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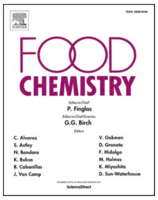

Isotopes Don’t Lie, differentiating organic from conventional banana (*Musa* AAA, Cavendish subgroup) fruits using C and N stable isotopes

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ABSTRACT

With the dramatic increase of organic banana production worldwide, it is essential to be able to monitor compliance with organic specifications. While the detection of pesticide fraud is routinely controlled by detecting pesticide residues in organic bananas, the detection of fertilizer fraud is much more complex. We compared the δ^{13}C and δ^{15}N isotopic values of green bananas from organic and conventional farms at seven sites around the world. In our whole dataset, the δ^{15}N values of banana fruits ranged between −1.25 and + 8.91‰. In all sites, δ^{15}N values of organic banana were significantly higher than conventional fruits (mean value of + 5.24‰ and + 2.342‰, respectively). Conversely, the type of fertilization did not significantly alter δ^{13}C values. Our results suggest that it is possible, upon arrival in importing countries, to differentiate bananas grown with synthetic fertilizer from those grown with organic fertilizer.

1. Introduction

With more than 97,000,000 tons produced yearly, bananas are the leading fruit produced and consumed in the world, providing an essential source of nutrients to the people living in producing and importer countries (ODEADOM, 2021). Bananas were also the first fresh fruit that is exchanged worldwide (CIRAD, 2021). The demand for healthy food that is also cultivated in an ’environmentally-friendly’ way is increasing dramatically. In particular, the demand for organic dessert bananas is growing very fast in Europe and in North America. For Europe, the market share of organic bananas has doubled between 2013 and 2020 from 6 to 12% (Dawson, 2021). On average, organic bananas are priced about 20 % higher than conventional bananas. Organic production specifications require the exclusion of synthetic conventional pesticides and mineral synthetic fertilizers. While the detection of pesticide fraud is routinely controlled by detecting pesticide residues in organic bananas, the detection of fertilizer fraud is much more complex. In the organic specifications, it is forbidden to use synthetic fertilizers, only organic fertilizers are allowed by European and American regulations (Department of Agriculture of USA, 2000; EU, 2018). Organic fertilization programs may be up to 6-times more expensive than those based on synthetic fertilizers (CIRAD, personal data), consequently some producers may be tempted to defraud by using synthetic fertilizers. Indeed, reducing production cost is a potential lever to fulfill an increasing demand while maintaining low sale prices. Furthermore, it is not possible to exclude that conventionally produced bananas can simply be substituted for organic ones in the supply chain.

Organic and synthetic fertilizers are manufactured using completely different processes. The nitrogen of synthetic fertilizers originates from the conversion of atmospheric N_2 into ammonia through the Haber-Bosch process that requires a high quantity of energy, high pressures and effective catalyst (Haber & Le Rossignol, 1913). Conversely, the nitrogen contained in organic fertilizers comes from organic matter from plant and animal materials, which are often composted (Senesi, 1989). Based on this difference, using stable isotopes, especially ^{15}N (versus ^{14}N), has been used to discriminate nitrogen sources in plant nutrition. This is based on the fact that δ^{15}N values of synthetic fertilizers is close to atmospheric nitrogen gas, while organic fertilizers are generally enriched in ^{15}N (Bateman & Kelly, 2007). The higher δ15N values for organic fertilizer are the result of the isotopic fractionation that occurs in many biological and physical processes, i.e. during the growth of plants or animals constituting organic matter (Deniro & Epstein, 1981;

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Minagawa & Wada, 1984). This isotopic method has been successfully used to discriminate between organic and conventional cultivation for lettuce (Šturm et al., 2011), diverse vegetables (Šturm & Lojen, 2011), and chicory (Sinkovič et al., 2020). In some cases, it was not possible to discriminate successfully between organic and conventional products, as for many vegetables (Schmidt et al., 2005). Nitrogen isotope analysis was also applied to animal products, such as eggs (Rogers et al., 2015) or lamb muscle (Cantalapiedra-Hijar et al., 2016). More recently, other isotopes have been used for tracing the sources of fertilizers in agriculture. For instance, Bontempo et al. (2016) showed that a combination of five stables isotopes is helpful to determine both the geographic origin and the type of cropping system (organic vs. conventional). There is currently a lack of data on the feasibility to apply this approach to banana. To date, there is very limited data that compare in the same sites real organic and conventional banana, one case in Brazil with 10 measures (Trapp et al., 2021) and one in Ecuador with 12 measures (Wang et al., 2021).

In dessert banana plantations the yearly nitrogen fertilization is usually between 300 and 600 kg.ha^{-1}.y^{-1} while losses of nitrogen are typically around 100 kg.ha^{-1}.y^{-1} (Dorel et al., 2008). A large part of this excess of nitrogen inputs is lost by leaching (Armour et al., 2013; Sansoulet et al., 2007). As many intensive agricultural systems, the health issues of mineral fertilizer use are becoming a growing concern, with the presence of cadmium in agricultural product (Carne et al., 2021) or nitrates in drinking water (Camargo & Alonso, 2006). In conventional banana cropping systems, fertilizer applications are generally made monthly with synthetic and mineral products. To comply with the specifications of organic agriculture, the fertilization can only be done with organic matters, usually mixing vegetal, animal and manure. In organic systems, biostimulants (with unstandardized compositions) are also often applied. The cost of organic fertilization programs is much higher than a conventional ones; organic matters are between 3 and 5-times more expensive and requires more labor to be applied in the field (CIRAD, unpublished data). Export banana plantations are located across tropical countries, mainly in Latin America, Caribbean, Africa, and South East Asia, with different soil types and climatic conditions. These soils participate indirectly to the nutrition of banana plants through the mineralization of their organic matters (Dorel et al., 2008). The isotopic signal of nutrients originated from the mineralization of soil organic matter and fertilizers immobilized in the living compartment are susceptible to have been altered compared to the signal of fertilizers applied (Amelung et al., 2008). This interference in the potential use of stable isotopes to separate organic from conventional fertilization systems implies testing this method in broad ranges of soil conditions.

In this study, we investigated for the first time how the type of fertilization, i.e. organic versus conventional, altered the δ^{13}C and δ^{15}N values of banana fruits across a wide range of conditions in West Africa and in the French West Indies. We analyzed fruits originating from 14 farms (seven organic and seven conventional) from seven sites in four countries with contrasted situations of soil and climate; allowing a one-to-one comparison of organic and conventional fruits. From our dataset that included 238 individual fruits measures, we quantified the differences of isotopic values between organic and conventional fertilization treatments. A global analysis of all situations aimed to determine if there is a threshold that can reliably separate the two types of fertilization. We also analyzed the sampling effort needed to provide an isotopic signature that is representative of a given type of fertilization in a given region. Finally, we discuss our results with the perspective of developing a standardized method.

2. Materials and methods

2.1. The banana fruits collection

We collected banana fruits from seven sites with one conventional and one organic farm for each site (14 farms sampled in total). Three sites were located in the French West Indies in the Caribbean (one site in Guadeloupe and two sites in Martinique). The four other sites were located in West Africa (three sites in Ivory Coast and one site in Ghana). The conventional farms were following typical fertilization programs with the monthly application of synthetic/mineral fertilizers. Importantly, we were certain that organic farms were strictly following organic regulations, i.e. they only applied organic fertilizers that were mixtures of plant, animal, and manure organic materials. The doses of nutrients applied in both conventional and organic programs were similar, leading to comparable yields (ranging between 40 and 60 tons. ha^{-1}.y^{-1}). For each farm, we randomly sampled 10 to 20 individual fruits (238 fruits analyzed, 17 fruits per farm on average). Banana fruits were sampled at arrival in France following the regular process of packing and transport (at 13 °C). Fruits were collected at a green stage (before ripening process) and were representative of the production of a given farm. All fruits were analyzed separately. To test the possibility of pooling fruit to reduce the number of analyses, we also analyzed the isotopic content of a composite sample combining ten fruits from the conventional farm in Site 1.

2.2. Sample preparation

After fruit collection, fruits were immediately prepared. On each fruit, a 1 cm large slice of the banana was cut in the middle of the fruit (including pulp and skin). It was frozen at −80 °C and then lyophilized for three days. Finally, all samples were ground into fine powder (Retsch MM400) and kept in hermetically closed Eppendorf tubes.

2.3. Isotopic analysis

All isotopic analysis were carried out in the isotopic laboratory of the B&PMP unit in Montpellier, France. Samples were analyzed by isotopic mass spectrometry using mass-spectrometer ‘isoprime precisION’ coupled to Vario-PYROcube elemental analyzer (Elementar, UK). Samples were packed in tin cup (1 mg) then injected into the elemental analyzer. After combustion at 920 °C in the presence of oxygen and CuO, the gas molecules from the sample (mainly H_2O, CO_2, N_2) were transported by a flow of carrier gas (ultrapure Helium) to a reduction oven where nitrogen oxides were reduced to N_2 in the presence of copper at 600 °C (Dumas reaction). The H_2O produced was trapped by SICAPENT columns (Merck). The N_2 and the CO_2 were then separated. The CO_2 was trapped at ambient temperature in a programmable temperature desorption column and released at 100 °C. A Thermal Conductivity Detector allowed the quantification of total N and C. The N_2 and CO_2 were analyzed with the mass spectrometer (IsoPrime Precision, Elementar, UK) to determine the isotopic ratio of ^{15}N/^{14}N and ^{13}C/^{12}C. All stable isotope values were reported in the δ notation, with δ^{13}C or δ^{15}N calculated as.

$[(R_{sample}/R_{standard}) - 1\ ]$, where R is ^{13}C/^{12}C or ^{15}N/^{14}N. Reference substances were PeeDee Belemnite (Peterson & Fry, 1987) and atmospheric air (Mariotti, 1983) for C and N, respectively. Isotope delta values within the manuscript are reporting using the permille notation.

2.4. Calculation and statistical analysis

We tested the significance of the type of fertilization (conventional vs. organic) on the δ^{15}N and δ^{13}C values using mixed effect linear models using the ‘lme4’ package (Bates et al., 2015) with the site as a random factor on the intercept of the model (fitted with Laplace approximation). Separately for each site, the difference between the mean values of δ^{15}N or δ^{13}C between the two types of fertilization were tested with a Student’s *t* test. All statistical analyses were performed with R 4.1.1 (R Core Team, 2021) and with an alpha level of 0.05.

We carried out a bootstrap analysis with R to identify the sampling effort required to establish the number of individual fruits to be analyzed to provide a mean δ^{15}N value consistent with the segregation between fruits from organic and conventional systems. For each farm (two farms per site, 14 farms at all), we analyzed all possible sampling efforts ranging between two and the total number of analyzes performed for that farm. Then, we did 10,000 iterations, each corresponding to the calculation of the mean value of n randomly chosen samples. Among these iterations, we calculated the difference between the smallest and the biggest mean value, which can be considered as an indicator of the potential of error done in the estimation of the real mean value.

3. Results and discussion

3.1. Variation of δ^{15}N values across sites and fertilization types

In our whole dataset, the δ^{15}N values of banana fruits ranged between −1.25 and 8.91‰. The percentage of nitrogen was extremely homogeneous in our whole dataset, with an average value per farm ranging between + 0.705% and + 0.897%, it was not related to the type of fertilization. The richness in nitrogen of fruit was thus not a factor that could have altered the δ^{15}N values. This δ^{15}N values range is comparable to what is usually found in plants across diverse conditions of fertilization (Hayashi et al., 2011). The average δ^{15}N values of each site (organic and conventional together) ranged from + 3.08 and + 6.34‰. The site 7 located in Ghana exhibited the lowest δ^{15}N values. Inversely, the three sites in Ivory Coast (sites 4, 5 and 6) exhibited the highest δ^{15}N values. We hypothesize that this difference between sites is mainly the result of the soils types, especially in terms of organic matter. Indeed, soils in site 7 were the poorest from our dataset, i.e. with lower organic matter content. The variation range of δ^{15}N values for a given fertilization type and site was relatively homogeneous, with a median value of + 4.50‰. There was a highly significant effect of the type of fertilization on the δ^{15}N values (Table 1). This difference was always significant when making the comparison among each site (Fig. 1). Our results are in-line with recent observations in a limited dataset (one site) in Brazil (Trapp et al., 2021) and with one in Ecuador (Wang et al., 2021). For each site, the mean δ^{15}N value of fruits from organic fertilization was always above the one of fruits from mineral fertilization treatment. The average difference between the two types of fertilization ranged between + 1.63 and + 4.78‰ with a mean value of + 2.83‰. These significant differences in mean δ^{15}N values indicate that these measures reliably segregate banana fruits grown organically from those grown in conventional systems for the geographical locations and farms studied in this project. Our results demonstrate that Cavendish banana is a plant for which the δ^{15}N value is strongly altered by the type of fertilization. The magnitude of this response is similar to those observed for potatoes (Gatzert et al., 2021), chicory plant (Sinkovič et al., 2020), olives (Benincasa et al., 2018), and orange (Rogers, 2008), and much larger than the one observed for wheat (Bontempo et al., 2016). The difference between δ^{15}N value of organic and conventional banana reflects the difference between organic and mineral fertilizers (Verenitch & Mazumder, 2012) applied in these two cropping systems, respectively. The site that exhibited the lowest δ^{15}N value difference between organic and conventional (Site 2 in Martinique) is corresponding to a recent (3 years) conversion to organic agriculture. This is consistent with previous studies that showed that δ^{15}N values of plants have continuously varied for four years after a change in fertilizer type (Hayashi et al., 2011).

Table 1
Result from linear mixed model that tested the effect of the type of fertilization on the δ^{15}N (A) and δ^{13}C (B) values, with the site as a random factor on the intercept. The estimate of the fertilization type is given for the organic treatment compared to the conventional treatment.

Predictors	Estimates	CI	p-value	d.f.
δ^{15}N model				
Intercept	2.25	1.22–3.283	0.001	6.55
Fertilization type	2.89	2.55–3.24	<0.001	230.08
Marginal R^2: 0.411; Conditional R^2: 0.641				
δ^{13}C model				
Intercept	−24.74	−25.11 to −24.37	<0.001	7.05
Fertilization type	−0.42	−0.59 to −0.26	<0.001	230.14
Marginal R^2: 0.072; Conditional R^2: 0.300				

With CI, the confidence interval of estimates, and d.f. the approximated degrees of freedom.

3.2. Variation of δ^{13}C values across sites and fertilization types

The δ^{13}C values followed a very different pattern compared to δ^{15}N values. In our dataset, δ^{13}C values of banana fruits ranged between −26.85 and −22.21‰. This range is corresponding to values usually measured for C3 plants (Balesdent et al., 1987; DeNiro & Epstein, 1978). The average δ^{13}C values of each site ranged from −25.40 and −24.21‰. The site 7 located in Ghana exhibited the highest δ^{13}C values. We hypothesize that the higher δ^{13}C values in Site 7 could be the result of the irrigation regime that is more intensive than in other sites due to dryer conditions, which is a factor that can alter the δ^{13}C values (Unger et al., 2010). There was a highly significant effect of the type of fertilization on the δ^{13}C values (Table 1). However, this difference was only significant in the cases of Site 2 and Site 4 when making the comparison among each sites (Fig. 2). These results show that δ^{13}C values is a poor tracer of the type of fertilization applied on banana culture. However, the δ^{13}C values have a potential at determining the geographical origin of banana fruits. It cannot be used alone but together with other variables tracer of the context of growth (Di Paola-Naranjo et al., 2011; Zhao et al., 2020). In future studies, it would be relevant to address the effect of climatic conditions on δ^{13}C values, which are known to fluctuate with climate and latitude (Körner et al., 1991).

3.3. Thresholds and sampling effort needed for the detection of the fertilization type

The issue in developing a standardized method to segregate organic and conventional products relies in the definition of thresholds in variables used as tracers. When δ^{15}N and δ^{13}C values are considered together (Fig. 3), it is clear that there is not a single threshold including one or the two isotopes that can segregate surely organic from conventional banana fruits. The threshold of + 4‰ for δ^{15}N values is interesting because there were no conventional banana fruits that were above this limit. However, it is not a threshold that indicates surely that a banana fruit is organic; indeed bananas from Site 7 had a mean δ^{15}N value of + 3.01‰. Interestingly, this threshold of + 4‰ for δ^{15}N values was also identified in the screening of a wide range of commercial products (Rogers, 2008). Based on our data, it is needed to consider a threshold of + 3‰ for δ^{15}N values to be sure that banana fruits were grown with conventional fertilization. Our main conclusion is thus that it is needed to establish a regional reference for organic and conventional δ^{15}N values of banana fruits.

In our dataset, we measured up to 20 individual fruits from a given region and with a given type of fertilization. Reducing the sampling effort would make the approach more applicable. The bootstrap analysis showed that at least 16 measures are needed to insure that the mean δ^{15}N value of a given condition of production (site and fertilization type) does not vary more than 1‰ (Fig. 4). According to our results, a precision of 1‰ should be sufficient to discriminate organic from conventional banana fruits. Aside the number of measures, needed to stabilize the mean value, pooling samples from different banana fruits from a given farm would probably be an option to reduce the number of measures, but not the fruit sampling effort. This is suggested by the comparison of measures made on individual fruits with repeated measures of a pooled sample of the same fruits (Fig. 5).

The difficulty of using isotopic signature, in particular δ^{15}N value, to segregate without doubt in all cases organic from conventional products is an increasing issue. Recently, Liu et al. (2020) had a similar issue with

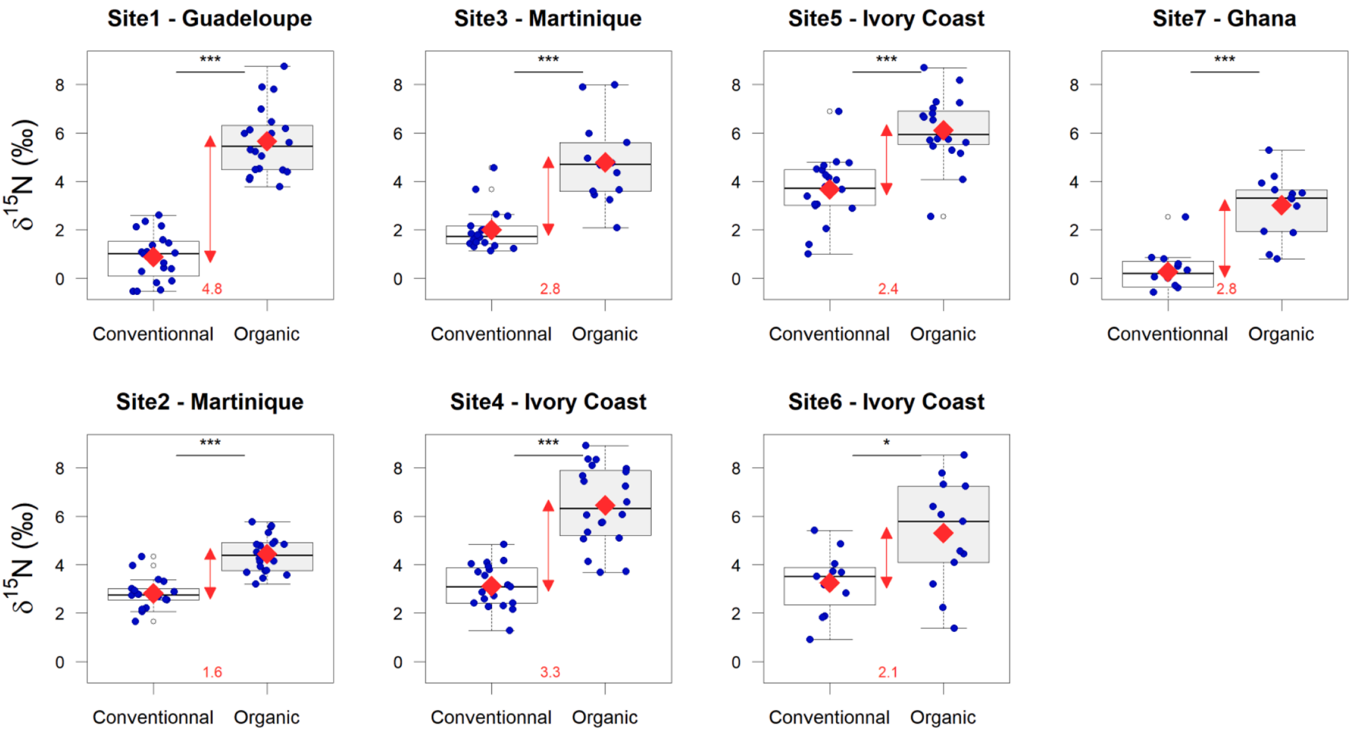

Fig. 1. Distribution of the δ^{15}N values according to the site and the type of fertilization. The blue dots show all the individual data (jittered on the x-axis for a better visualization). The red dots shows the mean values. Red arrows and the red numbers below show the average difference between fertilization treatments when significantly different. Asterisks at the top of each plot indicate the level of significance of the Student's t-Test that compared the mean value between conventional and organic fruits.

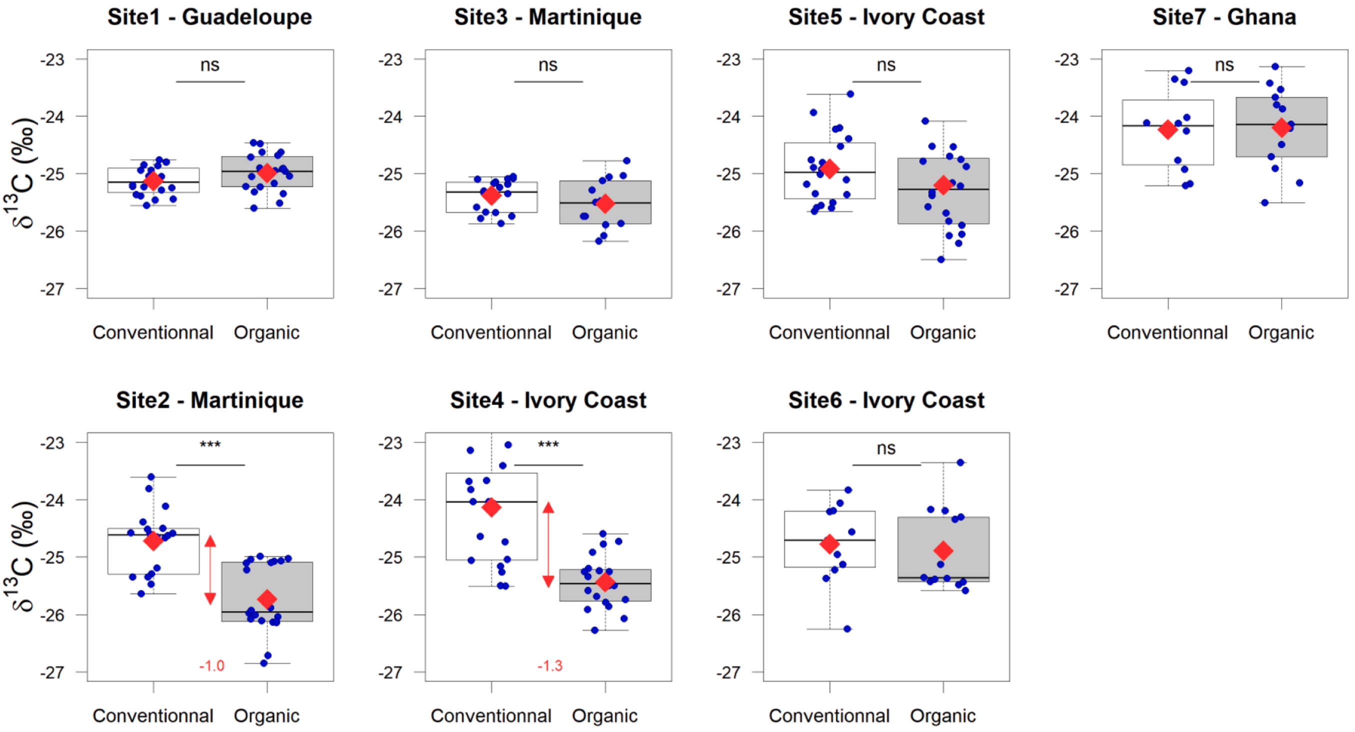

Fig. 2. Distribution of the δ^{13}C values according to the site and the type of fertilization. The blue dots show all the individual data (jittered on the x-axis for a better visualization). The red dots shows the mean values. Red arrows and the red numbers below show the average difference between fertilization treatments when significantly different. Asterisks at the top of each plot indicate the level of significance of the Student's t-Test that compared the mean value between conventional and organic fruits (ns for non-significant).

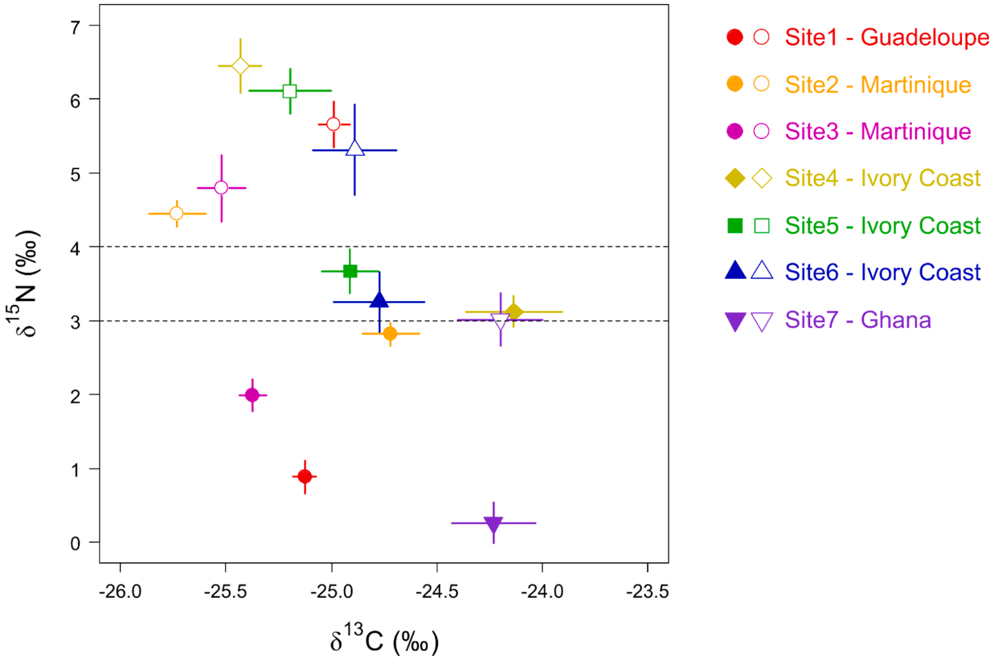

Fig. 3. Mean (±SE) δ^{15}N and δ^{13}C values according to the site and to the type of fertilization. Open and closed symbols represent organic and conventional fruits, respectively. The dash lines represents the thresholds discussed in this manuscript as potential limits between organic and conventional banana fruits.

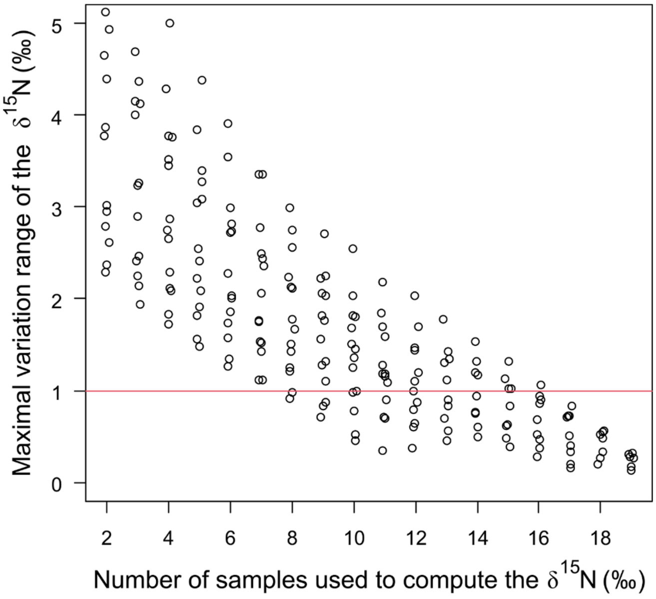

Fig. 4. Variation range (estimated with bootstrap analysis with 10,000 iterations) of the mean δ^{15}N values of a given farm according to the number of samples (slightly jittered to facilitate the visualization) used for the calculation for each situation.

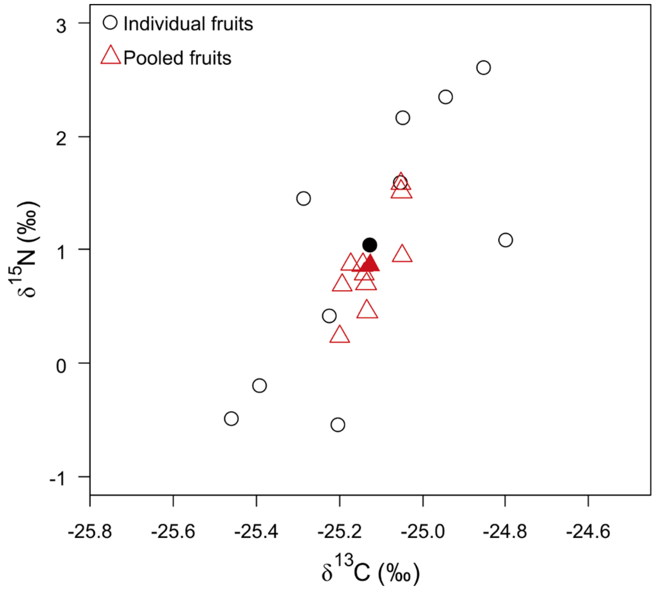

Fig. 5. Comparison of δ^{15}N and δ^{13}C values from ten individual fruits (open black points) and ten repetitions of the same fruits pooled (open red triangles). The filled black point and the filled red triangle show the mean values of individual fruits samples and pooled samples, respectively.

rice. Again, δ^{15}N values around + 4‰ were on the transition zone between surely organic and surely conventional products. Interestingly, in this later study, there was an intermediate product (green rice that was produced with a fertilization plan between organic and conventional). Detecting a drift in δ^{15}N signature with a change in fertilization is essential for being able to identify potential fraud to organic regulation. Using a multi-parameters analysis (including δ^{15}N and δ^{13}C values), Sinkovič et al. (2020) demonstrated that a mixed organic-conventional fertilization was well discriminated from pure organic and pure conventional fertilization. In this study, the mixed fertilization plan was close to the conventional plan on chicory plants, suggesting a good sensitivity to detect the integration of mineral to organic fertilizer. Future studies on banana should address this issue for instance by adding contrasted doses of mineral fertilizer to organic situations and then measure the evolution of the δ^{15}N values of fruits over the time. In the perspective to develop a routine method, it will also be interesting to test the robustness of the method with anonymized samples from different regions. Since storage conditions for bananas are highly standardized in the export industry and we performed our analyses on pre-ripening fruits, we can assume that storage and ripening conditions did not affect the δ^{15}N values of the fruit. However, it might be interesting to evaluate the method on post-ripening fruits, for example sampled directly from stores, to make the method available to any consumer.

Our results support the potential of δ^{15}N signature to discriminate organic from conventional banana fruits. To develop further a practical method to detect potential fraud in organic fertilization, it is essential i) to build a database in all production zone of interest, and ii) to test experimentally the sensitivity of the measure to the addition of mineral fertilizer in organic systems. It would be particularly useful to combine isotopic measurements with those of potential pesticide residues in order to detect any fraud of organic regulations.

CRediT authorship contribution statement

Philippe Tixier: Conceptualization, Investigation, Formal analysis, Writing – original draft, Visualization. **Denis Loeillet:** Conceptualization, Project administration. **Mathieu Coulis:** Conceptualization, Investigation, Writing – review & editing. **Thierry Lescot:** Conceptualization, Writing – review & editing. **Luc de Lapeyre de Bellaire:** Supervision, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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