

From production to consumption: tracing C, N, and S dynamics in Brazilian agroecosystems using stable isotopes

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Abstract – Brazilian scientists have played a pioneering role in developing and applying stable isotope methodologies, in terms of natural abundance and enriched levels, to trace carbon and nutrient flows in terrestrial ecosystems, including natural and agroecosystems. Significant contributions have been made in the areas of biological N₂ fixation, carbon dynamics in soil, synthesis and evaluation of labeled fertilizers, and food science. These contributions have originated from several decentralized units of Embrapa, from research institutions, and from federal or state universities. In order to capitalize the existing Brazilian expertise, it is necessary to provide, at an institutional level, analytical facilities for stable isotope research, aiming to strengthen national capacity and to maintain the international competitiveness of the research.

Index terms: biological N₂ fixation, fertilizer use efficiency, food science, labeled fertilizer, soil carbon, stable isotopes.

Da produção ao consumo: traçando a dinâmica de C, N e S nos agroecossistemas brasileiros com uso de isótopos estáveis

Resumo – Pesquisadores brasileiros têm tido um papel pioneiro no desenvolvimento e na aplicação das metodologias de isótopos estáveis, em termos de abundância natural e de níveis enriquecidos, para traçar os fluxos de carbono e nutrientes em ecossistemas terrestres, que incluem os naturais e os agroecossistemas. Contribuições significativas têm sido feitas nas áreas de fixação biológica de N₂, de dinâmica de carbono nos solos, de síntese e avaliação de fertilizantes marcados, e da tecnologia dos alimentos. Essas contribuições têm se originado de diversas unidades descentralizadas da Embrapa, de institutos de pesquisa e de universidades federais ou estaduais. Para capitalizar a experiência brasileira existente, é necessária a provisão, nas instituições, de instalações analíticas para pesquisa com isótopos estáveis, a fim de fortalecer a capacidade nacional e manter a competitividade internacional das pesquisas.

Termos para indexação: fixação biológica de N₂, eficiência de uso de fertilizantes, tecnologia de alimentos, fertilizante marcado, carbono do solo, isótopos estáveis.

Introduction

The Brazilian climate is predominantly tropical and subtropical, in marked contrast to the temperate or colder regions of Europe and North America. Therefore, the soils within the country are, in general, more highly weathered and support a greater variety of both intensive and extensive agricultural enterprises. Despite this, Brazilian soils share a similar set of problems encountered in their northern hemisphere counterparts, namely loss of soil organic matter and declining fertility under arable cropping. Stable isotope tracers have played a crucial role in gaining insight into carbon, nitrogen, and sulfur cycling in agroecosystems, including the estimation of fertilizer use efficiency and the contribution of biological N₂ fixation to

the N nutrition of legumes and C₄ grasses. In the globalization era of agricultural products of plant and animal origin, the public is demanding accountability and authenticity of the external description on food and beverage packaging. In this case, stable isotopes are being used to detect adulteration and to verify geographic origin and mode of production, such as organic vs. conventional systems.

The objective of this review was to highlight contributions that Brazilian science has made and continues to make to the global efforts to trace carbon and nutrient flows in terrestrial ecosystems; synthesize and evaluate the efficiency of labeled fertilizers; and authenticate foods and beverages. It is an overview of the significant accomplishments, seen through a small

window or aperçu, from the perspective of an outsider, who, nevertheless, has been fortunate to be involved in Brazilian stable isotope research and review since 1979.

This review addresses key examples, mainly but not exclusively, from agroecosystems, but does not attempt to make an extensive coverage of all relevant Brazilian literature. Recognition has been given to published papers in which the research was conducted and analytical services were provided in the country, irrespectively of the national or international affiliations of the scientists involved. Although considerable expertise exists in Brazil, this review does not cover aquatic environments (except for one example of fish production regime), microbial and plant ecology, plant and animal physiology, and pure and applied sciences, such as entomology, zoology, hydrology, geochemistry, paleontology, and related disciplines.

Absolute (x) and relative (δ) values of isotopic abundance

The lighter elements of interest in biological sciences, that is, H, C, N, O, and S, have naturally occurring stable isotopes: two, in the case of H, C, and N; three, in that of O; or four, in that of S (Table 1). The absolute abundance of each isotope, expressed as mole fraction, is given in Table 1. These values are equivalent to the atom fraction of an element, $x(^iE)$, which is defined as the amount of a specified atom

(isotope) of an element divided by the total amount of atoms of the element within the mixture (Coplen, 2011). Therefore, for an element with two stable isotopes, such as N, the absolute value is expressed by the equation:

$$\text{atom fraction } ^{15}\text{N}, x(^{15}\text{N}) = \frac{\text{amount of } ^{15}\text{N}}{\text{amount of } ^{14}\text{N} + ^{15}\text{N}}$$

If a sample is artificially enriched in an isotope, the excess over natural abundance is expressed as an excess atom fraction, x , which is the difference between the mole fraction of an isotope of an element in a substance and that of a reference (Coplen, 2011). For N, for instance, the isotopic enrichment is expressed by the equation:

$$\text{excess atom fraction } ^{15}\text{N}, x(^{15}\text{N})_{\text{sample/reference}} = x(^{15}\text{N})_{\text{sample}} - x(^{15}\text{N})_{\text{reference}}$$

in which the reference is air, which has a ^{15}N mole fraction equal to 0.003 663.

The range of natural variations in isotopic abundances, expressed as mole fraction, in terrestrial materials is also given in Table 1. However, it is more common to express small variations in natural abundance as relative (δ) rather than absolute (x) values. The relative value is the isotope ratio (R) of a sample, relative to the isotope ratio of the international standard for the element, as in the equation: $\delta(^iE) = (R_{\text{sample}}/R_{\text{standard}}) - 1$.

For N, R is the ratio of $^{15}\text{N}/^{14}\text{N}$. The international standard is air, with an R value equal to 0.003 676 47 and, by definition, a $\delta^{15}\text{N}$ or $x^E(^{15}\text{N})$ value equal to zero (Chalk et al., 2015b). In order to avoid fractions, δ values have traditionally been expressed in per mil (‰), by multiplying the right hand side of the equation of the isotope ratio by 1,000. Values of $\delta^{15}\text{N}$ can be negative or positive depending on whether the sample isotope ratio is, respectively, less than or greater than the isotope ratio of air.

A full description of the source, availability, and properties of primary and secondary international reference materials for isotope-ratio analysis is given in Brand et al. (2014).

Biological N₂ fixation (BNF)

Short-term measurements of the ability of legumes or actinorhizal plants to fix atmospheric N₂ can be performed by exposure of the whole plant or root

Table 1. Isotopic composition of the important elements in biological sciences. Adapted from Berglund & Wieser (2011).

Element	Atomic number	Stable isotope ⁽¹⁾	Mole fraction	
			Abundance ⁽²⁾	Natural variation ⁽³⁾
Hydrogen	1	¹ H	0.999 844 26	0.999 816–0.999 974
		² H	0.000 155 74	0.000 026–0.000 184
Carbon	6	¹² C	0.988 922	0.988 53–0.990 37
		¹³ C	0.011 078	0.009 63–0.011 47
Nitrogen	7	¹⁴ N	0.996 337	0.995 79–0.996 54
		¹⁵ N	0.003 663	0.003 46–0.004 21
Oxygen	8	¹⁶ O	0.997 620 6	0.997 38–0.997 76
		¹⁷ O	0.000 379 0	0.000 37–0.000 40
		¹⁸ O	0.002 000 4	0.001 88–0.002 22
Sulfur	16	³² S	0.950 407 4	0.944 54–0.952 81
		³³ S	0.007 486 9	0.007 30–0.007 93
		³⁴ S	0.041 959 9	0.039 76–0.047 34
		³⁶ S	0.000 145 79	0.000 13–0.000 19

⁽¹⁾Superscript is mass number. ⁽²⁾Best measurement from a single terrestrial source. ⁽³⁾Terrestrial materials.

system to an atmosphere containing enriched $^{15}\text{N}_2$ (De-Polli et al., 1977). Although the method is direct, it poses many practical difficulties in relation to the containment of the labeled gas and to the maintenance of plants in an enclosure, which is why it is seldom attempted.

Indirect methods, commonly used to estimate legume symbiotic dependence, such as the proportional contribution of biologically-fixed N_2 to the N nutrition of the legume, involve paired treatments of a legume and a non-legume reference plant. The latter is required to estimate the ratio of labeled to unlabeled N derived from the soil by the N_2 -fixing plant.

The ^{15}N enrichment (E) method involves the addition of a fertilizer or organic material artificially enriched in ^{15}N to the soil in the paired plots. The proportional dependence (P_{atm}) of the legume on biologically-fixed N is estimated by isotope dilution, using the following equation: $P_{\text{atm}} = 1 - (x^{\text{E}(^{15}\text{N})}_{\text{legume}}/x^{\text{E}(^{15}\text{N})}_{\text{reference plant}})$.

The E methodology was reviewed by Chalk & Ladha (1999).

The ^{15}N natural abundance (NA) method is based on the same principle, but isotopic discrimination, i.e., the B value, during N_2 fixation must be additionally measured, usually by growing the legume in an N-free medium. Symbiotic dependence is estimated according to the equation: $P_{\text{atm}} = [(\delta(^{15}\text{N})_{\text{reference plant}} - \delta(^{15}\text{N})_{\text{legume}})/\delta(^{15}\text{N})_{\text{reference plant}} - B]$.

The NA methodology was reviewed by Unkovich et al. (2008).

Endophytic BNF

Brazilian and visiting scientists working with Dr. Johanna Döbereiner, at Embrapa Agrobiologia, were the first to recognize the importance of diazotrophic bacteria in endophytic associations with tropical C_4

Gramineae. The first isotope dilution field measurement to quantify endophytic BNF was also carried out at Embrapa Agrobiologia with *Paspalum notatum* Flugge (Boddey et al., 1983). Since these pioneering studies, endophytic BNF has been quantified by both the E and NA methods in several C_4 forage grasses and in sugarcane (*Saccharum officinarum* L.) (Table 2). This research has unequivocally shown the importance of endophytic BNF to the N nutrition of C_4 plants. For example, in a four-year study, Urquiaga et al. (2012) found that P_{atm} values varied from 43 to 61% among nine varieties of sugarcane.

Legume BNF

In Brazil, symbiotic dependence has been estimated for grain and forage legumes in agroecosystems, using the E and NA methods, and for native leguminous shrubs and trees at undisturbed sites in the Cerrado and Caatinga biomes, using the NA method. Some examples are given in Table 3.

Hungria & Vargas (2000) suggested that high temperature, water stress, and soil acidity were the main abiotic constraints to BNF in tropical grain legumes in the country. However, this conclusion was based on an examination of the literature in which qualitative data, such as nodulation parameters or assays on acetylene reduction activity, were reported instead of quantitative data on legume symbiotic dependence. Isotope-based methodologies have played an important role in identifying both biotic and abiotic constraints to legume performance in Brazil. For example, the E methodology was used to identify the critical growth stages of common bean (*Phaseolus vulgaris* L.) during which water stress had a large negative impact on yield and BNF. Similarly, by reviewing the published studies in which the E methodology was used, Chalk

Table 2. Brazilian studies of Embrapa Agrobiologia, using ^{15}N -based methodologies to quantify endophytic N_2 fixation in C_4 forages and in sugarcane (*Saccharum officinarum*).

Species ⁽¹⁾	Reference plant	Scale	Method ⁽²⁾	Duration	$P_{\text{atm}} \times 100$ (%) ⁽³⁾	Chronological reference
'Batatais' Bahia grass	'Pensacola' Bahia grass	Field	E	357 days	12 (4 harvests)	Boddey et al. (1983)
Guinea grass, 25 ecotypes	'IRI 442' <i>Urochloa arrecta</i>	Pots	E	350 days	16–39	Miranda et al. (1990)
Signal grass, 5 species	Variety with $>x^{\text{E}(^{15}\text{N})}$	Field	E	5 months	4–23	Reis et al. (2001)
Elephant grass, 3 sites, 5 varieties	Weeds	Field	NA	2 years	18–70	Morais et al. (2012)
Sugarcane, 9 varieties	Weeds and <i>Sorghum</i> sp.	Field	NA	4 years	43–61	Urquiaga et al. (2012)

⁽¹⁾Bahia grass, *Paspalum notatum*; Guinea grass, *Panicum maximum*; signal grass, *Urochloa brizantha*, *U. decumbens*, *U. humidicola*, *U. ruziziensis*, and *U. arrecta*; elephant grass, *Pennisetum purpureum*; and sugarcane, *Saccharum officinarum*. ⁽²⁾E, ^{15}N enrichment; and NA, ^{15}N natural abundance. ⁽³⁾ P_{atm} , proportional dependence of the legume on biologically-fixed N.

et al. (2010) were able to identify temperature, water, salinity, sodicity, acidity, and mineral nutrition as major abiotic factors affecting legume BNF globally.

Moreover, biotic factors – such as the absence of indigenous rhizobia or arbuscular mycorrhizal fungi (Ibijbjen et al., 1996) – and legume genotype can also affect legume symbiotic dependence. A comprehensive review of this subject was published by Chalk et al. (2006).

BNF dynamics

Apart from the quantification of legume BNF, a suite of indirect ^{15}N methodologies has been developed to estimate the transfer of BNF or legume N (LN) to companion non-legume species, as reviewed by Chalk et al. (2014b). Other ^{15}N -based methods have been developed to quantify the transfer of LN or BNF to an array of crops in sequential rotations. A frequently used method involves the application of a ^{15}N -labeled crop residue obtained from a particular source to unlabeled plots in a subsequent experiment, which might be separated in space and time from the original source. Primo et al. (2014), for example, measured cumulative recoveries of ^{15}N -labeled *Gliricidia sepium* (Jacq.) Kunth biomass in a pot experiment, in three subsequent crops of corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), or cowpea [*Vigna unguiculata* (L.) Walp.], obtaining values of 28, 35, and 41%, respectively. In an attempt to overcome the obvious limitations of such

an approach, an in situ method based on ^{15}N natural abundance was proposed by Perin et al. (2006), who estimated that corn following sunn hemp (*Crotalaria juncea* L.) green manure derived 15% of its grain N content from N_2 fixed by the preceding sunn hemp crop.

Organic carbon dynamics in soil

For carbon, the relative isotopic abundance is expressed by the isotope ratio equation, in which R is the ratio of $^{13}\text{C}/^{12}\text{C}$. The international standard, in this case, is Vienna Pee Dee Belemnite (VPDB), which has a δ value of -0.030 031.

It is well known that ^{13}C isotopic fractionation occurs during photosynthesis, so that plant organic C is naturally depleted in ^{13}C , when compared with atmospheric CO_2 ($\delta^{13}\text{C}\text{O}_2 = -8\text{‰}$). Different CO_2 assimilation pathways between C_3 (the Calvin cycle), C_4 (the Hatch-Slack pathway), and C_{CAM} (Crassulacean acid metabolism) plants result in markedly different bulk $\delta^{13}\text{C}$ signatures of photosynthetic plants, mainly due to diffusion and enzymatic effects. The ranges in the $\delta^{13}\text{C}$ values for C_3 , C_4 , and C_{CAM} plants are -22 to -30‰, -9 to -13‰, and -10 to -20‰, respectively.

In undisturbed natural ecosystems, the $\delta^{13}\text{C}$ signature of surface soil organic matter is similar to that of the native vegetation from which it was derived. Many of the soils used in agriculture were originally under C_3 forest and, therefore, had very negative $\delta^{13}\text{C}$

Table 3. Brazilian field studies using ^{15}N dilution to quantify symbiotic N_2 fixation in legumes⁽¹⁾.

Legume species ⁽¹⁾	Reference plant ⁽²⁾	Method ⁽³⁾	Duration	$P_{\text{am}} \times 100$ (%) ⁽⁴⁾	Reference
Grain legumes					
Common bean, 5 varieties	Wheat	E	60–90 days	38–65	Ruschel et al. (1982)
Soybean	Nn soybean	E	92 days	81–85	Boddey et al. (1984)
Groundnut	Weeds, 3 species	NA	98 days	52–70	Okito et al. (2004)
Forage or green manure legumes					
<i>Centrosema</i> , <i>Galactia striata</i> , and <i>Desmodium ovalifolium</i>	C_4 grasses, 3 species	E	97 days	37–65	Viera-Vargas et al. (1995)
<i>Crotalaria juncea</i> and <i>Mucuna niveum</i>	Weeds, 5 species	NA	75 days	70–75 ⁽⁵⁾	Ramos et al. (2001)
Native legumes					
<i>Cratylia mollis</i> , dry and wet seasons, 4 sites	Neighboring dicots Nnlegume, 2 species	NA	Spot samples	8–22 and 42–52 ^(5, 6)	Teixeira et al. (2006)
<i>Mimosa blanchetii</i> , <i>M. gemmulata</i> , and <i>M. ulbrichiana</i>				38, 51, 60	Reis Junior et al. (2010)

⁽¹⁾Common bean, *Phaseolus vulgaris*; soybean, *Glycine max*; and groundnut, *Arachis hypogaea*. ⁽²⁾Wheat, *Triticum aestivum*; Nn, non-nodulating; Weeds, *Digitaria horizontalis*, *Sorghum arundinaceum*, and *Cenchrus echinatus*; C_4 grasses, *Urochloa brizantha*, *U. arrecta*, and *Panicum maximum*; Weeds, *Sorghum* sp., *Zea mays*, *Cynodon dactylon*, *Cyperus rotundus*, and *Sida glaziovii*; and Nnlegume, the non-nodulating legumes *Bauhinia cheilanta* and *Caesalpinia pyramidalis*. ⁽³⁾E, ^{15}N enrichment; and NA, ^{15}N natural abundance. ⁽⁴⁾ P_{am} , proportional dependence of the legume on biologically-fixed N. ⁽⁵⁾B value was assumed to be zero (Ramos et al., 2001), -1.3‰ (Teixeira et al., 2006). ⁽⁶⁾Stem tissue, low range is dry season, and high range is wet season.

values. With the introduction of sugarcane and tropical forage grasses, such as C_4 species (Table 2), the above-ground C input into the ecosystem had a much higher $\delta^{13}\text{C}$ signature, and, over time, the $\delta^{13}\text{C}$ value of the soil organic matter became less negative (Vitorello et al., 1989; Moraes et al., 1996; Pinheiro et al., 2010) (Table 4).

An innovative methodology to estimate the proportions of soil C from the original and introduced vegetation is based on the shift in the $\delta^{13}\text{C}$ signature of soil C and was developed by Cerri et al. (1985) at Centro de Energia Nuclear na Agricultura (Cena), the center for nuclear energy in agriculture of Universidade de São Paulo (USP). The proportion of organic C in a soil layer derived from the introduced C_4 vegetation ($P_{C_4\text{soil}}$) was expressed by the following equation: $P_{C_4\text{soil}} = \frac{\delta^{13}\text{C}_{C_4\text{soil}} - \delta^{13}\text{C}_{C_3\text{soil}}}{(\delta^{13}\text{C}_{C_4\text{plant}} - \delta^{13}\text{C}_{C_3\text{soil}})}$, in which $\delta^{13}\text{C}_{C_4\text{soil}}$ is the $\delta^{13}\text{C}$ value of a soil layer under C_4 vegetation; $\delta^{13}\text{C}_{C_3\text{soil}}$ is the $\delta^{13}\text{C}$ value of a soil layer under the original C_3 vegetation; and $\delta^{13}\text{C}_{C_4\text{plant}}$ is the $\delta^{13}\text{C}$ value of the C_4 vegetation. $P_{C_4\text{soil}}$ increased with increasing years under C_4 pasture or sugarcane (Table 4), and Moraes et al. (1996) reported that the original C_3 signal in the 0–10-cm soil layer had disappeared after 81 years under C_4 pasture.

The concentrations of soil organic C and $\delta^{13}\text{C}$ values are not constant with soil depth (Sisti et al., 2004). Organic C concentrations generally decrease with soil depth. However, in C_3 forest, soil $\delta^{13}\text{C}$ values increased with depth, although, in soil under C_4 pasture or sugarcane, these values also decreased with depth due to the decreasing effect of the C_4 pasture with depth (Vitorello et al., 1989; Moraes et al., 1996; Pinheiro et al., 2010).

Management factors can affect the dynamics of C when the system is undergoing interchange between C_3 - and C_4 -dominant vegetation. For instance, the tillage system (zero vs. conventional) under different long-term crop rotations was shown to affect the decay of the original C_3 -derived soil organic C to a depth of 100 cm (Sisti et al., 2004). Balieiro et al. (2008) showed that the stocks of soil C and $\delta^{13}\text{C}$ signatures to 10-cm depth were affected by the reintroduction of two C_3 -forest species, *Eucalyptus grandis* W.Hill and *Pseudosamanea guachapele* Harms, the latter being a N_2 -fixing tree. The site had formerly supported C_4 *Panicum maximum* Jacq. for ten years. The rate of replacement of soil C by new forest C after five years was much faster under a mixed plantation of trees rather than under pure plantations of either species (Balieiro et al., 2008).

There is an increasing interest worldwide in the use of soil additives, such as biochars, due to their potential to sequester soil C. In a 30-day laboratory incubation study with three biochars derived from C_3 oilseed residues added to a sandy soil containing C_4 carbon, Rittle et al. (2015) found that respired CO_2 came principally from the biochars and that these decelerated the mineralization of the indigenous C (negative priming effect). Therefore, there may be both direct and indirect effects of biochar addition on C sequestration.

Synthesis and evaluation of labeled fertilizers

Scientists at Cena of USP have been involved for many years in the synthesis of labeled fertilizers using ion exchange chromatography (Bendassolli et al., 1997). They have also developed new methods

Table 4. $\delta^{13}\text{C}$ signatures of Brazilian soils under forest and after a number of years of C_4 pasture or sugarcane (*Saccharum officinarum*) established on cleared forest.

Chronological reference	Location			Soil				$P_{C_4\text{soil}}^{(1)}$	
	Municipality	State	Coordinates	USDA order	Depth (cm)	Vegetation	Time (y)		$\delta^{13}\text{C}$ (‰)
Vitorello et al. (1989)	Piracicaba	São Paulo	22°43'S 47°38'W	Oxisol	0–30	Forest	-	-25.1	-
						Sugarcane	12	-22.8	0.17
Moraes et al. (1996)	Nova Vida	Roraima	10°10'S 62°49'W	Ultisol	0–5 0–10	Forest	-	-28.0	-
						C_4 pasture	81	-14.3	1.0
Pinheiro et al. (2010)	Usina Lagrisa, Linhares	Espírito Santo	19°18'S 40°19'W	Ultisol	0–10	Forest	-	-28.0	-
						Brachiaria + sugarcane	3 + 14	-18.0	0.56

⁽¹⁾Equation of proportional N recovery per plant.

for the isotope-ratio analysis of labeled plants and soils (Carneiro et al., 2008). Some examples of labeled fertilizers synthesized at Cena are given in Table 5. The isotopic enrichment of the product is largely determined either by the isotopic enrichment of the source materials or by the degree of isotopic fractionation during ion exchange chromatography.

By using isotopically-labeled fertilizers, it is possible to determine the recovery in the plant-soil system. The proportion of ^{15}N -enriched fertilizer recovered by a plant, for example, can be calculated according to the equation: $\text{proportional N recovery}_{\text{plant}} = (\text{mass N}_{\text{plant}} \times x^{\text{E}}(^{15}\text{N})_{\text{plant}}) / (\text{mass N}_{\text{fertilizer}} \times x^{\text{E}}(^{15}\text{N})_{\text{fertilizer}})$.

in which $x^{\text{E}}(^{15}\text{N})_{\text{plant}}/x^{\text{E}}(^{15}\text{N})_{\text{fertilizer}}$ is the fraction of plant N derived from the fertilizer. The same procedure is used to determine the recovery of fertilizer in the soil, and fertilizer loss from the plant-soil system can be calculated by mass balance.

Many studies have been undertaken with different crops and strategies of N fertilizer application, in order to estimate N fertilizer efficiency. Recovery by annual crops seldom exceeds 60%, and the cumulative residual value of the N fertilizer in two subsequent crops is usually <10%. Some examples of recoveries of ^{15}N - and ^{34}S -labeled fertilizers in plant tops are given in Table 5.

In addition to studies with labeled synthetic (industrially manufactured) N fertilizers, such as urea and ammonium-based forms, efficiency studies have also been performed with labeled organic sources, including composts (Chalk et al., 2013) and animal excreta (Primo et al., 2014), or with labeled slow- or controlled-release products (Chalk et al., 2015a). It is

also possible to trace the dynamics of N from source (fertilizer) to product (food) in agroecosystems using ^{15}N natural abundance (Chalk et al., 2014a).

Food and beverage authentication

In the globalization era of agricultural products of plant and animal origin, the public is demanding authenticity and accountability in food and beverage labelling. Stable isotopes are playing an expanding role in detecting adulteration and in verifying geographic origin and production regime. Some examples of research carried out in Brazil are summarized in Table 6, of which several will be outlined in some detail.

Adulteration

Marked differences are observed in the $\delta^{13}\text{C}$ signatures between sugars derived from C_3 and C_4 plants. This makes it possible to detect the presence of C_4 sugar or C_4 ethanol in a product ostensibly derived from a C_3 source. Therefore, it allows identifying, for instance, the sources of ethanol in Brazilian brandy (Pissinatto et al., 1999) or sparkling wine (Martinelli et al., 2003), or whether C_4 sugar has been added to freshly-squeezed orange juice (Pupin et al., 1998) or to honey (Padovan et al., 2003).

Honey is produced from the nectar collected by bees from flowering plants, mainly from C_3 plants, and, to a lesser extent, from C_4 and C_{CAM} plants. The $\delta^{13}\text{C}$ signature of the protein extract of honey will reflect the dominant source from which the honey was derived, e.g. -22 to -33‰ for C_3 plants, -10 to -20 for C_4 plants,

Table 5. Synthesis of labeled fertilizers at Centro de Energia Nuclear na Agricultura of Universidade de São Paulo, as well as fertilizer use efficiency.

Fertilizer	x^{E}	Crop ⁽¹⁾	Yield or recovery ⁽²⁾ (%)	Reference
Synthesis				
$\text{Ca}^{34}\text{SO}_4 \cdot 2\text{H}_2\text{O}$	0.058 5		95	Rossete et al. (2006)
$\text{CO}(^{15}\text{NH}_2)_2$		Not applicable	77	Sant Ana Filho et al. (2012)
$(^{15}\text{NH}_4)_2\text{SO}_4$	0.90		-	Máximo et al. (2013)
Use efficiency				
$\text{Na}_2^{34}\text{SO}_4$	0.143	Rice	27 (122)	Trivelin et al. (2002)
		Sunn hemp	15 (72)	
$(^{15}\text{NH}_4)_2\text{SO}_4$	0.0302	Sugarcane	29 (363)	Ambrosano et al. (2005)
	0.0207	Coffee	55 (366)	Fenilli et al. (2008)
			69 (731)	

⁽¹⁾Rice, *Oryza sativa*; sunn hemp, *Crotalaria juncea*; sugarcane, *Saccharum officinarum*; and coffee, *Coffea arabica*. ⁽²⁾Recovery in plant tops, in which data in parentheses represent growth periods in days.

and -11 to -13.5 for C_{CAM} plants (Padovan et al., 2003). Adulteration of pure honey with cheap C_4 sugars, such as cane or corn syrup, has become an international problem. It can be detected by $\delta^{13}C$ analysis of the bulk honey and of the protein extracted from the honey, although the latter is not affected by the adulteration. A difference of 1 δ indicates 7% of C_4 sugar added, which is the limit tolerated worldwide (Padovan et al., 2003).

Geographic origin

The most useful indicators of the geographic origin or provenance of terrestrial plants are the $\delta^{18}O$ or δ^2H values of stem water. At a given geographic location, the relationship between $\delta^{18}O$ and δ^2H of meteoric water, i.e., water from precipitation, is linear, referred to as the meteoric water line. $\delta^{18}O$ is defined by the isotope ratio equation, in which R is the ratio of $^{18}O/^{16}O$. The primary international standard, in this case, is Vienna Standard Mean Ocean Water (VSMOW), which, by definition, has $\delta^{18}O$ and δ^2H values of zero (Brand et al., 2014).

The isotopic composition of meteoric water is affected by physical phenomena such as condensation and evaporation, with temperature being the main variable that affects isotopic fractionation. The $\delta^{18}O$ and δ^2H composition of meteoric water follows a predictable geographic pattern that is related to latitude, altitude, amount of precipitation, and distance from the ocean. The annual mean $\delta^{18}O$ in meteoric water ranges from +2 to -2‰, in equatorial regions, to as low as -22‰ in the north-polar region. It should be noted that

there is no isotopic fractionation during water uptake by terrestrial plants and, therefore, stem water has the same signature as the water source, regarding access to deep vs. shallow water.

The three main wine producing regions in the state of Rio Grande do Sul could be differentiated on the basis of $\delta^{18}O$ signatures of water in the wines (Table 7) (Dutra et al., 2011), but not in the wine ethanol. These authors speculated that the variation in $\delta^{18}O$ among regions may be due to differences in local meteorological conditions, such as rainfall and temperature, but that altitude and distance to the ocean may also play a role. A more intensive data analysis of site-specific and regional climate and geography is necessary to separate these variables.

Geographic origin may also be differentiated by differences in $\delta^{13}C$ signatures. For example, a survey of $\delta^{13}C$ signatures of Big Mac hamburger patties from outlets in Brazil and Great Britain showed extreme median values of -11.1 and -25.4‰, respectively, indicating the predominant C_4 (tropical grasses) or C_3 (temperate species) diet of the beef cattle (Martinelli et al., 2011).

Both $\delta^{13}C$ and $\delta^{15}N$ signatures have also been successfully used to differentiate the regional production of the illegal drug marijuana (*Cannabis sativa* L.) in Brazil (Shibuya et al., 2006). From the known producing areas of the states of Pernambuco and Bahia (dry region) and of Mato Grosso do Sul and Pará (wet region), mean $\delta^{15}N$ values of the samples seized from the dry region varied from +1 to +2‰, whereas

Table 6. The use of stable isotope δ values to authenticate Brazilian foods and beverages.

Product	Isotope	Issue	Reference
		Adulteration	
Orange juice	$^{13}C, ^{18}O$		Pupin et al. (1998)
Brandy	^{13}C	Prohibited additions of water or C_4 -derived sugars	Pissinatto et al. (1999)
Sparkling wine	^{13}C		Martinelli et al. (2003)
Honey	^{13}C		Padovan et al. (2003)
		Geographic origin ⁽¹⁾	
Wine	$^{13}C, ^{18}O$	Certification of provenance	Dutra et al. (2011)
Big Mac patties	$^{13}C, ^{15}N$		Martinelli et al. (2011)
		Production regime	
Chicken (tissues)	$^{13}C, ^{15}N$	Offal in diet	Oliveira et al. (2010)
Freshwater fish ⁽²⁾	$^{13}C, ^{15}N$	Farmed vs. wild	Sant'Ana et al. (2010)
Beer	^{13}C	C_3 / C_4 fermentables	Mardegan et al. (2013)
Lettuce (<i>Lactuca sativa</i>)	^{15}N	N input sources	Inácio et al. (2015b)

⁽¹⁾The geographic origin of the seized samples of the illicit drug marijuana has also been determined by stable isotope ($^{13}C, ^{15}N$) analysis (Shibuya et al., 2006). ⁽²⁾Cachara, *Pseudoplatystoma fasciatum*.

those from the wet region were higher, varying from +5 to +6.8‰. The mean $\delta^{13}\text{C}$ values for the dry region were of -26 to -26.5‰, differing from those of the wet region, which varied from -29.2 to -30.2‰. This information provides the basis for the allocation of police resources to intercept shipments from the source to the largest population of consumers in the city of São Paulo, in the state of São Paulo.

Production regime

The $\delta^{13}\text{C}$ signature of the feces or body tissues of domestic animals is a robust indicator of diet composition. For instance, in another study, the ^{13}C natural abundance of cattle manure was an effective predictor of the proportion of legume in a diet consisting of legume (*Desmodium ovalifolium* Wall.) + grass [*Urochloa dictyoneura* (Fig. & de Not.) Veldkamp (Syn. *Brachiaria dictyoneura*)] forage (Macedo et al., 2010). Colleta et al. (2012) were able to separate barn vs. free-range chicken according to their diet, in which the latter had more C_4 in their diet and higher $\delta^{15}\text{N}$ values, which the authors attributed to the ingestion of animal protein.

The presence of poultry offal meal, i.e., a product derived from the entrails and internal organs of poultry, in broiler diets is a potential threat to the large Brazilian export market in chicken meat due to concerns regarding contamination with *Salmonella* or *Escherichia coli*. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ have shown promise under controlled experimental conditions as a diagnostic tool to identify the presence of offal in broiler diets (Oliveira et al., 2010), but, by themselves, cannot be used as a conclusive proof. Despite this, $\delta^{13}\text{C}$ values showed clear differences between a species of farmed and wild freshwater fish (Cachara) from the Alta Floresta region in the Amazon (Sant'Ana et al., 2010). This was observed both in the wet and dry

seasons, with mean values varying from -23.6 to -24‰ for farmed fish and from -29.1 to -30‰ for wild fish.

Furthermore, $\delta^{13}\text{C}$ composition is also a good index of the ingredients used in the brewing process of Brazilian beers (Mardegan et al., 2013). Large breweries use a relatively high proportion of C_4 corn resulting in a mean $\delta^{13}\text{C}$ value of -20‰, whereas small artisanal (boutique) breweries use a higher proportion of C_3 cereals, resulting in a mean $\delta^{13}\text{C}$ value of -25‰. These differences are driven by the need of small brewers to market a product distinctive in quality from mass-produced beer, and also by the need to use fewer ingredients of lower cost, more rapid fermentation, and higher alcohol content, which corn provides.

The use of stable isotopes to differentiate organic vs. conventional plant and animal products was reviewed by Inácio & Chalk (2015) and Inácio et al. (2015a), respectively. In a study of conventionally and organically produced lettuce (*Lactuca sativa* L.) in the state of Rio de Janeiro, Inácio et al. (2015b) concluded that the $\delta^{15}\text{N}$ signatures of lettuce were an indicator of the principal N inputs (BNF, organic or manufactured fertilizer) instead of a definitive indicator of the production regime.

Brazilian institutions involved in stable isotope applications in agroecosystems

The precise measurement of isotopic abundance of the lighter elements requires an isotope-ratio mass spectrometer (IRMS). Computer-controlled, automated sample processing and analysis is a feature of modern instruments, which are equipped with multiple inlet and collector systems, allowing for the simultaneous measurement of several ions differing in mass-to-charge ratio. IRMS is a mature but somewhat expensive technology, requiring a relatively large initial capital investment and on-going technical support and maintenance costs.

Several Brazilian research institutions have been involved in stable isotope research for many years, and their increasing number testifies to the importance of this technology. The known Brazilian institutions involved in stable isotope research are identified in Table 8.

Several educational institutions, including Cena of USP, or institutions affiliated with Universities, such as, Embrapa Agrobiologia and Universidade Federal Rural do Rio de Janeiro, have strong post-

Table 7. $\delta^{18}\text{O}$ in water and $\delta^{13}\text{C}$ in ethanol in wines from three regions of the state of Rio Grande do Sul, Brazil (Dutra et al., 2011)⁽¹⁾.

Region	$\delta^{18}\text{O}$ (‰)	$\delta^{13}\text{C}$ (‰)
Serra Gaúcha	+0.05±0.12a	+28.1±0.1a
Serra do Sudeste	+2.28±0.16b	+29.1±0.2b
Campanha	+1.29±0.24c	+29.2±0.1b

⁽¹⁾Means followed by equal letters do not differ by Tukey's test, at 5% probability.

Table 8. Brazilian institutions involved in stable isotope research in terrestrial and agroecosystems⁽¹⁾.

Acronym	Institution	IRMS ⁽²⁾	Location (City, State)
Unesp	Centro de Isótopos Estáveis Ambientais of Universidade Estadual Paulista	Yes	Botucatu, São Paulo
Cena-USP	Centro de Energia Nuclear na Agricultura of Universidade de São Paulo	Several	Piracicaba, São Paulo
Embrapa Agrobiologia	Embrapa Agrobiologia	Several	Seropédica, Rio de Janeiro
UFRRJ	Universidade Federal Rural do Rio de Janeiro	No	Seropédica, Rio de Janeiro
Embrapa Solos	Embrapa Solos	No	Gávea, Rio de Janeiro
USP	Universidade de São Paulo	Yes	Ribeirão Preto, São Paulo
UFP	Departament of Nuclear Energy of Universidade Federal de Pernambuco	Yes	Recife, Pernambuco
Embrapa Arroz e Feijão	Embrapa Arroz e Feijão	Yes	Santo Antônio de Goiás, Goiás
Embrapa Agrossilvipastoril	Embrapa Agrossilvipastoril	Yes ⁽²⁾	Sinop, Mato Grosso
UFV	Laboratory of stable isotopes from the Department of Soil Science of Universidade Federal de Viçosa	Yes	Viçosa, Minas Gerais
UB	Universidade de Brasília	Yes	Brasília, Distrito Federal
UFBA	Laboratory of stable isotopes of Universidade Federal da Bahia	Yes	Salvador, Bahia
Unicamp	Universidade Estadual de Campinas	No	Campinas, São Paulo

⁽¹⁾While endeavoring to be comprehensive, some institutions may have been inadvertently overlooked. ⁽²⁾IRMS, isotope-ratio mass spectrometer.

graduate research training and teaching programs in stable isotope applications. Therefore, Brazilian scientists are well equipped to participate not only in national programs but also in international ones involving stable isotope applications in soil, plant, food, and animal sciences. Among the opportunities for Brazilian scientists, are possible contributions to the research programs of the Division of Nuclear Techniques in Food and Agriculture of the Joint Food and Agriculture Organization of the United Nations/International Atomic Energy Agency (FAO/IAEA) and to the technical cooperation programs of IAEA, either as project participants or as institutional employees. The programs in food and agriculture cover the areas of plant breeding and genetics, soil and water management and crop nutrition, insect pest control, animal production and health, and food and environmental protection (Iaea, 2015). Participation in such programs raises the international profile of the important work being undertaken in Brazil.

Conclusions

Brazilian scientists have made significant original contributions, through stable isotope tracing, for better understanding of carbon and nutrient cycling in agroecosystems, as well as in food and beverage authentication. There is an increasing awareness of the importance of stable isotope research in many branches of science, for which IRMS is an essential piece of equipment that is best provided at institutional level. The less desirable and often impractical alternative

is to rely on expensive fee-for-service laboratories or to collaborate with a sister institution that has IRMS facilities. In this sense, one of the main impediments to a more innovative research in nutrient cycling in natural and agroecosystems in Brazil is the obstacle created by the current paucity of IRMS facilities in the national institutions.

New or emerging technologies in the agricultural sector will require the application of stable isotope techniques to follow the dynamics of C, N, and S in the soil-plant-animal-atmosphere continuum. For example, for the introduction of new legume species or cultivars, as well as of new crop rotations, including legumes or new inoculants, it will be necessary to assess the contribution of BNF to N balance in agroecosystems. Similarly, the efficacy of new slow- or controlled-release fertilizers or chemical inhibitors will need to be evaluated using stable isotope techniques. The effect of innovative agronomic practices, such as the use of biochar, will require the strategic application of stable isotopes to verify the sequestration of C, N, and S. The alternative use of radioactive tracers (e.g. ¹⁴C and ³⁵S) is generally precluded due to restrictions imposed by environmental and safety issues, whereas the half-life of ¹³N of 9.97 min is too short to be of practical value. Stable isotopes have a role in characterizing negative interactions between agriculture and the environment, such as methane emissions from ruminants and rice paddies, and also fertilizer-induced emissions of nitrous oxide. Scientists in Brazil are in a unique position to assess innovative technologies to enhance resource-use efficiency across a wide range of soils

and climatic conditions, extending from the humid tropics to temperate regions.

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