Nitrogen Use in Biofuel Production: A case study of U.S. Corn and Brazilian Sugarcane

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ABSTRACT

Ethanol produced from United States corn and Brazilian sugarcane dominates the liquid biofuels industry. Yet when determining the viability of these two feedstocks as sustainable and environmentally sound investments, the discussion focuses only on the net energy balances, life cycle analyses of greenhouse gas emissions, and food versus fuel debates. The impacts of growing corn and sugarcane for ethanol on the cycling of nitrogen are often overlooked, even though losses from the agroecosystem can create serious environmental issues, including acid rain, eutrophication of surface waters, and stratospheric ozone depletion.

This paper seeks to investigate nitrogen use in the cropping systems of corn and sugarcane from the physiological aspects of plant nitrogen nutrition to the movements of nitrogen in the soil-plant system at the landscape scale. Comparing the effects of plant physiology and crop management on nitrogen requirements and subsequent losses to the environment can highlight which feedstock creates the least disturbance to the nitrogen cycle and thus the surrounding environment. The 2008/2009 growing season provides a case study to examine the interdependency of the physiological nitrogen demands, soil nutrient management, and balanced nitrogen cycling on the yield of ethanol.

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CHAPTER 1. CURRENT PERSPECTIVES ON ETHANOL PRODUCTION

1.1 Ethanol: A growing industry

In July 2008, oil prices spiked to a record high of $147 a barrel, topping the petroleum prices witnessed during the 1973 OPEC oil embargo when adjusted to inflation equivalent dollar values (BBC News, 2009; William, 2007). Despite volatile oil prices, global energy consumption has grown from 35 quadrillion Btu in 1949 to 96 quadrillion Btu in 2008, primarily in the form of petroleum (EIA, 2009b). However, economies dependent on oil are vulnerable to severe, uncontrollable price increases as has been exemplified in the mid-1970s, early 1980s, and current period from 2004 to 2008 (UN Energy, 2007). Campbell and Laherrère predicted in 1998 that the peak of world petroleum production would be reached near 2010 with production rates declining then after. Most petrologists generally agree that current accessible petroleum reserves will be depleted within the next fifty years; even skeptics of the peak oil predictions admit that extraction costs, and thus prices, will continue to rise as more remote oil supplies are tapped, like the Canadian tar sands (Erickson and Carr, 2009). Oil-dependent nations must face the reality that increasingly scarcer petroleum resources are being consumed at an ever-quickening pace.

In order to diminish the reliance on petroleum and enhance national energy security, both the United States and Brazil have turned to the domestic production of biofuels to meet growing energy demands. In 2007, Alexander Iler of the FAO Sustainable Development Department stated, “The gradual move away from oil has begun. Over the next fifteen to twenty years, we may see biofuels providing a full twenty-five percent of the world’s energy needs” (UN Energy, 2007, p. 3). Since the turn of the millennium, the global production of biofuels has doubled, with the United States and Brazil dominating the liquid biofuels industry in the form of ethanol (UN Energy, 2007). In 2009 the global ethanol production reached 73.9 billion liters and the Global Renewable Fuels Alliance forecasts the 2010 production to reach 85.9 billion liters, displacing the equivalent of 370 million barrels of oil or about 59 billion liters (GRFA, 2010). Even the major petroleum refining companies have stacked their portfolios with holdings in biofuel technologies; BP holds a fifty percent stake in Brazil's Tropical Bioenergia, which produced 435 million liters of ethanol from sugarcane in 2008, while Royal Dutch Shell has rerouted funding for wind, solar, and hydro technologies into biofuel research in Brazil and the United States (Ernst & Young, 2009; Webb, 2009; 'The green slump', 2009)

The United States currently uses corn (*Zea mays*) as the primary feedstock for ethanol production while Brazil employs sugarcane (*Saccharum officinarum*). Outlooks for the U.S. energy profile to 2035 indicate that biofuel consumption will account for most of the total growth in liquid fuels, replacing six percent of the fossil fuel share of energy consumption in the U.S. as petroleum-based fuel use essentially stagnates (EIA, 2009a). The U.S. Energy Independence and Security Act of 2007 mandates a renewable fuels standard of 36.0 billion gallons of renewable fuel by 2022, consistently requiring an annual output of 15.0 billion gallons of ethanol from corn starting in 2015 (Rahall, 2007). In Brazil, ethanol production is regulated by the governmental Alcohol Interministrerial Committee, which set the ethanol-gasoline blend requirement at 22% in 2001. Growing from 382 million liters at the origins of the ethanol industry in 1948 to 27.6 billion liters in 2008, nearly half of Brazil's current sugarcane production is designated for ethanol

(Brazil Ministry of Agriculture, 2009; Bolling and Suarez, 2001). With higher yields per hectare for sugarcane, the U.S. Department of Agriculture (USDA) estimates that Brazil produced 76% more ethanol per hectare of land than the U.S. in 2007, an observation that led the debate surrounding ethanol to focus on food security and the energy balance of the fuel (ERS, 2008).

However, in determining which ethanol feedstock causes the least detriment to the environment or society, one critical component is often left out of the equation: nitrogen. Therefore, this paper seeks to characterize the nitrogen use in ethanol production in the context of the current political and environmental conditions under which ethanol is produced. The cases of ethanol derived from United States corn and Brazilian sugarcane will be specifically considered due to their dominance of the liquid biofuels industry. The impact on local and global nitrogen cycling will be analyzed from an ecological perspective of agriculture, considering the plant physiology, soil suitability, and anthropogenic manipulation of nitrogen inputs.

1.2 Political Context: Food versus fuel

The potential to use crops for producing energy creates an economic trade off against using corn or sugarcane for food. Population growth over the next three decades, as well as an expected one-third increase in demand for meat and dairy products, will inevitably require greater amounts of food and feed to be grown globally (UN Energy, 2007). The extent to which land, water, and other resources are diverted away from food production towards energy crops could compromise adequate food supplies in the future.

The United States is the largest producer and exporter of corn in the world, yet exports represent only 15% of U.S. corn harvested, making the global price of corn dependent upon the internal supply and demand of corn within the U.S (ERS, 2009). Shifting the allocation of corn acreage towards fuel could stress the supply of corn for food and feed. Some researchers argue that biofuel production from crops like corn will starve the poor by hiking up global corn prices as exports diminish (Runge and Senauer, 2007). Still, exports of corn for the U.S. are expected to rise from 81.9 million metric tons (MMT) in 2008 to 100.1 MMT in 2018, although these exports represent a decrease from 58.9% of the global share of corn exports to 56.5% on the same timeframe [Appendix: Figure A] (Backer and Allen, 2009). Additionally, corn accounts for 91% of grains used for livestock feed, so corn prices ultimately impact the cost of livestock production as well; although, the use of by-products from ethanol production as feed could reduce the impact of grain costs on the livestock industry (Stillman *et al.*, 2009). However, plant biologists as well as the USDA claim that the devaluation of the U.S. dollar, rising energy prices, increases in agricultural costs of production especially with regards to fertilizer, unfortunate weather, and protective import policies to mitigate inflation have led to the increase in grain prices, not an expansion of the biofuel industry (Armah *et al.*, 2009; ERS, 2008).

Similar to U.S. corn exports, Brazil has been the number one exporter of raw sugar for over ten years, exporting at least two million tons more than any other country annually since 1999 (Brazil Ministry of Agriculture, 2009). When ProAlcool (National Alcohol Programme) was formed late in 1975 though, sugarcane subsidies from the government rewarded the export of the commodity, even though a quarter of Brazilian citizens were considered undernourished at that time. Yet, it was the growth in biofuel production from sugarcane under ProAlcool that saved the Brazilian sugar industry from collapse as prices on the international market fell to record lows in the 1970s. Food shortages and food price increases in Brazil around 1985 were first blamed on the growth of the ethanol industry pushed by the government, but, retrospectively, years of agricultural policy emphasizing commodity export crops, unchecked inflation, deepening foreign debt, and adverse weather conditions seem to have been the primary causes of the price escalation and shortages of food (Rosillo-Calle and Hall, 1987). Thus, the large-scale sugarcane production and expansion in Brazil have not been shown to affect food production and have not diverted land from other crops like corn and soybeans, which have respectively twice and three times as large an area than sugarcane (Walter, 2009). A doubling of ethanol production in Brazil would mean that cane sugar would still only account for an additional 3% of the available agricultural land, so ethanol production could be expanded without much consequence to food supplies, especially since most new sugarcane development occurs on pastoral lands (Walter, 2009).

The concern for food security could be a limiting factor for the expansion of the ethanol industry. The strong growth of ethanol production in the short-term could perturb food exports for a limited period of time, but ultimately the market would adjust to the change in supply, either through corn or sugarcane acreage increasing in their respect countries or through other countries ramping up exports of corn and sugarcane to capture the temporarily higher prices. Any increase in crop acreage implies a concurrent increase in the total amount of nitrogen fertilizer used, yet the nitrogen component is rarely mentioned in the debate of food versus fuel.

1.3 Environmental Context: Net energy balance

The environmental focus on biofuels has mostly been concerned with the amount of fossil fuel energy used to produce them and related greenhouse gas emissions. Therefore, the justification for expanding the ethanol industry has hinged on the determination of a net positive or negative energy balance with regards to fossil fuel inputs and ethanol energy outputs as well as net greenhouse gas emissions. The U.S. Department of Agricultural claims that corn ethanol production yields a 34% energy gain for every Btu used to produce it, including growing, harvesting, transporting, and distilling the corn; the USDA attributes the 21,000 Btu per gallon energy surplus to high corn yields per acre, lower energy use in fertilizer production, and advances in fuel conversion technologies (Shapouri *et al*., 2002). However, ten years prior to this USDA study, Keeney and DeLuca (1992) reported a net energy loss of 8,440 Btu per gallon of corn ethanol. For ethanol derived from sugarcane, Baldani *et al*. (2002) estimate that the overall basic energy ratio is 2.43, a positive energy balance; however, if bagasse, the leftover biomass after juicing the cane in ethanol production, is used to power the factory (currently a universal practice in Brazil), then the energy ratio increases to 4.53, and if the plant is grown without nitrogen fertilizer, the value increases to 5.79. Various assumptions about crop yield, ethanol conversion technologies, fertilizer manufacturing efficiency and application rates, and co-product evolution significantly affect the calculation of the net energy value of ethanol, especially for corn where the balance can be positive or negative based on these assumptions. However, considering the improvements made to yield and ethanol conversion technologies, a positive net energy balance for both corn and sugarcane can be maintained in the future.

Additionally, estimations of greenhouse gas reductions from ethanol vary with scenarios of land use change and agricultural management practices. Due to the release of carbon dioxide, land use change may negate the benefit of ethanol in the short term. For instance, Searchinger *et al.* (2008) contend that if ethanol from Brazilian sugarcane only converted tropical grazing land for its expansion, the up front carbon emissions from land use change could be recovered in four years; however, if ranchers subsequently find new grazing land that encroaches on the rainforest, carbon emissions from land use change would not be balanced by the benefits of ethanol for over forty years since sugarcane harnesses significantly less carbon than rainforest vegetation. Similarly, land use change from the expansion of corn agricultural lands for ethanol will require over 160 years to establish a carbon balance, meaning carbon dioxide will continue to increase until the end of that period (Searchinger *et al.*, 2008). Even as carbon emissions become neutral over time, N_2O emissions will continue to rise due to the persistent requirement of fertilizer to support the growth of both feedstocks. Yet the effects of perpetually using fertilizer extend beyond just nitrous oxide emissions, but limited information exists that directly compares corn and sugarcane under the same lens.

1.4 Implications for the Nitrogen Cycle

The total mass of nitrogen in the atmosphere, waters, and soils equates to more than the mass of carbon, phosphorus, oxygen, and sulfur combined; yet of the 4×10^{21} grams of nitrogen, only about one percent is available in a reactive form usable by living organisms. The amount of reactive nitrogen (Nr) in the nitrogen cycle has increased over time mainly due to anthropogenic manipulation through the cultivation of nitrogen fixing crops, combustion of fossil fuels, and creation of reactive nitrogen products by the Haber-Bosch process. While fossil fuel combustion contributed 25 teragrams (Tg) of Nr in the year 2000 alone, the Haber-Bosch process introduced nearly four times as much reactive nitrogen in the same year with 85% used in the production of fertilizers (Galloway *et al.*, 2003).

Although Haber-Bosch derived fertilizer has enabled a significant increase in agricultural productivity per hectare, the injection of substantial amounts of reactive nitrogen into the agroecosystem has led to a series of unintended consequences. Increasing greenhouse gas levels from nitrous oxide emissions; increasing air and water pollution from ammonia, nitrogen oxides, and nitrates; and loss of biodiversity have all been documented in association with the augmented levels of reactive nitrogen (Hill *et al.*, 2010). Nitrous oxide has 298 times the global warming potential of carbon dioxide in a 100 year period; the atmospheric lifetime of N_2O is 114 years with a radiative efficiency of 3.03×10^{-3} W m^{-2} ppb^{-1} while CO_2 has a radiative efficiency of 1.4×10^{-5} W m^{-2} ppb^{-1} and an estimated atmospheric lifetime between 50 and 200 years, depending on ocean mixing rates and movement to other carbon reservoirs (Soloman *et al.*, 2007). Those forms of reactive nitrogen that do not contribute to greenhouse gas accumulation can still cause harm in the form of destroying stratospheric ozone, elevating ozone concentrations at ground level, acidifying precipitation, as well as eutrophying waters (Sutton *et al.*, 2009). Additionally, high levels of nitrate in groundwater can cause health impacts like methemoglobinemia, which is sometimes fatal in infants (Schlesinger, 2009).

Most of these negative environmental impacts result directly from the losses associated with excess reactive nitrogen in the agroecosystem. Nearly half of all the fertilizer applied to crop systems is lost to the atmosphere as emissions of NH_3, N_2O, or N_2 or to aquatic ecosystems as NO_3^- (Galloway and Cowling, 2002). Besides inorganic fertilizers, inputs of nitrogen to crop production may consist of the seeds, recycled crop residues, animal manures, N in irrigation water, atmospheric deposition, biological fixation, as well as indigenous sources of reactive nitrogen in the soil; nitrogen leaving the agroecosystem includes harvested biomass, gaseous emissions from plant and soil processes, volatilization of ammonia, leaching, and soil erosion (Smil, 1999). As denitrification fails to keep pace with the production of reactive nitrogen, reactive nitrogen accumulates in the atmosphere, soil, and water, perturbing the global nitrogen cycle.

Hence, crop agriculture represents the largest and most important interference in the nitrogen cycle, even beyond fossil fuel combustion; yet bioethanol, which utilizes both cropping and combustion in its lifecycle, remains poorly understood in regard to its nitrogen impact. The increasing demand for ethanol from crops could double the demand for Haber-Bosch derived nitrogen fertilizer, introducing 200 Tg Nr per year globally, with the potential for nitrogen pollution from the growing of ethanol feedstocks (Erisman *et al.*, 2008). Therefore, in order to accurately access the most environmentally sustainable feedstock for ethanol, the discussion must not be limited to global warming potential or the carbon cycle, but also seriously consider the effects on the nitrogen cycle as well.

1.5 Objectives and Scope

Most literature on ethanol only considers the cycling of nitrogen in so far as it relates to the emission of the greenhouse gas nitrous oxide (de Figueirdo *et al.*, 2010; Want *et al.*, 1997). Nitrogen use in ethanol production has been evaluated holistically on aggregated, macro levels but not by specific feedstocks (Erisman *et al.*, 2010). Inputs and losses of nitrogen for each feedstock are limited to disjointed studies and scantily quantify the overall impacts embodied in the production of a unit of ethanol. Additionally, little effort has been made to directly compare nitrogen use by corn and sugarcane, which remains a critical component for informing future bioethanol policies in order to ensure the greatest environmental benefit and sustainability. Understanding the role of the two largest biofuel feedstocks, corn and sugarcane, in the nitrogen cycle is therefore necessary to obtain an accurate assessment of the multi-faceted effects of biofuels on our environment beyond the typical scope of the carbon cycle.

This paper seeks to demonstrate the impact of producing corn and sugarcane for ethanol on nitrogen cycling and is driven by the question of which feedstock releases the least amount of nitrogen from the agroecosystem into the environment. Underlying the main question, this paper inquires whether the controlling mechanism for nitrogen loss per liter of ethanol produced relates primarily to the plant physiology or crop management. From this information, potential improvements to nitrogen use in the production of corn and sugarcane can be identified and a decision regarding which feedstock, if any, is preferable for the future expansion of the ethanol industry can be made.

It is hypothesized that Brazilian sugarcane will lose less nitrogen to the environment through erosion, runoff, volatilization, or denitrification than U.S. corn, and that historic and current sugarcane crop management is more responsible for this outcome than inherent plant physiology. It is also hypothesized that realistic changes to sugarcane crop management, particularly cultivar selection and harvest method, will reduce the ratio of nitrogen lost per liter of ethanol produced more so than the genetic modification of corn hybrids could reduce the same ratio by enhancing overall ethanol yield.

In order to deduce the reasons behind nitrogen use and loss from the agroecosystem of ethanol feedstocks, the plant physiologies and management strategies must be fully examined. As such, the physiological aspects of both corn and sugarcane will be investigated to highlight the utilization of nitrogen within each plant and to consider the plausibility of manipulating plant traits to enhance ethanol yield. Furthermore, characteristic environmental conditions and nutrient management practices surrounding crop production in the United States Corn Belt and Brazil will be evaluated to determine their effects on optimizing feedstock yield and on conserving soil nitrogen in the system. Finally, by utilizing the information about plant physiology and crop management, losses to the environment caused by producing a unit of ethanol from either U.S. corn or Brazilian sugarcane will be calculated for comparison.

The scope of this paper includes only the nitrogen cycling within the cropping system. The cropping system may be defined as the area in which corn or cane is planted for harvest as an ethanol feedstock. The nitrogen cycling in this cropping system refers to the inputs of nitrogen from fertilizer, biological fixation, and mineralization as well as the losses from denitrification, soil erosion, runoff, leaching, and volatilization. This paper will not consider life cycle carbon-equivalent emissions for ethanol production nor does this paper include nitrogen emissions from making nitrogen fertilizer, utilizing farming equipment, transporting the feedstock to the ethanol production facility, or vehicle emissions from gasoline-ethanol blends. The fundamental unit for comparison will be the hectare (ha). Unless otherwise noted, all values are quantified using the metric system (i.e. kilograms, kg; teragrams, Tg; metric tons, t; liters, L).

As advancements in hybrids of corn and sugarcane take place rather rapidly as well as the shifting of management practices, the calculations for nitrogen cycling will be considered for the most recent time frame with full data sets, the 2008-2009 growing season. The major areas of focus are the state of São Paulo in Brazil and the states of the U.S. Corn Belt (Iowa, Illinois, Indiana, Nebraska, Michigan, Minnesota, Ohio, and Wisconsin) as the majority of the feedstocks are produced in these areas. Driving this paper are two major assumptions: (1) every farmer strives, primarily, to maximize the yield of his feedstock and (2) maximizing yield of the feedstock will produce the greatest amount of ethanol.

CHAPTER 2. FEEDSTOCK PHYSIOLOGY

Underpinning both the American and Brazilian ethanol industries lays the cropping system of corn and sugarcane respectively. Within this paradigm, the physiological and molecular characteristics of these feedstocks ultimately define the nitrogen management and cycling within the crop agroecosystem. Understanding corn and cane on the most basic level provides a focused lens through which to view the potential benefits and pitfalls of each feedstock in terms of nitrogen demand and achievable yield. This chapter also offers insight as to whether opportunities exist to enhance biomass and ethanol yield through direct modifications to plant nitrogen utilization.

2.1 Corn Botany

About 9,000 years ago, modern corn was domesticated in Mexico from the annual grass *Balsas teosinte* [*Zea mays* L. ssp. *Parviglumis*] (Jeffe and Miguel, 1994). Today, dozens of varieties of commercial corn exist, mostly in the form of hybrids within the United States. These varieties vary by emergence date, duration of the grain-filling period, length of time to maturity, potential number of kernels, and final kernel size, but all follow the same orderly developmental stages (Kent, 2010). These developmental stages relate closely to the nitrogen requirements of the corn plant (Figure 1).

While development varies by hybrid and environmental field conditions, each corn plant usually develops approximately 20 leaves, silks about 65 days after emergence from the soil (VE) and matures about 125 after VE (Ritchie *et al.*, 1993). Leaves are numbered from emergence (VE) to tasseling (VT) as they grow out of the whorl from V1 to V(n) where n is the last leaf stage before VT. By V5, the tassel (male organ) has formed and ear shoots (potential ear) start to develop from every above ground node up to about six or eight nodes below the tassel. ,Eventually only the top one or two ear shoots nearest the tassel will develop into a harvestable ear. The number of kernel rows, kernels per row, and ovules (potential kernels) are determined around V12, and by V15, silks begin to develop with one silk for each ovule (Below, 1997). The flowering of the tassel (VT) begins about two to three days before the silks emerge from the husk, and pollen then sheds for one to two weeks (Ritchie *et al.*, 1993).

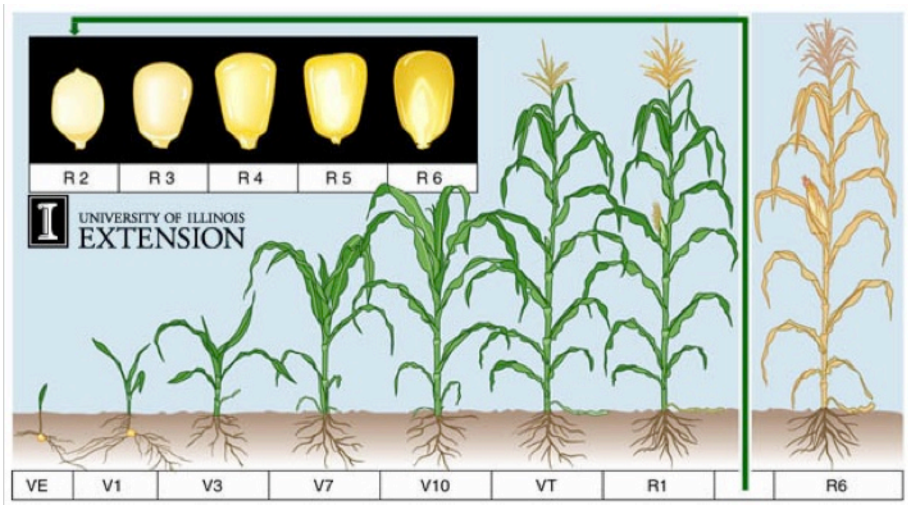

Figure 1. Corn Growth Stages. (Source: Ritchie *et al.,* 1993)

Once the silks are visible, the reproductive phases are numbered R1 through R6 and are generally referred to as the grain filling period for the 60 days after silk emergence. During silking (R1), pollen is blown from the tassels (male flowers at the top of the plant) and cross-pollinates the new, moist silks of ears located midway up the stalk. Each kernel develops from an individually pollinated silk, starting with development at the base of the ear first. Each silk remains receptive to germination for up to ten days after emergence, but if not fertilized during that time, no kernel will develop for that silk (Nielsen, 2001).

The degree of pollination during silking, as well as kernel abortion, affects the number of potential kernel ovules (Below, 1997). Kernel abortion from environmental stress like nutrient or water limitations occurs mainly during the kernel blister stage (R2) and milk stage (R3). During the dough stage (R4), starch accumulates in the endosperm, and in the dent stage (R5), stress can limit kernel dry weight accumulation if leaves senesce early and limit the source of nitrogen and carbon metabolites to the endosperm. Once kernel maturity (R6) is reached, the kernel has reached the maximum dry weight and neither stress nor frost will affect yield (Nielsen, 2001). Kernel characteristics (number, size, weight) will vary between hybrids and environmental field conditions, so careful selection of hybrids should be made for the particular environment in which they will be grown (Ritchie *et al.*, 1993).

The hybrid type and environmental conditions also affect the length of the reproductive growth period. The sink strength of developing kernels remains very high throughout the grain filling period, but despite the strong sink capacity, the extent to which kernels are filled depends on the length of the grain filling period (Hirel *et al.*, 2005). Seed fill duration controls yield even more so than biomass accumulation in vegetative plant parts since the maximum translocation of carbon and nitrogen metabolites from maturing leaves necessitates a longer grain filling period (Egli, 2004; Banzinger *et al.*, 2000). Compared to hybrids of the 1950s, hybrids today reach maturity about ten to fifteen days later, allowing for a longer grain filling period and greater accumulation of dry matter after tasseling, leading to higher grain yield in the newer hybrids (Zhang *et al.*, 2007).

From VE to VT, the main assimilate in corn exists as sucrose, while starch represents a temporary carbohydrate storage pool during the reproductive phases R1 to R6. In order to synthesize starch from sucrose, three enzymes are required: ADP-glucose pyrophosphorylase (ADPG-PPase) to initiate the conversion of glucose into starch, starch synthase (SS) to convert the glucose intermediary to amylose, and the starch braching enzyme (SBE) to combine amylose into amylopectin. Starch is then composed of two polymers, amylose and amylopectin (Zhang *et al.*, 2007). On a dry weight basis at harvest, starch makes up between 70 and 76% of kernel weight (Uhart and Andrade, 1995; Kheshgi *et al.*, 2000).

2.2 Nitrogen Take Up, Assimilation, and Use Efficiency: Corn

Nitrogen enters the corn plant in inorganic form, namely nitrate and ammonium. Most nitrogen assimilation in corn occurs in the roots, and the nitrogen source distinctly affects assimilation and transportation of nitrogen within the plant and thus growth. When only ammonium is available, the major site of nitrogen assimilation is in the roots; however, in nitrate-only fed corn, assimilation of nitrogen occurs mainly in the shoot and leaves (Cramer and Lewis, 1993). When ammonium and nitrate are both available for take up, most of the nitrogen is exported to the shoot as amino acids with the remainder as nitrate to be assimilated in the leaves, but ammonium is absorbed more rapidly than nitrate (Murphy and Lewis, 1987). Also, coupling ammonium and nitrate in fertilization stimulates growth more than just ammonium or nitrate sources alone, since more nitrogen reaches the shoots and leaves when both sources are present in the soil (Cramer and Lewis, 1993). Once assimilated, the structural components of corn, like the shoot, are not highly responsive to nitrogen, so nitrogen is incorporated into the photosynthetic process as carboxylating enzymes, proteins of membranes, and amino acids, mostly within chloroplasts (Moose and Below, 2009; Ding *et al.*, 2005).

Nitrogen take up from emergence until silking correlates directly with the leaf area index (LAI, leaf area per unit ground area) of the corn plant (Banzinger *et al.*, 2000). A greater LAI requires more nitrogen to sustain photosynthesis, and thus take up of N increases linearly with increases in LAI. Nitrogen taken up during this time funnels to the stalk, husk, cob, and shank, which act as first nitrogen sinks and then as sources for nitrogen after silk pollination (Plenet and Lemaire, 2000). The stalk represents the main depository for both carbon and nitrogen assimilates before silking, storing approximately 32% and 26% of total plant carbon and nitrogen respectively (Cliquet *et al.*, 1990). At tasseling the stalk contains about 40% of total plant nitrogen and contributes 45% of the nitrogen remobilized to the ear (Ta, 1991). The stalk provides less total nitrogen to the endosperm than the leaves. However, the stalk, with one-sixth the dry weight of the leaves, provides relatively more nitrogen as a percentage of dry weight than the leaves, making the stalk an important reservoir for nitrogen prior to grain filling (Herrmann and Taube, 2004). If sink limitations arise during grain filling though, carbohydrates and nitrogen continue accumulating in the stalk (Rajcan and Tollenaar, 1997). Ultimately, the uppermost leaves, especially above the ears, provide the highest amount of carbon and nitrogen assimilates to the grain, since the leaves at the bottom of the stalk have mostly senesced before grain filling (Hicks and Thomison, 2004).

The transportation of nitrogen from the leaves and shoot to the endosperm occurs mainly in the form of protein N and N compounds more complex than amino acids.

During the first twenty-five days after silking, both nitrogen take up and remobilization from leaves and shoot sustain the nitrogen demand of the growing ear, which requires large nitrogen concentrations while expanding the volume of the grain at this time. Twenty-five to thirty-five days after silking (R4), take up of nitrogen reaches a maximum as starch deposition in the grain related to carbon accumulation enhances nitrogen remobilization from the leaves and stalk (Plenet and Lemaire, 2000). Although, in supraoptimal nitrogen conditions, nitrogen take up can increase until silage maturity beyond the R4 stage (Herrmann and Taube, 2004). The remobilization of leaf nitrogen to the grain in the form of amino acids recycled from RuBisCo and chlorophyll initiates leaf senescence (Hirel *et al.*, 2005). Therefore, the amount of remobilized nitrogen depends more on leaf longevity than the level of soil nitrogen fertilization during the grain filling period (Hirel *et al.*, 2001).

The grain at maturity comprises between 40 and 70% of total nitrogen found in the whole plant because of the high level of N remobilization from the vegetative parts to the grain (Egli, 2004). Post-silking nitrogen take up, most of which is supplied by mineralization of soil N and not N fertilizer, contributes approximately 60% of the total grain nitrogen, depending on the hybrid, since remobilization from the leaves eventually becomes limited by the need to continue photosynthesis for the duration of the grain fill period (Hay *et al.*, 1951; Rajcan and Tollenaar, 1997).

The accumulation of maximum kernel weight depends on both carbon and nitrogen supplies. Nitrogen helps establish kernel sink capacity and stimulates enzymes relating to continued nitrogen take up and sucrose development, while carbon, in the form of sucrose, regulates the activities of ADPG-PPase (ADP- glucose pyrophosphorylase) in starch synthesis (Cazetta *et al.*, 1999). However, without sufficient supplies of nitrogen to the endosperm, ADPG-PPase activity is severely compromised despite the supply of carbon, and the final kernel dry weight realizes significant losses (Below, 1997). Thus, nitrogen usually poses as the limiting factor to final yield during grain filling over carbon, especially under low source-sink ratios (mg N assimilate/kernel) [Uhart and Andrade, 1995].

The ability of the corn plant to take up, assimilate, and utilize nitrogen to produce grain without causing limitations can be characterized as the nitrogen use efficiency of the plant. Nitrogen use efficiency (NUE) is a product of nitrogen take up efficiency (N-take up/N soil availability) and nitrogen utilization efficiency (yield/N-take up). NUE then measures the grain yield per unit of nitrogen available in the soil, including fertilizer (Gallais and Hirel, 2004). Agronomic NUE can then be simplified to grain yield per unit fertilizer applied. Agronomic NUE for corn has been continually documented between 30 and 50% efficiency, meaning that up to half the fertilizer applied is lost to the environment. However, over the past 30 years, grain yields have increased by nearly 20%, while applied fertilizer rates have hovered around 150 kg N ha^{-1}, implying some inherent improvement in the agronomic NUE of corn (Moose and Below, 2009).

Many physiological processes influence NUE including N take up from the soil by roots, N assimilation to amino acids by roots and shoot, N transport from leaves to grain, and utilization of N by the grain. To understand how nitrogen utilization efficiency (NUE) is controlled, the corn plant must be examined at the molecular level, specifically considering the synthesis of the major form of nitrogen transported in corn, glutamine (Ta, 1991).

Glutamine synthetase (GS) is the enzyme most responsible for assimilation of ammonium produced from nitrogen fixation, photorespiratory ammonium, or nitrate and ammonium take up. GS fixes ammonium in the amide-N of glutamine (Gln), which then enters the GOGAT[1] reaction to become glutamate; glutamate carbon funnels into chlorophyll, while glutamate nitrogen supplies most of the nitrogen for other amino acids (Oaks, 1994). Chloroplastic glutamine synthetase (GS2) relates to primary nitrogen assimilation, whereas cytosolic glutamine synthetase (GS1) plays a key role in leaf senescence and remobilization of nitrogen from the leaves to the endosperm and therefore affects yield. Two of the five genes encoding GS1, gln3 and gln4, are highly expressed regardless of the level of nitrogen fertilization or leaf age, suggesting that these two genes control nitrogen utilization efficiency in corn (Hirel *et al.*, 2005). Thus, GS1 could then be a target for marker-assisted breeding for improving nitrogen use efficiency while also improving yield (Miflin and Habash, 2002).

Marker-assisted breeding depends on defining quantitative trait loci (QTL), or the DNA associated with the genes that underlie a particular trait, and could allow improvements in grain yield and thus ethanol yield per hectare. Specifically, QTL analysis has identified the gln3 gene encoding cytosolic glutamine synthetase (GS1) as a candidate to influence grain filling. By overexpressing the gln3 gene, which is directly related to nitrogen translocation to the endosperm, kernel number may be increased up to 30% (Martin *et al.*, 2006). The ability to enhance remobilization could lead to lower nitrogen fertilizer demand because remobilization and take up are negatively correlated, such that reducing nitrogen take up then increases remobilization (Gallais and Hirel,

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[1] GOGAT reaction: glutamine + 2-oxoglutarate + NADPH + $H^+ \rightarrow$ 2 glutamate + $NADP^+$

2004). Thus, at high levels of nitrogen fertilization, nitrogen take up efficiency dominates and grain yield is positively correlated with nitrogen absorption, but under low N fertilization, nitrogen utilization efficiency becomes more important and grain yield becomes positively correlated to remobilization instead of take up. Over-expressing cytosolic glutamine synthetase could potentially lower demand for nitrogen fertilizer without affecting yield by increasing kernel number, remobilization, and nitrogen utilization efficiency (Murphy and Lewis, 1987). Trait selection for corn as an ethanol feedstock continues beyond NUE as discussed in the next section.

2.3 Trait Selection for Ethanol: Corn

Trait modification refers to the alteration of specific genes that control overlying crop characteristics like insect resistance or yield; often, trait stacking occurs in corn hybrids, combining many different desirable traits in one plant. Traits can be brought to prominence either through conventional breeding or genetic modification, although the latter has become more popular among the top seed manufacturers, namely Monsanto, Dupont, and Syngenta (Curtis, 2008). In the 2010 season, herbicide tolerance, insect resistance, or a combination of the two dominated United States cornfields with 83.6 million hectares (Mha), 21.7 Mha, and 28.7 Mha respectively (Rennie and Heffer, 2010). In addition to insect and herbicide tolerance, hybrids with limited biofuel processing traits are currently commercially available. Before 2015, the market will expand to include total yield enhanced traits and traits for the optimization of biofuel production, such as engineering hybrids to produce alpha-amylase, an enzyme necessary for ethanol production, inside the grain itself (Doering and Abbott, 2011). Only beyond 2015 will engineered traits offered encompass nitrogen use efficiency though (Rosegrand and Cavlieri, 2008). Traits molded for adaption to unfavorable environmental conditions like drought or acidic soil will only heighten the demand for nitrogen fertilizer as corn cropping expands to once undesirable geographies in order to support growing ethanol demands, yet nitrogen use efficiency enhancement remains only in the early stages of development (Rennie and Heffer, 2010).

Corn growers typically base hybrid selection on the highest potential yield given their field circumstances, but growers of corn specifically destined for ethanol may also highly consider the inherent fermentability of the grain in order to increase marketability upon harvest. In one seed catalog, offering all the hybrids bred by the two largest seed manufacturers, Monsanto and Syngenta, eleven of the thirty-five hybrids offered were either classified as highly fermentable corn (HFC), highly extractable starch (HES), or both (Kent, 2010). In order for a company like Monsanto to classify a hybrid as highly fermentable corn or highly extractable starch, a particular hybrid must have been tested in a variety of field conditions over multiple years for higher fermentability in dry mill production of ethanol or extractability in wet mill production. Though, the traits selected for HFC or HES hybrids realistically only increase the probability of producing higher ethanol yields per bushel and do not necessarily create consistent, measurable boosts in ethanol yield due to the strong influence of the growing environment on grain fermentability (Ream *et al.*, 2010).

While, at the current time, breeders have prioritized fermentability and extractability of starch and claim these traits increase ethanol yield 3 – 5%, no direct correlation between starch content of the grain and ethanol yield has been documented

(Singh, 2010; Ream *et al.*, 2010). Inter-annual variability in average ethanol yield has been noted up to 23% though, creating the hypothesis that environmental stress during corn growth, such as unfavorable temperature, disease, and water or nutrient limitations, may affect the non-starch components of the grain like oils and proteins, influencing ethanol yield (Sicklen-Pollack and Scott, 2005). Thus, when traits are selected in the future, starch granule packing, protein matrices, and yeast nutrition factors may become more prominent in gene modification (Ream *et al.*, 2010).

Additionally, focusing on the type of corn may reduce quantity of enzymes added during ethanol production. Alpha 1-4 and alpha 1-6 linkages string together individual units of glucose to form corn starch, with the amylose molecules comprising the 1-4 linkages and amylopectin encompassing the 1-6 linkages. Alpha-amylase enzymes are then required to hydrolyze the alpha 1-4 bonds in amylose in order to reduce the size of the starch polymer before the starch can be converted to glucose by glucoamylase, which can hydrolyze the remaining 1-6 bonds (Bothast and Schlicher, 2005).

In normal corn, amylose represents between 24 and 27% of the starch with amylopectin making up the remainder; however, in waxy corn varieties, all of the starch is amylopectin (Kweon *et al.*, 1997). The highest ethanol concentrations are derived from starch containing 100% amylopectin (i.e. waxy corn). Ethanol yields decline with increasing amylose content because more resistant starches are associated with high amylose content (Vivek, 2008). Starches with more amylose also require higher liquefaction temperatures and greater quantities of the alpha-amylase enzyme than strictly amylopectin starch because of the elevated levels of resistant starch and the higher peak viscosity of the slurry (Saunders *et al.*, 2011; Vivek, 2008). Waxy corn then provides another opportunity for improved marketability of harvested corn destined for ethanol plants as the 100% amylopectin starch of waxy corn is more susceptible to digestion during liquefaction and saves on enzyme and energy inputs. Alternatively, some normal dent corn hybrids have recently been genetically modified to over-express the starch hydrolyzing enzyme alpha-amylase, which may also reduce the amount of enzymes added during ethanol production (Ream *et al.*, 2010).

2.4 Sugarcane Botany

In 1532, the Portuguese introduced sugarcane, a tall perennial tropical grass, to the region of São Paulo State (Baldini *et al.*, 2002). Since this time, over 500 varieties of commercial sugarcane have been bred with the top twenty varieties occupying 80% of the planted land area in Brazil (Walter, 2009). Until the late 19th century, botanists had not realized sugarcane produced seeds, but in 1887, the first seeds of *S. officinarum* were crossed with the wild species of cane, *S. spontaneum* (Baldini *et al.*, 2002). *S. spontaneous* is considered an invasive species to South America, but the crossing of this highly vigorous, high fiber cane with the sucrose accumulating *S. officinarum* produced a hybrid with superior disease resistance and higher yields (Nemose, 2010). Most current varieties originate from this crossed hybrid of *S. officinarum* and *S. spontaneum*, but varieties differ in disease resistance, yield, juice quality, and time to maturity (Bull, 2000).

Although bred using seeds, sugarcane is propagated with pieces of the stem containing one or more buds, called setts (James, 2004). Buds are contained in the root band of the node and consist of embryonic shoots with a miniature stalk and small leaves, which sprout into the primary stalk. After the sprout emerges from the soil (germination), the seedling survives mostly on the sucrose and nutrients in the sett. Each sett also includes a band of root primordial in the node from which the sett roots originate, which supplies water and some nutrients to the seedling in the first weeks after germination in addition to sett assimilates (Nemose, 2010). Sett roots slowly die once shoots roots from the base of the stem are established, usually within five to seven days after plant emergence (Figure 2) [Rao and Vered, 2011]. Shoot roots become the main root system and proliferate where moisture, nutrients, and soil aeration are present, reaching an average depth of 1.6 meters but with 85% of the root mass residing in the top 60 centimeters of soil (Smith *et al.*, 2005; James, 2004).

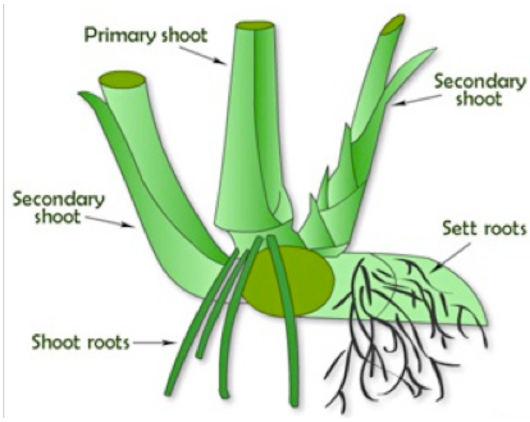

Figure 2. Roots and tillers of sugarcane.
(Source: Rao and Vered, 2011)

In the beginning growth stages, the primary shoot develops closely space nodes under the soil. Each node that develops on the cane stem (or stalk) contains a new bud and root primordial, regardless of placement on the stem. Thus, about 40 days after planting up until 120 days, tillering occurs whereby the nodes of the primary stem that remain underground sprout secondary shoots or tillers (Nemose, 2010). These tillers then establish their own shoot roots and become independent from the primary shoot and can even create tertiary shoots from their own underground nodes (Figure 2). Six to eight tillers occur per bud but only one or two form canes, thus tillering provides the crop with the appropriate number of cane stalks for a good yield (Smith *et al.*, 2005).

Following tillering, stem elongation, also known as the grand growth phase, occurs between 120 days after planting until 270 days depending on the variety (Nemose, 2010). Leaf production is frequent and rapid as the internodes are supplied with assimilates from their connecting leaves as the stem elongates between nodes. The grand growth phase creates the form of the cane, so sugar content of the cane remains low while fiber content of the stem increases (Bull, 2000). A hard, waxy rind forms around the soft inner parenchyma tissue and fibrous vascular bundles of the internodes, and, eventually, this parenchymatous tissue will store the juice containing the sucrose (James, 2004).

Finally, from 270 days after planting to around 360 days depending on environmental conditions and variety, the cane ripens and matures. The cane ripens up the stem from the internodes closest to the ground, rapidly accumulating sucrose in the storage parenchyma of each internode as vegetative growth is reduced (Nemose, 2010). Sucrose accumulation differs by cultivar due to morphological differences, with stalks high in moisture and low in fiber, like *S. officinarum*, generally collecting the most sucrose in the stem (Figure 3). Sucrose, a product of photosynthesis, is loaded into the phloem for transport through apoplast in the leaves; to be stored in the vacuoles of the parenchyma within mature internodes, invertases break the sucrose into hexoses (glucose and fructose) that are transported into the storage parenchyma cells where sucrose is then resynthesized (Appendix: Figure B) [Rae *et al.*, 2005]. Internodes function independently such that each internode may continue stem elongation and filling with sucrose as long as its green leaf remains attached (Bull, 2000). Harvesting of the cane usually transpires prior to flowering since the forming of a seed-filled tassel can reduce sugar yield in the cane (James, 2004).

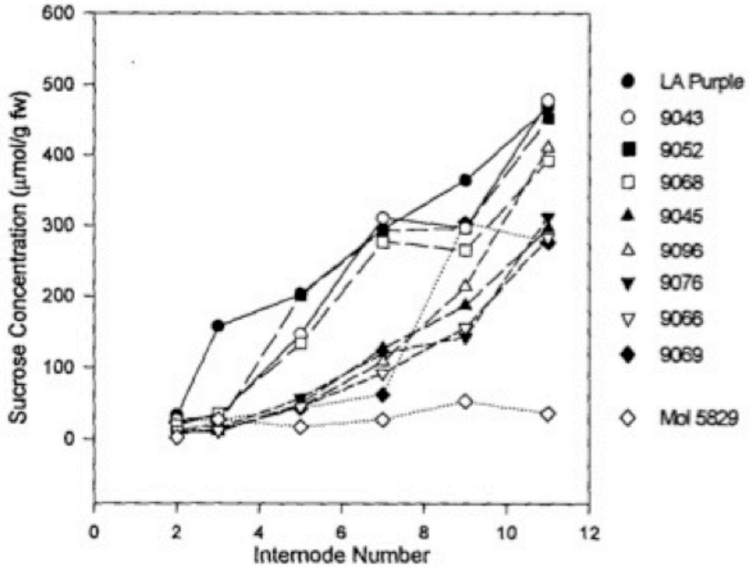

Figure 3. Difference in Sucrose Content by Cultivar and Internode. *S. officinarum* is LA Purple; *S. robustum* is Mol 5829; the remaining identifiers represent crosses between the two parents. Internode numbers proceed down the stalk, with 1 being the immature node at the top of the stalk. (Adapted from Zhu *et al.*, 1997)

After harvesting of the plant crop (cane derived from planted setts), the remaining portion of the cane stem may be allowed to germinate again into what is known as a ratoon crop (crop derived from stems of the previous crop). Ratoon crops generally sprout more tillers earlier in the growing season and have higher nutrient requirements than the plant crop. Ratooning may continue over four to six growing seasons, depending on environmental conditions, before the ratoons are ploughed under or removed with herbicide (Bull, 2000).

2.5 Nitrogen Take Up and Biological Nitrogen Fixation: Sugarcane

As in corn, sugarcane can take up nitrogen in both nitrate and ammonium forms from the soil, but sugarcane has also been noted to glean a significant portion of its nitrogen from biological nitrogen fixation (Boddey *et al.*, 1995). Sugarcane employs nitrogen for producing amino acids and proteins critical to photosynthesis and then almost immediately utilizes assimilated nitrogen for structural growth, unlike corn that circulates large portions of plant N in photosynthetic enzymes like RuBisCo until the N is remobilized to the grain in the latter half of the growing season (McCormick *et al.*, 2009). Sugarcane requires nitrogen to maintain structural growth, particularly for reaching the maximum height, number of leaves, girth, and accumulation of biomass (Lal, 1950). The effective use of nitrogen within the cane also determines leaf area expansion and green leaf duration (Kawamitsu *et al.*, 1999).

While sufficient nitrogen take up inspires rigorous photosynthesis and structural growth, higher photosynthetic rates do not imply greater sucrose accumulation. In fact, *Saccharum spontaneum*, noted for its high growth rates and low sugar content, photosynthesizes at a rate 30% greater than that of its high sucrose-storing cousin, *Saccharum officinarum* (McCormick *et al.*, 2009). Yet, nitrogen availability and take up remains critical from germination to stem elongation for proper stalk and leaf development; however, the continual take up of N past stem elongation is what leads to lower sucrose content and higher amounts of reducing sugars that lower juice quality (Lal, 1950). Withholding nitrogen during stalk maturation tends to increase sucrose content despite the possibility that lower leaf nitrogen content could reduce photosynthesis during stalk maturation.

Nitrogen accumulation in cane usually halts 140 to 210 days after planting or ratooning, during the stem elongation phase. Thus nitrogen take up, and perhaps BNF, occurs only within the first 6 months of cane growth. At the start of stem elongation, regardless of plant or ratoon crop or cultivar, about 50% of the plant nitrogen resides in the leaves and the rest splits evenly between the roots and stalk. By the time of stalk ripening and maturation, the leaves contain about 30% of the plant nitrogen while the stalk holds approximately 50%. At the time of harvest, the cane trash (senesced leaves) can contain as little as 5% of total plant nitrogen and the cabbage (unripe apex of the stalk and its leaves) can possess less than 10% due to volatilization of ammonia from maturing leaves (Wood *et al.*, 1996).

Since biomass accumulation extends beyond nitrogen accumulation, the nitrogen utilization efficiency (accumulated biomass/N-take up) continues to increase over the course of the growing period as biomass accumulates beyond the maximum nitrogen take up. Additionally, the plant crop take up of nitrogen continues longer into the growing season than ratoon crops, most likely due to a greater supply of non-fertilizer soil N resources. As such, ratoon crops depend more heavily on fertilizer than do plant crops but overall take up between plant and ratoon crops evens out to a similar amount over the course of the growing season (Gowariker *et al.*, 2009). For a typical Brazilian yield of 65 to 70 tons of cane per hectare, between 100 and 250 kg nitrogen is accumulated in the whole crop per hectare (Boddey *et al.*, 1995). Total nitrogen accumulation differs significantly between cultivars though, caused by differences in either take up efficiency of soil N or, more likely, the amount of N derived from biological nitrogen fixation (BNF) within the cane plant itself (Lima *et al.*, 1987).

Biological nitrogen fixation is catalyzed by the enzyme nitrogenase, found in diazotrophic bacteria (bacteria that can fix atmospheric nitrogen into a plant-usable form like ammonium) [Eady, 1996]. Between the 1950s and 1980s, diazotrophic bacteria from the genera *Beijerinckia* had been found in the rhizosphere and multiple N_2-fixing bacteria had been found in the roots, stems, and leaves of sugarcane, including species of *Erwinia,* *Herbaspirillum, Azotobacter, Derxia, Azospirillum,* and *Enterobacter* (Momose *et al.*, 2009; Boddey *et al.*, 1991). Yet, until the discovery of *Acetobacter diazotrophicus* (also known as *Gluconacetobacter diazotrophicus*), none of these bacteria occurred in large enough quantities to account for the level of biological nitrogen fixation observed in sugarcane (Boddey *et al.*, 1991). The pervasiveness of *A. diazotrophicus* has been noted in many top sugarcane varieties throughout Brazil (Stephan *et al.*, 1991).

A mutualistic relationship exists between sugarcane and *A. diazotrophicus*, which lives inside the plant tissue as an endophyte, such that the host cane plant provides a sucrose solution to the intercellular spaces of the stem apoplasts that closely matches the sucrose and pH requirements of the endophytic diazotroph in return for nitrogen. For optimal growth, *A. diazotrophicus* prefers a pH of 5.5 and a sucrose content of 10% (Dong *et al.*, 1994). However, *A. diazotrophicus* can operate at particularly low pH, continuing growth even at a pH of 3.0, and can tolerate up to 30% sucrose content in the stem (Momose *et al.*, 2009; Reis and Dobereiner, 1998). The ability to function at low pH seems to be a self-protecting trait since the bacteria acidifies its surrounding solution during N-fixing activity by forming acetic acid (Dong *et al.*, 1994).

In the presence of at least 10% sucrose in the stem, the nitrogenase activity of *A. diazotrophicus* is not affected by the presence of oxygen or ammonium (Reis and Dobereiner, 1998). In regards to ammonium, the reduction in assimilation of ammonium under high sucrose levels may protect against the inhibition of nitrogenase, but *A. diazotrophicus* may also possess a higher than average “switch off” point for its nitrogenase even when ammonium is available (Stephan *et al.*, 1991). Additionally, *A. diazotrophicus* does not contain nitrate reductase, so the presence of nitrate does not affect nitrogenase activity (Dong *et al.*, 1994). Lacking nitrate reductase and possessing the ability to regulate ammonium inhibition indicates that *A. diazotrophicus* can contribute to BNF even in the presence of N fertilizers, unlike most all other N_2-fixing bacteria (Stephan *et al.*, 1991). *A. diazotrophicus* also acts as an endophyte, only able to infect the cane plant if damaged tissues are present or if propagated in vesicular arbuscular mycorrhizae of planting material (Boddey *et al.*,1991).

The method by which N-fixing endophytes, including *A. diazotrophicus*, transfer nitrogen to the host plant remains unknown but two ways have been proposed: the live bacteria can excrete fixed N into the apoplast of the host plant whereby it can be absorbed by the plant, or the fixed N is released to the host cells after the death and disintegration of the bacteria (Momose *et al.*, 2009). Despite the mystery surrounding N transfer, both ^{15}N dilution and N balance methods show that some sugarcane varieties are capable of receiving between 60 to 80% of their total plant nitrogen from BNF, although many studies cite lower contributions from BNF between 40 and 60% (Appendix: Figure C) [Boddey *et al.*, 1991; Boddey *et al.*, 1995; Stephan *et al.*, 1991; Wheals *et al.*, 1999]. Although not every variety of Brazilian sugarcane has been shown to garner significant amounts of nitrogen from N-fixing bacteria like *A. diazotrophicus*, the historically high price of N fertilizers in Brazil has resulted in the preferential selection of high yielding cane varieties with low N inputs, which coincide with those varieties benefiting from high BNF (Dobereiner, 1997). Also, almost all of the top twenty varieties that comprise 80% of planted acres, as mentioned previously, have documented relationships with N-fixing bacteria (Walter, 2009; Boddey, 1995; Stephan *et al.*, 1991). Thus, it is assumed, for simplicity and lack of evidence to the contrary, that all acres harvested for ethanol consist of sugarcane varieties capable of significant biological N-fixation; where this assumption leaves an opportunity to overestimate nitrogen introduced to the agroecoystem through BNF, it simultaneously underestimates the quantity of nitrogen introduced as fertilizer, since N application rates cited in the literature for Brazilian sugarcane consider some notable contribution from BNF.

2.6 Sugarcane Use as an Ethanol Feedstock

The total reducing sugars index (measurement of glucose, fructose, and sucrose in cane juice) defines both the yield of the cane crop and of ethanol since sucrose serves as the fermentable base in ethanol production (Walter, 2009). Breeding for new cane cultivars not only focuses on the short-term goal of increasing the amount and fermentability of sugar content but also on the long-term goal of enhancing biomass yield and fiber content for use in second generation cellulosic biofuel programs, referred to as "energy cane" (Kennedy, 2010).

Since 1975, yields of sugar cane, in terms of fresh harvest weight, have expanded by nearly 60% with the breeding of new varieties (Walter, 2009). Yet despite the high potential for inheritability of sugar content, breeders have increased sugar yields via improvements in cane yield instead of sugar content (Jackson, 2005). If breeders could target the right functional markers through the mapping of quantitative trait loci (QTLs), the potential exists to increase the wet weight of sucrose by up to 25% (Waclawovsky *et al.*, 2010).

However, using functional traits for breeding as seen in corn proves to be extraordinarily difficult in sugarcane due to the polyploidic nature of the grass. QTLs often only explain 15% of the variation in a particular trait due to the multiplicity of genes representing any given trait (Aitken *et al.*, 2006). At most, breeders can attempt to duplicate observations of the genes expressed in hybrids with distinguished sugar accumulation in new cultivars. Observational studies have shown that high sugar content correlates to the down regulation of protein synthesis and processing, usually seen in N deficient canes (Casu *et al.*, 2005). Most studies attempting to map QTLs for marker-assisted breeding have limited success relegated to clustering QTLs into homologous groups (confounded by allelic forms of the same gene) and correlating these QTL groups to particular traits in an attempt to find any significance (Aitken *et al.*, 2008; Ming *et al.*, 2002).

With the difficulties in finding functional markers to assist breeding specifically for sugar content, cane varieties bred for sugar yield and biofuel processing traits are not likely to become available until after 2015 (Rosegrant and Cavalieri, 2008). In an effort to enhance sugar yield through external measures, products like “Atomic Grow” have been created, claiming to increase the ability of the leaves to produce more sugars by reducing the surface tension of soluble sugars and therefore increasing translocation of sugars from the leaves to the stem (‘Atomic Grow,’ 2009). With over a dozen diseases where varietal resistance remains the only control, past breeding efforts have focused mainly on instilling disease resistance in new varieties of cane. Varieties have also been modified for the ability of the stalk to drop old leaves (de-trash) and the ability to reach higher plant weights, but much work still remains to improve cane cultivars for the ethanol industry (James, 2004).

For the future, breeding programs should consider how best to expand both biomass and sugar yield in order to maximize the amount of ethanol derived from each hectare of sugarcane and thus reduce the total amount of nitrogen needed to sustain cane production by limiting the spatial expansion of the crop. Since the fibrous portion of the cane (bagasse) is commonly burned for energy in the distilleries after it has been juiced, increasing biomass production while maintaining or increasing sugar production per cane plant could improve the efficiency of ethanol production (Loureiro, 2007). Also, if bagasse hydrolysis becomes highly efficient, higher biomass yield could enhance the potential use of cane as a second-generation cellulosic biofuel and may become more valuable a trait than sucrose content in the ethanol industry. The establishment of more tillers, inspired by delayed stem elongation, can help to combine high biomass yield with high sugar content (MacColl, 1976). Improvements to tillering and delaying stem elongation are then crucial for future breeding of cane.

Production of rhizomes, associated with some varieties of *S. spontaneum*, may be a key trait to consider for enhanced tillering since rhizomes can extend far away from the primary shoot and incite intense tillering; rhizome production and the associated tillering can also extend the total number of ratoon crops, reducing the number of times a field must be ploughed for replanting and thus reducing soil erosion. Rhizomes also help to enhance drought tolerance (Matsuoka and Garcia, 2011). Since the cane growing regions of Brazil are expanding to the northeast and center-west regions where temperature and precipitation are not ideal, breeding for drought and cold tolerance by enhancing the germplasm from *S. spontaneum* in crosses would allow further growth of the ethanol cane plantings in Brazil without compromising yield (Waclawovsky *et al.*, 2010). Thus, selecting parent canes of *S. spontaneum* with demonstrated rhizome production could balance enhanced biomass yield with improved sugar content while also resisting drought, potentially leading to a new cultivar well suited for the ethanol industry in Brazil.

2.7 Conversion of Solar Energy to Biomass

Under optimal growing conditions, the maximum potential biomass any crop can accumulate over the course of the growing season is determined by the amount of solar energy reaching the cropland and how well the crop converts this energy into biomass and, thus, yield. Potential yield calculated as a function of solar radiation then provides a ceiling for maximum yield, unless improvements can be made in the way the plants capture and use radiation. The propinquity of current yields to theoretical maximum yields offers a metric to determine if genetic modification or altered crop management provides the greatest potential to improve yields.

Incoming solar radiation varies by latitude, geography, and day of the year though, and these variables affect potential yield. Furthermore, only about half of the incoming solar radiation lies within the absorption spectra of chlorophyll and carotenoid pigments, since chlorophyll absorbs violet-blue and red light waves and carotenoids absorb mainly blue light outside the range of chlorophyll (Sinclair and Weiss, 2010). The incoming solar energy that can be absorbed by chlorophyll and carotenoids is termed incident photosynthetically active radiation (iPAR). The leaves must then intercept the incident photosynthetically active radiation, and the interception efficiency (ε_i) depends directly on the leaf area index and the angle of the leaves. Even still, leaves only absorb between 85 and 92% of intercepted photosnythetically active radiation at maximum efficiency (Bergamaschi *et al.*, 2004). By the time radiation has been absorbed by the leaf, the storage of energy as biomass in both sugarcane and corn represents less than 1% of all the original incoming solar radiation realized during their growing periods (Kheshgi *et al.*, 2000). The ability of the crop to convert energy into biomass refers to the radiation use efficiency (RUE), measured in grams of dry biomass accumulated per megajoule (MJ) of intercepted radiation. Nitrogen supply not only manipulates RUE but can also augment LAI, green leaf duration (GLD), and crop growth rate. Consequently, maximum potential yield determined by incoming solar radiation is often unrealized due to nitrogen and water limitations.

In corn, approximately half of all nitrogen found in the leaf becomes directly involved with photosynthesis as either enzymes or chlorophyll. Therefore, photosynthetic rates become coupled to the nitrogen content of leaves, creating a strong dependence between leaf nitrogen content and radiation use efficiency (Figure 4). In corn, leaf N content directly relates to the soil nitrogen supply, increasing with the rate of N fertilization. The RUE increases indefinitely with the amount of N in leaves fifteen days before flowering

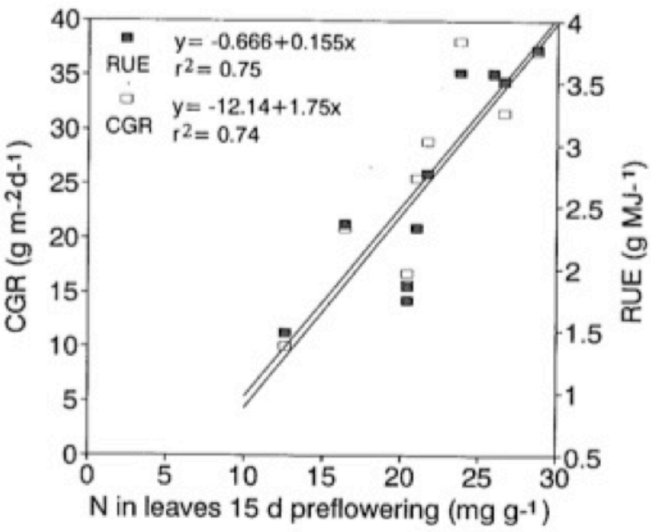

Figure 4. Effect of Leaf N Content on Crop Growth Rate and Radiation Use Efficiency.
(Source: Uhart and Andrade, 1995)

(about V18) in a linear relationship (Figure 4), but Banzinger *et al.* (2000) propose the benefits of leaf N content saturate around 20 mg per gram. Leaf N content also shows a positive linear relationship with the crop growth rate, but the gain in kernel number realized with higher growth rates reaches a plateau of 3600 kernels m^{-2} around 25 g m^{-2} d^{-1} of accumulated biomass (Uhart and Andrade, 1995; Lindquist *et al.*, 2005). A CGR of 25 g m^{-2} d^{-1} nearly corresponds to a leaf N content of 20 mg g^{-1} (Figure 4), suggesting that the relationships of leaf N content with RUE and CGR are interdependent and maximized at 20 mg N per gram of leaf biomass. Although a higher leaf N content improves RUE and CGR, these improvements may be at the expense of nitrogen use efficiency since obtaining high levels of NUE requires lower leaf N content (Gutschick, 1997). Thus, corn crops must find a balance between use efficiencies of their resources while optimizing growth when resources like nitrogen are insufficient. If leaf nitrogen does become scarce, nitrogen will be reallocated from older leaves and the stalk to younger leaves and storage organs, like the corn kernel. Reallocation of nitrogen from older to younger tissues leads to early leaf senescence of the older leaves and increases root growth over shoot growth, lowering above ground biomass and crop growth rate by 15 to 59% (Banzinger *et al.*, 2000; Uhart and Andrade, 1995).

In sugarcane, the same resource balance must be considered such that RUE does not needlessly increase at the expense of other resource use efficiencies. For example, breeding trends for enhanced aboveground biomass to improve RUE over the years may have come at the expense of root expansion. Limits on root penetration depth and shallow root breadth could then reduce water and efficiency of nutrient take up, especially for nitrogen sources like nitrate that are highly mobile in the soil profile (Smith *et al.*, 2005).

However, the leaf nitrogen content does not play as significant a role in determining RUE, unlike in corn. Leaf nitrogen content only influences the structural growth rate of cane and not sucrose creation or accumulation, although if the length of the internodes is severely reduced by the cane growth rate, a sink limitation can occur (Keating *et al.*, 1999). Leaf N contents above 1.2 g N m^{-2} keep RUE and photosynthetic rates operating without limitation, but RUE and the rate of biomass accumulation both decline at the end of the growing season before leaf N content dips below this critical N level, implying that the amount of leaf N does not control RUE (Wood *et al.*, 1996). Although Allison *et al.* (1997) suggest that photosynthetic rates are often restricted by low levels of specific leaf nitrogen (SLN), as specific leaf N frequently dips below the critical N level of 1.2 g N m^{-2} by 45 weeks in the growing season, yet this same study shows augmented N-fertilizer application serves little value for improving SLN or photosynthetic rates. Cultivars better adapted to take up nitrogen later in the growing period, after the usual six-month take up threshold, and able to partition this N to the leaves may realize greater photosynthetic rates throughout the duration of the growing season.

However, assuming nitrogen is supplied at optimal rates allows an investigation of how solar radiation directly affects the potential yield of corn and sugarcane. Assuming both water and nitrogen are readily available, potential yield (PY) may be determined by the amount of radiation captured by the plant using the formula (Banzinger *et al.*, 2000; Waclawovsky *et al.*, 2010):

$$PY = iPAR \text{ x } \varepsilon_i \text{ x } GLD \text{ x } RUE \text{ x } HI$$

Where iPAR is the incident photosynthetically active radiation per unit area each day, ε_i represents the interception efficiency of PAR by green leaves (integrated over the growing season in relation to LAI), GLD notes the number of days leaves stay photosynthetically active, RUE is the radiation use efficiency, and HI represents the harvest index (weight of harvested portion as a percentage of total plant weight of crop). Multiplying the potential yield (PY) by the portion of the yield that is fermentable for ethanol (PYF) gives an estimate of potential ethanol yield.

Because of their different latitudes, average incoming solar radiation for the U.S. Corn Belt and Brazil differ significantly, receiving 15 and 20 MJ m^{-2} d^{-1} respectively (Dohleman and Long, 2009; Waclawovsky *et al.*, 2010). However, in addition to latitude, solar radiation varies by season and year, so values for incoming solar radiation often differ (Table 1). Lindquist *et al.* (2005) report levels of incident solar radiation in Nebraska above that of Brazil at

Table 1. Review of values for incident photosynthetically active radiation (iPAR) and radiation use efficiency (RUE) for corn and sugarcane.

Source	Region of Study	iPAR (MJ m^{-2} d^{-1})	RUE (g MJ^{-1})
	CORN		
Dohleman and Long (2009)	Illinois	7.26	--
Lindquist *et al.* (2005)	Nebraska	10.70	3.7
Kiniry *et al.* (2004)	Texas	--	3.7
Andrade *et al.* (1993)	United States	--	2.7
	Average	8.98	3.4
	SUGARCANE		
Marin *et al.* (2008)	Brazil	9.30	--
Waclawovsky *et al.* (2010)	Brazil	9.94	--
Park *et al.* (2004)	Australia	--	1.8
Singels and Bezuidenhout (2002)	South Africa	--	5.68
	Average	9.62	3.74

21.4 MJ m^{-2} d^{-1}, most likely owing to a particularly cloudless growing season that would raise the average incident solar radiation per day.

The interception efficiency of iPAR (ε_i) is determined by the LAI of the crop. In order to maximize ε_i, the critical LAI for sugarcane should be at least 4 m^2 m^{-2}; reaching the necessary growth rate to establish an LAI of 4 m^2 m^{-2} requires 1 gram of nitrogen per square meter of leaf area, or 4 grams N per square meter of ground (Sinclair 2009). For corn, the critical LAI is 3 m^2 m^{-2}, but critical LAI can differ by corn hybrid depending on the leaf angle (erectophile or planophile) [Smith *et al.*, 1994]. Keeping the LAI above the critical LAI for the crop will maximize crop growth rate and biomass accumulation by heightening the interception efficiency. Maximum efficiency of photosynthesis would assume an interception efficiency of 0.95 when LAI is large, but the variation of ε_i with rising and falling LAI over the course of the growing season causes a significant departure from maximum interception values (Monteith, 1972). Potential yield, of course, decreases as the interception efficiency declines and ultimately lowers photosynthetic rates over the growing season. However, in order to propose a maximum yield based on solar radiation alone, this calculation will assume 100% interception of incident PAR as modeled by Sinclair (2009).

The duration of the green leaves also impacts LAI and the ability for plants to absorb radiation. Green leaf duration averages to approximately 95 days over the course of the growing period for corn (Hicks and Thomison, 2004; Dohleman and Long, 2009). GLD for sugarcane in a typical Brazilian 12-month crop is 295 days (Waclawovsky *et al.*, 2010).

Radiation use efficiency (RUE) may be defined as the amount of aerial biomass accumulated for the cumulative intercepted PAR in a given period or, more simply, the energy required to produce a unit of dry matter (Uhart and Andrade, 1995). RUE for corn generally increases with the plant population density, peaking between 8 and 9.5 plants per square meter with a RUE of 4.5 g MJ^{-1} (Kiniry *et al.*, 1989). RUE for corn, as discussed above, also correlates linearly to leaf N content and may also be impacted by water availability. With many environmental factors contributing to RUE, published values for corn differ widely among sources as outlined in Table 1.

RUE in sugarcane is mostly affected by the ratoon crop class (number of ratoons after initial planting) since consistent higher growth rates in the plant crop boost RUE above that of any subsequent ratoon crops (Park *et al.*, 2004). Still, little information on RUE in Brazilian cultivars has been documented in the literature, but with the highest production rates in the world per hectare, it would seem logical to infer RUE in Brazilian cultivars is most likely higher than those documented for other countries. For these calculations, RUE is assumed to be constant throughout the growing season since presented RUE values have already been averaged across the growing season for both corn and sugarcane.

Considering the harvest index, plant biomass weight and grain weight have increased proportionally with the evolution of corn hybrids from 1950 until today as a result of increased nitrogen take up after silking; therefore, the harvest index has remained fairly constant, hovering around 0.50 (Ding *et al.*, 2005; Hay *et al.*, 1951; Kiniry *et al.*, 2004). In sugarcane, biomass yield at harvest remains fairly even between plant and ratoon crops due to the loss of millable stalks during the maturation phase. The fraction of aboveground biomass representing the millable stalk at harvest is 80% (HI = 0.8) [Robertson *et al.*, 1996; Waclawovsky *et al.*, 2010].

Finally, the proportion of the yield that is fermentable (PYF) for corn is the starch content, which represents between 70 and 76% of the dry harvested grain biomass (Kheshgi *et al.*, 2000; Uhart and Andrade, 1995). For sugarcane, PYF is the sucrose, which accounts for about 50% of the dry harvested stalk biomass (Kheshgi *et al.*, 2000).

The values presented above consider corn and sugarcane under optimal growing conditions, without nutrient or water limitations, and thus can present the maximum potential yield that may be realized for the solar radiation in the U.S. Corn Belt and Brazil given current interception efficiency, green leaf duration, and radiation use efficiency of the plants. With an average iPAR of 8.98 MJ m^{-2} d^{-1}, GLD of 95 days, RUE of 3.4 g MJ^{-1}, and harvest index of 0.5, the potential grain yield of corn is 1450 g m^{-2} or 14.5 metric tons (t) per hectare. The maximum proportion of the yield that is fermentable as ethanol is then 11.02 t ha^{-1}. For a plant crop of sugarcane, the potential biomass yield is 8491 g m^{-2} or 84.91 t ha^{-1} and the fermentable yield of sucrose is 42.45 t ha^{-1}. These values for potential yield of corn and sugarcane are in the range of observed maximum yields realized in the U.S. and Brazil respectively (Sinclair, 2009). The realized yields of sugarcane in Brazil are closely approaching the calculated maximum potential yield of 84.91 t ha^{-1}, but U.S. corn still has a fairly wide margin before average annual yields draw near to the theoretical solar maximum (FAOSTAT, 2010).

Thus, Brazilian cane must intercept radiation with superior efficiency and subsequently use this radiation effectively to build biomass. To push cane yields even higher, breeding programs would need to focus on the longevity of green leaf duration by delaying trashing as well as improving the inherent ability of the cane to utilize radiation to produce each unit of biomass. Alternatively, cane can be managed to have more tillers and increase the density of cane being grown in any one hectare to further maximize the utility of solar radiation on each hectare of land and increase yields.

However, for corn, distinct possibilities exist to enhance yield through genetic modification in conjunction with management decisions. Since RUE relates directly to the leaf N content, RUE could be improved with the enhancement of nitrogen take up efficiency. Moreover, engineering corn plants to survive under harsh competition for light and nutrients would allow corn to be planted at higher densities without perpetually increasing N fertilization rates, leading to increases in LAI and thus interception efficiency. Currently, the management of the optimal plant population density is limited by the ability of corn to compete for nutrient and water resources but not necessarily light, so further enhancements can be achieved (Gorden, 2005).

CHAPTER 3. SOIL FERTILITY AND NITROGEN FERTILIZER USE

Without nitrogen, the growth of corn and sugarcane would be fundamentally constrained, as discussed in the previous chapter. The degree to which these feedstocks depend on applied nitrogen fertilizer for achieving optimal yields defines the management of these crops at a field level. The variables underlying the amount of nitrogen applied to corn and sugarcane, such as the soil substrate and other management decisions like crop rotation, are discussed below. Ultimately, the N fertilization rate affects the potential for losses from the agroecosystem, which will be considered in the following chapter.

3.1 The Creation of Nitrogen Fertilizer

Prior to 1913, the only nitrogen available for crops, outside of biological nitrogen fixation and N fixation from lightning, came from animal and vegetable wastes, Peruvian guano, Chilean saltpeter deposits, and sal ammoniac extracted from coal (Smil, 2001; Erisman *et al.*, 2008). Then, the discovery that simple atmospheric N_2 could be fixed into ammonia by Fritz Haber and the industrialization of this process by Carl Bosch completely transformed the potential for using reactive nitrogen in cultivation. One century later, the Haber-Bosch process has become responsible for the introduction of more than 100 Tg of nitrogen each year, 85% of which is used in the creation of nitrogen fertilizers for cultivation (Galloway *et al.*, 2003). If the demand for biofuels necessitates an expansion of global crop production, then nitrogen fixed in the Haber-Bosch process for fertilizer can be expected to double output to nearly 200 Tg N per year (Erisman *et al.*, 2008). For perspective, the combustion of fossil fuels contributed 25 Tg N to the global nitrogen cycle in the year 2000 (Galloway *et al.*, 2003).

The Haber-Bosch process uses high pressure and temperature to combine atmospheric N_2 and hydrogen gas (derived from natural gas) to yield anhydrous ammonia (NH_3), and carbon dioxide is expelled as a by-product (The Fertilizer Institute, 2011). Anhydrous ammonia, containing 82% N by volume, may then be used by itself as fertilizer or in the production of other nitrogen fertilizers (Mengel, 2010). Some examples of nitrogen-based fertilizers derived from anhydrous ammonia include urea (46% N), ammonium nitrate (34% N), and urea ammonium nitrate or UAN (32% N), although many more exist (Abram and Forster, 2005).

While the nitrogen emissions and losses related to the manufacturing of N fertilizer do affect the global N-cycle as well as the energy balance of ethanol fuel, these emissions are not direct contributors to the cycling of N in the agroecosystem. As such, only the fate of the Haber-Bosch nitrogen contained in fertilizers will be considered further. The perpetual demand for nitrogen fertilizers from corn and sugarcane will require more reactive nitrogen to be introduced to the global N-cycle with each growing season, which could cause significant downstream effects, like accumulating nitrous oxide in the atmosphere or eutrophying surface waters, as corn and sugarcane acreage expands. The extent to which these feedstocks require synthetic N fertilizers will determine how much unreactive N_2 will be fixed into reactive forms by the Haber-Bosch process in the future.

3.2 United States Corn Belt Soil Characteristics

Alfisols dominate as the main soil order in the Corn Belt, and the suborders of Aqualfs and Udalfs characterize the majority of the area (Figure 5A) [NRCS, 2009].

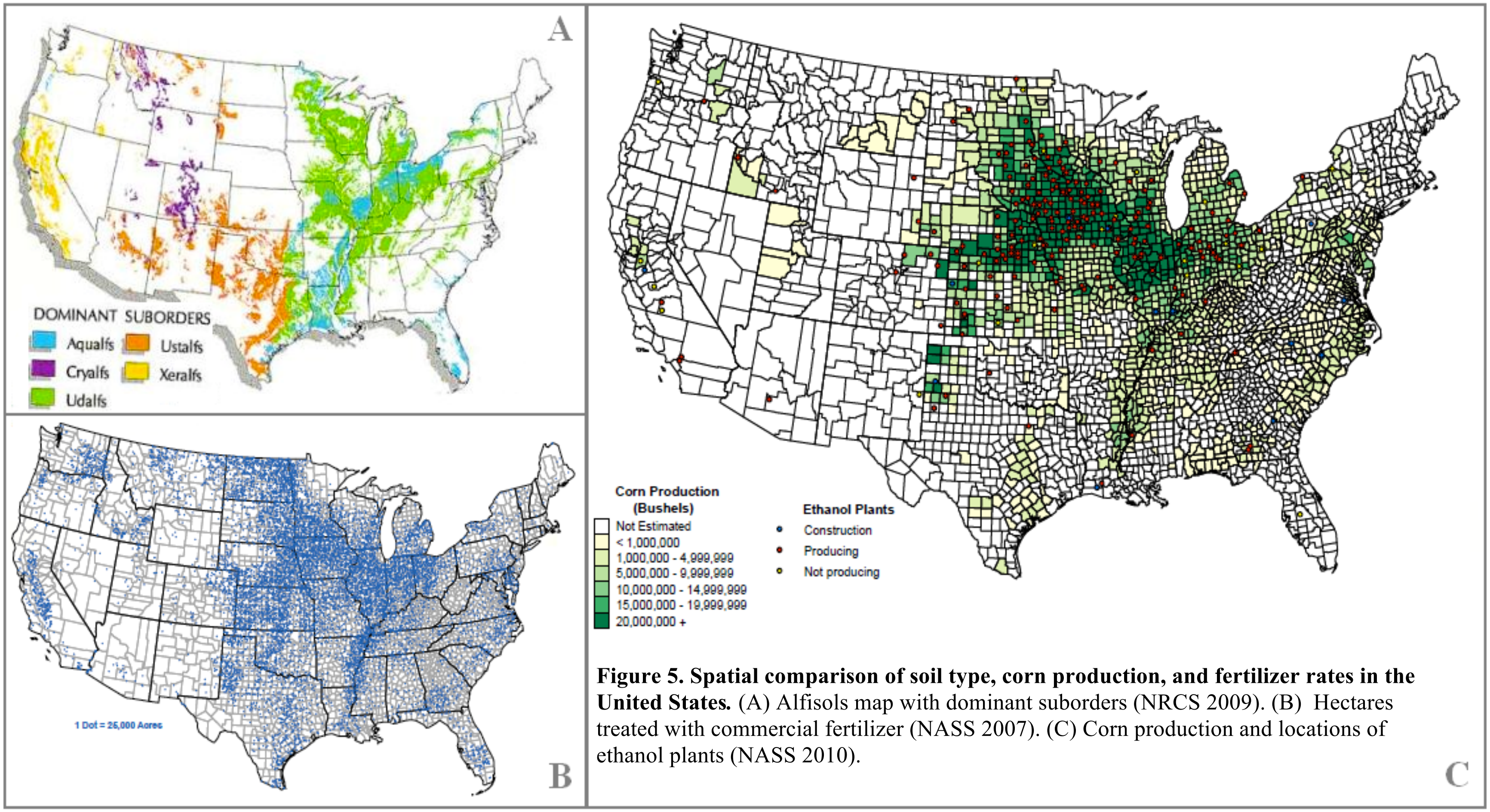

Figure 5. Spatial comparison of soil type, corn production, and fertilizer rates in the United States. (A) Alfisols map with dominant suborders (NRCS 2009). (B) Hectares treated with commercial fertilizer (NASS 2007). (C) Corn production and locations of ethanol plants (NASS 2010).

Udolls and Aquolls suborders also occupy a small portion of the region (Pierce *et al.*, 1984). Alfisols originated from historic deciduous forests and, as a prerequisite for classification, must contain an argillic horizon, which features high accumulations of clay and a high cation exchange capacity. Alfisols are also noted for their thin to non-existant O horizon and susceptibility to erosion (Grunwald, 1999). Suborders are distinguished by soil temperature and moisture. Aqualfs are the least well drained (wettest) of the suborders, usually with high groundwater tables, while Udalfs can vary significantly in their drainage but general have udic moisture regimes (NRCS, 2009). Corn requires well-drained, well-aerated soils for optimal root growth and performance, so some Corn Belt soils, especially Aqualfs, may need to be artificially drained for maximum productivity, even though this practice often necessitates higher nitrogen application rates (UN-Energy, 2007).

While Alfisols do not have a substantial humus layer in general, erosion from cultivation has stripped most all Udalf soils in the Corn Belt of their natural O horizon as well as parts of their eluvial horizon (Grunwald, 1999). Still nearly half of the land has negligible sloping of less than 2% that can help prevent erosion. While soil characteristics can vary greatly across the multiple states of the Corn Belt and among soil suborders, soils are generally deep, medium to moderately fine textured soils with loess uplands and glacial till plains occurring frequently (Pierce *et al.*, 1984). The most productive soils lay in western Ohio, central Indiana, northern Illinois and Iowa since these states are rich in soil organic matter (Jones and Durand, 1954). Since the best soils for corn cropping are well drained, deep loams or silt loams, the soil properties of the Corn Belt are superiorly suited for corn and thus explain the prevalence of corn cropping in the region (Khan *et al.*, 2005).

While indigenous soil nitrogen in the Corn Belt amounts to nearly 4000 kg N ha^{-1} in the upper 20 centimeters of the soil, mineralization rates limit availability to between 2 and 6% or 80 - 240 kg N ha^{-1} during the growing season (Galloway *et al.*, 2003; Cassman *et al.*, 2002; Bundy, 1998). Soil N varies significantly spatially and temporally based on soil type and characteristics as well as temperature and rainfall that can affect mineralization rates (Hirel *et al.*, 2005). The indigenous soil N represents a crucial N pool for corn plants over the growing season since the average N fertilizer recovery (NUE) of corn sits at about 40% of N applied (Moose and Below, 2009; Cassman *et al.*,2002).

While at the beginning of leaf development (from emergence until V8) the corn plant relies mostly on fertilizer N, nutrition from soil N begins to dominate by tasseling (VT) and far outweighs fertilizer N by harvest (Figure 6) [Sawyer *et al.*, 2006]. In the beginning of the growing season, mineralization rates are limited by cold soil temperatures, but once in full operation, microbes supply large amounts of N from the soil organic matter and

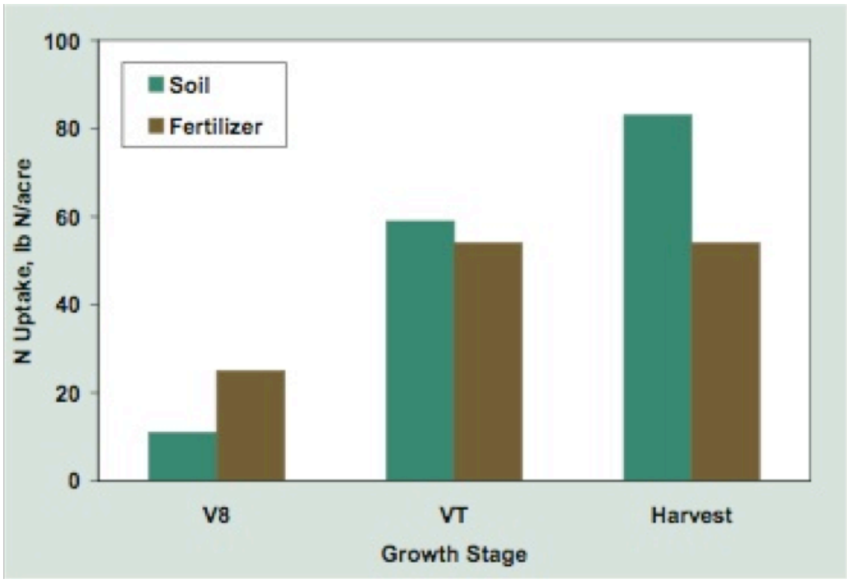

Figure 6. Corn N Take up by Growth Stage. 110 kg N ha^{-1} fertilizer applied to corn following soybeans in south-central Illinois. ^{15}N tracers used to observe take up from soil or fertilizer N. (Source: Sawyer *et al.* 2006)

immobilize some fertilizer N in the same pool. Since 60% of the nitrogen in the grain comes from N take up after VT, as discussed in Chapter 2, a large majority of grain N is supplied by indigenous soil N in addition to fertilizer N. Consequently, most of the N take up from fertilizer provides nutrition to the stalk and leaves as only 20% of grain N seems to originate from fertilizer according to ^{15}N tracers (Reddy and Reddy, 1993).

Table 2. Corn Production Practices of the Corn Belt in 2005 (Adapted from ARMS, 2005)

Practice	Specifics	Percentage of Planted Acres (%)
N Fertilization	Use of N fertilizer	97
Manure Treatment	Not treated with manure	90
	Treated with manure only	2
	Treated with manure and N fertilizer*	8
Soil N Testing	Tested for soil N*	15
	Not tested for soil N	85
Crop Rotation	Rotated with legumes*	82
	Continuous corn	18
N Application Timing	Fall Plow Down	45
	Spring Pre-Planting	69
	At Planting*	31
	Side- or Topdressing*	30
N Application Method	Incorporated into soil*	66
	No incorporation	34

Note: Methods considered ‘best management practices’ are marked with an asterisk.

The optimal soil pH for corn is 6.0-7.2, but corn has the capability to grow between 5.0 and 8.0 (Hoeft, 2010; Khan *et al.*, 2005). If corn is grown in rotation with alfalfa or soybean, the pH should be kept above 6.8 due to the sensitivity of these crops to soil acidity (Bundy, 1998). Liming is used when pH dips below 5.5 to maintain optimal pH levels in corn soils, but often corn irrigated with groundwater supplies sufficient lime to sustain the proper pH (Shapiro *et al.*, 2008). The soil pH can significantly impact the ability of the corn plant to take up nitrogen, which also implies that lower soil pH inspires greater amounts of N fertilizer to remain in the field unutilized. Of course, the spatial heterogeneity, even within one field, can affect the relative proportions of parameters like soil organic carbon, total nitrogen, and pH (Cambardella *et al*., 1994).

3.3 Nitrogen Fertilizer: Sources and Application Requirements for Corn

The United States used 12.5% of the world's nitrogen fertilizer supply in the 2006-07 growing season, 48.4% of which was dedicated to corn in the gross amount of 5.8 million metric tonnes of nitrogen (Heffer, 2009). Considering more than 85% of corn farms in the U.S. employ a high amount of fertilizer, it comes as no surprise that nearly half of all commercial fertilizer used in the United States today is applied to corn, even though corn represents less than one-third of total U.S. crop production (Groom *et al*.,2008; Christensen, 2002). The highest volume of this nitrogen fertilizer is applied in the Corn Belt nearest to ethanol production facilities, as can be seen by comparing Figure 5B and 5C. Furthermore, 97% of planted acres of corn employ N fertilizer (Table 2).

Still, states in the Corn Belt differ widely in their approaches to determining nitrogen fertilizer application rates. Illinois, Iowa, Minnesota, and Wisconsin estimate N fertilizer rates based on testing of soil nitrates with adjustments for soil N from the previous crop rotation and manure supplements. Indiana, Ohio, Michigan, and Nebraska tend to use the potential yield or yield-goal approach, assuming 1.2 pounds (0.54 kg) of N are required for each expected bushel of corn (25.4 kg of grain) in a continuous corn rotation and giving N credits for previous crops like soybeans or alfalfa and applied manure (Lauer, 2011; Shapiro *et al*., 2008). Fertilizer recommendations for corn that are based on the 1.2 lbs N bu^{-1} assumption do not include contributions of mineralized N in the soil though, since soil N testing is rarely completed under this model (Mulvaney *et* *al*., 2004; Sawyer *et al*., 2006). However, the yield-goal approach can be modified to include soil nitrate testing and soil organic matter in addition to other N credits, but the yield-goal method frequently results in over fertilization (Appendix: Figure D) [Shapiro *et al*., 2008). While soil testing can significantly reduce the chances of excessive fertilization, only 15% of soils are tested for N in a given year, leaving 85% of the area dedicated to corn prone to incorrect estimates of soil N availability and nitrogen demand (Table 2).

The majority of the N fertilizer applied in the Corn Belt is in the form of anhydrous ammonia (47% of planted acres), injected into the soil to avoid volatilization losses, with most of the remainder applied as urea-ammonium nitrate (27%) and urea (20%) [Hoeft and Peck, 1991]. Anhydrous ammonia contains 82.2% nitrogen, urea 46% N, and urea-ammonium nitrate (UCAN or UAN) between 28 and 32% N, but all form ammonium once in the soil (Mengel, 2010). Urea can be applied as granules or dissolved in irrigation water or foliar sprays, but once the urea contacts the moist soil or plants, a naturally occuring enzyme called urease begins to quickly convert the urea back to ammonia or, if H^+ ions are available in the soil, to ammonium ('Urea,' 2010). Urea-ammonium nitrate is applied as a liquid either through injections into the soil, spraying onto the soil surface, or additions to irrigation water. Since half of the nitrogen in UAN takes the form of urea, the same precautions against volatilization must be taken to avoid large losses of available N ('Urea-ammonium nitrate,' 2010). Even though large losses can occur for fertilizer left on the soil surface, 34% of the planted acres still are left in this state (Table 2).

Nitrogen may be applied at various times throughout the year including fall plow down, spring pre-planting, plant emergence, and side dressing or top dressing throughout the growing season (Lauer, 2011). The greatest amount of acreage receives nitrogen in the spring before planting (69%), followed by nitrogen incorporated during fall plow down (45%) as noted in Table 2. Unfortunately, at-planting and side-dressing treatments are the least common practices, even though these application times best meet the demand of the corn plant. For Nebraska, Lindquist *et al.* (2005) suggest 100 kg N ha^{-1} incorporated into the soil before planting with an additional 125 kg N ha^{-1} over the course of the growing season as side-dressings at V6, V10, and VT to gain maximum nitrogen use efficiency and yields.

Data on the average amount of nitrogen fertilizer applied in the Corn Belt varies greatly in the literature (Table 3). Even among farms in the same region of the Corn Belt in the same year, the deviation from the average N fertilizer rate can be up to 85 kg ha^{-1} based on timing, management practices, and soil characteristics (Cassman *et al.*, 2002). High yield corn hybrids can take up nearly 300 kg ha^{-1} of nitrogen, requiring substantially more N fertilizer to meet that demand (Sawyer *et al.*, 2006). With applications above 180 kg N ha^{-1}, leaf N content

Table 3. Average N Fertilizer Applied to Corn per Growing Season in the Corn Belt

Source	Average N Fertilizer Applied (kg N ha^{-1})	Region
Lindquist *et al.* (2005)	225	Nebraska
Lauer (2011)	180	Corn Belt
Hoeft and Peck (1991)	170	East Corn Belt
	145	West Corn Belt
ARMS (2005)	165	Corn Belt
Sinclair (2009)	155	United States
Moose and Below (2009)	150	United States
McSwiney and Robertson (2005)	130	Michigan
Average	**165**	**Corn Belt**

generally reaches a maximum, far above critical N levels, suggesting sufficient N availability at these rates (Schlegel and Havlin, 1995; Plenet and Lemaire, 2000). An average of cited literature values (Table 3) produces a N fertilizer application rate of 165 kg N ha^{-1}. This aggregated average of 165 kg N ha^{-1} is in close proximity to the suggested sufficient N value above and matches the documented value for real usage on corn fields by the U.S. Department of Agriculture, justifying its use as the baseline assumption for N application rates throughout this paper (ARMS, 2005).

Of course, yields may be optimized at significantly lower N levels with careful nutrient management and selection of hybrids suited for the growing environment, such as in southwest Michigan where 101 to 132 kg N ha^{-1} has been shown to optimize yields for the region (McSwiney and Robertson, 2005). Alternatively, nitrogen application rates can be excessive if fertilizer cost is not limiting and mitigating N losses is not a concern, as documented by Lindquist *et al.* (2005). Other studies specifically conducted in the Corn Belt region fall within the boundaries of these two studies, and five robust studies are listed in the table for comparison. At the absolute minimum, every hectare of corn must receive nitrogen applied at rates above 50 kg N ha^{-1} for the growing season, since even this amount is considered insufficient for corn crops and would lead to severe nitrogen deficiencies and thus grain yield reductions (Hirel *et al*., 2005; Uhart and Andrade, 1995).

Besides commercial fertilizers, nitrogen can also be supplied from manures, mineralization of soil organic matter and residual crop residues, and previous leguminous crops (O'Leary *et al*., 1994). When manures are used as fertilizer (10% of planted acres [Table 2]), five to ten tons per acre are usually spread, but the amount of N afforded by manure depends on the source, such as poultry or swine (Lauer, 2011). Calculating the contributions of N to the soil from mineralization is far more convoluted than documenting available nitrogen in manures though. The prediction of N supplied by soil mineralization is complicated by the fact that soils differ spatial and temporally, and different environmental conditions like soil pH and aeration, temperature, and precipitation can hugely influence the levels of microbial activity; because of these complications, the amount of N potentially contributed by mineralization of residues and soil organic matter often goes unaccounted when determining N fertilizer application rates in the Corn Belt (Sawyer *et al*., 2006). When mineralization is taken into account, most fertilizer recommendations assume 2% indigenous soil N, the lowest end of the range as discussed in the previous section (Bundy, 1998). Credits to previous crop rotation and manure integration are most always considered though, and soil N testing, which remains uncommon in the Corn Belt, is meant to account for the net mineralization prior to fertilizer application.

The previous crop, whether soybean or corn as typically seen in the Corn Belt, significantly affects the amount of N fertilizer required for optimum yield though (Table 2). While up to 140 kg N ha^{-1} may remain in the residues of corn left in the field after harvest, this residue has a higher C:N ratio than residue from soybeans (70:1 versus 40:1), both of which exceed the ideal microbial C:N ratio of 25:1 (Erickson and Carr, 2009; Chapping *et al*., 2002). The lower C:N ratio of soybean residues allows microorganisms to more quickly release N into the soil than with corn residues and leads to more available ammonium and nitrate in the soil after a rotation with soybeans than with continuous corn. Thus, after soybeans the amount of N fertilizer required for corn is less than continually planting corn. On average, after soybeans, if no fertilizer were applied, yields would reach 70% of those with N fertilizer, but after corn, yields would only reach 56% (Sawyer *et al*., 2006). Generally, for Corn Belt states, continuous corn rotations require an additional 45 to 55 kg N ha^{-1} compared to corn following soybean (Sawyer and Nafziger, 2005). Even then, yields from corn following soybeans are commonly 10 to 15% higher than continuous corn rotations (Figure 7) [Nafziger *et al*., 2003; Jagadamma *et al*., 2008; Hicks and Thomison, 2004]. Even though rotations with soybeans result in higher yields and less N fertilizer use, the pressure to supply enough corn to meet rising ethanol demand has forced a large portion of farmers into continuous corn cropping (Coulter *et al*., 2009).

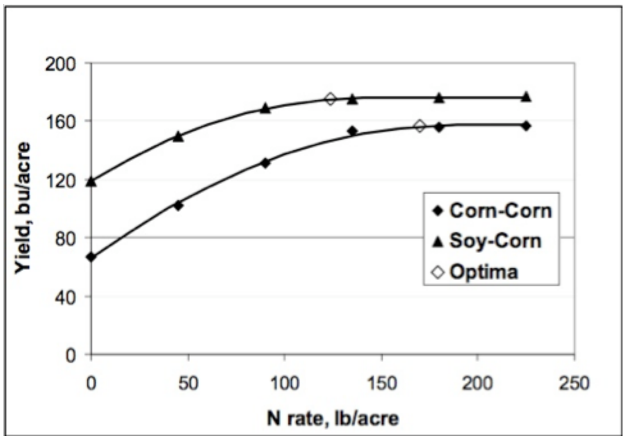

Figure 7. Effect of Crop Rotation on N Fertilizer Response and Yield. Averages for 20 sites in Illinois from 1999-2003. Optima based on N:corn price ratio of 0.1. (Source: Nafziger *et al.* 2003).

For determining conservative nitrogen fertilizer rates after leguminous crops, soybeans can be credited 45 kg N ha^{-1}, and alfalfa may be credited between 100 and 170 kg N ha^{-1} based on the hardiness of the crop in non-sandy soils; soybeans do not receive any N credits for the next season if grown in sandy soils, and the credits for alfalfa drop to between 45 and 110 kg N ha^{-1} (Bundy, 1998). Less conservative approaches to legume crediting may offer up to 35 kg N ha^{-1} for soybeans grown in sandy soils, if the soybean yield is greater than 75 bu ha^{-1} (Shapiro *et al*., 2008). Manure credits vary drastically by manure source and type (solid or liquid) applied, but rarely is N fertilizer applied in conjunction with manure, so credits are mostly irrelevant (Table 2) [Bundy, 1998].

Despite the results for N recommendations from prior credits and soil testing, the rate of nitrogen application still may be constrained by cost. Nitrogen typically represents the greatest fertilizer cost, and sometimes even one of the highest operational costs, for growing corn. To maximize profits, farmers consider the maximum return to nitrogen (MRTN) or the N application rate where the value of the corn grain minus the cost of N fertilization is greatest (Sawyer and Nafziger, 2005). Thus, even if yield could be increased further by higher N application rates, there would be no economic incentive to do so based on the price received for grain and price paid for fertilizer. The N fertilization rate when considering MRTN may be less than the amount required to maximize yield, but if corn prices are too low or nitrogen prices too high, maximizing the yield per hectare may not remain economically viable.

Agronomists in Iowa, Illinois, Minnesota, Ohio, and Wisconsin have all noted the importance of the relationship between the price of nitrogen in fertilizer (cost per pound N) and the selling price of a bushel of corn (N:corn price ratio) in regulating the N application rates in corn fields (Sawery *et al*., 2006). In Wisconsin, a low price ratio (0.05) results in N recommendations of 190 kg N ha^{-1} on high yield soils and 240 kg N ha^{-1} on sandy, irrigated soils; yet, a higher price ratio (0.20) reduces the N recommendations to 130 kg N ha^{-1} and 200 kg N ha^{-1} for the respective soil types (Nutrient and Pest Management staff, 2010). Generally, for the Corn Belt, application rates from anywhere between 110 and 270 kg N ha^{-1} can provide the maximum economic return for nitrogen applied (Sawyer *et al*., 2006).

Ultimately, the amount of fertilizer applied on corn acreage dedicated to ethanol contributes directly to the nitrogen use in biofuel production. Considering the physiology of the corn plant in assimilating N and the impact of nitrogen on yield, nitrogen will remain imperative to sustaining corn production for ethanol, even where overall energy efficiency in production can be improved. The yield in bushels per acre (or kilograms per hectare) harvested each year determines the amount of acres necessary to meet ethanol demand (Table 4). Increases in yield through NUE could potentially slow the growth of actual harvested acreage in ethanol production and lower fertilizer usage, but ethanol demand will more likely outstrip any gains realized in yield or reductions in N fertilizer by consuming upwards of 36% of harvested acres by 2016.

Table 4. Corn Yield, Production Area, and Total Fertilization for 2008/09. (Adapted from: USDA, 2010)

Yield (bu acre^{-1})	153.9
Yield (kg ha^{-1})	9653
Harvested Area (million ha)	31.8
Ethanol production (million ha)	9.67
Ethanol production as percentage of total harvested area (%)	30.4
Total N fertilizer applied to corn in ethanol production* (Tg N)	1.6

*Note: Calculated value based on average N fertilizer rate of 165 kg N ha^{-1} as calculated in Table 3 and the hectares harvested for ethanol production. Average N fertilizer rate used to provide a conservative estimate of total N applied to corn in ethanol.

The most reliable information available for both corn and sugarcane occurs for the 2008-2009 growing season, so this season will be used as the reference point to compare yield and nitrogen use. The U.S. Department of Agriculture has not been able to correctly project ethanol production, since reports in 2009 and 2010 do not anticipate ethanol production surpassing the 5 billion bushels mark until after 2017, but already the latest report suggests that the market will have surpassed this point by 2012 (USDA, 2010; USDA, 2011). As such, to avoid introducing additional errors by using projected data and to provide a solid base for comparison between feedstocks, the 2008/09 growing season data will be primarily considered. Bearing in mind that nitrogen use per acre has decreased only 6% over the last twenty-five years (0.24% per year), the results may be reasonably extrapolated to the future without revisiting fertilizer use statistics (Erickson and Carr, 2009).

A yield of 9653 kg ha^{-1} would require an take up of 180 kg N ha^{-1} according to the relationship between yield and take up outlined in Cassman *et al.* (2002). Cassman *et al.* also suggest that 115 kg N ha^{-1} would likely come from indigenous soil N, which is well within the range for soil N in the Corn Belt. This would leave 65 kg N ha^{-1} to be supplied by N fertilizer. Assuming a fertilizer recovery rate of 40%, as described previously, about 165 kg N ha^{-1} would need to be applied to meet crop N demand, matching the calculated average fertilizer rate from Table 3. Thus, with 9.67 million hectares in ethanol production and an average of 165 kg N applied to each of those hectares, approximately 1.6 Tg N was used to create the corn feedstock for ethanol produced in 2009. The subsequent cycling of this large sum of nitrogen in the agroecosystem will be considered in the next chapter.

3.4 Effect of Nitrogen on Corn Grain Yield

When considering the conversion of corn into ethanol, grain yield characterizes a significant determinant of ethanol volume per hectare of corn. Also, farmers increase profits per hectare if grain yield per hectare rises, since they are paid by the dry weight of their crop (Ream *et al*., 2010). Thus, the effect of nitrogen on grain yield must be carefully examined since potential yield drives major decisions within the agroecosystem.

Until the early 1950s, corn yield improvements had been made through advancements in engineering hybrids, but from the 1950s onward, yield enhancements have been made through the addition of N fertilizers (Jeffe and Miguel, 1994). Total potential grain yield may be approximated by multiplying the number of plants per hectare (plant population density), ears per plant, grains per ear, and weight per grain (Banzinger *et al*., 2000). Examining the impact of nitrogen fertilization on these yield components disaggregates the known phenomenon that grain yield increases with nitrogen application rates and allows a more directed focus for understanding how nitrogen affects grain yield and thus potential ethanol yield.

The optimal plant population density (PPD) will maximize the leaf area per square meter of ground area (leaf area index), leading to greater photosynthetic capacity and reduced evaporative losses from the soil while only marginally increasing transpiration (Smith *et al*., 1994). Mainly, the type of hybrid determines ears per plant, but lower plant population densities can increase the probability of prolific plants with more than one ear (Ritchie *et al.*, 1993; Uhart and Andrade, 1995). However, the optimal PPD will maximize yield for the entire field, even if individual plants do not produce as many ears or as much grain as when provided unlimited space (Lyon, 2009). The type of hybrid will determine the plant density, with earlier maturing hybrids obtaining greater yields with higher plant population densities (Rankin, 1991). Generally, optimal PPD for the central Corn Belt is around 75,000 plants per hectare for early maturing hybrids and 64,000 plants per hectare for late maturing hybrids (Lyon, 2009). Closer row spacing in zig-zag twin rows (diamond formation) may even enhance yields more so than plant population density augmentation alone due to higher LAI achieved with zig-zag twin rows instead of single, straight rows (Shakarami and Rafiee, 2009). The ability of plants to maximize yields at higher plant population densities relates mainly to the capacity of plants to survive under increasing levels of water, nutrient, and light stress, which has been achieved mostly through genetic modification of corn hybrids; thus, the effect of PPD on yield operates in parallel to nitrogen fertilization rates since the optimal PPD is meant to maximize available resources (Blumenthal *et al.*, 2003).

The main contribution of nitrogen fertilization relates to the number of kernel grains per ear and the weight per grain. Nitrogen-induced increases in overall kernel number are primarily a result of reducing kernel abortion rather than increasing potential kernel ovules with increasing applications of nitrogen (Figure 8) [Below, 1997). Thus, nitrogen fertilization specifically impacts yield by augmenting kernel number via the avoidance of kernel abortion. To put kernel abortion into perspective, approximately one bushel per acre is lost for every three kernels aborted per ear (Kent, 2010). Weight per kernel also slightly increases with increasing nitrogen application, but nitrogen fertilization affects kernel number far more than kernel weight (Figure 8) [Below, 1997; Gallais and Hirel,

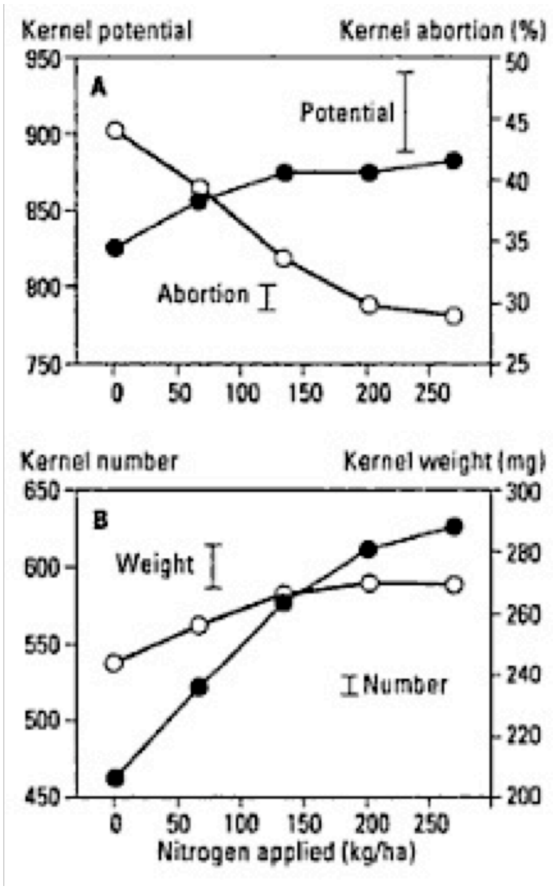

Figure 8. Effect of nitrogen application rate on kernel development. (Source: Below, 1997)

2004; Uhart and Andrade, 1995]. However, high leaf nitrate content has been shown to have a higher correlation with kernel weight than kernel number, implying that yield could be improved through kernel weight enhancement in hybrids capable of storing additional nitrate from fertilizer in their leaves (Hirel *et al*., 2001).

The timing of soil nitrogen availability, and thus the timing of N fertilization, strongly affects the impact of nitrogen on grain yield though. The corn plant begins rapidly absorbing N at the V6 stage. Yet, the largest yield reductions will result from water or nutrient stress bracketing silking (V15 to R2), since stress during this time will cause a lag between pollen shed from the tassels and silk elongation to the end of the year; any silks that emerge after pollen shed has finished will not be fertilized, and the kernel will not be filled (Ritchie *et al*., 1993). Additionally, nitrogen stress before tasseling decreases leaf area development and photosynthesis, which creates a source limitation and affects the potential number of kernel ovules (Banzinger *et al*., 2000). Similarly, nitrogen take up at silking determines the kernel abortion rate, directly moderating the ultimate kernel number (Gallais and Hirel, 2004). Since a high demand for nitrogen exists within the endosperm just after pollination of the silks, nitrogen deficiency post-tasseling can result in depressed growth rates and abrupt kernel abortion just before steady grain fill (R2), reducing the number of filled kernels by up to 20% (Uhart and Andrade, 1995; Below, 1997). Later in the grain filling period (R2 to R4), nitrogen supplies can affect kernel weight, since insufficient nitrogen may accelerate leaf senescence, shortening the duration of time C and N assimilates are available for remobilization to the kernel (Banzinger *et al*., 2000).

In regards to kernel weight, the activity of many enzymes involved in the metabolism of carbon, including starch synthase and ADPG-PPase, increases up to 2.5-fold with enhanced N supply (Singletary *et al*., 1990). Thus, nitrogen delivered to developing kernels enhances the capacity of the endosperm to synthesize proteins and starch via its impact on the controlling enzymes in those processes. The increases in kernel weight with higher levels of N fertilization may then be attributed, in part, to the positive response of kernel metabolism to N supply.

Nitrogen supply therefore remains a critical determinant of grain yield in corn due to its effect primarily on kernel number, and to a lesser extent, kernel weight. The V15 stage (about two weeks before tasseling) until the R2 stage (about two weeks after silking) represents the most imperative time for sufficient nitrogen supply in order to realize the highest yields. Some studies even suggest that nitrogen deficiencies prior to these two week period bracketing tasseling have little impact on yield as long as nitrogen is available during this crucial period (Uhart and Andrade, 1995).

3.5 Effects of Soil Nutrient Interactions on Corn Growth

While nitrogen represents the most important, and usually most limiting, nutrient in corn development, maximum yields cannot be achieved without sufficient phosphorus (P) and potassium (K). The rates of assimilation of P and K are fairly consistent throughout the growing season, but their durations of take up differ (Oaks, 1994). The take up of K essentially ceases soon after silking, but the take up of N and P can continue until grain maturity, mostly following the trend in the accumulation of biomass (Hoeft, 2010; Alley *et al*., 2009). Because of this trend, most of the N and P resides in the grain at harvest and is removed from the field while most of the K remains in the stover and returns to the soil (Ritchie *et al*., 1993). P and K are relatively immobile in the soil profile though, so most all of the remaining P and K in the soil after harvest will be available in the next growing season. The low leaching and erosion potential of P and K allow these nutrients to be applied any time during the year, unless applied on sandy soils, in which case the possibility exists that K may be leached out of the rooting zone and thus should be applied in the spring (Hoeft, 2010). The effects of N with P and K supplied in unison are often additive or at least synergistic.

Similar to nitrogen, phosphorus is taken up most intensely prior to tasseling until just after silking, and heightened take up begins when leaves start to quickly develop around the V10 stage (Skowronska and Filipek, 2010). The ability of the plant to take up P is directly related to nitrogen nutrition and vise versa. For example, ammonium-based fertilizers aid in an amplified take up of phosphorus more so than nitrate fertilization (Hoffmann *et al*., 1994). Alternatively, without phosphorus, leaves senesce more quickly to meet the N demand in younger tissues since N take up is restricted without sufficient soil P (Usuda, 1995). P deficiency severely reduces leaf growth, hindering the amount of intercepted PAR but not necessarily RUE (Plenet *et al*., 2000). Sufficient P, and also K, can then lengthen the green leaf and thus expand the grain filling period (Egli, 2004).

Furthermore, nitrogen interacts with phosphorus such that both grain yield and nitrogen use efficiency are substantially improved when the nutrients are supplied together instead of either applied alone (Aulakh and Malhi, 2005. For instance, when P is applied in conjunction with at least 135 kg N ha^{-1}, increased yields of up to 245 bushels per hectare may be realized over independent nitrogen or phosphorus fertilization regimes (Schlegel, 1995). Fertilizer recovery rates, as seen in the grain N, are twice as high with joint N-P fertilization as well (Schlegel and Havlin, 1995). As plant phosphorus subsequently affects the take up of nitrogen, the amounts of total reduced N, chlorophyll, and N proteins in the stalk and leaves depend on the P status of the plant (Usuda, 1995). Phosphorus fertilization directly enhances plant biomass accumulation through augmenting the number of rows and grains per cob and the grain weight; since the plant height also increases with P nutrition, the harvest index remains the same for all rates of P fertilization (Sawyer *et al*., 2006). However, a wide range of values exists where the N and P balance enhances yield, indicating that the boost in plant biomass accumulation from P depends heavily on the other macro and micro nutrients in the plant as well (Dumenil, 1961).

The addition of potassium (K) generally enhances the N x P benefits, mainly by increasing the take up of P, but interannual variability with K additions limits the predictability of the effectiveness of the nutrient; one year K may help significantly boost grain yield in combination with N and P while the next year additions of K have no effect on yield (Aulakh and Malhi, 2005). The interannual variability in K fertilizer effectiveness most likely results from residual K in the soil from previous years, since K fertilizer has little effect on the take up of N and P when soil K levels are already high (Skowronska and Filipek, 2010). Additionally, K functions as a growth stimulator by increasing protein production rates and improving water use efficiency.

Phosphorus and potassium are supplied in lesser amounts than nitrogen and the amounts are determined via soil testing (Alley *et al*., 2009). Corn plants only use between 10 and 20% of the P applied each growing season since mineralization rates of P are very slow, and the amount of K available in the next growing season depends on rainfall and soil type since K can be leached below the root zone with high levels of precipitation in sandy soils (Lauer, 2011). Generally, phosphorus fertilization rates fall within 45 – 80 kg P ha^{-1} and potassium within 85 – 110 kg K ha^{-1} (Lindquist *et al*., 2005; ARMS, 2005). P and K rates are almost always less than N fertilizer amounts, but this rule depends on the soil type and quality. Unfortunately, the lack of soil testing, insufficient past supplies of P and K, and erosion in the Corn Belt have lead to a universal decrease in P and, to a lesser extent, K across soils of the Corn Belt, reducing overall soil fertility (Fixen, 2010). As corn crops further mine P and K from the soil, fertilizer rates of these two nutrients must increase to replenish the soil and support nitrogen use efficiency and yield.

3.6 Brazilian Soil Characteristics

Sugarcane can thrive on most all soil textures from sandy to heavy clay soils, but soils must be well drained and deep with a bulk density that can support a water holding capacity[2] of at least 15% (UN-Energy, 2007). São Paulo State in the southeast and the northeastern coast of Brazil represent the two main sugarcane production areas (Figure 9). Approximately 90% of ethanol production occurs in the Center-South region of Brazil, with 60% of production in the southeastern district of São Paulo; the remaining 10% of cane for ethanol is grown in the Northeast but yields are generally much lower due to hilly terrain and poorer soils (Bolling and Suarex, 2001; Hartemink, 2008). Despite reduced yields, cane harvested from the northeastern region remains critical to the ethanol industry since the harvest season from September through April in the

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[2] Defined as the amount of water able to be held in the pore spaces of the soil against gravity

Northeast helps ease the ethanol supply gap while awaiting the harvest of cane in the Center-South that occurs from May to November (Bolling and Suarex, 2001).

Figure 9. Major Cane Growing Areas in Brazil. (Source: Bolling and Suarex, 2001)

In the major sugarcane growing regions of São Paulo State as well as the northeastern region of Brazil, soils are characterized by low activity clay (LAC) or clayey soils with low cation-exchange capacity (Appendix: Figure E) [Bernoux *et al.*, 2000). These LAC soils are mainly Latossolos (equivalent to Oxisols in U.S. soil taxonomy or Ferrasols in general agronomic terms). Both of these soils are deep to very deep, well drained due to high permeability, and contain a stable micro-structure that reduces erosion rates. The kaolinite clay texture leads to an accumulation of iron and aluminium oxides though, which may reach toxic levels frequently, and the low cation exchange capacity of the clay allows nutrient reserves to becomes easily exhausted (de Faccio Cavalho, 2007). Additionally, sugarcane nutrient demand often depletes the soil nutrient reserves, especially potassium and, to a lesser extent, nitrogen (Naumov and Prado, 2008).

Nearly all soils in tropical South America are prone to acidic conditions, which can inhibit root growth, reduce take up of nutrients, and lower biological nitrogen fixation in the rhizosphere (Correa *et al.*, 2001). Additionally, acidification of sugarcane soils has been reported in conjunction with the application of ammonium N fertilizer, with associated nitrification causing acidification (Silva *et al.*, 2005). However, the proliferation of soil microbes with fertilization may overcome the detriment caused by lower pH by increasing mineralization rates of N (Graham *et al.*, 2002). The optimal soil pH is approximately 6.5, but cane can tolerate high levels of both acidity and alkalinity in the soil ranging from pH 5 to 8.5; toxic acidity can be corrected with liming while alkalinity can be controlled with gypsum applications (Rao and Vered, 2011).

The high yielding capacity of São Paulo State results primarily from the highly suitable soils found in this region. Deep, clayey, well-drained soils that are flat and high in fertility are considered excellent soils for sugarcane cultivation. Soil aptitude for cane declines to good or regular as soils become shallower, sandier, erodible, and less well drained (Rao and Vered, 2011). Underlying the main sugarcane plantations of São Paulo are soils with either good or excellent ratings for their ability to support sugarcane growth, most of which are Oxisol soils (Figure 10) [Marin *et al.*, 2008]. The efficiency of water and nutrient take up is influenced by the quality of the soil, and Marin *et al.* (2008) contend that soil aptitude for cane growing explains between 36 and 44% of the efficiency in reaching potential maximum yields as influenced by water and nutrient status of the crop. As such, growing sugarcane in soils with optimal characteristics may prove more important to maximizing yield than even solar radiation or annual rainfall.

The highly porous nature of the deep, well-drained soils of São Paulo also leaves the soil susceptible to both surface and subsurface compaction. Soil compaction can severely limit water and nutrient take up of sugarcane, and the current transition in Brazil from manual to mechanical harvesting causes concern for degrading cane soils through compaction from machinery, especially those soils that can withstand little pressure without additional compaction (Silva *et al.*, 2005). Similarly, burning sugarcane before harvesting, as typically practiced in Brazil, increases topsoil bulk density by reducing the fraction of water stable aggregates and also creates impermeable surface crusting (Graham *et al.*, 2002). Additionally, each successive ratoon crop experiences soil with a higher bulk density than the original planting, since the soil is normally tilled prior to the first planting (Hartemink, 2008). Because increasing bulk density often reduces water intake of sugarcane, maintaining a sufficient bulk density around 1.2 g cm^{-3} for water availability is crucial for sugarcane sett germination and growth as well as optimal

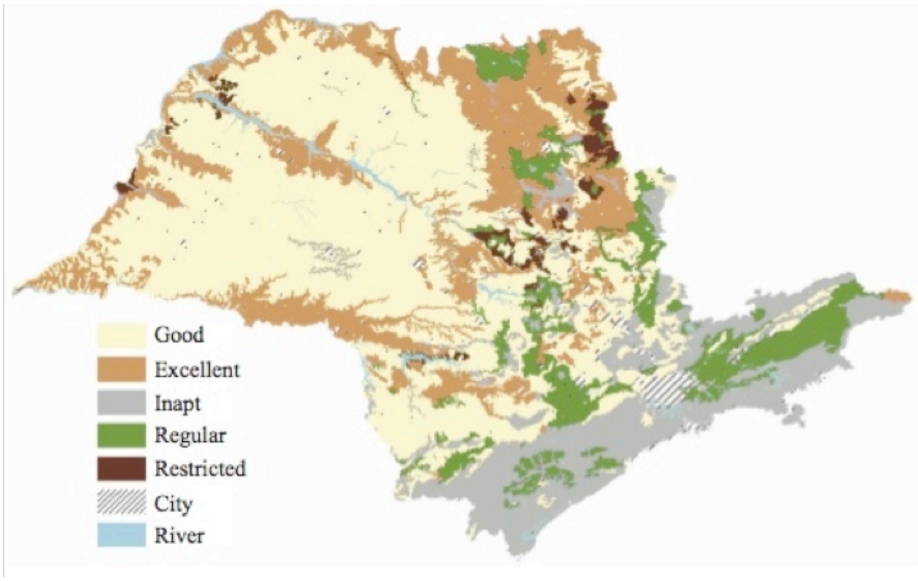


Figure 10. Soil aptitude for sugarcane in São Paulo, Brazil. (Marin *et al.,* 2008)

biological nitrogen fixation (Boddey *et al.*, 2003). Yet, compaction and subsequent sub-surface hard pans are difficult to correct once established (Rao and Vered, 2011). However, the elimination of trash burning (burning of dead leaves on the stalk and ground) can increase the productive longevity of cane fields from five years to seven or eight years, and the additional decomposition of trash left in the field can further increase longevity to twelve or fifteen years (Saidak, 2011).

The fertility added by the decomposing trash derives from the contribution of organic carbon to the soil. In tropical soils, the soil organic carbon content significantly impacts soil productivity and loss of significant amounts of organic C renders the soil unusable for cane cropping (Hartemink, 2008). In all types of Brazilian soils, sugarcane cultivation reduces organic soil C pools to nearly half the amount found in uncultivated soils and pH decreases significantly over time (Caron *et al.*, 1996; Cerri and Andreaux, 1990). However, the additions of filter cake, vinasse, and synthetic fertilizers greatly enhance the amount of organic C contributed to the soil by the sugarcane crop, especially where trash remains in the fields after harvest (Silva *et al.*, 2007). Amounts of N, P, and K in the soils generally depend directly on the fertilizer management practices (Hartemink, 2008). but the amount of residual nitrogen in the soils of São Paulo State are considerably high, with estimates reaching above 7600 kg N ha^{-1} (de Oliveira *et al.*, 2000).

3.7 Balancing BNF and Nitrogen Fertilization for Sugarcane

For over 100 years, some areas of Brazil have been continuously cultivating sugarcane without employing any nitrogen fertilizers, perhaps leaving a niche in which N-fixing bacteria could adapt and prosper (Neyra and Dobereiner, 1977). In fact, the majority of Brazilian sugarcane varieties show little or no yield response to nitrogen fertilization since nitrogen nutrition from synthetic fertilizers increases at the direct expense of biological nitrogen fixation (Reis *et al.*, 2000; Azeredo *et al.*, 1986).

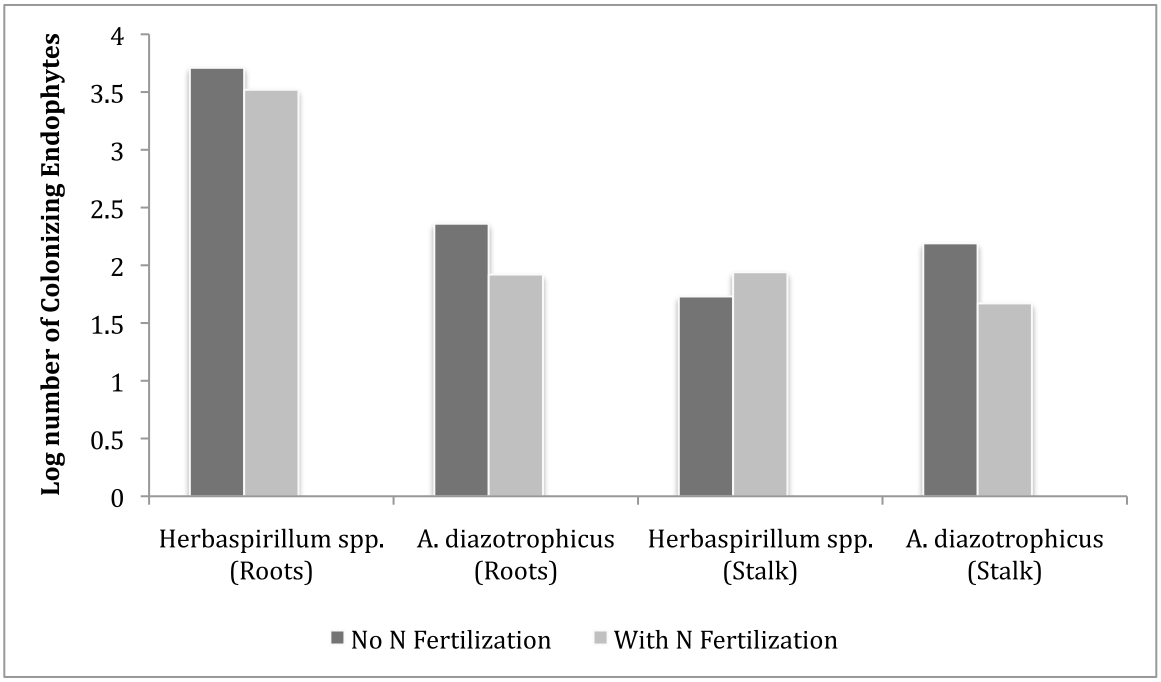


Figure 11. Interaction between N-Fixing Endophytes and N Fertilization. *Herbaspirillum* spp. and *A. diazotrophicus* were sampled from the Brazilian sugarcane cultivar SP 79-2312. Cane with N fertilization received 300 kg N ha^{-1}. Adapted from Baldani *et al.* (2002).[446]

Populations of dominant N-fixing bacteria associated with cane exhibit sensitivity to synthetic N fertilization; in particular, colonization by *A. diazotrophicus* decreases significantly with higher rates of N application (Figure 11) [Reis *et al.*, 2000; Fuentes-Ramirez *et al.*, 1999; Baldani *et al.*, 2002).

In addition to high concentrations of nitrogen fertilizer, extremes in soil temperature, moisture and pH or lack of micronutrients like iron and molybdenum can inhibit biological nitrogen fixation (Smil, 1999). BNF rates are highest in soils supplied with minimal N fertilizers and where P, Fe, and Mo are adequate (Hartemink, 2008). If BNF alone cannot meet the N demands of the plant, the plant will take up additional N from the soil.

Multiple studies report that 50% or more of the total N assimilated by sugarcane is obtained from BNF, representing an average of 90 kilograms of nitrogen fixed per hectare of cane harvested each season (Table 5). In the 2008/09 growing season, an approximate 0.391 Tg N was fixed by Brazilian sugarcane harvested specifically for ethanol (Table 6), which accounts for nearly 1.2% of the global total of human-induced biological nitrogen fixation (Galloway and Cowling, 2002). In cane varieties known to utilize BNF for over 60% of their N nutrition though, the optimization of P, K, and water supplies could render an opportunity to discontinue N fertilization for at least the plant cane and first two ratoons (Boddey *et al*., 1991). Still, ratoon crops may benefit from N applications if the previous crop has diminished soil N reserves, but even

Table 5. Total N Assimilated and Average N Fertilizer Applied to Sugarcane per Growing Season in Brazil

Source	**Total N Assimilated (kg N ha^{-1})**	**N from BNF (kg N ha^{-1})**
de Oliveria *et al.* (2000)	100 – 120	--
Bakker (1999)	200	--
Boddey *et al.* (1995)	100 - 200	60 - 120
Lima *et al.* (1987)	180	90
Average	**160**	**90**
Source	**Average Nitrogen Applied (kg N ha^{-1})**	
	Plant Cane	**Ratoon Cane**
Baldini *et al.* (2002)	50	100
Boddey *et al.* (1995)	60	100
Hartemink (2008)	50	--
Macedo *et al.* (2008)	50	80
Malavolta (1994)	65	80
Average	**55**	**90**

under these conditions, fertilizer use efficiency in Brazilian cane varieties remains low (Baldini *et al*., 2002).

On average at harvest, the total N in ratoon cane derived from N fertilizers fluctuates around 20% while fertilizers contribute less than 10% of total N in plant cane (Franco *et al*., 2011; de Oliveira *et al*., 2000). Despite ratoon crops taking up and benefiting from enhanced rates of N fertilization, fertilizer use efficiency averages less than 35% and at best reaches 60% of applied N due to fluctuating plant N demands (Boddey *et al*., 1995; de Oliveira *et al*., 2000). For instance, various levels of N fertilization can create different sucrose yield responses in the same cultivar; thus, for each cane varietal, an optimal fertilization rate exists that allows the plant to establish a leaf area index that promotes the greatest amount of growth without reducing the amount of sucrose in the cane juice (Franco *et al.*, 2011; Ashraf *et al.*, 2008). Yet, this optimal rate can range from zero if fertilization interferes with BNF to 100 kg N ha^{-1} in ratoon cane of the same varietal (Rosario and Chantha, 2001). Optimal N fertilization rates also vary between cultivars as cultivars differ in their ability to obtain N from BNF and soil and fertilizer N (Lima *et al.*, 1987).

With the diversity of cane cultivars complicating fertilizer management, the largest sugar and ethanol company in Brazil, Copersucar, suggests 50 kg N ha^{-1} for plant cane and 100 kg N ha^{-1} for ratoon cane each growing season, noting that higher doses of nitrogen fertilizer could negatively affect their profits by reducing sucrose yield (Inoue *et al.* 2009). Averages of applied N from multiple studies conducted in Brazil hover near Copersucar's recommendation level with 55 kg N ha^{-1} for plant cane and 90 kg N ha^{-1} for ratoon crops (Table 5). Considering these average N application rates, 0.360 Tg N would have been applied to areas harvested for ethanol production in the 2008/09 growing season (Table 6). Providing validation for this estimate, the International Fertilizer Industry Association accounted that Brazil consumed 2.4% of the world's nitrogen supply in 2008, with 23.3% of this nitrogen having been used for sugar crops (beet and cane), amounting to about half a million metric tonnes of nitrogen (Heffer, 2009). Combining total N fertilizer use with BNF, the production of sugarcane in Brazil for ethanol in the 2008/09 growing season has added an estimated 0.751 Tg of nitrogen to the agricultural landscape.

Table 6. Sugarcane Yield, Production Area, and N Use for 2008/09.
(Adapted from: Brazil Ministry of Agriculture, Livestock, and Food Supply, 2009)

Biomass Yield (ton ha^{1}) [a]	77.52
Sugar Yield (kg ton^{-1})	55.60
Harvested Area (million ha)	8.14
Ethanol production (million ha)	4.34
Ethanol production as percentage of total harvested area (%)	53
Total N fertilizer applied to cane in ethanol production (Tg N) [b]	0.360
Total N fixed by cane in ethanol production (Tg N) [c]	0.391

[a] Combines plant and ratoon crops harvested for the year
[b] Calculated value based on weighted average N fertilizer rate of 55 kg N ha^{-1} for plant cane and 90 kg N ha^{-1} for ratoon cane (Table 5), where 20% of the harvested hectares in ethanol production are plant cane and 80% are ratoon cane based on the average ratooning cycle of 5 years.
[c] Calculated value based on the average BNF rate of 90 kg N ha^{-1} derived in Table 5 and the number of harvested hectares in ethanol production.

3.8 Role of N-P-K in Sucrose Accumulation

Potassium is often highlighted as the main nutrient responsible for sucrose accumulation since nitrogen affects stalk weight more than sucrose yield (Muchovej and Newman, 2004). However, nitrogen contributes indirectly to sucrose accumulation by supporting the creation of sucrose storage space, including the lengthening of space between stalk internodes, the widening of stalk diameter, and an increased number of tillers per plant (Ilyas and Khan, 2010; Ashraf *et al.*, 2008). N deficiency does not affect the amount of sucrose produced; instead, lack of sufficient N suppresses stalk growth, creating a sink limitation that then prevents the translocation of sucrose from the leaves to the stalk (Hartt, 1970). Conversely, since nitrogen promotes growth of the ligneous stalk, excess additions of nitrogen fertilizer out of proportion with potassium and phosphorus applications can cause vigorous growth that leads to lodging (mid-stalk cane bending), destroying the millability of the cane (Bakker, 1999). However, sufficient availability of the mineral nutrient silicon (Si), may reduce lodging by improving plant tissue rigidity (Savant *et al.*, 1999). In addition, an overabundance of nitrogen added to the soil can delay ripening, lower juice sucrose content, and create conditions for soil N leaching (Bakker, 1999).

The storage of sugars depends primarily on potassium, but cane cannot take up or utilize potassium without the concurrent availability of nitrogen and phosphorus in the N-P-K ratio of 2-1-3 as well as moisture (Harrt, 1934; Maeda *et al.*, 2009; Ng Kee Kwong, 2001). For instance, if potassium is readily available in the soil matrix but soil moisture limits the take up of nitrogen and creates a nitrogen deficiency, potassium will not be taken up by roots or absorbed by plant tissues in order to maintain the proper balance of N, P, and K, and sucrose yield will be reduced (Bakker, 1999). Similarly, inadequate supplies of potassium that create an imbalance with N and P will also result in cane with low sucrose content, since adequate K levels are required to utilize unassimilated N in the plant before cane ripening can occur (Ng Kee Kwong, 2001). An oversupply of N early in the grand growth stage may even be amended by a sufficient supply of K, but late N application after 7 months of growth can cause sugar storage to decline. Furthermore, potassium deficiency limits the translocation of nitrogenous compounds and sucrose from the leaves to the stalk, stunting growth and sucrose storage (Rao and Vered, 2011). Inadequate supplies of K may also enhance soluble acid invertase (SAI) activity, a sucrose hydrolyzing enzyme, causing further degradation of sucrose storage (Ng Kee Kwong, 2001).

Although not well understood, potassium most likely influences sucrose accumulation through its effect on SAI. The activity of SAI directly affects the sucrose accumulation in internodes; high levels of SAI inhibit significant sucrose accumulation in cane storage parenchyma by hydrolyzing accumulated sucrose to reducing sugars (fructose and glucose), which are easily and rapidly diffused out of the storage vacuoles (Sacher *et al.*, 1963). High levels of nitrogen tend to increase total reducing sugars regardless of whether total sugar yield increases, suggesting that nitrogen levels also directly impact SAI activity and enhance sucrose hydrolysis (Robertson *et al.*, 1996).. The relationship between nitrogen and SAI activity explains the delay in cane maturation and ripening when N supply remains large. SAI levels are usually high during periods of rapid growth, driven by nitrogen, causing low sucrose accumulation; however, SAI activity decreases to nearly zero during stem ripening and maturation when stem growth slows, allowing for the collection of sucrose. (Zhu *et al.*, 1997). Sucrose concentrations also depend on the interaction between SAI and sucrose-phosphate synthase (SPS), whereby the largest positive difference between SAI and SPS yields the greatest sucrose accumulation (Gutierrex-Miceli *et al.*, 2002). The activity of SAI and SPS varies by cane genotype, and thus the inherent physiology of the cane variety determines much of the ability of the cane to store sucrose as described in Section 2.5. However, slow growth inspired by cool temperatures or limited water and nutrients, especially nitrogen, can lead to higher amounts of sucrose storage (Zhu *et al.*, 1997).

Although the demand for phosphorus is low in cane compared to nitrogen and potassium, phosphorus incites faster, fuller development of the root system and increases the rate of tillering (Bakker, 1999). Thus, the demand for P is greatest in the early, formative stages of the cane. The slow release of phosphorus from the soil organic matter pool and the potential of aluminum and iron to retain phosphate in acidic soils then necessitate P fertilizer to meet even the meager cane P demand. Phosphorus should be applied with nitrogen prior to planting and nearest the root zone as possible (Perez and Melgar, 1998). Potassium also should be applied in the furrow prior to planting in conjunction with nitrogen, since potassium enables the cane to more efficiently utilize N, boosting plant productivity (Ashraf *et al.*, 2008). However, due to the perennial nature of sugarcane and the ability of cane to mine K from the soil, plant cane and the first and second ratoons may not have a significant response to K fertilizer (Ng Kee Kwong, 2001). Thus the critical balance between N, P, and K necessary for maximum sucrose accumulation must be found by experimentation based on the soil type and cultivar planted.

Besides finding the proper N-P-K balance, the best way to manage nutrients for sugarcane is to apply them either at the time of planting in the furrow for plant cane or banded next to the root zone of ratoon cane. Unlike corn that has many management variables that can optimize yield and reduce nitrogen losses (Table 2), the perennial nature of sugarcane and need for the front-loading of nutrients at the beginning of the growing season limit the applicability of general ‘best management practices’; the most beneficial management decisions are based on field-level experimentation to find the proper balance between the nutrient ratios, harvest method, and type of cultivar to match the particular soil type of the plantation.

CHAPTER 4. NITROGEN CYCLING IN FEEDSTOCK PRODUCTION

The individual plant acquisition and assimilation of nitrogen, as well as the addition of nitrogen fertilizers to enhance yield, each play a particular role in the overall management of the cropping system. Management practices of the corn and sugarcane crop systems can further influence the use of nitrogen and its losses from the agroecosystem. As such, the inherent cycling of nitrogen in the agroecosystem should be considered to determine the impact of crop management on the environment. However, the nitrogen dynamics of the soil-crop interaction have not been holistically mapped, and fundamental process, like denitrification, cannot be precisely quantified (Stockdale *et al.*, 1997). These gaps in knowledge provide room for error and inconsistency in budgeting the N fluxes of the agroecosystem. Even still, the data presented confirm the hypothesis that the cultivation of sugarcane in Brazil releases significantly less nitrogen to the environment through erosion, runoff, leaching, volatilization, or denitrification than U.S. corn. Owing mainly to the contribution of BNF and subsequently lower applied nitrogen rates, Brazilian sugarcane realizes losses of 321,160 metric tons of nitrogen with pre-harvest burning and 147,560 metric tons of nitrogen without burning, compared to 918,650 metric tons of nitrogen lost to the environment from the corn agroecosystem.

4.1 The Nitrogen Cycle in the Agroecosystem

While 78% of the atmosphere is comprised of nitrogen, this nitrogen pool remains inaccessible to plants until N_2 is changed into one of the reactive forms of nitrogen. Reactive forms of N include ammonium (NH_4^+) and nitrate (NO_3^-) as ionic forms; nitrous oxide (N_2O), nitrogen oxides (NO_x), and ammonia (NH_3) as gaseous forms; and urea, amines, proteins, and nucleic acids as organic forms (Robertson, 1997). Nitrogen cycling within the cropping system (agroecosystem) of corn and sugarcane considers the interactions among the atmosphere, soil, and crop that move these forms of reactive nitrogen through the field system (Figure 12).

First, reactive nitrogen (Nr) can enter the cropping system naturally or by human influence. Lightning fixes a small amount of di-nitrogen into nitric oxide that then

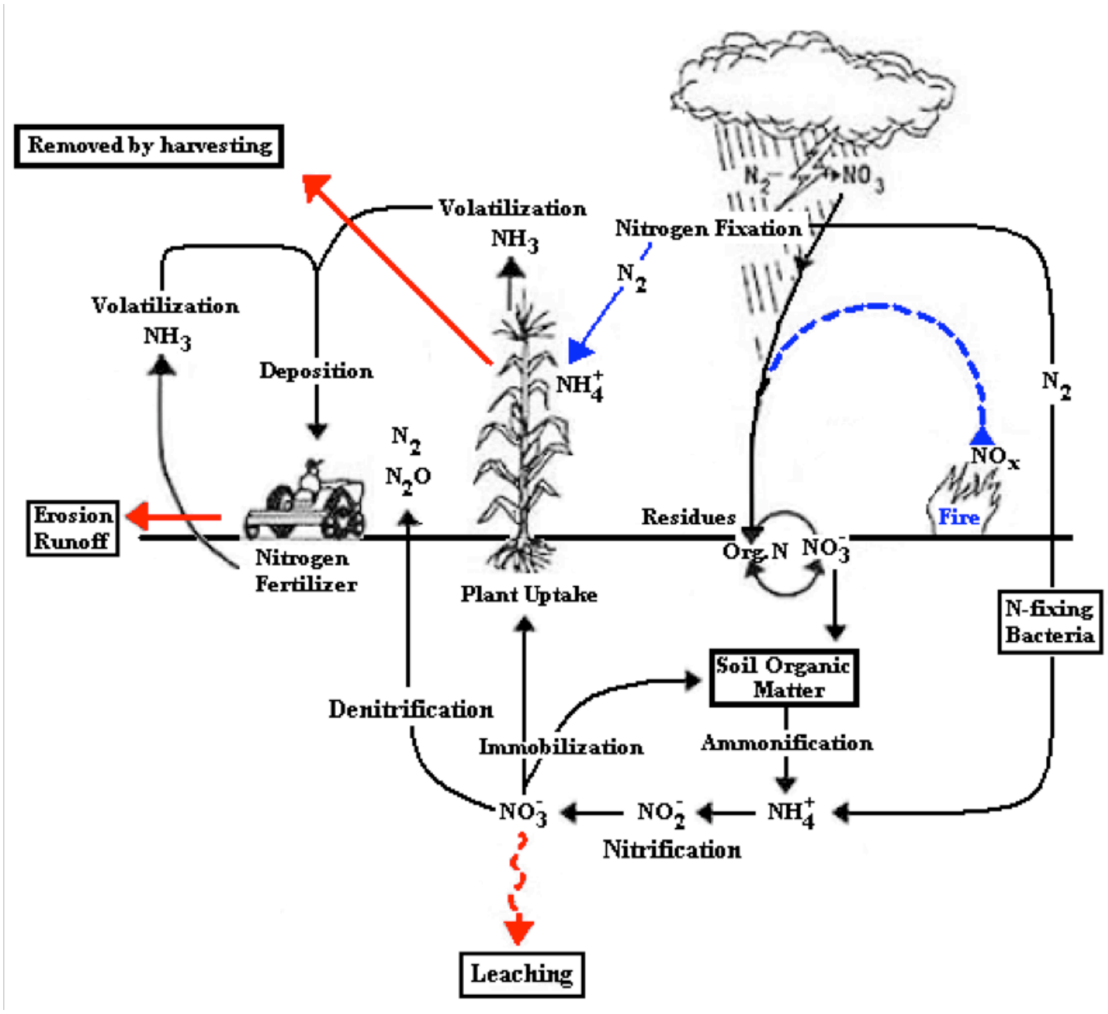


Figure 12. Nitrogen Cycle in the Agroecosystem. Black arrows apply to both corn and sugarcane cropping systems; blue arrows correspond to sugarcane only. Red arrows apply to both corn and sugarcane and represent nitrogen leaving the agroecosystem. Adapted from Johnson (2001) and Harrison (2003).

reaches the ground as nitric acid in rain, which readily forms NO_3^- in the soil (Asato, 2010). Also, N-fixing bacteria in the soil can convert N_2 to ammonium. The anthropogenic augmentation to the Nr entering the agroecosystem comes in the form of nitrogen fertilizer application as well as the planting of sugarcane, since sugarcane contributes large amounts of biological N fixation to the system. Although biological nitrogen fixation seems to be a natural contribution of Nr to the cycle, the Nr that accumulates in the cropping system due to the growth of sugarcane would not have otherwise entered the global nitrogen cycle if humans did not actively propagate and raise this nitrogen-fixing plant; thus, biological nitrogen fixation from sugarcane is a result of human influence on the agroecosystem.

Once Nr has become active in the nitrogen cycle of the agroecosystem, the various compounds can undergo a variety of processes. The soil microbes may immobilize N by utilizing nitrate and ammonium for their own growth, and when the microbes die, ammonium is released back into the soil where it may be taken up by plants or be converted to nitrate by the nitrification process in well aerated soils. Nitrate may then again be immobilized by soil microbes, taken up by plants, or denitrified. Denitrification occurs under anaerobic conditions, such as after heavy rains when soils are waterlogged for 2 to 3 days, and changes nitrate into mostly N_2 and a small amount of N_2O (Fageria and Baligar, 2005). Additionally, N fertilizer may be volatized into NH_3 and volatilization may occur from the tops of plants. The management practices of sugarcane in particular, with the burning of the cane fields prior to harvest, release gaseous N as nitrogen oxides (Harrison, 2003). Figure 12 diagrams these processes in the cycling of nitrogen through the agroecosystem for both corn and sugarcane.

Prior to human interference in the nitrogen cycle, reactive N did not accumulate in the environment as denitrification was able to balance the natural modes of nitrogen fixation from lightning and biological nitrogen fixation (Galloway *et al.*, 2003). However, the artificial fixation of di-nitrogen into nitrogen fertilizers through the Haber-Bosch process (as described in Chapter 3) as well as the continued expansion of crops capable of fixing nitrogen have brought enormous amounts of reactive nitrogen into the agroecosystem. This reactive nitrogen continues to circulate in various forms until it can be denitrified back into atmospheric N_2. The disruption of the natural balance between fixation and denitrification has allowed reactive nitrogen to not only accumulate in the agroecosystem but also to escape the boundaries of the immediate cropping system into ground and surface water and the atmosphere. While the accumulation potential of Nr in agroecosystems remains low to moderate, the transfer potential to outside the system is very high and thus has the potential to cause adverse environmental effects (Galloway *et al.*, 2003). The following sections will quantify the recycling and losses of reactive nitrogen from the corn and sugarcane cropping systems at the field level and discuss the implications of the losses on environmental quality.

4.2 Impact of N Management on Nitrogen Cycling: Corn

With high nitrogen fertilizer application rates and low nitrogen use efficiency by corn, management decisions regarding nitrogen become critical for reducing nitrogen losses from the agroecosystem. Most importantly, nitrogen availability needs to match crop nitrogen demand to improve the use efficiency of fertilizer. The timing of N fertilizer application must be well integrated with the application method and type of fertilizer to ensure the nutrient becomes available when crop demand is high. The tillage system and crop rotation pattern established in the fields also affect the N status of the soil, and their impacts on the soil need to be carefully managed to avoid over-fertilization and subsequent losses from the system.

Timing of nitrogen applications can significantly impact nitrogen retention in the corn agroecosystem. One-quarter of the area in the Corn Belt (5.2 Mha) receives nitrogen applications during the fall while plowing down the residues from the previous crop, while the rest of the Corn Belt practices nitrogen applications in the spring prior to planting. Although spring application of nitrogen tends to be superior in reducing N losses from leaching and denitrification, many farmers, especially in the northern parts of the Corn Belt, apply nitrogen as plow down in the fall so that corn can be planted as early as possible in the spring (Randall and Sawyer, 2010). With high loss potentials from fall plow down of nitrogen, the practice

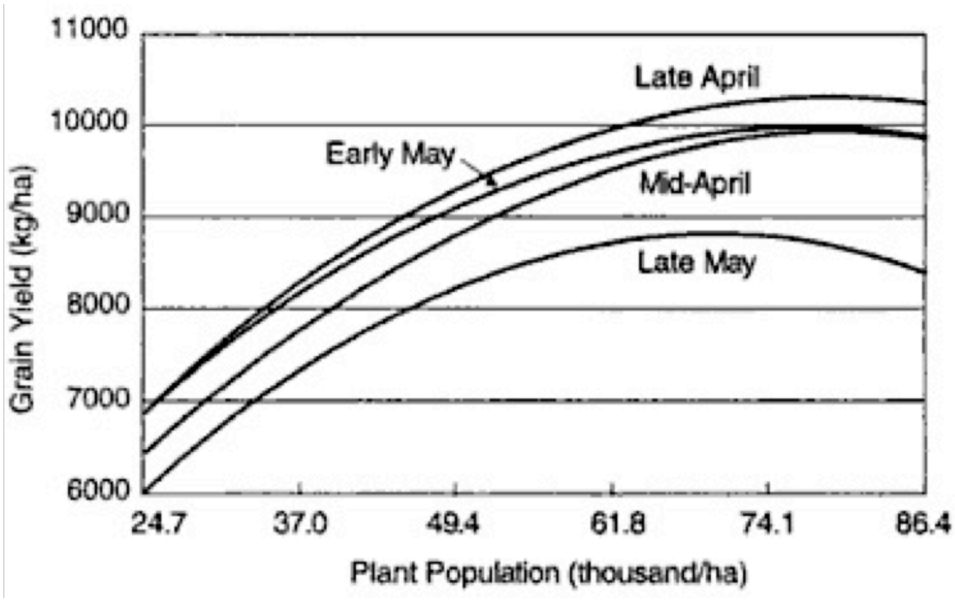


Figure 13. Effect of planting date on grain yield. Applications of fertilizer in the spring can delay planting by 1 – 2 weeks. Planting occurs from Mid-April to Late May. (Source: Hicks and Thompson, 2004)

would seem irrelevant. However, the spring planting date depends on the soil temperature, so later spring thawing can create an immediate need to sow the seeds as soon as the soil warms enough, and any further delays for fertilizer treatments in the spring can reduce the maximum achievable grain yield (Figure 13).

Despite the gamble with the spring thaw and planting date, fall plow down is not appropriate for all fields. Applications of nitrogen as fall plow down are only suggested for medium-textured, well-drained soils. Excessive nitrogen losses may be realized before the corn is even planted if nitrogen is applied as fall plow down on sandy, shallow, or finely-textured soils (leaching losses) or poorly drained soils (losses to enhanced denitrification). To limit the potential for nitrification and leaching or denitrification, nitrogen applications in the fall should only be applied after the temperature at the 6-inch soil depth is below 50˚F and should be used in conjunction with nitrification inhibitors (Bundy, 1998). Even with control measures in place to reduce N losses from fall plow down, 10 kg N ha^{-1} more nitrogen is lost each year than with nitrogen applied in the spring. Additionally, combining spring pre-planting applications of controlled-release nitrogen with side-dressing of N at appropriate stages of growth further improves yields and NUE by best matching the N demand of the corn plant (Randall and Sawyer, 2010).

Demand for nitrogen in the corn plant begins rising quickly at the V5-V6 stage, approximately 70 days after emergence, and continues until silking (Mulvaney *et al.*, 2004). Still, substantial N must be present in the soil at emergence so that the shallow and limited root system in the young corn plant may gain access to the nutrient, even if only a small amount is assimilated (Simpson, 2009). Thus, split applications prior to planting or at sowing with additional side-dressing at V6, V10, and VT have shown the greatest fertilizer use efficiency and highest grain yields, because this N regimen matches the needs of the corn plant (Lindquist *et al.*, 2005; Hall *et al.*, 2011). Thus, incorporating at least 30% of the total N applied into the soil or through fertigation as side-dressings can reduce the total amount of nitrogen required by 5% (Shapiro *et al.*, 2008). Keeping records of the stalk nitrogen content at harvest and the soil N just after harvest can effectively show if the proper amounts of nitrogen fertilizer were used over the growing season and provide a framework for adjusting nitrogen management in subsequent years (Schroder *et al.*, 2000; Blackmer *et al.*, 1997).

Soil testing to discern the potential N made available by mineralization over the course of the growing season is critical for reducing over-fertilization. Mineralization rates depend on the previous crop, residue management, and synthetic N fertilization (IFIA, 2001). Typically, the amount of N fertilizer applied is calculated using the proven-yield method (N rate equal to 1.2 times that of historical “proven” yields minus credits for previous legume crops or manure); yet, this method has been documented to overestimate the amount of N required by an average of 123 lb acre$^{=1}$ on continuous corn cropping, 66 lb acre^{-1} on corn after soybean rotations, and 58 lb acre^{-1} if soils had been manured within one year (Mulvaney *et al.*, 2004). Properly crediting leguminous cover crops, like alfalfa, and manure can help avoid over-fertilization in subsequent corn crops, but overfertilization may also be an artifact of not properly calculating the contribution from mineralized N in the soil, demonstrating the importance of post-harvest soil testing (El-Hout and Blackmer, 1990).

Applying the appropriate rate of N at the optimal time controls nitrate losses more than the tillage system, yet the type of tillage used can affect the amount of N required

(Randall and Mulla, 2001). In no-tillage operations, leaf, root, and stalk residues from the previous crop rotation are left in the field, and rows just wide enough for seed planting are uncovered for the next crop. Conventional tillage systems plough and disk the residues into the soil after the crop harvest. When first beginning a no-tillage regimen, the residues on the surface can raise the C:N in the soil organic matter, causing N to be immobilized (Hicks and Thompson, 2004). Over time though, no-tillage systems result in higher organic N in the first 10 centimeters of soil than conventional tilling practices due to the continual decomposition of residues left in the field. Additionally, in the western Corn Belt, no-till has helped preserve soil moisture during dry spells. However, in the eastern Corn Belt, the large amounts of residue in no-till keep the soil too cool and wet, reducing soil mineralization and delaying planting (Peterson, 2005).

Since no-till is not suitable for all locations, a compromise between conventional and no-till practices has arisen in the form of conservation tillage. Conservation tillage, where more than thirty percent of crop residues are left in the field, is slowly taking hold in the Corn Belt; no-till has gathered a 20% share of the corn acreage, and mulch-till, where the residues are plowed under just before planting, has gained a similar percentage (Peterson, 2005). However, conventional tillage and reduced tillage, where less than thirty percent residue cover remains after harvest, still dominate the Corn Belt with 60% of planted acres involved in these practices (Christensen, 2002). Conventional and reduced tillage can allow for significant losses of N to runoff and erosion, since the soil is left without protective cover.

4.3 Fate of Nitrogen in the Corn Agroecosystem

The amount of nitrogen lost to the environment or retained in the agroecosystem depends greatly on the soil type, crop management, and type of fertilizer applied as discussed above. Acknowledging these complexities, the fluxes below are based on averages from a variety of sources in the literature and, as such, contain errors associated with the particular studies from which they were taken as well as from aggregating them in this paper. The averages also homogenize a rather large and diverse system of cropping in the Corn Belt, creating a limitation on the precision of the data. It also should be noted that none of the nitrogen fluxes could be estimated precisely since the fate of applied nitrogen is generally accounted for by mass-balance studies instead of individual measurements (Schlesinger, 2009).

However, the discussion remains relevant to a general approximation of nitrogen cycling in the Corn Belt (Table 7) and the potential environmental degradation caused by nitrogen lost from the agroecosystem. The assumptions underlying the nitrogen budget constructed in Table 7 are discussed in the noted 'Source' sections.

Table 7. Nitrogen Budget for the Corn Agroecosystem

Inputs	**kg N ha^{-1} yr^{-1}**	**Source**
N Fertilizer	130 – 225	Section 3.3 (Table 3)
Deposition	13 – 36	Section 4.3.3
Total	143 – 261	
Outputs		
Erosion/Runoff	20 – 65	Section 4.3.1
Leaching	13 – 66	Section 4.3.2
NH_3-volatilization	11 – 41	Section 4.3.3
Denitrification	9 – 33	Section 4.3.4
Harvest	100	Section 4.3.5
Total	153 – 305	

Estimations of losses from erosion, runoff, leaching, and denitrification total 75 kg N ha^{-1} each year when N outputs of the budget were calculated using the average N fertilizer rate of 165 kg N ha^{-1}. Considering the 9.67 million hectares of corn harvested for ethanol in the 2008/09 growing season (Table 4), the production of corn for ethanol released an average total of 918,650 metric tons of nitrogen from the agroecosystem into the atmosphere, surface waters, and groundwater. Additionally, as outputs are greater than inputs, the budget suggests that corn cultivation depletes soil N, implying the crop requires more fertilizer over time, which has been noted by farmers and documented in the literature (Salem, 2010; Mulvaney *et al.*, 2009). Details of the N losses from the agroecosystem (outputs) and the related potential consequences are explained in the following sections.

4.3.1 Soil Erosion and Runoff

Half of the cropland in the Corn Belt occurs on slopes less than 2%, leading to low average erosion rates in this area of 1.9 to 10.6 t ha^{-1} yr^{-1}, but overall, the Corn Belt averages erosion losses of 15 t ha^{-1} yr^{-1} (Pierce *et al.*, 1984; Smil, 1999). Considering an erosion rate of 10.6 tons per hectare, 4.8 kg of soil is eroded for each liter of ethanol produced (Mubako and Lant, 2008). In heavily cropped areas of the Corn Belt, erosion is moderate to severe, equating to greater than 25% and 75% loss of the original A horizon respectively. This erosion changes many soil properties, including a significant decrease in soil nitrate with increasing erosion severity in all soil types (Cihacek and Swan, 1994). The loss of soil organic carbon to erosion reduces the C:N ratio in the soil above that which microbes can fully utilize available soil nitrogen, allowing a concurrent loss of nitrogen to runoff or leaching (Galloway *et al.*, 2004).

Runoff carries away high levels of nutrients from the soil surface since runoff also causes the majority of erosion through water erosion (Groom *et al.*, 2008). This nutrient-rich runoff results in a median concentration of nitrate in rivers and streams of 5 mg L^{-1} within the Corn Belt. The amount of fertilizer lost through erosion and runoff relates directly to the rainfall patterns for each growing season though. For example, in the eastern Iowa Basin, higher amounts of precipitation in 1998 led to a loss of 47 kg N ha^{-1} for the year, up from 19 kg N ha^{-1} in the 1996 growing season (Kalkhoff *et al.*, 2000). Averaging across years of varying precipitation intensities, nitrate losses to surface waters generally total 20 to 65 kg N ha^{-1} yr^{-1}, but segregating these losses between surface runoff and groundwater leaching proves difficult (Dinnes *et al.*, 2002; Mubako and Lant, 2008). Nitrogen lost through erosion and runoff can degrade surface water quality and lower the overall productivity of the soil.

4.3.2 Leaching

The amount of ammonium in the soil controls the rate of nitrification, which, in turn determines the availability of nitrate in the soil able to be leached (Stockdale *et al.*, 1997). Nitrates are highly mobile and move easily with water through the soil profile and into groundwater (O'Leary *et al.*, 1994). While leaching depends on soil thickness and permeability as well as rainfall, the quantity of nitrogen fertilizer applied can affect the total amount lost; fertilizer applications of less than 150 kg N ha^{-1} lose an average 10% of N applied to leaching while levels greater than 150 kg N ha^{-1} generally leach about 20% of N applied (Smil, 1999). Although, depending on the amount of annual rainfall, leaching can reach up to 40% of N applied (Kessavalou *et al.*, 1996). Leaching losses upwards of 50 kg N ha^{-1} are not unusual in the Corn Belt (Smil, 1999). Considering the range of N fertilizer rates in the Corn Belt of 130 – 225 kg N ha^{-1}, leaching potential could span from 13 – 45 kg N ha^{-1} yr^{-1}; under the worst leaching scenario of 40% of applied N, an average of 66 kg N ha^{-1} yr^{-1} could be lost. Even these figures may underestimate the amount of N leached since N mineralized by soil microbes can also be leached, especially just after harvest.

Leaching reaches maxima in the spring and fall, prior to planting and post harvest, as mineralization of nitrogen in the soil organic matter pool continues without concurrent take up by the crop (Robertson, 1997). Abating nitrification by adding nitrification inhibitors to the soil after harvest can reduce the nitrate pool while the land lies fallow over the winter. Nitrapyrin (N-Serve), the most commonly used nitrification inhibitor in the United States, can limit losses of nitrate to denitrification and leaching up to 14% (Randall and Sawyer, 2010). Though, only 12.7% of acres planted in 2005 used a nitrogen inhibitor, leaving room for unnecessary losses (ARMS, 2005).

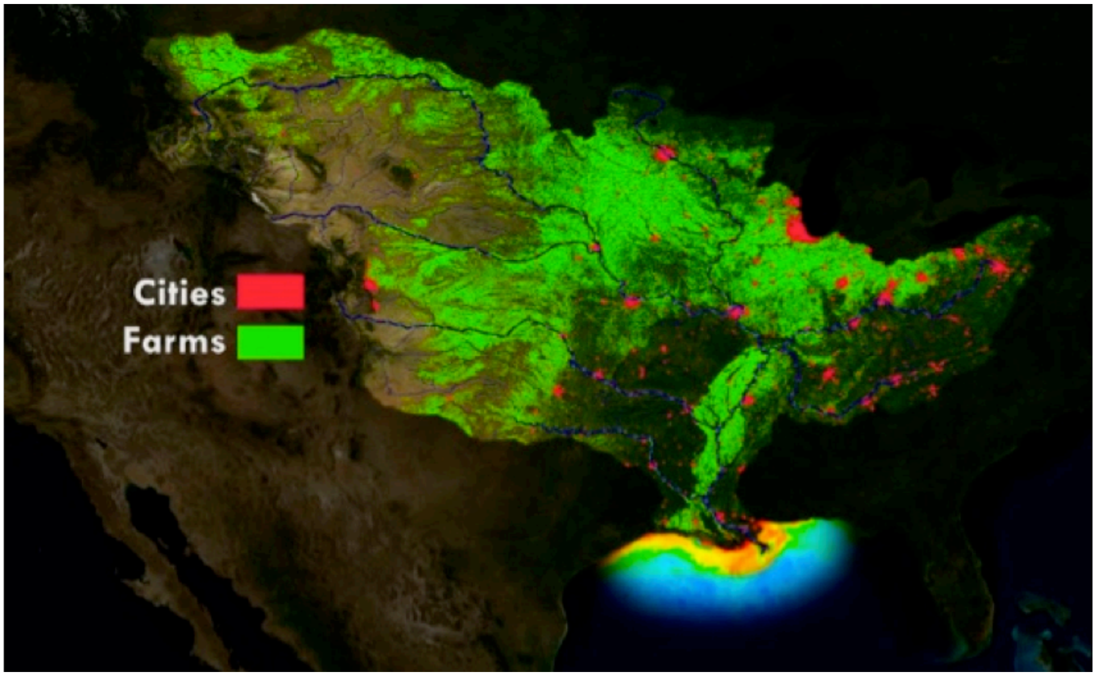


Figure 14. Nutrient Transport from the Mississippi Watershed. Phytoplankton concentrations represented from low (blue) to high (red). Source: NOAA (2011).

Consequences of nitrate leaching into groundwater can be severe from both an environmental and public health standpoint. In most all regions of the Corn Belt, the probability that groundwater nitrate exceeds 4 mg L^{-1} is at least 20 – 30%, and many areas in the heart of corn-growing regions have a 65 – 85% probability of groundwater contamination (Nolan *et al.*, 2002). Nitrates in groundwater can contaminate both public and private wells, with levels above 10 mg L^{-1} causing serious health conditions like methemoglobinemia (Blue Baby syndrome) in infants (Colorado Department of Public Health and Environment, 2010). Additionally, groundwater transports nitrates into streams and rivers that then can be exported from the Corn Belt region, which has caused major nutrient loading in the Gulf of Mexico.

About 1.5 million metric tons of nutrients, mainly nitrogen and phosphorous, are dumped into the Gulf of Mexico each spring, causing one of the largest phytoplankton blooms on Earth, second only to the bloom in the Baltic Sea (Figure 14) [NOAA, 2011; Larsen, 2004]. As the phytoplankton decomposes, oxygen levels drop and a hypoxic dead zone, 20,000 square kilometers in size, forms over the summer until the fall winds help mix the surface and deep waters to restore oxygen levels (Biello, 2008). With the Corn Belt dominating the headwaters of the Mississippi watershed, nitrogen contaminated runoff and, to a greater degree, nitrate leaching from corn fields ultimately contributes to the dead zone downstream in the Gulf of Mexico (Rabalais *et al.*, 2002; David and Gentry, 2000). Multiple studies propose that nitrogen transported by the Mississippi River to the Gulf of Mexico could increase as much as 34% by 2022 due to the increasing acreage in the river basin dedicated to corn for ethanol as well as the simultaneous inability of rivers to increase the denitrification rate of N transported in the watershed

(Biello, 2008; Simpson *et al.*, 2008). Donner and Kucharik (2008) calculate that such an increase in nitrogen from corn fields would essentially guarantee an expansion in the size of the dead zone (>95% confidence interval). With increasing intensity, size and duration of the dead zone, recovery of the northern Gulf may be stunted and permanent, long-term ecosystem shifting could occur (Rabalais *et al.*, 2002).

4.3.3 Volatilization of NH_3

Volatilization of ammonia occurs from fertilizers as well as senescing leaves of maturating corn plants. Typically, anhydrous ammonia fertilizer is injected into the soil at a depth of 10 to 20 centimeters to avoid volatilization; urea should also be incorporated into the soil within three days of application for the same reason (Hoeft and Peck, 1991). Urea applied to the soil surface without incorporation remains at high risk for volatilization if conditions include high temperatures (>70˚F), high soil pH (>7.0), moist soil, high humidity (>75%), layers of crop residue, or low cation exchange capacity (Jones *et al.*, 2007; Appendix: Figure F). Depending on these soil conditions, volatile losses between 15 and 20% of applied N can occur. Rainfall of at least 0.5 inches within two to three days of urea application, use of urease inhibitors, or incorporation of urea into the soil can significantly reduce losses as ammonia (Bundy, 1998). Thus, assuming ammoniacal fertilizers are applied judiciously, only between 4 and 7 kg N ha^{-1}, about 3% of applied N, would be lost to volatilization.   Limiting volatilization from fertilizers improves corn yield 5 – 40% by improving nitrogen use efficiency (Fageria and Baligar, 2005).

Volatilization of ammonia can also occur from the plant itself as leaves senesce. A sizable flux of NH_3 between 7 and 34 kg N ha^{-1} has been documented from the tops of corn plants two to three weeks after tasseling, depending on the N applied and total N take up for the corn hybrid (Francis *et al.*, 1993). Merging the volatilization loss from fertilizer with that of senescing leaves gives a total loss of 11 – 41 kg N ha^{-1}. Still, nearly three-quarters of the nitrogen volatilized from fertilizer or the tops of the corn plant stays within the agroecosystem and falls back on the agricultural land from which it originated, since ammonia has a short residence period in the atmosphere of hours to days (Su *et al.*, 2003; Galloway *et al.*, 2003). Considering that deposition from other sources like the burning of fossil fuels drops about 5 kg N ha^{-1} yr^{-1} on agricultural fields, total deposition within the agroecosystem equates to 13 – 36 kg N ha^{-1} (Smil, 1999). However, some of the nitrogen returned to the agroecosystem through deposition can fall in surface waters, potentially helping create conditions for eutrophication, or contribute to nitrogen loss as runoff.

4.3.4 Denitrification

Denitrification occurs in anoxic soil areas such as soil aggregates, dense organic matter particles, and saturated soils following precipitation or heavy irrigation, which cause bacteria to respire using nitrate instead of oxygen (Robertson, 1997). With high spatial and temporal variability, the amount of denitrification is difficult to quantify and remains poorly understood, especially since anoxic hot spots in well aerated soils can still contribute to significant N losses to denitrification (Galloway *et al.*, 2004). The amount of soil pore space filled with water seems to influence the type of nitrogen released during denitrification though, with N_2O peaking at 80 – 90% water-filled pore space and N_2 generally occurring with less than 30% water-filled pore space (Smil, 1999). Nitrogen may be denitrified within the agroecosystem or outside the boundaries once leached or eroded. For instance, between 30 and 70% of nitrogen entering rivers is denitrified along riverbanks during transport, further contributing to greenhouse gas accumulation in the form of nitrous oxide (Galloway *et al.*, 2003). Nitrogen that does not undergo denitrification continues to cycle through the agroecosystem and the greater global N cycle until the excess N becomes denitrified or stored in a reservoir with a long turnover rate (Erisman *et al.*,2008).

Of nitrogen applied to the agroecosystem, 6 to 12% is denitrified to N_2 (Smil, 1999). Based on the soil types of the U.S., Ogle *et al.* (2008) suggest that 2 to 2.5% of N added to soils is denitrified to N_2O either directly in the field or indirectly offsite after nitrate has been lost by leaching or runoff. Mosier *et al.* (1986) support this measurement for irrigated crops in the Corn Belt but widen the range to 1 – 2.5% of applied N fertilizer. Li *et al.* (1996) quantified the annual N_2O flux depending on the fertilizer amount, type, placement depth, and timing of application; N_2O emissions from a corn field sampled in Iowa ranged from 2.6 to 6.3 kg N ha^{-1} yr^{-1} with an average of 4.1 kg N ha^{-1} yr^{-1}, falling in the ranges identified above. Thus, combining emissions of N_2 and N_2O, between 7 and 14.5% of N applied is lost to denitrification, equivalent to 9 and 33 kg N ha^{-1} based on the range of 130 – 225 kg ha^{-1} applied N.

Due to the decomposition of leguminous winter cover crops and cover residues, no significant difference in N_2O emissions occurs between conventional, no-till, and organic corn cropping systems. Additionally, unmanaged land also creates N_2O

emissions, but fluxes from annual corn sites are, on average, three times greater than forested sites (Robertson *et al.*, 2000). Generally, background emissions of N_2O equate to 1 kg N ha^{-1} each year (Bouwman, 1996).

The emission of nitrous oxide from denitrification relates directly to the application of nitrogen fertilizer, so with increasing hectares dedicated to corn cropping, more fertilizer and thus higher emissions of N_2O can be expected. Not only is N_2O a greenhouse gas with 298 times the global warming potential of CO_2, but also, for every one molecule of N_2O oxidized in the stratosphere, two ozone-destroying nitrogen oxides (NO) are created, which contributes to the weakening of the ozone layer (Crutzen and Ehhalt, 1977).

4.3.5 Removal in Harvest

Typical corn varieties commonly take up about 180 kg N ha^{-1}, about half of which is derived from synthetic N fertilizer while the remaining portion is supplied from mineralization of soil N (Cassman *et al.*, 2002). Thus, with 55% of total plant nitrogen contained in the grain at harvest, about 100 kg N ha^{-1} are removed from the field in the grain (Hay *et al.*, 1951; O'Leary *et al.*, 1994). Due to the shedding of flowers, pollen, and leaves as well as the volatile losses of NH_3 from the tops of maturing plants, the corn residues typically contain 15 less N than at the peak total take up value (Smil, 1999). A 0.5 harvest index for corn implies that half of the biomass is left in the field as residues, representing the remaining 50 kg N ha^{-1} once the 15% reduction from peak N take up is considered (Erickson and Carr, 2009).

4.3.6 Retention and Recycling of Nitrogen

The crop residues left in the field after harvest allow an opportunity for microorganisms to decompose and release the 50 kg N ha^{-1} stored in the remaining leaves, stubble, and roots. About 50% of corn residues left in the field are broken down by microorganisms for the next growing season, supplying about 25 kg N ha^{-1}; although some of this newly released nitrogen may be lost to leaching or erosion depending on the soil conditions (Sawyer *et al.*, 2006). The remaining residues lock up nitrogen until decomposed into the soil organic matter pool.

As mentioned above, approximately half of the nitrogen in fertilizer becomes incorporated into the corn crop while the rest of crop N demand is met by soil N mineralization (Smil, 1999). Usually around 20% of applied nitrogen becomes immobilized in the soil and released in later growing seasons through mineralization, so the carbon content of the soil and the subsequent C:N ratio highly influence the amount of N from fertilizer available in a given growing season (Reddy and Reddy, 1993). The remaining amount of N fertilizer is lost directly to the environment along with a portion of the nitrogen released from soil mineralization (Sawyer *et al.*, 2006). Nitrogen retained from one growing season may be lost in subsequent seasons though.

4.4 Farming Practices' Impact on Nitrogen Cycling: Sugarcane

The two largest contributors to greenhouse gas emissions from the cropping of sugarcane are soil emissions and trash (dead leaves) burning (Figure 15) [Macedo *et al.*, 2008]. Yet emissions from these sources, as well as other losses of nitrogen from the cane agroecosystem, can be controlled through smart management of fertilizer application and harvesting methods.

Generally, soil N reserves are not exhausted, and cane productivity is maintained over the course of multiple ratoon cycles, even when N fertilizer is not applied (Orlando *et al.*, 1980; Boddey *et al.*, 1995; Smith *et al.*, 2005). Still, most all cane grown in Brazil today receives nitrogen fertilizer, usually in the form of urea, to guarantee maximum yield potential. While urea causes less soil acidification than ammonium sulfate, the large volume of sugarcane trash prevents the incorporation of urea into the soil, and thus most urea is lost to volatilization and runoff unless applied below the trash layer (Hartemink, 2008). Surface broadcasting of urea leads to less than 20% nitrogen use efficiency, but drilling the urea into the soil can enhance NUE to nearly 30%. The gains in NUE with subsurface application of urea result from significant reductions in volatilization from 37% to 5.5% of applied N, although nearly 20% of this nitrogen

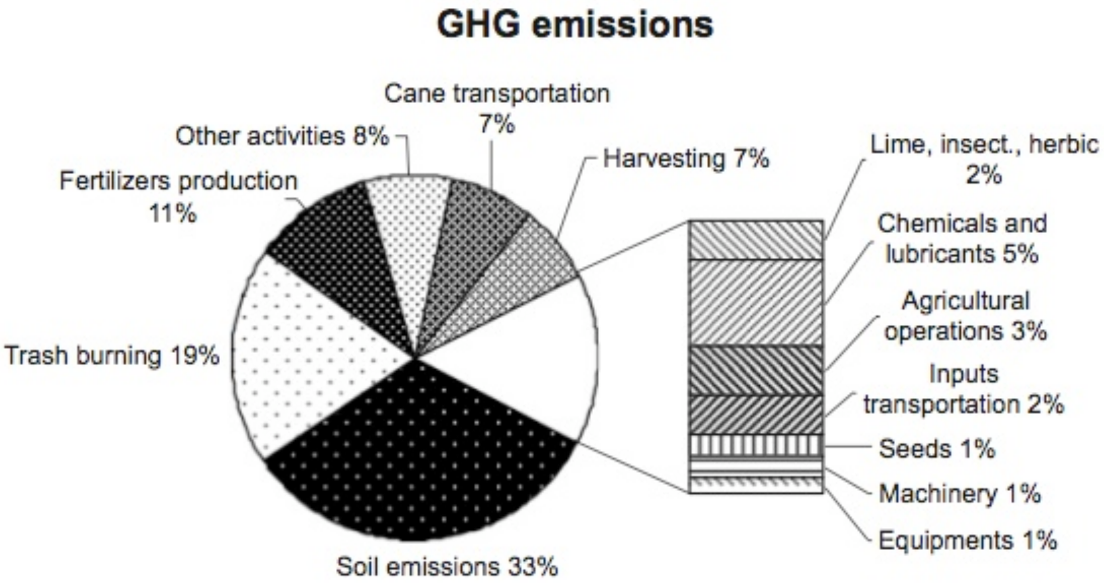


Figure 15. Greenhouse Gas Emissions Associated with Cane Production. Percentages are derived from comparison of CO_2-equivalent values. (Source: Macedo *et al.*, 2008)

retention is negated by subsequent losses to leaching and denitrification, leaving a net reduction in total N loss by switching application methods at 13.5% (Prasertsak *et al.*, 2002).

To minimize leaching and denitrification and ensure these losses do not cause N deficiency in the initial growth stages, the application of N fertilizer should be made in two doses during the grand growth period to match cane N demand (Gowariker *et al.*, 2009). Demand strength can be approximated by testing leaf nitrogen content between six to eight weeks after planting or ratooning in order to precisely time the second N fertilizer application. Nitrogen should be placed as close to the root zone as possible; for plant cane, the ideal first application would be placed in the furrow just prior to planting as long as adequate soil moisture is available to promote take up (Muchovej and Newman, 2004). For ratoon cane crops, the inability to closely place the nitrogen in the root zone as done with plant cane crops requires that ratoon crops are provided with 10% more nitrogen fertilizer than the plant cane. While ratoon crops have a greater need for and take up of nitrogen fertilizer, yield from plant crops still remains higher than succeeding ratoon crops, and N losses are lower for the plant cane over the growing season (Sisti *et al.*, 2003; Perez and Melgar, 1998). The boost in N fertilizer required by ratoon cane may also be attributed to the fact that the plant cane receives added N from the increase in the soil mineralization rate associated with the tilling and ploughing for planting the new setts (Saidak, 2011).

Even though tilling and ploughing increases soil mineralization and lowers the need for nitrogen fertilizer, these activities can contribute to significant topsoil organic matter loss in Brazil and create greater opportunities for widespread erosion (Sisti *et al.*, 2003). If the land is left fallow between the tilling of the last ratoon crop and ploughing for the planting of new setts, erosion becomes an even greater concern. To reduce erosion on fallow land without reducing soil N reserves, soybeans or other leguminous crops can be planted as cover crops (Saidak, 2011).

Erosion and runoff are also a concern just after harvesting, especially when the trash is burned prior to harvest to ease the manual harvesting process, leaving the soil exposed until the next ratoon emerges from the soil. Trash burning also contributes significant emissions of nitrogen oxides. As mechanized harvesting without burning overtakes manual harvesting, the trash left in the field may be used to enhance the nutrient status of the soil if managed intelligently. With the burning of sugarcane, little cane residues are left in the field, but the C:N ratio of the soil organic matter remains relatively low so nitrogen is available for the next ratoon (UNICA, 2008). However, mechanized harvesting leaves behind significantly more leaf residue in the field with a high C:N ratio between 80 and 120:1, resulting in immobilization of soil N in the short term (Thorburn, 2005). While in the long-term residues can improve soil N fertility by adding up to 900 kg N ha^{-1} over two decades, the immediate effect of residues reducing soil N availability may require a spike in levels of nitrogen fertilization in the future (Pinheiro *et al.*, 2010; Smil, 1999). At the very least, nitrogen fertilizer will need to continue for 6 – 15 years after the recycling of trash begins in order to provide enough soil N to support high cane yields (Robertson and Thorburn, 2007).

Additionally, the conservation of trash with the cessation of pre-harvest burning has been documented to increase cane sucrose yields up to 25% in the northeastern region of Brazil due to the propensity of trash to conserve soil moisture and contribute to a slow release of nitrogen; however, the benefits of trash conservation to cane yield in southeastern Brazil, where 90% of cane is grown, are muted since rainfall in this region is highly sufficient and mechanical harvesting associated with trash conservation causes severe soil compaction that reduces yields (de Resende *et al.*, 2006). Benefits may still arise though, such as the large macropores created by the trash layer, which could protect against N leaching by slowing the flow of water through the soil (Fageria and Baligar, 2005).

4.5 Fate of Nitrogen in the Sugarcane Agroecosystem

Beyond the potentials for error associated with creating a nitrogen budget from the literature outlined in Section 4.2, quantifying the nitrogen budget for Brazilian sugarcane proves extremely difficult. The large range of values cited in the literature regarding the same N flux, the diversity of crop management practices, and the lack of reliable literature published in English on the topic have prevented authors like Hartemink (2008) from completing a full nitrogen budget for Brazilian cane. Therefore, the nitrogen budget below (Table 8) should be regarded as a general approximation, since the data available only provide a rough sketch of the fluxes in the cane agroecosystem. The budget considers only the fluxes for the ratoon cane crop since the majority (80%) of cane is ratoon cane in any given growing season. For plant cane, the amount of inorganic fertilizer is less than that for ratoon cane, and losses from the agroecosystem are therefore smaller. The assumptions underlying the nitrogen budget constructed in Table 8 are found under the noted ‘Source’ sections.

Taking into account that 4.34 million hectares of cane were harvested for ethanol in the 2008/09 growing season (Table 6), average losses to the environment from the cane agroecosystem (excluding the harvest) amount to 321,160 metric tons of nitrogen with pre-harvest burning and 147,560 metric tons of nitrogen without burning under the average N fertilization rate of 90 kg N ha^{-1}. Conversely, nitrogen also accumulates in the agroecosystem under both harvest methods, mainly due to the contribution of BNF and the immobilization of N in residues and soil as discussed in section 4.5.7. However, the magnitude of accumulation may be an artifact of the errors associated with the budget as data on the outputs are scarce, and substantial accumulation of N in the soil would not be expected in tropical climates like Brazil. Still, it should be noted that without the input of BNF, soil nitrogen would be depleted or more inorganic N fertilizer would be necessary.

Table 8. Nitrogen Budget for the Sugarcane Agroecosystem (Ratoon Crop)

Inputs	**kg N ha^{-1} yr^{-1}** **With Burning**	**kg N ha^{-1} yr^{-1}** **Without Burning**	**Source**
Inorganic N Fert.	80 – 100	80 – 100	Section 3.7 (Table 5)
N Fixation	60 – 120	60 – 120	Section 3.7 (Table 5)
Deposition	13 – 27	13 – 27	Section 4.5.3
Total	153 – 247	153 – 247	
Outputs			
Erosion/Runoff	15	15	Section 4.5.1
Leaching	3 – 8	6 – 18	Section 4.5.2
NH_3-volatilization	11 – 29	11 – 29	Section 4.5.3
Denitrification	5 – 13	5 – 13	Section 4.5.4
Burning	25 – 55	--	Section 4.5.5
Harvest	50 – 125	55 – 138	Section 4.5.6
Total	98 – 245	93 – 213	

4.5.1 Soil Erosion and Runoff

Sugarcane in Brazil usually is cultivated on bench terraces that follow the contour in order to control runoff and soil erosion (Hartemink, 2008). The slopes and terraces essentially necessitate pre-harvest burning and manual harvesting in these plantations (Ellis and Merry, 2004). However, as bans against pre-harvest burning come into effect, farmers are being forced to convert to mechanical harvesting, which requires flat, straight rows of cane instead of terraced contours. Thus, soil erosion and runoff may increase with this change unless farmers begin to utilize reduced tillage and soil covers concurrently (Sparovek and Schnug, 2001a). Yet, since burning makes the topsoil hydrophobic and increases runoff, the switch to mechanical harvesting may reduce runoff more than the lack of terraced contours increases it (Robichaud and Hungerford, 2002). Additionally, with mechanized harvesting, residues left in the field can protect soil from erosion until the new ratoon emerges from the soil (UNICA, 2008). These tradeoffs are no yet quantified though.

Fields are most susceptible to erosion when land is fallow or just after ploughing of the final ratoon crop before planting new setts (Ellis and Merry, 2004). The level of soil loss, and thus nitrogen loss due to erosion, is relatively low in cane fields though since the soil is only tilled and replanted with new setts every sixth year in the typical 5-year ratoon cycle. Erosion rates in São Paulo are estimated at 31 Mg ha^{-1} yr^{-1} when areas of deposition and soil loss are averaged in the region. At this rate of erosion, soil productivity for supporting sugarcane cultivation can be maintained for over 300 years (Sparovek and Schnug, 2001b). Yet in a few regions of Brazil that have been intensively cropped with sugarcane for nearly a century, erosion can vary from severe to extremely severe due to poor management practices (Martinelli and Filoso, 2008). No data exist on the amount of nitrogen lost with soil erosion for Brazil, but estimates in Louisiana, United States, indicate 18 kg N ha^{-1} (Hartemink, 2008). Since erosion and applied nitrogen rates are both higher in Louisiana, the N removed by erosion and runoff in Brazil has been approximated to 15 kg N ha^{-1} as N from runoff is negligible.

Over-fertilization of nitrogen and subsequent runoff to aquatic ecosystems has not been a previous problem in Brazil. However, accumulation of nitrogen in the agroecosystem with the intensification of cane cropping has led to a slight increase in riverine export of N to reservoirs in the Piracicaba and Mogi watersheds from both erosion and runoff, which could cause future problems like eutrophication (Filoso *et al.*, 2003). Still, compared to other crops in Brazil like coffee and citrus, nitrogen application on cane fields is considered non-excessive and a fairly minimal contributor to runoff (Martinelli and Filoso, 2008). While runoff does transport small amounts of nitrogen to nearby surface waters, most nitrogen from fertilizer is immobilized in the soil organic matter pool and the main concern for runoff pollution lies in the high BOD vinasse (potassium-rich waste water from ethanol production used as organic fertilizer) [Gunkel *et al.*, 2007].

4.5.2 Leaching

The perennial nature of sugarcane and relatively continual take up of nitrogen from the soil organic matter pool significantly reduce opportunities for leaching, unlike in annual crops such as corn (Robertson, 1997). Multiple studies using ^{15}N labeled fertilizer have reported very low levels of leaching from sugarcane fields, between 1 and 3 kg N ha^{-1}, as soil microbes quickly immobilize N from fertilizer in the soil organic matter pool (Filoso *et al.*, 2003; Ghiberto *et al.*, 2009). The minimal leaching that does occur seemingly derives from the soil organic matter and sugarcane residues at average rates between 5 and 15 kg N ha^{-1} over the course of the growing season (de Oliveira *et al.*, 2000; Ghiberto *et al.*, 2009; de Oliveira *et al.*, 2002). As such, total leaching losses are approximated as 5 – 18 kg N ha^{-1} without burning and 3 – 8 kg N ha^{-1} with burning, since no sugarcane residues are left behind for decomposition but high rates of soil mineralization can still occur in tropical soils.

4.5.3 Volatilization

Most N fertilizer in Brazil is applied as urea, which can lead to large volatilization losses if not properly managed (Martinelli and Filoso, 2008). Volatilization of NH_3 from ammoniacal fertilizer incorporated into the soil below the residue layer varies between 0.6 and 9% of N applied (Schlesinger, 2009). Applied on the surface though, between 30 and 50% of the nitrogen in urea can be lost in the first week since the trash layer cannot bind the NH_3 released from the urea (Smil, 1999; Ralph, 1992). If, by chance, heavy rainfall forces the surface-applied urea below the residue layer, volatilization losses mimic those associated with subsurface incorporated fertilizer (Freney *et al.*, 1992). Volatilization losses can be reduced by greater than 20% by switching from urea to UAN or liquid residue enriched with N without significantly affecting yield (Costa *et al.*, 2003).

Volatilization from senescing plant leaves contributes large quantities of ammonia to the atmosphere though, with estimates reaching as high as 80 kg N ha^{-1} for the maturing cane plant and averages between 10 and 20 kg N ha^{-1} (Martinelli and Filoso, 2008; Hartemink, 2008). Volatilization from properly applied nitrogen fertilizer and senescing plant leaves can then vary between 11 and 29 kg N ha^{-1} based on the N fertilization rates of 80 – 100 kg N ha^{-1} for ratoon cane. Still, about three-quarters of ammonia lost to volatilization from fertilizer or senescing leaves is returned through wet and dry deposition to the same agroecosystem in a short period of time, equating to 13 – 27 kg N ha^{-1} when deposition from other sources like fossil fuel burning is also considered at a rate of 5 kg N ha^{-1} (Smil, 1999).

4.5.4. Denitrification

When fertilizer is broadcast on the surface, N_2O fluxes from denitrification represent 1.0 – 2.5% of applied N due to the heavy layer of residue cover, but incorporation of N into the soil reduces these emissions to between 0.03 and 0.5% of applied N in tropical soils (Matson *et al.*, 1996). Comparable to corn, denitrification as N_2 generally remains between 6 and 12% of N applied (Smil, 1999). The amount of denitrification from unfertilized cane soils still remains relatively the same as soils treated with nitrogen fertilizers due to the high quantity of nitrogen added to the soil by the biological nitrogen fixation associated with cane roots and stems. Although, denitrification from synthetic N fertilizer poses the chance for far higher N_2O emissions because of the potential for human error in N management (de Figueiredo *et al.*, 2010). Assuming proper incorporation of fertilizers into the soil, total denitrification in the forms of N_2 and N_2O is approximately between 5 and 13 kg N ha^{-1}.

Additional denitrification can occur outside of the agroecosystem from nitrogen that has been previously eroded or leached, similar to corn. Riverine transport of nitrogen in the Piracicaba River Basin, which covers the majority of São Paulo State, is reduced 25% by denitrification along river banks and in local reservoirs; N_2O emissions from denitrification in reservoirs of São Paulo have been particularly high though, estimated at 180 kg km^{-2} yr^{-1}, due to the anoxic conditions formed in the reservoirs from the climate, further contributing to the accumulation of greenhouse gases in the atmosphere (Filoso *et al.*, 2003).

4.5.5 Nitrogen Emissions from Cane Burning

Historically, the cane crop in Brazil has been harvested manually (Engelhaupt, 2008). In manual harvesting, the trash is burned for ease and safety of the workers entering the fields to cut only the matured stalks; however, mechanical harvesting does not require burning since the entire stalk, including attached leaves and part of the trash blanket on the ground are all scooped into the harvester and brought to the mill (Waclawovsky *et al.*, 2010).

The government of Brazil passed legislation in 2002 to ban all pre-harvest burning by 2031, mainly for environmental reasons but also since the change to green cane harvesting could increase ethanol yields 60 – 135 liters per hectare by eliminating sucrose losses from exudation caused by burning (Braunbeck *et al.*, 1999). Phasing in this change, all fields except those with slopes greater than 15° and with natural barriers (e.g. rocky areas) must cease pre-harvest burning and harvest raw (green) cane by 2020. By 2031, the remaining fields must have converted to green harvesting as pre-harvesting burning will be prohibited (Brehmer and Sanders, 2009). In 2007 though, the sugarcane industry union in São Paulo State signed an agreement with over 100 mills to end burning in the region by 2017 (Engelhaupt, 2008).

Still, in the 2007/08 growing season, 53% of cane fields in Brazil were burned to enable manual harvesting while the remaining 47% were unburned and harvested mechanically (UNICA, 2008). The 2008/09 season in São Paulo saw a 50-50 split between harvesting with and without burning, despite their pledge (de Aguiar *et al.*, 2010). With 20,000 kg of trash per hectare, approximately 100 million kilograms of organic matter is burned each year in São Paulo State alone (Lara *et al.*, 2001).

The pre-harvest burning of trash is estimated to emit 25 – 55 kg ha^{-1} of nitrogen in a variety of forms, depending on the amount of nitrogen taken up by the cane plant (Allen *et al.*, 2004; Hartemink, 2008; de Oliveira *et al.*, 2000). When burning crop residues, generally, between 60 and 70% of nitrogen is released as NO_x, N_2O and NH_3 while 30 to 40% is N_2 (Kuhlbusch *et al.*, 1991). In addition to nitrogen oxides, large concentrations of CO and O_3 are released during burning (Kirchoff *et al.*, 1991). NO_x can also create and elevate ground level ozone under the right conditions (Englehaupt, 2008). The main concern with nitrogen oxide emissions though relates to acidification.

Cane burning has a highly significant effect on the chemistry of rainwater in the Piracicaba River Basin that dominates São Paulo State, leading to high rates of acid rain and wet deposition of nitrogen in the region (Lara *et al.*, 2001). Returning the nitrogen to the ground as acid rain can lead to acidification of nearby waterbodies in a matter of a few years, which could result in fish kills (Galloway, 2001). Ironically though, streams and rivers in São Paulo have been protected from acidification in the short-term by the high buffering capacity afforded these waterways by the large discharge of untreated sewage; however, the acid rain may take a larger toll on the clayey soil structure by replacing cations with H^+ ions (Marinelli and Filoso, 2008).

Trash burning can also release N_2O, estimated by Macedo *et al.* (2008) as 1.8 kg CO_2-equivalent per ton of cane burned; yet N_2O emission estimates for denitrification of N fertilizers and residues are 5 times higher than from burning. Still, trash burning contributes the largest amount of CO_2-equivalent greenhouse gas emissions when nitrous oxide and methane emissions are combined, overshadowing emissions from soil N_2O, liming, and diesel used in farm equipment (de Figueiredo *et al.*, 2010).

4.5.6 Removal in Harvest

Ranges in the literature for total N assimilated by sugarcane vary from 100 kg N ha^{-1} up to 250 kg N ha^{-1}, but all the studies similarly report that about 50% of total assimiliated N resides in the stalk at harvest (de Oliveira *et al.*, 2000; Bakker, 1999; Lima *et al.*, 1987). Thus, estimates of nitrogen removed from the field in the stalk depend on the total nitrogen assimilated by the crop and fluctuate between 50 and 125 kg N ha^{-1} for burned stalks; when left unburned, about 5% of the leaves are harvested with the stalks, meaning 55 – 138 kg N ha^{-1} are removed in the harvest. The remaining N is split into nearly equal thirds amongst the dry leaves (trash), flag leaves (green leaves at the top of the cane at harvest), and the roots, with the trash containing slightly more N than either the flag leaves or roots. The trash may either be burned or left in the field as residue depending on the harvest method; flag leaves are often left in the field as are the roots from which the ratoon crop grows (Lima *et al.*, 1987).

4.5.7 Retention and Recycling of Nitrogen

If the harvest method does not include trash burning, all of the nitrogen not removed from the field in the stalks gets recycled as surface layer leaf residue or in the roots (50 – 125 kg N ha^{-1}). Because of the high C:N ratio of the residues though, nearly all of the nitrogen becomes immobilized in the soil organic matter pool in the short-term (Basanta *et al.*, 2003). With trash burning, usually only the roots are recycled for the next crop, representing 20 – 40 kg N ha^{-1} (Lima *et al.*, 1987).

Biological nitrogen fixation can also add to N retention. Even when no N fertilizer is applied, the soil N balance at the end of the growing season remains positive in the top 20 cm and highly positive in the top 60 cm, indicating the ability of BNF to support cane growth without depleting soil N reserves (de Resende *et al.*, 2006). Soils in Brazil have been shown to accumulate nitrogen in the soil from BNF at rates between 35 and 80 kg N ha^{-1} yr^{-1} based on a nine-year study in northeastern Brazil (de Oliveira *et al.*, 1994). The addition of nitrogen fertilizers to the soil actually establishes the conditions for nitrogen to be lost from the agroecosystem over time as nitrogen accumulates in the soil and enhance the potential for erosion, leaching, and denitrification (de Resende *et al.*, 2006).

Chapter 5: Consequences for Ethanol

The physiological ability of the feedstocks to efficiently use nitrogen, the capacity of the soil to retain and supply nitrogen to the plants, and the management of nitrogen fertilizer applications all interact to determine the consequence of nitrogen use in ethanol production. The previous chapters have detailed a comprehensive rendering of the nitrogen necessary to sustain the agricultural production of corn and sugarcane for ethanol as well as the potential for nitrogen to escape the agroecosystem and create environmental degradation. Although ethanol can off-set greenhouse gas emissions from the production and burning of gasoline and provide some measure of national energy security, life cycle analyses barely include the environmental damage caused by the nitrogen required to grow the feedstocks, mentioning only the global warming potential of nitrous oxides emitted from the soil and applied nitrogen fertilizer (Kojima *et al.*, 2007). Yet, each liter of ethanol stamps a nitrogen footprint, and the ethanol industries in the U.S. and Brazil need to consider the impact of growing corn and cane for ethanol on the nitrogen cycle as the industry rapidly expands and contemplate were improvements can be made.

5.1 Processes for Corn Ethanol Production: Dry and Wet Milling

Corn can be transformed into ethanol through either wet milling or dry milling. However, 80% of corn ethanol is produced in dry milling systems since wet milling facilities are cost prohibitive and are more conducive for high starch products like high fructose corn syrup (Wang *et al.*, 2007; Bothast and Schlicher, 2005; Mosier and Ileleji, 2006). At the end of the milling process, the ethanol is blended with gasoline at 10% (E10) or 85% (E85) by volume.

In the wet milling process, the grain is first soaked in water-diluted sulphur dioxide to soften the kernels and loosen the hulls (Wheals *et al.*, 1999). Kernels are then degermed in order to extract oil from the germ before being ground to separate out the starch from other co-products like gluten (the gluten meal can then be sold as poultry feed) [Wang *et al.*, 2007; 'Soybean and Corn Advisor,' 2010]. Cooking the starch slurry, the starch becomes gelatinized, after which time alpha-amylase is added to break down amylose chains that are not easily transformed into glucose (Quintero *et al.*, 2008). Finally, glucoamylase converts the starch into glucose that can then be immediately fermented by the yeast *Saccharomyces cerevisiae* to ethanol (Wheals *et al.*, 1999).

In dry milling, the whole kernel is ground into powder (meal), and, without separating the starch, the powder is combined with water and processed in the same manner as wet milling. The starch is fermented into ethanol and distilled; the remaining unfermented portion becomes a useful bi-product called distiller grains with solubles (DGS) that are then fed to animals (Wheals *et al.*, 1999). Future dry mill ethanol plants may even use DGS to fuel the energy needs of the facility; the amount of energy (Btu) in the DGS produced by one liter of ethanol is greater than the amount of coal necessary to fuel the production of one liter of ethanol. Most new plants are powered by natural gas but the price of natural gas has forced some plants to convert back to coal power (Wang *et al.*, 2007).

The type of hybrid and the handling of the grain can make a difference in fermentability. Natural variation in the type of corn grain can streamline milling; since the grain of waxy corn hybrids contains only amylopectin, unlike normal corn grain that consists of amylose and amylopectin, the addition of alpha-amylase can be skipped (Smith *et al.*, 1994). Additionally, the largest seed companies, like Monsanto, are genetically engineering biofuel specific traits. Highly Extractable Starch (HES) hybrids are advertised to streamline the wet milling process while Highly Fermentable Corn (HFC) hybrids are meant to obtain higher ethanol yields in the dry milling plants (Monsanto, 2010). While HFC hybrids conceivably increase volumetric ethanol output by 3 – 5%, the marginal value to the processor is not large enough to justify paying premiums on these hybrids yet (AgriGold, 2010). Some processors pay premiums for lack of stress cracks and ideal moisture content though, which relate to the handling of the grain after harvest (Smith *et al.*, 1994).

As outlined in Ream *et al.* (2010), the storage conditions and grain drying process can affect the fermentability of the corn grain and subsequent ethanol yields, creating a disconnect in the relationship between starch content and ethanol yield. Prior to storage, the grain is dried in an oven to the desired soil moisture, but high oven temperatures (above 140˚F) can over-dry the grain and cause stress cracks. Furthermore, prolonged storage of the grain can allow opportunities for insect damage that lowers starch content and fungal infections that inhibit yeast growth during the fermentation process. Some of the variability in ethanol yield remains out of the grower’s control though. The packing of starch in the grain can differ among hybrids, limiting the accessibility of hydrolyzing enzymes to break down the starch. The non-starch components of the grain (oils, amino acids, vitamins) can help or hinder fermentation depending on the hybrid and the way in which these variables interacted with any environmental stressors (water, temperature, disease, nutrient deficiencies) during the growing season. For example, the amino acid glutamine released from the kernel serves as a nitrogen source for the growth of yeast during the fermentation process (Thomas and Ingledew, 1990).

5.2 Field to Pump: Nitrogen Used to Create Corn Ethanol

Multiple studies site the conversion ratio of corn biomass to ethanol as 2.8 gallons of ethanol per bushel of corn or the metric equivalent (Bothast and Schlicher, 2005; Wheals *et al.*, 1999; Hirel *et al.*, 2001). Converting the average corn yield for the 2008/09 growing season, listed as 153.9 bushels per acre in Table 4, to liters of ethanol produced per hectare using the 2.8 gal bu^{-1} ratio gives 4030.8 L ha^{-1}. While each hectare of corn generates 4030.8 liters of ethanol at the mill, the agricultural production of corn for this same ethanol allows an estimated 75 kilograms of nitrogen to escape into the atmosphere, groundwater, and surface water (Table 7). Thus, at least 18.6 grams of nitrogen enters the external environment for every liter of ethanol produced.

The losses of nitrogen from the agroecosystem can be reduced with improvements in N fertilizer management to enhance nitrogen use efficiency as outline in Chapter 4; however, long-term gains in upgrading the NUE of corn will come from the genetic modification of the crop itself, which will not occur until after 2015 according to the major biotechnology companies (Hirel *et al.*, 2001). Higher yields could help mitigate the nitrogen losses per liter of ethanol by increasing the production of ethanol per hectare, but even genetic modifications to corn yield are not expected until 2015.

In the meantime, the U.S. ethanol industry has been growing exponentially since 2003 and will continue to consume more bushels of corn until production levels off at 15 billion gallons of ethanol per year in accordance with the 2007 Renewable Fuels Standard [USDA, 2011]. To support 15 billion gallons of ethanol, 5.3 billion bushels of corn will consistently need to be cropped. At the 2008 yield level of 153.9 bushels acre^{-1}, 34 million acres (13.94 million hectares) will be necessary. This land area represents a 4.26 million hectare increase over the hectares in ethanol production in 2008; in order to keep the land area dedicated to corn ethanol production at 2008 levels, the corn yield would need to be almost exactly the theoretical potential solar maximum (calculated in Chapter 1). Additional land area inherently implies an expanded need for nitrogen fertilizers, which creates further opportunities for nitrogen accumulation in the agroecosystem and consequent losses to the environment. If the average amount of fertilizer per hectare, 165 kg N ha^{-1}, is applied to the supplementary 4.26 million hectares, another 0.703 Tg N is incorporated into the nitrogen cycle with the potential for significant losses perturbing the surrounding environment.

5.3 Process for Sugarcane Ethanol Production

Sugarcane can be processed into ethanol in two types of distilleries, either a distillery annexed to a sugar mill or an independent distillery (Rolz, 1981). Because of the advantages afforded by simultaneously producing sugar and ethanol, most distilleries are annexed to a sugar mill (Macedo *et al.*, 2008). Upon arrival to the plant, regardless of the type, the cane is washed, chopped, shredded, and subsequently pressed to extract the sucrose-containing juice. The juice is filtered, creating a fluid called vinasse and particulates called filter cake (‘Soybean and Corn Advisor,’ 2010). In combined sugar-ethanol plants, the cane juice is concentrated until sucrose crystals form, which can then be removed by centrifugation, leaving only molasses behind. The molasses, containing 65% sucrose, can then be fermented with *Saccharomyces cerevisiae* to ethanol (Wheals *et al.*, 1999). Similar to corn grain, the availability and complexity of nitrogen sources in the form of amino acids affects the fermentation performance of *Saccharomyces cerevisiae* yeast (Junior *et al.*, 2009). In independent distilleries, the juice is simply fermented after having been filtered and sterilized. After fermentation, the water is removed from the ethanol through distillation, creating hydrous ethanol, which, despite being used in flex fuel vehicles and 100% alcohol-fueled cars, can contain up to 4.9% water; further dehydration can then generate anhydrous ethanol that is blended with gasoline at 24% by volume (E24) [Macedo *et al.*, 2008].

The processing of sugarcane into ethanol creates useful bi-products that allow the system to remain relatively self-sustaining, if managed properly. Firstly, the sugarcane bagasse (fibrous stalk material) is burned for electricity generation to fuel the sugar-ethanol plant (or individual ethanol facility), supplying all the required energy for both processes and often providing surplus energy to the grid (Ensinas *et al.*, 2008). In addition to the burning of bagasse to power the distillery, the conversion to green cane harvesting will allow the trash transported to the mill to be burned for fuel as well, further enhancing the energy surplus from sugarcane processing (Macedo *et al.*, 2008). Secondly, in the cane juice filtration process, one liter of ethanol generates 10 – 15 liters of vinasse (or sillage): a viscous, acidic, and high biological oxygen demand (BOD) bi-product (Cortez and Brossard Perez, 1997). Although against the law, it is not uncommon for small mills to discharge vinasse into local waterways or for larger mills to have leakage of vinasse into waterways from improper storage (Martinelli and Filoso, 2008).

Because of its high BOD, the emptying of vinasse directly into waterways can create anoxic conditions and significantly affect biodiversity, but the industry attempts to recycle most vinasse back to the cane fields to be used as a potassium-rich, yet nitrogen poor, fertilizer (Rosillo-Calle and Hall, 1987; Walter, 2009). Thirdly, the washing of cane prior to milling generates large quantities of warm wastewater with high levels of dissolved nitrogen, carbon, and particulate matter that is often discharged directly into waterways (Appendix: Figure G) [Martinelli and Filoso, 2008]. If recycled with vinasse back to the fields though, the wastewater can act as a low-grade nitrogen fertilizer.

5.4 Field to Pump: Nitrogen Used to Create Cane Ethanol

Over the last decade, Brazil has been able to completely met its domestic ethanol needs with cane ethanol and has beyond its own consumption by steadily increasing ethanol exports from 94.8 million liters in 2000/01 to 4.69 billion liters in 2008/09 (Brazil Ministry of Agriculture, Livestock, and Food Supply, 2009). Productivity, in tons per hectare, has had limited improvements, so major gains in overall yield have come from the dramatic increase in planted area devoted to sugarcane: when the ProAlcool Programme began in 1975, sugarcane was planted on 1.9 million hectares and yielded 46.82 tons ha^{-1}, and now, in 2008, 8.92 million hectares were planted with sugarcane and yielded 77.52 tons ha^{-1}, representing a 66% increase in yield and a 370% increase in planted area (Brazil Ministry of Agriculture, Livestock, and Food Supply, 2009).

Similar to corn production, an augmentation of the land area dedicated to cane production brings inherent increases in overall nitrogen applied as well as nitrogen fixed by the cane. In the short-term, nitrogen fixed by endophytes associated with sugarcane may recycle in the agroecosystem, but this source of reactive nitrogen still draws in unreactive N_2 into the reactive N cycle, where the nitrogen will continue to cascade through the environmental in the long-term until denitrified back to N_2. Thus, BNF introduces nitrogen into the system in the same way as the Haber-Bosch process with synthetic fertilizer.

Because the majority or all of cane N demand can be satisfied by biological nitrogen fixation, improving N fertilizer use efficiency may not have significant impacts on reducing nitrogen losses from the agroecosystem. Instead, to combat the extra BNF coupled to the increase in land area to support ethanol production, N fertilization should be nearly eliminated in favor of carefully selecting cultivars capable of satisfying N needs while maintaining yield for the given environmental conditions. Alternatively, the introduction of additional nitrogen and the potential for nitrogen losses could be simultaneously abated if ethanol yield was directly enhanced at the distillery by using the whole plant biomass for ethanol instead of only the sucrose. The hydrolysis of cane trash and bagasse in conjunction with sucrose, essentially combining first and second generation ethanol technologies, could lead to an output of 13,000 liters of ethanol per hectare, instead of the 6357 liters per hectare realized in the 2008/09 growing season in Brazil (conversion factor of 82 L per ton cane with a yield of 77.52 t ha^{-1}) [UNICA, 2008; Waclawovsky, 2010; Hartemink, 2008]. By attacking nitrogen losses per liter of ethanol produced from the angle of utilizing the whole plant biomass, losses from the agroecosystem can be diminished from 13.2 g N L^{-1} (with burning) and 5.3 g N L^{-1} (without burning) to only 2.6 g N L^{-1}.

5.5 Recommendations for the Future Ethanol Industry

Through a comprehensive analysis of plant nitrogen use and field-level nitrogen management, this paper has clearly answered the question of which feedstock least impacts the nitrogen cycle due to losses of N from the agroecosysem. As hypothesized, Brazilian sugarcane realizes significantly less losses to the environment per liter of ethanol produced than U.S. corn. Sugarcane performs better than corn in every category, from average yield of ethanol in liters per hectare to N applications per hectare to total and per liter losses of N from the agroecosystem (Table 9). While the association between N-fixing bacteria and the sugarcane plant reduces the overall demand for nitrogen fertilizer, this relationship is maximized through critical management decisions, such as appropriate selection of cultivars for the particular soil in which it is grown and finding the proper balance of N fertilizer to leverage BNF while still enhancing yields.

Table 9. Comparison of Ethanol Production from Corn and Sugarcane in 2008/09

	Corn	Sugarcane
Yield of crop (tons ha^{-1})	9.65	77.52
Ethanol conversion factor (liters ton^{-1})	417	82
Average Yield of Ethanol (liters ha^{-1})	4031	6357
Total Ethanol Yield (billion liters)	38.98	27.59
Average N fertilizer applied (kg N ha^{-1})	165	55 (P) 90 (R)[1]
Average N loss from 1 liter of ethanol (g N loss per L ethanol)	18.6	13.2 (B) 5.3 (NB)[2]
Average Total N loss from the Agroecosystem (Tg N)	0.919	0.321 (B), 0.148 (NB)[2]

[1] P = plant cane, R = ratoon cane
[2] B = pre-harvest burn, NB = no burning

Additionally, the harvest method of sugarcane greatly impacts the amount of nitrogen lost from the agroecosystem; the switch to mechanical harvesting from burning not only directly reduces the average N loss per liter of ethanol by more than half (Table

9), but also provides added indirect benefits. Biomass in the form of cane trash could be burned with bagasse to further improve the already highly positive net energy balance of ethanol from sugarcane or, eventually, could be turned into ethanol itself when cellulosic technologies are commercially available, increasing ethanol yield per hectare by up to 40% while still utilizing the same levels of nitrogen fertilizer [Goldemberg, 2008; Waclawovsky *et al.*, 2010].

These management decisions surrounding cultivar selection and harvest method for sugarcane exhibit possibilities for substantial improvements to N losses from the agroecosystem, even past what sugarcane offers as an N-fixing crop. Conversely, the improvement of N losses from the corn agroecosystem must depend on significant genetic modifications to the plant itself. Even though management decisions like plant population density and row spacing design can enhance yield per hectare, the hybrid selected must be capable of strongly competing for resources, which is genetically manipulated through components such as root structure and leaf angle. Advances in N management at the field level could be made with careful consideration of crop rotation, timing N application to meet plant demand, and soil N testing to avoid over-fertilization. Though, even these improvements would not alter the high N requirement and low nitrogen use efficiency of corn. Unfortunately, genetically engineering corn hybrids for high NUE has proved extraordinarily difficult, and the commercial availability of nitrogen-efficient corn hybrids is still at least 5 to 10 years away (Birger, 2011). Thus, genetic engineering has focused on improving ethanol yield from corn via starch fermenability and, as of 2011, through including processing enzymes in the grain itself.

Classically, the same types of corn grown for animal feed and processed foods are also the feedstock for ethanol, specifically dent and waxy corn varieties (Khullar *et al*., 2009). Only in February of 2011 did the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) deregulate the first corn specifically engineered for ethanol (Bell and McNally, 2011). Developed by Syngenta, the ethanol-specific 'Enogen' corn variety contains the alpha-amylase enzyme in the grain ("Enogen FAQ," 2011). While microbial alpha-amylase is typically added in the liquidfaction stage of ethanol production to hydrolyze the starch into glucose, expressing the enzyme directly in the grain makes the conversion process more efficient, increasing ethanol yield up to 8% while simultaneously reducing energy and water consumption (Pollack, 2011; Huberty, 2008).

While the U.S. Food and Drug Administration has approved the Enogen corn variety for human and animal consumption, milling and food-processing groups object to the use of a seed that could affect the quality of their operations ("Enogen FAQ," 2011). The North American Millers' Association contends that cross-pollination in the field or mixing at the mill of Enogen corn with corn used for food could degrade the quality of many products. Because alpha-amylase weakens the starch structure in the dough, even one kernel of Enogen corn in 10,000 conventional kernels could lead to "crumbly corn chips, soggy cereal, [and] loaves of bread with soupy centers" (Pollack, 2011, p. 1). In order to avoid contamination of corn for food processing, the distribution of Enogen seeds remains limited (<1% of U.S. corn acres), as this ethanol-specific corn must be surrounded by conventional corn hybrids dedicated to ethanol production ("Enogen FAQ," 2011). Thus, the highly fermentable corn varieties made by Monsanto for enhancing ethanol yield are more widely grown since the corn can be funneled towards animal feed, food processing, milling, or ethanol without any concerns.

While neither the HFC nor the Enogen seeds promise greater grain starch content, which would potentially require more nitrogen uptake to sustain, the effect of these genetic modifications on plant nitrogen requirements has yet to be determined. Currently, devoting more corn acres growing dent corn to ethanol will not vastly change nitrogen use in the Corn Belt. Firstly, dent corn is planted on over 80% of corn acres, with the remaining 20% consisting of specialty corns, like sweet and waxy corns, and silage corn (Huberty, 2008). Thus, as corn acres for ethanol expand, it is most likely that current dent corn acres dedicated to animal feed or milling would be re-assigned as ethanol acres instead of specialty and silage corn acres being switched to dent corn in large enough percentages to impact overall N use in the Corn Belt. Secondly, even though dent corn, the present feedstock for ethanol, typically takes up 20% more nitrogen than some other corn varieties like sweet corn, nitrogen fertilizer recommendations are still relatively uniform across corn types (Dahnke *et al.*, 1992). Despite lower nitrogen demand, average nitrogen applications for sweet corn range from 120 to 195 kg N ha^{-1}, which remains within the same range as N rates for dent corn but implies lower fertilizer use efficiency (Fritz *et al.*, 2010; Schultheis, 1998; Carter *et al.*, 1989). While nitrogen fertilizer rates do not vary significantly across typical corn types today, the potential for wide-spread substitution of general dent corn for ethanol-specific corns like Enogen commands further research on the nitrogen requirements of corn like Enogen and how its cultivation will affect fertilizer use in the Corn Belt.

Even as new ethanol-specific corn hybrids hit the market, the question remains if corn actually is the best ethanol feedstock option in the United States. Second generation feedstocks, such as switchgrass, algae, or corn stover, provide seemingly plausible alternatives for producing ethanol; yet, the technology for each of these feedstocks is either not ready at a commercial scale or extremely cost-prohibitive such that the actual implementation of second generation biofuels will be limited in the short term (Groom *et al.*, 2008). Still, each of the second generation feedstocks provides an opportunity to reduce the amount of reactive nitrogen produced through the Haber-Bosch process by replacing corn as the primary U.S. ethanol feedstock.

Grown in the Midwestern U.S., switchgrass can reach yields between 5 and 13 Mg ha^{-1} using only 55 – 120 kg N ha^{-1}, which would supply between 1,900 and 5,000 liters of ethanol per hectare, similar to the 4,000 liters ha^{-1} ethanol yield of corn (Sanderson and Reed 2000; Vogel *et al.*, 2002; Lemus *et al.*, 2008; Schmer *et al.*, 2007). Soil N levels remain constant with less than 120 kg N applied per hectare, unlike corn that mines N from the soil over time (Vogel *et al.*, 2002). Perhaps even more attractive as an ethanol feedstock, the perennial grass *Miscanthus* can reach yields upwards of 30 Mg ha^{-1} in the Midwestern U.S. using 25 kg N ha^{-1}, which could provide about 11,500 liters of ethanol per hectare, nearly quadrupling the ethanol yields derived from corn (Costello *et al.*, 2009). Corn acres could be rotated with switchgrass or *Miscanthus* in order to reduce N use in the Midwestern U.S. and improve ethanol yields, but these perennial grasses need three years in the ground to be profitable and the technologies for cellulosic ethanol production are not commercially available, giving corn the competitive advantage in the short-term (Kheshgi *et al.*, 2000).

Capable of using marginal lands and nutrient-rich wastewater for cultivation, algae also could be a beneficial alternative to corn for ethanol production (Clarens *et al.*, 2010). Although only the carbohydrates can be made into ethanol while the algal oils make biodiesel, which is not blendable with gasoline. Additionally, current technologies are extremely costly as 1 barrel of ethanol from algae costs between $300 and $2600. Yet algae has the potential to limit overall land usage for ethanol production while simultaneously lowering the amount of N fertilizer required to sustain the operation.

Since technologies for feedstocks requiring significantly less nitrogen than corn are not yet available, better utilizing the entirety of the corn plant through the collection of corn stover could improve ethanol yields per hectare for corn as a feedstock. Despite the availability of over 200 million dry tons of stover per year, which could triple ethanol production from corn, the use of stover as a feedstock is limited by the evolution of no-till cropping, farmers' resistance to participation, and expensive technologies for collecting the corn stover (Erickson and Carr, 2009).

Still, none of these alternatives show more promise for conserving nitrogen than sugarcane. Additionally, for 1000 liters of ethanol, the production costs are 2.7 times greater for U.S. corn than Brazilian sugarcane. While the heftiest costs are incurred from procuring the feedstock, the cost differential results from the higher prices for equipment, labor, and other operational costs in the United States. The sale of byproducts from ethanol production and government subsidies make ethanol from U.S. corn competitive with ethanol from Brazilian sugarcane, but net production costs still favor Brazilian ethanol by a margin of $10.00 (Goldemberg, 2008).

With a cost advantage over ethanol production from corn as well as substantially less impact on the nitrogen cycle through losses of N to the environment, Brazilian sugarcane should be the top ethanol feedstock over corn. The U.S. should prioritize the importation of Brazilian sugarcane until 2nd generation biofuel technologies are commercially available. Removing the 54 cent tariff on Brazilian sugarcane and the 38 cent subsidy for corn would allow the market price of ethanol to drive the source of the feedstock, which would favor Brazilian sugarcane (Eaves and Eaves, 2007). Still, Brazilian cane ethanol alone cannot meet all of the U.S. transportation fuel demand. Brazil is capable of fulfilling all of its own ethanol fuel needs using less than 2% of its arable land for cane devoted to ethanol, but producing enough ethanol for export to the U.S. just to meet the Renewable Fuel Standard of 57 billion liters (15 billion gallons) would require 110% of Brazilian sugarcane hectares and 13% of the arable land of the country (EIA, 2011; FAOSTAT, 2011). However, to meet the same goal using U.S. corn would require 14.1 million hectares, or 44% of U.S. corn acres and 8% of arable land. Thus, some combination of the two feedstocks would be necessary to meet the ethanol demand of both countries, but the real market price of ethanol should drive this ratio instead of the U.S. government.

If national energy security is motivating the choice for the use of corn ethanol in the United States, then serious research should be invested in planting tropical corn in the temperate Midwest to be grown and processed for ethanol like sugarcane. While cultivating sugarcane in the U.S. (i.e. Florida, Louisiana, Hawaii) requires upwards of 300 kg N ha^{-1}, growing tropical corn in temperate regions could produce a sucrose-rich stalk similar to sugarcane using less nitrogen than current corn hybrids (Baldani *et al.*, 2002; White, 2010). The long summer days of the U.S. Corn Belt would prohibit tropical corn from creating an ear as flowering would be delayed in the short-day tropical corn; without the grain as a sink for carbon and nitrogen assimilates stored in the leaves and stalk, the photoassimilates are stored as sucrose in the stalk (White, 2010). The lack of grain also means that the tropical corn requires less nitrogen since the focus is on biomass yield instead of grain yield. Additionally, the tropical corn stalks require less processing than corn grain and can be processed similar to sugarcane, burning the leftover biomass to power the ethanol facility after extracting the sucrose (Below and Biopact Team, 2007). Therefore, either revamping the corn landscape to include tropical corn hybrids or importing Brazilian sugarcane provide the best models for the future sustainability of the ethanol industry while minimizing the impact on the global nitrogen cycle.

APPENDIX

Figure A. End uses for U.S. Corn. (Source: Baker and Allen, 2009).

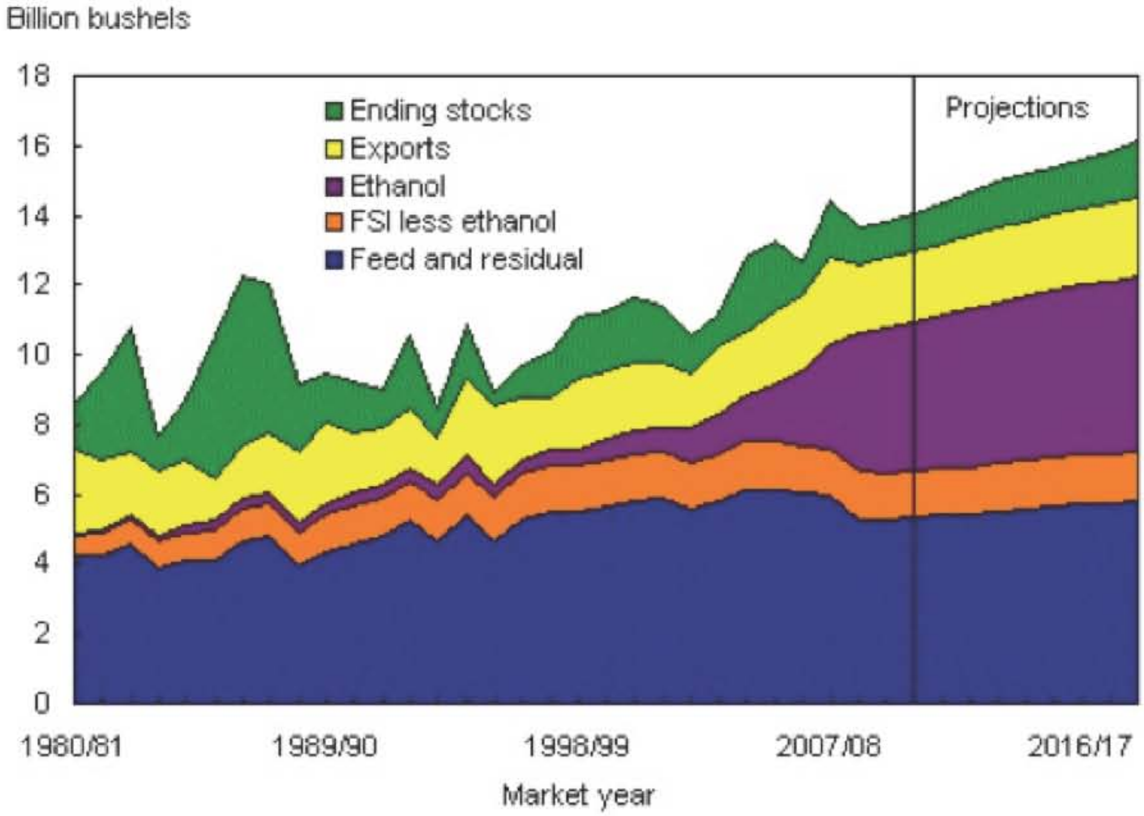


Figure B. Process of sucrose storage in sugarcane tissues.
(Source: Glasziou and Gayler, 1972).

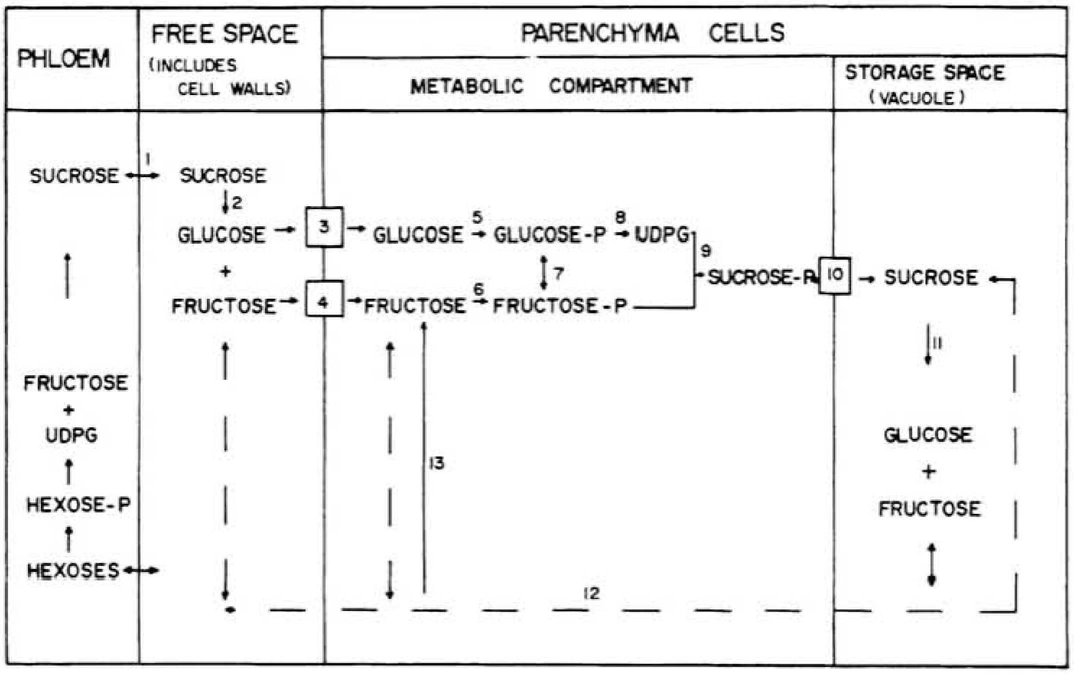


FIG. 1. The sugar cycle in sugar cane storage tissue. 1. Movement of sugars between phloem and free space (which includes cell walls). 2. Hydrolysis of sucrose in free space by acid invertase. 3. Carrier-mediated transfer of glucose into metabolic compartment. 4. Carrier-mediated transfer of fructose into metabolic compartment. 5, 6 and 7. Hexose phosphorylation and interconversion. 8. Synthesis of uridine diphosphate-glucose. 9. Synthesis of sucrose phosphate. 10. Transfer of sucrose moiety of sucrose phosphate into storage. 11. Hydrolysis of stored sucrose by acid invertase (immature tissue only). 12. Diffusional movement of sugars in the direction of the prevailing gradient. 13. Hydrolysis of sucrose by neutral invertase.

Figure C. Estimates of BNF associated with sugarcane using ^{15}N isotope dilution and N balance techniques. ^{15}N isotope dilution method used ^{15}N-labelled soil organic matter as the substrate for growing 10 varieties of sugarcane in concrete tanks. *Brachiaria arrecta* was the non-N_2-fixing control plant. N balance method described in table footnotes (Source: Boddey *et al.*, 1991).

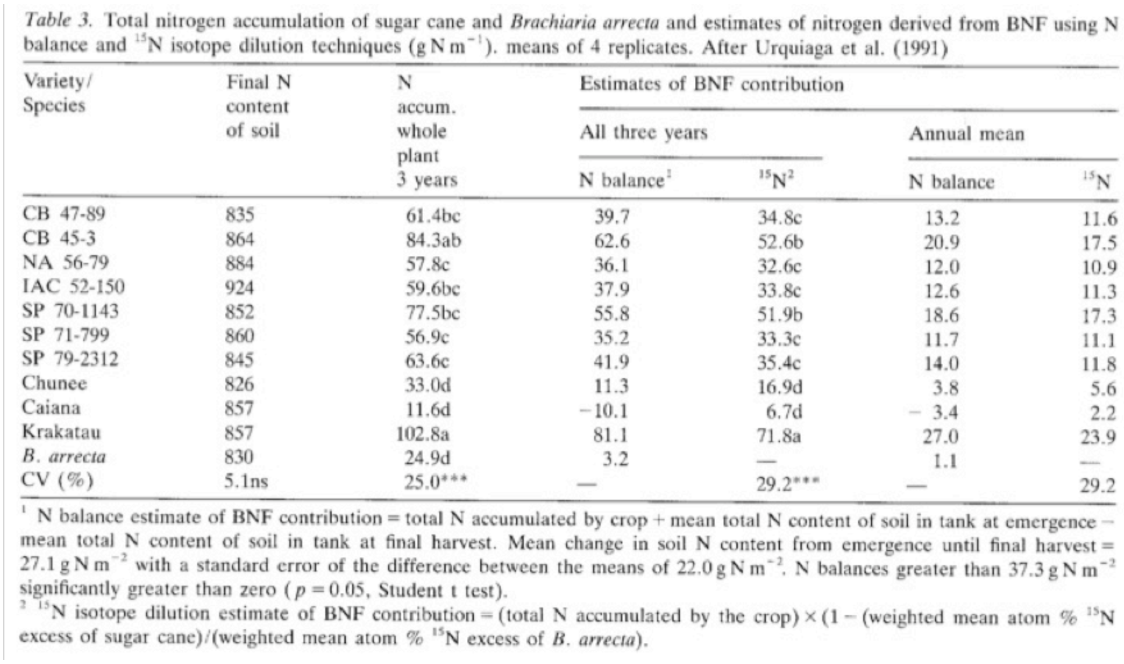


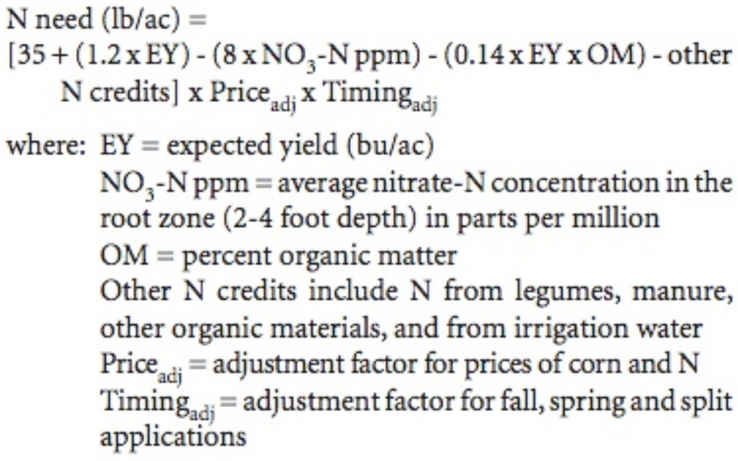
Table 3. Total nitrogen accumulation of sugar cane and *Brachiaria arrecta* and estimates of nitrogen derived from BNF using N balance and ^{15}N isotope dilution techniques ($g\,N\,m^{-1}$). means of 4 replicates. After Urquiaga et al. (1991)

Variety/ Species	Final N content of soil	N accum. whole plant 3 years	Estimates of BNF contribution			
			All three years		Annual mean	
			N balance[1]	^{15}N[2]	N balance	^{15}N
CB 47-89	835	61.4bc	39.7	34.8c	13.2	11.6
CB 45-3	864	84.3ab	62.6	52.6b	20.9	17.5
NA 56-79	884	57.8c	36.1	32.6c	12.0	10.9
IAC 52-150	924	59.6bc	37.9	33.8c	12.6	11.3
SP 70-1143	852	77.5bc	55.8	51.9b	18.6	17.3
SP 71-799	860	56.9c	35.2	33.3c	11.7	11.1
SP 79-2312	845	63.6c	41.9	35.4c	14.0	11.8
Chunee	826	33.0d	11.3	16.9d	3.8	5.6
Caiana	857	11.6d	−10.1	6.7d	− 3.4	2.2
Krakatau	857	102.8a	81.1	71.8a	27.0	23.9
B. arrecta	830	24.9d	3.2	—	1.1	—
CV (%)	5.1ns	25.0***	—	29.2***	—	29.2

[1] N balance estimate of BNF contribution = total N accumulated by crop + mean total N content of soil in tank at emergence − mean total N content of soil in tank at final harvest. Mean change in soil N content from emergence until final harvest = $27.1\,g\,N\,m^{-2}$ with a standard error of the difference between the means of $22.0\,g\,N\,m^{-2}$. N balances greater than $37.3\,g\,N\,m^{-2}$ significantly greater than zero ($p = 0.05$, Student t test).

[2] ^{15}N isotope dilution estimate of BNF contribution = (total N accumulated by the crop) × (1 − (weighted mean atom % ^{15}N excess of sugar cane)/(weighted mean atom % ^{15}N excess of *B. arrecta*).

Figure D. Yield-goal equation adapted for nitrate, SOM testing, price, and timing. $Price_{adj}$ refers to the effect of the N:corn price ratio on MRTN. $Timing_{adj}$ considers the loss of nitrogen from fall applications (factor 1.05) and gain from split applications (factor 0.95) [Source*:* Shapiro *et al.,* 2008]



N need (lb/ac) =

$$[35 + (1.2 \times EY) - (8 \times NO_3\text{-N ppm}) - (0.14 \times EY \times OM) - \text{other N credits}] \times Price_{adj} \times Timing_{adj}$$

where: EY = expected yield (bu/ac)

NO_3-N ppm = average nitrate-N concentration in the root zone (2-4 foot depth) in parts per million

OM = percent organic matter

Other N credits include N from legumes, manure, other organic materials, and from irrigation water

$Price_{adj}$ = adjustment factor for prices of corn and N

$Timing_{adj}$ = adjustment factor for fall, spring and split applications

Figure E. Soil types of Brazil. (Source: Bernoux *et al.*, 2000).

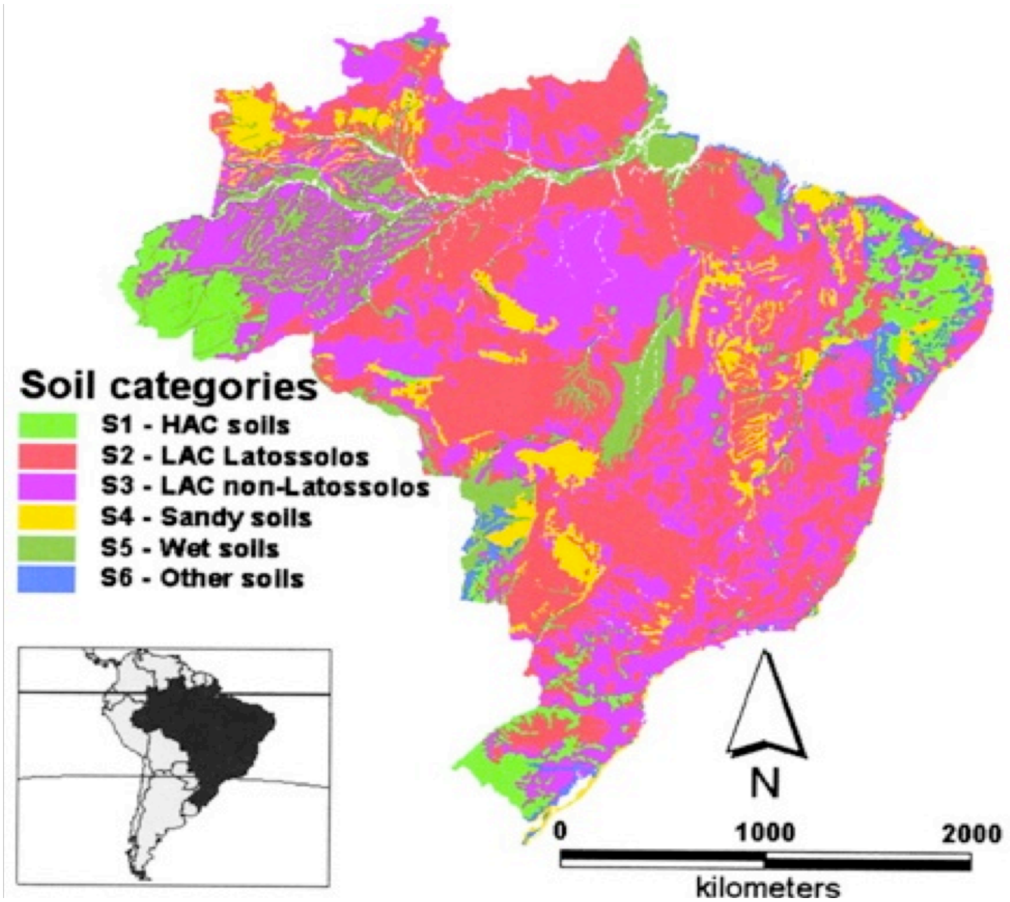
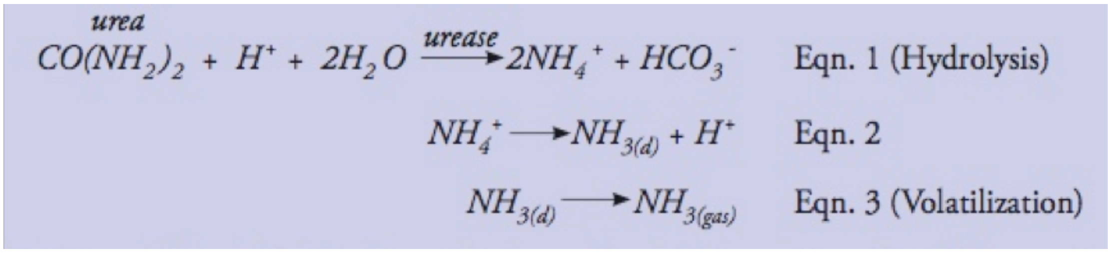


Figure F. Chemical reactions for the breakdown of urea fertilizer. When H^+ ions are available in the soil, urea hydrolyzes to ammonium (Eqn. 1) and can subsequently be converted to either nitrate or dissolved ammonia (Eqn. 2). If the reaction occurs on the soil surface, dissolved ammonia may be released as ammonia gas (Eqn. 3). When H^+ ions are scarce though (high pH), urea reacts with water to form ammonia and carbon dioxide directly [$CO(NH_2)_2 + H_2O + \text{urease} \rightarrow 2NH_3 + CO_2$] (Source: Jones, *et al.*, 2007).

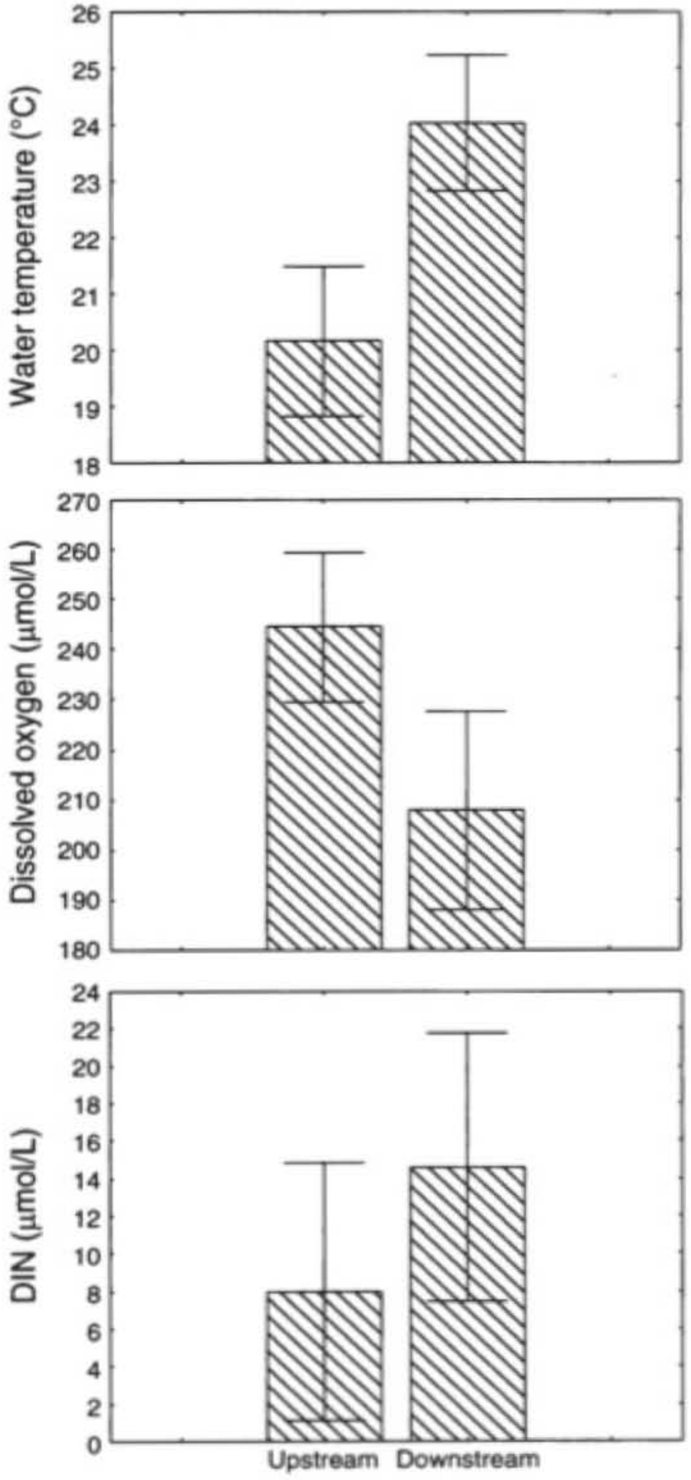


$$\overset{urea}{CO(NH_2)_2} + H^+ + 2H_2O \xrightarrow{urease} 2NH_4^+ + HCO_3^- \quad \text{Eqn. 1 (Hydrolysis)}$$

$$NH_4^+ \longrightarrow NH_{3(d)} + H^+ \quad \text{Eqn. 2}$$

$$NH_{3(d)} \longrightarrow NH_{3(gas)} \quad \text{Eqn. 3 (Volatilization)}$$

Figure G. Effect of a Sugarcane Mill in São Paulo on Water Parameters. Measurements taken from upstream and downstream of the mill. DIN = dissolved inorganic nitrogen (Source: Martinelli and Filoso, 2008).



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