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Comparison of mineral concentrations in commercially grown organic and conventional crops – Tomatoes (*Lycopersicon esculentum*) and lettuces (*Lactuca sativa*)

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ABSTRACT

Trace element concentrations and stable nitrogen isotope data ($\delta^{15}N$‰) from tomatoes (*Lycopersicon esculentum*) and lettuces (*Lactuca sativa*) were subjected to multivariate analysis in an attempt to distinguish between conventional and organic cultivation. This approach improved the correct classification of tomato samples but appears to have had a limited effect on lettuces. Our findings support the growing body of evidence which suggests that systematic differences in the concentrations of certain elements such as manganese, calcium, copper, and zinc may occur between crops cultivated under organic and conventional regimes possibly due to the presence of elevated levels of arbuscular mycorrhizal fungi (AMF) in soils cultivated organically. We assert that such differences in elemental composition may be useful as 'indicators of authenticity'. However, we recognise the limitation that this approach may be restricted to horticultural crops where there are significant differences in agricultural practice such as conventional-hydroponic versus soil-grown organic tomatoes.

1. Introduction

Price differentials or simply higher demand means there is an economic incentive to mislabel conventionally grown crops as 'organic' (Nelson, Giles, MacIlwain, & Gewin, 2004). Generally, organic products sell for higher prices and this reflects the additional costs associated with less intensive cultivation methods, maintaining exclusive supply chains and the costs associated with appropriate accreditation and inspection (van Elzakker & Neuendorff, 2005). Common triggers for fraudulent activity are a shortage of an agricultural product, especially of a satisfactory quality, leading to a product becoming relatively expensive (Dennis, 1997). Within the scope of a 'critical control point approach' to identifying organic fraud in international supply chains, it has been suggested that it would be advantageous to have analytical methods in place to verify product authenticity (Siderer, Maquet, & Anklam, 2005).

Previous studies have demonstrated that the nitrogen isotope composition of a crop may be exploited to distinguish between crops grown using conventional synthetic fertilisers and crops grown under organic conditions (Choi, Ro, & Lee, 2003). However, there is evidence that the timing of application (Choi, Lee, Ro, Kim, & Yoo, 2002) and the chemical form of a synthetic fertiliser (Evans, Bloom, Sukrapanna, & Ehleringer, 1996) are important in determining how fertiliser $\delta^{15}N$ impacts crop $\delta^{15}N$. Additionally, there are many factors, other than fertiliser $\delta^{15}N$ value, that are likely to be influential over crop $\delta^{15}N$ values. These include soil type, antecedent land-use, variability in atmospheric nitrogen deposition, variations in local agricultural practices, etc. For example, soil saturation levels were found by Choi et al. (2003) to be important in controlling the $\delta^{15}N$ of soil nitrate being produced from different fertiliser types. Nevertheless, if nitrogen isotope analysis is used with an appropriate database of representative values for comparison and consideration of its limitations are taken into account, it can provide a relatively reliable test to corroborate authenticity investigations (Bateman, Kelly, & Woolfe, 2007).

Previously, major and trace element profiling has been used to try and distinguish between organic and conventionally cultivated onions and peas (Gundersen, Bechmann, Behrens, & Stűrup, 2000). The Danish study demonstrated that it was possible to correctly separate 19 farms into organic and conventional regimes. However, the study was empirical and did not attempt to identify the reasons why specific elemental concentrations were useful in distinguishing between organic and conventional cultivation. A significant number of other direct comparisons between the concentrations of trace-elements in organic and conventional pro-

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duce have been made (Fernandes, Tagliaferro, Azevedo, & Bode, 2002; Ghidini et al., 2005; Jorhem & Slanina, 2000; Lecerf, 1995; Malmauret, Parent-Massin, Hardy, & Verger, 2002; Olsson, Jonsson, & Oskarsson, 2001; Ryan, Derrick, & Dann, 2004; Woese, Lange, Boess, & Bögl, 1997; Worthington, 2001). Generally these comparisons have been undertaken to investigate claims that organic foodstuffs contain higher concentrations of nutritionally beneficial trace metals and lower concentrations of potentially harmful heavy metals.

Consistently substantiating such claims is by no means a trivial task. However, there is evidence to suggest that systematic differences in the concentrations of certain elements such as manganese do occur between crops cultivated under organic and conventional regimes as a result of the presence of elevated levels of arbuscular mycorrhizal fungi (AMF) in organically cultivated soils (Gosling, Hodge, Goodlass, & Bending, 2006). Such potentially beneficial nutritional differences may be ascribed as a 'quality index' for organic crops and consequently we suggest that the same differences could also be used as an 'indicator of authenticity'. Therefore, combining trace element and nitrogen isotope data in a multivariate approach may improve the potential for discrimination between these two agricultural regimes for the purposes of authenticity control. The approach of combining stable isotope and trace element data to determine the origin of foodstuffs is gaining wider acceptance in many branches of forensic science including food forensics (Kelly, Heaton, & Hoogewerff, 2005). In this paper, we present the trace element results from this study combined with stable nitrogen isotope data ($\delta^{15}N$) data from a previous study (Bateman et al., 2007) and discuss the potential for using a multivariate approach to detect deception where conventional produce is being fraudulently sold as organic.

2. Materials and methods

2.1. Collection of samples

The collection of tomatoes and lettuces used in this study has been described previously (Bateman et al., 2007). Briefly, samples were obtained from different growers across a wide geographic area in order to ensure that the datasets contained crops grown in conditions with variability in the factors that may influence crop characteristics, for both the organic and conventionally grown samples. A representative database of authentic samples is one of the most important conditions if an end-product test is to be used as an officially acknowledged procedure for food quality control and any database should take into account factors such as regional origin, plant species, variety, season and climate (Rossman, 2001). Organic samples were obtained either directly from certified organic growers who were aware of the aims of the project and had agreed to supply samples or directly from organic farm shops selling their own produce. Although there is no absolute guarantee that all the samples analysed are genuine, we believe that this approach has minimised the chances of including in the datasets any conventional produce being fraudulently mis-sold as organic. The organic samples are dominated by samples from the UK (with a few EU samples) but are from a wide geographic spread encompassing a wide variety of soil types and crop varieties.

2.2. Stable nitrogen isotope ratio analysis

The analysis of the stable isotope ratios of nitrogen in the tomato and lettuce samples has been described in detail previously (Bateman et al., 2007).

2.3. Nitrate, phosphate and sulphate analysis of lettuce samples

Nitrate (NO_3^-), phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}), concentrations were determined for hot water extracts of all lettuce samples where sufficient sample was available. Measurements were also made on a test batch of tomato samples but ion-chromatographic complications (high chloride concentrations in the tomato extracts) meant that no further analyses were conducted.

Twenty milligrams (±10%) of homogenised, freeze-dried and finely ground lettuce sample were weighed out into a 15 ml centrifuge tube (Greiner®). Ten millilitres of deionised water were added and samples placed into a 90 °C hot water bath for 20 min. Samples were allowed to cool, filtered through a 0.2 μm cellulose acetate syringe filter and then diluted (15-fold) to a concentration appropriate for ion chromatography analysis.

Samples were analysed on a Dionex 2000 system (Dionex Ltd., Camberley, UK) with a 50 mm long (2 mm i.d.) Dionex AG18 guard column and a 250 mm long (2 mm i.d.) Dionex AS18 analytical column. A 25 μl injection loop was used with 12 mM potassium hydroxide at 0.25 ml/min as the eluent for the first 10 min of each analysis after which the concentration of the eluent was increased to 25 mM (Dionex 2000 system, Dionex Ltd., Camberley, UK). Replicates and a process blank were included in each batch. A range of mixed anion standards from 2 to 200 μM (NO_3^-, SO_4^{2-}, PO_4^{3-}) and a MQ blank were analysed at the beginning and end of each sample batch for calibration and to account for any instrument drift during a run. Replicate analyses of separately prepared samples typically gave concentrations for each anion with relative standard deviations of less than 3% and always less than 8% (where relative standard deviation = $100 \times \sigma^{n-1}/\bar{x}$, where σ^{n-1} = standard deviation, $\bar{x}$ = mean, $n = 3$).

2.4. Trace element analysis of tomato and lettuce samples

Samples for multi-element analysis were prepared in batches of 12. Approximately 0.2 g of freeze-dried vegetable material was weighed out into a quartz tube. In a fume cupboard, 2.5 ml of ultrapure nitric acid was added and the samples simmered in a hot water bath for 2 h. Samples were then placed in a UV digester for 2 h after which the solutions were colourless and residue-free. The volume of digest remaining in the quartz tubes was typically around 2 ml. The quartz tubes were triple rinsed with deionised water and samples transferred into pre-weighed 15 ml Greiner® tubes. The mass of the sample digest + deionised water used to rinse out the quartz tube was recorded. This typically resulted in a total volume of approximately 10 ml of solution in each Greiner® tube meaning that the neat sample digest was diluted approximately 5-fold by deionised rinse water at this stage. Sample digests then required further dilution before analysis by ICP-MS. 0.75 ml of each primary digest was pipetted into a second pre-weighed 15 ml Greiner® tube and the mass recorded. The density of the primary digest could therefore be calculated and therefore the total volume of the digest + deionised rinse water present in the first Greiner® tube determined. 15 ml of a 2% Ultrapure HNO_3 solution was then added to each Greiner® tube containing the 0.75 ml of primary digest. This represents approximately a 20-fold dilution (giving a total dilution of the original neat digest of approximately 100-fold).

For all ICP-MS measurements an internal standard was added in order to quantify the elemental composition of the samples and correct for any instrument drift during analysis. An internal standard of 10 ppm Rhodium, Germanium and Platinum was prepared. Seventy-five microlitres were added to each 15.75 ml sample giving an internal standard concentration equivalent to 47.4 ppb. In each batch of 12 samples, a National Institute of Standards and Technology (NIST) Certified Reference Material 1573a (Tomato Leaves) was included and processed through the entire digestion and subsequent dilution procedure. The processing of the NIST Reference Material enabled the accuracy of the digestion, dilution and ICP-MS measurement to be assessed. In each batch of 12 samples, 1 sample was analysed in triplicate. This permitted the repeatability of the method to be determined. Replicate analyses of the same sample typically gave concentrations for each element with relative standard deviations of less than 5% (where relative standard deviation = $100 \times \sigma^{n-1}/\bar{x}$, where σ^{n-1} = standard deviation, $\bar{x}$ = mean, $n = 3$). For all elements reported, the concentration measured for the NIST reference material in this study was usually less than 5% and always less than 20% different from the certified values for each element. ICP-MS conditions were as follows:

Brand: Agilent 7500ce
Nebuliser: Concentric PFA MicroMist
Spray chamber: Water-cooled Scott double-pass
Nebuliser gas flow-rate (L/min): 0.9
Aux gas flow-rate (L/min): 0.9
Plasma gas flow-rate (L/min): 15
Cones: Nickel
RF power (W): 1500
Collision gas 1: Hydrogen (3.5 ml/min)
Collision gas 2: Helium (3.5 ml/min)
Spray chamber temperature: 2 °C
Mass resolution: ~0.7 amu
Integration time (s/mass): 0.1

2.5. Data analysis

Canonical discriminant analysis (CDA) examines the differences between two or more groups (in our case, organic and conventional) with respect to 'predictor' variables (δ^{15}N, %N, nitrate, sulphate, phosphate and multi-elements). The CDA identifies which variables are most successful at enabling samples to be placed into their appropriate groups. Stepwise analysis was carried out because this permitted the model to be built up step by step, rather than all the predictor variables being entered together – this can help to avoid 'over-fitting' of the data. At each step, all variables were reviewed and evaluated to determine which ones were contributing most to the discrimination between groups. That variable was then included in the model, and the process repeated. Variable selection was based on Wilks' lambda. At each step, the variable that minimised the overall Wilks' lambda was selected. Additional variables were only entered into the model if the significance level of its F value was less than 0.05. A cross-validation procedure was applied to assess the model. In this procedure, each individual case was in turn omitted from the estimation of the model constants, and then its group membership (organic/conventional) determined from the resulting model and compared to its known group identity to calculate a classification success rate.

3. Results and discussion

3.1. Lettuce nitrate, phosphate and sulphate concentrations

Fig. 1 shows the means and standard deviations for nitrate, sulphate and phosphate concentrations for the conventionally and organically grown lettuces ($n = 55$ and 48, respectively). The mean nitrate concentration for the conventionally grown lettuces of ~30 g nitrate/kg dried sample is almost double the mean value for the organically grown lettuces (~16 g nitrate/kg dried sample). However, the error bars showing the standard deviation of the means indicate that there is a large range in nitrate concentrations for both sets of samples particularly for the conventionally grown

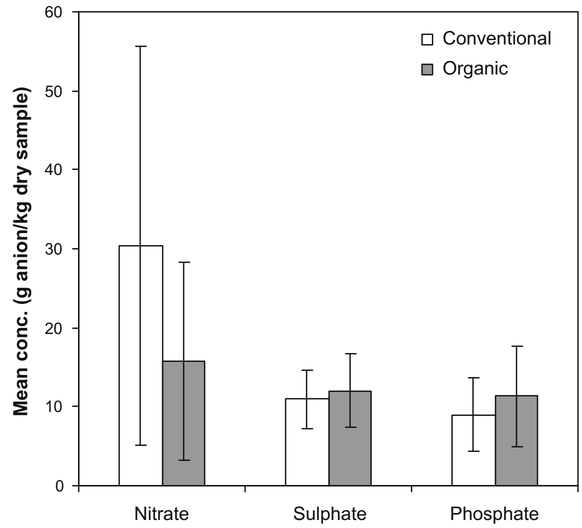

Fig. 1. Nitrate, sulphate and phosphate concentrations in conventional ($n = 55$) and organic ($n = 48$) lettuces. Bars show mean values and error bars indicate standard deviation (σ^{n-1}).

lettuces. A Levene's Test for equality of variances confirms that variances of the organic and conventional datasets are unequal and a t-test indicates a significant difference ($p = 0.000$) between the group means. Fig. 2 shows a cross-plot of the lettuce nitrate concentrations versus the corresponding δ^{15}N‰ value for each sample. There is a distinct group of conventional lettuces with nitrate concentration of greater than 60 g nitrate/kg dried sample and it is predominantly these samples that have resulted in the higher mean value for the conventional lettuces compared to the organically grown lettuces.

Siderer et al. (2005) comment that many reported literature studies find that conventionally cultivated (mineral fertilised vegetables) have a higher nutrient content than organically produced vegetables. This may in part be due to a greater availability and uptake of plant-available nitrogen when vegetables are grown with synthetic fertilisers in conventionally agricultural practices. However, Siderer et al. (2005) note that there is evidence that excessive fertilisation stimulates rapid growth and that this increases the yield of crops at least partly by simply swelling them with more

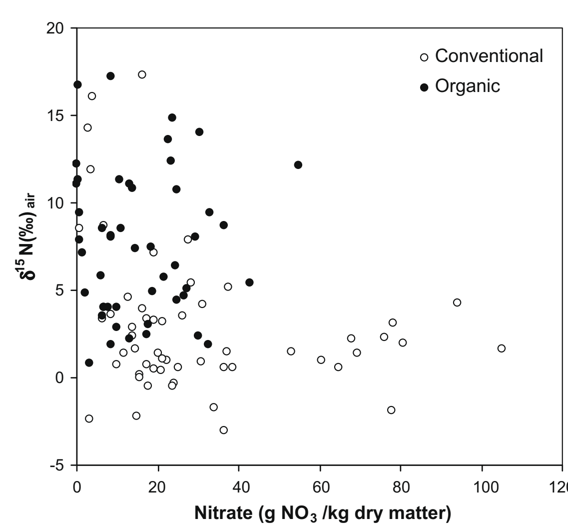

Fig. 2. Nitrate concentrations (mg NO_3/kg dry matter) versus δ^{15}N(‰) air for organically (Org) and conventionally (Conv) grown lettuces.

water. They go on to say that what is reported in the literature is often due to a positive correlation between the dry matter content and the nutrient content of crops. For example, if a conventionally grown lettuce with a particular nutrient content has a higher water content than an organically grown crop with the same nitrate concentration, then measurements of nitrate concentrations on a dry weight basis will result in higher reported values for the conventionally grown crop (even though on a fresh weight basis they have the same concentration). Siderer et al. (2005) argue that this means that it is important that nutrient and nitrate levels are reported and compared on a fresh weight basis. This is true if the intention is to compare the differences/dietary implications of consuming conventional/organic crops. However, for our purposes, we are primarily interested in differentiating between conventionally and organically grown crops.

Previous work has also shown that light is an important factor in determining lettuce nitrate concentrations. Lower light levels (during winter or cloudy spells at other times of year) may result in lower rates of photosynthesis which can cause an accumulation of nitrate in lettuce tissues (Food Standards Agency Report, FSIS 63/04). Variations in lettuce nitrate concentrations might also be related to whether lettuces are cultivated indoors or outdoors and the observed difference in the mean nitrate concentration between conventional and organic lettuces could be partly related to this. For example, it may be less usual to grow lettuces indoors in organic systems than in conventional systems and such a difference in cultivation practice could result in the observed difference in lettuce nitrate concentrations between the two datasets. We do not have sufficient information on the specific growing conditions for each sample to establish whether this may be an influential factor here.

Fig. 1 illustrates that there is little difference between the mean sulphate and phosphate concentrations in the organically and conventionally grown lettuces. A *t*-test confirms that there is no significant difference between the mean sulphate values for the organically and conventionally grown lettuces ($p = 0.182$). However, a *t*-test on the phosphate data reveals that there is statistically significant difference ($p = 0.038$) between the mean values for the two groups with the organic samples having a marginally higher mean phosphate content than the conventional lettuce samples.

3.2. Tomato major and trace element concentrations

The means and standard deviations for each variable are plotted in Fig. 3. Independent samples *t*-tests (SPSS 12.0.2) show that there are statistically significant differences between the mean values for the organic and conventional tomatoes for $\delta^{15}N$, calcium (Ca), manganese (Mn), copper (Cu), zinc (Zn) and rubidium (Rb). Differences in the nitrogen isotopic composition of these samples have been extensively discussed previously (Bateman et al., 2007). Calcium is an essential plant macronutrient whilst manganese, copper and zinc are considered important micronutrients. Interestingly, the observed higher mean concentrations of Ca, Cu and Zn and lower mean concentration of Mn in organic crops is a pattern that has been reported previously and has been attributed to higher concentrations of arbuscular mycorrhizal fungi (AMF) in organic soils (Gosling et al., 2006). The relationship between AMF and plant/soil-nutrient uptake is complex and in many cases the effect on different nutrients is inconsistent (Gosling et al., 2006). However, there appear to be general trends which are consistent with our findings for tomatoes and support the possibility of using these nutrients to assist in discriminating between organic and conventionally cultivated crops. For example, Ryan et al. (2004) observed only minor variations in nitrogen, potassium (K), magnesium (Mg), Ca, sulphur (S) and iron (Fe) concentrations between commercial grain cultivated on paired organic and conventional farms, however organic grain had higher Zn and Cu but lower Mn and phosphorus concentrations than conventional grain.

Other factors during cultivation that may effect nutrient concentrations should not be overlooked. The mean calcium concentration for the organically grown tomatoes is 2126 mg/kg dry sample compared to a mean value of 1027 mg/kg dry sample in the conventionally grown tomatoes. The standard deviation of the calcium concentrations for the conventionally grown tomatoes is smaller possibly reflecting tighter control on calcium concentrations (for example, under hydroponic conditions) when the tomatoes are cultivated conventionally.

Rubidium is normally considered a useful variable in the determination of geographical origin since levels in soil/plants are related to the underlying geology. The mean rubidium concentration for the organic tomatoes is nearly four times the mean value for the conventionally grown tomatoes. This could be due to differences in geographical origin of the organic and conventional samples or it might be a reflection of differences in organic and conventional cultivation practices. Conventionally grown tomatoes are often grown hydroponically and under these conditions the plants will not be exposed to rubidium in the soil and this could explain the lower rubidium levels in the conventionally grown tomatoes. The greater range in rubidium values in the soil-grown organic tomatoes may be related to the local rock/soil Rb levels – high Rb content in the tomatoes where rubidium levels in the underlying rocks are high, and lower levels where rocks contain low levels of rubidium.

Mean Cu and Zn concentrations are marginally (but statistically significantly) lower with smaller standard deviations for the conventionally grown tomatoes compared to those grown organically. Cu and Zn are common constituents of animal feed supplements and a possible explanation for the higher concentrations of Cu and Zn in the organic samples is that the application of manures, more typical of organic cultivation, may act as an additional source of Cu and Zn to the soil and the plants (Bolan, Khan, Donaldson, Adriano, & Matthew, 2003; Zhou, Hao, Wang, Dong, & Cang, 2005). However, it should be noted that metal bio-availability and especially plant-uptake is strongly dependent on the form of the metal on entering the soil, the physico-chemical properties of the soil and the crop type (Alloway, 1995). Bordeaux mixture (a copper sulphate and lime solution) is permitted in organic cultivation where the need is recognised by the certifying body. Tomatoes can be badly affected by blight which is caused by the fungus *Phytophthera infestans.* Bordeaux mixture is used to help control fungal and bacterial diseases (Brun, Maillet, Hinsinger, & Pepin, 2001). When Bordeaux mixture is sprayed onto plants, the copper sulphate tends to stay on the surface and is not readily washed off by rain. Organic certification bodies are currently re-examining the endorsement of copper-based fungicides. The reassessment is principally concerned with the potential harm to farm workers affected when spraying crops, although care is necessary to ensure Bordeaux mixture is wiped off tomatoes before eating. It might be possible that the higher copper concentrations found for the organic tomatoes could be due to residual Bordeaux mixture. However, copper would also be applied either in the hydroponic solutions or as liquid feed to tomatoes grown in conventional practices and copper-based fungicides may also be used by conventional growers to help control of blight.

The mean concentration of Mn in the conventionally grown tomatoes is 18 mg/kg dry sample compared to a mean value of 11 mg/kg dry sample for the organically grown samples. Soil is an important source of manganese to plants and perhaps in organic systems it is not considered necessary to supply additional Mn above the levels naturally present in the soil (although manganese is also commonly used as an additive to livestock feed supplements and this may be a source in organic systems where manures are applied).

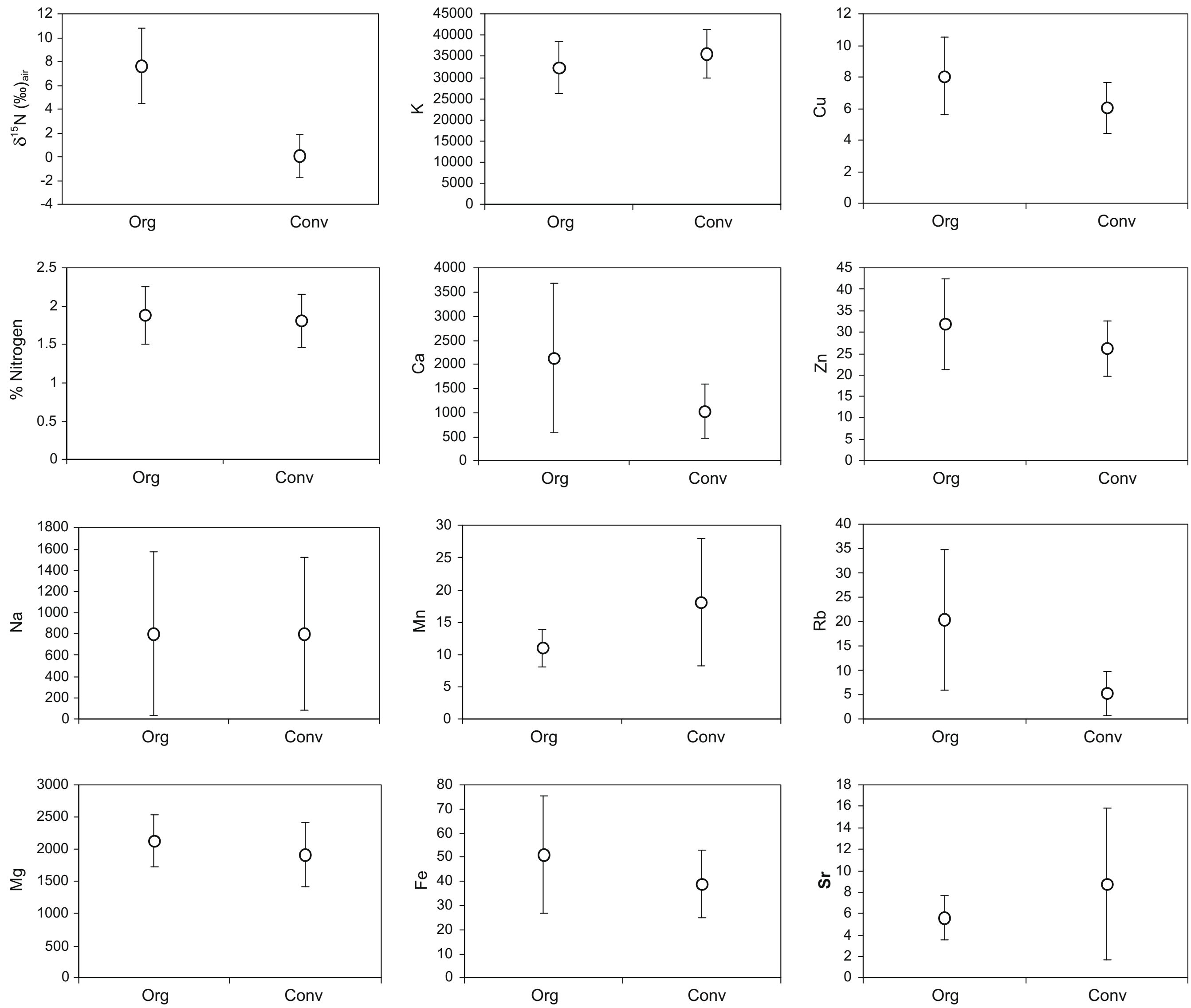

Fig. 3. Mean (circle) and standard deviations (error bars, σ^{n-1}) for tomato samples analysed for their multi-element, $\delta^{15}N(‰)_{air}$ and % nitrogen composition. Na = sodium, Mg = magnesium, K = potassium, Ca = calcium, Mn = manganese, Fe = iron, Cu = copper, Zn = zinc, Rb = rubidium, Sr = strontium. All units for multi-elements are mg element/kg dried sample. % Nitrogen is on a dry weight basis. Org = organic, Conv = conventional.

As an important micronutrient, it would be important that manganese is present in the hydroponic solutions used to grow tomatoes conventionally and it might be that the levels used in the hydroponic solutions, or the form in which the manganese is present, results in a more plant-uptake of Mn in conventional systems.

3.3. Lettuce major and trace element concentrations

The means and standard deviations for each variable are plotted in Fig. 4. Once again numerically higher mean concentrations of Ca, Cu and Zn and a lower mean concentration of Mn were observed in organic lettuces and may be due AMF associations. However, independent sample *t*-tests (SPSS 12.0.2) demonstrated that there were only statistically significant differences between the mean values for the organic and conventional lettuces for $\delta^{15}N$, Cu and Rb.

The mean Rb value for the organically grown lettuces is 21 mg/kg dry sample compared to a value of 9 mg/kg dry sample for the conventionally grown lettuces. In the same way as tomatoes, this could be reflecting a geographical bias between the organically and conventionally grown samples. However, it is the organic samples that have the larger standard deviation in their rubidium content and these samples are dominantly from the UK while the conventional samples are from a wider geographical spread from the UK and EU. As suggested for the tomato samples, the larger range in the rubidium values for the organic samples might be due to a greater proportion of the organic samples being soil-grown compared to module-grown or hydroponically grown conventional lettuces.

The means and standard deviations of the Cu concentrations in the organic and conventional lettuces exhibit the same pattern observed for the tomatoes. The arguments put forward to explain the differences between the organically and conventionally cultivated tomatoes may equally apply to the lettuces.

In summary, the multi-element and anion (NO_3^-, SO_4^{2-}, PO_4^{3-}) analysis has identified statistically significant differences between the mean values for some of the measured anions and elemental concentrations and possible explanations have been put forward for the observed differences. However, the datasets analysed are not large and analysis of further samples would be an appropriate next step. The additional discriminatory power that these variables offer compared to using $\delta^{15}N$ and %N data alone to distinguish organic and conventional cultivation is discussed in the following section.

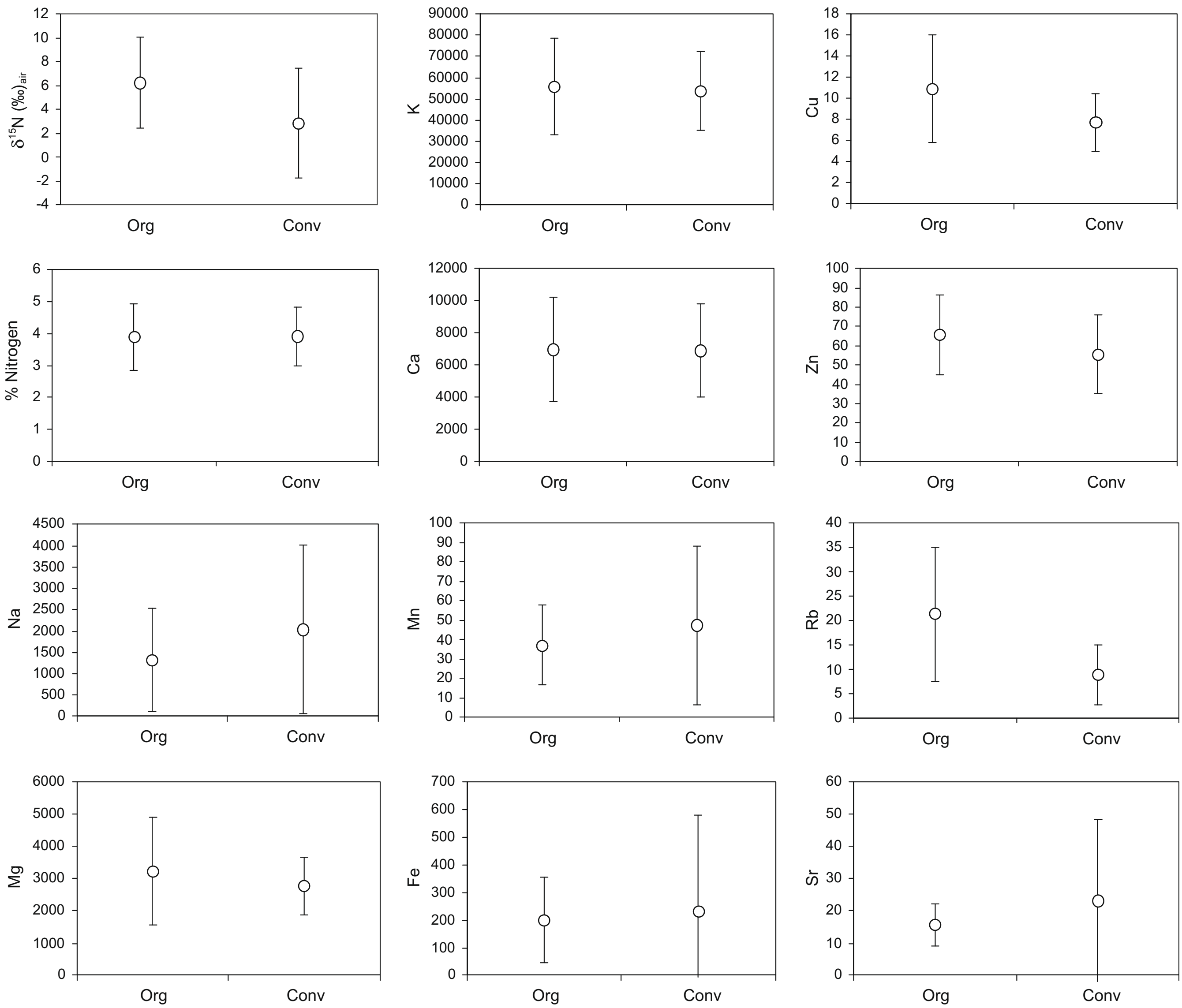

Fig. 4. Mean (circle) and standard deviations (error bars, σ^{n-1}) for lettuce samples analysed for their multi-element, $\delta^{15}N$(‰)$_{air}$, % nitrogen. Na = sodium, Mg = magnesium, K = potassium, Ca = calcium, Mn = manganese, Fe = iron, Cu = copper, Zn = zinc, Rb = rubidium, Sr = strontium. All units for multi-elements are mg element/kg dried sample. % Nitrogen is on a dry weight basis. Org = organic, Conv = conventional.

3.4. Canonical discriminant analysis (CDA) of tomato samples

Tomatoes collected in Year 1 of this study were analysed for $\delta^{15}N$, %N and their multi-element composition. This dataset consisted of 51 individuals (33 organic, 18 conventional). CDA was initially conducted with $\delta^{15}N$ and %N as the only variables. The CDA was repeated using $\delta^{15}N$, %N and the multi-elements as predictor variables for the Year 1 samples. Finally, the CDA was repeated for the Years 1 + 2 tomato samples (61 organic, 46 conventional) with $\delta^{15}N$ and %N as the variables entered into the model since the Year 2 samples were not analysed for their multi-element composition.

Results from entering $\delta^{15}N$ and %N as variables in the CDA for the Year 1 samples are shown in Table 1. Only $\delta^{15}N$ was selected by the CDA as a predictor variable. Overall, 94.1% of cases were classified correctly. Three organic samples were mis-classified as conventional. These samples were from an organic grower who had cultivated tomato plants in pots longer than anticipated because of a delay in building of a polytunnel in which they were to be grown. These samples may be slightly anomalous because it was the first year the land had been in cultivation. The unstandardised canonical discriminant function coefficients from the CDA are used to calculate discriminant scores for each case, where discriminant score (DS) = $(0.360 \times \delta^{15}N) - 1.791$. If the DS for an individual case is negative then the case is predicted to be a conventional sample and a positive DS means a case is predicted to be organic. The CDA also computes the probability of a case belonging to each group given its discriminant score and the case is assigned to the group for which the probability is greater than 0.5 (since there are only two groups in our analysis). Taking account of these probabilities is useful since it gives an indication of the confidence with which the model is assigning an individual case to the group.

When the $\delta^{15}N$, %N and the multi-element data for the Year 1 samples were entered into statistical analysis together, $\delta^{15}N$, rubidium, copper and %N were identified by the CDA as the best predictor variables for assigning cases as organic/conventional. Possible reasons for differences in rubidium and copper concentrations between organic and conventional samples have already been discussed. The results of the cross-validation of the model

Table 1
Summary of the results of the canonical discriminant analysis of tomatoes showing the predictor variables identified by the model and the number and % of cases from each group (organic/conventional) correctly classified by cross-validation.

	Variables entered	Predictor variables	Number of individuals	Conventional		Organic		Overall
Year 1	δ^{15}N, %N	δ^{15}N	51	18/18	100%	30/33	90.9%	94.1%
Year 1	δ^{15}N, %N, trace-elements	δ^{15}N, Rb, Cu, %N	51	18/18	100%	33/33	100%	100%
Years 1 + 2	δ^{15}N, %N	δ^{15}N	107	45/46	97.8%	57/61	97.8%	95.3%

in Table 1 show that 100% of the cases are correctly classified. The discriminant function coefficients for the model were δ^{15}N = 0.396 (1.099), %N = −1.312 (−0.480), Cu = 0.255 (0.564), Rb = 0.082 (0.980), constant = −2.654. Values shown first are the unstandardised values, values in brackets are the standardised values.

Results from CDA of the combined Years 1 and 2 tomatoes (n = 107) with δ^{15}N and %N as independent variables were entered into the CDA. Only δ^{15}N was selected by the model as a predictor variable. Table 1 shows the results of cross-validation in which each individual case is omitted from the model and then its group membership determined based on the measured variables for that case. The unstandardised discriminant function coefficients for the model was δ^{15}N = 0.363, constant = −1.652. The discriminant functions for the model have changed slightly and the % of samples cross-validated by the model has improved marginally compared with the results from the analysis of the Year 1 tomatoes only. There are four organic samples that are mis-classified by the CDA as conventional. These are the three Year 1 samples already discussed and one additional sample. As mentioned above, the CDA calculates the probability that each case belongs to the group to which it has been assigned. This information is useful when considering individual cases and the likelihood of their organic authenticity given a measured δ^{15}N value. The additional incorrectly assigned organic tomato had a Probability (conventional) of 0.502 only slightly higher than its probability (organic) of 0.498.

3.5. Canonical discriminant analysis (CDA) of lettuce samples

Samples collected in Year 1 of the study were analysed for their δ^{15}N, %N, anion concentrations and multi-element composition. This dataset consists of 42 individuals (14 organic, 28 conventional). CDA was run with (i) δ^{15}N only, (ii) δ^{15}N, %N + anions and (iii) δ^{15}N, %N, anions and multi-element data for the Year 1 sample. Samples collected in Year 2 of the study were only analysed for their δ^{15}N, %N and anion concentrations. The classification result is shown in Table 2. δ^{15}N was selected by the analysis as the only useful predictor variable.

When the CDA was repeated with δ^{15}N, %N and the anion data (nitrate, sulphate and phosphate), the predictor variables identified by the model were the anions. δ^{15}N and %N were not used in the analysis. The classification results were marginally improved in comparison with the CDA using δ^{15}N as the only variable with one additional conventional case correctly identified (see Table 2).

When the analysis was repeated with the complete set of variables (δ^{15}N, %N, anions and multi-elements), rubidium and δ^{15}N were selected as the predictor variables. Overall, the percentage of cross-validated cases grouped correctly was the same as for the CDA when only the anion data were used and only marginally better than when δ^{15}N was the only predictor variable. In addition, the improvement is due to a higher proportion of conventionally grown cases being correctly assigned and less of the organically grown cases have been correctly identified (Table 2).

Samples collected during the second year of sampling were not analysed for their multi-element composition. When only δ^{15}N and %N are entered into the CDA, only δ^{15}N is selected by the analysis as a predictor variable. The classification results are shown in Table 2. When the CDA was repeated with δ^{15}N, %N and the anion data (nitrate, sulphate and phosphate), the predictor variables identified by the model were δ^{15}N, NO_3^-, %N and SO_4^{2-}. Only PO_4 was not used in the analysis.

Overall there is little difference in the percentages of correctly cross-validated cases when δ^{15}N is used on its own as a predictor variable compared with when anion and multi-element data are used in conjunction with the δ^{15}N data. A key consideration is the proportion of authentic organic samples correctly assigned because it is particularly important that genuine organic produce is not erroneously flagged up by the method. For this reason, the overall greater proportion of correctly cross-classified cases when Rb and δ^{15}N are used as the predictor variables (for Year 1 data) should probably not be considered a better outcome than the 73.8% correctly cross-validated cases when only δ^{15}N was used since the number of correctly classified organic cases drops from 10/14 to 8/14.

That the CDA identified only NO_3^-, SO_4^- and PO_4^- as the predictor variables when δ^{15}N was also entered as an independent variable (for the Year 1 lettuces) is rather unsatisfactory since the CDA had already shown that δ^{15}N on its own was quite a good marker of whether a lettuce was grown conventionally/organically. This probably reflects one of the weaknesses of applying the CDA to this dataset. One of the ways in which the CDA identifies which variables are most appropriate at placing cases into their appropriate groups is by analysing the difference in the mean values for each of the groups for each variable. It has already been pointed out that whilst an organic grower is restricted in which soil improvers and fertilisers might be applied, the whole range of fertilisers, both conventional and organic can be used by a conventional grower. For tomatoes, the δ^{15}N data suggest that conventional growers tend to nearly always use synthetic fertilisers resulting in tomatoes with low δ^{15}N values. However, conventionally grown lettuces have δ^{15}N values across the whole range of values (probably due to conventional growers using organic-type fertilisers or to a greater proportion of the lettuces being soil-grown and thus subject to many more influences on their δ^{15}N composition compared to tomatoes). Whatever the reason, a more intelligent model might

Table 2
Summary of the results of the canonical discriminant analysis of lettuces showing the predictor variables identified by the model and the number and % of cases from each group (organic/conventional) correctly classified by cross-validation.

	Variables entered	Predictor variables	Number of individuals	Conventional		Organic		Overall
Year 1	δ^{15}N, %N	δ^{15}N	42	21/28	75%	10/14	71.4%	73.8%
Year 1	δ^{15}N, %N, NO_3, SO_4, PO_4	NO_3, SO_4, PO_4	42	22/28	78.6%	10/14	71.4%	76.2%
Year 1	δ^{15}N, %N, NO_3, SO_4, PO_4, multi-element data	Rb, δ^{15}N	42	24/28	85.7%	8/14	57.1%	76.2%
Years 1 + 2	δ^{15}N, %N	δ^{15}N	103	46/55	83.6%	30/48	62.5%	73.8%
Years 1 + 2	δ^{15}N, %N, NO_3, SO_4, PO_4	δ^{15}N, %N, NO_3, SO_4	103	45/55	81.8%	34/48	70.8%	76.7%

be one in which a minimum $\delta^{15}N$ value is expected for authentic organic samples.

4. Conclusions

The combination of trace element and nitrogen isotope data to attempt to distinguish between organic and conventional cultivation methods has improved the correct classification of tomato samples but appears to have had a limited effect on lettuces. Interestingly, our findings support the growing body of evidence which suggests that systematic differences in the concentrations of certain elements such as manganese, calcium, copper, and zinc do occur between crops cultivated under organic and conventional regimes as a result of the presence of elevated levels of arbuscular mycorrhizal fungi (AMF) in 'organic soils'. We assert that such differences in elemental composition may be useful as 'indicators of authenticity'. However, we recognise the limitation that this approach may be restricted to horticultural crops where there are significant differences in agricultural practice such as conventional-hydroponic versus soil-grown organic tomatoes. We strongly advocate that end-product tests such as the nitrogen isotope approach combined with trace element analyses cannot and should not be thought of as a replacement to organic certification and inspection schemes and that interpretation of analyses should be made carefully and sensitively. Whatever the current limitations of the proposed methods there is a place for end-product tests to corroborate paper traceability investigations especially for imported organic foods that have long supply chains where the risk of fraud and mis-labelling is greater. We suggest that any analytical techniques that assist in protecting consumers from fraud and help protect the interests of honest growers and traders should be viewed positively.

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