecosystems. In sugarcane plantations, the recovery of fertiliser-N applied as urea in crops is typically between 6 % and 37 % in the first harvest year (Chapman *et al.*, 1994, Isa *et al.*, 2006, Fortes *et al.*, 2010, Faroni 2008 *in* Fortes *et al.*, 2010). The N recovery of fertiliser-N applied as ammonium sulphate is typically higher than urea, being between 46 % and 76 % at the end of the first harvest year (Ambrosano *et al.*, 2011, Basanta *et al.* 2003, Isa *et al.*, 2006).

The ^{15}N recovery in the belowground biomass was 10.1 % at t1, and decreased to 8.4 % at t3. The belowground biomass is clearly not an insignificant component of the agroecosystem, as it constitutes 40 % of the total plant N at t1 and 36 % at t3. Coincidentally, a recent study on N recovery of sugarcane was conducted at the same site as ours, using the difference method. The N recovery of the sugarcane (aboveground biomass) was 39% at harvest at the end of the first year and 24% at the end of the second (Jean Paillat, eRcane, pers. comm). These values were higher than the N recovery in our study, one reason could be the difference in methods used for calculating N recovery. In the ^{15}N isotope method, ^{15}N can be exchanged with ^{14}N adsorbed on mineral surfaces (i.e. pool substitution bias) and not available for the crop (Harmsen & Moraghan, 1988).
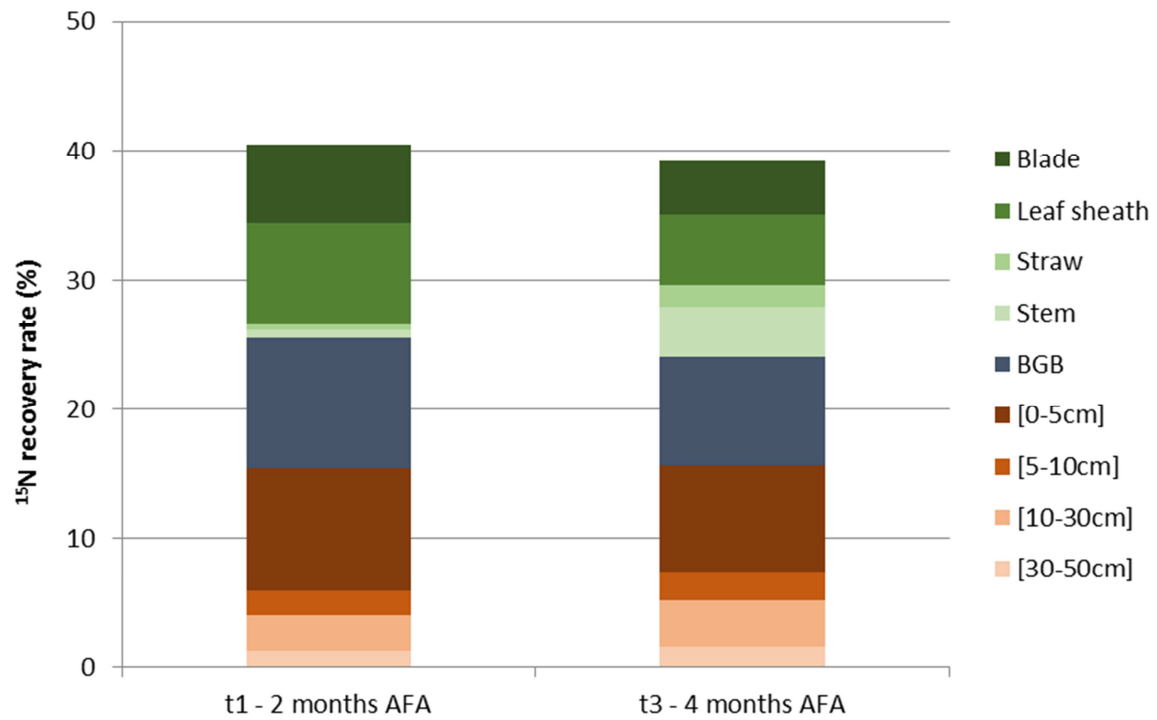
Figure 6. Urea-derived ^{15}N recovery rates in aboveground biomass, belowground biomass and soil compartment at sampling times t1 and t3.

Furthermore, additive soil N induced by priming-effect at the fertilisation was not accounted for in the ^{15}N isotope method, contrary to the difference method. N recovery values calculated by the ^{15}N isotope method are therefore generally less than those calculated by the difference method. Another possible explanation for the discrepancy between the results in two studies could be due to the timing of N recovery assessment, where in the present study, the N recovery was studied after two and then four months and in the other study, at the end of the crop year. Since there has already been a shift in ^{15}N between the two harvest dates, where a decrease in belowground biomass coincided with an increase in stem ^{15}N, this trend could continue. If so, the ^{15}N abundance of the aboveground biomass may increase to a value more similar to that of the other study, at the end of the crop year. This will be tested at the end of the crop cycle of this study's sugarcane plantation.

The soil N recovery remains constant between the two sampling dates, however the spatial distribution of ^{15}N appears to have changed between sampling dates. ^{15}N recovery decreases closest to the surface, and increases at each of the deeper soil horizons, indicating a downward transfer of ^{15}N. The total ^{15}N recovered in the plant (aboveground and belowground compartments), combined with ^{15}N recovered in the soil, was 40.1% 2 months after the urea application and 39.1% 4 months after the urea application (respectively 3 and 5 months after the beginning of the third ratoon, Figure 6). The 60 % of N not recovered by these agroecosystem components is typically lost from soil by ammonia volatilisation, denitrification and NO_3 lixiviation. In the experimental agroecosystem, the majority of N loss is likely to have been by the process of volatilisation. This is especially problematic for urea fertiliser, as the rate of volatilisation is further increased in conditions such as our study site, characterised by consistent high temperatures and wind, with a large surface area exposed (Genermont *et al.*, 2003).

6. Conclusion

The focus of the project was to develop and test non-destructive methods and sampling procedures for estimating sugarcane biomass, N and ^{15}N content at various phases of crop development. The application of these methods is the evaluation of NUE, by calculating the N recovery of the agroecosystem. In brief summary, ^{15}N abundance of the Leaf+3 was used as a representation of aboveground biomass; the ^{15}N abundance of cane in the zone next to the microplot and in the row opposite were incorporated into Ndff. Allometric relationships were used to predict cane biomass from cane height (TVD) using a power function with varying parameters, and a dilution curve was modified to estimate the N concentration of the sugarcane biomass. The total ^{15}N recovered in the plant (aboveground and belowground compartments), combined with ^{15}N recovered in the soil, was 40.1% 2 months after the urea application and 39.1% 4 months after the urea application (respectively 3 and 5 months after the beginning of the third ratoon).

7. References

Ambrosano, E. J., Trivelin, P. C. O., Cantarella, H., Ambrosano, G. M. B., Schammass, E. A., Muraoka, T., & Rossi, F., 2011. ^{15}N-labeled nitrogen from green manure and ammonium sulfate utilization by the sugarcane ratoon. Scientia Agricola, 68(3), 361-368.

Azevedo, M.C.B., Chopart, J.-L., Conti Medina, C., 2011. Sugarcane root length density and distribution from root intersection counting on a trench-profile. Scientia Agricola 68, 94–101

Barrios, E., 2007. Soil biota, ecosystem services and land productivity. Ecological Economics 64(2), 269–285

Basanta, M.V., Dourado-Neto, D., Reichardt, K., Bacchi, O.O.S., Oliveira, J.C.M., Trivelin, P.C.O., Timm, L.C., Tominaga, T.T., Correchel, V., Cássaro, F.A.M., Pires, L. F., de Macedo, J. R., 2003. Management effects on nitrogen recovery in a sugarcane crop grown in Brazil. Geoderma 116, 235-248.

Carnaúba, B.A.A., 1989. Eficiência de utilização e efeito residual de uréia-^{15}N em cana-de-açúcar (Saccharum spp.), em condições de campo. M.Sc. Thesis. Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, 130 pp. Carnaúba, B.A.A., 1990.

Chapman, L.S., Haysom, M.B.C., Saffigna, P.G., 1992. N cycling in cane field from ^{15}N labelled trash and residual fertiliser. Australian Society of Sugar Cane Technology 14, 84-89.

Chapman, L.S., Haysom, M.B.C., Saffigna, P.G., 1994. The recovery of ^{15}N from labelled urea fertilizer in crop components of sugarcane and in soil profiles. Australian Journal of Agricultural Research 45, 1577-1585.

Cassman, K.G., Dobermann, A.R., Walters, D.T., 2002. Agroecosystems, Nitrogen-use Efficiency, and Nitrogen Management. Ambio 31, 132-140.

Courtaillac, N., Baran, R., Oliver, R., Casabianca, H., & Ganry, F., 1998. Efficiency of nitrogen fertilizer in the sugarcane-vertical system in Guadeloupe according to growth and ratoon age of the cane. Nutrient Cycling in Agroecosystems, 52(1), 9-17.

Cowling, E.B. and Galloway, J.N., 2002. Challenges and opportunities facing animal agriculture: Optimizing nitrogen management in the atmosphere and biosphere of the Earth. Journal of Animal Science 80, 157-167.

Dillewijn, C.V., 1952. Botany of Sugarcane. Chronica Botanica, Waltham.

Dobermann, A.R., 2005. Nitrogen Use Efficiency – State of the Art. IFA International Workshop on Enhanced-Efficiency Fertilizers, Frankfurt, Germany, 28-30.

FAO, 1998. World reference base for soil resources. World Soil Resources Reports.

Fortes, C., Trivelin, P.C.O., Vitti, A.C., Ferreira, D.A., Franco, H.C.J., Otto, R., 2010. Recovery of nitrogen (^{15}N) by sugarcane from previous crop residues and urea fertilization under a minimum tillage system. Proceedings of the international society of sugar cane technologists 27, 1-8.

Franco, H.C.J., Otto, R., Faroni, C.E., Vitti, A.C., de Oliveira, E.C.A., Trivelin, P.C.O., 2011. Nitrogen in sugarcane derived from fertilizer under Brazilian field conditions. Field Crops Research 121, 29-41.

Galloway, J.N., Cowling, E.B., 2002. Reactive nitrogen and the world: 200 years of change. Ambio 31, 64-71.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320, 889-892.

Genermont, S., Morvan, T., Paillat, J.-M., Flura, D., Saint-Macary, H., 2003. Volatilisation d'ammoniac après épandage de lisier en conditions tropicales. Cas des prairies d'altitude et de la canne à sucre à l'île de la Réunion. Modélisation des flux de biomasse et des transferts de fertilité – cas de la gestion des effluents d'élevage à l'île de la Réunion. Restitution des travaux de l'Atp 60/99. Actes du séminaire des 19-20 juin 2002, Montpellier, France. Cirad, Colloques, Cédérom. F. Guerrin, J.-M. Paillat (Eds.)

Harmsen, K., & Moraghan, J. T.,1988. A comparison of the isotope recovery and difference methods for determining nitrogen fertilizer efficiency. Plant and Soil, 105(1), 55-67.

Isa, D.W., Hofman, G., van Cleemput, O., 2006. Uptake and balance of fertilizer nitrogen applied to sugarcane. Field Crops Research 95, 348-354.

IPCC, 2007. Climate Change 2007: The Physical Scientific Basis. Contribution of Working Group I to the fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New-York, USA

IPCC, 2014. Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New-York, USA

Kwong, K. N. K., & Deville, J., 1994. The course of fertilizer nitrogen uptake by rainfed sugarcane in Mauritius. The Journal of Agricultural Science, 122(03), 385-391.

Lemaire, G., Salette, J., 1984. Relation entre dynamique de croissance et dynamique de prélèvement d'azote pour un peuplement de graminées fourragères. 1. Etude de l'effet du milieu. Agronomie 4, 423–430.

Martiné, J.-F., 2003. Modélisation de la production potentielle de la canne à sucre en zone tropicale, sous conditions thermiques et hydriques contrastées. Applications du modèle. Institut National Agronomique Paris-Grignon, France, pp. 132.

Pouzet, D., Martiné, J.-F., Leinhart, B., 1999. Evaluation des conseils en fertilisation de la canne pour les hauts rendements. Variétés R570 & R579 en culture irriguée à satisfaction des besoins sur sol brun fertile. Rapport CIRAD CA Réunion, programme canne à sucre, 34p.

Sampaio, E.V.S.B., Salcedo, I.H., Victoria, R.L., Trivelin, P.C.O., 1988. Redistribution of the reserves of ^{15}N-enriched stem cuttings and dinitrogen fixed by 90-day-old sugarcane plants. Plant Soil 108, 275–279.

Smil, V., 2002. Nitrogen and food production: Proteins for human diets. Ambio 31, 126-131.

Smith, D.M., Inman-Bamber, N. G., Thorburn, P.J., 2005. Growth and function of the sugarcane root system. Field Crops Research 92, 169-183.

Takahashi, D.T., 1967. Effect of amount and timing on the fate of fertilizer nitrogen in lysimeter studies with [15]N. Hawaii Plant Rec 57, 292–308.

Trivelin, P.C.O., Lara Cabezas, W.A.R., Victoria, R.L., Reichardt, K., 1994. Evaluation of a ^{15}N plot design for estimating plant recovery of fertilizer nitrogen applied to sugar cane. Scientia Agricola 51, 226-234.

Vallis, I., Catchpoole, V. R., Hughes, R. M., Myers, R. J. K., Ridge, D. R., & Weier, K. L., 1996. Recovery in plants and soils of ^{15}N applied as subsurface bands of urea to sugarcane. *Crop and Pasture Science*, *47*(3), 355-370.

Versini, A., Zeller, B., Derrien, D., Mazoumbou, J.C., Mareschal, L., Saint-André, L., Ranger, J., Laclau, J.-P., 2014. The role of harvest residues to sustain tree growth and soil nitrogen stocks in a tropical *Eucalyptus* plantation. Plant and Soil 376, 245-260.

Vieira-Megda, M. X., Mariano, E., Leite, J. M., Franco, H. C. J., Vitti, A. C., Megda, M. M., ... & Trivelin, P. C. O., 2015. Contribution of fertilizer nitrogen to the total nitrogen extracted by sugarcane under Brazilian field conditions. Nutrient cycling in agroecosystems 101(2), 241-257.

Wassenaar, T., Doelsch, E., Feder, F., Guerrin, F., Paillat, J.-M., Thuriès, L., Saint Macary, H., 2014. Returning Organic Residues to Agricultural Land (RORAL) – Fuelling the Follow-the-Technology approach. Agricultural Systems 124, 60-69.

Acknowledgements

Supplementary materials

A. Root sampling

The root sampling procedure was adopted from Jean-Louis Chopart studies (Azevedo *et al.*, 2011) and was further validated by Christophe Jourdan, both root specialists at CIRAD. Soil cores were sampled with a 1 m gouge coupled with a percussion hammer (Cobra TT, SDEC). Three soil cores were taken at t1 and t3 at three distances relative to the sugarcane row: 0-25 cm; 25-50 cm and 50-75 cm. The cores were divided into 5 soil layers: 0-10 cm; 10-30 cm, 30-50 cm, 50-75 cm and 75-100 cm. Roots were separated from soil by adding water and swirling the sample in a bucket, thereby creating a vortex, and also by manually separating the clumps of soil to separate the roots. A 50 µm sieve was used to capture the floating roots which were cleaned using a pipette. The organic matter fragments, mainly observable in the 0-10 cm soil layer, were removed manually from the roots. Dead roots were selected qualitatively by bending them to see whether they snap. If the roots did not snap, this indicated there was sufficient living tissue and that roots were living at the time of harvest. Each original sample was finally divided into four categories: fine roots (Ø<1 mm); thick roots (Ø >1 mm); dead roots; organic matter fragments. The roots and organic material were dried in an oven at 60°C for 72 hours and weighed. The roots collected at the intermediate distance of 25-50 cm were sampled within the ^{15}N microplot and were used to test the root contribution to Ndff and NUE estimations.

N recovery in the root (RE$_{N\,root}$) was calculated using the Ndff of the aboveground biomass of cane at the centre of the microplots. The assumption is that the Ndff of the roots are not significantly different to that of the aboveground biomass. It would otherwise be impossible, to ascribe the root Ndff to a particular cane individual, as root from different cane are inevitably mixed together in the samples.

B. Soil sampling

Soil samples were taken at the 15 cm x 15 cm microplots at t1 and t3. Mulch on the surface of the soil was sampled according to two categories: mulch within the 22.5 cm^2 microplot and mulch outside of this area. Soil was then sampled at four different soil depths: 0-5 cm, 5-10 cm, 10-30 cm and 30-50 cm. A metal square was used to extract soil at the 0-5 cm and 5-10 cm depths. The 10-30 cm and 30-50 cm soil layers were sampled with a manual auger. An additional soil sample was taken, representing the border region of the 22.5 cm^2, taken at 3 cm from the perimeter of the microplot and at a depth of 0-10 cm. The samples were dried and initially ground using a large pestle, and then sieved at 2 mm, removing any remaining stones and organic material. A representative subsample was ground manually with an agate pestle and mortar, the dry mass measured, and the samples sent to the PTEF INRA laboratory in Nancy for ^{15}N analysis.

N recovery in the soil was calculated by this formula:

$$RS_N = (A^{15}N - A^{15}\mathrm{N}_{\mathrm{CTL}}) * \frac{N_{soil}}{N_{fertilizer}} \qquad (4)$$

where RS_N is the recovery of fertiliser N in the soil (%), $A^{15}N$ is the abundance of the soil sample (%), $A^{15}N_{CTL}$ is the natural abundance of a control sample (%), N_{soil} is the quantity of N in the soil layer (g.m^{-2}) and $N_{fertilizer}$ is the quantity of N applied with the fertilizer (g.m^{-2}).

Abstract

Nitrogen (N) is a fundamental nutrient in agroecosystems, but has considerable negative environmental impact when used in excess. Meeting N demand while reducing excess N can be achieved through improvements in N use efficiency (NUE) of a crop agroecosystem. Non-destructive methods and sampling procedures for estimating biomass, N and ^{15}N content during various phases of sugarcane crop development were tested to evaluate NUE. Allometric relationships appeared to be an effective method of estimating aboveground biomass from cane height. The third leaf below the top visible dewlap was found to have an ^{15}N content sufficiently representative of crop aboveground biomass. Further, the use of a dilution curve was found to be an effective means of estimating N content from cane biomass. Using these methods, average ^{15}N recovery 2 and 4 months after urea application was determined for the sugarcane aboveground biomass, belowground biomass and the soil compartment as 15.0±0.1 %, 9.2±1.2 % and 15.6±0.1 %.

Résumé

L'azote joue un rôle primordial dans la productivité des agroécosystèmes, mais peut en excès présenter des impacts environnementaux. Un moyen d'assurer les besoins azotés des cultures tout en limitant ces impacts est d'améliorer l'efficience d'utilisation de N (NUE). Différentes approches permettant d'estimer la biomasse, la concentration en N et l'enrichissement en ^{15}N ont été testés. L'utilisation de relations allométriques a été retenue afin d'estimer la biomasse aérienne des canne-à-sucre. La troisième feuille sous le premier ochréa visible peut être utilisée afin d'estimer l'enrichissement en ^{15}N de l'ensemble de la biomasse aérienne. L'utilisation d'une courbe de dilution apparait comme le meilleur moyen d'estimer la concentration en N de la biomasse aérienne. Le recours à ces méthodes a permis d'établir que 15.0±0.1 %, 9.2±1.2 % et 15.6±0.1 % du ^{15}N apporté lors de la fertilisation sous forme d'urée sont retrouvés dans la biomasse aérienne, souterraine et le compartiment sol en moyenne 2 et 4 mois plus tard.