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Recovery of ^{15}N Labeled Nitrogen Fertilizer by Fertigated and Drip Irrigated Greenhouse Vegetable Crops

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Abstract: The stable isotope ^{15}N was used to assess the recovery of mineral N fertilizer applied to fertigated and drip-irrigated spring muskmelon and autumn-winter sweet pepper crops grown in greenhouse soil plots. They received 92–96% of mineral N fertilizer as NO_3^-. ^{15}N-labeled Ca $(NO_3)_2$ fertilizer was applied to crops during vegetative growth and fruit production phases. Crops were grown with either conventional management or combined improved N and irrigation management. Improved management for both irrigation and N was based on the combined use of models, to estimate crop requirements, and of monitoring of soil parameters. In sweet pepper, from conventional management, ^{15}N recoveries from the ^{15}N applications made during vegetative growth and fruit production were 66% and 58%, respectively. With improved management in sweet pepper, the corresponding ^{15}N recoveries were 82% and 77%. In muskmelon, ^{15}N recoveries from conventional management from the ^{15}N applications made during vegetative growth and fruit production were 71% and 42%, respectively. With improved management, the corresponding ^{15}N recoveries were 68% and 44%, respectively. The results demonstrated that combined drip irrigation and fertigation systems with frequent irrigation and N fertilizer application can have very high recovery of applied N fertilizer, of 77–82%.

Keywords: muskmelon; melon; pepper; greenhouse; irrigation; nitrogen management; soil

1. Introduction

With close to 42,000 ha, the greenhouse vegetable production of southeast (SE) Spain is the largest concentration of greenhouses in Europe [1–4]. There are appreciable and expanding areas of similar greenhouses devoted mostly to vegetable production in other Mediterranean Basin countries [1,5]. There is currently a rapid expansion of similar greenhouses in Central America, particularly in Mexico [6]. In China, there are an estimated 4 million ha of greenhouses (Dr. Junjang Yang, Institute of Plant Nutrition and Resources, Beijing, China; personal communication). Two of the most commonly-grown species in the greenhouse-based vegetable production of SE Spain are sweet pepper (*Capsicum annum*) and melon (*Cucumis melo*, L.), which are commonly grown in sequence. Pepper with an autumn-winter growing cycle, and melon with a spring growing cycle. Muskmelon is the most commonly-grown type of melon.

Large additions of nitrogen (N) are characteristic of intensive vegetable production systems. Intensive vegetable production is commonly associated with appreciable nitrate (NO_3^-) leaching losses [7–11] because of the common tendencies to apply excessive N [7,12] and to over irrigate [13]. Additionally, many vegetable crops have shallow roots and short growing cycles, which facilitate NO_3^- leaching loss [12]. While often being associated with large NO_3^- leaching loss, intensive vegetable production systems, also commonly have characteristics that make them very well suited for the adoption of systems of improved N and water management [12,14].

Greenhouse-based vegetable production on the SE Mediterranean coast of Spain is an example. This system is associated with substantial NO_3^- contamination of underlying aquifers [15,16]. In numerous locations [16], aquifer NO_3^- concentrations exceed the European Union (EU) limit of 50 mg NO_3^- L^{-1} [17]. In some locations, aquifer NO_3^- concentrations are > 300 mg NO_3^- [16]. However, this system also has the technical capacity for precise nutrient and irrigation management. Approximately 90% of cropping is in soil, the rest in substrate. Soil-grown crops are commonly grown with combined drip irrigation and automatically-controlled fertigation systems that apply N and other nutrients, in all irrigations, every 1–4 days [18,19]. High-frequency drip irrigation applying specific concentrations and amounts of N in all irrigations provides growers with the capacity for precise N and irrigation management [14].

Currently, in this system, conventional N and irrigation management are based on collective experience using standard nutrient solutions [18]. Consequently, this large potential for improved N and irrigation management is not being effectively used. Simulation models such as VegSyst [20,21] and Nup [22] have been developed to calculate daily crop N uptake for vegetable crops in this system. To calculate crop N fertilizer requirements with practical decision support systems [12,14,23], knowledge of crop recovery of fertilizer N is required.

A number of studies have used ^{15}N to examine N recovery in vegetable crops [24–27]. Very few studies have been conducted with drip irrigation and fertigation, and they were conducted under open field conditions [26]. There are no reported studies under conditions of frequent fertigation using drip irrigation in greenhouse vegetable production.

The objectives of the present study were to: (1) determine the recovery of fertilizer N applied to conventionally-managed vegetable crops, using ^{15}N, (2) determine if improved crop management practices enhance the recovery of mineral fertilizer N, using ^{15}N, and (3) determine the relative distribution of N in plants at final harvest of previously-applied ^{15}N-labeled fertilizer. The crops examined were muskmelon and sweet pepper grown in soil under greenhouse conditions.

2. Materials and Methods

2.1. Location and Greenhouses

The work was conducted in two identical plastic greenhouses in the Research Station of Cajamar in El Ejido, Almería, in SE Spain (36°48′ N, 2°43′ W). The greenhouses were representative of those used in this region [18,19]. The greenhouses had an asymmetrical, shallow, inverted V-shaped roof, and the structure was of stainless-steel tubes and wires; the cladding was 200 µm-thick colorless low-density polyethylene film. Each greenhouse measured 24 × 18 m. The two greenhouses had an east–west orientation, and were adjacent to one another along the east–west axis. They had passive ventilation and no heating system. Each greenhouse was divided approximately in half along the east-west axis by a 2 m wide concrete path; this work was conducted in the northern half of each greenhouse.

The greenhouses had an artificial soil system known locally as “enarenado” soil, which is commonly used in this vegetable production system [18,19,28]. The soil consisted of a 30 cm layer of imported clay soil, obtained from a local quarry, which was placed over the naturally occurring loam soil; a 10 cm layer of coarse river sand was placed over the imported clay soil as a mulch. The 10 cm sand mulch layer over the soil surface substantially reduces evaporation and weed growth. Generally, in this artificial soil system, roots are mostly concentrated in the imported soil layer. Relevant properties of the soil at the beginning of the study are given in Table 1. All soil depths in Table 1, and referred to subsequently, are relative to the surface of the imported layer of clay soil. This artificial soil system was formed when the greenhouse was constructed in 1995.

Table 1. Selected soil properties for 0–60 cm soil depth at the beginning of the study. Soil depths are in relation to the surface of the layer of imported clay soil.

Soil Property	**Depth (cm)**				
	0–10	**10–20**	**20–30**	**30–40**	**40–60**
Clay content (%)	49	51	53	32	13
Silt content (%)	36	35	34	33	15
Sand content (%)	15	14	13	35	72
Texture classification (USDA)	clay	clay	clay	clay-loam	sandy loam
Bulk density (Mg m^{-3})	1.4	1.4	1.6	1.5	1.6
pH (soil:water; 1:1)	8.4	8.4	8.3	8.3	8.3
EC saturated extract (dS m^{-1})	2.7	2.4	2.3	2.7	2.0
Organic carbon (%)	1.05	0.67	0.29	0.80	0.15
Total N (%)	0.12	0.08	0.06	0.08	0.02
Total carbonates (%)	20.4	20.0	19.6	19.2	24.9

In June 2003, sheep manure was applied to the surface layer of imported clay soil at a rate of 73 t ha^{-1}, supplying 1270 kg N ha^{-1}. The application of manure at these rates, at the formation of the artificial soil system and thereafter every 2–5 years, is common practice in this vegetable production system in response to the low organic matter contents of the sub-soils that are imported, from quarries, into the greenhouses for cropping [18,19].

All plants were grown in north–south aligned rows, with 1 m spacing between rows, and 0.5 m between adjacent plants within rows. All plants were vertically supported using nylon guides.

2.2. Crops and Crop Management

Muskmelon (*Cucumis melo*, L., cv. 'Deneb') type Galia and sweet pepper (*Capsicum annum*, L., cv. 'Vergasa') crops were grown sequentially in both 2005 and 2006. The crops grown in 2005 are hereafter referred to as the 2005 muskmelon and 2005 sweet pepper crops, and those grown in 2006 as the 2006 muskmelon and 2006 sweet pepper crops. All crops were grown following transplanting as 6-week old seedlings.

The 2005 muskmelon crop was grown from 17 March to 1 June 2005 (76 days) and the 2006 muskmelon crop from 14 February to 18 May 2006 (93 days). The 2005 sweet pepper crop was grown from 21 July to 20 December 2005 (152 days), and the 2006 sweet pepper crop from 20 July 2006 to 2 February 2007 (201 days).

Above-ground drip irrigation was used with one 2.8 L h^{-1} emitter immediately adjacent to each plant and separated by approximately 5 cm. Complete nutrient solutions were applied by fertigation in all irrigations after the first 2 weeks following transplanting; previously, only water was applied. Examples of the nutrient solutions used for the conventionally managed muskmelon and sweet pepper crops are presented in Supplementary Table S1. All cultural practices (crop pruning and guiding, pollination, fruit harvesting) and pest management operations followed established local practices. Sweet pepper fruit, in both crops, was collected in five harvests conducted at 1–2 week intervals over approximately 2-month period. Muskmelon fruit was harvested at the end of the crop. Irrigation and N management are described subsequently in sub-Section 2.4, and in Tables 2 and 3. Monthly average climatic data inside the greenhouses from January 2005 to January 2007 are presented in Figure 1.

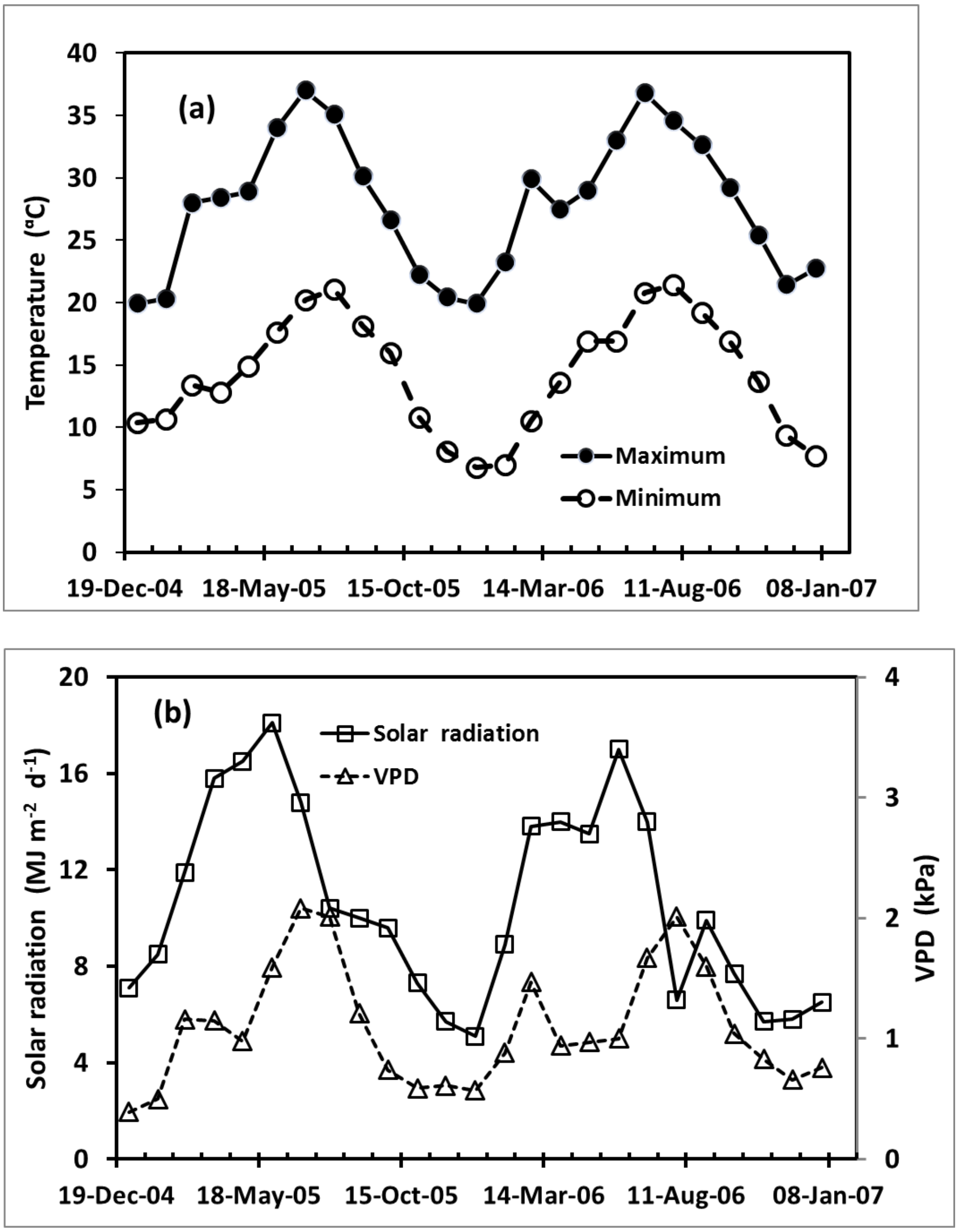

Figure 1. For the period, January 2005 to January 2007, (**a**) monthly data of average maximum and minimum daily air temperatures, and (**b**) mean daily vapor pressure deficit (VPD) and the integral of daily solar radiation inside the greenhouses.

Table 2. Muskmelon. Total amounts of irrigation (mm) and mineral nitrogen (N_{min}) (kg N ha^{-1}) applied to each treatment in the 2005 and 2006 muskmelon crops, and the average concentration of NO_3^- and NH_4^+ in the applied nutrient solution (m*M*). CM and IM are conventional and improved management crops, respectively. N_{min} is the sum of applied NO_3^-–N and NH_4^+–N.

Parameter	CM-2005	IM-2005	CM-2006	IM-2006
Irrigation applied (mm)	220	186	199	178
N_{min} applied (kg N ha^{-1})	364	298	326	235
NO_3^- concentration in solution (m*M*)	10.9	10.5	11.1	9.4
NH_4^+ concentration in solution (m*M*)	0.9	0.9	0.6	0.5

Table 3. Sweet pepper. Total amounts of irrigation (mm) and mineral nitrogen (N_{min}) (kg N ha^{-1}) applied to each treatment in the 2005 and 2006 sweet pepper crops, and the average concentration of NO_3^- and NH_4^+ in the applied nutrient solution (m*M*). CM and IM are conventional and improved management crops, respectively. N_{min} is the sum of applied NO_3^-–N and NH_4^+–N.

Parameter	2005-CM	2005-IM	2006-CM	2006-IM
Irrigation applied (mm)	307	254	344	290
N_{min} applied (kg N ha^{-1})	475	275	493	317
NO_3^- concentration in solution (m*M*)	10.4	7.4	9.7	7.4
NH_4^+ concentration in solution (m*M*)	0.6	0.3	0.5	0.4

2.3. Experimental Design

The working area in the northern half of each greenhouse of 20 rows of 9 plants was divided into four representative plots each of 5 rows with 9 plants per row. All measurements were made with four replications, with one measurement being made in each plot.

In each of the four crops, there were two treatments, being conventional N and irrigation management (CM), or an improved N and irrigation management system (IM). Each treatment was applied to an individual greenhouse. The treatments are fully described in the next sub-section.

2.4. Treatments—N and Irrigation Management

For the conventional management treatment, irrigation followed local practices with respect to volume and timing of irrigation. Published [29] and unpublished data (M.D. Fernández, Research Station of Cajamar, El Ejido, Almeria, Spain) relating irrigation volumes to calculated crop evapotranspiration (ETc) were used to characterize conventional irrigation management. Crop evapotranspiration was calculated using the PrHo model developed for vegetable crops in this system [30–32]. For conventional management, volumes of applied nutrient solution were in excess of crop evapotranspiration (ETc) until maximum crop coefficient (k_c) values [29] were reached, and then, were similar to ETc [29]. In the improved management systems, irrigation was based on estimated daily ETc [30–32] and adjusted to maintain soil matric potential (SMP) at 15 cm depth (measured with tensiometers) between −10 and −40 kPa; the timing of irrigation was in response to SMP.

N management in the conventionally managed treatments was based on applying fixed NO_3^- and NH_4^+ concentrations in the applied nutrient solutions following the recommendations of local technical advisors. N management in the treatments with improved management was based on a prescriptive-corrective management system [12,14,22,33,34] that was developed with these crops. The prescriptive component developed for N management was based on applying N to match simulated daily crop N uptake that was estimated using the Nup simulation model [22,34]. Nup calculates daily crop N uptake in vegetable crops grown in plastic greenhouses in SE Spain. Inputs are daily values of maximum and minimum air temperatures and the integral of solar radiation, within the greenhouse. It was calibrated and validated for muskmelon and sweet pepper [22,34]. Brief [22] and detailed [34] descriptions are available. The corrective component for N management was based on maintaining the soil solution NO_3^- concentration, in the immediate root zone, within a specified range [12,14,22,34]. The specific N management practices used in each crop are described subsequently.

In the 2005 IM muskmelon crop, the concentration of applied N in the IM treatment was similar to the CM treatment; the 16% reduction in irrigation volume was associated with an 18% reduction in the amount of N applied (Table 2). In the 2005 IM sweet pepper crop, N management was based on maintaining the soil solution NO_3^- concentration, at 15 cm depth, within the range of 8–12 m*M*. With the reductions of 17 and 30% in irrigation volume and N concentrations, the amount of applied N was reduced by 42% (Table 3).

In the 2006, IM muskmelon crop, N management was based on estimated crop N uptake, which was simulated using the Nup model [22,34]. With the reductions of 11% and 15% in irrigation volume and applied N concentration, the amount of applied N was reduced by 28% (Table 2). In the 2006 IM sweet pepper crop, N management was based on simulated crop N uptake using the Nup model [22,34] and maintaining soil solution NO_3^- concentration, at 15 cm depth at 8–12 m*M*. With the reductions of 16 and 24% in irrigation volume and N concentrations, the amount of applied N was reduced by 36% (Table 3).

The concentrations of the nutrients other than N applied in the complete nutrient solutions followed established local practice. From two weeks after transplanting, the crops received complete nutrient solutions in all irrigations, with 92–96% of N as NO_3^-, and the rest as NH_4^+ (Tables 2 and 3) following local practice. Irrigation volumes were measured daily with a volume meter. Samples of applied nutrient solutions were analyzed weekly for NO_3^- and NH_4^+ concentrations with an automatic continuous segmented flow analyzer (model SAN++, Skalar Analytical B.V., Breda, The Netherlands).

2.5. ^{15}N *Applications*

In each of the two treatments of each of the 2005 and 2006 muskmelon and sweet pepper crops, four replicate individual plants, one in each replicate plot, directly received nutrient solution containing ^{15}N-labeled $Ca(NO_3)_2$ (16–17 atom% excess ^{15}N in muskmelon crops, 18 atom% excess ^{15}N in sweet pepper crops) on each of three consecutive days. The ^{15}N-labeled nutrient solution was applied to the crops at two different developmental stages: (i) during rapid vegetative growth (VG) and (ii) during the fruit production phase (FP). Detailed descriptions of the ^{15}N applications in the muskmelon and sweet pepper crops are given in Tables 4 and 5, respectively.

Table 4. Muskmelon. Details of the ^{15}N applications made to the 2005 and 2006 muskmelon crops, the NO_3^- concentration and ^{15}N enrichment (atom% excess), and the total volume and total amount of N added in the three sequential daily applications of ^{15}N-labeled nutrient solution. CM: conventional management. IM: improved management. VG: vegetative growth phase. FP: fruit production phase.

^{15}N Treatments	Year of Application, Crop Management, Crop Growth Stage			
	2005 CM VG	2005 IM VG	2006 CM FP	2006 IM FP
Total volume (mm)	16.8	9.7	13.8	13.8
NO_3^- concentration (m*M*)	11.6	11.6	14.2	12.3
^{15}N enrichment (atom% excess)	17.1	17.1	16.0	16.5
Total N amount (kg N ha^{-1})	4.5	2.6	6.9	6.6
Percentage of total N_{min} applied (%) as ^{15}N labeled fertilizer	1.2	0.9	2.1	8.0

Table 5. Sweet pepper. Details of the ^{15}N applications made to the 2005 and 2006 sweet pepper crops, the NO_3^- concentration and ^{15}N enrichment (atom % excess), and the total volume and total amount of N added in the three sequential daily applications of ^{15}N-labeled nutrient solution. CM: conventional management. IM: improved management. VG: vegetative growth. FP: fruit production.

^{15}N Treatments	Year of Application, Crop Management, Crop Growth Stage			
	2005 CM VG	2005 IM VG	2006 CM FP	2006 IM FP
Total volume (mm)	11.2	11.2	6.2	6.2
NO_3^- concentration (m*M*)	13.1	8.7	13.6	8.5
^{15}N enrichment (atom% excess)	18.0	18.4	18.1	17.9
Total N amount (kg N ha^{-1})	3.4	2.3	1.6	1.1
Percentage of total N_{min} applied (%) as ^{15}N labeled fertilizer	0.7	0.8	0.3	0.3

The vegetative growth phase was defined as being when there was predominantly vegetative growth, and the fruit production phase when there was predominantly fruit growth. The fruit production phase was considered to start when there was the full canopy and the exponential growth of fruit had commenced. In muskmelon, the transition between the vegetative growth and fruit production phases occurred when fruit biomass was approximately 1% of final fruit production. In the sweet pepper crops, it occurred at 12–15% of final fruit production.

For both muskmelon and sweet pepper, the ^{15}N applications during vegetative growth were made in 2005, and during fruit production in 2006. The fruit production applications were made in 2006 and not in 2005, as was originally planned, because localized virus infections in the latter part of the 2005 muskmelon and sweet pepper crops created uncertainty about the value of applying ^{15}N in the latter part of the 2005 crops.

In muskmelon, the ^{15}N application during vegetative growth was applied to the 2005 crop, 41–43 days after transplanting (DAT), on 26, 27 and 28 April 2005. The ^{15}N application in the fruit production phase was made 71–73 DAT in the 2006 muskmelon crop, on 25, 26 and 27 April 2006. In sweet pepper, ^{15}N was applied during vegetative growth 70–72 DAT in the 2005 sweet pepper crop, on 27, 28 and 29 September 2005, and in the fruit production phase at 105–107 DAT in the 2006 sweet pepper crop, on 1, 2 and 3 November 2006.

The four individual plants that were directly labeled in each "labeling" of each treatment were in each of the four representative plots of the corresponding treatment. Within each plot, the locations of the ^{15}N applications were chosen to maximize the distance from any previous ^{15}N applications.

The ^{15}N labeled nutrient solutions were prepared firstly as nutrient solutions with a composition of nutrients, other than N, as close as possible to that of the nutrient solutions being applied to the crops. These initial nutrient solutions had 2.0–2.5 m*M* NO_3^- and no NH_4^+. ^{15}N labeling was conducted by adding sufficient ^{15}N labeled $Ca(NO_3)_2$ at 60 atom% ^{15}N and unlabeled $Ca(NO_3)_2$ to achieve the target NO_3^- concentrations and ^{15}N enrichments.

The ^{15}N analysis was conducted with elemental analyzer isotope ratio mass spectrometry (EA-IRMS) using an isotope ratio mass spectrometer (IRMS) (Model Europa Scientific 20–20, Sercon Ltd., Crewe, UK) coupled to a Europa Scientific elemental analyzer system (Sercon Ltd., Crewe, UK). Total N content was calculated as the total ion beam area generated by the IRMS. The ^{15}N analyses were conducted by Iso-Analytical Limited Sandbach, UK. ^{15}N enrichment was calculated, as ^{15}N atom% excess by subtracting the ^{15}N natural abundance values in equivalent materials (e.g., nutrient solution, fruit, leaf, stem, pruned material) that were unlabeled.

The nutrient solutions of ^{15}N-labeled Ca $(NO_3)_2$ were applied using inverted 1.5 L polyethylene bottles connected by silicon tubing to intra-venous drippers. The inverted bottles were supported on metal stakes and the drippers were positioned 3 cm above the sand layer on the soil surface, directly above where irrigation emitter was normally located. Each day, prior to applying the ^{15}N-labeled solution, unlabeled nutrient solution was applied through the drip irrigation system, to all plants in each greenhouse except those that were to directly receive the labeled nutrient solutions. For plants that were to be directly labeled, 7 L plastic trays were used to collect the unlabeled nutrient solution. This ensured that the only nutrient solution received during the application period of three consecutive days of labeling was the ^{15}N-labeled nutrient solution. The applied volumes and the nutrient concentrations of ^{15}N-labeled nutrient solution were identical to those being applied to the rest of the plants in each greenhouse during the three days labeling periods.

Sheet metal plates 1 m long × 22 cm high were inserted into the soil, to a depth of 22 cm, parallel to and 50 cm from the row of plants being labeled, to limit lateral movement, perpendicular to the rows, and prevent uptake of ^{15}N by plants in adjacent crop rows. One plate was inserted on each side of each labeled plant, parallel to the crop rows. The center part of each plate was adjacent to the directly labeled plant.

2.6. Determinations of Plant/Soil Parameters

2.6.1. Field Sampling and Handling of ^{15}N-Labeled Plant Material

The plants that directly received ^{15}N-labeled nutrient solution ("directly labeled plants"; DLP) together with the two immediately adjacent plants on either side, within the same plant row ("adjacent plants”; AP), were identified as “^{15}N labeled plants”. Following labeling, all pruned shoot material from each subsequent pruning of each ^{15}N labeled plant was collected and oven-dried at 65 °C until constant weight. Similarly, all mature fruit collected from each ^{15}N labeled plant was collected and oven-dried at 65 °C. At the end of each crop (crop maturity), each labeled plant was cut at the ground level, and separated into leaf, stem and immature fruit. Each of these three components was oven-dried at 65 °C until constant weight.

All dried pruned material collected after labeling was bulked for each plant. Similarly, all dried mature fruit was bulked for each plant. Fallen leaf collected from the soil surface below each plant was collected and bulked. For data analysis, the small amounts of ^{15}N in fallen leaf were included in that of pruned material. From each labeled plant, both from directly labeled (DLP) and adjacent (AP) plants, there were samples of bulked pruned material, bulked mature fruit, bulked fallen leaf, and the leaf, stem and immature fruit removed at the end of the crop.

For each labeled plant, the bulked pruned material, bulked mature fruit, bulked fallen leaf, and the leaf, stem and immature fruit, removed at the end of each crop, were individually ground with a knife mill (Model SM100 Comfort, Retsch, Germany). A representative sub-sample was then ground with a ball mill (Model MM200 Comfort, Retsch, Germany) until sufficiently fine to pass through a 0.2 mm mesh.

2.6.2. Calculation of ^{15}N Recovery

For each ^{15}N-labeled plant, the amount of recovered excess ^{15}N was calculated as the sum of excess ^{15}N in each of the components of leaf, stem and immature fruit at the end of the crop, and the bulked mature fruit and bulked pruned material. The percentage crop recovery of ^{15}N from ^{15}N-labeled nutrient solutions was calculated using a mass balance method using the equation:

$$R = \frac{^{15}N_U}{^{15}N_A} \times 100$$

In this equation, R is the percentage ^{15}N recovery of applied ^{15}N by the plant, $^{15}N_U$ is the uptake of enriched ^{15}N by the plant and $^{15}N_A$ is the enriched ^{15}N in the nutrient solution applied to the directly labeled plant. The total ^{15}N recovery by the crop was calculated as the sum of ^{15}N recovery by the DLP plant and of each of the two corresponding AP plants. For each plant, ^{15}N recovery was calculated for leaf, stem, fruit and pruned material to provide information on the relative distribution of recovered ^{15}N within plants. All ^{15}N data were the means of four replicates.

2.6.3. Fruit Production, Dry Matter Production, and Crop N Uptake of Unlabeled Plants

For each treatment of each crop, four areas of 4 m^2, each with eight plants were marked to determine fruit production, the mass of pruned material removed during the crop, and final standing biomass (after prior removal of prunings and harvested fruit) at the end of each crop. There was one group of eight plants in each replicate plot. There were several prunings in each crop, one harvest of mature muskmelon fruit and 5–8 harvests of mature sweet pepper fruit. At each fruit harvest, the fruit was separated into commercial and non-commercial fruit using local commercial criteria; fresh and dry weights were determined of both fruit categories. At each pruning, dry matter removed in leaves and stems was determined. Dry matter (DM) content of all crop components was determined by oven drying at 65 °C until constant weight.

At the end of each of crop, the four groups of eight plants in each treatment, were completely removed and separated into leaves, stem and fruit, which were weighed, and the dry matter content determined. Final total shoot dry matter production (final DMP) for each treatment was determined by summing the amount of DM of these sampled plants, the total amount of DM removed in the various prunings, and the total amount of DM harvested as fruit.

Representative samples of leaves, stems, and fruit from the final standing biomass sampling, and of harvested fruit and prunings were ground separately and sequentially with a knife mill and ball mill, as previously described for the equivalent ^{15}N labeled material. The total N content of each sample was determined using a Dumas-type elemental analyzer system (model EA 3000, EuroVector SpA., Milan, Italy). Total crop N uptake was calculated, as the sum of N in the final standing biomass, harvested fruit and prunings for each replicate group of plants for each treatment.

2.6.4. Soil Mineral N

For each treatment of each crop, soil samples were taken at the beginning and end of the crop, at three depths from the surface of the imported clay soil (0–20, 20–40 and 40–60 cm depth), from four replicate locations, one from each replicate plot. In each location of each treatment, the soil was sampled in three positions with respect to the plant and the irrigation emitter, being (1) very close to the plant and emitter, (2) between two adjacent emitters of the same drip-line, and (3) mid-way between adjacent emitters of adjacent lines of emitters. The inorganic N content of the soil was determined following extraction by potassium chloride (KCl) (40 g moist soil: 200 mL 2 *M* KCl). Concentrations of NO_3^- and NH_4^+ in the soil extracts were determined using the automatic segmented flow analyzer previously described for analyzing nutrient solutions. With respect to the sampling positions, soil mineral N (NO_3^-–N plus NH_4^+–N) was calculated as: (0.25 × position 1) + (0.25 × position 2) + (0.50 × position 3).

2.6.5. Soil Solution NO_3^-

Soil solution was obtained weekly using soil solution suction samplers (model 1900L12, Soil Moisture Equipment Co., Santa Barbara, CA, USA) installed at 15 cm depth from the surface of the imported clay soil, 5 cm from the emitter in the perpendicular direction of the drip-line and 8 cm from the plant in the same direction of the drip-line. There were four replicates per treatment. The soil solution samples were taken 24 h after applying vacuum (−60 kPa) to the suction cups. There was a period of at least 24 h after the previous irrigation (and application of nutrient solution) before vacuum was applied. The NO_3^- concentration in soil solution samples was analyzed using the procedures and equipment previously described for nutrient solutions.

2.7. Statistical Analyses

The following statistical analyses were conducted. Analysis of variance was conducted to examine the differences between treatments in the recovery of ^{15}N, in the fruit and total dry matter production and in the total N uptake, and standard errors of the means were calculated for all measured results.

3. Results

3.1. Muskmelon

Seventy-one and 68% of ^{15}N applied, during the vegetative growth phase (VG) in 2005, was recovered by the conventional and improved managed muskmelon crops, respectively (Table 6). Forty-two and 44% of ^{15}N applied, during the fruit production phase (FP) in 2006, was recovered by the conventional and improved managed muskmelon crops, respectively (Table 6). These results demonstrated high crop recoveries of ^{15}N applied during vegetative growth, and appreciably lower recoveries of ^{15}N applied during fruit production. The differences between the conventional and improved management practices applied during the muskmelon crops were insufficient to affect the recovery of ^{15}N.

In muskmelon, 23–37% of ^{15}N applied, during vegetative growth, was recovered in the two plants adjacent to the directly-labeled plants (Table 6). From the ^{15}N applied during fruit production in muskmelon, only 10–14% of applied ^{15}N was recovered in adjacent plants (Table 6).

Table 6. Muskmelon. Final crop recovery of ^{15}N by the muskmelon crops. Data are the mean and standard errors of the mean of four replications. CM: conventional management. IM: improved management. Recovered ^{15}N in prunings, also includes ^{15}N in fallen biomass. The results of ANOVA comparing the CM and IM treatments are presented. n.s.: no significant difference at $p < 0.05$.

Plant	Percentage Recovery of Applied ^{15}N					
	Applied during Vegetative Growth (2005)			Applied during Fruit Production (2006)		
	CM (%)	IM (%)	ANOVA Results	CM (%)	IM (%)	ANOVA Results
Directly labeled plant	33.3 ± 4.2	44.7 ± 2.6	n.s.	32.3 ± 9.1	30.7 ± 2.1	n.s.
Adjacent plants	37.2 ± 0.1	23.2 ± 3.0	n.s.	10.0 ± 2.8	13.7 ± 2.2	n.s.
Plant total recovery	70.5 ± 4.1	67.9 ± 1.0	n.s.	42.2 ±11.9	44.4 ± 1.5	n.s.

From the ^{15}N application to muskmelon during vegetative growth, 13–37% of the recovered ^{15}N was in fruit (Table 7), suggesting appreciable remobilization of N within the plants following crop uptake, as at the time of ^{15}N labeling only 1% of final fruit biomass had formed. Much of the rest of the recovered ^{15}N from the vegetative growth labeling was in leaves and stem (Table 7). From the ^{15}N application to muskmelon during fruit production, 47–48% of recovered ^{15}N was in fruit, and the rest in shoots, mostly in leaves (Table 7). These results demonstrate that approximately 50% of N absorbed during the fruit production phase was used in the production of shoot material.

For the 2005 and 2006 muskmelon crops, total fresh fruit production was 6.8–7.8 kg m^{-2} and total commercial fruit production was 6.3–7.5 kg m^{-2} (Table 8). These yields are similar to those for commercial production in the region, and there were no differences between conventional and improved management for either total or marketable fruit production in either the 2005 and 2006 muskmelon crops. Total crop N uptake was 239–266 kg N ha^{-1} for the 2005 muskmelon crops and 268–283 kg N ha^{-1} for 2006 muskmelon crops (Table 8).

Table 7. Muskmelon. Relative distribution of recovered ^{15}N in components of muskmelon plants. Data are the mean and standard errors of the mean of four replications. VG: vegetative growth. FP: fruit production. CM: conventional management. IM: improved management. Recovered ^{15}N in prunings, also includes ^{15}N in fallen biomass.

Period of ^{15}N Application	Crop Management System	Relative Distribution of Recovered ^{15}N in Plant Components (%)			
		Fruit	Leaves	Stem	Prunings
VG (2005)	CM	37.4 ± 2.6	38.7 ± 2.9	16.2± 1.0	7.7 ± 1.5
VG (2005)	IM	13.0 ± 1.5	53.6 ± 1.6	21.9 ± 1.9	11.6 ± 3.3
FP (2006)	CM	47.2 ± 2.8	34.8 ± 1.9	18.0 ± 2.0	0
FP (2006)	IM	48.3 ± 4.5	36.2 ± 3.2	15.5 ± 1.3	0

Table 8. Muskmelon and sweet pepper. Total fresh and total commercial fruit production and total crop N uptake for each treatment in the 2005 and 2006 muskmelon (M) and sweet pepper (SP) crops. Data are the mean and standard errors of the mean of four replications. CM: conventional management. IM: improved management. VG: vegetative growth. FP: fruit production.

Year of Application, Crop, Crop Management, Growth Stage at Labeling	Fruit Production		Total Dry Matter Production	Crop N Uptake
	Total Fresh	Total Commercial		
	(kg m^{-2})	(kg m^{-2})	(t ha^{-1})	(kg N ha^{-1})
2005-M-CM-VG	6.9 ± 0.4	6.7 ± 0.4	8.6 ± 0.2	* 266 ± 10
2005-M-IM-VG	6.8 ± 0.2	6.3 ± 0.2	8.1 ± 0.1	239 ± 4
2006-M-CM-FP	7.8 ± 0.3	7.5 ± 0.2	9.3 ± 0.2	283 ± 6
2006-M-IM-FP	7.1 ± 0.5	6.8 ± 0.5	8.7 ± 0.2	268 ± 3
2005-SP-CM-VG	6.8 ±0.4	6.2 ± 0.4	9.4 ± 0.7	236 ± 16
2005-SP-IM-VG	6.1 ±0.2	5.5 ± 0.1	8.8 ± 0.3	206 ± 8
2006-SP-CM-FP	9.8 ±0.4	9.1 ± 0.4	11.7 ± 0.5	316 ± 14
2006-SP-IM-FP	10.5 ± 0.3	9.9 ± 0.1	* 13.2 ± 0.2	335 ± 8

* This measure was significantly higher (ANOVA, $p < 0.05$) than the corresponding alternative treatment in the same crop.

The soil mineral N (NO_3^-–N + NH_4^+–N) content measured at 0–60 cm depth at the beginning and at the end of each treatment of the two muskmelon crops are given in Table 9. There were very high contents of soil mineral N in the 0–60 cm profile throughout all treatments of the 2005 and 2006 muskmelon crops. Nearly all the soil mineral N was in the form of NO_3^- and <5 kg N ha^{-1} was in the form of NH_4^+–N.

Table 9. Muskmelon. Soil N_{min} (NO_3^-–N + NH_4^+–N) content at the beginning and at the end of 2005 and 2006 muskmelon crops for 0–60 cm depth. Data are the mean and standard errors of the mean of four replications. Less than 5 kg N ha^{-1} was in the form of NH_4^+–N.

	Conventional Management		**Improved Management**	
Crop	**Beginning (kg N ha^{-1})**	**End (kg N ha^{-1})**	**Beginning (kg N ha^{-1})**	**End (kg N ha^{-1})**
Melon 2005	648 ± 43	558 ± 36	606 ± 66	627 ± 94
Melon 2006	511 ± 41	305 ± 65	449 ± 29	286 ± 55

The soil solution NO_3^- concentration ([NO_3^-]) over time, in the immediate root zone of the muskmelon plants, for each treatment of the 2005 and 2006 muskmelon crops is presented in Figure 2a,b, respectively. In both the conventional and improved management treatments of the 2005 muskmelon crop, there was a substantial on-going reduction in soil solution [NO_3^-] within the root zone immediate to the plant for the first 48 days after transplanting (DAT), from 28–33 to 4–10 m*M* (Figure 2a). This period corresponded to the phase of rapid vegetative growth. Similarly, in the conventional and improved management treatments of the 2006 muskmelon crop, there was a tendency of on-going reduction in soil solution [NO_3^-] until 56 DAT (Figure 2b). These data demonstrate that despite the large amounts of soil mineral N present during these crops and the constant application of fertilizer mineral N, that during the phase of rapid vegetative growth there were consistent reductions in NO_3^- within the immediate root zone. In the latter part of the two treatments of both the 2005 and 2006 muskmelon crops, there was an accumulation of NO_3^- in the immediate root zone. As general observations, the soil solution [NO_3^-] data suggest that within the immediate root zone, during the phase of rapid vegetative growth until 50–60 DAT there was an on-going negative balance of mineral N, and thereafter there was an on-going positive balance. During much of 2005 and 2006 muskmelon crops, the soil solution [NO_3^-] was consistently less in the improved compared to conventionally managed treatment.

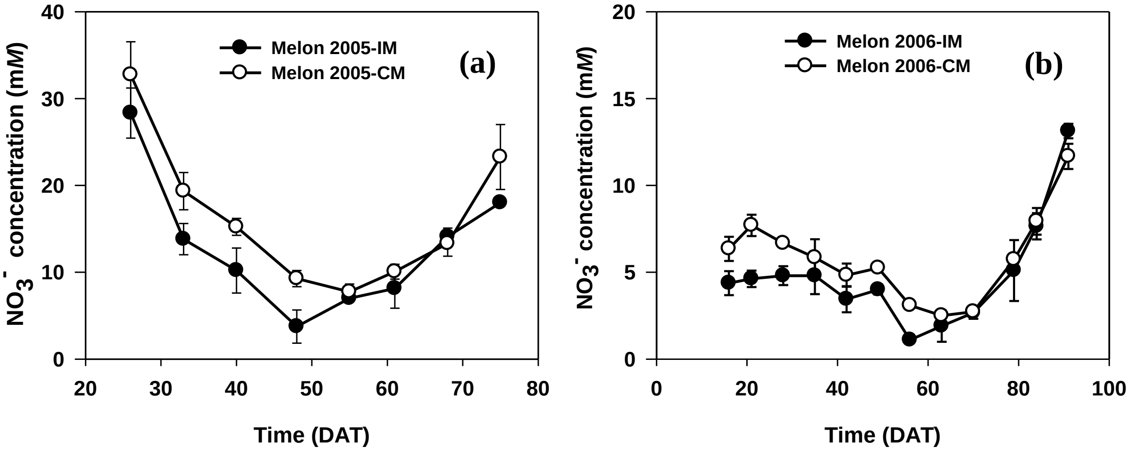

Figure 2. Evolution of the soil solution NO_3^- concentration (m*M*) at 15 cm depth throughout 2005 (**a**) and 2006 (**b**) muskmelon crops, for the conventional and improved management treatments in each crop. Data are the means and standard errors of four replicate determinations. CM: conventional management; IM: improved management. DAT: days after transplanting.

3.2. Sweet Pepper

Total recovery of ^{15}N applied during vegetative growth was 66% with conventional management and 82% with improved management (Table 10). Total recovery of ^{15}N applied during the fruit production phase was 58% with conventional management and 77% with improved management (Table 10). The recoveries in the improved management treatments, from the ^{15}N labeling at both vegetative growth and fruit production, were very high being 77–82%. These recoveries were appreciably higher than the recoveries from the corresponding ^{15}N applications in the conventionally managed treatments of 58–66%.

Table 10. Sweet pepper. Final crop recovery of ^{15}N by the sweet pepper crops. Data are the mean and standard errors of the mean of four replications. CM: conventional management. IM: improved management. The results of ANOVA comparing the CM and IM treatments are presented. n.s.: no significant difference at $p < 0.05$; *: significant difference at $p < 0.05$.

Plants	**Percentage Recovery of Applied ^{15}N**					
	Applied during Vegetative Growth (2005)			**Applied during Fruit Production (2006)**		
	CM (%)	**IM (%)**	**ANOVA Results**	**CM (%)**	**IM (%)**	**ANOVA Results**
Directly labeled plant	51.2 ± 3.7	69.9 ± 3.1	*	49.2 ± 2.3	70.9 ± 2.5	*
Adjacent plants	14.7 ± 0.7	12.4 ± 1.4	n.s.	8.4 ± 2.3	6.1 ± 0.7	n.s.
Plant total recovery	66.0 ± 4.4	82.3 ± 1.8	n.s.	57.6 ± 3.3	77.0 ± 2.7	n.s.

In sweet pepper, most of the applied ^{15}N was recovered in the directly-labeled plants (Table 10). Only 6–8% of applied ^{15}N during fruit production was recovered in the plants immediately adjacent to the directly labeled plants. From ^{15}N applied to sweet pepper during vegetative growth, 12–15% of applied ^{15}N was recovered in adjacent plants.

In sweet pepper, the relative distribution of recovered ^{15}N within plant components was similar between treatments and ^{15}N application times (Table 11). Between 44–57% of applied ^{15}N was recovered in fruit, 25–30% in leaves and 16–26% in stem (Table 11).

Table 11. Sweet pepper. Relative distribution of recovered ^{15}N in components of sweet pepper plants. Data are the mean and standard errors of the mean of four replications. VG: vegetative growth. FP: fruit production. CM: conventional management. IM: improved management. Recovered ^{15}N in prunings also includes ^{15}N in fallen biomass.

Period of ^{15}Napplication	**Crop Management System**	**Relative Distribution of Recovered ^{15}N in Plant Components (%)**			
		Fruit	Leaves	Stem	Prunings
VG (2005)	CM	57.2	25.2	15.5	2.0
VG (2005)	IM	57.4	25.5	15.9	1.2
FP (2006)	CM	43.6	30.0	26.2	0.2
FP (2006)	IM	51.6	26.6	21.1	0.7

For the 2005 sweet pepper crop, total fresh fruit production was 6.1–6.8 kg m^{-2} and total commercial fruit production was 5.5–6.2 kg m^{-2} (Table 8). For the 2006 sweet pepper crop, total fresh fruit production was 9.8–10.5 kg m^{-2} and total commercial fruit production was 9.1–9.9 kg m^{-2} (Table 8). The yields from the 2006 sweet pepper crop were notably higher than from the 2005 crop because of the longer growing season. There were no significant differences between conventional and improved management for either total or marketable fruit production in either the 2005 and 2006 sweet pepper crops. Total crop N uptake was 206–236 kg N ha^{-1} for the 2005 sweet pepper crops and 316–335 kg N ha^{-1} for 2006 sweet pepper crops (Table 8).

The soil mineral N (NO_3^-–N + NH_4^+–N) contents measured at 0–60 cm depth at the beginning and at the end of each treatment of the two sweet pepper crops are given in Table 12. There were very high contents of soil mineral N in the 0–60 cm profile throughout all treatments of the 2005 and 2006 sweet pepper crops (Table 12).

Table 12. Sweet pepper. Soil N_{min} (NO_3^-–N + NH_4^+–N) content at the beginning and at the end of 2005 and 2006 sweet pepper crops for 0–60 cm depth. Data are the mean and standard errors of the mean of four replications. Less than 5 kg N ha^{-1} was in the form of NH_4^+–N.

Crop	Conventional Management		Improved Management	
	Beginning (kg N ha^{-1})	End (kg N ha^{-1})	Beginning (kg N ha^{-1})	End (kg N ha^{-1})
Pepper 2005	487 ± 70	590 ± 74	418 ± 68	534 ± 46
Pepper 2006	395 ± 12	429 ± 24	352 ± 9	405 ± 29

The evolution of the soil solution NO_3^- concentration ([NO_3^-]) for each treatment of the 2005 and 2006 sweet pepper crops is presented in Figure 3a,b, respectively. Generally, there was an appreciably lower soil solution NO_3^- concentration in the root zone of the crops with improved management compared to those with conventional management.

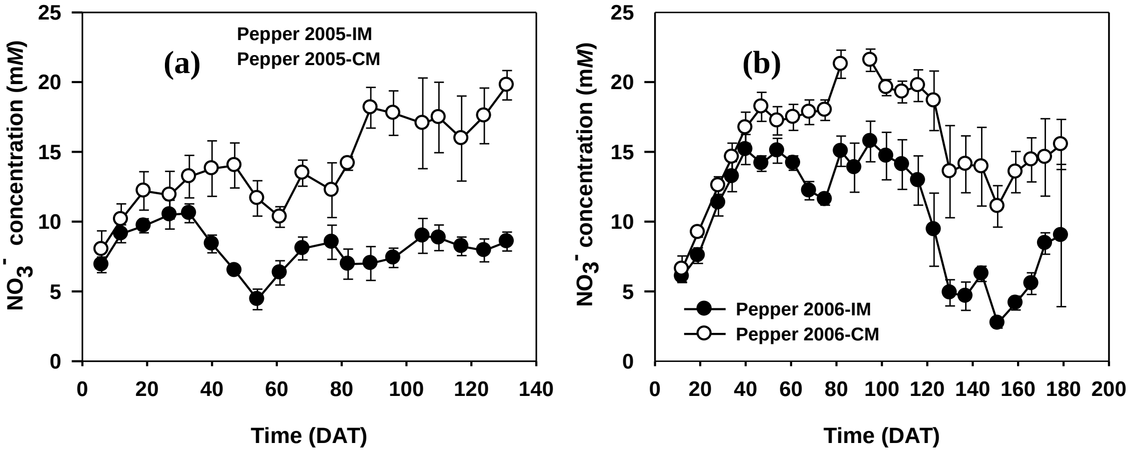

Figure 3. Evolution of the soil solution NO_3^- concentration (m*M*) at 15 cm depth throughout 2005 (**a**) and 2006 (**b**) sweet pepper crops, for the conventional and improved management treatments in each crop. Data are the means and standard errors of four replicate determinations. CM: conventional management. IM: improved management. DAT: days after transplanting.

4. Discussion

^{15}N recovery is considered to be a good indicator of N fertilizer efficiency [18]. The sweet pepper crops recovered 58–82% of ^{15}N applied during vegetative growth or during fruit production. These are high recovery values, given that N recoveries from mineral fertilizer N applications, in vegetable production, are generally <50% [12,35]. The recovery of ^{15}N applied to muskmelon during vegetative growth was also relatively high. However, the recovery, by muskmelon, of ^{15}N applied during fruit production, was notably lower. These relatively low recoveries can be explained by excessive N application during fruit production of muskmelon, as demonstrated by the on-going increases in soil solution NO_3^- during the latter part of the melon crops. These data suggest that applied N concentrations during the latter part of melon crops can be appreciably reduced, in relation to what is applied earlier in the crop. This is supported by recent modeling work, in the context of this system, that showed that applied N concentrations, to meet crop N requirements, decline from the vegetative to fruit production phases [20,21,23]. Common commercial management practice in this system is to maintain relatively constant applied N concentrations throughout crops [36].

In the sweet pepper crops, the improved N and irrigation management practices were associated with very high ^{15}N recoveries of 77–82% from ^{15}N applications during both vegetative growth and fruit production. These values suggest that the combination of (i) matching N supply to N demand, (ii) frequent small N applications made every 1–4 days, and (iii) the avoidance of excessive irrigation enabled very high recoveries of applied N. Essentially, N was applied when it was required and to where it could be most effectively obtained by the crop. Similarly, high crop recoveries of applied N, averaged over a complete crop, were obtained for optimally managed substrate-grown tomato grown with a similar combined fertigation and drip irrigation system [37]. While the growing media and the frequency of application of nutrient solution are different, it appears that within the drip irrigation bulb in both soil and substrate that precise and frequent N fertilization with optimal management can result in very high recoveries of fertilizer N.

These high ^{15}N recoveries were obtained in the presence of high soil mineral N contents. A major factor contributing to these high ^{15}N recoveries is likely to be the effect of frequent drip irrigation displacing NO_3^- from the inside to outside the drip irrigation bulb [38,39]. Ref. [39] demonstrated considerable displacement of NO_3^- from the irrigation bulb by combined fertigation and drip irrigation. Appreciable lateral movement of NO_3^- was demonstrated in the 2006 sweet pepper crop of the present study where soil $[NO_3^-]$ between the crop rows was generally 10–15 m*M* greater than in the immediate root zone [22,34]. The data of sweet pepper crops with improved N and irrigation management indicate that vegetable crops can obtain nearly of their N requirements from within the drip irrigation bulb, regardless of the amounts of available present outside the drip irrigation bulb. When there is appreciable mineral N present in the upper part of these soil profiles outside the immediate wet bulb, management systems will need to be developed, for drip irrigated and fertigated crops, so that they absorb mineral N from outside the bulb.

A notable feature of the results with the sweet pepper crops was the increase in ^{15}N recovery associated with improved management compared to conventional management. In the sweet pepper crops, the improved management practices resulted in a 36–42% reduction in the total amount of mineral N fertilizer applied. These reductions in applied N were associated with appreciable increases in the ^{15}N recovery of 16% for the vegetative growth application, and 19% for the fruit production application. The effect of substantial reductions in applied N was also apparent in consistently appreciably lower soil solution $[NO_3^-]$ in the immediate root zone of the sweet pepper crops with improved management.

In the muskmelon crops, the improved management practices did not enhance ^{15}N recovery compared to conventional management. The reductions in the total amount of fertilizer N of 18 and 28%, in 2005 and 2006, respectively, were apparently insufficient to affect a notable improvement in N efficiency. A factor contributing to the high N recovery of muskmelon during vegetative growth, in the present study, was presumably the very rapid vegetative growth of this crop. The associated high N demand was indicated by the substantial depletion in soil solution $[NO_3^-]$ in the immediate root zone during this growth phase.

The high amounts of soil mineral N in the current study have been observed in other studies conducted in this system [40]. They are consistent with the use of standard nutrient solutions, periodic large manure applications, and that soil mineral N and manure N are generally not considered when developing N management plans [18]. As previously discussed, the ^{15}N data indicate that the high amounts of soil mineral N had relatively little effect on ^{15}N recovery.

Optimal N and irrigation management when using fertigation and drip irrigation systems, enable the frequent application of small amounts of N and small volumes of water to accurately meet crop requirements. The current study demonstrated that with optimal N and irrigation management that very high recoveries of applied N fertilizer can be achieved. This greenhouse production system enhances the potential for high recovery of applied mineral because there are no rainfall-related NO_3^- leaching events, and the combination of the sand mulch and the use of NO_3^- based N fertilizers considerably reduces the possibility of NH_3 volatilization loss from these alkaline soils.

The use of ^{15}N-labeled fertilizer demonstrated that N recoveries from N applied were generally high for soil-grown vegetable crops grown in greenhouses using fertigation and drip irrigation. With optimal management practices, recoveries of 77–82% were obtained.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/5/741/s1, Table S1: Muskmelon and sweet pepper.

Author Contributions: Conceptualization, R.B.T. and C.M.-G.; methodology, R.B.T., C.M.-G., M.R.G., M.D.F.; software, M.R.G., M.D.F.; formal analysis, C.M.-G., M.R.G. and R.B.T.; investigation, C.M.-G., M.R.G. and R.B.T.; resources, R.B.T., M.D.F.; data curation, C.M.-G., M.R.G. and R.B.T.; writing—original draft preparation, C.M.-G.; writing—review and editing, R.B.T., M.G. and C.M.-G.; supervision, R.B.T. and M.G.; project administration, R.B.T.; funding acquisition, R.B.T. and M.G. All authors have read and agreed to the published version of the manuscript.

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