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Carbon and Nitrogen Isotopic Signatures and Nitrogen Profile To Identify Adulteration in Organic Fertilizers

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ABSTRACT: Recently it has been shown that stable isotopes of nitrogen can be used to discriminate between organic and synthetic fertilizers, but the robustness of the approach is questionable. This work developed a comprehensive method that is far more robust in identifying an adulteration of organic nitrogen fertilizers. Organic fertilizers of various types (manures, composts, blood meal, bone meal, fish meal, products of poultry and plant productions, molasses and seaweed based, and others) available on the North American market were analyzed to reveal the most sensitive criteria as well as their quantitative ranges, which can be used in their authentication. Organic nitrogen fertilizers of known origins with a wide δ^{15}N range between −0.55 and 28.85‰ (n = 1258) were characterized for C and N content, δ^{13}C, δ^{15}N, viscosity, pH, and nitrogen profile (urea, ammonia, organic N, water insoluble N, and NO_3). A statistically significant data set of characterized unique organic nitrogen fertilizers (n = 335) of various known origins has been assembled. Deliberately adulterated samples of different types of organic fertilizers mixed with synthetic fertilizers at a wide range of proportions have been used to develop the quantitative critical characteristics of organic fertilizers as the key indicators of their adulteration. Statistical analysis based on the discriminant functions of the quantitative critical characteristics of organic nitrogen fertilizers from 14 different source materials revealed a very high average rate of correct classification. The developed methodology has been successfully used as a source identification tool for numerous commercial nitrogen fertilizers available on the North American market.

KEYWORDS: *nitrogen organic fertilizers, synthetic fertilizers, nitrogen isotopic signature, adulteration, N profile, source identification tool*

INTRODUCTION

Globally, about 37.2 million hectares are under organic agricultural management,[1] which is only 0.85% of total agricultural area. The region with the most organic agricultural land is Oceania, with 12.15 million hectares, followed by Europe with almost 9.3 million hectares. North America has 2.7 million hectares under organic agricultural practices. Although relatively still low compared to the overall agricultural industry, the market for organic products has grown from nonexistent in 1990 to $55 billion in 2009.[1] This demand has driven a similar increase in organically managed farmland, which has grown over the past decade at a compounding rate of 8.9% per annum.

Organic agricultural methods are internationally and locally regulated. Organic farmers must adhere to stringent guidelines and be certified by recognized certifying agencies before they can sell their produce as "organic". The idea of organic farming is not about the use of certain methods and substances and the avoidance of others. It is about an implementation of a structure that is imitating the structure of a natural system that has integrity, independence, and a benign use of organisms. To sustain soil fertility, organic farmers rely in part on the use of such agricultural techniques as crop rotation, green manure, compost, and biological pest control. Although organic farming uses fertilizers and pesticides, it excludes or strictly limits the use of manufactured (synthetic) fertilizers, pesticides, plant growth regulators such as hormones, livestock antibiotics, food additives, genetically modified organisms,[2] human sewage sludge, and nanomaterials.[3]

The use of all fertilizers in organic farming has to be authorized by the appropriate inspection body, and the organic fertilizer has to be inspected and certified. The use of soil amendments such as organic fertilizers and soil conditioners is a necessary step in agricultural practices of organic farming primarily applied to improve fertility and tilth and to correct soil problems. A list of these substances permitted in organic production systems in Canada is regulated by the Canadian General Standards Board (CGSB) accredited by the Standards Council of Canada (SCC).[4] Regulatory bodies similar to CGSB exist in other countries, such as the National Organic Standards Board (NOSB) in the United States and Council Regulation (EEC) in the European Union.[5,6] The main categories of organic fertilizers permitted in organic cultivation in Canada and the United States are summarized in Table 1. The use of some of these fertilizers is often subject to additional review on a case-by-case basis. For example, the use of aquatic plant products is prohibited if they contain synthetic preservatives, such as formaldehyde, or are fortified with other prohibited plant nutrients. Natural (nonsynthetic) extracts of aquatic plant products are allowed. Extraction with synthetic solvents is prohibited except for potassium hydroxide or sodium hydroxide, provided that the amount of solvent used does not exceed the amount necessary for extraction.

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Table 1. Main Categories of Organic Fertilizers Permitted in Organic Cultivation in Canada and the United States

fertilizer group	origin and usage
alfalfa meal and pellets	use of organic alfalfa unless commercially unavailable; ensure nonsynthetic alfalfa is not a product of genetic engineering
amino acids, nonsynthetic	amino acids produced by plants, animals, and micro-organisms that are not from genetic engineering and that are extracted or isolated by hydrolysis or by physical or other nonchemical means
animal manure	heat-treated, mechanically and physically processed manures may be acceptable but shall be reviewed on a case-by-case basis
aquatic plant products	natural (nonsynthetic) extracts are allowed; no addition of synthetic preservatives allowed
ash	ash from plant or animal sources only; ash from burning minerals, manure, or prohibited substances is prohibited (manure ash is prohibited because burning manure wastes organic matter and nutrients)
clay	bentonite, perlite, and zeolite as a soil amendment or seed pellet additive; mined minerals and unprocessed mined minerals
compost, including mushroom compost	composting refers to the carefully managed process whereby organic substances are thermophilically digested; composted animal excrements, including poultry; natural substances or those derived from natural substances without use of chemicals or chemical treatment; spent mushroom and vermiculate substrate
guano, bat or bird	decomposed, dried deposits from wild bats or birds; domesticated fowl excrement is considered to be manure, not guano
products or byproducts of animal origin	blood meal, bone meal, feather, fur, and hair meals, fish and meat meals, dairy products; all products have to be further processed, sterilized, and guaranteed free of specific risk materials
products (vegetative) or byproducts of plant origin	those derived from natural substances without the addition of chemically synthesized substances or chemical treatment; organic sources shall be used unless commercially not available, for example, molasses, soy, corn, wheat
peat moss	shall not contain synthetic wetting agents

The production of most commonly used organic nitrogen fertilizers is a multiple-step process that can be very complex depending on the origin of the source material. Very often it involves such steps as filtration, biodigestion, concentration, and sterilization. Although being a target component, nitrogen very often accounts for only 1−4% by weight in a final product. As a result, adulteration of organic fertilizers has become a pressing issue in the organic agriculture industry.[4,5]

A few studies[7−12] have recently demonstrated the possibility of using nitrogen stable isotope in discriminating not only between organic and synthetic fertilizers but between crops being grown with their use as well. Synthetic nitrogen fertilizers have been well studied and documented in the literature.[13−16] Generally, nitrogen isotopic values of synthetic fertilizers lie in the range between −4 and 4‰ (per mil). Organic fertilizers have much higher values of δ^{15}N and a wider range of compositions than their synthetic counterparts, reflecting their more diverse origins. The range of δ^{15}N values of organic nitrogen fertilizers spreads from around 0 to >30‰.[7,17] The organic nitrogen fertilizer with the largest range of δ^{15}N in the study by Bateman[9] was farmyard manure, with δ^{15}N between 3.5 and 16.2‰ and a mean value of 8.1‰. Such a wide range can be explained by two factors. One of them is the source of the manure and the diet of the animals producing it. Another is that animal manure over time becomes isotopically heavier due to volatilization of ^{14}N ammonia. Seaweed-based fertilizers were found[9] to have a range of δ^{15}N values between 0.6 and 5.4‰ with a mean of 2.5‰. Organic fertilizers produced from animal byproducts such as blood meal and bone meal have shown quite narrow ranges for δ^{15}N values, from 4.1 to 6.8‰ with a mean at 5.9‰. The general rule is that the nitrogen isotope composition of animal proteins including blood, bone, hair, and muscle materials is determined by diet and is by 2−3‰ higher than the δ^{15}N of the protein composition of their diet.[18] For this reason, organic fertilizers based on fish products have a relatively large range of δ^{15}N depending on the trophic level of the seafood they are produced from. δ^{15}N of such sources could be as low as 2.1‰ and as high as >16‰. Such diversity in source material with a very wide range of nitrogen isotopic signatures makes the authentication of organic fertilizers very challenging, in particular when only one analytical parameter, such as δ^{15}N, is relied on.

The use of δ^{13}C isotopic signature in the process of authentication of organic fertilizers is based on the fact that there are three main natural plant photosynthetic cycles. The Calvin cycle (also called the C3 cycle) provides isotopic values between −22 and −32‰. The Hatch Slack cycle (or C4 cycle) is characterized by isotopic values between −8 and −20‰. Organic carbon of most land plants is in the range of the C3 group. Sugar cane and corn, on the other hand, belong to the C4 group. The isotopic composition of inorganic forms of carbon, such as carbonate, bicarbonate, and others, and other carbon-bearing materials varies quite significantly.[19] For this reason, carbon isotopic values of organic fertilizers can provide very useful information about the source of the material used in their production.

The main objective of our study is to develop a robust and effective method for verification of the source of organic nitrogen fertilizers and confirmation of their organic origin. Numerous commercial organic fertilizers of various origins (blood meal, fish meal, plant origin, manures, composts, etc.) from organic certified producers have been analyzed using N and C isotopic analyses as well as full N profile characteristics (urea, ammonia, organic N, water-insoluble N, and NO_3). On the basis of the compelling data set constructed, the most sensitive criteria as well as their quantitative range have been identified. A library database has been developed consisting of the identified most sensitive quantitative critical characteristics (QCC) of 335 unique organic nitrogen fertilizers from 14 different source materials. It is shown in the study that this library can be used as the tool in the identification of an adulteration of commercial fertilizers offered to organic agriculture. The developed methodology has been offered to the organic material certification agencies in North America, as well as the organic farming sector, as a robust and cost-effective method for authentication of commercial organic nitrogen fertilizers.

METHODS AND INSTRUMENTATION

Sample Collection. Most of the fertilizer samples analyzed in the study were supplied in a liquid form. All fertilizer samples were collected in clean, preferably new, small glass containers of the size between 5 and 10 mL, with secure, preferably screw top, closure. The samples were placed in a cooler with ice packs and shipped to the laboratory, where they were kept refrigerated at 4 °C until their analyses were performed within 3 weeks after their arrival. Organic

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nitrogen fertilizers of known origin, used for constructing the library data set, were provided by certified producers of organic fertilizers.

Carbon and Nitrogen Contents and Isotopic Signatures by EA-IRMS. Samples of fertilizers for N and C isotope analyses were analyzed without pretreatment. All liquid samples were shaken vigorously to achieve homogeneity, and an aliquot of 1.0−3.5 mg of liquid fertilizer was transferred into a tin capsule. The amount of sample or standard analyzed in the procedure was based on the criteria that the total amount of N in it must be within the range of 0.02−0.2 mg. Each sample capsule contained about 0.5 mg of Chemosorb for retaining the volatile ingredients of the sample. Nitrogen and carbon isotope compositions were determined using a thermal combustion elemental analyzer (EA) Costech ECS 4010 from Costech Analytical Instruments Inc. (Valencia, CA, USA) coupled via the flow-reducing interface ConFlow III with continuous flow isotope ratio mass spectrometer (IRMS) Thermo Finnigan Delta^{PLUS} Advantage from ThermoFinnigan Inc. (Bremen, Germany). Each batch of samples included quality assurance and quality control (QA/QC) samples: three types of secondary reference material in duplicate analyzed before and after each batch of samples, a sample duplicate, and a procedural blank. The N (total nitrogen) and C (total carbon) contents of fertilizers were determined on the basis of the EA-IRMS analysis of acetanilide used as a calibration standard. Nitrogen and carbon isotope data are reported in conventional δ notation in units of per mil (‰) with reference to atmospheric nitrogen (air) and Vienna Pee Dee Belemnite (VPDB) carbonate standard, respectively, according to the equation for nitrogen, for example

$$\delta^{15}N_{sample}\ (‰) = (R_{sample} - R_{standard})/R_{standard} \times 1000$$

where $R = {}^{15}N/{}^{14}N$ and the standard is atmospheric nitrogen with a ${}^{15}N/{}^{14}N$ ratio of 0.00368 and a $\delta^{15}N$ value of 0‰. The instrument was calibrated with the following international reference standards: IAEA-N1 (ammonium sulfate reference material certified by the International Atomic Energy Agency) with a $\delta^{15}N$ value of 0.43‰; IAEA-N2 with a $\delta^{15}N$ value of 20.32‰, used for N; sucrose ANU with a $\delta^{13}C$ value of −10.43‰; and NBS-22 oil with a $\delta^{13}C$ value of −29.74‰, used for C isotopic quantification. Long-term performance of the mass spectrometer was monitored by analysis of secondary reference materials in every batch: acetanilide with C and N contents of 71.09 and 10.36%, respectively; dorm with $\delta^{13}C$ and $\delta^{15}N$ values of −17.17 and 14.33‰, respectively; and caffeine with $\delta^{13}C$ and $\delta^{15}N$ values of −42.02 and −0.95‰, respectively. The long-term standard deviation of the values obtained from measurements of the secondary reference materials were 0.33 and 0.25‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Nitrogen Profile Analysis. The full N profile (urea, ammonia, organic N, water-insoluble N (WIN), and NO_3) was analyzed using the following procedures. One gram of well-shaken fertilizer was placed in 1 L of double-distilled deionized water. The sample was put in a refrigerator for overnight soaking. The next morning the solution was shaken again and filtered through a preashed glass microfiber filter (GF/F) of 47 mm diameter (pore size = 0.45 μm; Cole Parmer, QC, CA) and analyzed for total dissolved nitrogen (TDN), nitrite, and nitrate using a Lachat Autoanalyzer, QuickChem 8500 series (Lachat Instruments, Lincolnshire, IL, USA).[20] Briefly, determination of TDN involves a digestion of a filtered aliquot of aqueous solution of a fertilizer with potassium persulfate at pH 12 to oxidize all organic nitrogen, ammonia, and nitrite to nitrate. The concentration of nitrate in the digested sample is then measured using a cadmium reduction column, which converts all nitrate to nitrite form. The nitrite is then determined by diazotizing with sulfanilamide followed by coupling with *N*-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water-soluble dye has a magenta color, which is read at 520 nm. Nitrite concentration in a fertilizer sample is determined without the use of a digestion step with the cadmium column removed. The method detection limit for N is 2 μg/g of fertilizer. WIN is determined by subtraction of TDN from total nitrogen (TN) determined on EA-IRMS.

The aqueous solution of the fertilizer was used to analyze the concentrations of ammonia and urea. A Dionex ion chromatograph setup was used to carry out these analyses. The concentration of ammonia was determined directly, without sample pretreatment. The ion chromatographic conditions were the following: a pump of the GP 50 series was set at 0.36 mL/min; IonPac CS-16 3 × 250 mm ion chromatography column with CG-16 3 × 50 mm guard column; AS-40 automatic sampler; CD-25 conductivity detector; chemical suppressor CCMMS-300, 2 mm; 0.02 M aqueous solution of methanesulfonic acid used as eluent and 0.103 M aqueous solution of tetrabutylammonium hydroxide as regenerant. A five-point calibration curve with the standard concentrations at 0.5, 1.0, 5.0, 10.0, and 50.0 mg/L of N in NH_4^+ form was used for quantification. The determination of the concentration of urea in fertilizer samples was performed using the urease enzyme catalysis approach, which hydrolyzes any urea present to ammonium ion[21] with the following ion chromatography analysis for ammonia. The final concentration of urea is calculated by using a simple subtraction of original ammonia ions present in untreated sample from the total concentration of ammonia in the urease-catalyzed sample. The determination of organic nitrogen is performed by subtracting the concentration of ammonia, nitrite, and nitrate from the total nitrogen concentration.

Statistical Analysis. The results of C and N contents and their isotopic signatures along with nitrogen profile data were chosen as quantitative critical characteristics (QCC) in the authentication of organic nitrogen fertilizers. QCCs of 1258 commercial organic nitrogen fertilizers obtained from various fertilizer manufacturers in North America were subjected to cluster and discriminant function analysis using SPSS software, ver. 20 for Mac. In the previous study of different application,[22] it was shown that both library size and diversity of the library samples influence the accuracy of the statistical results in cluster analysis and discriminant function analysis. Taking this into account, the fertilizers within the same source with identical QCCs were not included in the library data set because statistically the presence of fertilizers with identical QCCs hinders the fidelity of the results of discriminant function analysis. Application of these criteria resulted in assembling the compelling library data set composed of 335 unique organic N fertilizers produced from different groups of animal and plant byproducts, covering a wide diversity of the sources of organic fertilizers available on the North American market.

Cluster analysis is an unsupervised pattern recognition technique that uncovers intrinsic structure or underlying behavior of a data set without making a priori assumptions about the data, to classify the objects of the system into categories or clusters based on their nearness or similarity.[23] Hierarchical clustering is the most common approach in which clusters are formed sequentially, by starting with the most similar pair of objects and forming higher clusters step by step. The Jaccard similarity coefficient is a statistical measure used for comparing the similarity and diversity of sample sets. It is defined as the size of the intersection divided by the size of the union of the sample sets[24] and can be represented by the "difference" between analytical values from both samples. The unweighted pair group method with arithmetic mean (UPGMA) is a simple agglomerative or hierarchical clustering method, which examines the data set in a pairwise distance matrix (or a matrix of similarity) and constructs a dendogram[25] or a tree of similarity. The cluster analysis performed using Jaccard similarity coefficients and the UPGMA algorithm was applied to the library data set constructed of 335 unique organic nitrogen fertilizers from known sources with a view to group the similar sources of the samples. This statistical analysis produced nine clusters, separating plant products such as molasses, corn, and wheat from soy/alfalfa, aquatic algae, compost, animal manure, guano (bat/bird), bone meal, feather meal, and fish products.

A discriminant function analysis (unstandardized function coefficients; unexplained variance method; all groups equal prior probabilities, within-groups covariance matrix, within-groups correlation matrix, leave-one-out classification) with jacknife algorithm was performed on the cluster analysis results to evaluate how accurately QCCs are able to predict the source of an organic nitrogen fertilizer within the library data set. For each known source, the percentage of fertilizers that were classified in the correct source category is named the rate of correct classification (RCC), whereas the weighted average

Table 2. Percentage of Organic Nitrogen Fertilizers in the Library Database Assigned to the Correct Source by Using Discriminant Analysis (Jacknife Algorithm)[a]

fertilizer group	subgroup	fertilizers in database	soy/ alfalfa	molasses, corn, wheat, etc.	aquatic algae	compost	animal manure	guano (bat/bird)	bone/ blood meal	feather meal	fish meal
plant products	soy/alfalfa	32	**71.4**	13	15.6	0	0	0	0	0	0
molasses, corn, wheat, etc.		78	3.6	**64.3**	0	25	0	0	0	7.1	0
	aquatic algae	22	0	0	**100**	0	0	0	0	0	0
compost		30	0	0	0	**100**	0	0	0	0	0
animal manure		16	0	25	0	0	**50**	25	0	0	0
guano (bat/bird)		12	0	0	0	0	0	**75**	0	0	25
animal (by)products	bone/ bloodmeal	36	0	0	0	0	0	0	**82.4**	0	17.6
	feather meal	24	0	0	0	0	0	0	0	**100**	0
	fish	85	0	0	0	0	0	0	23.5	0	**76.5**

[a]Values in boldface indicate the rate of correct classification (RCC). The ARCC was 74.3%. RCC, rate of correct classification; ARCC, average rate of correct classification.

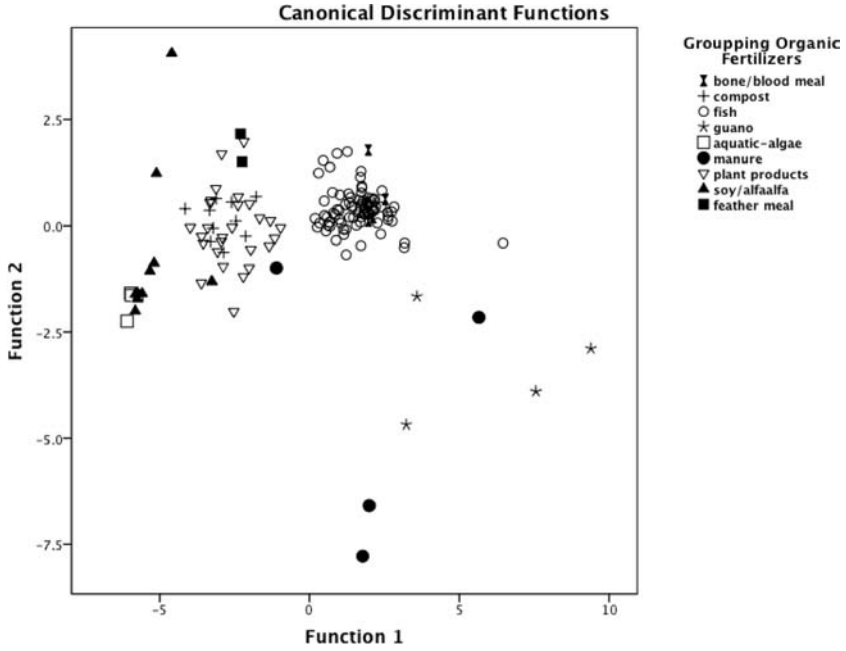

Figure 1. Clusters produced by canonical discriminant function analysis of various groups of organic nitrogen fertilizers included in the library database.

of the percentages of all fertilizers correctly classified in their source categories corresponds to the average rate of correct classification (ARCC). The leave-one-out classification or cross-validation is generated when the library fertilizers are self-crossed in both the calibration data set and test data set.

RESULTS AND DISCUSSION

A total of 1258 organic nitrogen fertilizers of different origins have been analyzed in the project. Isotopic signatures of C and N, as well as their contents and nitrogen profile characteristics (urea, ammonia, organic N, WIN, and NO_3), of nitrogen fertilizers were used as the most important critical quantitative criteria for their complete characterization and identification. These characteristics were used to construct a library data set consisting of the nitrogen fertilizers of known origin. The reproducibility of QCCs of the fertilizers included in the library database was checked by performing a complete analysis of each fertilizer in three replicates. Although the quantitative critical characteristics obtained on the replicates of the library fertilizers were not identical, the values of their relative standard deviations were <8%. For this reason, the value of 92% was chosen as the similarity threshold for the fertilizers of the same origin to be included or excluded in the library database. For example, organic nitrogen fertilizers from the same source group were included in the library database if, after the cluster analysis, their similarity value was <92%. This criterion applied to all nitrogen fertilizers analyzed in the study has narrowedthe group to 335 organic nitrogen fertilizers with unique QCCs. Nine main clusters were formed when a cluster analysis was performed on the constructed library data set. This analysis was carried out using Jaccard similarity coefficients and the UPGMA algorithm. A combination of cluster analysis with a discriminant function analysis usually provides results with additional confidence.[26,27] The nine clusters formed from 335 organic nitrogen fertilizers of the library data set by cluster analysis show the level of separation of sources as well as the relatedness of the fertilizers included in the library. The

percentage of fertilizers that has been correctly identified to its source (RCC) and the ARCC have been determined by discriminant function analysis performed on the library data set. ARCC was found to be 74.3% (Table 2; Figure 1). The groups of algae-based and humic acid-based nitrogen fertilizers (aquatic/algae), compost, feather meal, and animal byproducts such as bone meal and blood meal were highly classified with RCCs of 100, 100, 100, and 82.4%, respectively, whereas 64.3, 71.4, 50, 75, and 76.5% of plant products (molasses, wheat, corn, and green vegetables), soy/alfalfa based, manure, guano, fish-based nitrogen fertilizers, respectively, were classified correctly as their source groups (Table 2). The RCC value for animal manure fertilizers was the lowest (50%), which might be attributed to the fact that some of these fertilizers had high contents of hay causing 25% of these fertilizers to be classified as plant origin. The group of plant product fertilizers with RCC at 64.3% has 25% assigned to the compost group, reflecting in some cases a very similar source material of these two groups (see also Figure 1). Of the fish-meal nitrogen fertilizers, 23.5% were misclassified to the group of bone/blood meal fertilizers, probably due to the similar sources of both groups in some cases.

The constructed library was used as the tool in the material source identification of unknown fertilizers. A canonical discriminant function plot revealed that 335 organic nitrogen fertilizers included in the library database clearly clustered into their specific source groups (Figure 1). The two-function model showed significant group difference (χ^2 = 618.52, P < 0.005) with functions 1 and 2 having canonical correlation values of 0.940 and 0.725, respectively, and accounted for 82.9%. The details of the QCCs generated by analyzing the library's organic nitrogen fertilizers of known origin are presented in Table 3. Compiled data of our own results with the existing literature[9] nitrogen isotope data of synthetic nitrogen fertilizers are included in the table, as well. Synthetic nitrogen fertilizers were represented by synthetic commercial substances such as urea and various sorts of ammonium or nitrate salts containing various contents, forms, and isotopic values of nitrogen. This diversity of synthetic nitrogen fertilizers, when summarized in one group (Table 3), resulted in a wide range of standard deviations for most of their QCC parameters. The values of nitrogen isotopic signature of synthetic nitrogen fertilizers fit in a quite narrow range of 0.65 ± 1.64‰, reflecting the common source of nitrogen in these fertilizers, which is atmospheric nitrogen with a δ^{15}N value of 0.0‰.

The organic nitrogen fertilizers of plant origin (n = 132) have been clustered into three subgroups: plant products of such origin as molasses, corn, wheat, and green vegetables; soy/alfalfa group; and algae-based humic/fulvic acids (Tables 2 and 3). The QCC parameters of all three subgroups vary quite widely, reflecting the chemical, physiological, and environmental characteristics of their source materials. For example, the total nitrogen contents (Table 3) of both soy/alfalfa-based fertilizers and algae-based humic/fulvic acids are quite low, 3.05 ± 3.57 and 0.62 ± 0.69%, respectively, whereas their carbon contents differ quite significantly, 18.17 ± 19.06% for soy/alfalfa-based fertilizers and 1.34 ± 1.36% for algae-based humic/fulvic acids. It is worth noting that their C and N isotopic signatures are very similar, δ^{13}C = −26.02 ± 1.32‰ and δ^{15}N = −0.07 ± 0.30‰ and δ^{13}C = −24.50 ± 1.29‰ and δ^{15}N = −0.23 ± 0.33‰ for soy/alfalfa-based fertilizers and algae-based humic/fulvic acids, respectively. The water

Table 3. Summary of the Mean Values of Quantitative Critical Characteristics (QCC) of the Organic Fertilizers Included in the Library Database with Their Standard Deviations

fertilizer group	C, %	N, %	δ^{13}C	δ^{15}N	NH_4, mg/g	urea, mg/g	NO_3, mg/g	WIN, mg/g	organic N, mg/g
plant products (molasses, corn, etc.)	20.98 ± 14.31	4.40 ± 3.74	−20.67 ± 5.99	5.23 ± 3.94	4.00 ± 5.62	2.24 ± 4.13	1.22 ± 1.81	10.37 ± 11.86	34.42 ± 35.09
soy/alfalfa (plant)	18.17 ± 19.06	3.05 ± 3.57	−26.02 ± 1.32	−0.07 ± 0.30	0.66 ± 0.93	3.67 ± 7.62	0.01 ± 0.0004	17.76 ± 25.47	22.66 ± 29.00
aquatic/humic/fulvic acids	1.34 ± 1.36	0.62 ± 0.69	−24.50 ± 1.29	−0.23 ± 0.33	0.09 ± 0.08	1.74 ± 1.24	ND	4.06 ± 5.92	6.07 ± 6.82
compost	5.46 ± 1.74	0.60 ± 0.17	−18.21 ± 1.99	6.74 ± 2.55	0.06 ± 0.03	0.10 ± 0.04	0.01 ± 0.004	6.03 ± 0.86	6.31 ± 1.13
manure	10.61 ± 13.11	7.57 ± 5.84	−26.51 ± 7.03	9.50 ± 8.81	5.98 ± 1.28	0.60 ± 0.28	0.001 ± 0.0005	2.56 ± 0.58	14.72 ± 3.55
guano (bat and bird)	6.46 ± 5.92	8.24 ± 5.02	−12.55 ± 2.90	24.46 ± 6.62	12.38 ± 2.84	3.24 ± 1.64	0.004 ± 0.001	ND	3.24 ± 1.84
fish products	18.23 ± 5.49	4.43 ± 1.32	−18.58 ± 2.23	12.33 ± 2.99	4.68 ± 2.40	1.60 ± 1.55	0.35 ± 1.35	13.39 ± 12.46	39.70 ± 13.29
feather meal	55.47 ± 8.25	12.92 ± 4.32	−18.04 ± 3.04	3.55±1.54	5.62±2.04	1.25±1.22	ND	51.25 ± 3.28	110.21 ± 36.47
animal (by)products (bone/blood meal)	25.57 ± 15.53	6.43 ± 3.91	−18.46 ± 1.57	9.54 ± 4.77	6.39 ± 0.19	1.17 ± 0.52	0.02 ± 0.01	8.31 ± 2.12	36.15 ± 15.01
synthetic	4.68 ± 10.87	24.55 ± 15.05	−16.84 ± 8.95	0.65 ± 1.64	108.52 ± 76.58	276.47 ± 122.31	174.58 ± 110.32	ND	276.47 ± 122.31

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Table 4. Quantitative Critical Characteristics of Some Groups of Organic Nitrogen Fertilizers and Their Mixtures with Synthetic Chemicals

origin	mg of N added as chemical per g of mix	total C, %	total N, %	$\delta^{13}C$	$\delta^{15}N$	N as NH_4, mg/g	urea, mg/g	N as NO_3, mg/g	WIN, mg/g	organic N, mg/g
fish/urea	66.91	19.15	11.44	−21.06	5.31	1.58	67.77	0.02	7.25	114.28
fish/urea	55.42	19.09	10.29	−20.49	6.51	1.84	56.28	0.03	8.05	102.3
fish/$(NH_4)_2SO_4$	44.29	15.37	8.66	−16.94	7.66	47.28	0.38	0.03	8.76	23.54
fish/$(NH_4)_2SO_4$	32.76	16.77	7.86	−17.00	9.16	35.96	0.46	0.04	9.26	28.18
fish/$(NH_4)_3SO_4$	49.48	15.09	9.11	−16.97	6.64	53.12	0.36	0.02	8.04	20.68
fish/$(NH_4)_2SO_4$	15.36	17.87	6.39	−16.98	11.55	18.76	0.64	0.04	11.28	36.14
fish/KNO_3	10.55	17.18	5.69	−16.98	12.92	2.28	0.36	10.47	12.87	37.25
fiSh/KNO_3	18.74	16.63	6.49	−16.99	11.16	1.84	0.31	18.56	10.26	36.07
fish/KNO_3	35.02	14.94	7.78	−16.98	8.77	1.12	0.28	35.11	9.16	25.82
fish/KNO_3	36.33	14.33	7.69	−16.98	8.58	1.02	0.24	36.42	8.84	25.41
fish/$NaNO_3$	22.24	16.51	6.72	−16.94	10.33	1.76	0.26	22.31	9.94	29.63
fish/$NaNO_3$	11.78	17.79	5.99	−16.93	12.60	2.36	0.34	11.67	12.32	36.12
fish/$NaNO_3$	38.64	14.94	8.17	−17.00	7.71	1.22	0.28	38.52	8.04	25.04
fi3h/$NaNO_3$	43.7	14.52	8.46	−16.95	7.28	0.86	0.19	43.82	8.11	23.41
fish products	0	**18.23**±5.49	**4.43**±1.32	**−18.58**±2.23	**12.33**±2.99	**4.68**±2.40	**1.60**±1.55	**0.35**±1.35	**13.39**±12.46	**39.70**±13.29
2/3 molasses/1/3 fish-$NaNO_3$	18.64	13.48	5.67	−23.88	7.50	3.35	3.01	18.24	4.68	26.82
2/3 molasses/1/3 fish-$NaNO_3$	35.11	11.86	7.11	−23.75	5.89	2.17	1.25	34.88	3.08	21.84
plant products (molasses, corn, etc.)	0	**20.98** ± 14.31	**4.40** ± 3.74	**−20.67** ± 5.99	**5.23** ± 3.94	**4.00** ± 5.62	**2.24** ± 4.13	**1.22** ± 1.81	**10.37** ± 11.86	**34.42** ± 35.09
bone meal/KNO_3	0	20.82	5.30	−16.67	9.54	6.39	1.17	0.02	8.31	46.15
bone meal/KNO_3	12.14	18.24	6.08	−16.72	7.56	4.56	0.76	12.28	7.21	40.08
bone meal/KNO_3	38.61	13.081	7.68	−16.22	4.72	3.72	0.38	37.88	5.35	31.54
animal (by)products (bone/blood meal)	0	**25.57** ± 15.53	**6.43** ± 3.91	**−18.46** ± 1.57	**9.54** ± 4.77	**6.39** ± 0.19	**1.17** ± 0.52	**0.02** ± 0.01	**8.31** ± 2.12	**36.15** ± 15.01
poultry manure/feather meal-urea/$NaNO_3$	62.76-urea, 46.72-$NaNO_3$	3.15	11.29	−33.88	−0.41	3.7	62.9	47.00	ND	63.4
poultry manure/feather meal-urea/$NaNO_3$	29.80-urea, 30.00-$NaNO_3$	5.67	6.82	−25.67	3.21	1.38	29.84	30.25	0.52	31.63
manure	0	**15.61** ± 20.35	**2.88** ± 1.42	**−20.47** ± 0.87	**15.47** ± 9.43	**5.98** ± 1.28	**0.60** ± 0.28	**0.001** ± 0.0005	**2.56** ± 0.58	**14.72** ± 3.58

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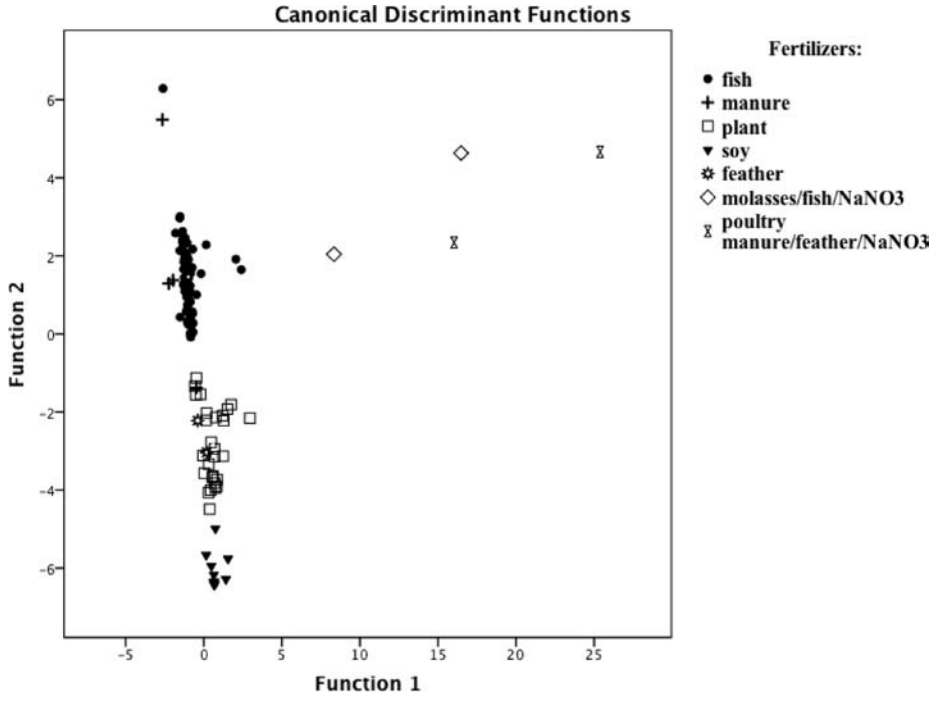

Figure 2. Identification of adulteration of organic nitrogen fertilizers (molasses/fish and poultry manure/feather meal) with synthetic chemicals using the library database and canonical discriminant function analysis.

solubility of these fertilizers varies quite widely, from very soluble subgroup such as algae-based humic/fulvic acids fertilizers to soy/alfalfa-based and plant products with WIN = 4.06 ± 5.92, 17.76 ± 25.47, and 10.37 ± 11.86 mg/g, respectively (Table 3).

All organic nitrogen fertilizers used to assemble the library database were received from certified producers. Most of the fertilizers were accompanied with their major ingredients and information about their production processes. On the basis of the information assembled in the library database and the literature data,[9−12] nitrogen fertilizers could be quite easily clustered into two groups, synthetic and organic origin, by using just their nitrogen isotopic signatures. Using canonical discriminant function analysis applied to these two groups, the χ^2 was found to be 1415.92 and the first two discriminant functions accounted for 99.1%. The canonical correlation coefficients of the first two functions were 0.998 and 0.940, respectively. Nevertheless, the proper identification of the source of nitrogen fertilizers without additional characteristics such as content and isotope values of carbon, as well as nitrogen profile parameters, becomes less accurate when adulteration (addition of synthetic nitrogen) is involved or when there is a need to distinguish within the group of organic fertilizers. For example, when a synthetic nitrogen compound is added in small portions to an organic nitrogen fertilizer, the value of $\delta^{15}N$ of the final product changes according to the formulas:

$$\delta^{15}N_{final}\,(‰) = \sigma_{fertilizer} \times \delta^{15}N_{fertilizer} + \sigma_{chemical} \times \delta^{15}N_{chemical}$$

$$\sigma_{fertilizer} + \sigma_{chemical} = 1$$

where $\delta^{15}N_{fertilizer}$ and $\delta^{15}N_{chemical}$ are the nitrogen isotopic signatures of the organic fertilizer and the chemical compound, respectively, and $\sigma_{fertilizer}$ and $\sigma_{chemical}$ are the contents of nitrogen in the mixture attributed to the organic fertilizer and the chemical, respectively. We have analyzed mixtures of various synthetic fertilizers with different types of organic fertilizers at a wide range of their proportions. The results of the analyses of these "adulterated" organic fertilizers are presented in Table 4. It is clear from these data that due to a wide diversity of the sources of the materials used in the production of organic nitrogen fertilizers, there is a lot of room for adulteration of their formula. For this reason, a complete characterization of organic nitrogen fertilizers, which involves a determination of a full spectrum of their QCC, becomes essential in their authentication. Besides QCC parameters, information about the source of the material used in manufacturing any specific organic nitrogen fertilizer has also essential value in helping to increase their RCC. For example, the specification of the sources of fertilizer material helped to increase the RCC of the mixtures of such fertilizers as molasses/fish-based fertilizers with small amounts of $NaNO_3$ and poultry manure/feather meal-based fertilizers with small amounts of urea/$NaNO_3$ (Table 4). The RCCs of these samples changed from 88.5 and 92.6% to 100 and 100%, respectively, confirming the identification of their adulteration with high confidence (Figure 2).

The results of this study have demonstrated the accuracy and reliability of the developed methodology in the identification of the sources of organic nitrogen fertilizers. A number of adulteration cases have been identified among 1258 commercial organic nitrogen fertilizers analyzed. The robustness of the developed methodology as well as the constructed compiled library data set of organic nitrogen fertilizers from various sources has allowed us to apply this technique in a certification procedure used by a few organic material certification agencies in North America.

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Notes

The authors declare no competing financial interest.

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