



Soil nitrogen availability indices as predictors of sugarcane nitrogen requirements



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ABSTRACT

Nitrogen recommendation systems for sugarcane (*Saccharum* spp.) generally does not consider the N supply from soil. Identifying a reliable soil test for estimating N availability is crucial to avoid yield losses or environmental pollution. Therefore, the objective of this study was to correlate and calibrate N availability indices with field-based measures of soil N supply. Between 2006 and 2013, 15 trials for rate–response to N fertilizer by sugarcane ratoons were performed in São Paulo, the main sugarcane-producing state in Brazil. The following indices were tested: KMnO_4 oxidizable C, hot KCl extractable N, phosphate–borate buffer distillable N, NaOH distillable N, Illinois Soil N Test, organic C, total N, mineral N, anaerobic incubation, soil respiration, substrate-induced respiration, microbial biomass C, metabolic quotient, microbial quotient, and gross N mineralization. The indices were then correlated with sugarcane yield (Y_{ON}) and N content of the crop (N_{ON}) in N-unfertilized plots, relative yield (RY), and the N rate predicted to achieve 90% of the RY (NR 90%RY). Although weak correlations were found between Y_{ON} with anaerobic incubation, total N, and soil respiration, as well as between N_{ON} and anaerobic incubation, no index correlated with RY or NR 90%RY. Grouping sites based on soil texture or byproduct management did not improve prediction of RY. Therefore, we concluded that none of the fifteen laboratory indices is a reliable predictor of soil N supply, and hence could not be used to adjust N fertilization rate for sugarcane.

1. Introduction

Sugarcane (*Saccharum* spp.) is one of the most robust feedstock for ethanol production because of its large positive energy balance and lower greenhouse gas emissions as compared to other crops such as corn (*Zea mays* L.), sugar beet (*Beta vulgaris* L.), and wheat (*Triticum aestivum* L.; Goldemberg et al., 2008; Long et al., 2015). To ensure optimum yields, N fertilizer additions are a common practice by farmers, with N rates varying from 60 to 120 kg ha⁻¹ in Brazil, 150–400 kg ha⁻¹ in China, and 100–755 kg ha⁻¹ in India, the three largest sugarcane producer countries (Spironello et al., 1997; Robinson et al., 2011). Although N fertilization may increase the crop yield (Otto et al., 2016), the accurate quantification of mineralizable N from organic fractions is fundamental because the soil, unlike fertilizer, is the main N source for sugarcane (Prasertsak et al., 2002; Franco et al., 2011). In addition, N recommendation systems (e.g., target yield concept) usually does not account for the soil N supply through organic matter mineralization, thus leading to yield losses and environmental

concerns associated with under- and overfertilization. Therefore, soil tests for predicting N availability have been the focus of research in past decades, but to date, the results have been heterogeneous and inconclusive (Bundy and Meisinger, 1994; Griffin, 2008; Ros et al., 2011).

Most laboratory indices developed for estimating soil N mineralization can be grouped into two categories: biological and chemical (Griffin, 2008; Ros et al., 2011; St. Luce et al., 2011). One of the most widely used biological indices is long-term aerobic incubation (Stanford and Smith, 1972). In this procedure, potentially mineralizable N (N_0) represents the soil N fraction susceptible to mineralization under optimal conditions of temperature and moisture. However, it is a time-consuming protocol (~8 mo of incubation) and therefore not suitable for routine soil analysis. In contrast, net N mineralization obtained through short-term anaerobic incubation (ANI–N) is performed by incubating soil samples under anoxic conditions for 7 d, followed by NH_4^+ analysis (Keeney and Bremner, 1966). The correlation between N_0 and ANI–N is usually strong (Soon et al., 2007; Mariano et al., 2013).

For chemical indices, acid and alkali solutions, as well as strong

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oxidizing agents are used to extract mineral and/or organic N fractions from the soil. Chemical indices developed until mid-1981, such as hot KCl extractable N (HKCl-N; Gianello and Bremner, 1986) and phosphate-borate buffer distillable N (PBB-N; Gianello and Bremner, 1988) have been extensively tested and satisfactorily estimated available and potentially mineralizable N (Cantarella et al., 1994; Jalil et al., 1996; Nayyar et al., 2006; Schomberg et al., 2009), although unsuccessful results were also reported (Hong et al., 1990; Sharifi et al., 2007a; Nyiraneza et al., 2012). It has been suggested that HKCl-N measures soluble and exchangeable NH_4^+ as well as hydrolyzable N (St. Luce et al., 2011). Regarding PBB-N, besides soluble and exchangeable NH_4^+ , some soil amino acids and amino sugars have also been quantified (Gianello and Bremner, 1988). Khan et al. (2001) proposed the Illinois Soil Nitrogen Test (ISNT-N), which estimates NH_4^+ and amino sugar content through direct diffusion of soil samples treated with NaOH in an adapted glass jar. Later, Bushong et al. (2008) and Roberts et al. (2009) developed an alternative protocol (NaOH distillable N – NaOH-N) to ISNT-N, replacing the use of glass jars with Kjeldahl distillation units. However, calibration results of ISNT-N and NaOH-N for predicting rice (*Oryza sativa* L.) and corn responses to N fertilization in the USA are highly heterogeneous, with cases of success (Klapwyk and Ketterings, 2006; Mulvaney et al., 2006; Williams et al., 2007; Roberts et al., 2011, 2013), but also failure (Barker et al., 2006; Laboski et al., 2008; Osterhaus et al., 2008; Spargo et al., 2009). For sugarcane, NaOH-N and ISNT-N were effective in identifying responsive and nonresponsive sites to N fertilization (Otto et al., 2013). Another promising chemical index is KMnO_4 oxidizable C ($\text{KMnO}_4\text{-C}$), which possibly quantifies highly labile fractions of soil organic C (OC) using permanganate as an oxidant (Culman et al., 2012).

Microbiological indices are less common than biological and chemical methods, but they have attracted recent interest (Franzuebbers, 2016). Although several protocols have been proposed to determine the microbial biomass C (MBC), the fumigation-incubation and fumigation-extraction techniques are the most widely used (Jenkinson and Powelson, 1976; Vance et al., 1987). Soil respiration (SR), in turn, involves the quantification of CO_2 produced by soil samples incubated under controlled conditions of moisture and temperature, and is directly associated with C mineralization (Anderson, 1982). Similarly, induction of microbial respiration through the addition of an easily decomposable carbohydrate (e.g., glucose) is defined as substrate-induced respiration (SIR; Anderson, 1982). Furthermore, other important parameters can be derived from MBC and SR, such as metabolic ($q\text{CO}_2$) and microbial quotients ($q\text{Mic}$; Insam and Domsch, 1988; Insam and Haselwandter, 1989). However, results of microbiological indices as predictors of soil N supply are heterogeneous and limited, particularly when crop parameters have been used for correlation and calibration (Franzuebbers et al., 2000; Sharifi et al., 2007a,b; Schomberg et al., 2009).

Unlike net N mineralization, the use of ^{15}N pool dilution techniques allows gross N mineralization (GNM) to be studied independently of the processes that consume NH_4^+ , such as volatilization, immobilization, nitrification, and plant uptake (Barraclough and Puri, 1995; Murphy et al., 2003). In this technique, the soil NH_4^+ pool is initially enriched in ^{15}N , with subsequent incubation of the sample for ~4 d. The GNM is then measured considering the decrease in ^{15}N enrichment and change in the size of the NH_4^+ pool. Although this index is not intended for routine use owing to its high cost and complexity, it might be used as a standard index for the evaluation of more economical and simple soil tests, if its ability for predicting crop N requirements is proven. Otto (2012) reported that among some indices, GNM was the best predictor of responsive and nonresponsive sites to applied N in sugarcane.

Calibration of N availability indices through crop parameters, primarily relative yield (RY), has been intensified after publication of the study on ISNT-N by Khan et al. (2001). In the past, the vast majority of studies have used aerobic and anaerobic soil incubations as reference indices for the correlation and calibration of potential soil N tests

(Keeney and Bremner, 1966; Gianello and Bremner, 1986; Wang et al., 2001). Although this procedure is still frequently adopted (Sharifi et al., 2007a,b; Soon et al., 2007; Schomberg et al., 2009; Nyiraneza et al., 2012; McDonald et al., 2014), a note of caution arises on using biological incubations as standard measures: they are somewhat inconsistent on predicting crop parameters (Khan et al., 2001; Griffin, 2008; Nyiraneza et al., 2012; Otto et al., 2013). Moreover, the following criticisms of incubation results have been reported: (i) a lack of fluctuations in temperature and dry-wet cycles; (ii) the absence of plant roots, as the rhizosphere alters the microbial diversity, soil moisture, and nutrient cycling; and (iii) values of N_0 and the mineralization rate constant (k) are dependent on incubation time, temperature, and moisture (Griffin, 2008; St. Luce et al., 2011).

A soil test to predict N fertilization requirements is crucial for sugarcane, since it can lead to economic, agronomic, and environmental benefits. Since this crop has a long growth cycle (~12 mo for ratoons), plant dependence on the soil N supply is irrefutable and essential to meet crop demand. Therefore, the objectives of this study were: (i) to assess the ability of biological, chemical, microbiological, and isotopic indices to correlate with sugarcane parameters; and (ii) calibrate N application rate using soil tests for sugarcane.

2. Material and methods

2.1. Experimental sites and soil sampling

Fifteen rate-response curve trials to N fertilization by sugarcane ratoons, under rainfed conditions, were performed in the main sugarcane-producing regions in São Paulo, Brazil, between 2006 and 2013 (Table 1; Suppl Fig. 1). Crop planting is performed *ca.* every six years and harvested annually. In a diversity of sites (7, 8, 9, 10, 11, and 12), the experiment was performed during two growing seasons (with reapplication of N fertilizer), totaling therefore 21 site-years. Prior to trial set up, sugarcane was cropped without burning at all locations, and the straw (dry and green leaves) resulting from harvests remained on the field. Several sites had a long history of vinasse (high amount of K and organic C) and press mud (moderate amount of N and P) application (Table 1), two byproducts originated from the sugar and bioethanol production. Similarly, at two sites (9 and 10), organomineral N fertilizer (a byproduct derived from the synthesis of amino acids and enriched with nutrients) amendment had occurred, as well as crop rotation with peanut (*Arachis hypogaea* L.; Site 10), a N-fixing legume. The sugarcane cultivars used varied according to the soil fertility and production potential of each site, defined by the soil type and historical average yield of the crop. The soil texture varied from sandy to clayey. Detailed information of the experimental sites is presented in Table 1. Precipitation and temperature recorded during each growing season is shown in Suppl Tables 1 and 2, respectively.

The experimental design at each location was a randomized complete block with four or five replications. The treatments consisted of N rates (50, 100, and 150 kg ha^{-1} at Sites 1, 2, 3, 8, 11, and 12; 50, 100, 150, and 200 kg ha^{-1} at Sites 4, 5, 6, 9, 10, 13, 14, and 15; 60, 120, and 180 kg ha^{-1} at Site 7;), applied as ammonium nitrate (32% N), calcium ammonium nitrate (26% N), or ammonium sulfate (21% N). Additionally, N-unfertilized plots (no N applied) were included at each site. The N rates were established to promote a crescent yield response of the crop to the nutrient until a plateau was reached, although yield decreases (through toxicity) owing to the application of high N rates were also expected. When necessary, limestone, agricultural gypsum, and nutrients (with exception of N) were applied according to Spironeo et al. (1997) to ensure optimum sugarcane growth. Fertilizers were surface band-applied 20 cm from the sugarcane row, 60–90 d after harvesting the previous crop cycle. The plot size ranged as follows: 8–15 m in length, 5–10 crop rows, and row spacing of 1.5 m, except for Site 12, with row widths of 1.0 m.

Soils were sampled using a Dutch auger in N-unfertilized plots

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