

# Nitrogen Use Efficiency for Sugarcane-Biofuel Production: What Is Next?

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**Abstract** Land area devoted to sugarcane (*Saccharum* spp.) production in Brazil has increased from 2 million to 10 million ha over the past four decades. Studies have shown that, from an environmental perspective, the transformation of nitrogen (N) fertilizers into N<sub>2</sub>O gases can offset the advantages gained by replacing fossil fuels with biofuels. Our objectives here were to review recent developments in N management for sugarcane-biofuel production and assess estimates of N use efficiency (NUE) and N losses based on future scenarios, as well as for life-cycle assessments of bioenergy production. Approximately 60 % of N-based fertilizer applied to sugarcane fields in Brazil is recovered by plants and soils, whereas N losses to leaching and N<sub>2</sub>O emissions can average 5.6 and 1.84 % of the total applied N, respectively. Maintenance of trash, rotation with N-fixing legume species, and optimization of byproducts usage have potential for reducing the N requirements of sugarcane cultivation in Brazil. Moreover, the development of sugarcane genotypes with higher NUEs, along with management systems that consider soil capacity of mineralization, is required for improving the NUE of sugarcane. Strategies to maintain N as NH<sub>4</sub><sup>+</sup> in sugarcane-cropped soils also have the potential to reduce N losses and enhance NUE. The development of second-

generation biofuels is important for increasing biofuel production while simultaneously maintaining N rates and improving NUE, and sugarcane systems in Brazil show potential for sustainable biofuel production with low N rates and limited N<sub>2</sub>O losses. Reducing N rates in sugarcane fields is thus necessary for improving sugarcane-based biofuel production and reducing its environmental impacts.

**Keywords** Review · Ethanol · NUE · N<sub>2</sub>O · Nitrogen fertilizer

## Introduction

Production of biofuel from sugarcane has numerous advantages over that from other crops such as maize, wheat, and sugar beets, which include lower energy demand over the course of the production process and highly positive energy balances [1–4]. This feedstock has a high photosynthetic efficiency in tropical regions, resulting in high biomass production, which, in association with the re-use of its byproducts, has environmental and economic advantages over fossil fuels [2, 5, 6].

Cultivation of feedstock is generally viewed as controversial due to the uncertainty regarding greenhouse-gas (GHG) emission reductions and its potential competition with land use for biodiversity and food [7]. N<sub>2</sub>O is an important N component for the net GHG balance of biofuels, as N<sub>2</sub>O is 300-fold more potent than CO<sub>2</sub> as a GHG and, therefore, a small increase in N<sub>2</sub>O emissions resulting from additional fertilizer use can offset large CO<sub>2</sub> reductions through the replacement of fossil fuels by biofuels [7, 8].

Production of ethanol from sugarcane is one of the most robust GHG-saving options based on first-generation biofuel production [9]. Optimization of NUE and development of second-generation technologies are also options for increasing

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sustainability of biofuel production [7]. Second-generation ethanol production by enzymatic hydrolysis of trash or bagasse is under development in Brazil, with potential to increase ethanol production per area without increasing application of N-based fertilizers. Brazil uses less N in its sugarcane production systems than all other large-scale producers worldwide [32]. Recent developments in N management for sugarcane production in Brazil include in situ quantification of N losses from leaching, denitrification, volatilization, and a series of N-response trials in the main areas of intense sugarcane cultivation.

Given their tendency to increase N rates, the rapid expansion of green-cane trash blanketing (GCTB) systems has posed challenges to traditional N management practices, especially if increasing N rates do not directly enhance yields in light of the positive correlation between fertilizer N rates and degree of N losses to the environment [7]. Reviewing recently developed N response curves for growing conditions in Brazil will be helpful to ascertain whether there is an increase in N response under GCTB cultivation in the country—information that is critical for the development of effective N management strategies.

Brazil is currently the largest sugarcane producer in the world, and the area under sugarcane cultivation is expected to continue to expand as domestic demand and consumption of ethanol products increase over the coming decades. As this expansion will occur primarily in areas with low fertility soils,—degraded grasslands, for instance—substantial N inputs will be required to generate high yields. Although bioenergy production has the potential to reduce CO<sub>2</sub> emissions from the burning of fossil fuels, these crops also bring the issues relating to increasing N requirements for generating high crop yields and the environmental concerns associated with the use of synthetic fertilizers to the attention of the scientific community.

Our objectives were therefore to review recent developments in N management for sugarcane-ethanol production and to provide reliable estimates of NUE and N losses that can be useful to future assessments of bioenergy production. The review discusses the most up-to-date information about the preferred forms of N uptake by sugarcane, NUE, in situ measurements of N losses, and the effects of trash and crop rotation on N requirements, as well as N-response trials for the development of N management guidelines for GCTB systems. Finally, we highlight the main advances required in terms of N management strategies to advance sugarcane production while reducing N usage.

### Sugarcane in Brazil: Current Scenario

Sugarcane production in Brazil generates over 1 million jobs directly and 3.6 million indirectly, and a foreign exchange of around US\$107 billion per year [10]. Approximately 45 % of

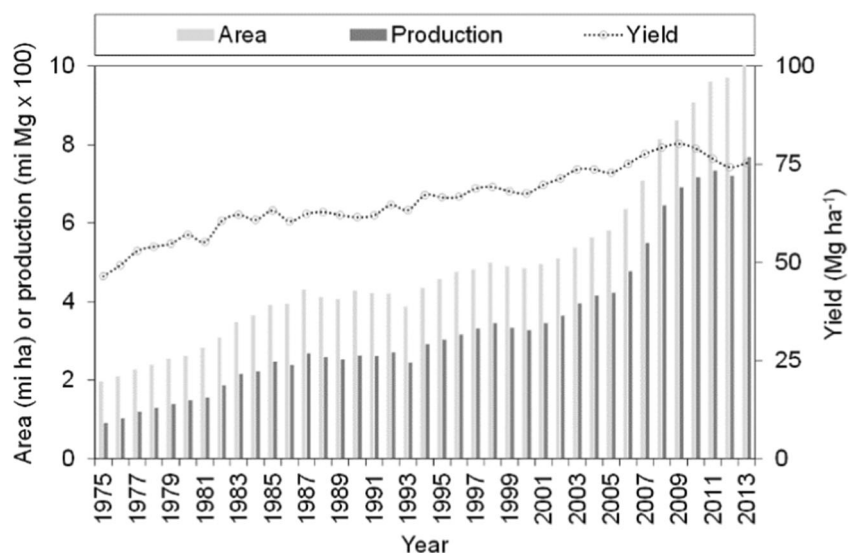
the total energy matrix in Brazil is composed of renewable energy sources [11, 12], with 42 % of renewable sources consisting of sugarcane biomass (used for ethanol or electricity production), 28 % hydroelectric, 20 % wood, and 10 % deriving from other sources [13]. Brazilian sugarcane biofuel production creates six times as many employment opportunities as does the country's petroleum sector [14].

Of the 27 million ha of sugarcane produced worldwide, the largest share, at 10.2 million ha, is found in Brazil, followed by India (5.1 million ha) and China (1.8 million ha) [15]. The government of Brazil developed the National Alcohol Program (Proálcool) in 1975 to promote large-scale substitution of petrol-derived motor fuels with ethanol, which consequently drove the rapid increase in the Brazilian sugarcane industry [2]. As a result, the area under sugarcane cultivation in Brazil expanded from 2 million ha in 1975 to 10.2 million ha in 2013 (Fig. 1) and annual production increased from 91 million Mg to 768 million Mg while the national average productivity increased from 46 to 75 Mg ha<sup>-1</sup>.

Some projections indicate that the land area under sugarcane cultivation in Brazil will reach 11.5 million ha by 2024, with sugarcane production rising to an estimated 884 million Mg [13]. From 2015 to 2024, sugar production could increase from 39 to 48 million Mg and ethanol production from 30 to 42.5 billion L [13]. Another advantage that the sugarcane industry in Brazil has compared to that in other countries is the flexibility that most sugarcane mills have for producing sugar or ethanol in the same plant, thereby allowing rapid transition from one product to another in response to changing economic conditions or market demand. Presently, around 53 % of sugarcane milled in Brazil is allocated for ethanol production, with the remaining 47 % intended for sugar production, by 2024, however, it is estimated that closer to 60 % of sugarcane will be processed for ethanol production following the expected increase in domestic consumption [13].

Approximately 90 % of the country's sugarcane yield is generated in southeastern Brazil, whereas São Paulo is the single largest producer state, with 56 % of national production [16]. The shift from burned to GCTB systems occurred rapidly over the past decade in Brazil. Approximately 66 % of the cultivated area in the state of São Paulo was burned in 2006, causing environmental problems due to emissions of air pollutants (particulate matter, carbon monoxide, and hydrocarbons) and GHG emissions (CH<sub>4</sub> and N<sub>2</sub>O), as well as affecting local fauna and flora. In 2014, 84 % of the cultivated area in the state was harvested under the GCTB system [17]. The preservation of sugarcane residues on the soil surface minimizes GHG emissions from the burning process and may promote carbon (C) storage in soils, and, over the long term, may also result in lower amounts of N application to the crop due to enhanced N release through trash mineralization, thus reducing GHG emissions from fertilizer N sources [18]. Maintaining crop residues over soil surfaces brings new

**Fig. 1** Evolution of cultivated area, production and average yield of sugarcane in Brazil from 1975 to 2013. Source: FAOSTAT [15]



challenges, however. The main difficulties caused by a thick layer of trash, when compared with sugarcane fields that were previously burned, related to the incorporation of fertilizers [19], loss of N due to  $\text{NH}_3$  volatilization from surface-applied urea [20, 21], and a higher incidence of pests [22]. Maintenance of trash on the soil surface may also delay sugarcane sprouting during the winter in colder regions, subsequently affecting yields [23].

In addition to struggling with problems associated with maintenance of surface trash, the Brazilian sugar and ethanol sectors have also sought to increase energy generation in an effort to boost profitability. One of the strategies they have adopted includes the use of trash left in the fields following mechanical harvesting. Sugarcane trash can be burned in boilers to generate steam for electricity production (co-generation) or it can be used for the production of second-generation ethanol [24]. Considering the large-scale production of cellulosic ethanol from ground bagasse and crop residues (i.e., trash), an important topic of discussion is how to effectively manage the sugarcane residues in the fields [25]. Related issues to be addressed include the proportion of trash to be removed from soil for this purpose and how much trash should be left in the fields to provide physical, chemical, and biological benefits to the soil-plant system.

### Nitrogen Forms and Nitrogen Use Efficiency by Sugarcane

The main forms of available N for plant uptake in the soil are ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ), which are either generated as products of the mineralization of soil organic matter (SOM) or added in the form of mineral fertilizers [26]. In environments where N availability is limited, plants and microorganisms are also able to absorb intact amino acid, small

peptides, and urea [27–29], as reported by Vinnall et al. [30] in a study demonstrating that sugarcane plants take up some amino acids. However, it is generally accepted that mineral forms are the main sources of N to plants in agricultural systems.

Research on sugarcane using a  $^{15}\text{N}$ -labeled nutrient solution has shown that N recovery is higher when the nutrient was provided as  $\text{NH}_4^+$  than  $\text{NO}_3^-$  [31, 32]. However, considering the short-term assessment performed by Robinson et al. [32], further research is required to ascertain the preferential forms of N for sugarcane over the entirety of the crop cycle under field conditions (~12 months). This is more apparent considering the rapid conversions of  $\text{NH}_4^+$  into  $\text{NO}_3^-$  through nitrification that occur under field conditions, as reported by D'Andréa [33], who assessed gross nitrification rates in 17 soils under sugarcane cultivation in São Paulo. If the observation made by Robinson et al. [32] occurs, to a large extent, under field conditions, the use of nitrification inhibitors such as dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) may be a temporary alternative for attenuating the conversion rate from  $\text{NH}_4^+$  into  $\text{NO}_3^-$  by soil-nitrifying bacteria [34] and, thus, may enhance sugarcane NUE.

Several studies using the  $^{15}\text{N}$  tracer through isotope dilution methods were undertaken in Brazil over the past several decades. In such studies, three indices are usually reported: (1) percentage of the fertilizer N assimilated by plants (i.e., NUE); (2) percentage of the fertilizer N remaining in the soil (NDFS); and (3) percentage of the total N content deriving from fertilizer in plants (NDFP). Values of sugarcane NUE in Brazil range between 7 and 40 % (average of 26 %) [35–40], whereas NDFS varies between 23 and 37 % (average of 32 %) [38, 40–42]. Together, the proportion of the fertilizer N taken up by plants and remaining in the soil (e.g., immobilized, soluble, or sorbed) is ~58 %, with losses by leaching, volatilization, denitrification, runoff, and  $\text{NH}_3$  and  $\text{N}_2\text{O}$  emissions via the

leaves accounting for the remaining 42 % or so. Furthermore, foliar emissions of ammonia occur in senescent tissues via the stomata due to decreases in the  $\text{NH}_3$  compensation point [43], and it seems to be the main pathway of N loss during advanced growth stages [44], in which losses as high as  $90 \text{ kg N ha}^{-1}$  have been reported in sugarcane fields [36].

NDFP at the time of crop harvest normally varies between 10 and 28 % [35, 37, 40, 41, 45–47], which means that 28 % of the N found in the plant (with the exception of the roots in most cases) derived from fertilizers and 72 % from other sources, such as SOM mineralization, biological N fixation (BNF), dry and wet deposition, and so on. A common finding in such studies is that NDFP levels are higher during initial growth stages and decrease through the final growing stages. Franco et al. [46] and Vieira-Megda et al. [47], for instance, observed that NDFP in sugarcane reached 60–70 % in the initial stages of the crop cycle but had dropped to 10–20 % at the time of harvesting, which demonstrates the importance of N fertilizer application for crop growth in the initial stages and the importance of soil N reserves for the remaining period. Immediate release of soluble N fertilizers explains optimal N fertilizer recovery by crops immediately following fertilizer application [48]; during crop growth, however, N derived from SOM proportionally increases as the root system expands, which does not occur with N from fertilizers [46]. This finding indicated that SOM, and not fertilizers, is the main source of N for sugarcane over the course of the entire crop cycle, as previously reported [46–49]. As such, soil N supply from mineralization must be considered in N management systems where increasing NUE is a primary goal.

Strategies for improving sugarcane NUE include adoption of best fertilizer management practices, which will be discussed later in this review. Reducing N rates without affecting yield is an alternative to increasing NUE in sugarcane systems, but a soil-based N management approach must be developed to determine under what conditions N fertilization can be reduced. Otto et al. [49], for example, designated sites were as highly, moderately or non-responsive to N fertilization. Although N fertilization should not be reduced in highly responsive sites to avoid yield limitations, it can be substantially reduced (from  $100 \text{ kg ha}^{-1}$  to  $50 \text{ kg ha}^{-1}$  N, for instance) in moderately responsive sites, or even eliminated in non-responsive sites. The strategy of reducing N rates while maintaining yields was proven to be the best option for increasing sustainability of biofuels production [50].

Another means of improving sugarcane NUE in Brazil is through the use of more N-efficient sugarcane genotypes. Identification and quantification studies have demonstrated the NUE-related differences among different sugarcane genotypes [51, 52], with some genotypes being highly efficient in using available N to produce biomass, whereas others are capable of storing N during the initial growth stages [32, 51–53]. Evaluating genotypic differences in terms of NUE

by sugarcane is an important strategy for enhancing NUE and hence decreasing the environmental risks that arise from N fertilization. Over the long term, breeding programs should focus on developing genotypes with improved NUE, thereby allowing growers to choose genotypes that deliver high yields with low N demand. However, in terms of genetically modified genotypes, current attention is focused mainly on breeding resistance to herbicides, insects, drought-tolerance, and high sugar content [54] rather than improving NUE. As such, further research on NUE-related genotypic differences among sugarcane genotypes is greatly needed.

### Nitrogen Losses in Sugarcane Fields

Globally, approximately 2.5 Tg of fertilizer N is applied to sugarcane fields every year, representing 2 % of total global fertilizer N use [32]. The magnitude of N losses and the low NUE of sugarcane translate into significant financial and environmental costs [55, 56], and there is growing concern about the economic and environmental sustainability of the increasing dependency on fertilizer N for the production of bioenergy crops [7].

Of the top producing nations worldwide, sugarcane cropped in Brazil uses N fertilizer more efficiently, given the low N rates applied, the relatively high yields, and the favorable growing conditions. Robinson et al. [32], for example, reported that N-application rates are considerably lower in Brazil ( $60\text{--}100 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ), than in India ( $150\text{--}400 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ) and China ( $100\text{--}755 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ), furthermore noting that Brazil produces approximately 42 % of the world's supply of sugarcane but consumes only 25 % of the N used for sugarcane production, whereas India and China collectively produce 31 % of global sugarcane and consume 50 % of N fertilizer applied to sugarcane fields. Nitrogen rates were reduced from 200 to  $160 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  in sugarcane fields in Australia [57] following an unprecedented scrutiny of the environmental impacts of N application rates and N losses attributed to the country's sugarcane industry [55]. Consequently, efforts have been made to develop best management practices aimed at improving NUE and reducing N losses in Australian sugarcane fields [57].

Following N fertilizer application, microbial oxidation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  in the soil releases  $\text{H}^+$ , resulting in soil acidification over the long term [58]. Nitrate can also be leached from the root zone since soil colloids and SOM do not hold  $\text{NO}_3^-$ . Leaching of  $\text{NO}_3^-$  is often accompanied by basic cations such as Ca, Mg, and K, resulting in  $\text{H}^+$  concentrations in the soil solution that increase acidification [59]. Soil acidification is of great concern to sugarcane producers in locations with more acidic soils, as it limits yields [60]. Leaching of  $\text{NO}_3^-$  can result in environmental problems, especially in regard to water quality; the magnitude of  $\text{NO}_3^-$  losses varies, depending on soil texture, soil charges in the subsoil, water

balance, and N-management practices (rates, timing, and method used; e.g., single or split N-fertilizer application), with the highest rates of leaching most likely to occur on coarse-textured, free-draining soils following periods of heavy precipitation [61].

To compare the influence of soil properties and management practices on the potential of leaching losses in sugarcane soils, we will compare the production systems in Brazil and Australia. Nitrogen rates adopted in Brazil are lower than in Australia, both in the plant cane and ratoon [32]. The sugarcane-cultivated area in Australia is largely located within wet tropical and humid subtropical climates separated by areas of unsuitable soils or unreliable rainfall [62]. In some areas, frequent flooding may occur during the wet season [55]. Not surprisingly, sugarcane cultivation was ranked as the largest source of anthropogenic dissolved mineral N in the Great Barrier Reef catchments [63]. In Brazil, sugarcane is cultivated mainly in deep, well-drained, and highly weathered soils [64], and the expansion of sugarcane in Brazil will occur mainly in areas of similar conditions. In subsurface horizons of highly weathered soils with low SOM, iron (Fe) and aluminum (Al) oxides prevail and, depending on soil pH, development of anion exchange capacity can sorb anions, such as  $\text{NO}_3^-$  [65]. Therefore, given the comparatively deep and weathered soils, only limited leaching of fertilizer N has been reported for the sugarcane production systems of Brazil (Table 1) and, thus, only relatively small amounts of  $\text{NO}_3^-$  enter water tables. Reductions in N leaching losses were also observed for sugarcane production systems in Argentina when moderate N rates were applied to the crop [70].

Denitrification is defined as the conversion of  $\text{NO}_3^-$  to gaseous forms of N (nitric oxide—NO, nitrous oxide— $\text{N}_2\text{O}$ , or di-nitrogen— $\text{N}_2$ ) by microorganisms under anaerobic conditions [71]. Among these three N gases, special attention shall be given to  $\text{N}_2\text{O}$ , a GHG with a global-warming potential 298-fold higher than that of  $\text{CO}_2$  [63]. Emissions of  $\text{N}_2\text{O}$  have raised concerns over the past several years in terms of the sustainability of biofuel production [7–9, 72–74]. The

Intergovernmental Panel on Climate Change (IPCC) has estimated that 1 % of applied N fertilizers is converted into  $\text{N}_2\text{O}$  in agricultural systems [75], but this factor could be even higher if indirect emissions are also taken into account. For example, Crutzen et al. [8], in deriving  $\text{N}_2\text{O}$  emission factors from global N budgets, fixed N inputs, and atmospheric concentrations of  $\text{N}_2\text{O}$ , calculated an overall emission factor closer to 3–5 %. Using these values in the estimations, they found that the production of biofuels from rapeseed and corn may, in fact, contribute to global warming via increased  $\text{N}_2\text{O}$  emissions as opposed to having a mitigating effect by substituting for fossil fuels. In the study of Crutzen et al. [8], ethanol from sugarcane was the only environmentally friendly option from the perspective of GHG emissions, even considering the relatively high emission factor of his estimations. However,  $\text{N}_2\text{O}$  emissions from sugarcane fields are highly heterogeneous [76–81]: a series of in situ measurements of  $\text{N}_2\text{O}$  emissions developed over the past several years show emission factors both higher and much lower than the values reported by Crutzen et al. [8] depending on the site.

High  $\text{N}_2\text{O}$  emissions can be expected from waterlogged soils with high C and/or  $\text{NO}_3^-$  contents, and high temperature [76]. GCTB systems also promote higher  $\text{N}_2\text{O}$  emissions, as they promote soil-moisture retention and increased microbial activity [77]. In sugarcane systems in Australia, it has been estimated that from 1 to 6.7 % of N fertilizer can be converted into  $\text{N}_2\text{O}$  [76]; under favorable conditions, denitrification losses can be as high as 21 % of fertilizer N [78, 79]. In a review, Lisboa et al. [79] calculated an emission factor of 3.9 % (without subtracting background values) using data from Australia, Hawaii, and Brazil. Measurements of denitrification losses under sugarcane cultivation in other large-scale producers (e.g., China and India) are notably lacking.

Rates of denitrification are lower on the well-drained and low-C-content soils on which sugarcane in Brazil are typically grown; Soares et al. [80] reported  $\text{N}_2\text{O}$  emissions of 0.7–0.75 % over two consecutive seasons under sugarcane cultivation in the state of São Paulo, for instance. Emissions of 1.1 % of N fertilizer applied over GCTB were observed, but combined vinasse application boosted  $\text{N}_2\text{O}$  emissions to 3 % [81]. Soil and climatic conditions following fertilizer application may affect the magnitude of losses, as demonstrated by Signor et al. [82], who found that losses of ammonium nitrate reached 1.2–1.5 % and that of urea 0.3–1.1 % at one experimental site, and under conditions more conducive to losses (e.g., maintenance of trash, rainfall events after fertilizer application), these values were as high as 2.9–6.7 % for urea and 0.8–13.0 % for ammonium nitrate [82]. Based on data compiled from recent studies carried out under Brazilian field conditions, an average 1.84 % emission factor was estimated (Table 2). In addition, replacing fossil fuels with ethanol production from sugarcane reduces overall GHG emissions even at  $\text{N}_2\text{O}$  emission levels of 3–5 % [8].

**Table 1** Leaching of N from native soil and  $^{15}\text{N}$ -labeled fertilizer in studies performed in sugarcane fields in Brazil

Crop cycle	N rates kg ha <sup>-1</sup>	Losses by leaching from		Reference
		Native soil-N kg ha <sup>-1</sup>	Fertilizer-N %	
Plant cane	0, 30, 60, 90	5.0, 2.5, 4.3, 3.9 <sup>1</sup>	0	[66]
Plant cane	120	18.3	0.02	[67]
Plant cane	120	1.1	0.05	[68]
Ratoon	0, 100, 150	3.9, 34.3, 21.6	22.5	[69]
Average	—	10.5	5.6	—

Mean values of sugarcane harvested with prior burning and under the green cane trash blanketing system

**Table 2** In situ measurements of N<sub>2</sub>O emissions in sugarcane fields in Brazil

Crop cycle	Fertilizer management <sup>a</sup>	N rates kg ha <sup>-1</sup>	N <sub>2</sub> O emissions <sup>b</sup> %	Reference
Plant cane	Urea	60	1.11	[81]
Plant cane	Urea + press mud	122	1.10	[81]
Plant cane	Urea + vinasse	87	2.65	[81]
Plant cane	Urea + press mud + vinasse	149	1.56	[81]
Ratoon (Piracicaba)	Urea	60, 90, 120, 180	0.8, 1.33, 6.21, 12.95	[82]
Ratoon (Piracicaba)	Ammonium nitrate	60, 120, 180	1.22, 1.53, 1.22	[82]
Ratoon (Goianésia)	Urea	60, 90, 120, 180	2.85, 3.59, 6.76, 4.31	[82]
Ratoon (Goianésia)	Ammonium nitrate	60, 120, 180	1.10, 0.63, 0.31	[82]
Ratoon	Urea	120	0.69 (y1) / 0.75 (y2)	[80]
Ratoon	Urea + DCD	120	0.04 (y1) / 0.14 (y2)	[80]
Ratoon	Urea + DMPP	120	0.01 (y1) / 0.00 (y2)	[80]
Ratoon	Urea + DCD-R	120	0.07 (y2)	[80]
Ratoon	Urea + DMPP-R	120	0.05 (y2)	[80]
Ratoon	PSCU	120	0.93 (y1) / 1.09 (y2)	[80]
Ratoon	Urea	120	0.68, 0.96, 0.76, 2.03 <sup>c</sup>	[81]
Ratoon	Urea + vinasse	142	0.59, 1.19, 1.89, 3.03 <sup>c</sup>	[81]
Average			1.84	

<sup>a</sup> DCD, dicyandiamide; DMPP, 3,4-dimethylpyrazole phosphate; PSCU, polymer-S coated urea; R, nitrification inhibitor reapplied in the same plot of previous cycle

<sup>b</sup> y1, year 1; y2, year 2

<sup>c</sup> Respectively for 0, 7, 14, and 21 Mg ha<sup>-1</sup> of trash

Nitrogen losses via denitrification may be reduced if adequate fertilization strategies are adopted, such as application rates based on the variable N demand of plants, as well as the ability of soil to supply N [83]. Another strategy for mitigating N<sub>2</sub>O emissions from N fertilization is by adding nitrification inhibitors directly to the fertilizers, thereby reducing rates of microbial oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> [34] and consequently reducing substrate availability for denitrifying bacteria. This strategy is interesting given that the addition of these inhibitors has been shown to suppress N<sub>2</sub>O emissions from urea by 90 % under Brazilian conditions [80]. However, while DCD and DMPP exhibited similar capacities for reducing N<sub>2</sub>O emissions, little potential for reducing N<sub>2</sub>O emissions was found for controlled-release fertilizers [80].

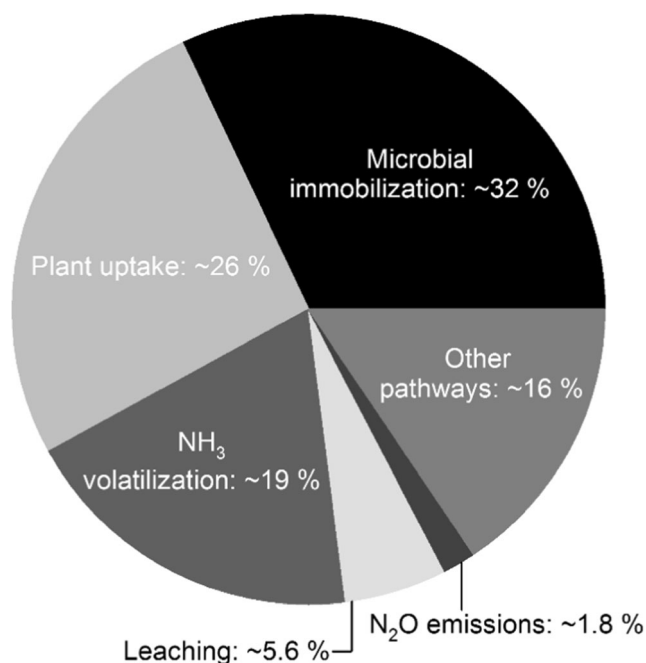
Considering the in situ measurements of leaching, denitrification, N uptake, immobilization, and volatilization losses under Brazilian field conditions that have been made available over the past several years, it is possible to develop a simplified N budget in the soil-plant system, as demonstrated in Fig. 2. Such generalizations can be useful in establishing future scenarios or life-cycle assessment of sugarcane production, but care must be taken in interpreting these data; for example, the mean value of 5.6 % for leaching losses does not include losses from native soil N (Table 1). Emissions of N<sub>2</sub>O are affected by numerous factors, such as the sources and rates of N [82] and soil moisture [84] and, thus, a single emission factor cannot be applied universally to all production

systems. Volatilization losses can be much higher than the value reported when urea is applied over GCTB [20, 21] and can be much lower when non-amidic sources are used [20, 85]. Such limitations must be considered when forming broader generalizations from the estimates shown in Fig. 2.

### Influence of Trash Blanket on Nitrogen Fertilization

Sugarcane cultivated in GCTB production systems adds approximately 10–20 Mg ha<sup>-1</sup> (dry mass) of crop residues annually, composed mainly of dry leaves and sugarcane tops [6, 88, 89], depending on yield and genotype characteristics. The amount of nutrients returned to the soil through trash also varies, ranging from 39–72 kg N ha<sup>-1</sup>, 4–23 kg P ha<sup>-1</sup>, 35–173 kg K ha<sup>-1</sup>, 9–81 kg Ca ha<sup>-1</sup>, 6–26 kg Mg ha<sup>-1</sup>, and 7–15 kg S ha<sup>-1</sup> [90–92]. In addition, the trash layer that accumulates over the soil surface influences sugarcane production by affecting yield, fertilizer management, weed control, soil erosion, and SOM dynamics [6].

Under the field conditions that occur in Brazil, trash can be strongly reduced (~73 % depletion) as a result of decomposition by microorganisms within 3 years after its initial deposition, and the nutrients released in larger amounts are K, Ca, and N [89]. However, given the high C/N ratio of the trash (varying from 80 to 100), its decomposition and subsequent release of N are low in one crop season. Simulations performed by Robertson and Thorburn [93] using data from Australia determined that organic



**Fig. 2** Fate of N from fertilizer in sugarcane-cropping soils based on measurements under Brazilian field conditions. Microbial immobilization: values gathered from Gava et al. [42] (37 %), Vitti [38] (32 %), Basanta et al. [41] (29 %), and Faroni [40] (29 %). Plant uptake: values gathered from Trivelin et al. [36] (12 %), Gava et al. [37] (17 %), Vitti [38] (26 %), Franco et al. [39] (28 %), Faroni [40] (37 %), and Trivelin et al. [35] (40 %); 3) NH<sub>3</sub> volatilization: values gathered from Costa et al. [20] (24 %), Cantarella et al. [85] (8 %), and Mariano et al. [21] (25 %). Leaching: values gathered from Table 1. N<sub>2</sub>O emissions: values gathered from Table 2. Other pathways: N derived from fertilizer not accounted in previous pathways (e.g., losses by runoff and foliar emissions of NH<sub>3</sub> and N<sub>2</sub>O)

C and total N in the soil could increase by 8–15 % and 9–24 %, respectively, whereas soil mineral N could increase by 37 kg ha<sup>-1</sup> y<sup>-1</sup> under GCTB, taking 20–30 years for the soils to reach a new steady state. According to their results, N fertilizer application should not be reduced within the first 6 years after adoption of GCTB, but small reductions may be possible in the long term (> 15 years). In a simulation performed for southeastern Brazil, Trivelin et al. [94] found that GCTB would increase soil N stocks and N recovery by sugarcane, reaching a dynamic equilibrium after 40 years, with N recovery by plants reaching ~40 kg N ha<sup>-1</sup> y<sup>-1</sup>.

The effects of GCTB on sugarcane yield are complex, with research studies reporting both negative [23, 41, 95] and positive [36, 96] effects on yield. In colder regions, trash reduces shoot sprouting from ratoon crops [97], which may account for some of the observed negative results. One option for avoiding decreasing yields in such regions is to remove trash mulch from crop rows but maintain it between rows, as demonstrated by Campos et al. [23]. In warmer climates, trash promotes higher yields by helping to conserve soil moisture and decreasing soil temperatures [98].

The large-scale adoption of GCTB in Brazil triggered two significant changes in fertilization management. The first involved alterations in the methods and sites of application; incorporation of fertilizer midway between rows, as was typically done in the previous management system when residues were largely absent, was now hindered by the trash layers that cover the soil surface [99], and for this reason, producers began to adopt the practice of surface applications in ratoon. The second modification refers to a trend in increasing N rates under GCTB as compared to the previous system (manual harvest with prior burning), to avoid yield losses associated with N immobilization during trash mineralization by microorganisms.

Another recent issue that has arisen in the sugarcane industry is the increasing interest in removing the trash blankets to use in the production of electricity or second-generation ethanol [24], a topic that is being widely discussed in the literature in Brazil [26]. Considering the advantages of GCTB for N cycling [94], removing trash may directly affect the sustainability of the agroecosystem over the long term. Although trash does not effectively supply N in the first growing season, its contribution to SOM maintenance is twice that of N fertilizers and, in addition, the long-term effect of crop residues in providing mineral N to the crop is greater than that of N fertilizers [48]. Therefore, research is required to assess the impacts of trash-blanket removal on sugarcane productivity under different climatic conditions, as well as on C and N storage in soils.

### Influence of Crop Rotation on Nitrogen Fertilization

Cultivation of N-fixing legumes (sunn hemp species: *Crotalaria juncea*, *Crotalaria spectabilis*, and *Crotalaria ochroleuca*; peanuts: *Arachis hypogaea* L.; and soybean: *Glycine max* L.) in rotation with sugarcane is becoming a common practice, especially in the southeastern region of Brazil. The main benefits of such rotations include improving N supply [100–103], weed control [104], reduction in the population of nematodes [105], and erosion control, and increasing yields [100, 102, 106].

Legumes typically accumulate large amounts of N and K, which are essential for sugarcane. The amount of N fixed from the atmosphere by bacteria associated with legumes may vary from 30 to 200 kg N ha<sup>-1</sup> [106–108]. However, the amount of N in legume residues available for subsequent crops depends on the internal processes of N-cycling in the soil, such as mineralization and immobilization, as well as whether legumes are cultivated exclusively for biomass production—and hence will be returned to the soil—or as grain crops such as soybeans and peanuts, which reduce the N contribution to the system owing to nutrient removal as a result of harvest. In general, the amount of N fertilizer applied to a cycle of plant cane might be reduced, or even suppressed, when rotation with N-fixing legumes is performed [109]. Nevertheless,

sugarcane growers normally maintain the usual N rates that are applied on ratoon crops, neglecting possible residual effects of N fixed by legumes on subsequent ratoon. Park et al. [110] simulated the residual effect of N fixed by soybean (in the absence of grain harvesting) during a sugarcane-growing season in Australia. They observed a potential for reduction of 100 % of N in the plant cane, and 60, 25, and 10 % in subsequent ratoon crops, respectively. The cultivation of legume crops in rotation with sugarcane certainly has the potential to reduce N rates under Brazilian conditions, based on the results of a study by Otto et al. [49] in which no response of ratoon to N fertilization in fields managed with legume rotation was found. However, the effects of legume crop rotation on the N requirements of subsequent crop cycles (ratoon) have yet to be examined in Brazil, even for the *Crotalaria* species that are being widely adopted in the country.

## Improvements in Sugarcane Nitrogen Fertilization: Recent Developments in Brazil

### Nitrogen Rates

There is a perception in Brazil that plant cane cycles show limited responses to N fertilization [111]. Recent studies have reported positive responses, but N rates that delivered high yields or net returns still range in the relatively low 40–60 kg N ha<sup>-1</sup> [112]. There is a clear relationship between soil tillage operations and plant cane responsiveness to N. When fields are established following conventional tillage operations (e.g., deep plowing, subsoiling, or disking), plant cane responsiveness to N is limited [108], whereas responsiveness increases under reduced tillage practices [113]. Such results indicate a clear relationship between conditions that enhance soil N mineralization and decrease the N requirements of sugarcane [114, 115], demonstrating that soil N mineralization plays a key role in supplying N to plants. Based on such findings, we expect that the usual N rates adopted for plant cane in Brazil will not be modified over the medium term.

Research undertaken in Brazil has suggested that BNF by diazotrophic bacteria might be an important source of N to sugarcane [116, 117]; Urquiaga et al. [117], for instance, estimated that sugarcane can obtain at least 40 kg N ha<sup>-1</sup> through BNF. Five species of diazotrophic bacteria [118] have been used in studies under greenhouse and field conditions, with a mix of positive and negative results. On the positive side, Schultz et al. [119, 120] obtained similar yields between inoculated and fertilized treatments in one site but not in another. However, Cantarella et al. [121] did not find positive results of inoculation in a comprehensive 13-site-years study in São Paulo. Evidence of BNF was also not observed for sugarcane in Australia [122] or South Africa [123]. More recent evidence has shown that the benefits may be associated with the

production of plant-growth promoting substances rather than BNF [120, 124, 125]. These results indicate a gap between the evidence of BNF obtained in the past (usually using micropropagated sugarcane) and studies performed under field conditions. Further research is required that focuses on transforming inoculation into a practical alternative for sugarcane production and its potential for reducing N requirements.

Ratoon N fertilization has been shown to improve sugarcane yields [126, 127] and can be effective in circumstances of limited soil disturbance, which results in lower soil N mineralization. However, the N rates adopted in Brazil are substantially lower than are the N rates adopted in other large-scale producers [32]. In São Paulo, for example, official recommendations for N fertilization on ratoon crops vary between 60 and 120 kg N ha<sup>-1</sup>, depending on the expected yield concept [128]. Such recommendations were formulated from experiments conducted at sites subjected to burning (i.e., no trash maintenance) and have resulted in an empirical and widely adopted recommendation of an N fertilizer requirement of 1.0 kg N per Mg of stalk.

Currently, ~80 % of the area under sugarcane cultivation in Brazil is harvested under GCTB systems [17, 129]. These systems promote alterations in soil C and N cycling, which may require further adjustment in the N recommendation system for sugarcane. On the one hand, the amount of trash maintained overtop of the soil can promote immobilization of N fertilizer during trash decomposition and may require an increase in the recommended N rates for burned sugarcane. On the other hand, the release of N from trash along with increases in soil C and N stocks over time may serve to reduce demand for N fertilization in GCTB systems. In the short term, no reduction in N rates are expected [93, 94], whereas in the medium- to long-term, gradual release of trash N may enhance soil N availability to sugarcane [89, 130–132].

It is not yet clear whether the GCTB system will require increases or reductions in N rates as compared to the previous burning system, but growers have shifted to an increase in N rates to avoid yield losses due to N deficiencies. The official recommendation of 1.0 kg N per Mg of stalk produced has been empirically replaced by the 1.2 kg N per Mg factor adopted by the majority of growers in southeastern Brazil. This approach is similar to the 1.2 factor used in the Six Easy Steps nutrient management program [133], and higher than the 1.0 factor used in the N Replacement System [127], both of which were developed for application under Australian conditions.

Aiming to elucidate the need for increased N rates adopted in GCTB systems, we surveyed 45 field trials carried out under the GCTB system over the past several decades in Brazil. We recorded the results of check-plot yields, and calculated the yield increase to N fertilization in each single trial (Table 3), and ranked the sites as non-responsive to N fertilization (no significant effect of N fertilization in the original study), moderately responsive (from 0 to 25 % yield increase),



**Table 3** Survey of N-response curve trials under the green cane trash blanketing system in Brazil

Trial	Check plot <sup>a</sup> Mg ha <sup>-1</sup>	Yield increase <sup>b</sup>		Responsiveness <sup>c</sup>	Details	Reference
		%	Mg ha <sup>-1</sup>			
1	117	0	0	Non-responsive	Year 1	[95]
2	100	0	0	Non-responsive	Ribeirão Preto	[126]
3	91	0	0	Non-responsive	Site 4	[49]
4	80	0	0	Non-responsive	Site 5 Year 1	[49]
5	142	0	0	Non-responsive	Site 5 Year 2	[49]
6	81	0	0	Non-responsive	Site 5 Year 3	[49]
7	91	0	0	Non-responsive	Site 7	[134]
8	108	0	0	Non-responsive	Site 9	[134]
9	91	0	0	Non-responsive	Site 10	[134]
10	109	0	0	Non-responsive	Site 15	[134]
11	93	0	0	Non-responsive	Site 21	[134]
12	128	5	7	Moderately responsive	Araras	[126]
13	104	5	6	Moderately responsive	Site 2 Year 2	[49]
14	88	6	5	Moderately responsive	Promissão	[126]
15	84	6	5	Moderately responsive	Site 17	[134]
16	117	7	8	Moderately responsive	Year 1	[135]
17	114	7	8	Moderately responsive	Site 2 Year 1	[49]
18	67	9	6	Moderately responsive	Orindiúva	[126]
19	79	10	7	Moderately responsive	Site 3	[49]
20	99	11	11	Moderately responsive	Piracicaba	[126]
21	74	11	8	Moderately responsive	Pradópolis	[126]
22	99	11	11	Moderately responsive	Guaira 2	[126]
23	84	11	9	Moderately responsive	Site 20	[134]
24	45	12	5	Moderately responsive	Planalto	[126]
25	90	12	11	Moderately responsive	Sertãozinho	[126]
26	64	14	9	Moderately responsive	Andradina	[126]
27	76	14	11	Moderately responsive	Year1	[136]
28	65	15	9	Moderately responsive	Year 2	[91]
29	65	15	9	Moderately responsive	Year 2	[135]
30	85	15	13	Moderately responsive	Santa Rita	[126]
31	90	15	14	Moderately responsive	–	[137]
32	90	16	15	Moderately responsive	Iracemópolis	[126]
33	100	19	19	Moderately responsive	Year 1	[138]
34	68	22	15	Moderately responsive	Araçatuba	[126]
35	55	25	14	Highly responsive	Year 2	[136]
36	103	27	28	Highly responsive	Mean of four seasons	[139]
37	95	28	27	Highly responsive	Site 16	[134]
38	69	30	21	Highly responsive	Site 1	[49]
39	84	36	30	Highly responsive	São João da Barra	[126]
40	61	36	22	Highly responsive	–	[140]
41	66	39	26	Highly responsive	Year 2	[138]
42	59	41	24	Highly responsive	Guaira 1	[126]
43	88	47	41	Highly responsive	First ratoon	[141]
44	83	48	40	Highly responsive	Second ratoon	[141]
45	54	51	28	Highly responsive	Third ratoon	[141]

<sup>a</sup> Check plot yield, stalk yield obtained in the control treatment (no-N applied)

<sup>b</sup> Calculated as ((high yielding treatment—check plot yield)/check plot yield x 100)

<sup>c</sup> Non-responsive (yield increases =0); moderate (yield increases between 0–25 %); high (yield increases >25 %)

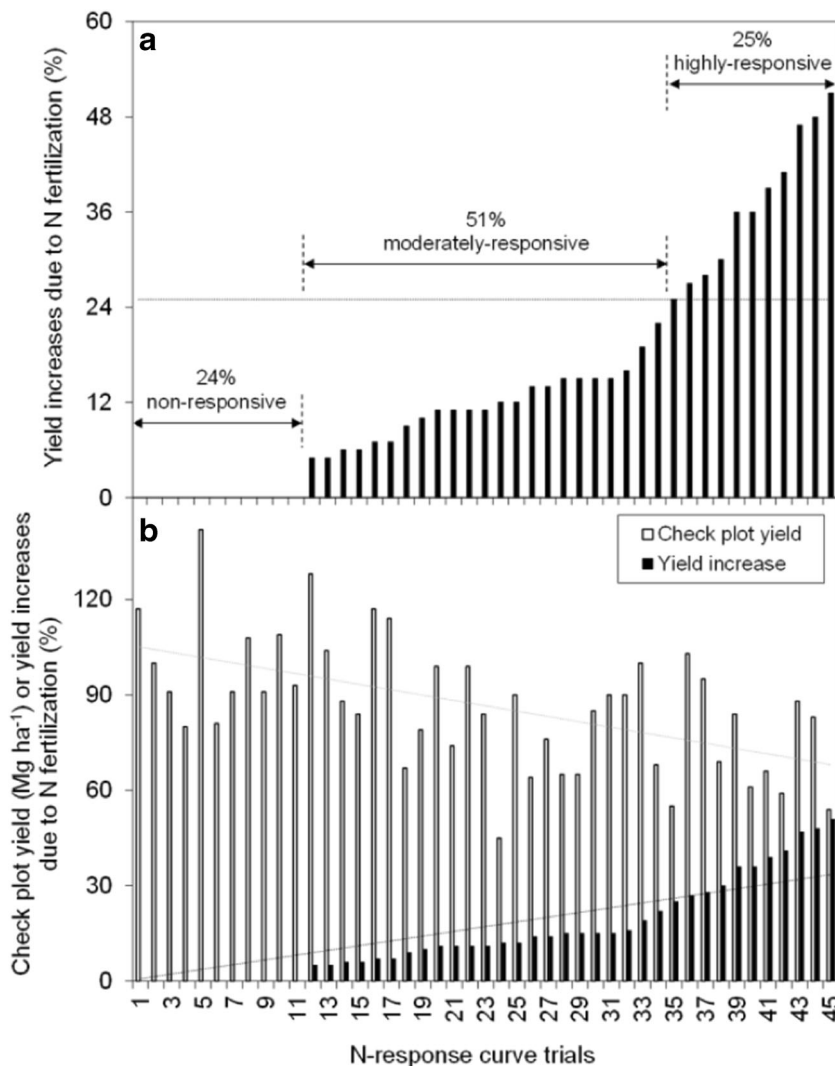
and highly responsive (> 25 % yield increase). An N requirement adjustment to sugarcane production in Brazil would be expected if most sites showed high levels of response to N fertilization after being shifted from burning to a GCTB system. However, as the results presented in Fig. 3 show, 75 % of the sites were non-responsive or moderately responsive to N fertilization, whereas only 25 % of the sites were highly responsive to N fertilization. These results suggest that increasing N fertilization will probably not result in yield increases in most cases, and that the change from 1.0 to 1.2 kg N per Mg of stalk produced requires further revision.

More interestingly, plotting data of N responsiveness against the check-plot yield for each site revealed a linear negative correlation ( $r = -0.49$ ;  $P < 0.001$ ), indicating a clear trend toward reduction in N responsiveness as check-plot yield increases (Fig. 3). The high check-plot yield indicates a high availability of mineral N or easily mineralizable organic N fractions in the soil. This is expected, given the gradual

release of N in GCTB systems [94], the optimization of press mud and vinasse usage by growers, and the expansion of areas using legume rotation. Such management approaches have the potential to increase soil N availability and reduce sugarcane response to N, as demonstrated by Otto et al. [49]. In contrast, low check-plot yields are indicative of plants growing in conditions of low soil N availability (limited N conditions) and, thus, exhibit significant responses to N additions. Indeed, the majority of sugarcane grown in Brazil is not subjected to limited N conditions (considering the byproducts usage, legume rotation, and usual practices of N fertilization) and, thus, it is expected that moderate responses to N fertilization of sugarcane will remain in the future.

Even with the low N rates adopted for sugarcane ratoon in Brazil as compared to other countries, lower profitability for growers may result from the N fertilization practice in situations where there is no substantial return on the investment and due to costs associated with overuse of N fertilizer, which

**Fig. 3** Yield increases due to N fertilization (a) and relationship between check plot yield and yield response (b) as obtained in 45 N-response curve trials carried out under GCTB systems in Brazil. In each single trial reported on Table 3, check plot yield represents the control treatment (no-N applied); increase in yield due to N fertilization was obtained as [(high yielding treatment – check plot yield)/check plot yield × 100]; yield response was further categorized as non-responsive (yield increases =0), moderately responsive (yield increases between 0–25 %), and highly responsive (yield increases >25 %). Dotted line in panel A represents the boundary line (yield increase =25 %) between moderate and highly responsive sites. Dotted lines in panel B indicates the curve fitting to a linear model for check plot yield and yield increase data



may also result in significant environmental impacts [7]. Regarding leaching losses, for example, whereas Ghiberto et al. [67, 68] reported limited N losses in the plant cane, N leaching in the ratoon crop cycle represented 22.5 % of the total N applied as fertilizer (100 kg N ha<sup>-1</sup>) [69]. Higher leaching losses are expected to occur in ratoons given the proximity between the period of fertilizer application and the rainy season. This is of particular concern considering that NO<sub>3</sub><sup>-</sup> leaching is usually highest at the onset of the wet season, when both water drainage and NO<sub>3</sub><sup>-</sup> are present simultaneously [68, 70]. Moreover, higher N rates also have the potential to increase N<sub>2</sub>O emissions [82].

### Fertilizer Nitrogen Sources

Urea is widely used as a fertilizer; in Brazil, it represents 66 % of N fertilizer consumption [142], despite the high potential for losses through volatilization [85]. However, reconciling the trend toward increasing use of urea and the large-scale adoption of GCTB systems with surface application of fertilizers presents a challenge, as surface-applied urea on trash blankets may result in increased NH<sub>3</sub> volatilization losses ranging between 24 to 37 % of applied N [21, 85, 86].

One possible strategy for reducing NH<sub>3</sub> losses from surface-applied urea is through the use of urease inhibitors. Under field conditions, Cantarella et al. [85] observed that NBPT [N-(N-butyl) thiophosphoric triamide]-treated urea reduced N losses via volatilization by 15–78 % when compared with urea-only losses. Faria et al. [87], in an assessment of B- and Cu-coated urea as well as S-coated urea as additional alternatives to reduce the volatilization process, found that although the coatings delayed loss peaks, accumulated N losses were similar to those reported for conventional granulated urea. Such results and concerns regarding the effectiveness of urea inhibitors in systems with large amounts of trash overtop the soil surface (such as sugarcane) is a limiting factor for their large-scale adoption by sugarcane growers in Brazil. For this reason, sources less subject to volatilization losses (i.e., ammonium nitrate and ammonium sulfate) are preferred to urea for superficial application, especially in dry periods. Adjustments in the quantity of NBPT to be added to urea under high-trash conditions, or improvements in the inhibitors themselves, are required before widespread use of urea-based fertilizer for sugarcane production in Brazil can be initiated.

Nitrification inhibitors can also be added to urea- or ammonium-based fertilizers in order to delay microbial oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup>. There are three main reasons to expect positive effects of using nitrification inhibitors in sugarcane cropped soils: (1) NO<sub>3</sub><sup>-</sup> is subjected to leaching in sugarcane fields in some circumstances [68]; (2) NO<sub>3</sub><sup>-</sup> is subjected to denitrification losses, especially under GCTB and high-moisture conditions [84]; and (3) the NO<sub>3</sub><sup>-</sup> form is generally the most prevalent in sugarcane-cropped soils, and

sugarcane has been shown to preferentially absorb NH<sub>4</sub><sup>+</sup> rather than NO<sub>3</sub><sup>-</sup> [32]. DCD has been the most commonly used product to inhibit nitrification [143, 144], despite the availability of alternative products, such as DMPP. Soares et al. [80], for instance, reported that DMPP- or DCD-treated urea reduced N<sub>2</sub>O losses from urea by 90 %. However, adopting urease and nitrification inhibitors simultaneously may lead to increased volatilization losses due to the retention of high NH<sub>4</sub><sup>+</sup> concentrations in the soil [86] and, therefore, should be avoided. Research evaluating the effects of nitrification-inhibitor treated urea in sugarcane systems is needed.

The use of ammonium chloride is not common in Brazil, and some studies have shown limitations in its effectiveness as a fertilizer for sugarcane. Similar levels of efficiency for ammonium chloride, ammonium nitrate, ammonium sulfate, and urea in the first year of application to sugarcane have been reported [137], but assessment of the residual effects of fertilizers on subsequent cycles suggested that ammonium chloride linearly reduced crop yield when compared to other N sources, most likely due to the cumulative salt effect of chloride ions [145]. Similarly, Mariano et al. [44] reported a low availability of mineral N in soils and a lower N content in sugarcane following ammonium chloride application.

Slow- or controlled-release fertilizers (also known as “enhanced efficiency fertilizers”) were developed that supply nutrients in a more gradual manner to better match the variations in plant nutritional demands. Organic or synthetic compounds are used as coating materials, but these types of fertilizers generally have positive or no effects when compared to conventional N sources [34]. In spite of favorable results in other crops [146], no studies were found regarding the effectiveness of such fertilizers in sugarcane systems in Brazil.

### Application Methods

Fertilizer application methods require further evaluation, as most experiments involving sugarcane are restricted to comparisons of fertilizer sources or application rates [147]. Due to growing concerns about GHG emissions, several studies have been carried out to examine how N application methods may influence N<sub>2</sub>O emissions [148]. Incorporated application of N fertilizer to soils at depths of 5 cm might reduce N<sub>2</sub>O emissions, especially in humid climates [149]. Lower N<sub>2</sub>O emissions were also observed when fertilizer granules were placed 10-cm deep, as deeper applications increase the residence time of N<sub>2</sub>O in soil and, thus, lower losses to the atmosphere [150]. Incorporation of N fertilizers into the soil also has the potential to eliminate NH<sub>3</sub> volatilization losses from urea [45, 151] which is of particular interest given that reducing volatilization losses has the additional benefit of increasing NUE [152, 153].

In the burning systems used in the past, fertilizers were normally incorporated into the soil during cultivation.

Cultivation was typically performed using a deep tillage cultivator that breaks up the soil between crop rows [154], making it possible to incorporate fertilizer in the middle-row. However, incorporation of fertilizer in crops under GCTB systems is more difficult [99], and it requires specialized equipment and climatic conditions to ensure successful operations. Moreover, incorporation of fertilizer has been shown to result in similar or even lower yields compared to single-side surface-banding application [155, 156].

In terms of fertilizer placement, Bianchini et al. [19] observed that banded application resulted in higher yields than did broadcast application, whereas Vitti et al. [151] found no differences between the two in terms of yield. These results suggest that, so long as N fertilizers are not subject to  $\text{NH}_3$  volatilization losses, there is little need for their incorporation in areas under GCTB, and that localized application (usually performed 20-cm distant from the crop row) has advantages because roots are concentrated in the superficial soil layer (0–20 cm) and adjacent to the sugarcane row [157, 158]. However, development of the specialized equipment that would allow for incorporated application next to the crop row has the potential to maintain sugarcane yield (due to proximity to the root system) and permits the use of urea-based fertilizers by mitigating  $\text{NH}_3$  and  $\text{N}_2\text{O}$  emissions.

### Timing of Fertilizer Application

In plant cane, N fertilization is usually applied at the bottom of the furrow, at depths of 0.15–0.30 m. Consequently, N losses caused by volatilization are insignificant even when urea is applied [151]. Despite official recommendations for split application of N on plant cane [128], in most cases N is applied once at planting due to uncertainties regarding yield gains when using a split application approach [159]. However, in some cases, growers are beginning to split N application following K application coupled with land-leveling (performed to facilitate mechanized harvesting), which is carried out at 90–120 days following planting. The results of split N application in such operations are still unclear and must be investigated.

There is asynchrony between the time of fertilizer application and crop nutritional demand in the ratoon crop cycle. In southeastern Brazil, sugarcane harvest occurs from April to November, and fertilizers are usually applied right after harvesting, but ~75 % of the total biomass and N content accumulated by sugarcane in the ratoon cycle occurs between December and March [45, 46, 160]. Thus, further research examining the viability of split application of N in the ratoon crop cycle is needed, as the evidence for the benefits of split application derive from studies of fertigated sugarcane, in which N is applied according to crop demand, resulting in high responsiveness to N [161, 162]. The use of slow- or controlled-release fertilizers may serve a similar purpose.

The development of specialized equipment enabling variable rate application creates new opportunities for the timing of fertilizer application using information obtained from canopy sensors [163]. The use of close remote sensing (optical sensors attached to agricultural machinery) assumes that leaf spectral properties, such as reflectance and transmittance, are affected by N deficiency [164]. The normalized difference vegetation index (NDVI) is commonly used to estimate N nutrition, yield, and chlorophyll content in leaves [165–167]. Depending on the methodology adopted, the use of NDVI facilitates the development of recommendations for variable-rate application of fertilizers, in either real-time or by following recommendation maps [168]. However, there are few studies that have assessed the effectiveness of using optical sensors (such as NVDI) on variable-rate N application for sugarcane and, thus, further research on calibrating vegetation indices with crop yields is required [169] prior to large-scale adoption of variable-rate technology in sugarcane fields.

### Concluding Remarks

Moderate N rates are generally used for sugarcane production in Brazil when compared to other major sugarcane-producing countries. Volatilization is one of the primary N-loss pathways in Brazilian sugarcane systems, but concerns about leaching loss and  $\text{N}_2\text{O}$  emissions that result from biofuel production are rising. Here, we found that approximately 60 % of the fertilizer N is recovered by plants and soils throughout the crop cycle, while leaching losses and  $\text{N}_2\text{O}$  emissions may reach as high as 5.6 and 1.84 % of the applied N, respectively.

The increasing shift from burning to non-burning (GCTB) systems in Brazil has led to modifications in the country's N-management strategies, with an increase in N rates from 1.0 kg N per Mg of stalk to 1.2 kg N per Mg of stalk being adopted by most growers. However, our review demonstrates that 75 % of the sites examined were non-responsive or only moderately responsive to N, indicating that increases in N rates are unlikely to promote higher yields, further reducing NUE. More interestingly, high-yielding systems exhibited very limited response to N fertilization, whereas low-yielding systems were highly responsive. Such findings indicate that sugarcane growing under conditions of an adequate supply of N require lower N rates.

This review identified strategies that can be adopted to enhance NUE for sugarcane-biofuel production. Such strategies include (i) reduction in N-fertilizer usage by most large-scale sugarcane producers in order to optimize plant uptake and reduce losses. Savings in N fertilizers without reduction on yield can be ensured by optimizing byproduct usage, growth of legume crops in rotation, and trash maintenance in sugarcane fields; (ii) adoption of best-management practices, including strategies to maintain N as  $\text{NH}_4^+$  in soils, as well as

adopting fertilizer and application methods that minimize losses; (iii) developing new N-recommendation systems that do not exclusively focus on the expected yield concept; (iv) developing second-generation biofuel production that increases ethanol production per unit area without requiring additional N fertilizer; and (v) evaluating N-internal use efficiency in breeding programs in order to propagate genotypes with improved NUE. All of these issues must be addressed in Brazil and other large-scale producing countries to deliver high sugarcane yields with minimum N usage and reduced environmental impacts.

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