agronomy

MDPI

Article

Soil Monitoring Methods to Assess Immediately Available Soil N for Fertigated Sweet Pepper

Alejandra Rodríguez [1,*], M. Teresa Peña-Fleitas [2], Francisco M. Padilla [2,3], Marisa Gallardo [2,3] and Rodney B. Thompson [2,3]

1. University of Costa Rica, Sede Regional de Guanacaste, Liberia 50101, Guanacaste Province, Costa Rica
2. Department of Agronomy, University of Almeria, Carretera de Sacramento s/n, La Cañada de San Urbano, 04120 Almeria, Spain; mtpena.fl@ual.es (M.T.P.-F.); f.padilla@ual.es (F.M.P.); mgallard@ual.es (M.G.); rodney@ual.es (R.B.T.)
3. CIAIMBITAL Research Centre for Mediterranean Intensive Agrosystems and Agrifood Biotechnology, University of Almeria, La Cañada de San Urbano, 04120 Almeria, Spain

* Correspondence: alrodcha@gmail.com; Tel.: +34-665-992-591

Received: 12 November 2020; Accepted: 15 December 2020; Published: 19 December 2020

Abstract: Excessive N application occurs in greenhouse vegetable production. Monitoring methods of immediately available soil N are required. [NO_3^-] in soil solution, sampled with ceramic cup samplers, and [NO_3^-] in the 1:2 soil to water (*v*/*v*) extract were evaluated. Five increasing [N], from very N deficient (N1) to very N excessive (N5) were applied throughout three fertigated pepper crops by combined fertigation/drip irrigation. The crops were grown in soil in a greenhouse. Soil solution [NO_3^-] was measured every 1–2 weeks, and extract [NO_3^-] every 4 weeks. Generally, for treatments N1 and N2, both soil solution and extract [NO_3^-] were continually close to zero, and increased with applied [N] for treatments N3–5. The relationships of both methods to the nitrogen nutrition index (NNI), an indicator of crop N status, were assessed. Segmented linear analysis gave R^2 values of 0.68–0.70 for combined data from entire crops, for both methods. NNI was strongly related to increasing [NO_3^-] up to 3.1 and 0.9 mmol L^{-1} in soil solution and extracts, respectively. Thereafter, NNI was constant at 1.04–1.05, with increasing [NO_3^-]. Suggested sufficiency ranges were derived. Soil solution [NO_3^-] is effective to monitor immediately available soil N for sweet pepper crops in SE Spain. The extract method is promising.

Keywords: *Capsicum annuum* L.; ceramic suction cups; greenhouse; nitrate leaching; NNI; soil solution; soil testing; soil-water extracts; sufficiency values; vegetable crops

1. Introduction

Approximately 170,000 ha of greenhouses and plastic tunnels [1] are used for intensive production of vegetables in the Mediterranean Basin. These greenhouse production systems are commonly associated with N applications that appreciably exceed what is required to ensure high yields [2,3]. Additionally, irrigation is often excessive to crop water requirements [3–5]. Consequently, large nitrate (NO_3^-) leaching losses [5–7], and associated aquifer NO_3^- contamination occur [8].

In southeast (SE) Spain, there are 42,000 ha of highly concentrated plastic greenhouses [9], of which 32,000 ha are located in the province of Almeria [10]. In approximately 90% of these greenhouses, crops are grown in soil [11]. Nearly all of the greenhouse production areas in Almeria have been declared nitrate vulnerable zones (NVZ) [12] in accordance with the EU Nitrates Directive [13]. Nitrate concentrations as high as 400 mg NO_3^- L^{-1} have been reported [14], and there is a general tendency to increase [8,14].

Given that most greenhouses in Almeria are within NVZs, there is an obligation to adopt management practices that reduce aquifer NO_3^- contamination [15,16]. These improved practices must ensure optimal, or at least considerably improved, crop N management. All growers have combined drip irrigation and fertigation systems that frequently apply small amounts of N [6,11,17]. Tools/systems that monitor crop N status or the immediate supply of crop available N will enable frequent N applications to be adjusted to ensure optimal crop N status [18,19]. The assessment of crop N status of greenhouse-grown vegetable crops has suggested that generally crop/plant monitoring approaches (proximal optical sensors, petiole sap analysis) are sensitive to crop N deficiency, but less sensitive to excessive N supply [18,19].

Previous studies in Almeria greenhouses have suggested that monitoring the immediately available soil N supply, as the NO_3^- concentration ($[NO_3^-]$) of a root-zone soil solution can indicate an excessive N supply [20,21]. In these studies, ceramic cup soil solution suction samplers were used. However, until now, suggested quantitative lower and upper limits have been tentative, being based on observation rather than on objective criteria [6,21]. A number of studies have examined various practical aspects of determining the $[NO_3^-]$ of soil solution, such as minimizing spatial variability [19,21], their use with rapid analysis systems [22], and providing general recommendations on their use [18,19]. However, only one study has quantitatively assessed sensitivity in relation to crop N status [20]. In that study, simple linear regression analysis was used. No studies have assessed sensitivity in relation to crop N status using the more comprehensive approach of segmented line analysis. No studies have quantitatively determined lower and upper sufficiency limits.

Another soil-based approach to assess the immediately available soil N supply is the 1:2 soil to water (*v*/*v*) extract method, which is routinely used in commercial greenhouse production in The Netherlands [18,23,24]. This method involves taking a composite soil sample and extracting one volume of fresh soil with two volumes of water. The $[NO_3^-]$ is determined in the decanted solution from the suspension [23,25]. Good agreement between N determined with the 1:2 soil to water (*v*/*v*) extract method and N extracted from mineral soils has been reported [26].

Lower and upper and sufficiency values are available for the 1:2 soil to water (*v*/*v*) extract method for vegetable and ornamental crops grown in soil in Dutch greenhouses [18,24,27]. Some work with this method has been conducted in Greek and Italian greenhouses [27,28]. However, sufficiency values for those conditions have not been published, although suggested unpublished sufficiency values are lower than those used in The Netherlands [29]. The 1:2 soil to water (*v*/*v*) extract method has not been evaluated in greenhouses in SE Spain. There is no available information as to its effectiveness or of sufficiency values for these conditions.

Quantitative evaluation of the sensitivity and quantitative determination of sufficiency values, for the $[NO_3^-]$ in soil solution and in the extract from the 1:2 soil to water (*v*/*v*) extract method, can be done by relating these $[NO_3^-]$ to an indicator of crop N status. The nitrogen nutrition index (NNI) is a reliable and established indicator of crop N status [30,31]. The NNI is the ratio between the actual crop N content and the critical N content of the crop [32]. An NNI value of 1.0 indicates N sufficiency for maximum dry matter production (DMP) (i.e., maximum growth), values of <1.0 indicate N deficiency, and values of >1.0 indicate N excess [30]. The relationship of soil solution $[NO_3^-]$ to NNI of fertigated tomato and melon was examined in one study [20]. These authors observed a tendency for increasing NNI with increasing soil solution $[NO_3^-]$ in melon, but little relationship in tomato. No studies have reported quantitative assessment of the 1:2 soil to water (*v*/*v*) extract method using NNI.

In Almeria greenhouses, sweet pepper is one of the main crops, being grown annually on approximately 10,000 ha [10]. While various crop monitoring tools have been thoroughly evaluated to determine N deficiency of sweet pepper (e.g., [33–35]), complementary soil monitoring tools are required that can also inform of excessive N supply.

The objectives of this study were, in the context of greenhouse-grown sweet pepper crops in SE Spain, to: (i) determine the response of $[NO_3^-]$ in soil solution and the 1:2 soil to water (*v*/*v*) extract to increasing N supply, (ii) evaluate the sensitivity of $[NO_3^-]$ in soil solution and the 1:2 soil to water (*v*/*v*) extract to assess crop N status, (iii) derive lower sufficiency values for maximum growth, and (iv) derive upper sufficiency values to identify excessive N supply. Objectives (ii) to (iv) used NNI. Additional objectives were to determine: (a) the relationships of [NO_3^-] in soil solution and in the 1:2 soil to water (*v*/*v*) extract to the applied N concentration, and (b) the relationship between [NO_3^-] in soil solution and [NO_3^-] in the 1:2 soil to water (*v*/*v*) extract.

2. Materials and Methods

2.1. Experimental Site and Crops

Three sweet pepper (*Capsicum annuum* L. "Melchor") crops were grown, in soil, in a plastic greenhouse at the Experimental Farm of the University of Almeria, in Retamar, Almeria (36°51′ N, 2°16′ W, 92 m elevation) in SE Spain. The crops were grown with autumn-winter growing periods in 2014–2015 (2014 crop), 2016–2017 (2016 crop), and 2017–2018 (2017 crop) (Table 1). Cropping conditions were very similar to those of local commercial production [9].

The greenhouse had a multi-tunnel structure with a galvanized steel frame. The roof consisted of low-density polyethylene (LDPE) tri-laminated film, and walls of polycarbonate. It was ventilated passively through flap roof windows and lateral side panels. Total cropped area within the greenhouse was 1327 m^2. The soil was an artificial layered "enarenado" soil that is commonly used in this region [3,36]. It consisted of a 30-cm layer of imported silty loam textured soil placed on the original loam soil; a 10-cm layer of fine gravel was placed on the imported soil as a mulch. Details of the soil are available in [37].

Irrigation was applied through above-ground drip tape. The drip tape was organized in paired lines, with 0.8 m separation between the lines of each pair, 1.2 m separation between adjacent pairs of lines, and 0.5 m spacing between drip emitters in the drip tape. The density of emitters was 2 emitters m^{-2}. The discharge rate was 3 L h^{-1}. The drip system had a coefficient of uniformity of >95%. Complete nutrient solutions were applied through the drip irrigation system in each irrigation, consistent with local fertigation practice [3,9,17].

The cropped area was divided into 24 experimental plots measuring 6 m by 6 m. In this study, 20 of the plots were used. In each plot, there were three paired lines of drip tape with 12 emitters in each line. The plots were hydraulically separated from one another, by 30 cm deep vertical barriers of polyethylene sheet (250 µm thickness). Individual plants were positioned 6 cm from and adjacent to each emitter. The plant density was two plants m^{-2}; there were 72 plants per plot. Plants were transplanted as five-week-old seedlings. Additional information of the layout is provided in [38].

2.2. Experimental Design and Treatments

The 20 experimental plots were allocated to five different irrigation/fertigation sectors using a randomized block design. Each sector received a different N treatment. Prior to each crop, the soil was leached, with large irrigation volumes, to homogenize the plots with respect to residual soil NO_3^- and salinity.

Throughout each crop, five different N treatments, of increasing N concentration, were applied in each irrigation. The five N treatments were: very N deficient (N1), N deficient (N2), conventional N management (N3), excessive N (N4), and very excessive N (N5), applied in the nutrient solution. The concentrations and amounts of N applied to each treatment in each crop are provided in Table 1. More than 90% of the mineral N was applied as NO_3^-, the rest as ammonium (NH_4^+). Other macronutrients and micronutrients were applied to the nutrient solution in concentrations that were sufficient to ensure they were not deficient. For several days after transplanting (DAT), the transplanted seedlings received only water (<0.04 mmol N L^{-1}). The different N treatments commenced at 1 DAT, 9 DAT, and 10 DAT in the 2014, 2016, and 2017 crops, respectively.

Table 1. Information of the three pepper crops and N treatments. Duration of the crops, total mineral N applied, irrigation amount, the average applied N concentration by nutrient solution, total dry matter production (DMP), and total yield (TY).

Crop Year	Date of Transplanting	Date End of the Crop (Duration)	N Treatment [a]	Mineral N Applied (kg N ha^{-1}) [b]	Irrigation Amount (mm) [c]	[N] in Nutrient Solution (mmol L^{-1}) [b]	DMP (t ha^{-1})	TY (t ha^{-1})
2014	12 August 2014	29 January 2015 (170 days)	N1	64	190	2.4	5.7	38.7
			N2	189	216	6.2	7.9	52.2
			N3	516	294	12.6	8.6	52.9
			N4	804	357	16.1	9.7	51.1
			N5	990	354	20.0	9.3	46.4
2016	19 July 2016	24 March 2017 (248 days)	N1	88	319	2.0	8.8	67.2
			N2	302	404	5.3	12.6	86.4
			N3	561	414	9.7	15.2	91.5
			N4	1052	557	13.5	14.4	94.2
			N5	1320	532	17.7	13.6	89.7
2017	21 July 2017	20 February 2018 (214 days)	N1	86	304	2.0	5.1	33.3
			N2	304	383	5.7	9.3	54.4
			N3	519	383	9.7	10.5	61.0
			N4	870	475	13.1	12.6	65.1
			N5	1198	513	16.7	12.6	68.9

[a] N1: very N deficient; N2: N deficient; N3: conventional N; N4: excessive N; N5: very excessive N. [b] For the period of N treatments, which commenced 1, 9 and 10 DAT in the 2014, 2016 and 2017 crops, respectively. [c] For the complete cropping cycle.

Irrigation/fertigation was conducted every 1–4 days to maintain the soil matric potential between −10 to −30 kPa. One tensiometer (Irrometer, Co., Riverside, CA, USA) was installed per plot at 12 cm depth where roots were most concentrated. Total irrigation volumes are presented in Table 1. During the crops, additional irrigation as nutrient solution or water was applied, to certain treatments, to reduce the build-up of soil salinity. In the 2014 crop, additional nutrient solution was applied to the N3, N4, and N5 treatments during 80–103 DAT; the total additional volumes were 23 mm, 44 mm, and 45 mm, respectively. In the 2016 crop, additional nutrient solution was applied for 66–71 DAT, 104–111 DAT, and 178–180 DAT for treatments N1 to N5 with total volumes of 62 m, 79 mm, 84 mm, 115 mm, and 107 mm, respectively. In the 2017 crop, additional irrigation was applied as water to the N3 to N5 treatments (72–110 DAT, 129–143 DAT, and 185–208 DAT); the total extra volumes applied were 31 mm, 39 mm, and 39 mm, respectively.

Crop management followed local commercial practice. The crops were physically supported with nylon cords placed horizontally along the side of the crop, using a local system known as "enfajado". Excessive greenhouse temperature was prevented by applications of $CaCO_3$ suspension to the greenhouse roof. Details of the timing and applied concentrations of $CaCO_3$ suspension, and of the resultant transmissivity of photosynthetically active radiation (PAR) are reported in [38].

2.3. Obtaining and Analysis of Samples of Soil Solution and 1:2 Soil to Water (v/v) Extract

2.3.1. Soil Solution

Soil solution [NO_3^-] was determined in samples collected every 1–2 weeks using ceramic cup suction samplers that were 3.1 cm in diameter and 35 cm long (Model SPS200 3, SDEC, Reignac Sur Indre, France). Sampling was weekly in the 2014 crop and every 2 weeks in the 2016 and 2017 crops. The suction samplers were placed within the drip irrigation bulb where most roots are located, 8 cm to the side of a plant, and 5 cm from the drip line, at 12 cm depth from the surface of the imported soil. One sampler was installed in each plot of each treatment. The locations of the suction samplers were carefully chosen to reduce spatial variability, by avoiding non-representative plants, the edges of plots, localized zones of rainwater infiltration, and shading.

Soil solution was collected after applying vacuum of −70 kPa for 24 h. No irrigation/fertigation was applied during the 24 h period of sample collection, nor during the 24 h before application of vacuum. The [NO_3^-] were analyzed using an automatic continuous segmented flow analyzer (model SAN++, Skalar Analytical B.V., Breda, The Netherlands). In the 2014, 2016, and 2017 pepper crops, sampling commenced at 14 DAT, 9 DAT, and 19 DAT, respectively. In these crops, there were nineteen, twenty-six, and sixteen samplings, respectively.

2.3.2. The 1:2 Soil to Water (*v*/*v*) Extract Method

The 1:2 soil to water (*v*/*v*) extract [18,24,25] was obtained from composite soil samples taken every four weeks, in the 2016 and 2017 crops. The composite samples were from the 0–20 cm soil depth of the imported soil. In each replicate plot of each treatment, the composite soil sample was obtained by mixing four 0–20 cm depth soil samples of equal volume; two samples were taken at both 8 cm and 20 cm adjacent to the plant. As with the location of suction samplers, care was taken to choose locations for soil sampling that minimized spatial variability. Immediately after sampling, the soil samples were mixed, and a 100 mL sub-sample of the composite soil sample was placed in a sealed plastic bag and refrigerated at 5 °C.

Within 24 h of sampling, the refrigerated sub-samples were extracted, following the method of [23]. Forty mL of soil was placed in a transparent container filled with 80 mL of demineralized water; field-moist soil was added until the total volume increased to 120 mL to obtain the 1:2 (*v*/*v*) soil to water combination. The mixture of soil and water was gently mixed in an agitator for 20 min at 48 rpm. The suspension obtained was allowed to sediment for 24 h while refrigerated at 5 °C, after which the supernatant was filtered. The [NO_3^-] of the filtered supernatant was analyzed using the automatic continuous segmented flow analyzer described in Section 2.3.1.

In the 2016 and 2017 pepper crops, the sampling commenced at 21 DAT in both crops. There were eight and seven samplings in the 2016 and 2017 crops, respectively.

2.4. Determination of Crop Nitrogen Nutrition Index (NNI)

Crop N status was determined as:

$$NNI = \frac{N_{act}}{N_c}, \quad (1)$$

where N_{act} is the measured N content of all shoot dry matter [31]. N_{act} values for the dates of soil solution and soil sampling were obtained by interpolating from measured crop N content values that were determined in biomass samplings conducted approximately every 21 days, for each N treatment in each crop [38]. N_c is the critical N content, for each sampling date, calculated using the critical N curve (CNC): $\%N_c = 4.71 \times DMP^{-0.22}$ (DMP is dry matter production) for greenhouse-grown sweet pepper determined by [38]. The DMP values for each sampling date, that were required to calculate the corresponding $\%N_c$ values, were interpolated from measured DMP from the regular biomass samplings.

To determine the amount of dry matter at transplanting, 100 seedlings were sampled. Subsequent biomass sampling, conducted approximately every 21 days, involved sampling one representative plant of each replicate plot, and dividing the plant into leaf, stem, and fruit. The dry matter content, of each component, was determined by oven-drying all material at 65 °C until constant dry weight. In each crop, eight plants were marked in each replicate plot; these were used to determine fruit production and the amount of pruned material. Total DMP, at each biomass sampling, was calculated as the sum of dry matter of leaf, stem, and fruit on that sampling date, plus that of all previously sampled pruned material and harvested fruit.

To determine the total crop N content (N_{act} as %N), representative sub-samples of leaves, stems, fruit, pruned material, and harvested fruit from each replicate plot were sequentially ground using knife and ball mills. The N content of each component was determined using a Dumas-type elemental analyzer system (Model Rapid N, Elementar Analysen systeme GmbH, Hanau, Germany). Total crop N content was calculated, for each replicate plot, as total crop N uptake divided by total DMP. Total crop N uptake (kg N ha^{-1}) in each replicate plot, at each biomass sampling, was the sum of N in all relevant components (leaf, stem, and fruit), including previously pruned material and harvested fruit, as was done for the calculation of total DMP.

2.5. Data Analysis

The relationships between soil solution [NO_3^-] and NNI were analyzed using data from individual entire crops, pooled entire crop data from the three crops, and pooled data for each phenological stage of the three crops. The relationships between [NO_3^-] in the 1:2 soil to water (*v/v*) extract and NNI were analyzed using data from individual entire crops, pooled entire crop data from the 2016 and 2017 crops, and pooled data for each phenological stage of the two crops. Three different phenological stages were considered, being (i) vegetative; (ii) flowering and early fruit growth; and (iii) harvest. For the three pepper crops, the vegetative stage was from 0 to 41 DAT. The flowering and early fruit growth stage included fruit set and development, from 42 to 97 DAT, 100 DAT, and 109 DAT in 2014, 2016, and 2017 crops, respectively. The harvest stage was from 98 to 170 DAT in 2014, from 101 to 248 DAT in 2016, and from 110 to 214 DAT in 2017.

Segmented linear regression analysis was used to analyze the relationships between NNI and soil solution or extract [NO_3^-]. The segmented linear regressions consisted of an inclined and a horizontal segment. The inclined segment was described by $y = ax + b \ (if\ x < x_0)$ and the horizontal segment by $y = c \ (if\ x \geq x_0)$, where y is NNI value, x is the [NO_3^-] in the soil solution or 1:2 soil to water (*v/v*) extract, a is the slope, b is the intercept of the inclined segment, and x_0 is the [NO_3^-] where the two segments intersect. The x_0 value is the [NO_3^-] in the soil solution or 1:2 soil to water (*v*/*v*) extract value that provides *c*, the maximum NNI value. Once the maximum NNI value is obtained, the response is constant. The segmented linear regression analysis was conducted using RStudio2 software (RStudio Inc., Boston, MA, USA).

To assess the spatial variability of the [NO_3^-] determined in soil solution and the 1:2 soil to water (*v*/*v*) extract, the coefficient of variation (CV) of these measurements was calculated. The CV was determined as:

$$CV = \frac{\sigma}{\overline{x}} \times 100, \tag{2}$$

where σ is the standard deviation and $\overline{x}$ is the average from the replicates (n = 4) of each treatment calculated for each sampling date. Average CV values were calculated for each crop for each soil NO_3^- monitoring method.

3. Results

3.1. Soil Solution [NO_3^-]

3.1.1. Responses of Soil Solution [NO_3^-] to N Treatments

The response of soil solution [NO_3^-] to the different N treatments was similar in each of the three pepper crops (Figure 1a,c,e). Immediately after transplanting, soil solution [NO_3^-] was similarly low (<5 mmol L^{-1}) in all of the different N treatments (Figure 1a,c,e). After 30 DAT, when the crops had received the N treatments for approximately 20 days, soil solution [NO_3^-] in the N1 and N2 treatments remained low with values of 0.0–2.2 mmol L^{-1} until the end of the crops. In contrast, after 30 DAT, soil solution [NO_3^-] progressively increased in treatments N3, N4, and N5, with the degree of increase being related to the applied [N].

In treatment N3, soil solution [NO_3^-] increased slowly and progressively, generally reaching approximately 15 mmol L^{-1} by the end of the crops (Figure 1a,c,e). In treatments N4 and N5, soil solution [NO_3^-] increased rapidly, with some fluctuations during crop growth (Figure 1a,c,e), reaching maximum values of 34–46 mmol L^{-1} (Figure 1a,c,e). The relative differences in soil solution [NO_3^-] between treatments N3–5, of N3 < N4 < N5, were consistently maintained throughout the three crops.

3.1.2. Response of NNI to N Treatments

The response of NNI to the different N treatments was similar during each of the three pepper crops (Figure 1b,d,f). There were consistent and clear differences between N1, N2, and N3 treatments, with N1 < N2 < N3 (Figure 1b,d,f). The ranges of NNI values for these treatments were 0.47–0.88, 0.68–0.99, and 0.80–1.11 for the N1, N2, and N3 treatments, respectively. The N3 treatment had NNI values that were consistently close to 1.0 during the three crops (Figure 1b,d,f). The N4 and N5 treatments had very similar values to each other throughout the crop with NNI values of 0.85–1.14, which were consistently slightly higher than the NNI values of the N3 treatment (Figure 1b,d,f). Generally, the NNI values from treatments N4 and N5 were slightly and consistently higher than 1.0.

The evolution of the NNI values during the crops indicated that the crops grown in the N1 and N2 treatment were, respectively, consistently very N deficient and slightly N deficient, given their consistent relative differences in relation to NNI of 1.0 (Figure 1b,d,f). The N3 treatment was generally N sufficient, with NNI values that were consistently very close to 1.0. The N4 and N5 treatments were very similar in that for both treatments there was continuously slight luxury N uptake, indicated by NNI values that were consistently slightly >1.0.

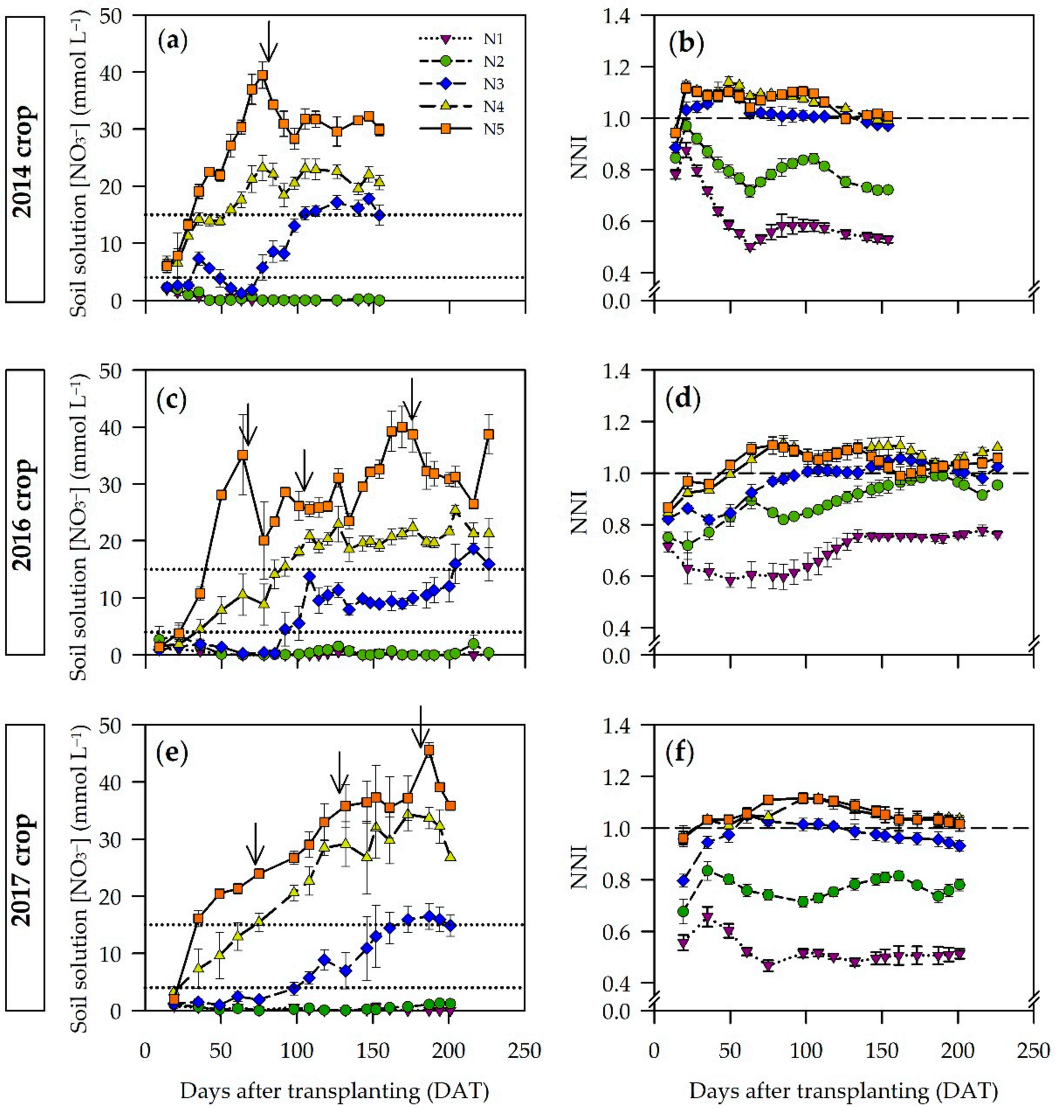

Figure 1. Evolution of the soil solution [NO_3^-] and nitrogen nutrition index (NNI) values as a function of time (days after transplanting (DAT)) during the 2014 (**a**,**b**), 2016 (**c**,**d**), and 2017 (**e**,**f**) pepper crops, each with five N treatments. Values are means (n = 4) ± standard error (SE). The horizontal dotted lines (Figure **a**,**c**,**e**) represent the sufficiency values of 5.0 mmol L^{-1} and 15.0 mmol L^{-1} determined in this work. Arrows (Figure **a**,**c**,**e**) indicate the date of the commencement of the additional irrigation or fertigation during 80–103 DAT (N3, N4, and N5) in the 2014 crop, during 66–71 DAT, 104–111 DAT, and 178–180 DAT (N1 to N5) in the 2016 crop, and during 72–110 DAT, 129–143 DAT, and 185–208 DAT (N3 to N5) in the 2017 crop. The horizontal long dashed lines (Figure **b**,**d**,**f**) represent NNI = 1.0. The NNI figures (**b**,**d**,**f**) were modified from [33]. The use of chlorophyll meters to assess crop N status and derivation of sufficiency values for sweet pepper. Sensors 19: 2949, published by MDPI and distributed as open access under the Creative Commons Attribution (CC BY) license.

3.1.3. Relationship between NNI and Soil Solution [NO_3^-]

The relationships between NNI and soil solution [NO_3^-], for the entire crop were described by segmented linear regression analysis, using data for the combined set of data from the three crops (Figure 2a; Table 2). There were similar strong relationships using segmented linear regression analysis for each phenological stage (Figure 2b; Table 2). In all of these segmented linear regression analyses, NNI increased rapidly to values of approximately 1.05 as soil solution [NO_3^-] increased from 0.0 to

3.1 mmol L^{-1} (Figure 2a,b). Thereafter, as soil solution [NO_3^-] further increased to values as high as 45.6 mmol L^{-1}, NNI remained constant at NNI = 1.05 (Figure 2a).

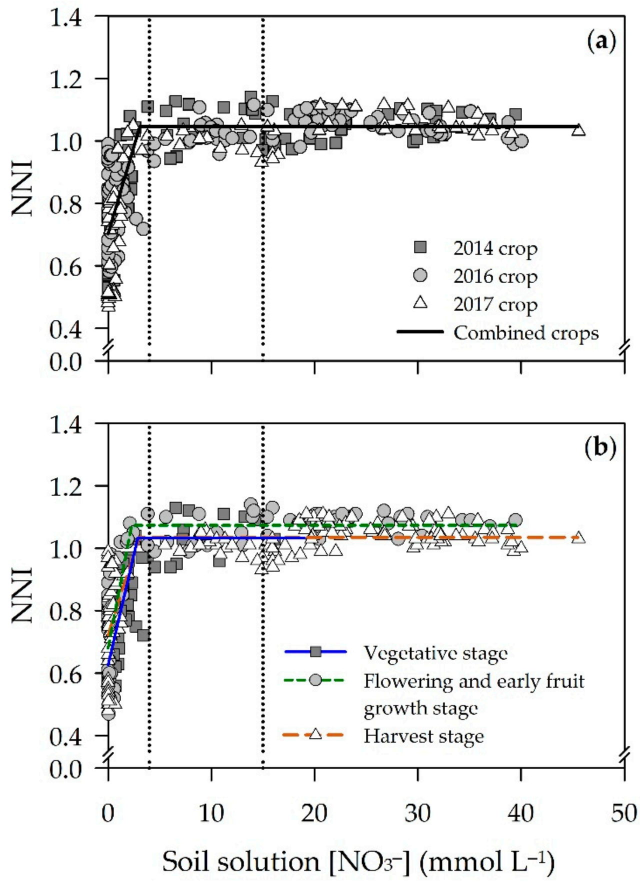

Figure 2. Relationships of NNI values with soil solution [NO_3^-] for (**a**) the entire 2014, 2016, and 2017 pepper crops considered together, and (**b**) the vegetative, flowering and early fruit growth, and harvest phenological stages using combined data from the three pepper crops. The vertical dotted lines represent the sufficiency values of 5.0 mmol L^{-1} and 15.0 mmol L^{-1} determined in this work. The segmented linear regression was represented by an inclined segment defined by $y = ax + b\ (if\ x < x_0)$ and the horizontal segment by $y = c\ (if\ x \geq x_0)$, where y is NNI value, x is soil solution [NO_3^-], a is the slope, b is the intercept of the inclined segment and x_0 is the intersection of the two segments. The x_0 is the soil solution [NO_3^-] required to reached c, the maximum NNI value. After reaching the maximum NNI value, the response is constant. The equations of the segmented regression are presented in Table 2.

The relationship for the combined entire crop data of the three crops was described by $y = 0.1116x + 0.7029\ (if\ x < x_0)$ and $y = 1.05\ (if\ x \geq x_0)$, with an R^2 of 0.70 (Figure 2a; Table 2). The segmented linear regression analyses for the 2014, 2016, and 2017 crops had R^2 values of 0.81, 0.66, and 0.81, respectively (Table 2). The R^2 values for the vegetative, flowering and early fruit growth, and harvest stages, for the three crops considered together, were 0.72, 0.76, and 0.68, respectively (Table 2). These results showed that the strong relationship between NNI and soil solution [NO_3^-] was consistent for each of the three crops, and was maintained throughout the three crops.

Table 2. Inclined linear equation of segmented linear regression relating NNI values to soil solution $[NO_3^-]$ (mmol L^{-1}) for each and combined dataset of the three pepper crops and for combined dataset of the three pepper crops for each phenological stage. Equation, x_0 value, maximum NNI value reached, coefficients of determination (R^2), standard error (±SE) and the number of data points (n) are shown.

Data	Segmented Equation	x_0 Value $[NO_3^-]$ (mmol L^{-1})	Maximum NNI Value	R^2	SE	n
Pepper crop						
2014	$y = 0.1408x + 0.6602$	2.8	1.05	0.81	0.09	93
2016	$y = 0.0302x + 0.7999$	8.4	1.05	0.66	0.10	130
2017	$y = 0.1915x + 0.5868$	2.3	1.03	0.81	0.09	80
Combined crops	$y = 0.1116x + 0.7029$	3.1	1.05	0.70	0.10	303
Phenological stage						
Vegetative	$y = 0.1412x + 0.6264$	2.9	1.04	0.72	0.10	45
Flowering and early fruit growth	$y = 0.1723x + 0.6804$	2.3	1.08	0.76	0.09	95
Harvest	$y = 0.1034x + 0.7229$	3.0	1.03	0.68	0.09	163

3.1.4. Sufficiency Values of Soil Solution $[NO_3^-]$ for Optimal N Nutrition

The x_0 values represented the minimum soil solution $[NO_3^-]$ to obtain NNI values very close to 1.0 (Table 2), which corresponded to the minimum supply of N that ensured maximum crop DMP. For the combined entire crop data set, for two of the three entire individual crops, and for each of the three phenological stages, x_0 was ≤3.1 mmol NO_3^- L^{-1} (Table 2). Above this soil solution $[NO_3^-]$ value, NNI remained constant indicating that crop N status did not change with increasing soil solution $[NO_3^-]$ (Figure 2a,b). These data suggest that soil solution $[NO_3^-]$ values somewhat larger than 3.1 mmol L^{-1} will ensure maximum DMP, i.e., a minimum sufficiency value, and that an appreciably larger soil solution $[NO_3^-]$ value can be used as a maximum sufficiency value.

It is proposed that 5.0 mmol NO_3^- L^{-1} be a minimum recommended value for soil solution $[NO_3^-]$ for greenhouse-grown sweet pepper in SE Spain. This value will ensure both DMP production and a margin of comfort to avoid N deficiency. The data of the N3 treatments in the three crops provide a framework for determining a maximum recommended value. Throughout much of the crops, the NNI values of N3 treatments were very close to 1.0 (Figure 1b,d,f), when soil solution $[NO_3^-]$ was <15.0 mmol NO_3^- L^{-1} (Figure 1a,c,e). Therefore, a maximum recommended value of 15.0 mmol NO_3^- L^{-1} is proposed. The proposed sufficiency range of 5.0–15.0 mmol NO_3^- L^{-1} provides a range of soil solution $[NO_3^-]$ that ensures growth is not limiting (Figure 2a,b; Table 2). Additionally, this range can be maintained in farming practice. Above this range (i.e., >15.0 mmol NO_3^- L^{-1}), the immediately available soil N supply is excessive and wasteful.

3.2. $[NO_3^-]$ in the 1:2 Soil to Water (v/v) Extract

3.2.1. Response of $[NO_3^-]$ in the 1:2 Soil to Water (*v*/*v*) Extract to N Treatments

The $[NO_3^-]$ values in the 1:2 soil to water (*v*/*v*) extract were generally similar in each of the 2016 and 2017 pepper crops for the different N treatments (Figure 3a,c). At approximately 20 DAT, all N treatments had similar values of <1.0 mmol NO_3^- L^{-1}. Thereafter, there were clear differences between the N2, N3, N4, and N5 treatments in both crops (Figure 3a,c). In the N1 and N2 treatments, in both crops, the extract $[NO_3^-]$ was constantly 0.0–0.8 mmol L^{-1}. Extract $[NO_3^-]$ for the N3 treatment increased slowly and consistently during the crops, reaching values of 0.3–2.0 mmol L^{-1}. Extract $[NO_3^-]$ in the N4 and N5 treatments increased more rapidly over time, reaching maximum values of 2.7–4.4 mmol L^{-1}. The relative differences in extract $[NO_3^-]$ of N3 < N4 < N5 were maintained consistently throughout the two crops (Figure 3a,c).

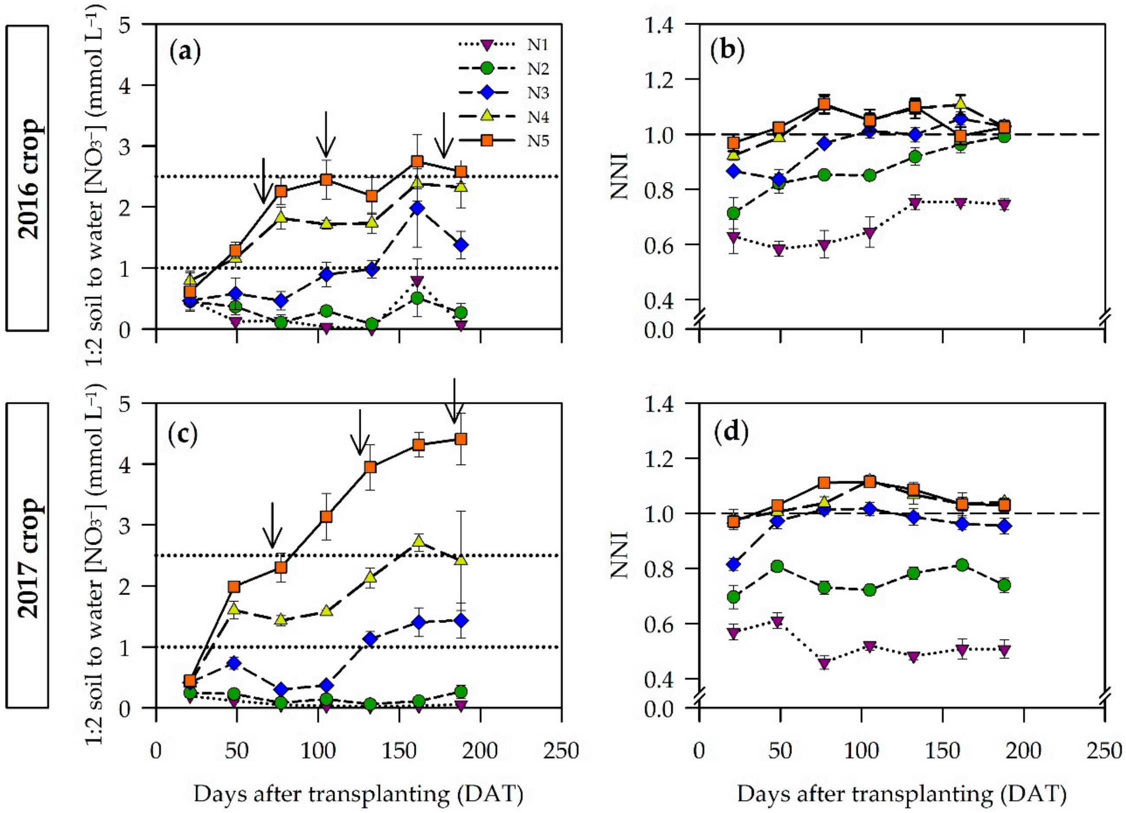

Figure 3. Evolution of the $[NO_3^-]$ in the 1:2 soil to water (*v/v*) extract and NNI values as a function of time (days after transplanting (DAT)) during 2016 (**a**,**b**) and 2017 (**c**,**d**) pepper crops, each with five N treatments. Values are means (n = 4) ± standard error (SE). The horizontal dotted lines (Figure **a**,**c**) represent the sufficiency values of 1.0 mmol L^{-1} and 2.5 mmol L^{-1} determined in this work. Arrows (Figure **a**,**c**) indicate the date of the commencement of the additional irrigation or fertigation during 66–71 DAT, 104–111 DAT, and 178–180 DAT (N1 to N5) in the 2016 crop, and during 72–110 DAT, 129–143 DAT, and 185–208 DAT (N3 to N5) in the 2017 crop. The horizontal long dashed lines (Figure **b**,**d**) represent NNI = 1.0. The NNI figures (**b**,**d**) were modified from [33]. The use of chlorophyll meters to assess crop N status and derivation of sufficiency values for sweet pepper. Sensors 19: 2949, published by MDPI and distributed as open access under the Creative Commons Attribution (CC BY) license.

3.2.2. Response of NNI to N Treatments

The responses of NNI to the N treatments for the 2016 and 2017 crops are described in Section 3.1.2.

3.2.3. Relationship between NNI and $[NO_3^-]$ of the 1:2 Soil to Water (*v/v*) Extract

There were strong relationship between NNI and $[NO_3^-]$ of the 1:2 soil to water (*v/v*) extract, described by segmented linear regression equations for the two crops, considered together and separately, and for each phenological stage (Figure 4a,b; Table 3). The segmented linear regression, for the combined entire crop data set, was described by $y = 0.4132x + 0.6588\ (if\ x < x_0)$ and $y\ =\ 1.04\ (if\ x \geq x_0)$, with a value of R^2 of 0.68 (Figure 4a; Table 3). For the 2016 and 2017 crops considered separately, the corresponding R^2 values were 0.64 and 0.83, respectively (Table 3). NNI values increased rapidly to 1.04 as $[NO_3^-]$ of the 1:2 soil to water (*v/v*) extract increased from 0 to 0.93 mmol L^{-1} (Figure 4a; Table 3). With increasing extract $[NO_3^-]$ from 0.93 mmol L^{-1} to 4.4 mmol L^{-1}, NNI remained constant at 1.04 (Figure 4a). The results for the segmented linear regression for the combined vegetative, flowering, and early fruit growth, and harvest phenological stages were generally very similar in terms of x_0, c and R^2 values (Figure 4b; Table 3).

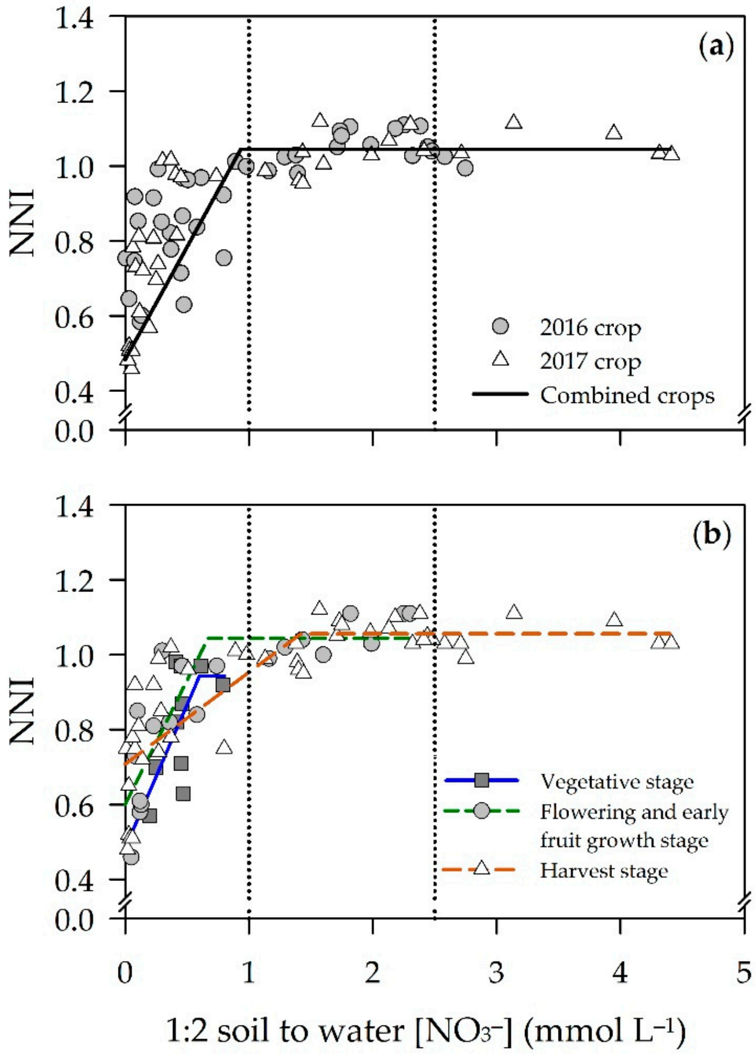

Figure 4. Relationships of NNI values with the [NO_3^-] of the 1:2 soil to water (*v*/*v*) extract for (**a**) the entire 2016 and 2017 pepper crops considered together, and (**b**) the vegetative, flowering and early fruit growth and harvest stage using combined data from the two pepper crops. The vertical dotted lines represent the sufficiency values of 1.0 mmol L^{-1} and 2.5 mmol L^{-1} determined in this work. The segmented linear regression was represented by an inclined segment defined by $y = ax + b\ (if\ x < x_0)$ and the horizontal segment by $y = c\ (if\ x \geq x_0)$, where *y* is NNI value, *x* is the [NO_3^-] of the 1:2 soil to water (v/v) extract, *a* is the slope, *b* is the intercept of the inclined segment and x_0 is the intersection of the two segments. The x_0 is the [NO_3^-] of the 1:2 soil to water (*v*/*v*) extract required to reached *c*, the maximum NNI value. After reaching the maximum NNI value, the response is constant. The equations of the segmented regression are presented in Table 3.

3.2.4. Sufficiency Values of [NO_3^-] of the 1:2 Soil to Water (*v*/*v*) Extract for Optimal N Nutrition

Using the same approach, as used in Section 3.1.4, the proposed sufficiency range for [NO_3^-] in the 1:2 soil to water (*v*/*v*) extract, for greenhouse-grown pepper is 1.0–2.5 mmol L^{-1}.

Table 3. Inclined linear equation of segmented linear regression relating NNI values to 1:2 soil to water (*v/v*) [NO_3^-] (mmol L^{-1}) extract method for each and combined dataset of the two pepper crops and for combined dataset of the two pepper crops for each phenological stage. Equation, x_0 value, maximum NNI value reached, coefficients of determination (R^2), standard error (±SE), and the number of data points (*n*) are shown.

Data	Segmented Equation	x_0 Value [NO_3^-] (mmol L^{-1})	Maximum NNI Value	R^2	SE	*n*
Pepper crop						
2016	$y = 0.2056x + 0.7471$	1.55	1.07	0.64	0.09	40
2017	$y = 1.0310x + 0.5278$	0.49	1.03	0.83	0.09	35
Combined crops	$y = 0.4132x + 0.6588$	0.93	1.04	0.68	0.10	75
Phenological stage						
Vegetative	$y = 0.7659x + 0.4835$	0.60	0.94	0.42	0.12	10
Flowering and early fruit growth	$y = 0.6619x + 0.6005$	0.67	1.04	0.74	0.10	20
Harvest	$y = 0.2452x + 0.7079$	1.42	1.06	0.68	0.10	45

3.3. Relationship between Soil Solution [NO_3^-] and Applied Nutrient Solution [NO_3^-]

Soil solution [NO_3^-] was linearly related to the applied nutrient solution [NO_3^-] (Figure 5a,b). These relationships were determined for combined data sets of the three crops and for the three crops considered individually, for (1) the entire crop, from transplanting to final harvest (Figure 5a), and (2) from 70 DAT (Figure 5b) onwards when the effects were more stable (Figure 1a,c,e). For the combined data set of the three entire crops, the relationship was described by $y = 1.7146x - 3.6156$ with an R^2 value of 0.62. For the 2014, 2016, and 2017 entire crops, the R^2 values were 0.71, 0.56, and 0.77, respectively (Table 4). For the three entire crops considered together or individually, the slope, intercept and R^2 values were generally similar (Figure 5a; Table 4).

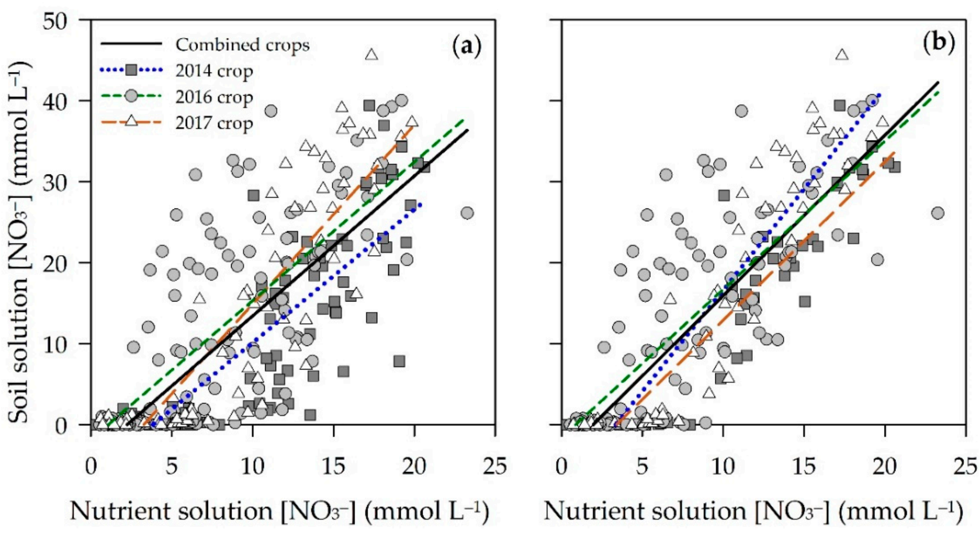

Figure 5. Linear relationship between soil solution [NO_3^-] and the applied nutrient solution [NO_3^-] during (**a**) the entire crop, considering the entire crops and the (**b**) crops after 70 days after transplanting (DAT). The linear regression of all dataset combining the three pepper crops of the soil solution [NO_3^-] is presented as a black solid line, the linear regression for 2014 (dotted line), 2016 (short dash) and 2017 (long dash) crops is shown. The equations of the linear regressions are presented in Table 4.

Table 4. Linear relationship relating soil solution [NO_3^-] and to the applied nutrient solution [NO_3^-] for sweet pepper crops, considering the entire crops and crops after 70 days after transplanting (DAT). The equation, coefficients of determination (R^2), standard error (±SE), and the number of data points (*n*) are presented.

Crop Year	Entire Crop	R^2	±SE	*n*	Crop after 70 DAT	R^2	±SE	*n*
2014	$y = 1.6565x - 6.3849$	0.71	6.2	93	$y = 1.9555x - 6.6405$	0.86	4.8	48
2016	$y = 1.6959x - 1.5405$	0.56	8.0	130	$y = 1.8308x - 1.5868$	0.61	7.6	110
2017	$y = 2.2113x - 7.2355$	0.77	6.8	80	$y = 2.4963x - 8.3451$	0.85	5.7	60
Combined crops	$y = 1.7146x - 3.6156$	0.62	7.6	303	$y = 1.9800x - 3.8567$	0.72	6.9	218

For the first 70 DAT, it appeared that the leaching of residual mineral N prior to each crop influenced the relationship between applied [NO_3^-] and soil solution [NO_3^-]. Compared to the entire crop relationships (Figure 5a; Table 4), for the equivalent data after 70 DAT (n = 218) there was an appreciable difference in the slope of the linear relationship between soil solution [NO_3^-] and nutrient solution [NO_3^-] and in the R^2 values (Figure 4b; Table 4). For the period after 70 DAT, the slope values increased by approximately 0.3 and the R^2 values by 0.05–0.15 (Table 4). After 70 DAT, the soil solution [NO_3^-] was approximately twice that applied in the nutrient solution.

3.4. Relationship between [NO_3^-] of the 1:2 Soil to Water (v/v) Extract and the Applied Nutrient Solution [NO_3^-]

The [NO_3^-] in the 1:2 soil to water (*v*/*v*) extract was linearly related to the applied nutrient solution [NO_3^-] (Figure 6a,b). The relationship was determined for data sets of the 2016 and 2017 crops, together and separately, for the entire crop (Figure 6a) and for the crop after 70 DAT (Figure 6b), as was done in Section 3.3.

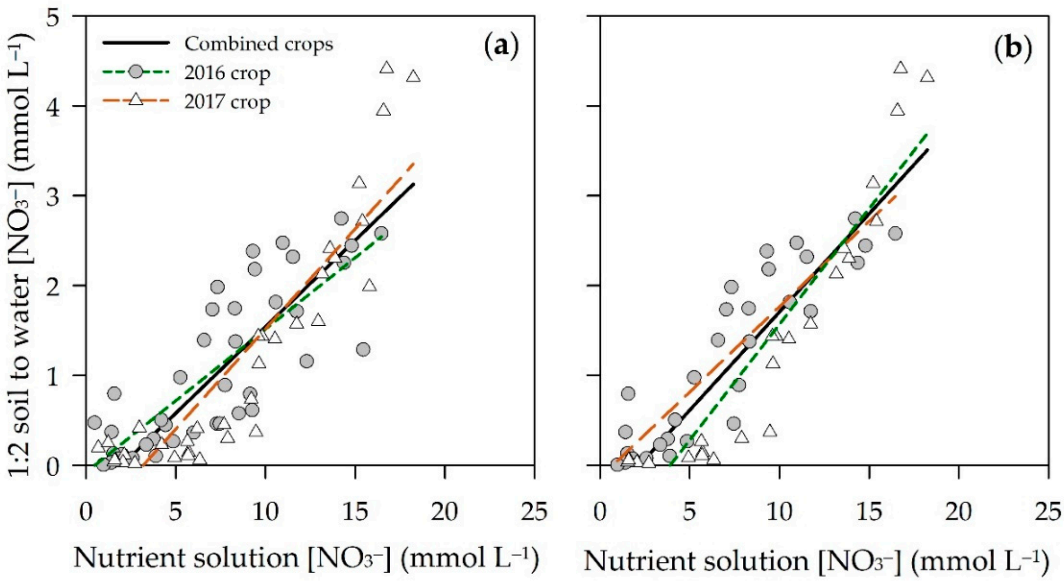

Figure 6. Linear relationship between 1:2 soil to water (*v*/*v*) extract method [NO_3^-] and the applied nutrient solution [NO_3^-] during (**a**) the entire crop, considering the entire crops and the (**b**) crops after 70 days after transplanting (DAT). The linear regression of all dataset combining the two pepper crops of the 1:2 soil to water (*v*/*v*) extract method [NO_3^-] is presented as a black solid line, the linear regression for 2016 (short dash) and 2017 (long dash) crops is shown. The equations of the linear regressions are presented in Table 5.

Using the combined data from the two entire pepper crops, the relationship was $y = 0.1923x - 0.3819$, with an R^2 value of 0.74 (Figure 6a; Table 5). For each of the entire 2016 and 2017 crops, the linear relationship had R^2 values of 0.66 and 0.82, respectively (Figure 6a; Table 5). For the two entire crops considered individually, the slope and intercept values were generally similar.

Table 5. Linear relationship relating 1:2 soil to water (*v*/*v*) extract method [NO_3^-] to the applied nutrient solution [NO_3^-] for sweet pepper crops, considering the entire crops and crops after 70 days after transplanting (DAT). The equation, coefficients of determination (R^2), standard error (±SE), and the number of data points (*n*) are presented.

Crop Year	Entire Crop	R^2	±SE	*n*	Crop after 70 DAT	R^2	±SE	*n*
2016	$y = 0.1591x - 0.0773$	0.66	0.5	40	$y = 0.1899x - 0.1338$	0.82	0.4	30
2017	$y = 0.2226x - 0.7094$	0.82	0.6	35	$y = 0.2576x - 1.0090$	0.87	0.5	25
Combined crops	$y = 0.1923x - 0.3819$	0.74	0.6	75	$y = 0.2185x - 0.4792$	0.82	0.5	55

After 70 DAT, the relationship between extract [NO_3^-] and applied [NO_3^-] was described by $y = 0.2185x - 0.4792$, with an improved R^2 value of 0.82. Compared to linear regressions for the entire crop, the slope values increased by approximately 0.03 mmol L^{-1} and the R^2 values by 0.05–0.16 (Table 5). After 70 DAT, the [NO_3^-] in the 1:2 extract was approximately 0.22 of that applied in the nutrient solution.

3.5. Relationship between Soil Solution [NO_3^-] and [NO_3^-] of the 1:2 Soil to Water (v/v) Extract

Combining data from the entire 2016 and 2017 crops, a single linear relationship $y = 0.0824x + 0.2408$ was derived relating the soil solution [NO_3^-] and the [NO_3^-] in the 1:2 soil to water (*v*/*v*) extract, with an R^2 value of 0.89 (Figure 7; Table 6).

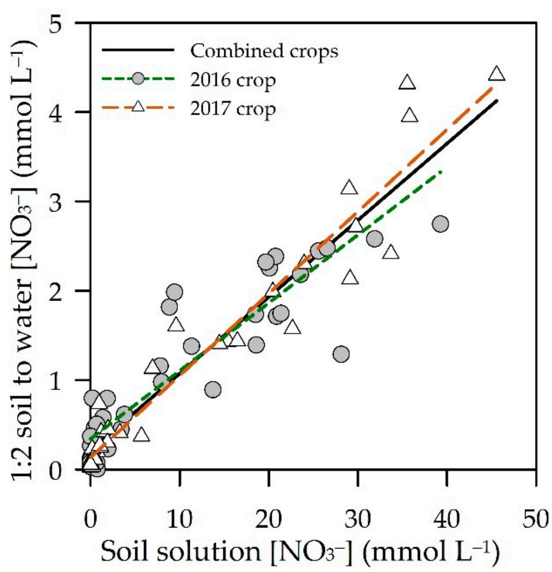

Figure 7. Linear relationship between 1:2 soil to water (*v*/*v*) extract method [NO_3^-] and soil solution [NO_3^-] during the cropping cycle. The linear regression for combining the 2016 and 2017 pepper crops is presented as a black solid line, the linear regression for 2016 (short dash) and 2017 (long dash) crops is shown. The equations of the linear regressions are presented in Table 6.

The linear relationship between the soil solution [NO_3^-] and the [NO_3^-] in the 1:2 soil to water (*v*/*v*) extract derived for each of the 2016 and 2017 crops was similar in terms of slope and intercept (Table 6). In the 2016 and 2017 crops, the R^2 values were 0.84 and 0.93, respectively, indicating strong relationships. These data suggest that the [NO_3^-] in the 1:2 soil to water (*v*/*v*) extract was approximately 0.08 that in the soil solution.

Table 6. Linear regressions analysis relating 1:2 soil to water (*v/v*) extract method [NO_3^-] and soil solution [NO_3^-] for 2016 and 2017 pepper crops and combined all dataset of the two pepper crops. The equation, coefficients of determination (R^2), standard error (±SE), and the number of data points (*n*) are presented.

Crop Year	Entire Crop	R^2	±SE	*n*
2016	$y = 0.0705x + 0.3621$	0.84	0.4	40
2017	$y = 0.0917x + 0.1337$	0.93	0.3	35
Combined crops	$y = 0.0824x + 0.2408$	0.89	0.4	75

3.6. Variability of the Measurements of the Soil Solution [NO_3^-] and [NO_3^-] of the 1:2 Soil to Water (v/v) Extract

The average coefficient of variation (CV) values determined for both [NO_3^-] extraction methods, for the individual crops were similar (Table 7). The average CV values calculated for soil solution [NO_3^-] were 11.4, 13.7, and 18.0% for 2014, 2016, and 2017 crops, respectively. For the [NO_3^-] of the 1:2 soil to water (*v/v*) extract, the average CVs were 17.0 and 11.0% for 2016 and 2017 crops, respectively. For both extraction methods, it is considered that the calculated CVs were relatively low.

Table 7. Coefficient of variation (CV) of the measurements of [NO_3^-] extract method of soil solution and 1:2 soil to water (*v/v*) for each pepper crop.

[NO_3^-] Extract Method	Coefficient of Variation (CV)	
	Average (%)	Range (%)
Soil solution		
2014 crop	11.4	1.6–49.3
2016 crop	13.7	0.2–65.2
2017 crop	18.0	0.1–51.4
1:2 soil to water (v/v)		
2016 crop	17.0	2.7–49.1
2017 crop	11.0	2.1–33.7

4. Discussion

4.1. Relationships of [NO_3^-] in Soil Solution and 1:2 Soil to Water (v/v) Extract, to N Supply and NNI

When assessing the response of the [NO_3^-] of the two soil N monitoring approaches in the present study, the leaching of soil NO_3^- prior to each crop must be considered, because all values for all N treatments were initially close to zero. Thereafter, the general responses of the two approaches, to different [N] applied throughout the crop, were similar. With both approaches, [NO_3^-] of the deficient treatments (N1 and N2) remained close to zero throughout the crops. With the conventional treatment (N3) and the two increasingly excessive treatments (N4 and N5), there were consistent tendencies, with both approaches, to increase during the crops; the degree of increase was positively related to the applied [N]. For the excessive treatments (N4 and N5), the increases occurred immediately. For the conventional treatments (N3), the increase was delayed until approximately 70–100 DAT. There were some fluctuations in the increasing tendencies of soil solution and extract [NO_3^-] in treatments N3–5 during the crop, because of the additional irrigations applied to reduce salinity during the crop.

In treatments N1 and N2, the very low soil solution and extract [NO_3^-] indicated that the crops absorbed all N applied throughout the crops, which was consistent with the deficient crop N status indicated by the corresponding NNI values. In treatment N3, the initial lack of soil NO_3^- accumulation until approximately 70–100 DAT, and subsequent on-going increase, were consistent with the NNI data. The NNI data of treatment 3 indicated a N deficiency until 30–90 DAT; subsequent NNI values were close to 1.0, indicating adequate crop N status. Treatments N4 and N5 accumulated soil NO_3^-

throughout, which was consistent with the corresponding NNI data that indicated consistent slight luxury N uptake.

The general tendencies in soil solution [NO_3^-] observed throughout the crops, in the present study, of no increase with deficient N supply, a slight increase with a sufficient N supply, and a rapid increase with excess N supply, are consistent with other studies in this system [20,21,39]. In these studies, as in the current study, irrigation was well-managed using decision support systems and/or tensiometers, and there was generally little drainage.

Segmented linear analysis effectively described the nature of the relationship between [NO_3^-] and NNI, for both soil solution [NO_3^-] and extract [NO_3^-]. Using combined data from the three entire pepper crops, soil solution [NO_3^-], and NNI were positively related until 3.1 mmol L^{-1} at NNI = 1.0; thereafter, NNI was close to 1.0 (average value of 1.05 mmol L^{-1}) despite the soil solution [NO_3^-] increasing to values of 45.6 mmol L^{-1}. Similarly, for the 1:2 soil to water (*v*/*v*) extract [NO_3^-], there was a positive relationship until 0.93 mmol L^{-1} at NNI = 1.0; thereafter, NNI was close to 1.0 (average value of 1.05 mmol L^{-1}), despite extract [NO_3^-] increasing to 4.4 mmol L^{-1}.

The authors of [20] were unable to strongly relate soil solution [NO_3^-] to NNI for greenhouse-grown melon and tomato. However, they only used simple regression analysis and not segmented linear analysis, as in the current study, and they had relatively small data sets from only one crop per species.

4.2. Sufficiency Values

A sufficiency range of 5.0–15.0 mmol L^{-1} for soil solution [NO_3^-] was proposed for greenhouse pepper crops in SE Spain. This lower value is consistent with the minimum soil solution [NO_3^-] values suggested by other authors. For vegetable crops in California, sufficiency values of 4.0–5.0 mmol L^{-1} were proposed by [40]. Reference [41] proposed 5.0 mmol L^{-1} as a general minimum value, but suggested with frequent N application using combined drip irrigation/fertigation systems that values <5.0 mmol L^{-1} may be sufficient. Similarly, minimum values of <5.0 mmol L^{-1} have been reported that could be used with well-managed, combined drip irrigation/fertigation systems [42]. The minimum value of 5.0 mmol L^{-1}, proposed here is a conservative value. A value of 4.0 mmol L^{-1} probably could be used. However, to avoid risk, 5.0 mmol L^{-1} is the proposed minimum sufficiency value, until further studies support a lower recommended minimum value. The suggested maximum sufficiency value of 15.0 mmol L^{-1} is consistent with values of 12.0–15.0 mmol L^{-1} suggested by [21,42] for greenhouse pepper crops in Almeria.

The present study proposed a sufficiency range of 1.0–2.5 mmol L^{-1} for the [NO_3^-] in the 1:2 extract for greenhouse pepper crops in SE Spain. These values are considerably less than the minimum value 4.5 mmol L^{-1} used in The Netherlands [24,27]. In the Mediterranean Basin, this method has been investigated in Italy [24] and Greece [28]. Minimum sufficiency values for Italy were less than those in The Netherlands [29], but not as low as in the present study. Additional work is required to validate the proposed sufficiency values for this method, obtained in the present study.

Appreciable spatial variability of soil solution [NO_3^-] has been reported [43], and could be a limitation for its use in practical management. Through careful placement and management, and replication, ref. [21] were able to reduce the coefficient of variation (CV) of soil solution [NO_3^-] measurement to 16%. The CVs in the current work of 11.4–18.0% were consistent with those reported by [21]. These CV values suggest that with careful placement and management that the spatial variability of soil solution [NO_3^-] is sufficiently low so as not to limit its use for N management.

The 1:2 soil to water (*v*/*v*) extract method should reduce spatial variability, because of the use of composite soil samples [18]. In the current work, the CVs for this method of 11.0–17.0% were similar to those for soil solution [NO_3^-].

4.3. General Considerations of the Use of the Soil Solution and 1:2 Soil to Water (v/v) Extract Methods

Greenhouse vegetable production is a suitable cropping system for the use of ceramic soil solution suction samplers. Frequent irrigation and N addition ensure constantly moist soil and reduce fluctuations in root zone NO_3^- content. Soil solution [NO_3^-] is an option for monitoring the readily available soil N supply in these systems. However, in other cropping systems, particularly outdoor systems with more infrequent irrigation and N application, it is probably less well suited. For this method to be most effective, considerable care should be taken to ensure that samples are as representative as possible and to minimize spatial variation. Relevant procedures are described in detail by [19,21]. Analysis of [NO_3^-] can be conducted on the farm using rapid analysis systems [19,22].

The variable water content in the soil samples for the extraction of [NO_3^-] in the 1:2 extract is not a limitation for the interpretation of the results. The variation in the water content of the soil did not affect the interpretation of results, for the ranges of soil water content commonly encountered in intensive vegetable production [28]. Practical advantages of the extract method are that it only requires a single visit to the greenhouse for each measurement, and that taking soil samples can be done rapidly.

4.4. General Application of Results

The soil solution [NO_3^-] effectively monitored the readily available soil N supply for greenhouse pepper crops in SE Spain. This method will likely be suitable for other species in this vegetable production system and in greenhouses throughout the Mediterranean Basin. Although, ideally, sufficiency values should be determined for each species and location, it is likely that the sufficiency values obtained in the present work will be applicable to other species in this and similar systems. The 1:2 soil to water (*v*/*v*) extract method is a promising and versatile method; however, more work is required before it can be recommended for use in vegetable production in SE Spanish greenhouses.

5. Conclusions

[NO_3^-] in soil solution and in the extract from the 1:2 soil to water (*v*/*v*) method were both sensitive to excess N application, in greenhouse pepper crops. Excess N application was indicated by increasing tendencies and clear differences between adequate, excessive, and very excessive N supply. Both methods were sensitive to deficient N application, which was apparent as values very close to zero. For both methods, segmented linear regression analysis described the relationship with NNI which was used a measure of crop N status. The results of the segmented linear regression analysis were generally consistent between phenological stages and between crops. A range of sufficiency values, for the entire crop, were determined for each method.

Soil solution [NO_3^-] effectively monitored the supply of readily available soil N for pepper grown in SE Spanish greenhouses. This method and the associated sufficiency range values are likely to be applicable to other vegetable species grown in this system. The 1:2 soil to water (*v*/*v*) extract is a promising method, but requires further assessment in this system.

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

Funding: This research was funded by the SPANISH MINISTRY OF ECONOMY AND COMPETITIVENESS (grant number AGL2015-67076-R) and by ZERAIM GEDERA, an affiliated company in the Syngenta Group of Companies, grant number (University of Almeria contract 401145). A.R. was funded by the UNIVERSITY OF COSTA RICA (UCR contract OAICE-180-2017). F.M.P by a Ramón y Cajal (grant number RYC-2014-15815) from the SPANISH MINISTRY OF ECONOMY AND COMPETITIVENESS.

Acknowledgments: We thank the various people who assisted with the field and laboratory work, and the staff of the Experimental Station of the University of Almeria. We thank Zeraim Gedera for giving us permission to publish data from the 2014 pepper crop.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Pardossi, A.; Tognoni, F.; Incrocci, L. Mediterranean Greenhouse Technology. *Chron. Horticult.* **2004**, *44*, 28–34.
2. Tei, F.; De Neve, S.; de Haan, J.; Kristensen, H.L. Nitrogen management of vegetable crops. *Agric. Water Manag.* **2020**, *240*, 106316. [CrossRef]
3. Thompson, R.B.; Martínez-Gaitan, C.; Gallardo, M.; Giménez, C.; Fernández, M.D. Identification of irrigation and N management practices that contribute to nitrate leaching loss from an intensive vegetable production system by use of a comprehensive survey. *Agric. Water Manag.* **2007**, *89*, 261–274. [CrossRef]
4. Fernández, M.D.; González, A.M.; Carreño, J.; Pérez, C.; Bonachela, S. Analysis of on-farm irrigation performance in Mediterranean greenhouses. *Agric. Water Manag.* **2007**, *89*, 251–260. [CrossRef]
5. Peña-Fleitas, M.T.; Thompson, R.B.; Gallardo, M.; Fernández-Fernández, M.D. Regional model of nitrate leaching for an intensive vegetable production system. In *Nitrogen, Environment and Vegetables, Proceedings of the NEV 2013, Turín, Italy, 15–17 April 2013*; Fontana, E., Grignani, C., Nicola, S., Eds.; Università degli Studi di Torino, 10095 Grugliasco: Turín, Italy, 2013; pp. 73–74.
6. Thompson, R.B.; Padilla, F.M.; Peña-Fleitas, M.T.; Gallardo, M. Reducing nitrate leaching losses from vegetable production in Mediterranean greenhouses. *Acta Hortic.* **2020**, *1268*, 105–117. [CrossRef]
7. Thompson, R.B.; Gallardo, M.; Fernández-Fernández, M.D. Measurement of Nitrate Leaching in Commercial Vegetable Production in SE Spain. In *Nitrogen, Environment and Vegetables, Proceedings of the NEV 2013, Turín, Italy, 15–17 April 2013*; Fontana, E., Grignani, C., Nicola, S., Eds.; Università degli Studi di Torino, 10095 Grugliasco: Turín, Italy, 2013; pp. 67–69.
8. Pulido-Bosch, A.; Rigol-Sanchez, J.P.; Vallejos, A.; Andreu, J.M.; Ceron, J.C.; Molina-Sanchez, L.; Sola, F. Impacts of agricultural irrigation on groundwater salinity. *Environ. Earth Sci.* **2018**, *77*, 1–14. [CrossRef]
9. Valera, L.D.; Belmonte, L.J.; Molina, F.D.; López, A. *Greenhouse Agriculture in Almeria. A Comprehensive Techno-Economic Analysis*; Cajamar Caja Rural: Almeria, Spain, 2016.
10. Junta de Andalucía. Cartografía de Invernaderos en Almería, Granada y Málaga. Año. 2019. Available online: https://www.juntadeandalucia.es/export/drupaljda/Cartografia_inv_AL_GR_MA_180725.pdf (accessed on 13 May 2020).
11. García, M.C.; Céspedes, A.J.; Pérez, J.J.; Lorenzo, P. *El Sistema de la Producción Hortícola Protegido de la Provincia de Almería*; IFAPA: Almeria, España, 2016.
12. BOJA. Orden de 7 de Julio de 2009, por el que se Designan las Zonas Vulnerables y se Establecen Medidas Contra la Contaminación por Nitratos de Origen Agrario. Boletín Oficial de la Junta de Andalucía. N°157, 48–51. Año. 2009. Available online: http://www.juntadeandalucia.es/agriculturaypesca/cocow/archivos/orden_7_julio_2009_modificacion_decreto_36_2008.pdf (accessed on 22 October 2020).
13. Anonymous. Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources. *Off. J. Eur. Commun.* **1991**, *L375*, 1–8.
14. Domínguez, P. Estado Actual de los Acuíferos del Sur de la Sierra de Gádor-Campo de Dalías. Instituto Geológico y Minero de España. Ministerio de Economía y Competitividad. Año. 2014. Available online: http://info.igme.es/ConsultaSID/presentacion.asp?Id=166757 (accessed on 21 May 2019).
15. BOJA. Orden de 1 de Junio de 2015, por la que se Aprueba el Programa de Actuación Aplicable en las Zonas Vulnerables a la Contaminación por Nitratos de Fuentes Agrarias Designadas en Andalucía, Boletín Oficial de la Junta de Andalucía. N°111. Año. 2015. Available online: https://www.juntadeandalucia.es/boja/2015/111/index.html (accessed on 22 October 2020).
16. Thompson, R.B.; Incrocci, L.; van Ruijven, J.; Massa, D. Reducing contamination of water bodies from European vegetable production systems. *Agric. Water Manag.* **2020**, *240*, 106258. [CrossRef]
17. Thompson, R.B.; Incrocci, L.; Voogt, W.; Pardossi, A.; Magán, J.J. Sustainable irrigation and nitrogen management of fertigated vegetable crops. *Acta Hortic.* **2017**, 363–378. [CrossRef]
18. Thompson, R.B.; Tremblay, N.; Fink, M.; Gallardo, M.; Padilla, F.M. Tools and Strategies for Sustainable Nitrogen Fertilisation of Vegetable Crops. In *Advances in Research on Fertilization Management of Vegetable Crops*; Tei, F., Nicola, S., Benincasa, P., Eds.; Springer: Heidelberg, Germany, 2017; pp. 11–63.
19. Padilla, F.M.; Farneselli, M.; Gianquinto, G.; Tei, F.; Thompson, R.B. Monitoring nitrogen status of vegetable crops and soils for optimal nitrogen management. *Agric. Water Manag.* **2020**, *241*, 106356. [CrossRef]

20. Peña-Fleitas, M.T.; Gallardo, M.; Thompson, R.B.; Farneselli, M.; Padilla, F.M. Assessing crop N status of fertigated vegetable crops using plant and soil monitoring techniques. *Ann. Appl. Biol.* **2015**, *167*, 387–405. [CrossRef] [PubMed]
21. Granados, M.R.; Thompson, R.B.; Fernández, M.D.; Martínez-Gaitán, C.; Gallardo, M. Prescriptive-corrective nitrogen and irrigation management of fertigated and drip-irrigated vegetable crops using modeling and monitoring approaches. *Agric. Water Manag.* **2013**, *119*, 121–134. [CrossRef]
22. Thompson, R.B.; Gallardo, M.; Joya, M.; Segovia, C.; Martínez-Gaitán, C.; Granados, M.R. Evaluation of rapid analysis systems for on-farm nitrate analysis in vegetable cropping. *Spanish J. Agric. Res.* **2009**, *7*, 200–211. [CrossRef]
23. Sonneveld, C.; Van den Ende, J.; De Bes, S.S. Estimating the chemical compositions of soil solutions by obtaining saturation extracts or specific 1:2 by volume extracts. *Plant Soil* **1990**, *122*, 169–175. [CrossRef]
24. Sonneveld, C.; Voogt, W. *Plant Nutrition of Greenhouse Crops*; Springer: Dordrecht, The Netherlands, 2009.
25. Sonneveld, C.; Van den Ende, J. Soil Analysis by Means of a 1:2 Volume Extract. *Plant Soil* **1971**, 505–516. [CrossRef]
26. Gysi, C. A simple procedure to express results of soil analysis (1:2 volume extract) on a volume basis. *Plant Soil.* **1981**, *63*, 523–526. [CrossRef]
27. Incrocci, L.; Massa, D.; Pardossi, A. New trends in the fertigation management of irrigated vegetable crops. *Horticulturae* **2017**, *3*, 37. [CrossRef]
28. De Kreij, C.; Kavvadias, V.; Assimakopoulou, A.; Paraskevopoulos, A. Development of Fertigation for Trickle Irrigated Vegetables under Mediterranean Conditions. *Int. J. Veg. Sci.* **2007**, *13*, 81–99. [CrossRef]
29. Incrocci, L.; University of Pisa, Pisa, Italy. Personal communication, 2020.
30. Lemaire, G.; Jeuffroy, M.H.; Gastal, F. Diagnosis tool for plant and crop N status in vegetative stage. Theory and practices for crop N management. *Eur. J. Agron.* **2008**, *28*, 614–624. [CrossRef]
31. Lemaire, G.; Gastal, F. N uptake and distribution in plant canopies. In *Diagnosis of Nitrogen Status in Crops*; Lemaire, G., Ed.; Springer: Berlin, Germany, 1997; pp. 3–41.
32. Greenwood, D.J.; Lemaire, G.; Gosse, G.; Cruz, P.; Draycott, A.; Neeteson, J.J. Decline in Percentage N of C3 and C4 Crops with Increasing Plant Mass. *Ann. Bot.* **1990**, *66*, 425–436. [CrossRef]
33. de Souza, R.; Peña-Fleitas, M.T.; Thompson, R.B.; Gallardo, M.; Grasso, R.; Padilla, F.M. The Use of Chlorophyll Meters to Assess Crop N Status and Derivation of Sufficiency Values for Sweet Pepper. *Sensors* **2019**, *19*, 2949. [CrossRef] [PubMed]
34. Padilla, F.M.; de Souza, R.; Peña-Fleitas, M.T.; Gallardo, M.; Giménez, C.; Thompson, R.B. Different responses of various chlorophyll meters to increasing nitrogen supply in sweet pepper. *Front. Plant Sci.* **2018**, *9*, 1–14. [CrossRef] [PubMed]
35. Padilla, F.M.; Peña-Fleitas, M.T.; Gallardo, M.; Thompson, R.B. Determination of sufficiency values of canopy reflectance vegetation indices for maximum growth and yield of cucumber. *Eur. J. Agron.* **2017**, *84*, 1–15. [CrossRef]
36. Gázquez, J.C.; Pérez, C.; Meca, D.E.; Segura, M.D.; Domene, M.A.; De La Cruz, E.; López, J.C.; Buendía, D. Comparative study of tomato production strategies for long-cycle crop in enarenado and for inter-planting in different substrates systems in the Mediterranean area. *Acta Hortic.* **2017**, *1170*, 773–776. [CrossRef]
37. Padilla, F.M.; Peña-Fleitas, M.T.; Gallardo, M.; Thompson, R.B. Evaluation of optical sensor measurements of canopy reflectance and of leaf flavonols and chlorophyll contents to assess crop nitrogen status of muskmelon. *Eur. J. Agron.* **2014**, *58*, 39–52. [CrossRef]
38. Rodríguez, A.; Peña-Fleitas, M.T.; Gallardo, M.; de Souza, R.; Padilla, F.M.; Thompson, R.B. Sweet pepper and nitrogen supply in greenhouse production: Critical nitrogen curve, agronomic responses and risk of nitrogen loss. *Eur. J. Agron.* **2020**, *117*, 126046. [CrossRef]
39. Gallardo, M.; Thompson, R.B.; López-Toral, J.R.; Fernández, M.D.; Granados, R. Effect of Applied N Concentration in a Fertigated Vegetable Crop on Soil Solution Nitrate and Nitrate Leaching Loss. *Acta Hortic.* **2006**, *700*, 221–224. [CrossRef]
40. Hartz, T.K.; Hochmuth, G.J. *Fertility Management of Drip-Irrigated Vegetables*; UC Davis, Vegetable Research and Information Center: Davis, CA, USA, 1991.
41. Burt, C.; O'Connor, K.; Ruehr, T. *Fertigation*; Irrigation Training and Research Center, California Polytechnic State University: San Luis Obisbo, CA, USA, 1995.

42. Magán, J.J.; Gallardo, M.; Fernández, M.D.; García, M.L.; Granados, M.R.; Padilla, F.M.; Thompson, R.B. Showcasing a fertigation management strategy for increasing water and nitrogen use efficiency in soil-grown vegetable crops in the FERTINNOWA project. *Acta Hortic.* **2019**, *1253*, 17–24. [CrossRef]
43. Hartz, T.K. The assessment of soil and crop nutrient status in the development of efficient fertilizer recommendations. *Acta Hortic.* **2003**, *627*, 231–240. [CrossRef]