Isotopic Discrimination as a Tool for Organic Farming Certification in Sweet Pepper

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Organic farming is a form of agriculture that excludes the use of synthetic fertilizers, pesticides, and genetically modified organisms. These fertilizers have been traditionally overused in conventional farming to avoid lost revenue, but this often not does not take into account the potential contamination of aquifers and river due to nitrate leaching. Transition to organic farming practices could provide an instrument to reduce contamination and increase potential income. It is difficult to determine to what extent those fertilizers could have been used within a complete traceability of the production process. In this experiment, we evaluated the use of $^{15}N/^{14}N$ isotopic discrimination in sweet pepper plants to test the hypothesis that synthetic fertilizers significantly reduce $^{15}N/^{14}N$ compared with exclusively organic practices. Therefore, three common types of organic manures (sheep, hen, or horse) were applied at a rate of 8 kg m^{-2} with or without synthetic fertilizer amendments under fully controlled environmental and irrigation conditions. Results indicate that (i) use of synthetic fertilizers significantly reduced $^{15/14}N_2vsN_2atm$ compared with treatments that only received water; (ii) with respect to the plant organs, old leaves and fruits were more sensitive to the synthetic fertilizer additions with reductions in $^{15/14}N_2vsN_2atm$ of 24.1 and 27.8%, respectively; and (iii) independently of the organic manure used, no additional fertilization (synthetic or organic) is required before 106 days after transplanting at that dosage because plant fresh weight was not reduced.

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There is increasing concern about food quality and safety. Consumers are interested in organic products because they are considered to be safer for health due to the absence of pesticide residues and because they are produced in a more environmentally compatible manner (Brandt and Mølgaard, 2001). According to the USA and European Union regulations on organic farming, organic products are subject to controls by an accreditation and certification system. In particular, they must be recorded at each step of production, thereby ensuring the complete traceability at all stages of production, processing, and marketing. In addition to the biological control for pest and diseases, soil fertilization in organic farming is only possible with organic fertilizers and amendments such as composted or uncomposted cattle manure or other organic waste, whereas in conventional production systems, synthetic fertilizers are used (Rapisarda et al., 2005). However, in managing organic farming, it could be difficult to establish a correct fertilization because of differing compositions of the manure used. Furthermore, we should take into account net mineralization of soil organic N under different field conditions, which arises from the effects of temperature and moisture supply on the N-cycle processes (Mulvaney et al., 2001). Thus, in organic farming, it is difficult to determine the availability of this N to the crop during its cycle, and plant growth could be reduced if appropriate monitoring techniques are not applied (del Amor, 2006). To overcome these problems while maintaining high yields, farmers could use synthetic fertilizers to avoid lost revenue.

Conventional farming, especially in intensive horticultural crop production, is the main source of nitrate contamination to aquifers and rivers. Transition to a more sustainable production systems is therefore required especially in the nitrate-vulnerable zones, and the extra income gained through selling surplus (premium prices for certified organic) could be an attractive incentive to reduce contamination. However, managing organic farming could be more difficult than conventional farming, and systems to control and monitor N inputs or N leaching for each farm are costly and often not possible. Therefore, we evaluated a technique to determine the use of chemical fertilizers in horticultural crops with two goals: (i) to identify the use of chemical fertilizers and therefore the increased potential contamination via N leaching and (ii) to develop a tool to detect fraud in organic farming production and thus to help genuine organic farmers, consumers, and certification policies.

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Abbreviations: DAT, days after transplanting; OM, organic matter; TN, total nitrogen.

The hypothesis of use of N-isotopic composition is as follows: The N in synthetic N fertilizers is derived from atmospheric N_2 by high-temperature catalytic reaction with hydrogen to form ammonia, which is subsequently oxidized to nitrate. This process results in little change in the original atmospheric N isotope ratio. Therefore, ammonia and nitrate derived from such a fertilizer source has a ^{15}N value close to 0‰, the value of atmospheric N_2 (Shearer et al., 1974; Vitoria et al., 2004). By contrast, nitrate derived from sewage sources can have values greater than +20‰, signifying a ^{15}N enrichment of 2% over the original $^{15}N/^{14}N$ ratio in starting N_2. This is significantly different from the fertilizer N isotopic value (Jeffrey et al., 2002).

We tested this hypothesis about the use of isotopic discrimination in crops to detect addition of chemical N-fertilizers in sweet pepper plant with three common manures, with or without the addition of chemical fertilizers. If a significant reduction in $^{15}N/^{14}N$ in any plant organ could be observed, then this reduction would provide us with a tool to identify nonorganic procedures and to reduce contamination through the use of certified organic production practices.

Materials and Methods

Plant Material and Growth Conditions

Sweet pepper plants, cv. Cierva, were transplanted from a commercial nursery on 18 Jan. 2006. The greenhouse was divided in three independent zones corresponding with the three manure treatments. Manure was applied at preplant. During the crop cycle, half of the plants in each manure zone received only water, and the other plants received chemical fertilizers as commonly used in conventional cultivation. Thus, six treatments were studied corresponding to three manure types and two drip-irrigation regimens (only water or addition of chemical fertilizers) per each type of manure.

Manure (8 kg m^{-2}) was applied for each treatment. The manures had the following composition in dry weight (%): Sheep manure: organic matter (OM), 73.8; total N (TN), 2.01; C/N, 21.13; P_2O_5, 1.04; K_2O, 3.78; CaO, 5.86; MgO, 1.52. Horse manure: OM, 70.3; TN, 1.77; C/N, 23.04; P_2O_5, 1.5; K_2O, 3.08; CaO, 7.18; MgO, 0.87. Hen manure: OM, 57.4; TN, 2.41; C/N, 13.82; P_2O_5, 3.24; K_2O, 3.57; CaO, 16.54; MgO, 1.52. Chemical fertilizers were applied with the following composition in the nutrient solution in mmol L^{-1}: 4.2 NO_3^-; 1.7 $H_2PO_4^-$; 3.4 SO_4^{2-}; 3.8 Ca^{2+}; 1.2 K^+; 2.0 Mg^{2+}. All treatments received the same amount of irrigation (water or fertigation). Each treatment had four rows with 78 plants each. The rows at both ends were not controlled and were considered as border plants; thus, each treatment had four blocks with 16 plants per block in the central two rows. All sampled plants or fruits were surrounded by plants of the same treatment.

Plant Biomass and Gas Exchange Parameters

At 106 days after transplanting (DAT), plants were harvested for plant biomass, CO_2 assimilation, and isotopic discrimination. Plants were harvested and separated into young and old leaves, stems, roots (only the upper part due to the difficulty in extracting all the organs), and mature fruit. Plants organs were dried for at least 72 h at 65°C.

Net CO_2 assimilation and leaf transpiration were determined with a LI-6400 (Li-COR Biosciences, Lincoln, NE). Light intensity (photosynthetically active radiation) was fixed at saturated light conditions (1500 μmol m^2 s^{-1} photon flux density) and 370 mg L^{-1} CO_2 inside the leaf chamber. Four measurements were made per treatment on the youngest and fully expanded leaves.

Isotopic Determination

From each sample, 5 to 8 mg of powdered plant material was packed in tin capsules and analyzed by isotope ratio mass spectrometry for the $^{15}N/^{14}N$ (Continuous Flow Isotope Ratio Mass Spectrometer-CF-IRMS, Thermo-Quest Delta plus). Abundance of ^{15}N is expressed by the ratio $^{15}N/^{14}N$. Natural ^{15}N abundance in the atmosphere, the largest N reserve in the world, is at the level of 0.0036765 and remains constant around the world (Ehleringer and Cerling, 2002). Natural ^{15}N abundance in samples is normally related to ^{15}N abundance in the atmosphere by the following expression (Robinson 2001):

$$\delta^{15}N\ (‰) = 1000 \times \{[(^{15}N/^{14}N\ \text{sample})/(^{15}N/^{14}N\text{atmosphere})] - 1\}$$

Several physical, chemical, and biological processes and reactions present different affinities for ^{15}N or ^{14}N isotopes. Due to this discrimination, products resulting from these reactions are usually enriched or impoverished in ^{15}N in comparison with the original. For batch calibration in the isotope ratio analysis, plant materials, previously calibrated against standard material of known isotope composition, were used as working standards.

Statistical Analyses

Analysis of variance was performed on main effects (manure type, fertilization post-transplant, and organ) with Statgraphics Plus 5.1. When the interaction between organs and fertilization was found ($P \leq 0.05$), mean values for each organ were separated by LSD at $P \leq 0.05$.

Results and Discussion

Sheep manure significantly increased ($P \leq 0.05$) shoot biomass compared with hen or horse manure (Table 1). No significant effect was found due to the irrigation treatments (only water or with chemical fertilizers amendment). At later stages of crop production (164 DAT), only water application reduced growth in all manures (data not shown). Net CO_2 assimilation rate and transpiration were not affected by the addition of chemical fertilizers. However, transpiration was higher with sheep manure rather than hen or horse manure.

Organic carbon additions from farm manures affect soil physicochemical and biophysical properties; therefore, manures can increase soil porosity and plant-available water capacity, which results in better soil structural conditions (Bot and Benites, 2005). Therefore, these soil conditions and characteristics provided by manures may affect the leaf transpiration. Sheep manure had higher OM than horse or hen manure and could improve

Table 1. Biomass (leaves and stems), net CO_2 assimilation (A_n), and leaf transpiration (E).

Irrigation	Manure	Plant fresh weight	A_n	E
		g	μmol CO_2 m^{-2} s^{-1}	mmol H_2O m^{-2} s^{-1}
Water	sheep	635.6	22.58	9.27
	hen	351.3	23.10	8.63
	horse	367.7	21.83	7.87
Fertilizer	sheep	464.9	26.25	10.05
	hen	365.1	22.77	8.66
	horse	400.5	22.73	7.94
			ANOVA	
Main factors				
Manure	*p* value	0.036	0.164	0.0001
	F ratio	3.92	1.91	12.9
	$LSD_{95\%}$	155	3.37	0.74
Irrigation	*p* value	0.50	0.177	0.368
	F ratio	0.46	1.90	0.83
	$LSD_{95\%}$	126	1.98	0.60

soil water capacity better than hen or horse manures. Additionally, plants with sheep manure had higher fresh weight; thus, not only OM but also textural and microbiological proprieties of this manure could be implicated in this response.

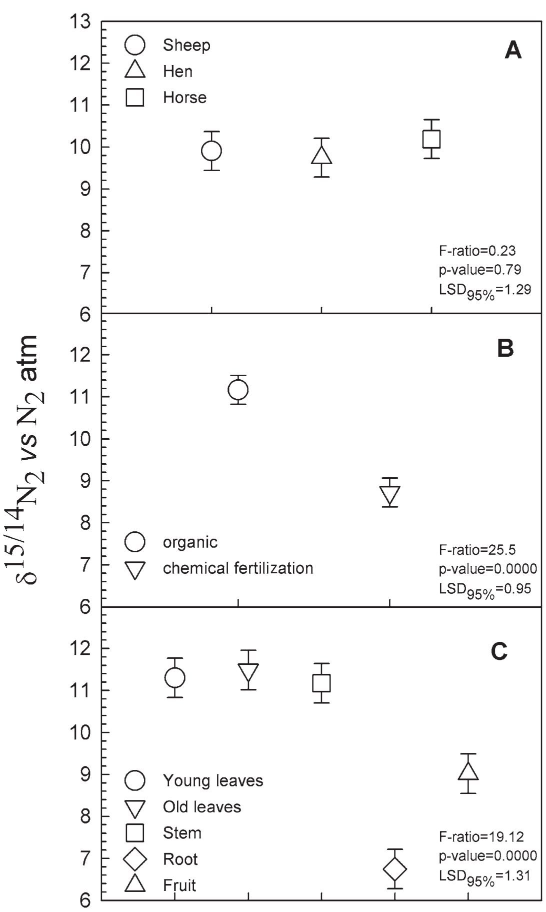

Fig. 1. Isotopic composition according to (A) different organic manures, (B) irrigation (water or with additional chemical fertilizers), and (C) different plant organs of sweet pepper plants. Vertical bars indicate LSD values ($P \leq 0.05$) for treatment comparisons.

This result indicates that proper manure management in sweet pepper is more important than chemical fertilization. This result agrees with other findings where animal manure is a valuable resource in crop nutrition, but its efficient management is a greater challenge than when using mineral fertilizers (Thomsen, 2005; del Amor, 2006).

Taking into account the main effects (manure, fertilization, and plant organ) on the isotopic composition of sweet pepper (Fig. 1), our results show that there is no significant effect ($P \leq 0.05$) of the type of manure; however, an important reduction in $\delta^{15/14}N_2$vsN_2atm was observed related to the fertilization type (water or with chemical fertilizer amendment). Thus, when organic manure was irrigated with chemical fertilizers, $\delta^{15/14}N_2$vsN_2atm was significantly reduced from 11.16 to 8.72 (21.8%) with respect to the addition of water alone. When all plant organs were analyzed, roots and fruits of sweet pepper plants showed in general a lower $\delta^{15/14}N_2$vsN_2atm than young or old leaves or stems. Choi et al. (2003) proposed the possibility of using the $\delta^{15}N$ of an organic product as a tool for verifying the organic or inorganic nature of N fertilizers used in organic farming. For this to happen, two requirements need to be satisfied: (i) The $\delta^{15}N$ of organic and inorganic fertilizers should be largely different, and (ii) plant $\delta^{15}N$ should reflect the $\delta^{15}N$ of received fertilizers. Evans (2001) asserted that whole-plant and leaf N isotope composition is determined by the isotope ratio of the external N source and by physiological mechanisms within the plant. Whole-plant isotope composition can reflect that of the N source when plant demand exceeds N supply. Recent studies have reported that N-isotope studies can provide unique information on features such as nutrient sources, nutrient cycling, mixing regime, water column stability, and sediment provenance (Naseeb et al., 2006).

Any biological processes implicated in the synthesis and transformation of organic compounds could show a different affinity for ^{15}N and for ^{14}N. Thus, the $\delta^{15/14}N$ of the resulting compounds could be altered (Evans 2001). Organic fertilizers of animal origin show the highest $\delta^{15/14}N$, followed by fertilizers of plant origin. This is similar to the $\delta^{15/14}N$ of nonfertilized soil. This could be due to the fact that most of the N present in nonfertilized soils comes from the decomposition of vegetable residues. On the other hand, the $\delta^{15/14}N$ of synthetic fertilizers is similar to atmospheric $\delta^{15/14}N$. Our results clearly show this fact and that the addition of synthetic fertilizers to any of the studied manures significantly reduced δ15/14N2vsN2atm. No significant differences were found between organic manures; this fact proves the wide range of this technique in organic systems where different types of animal manures are applied.

Table 2. Isotopic composition of different plant organs for plants irrigated with water or with chemical fertilizers management.

	$\delta^{15/14}$ N vs N_2 atm				
Irrigation	Young leaves	Old leaves	Stem	Root	Fruit
Water	12.41	13.06	12.34	7.53	10.47
Fertilizer	10.18	9.91	9.99	5.95	7.55
		ANOVA			
p Value	0.003	0.0001	0.042	0.051	0.0005
F ratio	10.84	24.33	4.64	4.22	16.55
$LSD_{95\%}$	1.40	1.32	2.26	1.59	1.48

The ANOVA considering the fertilization type effect on $\delta^{15/14}N_2 vsN_2 atm$ in each plant organ (Table 2) showed the highest significance values for old leaves ($p = 0.0001$) and fruits ($p = 0.0005$) rather than young leaves ($p = 0.003$) or stem ($p = 0.004$), and it was not significant for roots. This is especially relevant for monitoring organic crops in the field and for organic certification of fruits.

Conclusions

Agencies for organic farming certification require techniques to verify the organic nature of the N fertilizers applied to crops. Results show that significant differences have been found between organic and not fully organic practices. Thus, this study demonstrates that with N-isotopic techniques it is possible to discriminate the use of chemical fertilizers in the organic production of sweet peppers with respect to strictly nonorganic crops. Further studies including the effects of different soils characteristics, climate, and biotic or abiotic stress could be useful in determining the proper interval of $^{15}N/^{14}N$ ratio to exclude nonorganic fertilization practices.

The transition to organic farming could significantly reduce nitrate contamination in this crop. Additionally, it has been found that regardless of the organic manure used, no additional fertilization (synthetic or organic) is required before 106 DAT because plant fresh weight was not reduced. This conclusion is important for conventional farmers who apply manure preplanting for biofumigation with solarization because the use of methyl bromide (a gas that has been used as a structural fumigant to control a wide variety of pests in the soil) is banned. Thus, the requirement for biofumigation in conventional farming makes unnecessary the use of synthetic fertilizers during a significant period of the crop, avoiding nitrate contamination.

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References

Bot, A., and J. Benites. 2005. The importance of soil organic matter: Key to drought-resistant soil and sustained food production. FAO Soils Bull. 80. FAO, Roma.

Brandt, K., and J.P. Mølgaard. 2001. Organic agriculture: Does it enhance or reduce the nutritional value of plant foods? J. Sci. Food Agric. 81:924–931.

Choi, W.J., H.M. Ro, and E.A. Hobbie. 2003. Patterns of natural ^{15}N in soils and plants from chemically and organically fertilized uplands. Soil Biol. Biochem. 35:1493–1500.

del Amor, F.M. 2006. Growth, photosynthesis, and chlorophyll fluorescence of sweet pepper plants as affected by the cultivation method. Ann. Appl. Biol. 148:133–139.

Ehleringer, J.R., and T.E. Cerling. 2002. Stable isotopes. p. 544–550. *In* Encyclopedia of global environmental change. John Wiley & Sons, New York.

Evans, R.D. 2001. Physiological mechanisms influencing plant nitrogen isotope composition. Trends Plant Sci. 63:121–126.

Jeffrey, A., I. Kaplan, D. Zhang, S.T. Shan-Tan, and J. Nielsen. 2002. Environmental tracers: Identifying the sources of nitrate contamination in groundwater. Soil Sed. Waters 6:15–18.

Mulvaney, R.L., S.A. Khan, R.G. Hoeft, and H.M. Brown. 2001. A soil organic nitrogen fraction that reduces the need for nitrogen fertilization. Soil Sci. Soc. Am. J. 65:1164–1172.

Naseeb, H., A. Akber, A. Alhadad, E. Al-Awadi, and A. Bushihri. 2006. Identification of nitrogen sources in the groundwater of Kuwait using nitrogen isotopes. Eur. J. Sci. Res. 15:220–234.

Rapisarda, P., M.L. Calabretta, G. Romano, and F. Intrigliolo. 2005. Nitrogen metabolism components as a tool to discriminate between organic and conventional citrus fruits. J. Agric. Food Chem. 53:2664–2669.

Robinson, D. 2001. Delta ^{15}N as an integrator of the nitrogen cycle. Plant Soil 16:153–162.

Shearer, G.B., D.H. Kohl, and B. Commoner. 1974. The precision of determinations of the natural abundance of nitrogen-15 in soils, fertilizers, and shelf chemicals. Soil Sci. 118:308–316.

Thomsen, I.K. 2005. Crop N utilization and leaching losses as affected by time and method of application of farmyard manure. Eur. J. Agron. 22:1–9.

Vitoria, L., N. Otero, A. Soler, and A. Canals. 2004. Fertilizer characterization: Isotopic data (N, S, O, C, and Sr). Environ. Sci. Technol. 38:3254–3262.