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par

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Effet de l'apport de produits résiduaires organiques sur le cycle biogéochimique de l'azote en culture de canne à sucre à la Réunion

The Effect of organic fertiliser application on the nitrogen biogeochemical cycle of sugarcane crops in Réunion Island

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Thesis Abstract

Nitrogen fertilisers have contributed substantially to global food security and nutrition. However, when accumulated in excessive amounts in ecosystems, and the atmosphere, they lead to significant negative environmental impacts. There is frequently a large disparity between what is supplied by fertilisation and what is used by crops, leading to low nitrogen use efficiencies (NUE) of fertilisers. The recycling of organic residues in agroecosystems could be a promising alternative or complement to synthetic fertilisers, and a means to promote circular economic and agricultural sustainability. The overall aim of this PhD thesis was to evaluate and provide a detailed quantitative and temporal account of N inputs and outputs into and from a highly monitored experimental site cultivated with sugarcane. Secondly, it was to determine the fate of N from two types of organic fertilisers (pig slurry and sewage sludge) as compared to mineral fertiliser, in the soil-sugarcane system.

The evolution of sugarcane biomass and total N mass accumulation was measured monthly over 24 months for four distinct fertiliser treatment types: unfertilised, urea, pig slurry, and sewage sludge. The evaluation of different sugarcane biomass components revealed that while the N in tillers, strawfall and belowground stools remains limited, the proportion of plant N contained in the roots could be considerable and represented up to 65 and 104 % of the N measured in the aboveground biomass of the urea and unfertilised treatments respectively.

A combination of minimally destructive methods is proposed to determine the NUE throughout the sugarcane growth-cycle. Microplots containing ^{15}N labelled urea or mulch were assessed over the two experimental years to study the respective contributions of different fertiliser sources to sugarcane N content. The mulch and previous fertiliser applications provided a constant but low contribution of less than 5 %, with mineral and organic fertilisers contributing 9.6-17.8 % and 4.4-7.1 % of the sugarcane N respectively. The soil was by far the largest source of N, providing a minimum of 74 % of the sugarcane N content. Calculations of fertiliser NUE were evaluated on a monthly basis with the difference and isotopic dilution methods, highlighting 1/ a difference between the values calculated using the two methods, which is reduced when the root component is considered, 2/ a pronounced decrease in the NUE over the last 6 months of the sugarcane growth-cycle when using the isotopic method suggesting a deficit in ^{15}N which should be further interrogated, 3/ a particularly low NUE of 9.2 – 16.1 % for the reference fertiliser urea, partly as a result of a particularly high level of N loss via volatilisation.

In terms of N outputs from the sugarcane-soil system, particular attention was paid to the leaching of fertiliser N with the use of porous cups and TDR probes to determine the N content in soil solutions and the corresponding water flux at three soil depths for the four fertiliser treatment types. Despite substantial quantities of N observed at a soil depth of 10 cm, the estimated losses at a

depth of 100 cm did not exceed 18.3 $kgNha^{-1}$ for the different fertiliser treatments. This result is probably in part as a result of the soil's capacity to retain nitrates, as well as importantly due to the effective N uptake of the sugarcane after fertiliser N application, for which its extensive roots and early foliar activity enable active N uptake from 2-3 months after the start of the ratoon onwards.

A complete budget of N flux at the scale of the agroecosystem established that of the N applied with the urea fertiliser, 22 % was absorbed, 36 %, 1.4 % and 3 % lost via volatilisation, denitrification and leaching respectively, and 37 % immobilised in the soil. Of the N applied with pig slurry, 7 % was absorbed, 63 %, 3.6 % and 2 % lost via volatilisation, denitrification and leaching respectively, and 27 % immobilised in the soil. Finally, of the N applied with sewage sludge, 9 % was absorbed, 8 %, 0.7 % and 5 % lost via volatilisation, denitrification and leaching respectively, and 70 % immobilised in the soil.

In conclusion, this thesis highlights 1/ the central role of soil as a major source of N, and 2/emphasised the important role of the root component of sugarcane, and 3/ the need to improve the efficiency of fertiliser use by lowering the level of volatilisation in Réunion. The use of organic fertilisers as a substitute, or partial substitute, for mineral fertilisers appears to be a good means to supply additional N to sugarcane and to the soil, while limiting environmental pollution.

Keywords: *Nitrogen, Nitrogen Use Efficiency, Organic fertilisers, Sugarcane, Leaching, Nitrogen budget*

Resumé de Thèse

Les engrais azotés ont contribué de manière substantielle à la sécurité alimentaire et à la nutrition mondiales. Toutefois, l'azote qu'ils contiennent peut être accumulé en quantités excessives dans les écosystèmes ou dans l'atmosphère; il entraîne alors des impacts environnementaux négatifs. Il existe souvent une grande disparité entre ce qui est fourni par la fertilisation et ce qui est utilisé par les cultures, ce qui entraîne de faibles rendements d'efficience de l'utilisation de l'azote (NUE) des engrais. L'amélioration de la NUE des cultures permettra de répondre aux demandes d'azote (N) des cultures tout en réduisant l'offre de N, et donc l'excès de N et les implications négatives sur l'environnement. Le recyclage des résidus organiques dans les agroécosystèmes pourrait être une alternative ou un complément prometteur aux engrais synthétiques, et un moyen de promouvoir une durabilité économique et agricole circulaire. L'objectif général de cette thèse de doctorat était dans un premier temps de dresser un bilan complet et dynamique des entrées et sorties d'azote dans un site expérimental fortement instrumenté cultivé en canne à sucre. Dans un second temps, il a s'agit d'étudier le devenir de l'azote apporté avec deux types d'engrais organiques (lisier de porc et boues d'épuration méthanisées chaulées séchées) dans ce système sol-plante en comparaison d'un apport d'engrais de référence (urée), pour la canne à sucre à la Réunion.

L'évolution de la biomasse et de la minéralomasse de N a été mesurée au pas de temps mensuel au cours des 24 mois de l'étude dans 4 traitements distincts (non fertilisé, urée, lisier de porc, boues de STEU méthanisées chaulées séchées pelletisées). Le suivi de différents compartiments de biomasse a révélé, que si l'azote contenu dans les talles, les pailles et la souche restait limité, la part de l'azote de la plante contenu dans les racines pouvait être considérable et représenter jusqu'à 65 % et 104 % de l'azote mesurée dans la biomasse aérienne des traitements non-fertilisé et fertilisé.

Un ensemble de méthodes peu destructives a été proposé afin d'estimer l'efficience d'utilisation de l'azote tout au long du cycle de croissance de la canne à sucre. Les contributions respectives de différentes sources de N pour la nutrition de la canne ont été déterminées au cours de ces deux années de suivi à l'aide de microplacettes contenant initialement de l'urée ou du paillis enrichis en ^{15}N. Le paillis et les apports précédents d'engrais présentaient une contribution constante mais inférieure à 5 %, les engrais, qu'ils soient minéraux ou organiques, représentaient environ 4.4-17.8 % ; c'est donc le sol qui représentait de loin (>74 %) la principale source de N à la nutrition de la canne à sucre.

Des calculs d'efficience d'utilisation de l'azote des engrais ont été élaborés avec deux méthodes, par différence et isotopique, mettant en évidence 1/ un écart de résultats entre méthodes que la prise en compte du compartiment racinaire permet de corriger, 2/ une baisse au cours des 6

derniers mois avec l'approche isotopique uniquement suggérant un déficit de ^{15}N qu'il reste à élucider, 3/ une efficience faible autour de 9.2 – 16.1% pour l'engrais de référence en raison notamment d'un fort niveau de volatilisation.

Une attention particulière a été accordée à la lixiviation de l'azote apporté avec les engrais grâce à un dispositif de bougies poreuses d'une part permettant le dosage de N dans les solutions de sol à trois profondeurs et dans 4 traitements, et des sondes TDR d'autre part rendant possible la modélisation des flux hydriques. Malgré des quantités importantes de N observées à 10 cm, les pertes estimées à 100 cm n'ont pas dépassé 18.3 kgN/ha quel que soit le traitement. Ce résultat est probablement à mettre au compte d'une capacité des sols à retenir les nitrates mais surtout à la dynamique de croissance de la canne dont les racines profondes et l'activité foliaire précoce garantissent un prélèvement actif dès 2-3 mois.

Un bilan complet des flux à l'échelle de l'écosystème a permis d'établir que le N de l'urée était à 22 % absorbé par la canne, à 36 %, 1 % et 3 % perdu via volatilisation, dénitrification et lixiviation respectivement et à 37 % immobilisé dans le sol. Le N du lisier de porc était à 7 % absorbé, à 63 %, 3.6% et 2 % perdu via volatilisation, dénitrification et lixiviation respectivement et à 27 % immobilisé dans le sol. Enfin le N des boues de STEU était à 9 % absorbé, à 8 %, 0.7 % et 5 % perdu via volatilisation, dénitrification et lixiviation respectivement et à 70 % immobilisé dans le sol d'après différents modes de calculs.

En conclusion, ces travaux ont mis en évidence le rôle central du sol en tant que pourvoyeur de N. Ils ont mis en lumière le rôle de premier plan du compartiment racinaire ainsi que la nécessité d'améliorer l'efficience d'utilisation des fertilisants en abaissant le niveau de volatilisation à la Réunion. Le recours à des engrais organiques en substitution des engrais minéraux apparait comme un bon moyen de nourrir les cultures et d'amender les sols, tout en limitant les pollutions environnementales.

Mots-clés: *Azote, Efficience d'utilisation de l'azote, Engrais organiques, Canne à sucre, Lixiviation, Bilan d'azote*

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South Africa and abroad

To my parents for their love and constant support. To my mom, for her strength, love, resilience, intellect, quirkiness, and her own life as a researcher. My dad, for our trips into the rural wild since I was a baby, him having followed an atypical course of life and work and travel, and his genuine interest in my work. Jenah and Josephine for their love. And Jeda, Valeska and Mo.

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With my family history and lineage in mind, I like to think there is a certain continuity of the legacy of my grandparents as farmers, and the generations before, starting with the first settler, Johannes Pretorius, who described the fauna and flora of Mauritius[1] (likely with the same language barrier and in the same Mascarene region as my PhD study) before later settling in South Africa in 1669, and becoming a farmer after a few years.

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"The research scientist is a solver of puzzles, and the puzzles upon which he concentrates are just those which he believes can be both stated and solved within the existing scientific tradition" Thomas S. Kuhn. The Structure of Scientific Revolutions[2]

[1] Hume, J. P., & Winters, R. (2016). Captive birds on Dutch Mauritius: bad-tempered parrots, warty pigeons and notes on other native animals. *Historical biology*, *28*(6), 812-822.

[2] Kuhn, T. S. (2012). *The structure of scientific revolutions*. University of Chicago press. Original text, 1962.

Preface

Timeframe of study

The PhD study took place over a three-year period (October 2017 – September 2020), with a three-month extension granted in the third year due to the impact of the Covid pandemic. The PhD study was prefaced by a 6-month internship which formed part of my master's degree at the University of Montpellier, and completed in July 2017. The project undertaken during the internship laid the foundations for the PhD project, and contributed to two published papers presented here; the first is the first chapter of the thesis, and the second the appendix paper.

Congresses

- N workshop, Rennes, France, June 2018. Poster presentation, "Effects of organic waste application on nitrate leaching in sugarcane agroecosystems in Reunion Island."
- International Society of Sugarcane Technologists (ISSCT) workshop La Réunion, September 2018. Oral presentation "Non-destructive sampling procedures to study Nitrogen Use Efficiency throughout the crop development of sugarcane plantations". Received the Young Researcher's Award for this presentation.
- ISSCT 30th International Congress on Sugarcane. Tucuman, Argentina, September 2019. Proceedings' paper presented as an oral presentation, "Contribution of organic fertilisers to nitrogen nutrition in sugarcane and nitrate leaching in sugarcane agroecosystems in Reunion". Again received the Young Researcher's Award.

Articles published

Two papers were published, the first presents the methodology used throughout the PhD study (Chapter 1); the second is included as an appendix, given that the root compartment of sugarcane is an overarching theme for the different chapters in the thesis:

Poultney, D. M. N., Christina, M., & Versini, A. (2020). Optimising non-destructive sampling methods to study nitrogen use efficiency throughout the growth-cycle of giant C4 crops. *Plant and Soil*, *453*(1), 597-613.

Versini, A., Poultney, D., Bachir, H., Février, A. & Paillat, J. (2020). Effect of Nitrogen Fertilisation on Sugarcane Root Development and Nitrogen Accumulation in Ratoon Crops of Reunion Island. *Sugar Tech*, 1-12.

Structure of the thesis

The thesis is based on articles, and each chapter is therefore presented in this format. However, in order to avoid repetition, parts of the material and methods (e.g. the experimental site which remains the same for the different experiments throughout the study), are presented at the start of the thesis in the second section, Experimental Site, following directly after the General Introduction.

Summary of terms and abbreviations

Mulch	Post-harvest residue retained on the soil surface after harvest, also referred to as "trash"
NRE	Fertiliser (or other N source) recovery efficiency. See summary of different terms used in literature to describe NRE (Annexure, Section 7.1)
dNRE	Fertiliser-N recovery efficiency determined using the difference method
iNRE	Fertiliser N-recovery efficiency determined using the ^{15}N isotopic dilution method
NdFs	Nitrogen derived from source (fertiliser N or other N pool). This applies to fertilisers (NdFf), mulch (NdFm) and soil (NdFsoil).
N mass	In French this is referred to as "Minéralomasse", whereas several different terms are used in English. N mass is the N content corresponding to sugarcane biomass at a plot scale (N content multiplied by sugarcane biomass). Also referred to as "total N" (Vieira-Megda et al. 2015), "N accumulation" Boschiero et al. (2020), "total crop N accumulation" (Wood et al. 1996)
OFs	Organic fertilisers (pig slurry and sewage sludge in this study)
Ratoon	Sugarcane "resprout crop" after harvest
Stool	Base of the plant, which remains in the soil over the sugarcane plantation and subsequent ratoons. Comprises branched secondary shoots (tillers) with underground buds and the associated fibrous root system (Rae et al. 2013). Sometimes referred to as "pseudo-rhizome"
Strawfall	Dry sugarcane leaves which fall to the soil from approximately mid- growth cycle until harvest. The equivalent would be "litterfall" in forestry
Sugarcane growth-cycle	In this study, each plantation or single ratoon year — i.e. from the start of the ratoon until the following harvest (approximately 12 months after the start of each ratoon). Some sugarcane specialists (e.g. Meier & Thorburn 2016) also refer to a sugarcane "crop cycle" as the combination of plant crop (i.e. the year after plantation); ratoon crops (each of the "resprouting" years between harvests); and a fallow period, before a new plantation begins (typically 4-6 months after the fallow period)
Tillers	Tillers are secondary shoots which emerge from the axillary buds of an existing culm to form additional culms (Bonnett 2014)

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General Introduction

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 (long chains of N-containing amino acids residues) that drive the metabolic machinery of every living cell (Galloway & Cowling, 2002). About 80% of the Earth's atmosphere is composed of N in the form of N_2 making it the largest pool of N. However, in this form N is not biologically available to most organisms. N_2 needs to be broken down and the resulting single-N atoms bonded chemically with one or more of three other essential elements: oxygen and/or hydrogen through N-fixation processes and carbon through N-assimilation processes. Non-biological fixation occurs in the air by means of lightning while 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 N was made available to living organisms (Galloway & Cowling, 2002). This has changed radically 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 anthropogenically produced reactive N (Galloway & Cowling, 2002). Reactive N can be defined as all biologically, photochemically, and/or radiatively active forms of N which comprises a range of nitrogeneous compounds that includes organic compounds, mineral N forms (e.g. NO_3^- and NH_4^+), and gases that are chemically active in the troposphere (NOx, NH_3, N_2O) and which contribute to air pollution and the greenhouse effect (Galloway et al. 2005). 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) (Galloway et al. 2008). Nitrogenous fertilisers used for food production have played a crucial role in contributing to this substantial increase (Smil, 2002), accounting for 63 % of all anthropogenic sources of reactive N.

Nitrogen fertilisers have had, in part, a largely positive impact on society by contributing to food security and adequate nutrition (Tilman et al. 2002, Doberman, 2005). In addition, their positive environmental impact has been in increasing crop productivity which has reduced the rate of agricultural expansion into natural ecosystems, providing critical habitat for protecting biodiversity and ecosystem functioning (Cassman et al. 2003, Tilman et al. 2002). However, when accumulated in excessive amounts in terrestrial and aquatic ecosystems, and in the atmosphere, N leads to a significant impact on environmental quality, ecosystems, biodiversity and human health

(Galloway et al. 2003, Schlesinger, 2009). The greatest overall challenge, therefore, as posed by Galloway et al. (2008), is to find the means to maximise the benefits of anthropogenic N while minimising its unwanted consequences.

Crop production in agricultural systems is the single largest contributor to the production of reactive N (Smil, 1999). It follows that there would therefore be a strong interest in evaluating the inputs and outputs of N in and from agricultural systems. Such a systemic understanding and approach, at temporal and quantitative levels, would make it possible to inform ways to minimise unnecessary inputs and excess outputs with their resultant environmental implications.

Nitrogen biogeochemical cycles in agroecosystems

Nitrogen is one of the most important elements required in agricultural systems to produce food and to supply a continuously growing world population (Follet & Hatfield, 2001). It is essential for plant growth and development, and therefore crop productivity (Barrios, 2007). In agroecosystems, the crop N requirement can be supplied by several sources: microbial-mediated mineralisation of soil organic matter, mulch post-harvest and root residues, the application of mineral and organic 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 increasing crop demand for N with the application of various forms of organic and inorganic N (Galloway et al. 2008).

There are several pathways of N loss from agricultural systems, each with environmental consequences. Nitrogen can be lost to the atmosphere via NH_3 volatilisation, which can impact 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 which at high enough levels would lead to dead zones.

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 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). At a local scale, 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 (Hansen et al. 2017).

The single largest input into the global N cycle are mineral (or inorganic) fertilisers (Smil 1999). The total use of mineral fertilisers in the last global survey was 109 million tonnes of N (58% of total chemical input) in 2018, which was 35% higher than the survey before in 2000, corresponding to an average global input into cropland area of 70 kh.ha^{-1} per year (FAOSTAT, 2020). At a global scale there are vast spatial disparities in the use of fertilisers. Even though N compounds are the single largest input of nutrient in the world's crop production, certain crop fields have received little or even no inorganic fertiliser, particularly in certain regions of Africa (Smil, 1999). Only 50 countries globally consume 95 % of synthetic N fertiliser (Uwizeye et al. 2020). In 2018, Asia represented 55 % of world total chemical fertiliser use, followed by the Americas (27 %), Europe (12 %), Africa (4 %) and Oceania (2 %) (FAOSTAT, 2020). Given the increased demand for crop production globally, there is concurrently an increased demand for N fertilisers, including in previously low mineral fertiliser-input regions. Between 2000 and 2018, the fastest increase in fertiliser use (of N but also phosphorous P and potassium K) took place in Africa where there was an increase of 74 % (which represents only 3 million tonnes per year given its low starting level). The highest increase in absolute terms was in Asia, with an increase of 32 million tonnes per year (or 44 %) between these time periods (FAOSTAT, 2020).

The substantial global increase in fertiliser N consumption in meeting the demands for increased global crop production is likely to continue to rise. If the rate of loss per unit N fertiliser applied is not improved, there will be major environmental consequences through the continued accumulation of different forms of reactive N (Galloway et al. 2003, Galloway et al. 2008). Consequently, crop nutrition, and in particular N fertilisation in agroecosystems, should be optimised to sustain crop productivity while limiting N contaminations at local, regional and global scales. In order to achieve this balance, it is important to have a holistic understanding of the biogeochemical N cycling of the given soil-crop system, in order to evaluate (quantitatively and temporarily) the N inputs and outputs of this agricultural system. A systemic approach would help minimise unnecessary N inputs and excess outputs, and the resultant environmental implications.

The development of nitrogen budgets is a useful approach to evaluate system-level N use efficiency and to understand N cycling by estimates of input, storage and export processes by mass balance. N budgets can be constructed for different time periods at any scale, ranging from an agricultural management unit (i.e. at a plot scale) to regional and continental scales. The degree of detail depends on the purpose of budgeting and on the resources available to collect the information. For example, certain N budgets used for guiding agricultural management or government policy decisions use simple budgets that consider only certain major fluxes into and out of the agroecosystem (Dobermann, 2005). In these scenarios, there will inevitably be

substantial portions of unaccounted for N, which may be lost through unstudied pathways, or retained in the soil-crop system. The more simplistic the budget, the more difficult it would be to construct an accurate understanding of what is lost and what is retained in the system. In order to acquire a detailed understanding of an agroecosystem, in this case a soil-crop system, a more complete budget needs to be evaluated, quantifying the relative role of N inputs and outputs, as well as the distribution and turnover of N among internal components of the system.

Evaluating and improving nitrogen use efficiency

It is estimated that globally only between 30 % and 50 % of applied fertiliser N is taken up by crops (Cassman et al. 2002, Smil, 1999 & Tilman et al. 2002). There is thus a substantial disparity between what is applied and what is actually used by crops. A portion of what is not immediately used is immobilised in the soil and has the potential to be mineralised, thereby contributing to crop nutrition in subsequent growth cycles which is clearly advantageous. The remainder which is lost from crop systems, such as sugarcane which is the focus of this thesis, can contribute significantly to reactive N enrichment of the atmosphere, surface, and ground water (Smil, 1999). One important means to decrease this disparity and optimise N cycling, and to reduce the portion of N lost from agricultural systems, 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 optimising trade-offs between yield, profit and environmental protection (Cassman et al. 2002).

Nutrient use efficiency is the ability of a crop to acquire nutrients from a growth medium and to incorporate or use these nutrients in the production of harvestable plant material, aboveground biomass or total plant biomass (including belowground biomass) (Baligar et al. 2001; Blair, 1993). Some key factors which have contributed to the improvements of NUE for certain crops have been modern cultivar development with higher stress tolerance and greater resultant yields, improved production factors other than N (for example optimising the use of other essential macronutrients and better water management), as well as better N fertiliser management. Some suggested approaches of improving fertiliser management are either by using better fertilisers and NUE enhancing products (for example coated urea, or nitrification inhibitor treated urea), which can be expensive and is often not a viable option in developing contexts, or better application methods and strategies (Dobermann, 2005).

Nitrogen use efficiency is quantified with the index “N-Recovery Efficiency” or NRE, which is a measure of the efficiency of crops to use N applied to soil through fertiliser. The NRE is calculated as the percentage of fertiliser-N recovered in the crop biomass during the crop-growing season

(Cassman et al. 2002). A table of the different terms and abbreviations, used by different authors, and sometimes with slightly different interpretations, is shown in the in Annexure A of this manuscript. Two primary methods are used in agronomic research to determine the NRE. The first approach, the "difference method", corresponds with the broadest measures of NUE and 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 difference method is simple and cost-effective and is particularly well-suited to on-farm research sites. The second approach to studying NUE uses ^{15}N-labelled fertilisers to estimate the crop recovery of applied N (*coefficient réel d'utilisation* or CRU in French). One significant advantage of using the ^{15}N tracer method is that the fate of N can be evaluated in a quantitative manner in the distinct N components of an agroecosystem (the plant and its various compartments, soil and soil solutions), and the contribution of an N source to the N stocks of a given compartment to be evaluated (Versini *et al.* 2014). This is essential to a detailed, systemic soil-crop evaluation as in this study.

At a global, more general scale, NRE may be more practical and cost-effective to be evaluated using the difference method (Cassman et al. 2002). There is frequently a discrepancy between the NRE values determined using these two methods, however. NRE values calculated using the ^{15}N tracing method are typically lower than values calculated using the difference method (Doberman et al. 2005). There is debate as to why this may be the case, but it remains controversial. One suggested reason is that the discrepancy is a result of pool substitution, which is essentially the immobilisation of ^{15}N fertiliser in microbial biomass and the initial release of microbial derived ^{14}N (Jenkinson et al. 1985, Krupnik et al. 2004). An example of this discrepancy at a global level is an estimated global average NRE for cereal research trials as 51 % evaluated using the difference method and 44 % using the ^{15}N method (Ladha et al. 2005).

Whichever approach is used, the calculation of the NRE 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 NRE at different stages of crop growth, as the biomass is usually determined at the end of the crop growth-cycle when the crop is harvested. The few studies which have determined NRE at different stages of the crop cycle have frequently found NRE to be highly variable over the growth-cycle, and a tendency for a substantial decrease in NRE over the duration of the crop growth cycle (e.g. Ng Kee Kwong & Deville, 1994 and Courtaillac et al. 1998). These results point to the importance of considering the whole crop cycle when studying fertiliser NUE in agroecosystems. In particular, destructive methods of NRE could be minimised, allowing for further measurements to be made in the same treatment plots at further time intervals over the crop development cycle.

The evaluation of NRE of organic fertilisers and other N sources is not straightforward, when using the ^{15}N isotopic method. Synthetic fertilisers can be enriched in ^{15}N and are purchased as

such. In the current study a way of dealing with this was the use of mixed organic fertiliser -^{15}N mineral microplots and comparing this to a reference ^{15}N mineral microplot, in order to determine the N contribution of the organic fertilisers by deduction. The approach by deduction is described in more detail in the Experimental Site section and Chapter 3 of the thesis manuscript.

Organic fertilisation in agroecosystems

Organic fertilisers have the potential to increase the soil organic matter (SOM) pool. One potential advantage over most inorganic or mineral fertilisers in that many organic fertilisers typically favour a slow-release of N (Nieder & Benbi, 2010). SOM generally exceeds crop-applied fertiliser N by at least one order of magnitude (Robinson et al. 2014) and supplies at least 50-80 % of a crop's N content (Dourado-Neto et al. 2010, Stevens et al. 2005). One proposed means of improving N supply to crops would therefore be to focus on N *soil* supply to meet crop demands and shift (at least partially) from "readily dissolvable, rapid-turnover inorganic fertilisers" to "slower-turnover, organic N-based fertilisers" (Robinson et al. 2014). The soil N is primarily contained in the soil organic N pool (SON), which comprises soluble, insoluble, and dissolved organic polymers and oligomers (proteins, peptides) (Robinson et al. 2014).

The use of certain organic fertilisers is as old as agriculture itself but for many crops is often not used on a commercial scale. As a large portion of the fertiliser N is organic and therefore not immediately available for uptake by plants, it is challenging to evaluate quantitatively its contribution to N nutrition, efficiency and potential loss from soil-crop systems, as well as to meet the short-term N requirements of crops with organic fertiliser supply. Organic fertilisers are mainly applied as manure and sewage in solid and liquid form in a raw state, or applied after transformation of the material, as well as crop residues. The feces of farm animals consist mostly of undigested food that has escaped bacterial action during its passage through the body, with a resultant high cellulose content. Liquid manure, such as pig slurry in this study, may also contain significant amounts of NH_4^+ which has been formed from urea through hydrolysis. Sewage sludge is often considered a more contemporary human waste byproduct used as an organic fertiliser. However, in certain contexts it was used historically as fertiliser, such as in eighteenth century Japan. During this period, many of the European and North American countries had fertile soils and forests, whereas large parts of Japan had sandy soils that were low in nutrients (Zeldovich 2019). With an increasing population size and food demands, the country needed fertiliser to increase harvest, and sewage became a prized item.

Todays' sewage is usually transformed into a sludge from the biological treatment of domestic sewage and which does not contain raw, undigested solids (Nieder & Benbi, 2010). The sewage sludge can be produced in a digested, limed, dried and pelleted form, as was applied in our study. The organic component of the sludge is a complex mixture consisting of digested constituents that are resistant to anaerobic decomposition, dead and live microbial cells, and

compounds synthesised by microbes during the digestion process (Nieder & Benbi, 2010). The organic matter is relatively rich in N, P and S, and the C:N ratio of digested sludge ranges between 7-12. N (short-term) availability in sludge decreases as the content of NH_4 and NO_3 decreases and as the organic N becomes more stable as a result of digestion during biological waste treatment (Nieder & Benbi, 2010). Sewage sludge increases the soil organic matter content in soils and, since it is rich in certain macro (N, P, K) and micronutrients (e.g. Cu and Zn), promotes plant development, as well as certain plant- and soil-associated faunal communities (Carvalho et al. 2020).

Mineral fertilisation, which is the addition of inorganic fertilisers to agricultural systems, enhances plant growth and therefore crop production. The mineral fertiliser N, such as when applied as urea, is rapidly converted to ammonium and nitrate by soil microbes, which are forms of N readily available for plant uptake (Robinson et al. 2014). It is for this reason that it is often simpler to cater for the immediate and short-term N requirements of crops with mineral fertilisers. However, in these forms of N (ammonia and nitrate), there is a direct risk of loss of NH_3 via volatilisation, and of NO_3^- via leaching. A certain proportion of organic fertilisers (a relatively high proportion of certain slurries and a lower proportion of sewage sludge) are also in these inorganic forms, and risk loss after application by the same processes.

Regular addition of organic residues has been found to increase soil physical fertility, primarily by improving aggregate stability and decreasing soil bulk density (Diacono & Montemurro, 2010). These benefits of improving soil structure and soil chemical properties in turn enhance crop productivity and quality over subsequent growth-cycles (Tang et al. 2019). There is also often a higher capacity for soil-plant systems to "recall" the history of previous organic fertiliser (OF) application longer than that of mineral fertilisers, as is reflected in the longer-term N supply (over subsequent growth-cycles) of OFs which typically act via the soil N pool (Gutser et al. 2005). In a review of the longterm effect of organic amendments on soil fertility by Diacono & Montemurro (2010), repeated application of composted matter was found to enhance soil organic N content by up to 90 %, storing it for mineralisation in future cropping seasons, often without increasing the risk of nitrate leaching.

The use of organic fertilisers in substitution or partial substitution of mineral fertilisers may have the potential to alter N losses from soil-plant systems, for example the potential reduction of ammonia volatilisation and N runoff and leaching, as documented by Tang et al. (2019). In a global meta-analysis on substituting synthetic N fertiliser with different types of livestock manure and in different crops, Xia et al. (2017) found that substituting mineral fertiliser with organic fertiliser significantly reduced leaching (28.9 %) and runoff (26.2 %) by increasing the microbial immobilisation of mineral N.

However, one potentially significant risk of organic fertilisers is their content of certain heavy metals, which may occur in quantities sufficient to adversely affect plants and soils. The availability of any given metal in the soil will be influenced by pH, SOM content, type and amount of clay, content of other metals, cation exchange capacity, variety of crops grown, and others (Nieder & Benbi, 2010).

A central question is, therefore, what are the realistic advantages and shortcomings of using recycled agricultural residues as fertiliser, with respect to both crop nutrition and environmental impact, compared to conventional (i.e. mineral) fertiliser applications? Sugarcane plantations are most commonly fertilised using mineral fertiliser. However, there is limited published literature in terms of organic fertilisation of sugarcane agricultural systems.

Sugarcane crop

Sugarcane (*Saccharum officinarum. L.*) can be described as large, perennial, sucrose-storing tropical or subtropical C4 grasses, which have evolved and continue to grow under conditions of high sunlight, high temperatures, and large quantities of water (Moore et al. 2014). Sugarcane is produced by nearly 100 countries and is a significant component of the economy of many countries in the tropics and sub-tropics (Moore et al. 2014). It is produced over 23.8 million hectares, which may only be 1.5 % of the total world cropland area (FAO-STA, 2009), but given its high levels of productivity, is responsible for the third highest quantity of human consumed plant calories (152 kcal/capita/day), following rice and wheat (Moore et al. 2014). In addition, sugarcane is one of the most successful crops for bioenergy production (Goldemberg et al. 2008). It has numerous advantages over other crops such as maize, wheat and sugar beets due to its lower energy demand over the course of the production cycle (Otto et al. 2016). In addition, the production of ethanol from sugarcane is one of the most robust greenhouse gas-saving options based on first-generation biofuel production (Smeets et al. 2009).

Sugarcane production is dependent on large amounts of N fertiliser application, which can lead to substantial N losses to the environment (Thorburn et al. 2017). With these high rates of N fertilisation globally, there are frequently substantial imbalances between N-input and N-output ratios in most sugarcane producing nations (Robinson et al. 2014). China and India, among the world's largest sugarcane producers, have for example up to nine times more N applied than is removed by the sugarcane crops (Robinson et al. 2014). Sugarcane tends to have a particularly low NUE, typically between 20-40 % of the N it requires from fertiliser, and a much as 60 % of fertiliser N may be lost from the soil-crop system (Vallis et al. 1996, Otto et al. 2016). Comparatively, it is lower than the average global values of other crops, such as cereals, which have average efficiencies closer to 50 % for maize, rice and wheat (Ladha et al. 2005, Ladha et al. 2016).

In Brazil, the world's largest producer of sugarcane, crops absorb barely 20 % of the N-fertiliser applied (Vieira-Megda et al. 2015). The reason for this low recovery has been attributed to N losses from the soil-crop system after fertiliser application to the soil surface, and to a certain portion being immobilised in the soil. There is therefore a need to improve the NUE of sugarcane agricultural systems to meet N demands, while reducing the quantity of N fertiliser applied, and improving the coordination of N application timing with the nutrient requirements of sugarcane crops.

Sugarcane nutrition has long been studied. Traditional approaches to crop nutrition have focused on crop productivity, economic yield and product quality, whereas in recent years the focus has shifted to the sustainability of production and ecological resources (Kingston, 2014). In this shift in approach, there are still considerable doubts about the N requirements of sugarcane crops (Robinson et al. 2014).

As with other crops, the soil is responsible for a large portion of N nutrition in sugarcane. A substantial proportion of N fertiliser that is not used by the plant is likely immobilised by microbial communities in the soil (Otto et al. 2016, Joris et al. 2020) and it has been suggested that long-term N fertilisation has the potential to increase the contribution of soil N to sugarcane N nutrition (Joris et al. 2020).

The relative importance of certain biomass components contributing to the nitrogen nutrition of the sugarcane system, and their respective influence on the accumulation of N in the biomass of the crop over its growth-cycle, are still not well understood. For example, a better understanding of sugarcane root development can improve crop management, in terms of allowing a more complete evaluation of the nutrient composition and temporal requirement of crops for example, and enhance agroecosystem productivity (Eshel & Beeckman, 2013). But studies on the root compartment remain scarce since it is methodologically challenging to study (Smith, 2005, Bell et al. 2015). This is due to the time-constraining nature of root sampling and processing, as well as the high uncertainty in results due to spatial and temporal variability (Versini et al. 2020). Other components of the soil-sugarcane system remain understudied, such as the loss of biomass of tillers (secondary shoots) due to senescence over the crop growth-cycle (Bell & Garside, 2005). A potentially important N source is the decomposition of trash, and the movement of this trash to the soil or plant over time (Meier et al. 2006). In addition, sugarcane trash (or 'mulch') is typically studied as what remains post-harvest, and the re-integration of strawfall (the equivalent to litterfall or leaf-fall but of dry, dead leaves) into the sugarcane system, which typically occurs from 6 months after harvest till the following harvest, appears to be rarely considered in sugarcane plantations.

Organic fertilisation in sugarcane agroecosystems in Réunion Island

The volcanic island of Réunion is situated in the Indian Ocean. It has a growing population of about 850,000 which is increasing by over 10,000 a year, and a small portion of arable land (17 % of the 2,5 000 km2 area) dominated by sugarcane production (Wassenaar et al. 2014). Réunion Island has a humid tropical climate with certain world records with respect to daily and yearly precipitation. Soils originating from volcanic materials generally have high clay and silt contents and an aggregated structure with high porosity and hydraulic conductivity values (Payet et al. 2009). This combination of pedo-climatic conditions could potentially lead to high N losses via volatilisation and leaching, for example.

Since the end of the 1980s, Réunion Island has developed intensive livestock farming to increase its self-sufficiency in food production and to create more employment through agriculture. Local food production has increased, but the consequence has been a large production of livestock effluents and a need to better manage these effluents, especially given the limited space in this context (Aubry et al. 2006). Despite certain improvements in self-sufficiency, there is still a strong and increasing dependence on import, specifically related to inputs for agricultural production or food consumption. This means, on the one hand, that smallholders depend on increasingly expensive inputs (e.g. fertilisers) from the global market, thus threatening the competitiveness of their produce. On the other hand, the situation is exacerbated by stringent European Union (EU) regulation, which leads to a pressing need for solutions to the rapidly increasing organic waste management problems (Wassenaar et al. 2014).

The recycling of organic residues in agricultural land appears therefore to be one potentially promising alternative, and a means to promote circular economic and agricultural sustainability in context of this agroecological transition. Rehabilitating disrupted nutrient cycles through organic residue recycling may carry the plausible promise of enhancing the eco-efficiency and resilience of agriculture while reducing environmental pressure (Wassenaar et al. 2014). At the same time, a high priority in this context is the better management of animal and human wastes to alleviate the imbalance in application within and between agricultural zones, and to reduce environmental risks (Aubry et al. 2006). From an economic and environmental point of view, a more comprehensive perspective than purely an interest in crop yield would be to evaluate the value of ecosystem services related to the partial substitution of mineral fertiliser with organic fertiliser. Such an approach would need to consider the range of environmental impacts and human benefits (Tang et al. 2019).

In this context, it is important to investigate the outcomes of N applied by these recycled organic fertilisers in the soil-crop system. The approach here is to use a systemic approach to evaluate the N cycling in the soil-sugarcane system, its N inputs, including the mineral and organic

fertilisers, their relative contributions, and the N outputs and the potential losses of N from the soil-crop system.

The overall aim of the thesis was to evaluate and provide a comprehensive and detailed quantitative and temporal account of N inputs and outputs into and from a highly monitored soil-sugarcane-experimental site. Secondly, it was to determine the fate of N from two types of organic fertilisers (pig slurry and sewage sludge) in the soil-sugarcane system with its established N biogeochemical cycle, compared to the application of the mineral fertiliser's (urea) N in the soil-sugarcane system, which is typically used in sugarcane agroecosystems in Réunion Island.

Chapter aims and objectives

Chapter 1. Optimising non-destructive sampling methods to study nitrogen use efficiency throughout the growth-cycle of giant C_4 crops

The aim of this chapter was to propose a method that minimises destructive sampling to quantify NUE over the crop growth cycle of sugarcane plantations, using the quantitative NRE index. The objectives were, therefore: 1/ to test whether the biomass of sugarcane can be determined non-destructively at a plot scale by using allometric relationships; 2/ to minimise the number of harvested cane required to construct an N-dilution curve; 3/ to determine the most relevant leaf for determining ^{15}N concentration in the aboveground biomass; and 4/ to assess the sensitivity of the NRE calculation depending on the chosen methods. Published in *Plant and Soil* (2020).

Chapter 2. Relative importance of distinct biomass components throughout the growth-cycle of sugarcane ratoons in N nutrition studies

The aim of this chapter was to investigate the relative importance of distinct biomass compartments in estimating the accumulation of N and N use efficiency throughout the growth-cycle of a sugarcane ratoon. The respective biomass and N mass of shoot, tiller, strawfall, root and stool compartments were measured monthly in the aboveground compartment of the system, and annually for belowground compartments, in unfertilised and fertilised treatments throughout two successive ratoons.

Chapter 3. Relative contributions to sugarcane nutrition and agronomic efficiency of distinct nitrogen sources: mineral fertiliser, organic fertilisers, past-fertilisation, mulch and soil organic matter

The aim of this chapter was firstly to determine the contribution of different N sources to sugarcane nutrition; and secondly, to determine the N use efficiency of mineral and two organic fertilisers (pig slurry and sewage sludge) over the growth-cycle of two sugarcane ratoons. More specifically, the objectives were: 1/ to determine the N mass of sugarcane subject to mineral and OF application, while considering the different biomass compartments of the sugarcane; 2/ to

determine the relative contributions of the sugarcane derived from different sources of N; 3/ to determine the fertiliser N-recovery efficiency of the mineral and two OFs over the two growth-cycles of sugarcane.

Chapter 4. Soil solution nitrogen transfers in mulched sugarcane ecosystems fertilised with mineral and organic fertilisers

The aim of this chapter was to monitor the leaching transfers of N at different soil depths in agroecosystems supplied with mineral or organic fertilisers as compared to unfertilised sugarcane over the two-year study. More specifically, the objectives were, firstly, to verify the risk of losing N by leaching throughout the crop cycle; and secondly, to investigate whether the nature of the fertilisers had an influence on the amplitude and temporal dynamics of soil solution N transfers.

Chapter 5. The fate of fertiliser-N in sugarcane agroecosystems: synthesis and perspectives

The aim of this chapter was to establish a comprehensive account of all the major N fluxes of the soil-sugarcane system, as well as to evaluate in detail the fate of N from the mineral fertiliser (urea) as well as two types of organic fertilisers, pig slurry and sewage sludge. In this concluding chapter, the N inputs and outputs are summarised in a visual nitrogen budget for the sugarcane-soil system.

Experimental Site

Study context

Réunion Island is located 700 km east of Madagascar, in the southern Indian Ocean region (Figure 1). It is a relatively young island geologically, with the main peak having emerged 3 million years ago.

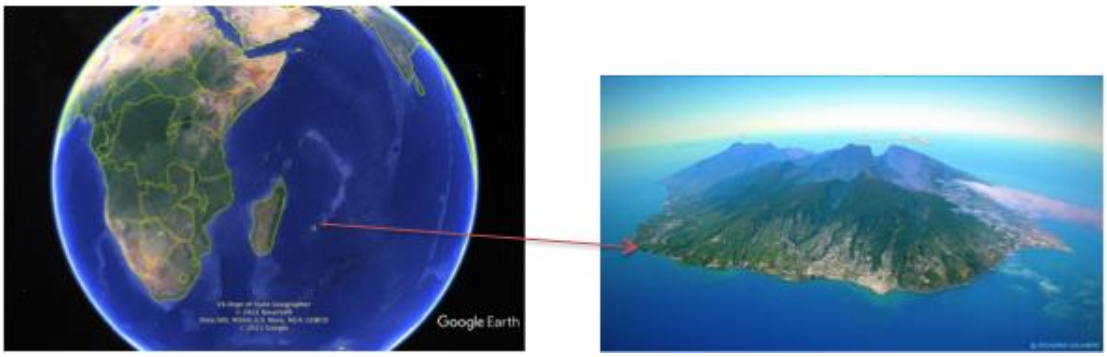

Figure 1. Geographical location of Réunion Island

It is a volcanic tropical island with a diversity of soils and pedoclimatic conditions. There are six primary soil types on the island: Non-Perhydrated Andosol, Perhydrated Andosol, Cambisol, Andic Cambisol, Nitisol, Vertisol (Figure 2).

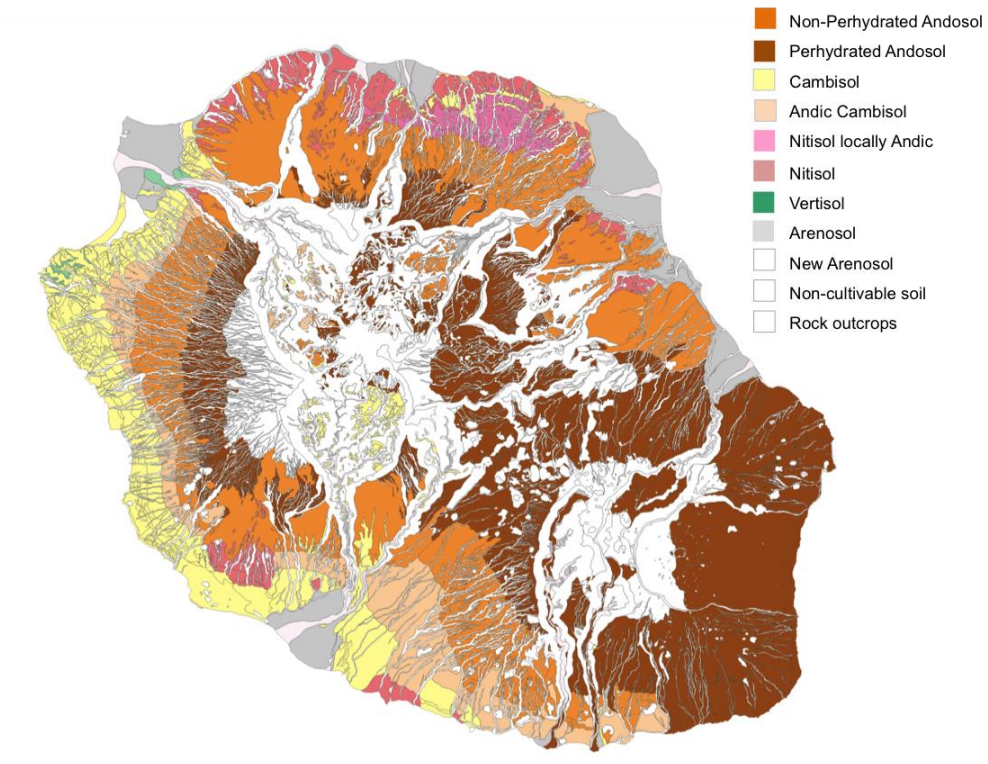

Figure 2. Soil type and distribution on Réunion Island (Pouzet et al. 2003)

The rainfall patterns are spatially very variable over the island, with the East coast typically having far higher rainfall than the West coast.

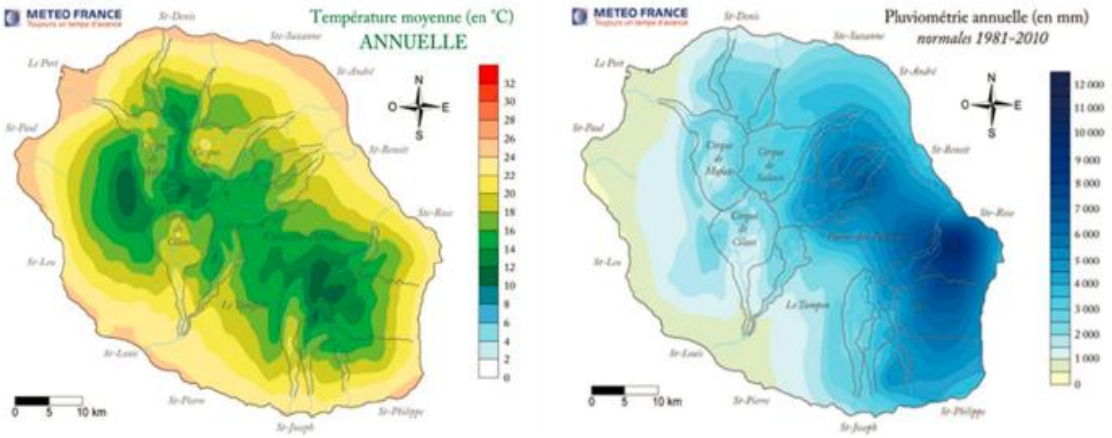

Figure 3. Average rainfall (Meteofrance, normalised continous data 1981-2010)

Agriculture covers approximately 17 % of the territory (43,692 ha) with 9272 farms, most of them less than 5 ha in size (Anon, 2001). Sugarcane is the primary crop covering 25,923 ha (55 %) and is mainly located in the lowlands (<800m) (Aubry et al. 2006).

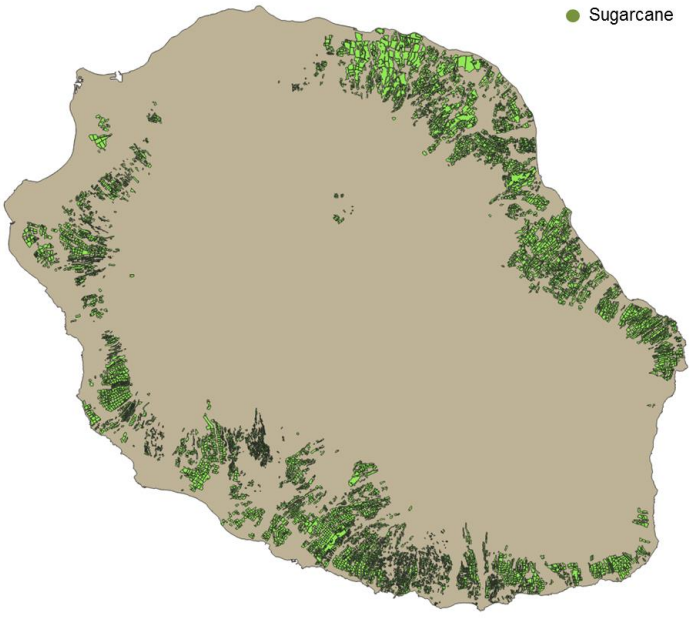

Figure 4. Sugarcane distribution on Réunion Island (Pouzet et al. 2003)

Sugarcane is a perennial crop, typically sown from vegetative cuttings. In Réunion Island, the first "plant" crop is typically sown between August and November and harvested the following year. To establish the sugarcane plantation, the soil is plowed to a depth of 30 cm. The sugarcane stalks are planted in the ground as seedlings or vegetative cuttings manually or mechanically at different interrow spacings. The first harvest is typically 10-12 months after planting. Thereafter, a succession of "ratoon" crops sprout from the stools (or stumps, or "pseudo-rhizomes") of each harvested crop and are grown for approximately 12 months each. In certain regions, the crop is replanted following a 4-6 month fallow period after 3-5 ratoons, as is considered to have lost vigour over this duration (Meier & Thorburn, 2016). However, in Réunion Island, the crop is typically replanted after up to 7-8 ratoons. Herbicide (usually glyphosate) is typically applied before bud sprouting to eliminate liana seeds in the soil, allowing the sugarcane shoots to outcompete weeds at the beginning of the cropping season. At certain plantations, there is a second application of herbicide during the growth period of the sugarcane. The sugarcane has several phenological stages, from the planting of stalk pieces, to bud sprouting and rooting, to tillering, and maturation of the sugarcane, as shown in Figure 5 (Cheavegatti-Gianotto et al. 2011).

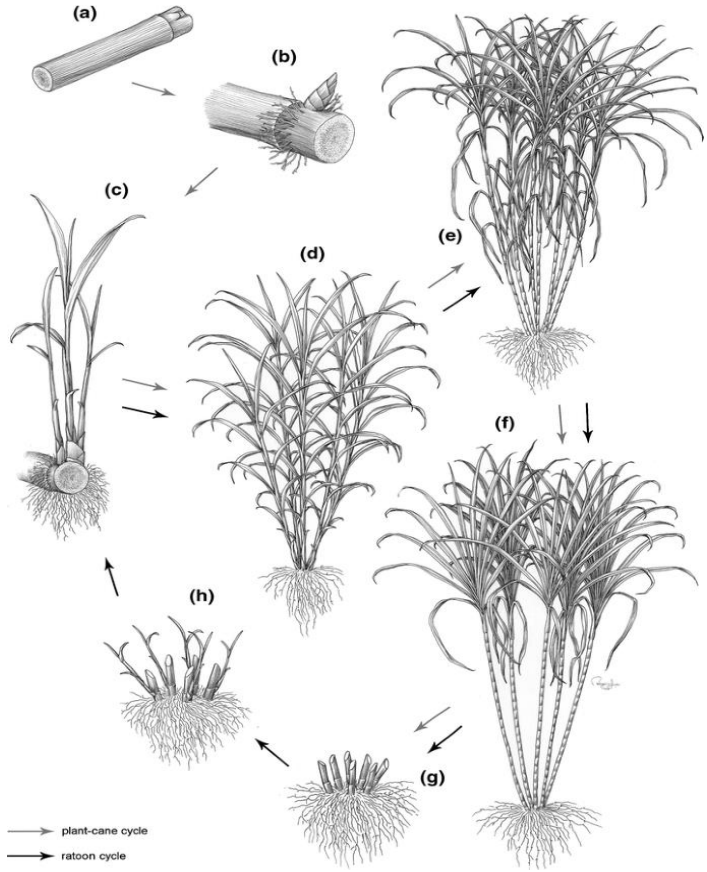

Figure 5. Sugarcane phenological cycle (Cheavegatti-Gianotto et al. 2011): (a) stalk pieces used in planting; (b) beginning of bud sprouting and rooting; (c) tillering initiation; (d) intense tillering; (e) beginning of maturation; (f) manufacturable stalks in optimal sucrose concentration; (g) harvesting; (h) ratoon sprouting.

Study site

The experimental study site for all experiments in this doctoral study were located at the La Mare experiment station, near Saint-Denis on Réunion Island (20°54'12.2"S, 55°31'46.6"E). The experimental trial took place in a highly monitored site belonging to the SOERE-PRO (best translated in English as *System of Observations, Experiments and Environmental Research on Organic Residual Waste*) network (https://www6.inrae.fr/valor-pro_eng/French-Observatory-on-Organic-Residues). The overall objective of this international network of experimental sites is to evaluate the long-term impact of organic fertilisation on the different components of different crop agroecosystems. Most of the SOERE-PRO sites are in France, but there is one experimental site in West Africa (Senegal) and in the French overseas territory of Réunion Island. The Soere-PRO site in Réunion Island is heavily instrumented with experimental equipment to measure the fluxes of nutrients, among other experiments. It is characterised by a tropical climate with an average annual temperature of 25°C and annual precipitation of 1650 mm.

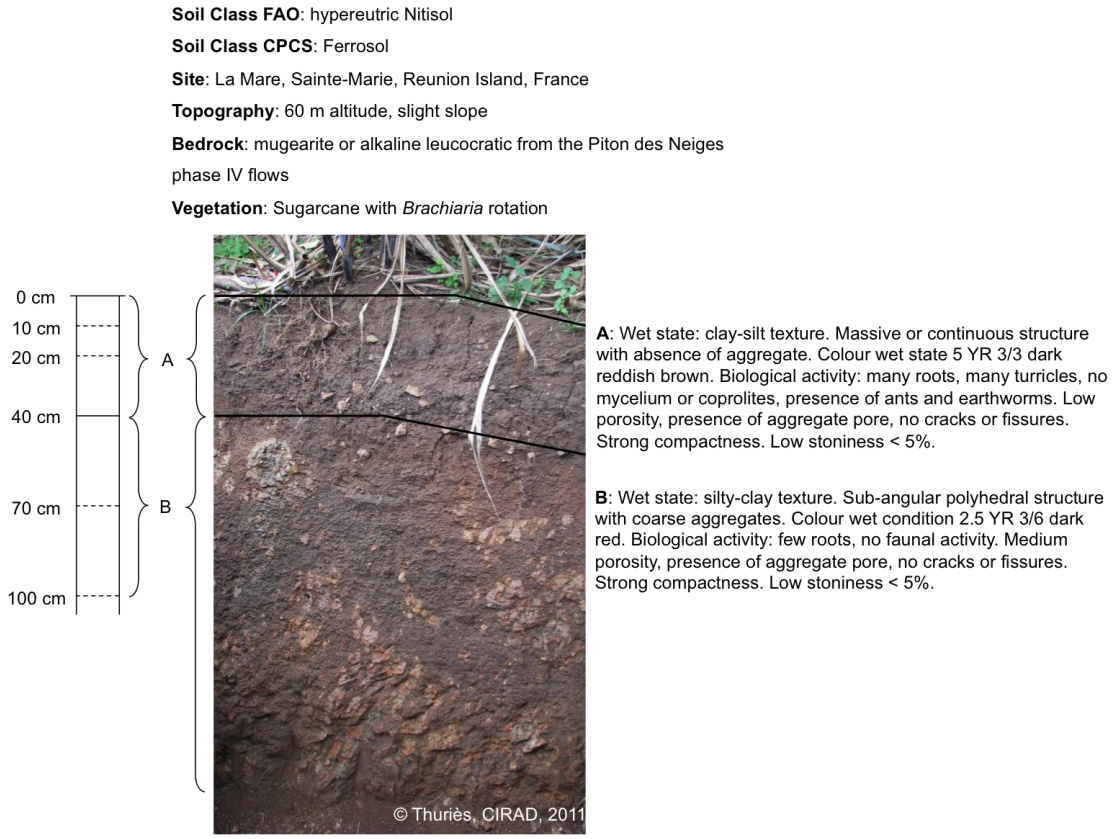

Figure 6. Soil profile distribution at La Mare (Versini, Feder pers. comm)

The soil is a silty clay Nitisol (FAO, 1998) with a cation exchange capacity (CEC) of 108.6 mmol/kg and a topsoil organic carbon content of 2 %. The soil profile is shown in more detail in Figure 6, above, where it is split into two primary descriptive categories: the soil horizon between the surface and a depth of 40 cm (A), and the soil horizon between 40 cm and 100 cm (B).

Table 1. Soil properties per soil horizon (Versini, pers. Comm,and Feder et al. 2015)

Horizon	Depth	Clay	Silt	Sand	Bulk density	Wp pF2.0	Wp pF3.0	Wp pF4.2	C	N	C:N	AEC	CEC	pH_{water}	pH_{KCL}	CEC	K^+	Na^+	Ca^{2+}	Mg^{2+}	P*
	cm	*%*	*%*	*%*	$g.cm^{-2}$	*dry weight %*	*dry weight %*	*dry weight %*	$g.kg^{-1}$	$g.kg^{-1}$	*ratio*	$/mol_{(-)}kg^{-1}$	$/mol_{(+)}kg^{-1}$			*mé/100g sec*	*mé/100g sec*	*mé/100g sec*	*mé/100g sec*	*mé/100g sec*	$mg.kg^{-1}$
	0-10	43	46	11	1.36	36	28	22	21.4	1.8	12.0		0.1963	6.1	4.8	10.6	0.7	0.2	6.7	2.9	117
A	10-20	42	47	11	1.29	38	29	23	18.7	1.6	11.4	0.0054	0.2002	6.1	4.7	10.1	0.6	0.1	6.6	2.7	90
	20-40	45	43	12	1.34	42	32	26	11.7	1.1	10.5		0.1925	6.1	4.8	8.8	0.2	0.3	5.8	2.4	39
	55-65	37	44	18	1.31	49	37	27	5.0	0.5	9.9	0.0090	0.1614	6.4	5.0	8.2	0.0	0.5	5.2	2.1	19
B	75-85	31	45	25	1.28	49	37	26	3.5	0.4	9.8		0.1574	6.4	4.9	8.0	0.0	0.5	4.9	2.1	19

The experiments of this study were centred on this SOERE-PRO experimental site, which held considerable value in terms of the variety and spectrum of experimental apparatus available. At the same time there were certain limits that constrained the type of experiments that could be undertaken and the protocols possible at the experimental site. This was in part why the study took place on a single site, which in one sense may limit the repetitions or variety of pedo-climatic conditions, for example. However, the site allowed the measurements of a large variety of N fluxes into and out of the soil-sugarcane system. In addition, the experimental site allowed in-situ field experiments and manipulations. As already outlined, there is great potential use in establishing a detailed N budget to better understand the inputs and outputs, and to quantify more precisely through the higher resolution and detail of different fluxes, of what is in many other studies "unaccounted for" N.

The sugarcane cultivar R579 was planted at the experimental trial site in March 2014 within a 1.5 m row-spacing configuration. The trial was irrigated throughout the crop cycle (29 mm/week) except for the last two months before harvest. The trial included six treatments, each with a different fertiliser, replicated in five blocks (Figure 7). Each plot contained six rows of sugarcane 28 m long that resulted in a total plot area of 250 m^2.

The measurements described in Chapter 1 were conducted over the third ratoon of the sugarcane (January to June 2017). The rest of the study (Chapters 2-5) was conducted over two experimental years, corresponding to the fourth and fifth ratoons of the sugarcane agrosystem.

Experimental design

The investigation reported here was conducted in three specific plots (Figure 7). The treatments in these plots were: 1) annual split applications of urea (plot T); 2) annual split-application of urea and a single application of pig slurry complement (plot LP); and 3) split application of urea and a single application of the sewage sludge (plot BA). The experimental design remained the same over the two experimental years, with a single exception: the 100 % urea microplots in the organic fertiliser plots moved position at the start of the second year, in order to avoid the effects of accumulated ^{15}N labelled-urea N from preceding applications.

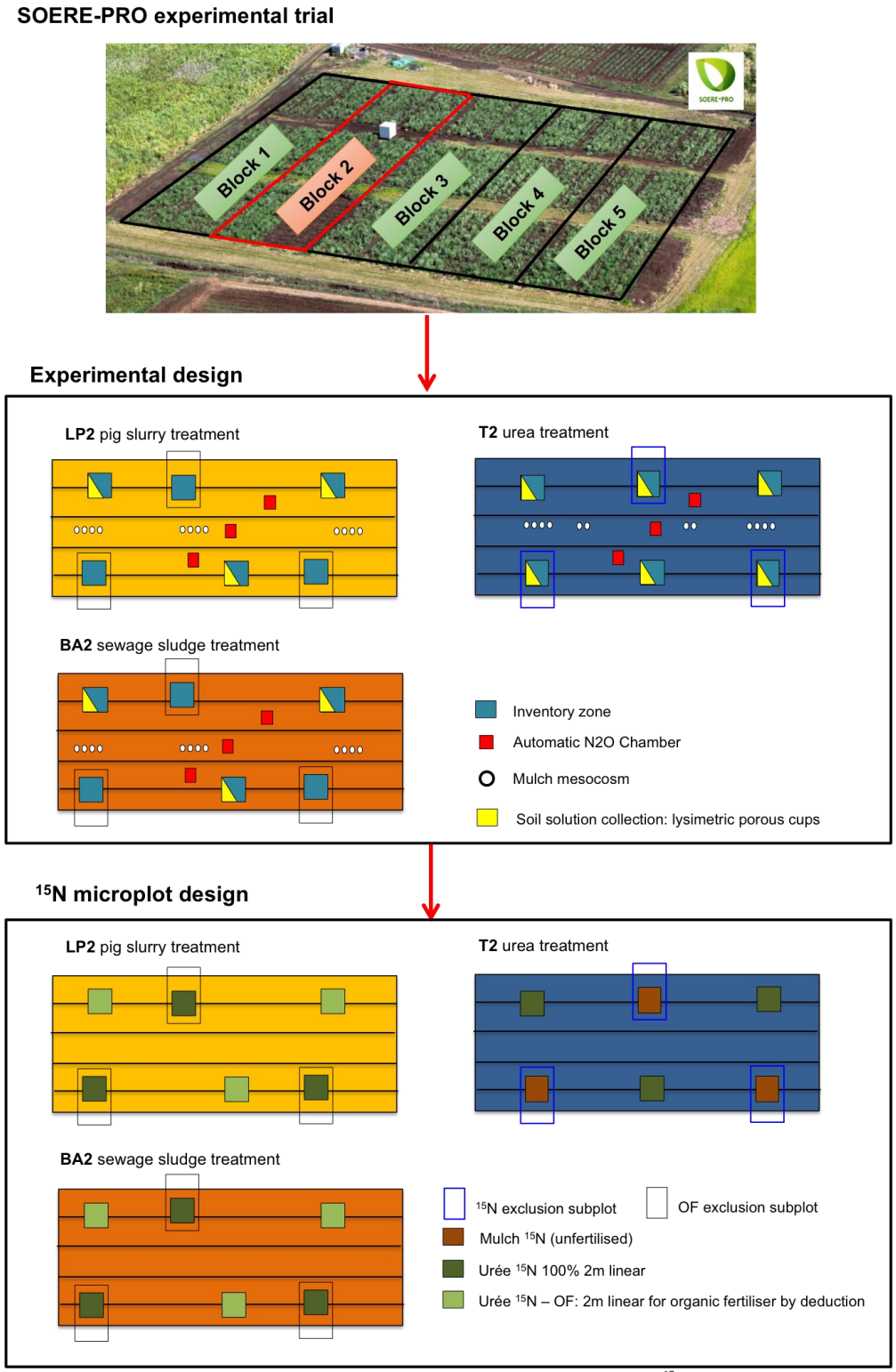

Figure 7. *Soere-PRO experimental site (top), experimental design (middle) and ^{15}N microplot design (below) for the different fertiliser treatment plots (LP2 with pig slurry application, BA2 with sewage sludge application and T2 with urea application) .The 2 m linear inventory zones, placement of automatic N_2O chambers, mulch mesocosms, and the positions of lysimetric porous cups (placed at soil depths of 10 cm, 40 cm and 100 cm at each position) are shown in the middle "Experimental design" diagram. The different ^{15}N microplot modalities and their positioning in the treatment plots are shown in the bottom diagram "^{15}N microplot design".*

The quantity of fertiliser-N for the different treatment types and their respective application periods, are summarised in Figure 8. This is explained in more detail in the Methods and Materials of Chapter 3. The pig slurry applied can be described as a clear, thick liquid with a high mineral N content. The sewage sludge applied was in the form of methanised, limed and dried pellets. The quantity of total N applied for each organic fertiliser was determined at the start of each ratoon using nitrogen mineral equivalent efficiency units.

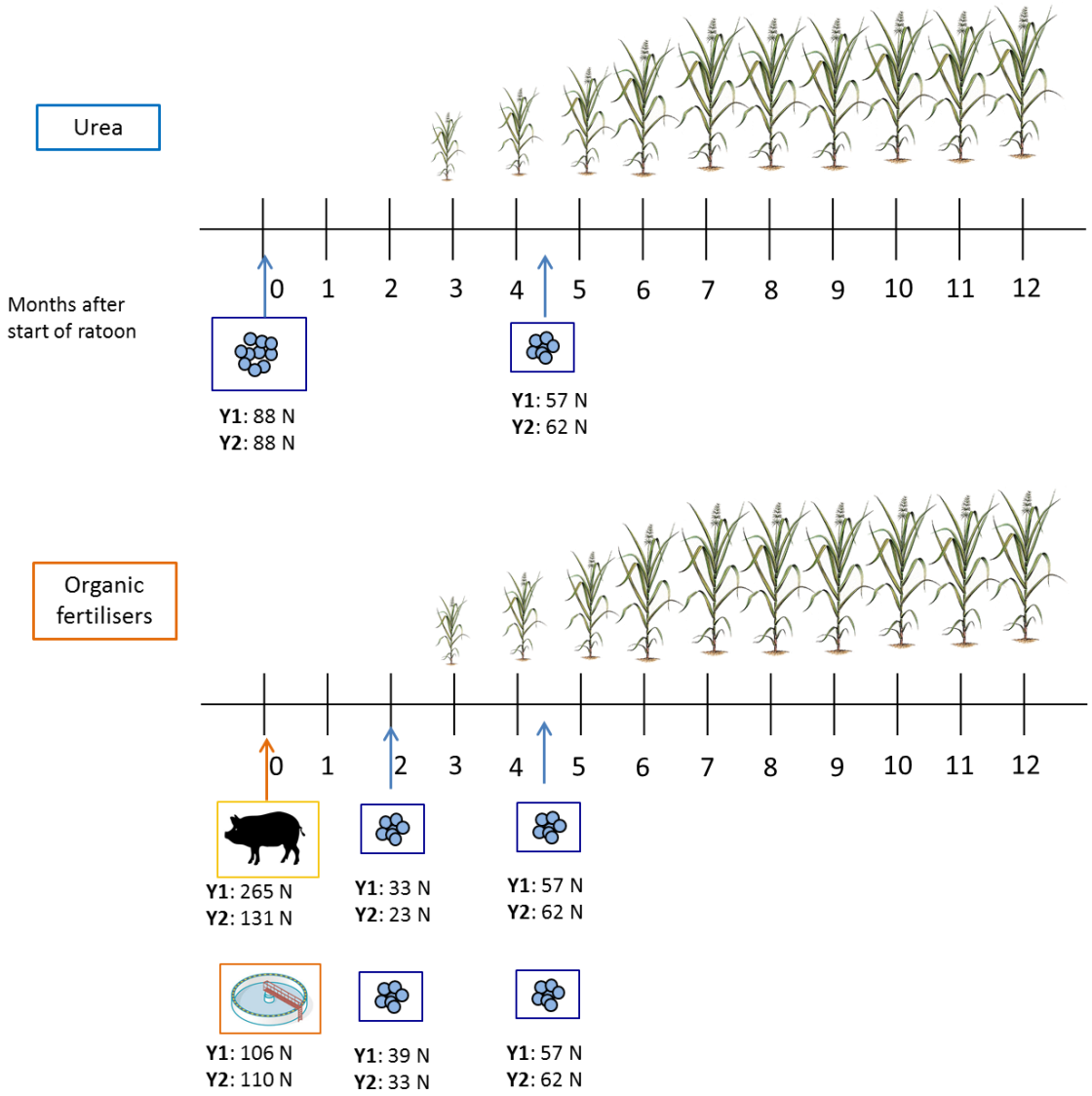

Figure 8. *Fertilisation for the first (Y1) and second (Y2) experimental years. Fertiliser doses are given as total N supplied, in units of kgN.ha-1 (labelled "N").*

Overall Material and Methods

Microplots for ^{15}N-labelling experiment

Each of the fertiliser treatment plots had 6 microplots of 2 m linear (2m x 1.5m) (Figure 7, above), based on the optimised single-row 2 m linear ^{15}N-enriched microplot design of Trivelin et al. (1994). Among them, 3 microplots corresponded to the same fertilisation design than the overall plot (Photo 1). In these 3 microplots, ^{15}N-labelled urea was applied, in the same dose and at the same time as the unlabelled urea in the rest of the plot. From the centre of each of these microplots, the sugarcane "leaf+1" (first leaf below the top visible dewlap) was harvested from two central sugarcane stalks at the start of each month. These leaves were shown to be representative of the sugarcane plant ^{15}N in Chapter 1 and are therefore used to calculate the fertiliser N recovery efficiency (NRE).

Photo 1. Urea ^{15}N-OF microplots

In each of the organic fertiliser plots, LP2 (pig slurry) and BA2 (sewage sludge), there were 3 "exclusion subplots", subject to the same urea fertiliser application as that of the T urea plot (Photo 2). These served to determine the N content of sugarcane subject to urea fertilisation in this treatment plot (i.e. subject to organic fertilisation in previous years).

Photo 2. Exclusion zone in organic fertiliser treatments

In addition, at the start of the first experimental year, ^{15}N enriched mulch was placed in three 2 m x 1. 5 m unfertilised microplots in the T2 plot (Photo 3). The previous growth-cycle's ^{15}N microplot sugarcane was harvested and the ^{15}N enriched green tops and straw were placed into these microplots, where existing unlabelled mulch was removed beforehand.

Photo 3. ^{15}N mulch microplots

Organic fertiliser N recovery efficiency calculated by deduction

The proportion of N derived from soil was calculated by deduction in the urea treatment and the 100 % urea microplots in the organic fertiliser plots, using the values of the ^{15}N enriched urea and mulch (Figure 9).

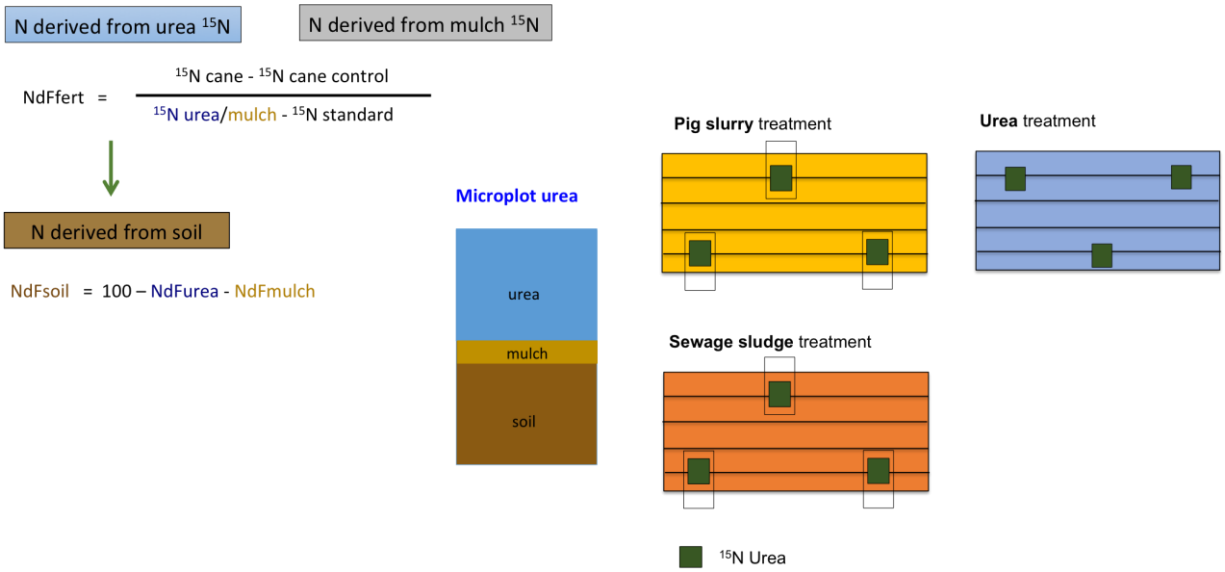

Figure 9. Calculation of nitrogen derived from ^{15}N enriched urea, ^{15}N enriched mulch, and from the soil as calculated by deduction.

With this information, the N derived from the organic fertilisers was calculated as shown in Figure 10. The soil value, as already calculated by deduction from the 100 % urea microplots (see above) was considered to be the same in the mixed urea-OF microplots, based on the assumption that the soil N contribution is homogenous over the whole treatment plot.

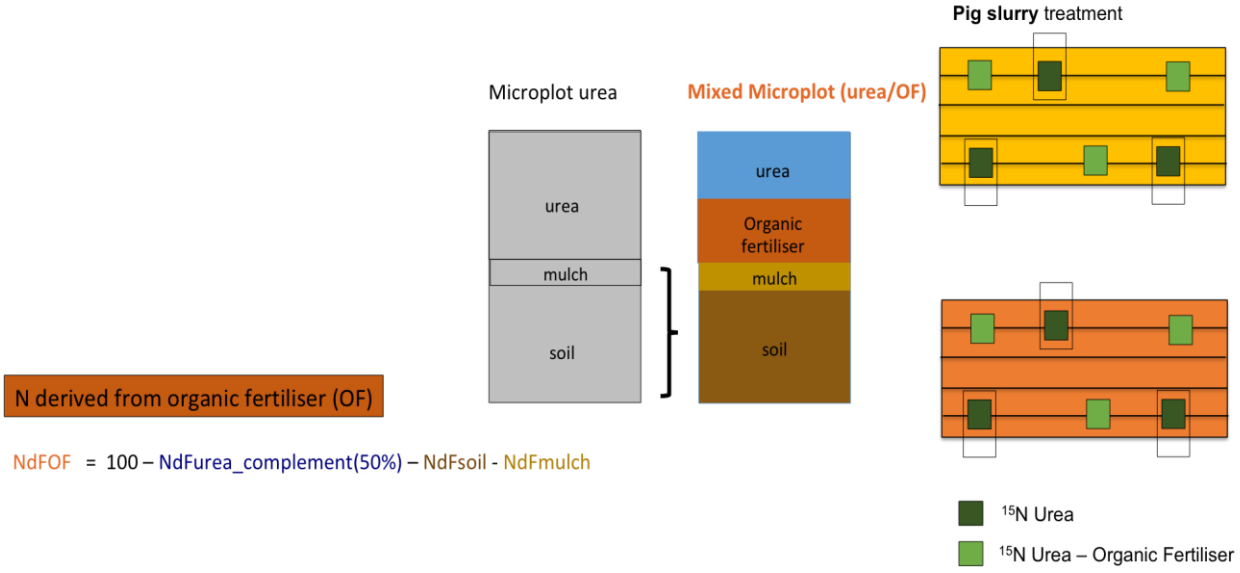

Figure 10. Calculation of nitrogen derived from the organic fertiliser portion of the mixed microplots.

The 50 % ^{15}N urea complement was calculated in the same way as in Figure 9. With these values of the soil, mulch and 50 % urea complement contributions, the N derived from the 50 % OF in the mixed-fertiliser microplot was calculated by deduction. The calculation is shown in more detail in the Methods and Material section of Chapter 3.

The reason for calculating the contribution of organic fertiliser to sugarcane N by deduction was that it would have been complicated to enrich the organic fertilisers directly in ^{15}N. In terms of pig slurry, an initial proposition was to enrich maize in hydropony with ^{15}N and to feed this to pigs, and to use these faeces enriched in ^{15}N in the microplots. However, an important constraint is that this slurry would not be homogenous and the same as the rest of the pig slurry produced industrially and applied in the rest of the plot. One means of enriching sewage sludge that was initially proposed, would have been to combine the sewage with ^{15}N marked solution during the methanisation process. However, the sewage sludge physical as well as N-type composition properties may well be altered during this process, and again differ from the rest of the pelleted sewage sludge applied in the rest of the plot.

^{15}N microplot inventories to monitor sugarcane growth

In total, there were eighteen 2 m linear microplots where inventories were taken on a monthly basis. There are typically 20-40 sugarcane stalks in a microplot, with a higher number early on in the growth-cycle and few later on due to tiller (i.e. secondary shoot) senescence. The height (from soil to the top visible dewlap, as explained in Chapter 1) and corresponding basal diameter was measured for each individual sugarcane stalk every month in each of these microplots. The sugarcane height and basal diameter were then used to calculate the sugarcane aboveground biomass using allometric relationships, as explained in more detail in Chapter 1. Briefly, allometric relationship use other measurable traits, from which the corresponding biomass can be used, and is a technique often used in forestry.

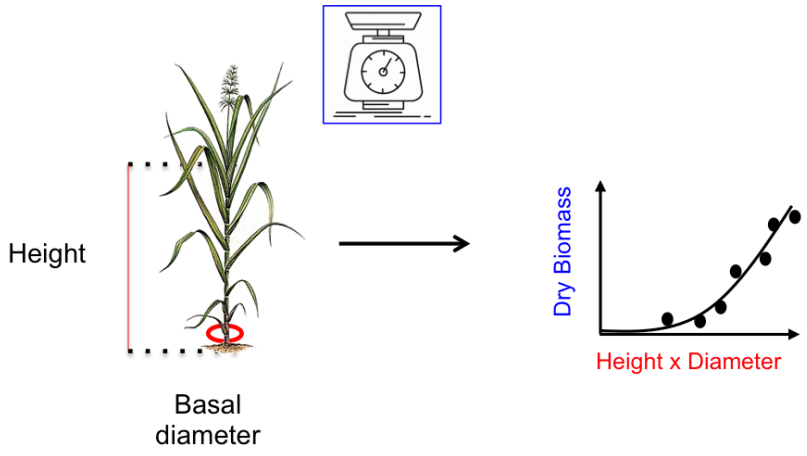

Figure 11. Measures taken to calculate sugarcane biomass using allometric relationships

Nitrogen use efficiency campaign

At the beginning of each month, starting at three months after each harvest, sugarcane inventories and the harvesting of certain sugarcane shoots, as well as ^{15}N leaves took place, in each of the different fertiliser treatment plots (of Block 2, Figure 7). This was essentially to determine the sugarcane biomass, its respective N content and ^{15}N which allows the N fertiliser efficiency (NRE) to be determined. The method is detailed in the Materials and Methods section of Chapter 1. Briefly, sugarcane measurement inventories were taken at each of the subplots (Figure 7). Height and basal diameter measurements were taken for each of the sugarcane shoots to estimate the biomass of the plot. Six sugarcane stalks were harvested from each of the treatment plots to establish an N dilution curve and to calculate the total N mass at a plot scale for each treatment type. Two sugarcane leaves (from separate plants) were harvested at the centre of each ^{15}N subplot and in the row adjacent to the subplot, to determine the N derived from fertiliser index. With these measures, the NRE is determined at a monthly interval over each of the two experimental years.

Strawfall sampling

Strawfall was collected using 1.5 m x 1.5 m "catchment" nets that were placed on the soil between sugarcane rows in each treatment plot (detailed in the Material and Methods section of Chapter 2) (Photo 4). From 6 months after the start of each experimental year (when leaves began to fall) until harvest, sugarcane leaves that fell onto this catchment net, and the portion of dry leaves that made contact with the net, were harvested twice every month. The biomass and N content was determined for the strawfall at each of these sampling dates.

Photo 4. Strawfall catchment

Mulch decomposition mesocosms

Mulch (i.e. residue or "green trash" after harvest) decomposition was measured using 3 repetitions of 4 mesocosms placed in the different treatment plots (Figure 7). The mesocosms were cylindrical PVC rings with a diameter of 40 cm and a height of 10 cm (Photo 4) (method detailed in Chapter 5). Holes were drilled into the base of the mesocosms to allow for the passage of soil fauna, possibly involved in the process decomposing the plant matter. A plastic net with a relatively large netting (>1 cm) was used to cover the mesocosms, to keep the mulch from blowing away, but without affecting the entry of water by rainfall or irrigation. Every three months, the mulch from three mesocosms of each fertiliser plot were harvested, to determine the biomass and N content of the remaining mulch at each of these dates.

Photo 5. Mulch mesocosms

Belowground biomass sampling

Sugarcane roots were sampled once over each experimental year, directly after harvest at the end of each ratoon. Soil cores were taken from each of the ^{15}N subplots (as detailed in the Material and Methods section of Chapter 2). Root biomass was estimated from 9 soil cores corresponding to 3 repetitions and 3 positions relative to the sugarcane row (0-25 cm, 25-50 cm and 50-75 cm) to a depth of 50 cm.

The reason roots were only sampled to a soil depth of 50 cm was because the vast majority of sugarcane roots at this experimental site are found within the first 50 cm, as observed in my internship study preceeding the PhD study, and corroborated in the appendix Chapter. As seen in Figure 1C of the Appendix paper (pp. 180), 70 % of the sugarcane root biomass was found in the top 30 cm of the soil profile. In certan studies, sugarcane roots have been found to extend deeper than

100 cm (e.g. Ball-Coelho et al. 1992 in Brazil), however the bedrock at the SOERE-PRO experimental site is found at a soil depth of 100 cm (as seen above in Figure 6) and therefore this would not be possible in our study.

In the field, the soil cores were divided into three layers: 0-10 cm, 10-30 cm and 30-50 cm. These soil-root samples were then taken to the laboratory, where roots were separated from soil by placing each sample into a bucket of water and swirling the water to create a vortex. The roots would float to the surface. These roots were then dried and analysed for their N and ^{15}N contents. At harvest at the end of the second experimental year, sugarcane stools were harvested. The sugarcane stool was dug out from the centre of each of the different microplots. The fine roots were cut off the stool, and the stool was dried, weighed and sampled for its N content.

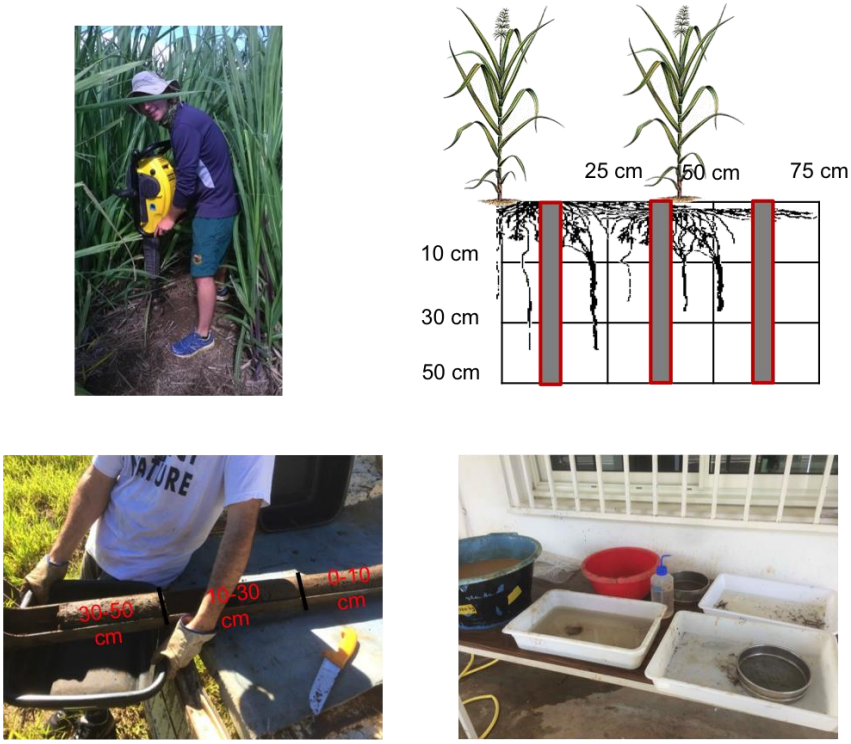

Photo 6. Root sampling (automatic auger and the positioning of soil cores above, soil core and root liberation from soil below).

Soil sampling

Soil samples were taken at the centre of the ^{15}N microplots also directly after harvest at the end of each ratoon. Soil was sampled at four different 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. These samples were then taken to the laboratory to determine their N and ^{15}N contents. This is detailed in Chapter 5.

Photo 7. Soil sampling

Lysimetric system to monitor soil solutions

Porous suction cup lysimeters were installed at the experimental site at soil depths of 10 cm, 40 cm and 100 cm. Soil solutions were collected using the porous suction cups, which were connected to a self-driven vacuum pump and maintained manually, using the vacuum pump to create a vacuum of approximately 70 kPa twice a week. This soil solution was filtered and analysed for N (in its different forms). The protocol is covered in more detail in the Material and Methods of Chapter 4.

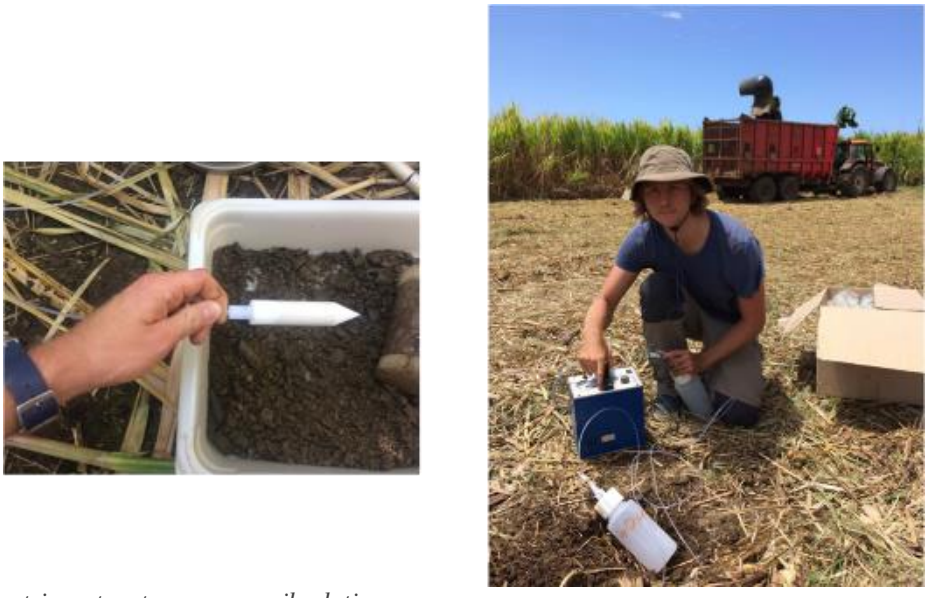

Photo 8. Lysimetric system to measure soil solutions

NH_3 volatilisation

The gas emissions were monitored and analysed by Charles Detaille, the field-engineer of the Soere-PRO experimental site.The NH_3 emissions were measured using "ALPHA" badges, which are low-cost ammonia diffusion samplers, coupled with the "FIDES" model which uses meteorological and wind turbulence data gathered at the experimental site. This is explained in more detail in Chapter 5. After the annual harvest, the geometry of the plots was traced using a high-precision GPS. Then, three ALPHA badges were placed on a mast at the center of each of the experimental plots, suspended at a height of 50 cm. The ALPHA badges were removed 6-7 after N fertiliser application to measure the ammonium concentration at the Cirad laboratory in Saint-Denis, Réunion Island.

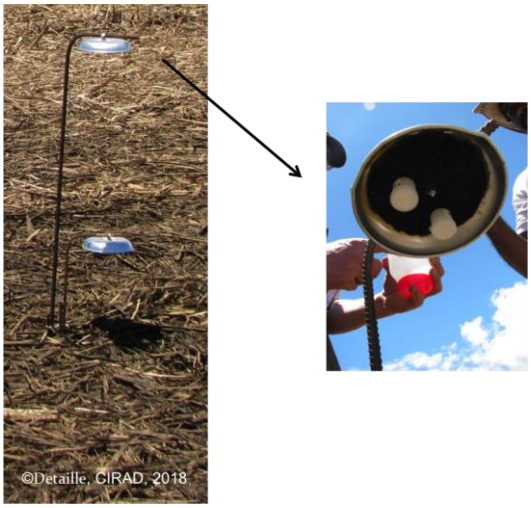

Photo 9. Alpha badge

N_2O emissions

N_2O emissions were measured using three automatic gas chambers placed centrally in each of the different treatment plots (Figure 7, above). The chambers would close for 20 minutes four times each day, on a 6-hour rotation. Measurements of the N_2O emissions were taken in the central station using an in-situ gas chromatograph.

Photo 10. Gas emissions monitoring (automatic chambers in plot T2 on the left and a single automatic chamber measuring N2O on the bottom-right).

CHAPTER ONE

Abstract

Aims

The improvement of nitrogen use efficiency (NUE) of crops allows crop nitrogen (N) demands to be met while reducing N supply, and so reducing excess N which has potential negative environmental implications. NUE is often determined destructively at the end of crop growth-cycles without considering temporal variability. Here we present a methodological study which optimises the determination of NUE throughout the sugarcane growth-cycle using minimally destructive methods.

Methods and results

The determination of the NUE relied on the optimisation of three methods: the estimation of aboveground biomass, N content and N derived from fertiliser (NdfF). First, the ability of different allometric relationships to estimate sugarcane biomass was investigated by selecting a relationship based on height and diameter to estimate aboveground biomass along the crop growth-cycle. Secondly, we assessed the minimum number of harvested sugarcane required to construct a dilution curve to predict N content from biomass and found that a sampling of 5 sugarcanes at 3 dates was sufficient to represent aboveground N content over the growth-cycle. Finally, the ability of ^{15}N content of individual leaves to represent the NdfF in ^{15}N-fertilised cane was tested. The first and second leaf below the top visible dewlap were the most representative. Based on a variance analysis, we assessed the level of influence of each method on the NUE calculation. Crop age accounted for 54% of the variance of NUE, the choice of ^{15}N leaf 13 %, with the choice of model to estimate biomass and the number of plants harvested for the N dilution curve, each accounting for less than 2 % over the four sampling dates.

Conclusions

This study highlighted the importance of evaluating NUE not only at the point of harvest. We propose, therefore, a set of methods to study NUE throughout the sugarcane growth-cycle by using minimally destructive sampling. The use of these methods could also potentially be used for other giant C_4 crops.

Keywords: *Allometric relationships, Dilution curves, Nitrogen Use Efficiency, ^{15}N labelling, N-fertiliser Recovery Efficiency, Sugarcane*

Résumé

Objectifs

L'amélioration de l'efficience d'utilisation de l'azote (ici le « coefficient réel d'utilisation de l'azote » ou CRU) par les cultures permet de satisfaire les besoins en azote (N) des cultures tout en réduisant les apports de N, réduisant du même coup le N excessif qui peut avoir des conséquences négatives sur l'environnement. Le CRU est souvent déterminé de manière destructive à la fin du cycle de croissance des cultures sans tenir compte de la variabilité temporelle. Nous développons dans ce chapitre une approche peu destructive permettant d'estimer l'efficience d'utilisation de N tout au long du cycle de croissance de la canne à sucre.

Méthodes et résultats

La détermination de l'efficience d'utilisation de N s'est appuyée sur l'optimisation de trois méthodes : l'estimation de la biomasse aérienne, de la teneur en N et du N issu des fertilisants (NdfF). Premièrement, la capacité de différentes relations allométriques à estimer la biomasse de la canne à sucre a été validée en sélectionnant une relation basée sur la hauteur et le diamètre pour estimer la biomasse aérienne le long du cycle de croissance de la culture. Ensuite, nous avons évalué le nombre minimum de cannes à sucre à récolter afin de construire une courbe de dilution permettant de prédire la teneur en N à partir de la biomasse. Nous avons mis-en-évidence qu'un échantillonnage de 5 cannes à sucre à 3 dates était suffisant pour estimer la teneur aérienne en N tout au long du cycle de croissance. Enfin, la capacité de la teneur en ^{15}N des feuilles individuelles à représenter le NdfF dans la canne fertilisée en ^{15}N a été testée. La première et la deuxième feuille sous le premier ochréa visible se sont montrés les plus représentatives de la teneur globale du compartiment aérien. En se basant sur une analyse de variance, nous avons évalué le niveau d'influence de chaque sous-méthode sur le calcul de l'efficience d'utilisation du N. L'âge de la culture représentait 54 % de la variance de l'efficience d'utilisation du N, le choix de la feuille ^{15}N comptait pour 13 %, le choix du modèle pour estimer la biomasse et le nombre de cannes récoltées pour la courbe de dilution de l'azote représentant chacun moins de 2 % de la variance.

Conclusions

Cette étude a mis en évidence l'importance d'évaluer l'efficience d'utilisation de N pas uniquement au moment de la récolte mais tout au long du cycle de culture. Nous proposons ainsi un ensemble de méthodes permettant d'étudier l'efficience d'utilisation de N tout au long du cycle de croissance de la canne à sucre en utilisant un échantillonnage peu destructif. Cette méthode pourrait également être mobilisée pour d'autres cultures géantes en C4.

Mots-clés: *Relations allométriques, Courbes de dilution, Efficience d'utilisation de l'azote, Marquage ^{15}N, Coefficient réel d'utilisation d'azote, Canne à sucre*

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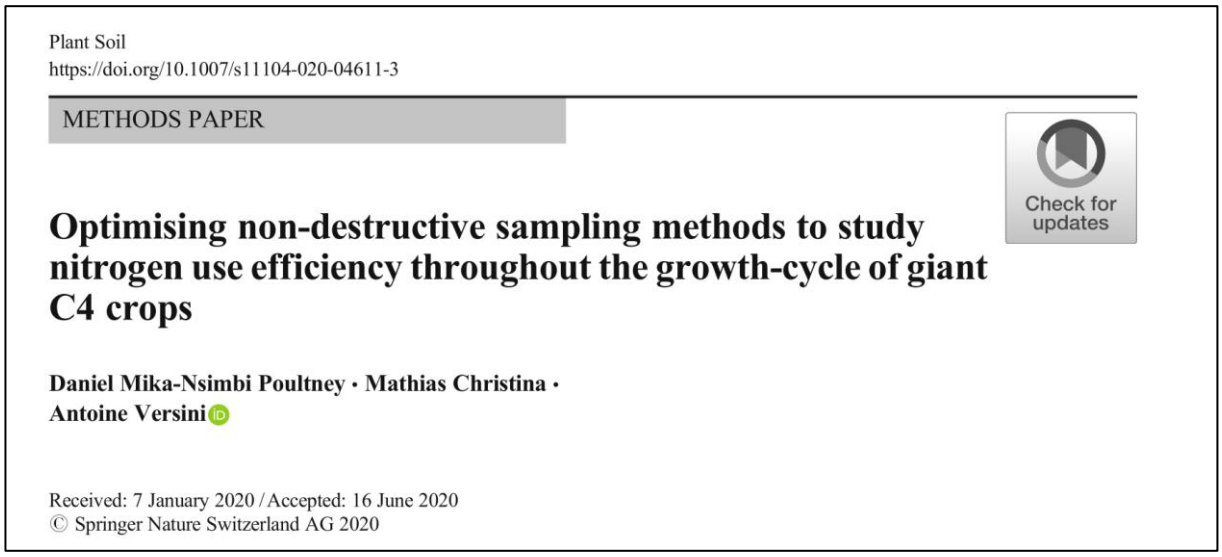

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METHODS PAPER

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Optimising non-destructive sampling methods to study nitrogen use efficiency throughout the growth-cycle of giant C4 crops

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Chapter 1. Optimising non-destructive sampling methods to study nitrogen use efficiency throughout the growth-cycle of giant C4 crops

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1.1 Introduction

Nitrogen (N) is the "very stuff of life" in that it drives the machinery of every living cell and is required by all living organisms (Galloway and Cowling, 2002). Nitrogen availability directly influences crop growth in a soil-plant system, and accounts for 80 % of soil derived essential nutrients acquired by plants (Robinson et al. 2013). The crop N requirement can be supplied by several sources. However mineral and organic N fertilisers have increasingly played major roles in meeting N crop demand, given the rapidly rising human population and associated rise in food and energy production (Galloway et al. 2008; Smil, 2002). Without N fertilisers, it is estimated that only half of today's world population would be fed by pre-fertiliser farming (Smil, 2002). However, despite the substantial benefits of using N fertilisers, excessive amounts accumulated in terrestrial and aquatic ecosystems can lead to a significant impact on environmental quality, ecosystems, biodiversity and human health (Dobermann, 2005).

Globally, it is estimated that between 30 % and 50 % of applied N-fertiliser is taken up by crops (Cassman et al. 2002; Smil 1999; Tilman et al. 2002). Similarly, to other major crops, sugarcane plantations have high N fertilization rates globally (Robinson et al. 2013). China and India, some of the largest sugarcane producers, have up to nine times more N applied than is removed by sugarcane crops (Robinson et al. 2013). Furthermore, there is frequently a lack of synchronization between sugarcane N needs and the quantity of N fertiliser applied. In Brazil, the world's largest producer of sugarcane, sugarcane crops absorb barely 20 % of the N-fertiliser applied (Vieira-Megda et al. 2015). The reason for this low recovery has been mainly attributed to high microbial immobilisation and to N losses from the soil-plant system after urea application to the soil surface. Consequently, there is a need to improve nitrogen use efficiency (NUE) of sugarcane agroecosystems to meet N demand while reducing N applications.

Nutrient use efficiency is generally defined as the ability of a crop to acquire nutrients from a growth medium and to incorporate or utilise these nutrients in the production of harvestable plant

material, aboveground biomass or total plant biomass (including belowground biomass) (Baligar et al. 2001; Blair, 1993). One index which quantifies NUE, as the efficiency of crops to use nitrogen applied to soils, is the N-Recovery Efficiency (NRE). This is the same, but termed differently, as "Crop recovery efficiency of applied N" (RE_N) by Cassman et al. (2002), as well as Dobermann (2005); "Fertiliser N uptake efficiency" ($NUpE_{Fert}$) by Bell et al. (2015); and "Apparent nitrogen recovery" (AR) by Good et al. (2004). This NRE is the calculated percentage of fertiliser-N recovered in the crop aboveground biomass during the crop-growing season. Two main approaches have previously been used to assess NRE. The first approach, the "difference method", corresponds to the broadest measures of NRE. It is based on the difference between the amount of N accumulated in the aboveground biomass (N content per unit biomass) of N-fertilised and non-fertilised crops (Cassman et al. 2002; Ferchaud et al. 2016). The second approach uses ^{15}N-labelled fertilisers to estimate the crop recovery of applied N (Hauck and Bremner, 1976). For both approaches, the calculation of the NRE indices requires a precise estimation of the crop biomass and the amount of N contained in the aboveground biomass. It is for this reason that it remains challenging to study NUE at different stages of crop growth, as the biomass is usually determined at the end of the crop growth-cycle when the crop is harvested.

Most studies using the ^{15}N method to estimate the N-fertiliser recovery in sugarcane agroecosystems have focused on fertiliser NRE at the end of the crop cycle (Ambrosano et al. 2011; Basanta et al. 2003; Chapman et al. 1994; Fortes et al. 2011; Isa et al. 2006). Only a few studies have investigated NRE during the crop cycle and crop development for sugarcane crops (Courtaillac et al. 1988; Ng Kee Kwong and Deville, 1994), and for certain other crops such as during different growth stages of different cultivars of wheat (Ma & Dwyer, 1998); or the use of N derived from fertiliser (NdfF) at different phases of the crop cycle (Franco et al. 2011; Vieira-Megda et al. 2015). Although NdfF is not directly related to NUE, it provides information on the relative importance of particular N sources on plant nutrition. Studies by Franco et al. (2011) and Vieira-Megda et al. (2015) found that NdfF decreases from 40-70 % at initial stages of sugarcane crop development, to 10-30 % before crop harvest. These studies have highlighted the crucial role of N fertilization for sugarcane nutrition over the crop growth cycle, with a particular importance at early stages of development. Furthermore, a study evaluating NRE specifically, by Ng Kee Kwong and Deville (1994), showed that NRE decreased from 20-40 % during the cane growth cycle to 13-18 % at the crop harvest using the ^{15}N isotopic method, which highlights the temporal variability of NRE. These results point to the importance of considering the whole crop cycle when studying fertiliser NUE in sugarcane agroecosystems. In particular, destructive methods of NRE, could be minimised, allowing for further measurements to be made in the same treatment plots at further time intervals over the crop development.

To assess the NUE in sugarcane agroecosystems, it is necessary to assess i) sugarcane biomass, ii) N concentration and iii) the proportion of cane-N derived from fertiliser. Sugarcane biomass was classically obtained from destructive sampling or simulated with sugarcane growth models (Lisson et al. 2005; O'Leary et al. 2000). One alternative to destructive sampling is the use of remote sensing to determine crop biomass and its corresponding N content. This method allows for the estimation of crop biomass through LAI estimation, as well as crop N content, through crop chlorophyll content estimation (Lemaire et al. 2008). Another alternative way of calculating sugarcane biomass non-destructively, without these constraints, is the use of allometric relationships; that is, measuring the relationship between plant biomass and other morphological traits (such as height, stem diameter and canopy volume) without harvesting the plant. These relationships are typically used in forestry (Chave et al. 2014; Paul et al. 2013; Parresol et al. 1999), but have been used for certain giant C4 crops, which tend to have a cylindrical morphology, such as for various maize cultivars (Tittonell et al. 2005); sorghum (Martin et al. 2013) and for various tall-grass species such as miscanthus (Anten & Hirose. 1999). The use of allometric relationships to estimate sugarcane biomass was only recently reported by Youkhana et al. (2017) using cane diameter and height as separate measures. The N content of sugarcane has traditionally been estimated with dilution curves representing the quantity of crop N as a function of crop biomass (e.g. Oliveira et al. 2013). The concept of the N dilution curve was developed by Lemaire and Salette (1984) for tall fescue and was primarily used to monitor and fine-tune fertiliser input in agroecosystems. Finally, when the ^{15}N isotopic approach is used for sugarcane, a single leaf with a ^{15}N signature representative of the entire plant can be sampled to assess the proportion of cane-N derived from fertiliser (Trivelin et al. 1994). As Dillewijn et al. (1952) initially proposed that the leaf+3 (relative to the top visible dewlap or TVD) was representative of the entire sugarcane aboveground biomass, it has subsequently been used in multiple studies determining NdfF (Franco et al. 2011; Trivelin et al. 1994; Vieira-Megda et al. 2015).

The aim of our study was to propose a method that minimises destructive sampling to quantify NUE over the crop growth cycle of sugarcane plantations, using the quantitative NRE index. The objectives were therefore: 1/ to test whether the biomass of sugarcane can be determined non-destructively at a plot scale by using allometric relationships; 2/ to minimise the number of harvested cane required to build an N-dilution curve; 3/ to determine the most relevant leaf for determining ^{15}N concentration in the aboveground biomass; and 4/ to assess the sensitivity of the NRE calculation depending on the chosen methods.

1.2 Materials and methods

1.2.1 Study site

Refer to the study site in the Experimental design and general methodology. In this plot, 97 kg N ha^{-1} was applied following the annual harvest in December 2016 and 44 kg N ha^{-1} in March 2017.

1.2.2 Cane biomass and allometric relationship with measurable parameters

Twenty individual sugarcanes were harvested at 6 sampling dates in order to establish allometric relationships (Table 1). Four individual cane shoots (dry leaves, tops and stalk), were collected within 5 height classes representative of the height distribution in the experimental plot. The 5 height classes were determined as the mean of each quintile of sugarcane heights measured in four 2 m linear subplots. The dry biomass of the 20 canes were weighed after they were oven-dried at 60°C for 72 hours.

Different allometric relationships were tested, by fitting models that included plant aboveground dry biomass and a range of measurable cane traits. The cane traits included height to the top visible dewlap (h_{TVD}) and the diameter of the base of the cane stalk (D_b) measured for each of the 20 sugarcane collected at each date. Various functions were tested (data not shown for this preliminary test) to determine the best fit; that is, linear, power, exponential and second degree polynomials, using the *nls* function to fit the function to the data points; and the aic (Akaike Information Criterion) criteria to rank the best fitting functions with R version 3.3.2. software (R Development Core Team 2016). The power function between the plant aboveground dry biomass and cane traits was used as allometric relationship because it showed the lowest AIC.

We compared allometric relationship, depending on cane traits and sampling dates, for estimating sugarcane biomass along the crop growth-cycle. Two types of models were tested: local models (n=20), where allometric relationships were established for each specific date, and global models (n=120), where a unique allometric relationship was established across all sampling dates. Consequently, we compared four types of allometric relationships to predict plant aboveground dry mass: 1/ a local model using only h_{TVD}; 2/ a global model using only h_{TVD}; 3/ a local model using h_{TVD} combined with D_b; 4/ a global model using h_{TVD} combined with D_b (models summarized in Table A).

To test these allometric relationships, an additional 2-metre linear subplot of sugarcane (3 m^2) was collected at each sampling date. Dry mass, h_{TVD} and D_b were determined for each cane shoot and the sum taken to estimate the plot aboveground dry biomass (kg m^{-2}). Similarly, the sugarcane dry biomass (kg m^{-2}) was estimated at each date from the four models. The mean

difference between estimated and observed plot aboveground biomass (Δ%), was finally used to select the best allometric relationship model.

1.2.3 Sugarcane nitrogen content and dilution curve

The 20 cane shoots (dry leaves, tops and stalk) collected on the 5 sampling dates (Table 1) from different locations in the plot to build allometric relationships were analysed for their N concentration. All dried samples were ground to pass a 1 mm mesh using a Universal Cutting Mill (PULVERISETTE 19, Fritsch) and analysed for N with an elemental analyzer (Vario Max Cube CNS, Elementar, Hanau, Germany) in the CIRAD laboratory in Saint-Denis (La Réunion, France).

A reference dilution curve was constructed between biomass estimations at the plot scale over the 5 sampling dates and the N content of the 20 sampled canes per dates (power function, *nls* function). The sum square error was calculated and defined as SSE_{20}. The minimum number of cane shoots required to estimate the N content was investigated to minimize the number of harvested cane. To do this, we compared the reference N dilution curves with dilution curves built with a lower numbers of canes. As an example, we built a new dilution curve using 5 canes per dates and calculated the SSE of this new regression (in that example SSE_5). Then we estimate the SSE from the nested model including data from the 20 original canes plus the 5 sampled canes, named SSE_{20+5}. Finally, we calculated the Fisher statistics as follows:

$$F = (SSE_{20+5} - SSE_{20} - SSE_5)(p_{20} + p_5 - p_{20+5})(SSE_{20} + SSE_5)(n - p_{20} - p_5)$$

Where p is the number or parameters of each regression (2 in this case) and n the number of data (here 5 x 20). This F statistic was compared to the Fisher-Snedecor tables (α=5%). If not statistically different, the dilution curve was defined as "successful". For a 5 shoot sample, the operation was repeated 5000 times to take account of variability in the reference sampling. The probability of obtaining a dilution curve different from that of the reference curve (P_{DIFF}) was determined for 15, 10, 5 and 2 sugarcanes respectively at the 5 dates. For 'non-successful' dilution curves, which were not identical to the reference curve, a 95 % confidence interval was established ($CI_{95\%}$). This was the interval where there was a 95 % probability of obtaining the reference curve that differed from the reference curve. The same approach was used to test whether the same dilution curve would be obtained for sugarcane sampled on 3 dates rather than 5 dates.

1.2.4 Nitrogen derived from fertiliser

The N derived from fertiliser was estimated using sugarcane leaf samples collected from microplots that have received fertiliser labeled ^{15}N for the determination of ^{15}N abundance (% atoms ^{15}N) (Takahashi 1967; Trivelin et al. 1994; Franco et al. 2011). Within the experimental plot, three microplots of 2.25 m^2 (1.5 m x 1.5 m) received labeled urea (3 atom% ^{15}N excess) in the same

quantity as the conventional non-labeled urea. It was therefore applied homogeneously across the microplots on December 2016 and March 2017.

On four of the sampling dates (Table 1.1), one individual cane shoot was harvested at the center of each of the three microplots to test the reliability of each leaf to represent the overall biomass ^{15}N. The first 5 leaf blades were separated from the sugarcane plant to test which leaf had a ^{15}N signature closest to the aboveground biomass. The numbering of leaves followed the Kuijper's leaf numbering system (1915), where "leaf+1" is considered as the first leaf connected to the top visible dewlap (TVD) and the leaf number increases moving down the cane stalk (*i.e.* "leaf+2" is the second leaf below the TVD, etc.).

Table 1.1. Sampling dates to estimate cane biomass with allometric relationships, N content from dilution curves, NdfF from ^{15}N enrichment and Nitrogen Recovery Efficiency (NRE) along the crop cycle in sugarcane agroecosystems.

Date	1 Feb 2017	1 March 2017	29 March 2017	4 May 2017	7 July 2017	13 Sept 2017
Month after planting	3	4	5	6	8	11
Biomass	X	X	X	X	X	X
Dilution Curve	X	X	X	X	X	-
NdfF	X	-	X	-	X	X
NRE	X	-	X	-	X	X

To assess the ^{15}N signature of each of the leaves or aboveground biomass, all samples were ground to pass a 500 µm screen using a Cyclotec grinder (CT Tecator Cyclotec Sample Mill, Foss), sent to the PTEF laboratory in Nancy (Plateforme Technique d'Ecologie Fonctionnelle, INRA, France), where samples were further ground to pass a 100 µm screen 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 fertiliser (NdfF, the proportion of N in the plant derived from fertiliser, %) was determined by the following formula:

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

where a is the abundance of ^{15}N atoms in the plant (%), b is the natural abundance of ^{15}N atoms in a control unlabeled plant sample (%), c is the abundance of ^{15}N atoms in the fertiliser (%) and d is the natural abundance of ^{15}N atoms of a standard (0.366%).

The difference (%ΔNdfF) between NdfF of each of the plant leaves and the aboveground biomass was determined for each sampling date. The average aboveground NdfF was compared with the average NdfF estimated from each leaf using a t.test.

1.2.5 Computation and variability of NRE

The nitrogen recovery efficiency (NRE) was calculated at four dates (Table 1) from aboveground biomass, crop N content and NdfF. Due to sugarcane's ability to uptake N via roots at considerable distances from the stem of the plant (Smith et al. 2005), the NdfF of sugarcane outside of the microplot was also considered:

$$NdfF_T = NdfF_M + 2 \times NdfF_{AR} \quad (2)$$

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 was that the uptake of N by sugarcane in the row adjacent to the microplot would be the same as the N uptake of the sugarcane inside the microplot, taken from the row adjacent (Trivelin et al. 1994).

Nitrogen recovery in the plant biomass was calculated according to:

$$\text{NRE} = \frac{Ndff * N_{plant}}{N_{fertiliser}} \quad (3)$$

where NRE is the recovery efficiency of fertiliser N in the plant (%), N_{plant} is the quantity of N in the plant ($g.m^{-2}$) and $N_{fertiliser}$ is the quantity of N applied with the fertiliser ($g.m^{-2}$).

We assessed the influence of the different methods of estimation of biomass, N content and NdfF, on the NRE estimation. A reference method was defined based on 1) the best allometric relationship in estimating sugarcane biomass at a plot scale, 2) the reference dilution curve based on 20 harvested canes and 3) the NdfF calculated with the aboveground ^{15}N signature. NRE was additionally determined using other methods to assess the influence of biomass, N content and NdfF. Considering biomass, the tested methods included the local and global models based on height, diameter and height x diameter. Considering the N concentration, N dilution curves built using 5 and 2 cane shoots were tested. Considering the NdfF, the methods tested included the use of the leaf+1, the leaf+2, the leaf+3, the leaf+4 and the leaf+5 (the leaf number referring to the position of the leaf below the top visible dewlap).

The effect of the different methods (biomass, N and NdfF) and their interaction on the NRE estimation was assessed using a linear variance analysis (ANOVA). The percentage variance of each estimation method was then calculated using the following equation:

$$var_{method} = \frac{SSE_{method}}{\Sigma \text{SSE}} \quad (4)$$

where var_{method} is the percentage variance of a method; SSE_{method} is the sum of squares of the respective method and ΣSSE is the sum of the sum of squares of all the ANOVA parameters.

1.3 Results

1.3.1 Biomass estimation with allometric relationships

The first step in determining the N-fertiliser recovery efficiency (NRE) over the sugarcane growth-cycle, was the estimation of the sugarcane aboveground biomass at a plot scale, using allometric relationships. The accumulation of aboveground dry biomass as the growth-cycle progressed followed a similar trend for all of the allometric relationship models except for the global diameter model (Figure 1.1). The measured dry biomass varied from 0.684 kg.m^{-2} at 3 months to 3.960 kg.m^{-2} at 11 months (Figure 1.1).

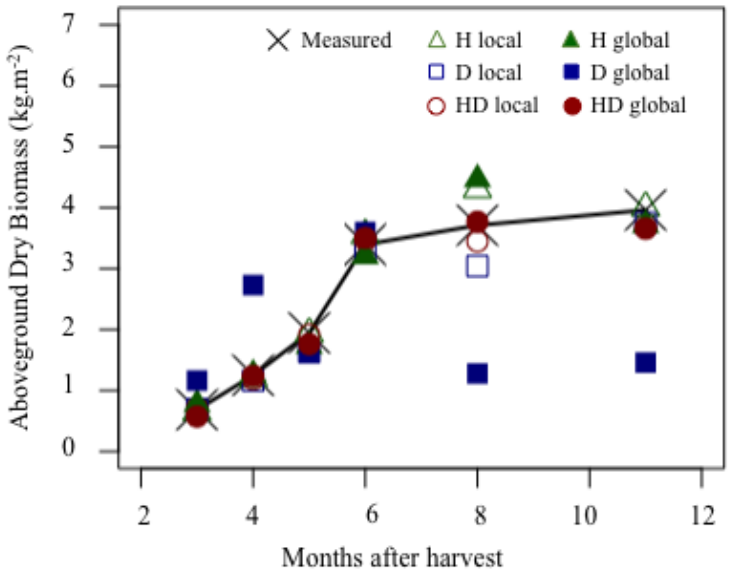

Figure 1.1 *Aboveground biomass (kg m-2) measured in a 2 m linear plot and estimated using a local model of cane height (H local, n=20 cane); a local model of cane diameter (D local, n=120 cane); a local model of cane height coupled with basal diameter (HD local, n=120 cane); a global model of cane height (H global, n=20 cane); a global model of cane diameter (D global, n=20 cane); and a global model of cane height coupled with basal diameter (HD global, n=120 cane).*

However, the ability of the different allometric relationship models to estimate biomass was variable. The local models estimated cane dry biomass using different equations at each crop age to best approximate the measured biomass (Figure 1.2, Table 1.2, Table 1.3). When using only cane height to estimate cane biomass in a local model, the mean difference (Δ%) across sampling dates between measured values and estimated biomass values was 5 ± 4 % with a mean R^2 of 0.86. When only basal diameter was used to estimate biomass in a local model, the mean difference was 8 ± 6 % with a mean R^2 of 0.74. In turn, when height was coupled with basal diameter, the mean difference was 4 ± 2 % with a mean R^2 of 0.93. The global models used the same model with the same parameters across sampling dates (Figure 1.2, Table 1.2). The mean differences (Δ%) across sampling dates between measured values and estimated biomass values with the global models

were 10 ± 5 %, 57 ± 30 % and 9 ± 4 % with models based on cane height (R^2=0.95), basal diameter (R^2=0.79) and combined height and diameter (R^2 = 0.98), respectively (Fig. 2b,d,f).

Table 1.2 Local and global allometric models using height (hTVD); basal diameter (Db); and height combined with diameter to estimate sugarcane biomass

Model	Equation	R^2	Mean ΔB
H local	Variable each month (Table S1)	Variable each month (Fig. 1)	5 ± 4 %
D local	Variable each month (Table S1)	Variable each month (Fig. 1)	8 ± 6 %
HD local	Variable each month (Table S1)	Variable each month (Fig. 1)	4 ± 2 %
H Global	$B = 0.658\ h_{TVD}^{1.180}$	0.95	10 ± 5 %
D Global	$B = 0.0024\ D_b^{3.323}$	0.79	57 ± 30 %
HD Global	$B = 0.021(h_{TVD} * D_b)^{1.113}$	0.98	9 ± 4 %

*$B = Biomass\ (g)$; h_{TVD} = Height to top visible dewlap; D_b = Basal diameter

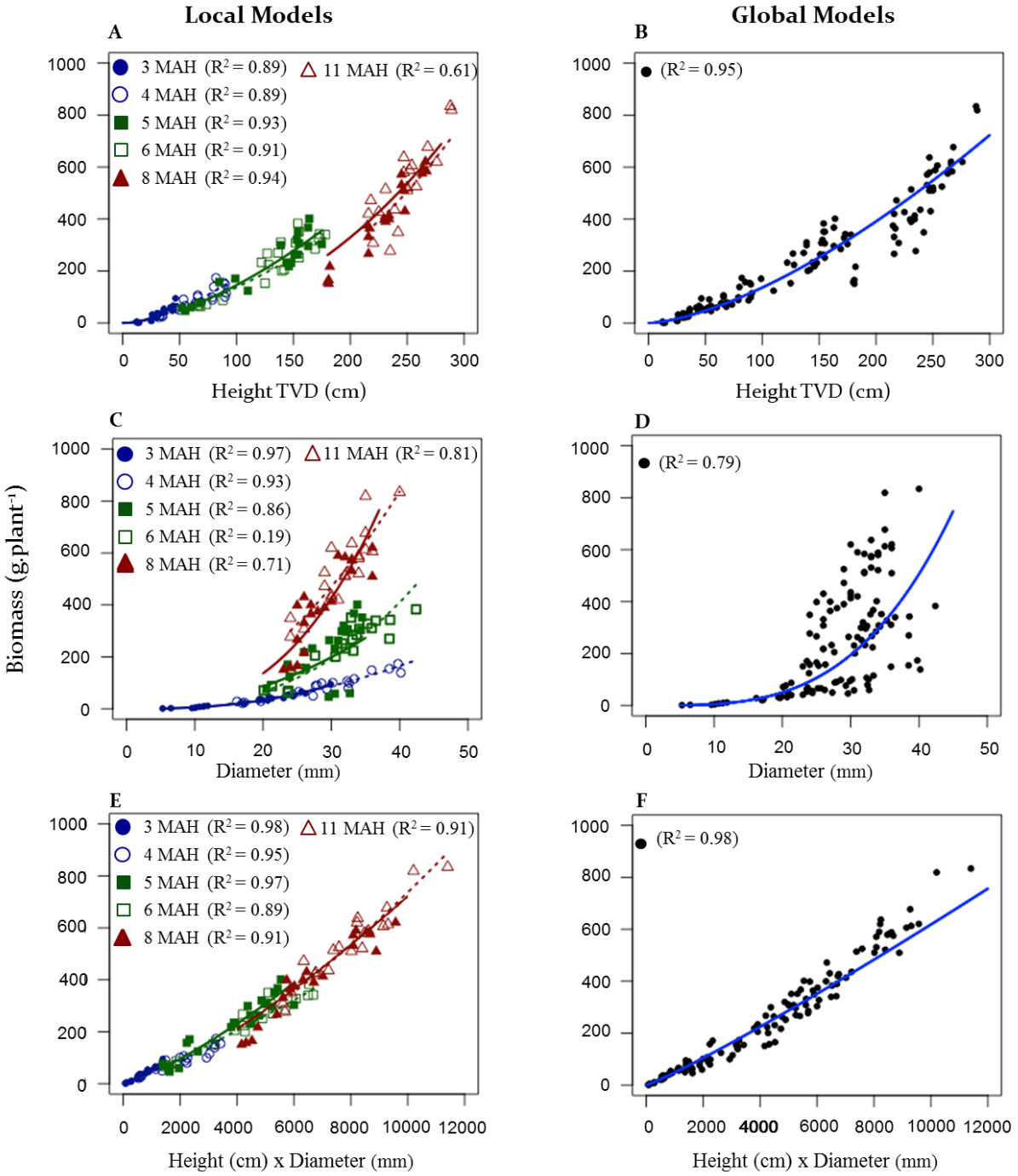

Figure 1.2 *Relationship between aboveground dry biomass (kg.plant-1) and measurable traits (cane TVD height H and basal diameter D) using local (a, c, e) and global (b, d, f) models. The relation between biomass and H (a,b), D (c,d) or H x D (e,f) are represented. Allometric relationships are represented by lines while measurements are represented by points. R-squared of each allometric relationship are indicated.*

Table 1.3 Local and global allometric models using height and height combined with diameter to estimate sugarcane biomass

Months	3	4	5	6	8	11
H (local)	$y = 0.0222 \times H^{2.0913}$	$y = 0.09 \times H^{1.6423}$	$y = 0.0934 \times H^{1.596}$	$y = 0.1278 \times H^{1.514}$	$y = 0.0310 \times H^{2.8012}$	$y = 0.001 \times H^{2.3785}$
D (local)	$y = 0.0178 \times D^{2.5148}$	$y = 0.0484 \times D^{2.2056}$	$y = 0.1776 \times D^{2.0642}$	$y = 0.0246 \times D^{2.6349}$	$y = 0.0031 \times D^{1.3417}$	$y = 0.5140 \times D^{2.0045}$
HD (local)	$y = 0.0114\ (HD)^{1.2314}$	$y = 0.0546(HD)^{0.9767}$	$y = 0.0058(HD)^{1.2771}$	$y = 0.0186(HD)^{1.1218}$	$y = 0.6582(HD)^{1.1803}$	$y = 0.0013(HD)^{1.4383}$
H (global)			$y = 0.6582 \times H^{1.1803}$			
D (global)			$y = 0.0024 \times H^{3.3232}$			
HD (global)			$y = 0.021 \times (HD)^{1.1131}$			

1.3.2 Nitrogen estimation from dilution curves

The following step in determining the NRE, was to determine the N content of the sugarcane aboveground biomass at a plot scale using an N dilution curve. This was optimised to test for the minimum number of cane required to construct an effective N dilution curve. Based on a reference N dilution curve constructed with 20 canes sampled on 5 dates, aboveground N content was estimated as 9.0 $mg.g^{-1}$, 5.3 $mg.g^{-1}$, 4.0 $mg.g^{-1}$, 3.7 $mg.g^{-1}$ and 2.4 $mg.g^{-1}$ at 3, 4, 5, 6 and 8 months, respectively (Figure 1.3).

When sampling fewer than 20 cane on 5 dates to construct an N dilution curve, the probability of obtaining a dilution curve that differed from the reference curve of 20 canes (P_{DIFF}), was 0 %, 0.9 %, 3.2 % and 4.9 % for 15-, 10-, 5- and 2-canes sampled respectively (Figure 1. A). In the instances where different N dilution curves were obtained, the estimation error ($CI_{95\%}$) on aboveground N content reached ±2 mg g^{-1} for 10- and 5-canes sampled, and ±4 mg g^{-1} for 2-canes sampled, at the beginning of the growth (i.e. where cane has a corresponding low aboveground dry biomass, Figure 1.3 A). Considering older crops (i.e. high aboveground dry biomass), the estimation error was similar (± 1 mg g^{-1} from 5 months after harvest) whatever the number of cane sampling (Figure 1.3 A).

When sampling fewer than 20 cane on 3 dates to construct an N dilution curve, rather than 5 dates, the probability of obtaining a dilution curve that would differ from the reference curve was 0.52 %, 2.96 %, 5.22 % and 5.5 % for 15-, 10-, 5- and 2-cane sampled respectively (Figure 1.3 B). In the instance where different dilution curves were obtained from the reference curve, the error in estimating aboveground N content was similar to the previous scenario where cane was sampled on 5 dates.

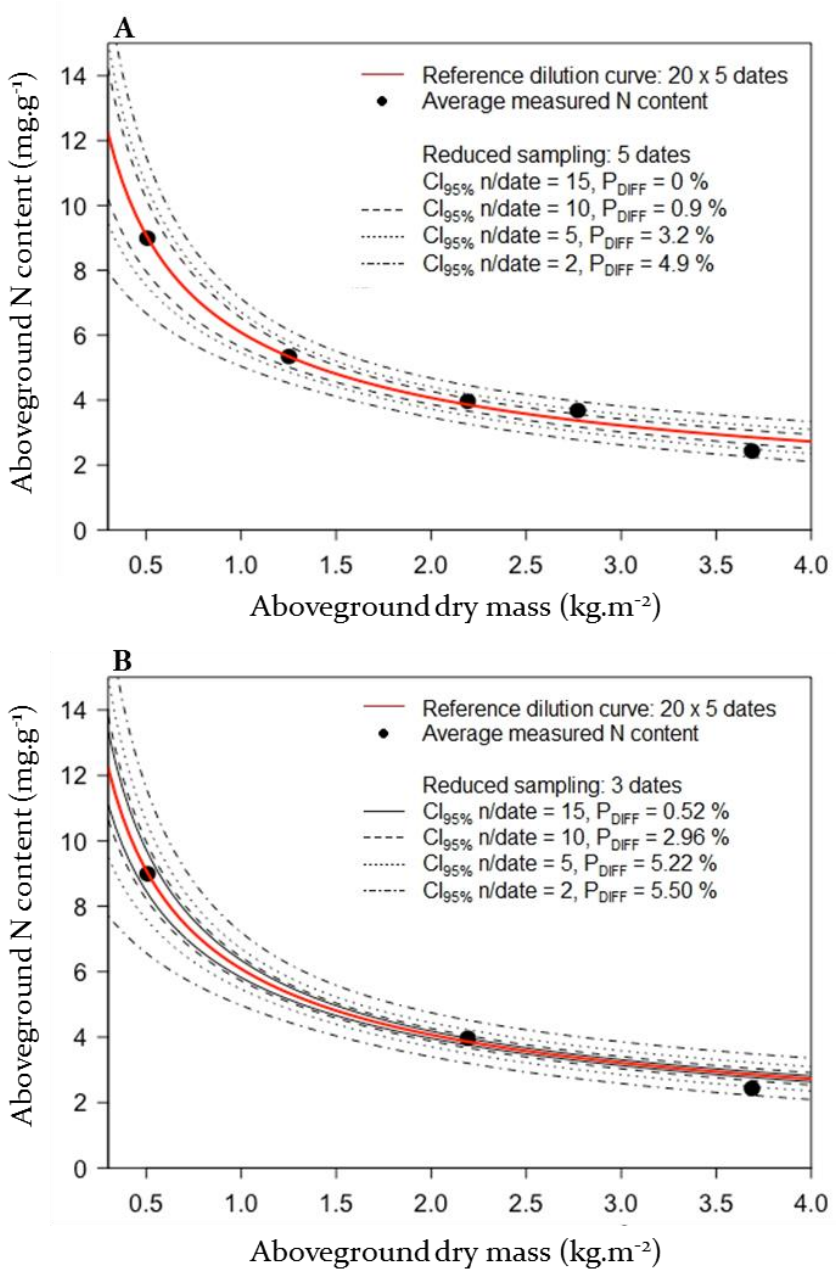

Figure 1.3 *Change in aboveground N content depending on aboveground dry mass. The average measured N content are presented by points and the reference dilution curve built with 20 canes at 5 dates is presented with the red line. The number of cane used to construct N dilution curve was reduced from 15 to 2 canes (multiple random sampling). For each sample, the probability to obtain a dilution curve statistically different than the reference one is indicated by PDIFF. The black dashed line represented the 95% confidence interval of the dilution curves that differed from the reference one. Dilution curves built with canes sampled at 5 and 3 dates are represented in (a) and (b), respectively.*

1.3.3 Isotopic ^{15}N enrichment of sugarcane leaf and whole cane

The final step in determining the NRE, was the calculation of the NdfF using a sugarcane leaf with an ^{15}N signature most representative of that of the aboveground biomass. The NdfF of the whole aboveground sugarcane decreased steadily with cane age, being 40 % three months after harvest, decreasing to 22 %, 20 % and finally to 15 % at 5, 8 and 11 months after harvest, respectively (Table 1.3). There was no significant difference between the NdfF calculated from ^{15}N signature of each of the leaves sampled and that of the entire aboveground sugarcane on each sampling date (Figure 1.3).

However, the proximity of the ^{15}N signature of the leaves to that of the whole cane shoot varied between the different leaves on the four sampling dates (Figure 1.3). The first and second leaves below the top visible dewlap (L+1 and L+2) had the closest ^{15}N value to the aboveground biomass over the sugarcane growth period, with an average relative difference (ΔNdfF %) of 9 % for leaf+1 and 10 % for leaf+2. In comparison, the average relative difference was higher for the third, fourth and fifth leaves, with an average ΔNdfF % of 23, 25 and 27 % for leaf +3, +4 and +5, respectively.

Table 1.4 Nitrogen derived from fertiliser (NdfF) of the first five leaves and whole cane at four sampling dates. Different letters indicated significant differences (n=3). The relative differences between NdfF of the different leaves and the aboveground biomass is given (ΔNdfF, %).

Sugar cane organ	3 months		5 months		8 months		11 months	
	NdfF %	ΔNdfF(%)	NdfF %	ΔNdfF (%)	NdfF %	ΔNdfF (%)	NdfF %	ΔNdfF (%)
Leaf + 1	37 ± 16 [a]	7.5	20 ± 4 [a]	9	20 ± 6 [a]	0	18 ± 4 [a]	20
Leaf + 2	42 ± 19 [a]	5	21 ± 4 [a]	5	21 ± 6 [a]	5	19 ± 4 [a]	27
Leaf + 3	53 ± 27 [a]	32.5	21 ± 5 [a]	5	26 ± 9 [a]	30	19 ± 4 [a]	27
Leaf + 4	51 ± 18 [a]	27.5	24 ± 6 [a]	9	17 ± na	15	22 ± 4 [a]	47
Leaf + 5	50 ± 16 [a]	25	26 ± 8 [a]	18	18 ± na	10	23 ± 1 [a]	53
Whole cane	40 ± 15 [a]		22 ± 4 [a]		20 ± 7 [a]		15 ± 3 [a]	

1.3.4 N-fertiliser recovery efficiency

The N-fertiliser recovery was variable over time, being 19 % at 3 and 5 months, and then decreasing to 14 % at 8 months and 12 % at 11 months after harvest, using the reference method [height x diameter global model for estimating the aboveground dry biomass, 20 cane harvested for N content and the whole plant ^{15}N content for NdfF (Figure 1.4)]. The relative influence of each estimation method for aboveground biomass, N content and NdfF on the NRE assessment was tested by taking account of the variability in estimation methods (Figure 1.4 A, B, C). The percentage variance of the NRE for the four sampling dates over the crop cycle, explained by each respective method, was 13 % in leaf choice for NdfF and less than 2 % for biomass estimation and N content estimation (Figure 1.4 D). The crop age explained 54 % of NRE variability over the growth cycle. The interactions crop age x biomass and crop age x NdfF explained 10 % and 19 % of NRE variability respectively, highlighting the influence of these estimation methods at the beginning of the growth (3 months, Figure 1.4 A, C).

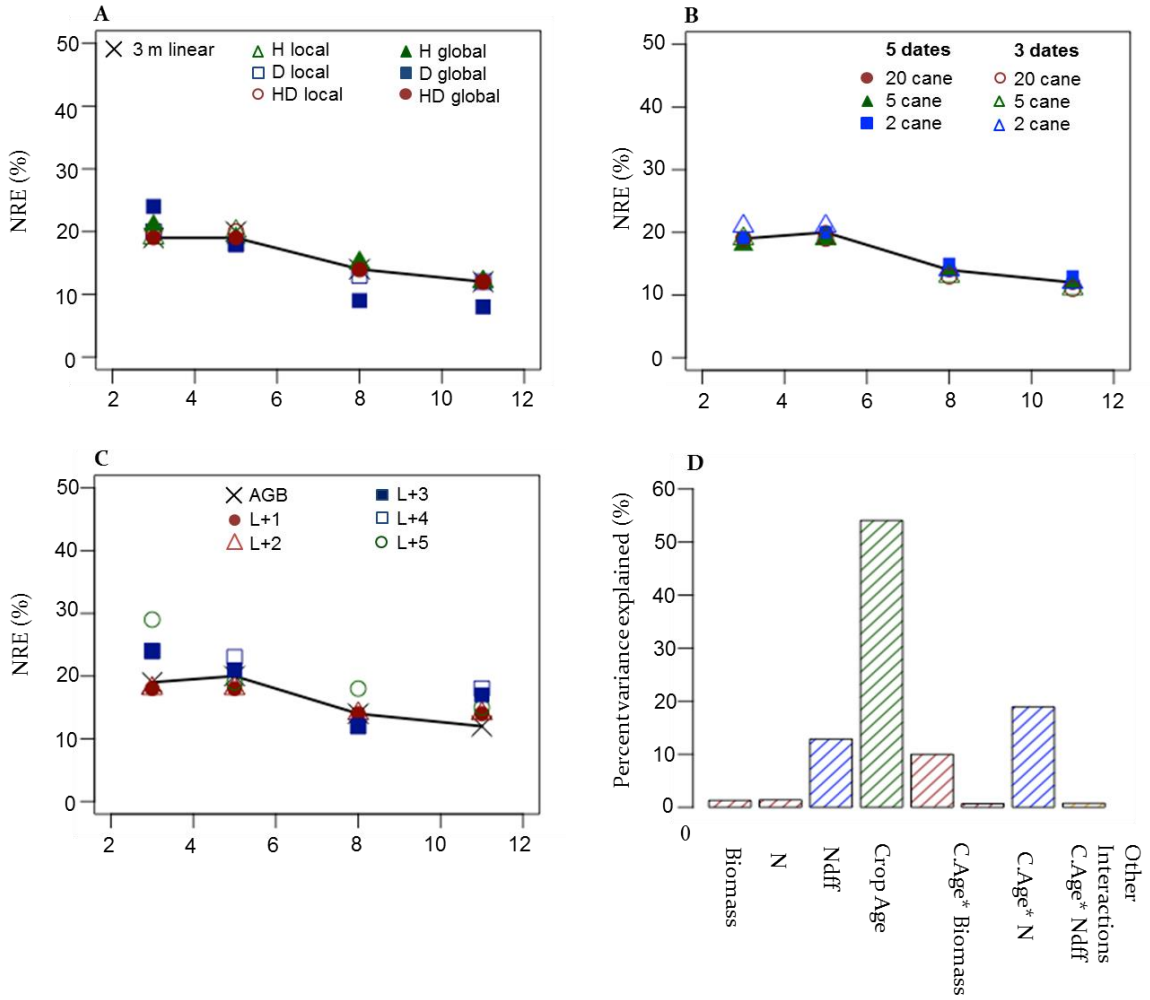

Figure 1.4 *Change in N-recovery efficiency (NRE) with time according to choice of method used for biomass, nitrogen content and Nitrogen derived from fertilizer estimations; (a) NRE as a function of the method used to calculate biomass (3 m harvest, height (H) local model, diameter (D) local model, height x diameter local model, height global model, diameter global model, height x diameter global model); (b) NRE as a function of N content calculated using different numbers of cane harvested for the dilution curve (20, 5 and 2 cane harvested per month using five harvested dates and using three harvesting dates respectively); (c) NRE as a function of plant organ used to calculate NdfF (aboveground biomass (AGB), leaf+1, +2, +3, +4 and leaf+5). (d) Percentage variance of N-recovery efficiency explained by the different methods (i/ Biomass calculation; ii/ nitrogen content calculation; iii: choice of plant organ in calculating NdfF; iv / Crop age at which sugarcane was sampled and v/ interaction between crop age and biomass; vi/ interaction between crop age and NdfF; vii/ other interactions between variables.*

1.4 Discussion

1.4.1 A non-destructive method to estimate biomass

Allometric relationships, as a non-destructive approach, were an effective method to estimate sugarcane biomass. In the present study, aboveground biomass was best estimated using height coupled with basal diameter. Height was also an effective estimator of biomass, but deviated more from the measured biomass at certain dates than height coupled with diameter. Diameter on its own, however, was not an effective estimator of biomass when a single, global model was used across dates.

This finding differs from that of Youkhana et al. (2017), which appears to be the single published study where allometric relationships were used to estimate biomass for sugarcane, and where stalk diameter was found to be the best estimator of sugarcane aboveground biomass. Although different sugarcane cultivars subject to different pedo-climatic conditions were used in the two studies, the more likely reason for the disparity in findings is due to the temporal variability considered only in this study. If sugarcane biomass was only considered at harvest in our study, biomass estimated using only diameter (which would imply using the local diameter model at the last sampling date in this study) would also have given a value close to that of the measured biomass.

There is a large amount of variation in sugarcane morphological forms depending on plant cultivars and pedo-climatic conditions. Culm diameter, for example, is a morphological trait that varies widely between sugarcane plants (Rae et al. 2014). Using cane height combined with diameter, is more likely to account for this morphological variability and hence be a better predictor of the corresponding biomass.

The advantage of using allometric relationships to estimate biomass is that they can be used at multiple scales; from a linear meter (rather than harvesting individual canes) to the plot scale (rather than being limited to a harvested subplot). In addition, given that the method is non-destructive, the growth of biomass may be followed throughout the sugarcane growth cycle. This would allow for the study of biomass in parallel with other biogeochemical parameters without affecting the agroecosystem.

In a nearby experiment with the same cultivar (to the current study), these same allometric relationships were tested in their ability to estimate sugarcane biomass for different fertiliser treatment types – in fertilised and non-fertilised plots. The allometric relationships appeared to work effectively for both treatment types, differing by 6 % from the measured (cut & weighed) cane for the fertilised plot and by 10 % for the unfertilised plot. This suggests allometric relationships would indeed be applicable to other sites and fertiliser management conditions.

1.4.2 A reduced number of cane required to estimate N content at a plot scale

This study successfully managed to reduce the number of sugarcane shoots sampled per date in order to construct an N dilution curve and effectively estimated the total N of sugarcane at a plot scale throughout the crop cycle. Sampling 5 sugarcane shoots on 3 dates over the sugarcane crop growth-cycle was sufficient to establish an N dilution curve, from which N content at a plot scale could be calculated using the corresponding plot biomass. To our knowledge, this is the first study to assess the required number of harvested sugarcane shoots to establish a dilution curve. In comparison, the number of cane shoots harvested in a previous study to determine an N dilution curve, was approximately 2 m per sampling date (Oliveira et al. 2013).

Sampling 5 sugarcane shoots per month had a low probability of differing from sampling 20 shoots and could therefore be considered sufficient to construct an N dilution curve. Using 3 rather than 5 sampling dates did not change the shape of the dilution curve and its ability to estimate sugarcane total N at the plot scale. This greatly reduced the amount of destructive sampling required and was shown to be sufficient in establishing a dilution curve to determine the mean N content of sugarcane at a plot scale, while limiting the risk of errors. This would be especially important in contexts where the crop consists of multiple cultivars and/or fertiliser treatments, whereby each cultivar and/or plot subject to a different fertiliser type would require separate dilution curves to estimate the total sugarcane N.

1.4.3 ^{15}N representativity of leaves in determining N derived from fertiliser

Our study confirmed the use of a single leaf as a proxy for the ^{15}N content of the entire aboveground biomass of the corresponding sugarcane (Franco et al. 2011; Trivelin, 1994). This enabled an effective means of minimising the extent of destructive sampling in estimating the NdfF required to calculate the NRE. The first and second leaves below the top visible dewlap (L+1 and L+2) had the closest ^{15}N contents to the aboveground biomass in this study, and were therefore the most representative of the ^{15}N of the aboveground biomass. This differed from past studies, where the third leaf below the top visible dewlap (L+3) was proposed as the reference leaf for ^{15}N arguing that no significant difference was observed between the L+3 and the aboveground biomass (Franco et al. 2011; Trivelin et al. 1994). However, the variability between repetitions is typically very high in ^{15}N-pulse-labelling experiments. This explains why in the present study, as well as in others such as Franco et al. (2011), no significant differences were observed, whichever leaf was selected. A better approach for selecting the most representative leaf is to focus on the difference between the ^{15}N values of the sampled leaf and the aboveground biomass of the entire sugarcane shoot.

In general, leaves have a more rapid turnover in N cycling than other plant organs, and the selection of a single leaf can therefore lead to inaccuracies in the estimation of NRE. If younger leaves were selected following a pulse of ^{15}N, this would inevitably lead to an overestimation of the

fertiliser N contribution to the crop and should therefore be avoided. Conversely, towards the end of the sugarcane growth-cycle, ^{15}N is progressively lost from the lower leaves that have returned to the ground as litterfall, and the selection of an older leaf can potentially lead to an overestimation of the NRE. Younger leaves are supplied with N from both the soil and old plant organs. The translocation of N from old plant organs, including the stem and older leaves, to the newly emerging leaves can explain why the first and second leaves below the top visible dewlap remained the best predictors of the aboveground biomass in our study.

1.4.4 An integrated procedure to study N-fertiliser recovery efficiency

The N-fertiliser recovery efficiency decreased from 19 to 12% over the sugarcane crop growth-cycle in the present study and demonstrated that values of fertiliser NRE can be underestimated by 37% when considered only at harvest. Ng Kee Kwong and Deville (1994) found that the fertiliser N recovery efficiency decreased from 20-40 % in earlier phases of the cane growth-cycle to 13-18 % at the crop harvest. Courtaillac et al. (1998), found the fertiliser N-recovery efficiency also decreased over the ratoon crop growth-cycles. When considering the parameters which most strongly influenced the NRE, the effect of "crop number", or the effect of when the NRE is determined at different time intervals over the cane growth-cycle, has the largest effect on its value. These consistent results reinforced the importance of studying NUE throughout the crop growth-cycle and therefore warranted the use of a minimally destructive sampling method to estimate NRE.

In order to determine the NRE at different time intervals over the sugarcane growth-cycle, and therefore to capture its variability, an integration of different non-destructive methods was required. Here, the attempt has been to optimise these methods, by testing several scenarios where both the level of destruction was minimised and the NRE value remained as close as possible to the reference. The different methods of estimating the parameters (biomass, N content, NdfF) needed for calculating the NRE do not, however, impact the NRE to the same extent. Following crop age, the NRE was most strongly influenced by the choice of leaf used to represent the ^{15}N content of the entire aboveground biomass in calculating the NdfF. In our study, the use of "older leaves" (leaf +3, +4, +5) would have resulted in an average over-estimation of NRE by 33%, 8% and 39% at 3, 5 and 11 months, respectively. This is why leaf choice combined with crop age was also responsible for a high percent variance in our study, since NRE is impacted more by leaf choice at certain phases of the growth-cycle than others. The different methods of estimation of the biomass and the N content showed limited impact on the NRE computations. We therefore propose an integrated procedure, involving an allometric model, a dilution curve and "young leaves", to study NUE throughout the crop growth-cycle in sugarcane agroecosystems.

Although consistent with Ng Kee Kwong and Deville (1994), the range of NRE values in our study remained quite low, with a maximum of 19 % during the developmental phases of the ratoon

and a minimum of 12 % at harvest. Here, we have presented the findings only for the aboveground biomass but the root compartment was measured in a parallel study on the same plot at the corresponding sampling dates. When the N content of the belowground biomass was also considered, the NRE increased to 39 % at the first date and to 19 % at harvest. The omission of the root compartment might be problematic when the ^{15}N isotopic method was chosen as up to half of the N accumulated in the cane is ignored in such circumstances. This feature may explain why the N recovery efficiency was half as much when calculated with the ^{15}N isotopic method as with the difference method (similar disparity between values as found by Ng Kee Kwong and Deville, 1994). Integrating the root biomass and N content appears crucial in getting closer to actual values of NUE that could be used to fine-tune the N fertilisation practices in sugarcane agroecosystems.

1.4.5 Potential scope of application

Although optimised for sugarcane, our integrated set of methods should be applicable to all sugarcane varieties, as well as to most giant C4 crops. We have used the term "giant C4 crops" to categorise crops which have an architecture that lends itself well to the construction of allometric relationships. The cylindrical morphology of these crops, with a long stem and sufficiently large width, resembles a structural form between that of trees and grasses, hence the term "*giant*" C4 crops. It is primarily C4 crops which fall under this category, but certain C3 crops, such as *Arundo donax*, which is supposedly a "promising energy crop" of the Mediterranean regions (Mariani et al. 2010), has a similar cylindrical morphology to the giant C4 grasses, and could potentially also be considered. Allometric relationships have been used infrequently for non-forest crops (Youkhana et al. 2017), but appear to have been an effective method of determining crop aboveground biomass for certain other C4 crops, across different geographical locations, soil-types and pedo-climatic conditions. For example, plant height was found to be the best predictor of aboveground biomass for different varieties of maize (Tittonell et al. 2005), stalk height was also found to be an effective predictor of aboveground biomass for *Miscanthus sinensis* and certain other tall-grass species (Anten & Hirose, 1999), and height combined with stalk radius for *Sorghum bicolor* (Martin et al. 2013).

The spatial configuration of these crops favours the use of N dilution curves. In other words, there is a high density of crops, and the harvesting of a few stalks will have a minimal impact on the rest of the plantation. At the same time, a spatial configuration with interrows better enables the use of ^{15}N microplots. Nitrogen dilution curves have been documented only more recently for sugarcane (de Oliveira et al. 2013), but have been used more frequently for other giant C4 crops such as maize (Plénet and Lemaire 2000; Zhao et al. 2018), sorghum (Barbanti et al. 2011) and miscanthus (Zapater et al. 2016) and other C4 tropical grasses (Duru et al. 1997).

When considered independently, allometric relationships and N dilution curves are therefore not novel methods. The originality in this approach is the use of these methods together, while minimising the number of crops harvested, in conjunction with the use of a ^{15}N representative plant organ, to determine the NRE at different stages of the growth cycle of giant grass crops in a minimally destructive way.

This methodological procedure could feasibly be adopted by research teams working on the NUE of giant C_4 grass crops at different geographical locations. Soil type and pedo-climatic conditions do not fundamentally call into question the approach of our set of methods, but require local calibration for the allometric relationships and punctual validations for the other two methods, for different crops, cultivars and sites. Allometric relationships and dilution curves could be used to study NUE with the difference method, however these two methods combine best with the isotope method using ^{15}N-labeled fertilisers, in that each of these methods could be conducted in ^{15}N enriched microplots. In other words, at each ^{15}N microplot, this would consist of an inventory of sugarcane heights and basal diameters to estimate crop biomass at each sampling date, the harvesting of three sugarcane plants (which could take place outside the subplot if subject to the same fertiliser application), and the harvesting of a ^{15}N representative plant organ.

^{15}N labeled microplots is still a method used frequently in contexts such as Brazil (Vieira-Megda et al. 2015; Fortes et al. 2011; Franco et al. 2011), and Australia for sugarcane (Thorburn et al. 2017). For other crops, ^{15}N tracers are still used frequently for maize in China (e.g. Li et al. 2019), France (e.g. Gallais et al. 2006) and Argentina (Rimski-Korsakov et al. 2009); for example, sorghum with ^{15}N trials in France (Ferchaud et al. 2016) and Italy (Barbanti et al. 2011) and for miscanthus in France (Ferchaud et al. 2016). The combination of these methods could therefore feasibly be considered in these different contexts.

An alternative non-destructive method of determining crop biomass and corresponding N content is by using remote sensing. Several methods have been used to predict crop production parameters from aerial images. The performance of remote sensing is variable depending on the crop, with good estimations of biomass and N content for canola and corn for example (Dong et al. 2019), but was not able to accurately predict N content of sugarcane (Amaral et al. 2015). A reason for proposed for this poor correlation for sugarcane as a semi-perennial crop, is the variability of the crop population and 'skips' between rows which interfere with canopy sensor readings.

Another possibility is to couple remote sensing with 3D imaging. This has not yet been tested for sugarcane, but a parallel could be drawn to wheat, for which a recent study found an r^2 of 0.79 between the 3D imaging estimation of aboveground biomass and biomass measured by harvesting (Walter et al. 2018). Although our study focused on sugarcane, the best performing allometric

relationship predicted aboveground biomass with an r^2 of 0.98, similar to that of Youkhana et al. (2017), with an r^2 of 0.97, which was a far better performance.

One promising method seems to be the use of a radiative transfer model that would improve robustness and transferability of remote sensing approaches (Castaldi et al. 2015; Dong et al. 2015). However, to date these remote sensing approaches are mainly used for studies at large scales and developed for crop management purposes. Even if remote sensing is used, ^{15}N enriched microplots would still be required to determine the NRE of the crop, and crop plant or representative organs (e.g. ^{15}N leaf) will still need to be harvested if remote sensing is used. The advantage of using allometric relationships coupled with a dilution curve (constructed with a minimal number of crop plants) over remote sensing, it uses simple, accessible and inexpensive technology which can be effectively used at fine scale of a plot, to a larger scale of a plantation.

1.5 Conclusion

This study highlighted how NUE varies along the sugarcane crop growth-cycle. If it is only considered (as is usually the case) at crop harvest, the fate of fertilizer N could be incorrectly interpreted, highlighting the necessity to estimate NUE at various stages along the crop growth cycle. In order to calculate NRE (the quantitative index of NUE) along the sugarcane growth-cycle, the proposed methods gave the best estimate of NRE with minimal destruction, and were an integration of the following: 1/ the use of a global allometric model, using both cane height and diameter to estimate corresponding biomass at a plot scale; 2/ the use of an N dilution curve using a minimum of five sugarcane at a minimum of three dates; 3/ determining the NdfF using the ^{15}N content of the first or second leaf below the top visible dewlap as a proxy for the ^{15}N content of the cane aboveground biomass. The NRE calculation was mostly impacted by crop age, and secondly, by the choice of the most appropriate sugarcane component in the NdfF calculation. Given the importance of temporal variability in NRE, this proposition of minimally destructive methods could provide an effective means of evaluating the NUE over the crop growth-cycle, hence allowing sugarcane N demands to be better synchronized with fertilizer N-supply. The originality in this approach is the use of these methods together, while minimising the number of crops harvested, in conjunction with the use of a ^{15}N representative plant organ, to determine the NRE at different stages of the growth cycle of giant C_4 crops in a minimally destructive way. The use of this approach should be calibrated for the crop and site of interest for it to function optimally.

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CHAPTER TWO

Abstract

The primary objective of this chapter was to establish which components of the sugarcane crop are important to consider when evaluating the accumulation of sugarcane N mass, and when calculating the fertiliser N-use efficiency in sugarcane systems. The respective biomass, N mass, and fertiliser N recovery efficiency using the difference method (dNRE) of sugarcane shoots, tillers, strawfall, root and stool components were evaluated. The aboveground components (shoots, tillers, strawfall) were measured monthly, and the belowground components (roots, stools) were measured at harvest at the end of each ratoon. This was done in fertilised (urea application) and unfertilised treatments, throughout the sugarcane growth-cycle over two successive ratoons.

The active N uptake period was between 3 and 6 months after the start of the ratoon for the two experimental years in our study. The N mass increased considerably when root biomass was added, as the N mass of the roots comprises approximately two-thirds (65 %) and half (57 %) of the N mass of the aboveground sugarcane N mass at the final harvest for the two respective years of the fertilised treatment, and was approximately equal (104 %) to, and three quarters (74 %) of the unfertilised sugarcane aboveground N at the final harvest for the two experimental years, respectively. When the stool component is considered, a far smaller amount of N mass was added to the belowground biomass compartment and total crop N mass than the roots.

The baselined dNRE was on average 34 % over the first experimental year, and 21 % over the second experimental year. The dNRE decreased slightly with tiller senescence, increased slightly later in the cycle with strawfall, and roots had a small, variable effect.

Typically, only the aboveground biomass (without tillers or strawfall) is considered when evaluating the effect of N input from mineral and organic fertilisers on the accumulation of N in sugarcane. Here it is shown that the belowground biomass is very important to evaluate, in addition to the aboveground biomass, when determining the biomass and N mass of the sugarcane crop. Furthermore, the strawfall which falls from the plant from 6 months after the start of the ratoon until the following harvest, as well as the senescence of tillers over the sugarcane ratoon, should be studied when evaluating the evolution of N mass and N-use efficiency.

Resumé

L'objectif principal de ce chapitre était d'établir quels compartiments de la canne à sucre étaient importants à considérer lors de l'estimation de la minéralomasse N de la canne et des calculs d'efficience d'utilisation d'azote des engrais. Les biomasses, minéralomasses, et le coefficient réel d'utilisation d'azote de la canne en considérant les compartiments de canne, de talles, de paille, de racines et de souches ont été mesurées mensuellement concernant les compartiments aériens et annuellement concernant les compartiments souterrains, dans les traitements non fertilisés et fertilisés au cours de deux repousses successives.

La période d'absorption active de N était comprise entre 3 et 6 mois après le début de la repousse pour les deux années expérimentales de notre étude. La minéralomasse a considérablement augmenté lorsque la biomasse des racines a été ajoutée, car la masse d'azote des racines représente environ deux tiers (65 %) et la moitié (57 %) de la minéralomasse de la canne à sucre à la récolte finale pour les deux années respectives du traitement fertilisé. Pour le traitement non-fertilisé, le minéralomasse des racines étaient approximativement égale (104 %) et trois quarts (74 %) de minéralomasse de la canne à sucre non fertilisée à la récolte finale pour les deux années expérimentales, respectivement. Si l'on considère la composante des pseudo-rhizomes, une quantité de minéralomasse beaucoup plus faible que celle des racines a été ajoutée au compartiment de la biomasse souterraine et à la masse totale d'azote de la culture.

Le coefficient actuel d'utilisation d'azote (CAU) de base était en moyenne de 34 % au cours de la première année expérimentale, et de 21 % au cours de la deuxième année expérimentale. Les CAU ont légèrement diminué avec la sénescence des talles, ont augmenté légèrement plus tard dans le cycle avec la chute de la paille, et les racines ont eu un petit effet et variable.

En règle générale, seule la biomasse de canne est prise en compte pour évaluer l'effet d'engrais minéraux et organiques sur l'accumulation d'azote. Il est montré ici que le compartiment souterrain est important à évaluer en plus de la biomasse et de la minéralomasse aérienne de N en culture de canne à sucre. En outre, il faut également tenir compte de la paille qui tombe de la plante à partir de 6 mois après le début de la repousse jusqu'à la récolte suivante, ainsi que de la sénescence des talles de la canne à sucre.

Chapter 2: Relative importance of distinct biomass components throughout the growth-cycle of sugarcane ratoon crops in N nutrition studies

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2.1 Introduction

Nitrogen is critical to sugarcane growth and productivity. It is the most important soil-derived nutrient in terms of its quantitative contribution to sugarcane dry biomass over the crop growth-cycle (Robinson et al. 2013). However, N fertilisers are frequently overused leading to the application of excess N to agroecosystems. This has negative environmental consequences (Dobermann 2005) such as the leaching of nitrates and greenhouse gas emissions, as well as adverse economic implications (i.e. paying for excess fertiliser which does not contribute to crop productivity). Sugarcane crops typically obtain only 20–40 % of the nitrogen (N) they require from fertiliser, and as much as 60 % of fertiliser N may be lost from the soil–crop system (Vallis et al. 1996). A better synchronisation of crop N requirements and crop N supply with respect to the timing and quantities of fertiliser N used can lead to an improved nitrogen use efficiency (NUE) (Cassman et al. 2002). Evaluating the responses of sugarcane biomass and total N mass accumulation to N fertiliser application, as well as the NUE of the sugarcane, is not only relevant in terms of decreasing excess N and thereby minimising negative environmental consequences, but also has implications for sugarcane biomass production and sugar yield (Robinson et al. 2013). For example, higher N application rates generally increase cane yield, but can result in a reduction in the commercial cane sugar content of the sugarcane juice and sugar quality (Stevenson et al. 1992, Wiedenfeld, 1995, Muchow et al. 1996).

The NUE is the ability of a crop to acquire N from a growth medium and to use this N in the production of harvestable plant material, aboveground biomass or total plant biomass (including belowground biomass) (Baligar et al. 2001, Blair, 1993). The index used to quantify the NUE is the fertiliser N-recovery efficiency or NRE, which is the calculated percentage of fertiliser-N recovered in the crop aboveground biomass during the crop-growing season. It is typically determined at harvest (Chapman et al. 1994, Isa et al. 2006, Fortes et al. 2010), but given its temporal variability at different stages of the sugarcane growth-cycle merits an evaluation over the growth-cycle (Poultney et al. 2020). The concept of NUE and its indices have been explained in more detail in Chapter 1's

Introduction. In the current study, the NRE is determined by using the "difference" method, sometimes also called "Apparent nitrogen recovery" (Good et al. 2004) and is referred to here as "dNRE". It corresponds to the broadest measure of NRE and is based on the difference between the N mass accumulated in the aboveground biomass of N-fertilised crops (at different application levels), and non-fertilised crops (Harmsen & Moraghan, 1988, Cassman et al. 2002).

The total N mass is an indication of the uptake of N by sugarcane. Evaluating its accumulation over a crop growth-cycle gives an indication of when N is being taken up by the plant, as well as the "active" N uptake period, where the N mass accumulation gradient is greatest (Ng Kee Kwong et al. 1994). In contrast, the NRE is rather an indication of the proportion of fertiliser-N absorbed by the plant.

The NRE is variable over the sugarcane growth-cycle, as is shown in Chapter 1, as well as in the few studies which have evaluated the NRE at different periods of the sugarcane growth-cycle (Ng Kee Kwong & Deville, 1994, Courtaillac et al. 1998). Most studies consider only the NRE at harvest, which is problematic given its temporal variability over the crop growth-cycle (Poultney et al. 2020). The evolution of sugarcane biomass and total N mass should therefore also be assessed throughout the sugarcane growth-cycle (over the plantation or ratoon) in order to determine the active N uptake period. The use of allometric relationships to determine crop aboveground biomass over the ratoon, combined with the use of a dilution curve to determine the sugarcane N content at a plot scale, are minimally destructive methods proposed and valourised in Chapter 1. In this way the total N mass can be determined throughout the growth-cycle.

Despite the importance of synchronising nitrogen demand with supply, the relative importance of certain biomass components contributing to the nitrogen nutrition of the sugarcane system appear to have been rarely studied. Typically, only the sugarcane aboveground biomass is studied, partly due to the methodological challenge of sampling the belowground biomass (Versini et al. 2020). Along with the belowground biomass, the loss of N from the sugarcane crop via the senescence of tillers is seldom considered, and neither the N in dry leaves which fall from the plant as "strawfall" over the growth-cycle of the sugarcane.

The belowground biomass component of sugarcane affects sugarcane growth and production, yet there is an abundance of "gaps and misconceptions" with regards to this sugarcane component (Smith et al. 2005). The reason in part is that the knowledge of sugarcane root systems which currently exists, has not been widely applied to crop management or selection in that knowledge is "patchy" and uncertain (Smith et al. 2005). The sugarcane belowground compartment is suspected to accumulate N throughout the growth cycle and to supply N back to the sugarcane over subsequent ratoons. However, there is very limited information documenting the effect of N

fertilisation on root biomass and on belowground biomass accumulation (Robinson et al. 2013, Bell et al. 2015, Versini et al. 2020).

As recommended in the perspectives of a study by Wood et al. (1996), accurate estimations of N accumulation "must consider the losses of N in trash and losses of N due to stalk death under high-yielding situations." In order to address this gap in understanding, we have evaluated these sugarcane system components, and furthermore, the belowground biomass component which may also be of considerable importance.

Tillers are secondary shoots which emerge from the axillary buds of an existing culm to form additional culms (Bonnett 2014). The increased availability of N has been shown to stimulate tillering in sugarcane (Ng Kee Kwong et al. 1999, Garside et al. 2000). The survival of tillers until harvest is strongly dependent on the density of established primary shoots, since excessive tillering results in competition between culms for light and nutrients, which leads to the death of certain culms (Bell & Garside, 2005, Singels et al. 2005). Tillers are a component of the sugarcane system which has received little attention in terms of loss of biomass of a sugarcane plantation (Bell & Garside, 2005), and even less so in terms of the loss of N mass via tiller senescence over the sugarcane growth-cycle.

Sugarcane crop residue, also referred to as "trash" or "post-harvest residue", retained on the soil surface after harvest represents an additional N source, which can be up to 30-60 kg N ha^{-1} (Meier et al. 2006) and which should be incorporated into the crop N budget to avoid over-fertilisation. Tropical, humid regions may increase the rate of decomposition (Meier et al. 2006), making this a component of the sugarcane system potentially even more important in many of the large producing regions of the world (e.g. Brazil, India, Thailand). Few experiments in the wet tropics have investigated the decomposition of trash and the movement of N from the trash to the soil and plant over time, or as affected by trash incorporation (Meier et al. 2006). Moreover, this sugarcane trash (or "mulch") is typically studied as what remains post-harvest, and the re-integration of strawfall into the sugarcane system, which fall from the sugarcane plant over the growth-cycle and before harvest, appears to be rarely considered in sugarcane plantations. The leaves that fall over the growth-cycle are also possibly an overlooked source of N to the sugarcane. This component of the sugarcane system is evaluated in this study and is termed "strawfall", referring to the (dry) leaf-fall which falls to the ground from approximately mid-growth cycle until the following harvest. The equivalent and well-documented component of N biogeochemical cycles in forestry for example, would be "litterfall" (Vitousek, 1984).

One means of better coordinating fertiliser-N with sugarcane's N needs would be a better understanding of these sugarcane system components and the sugarcane N requirements with regards to the quantity and timing of fertiliser N inputs.

The aim of this study was therefore to investigate the relative importance of distinct biomass compartments in estimating the accumulation of N and N use efficiency throughout the growth-cycle of sugarcane ratoon. The respective biomass and N mass of the shoot, tiller, strawfall, root and stool compartments were measured monthly for aboveground compartment, and annually for belowground compartments, in unfertilised and fertilised treatments throughout two successive sugarcane ratoons.

2.2 Material and methods

2.2.1 Study site and experimental design

This is explained in detail in the Study site section of the Experimental Site section . The measurements in the present study were conducted over two years and sugarcane growth cycles, during the fourth and fifth ratoons of the sugarcane experimental plantation. In this trial, there were fertilised and unfertilised treatments (Figure 2.1, extracted from Plot T2 in the Experimental trial). In the fertilised treatment, 88 kg N ha^{-1} was applied two months after harvest of both ratoons. A further 57 kg N ha^{-1} at five months after the start of the fourth ratoon (referred to as the "first experimental year") and 62 kg N ha^{-1} at four months after the start of the fifth ratoon or "second experimental year". (See the "urea" treatment in Figure 7 summarising fertilisation in the Experimental design and general methods section).

2.2.2 Shoot estimation from inventories and sampling

The shoot biomass was estimated monthly from inventories and allometric relationships according to the methodology outline in Chapter 1. Monthly inventories of sugarcane were taken at 2 m linear (2 m x 1.5 m) subplots, starting at three months after harvest. There were three 2 m linear unfertilised subplots (in a single plot) and nine 2 m fertilised subplots, with 3 repetitions in 3 different plots of urea (Figure 2.1). In each 2 m subplot, sugarcane height was measured to the top visible dewlap for each sugarcane stalk, and the corresponding basal stalk diameter (taken between the soil and the first stalk node).

For the aboveground biomass, an N dilution curve was constructed by harvesting sugarcane stalks each month from the unfertilised microplot and the fertilised treatments, starting at three months after harvest. Six sugarcane plants were harvested from the non-fertilised subplot, 2 cane stalks from the row adjacent to each of the 2 m linear subplot (in the fertiliser exclusion zone) and six sugarcane were harvested from the fertilised treatment, 2 from the row adjacent to each of the urea subplots (Figure 2.1).

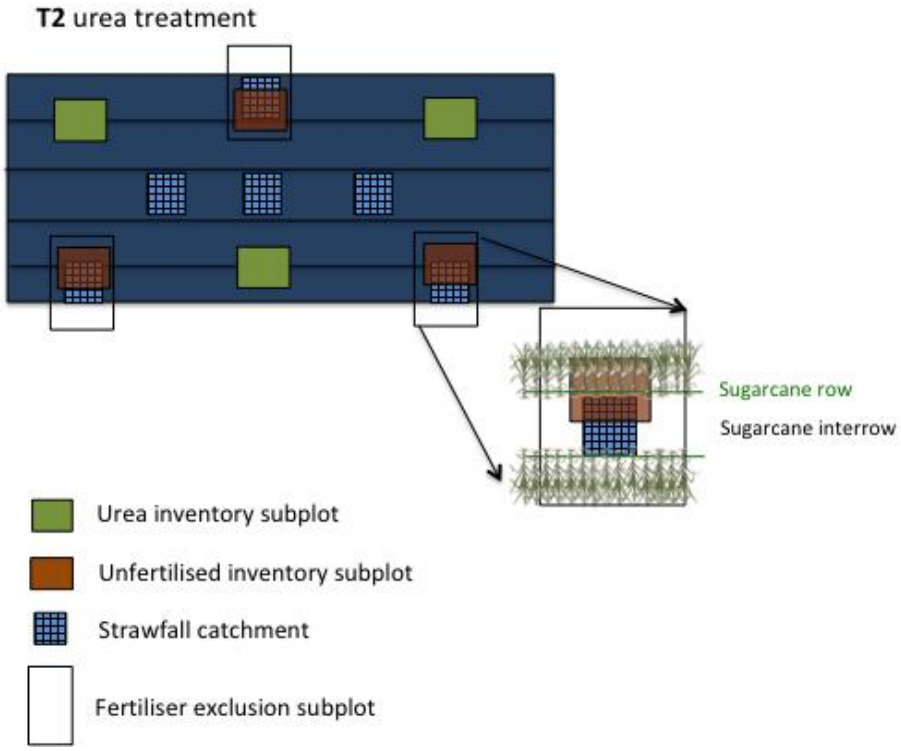

Figure 2.1 Experimental design of fertilised and unfertilised inventory subplots, and the strawfall catchment subplots in the T2 urea treatment.

2.2.3 Tiller estimation from inventories and sampling

The tillers were considered as the secondary shoots that did not survive from the start of the ratoon to the following harvest. This corresponds to studies by Bell & Garside (2005) and Singels et al. (2005), where primary shoots were found to occur up until 2 months after harvest, followed by a rapid addition of secondary and higher order tillers until approximately 3 months after harvest (where our sampling begins). Bell & Garside (2005) found that from 3 months until between 6 and 7 months after the start of the ratoon, there was a progressive loss of shoots until numbers stabilised at or near the final stalk population density recorded at harvest. The assumption in our study was that the tillers were the smallest sugarcane stalks (in terms of height and biomass), in that they sprouted later than the primary shoots over the ratoon, and were the most likely to be outcompeted for light (and other resources e.g. water, nutrients) over the ratoon.

For each month in our study, the number of tillers were considered as the difference between the number of sugarcane stalks at that month and at the final sampling date before harvest. At each month, the inventory of sugarcane height and diameter for each of the 2 m subplots was ordered from the plant with the lowest to highest biomass. The tiller biomass was the sum of the biomass of the lowest biomass sugarcane stalks at each month for each treatment.

2.2.4 Strawfall sampling from catchment

The strawfall component in this study was measured using 1.5 m x 1.5 m "catchment" nets that were placed on the soil between sugarcane rows in each treatment plot (see Figure 2.1 above). Sugarcane leaves that fell onto this catchment net, and the portion of dry leaves that made contact with the net, were harvested twice every month.

2.2.5 Root and stool sampling

The sugarcane root biomass was estimated with the auger method (Oliveira, 2000, Otto et al. 2009). Soil cores were sampled using a mechanic auger (inner diameter of 90 mm) composed of gouges coupled with a percussion hammer (Cobra TT, SDEC). Root biomass was estimated from 9 soil cores corresponding to 3 repetitions and 3 positions relative to the sugarcane row (0-25 cm, 25-50 cm and 50-75 cm) to a depth of 50 cm. In the field, the soil cores were divided into three layers: 0-10 cm, 10-30 cm and 30-50 cm. In the laboratory, roots were separated from soil by placing each sample into a bucket of water and swirling the water to create a vortex. Soil was manually disaggregated from the roots, and the roots would float to the surface of the water in the bucket. The floating roots were then collected using a 500 µm sieve.

The stool or pseudo-rhizome was collected only at the end of the growth-cycle of the second experimental year. A single, entire sugarcane plant was harvested at the centre of the ^{15}N subplots, the stalks and the rest of the aboveground biomass components were removed, as well as the roots attached to the stool. Soil was also separated from the stool by swirling the stool in a bucket of water, as for the roots, as well as by scrubbing out the soil using a brush.

2.2.6 Nitrogen concentration, N mass and N-fertiliser recovery efficiency

All sugarcane biomass components (stalks, tillers, strawfall, roots and stools) were cut into small pieces (approximately 10 cm x 1 cm) and dried at 60°C for a minimum of 72 hours until a constant weight was obtained on an analytical scale. Dried, sugarcane biomass components were then ground to 1 mm using a Universal Cutting Mill (Pulverisette 19, Fritsch) and analysed for N with an Elemental analyzer (Vario Max Cube CNS, Elementar, Hanau, Germany).

These N concentration values were plotted against the corresponding biomass values, estimated at a plot scale for both the fertilised and unfertilised treatments, from which the N dilution curve was established. This is also described in more detail in the Methods and Materials of Chapter 1. The N mass of each of these biomass components was then calculated as the biomass multiplied by the corresponding N concentration.

The N-fertiliser recovery efficiency by the difference method (dNRE) was then calculated as the difference between the N mass of the fertilised treatment, and the N mass of the unfertilised

treatment, divided by the total N applied by fertiliser. The N mass and dNRE was determined step by step introducing each of the sugarcane components, the "stalks" (which was the shoot minus tillers), the shoot (the conventional measure with tillers), the aboveground biomass (shoot with strawfall added) and the total biomass (aboveground biomass with the root biomass added, as well as the stool biomass for the second experimental year).

2.3 Results

2.3.1 Sugarcane aboveground biomass and nitrogen mass over the growth cycle

The sugarcane aboveground biomass reached a similar dry biomass at harvest for the two years, for both the fertilised and unfertilised treatments respectively (Figure 2.2 A). The fertilised treatment reached an aboveground biomass of 31.2 t.ha^{-1} at the end of the first year; and 32.9 t.ha^{-1} at the end of the second year at harvest, respectively. The unfertilised treatment reached 24.1 t.ha^{-1} at the end of the first year, and also 24.1 t.ha^{-1} at the end of the second year.

The aboveground N mass is close to double for the fertilised compared to the unfertilised treatments over the first year (Figure 2.2 B), and is approximately 1.5 times greater over the second year, on average.

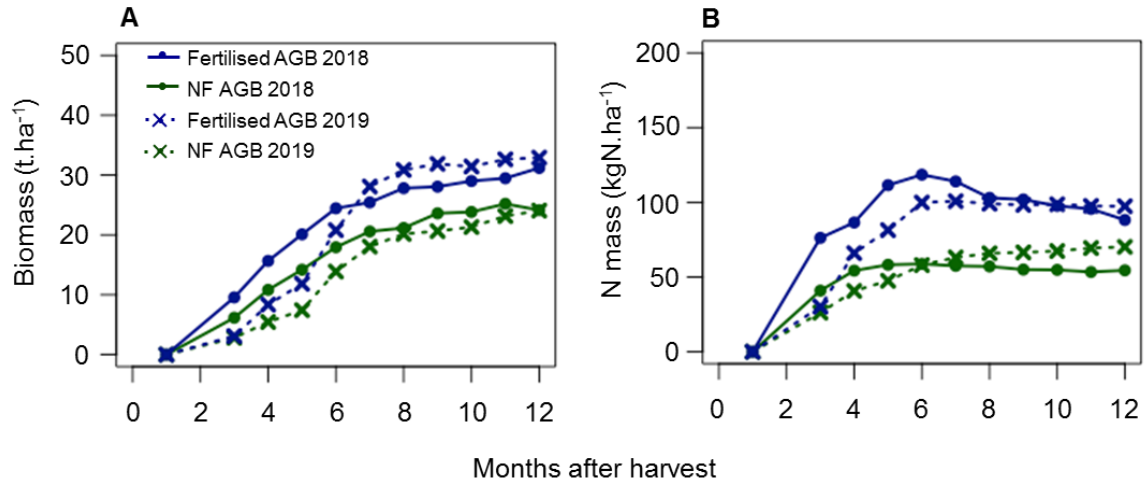

Figure 2.2 Aboveground biomass over sugarcane growth-cycle for unfertilised (NF AGB) and urea-fertilised (Fertilised AGB) treatments for the first (2018) and second experimental years (2019) (A) and the total aboveground N mass over the sugarcane growth-cycle for the two respective treatments over the first and second experimental years (B).

The final N mass was slightly higher for the second experimental year than the first, for both the unfertilised and fertilised treatments, with the fertilised treatment reaching an N mass of 88.2 kgN.ha^{-1} and 97.4 kgN.ha^{-1} at the end of the first and second years, respectively. The unfertilised treatment reached an N mass of 54.5 kgN.ha^{-1} and 70.2 kgN.ha^{-1}, at the end of the first and second years, respectively.

Although the aboveground biomass values at harvest converge at the end of the first and second experimental years, the pattern and timing of aboveground biomass accumulation is very different between the two experimental years, for both the unfertilised and fertilised treatments. There is a strong lag in growth of the sugarcane over the second experimental year, in comparison to the first. The second year aboveground biomass (fifth ratoon) only reaches that of the first year (fourth ratoon) at 7 months for the fertilised and at 8 months for the unfertilised treatments. There is a similar tendency in the accumulation of N mass to biomass over the growth-cycle, which differs substantially between experimental years. The N mass over the second experimental year reaches the values of the first year only at 8 months for the fertilised and 6 months for the unfertilised treatments.

For the fertilised treatment, there is a steep gradient in N mass accumulation indicating an active N uptake period until 5 months and 6 months after the start of the ratoon, over the first and second experimental years, respectively. Thereafter, a plateau is reached for the N mass accumulation. For the unfertilised treatment, the active N uptake period occurs until 5 months after the start of the ratoon over the first experimental year, but is more gradual over the second experimental year and continues, without reaching a plateau, over the growth-cycle.

2.3.2 Tiller biomass component

There are substantially more tillers over the first experimental year than the second experimental year for the fertilised treatment (Figure 2.3). For the fertilised treatment, at 3 months after the start of the ratoon, there are 28 and 8 tillers per 2 m linear subplot which do not survive to the following harvest, for the first and second experimental years respectively. At 6 months after the start of the ratoon, there are 10 and 4 tillers which do not survive till harvest per 2 m linear subplot, for the first and second years respectively. There was less difference between the two years for the unfertilised treatment, and the number of tillers was far lower than the fertilised treatment over the first year, but this difference was far less pronounced over the second year. At 3 months after the start of the ratoon, there were 8 and 6 tillers per 2 m linear subplot which did not survive to harvest for the unfertilised treatment over the first and second experimental years, respectively. At 6 months after the start of the ratoon, this decreased to 1 and 2 tillers per 2 m linear subplot which did not survive to harvest for the unfertilised treatment over the first and second years, respectively.

The biomass of the tillers, as well as the corresponding accumulated total N biomass, were therefore also higher for the fertilised treatment over the first experimental year than the second, with the peak difference between 3 and 8 months after the start of the ratoon. For the fertilised treatment at 3 months after harvest, there was an average tiller biomass of 2.4 $t.ha^{-1}$ and 0.3 $t.ha^{-1}$ for the first and second years, and this increased to a maximum of 4.2 $t.ha^{-1}$ and 1.3 $t.ha^{-1}$ at 6 months

after the start of each of the ratoons. Given that there were few tillers in the unfertilised treatments over the two experimental years, the average tiller biomass was 0,7 t.ha^{-1} and 0,5 t.ha^{-1} at 6 months after harvest, for the first and second experimental years, respectively. The tiller N mass followed a similar trend to that of the biomass over the two experimental years. At 3 months after the start of the ratoon, the fertilised treatment tillers had an average N mass of 16.8 kgN.ha^{-1} and 1.9 kgN.ha^{-1}, peaking at 4 months with an N mass of 21.8 kgN.ha^{-1} and 2.0 kgN.ha^{-1}, and decreasing thereafter until the final harvest.

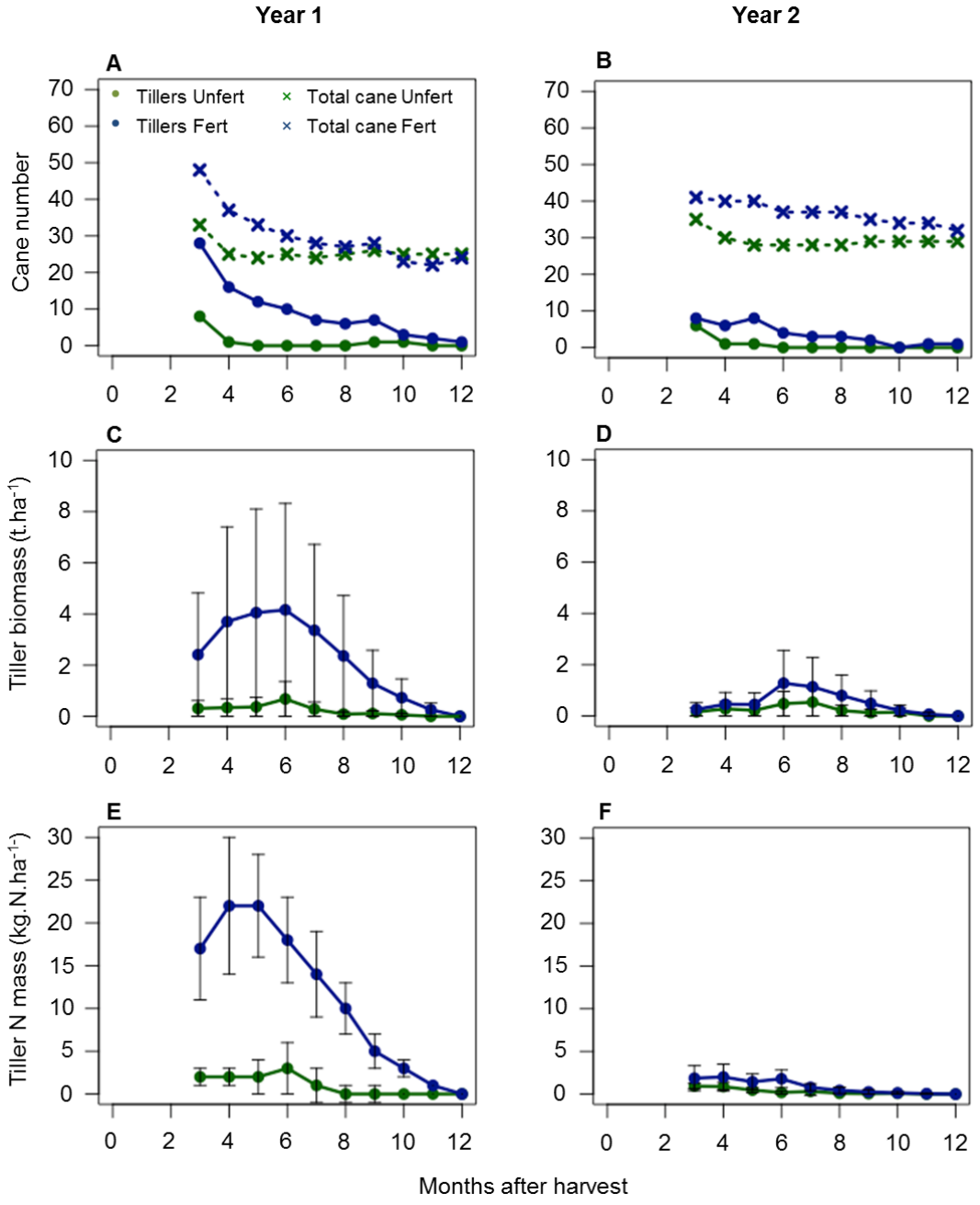

Figure 2.3. Tiller component for the first experimental year (left) and the second experimental year (right); for fertilised and unfertilised treatments. A & B Number of tillers and number of cane stalks per 2m linear microplot; C & D Tiller biomass; E & F N biomass of the tiller component.

2.3.3 Strawfall biomass component

The strawfall began at a similar point in time in the growth-cycle of both ratoons, where dry leaves began to fall from the sugarcane shoot at 6 and 7 months after the start of the fourth and fifth ratoons, respectively. The accumulated strawfall biomass and N mass was higher for both the fertilised and unfertilised treatments over the second experimental year than the first (Figure 2.4). However, this was especially the case for the fertilised treatment, where the accumulated strawfall at 12 months (just before harvest), was 5.0 $t.ha^{-1}$ and 2.6 $t.ha^{-1}$ for the first and second experimental years, respectively. For the unfertilised treatment, accumulated dry biomasses at 12 months were 3.8 $t.ha^{-1}$ and 2.9 $t.ha^{-1}$ respectively. For the fertilised treatment, the accumulated N mass was 24.2 $kgN.ha^{-1}$ and 9.9 $kgN.ha^{-1}$ at 12 months after the start of the ratoon for the first and second years, respectively. For the unfertilised treatments, the accumulated N mass was 16.5 $kgN.ha^{-1}$ and 11.2 $kgN.ha^{-1}$ at 12 months, respectively. The N mass was considerably higher over the first experimental year than the second.

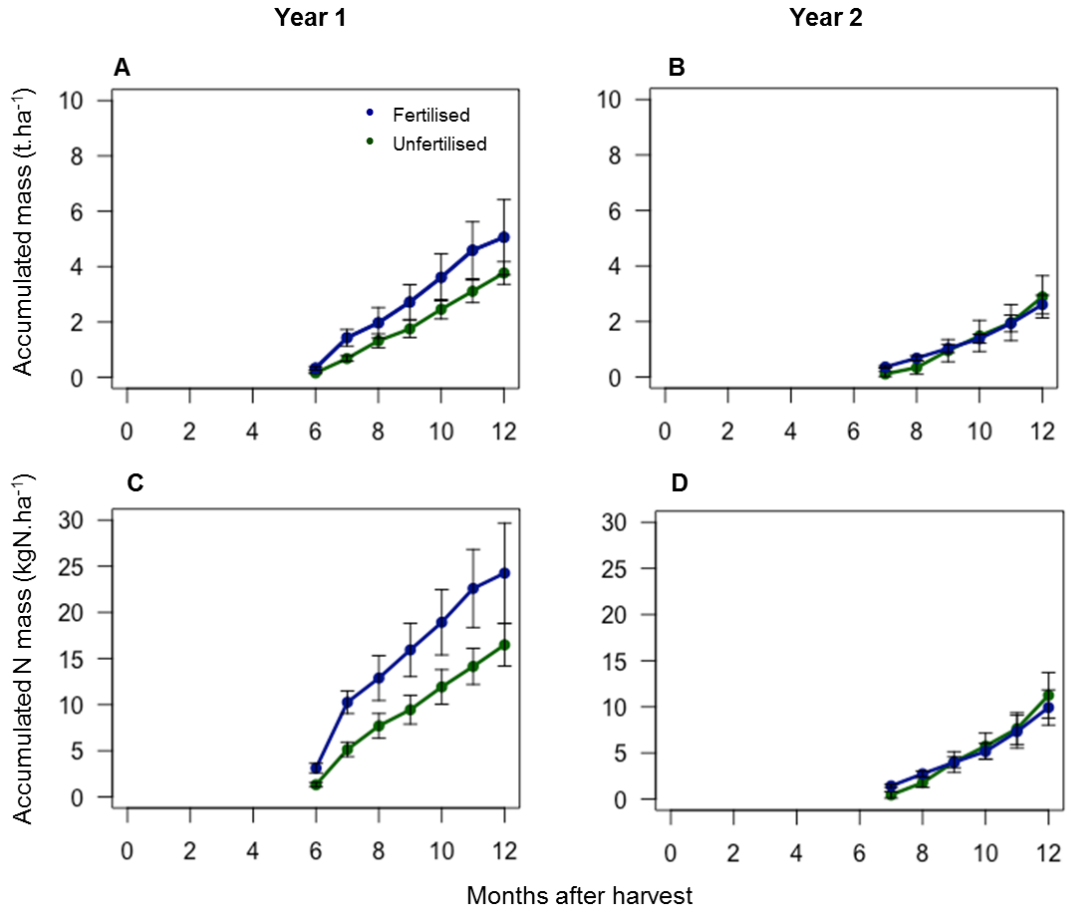

Figure 2.4 *Strawfall accumulated N mass for the first experimental year (left) and the second experimental year (right). A & B Accumulated strawfall biomass; C & D Accumulated strawfall N mass.*

2.3.4 Belowground biomass component

The root biomass at final harvest was slightly higher at the end of the second experimental year than the first for the fertilised treatment, with dry masses of 9.2 t.ha^{-1} and 10.9 t.ha^{-1} respectively (**Table 2.1**). Roots were harvested from the unfertilised treatment only at the end of the second experimental year, and had a higher dry biomass of 12.8 t.ha^{-1} than the fertilised treatment. The root N mass was similar for the fertilised treatment at final harvest of both experimental years, being 57.7 kgN.ha^{-1} and 55.5 kgN.ha^{-1} at the end of the first and second years respectively. The N mass was 61.2 kgN.ha^{-1} for the unfertilised treatment at the end of the second experimental year, which was higher than the fertilised treatment.

The sugarcane stool (or rhizome) was only harvested at final harvest at the end of the second experimental year. The stool component contributes less to the belowground biomass than the roots, but still has a substantial contribution of 2.5 t.ha^{-1} and 3.8 t.ha^{-1} in the fertilised and unfertilised treatments, respectively. This corresponds to a substantial N mass (although again lower than the roots) of 20.8 kgN.ha^{-1} and 17.5 kgN.ha^{-1} for the fertilised and unfertilised treatments, respectively.

Table 2.1 *Dry biomass and nitrogen mass of the belowground biomass component (roots and rhizome) at final harvest for the second experimental year. The root to shoot ratio is also given, for roots only and for roots and rhizomes combined.*

Treatment	Root biomass (t.ha^{-1})	Shoot biomass (t.ha^{-1})	R:S ratio (biomass)	Root N mass (kgN.ha^{-1})	Shoot N mass (kgN.ha^{-1})	R:S ratio (N mass)	Stool mass (t.ha^{-1})	Stool N mass (kgN.ha^{-1})
Fertilised	10.9 ± 1.5	32.9	0.33	55.5	97.4	0.57	2.5 ± 0.4	20.8
Unfertilised	12.8 ± 1.0	24.1	0.53	61.2	70.2	0.74	3.8 ± 0.9	17.5

2.3.5 Total nitrogen mass for different sugarcane components

The evolution of the total N mass of the sugarcane changed when the various biomass compartments were taken into account over each sugarcane growth-cycle (Figure 2.5). Over the first experimental year, when the tiller component of the sugarcane system was considered, the sugarcane total N mass decreased by 12 kg.ha^{-1} and 1 kg.ha^{-1} for the fertilised and unfertilised treatments, respectively. When the strawfall component was added to the baseline total crop N for the two treatments, the sugarcane total N mass increased progressively from 6 months until harvest. The sugarcane total N mass increased up to 15 kg.ha^{-1} at the end of the first year for the fertilised treatment, and up to 10 kg.ha^{-1} for the unfertilised treatment. The total sugarcane biomass increased substantially over the first experimental year when root biomass was also considered, by approximately 9.2 t.ha^{-1} for the fertilised treatment (i.e. increased by a factor of 1.5). There was not an unfertilised root sample for the first experimental year. The total N mass increased by 56.4 kg.ha^{-1} for the unfertilised treatment and by 57.7 kg.ha^{-1} for the fertilised treatment.

The second experimental year follows a similar trend to the first year, for both treatments. The baseline total N mass is more than double for the fertilised than the unfertilised treatments (Figure 2.5). When the tiller component of the sugarcane system is considered, there is far less of a decrease over the second year than the first year for the fertilised treatment. Between 3 and 10 months after the start of the ratoon, there was an average decrease in N mass of 3 $kg.ha^{-1}$ for the fertilised and 1 $kg.ha^{-1}$ for the non-fertilised treatments. When strawfall is considered over the second experimental year, the total N mass increased by an average of 3 $kg.ha^{-1}$, for both the fertilised and unfertilised treatments, returning to the baseline total N (i.e. before the N mass of the tiller component is considered). When the belowground biomass component was considered, the total crop biomass increased by 10.9 $t.ha^{-1}$ at harvest at the end of the second experimental year for the fertilised treatment (increased by a factor of 1.5) and 12.8 $t.ha^{-1}$ for the unfertilised treatment, which was similar to final harvest at the end of the first experimental year. The total N mass increased by 61.2 $kg.ha^{-1}$ for the unfertilised treatment (i.e. more than doubles) and by 55.5 $kg.ha^{-1}$ for the fertilised treatment (i.e. increased by a factor of 1.6).

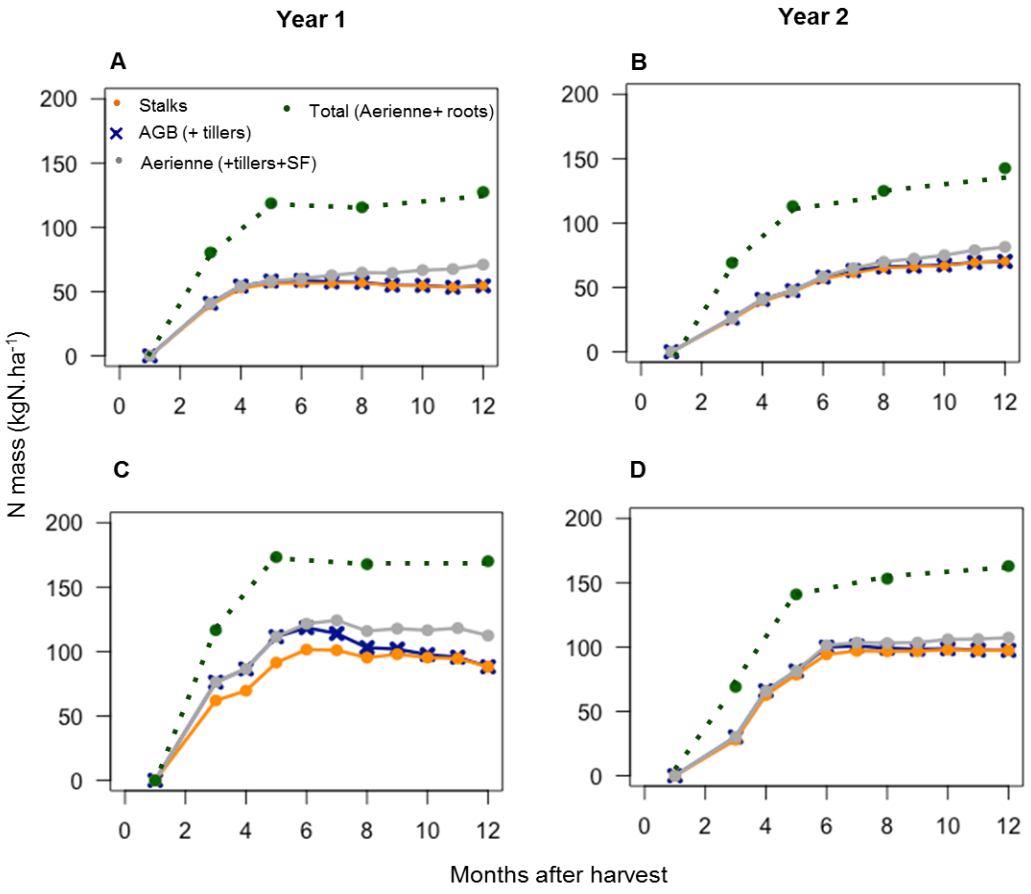

Figure 2.5 *Nitrogen mass for the first experimental (left) and second experimental year (right). The N mass for the unfertilised treatment is displayed with the top graphs (A & B); the N mass for the urea-fertilised treatment is shown by the two bottom graphs (C & D).*

2.3.6 N-fertiliser recovery efficiency

The baseline N-fertiliser recovery, when the tiller, strawfall and belowground biomass components were not considered, was on average 34 % over the first experimental year, and 21 % over the second experimental year (Figure 2.6). There was a decrease in the dNRE over the growth cycle of both experimental years. Over the first experimental year, dNRE begun quite high at 40.0 % at 3 months after the start of the ratoon and remained relatively constant until 7 months, and then declined to 31.9 % at 8 months and further to 23.4 % at final harvest. Over the second experimental year, the dNRE was 4.6 % at 3 months after the start of the ratoon (also being 3 months after the first fertilisation), which increased after the second fertilisation to a maximum of 29.1 % at 6 months after the start of the ratoon. The dNRE then declined to 18.9 % at final harvest of the second experimental year. When sugarcane stalks were considered over the first experimental year, and the tiller component N mass was removed from the aboveground N mass, the dNRE decreased by a maximum of 14.2 % at 3 months, lowering to a decrease of 2.5 % at 10 months, with an average decrease of 7.1 % over the growth-cycle. The impact of the tiller component on the dNRE was considerably lower over the second experimental year, with a decrease of 1.1 % at 3 monthsand 0.7 % at 9 months after the start of the ratoon, and an average decrease of 0.9 % over the growth-cycle. When the strawfall component was considered, the NRE increased by 5.4 % at the final harvest of the first experimental year, but by only 0.9 % at the end of the second experimental year. When the belowground biomass component was considered, there was an increase in NRE of 6.2 % at final harvest over the first experimental year. Over the second experimental year, there is a decrease of 4.9 % at harvest.

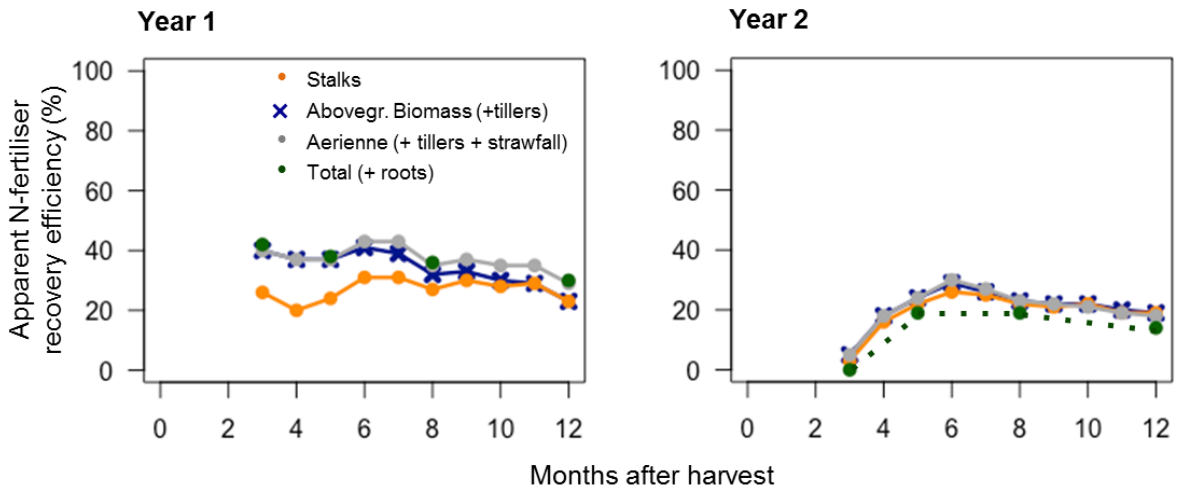

Figure 2.6 Nitrogen use efficiency using the difference method for the first (A) and second (B) years of experiment, for the different N biomass components (aboveground biomass, stalk biomass, aerienne biomass and total aboveground biomass.

2.4 Discussion

2.4.1 Aboveground biomass and N mass accumulation over the sugarcane growth-cycle

The fourth and fifth ratoons had a similar yield at harvest for the fertilised and unfertilised treatments. However, the trends in aboveground biomass evolution over the sugarcane ratoon were very different between the two years. The first experimental year had a far higher aboveground biomass during the initial growth phases of the ratoon (sprouting and emergence growth stages). However, the gradient of biomass accumulation between 3 and 8 months of the second experimental year was far higher and the sugarcane aboveground dry biomass eventually caught up with that of the first experimental year. The second experimental year followed the more traditional "S-curve" or logistic growth form, similar to the trends of sugarcane aboveground dry biomass accumulation in Ng Kee Kwong & Deville (1994), Wood et al. (1996), and for certain N fertiliser doses in Franco et al. (2011). By contrast, the aboveground biomass followed a logarithmic growth form over the first experimental year, with a far more gradual aboveground biomass accumulation.

The reason for the lag in biomass accumulation over the second experimental year was two-fold. At harvest, at the start of the ratoon, the sugarcane harvester cut the sugarcane too low at the base of the stalks, and there was a longer duration of the sprouting and emergence developmental phases of the sugarcane. Secondly, the sugarcane was impacted by caterpillars during the emergence stage of the growth-cycle, which affected the growth. The caterpillar infestation dissipated after approximately a month and the sugarcane returned to its normal, healthy state following which there was a steep increase in aboveground biomass over the second year, and growth caught up to the aboveground biomass of the first year at the end-year harvest.

This difference in trends in aboveground dry biomass over the sugarcane growth-cycle is then again reflected in the total N mass evolution with time, since the N mass measure is strongly dependent on the biomass. Second to biomass, the total N mass depends on the N concentration of the sugarcane which decreases slightly over the growth-cycle, typical for crops and from which N dilution curves can be developed (de Oliveira et al. 2013, Poultney et al. 2020). However, quantitatively, biomass has a larger contribution to the N mass value.

The final crop aboveground biomass and corresponding accumulated N mass at the end of the growth-cycle is lower than that of high-N supplied sugarcane plantations (>200 $kgN.ha^{-1}$) in Australia and Brazil, for example, which have crop yields of 60-150 $t.ha^{-1}$ of dry aboveground biomass, and corresponding accumulated N mass of 250 $kgN.ha^{-1}$ in the shoots and up to 380 $kgN.ha^{-1}$ in the total biomass (when belowground biomass is included) (Chapman et al. 1994, Muchow & Robertson, 1994, Wood et al. 1996, Basanta et al. 2003, Robinson et al. 2013). These

studies only report N mass values of the aboveground sugarcane biomass, without taking into account strawfall or tillers. However, other sugarcane plantations subject to lower N fertiliser applications have been reported in Australia, with a far lower N accumulation of approximately 66 $kgN.ha^{-1}$ (Thorburn et al. 2009, Bell et al. 2010). In our study, the biomass and N mass accumulation over the two ratoons is somewhere between these two scenarios.

The active N uptake period is where the gradient of N mass accumulation is at its greatest, and thereafter the N mass accumulation reaches a plateau, implying a far lower rate of N mass accumulation. The active N uptake period is between 3 and 6 months after the start of the ratoon for the two experimental years in our study. This is coherent with the active N uptake period in the studies of Ng Kee Kwong and Deville (1994), Wood et al. (1996) and Franco et al. (2011), where the active N uptake occurred up to between 5 and 7 months after the start of the ratoon.

Although the aboveground biomass continued to increase until harvest at the end of each ratoon, the baseline aboveground biomass (i.e. when tiller and strawfall components are not considered) began to reach a plateau approximately 2-3 months after the cessation of the N uptake period. This is coherent with Wood et al. (1996), where N accumulation ceased around 100-140 days before maximum biomass accumulation. The study by Wood et al. (1996), identified a need to determine the sources of variation in N accumulation for a given level of biomass, as well as the causes of the early plateau in N accumulation, and hypothesised that this plateau may be a consequence of an exhaustion of the soil N supply, reduced root activity or lowered crop N requirement. It is not clear from their study whether the cessation of N accumulated in the latter half of the season was associated with a slowing of biomass accumulation.

In our study, however, the sugarcane total N mass reached a plateau due to a lower rate of biomass accumulation from 6 months after the start of the ratoon onwards, coupled with a slight decrease in N concentration of the sugarcane aboveground biomass, which is a typical trend when considering N dilution curves. This is typically the case, according to studies such as that of de Oliveira et al. (2013), where during initial growth stages, the increases in leaf area and high rate of photosynthesis increases the demand for N and when soil N is readily available (typically the case after fertilisation, the N concentration in the tissues increase). There is a decrease in N content later in the growth-cycle since there is a higher proportion of stalk to leaf biomass (stalks have a low N content) and there is an increase in the cellulose and lignin concentrations of the older stalk tissues, with a lower concentration (Lemaire et al. 1992, Marino et al. 2004, de Oliveira et al. 2013).

2.4.2 Considering the different biomass components

The evaluation of sugarcane biomass components in our study, which are often overlooked, appear to have a considerable impact on the biomass and the total N mass over the sugarcane growth-cycle, and therefore give a more complete understanding of N nutrition in sugarcane. However, the level of impact was different between the two experimental years.

The tiller component of our sugarcane system had a far greater influence on the sugarcane biomass and N mass over the first experimental year than over the second, for the fertilised treatment. Or, more specifically, there was a far higher senescence of stalks over the sugarcane growth-cycle of the first experimental year than in the second. The first year trend of tiller senescence was coherent with studies such as Bell & Garside (2005) and Singels et al. (2009), where there was a progressive loss of stalks from 3 months after the start of the ratoon (peaking between 3 and 5 months) until stalk numbers stabilised at or near the number recorded at harvest. The second experimental year in our study, however, was quite different. There were very few stalks, once established, which did not survive until harvest.

There were not necessarily fewer tillers over the second experimental year, but rather it is likely that there was a higher senescence of the existing tillers, leading to a similar yield at the end of the crop growth-cycle for both experimental years. It has been observed that there is typically a negative relationship between the density of established primary shoots and the number of surviving tillers (Bell & Garside, 2005), since tiller survival depends on the shading and competition for light (Bell & Garside, 2005, Singels et al. 2005). By this logic, the fact that there was a higher initial number of stalks in the fertilised treatment at 3 months after the start of the ratoon during the first experimental year compared to the second, is likely to be at least partly why, in our study, there was a higher corresponding senescence of tillers over the first experimental year.

The N mass accumulation reflected this decrease in total aboveground biomass between 3 and 8 months for the fertilised treatment over the first experimental year, but with little effect on the total N mass over the second experimental year.

It appears that over the second experimental year, the sugarcane N biomass system had a more “conservative” functioning, with a lower initial aboveground biomass but a steeper biomass accumulation over the growth-cycle, and a low level of stalk senescence. In a sense, it is as if the sugarcane tried to retain what it already had as best as possible as a survival strategy over the second year. This is possibly related to the initial perturbation to the sugarcane system during the initial growth stages over the second experimental year.

The strawfall component was coherent with our standing hypothesis, i.e. that there was a higher biomass and N mass “turnover” over the first experimental year and that the sugarcane

system had a more conservative functioning over the second experimental year. In addition, the strawfall began to fall slightly earlier over the first experimental year (at 6 months after the start of the ratoon) than the second (at 7 months). The discrepancy between strawfall quantities over the two years was especially high for the fertilised treatment, with accumulated strawfall dry biomasses of 5.0 $t.ha^{-1}$ and 2.6 $t.ha^{-1}$ over the first and second experimental years respectively. This is lower than the strawfall dry biomass at harvest in what was reported on by Basanta et al. (2003) and Carvalho et al. (2013), which were 14.0 $t.ha^{-1}$ and 11.5 $t.ha^{-1}$ respectively.

The resultant accumulated N mass of the strawfall component was therefore also higher over the first experimental year than the second, with an accumulated N mass of 24.2 $kgN.ha^{-1}$ and 9.9 $kgN.ha^{-1}$ respectively. This was lower than the N mass values of straw at final harvest reported by Basanta et al. (2003), which was between 35 $kgN.ha^{-1}$ and 50 $kgN.ha^{-1}$ for their four different sites.

The estimated root N mass increased the total crop N mass massively, over both experimental years. The root biomass and N mass were relatively high, compared to other studies, such as that of Otto et al. (2009) and Carvalho et al. (2013). The root compartment comprised approximately a third of the total crop biomass, and a half of the total crop biomass, for the fertilised and unfertilised treatments at the end of the ratoon of both experimental years, respectively. The root dry biomass ranged between 9.2-12.8 $t.ha^{-1}$, which was coherent with the root biomass of 9-11 $t.ha^{-1}$ measured by Ball-Coelho et al. (1992) and 6.8-11.5 $t.ha^{-1}$ measured by Versini et al. (2020), but was considerably higher than the biomass range of 3-5 $t.ha^{-1}$ reported by Otto et al. (2009), from three Brazilian studies for the plant crops. The dry biomass root-to-shoot ratio of 0.30 and 0.33 for the fertilised treatment at final harvest at the end of the first and second experimental years, was consistent with that of Versini et al. (2020), who had a ratio of 0.29 for this same site, but for the third ratoon, as well as with that of Silva-Olaya et al. (2017), who also reported a ratio of 0.29 for the fourth ratoon of their experimental site. A study by Carvalho et al. (2013), found the belowground biomass to comprise 35 % of the total sugarcane dry biomass for the plant cane, but this declined to 20 %, which is somewhat lower than in our study.

The stool or "pseudo"-rhizome component in our study had a lower dry biomass than the root component, but this was still quite substantial, ranging between 2.5 $t.ha^{-1}$ and 3.8 $t.ha^{-1}$ for the fertilised and unfertilised treatments. This was in the same range as for Carvalho et al. (2013), with rhizome biomasses ranging between 1.8 $t.ha^{-1}$ and 6.3 $t.ha^{-1}$ across their 4 sites. However, they reported that the rhizomes had a greater contribution to the total sugarcane biomass than the roots, which was not the case in our study.

The N mass of the roots comprises approximately two-thirds (65 %) and half (57 %) of the N mass of the aboveground sugarcane N mass at the final harvest for the fertilised treatment, at the end of the first and second years, respectively. This was higher for the unfertilised treatment, where

the root N mass was approximately equal (104 %) to, and three quarters of (74 %) the aboveground N at the final harvest. This was consistent with Versini et al. (2020) (presented in Appendix 7.2), where for the same experimental site as our study (but the third ratoon), the N mass in the roots accounted for 70 % of the N mass of the aboveground component for the fertilised treatment, and between 67 % and 54 % of the N mass of the aboveground component at another experimental site for two fertiliser doses, slightly lower (99 $kgN.ha^{-1}$) and higher (165 $kgN.ha^{-1}$) than the fertiliser application dose for our experimental site. However, the study by Vieira-Megda et al. (2015), had a far lower proportion of root N mass, which accounted for only 7-19 % of the shoot N mass at harvest at an experimental site in Brazil.

When the stool component is considered, a far smaller amount of N mass was added to the belowground biomass compartment and total crop N mass than the roots.

2.4.3 Fertiliser nitrogen recovery considering the different biomass components

The N fertiliser recovery efficiency was not high for this sugarcane system, but rather in a midrange of values, with the NRE being slightly higher over the first experimental year than in the second.

The N fertiliser recovery efficiency decreased over the sugarcane growth-cycle, for both experimental years, from approximately 40.0 % to 23.4 % for the first, and from a maximum of 29.1 % (at 6 months) to 18.9 % for the second experimental year. This tendency for dNRE to decrease over the crop growth-cycle, has been documented for the iNRE calculated using ^{15}N isotopes, in the few studies on iNRE over the sugarcane growth-cycle (Ng Kee Kwong & Deville 1994, Courtaillac et al. (1998)), but not for the dNRE determined using the difference method.

The total dNRE (aboveground and belowground biomass combined) was lower than the aboveground biomass dNRE for the second year. This was because the N mass of the belowground biomass was higher for the unfertilised than the fertilised treatments, which is consistent with studies such as that of Versini et al. (2020). The N concentration/ biomass was higher leading to the higher N mass of the belowground biomass component of the sugarcane over the second experimental year.

The conclusion therefore is that the consideration of biomass components other than just the aboveground biomass is important when evaluating the NRE, especially over the first experimental year. If the strawfall is not considered over the experimental year, the dNRE could be underestimated by approximately 5.4 % at harvest. However, for the second experimental year, the difference is negligible (decrease by < 1 %). The consideration of the tiller component is most important over the first experimental year for the fertilised treatment, and the dNRE would be underestimated by approximately 15 % at 3 months after harvest, 8 % at 6 months after harvest.

As suggested by Robinson et al. (2009), sugarcane genotypes with a high leaf turnover rate and inefficient N remobilisation may have a lower NRE than genotypes which maintain green leaves for longer. Their study only considered strawfall just before the final harvest. The impact on NRE is greater when considered over the portion of the growth-cycle where leaves fall from the plant (i.e. strawfall from approximately 6 months after the start of the ratoon until the final harvest). This is likely to not only be genotype-specific, but applicable across sugarcane varieties.

In the study by Meier et al. (2006), the NRE was 2-4 % from surface trash. They suggested further that if 30 % of plant N is assumed to occur in the roots (van Dillewjin 1952, as cited by Meier et al. 2006), the NRE would increase to 6-8 % for the fertiliser and 3-6 % for the trash. A limitation of their study, as they suggested, was that "^{15}N in early-detached leaves was not measured", as was evaluated in our study.

Our study does not investigate the fate of the N of senescent tillers and strawfall, and whether (and the timing of when) the N from these components is immobilised in the soil and later mineralised to become available to the sugarcane system. Evaluating the fate of N from the components may give more insight into better synchronising the N fertilisation with crop N demand.

CHAPTER THREE

Abstract

The primary aim of this chapter was to evaluate the different sources of nitrogen in the soil-sugarcane system, and to quantify the relative contributions of these respective N sources. This was evaluated by using the methodology reported on in Chapter 1, in order to determine the fertiliser N-recovery efficiency (NRE) throughout the sugarcane growth-cycle. The sugarcane biomass components identified as important to consider when studying sugarcane N nutrition, reported on in Chapter 2, were considered when the relative proportions of N in the sugarcane derived from different N sources was evaluated.

The organic fertilisers, pig slurry and sewage sludge, as well as the mineral fertiliser urea, are assessed in this chapter, as well as the residual effect of urea, the mulch and soil N sources. This is one of the core themes of the overall study.

The fertiliser N-recovery efficiency (NRE) of urea ranged between 6.7 % and 26.1 % over the two years, with respective NRE averages of 9.2 % and 16.1 % for the first and second years. The pig slurry treatment had an NRE ranging from 0.9 – 10.5 % over the two years, with an average NRE of 4.3 % and 3.6 % over the first and second years. The sewage sludge treatment had an NRE ranging between 1.1 % and 12.2 % over the growth-cycle of the two years, with an average NRE of 6.7 % and 4.4 % over the first and second years. The mulch had a consistent contribution with an average of 4.7 % over the crop cycle.

A holistic vision of sugarcane N nutrition, and the various sources of N and their relative contributions, could be a better approach to synchronising crop requirements with the level of N fertiliser application. The form of N-fertiliser application is important, and the use of a combination of organic and mineral fertilisers may be an effective approach in sugarcane fertilisation, in that mineral fertilisers have a higher initial N use efficiency and can be used to plan the supply of nutrients to the plant as and when required. Certain organic fertilisers (especially the sewage sludge in this study with its high organic N content) may have a lower initial NUE but have a higher capacity for N immobilisation and is likely to become available during subsequent crop cycles, and have a higher contribution to soil fertility.

Resumé

L'objectif principal de ce chapitre était d'évaluer d'où vient l'azote dans le système sol-canne à sucre et de quantifier les contributions relatives de ces sources d'azote respectives. Basé sur la méthodologie décrite dans le chapitre 1 et sur les compartiments de la canne à sucre identifiés au chapitre 2, nous avons dans ce chapitre déterminé les proportions de N dans la canne à sucre provenant des différentes sources de N. Les engrais organiques (lisier de porc et boues d'épuration), ainsi que l'urée, un engrais de synthèse, sont pris en compte, de même que l'effet résiduel de l'urée, le paillis et le sol.

L'efficacité d'utilisation de l'azote (estimée par la mesure du « Coefficient Réel d'Utilisation de l'azote » ou CRU)) de l'urée a varié entre 6,7 % et 26,1 % sur les deux années, avec des moyennes respectives de CRU de 9,2 % et 16,1 % pour la première et la deuxième année. Le traitement lisier de porc a eu un CRU compris entre 0,9 et 10,5 % sur les deux ans, avec un CRU moyen de 4,3 % et 3,6 % la première et la deuxième année. Le traitement boues d'épuration présentait un CRU compris entre 1,1 % et 12,2 % sur le cycle de croissance des deux années, avec un recouvrement moyen de 6,7 % et 4,4 % la première et la deuxième année. Le paillis a eu une contribution constante de 4.7 % en moyenne sur le cycle de culture.

Une vision holistique de la nutrition de l'azote de la canne à sucre, et des différentes sources d'azote et de leurs contributions relatives, pourrait être une meilleure approche pour synchroniser les besoins des cultures avec le niveau d'application des engrais azotés. La forme d'application de N est importante, et l'utilisation d'une combinaison d'engrais organiques et minéraux peut être une approche pertinente pour la fertilisation de la canne à sucre, dans la mesure où les engrais minéraux ont une NUE initiale plus élevée et peuvent être utilisés pour planifier l'apport de nutriments à la plante en fonction des besoins. Certains engrais organiques (en particulier les boues d'épuration de cette étude avec leur forte teneur en N organique) peuvent avoir une NUE initiale plus faible mais ont une plus grande capacité d'immobilisation de l'azote et sont susceptibles d'être disponibles au cours des cycles de culture suivants, et contribuent plus fortement à la fertilité des sols.

Chapter 3: Relative contributions to sugarcane nutrition and agronomic efficiency of distinct nitrogen sources: mineral fertiliser, organic fertilisers, past-fertilisation, mulch and soil organic matter

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3.1 Introduction

Nitrogen (N) is an essential nutrient for plant growth and development and is frequently a limiting factor in agroecosystems. However, excess nitrogen lost from agricultural systems has significant environmental consequences (Dobermann, 2005). The challenge of maintaining or increasing yields while reducing the cost (both environmental and economic) of agricultural production has become a major global concern of the scientific community and policy-makers (Tang et al. 2019).

An effective approach to managing nitrogen inputs and reducing the extent of N loss would be not only a focus on the optimal use of fertiliser N in terms of the dose and timing of application, but also the evaluation and management of all the N sources in the soil-crop agroecosystem. Rather than merely being a matter of applying the right dose of mineral fertiliser at the right time, a more holistic approach would be to consider the partial replacement of some of the mineral fertiliser with agricultural or industrial (e.g. sewage) waste recycled as organic fertiliser. This would promote a circular economy, and is a "smart" agricultural method of nourishing the soil and supplying mid- to long-term crop N requirements. A more holistic approach would also consider the contribution of other N sources, such as mulch (crop residue remaining after harvest), the residual effect of previous fertiliser applications, and the N supply from soil organic matter (SOM), as a large N pool (Dourado-Neto et al. 2010). Ideally this would be assessed dynamically, i.e. over the crop growth-cycle, given the temporal variability of crop N needs as well as of N availability of the various sources.

The recycling of organic residues in agroecosystems could be a promising alternative or complement to the use of mineral fertilisers. This would also provide a means of promoting a circular economy and agricultural sustainability in the re-use and recycling of what otherwise would be considered "waste" which needs to be disposed of. The recycling of organic residues in agricultural land is also a means of rehabilitating disrupted nutrient cycles and has the potential to

enhance the resilience of agriculture while reducing environmental pressures (Wassenaar et al. 2014). From an economic as well as environmental point of view, a more comprehensive perspective than purely an interest in crop yield, would be to evaluate the value of ecosystem services related to the partial substitution of mineral fertiliser with organic fertiliser, which will consider the range of environmental impacts and human benefits received (Tang et al. 2019).

In sugarcane agroecosystems, the crop-N requirement may be supplied by several sources: the application of mineral and/or organic fertilisers, residues of harvested crops and associated roots, mineralisation of soil organic matter, biological N-fixation, and atmospheric deposition (Franco et al. 2011). The majority of N is typically supplied by soil organic matter, which frequently supplies at least 50-80 % of sugarcane's N content (Dourado-Neto et al. 2010, Stevens et al. 2005). Nonetheless, mineral and organic fertilisers have been found to play a key role in sugarcane N nutrition, particularly at the early development stages of the crop (Franco et al. 2011).

Mineral fertilisation, which is the addition of inorganic fertilisers to agricultural systems, enhances plant growth and therefore crop production. The mineral fertiliser N, such as when applied as urea, is rapidly converted to ammonium and nitrate by soil microbes, which are forms of N readily available for plant uptake (Robinson et al. 2013). It is for this reason that it is often simpler to cater for the immediate and short-term N requirements of crops with mineral fertilisers. However, in these forms of N (ammonia and nitrate), there is a direct risk of loss of NH_3 via volatilisation, and of NO_3^- via leaching (Nieder & Benbi 2010).

Organic fertilisers (OFs) have been shown in several studies to have beneficial effects on crop yield and quality through the improvement of soil structure and soil chemical properties (Tang et al. 2019). There is often a higher capacity for soil-plant systems to "recall" the history of previous OF application longer than that of mineral fertilisers, as is reflected in the longer-term N supply (over subsequent growth-cycles) of OFs which typically acts via the soil N pool (Gutser et al. 2005). There has been evidence to show that continuous applications of organic wastes can increase the physical fertility of soil, primarily by improving soil porosity (Tejada et al. 2009) and aggregate stability (Diacono & Montemurro, 2010), as well as soil biological fertility after the application of organic wastes such as sludge (Mats & Lennart, 1999). Certain OFs have a low mineral N content and may have less of an impact (and a lower N-fertiliser recovery efficiency) on crop growth directly after application. However, there is frequently a "residual" effect and a release and contribution of N to crop nutrition over subsequent growth cycles (Gomez-Munoz et al. 2017), as a result of the slow-release characteristics of organic N in certain OFs (Gutser et al. 2005).

Mulch (sometimes referred to as "green trash") is essentially the crop residue left after harvest, consisting primarily of straw (i.e. dry sugarcane leaves) and the green tops of the sugarcane plant. The mulch therefore contains a substantial proportion of nutrients from the previous ratoon,

and the maintenance rather than removal of mulch has been adopted as an agricultural practice in most sugarcane plantations (Carvalho et al. 2017) in order to favour nutrient restitution from one ratoon to the next. In sugarcane cropping systems such as the Australian wet tropics, mulch sometimes contains up to one third of the N applied in fertiliser (Meier et al. (2006)). However, mulch has been shown to supply N slowly with a large portion being immobilised in the soil organic matter. The implication is that mulch contributes to nitrogen nutrient storage. Thus while the direct contribution of mulch to sugarcane N nutrition during the growth-cycle following its application may be relatively low (Meier et al. 2006, Meier & Thorburn, 2016), a larger proportion of N nutrition could become available to crops over subsequent growth-cycles.

The relative importance of these multiple N sources other than fertilisers, such as mulch, as well as the residual effect of mineral fertilisation and the contribution of soil on sugarcane nutrition can be determined by using sources enriched with nitrogen isotope 15. Studies show that the relative contribution of a source can vary widely over time, and dynamic monitoring from non-destructive methods should therefore be favoured (Poultney et al. 2020, Chapter 1).

One important means of optimising N cycling and minimising the use of excess N in agroecosystems is through improvements in fertiliser N-use efficiency (NUE), resulting in less N fertiliser being used per unit of crop production. The NUE and its various indices are explained in detail in Chapter 1. The quantitative index used to evaluate the NUE is the N fertiliser recovery efficiency (NRE) (Cassman et al. 2002), which is essentially the percentage of fertiliser-N recovered by a crop during the crop growing season. In this chapter, the NRE is determined using the isotopic ^{15}N dilution method (iNRE), where ^{15}N labelled fertilisers are used to estimate the sugarcane recovery of applied N. The NRE is most often determined at harvest (Chapman et al. 1994, Isa et al. 2006, Fortes et al. 2010), but given its temporal variability at different stages of the sugarcane growth-cycle (Ng Kee Kwong & Deville, 1994, Courtaillac et al. 1998, Poultney et al. 2020), merits an evaluation over the growth-cycle. The use of ^{15}N tracer technology allows the different N-pools to be quantified and followed in sugarcane and other crop agroecosystems (Trivelin, 1994, Versini et al. 2014). More specifically, it allows the distinction in the plant N, between fertiliser N and the other sources of N. The ^{15}N isotope ratio of the plant allows the N derived from different N sources to be determined.

The evaluation of N derived from organic fertilisers (OFs), as well as other N sources, is less evident. In terms of OFs, since this is not produced synthetically, the N component cannot directly be enriched isotopically in a simple process, as in the case with mineral (i.e. chemically synthetic) fertilisers. The way of dealing with this in our study, was the use of mixed OF -^{15}N mineral microplots and comparing this to a reference ^{15}N mineral microplot, to determine the N contribution of the OF's by deduction, as explained in more detail in the Material & Methods section of this chapter.

Sugarcane biomass compartments which have a potential impact on the N mass and NRE of the sugarcane system are strawfall, the senescence of tillers over the growth-cycle and the belowground biomass compartments, as described in Chapter 2. The strawfall occurs from approximately mid-growth-cycle until the following harvest, and comprises the leaves which fall from the sugarcane to the ground, which is a potential N source often not measured when considering the N mass of the crop. Further, tillers are secondary shoots, for which little attention has been paid in terms of the loss of biomass of sugarcane plantations (Bell & Garside, 2005), and in terms of the loss of N mass via tiller senescence over the crop growth-cycle. Also important is the root system of sugarcane which appears to be a major pool of N that should be considered in studies dealing with fertiliser N use efficiency and N cycling in sugarcane agroecosystems (Versini et al. 2020). The decline in iNRE, which is typically observed from mid-crop growth-cycle until harvest, may be influenced by evaluating and taking into account these three additional biomass components into the NRE calculation, i.e. strawfall, tillers and root system.

The aim of our study was firstly to determine the contribution of different N sources to sugarcane nutrition, and secondly, to determine the N use efficiency of mineral (urea) and two organic fertilisers (pig slurry and sewage sludge) over the growth-cycle of two sugarcane ratoons. More specifically, the objectives were: 1/ to quantify the nitrogen stored in the different biomass compartments over time, 2/ to assess the origin of this nitrogen among the different potential sources (fertiliser, organic fertiliser, mulch and soil), and 3/ to estimate their level of contribution by calculating the recovery efficiency of these sources in sugarcane.

3.2 Material and methods

3.2.1 Study site

Refer to the Study site in the Experimental site section.

3.2.2 Experimental design

The investigation reported here was conducted in three specific plots (Figure 6, Experimental design) within the overall trial during the fourth-ratoon and fifth-ratoon crop (orfirst and second experimental years). The treatments in these plots were: 1) annual split applications of urea (plot T); 2) annual application of pig slurry and its urea complement (plot LP); and 3) annual application of sewage sludge and its urea complement (plot BA). The quantity and timing of fertiliser N application for the different treatment types is summarised in Figure 7 in the Experimental design and general methods.

The organic fertilisers, pig slurry and sewage sludge, were applied using 50 % of the applied dose N as organic fertiliser, quantified in terms of equivalent mineral efficiency units of N, and 50 % N as a urea complement. The pig slurry applied was composed of 57 % and 75 % NH_4 at the start

of the first and second experimental years, with the rest being organic N. The sewage sludge was composed of only 10 % NH_4 and 90 % was organic-N for both experimental years.

In summary, the first and largest fertiliser application occurs directly after harvest for each of the fertiliser types. In the OF plots, it is the OF treatment (i.e. pig slurry and sewage sludge) which is added at this point. Two months later, the 50 % N urea complement is added to the OF plots. Finally, four to five months after harvest, the split application of urea is added to each of the different plots.

3.2.3 Nitrogen mass of sugarcane biomass compartments subject to different fertiliser types

The protocol followed in determining the N mass of the different sugarcane biomass compartments was the same as that of Chapter 2's Material and Methods (Section 2.2.6). The single difference is that in Chapter 2, the only fertiliser treatments used were urea and the unfertilised treatments. In this chapter, the two organic fertilisers pig slurry and sewage sludge are also used, and the method of N mass evaluation is the same as that of urea and the unfertilised treatments.

3.2.4 ^{15}N labelling of N sources

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 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.

Within each experimental plot, three microplots of 2.25 m^2 (1.5 m x 1.5 m) received labelled urea (3 atom % ^{15}N excess) in the same quantity as the conventional non-labelled urea. It was therefore applied homogeneously across the microplots at the same time as the application of the unlabelled fertilisers.

In each of the organic fertiliser plots, LP and BA, there were three "exclusion subplots", subject to the same urea fertiliser application as that of the T urea plot, and without OF fertiliser application. Over the first experimental year, a split application of urea was applied to the 100 % urea subplots at the same time as in the T plot, i.e. at October 2017 and the second application in March 2018.

Over the second experimental year, the split application of urea was applied to the 100 % urea subplots 2 months later than the first, at the same time as the mixed organic-^{15}N labelled urea microplots, i.e. December 2018 and the second application in February 2019.

^{15}N enriched mulch was placed in three 2 m x 1.5 m unfertilised subplots at the start of the first experimental year (after harvest in October 2017). The existing mulch after harvest was removed from these subplots and replaced with the ^{15}N mulch which excluded the cane stalks. The location in the experimental site was the "NF" fertiliser exclusion subplots in the urea plot T (Figure 7 in the Experimental Site section). At the start of the second experimental year (October 2018), ^{15}N enriched mulch was place in an adjacent unfertilised plot. The ^{15}N enriched mulch was prepared at end of each ratoon by harvesting the ^{15}N fertiliser enriched microplots. The sugarcane stems were kept apart, and the rest of the aboveground biomass (green tops, leaves and straw) were ground (using the apparatus described in Chapter 2 Material and Methods) and mixed thoroughly by hand. This was divided in three for each subplot and a subsample was taken from each of the three repetitions to dose the ^{15}N content (as explained in more detail in Chapter 2).

3.2.5 Contribution of different sources to sugarcane N nutrition and nitrogen use efficiency

The sources of N were studied using ^{15}N enriched 2 m x 1.5 m microplots (Figure 7, Experimental Site). There were three microplots per treatment in which ^{15}N representative leaves were sampled monthly to determine the contribution of sources to sugarcane N. The ^{15}N representative leaves harvested were the first leaves below the top visible dewlap of the sugarcane (as determined in Chapter 1 and valourised as a paper by Poultney et al. 2020).

Nitrogen derived from fertiliser (*NdFf*) and mulch (*NdFm*) were determined, using ^{15}N-labeled urea and ^{15}N-labeled mulch, using the formula:

$$NdFf \text{ or } NdFm = \left[\frac{a-b}{c-d}\right].100 \quad (1)$$

where *NdFf* and *NdFm* are the proportion of N in the plant derived from fertiliser and mulch respectively (%), *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 urea or mulch, and *d* is the natural abundance of ^{15}N atoms of a standard (0.366%).

Nitrogen derived from soil (*NdFsoil*) was deduced by the formula:

$$NdFsoil = 100 - NdFf_{100} - NdFm \quad (2)$$

$NdFf_{100}$ was determined from 100% urea microplots in each treatment plot.

Nitrogen derived from the OFs pig slurry and sewage sludge (NdF_{OF}) was deduced, from the mixed fertiliser microplots receiving both ^{15}N-labeled urea and unlabelled OF, by the formula:

$$NdF_{OF} = 100 - NdFf_{comp} - NdFsoil - NdFm \quad (3)$$

where $NdFf_{comp}$ is determined for a complementary urea application in mixed fertiliser microplots from equation 1, *NdFsoil* is determined from 100 % urea microplots in the same plot according to equation (2), and *NdFm* from equation (1).

3.2.6 Nitrogen Recovery Efficiency of different N sources

Nitrogen recovery of a given source in the plant biomass was calculated by the formula:

$$NRE = \frac{NdFx * N_{plant}}{N_{fertiliser}} \quad (4)$$

where *NRE* is the recovery efficiency of a N source in the plant (%), *NdFx* is the proportion of N in the plant derived from the respective N source, N_{plant} is the amount of N in the plant (gN/m^2) and $N_{fertiliser}$ is the quantity of N applied with the fertiliser (gN/m^2).

When the NRE is calculated for the different sugarcane biomass compartments, *i.e.* aboveground biomass, aerienne biomass (with the tiller and strawfall compartments considered), and the belowground biomass compartment), the ^{15}N content was assumed to be homogenously distributed in the plant. The root biomass was therefore considered to have the same ^{15}N content as the aboveground biomass.

The NRE values were plotted against time (months after harvest), using the "Loess smoothing" function of the package *ggplot2* in R version 3.3.2. software (R Development Core Team, 2016).

3.3 Results

3.3.1 Nitrogen mass of sugarcane compartments subject to mineral and organic fertilisation

There was a decrease in the sugarcane N mass when the tiller compartment was considered, which peaks approximately midway over the growth-cycle, although this effect was more pronounced over the first year than the second (Table 3.1). When the strawfall compartment was considered, there was a gradual increase in the N mass from 6 and 7 months to the end of the growth-cycle over the first and second experimental years, respectively. The impact of this N mass compartment was again far more pronounced over the first experimental year than the second.

For the urea treatment, the N mass reached a plateau at 4 months and 6 months after the start of the ratoon, for the first and second experimental years, respectively, with respective peak values of 125.4 $kg.N.ha^{-1}$ and 100.8 $kg.N.ha^{-1}$. When the stalk compartment was considered (*i.e.* the tiller compartment removed), there was an average decrease of 12.5 $kg.N.ha^{-1}$ over the first year, but of only 2.3 $kg.N.ha^{-1}$ over the second year. When the strawfall compartment was considered, there was a maximum accumulative increase at the end of the ratoon of 24.3 $kg.N.ha^{-1}$ for the first year, but of only 9.9 $kg.N.ha^{-1}$ for the second year. When the root compartment was considered, there

was a very large increase in N mass, with an average increase of 58.3 kg.N.ha^{-1} and 51.0 kg.N.ha^{-1} over the first and second years, respectively, which resulted in an increase in N mass, from the initial aboveground biomass, by a factor of 1.6 and 1.8 for the first and second years, respectively.

The pig slurry N mass also began to reach a plateau at 4 and 6 months after the start of the ratoon, for the first and second years, respectively. The maximum N mass reached was slightly higher than that of the urea treatment, and was substantially higher over the first experimental year than the second, with values of 144.7 kgN.ha^{-1} and 103.4 kg.N.ha^{-1} over the two respective years. When only the stalks were considered, there was an average decrease of 19.6 kg.N.ha^{-1} and only 1.5 kg.N.ha^{-1} over the first and second years. When the root compartment was considered, again there was a very large increase in N mass (but slightly lower than the urea treatment), with an average increase of 43.9 kg.N.ha^{-1} and 50.8 kg.N.ha^{-1} over the first and second years, respectively, corresponding to an average increase in N mass by a magnitude of 1.4 and 1.8 over the two respective growth-cycles.

The sewage sludge N mass also begins to reach a plateau at 4 and 6 months after the start of the ratoon, for the first and second years, respectively. The maximum N mass reached was slightly higher than that of the urea treatment, with values of 132.1 kg.N.ha^{-1} and 109.0 kg.N.ha^{-1} over the first and second experimental years, respectively. When only the stalks were considered (i.e. the tiller compartment removed), there was an average decrease of 17.5 kg.N.ha^{-1} and 6.9 kg.N.ha^{-1} over the first and second years. When the root compartment is considered, again there was a very large increase in N mass (but slightly lower than the urea treatment), with an average increase of 40.1 kg.N.ha^{-1} and 44.5 kg.N.ha^{-1} over the first and second years, respectively, corresponding to an average increase in N mass by a magnitude of 1.4 and 1.6 over the two respective growth-cycles.

Table 3.1 Nitrogen mass for the different biomass compartments for the months after harvest (MAH) over the first and second experimental years, subject to the different fertiliser treatment types.

Year	Compartment	N mass at (kgN.ha-1)									
		3 MAH	4 MAH	5 MAH	6 MAH	7 MAH	8 MAH	9 MAH	10 MAH	11 MAH	12 MAH
Unfertilised											
Year 1	Aboveground	41.2	54.5	58.3	58.8	57.5	57.1	55.0	54.8	53.4	54.5
	Stalks	39.5	52.4	56.3	56.4	56.5	56.7	54.6	54.6	53.4	54.5
	Aerienne	41.2	54.5	58.3	60.1	62.6	64.8	64.4	66.7	67.5	71.0
	Total	101.7		118.8			115.6				127.5
	Total + stools	-	-	-	-	-	-	-	-	-	-
Year 2	Aboveground	25.1	41.5	50.3	63.8	64.6	63.6	63.3	62.8	61.1	60.1
	Stalks	23.9	39.8	49.1	62.2	62.5	62.9	62.9	62.2	61.1	60.1
	Aerienne	25.1	41.5	50.3	64.3	66.4	67.7	69.0	70.4	70.5	71.3
	Total	68.0		115.9			122.8				132.6
	Total + stools	73.8		124.8			130.2				140.8
Urea											
Year 1	Aboveground	103.1	121.6	125.4	123.3	122.1	118.4	117.9	116.0	115.0	110.9
	Stalks	81.1	98.2	104.8	104.4	110.0	107.2	105.6	113.1	113.1	110.8
	Aerienne	103.1	121.6	125.4	126.4	132.4	131.3	133.8	134.9	137.6	135.2
	Total	164.8		187.2			183.2				192.8
	Total + stools	-	-	-	-	-	-	-	-	-	-
Year 2	Aboveground	30.2	66.0	81.4	99.9	100.8	99.1	98.3	98.7	97.6	97.4
	Stalks	28.0	62.7	78.6	94.3	97.0	96.7	96.8	98.0	97.4	97.4
	Aerienne	30.2	66.0	81.4	101.3	103.5	103.1	103.5	106.0	106.2	107.3
	Total	69.1		140.9			153.1				162.8
	Total + stools	73.5		147.6			158.8				169.1

Year	Compartment	N mass at (kgN.ha-1)									
		3 MAH	4 MAH	5 MAH	6 MAH	7 MAH	8 MAH	9 MAH	10 MAH	11 MAH	12 MAH
Pig slurry											
Year 1	Aboveground	103.9	122.2	136.4	144.6	144.7	144.6	144.4	144.2	143.8	143.3
	Stalks	78.5	94.2	102.6	113.4	114.0	125.3	129.9	135.3	139.2	143.3
	Aerienne	103.9	122.2	136.4	150.6	157.7	161.9	164.4	167.2	171.1	172.2
	Total	150.4		182.9			201.0				215.6
	Total + stools	-	-	-	-	-	-	-	-	-	-
Year 2	Aboveground	29.7	55.5	76.3	100.0	103.4	102.9	102.0	100.7	100.1	98.8
	Stalks	27.2	53.3	74.2	97.1	101.4	101.5	101.4	100.4	99.5	98.8
	Aerienne	29.7	55.5	76.3	101.0	106.3	107.5	108.2	108.9	109.5	109.4
	Total	68.4		135.6			157.3				164.8
	Total + stools	-	-	-	-	-	-	-	-	-	-
Sewage	sludge										
Year 1	Aboveground	96.6	125.6	131.8	131.8	132.1	130.7	131.1	130.0	128.7	127.9
	Stalks	73.4	93.1	99.0	103.7	108.2	114.9	122.1	123.2	125.9	127.9
	Aerienne	96.6	125.6	131.8	135.9	143.1	148.0	153.0	154.7	156.4	158.0
	Total	137.0		172.3			181.9				203.8
	Total + stools	-	-	-	-	-	-	-	-	-	-
Year 2	Aboveground	41.6	82.1	103.4	109.0	102.8	100.6	97.9	97.4	97.5	97.2
	Stalks	34.9	72.4	91.7	96.1	92.9	94.1	92.8	94.0	94.8	97.2
	Aerienne	41.6	82.1	103.4	111.0	106.9	107.1	106.9	109.0	110.3	111.4
	Total	75.5		155.3			150.8				159.8
	Total + stools	-	-	-	-	-	-	-	-	-	-

3.3.2 Nitrogen derived from source

The greatest contribution of N across treatments and over the 2 experimental years, was the "rest" compartment, which had a contribution of at least 74 % for each scenario (Figure 3.1).

The total N mass was relatively constant from 3 months after the start of the ratoon until harvest over the first experimental year, but only reached this plateau of total N mass at 7 months after the start of the ratoon, over the second experimental year.

In the urea plot (T), there was a maximum NdFf of 30.3 % at 6 months after the start of the ratoon, with an average NdFf of 16.7 % over the growth-cycle. Over the second year, the urea reached a maximum of 28.7 % of the sugarcane N content at 6 months, with an average NdFf of 17.2 % over the second year's growth cycle. The "rest" compartment had the highest contribution to the sugarcane N content, with average contributions of 83.3 % over the first year and 82.8 % over the second year.

In the pig slurry plot (LP), the mixed pig slurry-urea complement treatment had a higher initial contribution from pig slurry than from urea over the first year from 3 to 6 months, but the balance shifts to urea being the dominant fertiliser N supply after the second urea application.

The pig slurry fertiliser had average contributions of 7.1 % and 4.4 % to the sugarcane N content over the first and second years, respectively. The maximum contribution of the pig slurry fertiliser was 14.9 % at 6 months after the start of the first year, and 7.9 % at 8 months after the start of the second year.

The average sugarcane N content derived from the urea (50 % N) complement, was 9.6 % over the first year, and 17.8 % over the second year. The "rest" compartment had the highest contribution, with an average of 83.3 % of the plant N content over the first year, and an average of 77.8 % over the second year.

In the sewage sludge plot (BA), the mixed urea-sewage sludge treatment had a higher initial contribution to the sugarcane N nutrition from the sewage sludge and after the second application of urea, there is a higher contribution of urea. Over the second year, there is a consistently higher contribution from urea than sewage sludge to the N content over the growth-cycle.

The average contribution of the sewage sludge fertiliser to sugarcane N was 6.2 % over the first year, and 5.5 % over the second year. The treatment's maximum contribution over the growth-cycle was 11.2 % at 4 months after the start of the first year, and 9 % at 5 months after the start of the second year. The urea complement contributed an average of 10.5 % and 16.8 % over the first, and second years, respectively.

Again, the "rest" compartment had the highest contribution to the sugarcane N content, with an average contribution of 83.3 % over the first year, and 78.1 % over the second year.

The other organic matter compartments (apart from soil) evaluated had a relatively low but constant contribution to the sugarcane N content. The mulch had average contributions of 3.8 % and 5.5 % over the first and second years, respectively. The residual urea contributed an average of 3.5 % over the first experimental year. The average residual mulch contribution was 3.6 % over the second year.

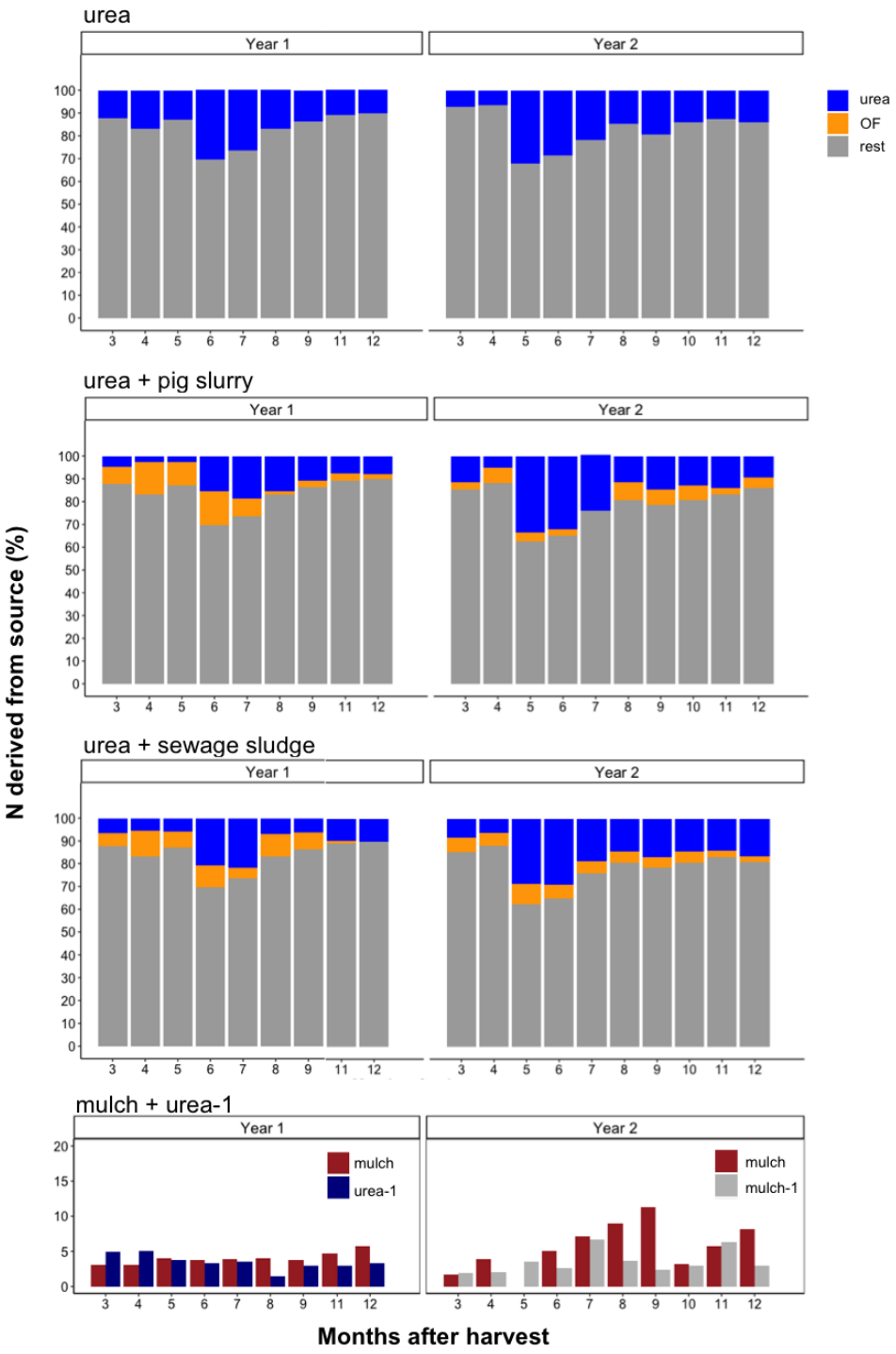

Figure 3.1 *Nitrogen derived from source (in units of percentage sugarcane N, %) over the two experimental years for the three different treatment plots: urea, pig slurry and sewage sludge. The biomass compartments considered were urea, OF (organic fertiliser, being pig slurry and sewage sludge respectively), urea-1 (from the previous year's application), mulch, mulch-1 (from the previous year's application), and soil.*

3.3.3 Nitrogen use efficiency

The NRE was highest for urea over the two experimental years, and when the often overlooked sugarcane biomass compartments were considered (Figure 3.2). The two organic fertilisers had NRE values between that of urea and the unfertilised treatment. The sewage sludge had a slightly higher NRE than the pig slurry over the first year (the pig slurry decreases close to 0 % at 8-9 months after the start of the ratoon). Over the second year, the NRE values of the two organic fertilisers were similar and converged at 7 months after the start of the ratoon.

Urea had an average NRE of 16.1 % and a maximum NRE of 26.1 % at 6 months over the first year, which was lower over the second year with an average NRE of 9.2 % and a maximum NRE of 16.7 % at 6 months after harvest. When the tiller compartment was considered, urea NRE decreased to an average of 13.9 % over the first year, and 7.8 % over the second year. When the strawfall compartment was considered, the average NRE increases again to an average of 14.9 % over the first year and 8.1 % over the second year. When the root compartment was considered, the NRE increased substantially to an average of 27.4 % with a maximum value at 6 months of 41.4 % over the first year, and an average of 14.6 % and a maximum NRE of 24.5 % at 6 months over the second year.

Pig slurry had an average NRE of 4.3 % and a maximum NRE of 10.5 % at 7 months over the first year, with similar values over the second year with an average NRE of 3.6 % and a maximum NRE of 5.2 % at 5 months after harvest. When the tiller compartment was considered, the pig slurry NRE decreased to an average of 3.4 % over the first year, and very slightly to an average of 3.5 % over the second year. When the strawfall compartment was considered, the average NRE increases again slightly to an average of 3.7 % over the first year and 3.6 % over the second year.

When the root compartment was considered, the NRE increased substantially to an average of 6.7 % with a maximum value at 7 months of 16.4 % over the first year, and an average of 6.4 % and a maximum NRE of 9.4 % at 5 months over the second year.

Sewage sludge had an average NRE of 6.7 % and a maximum NRE of 12.2 % at 8 months over the first year, with lower values over the second year with an average NRE of 4.4 % and a maximum NRE of 8.5 % at 5 months after harvest. When the tiller compartment was considered, the sewage sludge NRE decreased to an average of 5.6 % over the first year, and to an average of 3.6 % over the second year. When the strawfall compartment was considered, the average NRE increased again slightly to an average of 6.0 % over the first year and 3.8 % over the second year.

When the root compartment was considered, the NRE increased substantially to an average of 11.0 % with a maximum value at 8 months of 21.0 % over the first year, and an average of 6.8 % and a maximum NRE of 12.2 % at 5 months over the second year.

The other sugarcane system N components, being the mulch, residual mulch application, residual urea application and the soil component, mostly had similar NRE values over the two years. The mulch had an average NRE of 4.5 % and 10.3 % over the first and second years, respectively. The residual mulch had an NRE of 3.8 % over the second year. The soil component had an average NRE of 3.1 % and 2.0% over the first and second years, respectively.

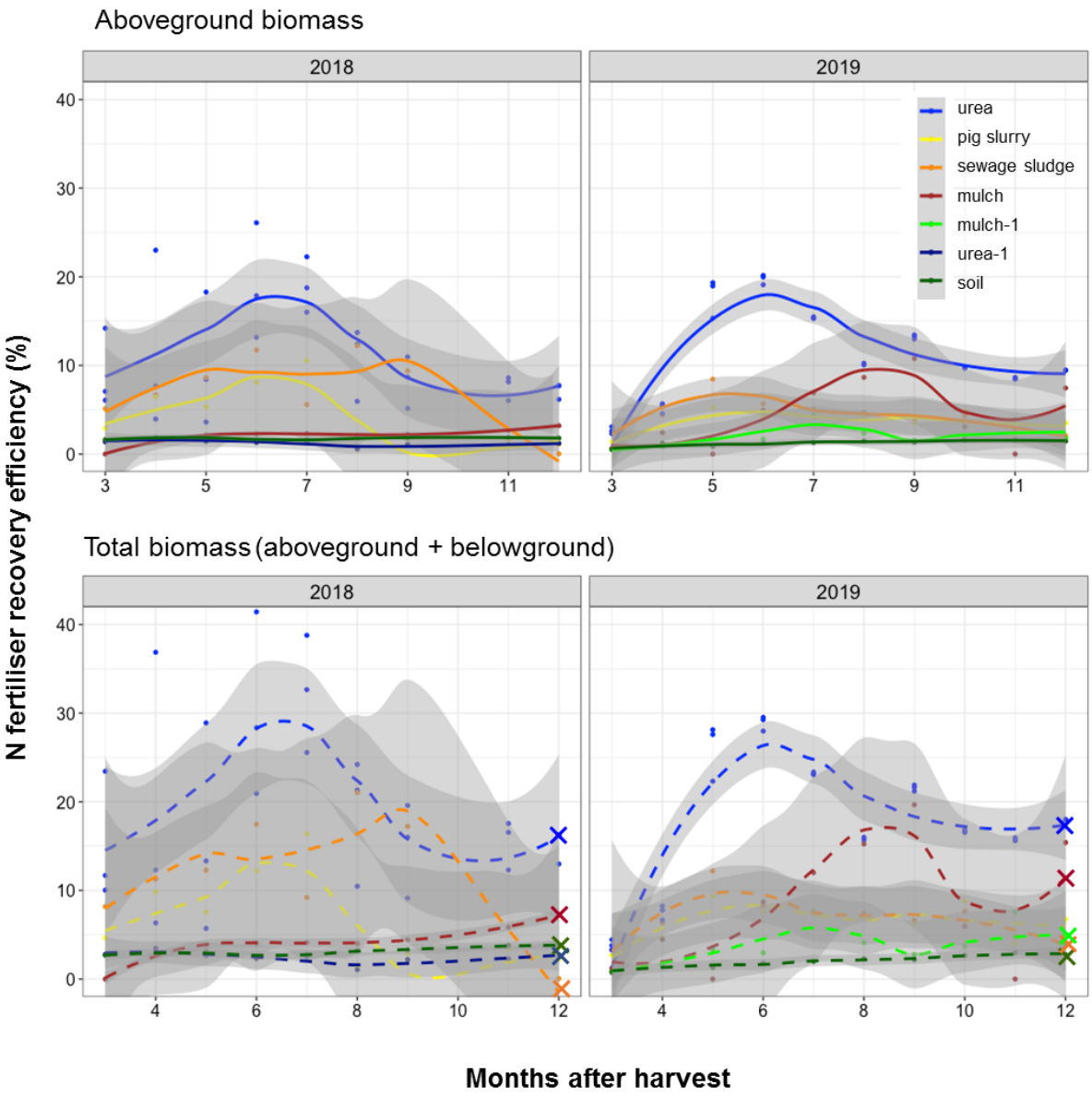

Figure 3.2 *Nitrogen recovery efficiency over the two experimental years for the different fertiliser treatments: urea, pig slurry, sewage sludge mulch, mulch-1 (from the previous year's application), urea-1 (from the previous year's application) and soil.*

3.4 Discussion

3.4.1 Computing sugarcane N nutrition considering different biomass compartments

The N mass accumulation has a similar pattern across fertiliser treatments and over the two experimental years. This is reassuring since the fertilisation design of the Soere-PRO experimental site is such that the sugarcane nutrient requirements are met optimally. As explained in the Materials and Methods section, organic fertiliser N doses were determined using equivalent mineral efficiency units of N.

For these different fertiliser treatment types, the sugarcane N mass increases over the first few months of the ratoon, after which the N mass reaches a plateau, signalling the end of the active N uptake period. This occurs early on over the first experimental year, already at 4 months after the start of the ratoon for the mineral treatment and for sewage sludge, and slightly later, at 6 months for the pig slurry. The active N uptake period continues for longer until 6 months after the start of the ratoon over the second year, for all of the fertiliser treatments.

This is in a timeframe coherent with studies such as that of Wood et al. (1996) and Franco et al. (2011), where N mass accumulation (for mineral fertilisers) reached a plateau at 5 and 6 months after the start of the ratoon, respectively. Franco et al. (2011), proposed that the active N uptake period at early stages of growth development could be linked to root system development, where the increase in N accumulation in aboveground biomass is related to large increases in root expansion over this period. This is coherent with what was found in our parallel study, in the Appendix Chapter published by Versini et al. (2020), as well studies such as as Otto et al. (2009). Franco et al. (2011), suggest that the expansion of roots during these developmental phases of the sugarcane could have favoured the absorption of N from the soil profile, and increased the amount of N accumulated in the aboveground (and in our study, belowground) biomass. One reason for why the sugarcane N mass reaches a plateau, and the N active uptake period ends, is potentially due to fertiliser N being immobilized in the soil, rendering part of the fertiliser N unavailable to the plants (Franco et al. 2011).

The difference between years in our study, however, is that there is a higher total N mass accumulation over the first experimental year than the second, and this occurs across fertiliser treatments. It is primarily a lower biomass (rather than the sugarcane N concentration which is relatively constant) over the second experimental year which results in the lower N mass over the second year, particularly over the first 6 months of crop growth. The reason for this lower biomass over the growth-season was likely for the two reasons mentioned in Chapter 2 when only the urea and unfertilised treatments were considered: during harvest, the harvester cut the sugarcane very low at the start of the second year, and there was a lag-phase in the sprouting of the sugarcane.

Secondly, early on over the growth-cycle of the second year (1-2 months after harvest), the sugarcane was infested by caterpillars, which impacted the sugarcane growth. This subsided, however, and the sugarcane returned to regular health, but with a lag phase in its biomass growth and plant development over the growth-cycle.

When the senescence of tillers is taken into account over the growth-cycle, there is a decrease in the N mass over the growth-cycle across fertiliser treatment types, and this decrease peaks approximately mid-way through the growth-cycle. There appeared to be far less tiller mortality over the second year, however, and the consideration of this compartment had far less of an impact on the sugarcane aboveground N mass, for the different treatment types. The peak tiller mortality around mid-growth cycle is coherent with studies of Bell & Garside (2005) and Singels & Smit (2009) for mineral fertilisers.

Taking into consideration the strawfall compartment resulted in a slight increase in the overall aboveground N mass, midway over the growth-cycle onwards, across fertiliser treatment types. Far less straw fell to the soil over the second year, and therefore the strawfall compartment again had less of an impact on the total N mass again for each of the different types of fertilisers.

It appears that since there was a large lag-effect in biomass and N mass accumulation over the second experimental year, the sugarcane system had more of a "conservative" functioning over the second year, with a tendency to retain its overall N mass through low tiller senescence and very little in terms of leaves falling from the plant and constituting the strawfall compartment. This is likely related to the fact that there was more perturbation to the sugarcane system at the start of the second year, which initially affected the growth and development of the sugarcane, before the crop returned to regular health. Although quite distantly related, the leaf lifespan of evergreen canopies has been shown to depend on the water availability of the previous season, and when preceding years have been water-limiting, trees retain a higher proportion of their leaves the next year (Limousin et al. 2012). Perhaps in a similar manner, if the sugarcane development has been perturbed, this more "conservative" functioning, where fewer leaves and tillers are lost, occurs.

The belowground biomass had a large impact on the N mass across treatments and over the two experimental years, increasing the N mass by a factor of between 1.4 and 1.8 across treatment types and over the two experimental years. The N mass increased substantially over the whole growth-cycle across fertiliser treatments, which was the case over both experimental years. This further substantiates the proposition in the Appendix Chapter 7.2 (Versini et al. 2020), that the "root system of sugarcane appeared to be a major pool of N that should be considered in studies dealing with fertiliser N use efficiency and N cycling in sugarcane agroecosystems." However, when the stools were considered for the urea and unfertilised treatments at the end of the second experimental year, there was only a very slight increase in the total sugarcane N mass (between 7

and 8 kgN.ha^{-1} for the two treatment types). This increase was minimal in comparison to the very large increase in sugarcane N mass, as a result of incorporating the root N mass.

3.4.2 Sources of nitrogen for sugarcane nutrition

The largest N contribution to sugarcane N nutrition was from the soil, across the different treatment types. This has been classified as "rest" in the Results section (Figure 3.1) since there are various N biomass pools that were considered: the mineraliszation of soil organic matter (likely the major source of soil N), mulch decomposition, decomposition of old cane roots, or internal N translocation of endophytic biological N fixation for example. The mulch compartment, studied in another experimental plot, contributed 5-10 % of the sugarcane N over the two experimental years, and a similar range of values is likely to apply to the OF plots. The mulch contribution was small, but nevertheless provided a regular N supply over the growth-cycle, for the two years. This was a similar value to that of Meier et al. (2006), who found that approximately 6-7 % of the N in the sugarcane aboveground biomass was derived from mulch in wet tropics of Australia.

Over the second year, there is a residual mulch N contribution over the second year, which is in a similar range to that of the first year, corroborating the idea that the mulch is a slow-release source that still has a contribution in subsequent sugarcane cycles. This has also been suggested in studies such as that of Meier et al. (2006) and Trivelin et al. (2013). The residual effect in the study by Meier et al. (2006) was, however, slightly lower than in our study, with a contribution of 2.1 %, compared to 3.6 % in our study, to the sugarcane leaf N over their second experimental year.

The residual urea appears to have had a similar nutritional role to that of the residual mulch (this was only studied over the first experimental year), and has a low (3-5 % of sugarcane N) but constant contribution to the sugarcane N content.

A major contribution of at least 70 % is therefore from the soil compartment (between 74 % and 85 % across treatments and over the two experimental years). This is coherent with studies such as that of Dourado-Neto et al. (2010) that found an average of 79% of N originated from soil in a diverse range of N-fertilised crop agroecosystems; and that of Stevens et al. (2005), where soil-N accounted for 54-83 % of total plant-N.

The mineral and organic fertiliser contributions were substantial sources of N for the sugarcane over each growth-cycle, even if their contributions were far lower than the soil compartment. The mineral fertiliser contribution had average values of 16.7 % and 17 %, and peak values of 30.3 % and 29 % over the two respective years. This is coherent (but slightly lower) than values for sugarcane observed by Franco et al. (2011), which (for ammonium sulfate) had an average contribution of approximately 37 % over the growth cycle. The contribution of ^{15}N fertilisers were particularly high initially in their study, with a range of 70 % during initial growth stages,

decreasing to 30 % at harvest for the ratoon crop, and ranging from 40 % at initial stages to 10 % at harvest for the plant cane. Vieira-Megda et al. (2015), found mineral fertiliser to contribute 60 % of the sugarcane N content at initial growth stages, which decreased to 20 % close to harvest.

When the mixed OF-mineral fertiliser plots were evaluated, the mineral fertiliser (i.e. urea complement) contributions to sugarcane N content were higher than the OF N contribution from mid-growth-cycle onwards, which was due to the second split-application dose of urea applied 4-5 months after the start of the ratoon. It is after the second application that the N nutrition balance tips in favour of the mineral over OF fertilisers. The sewage sludge had an average contribution to the sugarcane N content of 5.5 – 6.2 % with a maximum contribution of 9 – 11.2 %. The pig slurry had an average contribution of 4.4 – 7.1 % to the sugarcane N content with a maximum contribution of 7.9 – 14.9 %. This is in a similar range to a study by Chantigny et al. (2004), on maize, where pig slurry was found to contribute an average of 11 – 15 % of the N content of the crop.

3.4.3 Nitrogen use efficiency of different N sources

The NRE of urea ranged between 6.7 % and 26.1 % over the two years, with respective NRE averages of 9.2 % and 16.1 % for the first and second years. This was coherent with values from an earlier ratoon in the same site, which had an NRE of 12-19 % over the crop growth-cycle (Chapter 1). This is also coherent with NRE values of sugarcane in several other studies, where the NRE of urea in sugarcane is typically between 5 % and 40 % (Basanta et al. 2003, Fortes et al. 2010, Meier et al. 2006).

The pig slurry treatment had a lower NRE than urea, ranging from 0.9 – 10.5 % over the two years, with an average NRE of 4.3 % and 3.6 % over the first and second years. This was far lower than a study by Chantigny et al. (2004), where maize was fertilised with pig slurry, which found an NRE of 29 % in a clay soil at the end of the crop cycle. However, NRE values depend strongly on crop type, the amount of N applied, and the type of fertiliser application management practice (e.g. surface-applied vs incorporated) (Chantigny et al. 2004).

The sewage sludge treatment had an NRE ranging between 1.1 % and 12.2 % over the growth-cycle of the two years, with an average NRE of 6.7 % and 4.4 % over the first and second years. This is in a similar range to studies such as the long-term study of Börjesson & Kätterer (2018), which was determined using the difference method for wheat, sugar beet, barley and oats, and which ranged between 3-8 %. The sewage sludge NRE values in our study were also in a comparable range to a study by Gomez-Munoz et al. (2017), which found an NRE using the difference method (referred to in their study as an “apparent N uptake efficiency”), of 11 % for sewage sludge after the first year of application; and 7 % for a higher dose application. N from many organic fertilisers often shows little effect on crop growth in the year of application, because of the slow-release characteristics of organically bound N (Gutser et al., 2005).

The short-term N availability of organic materials depends largely on the N content of organic substances, as well as the mineral N content of the fertiliser applied (Gutser et al., 2005). For example, high mineral-N contents usually lead to good short-term N availability (Gutser et al., 2005). It is therefore likely that this effect would be more pronounced in the sewage sludge than the pig slurry, since their compositions are very different. The majority of N in the pig slurry fertiliser is in a mineral form (57 % of what was applied the first year and 75 % the second year), and the majority (90 %) of sewage sludge is organic N. There is therefore a lower proportion of sewage sludge N in a form immediately available to plants, but with a higher potential for N to become available in subsequent growth cycles after mineralization.

The mulch recovery efficiency in our study was on average 2.3 % for the first experimental year, and 4.5 % for the second. This is in a range coherent with studies evaluating mulch N recovery efficiency such as Basanta et al. (2003) with an average recovery of 3.1 %; Meier et al. (2006) with average recoveries of 2-4 % for surface-applied mulch, Dourado-Neto et al. (2010) with average recoveries ranging between 0.6 % and 3.7 % over the four-year period; and Fortes et al. (2013) with recoveries ranging between 1.8 % and 5.4 % (without N fertiliser application).

In our study, when the belowground biomass is considered, the mulch-NRE increases to 4.6 % over the first year and 8.3 % over the second year. A rare other study which considered mulch-NRE of sugarcane aboveground biomass *and* roots, was that of Meier et al. (2006), which proposed a hypothetical rather than experimental situation of incorporating roots. Their hypothesis was that 30 % of plant N to be in the roots (according to Dillewjin 1952), and estimated the mulch NRE to increase to 3-6 % when the root N mass is also considered, which is in a similar range to our study when roots are considered.

When the tiller and strawfall biomass compartment are considered, there is a slight "smoothing" of the NRE pattern over the two growth-cycles. Generally speaking, there is a peak in the NRE around mid-growth cycle, and a tendency to decrease towards the end of the crop growth-cycle (Ng Kee Kwong & Deville, 1994 and Poultney et al. 2020). When the tiller compartment is considered, there is a slight decrease in the NRE at mid-growth-cycle, which flattens the peak slightly. When the strawfall compartment is considered, there is a slight increase in the NRE towards the end of the growth-cycle. The consideration of these two compartments therefore evens out the NRE tendency slightly over the crop growth-cycle. This is as a result of the changes in N mass evolution when considering these biomass compartments, since the ^{15}N signal is assumed to be distributed homogenously in the sugarcane plant.

When the belowground biomass compartment is considered, the NRE almost doubles over the two years. This is again due to the N mass almost doubling when the biomass and N content of the belowground biomass is considered. The consideration of the belowground biomass

compartment therefore changes the interpretation of the NRE quite substantially, and could be important to consider for a more global vision of the fate of N from mineral and organic fertilisers, as well as other sources of N.

3.4.4 Conclusion

A holistic vision of sugarcane N nutrition, and the various sources of N and their relative contributions, could be a better approach to synchronising crop requirements with the level of N fertiliser application. The form of N-fertiliser application also appears to be important, and a combination of organic and mineral fertilisers may be an effective approach, in that mineral fertilisers have a higher initial N use efficiency and can be used to plan the supply of nutrients to the plant as and when necessary. Organic fertilisers (especially the sewage sludge with a high organic N content) may have a lower initial NUE but have a higher capacity for N immobilisation and is likely to become available during subsequent crop cycles and have a higher contribution to soil fertility (Börjesson & Kätterer, 2018). A longer-term experiment is likely to give greater insight into the contribution to sugarcane and soil N nutrition, and an evaluation of N balance in the soil-sugarcane system will be an effective means of weighing up the advantages of the different N fertilisers and their relative contributions to sugarcane N nutrition, as well as the disadvantages (and their relative extents) in terms of potential N losses from the sugarcane-soil system.

CHAPTER FOUR

Abstract

The aim of this chapter was to monitor the leaching transfers of N at different soil depths in agroecosystems treated with mineral or organic fertilisers as compared to unfertilised sugarcane over the two-year period, and to evaluate the quantity of N lost from the soil-sugarcane system via leaching.

There was a distinct pattern in N quantities in solution close to the surface at a soil depth of 10 cm, and lower down at a soil depth of 40 cm, where N in solution increased rapidly after the initial fertiliser application, which occurred directly after harvest. This occurred in a similar manner over both experimental years. Over the first year, there was little impact on N in solution at the different soil depths after the subsequent fertiliser applications, likely due to the fact that the sugarcane biomass and N mass was sufficiently developed at these points in time to absorb and use the mineral applied directly. Over the second year, the N in solution increased at the different soil depths after subsequent fertiliser applications since the sugarcane biomass was insufficiently developed over the initial few months after the start of the ratoon to effectively take up the N in solution in the soil at this point (due to a lag in growth, see Chapter 2).

Over the first experimental year, N in solution was negligible (less than 1 % of fertiliser N applied, across treatment types). Over the second experimental year, there was a higher level of N loss via leaching, with accumulated N losses of 7.8, 18.3, 10.2, and 17.9 $kgN.ha^{-1}$ for the unfertilised, urea, pig slurry and sewage sludge treatments, respectively.

It is likely for this reason that there was a higher level of leaching over the second experimental year for the different fertiliser treatments, whereas leaching was almost negligible for the different treatments over the first year. This suggests that in addition to variables such as soil type, the extent and timing of drainage, attention should be paid to the timing and doses of different fertiliser N applied with respect to the stage of growth of the crop and its root development.

Resumé

L'objectif de ce chapitre était de suivre, au cours des deux années de l'étude, les transferts de N par lixiviation à différentes profondeurs du sol d'une culture de canne à sucre fertilisée avec des engrais minéraux ou organiques, par rapport à la canne à sucre non fertilisée et d'évaluer la quantité de N perdue par lixiviation.

Nous avons observé une tendance distincte dans les quantités d'azote en solution près de la surface à une profondeur de 10 cm, et plus bas à une profondeur de 40 cm, où l'azote en solution augmente rapidement après l'application initiale d'engrais, qui a lieu directement après la récolte. Cela se produit de manière similaire au cours des deux années expérimentales. Au cours de la première année, il y a peu d'impact sur l'azote en solution aux différentes profondeurs du sol après les applications d'engrais précédents, probablement parce que la biomasse de la canne à sucre est suffisamment développée à ces moments pour absorber et utiliser l'azote minéral appliqué directement. Au cours de la deuxième année, l'azote en solution augmente aux différentes profondeurs du sol après les applications d'engrais ultérieures, car la biomasse de la canne à sucre n'est pas suffisamment développée pour un prélèvement efficace d'azote en solution dans le sol (en raison d'un retard de croissance, voir le Chapitre 2).

Au cours de la première année expérimentale, l'azote en solution était négligeable (moins de 1 % de l'azote des fertilisants appliqués, pour tous les traitements). Au cours de la deuxième année expérimentale, les pertes d'azote par lixiviation ont été plus élevées, avec des pertes cumulées de 7,8, 18,3, 10,2 et 17,9 kgN.ha-1 pour les traitements non fertilisés, urée, lisier de porc et boues d'épuration, respectivement.

C'est probablement pour cette raison qu'il y a un niveau de lessivage plus élevé au cours de la deuxième année expérimentale pour les différents traitements d'engrais, alors que les pertes par lixiviation sont presque négligeables pour les différents traitements au cours de la première année. Cela suggère qu'en plus de variables telles que le type de sol, le niveau et le moment du drainage, une attention particulière doit être apportée à la période d'apport et aux doses des différents engrais N appliqués en fonction du stade de croissance de la culture et de son développement racinaire.

Chapter 4. Soil solution nitrogen transfers in mulched sugarcane ecosystems fertilised with mineral and organic fertilisers

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4.1 Introduction

Nitrogen is essential to crop growth and development, and fertiliser N input has an important contribution to crop productivity. As has been illustrated in the previous chapters, mineral and/or organic fertiliser (OF) N clearly plays an important nutritive role in sugarcane production systems. In addition, sugarcane production systems have a tendency for high fertiliser N input, often with a low N-fertiliser recovery efficiency (Robinson et al. 2013). When applied in excess, one of the potentially important pathways of N loss is via leaching from the crop-soil system. Leaching is the transfer of ions in a soluble form, combined with the movement of water, which transports these ions to different regions of the soil profile and is essentially the proportion of ions which are removed in solution from the soil (SSSA, 2008, Ghiberto et al. 2009). Nutrients are lost via leaching when these ions in solution are transferred below the crop sub-root zone (Benoit et al. 2016). This leached N risks entering groundwater, where it is a contaminant. This is problematic from an agronomic point of view, in terms of loss of fertiliser N applied, as well as from an environmental point of view, especially with respect to the risk of groundwater contamination. Nitrate is considered to be one of the most problematic and widespread contaminants of water (Howden & Burt, 2008). Water containing high levels of N, most often in the form of nitrates, are a potential hazard to human health as well as to marine or freshwater ecosystems as a result of algal blooms and eutrophication (Thorburn et al. 2013, Howarth, 2008). Europe, the USA and China have substantial proportions of groundwater below cultivated land which have nitrate concentrations exceeding the international (WHO, 2017) recommendations for drinking water (50 mg/l) (Laegreid et al. 1999).

The major global concern with nitrate leaching and water contamination is as a result of the intensification of agricultural production involving the application of N fertilisers and organic wastes over the past 50 years (Di & Cameron, 2002). Agriculture is considered to be the major contributor to nitrate contamination of groundwater (Fraters et al. 1998, Sieling & Kage, 2006).

The fertiliser N which is not volatilised or denitrified, and taken up by the crop, risks being lost by leaching (Sieling & Kage, 2006).

The loss of chemicals and sediments from agricultural lands is well documented in the USA, Europe and Asia, with typically temperate climates (Thorburn et al. 2013). Tropical agroecosystems are not as well documented, despite their importance in global crop production, and the fact that agricultural development and intensification are accelerating in these regions (Thorburn et al. 2013, Ghiberto et al. 2015). However, there has been some pivotal research done in tropical regions in countries such as Australia and Brazil. In Australia, there has been great concern with regards to the loss of nutrients from surrounding agricultural land into the aquatic and marine ecosystem of the World Heritage-listed Great Barrier Reef and its catchments (Brodie et al. 2008, Armour et al. 2013). Sugarcane is one of the dominant forms of land use in catchments draining into the Great Barrier Reef lagoon (Thorburn et al. 2011, Armour et al. 2013). The production systems of sugarcane are intensive and rely on large inputs of N fertilisers, and in the context of this region of Australia, tend to be located on the coastal floodplains adjacent to the Great Barrier Reef lagoon (Thorburn et al. 2011). In one study based on wet-tropical regions next to the Great Barrier Reef, Rasiah et al. (2010), found that approximately 62 % of the nitrate-N that leached below the crop root-zone was exported to groundwater.

Brazil is the largest sugarcane producing country. Given the increasing area of cultivated sugarcane in regions such as the state of Sao Paulo and the associated risk of potential increases in environmental impacts, there have been several studies conducted on the leaching of macronutrients from sugarcane systems (Ghiberto et al. 2009, Ghiberto et al. 2015).

The majority of arable land in a tropical context such as Reunion Island is covered by sugarcane. A variety of mineral and organic fertilisers are used in agricultural zones on the island, and often in combination. The management of organic waste production, especially pig manure, is a major challenge (Feder & Findeling, 2007). N volumes derived from organic waste have been estimated at 2325 $t.year^{-1}$, with approximately 17 % of the island (representing 43 692 ha) having been designated as agricultural terrain (Aubry et al. 2006). The organic waste is frequently recycled by applying it to crop fields, and since crop production areas are limited in space, and given the high rainfall on the island, there is a potential risk of nitrate contamination of groundwater (Payet, 2005). If leaching is problematic in sugarcane production systems, which is not yet clear in the context of Reunion Island, it could have an especially important negative environmental impact in this type of context.

Sugarcane production systems are agriculturally intensive and rely on large inputs of fertiliser and water (Thorburn et al. 2011). As such, they may be at a high risk of N loss via leaching. In addition, there is not an abundance of information about N loss from sugarcane production

systems, especially irrigated systems (Thorburn et al. 2011). What has been documented in terms of losses of nitrates via leaching from the rooting zone of sugarcane production systems range widely. For example, in Mauritius, Brazil and Australia, the quantities of nitrates leached range from less than 1 to 70 $kgN.ha^{-1}$ (Ng Kee Kwong & Deville, 1984, Oliveira et al. 2002, Ghiberto et al. 2009, Rasiah et al. 2005, Rasiah et al. 2010, Thorburn et al. 2011, Armour et al. 2013).

Nutrient and more specifically N leaching depends on a combination of various factors: the dose, solubility and timing of N fertilisers applied, precipitation levels and the irrigation water regime and the consequent volume of drained soil solution, soil type and texture, the composition of crop residues incorporated into the soil, pedoclimatic factors, as well as the development of the crop root system (Oliveira et al. 2002, Ghiberto et al. 2015). The magnitude of the nutrient losses by leaching in soil systems has been shown to be proportional to the concentration of nutrients in the soil solution and the amount of drained solution (Ghiberto et al. 2009). Over-applied N has a tendency to leach readily below the root zone. This occurs more readily in well-aerated soils with a fast rate of nitrification, as well as weak interactions between $N\text{-}NO_3^-$ and soil colloids (Blum et al. 2013).

The timing of fertiliser N input relative to crop growth and the active N uptake periods of a crop should be considered, since the risk of nutrient leaching is higher when the input or mineralisation of N does not coincide with the nutrient uptake by plants (Oliveira et al. 2002, Sieling & Kage, 2006). This is why the consideration of crop biomass, as well as the periods of N uptake by the crop, would be useful indices to determine in conjunction with the dynamics of N in solution, even though leaching appears to be rarely studied relative to sugarcane growth and N uptake. Studying the dynamic of N in solution relative to the timing and dose of fertiliser N application, as well as to the amount of drainage (based on irrigation and rainfall patterns), is also important in determining the risk of leaching.

In this chapter, the study considers the dynamics of N in solution and the N leaching of both mineral fertilisers (urea) and organic fertilisers (pig slurry and sewage sludge), which are N fertilisers potentially used on sugarcane plantations in Reunion Island. Few studies have dealt with the dynamics of nitrate leaching of applied OFs to sugarcane plantations (Feder et al. 2007, Feder et al. 2015).

The aim of this two-year study was to monitor the transfers of N at different soil depths and the leaching of N from the sugarcane rooting zone in agroecosystems supplied with mineral or organic fertilisers compared to non-fertilised sugarcane. More specifically, the objectives were firstly to assess the risk of losing N by leaching throughout the crop growth-cycle; and secondly, to investigate whether the type of fertilisers had an influence on the extent and temporal dynamics of soil solution N transfers.

4.2 Material and methods

4.2.1 Study site

Refer to the description of the study site in Figure 7 of the Experimental site section.

4.2.2 Fertilisation

The experiment took place in three plots within the overall SOERE-PRO experimental trial, over the fourth and fifth crop ratoons, referred to in this study as the first and second experimental years.

The treatments in these plots were: 1/ urea (split) fertilisation; 2/ pig slurry fertilisation complemented with the split-application of urea; and 3/ sewage sludge fertilisation complemented with the split application of urea. The pig slurry applied was composed of 57 % and 75 % NH_4 at the start of the first and second experimental years, with the rest being organic N. The sewage sludge was composed of only 10 % NH_4 and 90 % was organic-N for both experimental years.

The first urea application (88 kg N/ha) to the urea treatment plot occurred directly after harvest of the third-ratoon crop in October 2017 and October 2018 for the first and second experimental years, respectively (Table 4.1). The organic fertilisers – pig slurry and sewage sludge – were also applied at this time, in their respective plots. These fertilisers used a 50% - 50% complement of organic fertiliser, quantified in terms of equivalent mineral efficiency units of N, followed by urea, to make up the equivalent dose as the urea treatment. The exact N dose applied for pig slurry was 265 $kgN.ha^{-1}$ and 131 $kgN.ha^{-1}$, applied at the start of the first and second years respectively, and for sewage sludge was 106 $kgN.ha^{-1}$ and 110 $kgN.ha^{-1}$ at the start of each respective ratoon (as specified in Table 1). The 50 % N of organic fertiliser was applied at the same time as the first urea application to the urea plot directly after harvest, and the first supplementary applications of urea were added to these organic fertiliser plots 2 months after the start of each ratoon, in December 2017 and December 2018 respectively. The second split applications of urea were added across the three plots 5 months after the start of the first experimental year in March 2018 and 4 months after the start of the second experimental year in February 2019, each with a dose of 57 kg N/ha.

Table 4.1 Fertiliser application for each of the treatment types (urea, pig slurry and sewage sludge) in their respective quantities and at their respective dates of application.

Fertiliser application	Months after harvest					
	Year 1			Year 2		
	1	2	5	1	2	4
Urea						
Urea ($kgN.ha^{-1}$)	88	-	57	88	-	62
Pig slurry						
Urea ($kgN.ha^{-1}$)	-	33	57	-	23	62
Org fert ($kgN.ha^{-1}$)	265	-	-	131	-	-
Sewage sludge						
Urea ($kgN.ha^{-1}$)	-	39	57	-	33	62
Org fert ($kgN.ha^{-1}$)	106	-	-	110	-	-

4.2.3 Lysimetric system

A mixed lysimetric system was used to sample the solution in the soil at different depths over the first experimental year, and a uniform porous ceramic suction cup system was used over the second experimental year. Lysimetric plates were installed before the start of the global experimental trial at a depth of 100 cm in November 2013. PTFE (Teflon) porous suction cup lysimeters (PRENART Equipment ApS, Denmark) were installed in the field at the end of September 2017, just before the annual harvest preceding the two ratoons studied in this experiment, at soil depths of 10 cm and 40 cm. The PTFE porous cups are inert, and when installed in the soil, silica flour (which has good hydrological properties) in solution was poured around the head of the cup to ensure effective capillary contact with the pore water in the soil.

Additional porous cups were installed at a soil depth of 100 cm before the start of the second experimental year, in September 2018. Over the second experimental year, samples were preferentially collected from the porous cups at 100 cm rather than from the lysimetric plates. The positioning of the porous cups is shown in Figure 7 of the Experimental Site section, For each fertiliser treatment, there were three repetitions of porous cups at each soil depth (10 cm, 40 cm and 100 cm). Soil solutions were collected using the porous suction cups, which were connected to a self-driven vacuum pump (PAV 2000, SDEC, France) and maintained manually, using the vacuum pump to create a vacuum of approximately 70 kPa twice a week. This vacuum resulted in the soil solution moving upward, against gravitational pressure, via tubes into polypropylene collecting bottles on the soil surface. This soil solution was collected from the bottles on a weekly basis. These were poured into 250 ml flasks and stored in a fridge at +5 °C. Samples were filtered (0.45 µm) and pooled proportionally to the volumes collected each week for a single monthly sample per lysimeter. Three subsamples of 10 ml were taken from each sample, for the samples where there was sufficient solution. Time-domain reflectometry (TDR) soil humidity probes

(Trase, Soil moisture, USA) had previously been installed (before the beginning of the experimental trial in November 2013) at soil depths of 10 cm, 30 cm, 40 cm, 60 cm, 80 cm, 100 cm and 120 cm.

4.2.4 Water sample analyses

Nitrate and NH_4 concentrations were analysed on a weekly basis over the first 3 months of each experimental year, and thereafter on a monthly basis, using the monthly solution composites by colorimetry (Evolution II, Alliance instruments) at the CIRAD laboratory in Reunion. Dissolved organic nitrogen (DON) concentrations were estimated by subtracting inorganic N concentrations (NO_3 + NH_4) from total N concentrations. The second series of samples (as monthly composites) were analysed for total N concentration using a TOC/TN analyser (TOC-VCSN, TNM-1, Shimadzu, Kyoto, Japan), at the Cirad laboratory in Montpellier.

4.2.5 Water flux

A model based on Richard's equation for simulating one dimensional water flow (Hydrus 1D, version 3.01) was calibrated to quantify water flow at the depths where the porous cups were installed (Simunek et al. 2008). The water flux (i.e. volume per unit of time) was determined using data on the level of evapotranspiration of the sugarcane, which was determined by using previous measurements of the leaf area index (LAI) of the sugarcane, coupled with values of irrigation of the experimental site as well as precipitation data accessed from the meteorological data taken from the experimental site. At certain dates, where there was a lack of water input from rainfall and irrigation, there was little or no soil solution extracted in the suction cups, and missing N concentrations were estimated as the mean of the values measured in the same suction cups at the sampling dates immediately before and after the required date.

4.2.6 Sugarcane N mass

The sugarcane aboveground N mass was estimated at each month using the allometric relationships to estimate the aboveground biomass, combined with an N dilution curve to determine the corresponding sugarcane N content (as described in the methodological Chapter 1). The values used were those determined in Chapter 2.

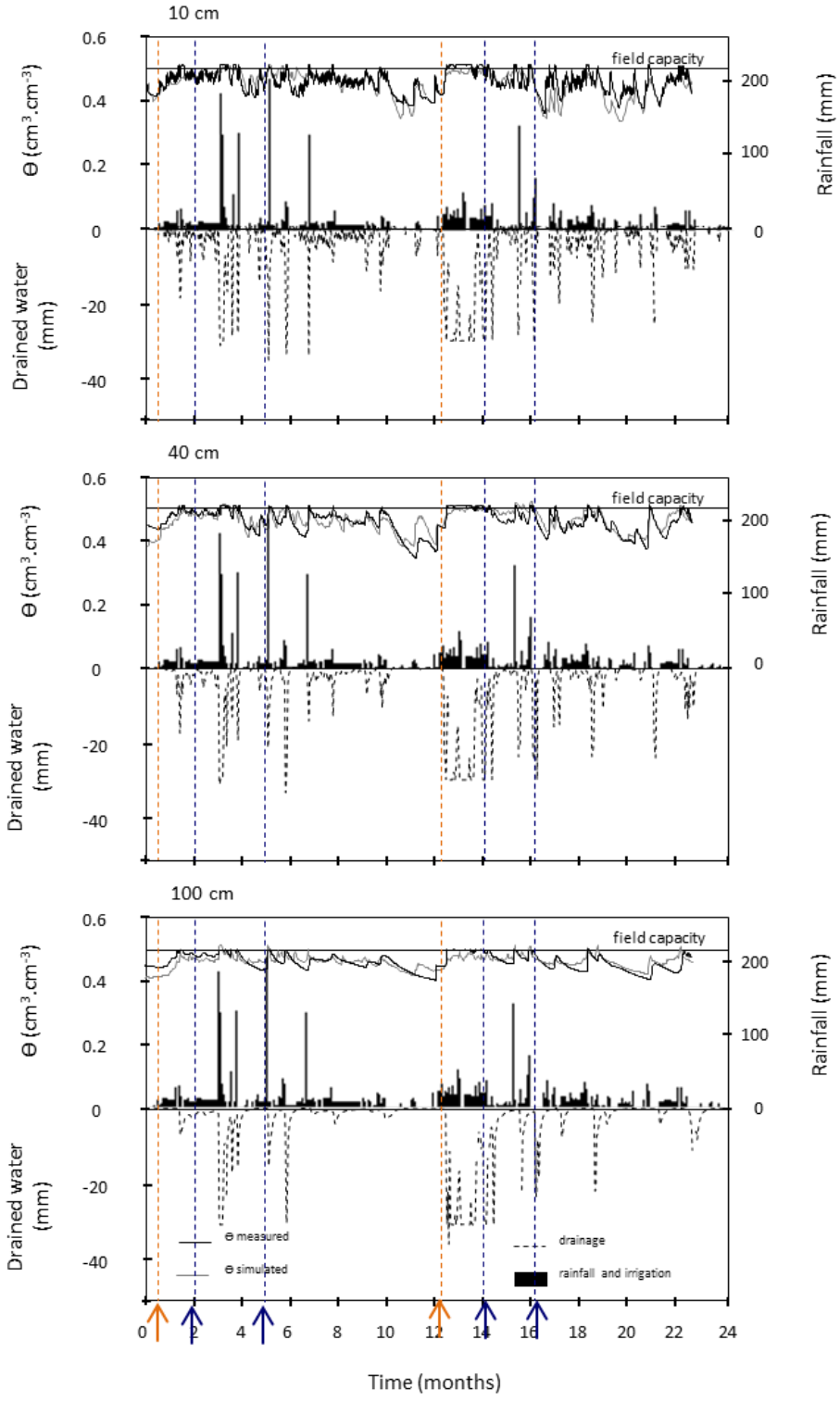

Figure 4.1 *Water flux from rainfall and irrigation and the consequent water drainage at soil depths of 10 cm, 40 cm and 100 cm over the two-year experimental period. The corresponding water flux input into the soil (irrigation and precipitation combined) is indicated for each graphic using a dotted line.*

4.3 Results

4.3.1 Nitrate concentrations in soil solutions

There is a higher level of drainage over the first two months after the start of the second experimental year than after the start of the first experimental year (Figure 4.1). This drainage is consistently higher over each of the soil horizons. This is primarily due to a consistently higher irrigation over the first two months of the second year. The higher irrigation at the start of the second year was a deliberate strategy over the larger experimental trial to reduce N losses via volatilisation.

At the start of each experimental year, there is a strong peak in the nitrate (NO_3^-) concentration after the first fertiliser application, across fertiliser treatment types and at the soil depths of 10 cm and 40 cm (Figure 4.2). The nitrate concentration generally has a similar tendency at 40 cm to that at 10 cm, but with lower concentrations. The NO_3^- concentrations are far higher after the first fertiliser application at soil depths of 10 cm and 40 cm over the first year than over the second, across treatment types.

Over the first year, the nitrate concentration at 10 cm in the pig slurry treatment is especially high after the initial application (which was 265 $kgN.ha^{-1}$) at the start of the ratoon, reaching a maximum NO_3^- concentration 42 days after the start of the ratoon, of 126 mg/l at 10 cm and a corresponding concentration of 39 mg/l at 40 cm, with insufficient soil solution to measure the NO_3^- concentration at 100 cm. Thereafter (from 50 days after the start of the ratoon), there is a decrease in the nitrate concentration across treatment types, which coincides with an increase in water influx from an increase in precipitation levels. The tendency is similar for the pig slurry over the second experimental year, except that the NO_3^- concentrations are far lower after the initial fertiliser application (which was lower, with an applied dose of 131 $kgN.ha^{-1}$), with soil concentrations reaching 15.1 mg/l, 4.9 mg/l and 3.8 mg/l at 10 cm, 40 cm and 100 cm respectively, 55 days after the start of the ratoon.

After the urea complement application to the organic fertiliser treatments 60 days after the start of the first year, there is little effect on the nitrate concentration at the different soil depths, for the pig slurry and sewage sludge treatments. The pig slurry NO_3 continues to decline to 13.3 mg/l, 11.3 mg/l and 0.0 mg/l at 10 cm, 40 cm and 100 cm soil depths, respectively at 89 days after the start of the ratoon, and to 1.2 mg/l, 0.0 mg/l and 0.0 mg/l at 149 days after the start of the ratoon. The sewage sludge follows a similar trend, decreasing to 3.2 mg/l, 3.4 mg/l and 0.0 mg/l at the same respective soil depths of 10 cm, 40 cm and 100 cm, and then decreased further to 0.6 mg/l, 0.1 mg/l and 0.0 mg/l at 149 days after the start of the ratoon of the first year. The trend was different at this point for the second experimental year. After the urea complement to the organic

fertiliser treatments 63 days after the start of the second year, the pig slurry treatment (i.e. pig slurry and urea complement) nitrate concentrations increased slightly to 9.9 mg/l, 14.4 mg/l and 1.0 mg/l as well as the sewage sludge treatment (i.e. sewage sludge and its urea complement) NO_3 concentrations (to 15.1 mg/l, 4.9 mg/l and 3.8 mg/l at soil depths of 10 cm, 40 cm and 100 cm, respectively. The urea and unfertilised treatments remain relatively constant at this point. For the organic fertiliser treatments, this continues to increase after the urea complement 60 days after the preceding harvest, which is more evident for the pig slurry treatment than the sewage sludge treatment.

After the split application of urea (at 157 days after the start of the first year and 132 days after the start of the second for all fertiliser treatments), there was little effect on the concentration of nitrates across the treatment types over the first experimental year, but by contrast, there was a substantial effect on NO3 content at 10 cm and 100 cm for certain fertiliser treatments (urea and pig slurry) over the second year. Over the first year, there is a very slight increase in NO_3 concentrations in the pig slurry treatment after this application at 10 cm and 40 cm, but the effect is negligible for the other treatments. At 180 days after the start of the first year, at soil depths of 10 cm, 40 cm and 100 cm respectively, the NO_3 concentrations for the pig slurry treatment increase slightly to 5.9 mg/l, 4.4 mg/l and 0.4 mg/l, the sewage sludge treatment remains relatively constant at 0.6 mg/l, 0.6 mg/l and 0.7 mg/l, and urea also remained relatively constant with concentrations of 2.3 mg/l, 1.7 mg/l and 0.2 mg/l at the respective soil depths. Thereafter, there is a continual decline in nitrates at the different soil depths for the rest of the first year.

The tendency is quite different over the second experimental year, coinciding with the higher levels of drainage at the start of the second experimental year. After the second (split application) of urea 132 days after the start of the ratoon, there is a substantial increase in NO_3^- concentrations at 10 cm and 40 cm, for the urea and pig slurry treatments, however this was not observed for the sewage sludge treatment. At 154 days after the start of the second year's ratoon, the nitrate concentrations at 10 cm, 40 cm, and 100 cm respectively, are 73.8 mg/l, 20.1 mg/l, 6.8 mg/l for the urea treatment; 74.5 mg/l, 30.5 mg/l and 2.5 mg/l for the pig slurry treatments; 1.2 mg/l, 3.5 mg/l and 1.1 mg/l for the sewage sludge treatment; and 2.5 mg/l. 0.4 mg/l and 0.3 mg/l for the unfertilised treatment.

After this point, there is a steady decline in nitrate concentrations at the different soil depths across treatment types until the end of the ratoon. This steady decline occurs when there is a steady increase in water flux and in sugarcane N mass (Figure 4.3). At 216 days after the start of the ratoon, at the respective soil depths of 10 cm, 40 cm and 100 cm, the NO_3 are 0.1 mg/l, 0.0 mg/l and 2.3 mg/l for the urea treatment; 1.7 mg/l, 0.1 mg/l and 0.9 mg/l for the pig slurry treatment; 0.0

mg/l, 0.3 mg/l and 0.0 mg/l for the sewage sludge treatment; and 0.0 mg/l at each of the soil depths for the unfertilised treatment.

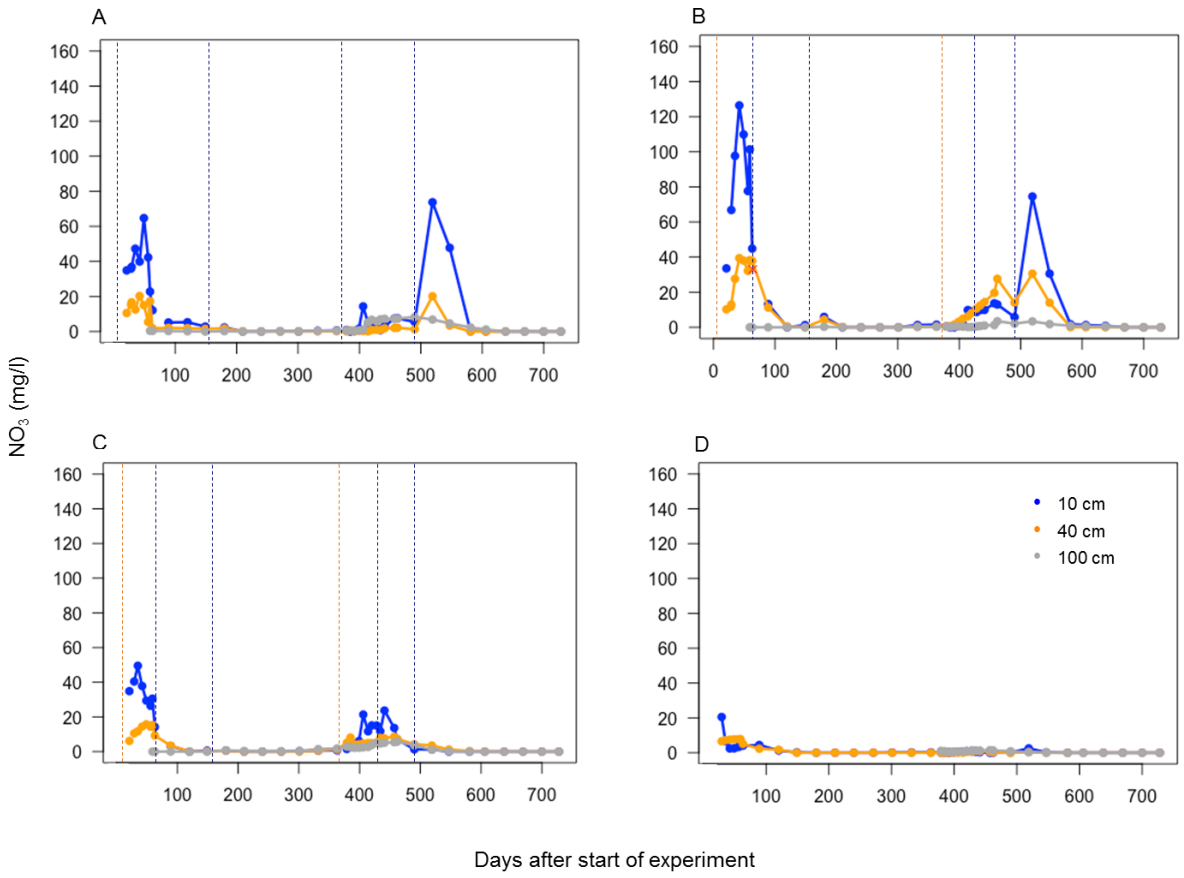

Figure 4.2 *Nitrate concentrations at different soil depths (10 cm, 40 cm, 100 cm), for the different fertiliser treatments: urea (A), pig slurry (B), sewage sludge (C), and the unfertilised treatment (D) over the two-year experimental cycle. Fertilisation is indicated using vertical dotted lines at the date of application, urea in blue and organic fertilization in orange.*

4.3.2 Nitrogen fluxes in soil solution

Globally, there is a similar tendency between the temporal dynamics of the N amount and of the NO_3 concentrations. Over the first experimental year, there is an initial steady increase in the N amount in solution at soil depths of 10 cm and 40 cm until 2 months after the start of the ratoon, and thereafter there is a steady decline in the N amount (Figure 4.3). Two months after the start of the first year, at soil depths of 10 cm, 40 cm and 100 cm respectively, the N content was 2.3, 4.0 and 0.0 $kgN.ha^{-1}$ for the unfertilised treatment; 10.6, 8.5, and 0.2 $kgN.ha^{-1}$ for the urea treatment; 46.6, 18.1, 0.0 $kgN.ha^{-1}$ for the pig slurry treatment; and 14.0, 7.5, 0.3 $kgN.ha^{-1}$ for the sewage sludge treatment.

This trend is similar over the second experimental year, with an initial peak 2 months after the start of the ratoon, as well as after the first fertiliser application, across treatment types. At soil depths of 10 cm, 40 cm and 100 cm respectively, the N amount was 3.0, 2.2 and 2.4 kgN.ha^{-1} for the unfertilised treatment; 5.8, 2.4, and 8.3 kgN.ha^{-1} for the urea treatment; it was far lower for the pig slurry treatment than the first year with concentrations of 8.5, 11.9, 2.4 kgN.ha^{-1}; and a higher N amount for the sewage sludge treatment to the first year of 18.9, 11.7, 6.9 kgN.ha^{-1}.

Thereafter, there is a steady decline in N content at the different soil depths over the first year, across treatment types. This coincides with the active N mass period of the sugarcane (see the N mass evolution in more detail in chapter 2), which is between 2 and 4-5 months after the start of the ratoon. The urea complement which is added to both organic fertiliser plots 2 months after the start of the ratoon does not result in an increase in the N amount in solution at the different soil depths, and the N amount continues to decline over this active N uptake period for the sugarcane biomass.

At 4 months after the start of the first year's ratoon, just before the second application of urea across treatments, at soil depths of 10 cm, 40 cm and 100 cm respectively, the N content was 3.1, 1.1, and 0.2 kgN.ha^{-1} for the urea treatment; 0.0, 0.3, 0.0 kgN.ha^{-1} for the pig slurry treatment; 0.0, 0.1, 0.0 kgN.ha^{-1} for the sewage sludge treatment; and 0.7, 1.0 and 0.0 kgN.ha^{-1} for the unfertilised treatment.

At 4 months after the start of the second year, the N content values were similar to that of the first year. At soil depths of 10 cm, 40 cm and 100 cm respectively, the N content was 0.7, 3.0 and 0.9 kgN.ha^{-1} for the unfertilised treatment; 2.9, 1.3, and 3.4 kgN.ha^{-1} for the urea treatment; 2.5, 9.2, 1.5 kgN.ha^{-1} for the pig slurry treatment; and 1.5, 2.9, 1.9 kgN.ha^{-1} for the sewage sludge treatment.

With the second portion of split urea application at 4-5 months after the start of the ratoon of the first experimental year, there is a slight increase in the N amount in solution at 10 cm and 40 cm over the following month and again a decline. At 5 months after the start of the ratoon of the first year, at soil depths of 10 cm, 40 cm and 100 cm respectively, the N content was 0.2, 0.0 and 0.0 kgN.ha^{-1} for the unfertilised treatment; 1.1, 0.3, and 0.0 kgN.ha^{-1} for the urea treatment; 0.4, 0.1, 0.0 kgN.ha^{-1} for the pig slurry treatment; and 1.9, 0.0, 0.0 kgN.ha^{-1} for the sewage sludge treatment.

At the corresponding 5 months after the start of the ratoon of the second year, at soil depths of 10 cm, 40 cm and 100 cm respectively, the N content was 1.0, 0.8 and 0.2 kgN.ha^{-1} for the unfertilised treatment; had far higher values for urea of 16.9, 3.4, and 0.7 kgN.ha^{-1}; far higher values than the first year for the pig slurry treatment of 18.2, 4.7, 0.5 kgN.ha^{-1}; and slightly lower values in the sewage sludge treatment of 0.6, 0.5, 0.2 kgN.ha^{-1}.

After this point in time over both experimental years, there is a decline in the NO_3^- concentrations across fertiliser treatments and soil depths (especially at 10 cm and 40 cm since 100 cm is already close to 0 kgN.ha^{-1}) over the rest of each respective experimental year.

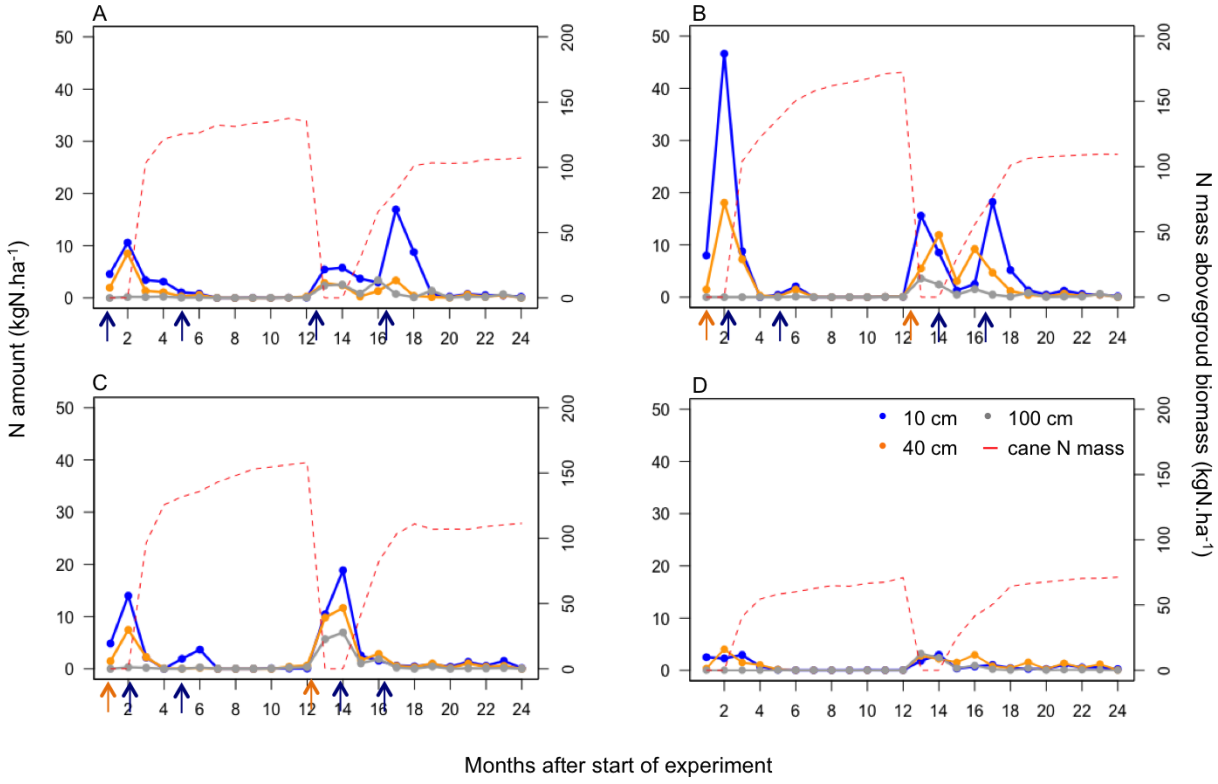

Figure 4.3 *Nitrogen amount at different soil depths (10 cm, 40 cm, 100 cm), for the different fertiliser treatments: urea (A), pig slurry (B), sewage sludge (C), and the unfertilised treatment (D). The corresponding water flux input into the soil (irrigation and precipitation combined) is indicated for each graphic using a dotted line. Fertilisation is indicated using arrows at the date of application, urea in blue and organic fertilisation in orange.*

4.3.3 N accumulation and percent fertiliser N at soil depths

In the urea treatment, at 2 months after the first fertiliser application, 11.8 %, 6.8 % and 0.2 % of the fertiliser N were found at 10 cm, 40 cm and 100 cm depths, respectively (Table 4.2). At 2 months after the start of the second experimental year, a slightly lower proportion of N applied moved down the soil profile, and was found at depths of 10 cm and 40 cm. 7.8 % and 0.5 %, of urea-N was measured at a soil depth of 10 cm and 40 cm respectively, and 6.1 % of fertiliser N applied at 100 cm.

At 12 months after the start of the first experimental year, the accumulated proportion of fertiliser N applied is 10.4 %, 4.8 % and 0.6 % at the three respective soil depths, and far higher overall values at 12 months after the start of the second experimental year, with values of 25.2 %, 0.0 % and 7.3 % of fertiliser N at the each of these respective soil depths.

For the pig slurry treatment, at 2 months after the start of the first experimental year, 18.8 %, 5.7 % and 0.0 % of the pig slurry N applied is observed at the 10 cm, 40 cm, and 100 cm soil depths. This is similar to 2 months after the start of the second year (where the pig slurry N applied at the start of the year was approximately half of that applied at the start of the first year), which was 14.8 %, 9.6 % and 0.3 % of the pig slurry N applied, at the same respective soil depths. At 12 months after the start of both experimental years, 16.2 %, 6.1 %, 0.1 % of accumulated N applied was measured at each of the soil depths at the end of the first year, and a slightly higher 21.0 %, 10.2 % and 1.1 % of N applied at the respective soil depths of 10 cm, 40 cm and 100 cm, was found at the end of the second year.

For the sewage sludge treatment, at 2 months after the start of the first experimental year, 13.3 %, 4.2 % and 0.3 % of the applied sewage sludge N was measured at depths of 10 cm, 40 cm and 100 cm. At 2 months after the start of the second year, the N in solution at these soil depths were slightly higher, being 22.3 %, 15.1 % and 7.6 % of the sewage sludge N application. At 12 months after the start of both experimental years, 9.0 %, 2.6 %, 0.7 % of accumulated N applied at each of the soil depths at the end of the first year, and slightly higher 14.1 %, 7.1 % and 4. % of N applied at the respective soil depths of 10 cm, 40 cm and 100 cm at the end of the second year.

Table 4.2 N accumulated (N accum) and percent fertiliser N (Percent N applied) at the different soil depths (10 cm, 40 cm, 100 cm) for the respective fertiliser treatments (urea, pig slurry & sewage sludge).

Measure type	Soil Depth (cm)	Months after harvest							
		Y1				Y2			
		1	2	5	12	1	2	4	12
Unfertilised									
N accum ($kgN.ha^{-1}$)	10 cm	2,5	4,8	8,5	8,6	1,8	4,8	5,9	10,2
	40 cm	0,4	4,4	7,1	7,1	2,7	4,9	9,4	15,4
	100 cm	-	-	-	-	3,2	5,6	6,9	7,8
Urea									
Fertilisation ($kgN.ha^{-1}$)	-	88	-	57	-	88	-	62	-
N accum ($kgN.ha^{-1}$)	10 cm	4,6	15,1	22,8	23,7	5,5	11,2	17,8	46,5
	40 cm	1,9	10,4	13,2	14,1	2,9	5,3	6,9	12,3
	100 cm	0,0	0,2	0,6	0,8	2,4	10,7	14,9	18,3
Percent N applied (%)	10 cm	2,3	11,8	9,8	10,4	4,4	7,8	8,3	25,2
	40 cm	1,8	6,8	4,2	4,8	0,1	0,5	0,0	0,0
	100 cm	-	0,2	0,4	0,6	0,0	6,1	5,5	7,3
Pig slurry									
Fert urea ($kgN.ha^{-1}$)	-	-	33	57	-	-	23	62	-
Fert slurry ($kgN.ha^{-1}$)	-	265	-	-	-	131	-	-	-
N accum ($kgN.ha^{-1}$)	10 cm	8,0	54,6	63,8	66,0	15,6	24,1	27,9	55,6
	40 cm	1,5	19,5	27,1	28,8	5,5	17,4	29,7	37,5
	100 cm	0,0	0,0	0,0	0,2	3,6	6,0	8,0	10,2
Percent N applied (%)	10 cm	2,1	18,8	15,6	16,2	10,5	14,8	10,2	21,0
	40 cm	0,4	5,7	5,6	6,1	2,1	9,6	9,4	10,2
	100 cm	-	0,0	0,0	0,1	0,3	0,3	0,5	1,1
Sewage sludge									
Ferturea ($kgN.ha^{-1}$)	-	-	39	57	-	-	33	62	-
Fert sludge ($kgN.ha^{-1}$)	-	106	-	-	-	110	-	-	-
N accum ($kgN.ha^{-1}$)	10 cm	4,8	18,8	22,9	26,7	10,5	29,3	33,4	39,2
	40 cm	1,4	8,9	11,2	12,4	9,8	21,5	26,0	30,0
	100 cm	0,0	0,3	0,5	1,4	7,0	14,0	16,9	17,9
Percent N applied (%)	10 cm	2,2	13,3	7,1	9,0	7,8	22,3	13,4	14,1
	40 cm	1,0	4,2	2,0	2,6	6,4	15,1	8,1	7,1
	100 cm	-	0,3	0,2	0,7	3,5	7,6	4,9	4,9

4.4 Discussion

4.4.1 Soil solution N transfers in sugarcane agroecosystems

During the 2-3 months after the first fertilisation at the start of each ratoon, there was a consistent increase in the N content in solution, for each of the different fertiliser treatment types. This coincides with high moisture periods, both from high seasonal rainfall, as well as irrigation applied in high volumes deliberately over this period. This was especially the case at this period over for the second experimental year with the aim of decreasing N loss via volatilization. High soil moisture, due to high rainfall and/or irrigation, leads to favourable conditions for soil solution drainage and this, combined with high N availability (due to mineral N fertilisation for example), favours the loss of N in solution via leaching from the crop rooting-soil system (Ghiberto et al. 2015).

The N in solution was transferred steadily from the soil surface to the different depths of the soil profile after the initial fertiliser applications. There was a marked decrease in the amount of N that reaches a soil depth of 40 cm, and eventually a very small portion of this which reached a soil depth of 100 cm. This was especially the case over the first experimental year. This suggests that N gradually migrates into the soil over the soil vertical profile, and that a substantial portion of the nitrates in solution were retained by adsorption into the soil. In a study by Feder et al. (2007), on the leaching of nitrates in the context of Reunion Island, approximately 85 % of mineral N was adsorbed on soil solids, over the soil profile until a depth of 100 cm. The reason suggested for this retention was due to the high anion exchange capacity of the soils in this context (Feder et al. 2007). In general, tropical soils have been found to frequently have an anion exchange capacity that reduces the transfer of nitrates and accumulates them in the soil (Feder et al. 2020, Feder et al. 2021).

At the start of each experimental year (i.e. following the previous ratoon's harvest), the sugarcane was in its early developmental stages and could not take up the majority of available N in soil solution at this point, since the root system was not sufficiently developed. The highest risk of loss of N in solution was therefore during this period for each of the different fertiliser types. Even for the unfertilised treatment, the highest amounts of N in solution at the different soil depths were during this period. The period when crop demand from N is low or non-existent therefore poses a particular risk for leaching (Sieling & Kage, 2006). The sugarcane biomass and total N mass evolution was however rapid, as was reported on in more detail in Chapter 2. The active N uptake period occurred until 4-5 months after the start of the first year, and until approximately 6 months after the start of the second year. Rapid root development occurred over this period, as can be seen in more detail in the Appendix Chapter 7.2. Over the first experimental year, there was little influence of the urea complement (for the organic fertilisers) and the second

split application of urea at 4-5 months after fertiliser application. This was very likely to be as a result of rapid N uptake by the sugarcane during this period, when the sugarcane and its rooting system are sufficiently developed. When evaluating the evolution of the sugarcane total N mass over the first experimental year, the urea split application occurs exactly during the N active uptake period.

The urea complement and second split application were however far more noticeable over the second year, where there was an observable increase in the N content in soil solution at the different soil depths after these fertiliser applications. The likely reason for this is that the sugarcane was less developed at this point in time, in terms of its biomass and total N mass, as well as its rooting system, as compared to the first experimental year (see Chapter 2). This is therefore probably why there was a higher quantity of N lost via leaching over the second year.

The evolution of the sugarcane biomass and total N mass is rarely evaluated in studies that assess N in soil solution and N loss via leaching, even though this is clearly an important element to take into account when studying N loss via leaching. Some studies state the potential importance of considering the N uptake periods of crops when studying leaching (e.g. Oliveira et al. 2002, Sieling & Kage, 2006), but without studying the evolution of the total N mass and dynamic of N uptake of crops. The timing of fertiliser N input relative to crop growth and the active N uptake periods of a crop should be considered, since the risk of nutrient leaching is higher during these periods.

The risk of N loss by leaching primarily in the form of nitrates, was therefore highest over the first 2-3 months after the start of the ratoon. This is when there is a high availability of N after fertilisation, as well as sufficient soil moisture leading to high drainage from irrigation and high rainfall, as well as when the capacity of the sugarcane to absorb N is limited by its low aboveground and belowground biomass. Fortunately, it appears that the soil in the context of our study has a high potential to absorb these nitrates and the majority was therefore retained over the soil profile, with a low portion of nitrates reaching a soil depth of 100 cm in solution, with the risk of being leached from the soil-root system.

In order to limit the losses of N via leaching, the fine-tuning of fertilisation should be recommended based on the following principles: 1/ the right dose, taking into account the N requirements of the crop and the efficiency of the fertiliser; 2/ the right timing, i.e. at least one month after harvest where the sugarcane can begin to actively take up N applied, and the fertilisation should be divided into at least two applications in order to limit the immobilisation of the applied N and to minimise losses which occur earlier on in the ratoon when the sugarcane is still underdeveloped.

4.4.2 Influence of fertiliser types on soil solution N transfers

The primary source of N in solution is from the fertilisers, both mineral (urea) as well as organic (the pig slurry and sewage sludge treatments). However, given the relatively high value of N leaching in the unfertilised treatment, a large proportion of leaching in the fertiliser treatments is derived from N sources other than the fertiliser, the majority likely derived from soil organic matter, as this is a major N pool (see the N derived from soil in Chapter 2 and the N budget in Chapter 5). Over the second experimental year, when there was a higher level of N loss via leaching, there was an accumulated N loss of 7.8 $kgN.ha^{-1}$ from the unfertilised treatment. The N derived from the pig slurry fertiliser (after the unfertilised derived-N is been deducted), was only slightly higher than the unfertilised treatment with an accumulated N of 10.2 $kgN.ha^{-1}$. The urea and sewage sludge treatments, however, had more than double the amount of accumulated N which reached a soil depth of 100 cm and which was thus considered to be leached from the soil-rooting zone, with N accumulated values of 18.3 $kgN.ha^{-1}$ for urea and 17.9 $kgN.ha^{-1}$ for sewage sludge. Studies such as that of Ghiberto et al. (2009) found a greater amount of N loss via leaching from soil than the fertiliser N applied. In the study by Oliveira et al. (2002), the total amount of leached N was 4.5 $kgN.ha^{-1}$, none of which was derived from the urea fertiliser, and 53 % of which occurred over the first three weeks, which they attributed to the high rainfall and insufficient sugarcane root development at this early stage of the crop growth-cycle (although this was not evaluated in the study by Oliveira et al. (2002)).

The temporal trends of N in solution passing down the soil profle and with respect to the dates of fertilisation, were similar between the mineral and OF treatments. There is a sufficiently high rate of mineralisation of the organic fertilisers resulting in a high concentration of nitrates shortly after fertiliser application (Feder & Findeling, 2007). In the pig slurry treatment, the majority of N in the pig slurry was in fact mineral (approximately 57 % and 75 % in its composition for the first and second year fertiliser treatments applied). In soil solution, N is transferred primarily as NO_3^- due to the low affinity for clay materials and negatively charged organic matter and to the fast nitrification rate in tropical soils (Blum et al. 2013). Blum et al. (2013), found that 92.3 % of the leached mineral N was in the form of NO_3^-, which is coherent with our study.

There was a similar trend between the dynamic of the total N amount in solution (when the volume of water drained through the soil profile is taken into account) over the 2 experimental years, to that of the NO_3^- concentration (without taking into account water flux). However, when the water flux was considered in calculating the N amount in solution, there are some differences over the second year. The N amount in soil solution was high after the initial fertiliser application at the start of the second year similar to that of the first, but the NO_3^- concentrations did not reflect this. This is likely to be so since there was a higher volume of water passing through the soil

profile and a higher level of drainage at the start of the second year, which would mean that these concentrations are diluted at this stage of the ratoon.

For each of the fertiliser treatment types, there was a higher proportion of fertiliser-N found in the top 10 cm of the soil, and also a far higher proportion that reached a soil depth of 100 cm and which was considered leached from the crop-rooting zone. For the mineral fertiliser treatment, 10.4 - 25.2 % of urea-N applied was measured within the first 10 cm of the soil, and 0.8 - 7.3 % of the urea-N then reached a soil depth of 100 cm corresponding to a loss of 0.8 - 18.3 $kgN.ha^{-1}$ of fertiliser-N via leaching over the two ratoons. For the first of the OFs, 16.2 - 21.0 % of the pig slurry-N was measured over the first 10 cm of the soil profile over the first year, and only 0.1 – 1.1 % reached a depth of 100 cm corresponding to a loss of 0.2 - 10.2 $kgN.ha^{-1}$ of fertiliser-N via leaching over the two ratoons. For the sewage sludge fertiliser, 9.0 – 14.1 % of the fertiliser treatment-N was found over the first 10 cm of the soil profile, and 0.7 – 4.9 % reached a soil depth of 100 cm, which corresponds to a loss of 1.4 -17.9 $kgN.ha^{-1}$ of fertiliser-N via leaching over the two ratoons. For example, in Mauritius, Brazil and Australia, the quantities of nitrates leached range from less than 1 to 70 $kgN.ha^{-1}$ (Ng Kee Kwong & Deville 1984, Oliveira et al. 2002, Ghiberto et al. 2009, Rasiah et al. 2005, Rasiah et al. 2010, Thorburn et al. 2011, Armour et al. 2013). The values in our study, were in the lower range of this relatively wide range.

More specifically, the values for the mineral fertiliser in our study were in a similar range to, but slightly lower than various studies which were conducted on sugarcane plantations in a tropical savanna ultisol region in Brazil. In one of their earlier studies, Ghiberto et al. (2009), found a total N loss via leaching of 18 $kgN.ha^{-1}$, which corresponded to 15 % of the applied N (120 $kgN.ha^{-1}$ urea application). In their later study, it was found that between 3.9 and 34.9 $kg.ha^{-1}$ of N was leached (from a soil depth of 0.9 m) for the different fertiliser N doses, ranging from unfertilized to 150 $kg.ha^{-1}$ (Ghiberto et al. 2015). For the fertiliser application of 100 $kgN.ha^{-1}$ in their study, 22.5 $kgN.ha^{-1}$ was leached from the sugarcane system (Ghiberto et al. 2015). However, between these two studies, Ghiberto et al. (2011), performed a similar experiment in an oxisol cultivated with sugarcane, and found a far lower level of leaching, of only 1.1 $kgN.ha^{1}$. These low N losses via leaching were attributed to a high demand of N by the sugarcane crop, as well as a lower level of rainfall than normal over the high rainfall season. Oliveira et al. (2002), also had lower values of 5 $kgN.ha^{1}$ leached corresponding to between 0 and 5 % of urea-N applied. In Australia, Armour et al. (2013), also had a lower range of leaching from urea, with 0.6 – 9.2 $kgN.ha^{1}$ of N in solution leached, corresponding to 1-6 % of urea-derived N. These values are more comparable to values across the fertiliser treatments over our first experimental year.

There is not a great difference in the amount of N leached between the different fertiliser treatment types in our study. This is different from studies such as that of Sieling & Kage (2006),

which measured N leaching from oilseed rape, wheat and barley rotations. In their study, mineral N fertilisation only increased N leaching slightly (as compared to an unfertilised treatment), whereas when pig slurry was added, N losses via leaching were boosted. However, for the crops studied in their study, the growth patterns, and especially the timing and extent of root development, may be quite different from that of sugarcane.

The values of nitrate leaching for sewage sludge in our study can be compared to that of Vieira et al. (2005), which found a slightly higher level of leaching for sewage sludge at 5 months after the initial fertiliser application, with 25.3 kgN.ha^{-1} leached from a tropical oxisol. This was slightly higher, at 5 months after the first fertiliser application, than the leaching of the mineral fertiliser N in their study (18.7 kgN.ha^{-1}) and the unfertilised treatment (9.3 kgN.ha^{-1}); which were both values similar to the leaching of these treatments at the end of the second year in our study. A study by Blum et al. (2013), which used treated sewage effluent applied as irrigation in a sugarcane plantation, had far higher values of leaching than the sewage sludge treatment in our study, with a total N leached of 39.5 kgN.ha^{-1} and 88.8 kgN.ha^{-1} for irrigation applied at 100 % and 150 % of crop water demand, respectively. However, 16.4 kgN.ha^{-1} was leached from their unfertilised treatment, which was also slightly higher than our study, suggesting higher leaching from N sources other than fertiliser in the soil-crop system. The fact that the sewage effluent was in a different physical form, and applied in solution via irrigation by Blum et al. (2013), is also likely to impact their higher levels of N leaching. The N leached from a non-irrigated treatment (with half of the suggested mineral dose applied 50 kgN.ha^{-1} per year was applied as ammonium nitrate, and the rest as treated sewage effluent applied in the irrigation) in the study by Blum et al. (2013), was 16.4 kgN.ha^{-1} (and a total of 320 mm of leached solution) after 691 days, or to sugarcane growth cycles. This was lower than the study by Ghiberto et al. (2011).

4.5 Conclusion

Overall, there was a relatively low level of N loss via leaching from the soil-sugarcane system, over the two experimental years. Over the first year, there was close to zero loss, but the losses were higher over the second year. The reason for the overall low extent of N loss via soil solutions is potentially due to the high Anion Exchange Capacity of soils in this context (Feder et al. 2007, Feder et al. 2021). It is very likely that the difference between the two years is as a result of the lag in sugarcane growth and root development over the initial months during the second year, where the sugarcane is unable to capture all of the N passing through the soil in solution. This suggests that in addition to variables such as soil type, the extent and timing of drainage, attention should be paid to the timing and doses of different fertiliser N applied with respect to the stage of growth of the crop and its root development.

CHAPTER FIVE

Abstract

This study has attempted to establish a comprehensive account of all the major N fluxes of the soil-sugarcane system, as well as to evaluate in detail the fate of N from the mineral fertiliser urea as well as two types of organic fertilisers, pig slurry and sewage sludge. In this concluding chapter, the N inputs and outputs are summarised in a visual nitrogen budget for the soil-sugarcane system.

Thereafter, the fate of N from fertilisers is followed, and discussed in the order of its passage through the soil-sugarcane biogeochemical cycle: 1/ N loss via gas emissions of NH_3, 2/ N loss via N_2O emissions, 3/ passage of fertiliser N through mulch (i.e. residue after harvest) is discussed, 4/ absorption of N by the sugarcane and discrepancies between methods used to calculate N fertiliser recovery efficiency, 5/ the leaching of N in solution after N losses via NH_3 have been considered, 6/ the quantity of fertiliser N input, which is not lost from the soil-sugarcane system, but rather stored in the soil, is evaluated using four different approaches. The first uses the N budget of each fertiliser (i.e. organic fertilisers considered separately from the urea complement); the second approach uses ^{15}N-labelled urea to determine the quantity of urea-derived N in the soil; the third approach uses the flux of N inputs and outputs for each fertiliser treatment plot (i.e. OF and its complementary urea); and the fourth approach uses data from the SOERE-PRO experimental trial to determine the average N stored in the soil.

A complete budget of N flux at the agroecosystem scale established that of the N applied with the urea fertiliser, 30 $kgN.ha^{-1}$ was absorbed, 52, 2 and 5 $kgN.ha^{-1}$ lost via volatilisation, denitrification and leaching respectively, and 53 $kgN.ha^{-1}$ immobilized in the soil using the first approach's calculation. This corresponds to 22 % of urea-N absorbed, 36 %, 1.4 % and 3 % lost via volatilization, denitrification and leaching respectively, and 37 % immobilised in the soil

Of the N applied with pig slurry, 11 $kgN.ha^{-1}$ was absorbed, 128, 7 and 2 $kgN.ha^{-1}$ lost via volatilization, denitrification and leaching respectively, and 53 $kgN.ha^{-1}$ immobilised in the soil. This corresponds to 7 % of pig slurry-N absorbed, 63 %, 3.6 % and 2 % lost via volatilization, denitrification and leaching respectively, and 27 % immobilized in the soil

Finally, of the N applied with sewage sludge, 11 $kgN.ha^{-1}$ was absorbed, 8, 1 and 5 $kgN.ha^{-1}$ lost via volatilization, denitrification and leaching respectively, and 76 $kgN.ha^{-1}$ immobilised in the soil. This corresponds to 9 % absorbed, 8 %, 0.7 % and 5 % lost via volatilization, denitrification and leaching respectively, and 70 % immobilised in the soil.

The chapter ends with the global conclusions and perspectives of this doctoral study.

Resumé

Ce chapitre représente une tentative de synthèse des principaux flux d'azote du système sol-canne à sucre, et d'évaluation du devenir de l'azote de deux engrais organiques (lisier de porc et boues d'épuration) et d'un engrais de synthèse (urée). Dans ce dernier chapitre, les entrées et sorties d'azote sont résumées dans une synthèse graphique de l'azote à l'échelle du système sol-canne.

Le devenir de l'azote des engrais est suivi et discuté dans l'ordre de son passage dans le cycle biogéochimique sol-canne à sucre : 1/ perte d'azote par volatilisation ammoniacale, 2/ perte d'azote par les émissions de N2O, 3/ passage de l'azote des engrais à travers le paillis (c'est-à-dire après la récolte), 4/ l'absorption de N par la canne à sucre et les différences entre les approches différentielles et isotopiques pour calculer le coefficient réel d'utilisation d'azote (CRU) des engrais, 5/ les flux de lixiviation ont été examinés après déduction des pertes par volatilisation, 6/ la quantité d'azote apportée par les engrais qui se retouve dans le sol a été évaluée selon quatre approches différentes. Les quatre approches sont: la première utilise le bilan N de chaque engrais (c'est-à-dire les engrais organiques considérés séparément du complément d'urée) ; la deuxième approche utilise l'urée marquée au ^{15}N pour déterminer la quantité d'azote dérivée de l'urée dans le sol ; la troisième approche utilise le flux des entrées et sorties d'azote pour chaque traitement (i. e. engrais organique et son complément d'urée) ; et la quatrième approche utilise les données du site expérimental SOERE-PRO (de 2013 à 2019) pour déterminer l'azote moyen stocké dans le sol, déduit par l'augmentation de l'azote dans le sol sur les 6 années de l'essai.

Un bilan complet du flux d'azote à l'échelle de l'agroécosystème a établi que, sur l'azote appliqué avec l'urée, 30 kgN.ha-1 ont été absorbés, 52, 2 et 5 kgN.ha-1 perdus par volatilisation, dénitrification et lixiviation respectivement, et 53 kgN.ha-1 immobilisés dans le sol selon le calcul de la première approche. Cela correspond à 22 % de l'azote de l'urée absorbé par la canne, 36 %, 1,4 % et 3 % perdus par volatilisation, dénitrification et lixiviation respectivement, et 37 % immobilisés dans le sol.

Sur l'azote appliqué avec le lisier de porc, 11 kgN.ha-1 ont été absorbés, 128, 7 et 2 kgN.ha-1 perdus par volatilisation, dénitrification et lixiviation respectivement, et 53 kgN.ha-1 immobilisés dans le sol. Cela correspond à 7 % de l'azote du lisier de porc absorbé par la canne, 63 %, 3,6 % et 2 % perdus par volatilisation, dénitrification et lixiviation respectivement, et 27 % immobilisés dans le sol.

Enfin, sur l'azote appliqué avec les boues d'épuration, 11 kgN.ha-1 ont été absorbés, 8, 1 et 5 kgN.ha-1 ont été perdus par volatilisation, dénitrification et lixiviation respectivement, et 76 kgN.ha-1 se sont immobilisés dans le sol. Cela correspond à 9 % absorbés par la canne, 8 %, 0,7 % et 5 % perdus par volatilisation, dénitrification et lixiviation respectivement, et 70 % immobilisés dans le sol.

Le chapitre se termine par les conclusions et les perspectives globales de mes travaux de thèse.

Chapter 5. The fate of fertiliser-N in sugarcane agroecosystems: synthesis and perspectives

This study has attempted to establish a comprehensive account of all the major N fluxes of the soil-sugarcane system and to evaluate in detail the fate of N from mineral fertiliser (urea), as well as two types of organic fertilisers (pig slurry and sewage sludge). In this concluding chapter, a visual summary is presented of the results in a nitrogen 'budget' or 'balance' of all the N inputs and outputs from the soil-sugarcane system.

The primary N inputs into the soil-sugarcane system have been evaluated in detail in Chapters 2 and 3. Additional N inputs into and outputs from the soil-sugarcane system not covered in the preceding chapters are also included here in the N budget. In terms of N outputs from the soil- sugarcane system, the major pathways which have thus far been evaluated are N export via sugarcane harvested, N in soil solution at different soil horizons, and the proportion of N in soil solutions that is eventually lost from the rooting zone via leaching and which can effectively be considered as N output (Chapter 4). Two important N outputs which have not yet been covered, and for which measurements were taken at this experimental site and which will be presented in this chapter, are the gas emissions of NH_3 via volatilisation and N2O emissions via denitrification. Finally, the proportion of fertiliser N from the different treatments which is neither taken up by the sugarcane nor lost from the soil-sugarcane system, but which is rather returned to the soil, is discussed.

The chapter starts with the graphic representation of the N budget for each of the treatment types (Figures 5.1 – 5.4). This N budget has values for certain components of the N cycle which have not yet been covered, but which will be described in this chapter. The soil N values at different depths were determined as presented in the Experimental Site section (Photo 7). In the organic fertiliser plots, the fate of the OFs and their urea complement are presented separately. For the soil solution N content at the different soil depths, the unfertilised treatment (in plot T2) was assumed to have the same impact in the other treatment plots. The N collected weekly in rainfall samples (representing atmospheric deposition), as well as N from irrigation, also collected weekly over the two years, is presented in the N budgets of the different fertiliser treatments.

These N inputs and outputs which have not been covered in the preceding chapters (but which are displayed in the N budget) are discussed in the order that follows the fate of fertiliser N in the soil-sugarcane system: 5.1 Nitrogen loss via NH_3 volatilisation; 5.2 Nitrogen loss via N_2O emissions; 5.3 Mulch as a temporal sink of fertiliser-N; 5.4 Fertiliser-N uptake by sugarcane; 5.5 Leaching after considering N loss via volatilisation; 5.6 Remaining N ending up in the soil.

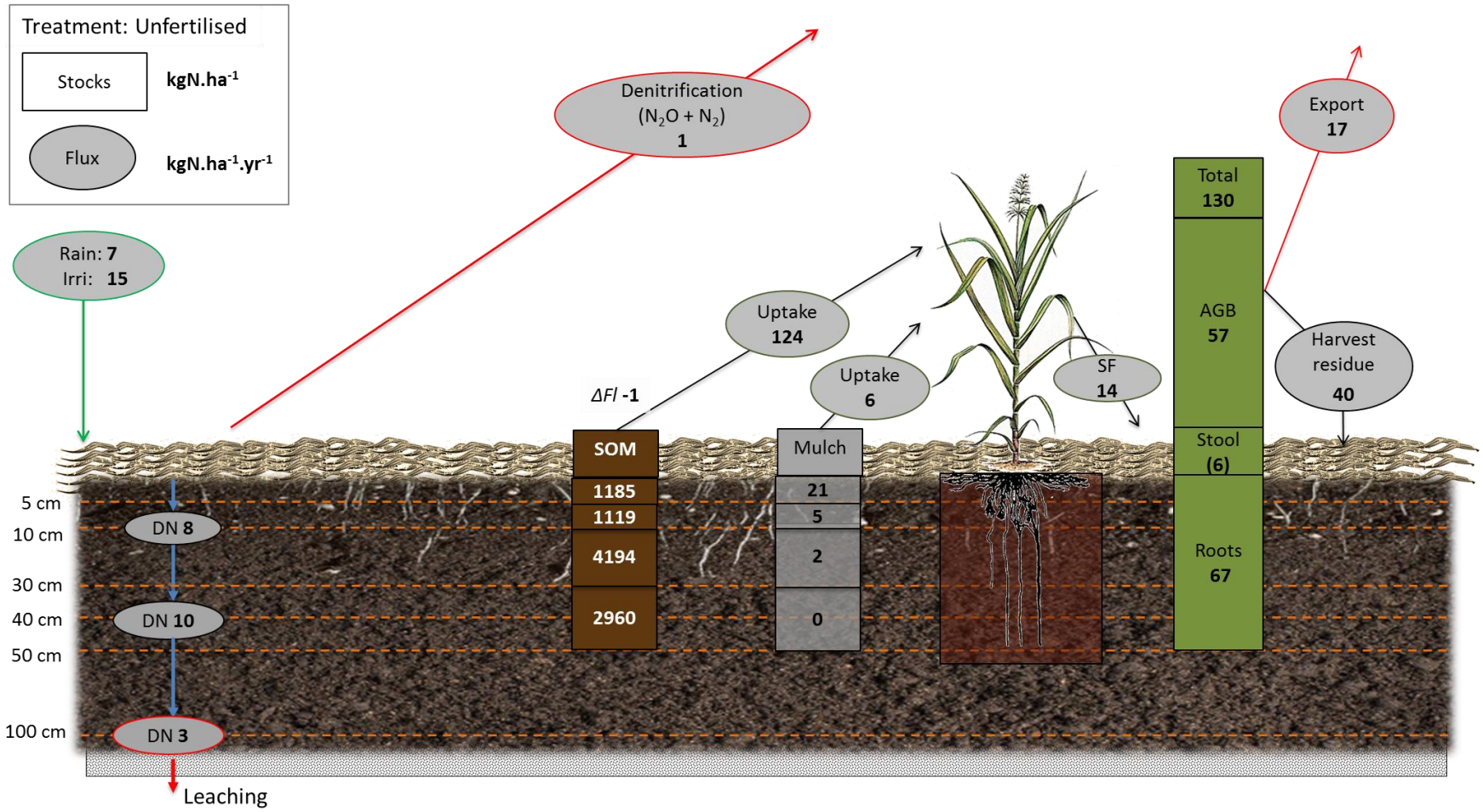

Figure 5.1 *Nitrogen budget for the unfertilised treatment, averaged over the two experimental years. Summarised are the N inputs of rainfall (Rain) and irrigation (Irri), and sugarcane green tops and straw left after harvest (Harvest Residue), and strawfall (SF) that falls to the soil over the sugarcane growth-cycle. The outputs of harvested sugarcane (Export), N emitted in the form of N2O and N2 gases via denitrification (Denitrification), dissolved nitrogen in soil solution (DN), with the portion at a soil depth of 100 cm lost from the soil-sugarcane system via leaching. The stocks of soil organic matter (SOM) and mulch (Mulch) and the relative uptake (Uptake) of these sources by the sugarcane are displayed. The N stocks in the different compartments of the sugarcane are shown, in the aboveground biomass (AGB), the stools (Stools), roots (Roots) and total N for the entire plant (Total).*

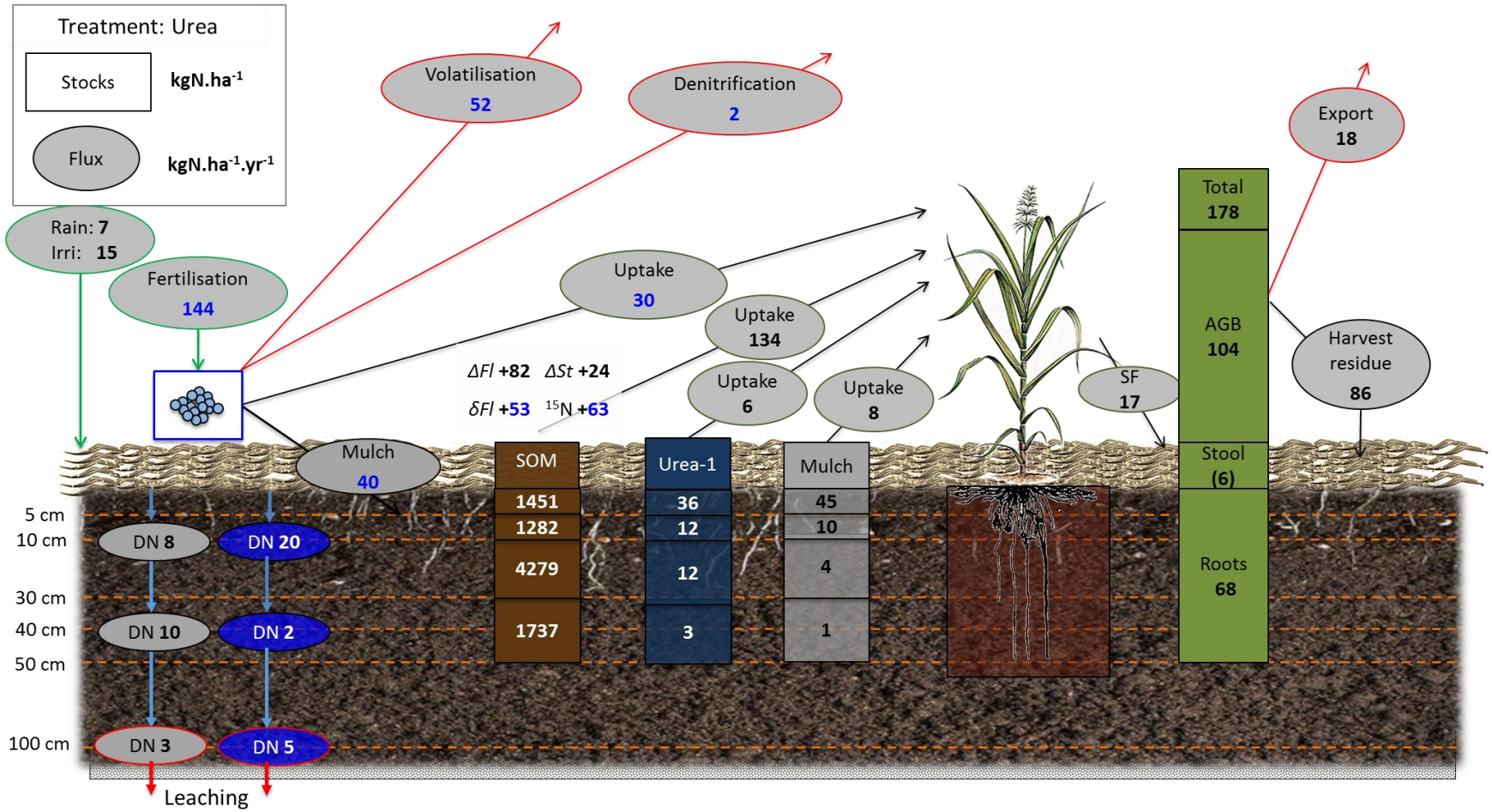

Figure 5.2 *Nitrogen budget for the urea treatment, averaged over the two experimental years. In addition to the N inputs of Figure 1, the urea fertilisation (Fertilisation) and its N uptake by the sugarcane (Uptake) as well as its content passing through the mulch (Mulch) is summarised. The N outputs via gas emissions of NH_3 (Volatilisation) and N_2O and N_2 via denitrification (Denitrification) are showed with N derived from urea in blue. The dissolved nitrogen in solution (DN) derived from the urea fertiliser is displayed in the blue bubbles. The stock of the previous ratoon's residual urea (Urea-1) and its N uptake by the sugarcane (Uptake) is shown. The amount of N stored in the soil from the urea fertiliser, by deduction from all the N inputs and outputs is displayed (Δ N flux), as well as its value when calculated using the ^{15}N content of the soil (^{15}N).*

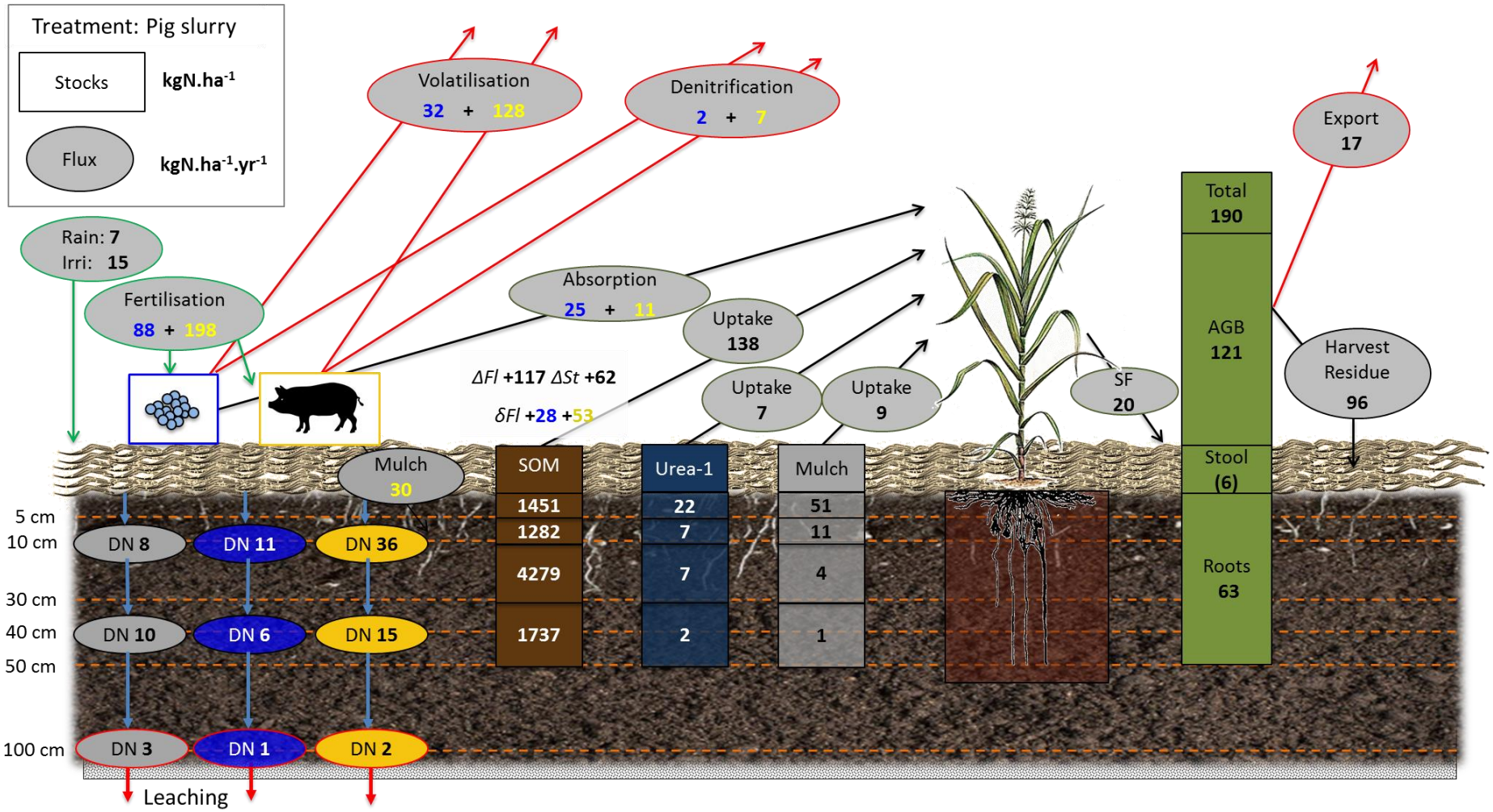

Figure 5.3 *Nitrogen budget for the pig slurry treatment, averaged over the two experimental years. In addition to the N inputs summarised in Figure 5.1 & 5.2; the N inputs and outputs derived from the pig slurry fertiliser are displayed in yellow (and that of its urea complement in blue).*

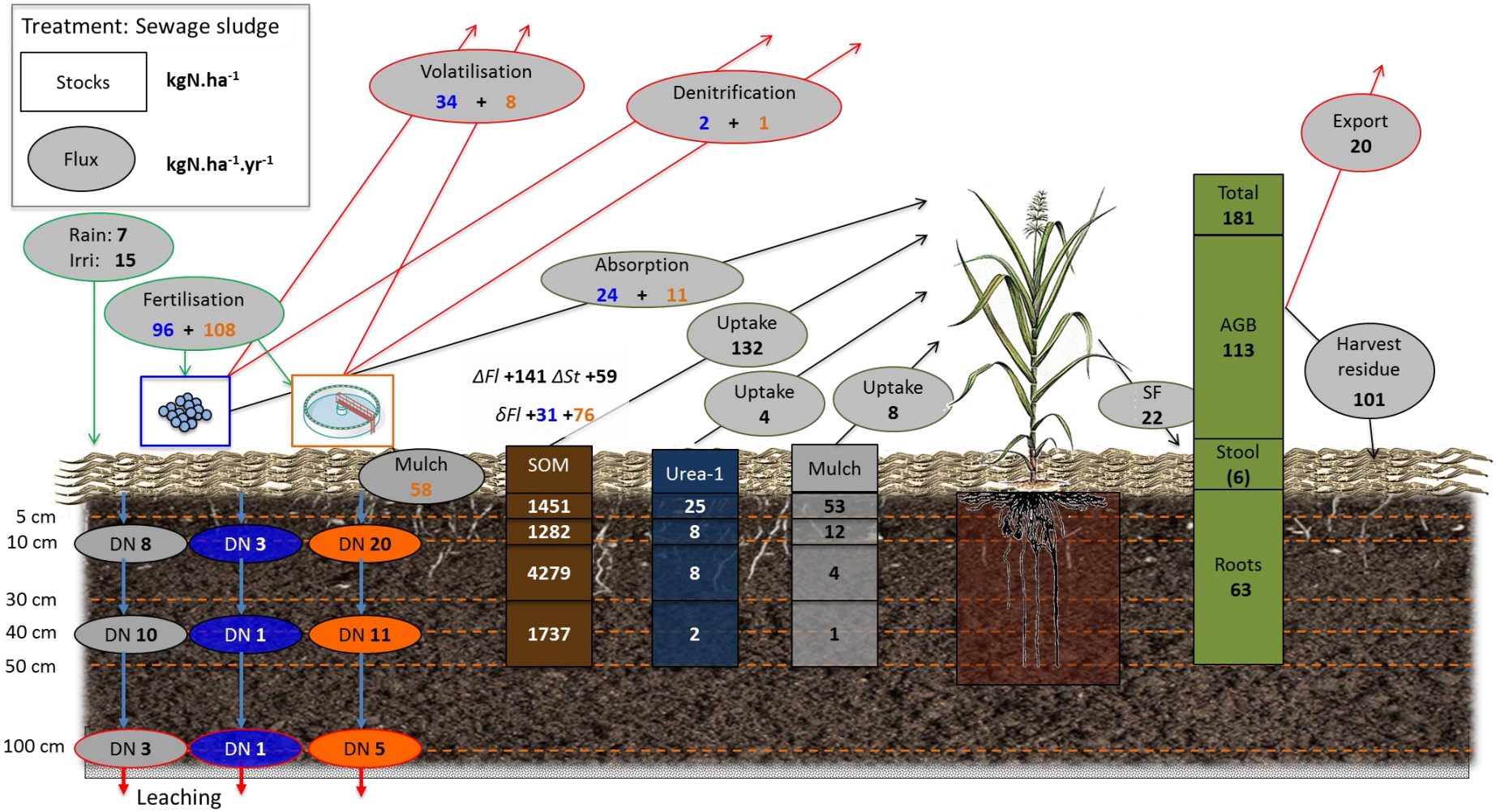

Figure 5.4 *Nitrogen budget for the sewage sludge treatment, averaged over the two experimental years. The N inputs and outputs follow the same logic and labelling as in Figure 5.3, except that sewage sludge-derived N values are in orange, and again the urea-complement values are in blue.*

5.1 Nitrogen loss via NH_3 volatilisation

One of the major N loss pathways from the soil profile after the application of fertiliser N is via gaseous ammonia (NH_3) emissions (Dattamundi et al. 2016). Agricultural application of N fertilisers is one of the major contributors to global atmospheric NH_3 emissions (Dattamundi et al. 2016, Loubet et al. 2018). While ammonia itself is not considered a major air pollutant, it plays an important role in atmospheric chemistry by neutralising precipitation and aerosol formation. This can have important environmental consequences (Behera et al. 2013), such as contributing to acid rain and indirectly to N_2O emissions (Cameron et al. 2013).

The majority of NH_3 emissions occur within a few days of fertiliser application, either from the soil profile or directly from the surface (Turner et al. 2012). This leads to a substantial reduction of N use efficiency of crops (Bouwman & Boumans, 2002; Dattamudi et al. 2016), and therefore has large agronomic and economic impacts for farmers (Loubet et al. 2018). The magnitude of NH_3 emissions is influenced by the type and dose of fertilisers used, the timing and technique of their application, as well as soil and meteorological conditions, especially soil moisture content (Dattamundi et al. 2016).

The NH_3 emissions were measured using ALPHA badges which are low-cost ammonia diffusion samplers that can be used to infer emissions from multiple agronomic plots (Loubet et al. 2018). These ALPHA badges were placed in the field directly after each harvest, and just before the application of the N fertilisers for a period of 6-7 weeks. At each of the fertiliser treatment plots, four ALPHA badges were placed on two masts at the centre of each plot, two badges each at 30 cm and 1 m above the soil. Four additional ALPHA badges were placed at each of the peripheral corners of the experimental trial. The geometry of the plots was traced using a high-precision GPS at the start of the experiment. The meteorological conditions, as well as wind turbulence, were monitored at a central small station at the centre of the experimental trial.

The ALPHA badges contain a solution of citric acid in ethanol which reacts in the air over a defined exposure period. Following the exposure period, the ammonium (NH_4^+) content in the badges was used to determine the average concentration of ammonia (NH_3^-) in the air in the centre of the plot during the period of exposure.

The ammonia concentrations, geometry and ultrasonic anemometer were then combined to infer emissions and their respective uncertainties using the FIDES model, which is a Eulerian dispersion model that has been validated for large field trials with high frequency ammonia concentration measurements (Loubet et al. 2010).

At our study site, the NH_3 gas emissions via volatilisation were responsible for a very large proportion of fertiliser N loss from the soil-sugarcane system. This was especially the case for the

urea and pig slurry fertilisers, both with substantial proportions of mineral N. Between 26 % and 66 % of urea-applied N was lost over the first 2 months, and 65 % to 85 % of pig slurry N was lost within the span of 2 months after fertilisation (Years 2017, 2018; Figure 5.5). Organic fertiliser NH_3 emissions have been rarely studied in the context of sugarcane. However, in a study on mineral fertiliser NH_3 emissions from sugarcane in Brazil, Costa et al. (2003) found 36 % of the urea-applied N was lost via volatilisation, which is the same as the mean N loss for the urea treatment in our study, over the two experimental years (Figure 5.2 above). For the sewage sludge treatment, however, far less of the applied-N was lost by this process. Only 5 % to 10 % of the applied sewage sludge N was lost via volatilisation over the 6-7 week duration after application.

Over the second year (Year 2018; Figure 5.5), there was a lower level of fertiliser applied-N loss via volatilisation, across certain fertiliser treatments. The reason for this lower level of N loss is likely because there was a high rate of irrigation over the first few months. Given that soil moisture is one of the notable factors influencing the extent of volatilisation, irrigation appears to have led to a decrease in volatilisation. In turn, with the high soil moisture and lower volatilisation, this is likely to be partly why there was a higher rate of N loss via leaching over the second year than the first year. As can be seen, for the five years of the experimental trial (Figure 5.5), there is quite a large variability in the NH_3 emissions for the different years. This is possibly a result of differences in pedoclimatic conditions and soil moisture, for example. In addition, for the pig slurry, the mineral-organic N composition can be very variable between applications. When there is a higher mineral N content, there is a higher risk of loss via volatilisation. The composition of sewage sludge is far more consistent between years, due to the process in which it is processed, leading to a more predictable and less variable product.

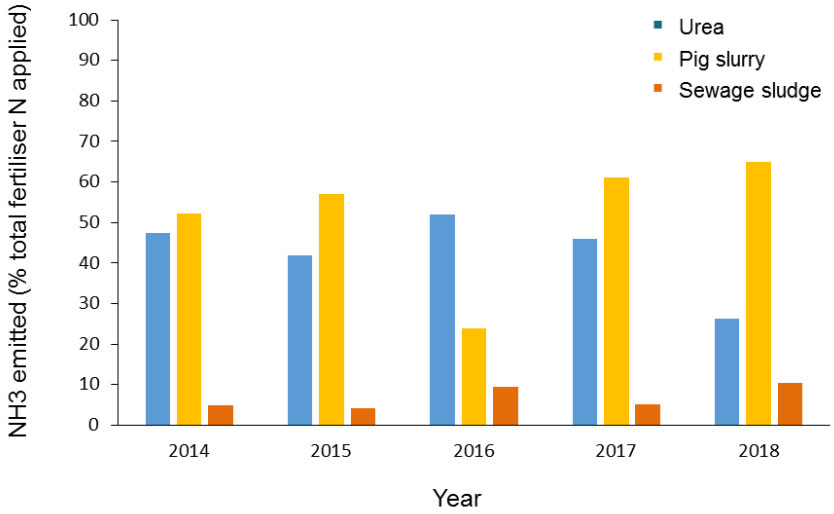

Figure 5.5. Fertiliser N lost via volatilisation for the different fertiliser treatments of the Soere-PRO experimental trial between 2014 and 2018 (Detaille, Versini, pers comm.)

5.2 Nitrogen loss via N_2O emissions

Nitrous oxide (N_2O) emissions are generally not responsible for a large proportion of the N budget but have a considerable environmental impact. From a meta-analysis of global sugarcane N_2O emissions, Yang et al. (2021) found that mean yearly global sugarcane N_2O emissions are 2.26 kg $N2O\text{-}N.ha^{-1}yr^{-1}$. It is, however, a major greenhouse gas contributing to global warming, and agricultural soils are the most significant anthropogenic source of N2O (Stehfest & Bouwman, 2006). In addition, humid tropical soils, such as at the experimental site of this study, favour the production of N_2O (Weitz et al. 2001).

In the experimental site of this study, automated gas chambers were used. Automation has several advantages, including that this allows gas emissions to be taken at frequent intervals and to define the shape of the N_2O response curve to N fertilisation and irrigation. Unforeseen episodic events (e.g. storms with high rainfall) are also more likely to be captured with these regular and frequent measures, which may otherwise be difficult to detect in time with the use of manual measurements (Grace et al. 2020). Additionally, when sampling at intervals of several days, for example (which is shown to be common in manual gas emission assessments according to meta-analyses such as Barton et al. 2015), there is the possibility of missing, or underestimating, significant emissions (Smith & Dobbie, 2001). Part of the reason for the uncertainty of current global estimates of N_2O emissions from agricultural soils may be as a result of the sampling frequency of datasets used (Barton et al. 2015). Infrequent sampling, which is often the case with manual gas emission collection studies, has the potential of overlooking both diurnal variability as well as day-to-day variability in N_2O emissions (Grace et al. 2020).

In Block 2 of the SOERE-PRO experimental trial, 3 automatic gas chambers were placed centrally in each of the different treatment plots (12 chambers in total). At the same time, air conditions were measured for the CO_2 and N_2O ambient concentrations. These automatic chambers were placed in the plots directly after each harvest. The chambers were placed at a soil depth of approximately 5 cm to ensure that there was no gas exchange between the inside and outside of the chambers when the chambers were closed, and when gas was collected. The chambers would close for 20 minutes four times each day, on a 6-hour rotation. The different gases emitted from the soil were trapped in the chambers and then, through a circuit of capillaries, the air was pumped to gas analysers. Each chamber had nylon tubes extending from the chamber connected to a central station between the plots. A pump would drive the air from the chamber to the central measurement station via these nylon tubes. Measurements of the N_2O emissions were taken in the central station using an in-situ gas chromatograph. Every 10 seconds, a measure of the concentration of N_2O and CO_2 was taken by the devices measuring the infrared absorbance of the

gases to measure their respective concentrations. The concentrations were stored in a Cambell Scientific CR3000 data logger.

In addition, the N_2 emissions via denitrification were estimated. N_2 was roughly estimated to be 10 times the value of N2O emissions for each fertiliser treatment. This ratio appears to be highly variable (Thorburn et al. 2010), The ratio was estimated based on several emissions run by another PhD student in the Cirad Recyclage & Risque unit, using the crop model STICS (Simulateur mulTIdisciplinaire pour les Cultures Standard). Similarly, Thorburn et al. (2010) found an N2/N2O ratio of 8.7 when residue was retained from diverse sugarcane production systems in Australia. In another study by Friedl et al. (2016) for an intensively managed sub-tropical pasture, this ratio was between 8 and 17, for 80 % and 100 % water-filled pore space sites.

The N_2O emissions for SOERE-PRO experimental site for the year 2019-2020, as determined by Detaille et al. (pers comm.) is presented in Figure 5.6. The daily emissions, as well as the accumulation of daily emissions for each fertiliser over the ratoon are shown. In the calculations for the N budget, the average cumulative N_2O emissions over the year 2017-2018, as well as the ratoon two years after over 2019-2020, were used. For the second experimental year (October 2018 – end September 2019), the automatic chambers were placed in other fertiliser treatment types (not evaluated in my study) in the experimental trial.

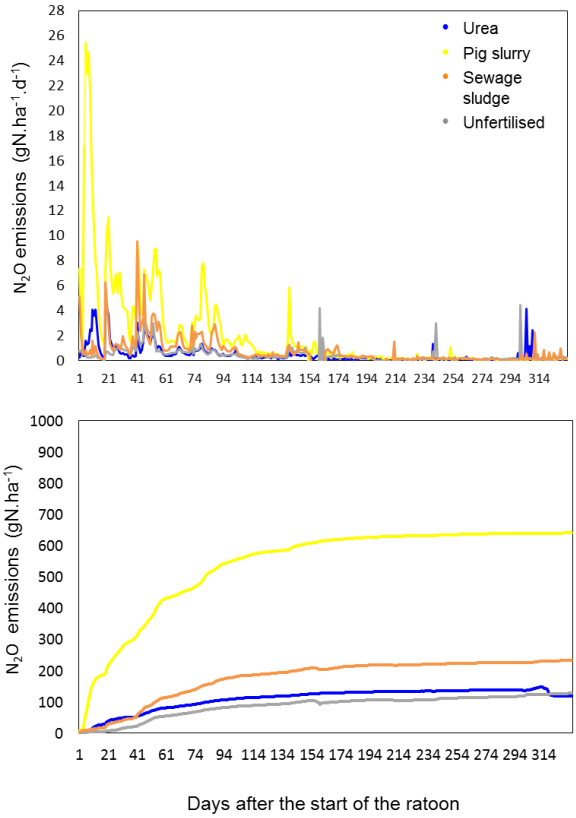

Figure 5.6 Daily N2O emissions (above) and cumulative emissions (below) for each of the fertiliser treatment types over a single sugarcane ratoon growth-cycle (2019-2020) (Detaille, pers. Comm.).

5.3 Mulch as a temporal sink of fertiliser-N

In terms of N input into the sugarcane-soil system, the mineral and organic fertiliser inputs have been evaluated in detail. An additional component which was also studied, but which was too small an experiment in this study to constitute a full chapter, was sugarcane mulch (or "residue" consisting of sugarcane straw and green tops) left after harvest. This is potentially an important N source in sugarcane agroecosystems, which appears to have a low contribution to sugarcane N content in the short-term, but may be beneficial in terms N contribution to the sugarcane-soil system over the long-term (Trivelin et al. 2013, Meier & Thorburn, 2016). In addition to nutrient recycling, mulch contributes to various other agroecosystem services such as water storage and the retention of soil humidity, carbon accumulation, the reduction of soil erosion, as well as reducing weed infestation (Carvalho et al. 2017).

Directly after each harvest, the dry mass of mulch per unit square metre was determined for the whole experimental trial. Three wooden quadrats of 50 cm^2 were placed in central positions in each of the plots of the SOERE-PRO experimental trial. These were weighed directly after the mulch was collected, and one of the mulch samples from each plot was kept in the laboratory to be dried at 60°C for 72 hours, and to determine the percentage moisture and dry mass. These samples were later ground and analysed for N using the apparatus described in Chapter 2.

With the mean mass of mulch per unit area calculated for the experimental trial, 3 repeats of 4 mesocosms were placed in the four different fertiliser treatment plots; 1/ unfertilised; 2/ urea; 3/ sewage sludge; 4/ pig slurry. The mesocosms were cylindrical PVC rings with a diameter of 40 cm and a height of 10 cm. Holes of approximately 2 cm diameter were drilled into the mesocosms close to the base, to allow for macrofaunal interaction and passage between the inside and outside of the mesocosms, as shown in the Experimental Site description (Photo 5, p27).

There are four sampling dates over the ratoon (i.e. samples collected every 3 months), with three repetitions per sampling date. At each date, the mulch remaining in each of the three mesocosms per fertiliser treatment were removed. The mulch was teased by hand to remove mud clusters and any stones in the sample. The mulch was dried in an oven at 60°C for 72 hours and then weighed to obtain the dry mass of each sample. All dried samples were ground to pass a 1 mm mesh using a Universal Cutting Mill (PULVERISETTE 19, Fritsch) and analysed for N with an elemental analyzer (Vario Max Cube CNS, Elementar, Hanau, Germany) in the CIRAD laboratory in Saint-Denis (La Réunion, France).

The fertiliser N decreased far more rapidly over the second year and then stabilized already at 1 month after the start of the ratoon. The fertiliser N loss in the first experimental year was slightly more progressive at first, but then the N fertiliser reached close to zero already at 4 months after the start of the ratoon, which was earlier than over the second year. It is possible that the

reason for the initial rapid lowering of fertilisers in the mulch over the second year was due to the consistently higher quantity and frequency of irrigation applied to the trial over the months following harvest over the second year, with the intention of lowering N losses due to volatilisation. The majority of the initial fertiliser N was therefore possibly transported in solution directly to the soil after the application, and the remainder immobilised by microorganisms in the mulch, hence the stabilisation of fertiliser N thereafter. One difference between the rates of transfer of the fertiliser types may be as a result of their different physical aspects, and certain fertilisers (e.g. pig slurry) may have a tendency to flow more readily through the mulch, whereas sewage sludge pellets may temporarily be trapped in the mulch (Kyulavski et al. 2019).

The C:N ratio decreases rapidly across treatment types until reaching a value of approximately 30-35 between 4 – 7 months after the start of the ratoon (Figure 5.7 E & F), for the two experimental years. This is in a coherent range to Meier et al. (2006) who found a C:N ratio of 30 after approximately 200 days. At this point in the growth-cycle, the fertiliser N has already passed through the mulch (Figure 5.7 E & F), further suggesting that the N mass which is "released" from the mulch originates from the mulch N source.

The mulch has a small but consistent contribution to the sugarcane nutrition, as explained in more detail in Chapter 3 (2.3 – 4.5 %), which is in line with several other studies on sugarcane mulch contribution to sugarcane N content, with values ranging from 0.6 % – 5.4 % (Basanta et al. 2003, Meier et al. 2006, Dourado-Neto et al. (2010), Fortes et al. (2013)). This finding has led to a further study currently taking place in the Cirad Recyclage & Risque (by Carol Tanner) on 9 sites around the island, which has found an average mulch NRE of approximately 4% (Tanner & Versini, pers. comm).

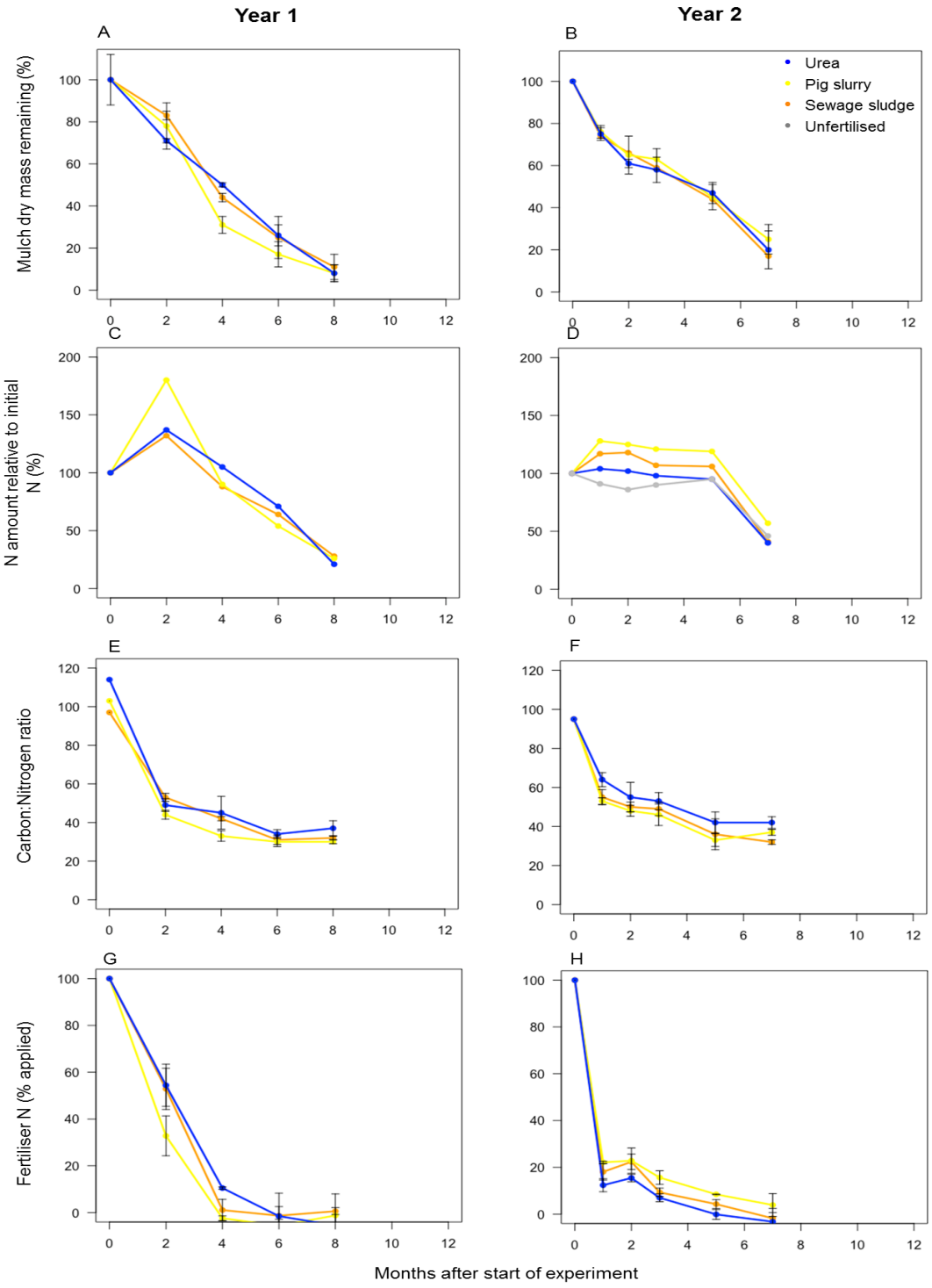

Figure 5.7 *Mulch degradation dynamic over the two experimental years for each of the fertiliser treatments (urea, pig slurry, sewage sludge). The top row is the dry mass of mulch remaining in the mesocosms over each experimental year (A, B). The second row is the percent N amount of the remaining mulch relative to the starting applied mulch N mass (C, D). The third row is the carbon:nitrogen ratio in the mulch over the sugarcane ratoons (E, F), and the bottom row is the percent fertiliser N found in the mulch over each of the experimental years (G,H).*

5.4 Fertiliser-N uptake by sugarcane

5.4.1 Filling the gap between the difference and ^{15}N isotopic methods

In Chapters 2 and 3, the difference in the values obtained for the NRE was described, when calculated using the difference method (dNRE, Chapter 2) and the isotopic method (iNRE, Chapter 3). When considering only the aboveground biomass (without strawfall or tiller senescence), as in most other studies evaluating NRE, the dNRE for the urea treatment was on average 34 % and 21 % over the first and second years, respectively. The iNRE calculated for the aboveground biomass of this same urea treatment was lower for both years, with a mean iNRE value of 16 % and 9 % over the first and second years, respectively.

This difference between NRE values determined by using the two different methods is consistent with other studies. The NRE calculated using the isotopic method (iNRE) has also been found in other studies to be typically lower than that of the difference method (dNRE) (Krupnik et al. 2004, Dobermann et al. 2005, Ladha et al. 2005). For example, in a review of global N use efficiencies in cereal production, Ladha et al. (2005) found that iNRE values were on average 7 % lower than dNRE values, across all regions and cereal crops.

The difference in NRE values has often been attributed to "pool substitution", where a portion of the initial ^{15}N applied is hypothesised to be immobilised in microbial biomass, coinciding with the early release of ^{14}N from the microbial biomass; or that the ^{15}N in the form of $^{15}NH_4$ is adsorbed on the mineral surface and exchanged with $^{14}NH_4$ which is desorbed and taken up by the plant (Hauck & Bremner, 1976; Jenkinson et al. 1985; Roberts & Janzen, 1990, Krupnik et al. 2004). This would result in a lower ^{15}N fertiliser recovery in the crop, since a lower proportion of applied ^{15}N reaches the plant, which may explain a consequent underestimation of the NRE with the use of the isotopic method. Pool substitution is frequently cited as a reason for this discrepancy in values between the two methods, but this is often not tested. And in the experiments where it is tested, it is typically performed in laboratory conditions in temperate regions, and over a short duration (of a few weeks, for example).

Given that my study takes place *in situ* in the sugarcane agroecosystem over a duration of one year for each ^{15}N application, and that the field site is subject to tropical conditions which could accelerate the functioning and N exchanges of the soil-plant system, it is also possible that the inverse process happens. This would mean that ^{15}N immobilised by soil microbial bacteria is again mineralised and taken up by the plant, which would lessen the impact of pool substitution on its suggested potential to decrease iNRE.

Another explanation for this difference between methods of calculating NRE, is that the addition of N fertiliser leads to an increase in crop-available N resulting in an over-estimation of

the dNRE calculated with the difference method as compared to the isotopic method iNRE (Cassman et al. 2002, Ladha et al. 2005). The "added-N effects" could occur if the root development of an N-fertilised crop were to increase, leading to the plant accumulating N from a deeper soil depth than the unfertilised crop (Krupnik et al. 2004, Ladha et al. 2005).

At our study site, N fertiliser application does not stimulate greater root development, nor the ability to colonise deeper into the soil profile (as shown in more detail in Table 4 of the paper included in Appendix 7.2, p163). In fact, the unfertilised treatment sometimes had a higher root biomass. Increasing crop-available N through N fertilisation therefore did not increase the plant root system to acquire N from the soil, and it therefore seems unlikely that "added-N effects" could lead to errors in the NRE calculation by this method.

Another added N effect would be if the rate of soil organic matter N mineralisation were to increase due to N fertilisation. Organic fertilisation is a common practice leading to considerable input of relatively labile C into agricultural soils and the input of organic fertilisers could therefore increase the mineralization of native OM (i.e. priming effect). Despite the large number of studies on the priming effect over the last 20 years, the presence of such a process in the context of organic fertilisation practices remains largely unknown.

In our study, when considering the root compartment, this results in almost a doubling of the iNRE, which results in values far closer to that of dNRE. Thus the consideration of this mostly disregarded sugarcane compartments appears to create more of a convergence between values obtained by the two approaches. In Chapter 2, there was not a consistent trend when the root compartment was considered, in terms of its effect on the calculated dNRE. The first year aboveground biomass dNRE of 34 % increased to 36 % when the root compartment was also considered, and the second year dNRE of 21 % decreased to 17 % when the root compartment was considered. In Chapter 3, the average iNRE of 16 % for the first year increased to 27 % when the root compartment was considered, and the iNRE of 9 % over the second year increased to 15 % when the root compartment was considered.

The root compartment clearly has a very large impact for the isotopic method, but far less so for the difference method. In addition, the difference in values between the two methods was smaller when the roots were considered, given the substantial resultant increase in the iNRE. The reason why the root compartment has less of an impact on the dNRE values was because the root N mass is not necessarily higher for the fertilised treatment than the unfertilised treatment, as can be seen in the Appendix Chapter (Versini et al. 2020).

This finding led the research unit to propose a new experiment conducted within the framework of the PhD of Marion Ramos, also in the CIRAD Recyclage et Risque research unit. In this parallel study, a number of experimental sites were evaluated at different locations around

Reunion Island, with different soil types and subject to a range of different pedo-climatic conditions. The NRE was determined for sugarcane at these experimental sites using both the ^{15}N isotopic and difference methods. In addition, both the aboveground and belowground biomass compartments were sampled and NRE was calculated for both of these compartments in Ramos's study. When only the aboveground biomass is considered, there is a tendency for the calculated iNRE to be lower than the dNRE (Figure 5.8). When considering both the belowground and aboveground compartments, there is more of a convergence between the two methods for many of the experimental sites. However, there are exceptions. For certain sites, the opposite trend was found, with higher values obtained using the isotopic than difference method.

There does not therefore seem to be a specific "rule" in establishing a congruency between the two methods, at least not in the data explored here. This is coherent with the argument of Krupnik et al. (2005) that sometimes the NRE for grains was higher using the difference method than the isotopic method, but sometimes the opposite was true, varying with regards to whether the data was in its upper or lower ranges of NRE, as well as whether the crop straw was included in the calculation. This being said, there is a clear benefit in evaluating the root compartment, especially when using the isotopic method.

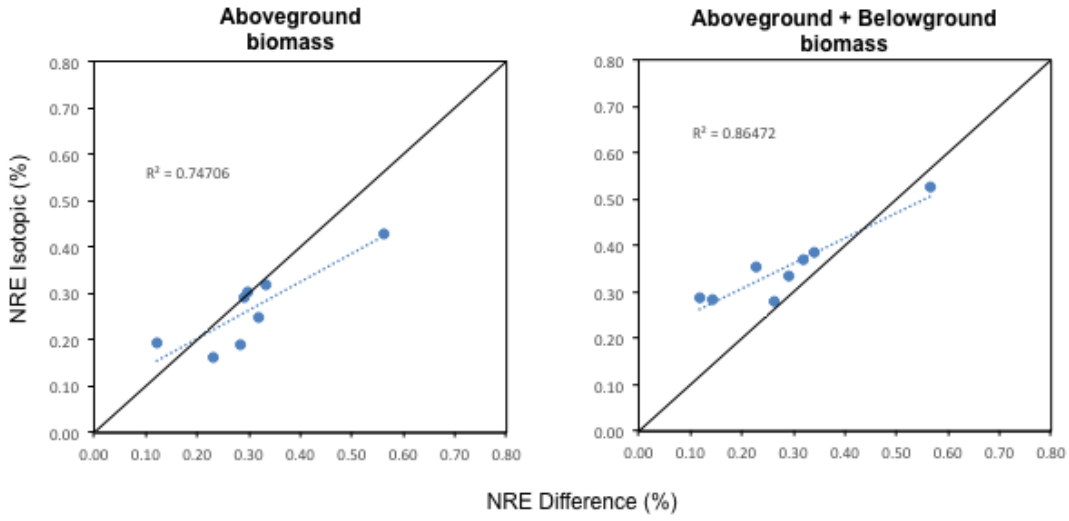

Figure 5.8. Regression between NRE using the difference and ^{15}N isotopic methods, for the aboveground biomass and the aboveground biomass combined with the belowground biomass (Ramos, pers comm).

An ideal situation would be to have a congruency between the difference and isotopic methods, since each has its merits. The difference method is simple and cheap to use and requires fewer analytical facilities, making it easy to calculate using on-farm measurements (although these farms do not often have many unfertilised plots), and on a global scale, is more frequently used (Dobermann et al. 2005, Ladha et al. 2005). However, more precise estimates of N use efficiency

can be determined using the isotopic dilution method (Smil, 1999, Smil, 2002, Ladha et al. 2005). In addition, the ^{15}N isotopic method allows the passage and fate of N to be traced from the source (^{15}N marked fertiliser or mulch, for example) and quantified in its various N pool "destinations", being the crop (and its different aboveground compartments), as well as in the soil for example (Versini et al. 2014).

5.4.2 Tackling the late-cycle ^{15}N deficit of the iNRE method

There was a decrease in the NRE values over the sugarcane growth-cycle for both methods in my study. However, there was more of a decrease for the iNRE described in Chapter 2 than the dNRE in Chapter 3.

In the few studies that consider the temporal variability of iNRE over the crop growth-cycle, there is a far more noticeable decrease in the iNRE over the crop growth-cycle when determined using the isotopic rather than difference method. This has been observed in the few studies evaluating iNRE over the sugarcane growth-cycle (Ng Kee Kwong & Deville, 1994, Courtaillac et al. 1998). By contrast, the dNRE supposedly remains more stable over the growth-cycle (Ng Kee Kwong & Deville, 1994), or may tend to decrease, but not to the same extent as the isotopic method.

One set of hypotheses in my study, was that a portion of the applied ^{15}N was lost from the sugarcane plant during the senescence and turnover of aboveground and/or belowground compartments. The first hypothesis was that if there was a sufficient amount of N from fertilisers which ended up in the dead leaves that fall from the plant as strawfall from mid-growth cycle to the following harvest, this would potentially "dampen" or reduce the decrease in NRE which also begins from approximately mid-growth cycle and continues until harvest. However, when incorporating the strawfall (as seen in Chapter 3), this did reduce the decrease in dNRE but the decrease in iNRE was only reduced slightly, and not sufficiently to counter-balance the tendency of the iNRE to decrease steadily over the crop growth-cycle.

The second hypothesis was that a portion of the ^{15}N in the sugarcane is translocated from the aboveground biomass and stored in the root compartment and therefore not detected in most studies evaluating iNRE, which only consider the aboveground biomass, leading to this decrease in iNRE later in the cycle. We have already demonstrated in Chapters 2 & 3 and the Annexure Chapter 7.2 that a major portion of the sugarcane N is in the root biomass. When calculating the iNRE, the assumption we used was that the ^{15}N in the plant was distributed homogenously, i.e. that the belowground biomass would have the same ^{15}N signature as the aboveground biomass. However, root N storage following a ^{15}N "pulse" after application could lead to the preferential storage of ^{15}N in the root compartment.

In a greenhouse experiment conducted during my study, the hypothesis of ^{15}N relocated from the aboveground biomass and stored in the belowground biomass was tested (Figure 5.9). Sugarcane was planted in a container with inert river sand (i.e. without soil organic matter). The sugarcane was fertilised with ^{15}N-labelled urea. Over the growth-cycle, there was a progressive translocation of ^{15}N from the leaves to the roots over the sugarcane growth-cycle. This could contribute to why, in most studies which do not consider the crop roots, there is a steady decrease in iNRE. The decrease in ^{15}N in the aboveground biomass would lead to a decrease in the calculated iNRE when only the aboveground biomass is evaluated, where in fact this would be an underestimation since the ^{15}N remains in the plant, but it has been translocated to the roots. This again emphasises the need to evaluate the belowground biomass in sugarcane NRE studies.

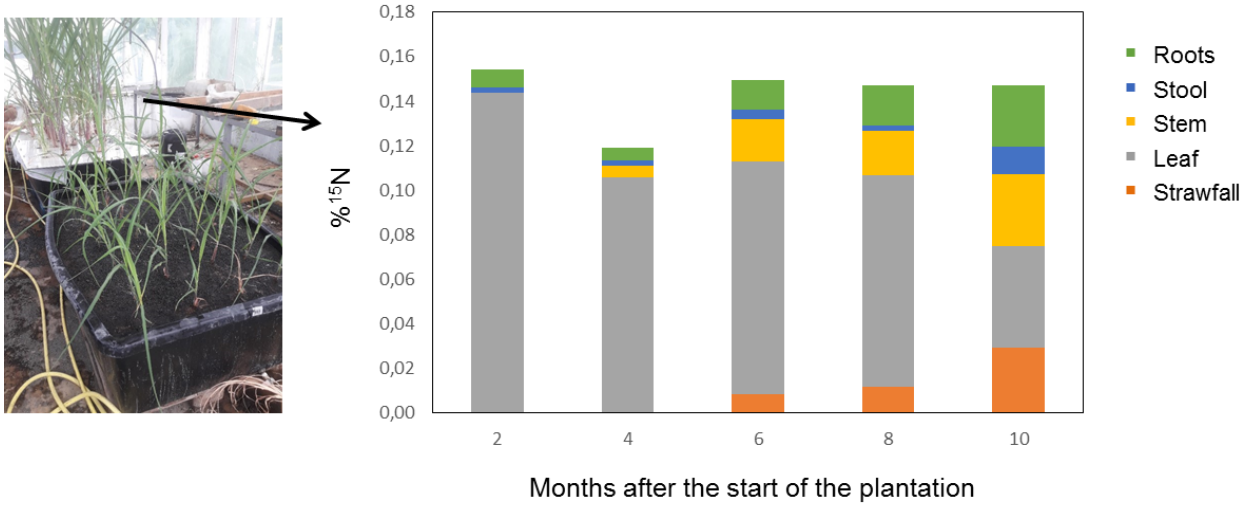

Figure 5.9. ^{15}N amount distribution in each of the compartments of the sugarcane plant in a greenhouse experiment.

To test this further, at the end of my second experimental year, I harvested sugarcane at the centre of the microplots, including the stool and roots. However, the ^{15}N of the belowground biomass was still not sufficient to eradicate the decrease in NRE.

A hypothesis could be that during the process of root turnover with the senescence of roots, a certain proportion of ^{15}N is lost with these roots, and replaced by new roots enriched in ^{14}N (derived from the SOM for example) and not the labelled ^{15}N. If there is a substantial root turnover between mid-growth-cycle and the following harvest, this would lead to an underestimation of ^{15}N in the plant and a decrease in total sugarcane iNRE during the process of root turnover.

Little is known with regards to root mortality and its rate of turnover is not known for sugarcane (Smith et al. 2005, Robinson et al. 2013). Investigating sugarcane root turnover would be a very interesting and informative topic of research.

In an evaluation of global patterns of root turnover for different plant species in terrestrial ecosystems, Gill & Jackson (2000) found average yearly root turnovers to be 1.4 for small diameter roots (5 mm or less), 1.6 for grassland fine roots (perhaps the closest morphologically to sugarcane) and 1.9 for shrublands. For certain species of trees, values of 1.3 to 1.8 have been reported for *Eucalyptus* for example (Jourdan et al. 2008), and 2.0 for Acacia for example (Lehmann & Zech, 1998). Using these known values, if we use a rough estimate based on other terrestrial plants, we could use a hypothetical rate of 1.7 year^{-1}. If this is considered, and the total root N mass was increased by 1.7, the sugarcane total N mass would again increase substantially (Figure 5.10). If the total sugarcane N mass increased, as is suggested in this theoretical scenario, the iNRE would likely increase as well, possibly resulting in even less of a discrepancy between iNRE and dNRE.

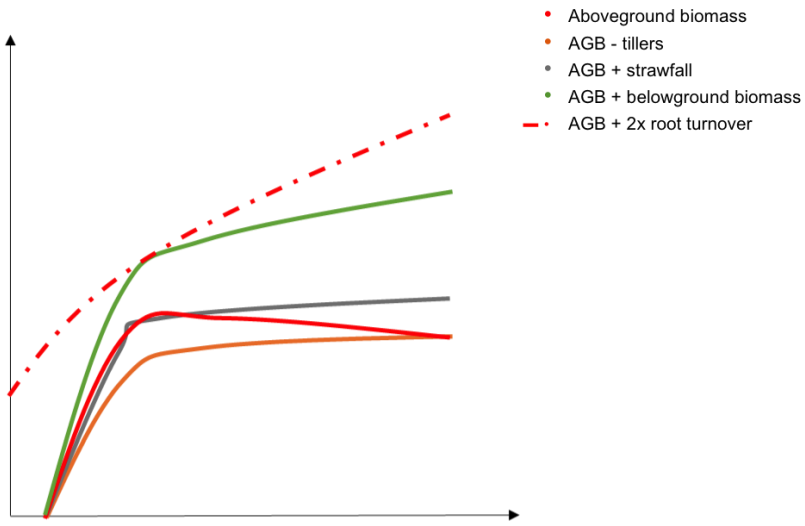

Figure 5.10. Theoretical figure showing sugarcane N mass considering different N mass components for the urea fertilised treatment. This is shown for the baseline aboveground biomass (Aboveground biomass), as well as the aboveground biomass after tiller senescence has been subtracted (AGB-tillers), after strawfall has been added (AGB + strawfall), after belowground biomass is added (AGB + belowground biomass measured) and after belowground biomass with root mortality has been added (AGB + 2 x theoretical root turnover)

5.4.3 Revisiting N absorption

As already discussed, it appears that a certain portion of N from the aboveground biomass is stored in the roots over the sugarcane growth-cycle. The question of interest now, is whether there is a remobilisation of this N in the following ratoon crop after harvest?

There have been certain suggestions that there may be N remobilised from the belowground compartments, and which becomes a source of N for the subsequent sugarcane ratoon (Robinson et al. 2009, Robinson et al. 2014), but this appears not to have been tested yet. For certain other crops, such as *Miscanthus* x *giganteus*, there is a large translocation of N to the belowground compartment (especially the rhizome) at the end of the growth-cycle, which forms a substantial N reserve for the resprouting aboveground biomass after harvest (Ferchaud et al. 2016).

In the greenhouse experiment already described, the hypothesis of ^{15}N remobilisation from the belowground biomass to the aboveground biomass compartment was tested (Figure 5.11). There was a clear remobilization of ^{15}N in the plant at the end of the plantation (i.e. when the cane was harvested) and at 2 months after the start of the first ratoon. No additional fertiliser is added at this point, and the ^{15}N in the whole plant at 2 months after the start of the following ratoon is almost exactly equal to that of the belowground compartment (roots & stool) close to harvest at the end of the previous plantation. This strongly suggests a translocation of ^{15}N from the roots to the leaves when the sugarcane is cut at the end of the growth-cycle. This supports the hypothesis that there is remobilisation and that there is potentially a higher role of previous ^{15}N fertiliser applications than previously estimated.

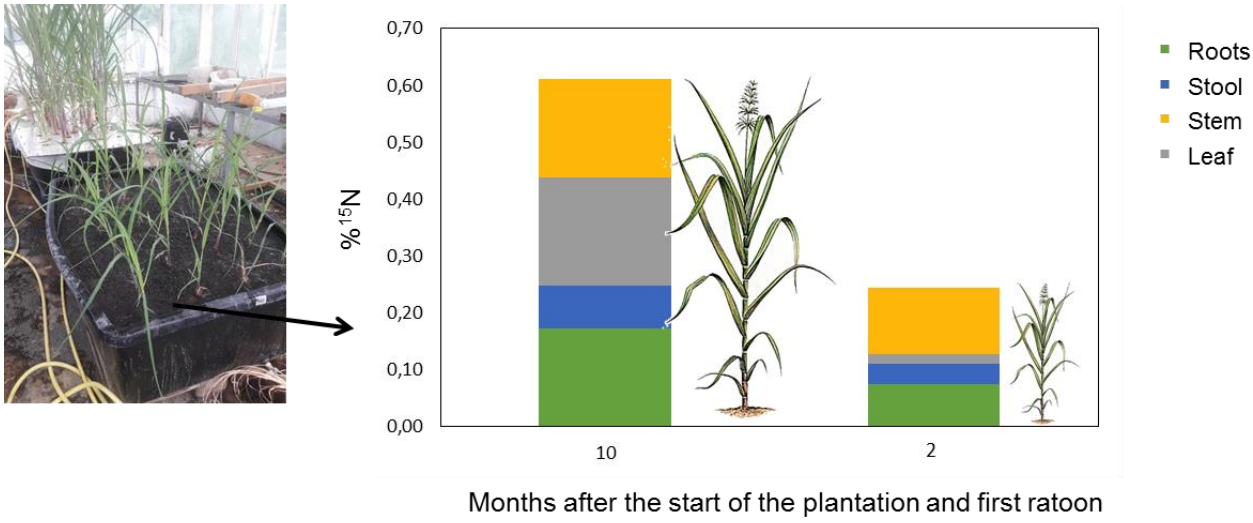

Figure 5.11. Amount of ^{15}N distributed in each of the compartments of the sugarcane plant in a greenhouse experiment, at 10 months after the start of the plantation and at 2 months after the start of the first ratoon.

In considering the remobilisation, or translocation of ^{15}N from the roots at the end of one growth-cycle, to the aboveground biomass of the new shoots after harvest, this might well change the calculation of the contribution of previous fertiliser applications to the sugarcane N content. If the ^{15}N enrichment from previous urea fertilisation is considered rather as what is stored in the roots at the end of the previous growth-cycle, the ^{15}N enrichment is approximately 0.5 % rather than 3.5 % (of the applied ^{15}N labelled urea), and previous fertiliser application has far more of a contribution to sugarcane N than previously calculated (Figure 5.12). This also suggests that fertiliser NRE may be underestimated to an extent. This would support the ideas suggested by research papers such as that of Krupnik et al. (2004) and Ladha et al. (2005) who suggested that along with the root compartment which is often not evaluated, the influence of previous fertiliser applications on N use efficiency is often overlooked, and may lead to global underestimations of NRE (Krupnik et al. 2004, Ladha et al. 2005).

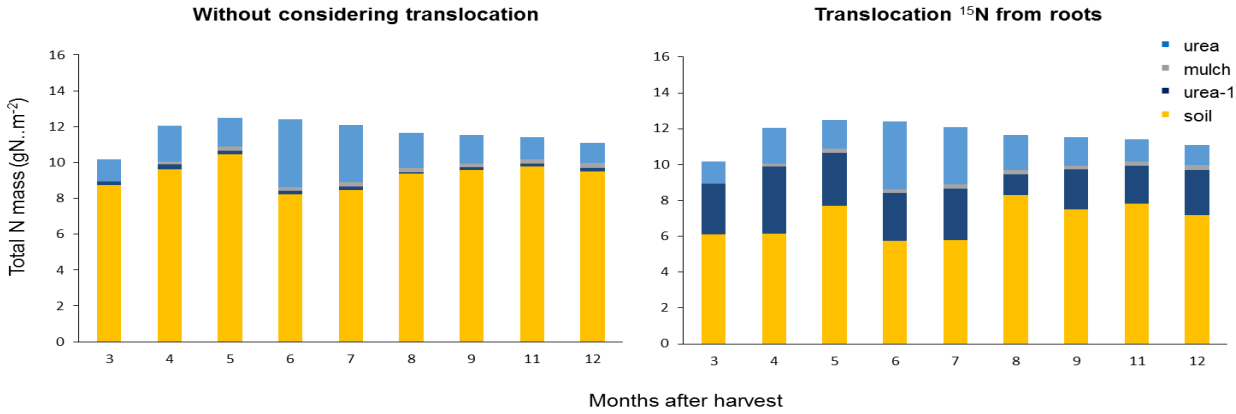

Figure 5.12. Total N mass derived from different N sources. On the left, when translocation of ^{15}N from the root compartment is not considered and on the right where it is considered.

5.5 Leaching after considering N loss via volatilisation

There appears to be an important relationship between the processes of N-loss via volatilisation and the quantity of N derived from fertiliser N input in soil solution, as well as what is eventually lost from the soil-plant system via leaching. Essentially, with higher initial volatilisation, there is a lower remaining fertiliser N quantity in solution which reaches the different soil depths. This is potentially because after a high loss via volatilisation, there is a lower proportion of fertiliser derived N in an available (mineral) form which can be lost readily via leaching (Robinson & Röper, 2003). By the same logic, when there are lower losses of N from volatilisation, there is likely to be a higher potential for N loss via leaching.

In Chapter 4, fertiliser N in solution was considered without taking volatilisation into account. When the proportion of fertiliser N in solution is evaluated after considering N losses via volatilisation, quite a different light is shed on the level of fertiliser-N loss via leaching for the different fertiliser treatments (Table 5.1).

Before considering loss via volatilisation, it appeared that the proportional loss of the different fertiliser derived N via leaching, was similar between treatments. However, given that there is a very large disparity between the proportions of fertiliser-N lost via volatilisation; this suggests that the rate of N loss from the different fertilisers is in fact quite different. For example, after accounting for loss via volatilisation, a far higher proportion of the remaining fertiliser N is found at the different soil depths for urea and pig slurry, than before volatilisation was considered. There is far less disparity for sewage sludge, since a far lower quantity is lost via volatilisation.

This suggests that one of the reasons the levels of loss via leaching are relatively low for urea and pig slurry, is because the majority of fertiliser-N has already been lost via volatilisation. Had there been lower levels of volatilisation (as was the case over the second year), it is likely that a higher amount of N would be lost via leaching for these treatments (which was the case over the second year). However, for the sewage sludge, there was a very low level of loss via volatilisation. Therefore, the relatively low loss of N via leaching is possibly because there is inherently this lower loss of N via soil solution, likely given the very low mineral N content of this fertiliser. In addition to the risk of fertiliser-N loss via leaching potentially being related to the extent of N loss via NH_3 volatilisation directly after fertiliser application, the risk of loss via leaching appears to be strongly related to the properties of the soil (i.e. level of retention of nitrates). This is in addition to the timing of N application relative to the sugarcane's ability to take up the N in solution, which is as a function of its growth and root development, as seen in Chapter 4.

Table 5.1. Nitrogen accumulated and percent of fertiliser nitrogen measured in soil solution at the different soil depths. Shown for each of the different fertiliser treatments, before (N applied) and after fertiliser N loss via volatilisation (N applied after volat).

Measure type	Soil Depth (cm)	Months after harvest			
		Y1		Y2	
		2	12	2	12
		Urea			
N applied (%)	10 cm	11,8	10,4	4,2	21,3
	40 cm	6,8	4,8	0,3	0,0
	100 cm	0,2	0,6	3,2	6,4
N applied after volat (%)	10 cm	34,7	30,7	10,6	34,2
	40 cm	20,1	14,2	0,7	0,0
	100 cm	0,6	1,7	8,4	9,9
		Pig slurry			
N applied (%)	10 cm	18,8	16,2	8,1	15,7
	40 cm	5,7	6,1	5,2	6,7
	100 cm	0,0	0,1	0,2	1,1
N applied after volat (%)	10 cm	129,3	83,8	42,2	61,2
	40 cm	39,2	31,7	27,3	29,9
	100 cm	0,0	0,3	0,8	3,2
		Sewage sludge			
N applied (%)	10 cm	13,3	9,0	11,9	8,3
	40 cm	4,2	2,6	8,2	3,3
	100 cm	0,3	0,7	4,6	3,4
N applied after volat (%)	10 cm	14,0	13,7	24,9	22,3
	40 cm	4,5	4,0	16,8	11,2
	100 cm	0,3	1,0	8,5	7,8

5.6 Remaining fertiliser N ending up in the soil

5.6.1 Focusing on the fate of N fertilisers

There were two approaches to estimating the fertiliser-N for the different fertiliser types which is not taken up by the sugarcane, or lost from the soil-sugarcane system, and which ends up in the soil. This "stored" N essentially becomes a part of the soil organic matter pool, mainly via immobilisation by microbial communities, and which has the potential to be taken up by crops at a later point (Jansen & Person, 1982, Krupnik et al. 2004).

The first approach (δ Fl in the Figures 5.1- 5.4) is by deduction from the N budget or "balance", which summarises all the N inputs and outputs from the soil-sugarcane system. Since the interest here is to determine the fate of fertiliser-N specifically, and the proportion of fertiliser N which is stored in the soil, the N inputs and outputs pertaining only to the different fertiliser types are evaluated, and not the other N sources (e.g. mulch). For the pig slurry and sewage sludge treatments, the organic fertiliser and its urea complement are calculated separately here. The first approach, which uses the N inputs and outputs in an N budget to determine N stored in the soil, is calculated with the following formula:

$$\delta Fl = N_{fert} - (N_{volat} + N_{leaching} + N_{denit} + N_{plant}) \qquad \text{Approach 1}$$

where N stored in the soil (δ Fl) is a function of the input from the respective fertilisers (N_{fert}), minus the N outputs, being N lost via volatilization (N_{volat}), N lost via leaching ($N_{leaching}$), N lost via denitrification (N_{denit}) and N taken up by the plant (N_{plant}).

The second approach (^{15}N in Figures 5.1 – 5.4) is by evaluating the ^{15}N from labelled fertiliser, which is found in different soil horizons in the ^{15}N microplots. The second approach was used specifically for the urea treatment, since only the urea fertiliser was labelled directly with ^{15}N. This approach is therefore also a means of testing whether the values of N stored in the soil determined by deduction from the N budget of the soil-sugarcane system were reasonably accurate (Approach 1). For this second approach, soil samples were taken at the centre of the ^{15}N microplots a few days before the final harvest. The sugarcane in these microplots were first harvested and analysed for their N and ^{15}N content (as used in Chapters 1-3). 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. 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:

$$NRE_{soil} = (A^{15}N - A^{15}\mathrm{N}_{\mathrm{CTL}}) * \frac{N_{soil}}{N_{fertiliser}} \qquad \text{Approach 2}$$

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

Table 5.2. Nitrogen storage in the soil determined using the N balance approach for the two experimental years. For the organic fertiliser treatments, the N storage derived from the organic fertiliser (OF) and its urea complement are given.

Treatment	Year 1		Year 2		Mean	
	Urea	**OF**	**Urea**	**OF**	**Urea**	**OF**
	(kgN.ha^{-1})	(kgN.ha^{-1})	(kgN.ha^{-1})	(kgN.ha^{-1})	(kgN.ha^{-1})	(kgN.ha^{-1})
Urea	46		60		53	
Pig slurry (LP)	21	85	35	22	28	53
Sewage sludge (BA)	26	87	36	66	31	76

Table 5.3. Nitrogen amount derived from urea at each of the soil depths for the two experimental years.

Soil layers	**Year 1**	**Year 2**	**Mean**
cm	*kgN.ha^{-1}*	*kgN.ha^{-1}*	*kgN.ha^{-1}*
0-5 cm	24 ± 9	47 ± 19	36 ± 16
5-10 cm	11 ± 5	13 ± 6	12 ± 2
10-30 cm	14 ± 6	9 ± 2	12 ± 4
30-50 cm	4 ± 3	2 ± 2	3 ± 1
Total	54	72	63 ± 13

The N stored in the soil was relatively high (Table 5.2). The extent of N storage in the soil does indeed vary between the fertiliser treatments in this study. Sewage sludge has the highest quantity of N storage in the soil from fertiliser N input. Urea and pig slurry had similar rates of immobilisation to each other. This is likely as a result of the high mineral N content of pig slurry and evidently that of urea, and as a consequence, the high loss of N, the majority of which is due to high rates of volatilisation. Sewage sludge, in the form it is applied in this study (i.e. digested, limed, dried and pelleted) appears to be a slow-release N fertiliser, with a relatively low N-use efficiency, but leading to a higher level of N stored in the soil. It therefore appears to be beneficial in "nourishing" the soil in its supply of N, and hence the crop over subsequent growth-cycles (i.e. over the medium-long term), rather than as an "immediate-action" fertiliser. A useful strategy could be the complimentary application of urea as a higher efficiency, "quicker action" fertiliser in supplying the sugarcane with N in a useable form during its earlier developmental stages, and in addition, sewage sludge, which is likely to be beneficial in adding to the N stocks of the soil and benefiting the crop over the medium- to long-term.

When the second, isotope-labelling approach is used (Table 5.3), a similar amount of urea-derived N is found over the soil profile as was calculated by deduction from the N budget method. This is reassuring, in that the quantitative ^{15}N labelling method corroborates the summary of N inputs and outputs calculated for the N budget.

5.6.2 Considering the fertiliser treatment plot at the experimental trial scale

A similar type of soil-sugarcane budget can be assessed considering the whole agroecosystem with two additional approaches: a complete input-output N balance in the treatment plot (i.e. flux-based approach), which is not only from the perspective of the fertiliser-derived N, but rather the global N inputs and outputs from the soil-sugarcane system for each treatment plot (where OF and the urea complement are combined in the pig slurry LP and sewage sludge BA plots). The second approach is the difference in soil N stocks over time (i.e. stock-based approach) for these same fertiliser plots.

The third approach (ΔFl in Figures 5.1-5.4):

$$\Delta Fl = (N_{OF} + N_{urea} + N_{rf,irr} - (N_{v,OF} + N_{v,urea} + N_{l,OF} + N_{l,urea} + N_{d,OF} + N_{d,urea} + N_{export})$$

where N stored in the soil (Δ Fl) is a function of the input from the organic fertiliser and its urea complement (N_{urea}), as well as N inputs from rainfall and irrigation combined ($N_{rf,irr}$). In each instance, the organic fertiliser (OF) component and urea complement are separate elements of the calculation. For the urea treatment plot, it is evidently only the urea component which is taken into account.

The N outputs are subtracted, being OF-N and urea-N lost via volatilisation $N_{v,OF}$ and $N_{v,urea}$ respectively, N lost via leaching, $N_{l,OF}$ and $N_{l,urea}$ respectively, N lost via denitrification, $N_{d,OF}$ and $N_{d,urea}$ respectively and N exiting via sugarcane stalks exported after harvest (N_{export}).

The fourth approach (ΔSt in Figures 5.1 – 5.4) is by evaluating the changes in N stocks in the different soil layers, and assessing whether there were changes due to fertiliser application over a longer period of time. For this approach, data from the whole SOERE-PRO experimental site was used, from the start of the experimental trial in 2013, until the end of harvest in 2019.

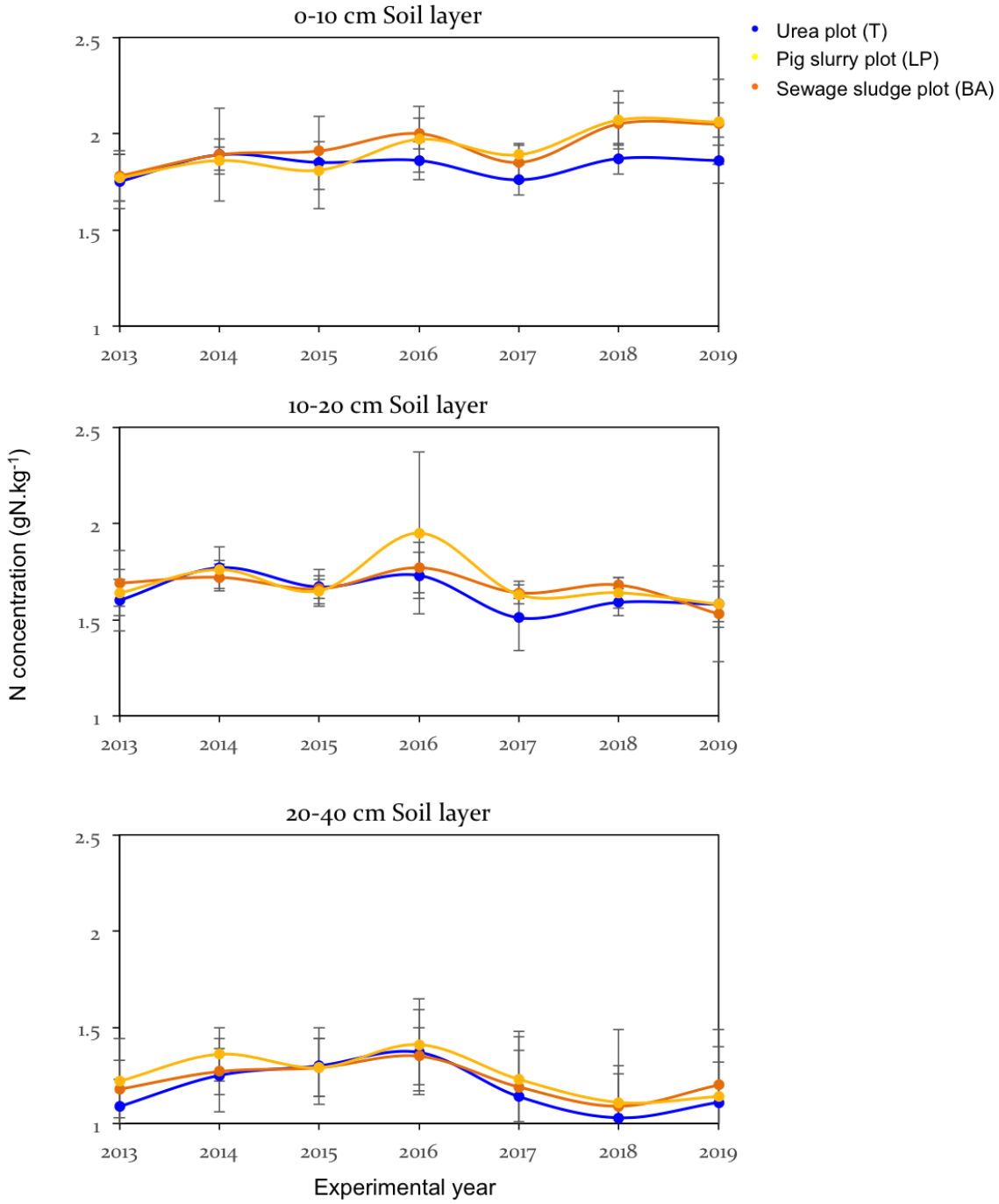

Figure 5.13 *Evolution of the N concentration of three soil layers (0-10 cm, 10-20 cm, 20-40 cm) in the urea treatment (T), the sewage sludge treatment (BA) and the pig slurry treatment (LP).*

Table 5.4 Nitrogen concentration and stock in 2013 and 2019, and annual Nitrogen accumulated in the 0-10cm soil layer in the three fertiliser treatments.

Treatment	[N] 2013	[N] 2019	N stock 2013	N stock 2019	Diachronic Δ Stock
	$g.kg^{-1}$	$g.kg^{-1}$	$kgN.ha^{-1}$	$kgN.ha^{-1}$	$kgN.ha^{-1}.y^{-1}$
Urea	1.75	1.86	2275	2418	24
Pig slurry	1.78	2.05	2314	2665	59
Sewage sludge	1.77	2.06	2301	2678	63

Over the 6-year period in the experimental trial, there was a clear increase in the soil N content in the top 0-10 cm of the soil for both OF treatments (pig slurry and sewage sludge) (Figure 5.9). This was not the case for the urea treatment, however. The deeper soil layers (10-20 cm and 20-40 cm) did not show this effect, however. This indicates that both OFs contribute to a real, observable increase in soil N at the top soil horizon.

When considering the N storage relative to the different treatment types, the values determined with the N inputs and outputs of the soil-sugarcane system (ΔFl) were higher than when calculated using the annual calculated increase in soil N stocks for the different treatment types (ΔSt, Table 5.5). The reason for this difference could either be as a result of the overestimation of certain N outputs from the agroecosystem, or that there are certain outputs missing from the N budget.

In terms of potential underestimations of N outputs, N loss via leaching seems unlikely to be largely underestimated, but should continue to be studied over following years in the experimental trial, to cover a wider range of different seasonal variations, and different levels of water drainage for example, over a longer period of time. The estimation of N_2 emissions based on an estimated ratio from N_2O emissions could certainly be interrogated, and either direct measurements of N_2 taken or more precise estimations of N_2 based on N_2O investigated.

In terms of overlooked N outputs from the soil-sugarcane system, this study has been as exhaustive as possible. One output which has not be quantified, however, was the N loss via runoff, which would imply lateral losses of N from the soil surface from the different fertiliser treatment plots. It appears that N loss via surface runoff is very variable in different soil-sugarcane systems. Runoff is a function of rainfall distribution, slope gradient, the timing of fertiliser application, as well as the extent of groundcover over different sugarcane growth stages (Li et al. 2020). In a study on N loss via runoff from sugarcane cultivated in Mauritius subject to similar pedoclimatic conditions as our study (in terms of the subtropical Mascarene region, rainfall levels and humid tropical soils), Ng Kee Kwong et al. (2002) found that the N load transported off-field by surface runoff ranged only between 2 to 7 kgN. ha^{-1}. Somewhat higher N losses were found in studies such

as that of Webster et al. (2012) and Davis et al. (2016), where N loss via surface runoff ranged between 10-15 kgN. ha^{-1} and 16 kgN. ha^{-1} (or 8-9 % of applied urea-N) respectively.

Higher proportions of the N loss via runoff occurs shortly after fertiliser application, particularly if there is high rainfall or irrigation-driven soil-surface runoff (Davis et al. 2016) and where sugarcane is in its early establishment stages of the growth-cycle and crop cover remains limited (Li et al. 2020). In our study, the vast majority of N appears already to have been accounted for in the N budget, with very high losses via volatilisation for the urea and pig slurry treatments, and a relatively large proportion that is found in soil solution within the first 10 cm of the soil, suggesting that if there are losses via surface runoff, they are likely to be low.

However, it may be of use to assess this flux in the SOERE-PRO experimental trial.

5.7 Conclusion and perspectives

5.7.1 Conclusions

- In order to calculate NRE (the quantitative index of NUE) throughout the sugarcane growth-cycle, the proposed methods gave the best estimate of NRE with minimal destruction, and were an integration of the following: 1/ the use of a global allometric model, using both cane height and diameter to estimate corresponding biomass at a plot scale; 2/ the use of an N dilution curve constructed with a minimum of five sugarcane stalks per fertiliser treatment and collected at a minimum of three dates over each sugarcane growth-cycle; and 3/ determining the N derived from fertiliser using the ^{15}N content of the first or second leaf below the top visible dewlap as a proxy for the ^{15}N content of the cane aboveground biomass, for iNRE calculations using the isotopic dilution method.
- When evaluating the biomass and N mass accumulation of sugarcane, the consideration of the mostly neglected sugarcane components of tillers, strawfall, belowground stools and most importantly roots, gives a more complete understanding of N accumulation and nutrition in sugarcane. The sugarcane total N mass increases substantially when the roots are considered in conjunction with the aboveground biomass and this in turn increases the N-fertiliser recovery efficiency when determined using the isotopic dilution method.
- The evaluation of the different sources of N and their relative contributions to sugarcane N is a more holistic approach to evaluating sugarcane N nutrition, and could be a better approach to synchronising crop requirements with the level and timing of N fertiliser application. The largest contribution to sugarcane N nutrition was from soil organic matter. The second highest contribution was from both the mineral and organic fertilisers applied. The mulch component, previous fertiliser and mulch applications, provided a low but relatively constant supply of N to the sugarcane.
- When evaluating nitrogen use efficiency, the fertiliser NRE is variable over the sugarcane cycle, as shown in Chapter 1. If it is only considered (as is usually the case) at crop harvest, the fate of fertiliser N could be interpreted incorrectly, which highlights the necessity to estimate NRE at various stages along the crop growth-cycle.
- The fertiliser iNRE was higher for the mineral fertiliser than the two organic fertilisers over the two sugarcane growth-cycles. This was expected, since mineral fertiliser provides N in a form immediately available to plants. Sewage sludge (especially in its digested form in this study after the process of methane production, and having been limed, dried and pelleted), has a very low mineral N content, and supplies less N to the sugarcane immediately after application. It has a slower release of N, which appears beneficial in contributing to soil organic matter and to the sugarcane over the mid- to longer-term.

- The N output potentially leading to N loss focussed on in detail in Chapter 4 was the leaching of fertiliser N in soil solutions from the sugarcane-rooting zone. There was a relatively low level of N loss via leaching from the soil-sugarcane system over the two experimental years. This result is probably in part due to the soil's capacity to retain nitrates at this study site. As well as importantly due to the effective N uptake of the sugarcane after fertiliser N application, for which its deep roots and early foliar activity enable active uptake from 2 months after the start of the ratoon onwards.
- A further primary N loss pathway that was discussed in relation to the N budget was via gas emissions. The volatilisation of NH_3 was responsible for the loss of very large proportions of urea and pig slurry applied N, but this was not the case for sewage sludge, probably due to its low mineral N content. N losses via the denitrification of N_2O were low (as expected) for the different fertiliser treatment types. However, this is an important pathway of N loss to consider, given its potency as a greenhouse gas.
- This study had certain limitations, as well as certain major benefits in terms of the various experiments being centered around one block of an experimental trial. This was limiting with regards to the number of statistically meaningful repetitions possible for example. Ideally, multiple blocks in the experimental trial, or even multiple sites would have been evaluated. However, the reason for this experimental design, was the access to a wide variety of equipment stationed centrally in the experimental trial at Block 2, which allowed for an extensive N budget of N inputs and outputs into and from a soil-sugarcane system to be evaluated for the mineral and organic fertilisers. This access to experimental equipment and the resultant N budget being generated as exhaustively as possible, is rare, especially for organic fertilisers in sugarcane agroecosystems.

5.7.2 Perspectives: Partial replacement of mineral fertilisers with organic fertilisers?

Recommendations for the sugarcane industry

This study has highlighted that urea NUE can be low for sugarcane in Réunion Island. The major pathway of N loss for mineral fertilisers and organic fertilisers with a high mineral N content (urea and pig slurry in this study respectively) was via the volatilisation of NH_3. Conditions at the experimental site, and more broadly for most of Réunion Island, are windy and sunny, and a high quantity of mulch is left in the sugarcane plantations after harvest. These conditions are conducive to high rates of N loss via volatilisation.

Potential solutions would include:

- Replacing urea with other mineral N fertilisers such as Ammonium. However, a negative consequence is that this type of fertiliser treatment could acidify the soil, and 50% of Réunion

sugarcane soils already have a pH of less than 5.5. Ammonitrate could be used but the import of this fertiliser is prohibited because of the risk of explosion during the storage phase.

- Another solution could be alternative forms of urea, for example coated urea, or urease inhibitor treated urea. These could be effective but are expensive, especially in a context like Réunion Island where there are many smallhold farmers.
- A further possibility is changing the way fertiliser is applied, for example by applying it directly to the sugarcane row, or beneath the mulch, or into the soil

Whatever alternative fertiliser or methods of application are used, it will remain important that good and effective fertilisation practices are adhered to. This firstly means using optimal doses of N application, which in the context of Réunion Island is determined by using the fertilisation tool "Serdaf" which takes into account parameters such as expected crop yield, soil N supply and fertiliser NUE for different soil types. A current PhD student (Marion Ramos) is working on improving this tool so that it would generate better fertilisation guidelines.

Secondly, good and effective practices imply the right timing of fertilisation. For example, the split application practice of fertilisation appears effective in reducing N losses which is highest at the beginning of the sugarcane growth-cycle. In the 3-6 months after the start of the ratoon, the sugarcane N uptake and requirements are high, and the split application at this point appears to be an effective practice. Shifting the first fertilisation application a month later may be an interesting option to reduce N losses further, especially if there is indeed a translocation of N from the sugarcane roots of the previous ratoon or plantation, to the aboveground biomass of the emerging sugarcane of the following ratoon.

Sewage sludge pellets "FertiPei"

This study supports the use of organic fertilisers for sugarcane fertilisation in Réunion Island.

- Sewage sludge in this form (methanised, limed, dried and pelleted) as applied in the study, is a good way to fertilise sugarcane and to amend the soil in terms of its nutritional input into the soil-sugarcane system while limiting the environmental impact of fertilisation practices.
- However, about half of the N should be applied as urea to complement sugarcane nutrition and to supply a higher N quantity early on in the sugarcane growth-cycle, as a large part of the mineral N is removed during the wastewater treatment process that leads to the production of sludge.

The sludge from Grand-Prado is now approved by ANSES (French Agency for Food, Environmental and Occupational Health & Safety) under the name Fertilpéi. Other solutions to the industrial production process could be found to conserve the N and to propose a N-P balance corresponding to a better fit with the sugarcane requirements.

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Appendices

7.1 Appendix A: Summary of nitrogen use efficiency terminology

Terms in defining nitrogen use efficiency

	Plant biomass / Plant N mass	**(Plant biomass-Plant biomass oN) / (Plant N mass-Plant N mass oN)**	**Plant biomass / ferti N**	**(Plant biomass-Plant biomass oN) / ferti N**	**Plant N mass / ferti N**	**(Plant N mass-Plant N mass oN) / ferti N**
	kgDM.kgN-1	kgDM.kgN-1	kgDM.kgN-1	kgDM.kgN-1	kgN.kgN-1	kgN.kgN-1
Good et al. 2004	Utilization efficiency			Agronomic efficiency	Uptake efficiency	Apparent Nitrogen Recovery
	UtE			AE	UpE	AR
Dobermann et al. 2005		Physiolog c al Efficiency	Partial factor Productivit y or NUE	Agronomic Efficiency		Crop Recovery Efficiency
		PEN	PFPN	AEN		REN
Burzaco et al. 2014	Nitrogen Internal Efficiency			Nitrogen Use Efficiency		Nitrogen Recovery Efficiency
	NIE			NUE		NRE
Robinson et al. 2013	internal NUE				external NUE	
	iNUE				eNUE	
Bell et al. 2014	Nitrogen Utilization Efficiency			Agronomic Efficiency of fertilizer N	Nitrogen Uptake Efficiency	Fertilizer Nitrogen Uptake Efficiency
	NUtE			AgronEffFER T	NUpE	NUpEFERT
Chosen term	Nitrogen Internal Efficiency			Agronomic Use Efficiency		Recovery Efficiency
	NIE					NRE

7.2 Appendix B: Paper published on the effect of Nitrogen fertilisation on sugarcane root development and Nitrogen accumulation

Effect of Nitrogen Fertilisation on Sugarcane Root Development and Nitrogen Accumulation in Ratoon Crops of Reunion Island

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RESEARCH ARTICLE

Effect of Nitrogen Fertilisation on Sugarcane Root Development and Nitrogen Accumulation in Ratoon Crops of Reunion Island

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Abstract Most of the world's sugar is produced from a semi-perennial plant whose root system, although being one key to its success, remains poorly understood. In this study, we sought to describe how nitrogen fertilisation is likely to affect the development of the sugarcane root system and can have significant agronomic implications. We studied sugarcane root distribution, root biomass production and root N accumulation from soil cores sampled down to a depth of 1 m throughout the growth cycle of a ratoon crop in a 144 kgN ha^{-1} year fertilised plot and at crop harvest in six ratoon plots with fertilisation ranging from 0 to 330 kgN ha^{-1} year. The development of the root system in the fertilised sugarcane plot showed 1/homogeneous colonization of the topsoil by fine roots from the early phase, followed by 2/a progressive development of thicker roots mainly localised under the sugarcane row. The results suggested that nitrogen fertilisation could reduce root density in the topsoil layer. The root-to-shoot ratio of biomass and N mass decreased, respectively, from 2.1 to 0.3 and from 1.2 to 0.7 throughout an annual crop growth cycle in a fertilised plot. When sugarcane was not fertilised, an increase of 70% root biomass was observed as compared to fertilised sugarcane. In addition, approximately half of the cane N mass was found in the root compartment of the non-fertilised crop. The root system of sugarcane appeared to be a major pool of N that should be considered in studies dealing with fertiliser N use efficiency and N cycling in sugarcane agroecosystems.

Keywords Sugarcane · Root development · Nitrogen fertilisation · Belowground biomass · Belowground nitrogen accumulation · Root-to-shoot ratio

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Introduction

Plant roots represent a direct interface between the plant and the soil and have been recognised as an important focus for investigations in field crop research. Improving our understanding of the root development is fundamental to improve crop management and to enhance agroecosystem productivity (Eshel and Beeckman 2013). In particular, a better understanding of the root growth dynamic throughout the crop cycle may lead to more efficient use of water and nutrients through optimised irrigation and fertilisation practices (Garnett et al. 2009). Despite the need to investigate root development, studies are rare and valuable due to the methodological difficulties to study this 'hidden' compartment (van Noordwijk et al. 1993). Root sampling and processing is time-consuming, and the results are often subject to high uncertainty due to spatial and temporal variability. In addition to methodological constraints, studies on crop root development must deal with agronomic feedbacks, since practices such as fertilisation will in turn influence the root development.

Fertilisation practices increase nutrient availability for plants and stimulate aboveground productivity. Conversely, nutrient deficiency can lead to the plant increasing

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photosynthate allocation to roots in order to aid the plant in obtaining more nutrients (Fageria and Moreira 2011). As a result, plant root-to-shoot ratios generally increase when soil chemical fertility decreases, and vice versa (Eghball and Maranville 1993; Lu et al. 2011). Nitrogen availability favoured root branching and localised densification (Drew 1975; Walch-Liu et al. 2006; Postma et al. 2014), but its effect on root distribution in the soil profile is less clear. Nitrogen application can stimulate root concentration in the topsoil (Hoad et al. 2001; Costa et al. 2002), but roots of unfertilised plants tend also to proliferate in the nutrient-rich topsoil (Vitousek and Sandford 1986).

Sugarcane is cultivated in about 27 million hectares in more than 90 countries for sugar and energy purposes. Sugarcane is a semi-perennial crop which is in most cases harvested annually without uprooting. The belowground biomass (i.e. the stool and the roots) is therefore the source of new shoots (i.e. ratoons) that emerged from the base of the crop plant and that will produce a fresh crop in the next season. Despite the clear importance of the root compartment for a perennial crop such as this, studies dealing with the sugarcane root compartment remain scarce mainly due to the methodological difficulties mentioned above (Smith et al. 2005, Bell et al. 2015). The development of the sugarcane root system is known to be influenced by water availability (Laclau et al. 2009) and by soil physical properties (Otto et al. 2011), but the effect of chemical fertility is less clear. Several studies suggest that nutrient availability in the soil, as affected by natural nutrient distribution and by fertilisation practices, may affect sugarcane rooting patterns (Otto et al. 2009). While the sugarcane belowground compartment is suspected to accumulate N throughout the growth cycle and to supply N to the subsequent cycles (Robinson et al. 2014), there is limited information documenting the effect of N fertilisation on root biomass and almost no data on belowground N accumulation (Bell et al. 2015).

We set out to improve our knowledge of the sugarcane root system and to gain insights into sugarcane root development as affected by N fertilisation. The root development was monitored in a fertilised plot throughout a ratoon crop in order to study the dynamic of root distribution in the interrow, by root type, by soil layer, and relative to the shoot compartment over a complete year. The effect of N fertilisation on the root development was specifically investigated through measurements of root and shoot biomass and N mass at crop harvest in six plots with fertilisation ranging from 0 to 330 kgN ha^{-1} year.

Materials and Methods

Study Site Description

The experimental station of La Mare is located on the northern coastal area of Réunion Island (20° 54′ S, 55° 31′ E, 65 m elevation). The climate is tropical with a rainy season from January to March and a dry season from May to November. Mean annual rainfall is approximately 1800 mm, and mean annual temperature is 24 °C with seasonal variations of approximately 5 °C. The soil has developed over volcanic rocks (olivine basalts, series of oceanites over 340,000 years BP) and is classified as Hypereutric Nitisol (USS Working Group WRB 2015). Briefly, this soil is characterised by a clayey texture greater than 30% down to the depth of one meter and an average bulk density of 1.3 g cm^{-3}. This Nitisol is slightly acidic and has relatively high contents of carbon, nitrogen and phosphorus (Table 1). The soil mineralogy is dominated by kaolinite and iron and aluminium oxides.

The study was carried out in two trials 200 m apart planted in 2014 at the experimental station of La Mare (Table 2). The first trial was 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 environmental impacts of organic waste recycling in sugarcane field crops over a long time scale. The trial was planted in March 2014 from viable buds of the R579 sugarcane variety placed with 1.5 m spacing between rows. The trial consisted of 6 plots, each with a different fertiliser treatment, repeated in 5 blocks, with each plot made up of 6 sugarcane rows of 28 m, constituting a total plot area of 290 m^2. The second trial was one of the four sites of the TERO network designed to determine the fertilising value of organic wastes recycled in sugarcane field crops. The trial was planted on September 2014 from viable buds of the R582 sugarcane variety placed with 1.4 m spacing between rows. The trial consisted of 72 plots, comprising 2 to 5 doses of 7 distinct fertiliser treatments, which were repeated in 3 replicate blocks, with each plot comprising 5 sugarcane rows of 12 m, with a total plot area of 84 m^2. The two trials were managed to prevent weed development and ensure an optimal water regime through irrigation.

Study Plots

The experiment was conducted during the third ratoon of sugarcane in two plots of the Soere-PRO Trial and during the second ratoon in four plots of the TERO trial (Table 2).

In the Soere-PRO trial, two different fertilisation treatments were selected: an unfertilised plot and a plot

fertilised with 144 kgN ha^{-1} (70% in December 2016 and 30% in March 2017). In the TERO trial, the four plots selected were: an unfertilised plot and 3 plots fertilised, respectively, with 99 kgN ha^{-1}, 165 kgN ha^{-1} and 330 kgN ha^{-1}. All these plots were fertilised using split application of urea granules (50% in September 2016 and 50% in December 2016).

The unfertilised plots were fertilised with 144 kgN ha^{-1} and 198 kgN ha^{-1} the previous year in the Soere-PRO and TERO trials, respectively (Table 2).

Sugarcane root development was studied at four main dates in the fertilised plot of the Soere-PRO trial: 1 February, 1 April, 6 July and 5 October 2017, or 3, 5, 8 and 11 months after the last harvest (MAH) and the beginning of the 3rd ratoon. The effect of fertilisation was studied at final harvest in all plots, corresponding to the 5 October 2017 in the Soere-PRO trial and to the 1 September 2017 in the TERO trial.

Root Biomass Sampling

The sugarcane root biomass was quantified down to a depth of 50 cm in all plots with the auger method (Oliveira et al. 2000; Otto et al. 2009). This soil layer is known to be densely occupied by roots and to account for most of their biomass. Soil cores were sampled using a mechanic root auger (inner diameter of 9 cm) composed of gouges coupled with a percussion hammer (Cobra TT, SDEC). Root biomass was estimated in both trials from 9 soil cores corresponding to 3 replications and 3 positions relative to the sugarcane row (0–25 cm, 25–50 cm and 50–75 cm). Stools were not sampled, but the surrounding roots were quantified in the 0–25 cm position. In the field, the soil cores were divided into 3 layers (0–10 cm, 10–30 cm and 30–50 cm), placed in plastic bags and stored at − 10 °C until processing. In the laboratory, soil cores were placed into a bucket of tap water and soil was disaggregated manually using the water stream. The roots were thereby separated from soil particles and easily collected by floatation with a sieve (500 µm mesh diameter). Plant fragments such as pieces of straw have been removed manually.

In the fertilised plot of the Soere-PRO trial, special attention was paid to temporal root development with two additional soil layers (50–75 cm and 75–100 cm) and a separation into three root classes (fine roots, thick roots and dead roots). The temporal monitoring of root development was thus based on 540 samples combining 3 interrow positions, 5 soil layers and 3 root classes on 4 dates with 3 replicates. Roots were manually separated into three classes: fine roots (diameter < 1 mm), thick roots (diameter > 1 mm) and dead roots (diameter > 1 mm). The first class corresponded to clearly identifiable roots and of an

Table 1 Chemical and physical characterization of the soil at the launch of the trials in 2014

Trial	Layer (cm)	pH_{water}	pH_{KCL}	Total N (g kg^{-1})	C (g kg^{-1})	Total P (mg kg^{-1})	CEC (mé/100 g s)	K^+ (mé/100 g s)	Na^+ (mé/100 g s)	Ca^{2+} (mé/100 g s)	Mg^{2+} (mé/100 g s)	Clay (%)	Bulk density (g cm^{-3})
Soere-PRO	0–10	6.1 ± 0.2	4.8 ± 0.1	1.8 ± 0.1	21.4 ± 1.1	117 ± 50	10.6 ± 1.0	0.70 ± 0.14	0.16 ± 0.01	6.7 ± 0.7	2.9 ± 0.3	43 ± 3	1.4 ± 0.1
	10–20	6.1 ± 0.2	4.7 ± 0.2	1.6 ± 0.1	18.7 ± 1.4	90 ± 28	10.1 ± 0.9	0.56 ± 0.13	0.12 ± 0.02	6.6 ± 0.8	2.7 ± 0.3	42 ± 4	1.3 ± 0.1
	20–40	6.1 ± 0.2	4.8 ± 0.2	1.1 ± 0.2	11.7 ± 2.7	39 ± 14	8.8 ± 0.6	0.16 ± 0.13	0.28 ± 0.09	5.8 ± 0.5	2.4 ± 0.3	45 ± 2	1.3 ± 0.1
TERO	0–15	6.1 ± 0.5	4.9 ± 0.4	1.8 ± 0.4	22.1 ± 4.1	90 ± 35	10.2 ± 1.7	0.59 ± 0.24	0.22 ± 0.06	5.1 ± 1.1	3.0 ± 0.6	–	–
	15–30	6.1 ± 0.5	4.9 ± 0.4	1.3 ± 0.4	14.1 ± 4.8	41 ± 21	8.1 ± 1.2	0.15 ± 0.13	0.35 ± 0.10	4.5 ± 1.1	2.5 ± 0.6	–	–
	30–60	6.5 ± 0.4	5.2 ± 0.4	0.8 ± 0.3	7.9 ± 3.1	21 ± 13	7.3 ± 1.0	0.04 ± 0.05	0.40 ± 0.08	3.9 ± 0.9	2.2 ± 0.6	–	–

Values represent means and standard variation of 30 samples in the Soere-PRO trial and from 72 samples in the TERO trial

Total N was analysed with the Dumas method with an elemental CN analyzer. Total P with the Olsen–Dabin method

Table 2 Technical information of the studied plots in the two trials

Trial	Ratoon	Plantation date	Harvest date of previous crop	Harvest date of current crop	Variety	Row spacing (m)	Fertilisation treatment (kgN ha^{-1})	Fertilisation the previous year (kgN ha^{-1})	Sampling dates Months after harvest
Soere-PRO	3R	March 2014	2 November 2016	2 October 2017	R579	1.5	0	144	11
							144	144	3, 5, 8, 11
TERO	2R	September 2014	4 September 2016	23 August 2017	R582	1.4	0	198	12
							99	165	12
							165	110	12
							330	330	12

organic matter fraction that we have visually identified as being mainly composed of very fine roots. In order to validate this observation, the plot was fertilised with ^{15}N-labelled urea on December 2016 and all the root fractions were analysed for ^{15}N on February 2017. The isotopic signature of the organic matter fraction ($\delta^{15}N$ = 590‰) was similar to that of the fine roots ($\delta^{15}N$ = 503‰) and the two fractions were therefore accounted for together in the fine root class. Diameter of root higher than 1 mm (thick and dead roots) was determined visually using a graduated reference. Dead roots were selected qualitatively by bending them to see whether they snapped. If the roots did not snap, this indicated there was sufficient living tissue and that roots could be considered living at the time of harvest. The quantification of dead roots was not possible for fine roots, but we considered that this class should be mostly made up of living roots since decomposition rate of fine roots should be very high in such tropical clayey soil conditions.

Shoot Biomass Estimation and Sampling

In the fertilised plot of the Soere-PRO trial, shoot biomass was nondestructively quantified 3, 5 and 8 MAH using an allometric relationship applied to cane inventories (Poultney et al. *accepted*). Briefly, 240 canes had been previously collected in the same trial to build a model able to predict cane shoot biomass from cane height (length from the base to the Top Visible Dewlap) and basal diameter (first internode from the ground). This allometric relationship was applied to cane inventories applied to each of four 3 m linear plots of sugarcane at each date in order to estimate shoot biomass at the plot level.

At the end of the crop cycle, the shoot biomass was harvested and weighed in the Soere-PRO and TERO trials.

On each date, 6 canes were sampled in each plot, weighed, ground together and two representative subsamples of 1 kg were constituted for the determination of the dry matter and of the N content.

Chemical and Data Analyses

The root and shoot samples were oven-dried at 60 °C for 72 h until a constant weight was obtained on an analytical scale. The dry samples were weighed to obtain the dry matter content. The dry root samples were composited by repetition in such a way that all depths and row distances were mixed together to obtain a sufficient sample mass for N analysis. The root and shoot subsamples were ground with a power cutting mill (Pulverisette 19, Fritsch, Idar-Oberstein) and analysed for N with an elemental CN analyzer (Vario Max Cube CNS, Elementar, Hanau, Germany). The root fractions for ^{15}N analysis were ground to pass a 500 µm screen using a Cyclotec grinder (CT Tecator Cyclotec Sample Mill, Foss), sent to the PTEF laboratory in Nancy (Plateforme Technique d'Ecologie Fonctionnelle, INRA, France), where samples were further ground to pass a 100 µm screen 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).

ANOVA test cannot be performed in the absence of replication in distinct plots. *T* test was used to determine significant differences of root biomass, N concentration and N accumulation along the sugarcane growth cycle in the fertilised plot of the Soere-PRO trial. At the end of the crop cycle, t test was used in both trials to test the effect of N fertiliser application on root biomass, N concentration and N accumulation thanks to pairwise comparisons between plots. The probability level used to determine significance was $P < 0.05$. Data were processed using the R free software (R Core Team 2016).

The nutritional crop N status in each fertilisation treatment was determined at harvest in both trials with the nitrogen internal efficiency (NIE) calculated according to:

$$\mathrm{NIE}\left(\mathrm{kgDM\ kgN^{-1}}\right) = \mathrm{Shoot\ biomass}\ \left(\mathrm{kgDM\ ha^{-1}}\right) / \mathrm{Shoot\ N\ mass}\left(\mathrm{kgN\ ha^{-1}}\right) \quad (1)$$

The crop efficiency to take up fertiliser nitrogen was evaluated with the fertiliser nitrogen recovery efficiency (NRE) calculated according to:

$$\mathrm{NRE} = (\mathrm{Crop\ N} - \mathrm{Crop\ N\ CTL})/\mathrm{FERT\ N} \quad (2)$$

where Crop N is the quantity of N either in the shoot or in the entire plant of fertilised crops (kgN ha^{-1}), Crop N CTL is the quantity of N either in the shoot or in the entire plant of unfertilised control crops (kgN ha^{-1}), and FERT N is the quantity of N applied with the fertiliser (kgN ha^{-1}).

Results

Root Distribution Throughout the Crop Cycle of a Fertilised Sugarcane Plot

The percentage of root biomass present as fine roots (diameter < 1 mm) decreased from 75% at 3 months after harvest (MAH) to 53% at 11 MAH in the fertilised plot of the Soere-PRO trial (Fig. 1a). Conversely, the proportion of root biomass formed by thick roots (diameter > 1 mm) increased from 12 to 43% over the same period. The proportion of dead roots was about 15% over the first three dates and dropped to 5% at the end of the studied year.

The root system was equally distributed among the three interrow positions 3 MAH (Fig. 1b). During the next 6 months, the proportion of roots contained in the 25 cm closest to the cane row progressively increased to account for about 55% of the roots at the last two dates. In return, the root located at the centre of the interrow (50–75 cm) decreased from 35% to 21% throughout the crop cycle, while the intermediate position (25–50 cm) remained at around 25% of the roots from the 5th month of growth onwards.

About 80% of the roots were located in the first 30 cm of the soil profile 3 MAH (Fig. 1c). The vertical distribution of roots appeared constant over the next three dates with about 70% of the root mass in the topsoil.

Root Biomass, N Accumulation and Root-to-Shoot Ratio Throughout the Crop Cycle

As expected, the shoot biomass increased throughout the annual growing season, reaching 36.4 t DM ha^{-1} at the end of the year (Table 3). By contrast, the largest root biomass was observed 5 MAH, but there was no trend for increasing or decreasing root biomass across the four sample dates. The biomass root-to-shoot ratio progressively decreased from 2.1 at 3 MAH to 0.3 from 8 MAH onwards (Table 3, Fig. 1d).

The shoot and root compartments showed opposite trends in terms of nitrogen concentrations throughout the crop cycle (Table 3). The N concentration of the shoot

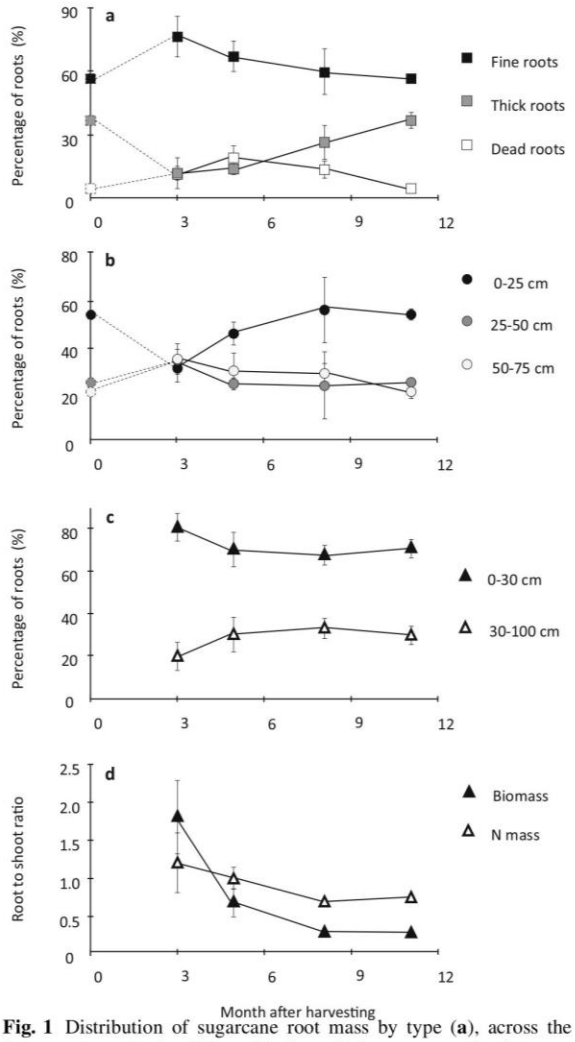

Fig. 1 Distribution of sugarcane root mass by type (**a**), across the interrow to a depth of 1 m (**b**), by soil layer across the interrow positions (**c**) and root-to-shoot ratio based on root biomass and mass of N in roots (**d**) throughout the ratoon of a fertilised plot in the Soere-PRO trial. All these results are based on root samples down to a depth of 1 m. Values represent means of 3 samples from each date, with vertical bars indicating standard deviation of the mean. Values presented on month 0 correspond to the values measured at the end the studied ratoon

compartment significantly decreased from 9.0 to 2.6 kg N t DM^{-1}, while the N concentration of the root compartment tended to increase from 5.2 to 6.7 kg N t DM^{-1} during the studied period.

The amount of N mass accumulated in the shoot biomass increased from 41 kg N ha^{-1} at 3 MAH to reach approximately 93 kg N ha^{-1} from 8 MAH onwards (Table 3). The root N mass increased from 49 at 3 MAH to 63–75 kg N ha^{-1} from 5 MAH. More than half of the N was contained in the root compartment 5 MAH, while the amount of N accumulated in the root compartment corresponded to about 70% of the shoot N mass 8 and 11 MAH (Table 3, Fig. 1d).

Nitrogen Fertiliser Effects on Root Distribution, Biomass and N Accumulation

The root distribution across the interrow was not affected by N fertilisation in either trial at harvest (Fig. 2a). The greatest concentration of root biomass was found in the 25 cm zone adjacent to the crop row (51%). The remainder of the root biomass was distributed relatively evenly between the 25–50 cm (27%) and 50–75 cm (22%) interrow zones.

Increasing rates of applied N fertiliser resulted in the proportion of root mass located in the 0–10 cm topsoil layer decreasing from 40 to 29% in the Soere-PRO trial and from 51 to 38% in the TERO trial (Fig. 2b).

The sugarcane crop yield increased by 49% and 38% in response to N fertiliser addition in the Soere-PRO and TERO trials (330 kgN ha^{-1} treatment), respectively (Table 4, Fig. 2d), although there was very little N response evident in the latter until very high N rates (330 kg N ha^{-1}) were applied. Root biomass was not significantly affected by fertilisation in both trials. Although not statistically significant, root biomass tended to decrease linearly with increasing fertiliser N rate at TERO (Table 4, Fig. 2c). The combination of these trends regarding shoot and root response to applied N resulted in biomass root-to-shoot ratio decreasing from 0.39 to 0.23 with fertilisation in the Soere-PRO and from 0.25 to 0.11 in the TERO (Table 4, Fig. 2f).

The N concentration increased with fertiliser application whatever the biomass compartment (Table 4, Fig. 2c, d). The concentration was increased by 12% in the Soere-PRO and by 55% in the TERO (330 kgN ha^{-1} treatment) on average for the two compartments (Fig. 2c, d).

The amount of N that accumulated in the root compartment tended to be higher in treatments supplied with intermediate doses of fertiliser, reaching 68 ± 18 kg N ha^{-1} in the TERO's plot fertilised with 165 kg N ha^{-1} of urea. The root compartment of the unfertilised plots of the Soere-PRO and TERO as well as the overfertilised plot of

Table 3 Biomass, N concentration and N mass of root and shoot compartments in the fertilised plot of the Soere-PRO trial throughout the ratoon cycle

Trial	Biomass					[N]		N mass		
	Dose (kg N ha^{-1} y^{-1})	Date (months)	Root (R) (t DM ha^{-1})	Shoot (S) (t DM ha^{-1})	R/S ratio	Root (kg N t DM^{-1})	Shoot (kgN tDM^{-1})	Root(R) (kgN ha^{-1})	Shoot(S) (kgN ha^{-1})	R/S ratio
Soere-PRO	144	3	9.5 ± 3.0^{a}	4.6 ± 4.0^{a}	2.1 ± 0.5^{b}	5.2 ± 0.6^{a}	9.0 ± 0.9^{c}	49 ± 21^{a}	41 ± 3^{a}	1.2 ± 0.4^{a}
	144	5	13.6 ± 3.8^{a}	19.2 ± 0.7^{b}	0.7 ± 0.2^{a}	5.6 ± 0.6^{ab}	4.0 ± 0.7^{b}	75 ± 11^{a}	76 ± 3^{b}	1.0 ± 0.1^{a}
	144	8	9.7 ± 0.8^{a}	33.9 ± 5.4^{c}	0.3 ± 0.0^{a}	6.4 ± 0.3^{ab}	2.7 ± 0.4^{a}	63 ± 4^{a}	92 ± 13^{b}	0.7 ± 0.1^{a}
	144	11	10.4 ± 1.4^{a}	36.4 ± 3.4^{c}	0.3 ± 0.0^{a}	6.7 ± 0.3^{b}	2.6 ± 0.4^{a}	70 ± 8^{a}	94 ± 9^{b}	0.7 ± 0.0^{a}

Values represent means and associated standard deviation of 3 samples from each date. Pairwise *t* test were used to determine significant differences of root biomass, N concentration and N accumulation between dates

Different letters indicated significant differences of root biomass, N concentration and N accumulation between dates obtained from a Pairwise *t* test

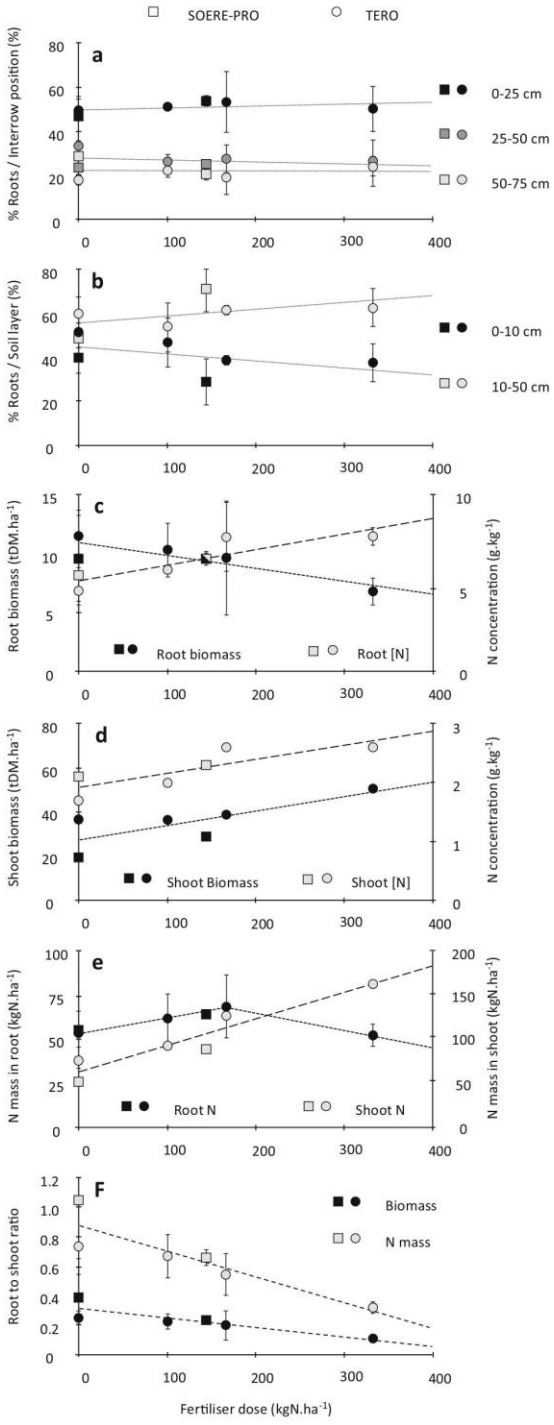

Fig. 2 The effects of amount of fertiliser N applied in the Soere-PRO (squares) and TERO (circles) trials on the distribution of the mass of sugarcane roots at harvest across the interrow to a depth of 50 cm (**a**), by soil layer across the interrow positions (**b**), the total root biomass and N concentration (**c**), the total shoot biomass and N concentration (**d**), the mass of N found in roots and shoots (**e**) and root-to-shoot ratio (**f**). All these results are based on root samples down to a depth of 50 cm. Values represent the mean of three samples from each plot, with the vertical bars representing the standard deviation of the mean

the TERO accumulated approximately the same amount of N, i.e. 54 ± 20 56 ± 10 kg N ha^{-1} (Table 4, Fig. 2e). The N mass root-to-shoot ratio decreased in response to N fertilisation for both sites (Table 4, Fig. 2f). In the Soere-PRO, about the same amount of N was contained in the shoot and the root compartments when sugarcane was not fertilised.

The NIE tended to decrease linearly with increasing doses of N fertiliser application for both the Soere-PRO and TERO trials (Table 4).

The NRE was variable with different N dose applications, for the two trials. The addition of the root compartment increased the NRE for all N dose applications for the two trials, except for the highest N dose of the TERO trial. The NRE of the Soere-PRO trial increased by 7% when the roots were added. For the TERO trial, the addition of roots resulted in an NRE increase of 6% and 8% for the 99 kg N ha^{-1} and 165 kg N ha^{-1} dose applications, respectively.

Discussion

Root Development in Relationship to Fertilisation

The topsoil was colonised by a dense and homogeneous network of fine roots early in the growth cycle of the fertilised ratoon in the Soere-PRO trial. The same biomass of roots was measured 3 and 11 MAH, while shoot biomass at 3 MAH represented only about 10% of that recorded at 11 MAH in this plot. The high amount of roots observed early in this 2nd ratoon can be explained by the survival of roots from the previous ratoon since variable fraction of them remain alive after the harvest and can still be active for the following ratoon (Glover 1968; Ball-Coelho et al. 1992). However, the pattern of root distribution by type across the interrow and by soil layer changed substantially between 3 and 11 MAH, suggesting that the system had been at least partly 'reset' between the two ratoons. The very low proportion of thick roots present at the beginning of the ratoon suggested that a considerable proportion of the roots of the previous ratoon (i.e. thick roots and associated fine roots) had died at harvest. A significant proportion of the fine roots observed at 3 MAH should therefore be associated with new shoots. The 'superficial roots' are indeed known to emerge very rapidly from new shoots and to extend

laterally to form a dense network of fine and branched roots (Smith et al. 2005). Lateral root development appeared to be quite rapid in the 3 months after crop harvest, as evidenced by the similar distribution of roots across the interrow at that time. Although the historical view that the root system of sugarcane is totally replaced after harvest had been denied, the survival and the reactivation of the root system between ratoons remains a necessary topic of research. In particular, further studies should be carried out to assess the relative proportions of old and new roots during the ratoon growth cycle.

Thicker roots growing deeper and primarily close to the row were increasingly observed from 3 to 11 MAH. The fine roots (diameter < 1 mm) were progressively replaced by thicker roots (diameter > 1 mm) supporting their own cohort of fine roots. These thicker roots may correspond to 'buttress roots' since the plant's anchoring must be strengthened as the stalk grows (Smith et al. 2005). In this way, approximately 55% of the root mass was found close to the row rather than in the middle of the interrow which was in agreement with most of the previous studies (Ball-Coelho et al. 1992; Otto et al. 2009; Azevedo et al. 2011; Otto et al. 2011). It is also well known that most sugarcane root biomass is found close to the surface and then declines approximately exponentially with depth (Smith et al. 2005). Our results, showing that 70% of the roots were located in the first 30 cm of the soil profile, were consistent with 70–74% in the 0–30 cm soil layer by Evans (1938), 62–69% in the 0–50 cm soil layer by Ball-Coelho et al. (1992), 60% and 80% found, respectively, in the 0–20 and 0–40 cm soil layers by de Sousa et al. (2013), 70% in the 0–20 cm soil layer by Otto et al. (2009) and to that of 70% for annual crops and 57% for tropical grasslands (Jackson et al. 1996).

Studies dealing with the effect of N fertilisation on the vertical distribution of the sugarcane root biomass are very scarce, but Otto et al. (2011) observed that N fertilisation led to intensified root growth in the surface layer and reduced root growth in the deeper layers. The proportion of the roots sampled in the 0–20 cm top layer was 50% and 70% of the total root biomass in the unfertilised and the fertilised treatments, respectively. They hypothesised that roots were concentrated in the topsoil where fertilisers were supplied, while the root system colonised a greater volume of the soil profile to meet the cane N demand in the unfertilised treatment. In our study, the opposite trend was observed where a decreasing level of N fertilisation led to root intensification in the 0–10 cm topsoil layer. In contrast to the hypothesis advanced by Otto et al. (2009), we hypothesise that the root system colonised the topsoil more densely in unfertilised treatments because this layer contained the greatest source of N available to plants (Weaver 1926). High N content and pedoclimate favourable to

Table 4 Biomass, N concentration and N mass of root and shoot compartments and NUE indexes in the unfertilised and fertilised plots of the two trials at the end of the ratoon cycle

Trial	Biomass					[N]		N mass		NIE	NRE		
	Dose (kgN ha^{-1})	Date (days)	Root (tDM ha^{-1})	Shoot (tDM ha^{-1})	R/S ratio	Root (kgN tDM^{-1})	Shoot (kgN tDM^{-1})	Root (kgN ha^{-1})	Shoot (kgN ha^{-1})	R/S ratio	Shoot (kgDM kgN^{-1})	Shoot (%)	Shoot + Root (%)
Soere-PRO	0	337	9.4 ± 3.8^{a}	24.4	0.39 ± 0.16^{a}	5.8 ± 0.4^{a}	2.1	54 ± 20^{a}	51	1.05 ± 0.40	478		
	144	337	9.8 ± 0.4^{a}	36.4	0.23 ± 0.02^{a}	6.6 ± 0.3^{a}	2.3	64 ± 1^{a}	89	0.66 ± 0.05	409	26	33
TERO	0	364	11.5 ± 2.2^{a}	45.9	0.25 ± 0.05^{a}	4.9 ± 0.5^{a}	1.7	56 ± 10^{a}	76	0.73 ± 0.13^{a}	604		
	99	364	10.3 ± 2.3^{a}	45.5	0.23 ± 0.05^{a}	6.0 ± 0.1^{ab}	2.0	62 ± 14^{a}	93	0.67 ± 0.15^{a}	489	17	23
	165	364	9.6 ± 4.8^{a}	48.7	0.20 ± 0.10^{a}	7.7 ± 1.8^{b}	2.6	68 ± 18^{a}	126	0.54 ± 0.14^{ab}	387	30	38
	330	364	6.8 ± 1.1^{a}	63.5	0.11 ± 0.02^{a}	7.8 ± 0.5^{b}	2.6	52 ± 6^{a}	163	0.32 ± 0.04^{b}	390	26	25

Values represent means and associated standard deviation of 3 samples from each date. Replications were not available for the shoot compartment. Pairwise *t* test were used in both trials to test the effect of N fertiliser application on root biomass, biomass R/S ratio, N concentration, N accumulation and N R/S ratio between plots

Different letters indicated significant differences of root biomass, N concentration and N accumulation between dates obtained from a Pairwise *t* test

microbial activity may promote N mineralisation and N uptake by sugarcane plants in the topsoil layer. In summary, these contradictory results highlight the importance of conducting further studies on the sugarcane root compartment since modification of their vertical distribution could have significant implications on crop productivity (water stress) and soil health (carbon inputs, soil density modification).

Root Biomass in Relationship to Fertilisation

The biomass of the root compartment was about twofold higher than that of the shoot compartment at the beginning of the ratoon. Smith et al. (2005) found for pot-grown sugarcane plants that the maximal root-to-shoot ratio of 0.42 was observed during early growth. Herbaceous species are known to invest primarily in the root system before developing their aerial organs (Wilson 1988). Furthermore, Silva-Olaya et al. (2017) observed root-to-shoot ratios of 0.15 and 0.29 for harvestable planted cane and 4-year ratoon, respectively, suggesting that ratooning may promote rooting. Together, root survival from the previous ratoon and the development of the new root system during early growth may be the reason for the high root-to-shoot ratio of 2.07 observed 3 MAH in the present study. As the cane grew, the root-to-shoot ratio dropped down to 0.29 at the end of the year in the fertilised treatment of the Soere-PRO trial, which was similar to the results of Silva-Olaya et al. (2017). According to the review of Mokany et al. (2006), the root-to-shoot ratio of a sugarcane ratoon was actually approaching that of tropical grasslands at the beginning of the ratoon (1.89) and to that of tropical forests at the harvest (0.21).

Fertiliser N application led to a substantial decrease in the root-to-shoot ratio from 0.39 to 0.23 at harvest in the Soere-PRO trial and from 0.25 to 0.11 in the TERO trial. Root-to-shoot ratio is commonly lower with high levels of nutrition (Marschner 1995), as N fertilisation commonly stimulates aerial yield as recorded in this study. Nevertheless, this effect was exacerbated by a trend towards less root biomass when the crop was fertilised in the TERO trial. Indeed, there was a 70% greater root biomass when not fertilised compared to the overfertilised plot in this trial. Such an effect, sometimes described for other species, has not been observed before for sugarcane (Comfort et al. 1988, Nadelhoffer 2000, Jourdan et al. 2008). Indeed, the few Brazilian studies that have addressed this issue have either found a positive effect of N fertilisation on root mass (Sampaio et al. 1987) or no effect of N fertilisation (Otto et al. 2009). The inconsistency between these results points to the difficulty of studying the root compartment and drawing generalisable observations. The auger method is well adapted to estimate the root biomass (Otto et al. 2009) but appeared less suitable to highlight significant differences between treatments. Roots are by nature heterogeneously distributed in the soil, while the sampling area is often restricted to a few centimetres. Root sampling with the auger method can therefore result in misestimations, especially under the row where root patches developed. Our nine soil cores seemed to do a reasonable job of minimising the variability in root biomass measures, but further sampling of the other replicate plots would have led to greater confidence in the significance of fertiliser effects. Further studies integrating more repetitions and involving more situations are therefore required to statistically establish whether the reduction in N fertilisation can lead to an increase in root biomass as observed in the present study.

Overall, the range of root biomass observed for sugarcane in this study (6.8–11.5 t ha^{-1}) was consistent with those of 9–11 t ha^{-1} measured by Ball-Coelho et al. (1992). These root biomass values measured during the ratoon cycle were higher compared to the values of 7.5 t ha^{-1} obtained by Ball-Coelho et al. (1992) and of 3–5 t ha^{-1} reported by Otto et al. (2009) from three Brazilian studies for planted crops. In plant cane, root development is intense because the crop establishes its entire root system over a short period of time. However, the observed biomasses seem to be higher in ratoon crops (Ball-Coelho et al. 1992), perhaps due to the coexistence of an old root system (associated with the stool) and of a newly established root system (associated with new shoots).

There are several potential methodological biases regarding root estimation, but the sieving mesh size of 0.5 mm used in our study as compared to the 2 mm in others studies could explain the rather high values observed in the present study. Livesley et al. (1999) demonstrated for maize roots that only 66% and 37% of the total root biomass were recovered at 0–15 cm and 30–45 cm soil layers, respectively, in the 2.0 mm sieve compared to almost 95% in the 0.5 mm sieve. While a finer mesh size may allow a larger proportion of roots to be recovered, it may, on the other hand, lead to root biomass overestimation by retaining extraneous organic matter (Hirte et al. 2017). At the first sampling date, a fourth class was separated corresponding to organic matter fragments that we have visually identified as being mainly very fine roots. Using root recovery of ^{15}N-labelled urea (data not shown), we were able to confirm that this fraction corresponded mainly to currently growing fine roots rather than dead roots or plant residues from previous ratoons, organic soil amendments or remnants of soil fauna.

Fertilisation Effects on Root N Accumulation

The root system of sugarcane appeared to be a major sink of N in these ratoon crops. The amount of N accumulated in the root compartment was greater than that accumulated in the shoot during the first 5 months of growth and corresponded to about 70% of the mass of aboveground N at harvest in the Soere-PRO. As expected, N fertilisation increased the proportion of N accumulated in the shoot relative to the root compartment. The root N compartment represented 90% of the shoot N mass in unfertilised treatments but only 32% in overfertilised treatments at harvest in the TERO. The only study directly comparable found a much slighter discrepancy with N mass root-to-shoot ratio of 50% and 39% for unfertilised and fertilised treatments (0 or 100 kg N/ha, respectively) in a second ratoon crop (Robinson et al. 2009). There have been few studies focusing on the mass of N contained in the root compartment mainly due to methodological difficulties, but presumably also because the root compartment had been considered negligible and not influenced by N fertilisation. As an example, the root N mass accounted only for 7–19% of the shoot N mass at harvest in the Brazilian study of Vieira-Megda et al. (2015), considering both fertilised and unfertilised treatments. Some authors like Meier et al. (2006) use the historical reference proposed by van Dillewjin (1952) giving 30% of the crop N contained in the roots, corresponding therefore to a N mass root-to-shoot ratio of 43%. A recent study showed that the N mass root-to-shoot ratio of 26 crop species grown in subtropical conditions was on average 24% but reached 67, 63 and 53% for rye, corn and barley, respectively (Redin et al. 2018). It should be added that only root N mass was estimated in our study, without considering the stool which may represent a significant part of the total belowground biomass (Sampaio et al. 1987; Bell et al. 2010; Fortes et al. 2010; Garside and Bell 2012). Including this compartment should further accentuate the need to pay attention to the belowground compartment of sugarcane in studies dealing with fertiliser N use efficiency and N cycling in sugarcane agroecosystems. Such a quantity of N stored in roots raises questions regarding the role of the belowground compartment in sugarcane nutrition, especially during the crucial period of regrowth. Several studies have mentioned the assumed importance of the root compartment on sugarcane N nutrition based on experimental suggestions (Trivelin et al. 2002; Vitti et al. 2007) or comparisons with other crop species like *Miscanthus* or *Panicum* (Nassi o Di Nasso et al. 2011; Strullu et al. 2011; Dohleman et al. 2012). Nutrient storage in the belowground compartment (roots + stool) during the sugarcane growth and nutrient remobilization at early growth stage, both via internal translocation, deserve particular attention in the future as these processes could explain the relatively good yield of sugarcane even in nutrient-poor soils.

The greater amount of N accumulation in roots observed at moderate levels of N fertilisation was the result of the opposing evolution of the root biomass and of its N concentration. The root biomass was low but was characterised by high N concentrations when N was supplied by fertilisation. Conversely, the root biomass was high but contained a low N concentration in unfertilised plots. As a result, the fertiliser N uptake efficiency was improved when the root compartment was considered according to NRE calculations in moderately fertilised treatments. By contrast, approximately the same amount of N accumulated in the non- and overfertilised plots and NRE had therefore not been influenced. To conclude, differences of N accumulation in roots may have significant implications in studies based on difference approach and could strongly influence the fertiliser N use efficiency of high root biomass crops estimated with isotope approach.

Conclusion

The development of the root system in a fertilised sugarcane plot can be summarised as follows: 1/homogeneous colonization of the topsoil by the fine roots during an early phase, 2/progressive development of thicker roots mainly localised under the sugarcane row during the mature phase. Most of the roots are located in the first 30 cm of the soil profile, but N fertilisation may have contradictory effects on root intensification in the topsoil layer, decreasing root density in this study.

A significant part of the biomass produced in sugarcane agroecosystems was found underground. The biomass of the root compartment was approximately twofold higher than that of the shoot compartment at the beginning of the ratoon. Application of N fertiliser led to a substantial decrease in the root-to-shoot ratio at harvest, notably due to a decreasing trend of the root biomass. These results need to be confronted in further studies since the fertiliser effect on roots could have significant implications on crop productivity (water stress) and soil health (carbon inputs, soil density modification).

The root system of sugarcane appeared to be a major pool of N that should be considered in studies dealing with fertiliser N use efficiency and N cycling in sugarcane agroecosystems.

Finally, this study highlighted the importance of further research focusing on the survival and the reactivation of the root system between ratoons as well as on nutrient storage in and remobilization from the belowground compartment via internal translocation.

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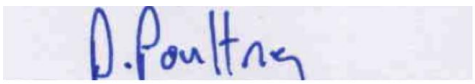
Signature : 

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