Title: Sweet pepper and nitrogen supply in greenhouse production: critical nitrogen curve, agronomic responses and risk of nitrogen loss

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Abstract

Intensive vegetable production in soil is often associated with large N losses to the environment. To contribute to improved N management of sweet pepper, this work developed a critical nitrogen curve (CNC). It also developed N recommendations and examined N use efficiency (NUE) and potential NO_3^- leaching loss in relation to increasing total available nitrogen (TAN). TAN is the sum of the soil mineral N at planting, N mineralized from soil organic material, and mineral N fertilizer. Three sweet pepper crops were grown in soil with autumn-winter cropping cycles in greenhouse conditions. Five different N concentrations in the nutrient solution were applied throughout the crop cycle: very N deficient (N1), N deficient (N2), conventional N management (N3), excessive N (N4) and very excessive N (N5). A critical N curve of $\%Nc = 4.71 \times DMP^{-0.22}$ was determined for sweet pepper. Relative yield of the three crops had a strong linear-plateau relationship (R^2 = 0.66) with integrated nitrogen nutrition index (NNI*i*). Maximum yield was associated with an NNI*i* of 0.86. In the three crops, total yield, dry matter production (DMP) and crop N uptake were generally strongly related to increasing TAN. An optimal TAN value (minimum TAN for maximum yield) of 425 kg N ha^{-1} was determined using a linear-plateau regression model. N uptake efficiency ($N_{upt}E$) decreased exponentially with increasing TAN, from almost 0.90 kg kg^{-1} in the N1 treatment to 0.30 kg kg^{-1} in the N5 treatment. The sum of residual mineral N and leached NO_3^-–N was considered to be potential NO_3^- leaching loss. Potential NO_3^- leaching loss increased exponentially, with increasing TAN, to 686–1034 kg N ha^{-1} in the highest N treatments. For the optimal TAN value, $N_{upt}E$ was 0.63 kg kg^{-1} and the potential NO_3^- leaching was 125 kg N ha^{-1}. The CNC and derived NNI values provide valuable information for N management of pepper. Consideration of TAN as the crop N supply enables maximize yield with less fertilizer N and less risk of N loss.

Keywords:

Capsicum annuum, crop N status, nitrate leaching, nitrogen nutrition index, NUE, optimal N management, production, yield.

1. Introduction

Large amounts of N mineral fertilizer are applied in intensive vegetable production in greenhouses, and are commonly associated with appreciable N loss and consequent negative environmental impacts (Thompson et al., 2017a). There is increasing legislative and societal pressure to reduce the risk of NO_3^- contamination of natural water bodies from these and other intensive agricultural systems (Thompson et al., 2017a).

In the Mediterranean Basin, there are approximately 170,000 ha of greenhouses and large plastic tunnels (Pardossi et al., 2004). Nearly all of these greenhouses are used for intensive vegetable crop production. The 42,000 ha of plastic greenhouses in the coastal regions of southeast (SE) Spain (Valera et al., 2016) are representative of Mediterranean greenhouses. In SE Spain, depending on prices, sweet pepper occupies either the largest or second-largest area of greenhouse cropping surface (Valera et al., 2016). Ninety percent of vegetable crops in greenhouses in SE Spain are grown in soil, the rest in free-draining soilless systems (García et al., 2016). All soil-grown crops are grown with drip irrigation and fertigation, which occur every 1–4 days (Thompson et al., 2017a, 2017b). Nutrients are generally applied in all irrigations throughout a crop (Thompson et al., 2007).

All of the greenhouse production areas in SE Spain have been designated Nitrate Vulnerable Zones in accordance with the European Union (EU) Nitrates Directive (Anonymous, 1991). Nitrate (NO_3^-) concentrations in underlying aquifers can exceed 200–300 mg NO_3^- L^{-1} (Domínguez, 2014), which is 4–6 times the EU limit (Anonymous, 1991). NO_3^- leaching losses from commercial vegetable production in this system, are often appreciable (Thompson et al., 2013). Research studies have measured NO_3^- leaching losses of 100–200 kg N ha^{-1} from sweet pepper crops grown with commercial N and irrigation management practices (Gallardo et al., 2006; Granados et al., 2007, 2013; Thompson et al., 2013). There is a strong requirement for adoption of improved N management practices to reduce these N losses in response to the applicable regional legislation (Anonymous, 1991; BOJA, 2015) and consumer pressure (Thompson et al., 2017a, 2017b).

Improved knowledge of crop response to N supply will greatly assist in improving N management of sweet pepper. A fundamental tool for the development of improved N management practices is the critical N curve (CNC) (Greenwood et al., 1990; Lemaire and Gastal, 1997). The CNC enables determination of the Nitrogen Nutrition Index (NNI), which is an effective and widely-used indicator of crop N status (Lemaire and Gastal, 1997). The NNI is used to develop optimal N management practices (Lemaire and Gastal, 1997; Lemaire et al., 2008) and to assess methods that monitor crop N status (Tremblay et al., 2011; Peña-Fleitas et al., 2015; Padilla et al., 2016; Thompson et al., 2017a). Specific CNCs have been determined for various crop species such as wheat (Justes et al., 1994), rice (Huang et al., 2018), maize (Yue et al., 2014), potato (Giletto and Echeverría, 2015; Abdallah et al., 2016), tomato (Tei et al., 2002; Padilla et al., 2015) and cucumber (Padilla et al., 2016). A species-specific CNC is a required tool for the development of improved N management practices for intensively managed pepper crops.

Optimal N management of sweet pepper crops requires recommendations of the optimal N supply, which maximizes fruit production with minimal N supply. To be most effective, this should consider the amount of total available N (TAN), that is the combined supply of N mineralized from manure, the mineral N in the root zone at the beginning of the crop and mineral N fertilizer (Soto et al., 2015; Thompson et al., 2017a). In the greenhouse system of SE Spain, manure can make a substantial contribution to the total amount of N suppled to a crop (Thompson et al., 2007; Jadoski et al., 2013), and large amounts of soil mineral N can be present at planting (Granados et al., 2013). Commercial N management practice in this greenhouse system is to apply a standard concentration of N, throughout a crop by fertigation, without considering other sources of N (Thompson et al., 2007). All mineral N fertilizer is applied by fertigation (Thompson et al., 2007). Studies of N balances of vegetable crops in commercial greenhouses in SE Spain, have demonstrated that the total N supply, considering all N sources (manure, soil organic N, soil mineral N, mineral N fertilizer) greatly exceeds crop N uptake (Thompson et al., 2007; Jadoski et al., 2013).

To fully understand crop response to increasing N supply, a range of parameters must be considered. Relevant parameters are total yield, dry matter production (DMP), crop N uptake, residual soil mineral N, N loss, and the various Nitrogen Use Efficiency (NUE) indices. In addition to Nitrogen Use Efficiency (NUE_{Yield} or NUE_{DMP}); (yield or dry matter production per unit of N supplied), the various component indices of N Uptake Efficiency ($N_{upt}E$; N uptake per unit of N supplied), N Utilization Efficiency ($N_{ut}E_{Yield}$ or $N_{ut}E_{DMP}$; yield or dry matter production per unit of N uptake) (Moll et al., 1982; Gastal et al., 2015; Milroy et al., 2019) should also be considered.

The objectives of the present study were to examine the response of sweet pepper to increasing amounts of TAN, in order to (i) determine the CNC, (ii) determine crop response to NNI i.e. to crop N status, (iii) determine the minimum amount of TAN required for maximum yield, (iv) determine the responses of total yield, dry matter production (DMP), crop N uptake, NUE and component NUE indices to increasing TAN, and (v) assess the risk of NO_3^- leaching loss with increasing TAN.

2. Material and methods

2.1. Experimental site

Three sweet pepper (*Capsicum annuum* L. "Melchor") crops were grown in soil in a greenhouse, under similar conditions to those of commercial intensive vegetable production, in southeastern (SE) Spain, at the Experimental Station of the University of Almeria located in Retamar, Almeria, SE Spain (36°51′ N, 2°16′ W and 92 m elevation). The three crops were grown with an autumn-winter cropping cycle in 2014–2015 (2014 crop), 2016–2017 (2016 crop) and 2017–2018 (2017 crop).

The greenhouse had a multi-span structure of galvanized steel with polycarbonate walls and a roof of low-density polyethylene (LDPE) tri-laminated film (200-µm thickness) with transmittance to photosynthetically active radiation (PAR) of approximately 60% (Padilla et al., 2014). The greenhouse had passive ventilation with lateral side panels and flap roof windows, an east-west orientation, with crop rows aligned north-south. The cropping area was 1327 m^2. The greenhouse had an artificial layered "enarenado" soil typical of the region (Bretones, 2003; Thompson et al., 2007; Gázquez et al., 2017) consisting of a 30-cm layer of imported silty loam textured soil placed over the original loam soil and a 10-cm layer of fine gravel (mostly 2- to 5-mm diameter) placed on the imported soil as a mulch (Padilla et al., 2014).

At greenhouse construction in July 2007, before adding the final gravel layer, 200 m^3 ha^{-1} of sheep manure (63% dry matter, 1.7% N content and 0.7 t m^{-3} density) was mixed into the top layer of the imported soil following local practices (Thompson et al., 2007). Above-ground drip irrigation was used for combined irrigation and mineral fertilizer application (i.e. fertigation). Drip tape was arranged in paired lines with 0.8-m spacing between lines within each pair, 1.2-m spacing between adjacent pairs of lines, and 0.5-m spacing between drip emitters within drip lines, giving an emitter density of 2 emitters m^{-2}. The drip emitters had a discharge rate of 3 L h^{-1}. The coefficient of uniformity of the drip system was >95%.

The greenhouse was organized into 24 plots, each measuring 6 m by 6 m; 20 plots were used in this study. Each plot contained three paired lines of drip tape with 12 drip emitters in each line. Sheets of polyethylene film (250-µm thickness) buried to 30-cm depth acted as a hydraulic barrier between plots. Individual plants were positioned 6 cm from and immediately adjacent to each emitter, giving a plant density of 2 plants m^{-2} and 72 plants per replicate plot. The greenhouse was divided longitudinally into northern and southern plots by a 2-m wide path along its east-west axis, with two plots of each N treatment in the northern and southern sectors. There were border areas along the edges of the greenhouse.

2.2. Pepper crops and experimental N treatments

Plants were transplanted as five-week-old seedlings. Dates of transplanting and of the end of each pepper crop are given in Table 1. For the first days after transplanting (DAT), seedlings were irrigated with water (<0.04 mmol N L^{-1}) until the different N treatments commenced at 1, 9, and 10 DAT in 2014, 2016 and 2017 crops, respectively.

In each of the three crops, five experimental treatments of increasing N concentration were applied by fertigation in every irrigation. The different N concentrations were applied as part of complete nutrient solutions, the composition of which ensured that all other macronutrients and micronutrients were not limiting. Before transplanting each crop, a series of large irrigations were applied to leach residual NO_3^- present in the soil, and to homogenize the soil profile between plots. The N treatments had increasing N concentration from very N deficient (N1), N deficient (N2), conventional N management (N3), excessive N (N4) to very excessive N (N5) (Table 1). The total amounts of irrigation and N applied to each treatment are presented in Table 1. Most of the mineral N was applied as NO_3^- (92% of applied), the rest was applied as ammonium (NH_4^+).

Table 1. General description for the three pepper crops and N treatments, including dates of transplanting and end of crop, total irrigation and drainage volumes, soil mineral N at transplanting, N fertigation treatments defined on the basis of N concentration of the applied nutrient solution, total amount of N applied and total available N (TAN) supplied to the crop. Apparent N mineralization, included in the calculation of TAN, was 24.3, 43.2 and 1.7 kg N ha^{-1} in the 2014, 2016 and 2017 crops, respectively.

Crop year	Date of transplanting	Date end of the crop (duration)	N treatment	Irrigation amount (mm)[a]	Drainage (mm)	Mineral N at planting (kg N ha^{-1})	N concentration in nutrient solution (mmol L^{-1})[b]	Total N applied (kg N ha^{-1})[a]	TAN (kg N ha^{-1})	N leached (kg N ha^{-1})
2014	12/08/2014	29/01/2015	N1	190	14	16	2.3	64	104	1
		(170 days)	N2	216	18	14	6.0	189	227	4
			N3	294	45	6	12.3	516	547	18
			N4	357	97	14	15.8	804	842	90
			N5	354	113	20	19.6	990	1035	168
2016	19/07/2016	24/03/2017	N1	319	16	87	2.0	88	218	3
		(248 days)	N2	404	20	81	5.3	302	426	4
			N3	414	14	85	9.7	561	689	8
			N4	557	143	74	13.5	1052	1169	165
			N5	532	144	119	17.7	1320	1483	250
2017	21/07/2017	20/02/2018	N1	304	5	34	2.0	86	122	0
		(214 days)	N2	383	5	46	5.7	304	351	1
			N3	383	3	51	9.7	519	571	0
			N4	475	37	49	12.1	870	921	10
			N5	513	56	85	15.7	1198	1284	46

[a] For the complete cropping cycle.

[b] For the period of N treatments, which commenced 1, 9 and 10 days after transplanting in the 2014, 2016 and 2017 crops, respectively.

Irrigation was scheduled to maintain the soil matric potential in the root zone, at 15-cm depth, within -10 to -30 kPa; one tensiometer (Irrometer, Co., Riverside, CA, USA) per plot was used to measure soil matric potential. Irrigation was applied every 1–4 days, with irrigation being more frequent during warmer periods, and less frequent during cooler periods. To avoid excessive accumulation of salinity in the soil solution, additional irrigations were made at particular times when considered necessary. In the 2014 crop, additional nutrient solution was applied during 80–103 DAT to treatments N3, N4 and N5, the respective totals of additional volumes were 23, 44 and 45 mm. In the 2016 crop, additional nutrient solution was applied during 66–71, 104–111, and 178–180 DAT for treatments N1 to N5; the respective total additional volumes were 62, 79, 84, 115 and 107 mm. In the 2017 crop, additional irrigation was applied as water only during 72–110 DAT to the N4 and N5 treatments, and during 129–143, and 185–208 DAT to the N3 to N5 treatments; the total volumes applied were 31, 39 and 39 mm, respectively.

The crops were managed following local practices. The crops were physically supported using a system of nylon cords placed horizontally along the side of the crop, a system known locally as “enfajado”. Periodic pruning was conducted, in the early part of each crop cycle, to create a more open canopy to reduce the risk of fungal infection. High temperature within the greenhouse was controlled by white-washing the plastic cladding of the greenhouse with applications of $CaCO_3$ suspension. $CaCO_3$ suspension was applied in three separate applications to each crop; at planting and at 8 and 34 days after transplanting (DAT) in the 2014 crop, at 6 days before transplanting and at 10 and 36 DAT in the 2016 crop, and at 8 days before transplanting and at 12 and 62 DAT in the 2017 crop. The first two applications in the 2014 and 2016 crops were 0.65 kg L^{-1} and in the 2017 crop were 0.50 kg L^{-1}; the third application was 0.20 kg L^{-1} in the 2014 crop, and 0.40 kg L^{-1} in the 2016 and 2017 crops. Following these $CaCO_3$ suspension applications, PAR transmissivity was 15–50%.

2.3. Measurements

2.3.1. Climatic data

Average values for daily minimum, mean and maximum air temperature and relative humidity, average values for the duration of the crop of the daily integral of solar radiation and daily reference evapotranspiration (ET_0), inside the greenhouse, for the three crops, are presented in Table 2.

Air temperature and relative humidity were measured inside the greenhouse with a relative humidity/temperature probe (Model 41382V, R.M. Young Company, MI, USA) encased in an aspirated protective radiation shield (Model 43502, R.M. Young Company, MI, USA). Solar radiation was measured with a pyranometer (Model SKS 1110, Skye Instruments, Llandrindod Wells, Wales, UK). All data were recorded and stored using a data logger (Model CR10X, Campbell Scientific Inc., Utah, USA).

The climatic conditions during the three pepper crops were similar and were within the normal range of values for autumn-winter crops grown in plastic greenhouses on the Mediterranean coast.

Table 2. For each pepper crop, means values for the cropping period of 24-h minimum, average and maximum air temperature and relative humidity (RH) values, and average values for the duration of the crop of the daily integral of solar radiation and of daily reference evapotranspiration (ET_0), inside the greenhouse.

Crop year	Temperature (°C)			RH (%)			Integral of solar radiation	ET_0
	Minimum	Average	Maximum	Minimum	Average	Maximum	(MJ m^{-2} d^{-1})	(mm d^{-1})[a]
2014	14.4	19.2	26.3	45.6	73.7	90.9	6.0	1.2
2016	14.1	19.0	26.0	54.4	75.7	89.9	6.1	1.2
2017	13.8	18.9	26.3	49.0	72.2	88.1	6.7	1.3

[a] Calculated using the modified FAO radiation equation of Fernández et al. (2010, 2011).

2.3.2. Soil mineral N

The soil was sampled and analyzed immediately before planting and the end of each crop, for mineral N (NO_3^-–N plus NH_4^+–N). To deal with heterogeneity associated with combined drip irrigation and fertigation, each soil sampling in each plot was made in two associated sampling positions in relation to a representative emitter and plant; the first at 5 cm from the drip emitter and the second mid-way between two paired lines. Soil mineral N was calculated as: (0.65 × position 1) + (0.35 × position 2). Soil was sampled in each position to a depth of 60 cm relative to the surface, in four depth intervals (0–15, 15–30, 30–45, 45–60 cm) in the 2014 crop, and at three depth intervals (0–20, 20–40, 40–60 cm) in the 2016 and 2017 crops. Each depth increment from each sampling position within each replicate location was treated as a separate sample.

Soil mineral N content was determined following extraction with potassium chloride (KCl) solution (40 g moist soil: 200 mL 2 mol L^{-1} KCl). NO_3^- and NH_4^+ concentrations in the extracts were determined with an automatic continuous segmented flow analyzer (Model SAN++, Skalar Analytical B.V., Breda, The Netherlands).

2.3.3. Irrigation volume, drainage, and nitrate leaching

Irrigation volume was measured in each treatment with volume meters. Three times per week, two replicate samples of applied nutrient solutions for each treatment were collected from separate drip emitters, to determine the concentration of NO_3^- and NH_4^+ in the applied nutrient solution. Drainage was collected from each treatment using two replicate, free draining, re-packed lysimeters (4 m long × 2 m wide × 0.7 m deep) located in the southern side of each greenhouse, the bottom and walls of the lysimeters were lined with butyl rubber. The soil profile in the lysimeter reproduced that of the outside area described above to a depth of 0.7 m, with a layer of gravel placed between the butyl rubber sheet and the layered soil.

Accumulated lysimeter drainage volumes were measured three times per week; representative sub-samples from each lysimeter were analyzed to measure the NO_3^- concentration using the automatic segmented flow analyzer system described previously; the concentration of NH_4^+ was negligible. NO_3^- leaching was calculated for each lysimeter by multiplying NO_3^- concentration by drainage volume.

2.3.4. Determination of crop dry matter production and N uptake

Above-ground dry matter production (DMP) was measured by sampling approximately every 21 days, by removing one complete and representative plant in each replicate plot. The dry matter content of each biomass component (stem, leaf, and fruit) was determined by oven-drying all the material at 65°C until constant weight. At transplanting, dry matter mass was determined in 100 seedlings. At each pruning during the crop, pruned dry matter mass was determined as described previously, from eight selected plants marked in each replicate plot. The same eight plants were used for all prunings. The final biomass sampling at the end of the crop was conducted by sampling and weighing the eight marked plants, to determine total fresh weight. The percentage of leaf, stem, and fruit was determined in two representative plants from these eight plants.

Representative samples (approximately 20% of fresh weight) of each component (leaf, stem and fruit) from each plant were used to determine the dry matter content by oven-drying at 65°C until constant weight. Dry matter of the whole sample was calculated by multiplying the fresh weight and dry matter percentage of each component and then summing the mass of dry matter of the three biomass components. Total dry matter production (DMP), at each biomass sampling, was calculated as the sum of dry matter mass of leaf, stem and fruit on that sampling date plus all previously sampled pruned material and harvested fruit.

All fresh fruit were harvested periodically from same eight marked plants that were used for collection of pruned material, in each replicate plot. Fresh and dry weights were determined for all fruit harvested from each plot. Once harvests commenced, they were generally conducted every 7–14 days. In the 2014, 2016 and 2017 crops, harvests commenced at 98, 101 and 110 DAT, respectively, and there were, respectively seven, sixteen and eleven harvests. Total yield at the end of each crop was calculated as the cumulative fruit production of all harvests, including fruit that were not considered suitable for the commercial market because of size and imperfections.

Representative sub-samples of leaves, stems, and fruit from each biomass sampling, and of pruned material and harvested fruit, from each replicate plot, were each ground sequentially in knife and ball mills. Total N content (%N) of each sub-sample was determined using a Dumas-type elemental analyzer system (Model Rapid N, Elementar Analysen systeme GmbH, Hanau, Germany). The mass of N in each relevant component was calculated from the %N of the sub-sample and corresponding dry matter of the sample.

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 including previous pruned material and harvested fruit as was done for the calculation of total DMP. Total crop N content (%N) was calculated, for each replicate, as total crop N uptake divided by total DMP. Harvest index (HI) was calculated as the ratio between dry matter in fruit and total above-ground biomass, and nitrogen harvest index (NHI) was calculated as the ratio between N uptake in fruit and total N uptake.

2.3.5. Determination of the critical N curve (CNC) for sweet pepper

A critical N curve (CNC) that related total crop N content to total crop DMP was calculated using data of the three crops, following the methodology of Greenwood et al. (1990). For each biomass sampling date, an analysis of variance was conducted to determine the treatment with the largest total DMP with the lowest applied N; total crop N content (%N) of the selected treatment was used for the derivation of the CNC. Where the largest total DMP occurred in more than one N treatment, the one with the lowest total crop N content was selected. These points were used to fit a negative power relationship between critical total crop N content (%Nc) and total crop dry matter production (DMP): $\%Nc = a \times DMP^{-b}$, where Nc is the minimum total crop N content (as %N) associated with maximum crop growth (total DMP), coefficient a represents the N concentration in the DMP when total crop DMP was 1 t ha^{-1}, and coefficient b is a statistical parameter governing the slope of the relationship (Greenwood et al., 1990). The curve cannot be applied to dry matter biomass of <1 t ha^{-1} (Justes et al., 1994; Ziadi et al., 2010). This curve was derived from the DMP of the total crop and the N content of total crop DMP; total crop DMP being the sum of the dry matter mass of leaves, stems, and fruits.

2.3.6. Evaluation of the contribution of fruit to the CNC of sweet pepper

To evaluate the contribution of fruit to N dilution in the CNC (in section 3.1.3), two approaches were used. Firstly, a CNC for only vegetative growth was determined for the three pepper crops. This vegetative growth CNC was derived from the DMP and N content of the sum of leaves and stems (vegetative DMP) for the entire crop, excluding DMP from the fruit. The vegetative DMP CNC was determined using the same criteria as for the whole crop CNC that included stems, leaves, and fruit. For each biomass sampling date, an analysis of variance was conducted to determine the treatment with the largest vegetative DMP (of stems and leaves) with the lowest applied N.

The second approach to evaluate the contribution of fruit to the dilution of N in the whole crop CNC was to consider the DMP and N content of the whole crop (stems, leaves and fruit) for two periods, which were before and after the commencement of fruit harvest. This was to examine whether the large contribution of fruit to total crop DMP during the fruit harvest period affected the dilution of the whole crop CNC. Natural logarithm (LN) values of the critical values of DMP and of crop N content of the whole crop (1) for the full duration of the crop, (2) until the commencement of fruit harvest, and (3) after the commencement of fruit harvest, were used.

Linear regression analyses were conducted to examine the relationship of LN of crop N content to LN of crop DMP, for (i) the entire crop cycle, (ii) the period of vegetative growth before harvest, and (iii) the fruit harvest period. Combined data from the three crops were used.

The vegetative growth period preceding the first harvest was from 42–84, 43–83 and 39–101 DAT for the 2014, 2016 and 2017 crops, respectively. The harvest period was from the first fruit harvest to the end of crop, being 98–170, 101–248 and 110–214 DAT the 2014, 2016 and 2017 crops, respectively. The dates referred to here are the dates of the biomass samplings in vegetative and harvest periods.

The precision of the three linear regression equations was evaluated by the root mean square error (RMSE). RMSE was calculated as follows: $RMSE = \sqrt{\frac{\sum_{i=1}^{n}(Yi-Oi)^2}{n}}$, where n is the number of samples, Yi is the estimated value of the relationship, and Oi is the observed value. A value close to zero indicates an excellent fit.

2.3.7. Calculation of nitrogen nutrition index and critical N uptake amount values

The nitrogen nutrition index (NNI) was used as a measure of crop N status. The NNI values for each treatment at each biomass sampling date, were determined as: $NNI = \frac{N_{act}}{N_c}$, where N_{act} is the total nitrogen content measured and N_c is the critical N content corresponding to the amount of shoot dry matter produced (Lemaire and Gastal, 1997).

In addition, NNI values were calculated as an integrated NNI value (NNI*i*) to characterize the treatments in terms of crop N status during the entire growing cycle of the crops. Integrated values were calculated as: $\mathrm{NNI}i = 1/D \times \sum NNIs \times ds$, where D was the total number of days of each pepper crop, $NNIs$ was the NNI value determined at each biomass sampling measurement date, and ds was the interval between two successive biomass samplings (Lemaire and Gastal, 1997; Lemaire et al., 2008).

The CNC for sweet pepper was used to calculate the critical N uptake amount (N_{Cupt}). The N_{Cupt} was calculated using the equation: $N_{Cupt} = 10a \times DMP^{(1-b)}$, where the term $10a$ represents the crop N uptake (kg N ha^{-1}), for crop biomass of 1 t DMP ha^{-1} (Sadras and Lemaire, 2014).

2.3.8. Total Available Nitrogen

In each crop, total available N (TAN) was calculated as the sum of soil mineral N at planting, mineral N applied by fertigation, and N mineralized from applied manure and soil organic matter. Mineralized N was calculated, for each crop cycle using an N balance approach (Feller and Fink, 2002), from the experimental data of treatment N1, where very little N was applied, using the equation: $mineralized\ N = (N\ uptake + N\ leached + N\ residual) - (N\ initial + N\ fertigation)$; where N uptake is the total crop N uptake, N leached is the total NO_3^-–N leached during the crop, and N residual is the residual mineral N at the end of the crop.

For the N balance calculation, it was assumed that gaseous N losses in the N1 treatments were negligible. It was assumed that N mineralized was equal for all treatments in each pepper crop. Using this N balance calculation, N mineralized was determined to be a 24.3, 43.2 and 1.7 kg N ha^{-1} for 2014, 2016 and 2017 crops, respectively. The larger N mineralization during the 2016 crop was likely related to the cultivation of the imported soil to 20 cm depth (after removing the sand mulch) one month prior to transplanting. This was the first and only cultivation of the greenhouse soil, since greenhouse construction in 2007.

2.3.9. Nitrogen use efficiency indices

Nitrogen use efficiency (NUE) was calculated for each treatment in each crop as the ratio between yield (NUE_{Yield}) or DMP (NUE_{DMP}) and total available N (TAN) (Moll et al., 1982; Huggins and Pan, 1993). The components of NUE, namely N uptake efficiency ($N_{upt}E$) and N utilization efficiency ($N_{ut}E$) were calculated following Moll et al. (1982). $N_{upt}E$ was calculated as the ratio between crop N uptake and TAN. N utilization efficiency for total yield ($N_{ut}E_{Yield}$) was calculated as the ratio between total yield and crop N uptake, and N utilization efficiency for biomass ($N_{ut}E_{DMP}$) as the ratio between DMP and crop N uptake (Caviglia et al., 2014).

2.4. Data analysis

The experimental data of the three crops were examined using analysis of variance (ANOVA) after verifying assumptions of normality and equal variance. If the main effects or interactions were significant at $P < 0.05$, the least significant difference (LSD) test was conducted for multiple comparisons of means. The results of the analysis of variance are presented as: no significant difference at $P > 0.05$ (ns), significant at $P < 0.05$ (*), very significant at $P < 0.01$ (**) and highly significant at $P < 0.001$ (***). All statistical procedures were performed with Statistica 13 (TIBCO Software Inc., Palo Alto, CA, USA).

The response of total yield to TAN and NNI*i* for the 2014, 2016 and 2017 crops was examined using the linear-plateau regression model. The linear-plateau regression model was defined by the equation: $Y = a + b\mathrm{N}$, if $\mathrm{N} < \mathrm{N}_0$, and $Y = P$, if $\mathrm{N} > \mathrm{N}_0$, where Y was total pepper yield (t ha^{-1}), N was TAN (kg N ha^{-1}), a was the minimum yield achieved when no N was applied to the crop (intercept), and b was the increase in yield in response to each kg of TAN (kg N ha^{-1}) (the slope); N_0 was the critical value of TAN, which occurred at the intersection of the inclined linear segment and the horizontal segment of the linear-plateau regression (Gianquinto et al., 2011; Li et al., 2015).

To determine the N_0 value of TAN in the soil (kg N ha^{-1}), total yield was examined as either absolute or relative values. N_0 values were determined for each of the three pepper crops using total yield (t ha^{-1}) data. To determine a single N_0 value for the three crops, relative yield (%) values were used; these were calculated as the percentage of the maximum yield of the given treatment in each crop. The linear-plateau regression model was examined using the software program RStudio2 (RStudio, Inc., Boston, MA, USA).

3. Results

3.1. Determination and evaluation of a critical nitrogen dilution curve for sweet pepper

3.1.1. Crop biomass and crop nitrogen content

The range of total crop DMP (leaves + stems + fruit) data, that fitted the statistical criteria for determining the Nc dilution curve for sweet pepper (defined in section 2.3.5), using combined data from the 2014, 2016 and 2017 crops, was 1.0 to 15.8 t ha^{-1}. The corresponding range for the vegetative DMP (leaves + stems, excluding fruit, see section 2.3.6) for the three crops was 1.0 to 8.2 t ha^{-1}. Crop N content decreased during the growing season and with increasing shoot biomass, from 5.0 to 2.6 %N for total crop DMP (leaves + stems + fruit) and from 5.0 to 2.8 %N for vegetative DMP (leaves + stems).

3.1.2. Critical N dilution curve for sweet pepper

Generally, throughout the 2014, 2016 and 2017 crops, for each biomass sampling, the N3 treatment was associated with the lowest crop N content required for maximum crop growth (Supplementary Table 1). Combining the data of the total crop critical N content and associated total crop DMP for each biomass sampling of the three crops, the following total crop CNC was obtained for sweet pepper: $\%Nc = 4.71 \times DMP^{-0.22}$ (R^2 = 0.94) (equation 1), where $\%Nc$ is the critical crop N content, 4.71 is the total crop N content (%N) for total DMP of 1 t ha^{-1}, and -0.22 is the value of a dimensionless parameter that describes the slope of the relationship with which %N declines with increasing DMP, the dilution coefficient.

This total crop CNC for sweet pepper has a notably lower N dilution with increasing biomass compared to the general CNC for C3 crops ($\%Nc = 4.80 \times DMP^{-0.32}$) of Lemaire and Gastal. (1997), (Fig. 1a).

3.1.3. Assessment of N dilution in sweet pepper CNC

To examine if fruit production contributed to the lesser dilution of the total crop sweet pepper CNC compared to the general CNC for C3 crops of Lemaire and Gastal. (1997), an additional CNC for vegetative DMP of sweet pepper was derived. The CNC for vegetative DMP, of the three crops, only used leaves and stems (i.e. fruit DMP was excluded) for the duration of the crops.

The CNC for vegetative DMP was $\%Nc = 4.82 \times DMP^{-0.26}$, ($R^2 = 0.90$) (Fig. 1a). The CNC for vegetative DMP was very similar to the CNC for total crop DMP (Fig. 1a) and different to the general CNC for C3 crops of Lemaire and Gastal. (1997) (Fig. 1a). The dilution coefficient of the vegetative DMP CNC was 0.26 compared to 0.22 of the CNC for total crop DMP determined for sweet pepper, and to the value of 0.32 of Lemaire and Gastal. (1997).

The possible contribution of fruit production to the relatively limited N dilution of the total crop CNC (based on total DMP) for sweet pepper was further examined by plotting the natural logarithm of the critical N content (of the total crop) against the natural logarithm of total DMP (of the total crop) for (i) the entire duration of the crop, (ii) the vegetative growth period (i.e. prior to the first harvest), and (iii) the harvest period (when fruit were harvested), for the three crops considered together (Fig. 1b). For the duration of the entire crop, the vegetative growth period, and the harvest period, these three relationships were all described by negative linear regressions (Fig. 1b). The linear regression for the duration of the entire crop had a slope of -0.22 and an RMSE of 1.13, while that for vegetative growth period had a slope of -0.24 and an RMSE of 0.82, and that for harvest period had a slope of -0.16 and an RMSE of 1.26 (Fig. 1b). The similarity of the slopes and of the RMSE values indicate that there would be no clear advantage from separate CNCs for vegetative growth and harvest periods, compared to a single whole crop CNC, for assessing crop N status of sweet pepper.

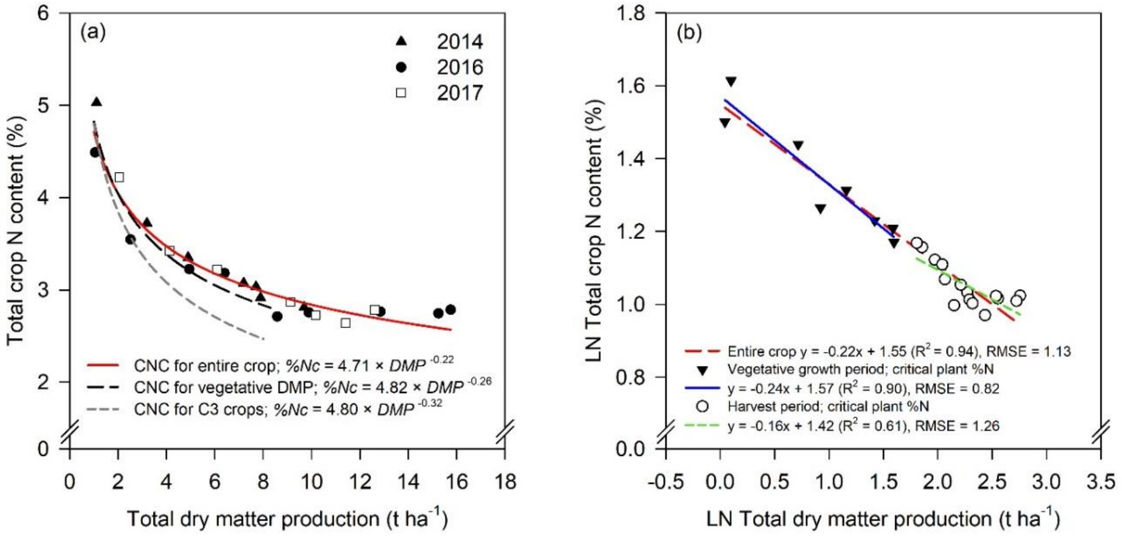

Fig 1. (a) Critical N curve for pepper using (i) total crop N content and total DMP (red), (ii) vegetative DMP and vegetative crop N content (black broken line) from the 2014, 2016 and 2017 crops and (iii) CNC for C3 crops of Lemaire and Gastal, (1997) (gray broken line), and (b) relationship between the natural logarithm (LN) of total crop N content and the LN of total DMP for (i) the entire crop cycle (red), (ii) until harvest (blue), and (iii) harvest period (green). The CNC derived for total and vegetative DMP and N content was calculated following the methodology of Greenwood et al. (1990). Each data point is the critical treatment that maximized the production with the lowest amount of N. The values represented are means from four replicate plots. The lines and equations represent the best-fit equations.

The validity of using a single CNC for sweet pepper was verified by comparing NNI values derived from a double CNC with those derived from the single CNC obtained from the data of the three crops. The single CNC was $\%Nc = 4.71 \times DMP^{-0.22}$ (i.e. equation 1), and the double CNC consisted of $\%Nc = 4.82\ \times DMP^{-0.24}$, ($R^2$ = 0.90) (equation 2) for the vegetative growth period; and $\%Nc = 4.13\ \times DMP^{-0.16}$, ($R^2$ = 0.61) (equation 3); for fruit harvest period, as represented in Fig. 1b in an NL-NL form. NNI values from the double CNC were compared to NNI values from the single CNC for (i) the vegetative growth period, until first fruit harvest, (ii) the period after first fruit harvest, and (iii) for the duration of the crop. Equation 2 was used for period (i), and equation 3 for period (ii); for the entire crop, the results of periods (i) and (ii) were combined. The linear regressions comparing NNI values calculated using the double CNC compared to using the single CNC were $y = 1.01x - 0.02$, (R^2 = 0.99) for the vegetative growth period until first harvest; $y = 0.95x + 0.05$, (R^2 = 0.99) after first harvest; and $y = 0.99x + 0.01$, (R^2 = 0.99) for the entire crop (Supplementary Figs. 1a, 1b and 1c).

3.1.4. Nitrogen nutrition index

The NNI values were calculated using Nc values derived from the single CNC for the duration of the crop. In each of the three pepper crops, there were highly significant ($P < 0.001$) differences in integrated nitrogen nutrition index (NNI*i*) values between the N treatments (Table 3). In the three crops, NNI*i* increased with increasing N supply (i.e. TAN) from N1 to N4, and thereafter was relatively constant (Table 3). In the three crops, there were statistically significant differences between treatments N1, N2, N3 and N4, and no significant differences between treatments N4 and N5 (Table 3). Treatment N3 had NNI*i* values that were very close to one in each of the three pepper crops.

Table 3. Integrated nitrogen nutrition index (NNI*i*) values for different N treatments for each of the three sweet pepper crops. Different letters indicate significant differences ($P < 0.05$) between means within each crop year, according to the procedure of least significant difference (LSD). A summary of the analysis of variance is presented as highly significant at $P < 0.001$ (***). The NNI*i* data are means ± SE over all sampling dates, from four replicate plots.

Treatment	Integrated NNI		
	2014 crop	2016 crop	2017 crop
N1	0.56 ± 0.01 a	0.69 ± 0.01 a	0.52 ± 0.01 a
N2	0.77 ± 0.01 b	0.89 ± 0.01 b	0.80 ± 0.01 b
N3	1.01 ± 0.01 c	0.96 ± 0.02 c	1.02 ± 0.02 c
N4	1.05 ± 0.01 d	1.04 ± 0.02 d	1.09 ± 0.01 d
N5	1.04 ± 0.00 d	1.02 ± 0.01 d	1.10 ± 0.02 d
Significance	***	***	***

The relationship between relative yield and NNI*i* was described by a linear-plateau regression model for combined data from the three pepper crops (Fig. 2). The NNI*i* value for maximum relative yield, under non-limiting N conditions, was 0.86 (Fig. 2).

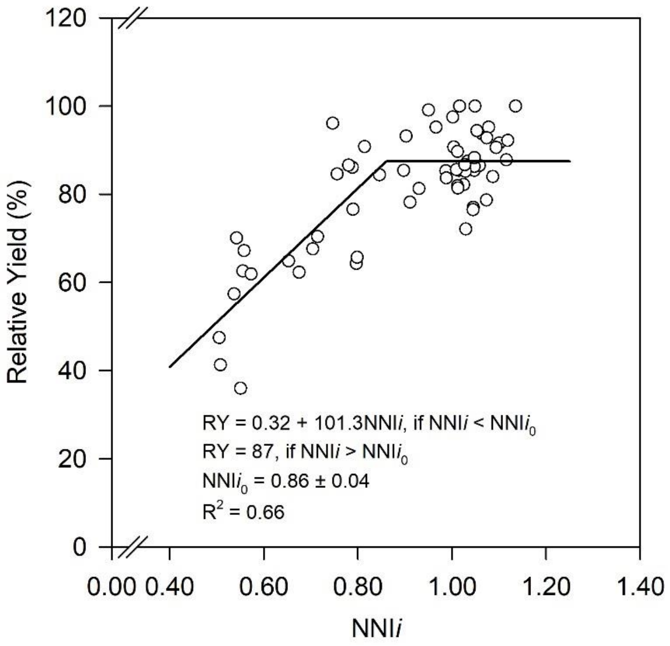

Fig 2. Relationship between relative yield and the integrated nitrogen nutrition index (NNI*i*) of sweet pepper from 2014, 2016 and 2017 crops. The inclined line was described by $Y = a + b\text{NNI}i$ (if $\text{NNI}i < \text{NNI}i_0$), the horizontal line by $Y = P$ (if $\text{NNI}i > \text{NNI}i_0$); *a* is the intercept, *b* is the slope, NNIi_0 is the critical NNI*i* value (the intersection of the inclined and horizontal line). NNI$\underline{i}$ data were the NNI over all sampling dates, from four replicate plots. The line and equation represent the best-fit equation.

3.1.5. Critical N uptake

The relationships between measured crop N uptake and the estimated critical N uptake amount (N_{Cupt}, the minimum amount of crop N uptake for maximum DMP), for the five N treatments in each of the three pepper crops are presented in Fig. 3. Measured crop N uptake and critical N uptake were initially very similar in young plants with crop N uptake of 0−50 kg N ha^{-1}, for all treatments in the three crops (Fig. 3). Thereafter, as the crops grew, the following general tendencies were apparent in each crop: in treatment N1, crop N uptake was appreciably below N_{Cupt}; in treatment N2, crop N uptake was below N_{Cupt}; in treatment N3, crop N uptake was consistently very similar to N_{Cupt}; and in treatments N4 and N5, crop N uptake was generally slightly higher than N_{Cupt}, particularly in the latter parts of the growing seasons. The very small difference between crop N uptake and N_{Cupt} for treatments N4 and N5 indicates that there was only a small amount of luxury N consumption with the highest N supply (i.e. TAN).

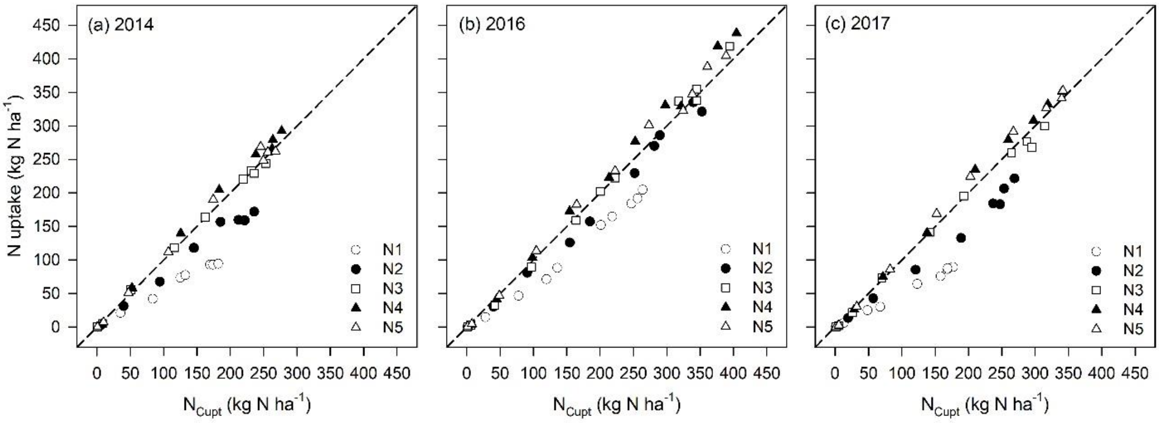

Fig 3. Measured crop N uptake and critical N uptake (N_{Cupt}) calculated for DMP values following the equation $N_{Cupt} = 47.1a \times DMP^{0.78}$ for the five N treatments in the (a) 2014, (b) 2016, and (c) 2017 pepper crops. The values represented are the means over all sampling treatment date. Dashed line represents the 1:1 line.

3.2. Agronomic response

3.2.1. Effect of N treatments on yield, dry matter production, and N uptake

There were highly significant ($P < 0.001$) or very significant ($P < 0.01$) effects of the N treatments on total yield in the 2014, 2016 and 2017 crops (Table 4). Total yield tended to increase with increasing N supply (i.e. TAN). In the 2014 and 2016 crops, treatments N2, N3, N4, and N5 were very similar to each other; treatment N2 had the highest yield with the lowest N supply (as TAN) in both years. For yield, in the 2017 crop, statistically N4 = N5, and N3 = N4, but N5 > N3; treatment N3 had the highest yield with the lowest N supply.

Total dry matter production (DMP) increased with increasing N supply (Table 4). However, there were differences between crops in the DMP response to increasing N. The highest DMP with minimum N supply occurred with treatment N2 in the 2014, treatment N3 in 2016 crop, and with treatment N4 in the 2017 crop. The DMP of treatment N1 was significantly lower than that of the other N treatments, in each crop. There were no significant differences between treatments in Harvest Index (HI) in the three crops; however, there was a tendency for HI to decrease with increasing N application (Table 4).

Total crop N uptake was strongly affected by the N treatments in each of the three pepper crops (Table 4). Crop N uptake increased from N1 to N4 in 2014 and 2017, and increased from N1 to N3 in 2016; thereafter, despite additional N, crop N uptake remained relatively constant or declined. In 2014, the following differences in crop N uptake were significant, N4 > N3 = N5 > N2 > N1. In 2016, the following differences were significant, N5 = N4 = N3 > N2 > N1. In 2017, the following differences were significant, N5 = N4 > N3 > N2 > N1.

In the 2014 crop, there was a clear tendency for Nitrogen Harvest Index (NHI) to decrease with increasing N from N1 to N4, after which it remained constant (Table 4). In the 2016 crop, there was a tendency for NHI to decrease from N1 to N3, after which it remained relatively constant. In the 2014 and 2016 crops, the majority of these differences were statistically significant. In the 2017 crop, the effect of N supply on NHI was not significant.

Table 4. Total yield, total dry matter production (DMP), total crop N uptake, harvest index (HI) and nitrogen harvest index (NHI) for each treatment in the 2014, 2016 and 2017 pepper crops. Different letters indicate significant differences ($P < 0.05$) between means within each crop year, according to the procedure of least significant difference (LSD). A summary of the analysis of variance is presented as: no significant at $P > 0.05$ (ns), significant at $P < 0.05$ (*), very significant at $P < 0.01$ (**) and highly significant at $P < 0.001$ (***). Data are means of four replicate plots.

Crop year/ Treatment	Total yield ($t\ ha^{-1}$)	Total DMP ($t\ ha^{-1}$)	Total crop N uptake ($kg\ N\ ha^{-1}$)	HI	NHI
2014					
N1	38.7 a	5.7 a	95 a	0.64	0.64 a
N2	52.2 b	7.9 b	172 b	0.63	0.59 ab
N3	52.9 b	8.6 bd	244 c	0.63	0.56 bd
N4	51.1 bc	9.7 c	292 d	0.56	0.48 c
N5	46.4 c	9.3 cd	262 c	0.59	0.52 cd
Significance	***	***	***	n.s	**
2016					
N1	67.2 a	8.8 a	192 a	0.53	0.56 a
N2	86.4 b	12.6 b	335 b	0.50	0.50 b
N3	91.5 b	15.2 c	419 c	0.46	0.44 c
N4	94.2 b	14.4 cd	419 c	0.47	0.45 bc
N5	89.7 b	13.6 bd	388 c	0.51	0.45 bc
Significance	**	***	***	n.s	***
2017					
N1	33.3 a	5.1 a	88 a	0.54	0.49
N2	54.4 b	9.3 b	222 b	0.50	0.43
N3	61.0 c	10.5 c	268 c	0.51	0.44
N4	65.1 cd	12.6 d	351 d	0.46	0.41
N5	68.9 d	12.6 d	341 d	0.47	0.44
Significance	***	***	***	n.s	n.s

3.2.2. Total yield response to total available nitrogen (TAN)

In the three pepper crops, total yield and relative yield responded to increasing TAN (Figs. 4a, 4b). The relationships between total yield (expressed in absolute values) and TAN was described by the linear-plateau regression model for the 2014, 2016 and 2017 pepper crops (Fig. 4a). Using relative yield values (expressed as a percentage of maximum total yield), the relationships between relative yield and TAN for the three crops were described by a single relationship using the linear-plateau regression model, with a R^2 value of 0.57 (Fig. 4b).

Using the linear-plateau regression model with absolute yield values, the corresponding values for maximum total yield were 50, 93 and 67 t ha^{-1} for the 2014, 2016 and 2017 crops, respectively. The corresponding minimum TAN values associated with these maximum yield values were 187 kg N ha^{-1}, 492 kg N ha^{-1} and 457 kg N ha^{-1}, respectively (Fig. 4a).

Using the linear-plateau regression model with relative values of total yield and combined data from the three crops, the maximum relative yield value was estimated to be 87%, and the minimum associated TAN value for this yield value was 425 kg N ha^{-1} (Fig. 4b). The R^2 value for fitting the linear-plateau regression model to these data from the three crops was 0.57 (Fig. 4b). The TAN value of 425 kg N ha^{-1} corresponds to TAN values between treatments N2 and N3 in the three crops (Table 1).

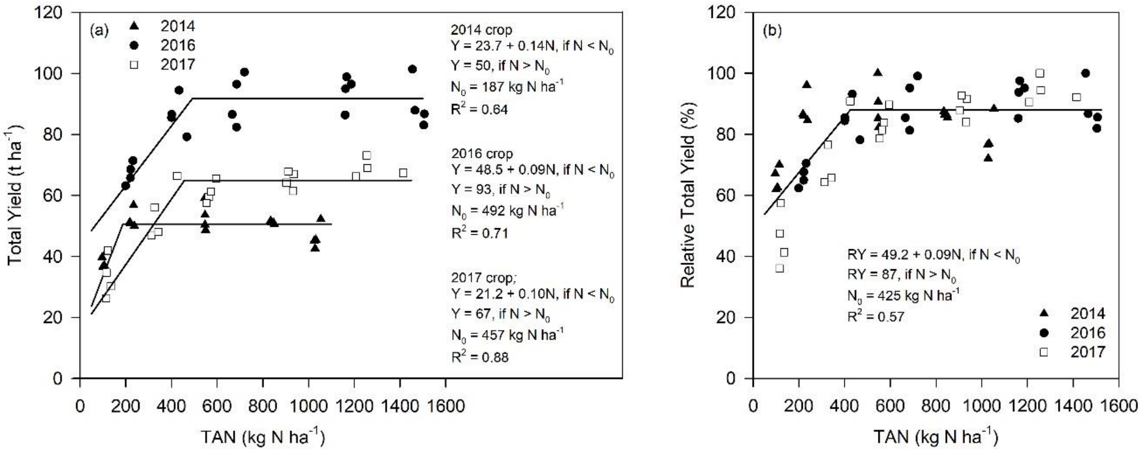

Fig 4. Total yield in response to total available nitrogen (TAN) for 2014, 2016 and 2017 pepper crops applying a linear-plateau regression model with (a) absolute total yield and (b) relative yield values. The inclined line was described by $Y = a + b\mathrm{N}$ (if $\mathrm{N} < \mathrm{N}_0$), the horizontal line by $Y = P$ (if $\mathrm{N} > \mathrm{N}_0$); *a* is the intercept, *b* is the slope, N$_0$ is the critical TAN value (the intersection of the inclined and horizontal lines). The values represented are individual replications. The lines and equations represent the best-fit equations.

The relationship between the total N supply, as TAN, and the means NNI*i* throughout the crop for all N treatments in the three crops is presented in Fig. 5. It was described by an exponential equation with a R^2 value of 0.73. For the NNI*i* value of 0.86 for maximum relative yield (from Section 3.1.4 and Fig. 2), the corresponding TAN value was 463 kg N ha^{-1} (Fig. 5). This optimal TAN value is similar to the TAN value of 425 kg N ha^{-1} obtained from the linear-plateau analysis between relative yield and TAN reported in the previous paragraph (Fig. 4b). For the pepper crops in this study, at NNI*i* values >0.86, the exponential increase in associated TAN values suggested a substantial increase in the risk of applying excess N once optical crop N status has been achieved.

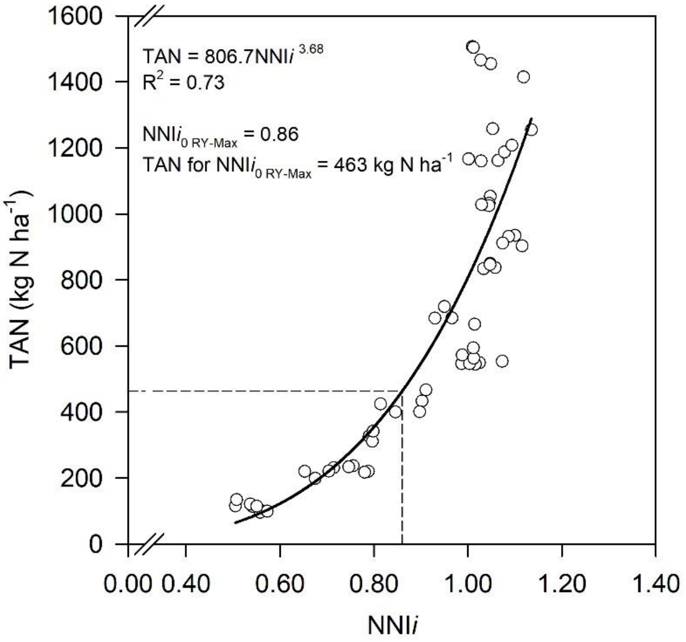

Fig 5. Relationship between total available nitrogen (TAN) and the integrated nitrogen nutrition index (NNI*i*) of pepper from 2014, 2016 and 2017 pepper crops. NNI$i_{0\ RY\text{-}Max}$ is the maximum value of NNI*i* associated with maximum relative yield from Fig. 2. NNI*i* data were the NNI over all sampling dates, from four replicate plots for each crop. The line and equation represent the best-fit equation.

3.2.3. Nitrogen use efficiency

The nitrogen use efficiency (NUE) values for each of the five N treatments of the 2014, 2016 and 2017 pepper crops are presented in Table 5. In the three crops, NUE_{Yield} and NUE_{DMP} decreased appreciably with increasing N supply. NUE_{Yield} values in the 2014, 2016 and 2017 crops decreased from 372 to 45, 308 to 61, and 274 to 54 kg kg^{-1} respectively (Table 5). NUE_{DMP} values decreased from 54 to 9, 40 to 9, and 42 to 10 kg kg^{-1} for the 2014, 2016 and 2017 crops, respectively (Table 5). For both NUE_{Yield} and NUE_{DMP}, there were highly significant differences (P < 0.001) between treatments. For both NUE_{Yield} and $NUE_{DMP,}$ there were generally the following significant differences between treatments: N1 > N2 > N3 > N4 = N5 (Table 5).

Nitrogen utilization efficiency in relation to total yield ($N_{ut}E_{Yield}$) and to DMP ($N_{ut}E_{DMP}$) decreased with increasing crop N uptake (Table 5). In the 2014 and 2016 crops, there were the following respective statistical differences between treatments: N1 > N2 > N3 = N4 = N5, and N1 > N2 = N3 = N4 = N5. In the 2017 crop, treatments N2 to N5 had generally similar values. A general observation, for the three crops, is that with increasing crop N uptake, $N_{ut}E_{Yield}$ decreased from values of approximately 350−400 kg kg^{-1} to values of approximately 200 kg kg^{-1}, after which $N_{ut}E_{Yield}$ remained relatively constant despite increasing crop N uptake (Table 5, Fig. 6a). The relatively constant $N_{ut}E_{Yield}$ values generally coincided with the N3 treatment in each of the three crops (Table 5). The relationship between $N_{ut}E_{Yield}$ and crop N uptake was described by an inverse linear-plateau regression model (Fig. 6a); the corresponding minimum value of crop N uptake at which the plateau value of $N_{ut}E_{Yield}$ first occurred was 262 kg N ha^{-1} (Fig. 6a).

$N_{ut}E_{DMP}$ values ranged from 60 to 35, 46 to 35, and 58 to 37 kg kg^{-1} in the 2014, 2016 and 2017 crops, respectively (Table 5). As with $N_{ut}E_{Yield}$, the relationship of $N_{ut}E_{DMP}$ with increasing crop N uptake, for the three crops, was described by an inverse linear-plateau regression model (Fig. 6b). The plateau value of $N_{ut}E_{DMP}$ to increasing crop N uptake was 35 kg kg^{-1} (Fig. 6b). The minimum value of crop N uptake at which the plateau value of $N_{ut}E_{DMP}$ occurred was 257 kg N ha^{-1} (Fig. 6b).

N uptake efficiency ($N_{upt}E$) decreased exponentially with increasing TAN, from almost 0.90 kg kg^{-1} in the N1 treatment to approximately 0.25 kg kg^{-1} in the N5 treatment Fig. 7; Table 5). For the minimum TAN value for maximum relative yield of 425 kg N ha^{-1}, the corresponding $N_{upt}E$ value was 0.63 kg kg^{-1} (Fig. 7). The relationship of $N_{upt}E$ to TAN was described by an inverse polynomial equation with a R^2 of 0.80 (Fig. 7).

Table 5. Nitrogen use efficiency for total yield ($\mathrm{NUE}_{\mathrm{Yield}}$) and for dry matter production ($\mathrm{NUE}_{\mathrm{DMP}}$), N uptake efficiency ($\mathrm{N}_{\mathrm{upt}}\mathrm{E}$), and N utilization efficiency for total yield ($\mathrm{N}_{\mathrm{ut}}\mathrm{E}_{\mathrm{Yield}}$) and for dry matter production ($\mathrm{N}_{\mathrm{ut}}\mathrm{E}_{\mathrm{DMP}}$) for the different N treatments during the 2014, 2016 and 2017 pepper crops. Different letters indicate significant differences ($P < 0.05$) between means within each crop year according to the procedure of least significant difference (LSD). A summary of the analysis of variance is presented as highly significant at $P < 0.001$ (***). Data are means from four replicate plots.

Crop year/ Treatment	$\mathrm{NUE}_{\mathrm{Yield}}$ (kg kg^{-1})	$\mathrm{NUE}_{\mathrm{DMP}}$ (kg kg^{-1})	$\mathrm{N}_{\mathrm{upt}}\mathrm{E}$ (kg kg^{-1})	$\mathrm{N}_{\mathrm{ut}}\mathrm{E}_{\mathrm{Yield}}$ (kg kg^{-1})	$\mathrm{N}_{\mathrm{ut}}\mathrm{E}_{\mathrm{DMP}}$ (kg kg^{-1})
2014					
N1	372 a	54.4 a	0.91 a	409 a	59.8 a
N2	230 b	34.6 b	0.76 b	304 b	45.8 b
N3	97 c	15.8 c	0.45 c	217 c	35.4 c
N4	61 d	11.5 cd	0.35 cd	175 c	33.1 c
N5	45 d	9.0 d	0.25 d	177 c	35.4 c
Significance	***	***	***	***	***
2016					
N1	308 a	40.2 a	0.88 a	350 a	45.7 a
N2	203 b	29.6 b	0.79 a	258 b	37.6 b
N3	133 c	22.1 c	0.61 b	218 b	36.4 bd
N4	81 d	12.3 d	0.36 c	225 b	34.3 ce
N5	61 d	9.2 d	0.26 c	231 b	35.0 de
Significance	***	***	***	***	***
2017					
N1	274 a	42.1 a	0.72 a	380 a	58.5 a
N2	155 b	26.6 b	0.63 b	245 b	42.1 b
N3	107 c	18.4 c	0.47 c	227 bc	39.2 bd
N4	71 cd	13.7 d	0.38 d	186 cd	35.9 c
N5	54 d	9.8 d	0.27 e	202 bd	36.9 cd
Significance	***	***	***	***	***

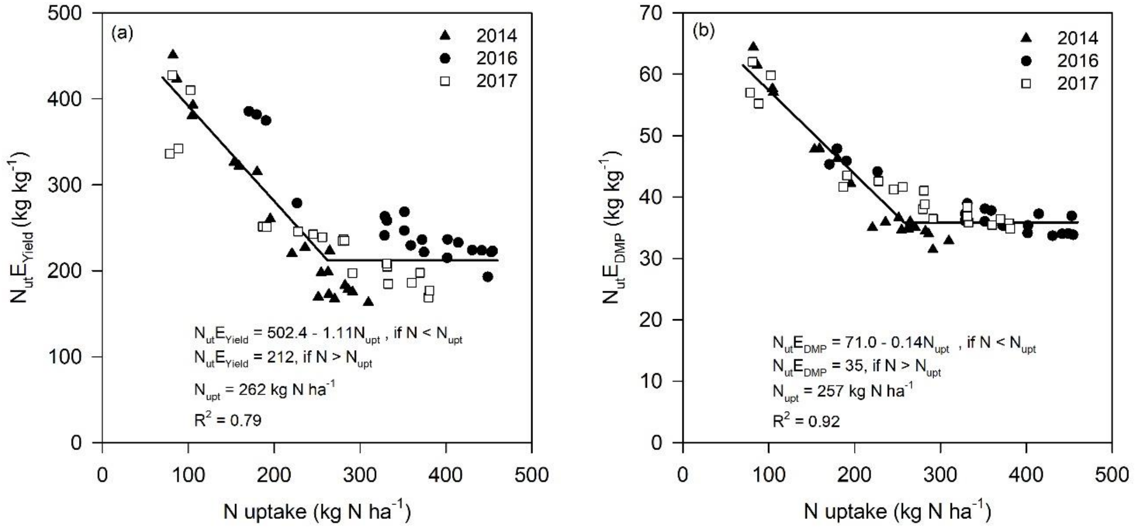

Fig 6. Nitrogen utilization efficiency for (a) yield per unit of N uptake ($\mathrm{N_{ut}E_{Yield}}$), and for (b) dry matter production per unit of N uptake ($\mathrm{N_{ut}E_{DMP}}$), in relation to crop N uptake in the 2014, 2016 and 2017 pepper crops using a combined data set for the 2014, 2016 and 2017 pepper crops. The inclined line was described by $Y = a + b\mathrm{N}$ (if $\mathrm{N} < \mathrm{N_{upt}}$), the horizontal line by $Y = P$ (if $\mathrm{N} > \mathrm{N_{upt}}$); *a* is the intercept, *b* is the slope, $\mathrm{N_{upt}}$ is the critical N uptake value (the intersection of the inclined and horizontal lines). The values represented are individual replications. The lines and equations represent the best-fit equations.

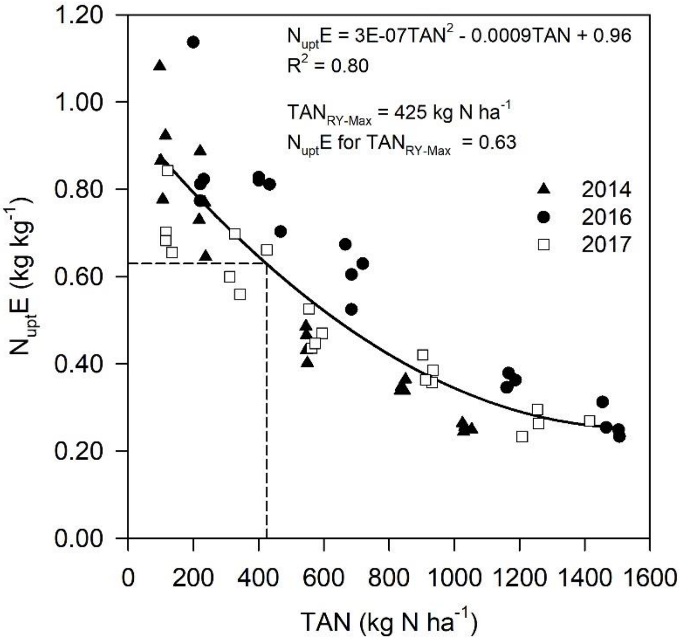

Fig 7. Relationship between N uptake efficiency ($\mathrm{N_{upt}E}$) and total available N (TAN) for the different N treatments in the 2014, 2016 and 2017 pepper crops. $\mathrm{TAN_{RY\text{-}Max}}$ is the maximum amount of TAN associated with maximum relative yield from Fig. 4b. The values represented are individual replications. The line and equation represent the best-fit equation.

3.2.4. Effect of N treatments on N loss and residual soil mineral N

In the three pepper crops, NO_3^- leaching increased with increasing TAN (Table 1), reaching maximum values of 168, 250 and 46 kg N ha^{-1} in the N5 treatment in the 2014, 2016 and 2017 crops, respectively. The maximum amounts of NO_3^- leached in 2017 were lower than in 2016 and 2014 due to lower drainage volumes, which reflected the combined salinity and irrigation management practices used (Table 1).

Residual soil mineral N increased with TAN, the relationship being described by a polynomial equation with a R^2 of 0.90 (Fig. 8a). In the N5 treatments with TAN values of 1035, 1483 and 1284 kg N ha^{-1}, the residual N values were 510, 893 and 713 kg N ha^{-1} for 2014, 2016 and 2017 crops, respectively. For the TAN value of 425 kg N ha^{-1}, which was the minimal TAN value for maximum relative yield (Fig. 4b), the corresponding residual N was 119 kg N ha^{-1} (Fig. 8a).

The relationship between residual soil mineral N and NNI*i* was described by a power equation with a R^2 of 0.60 (Fig. 8b). For optimal crop N status at NNI*i* = 0.86 (Fig. 2), the corresponding residual mineral N was 128 kg N ha^{-1}, which represents a relatively small potential NO_3^- leaching loss.

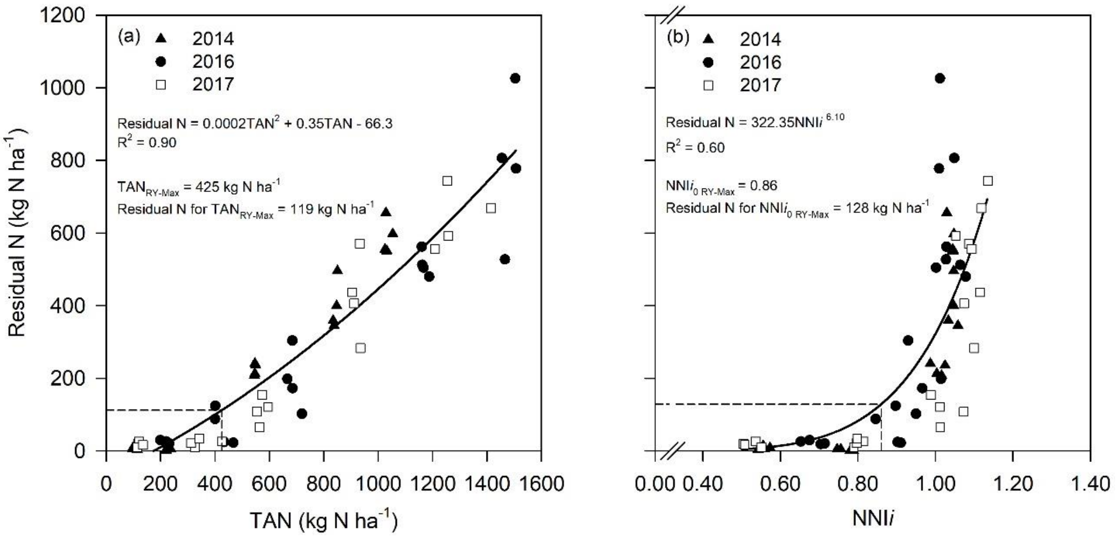

Fig 8. Relationship between residual mineral N in the soil at the end of the 2014, 2016 and 2017 crops, and (a) total available nitrogen (TAN) and (b) integrated nitrogen nutrition index (NNI*i*). $TAN_{RY\text{-}Max}$ is the maximum amount of TAN associated with maximum relative yield from Fig. 4b and $NNI\textit{i}_{0\ RY\text{-}Max}$ is the maximum value of NNI*i* associated with maximum relative yield from Fig. 2. The values represented are individual replications. The lines and equations represent the best-fit equations.

At the TAN value of 425 kg N ha^{-1} for maximum production with minimum N supply, the potential NO_3^- leaching loss (i.e. the sum of the amount of N leached and the residual soil mineral at the end of each crop) was 125 kg N ha^{-1} (Fig. 9). Thereafter with increasing TAN, the potential NO_3^- leaching loss increased exponentially, reaching values of 757, 1034 and 686 kg N ha^{-1} in the 2014, 2016 and 2017 crops, respectively.

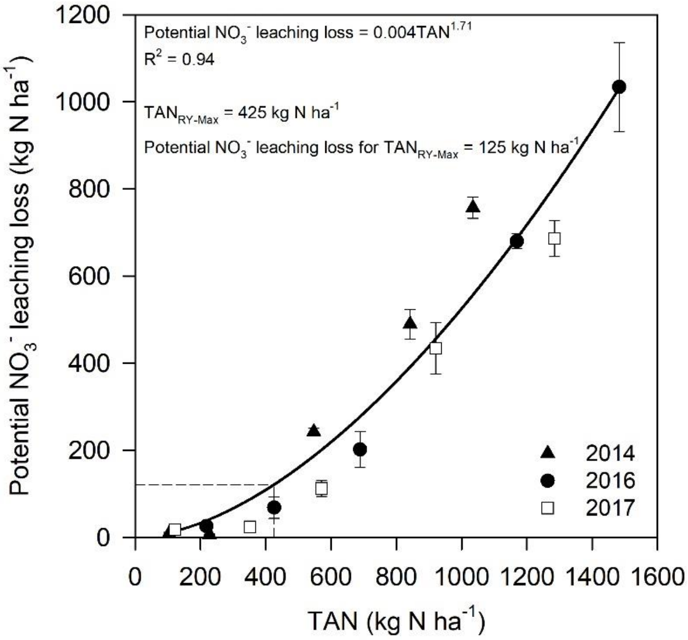

Fig 9. Relationship between potential NO$_3^-$ leaching loss at the end of the 2014, 2016 and 2017 crops, and total available nitrogen (TAN). TAN$_{RY-Max}$ is the maximum amount of TAN associated with maximum relative yield from Fig. 4b. The values represented are means ± SE of four replicate plots. The line and equation represent the best-fit equation.

4. Discussion

4.1. Critical N curve

4.1.1. Critical N dilution in sweet pepper

The critical N curve (CNC) equation of $\%Nc = 4.71 \times DMP^{-0.22}$ determined from the three sweet pepper crops in the present study has a notably lower dilution of N with increasing dry matter production (DMP) (Fig. 1a) than the general CNC for C3 crops of Lemaire and Gastal. (1997). It was demonstrated that the CNC determined for sweet pepper, in the current work, was valid for the duration of the whole crop, including both the vegetative and fruit production phases. Two factors may have contributed to the lower N dilution, of these pepper crops, compared to the general CNC equation for C3 crops of Lemaire and Gastal. (1997). The two factors are: (1) the indeterminate nature of greenhouse-grown sweet pepper crops, and (2) the relatively low planting density. Being indeterminate, sweet pepper crops continually produce new shoot and fruit tissue, simultaneously. This is in marked contrast to determinate cereal crops where (i) shoot growth ceases at flowering, (ii) N is remobilized from ageing shoots to developing grain, and (iii) all grain development is homogenous for a crop. The on-going production of new green photosynthetically active tissue in indeterminate pepper crops results in the maintenance of a relatively high N content throughout the crop. In high-density cereal crops (≥10 plants m^{-2}), there is a rapid reduction in light availability within the canopy, and consequently in photosynthesis, which contributes to a rapid decline in crop N content (Greenwood et al., 1990; Lemaire and Gastal, 1997). In low-density crops such as sweet pepper (2 plants m^{-2}), the greater light availability within the canopy, likely contributes to the relatively limited dilution of crop N content as the crop grows. In other low-density greenhouse-grown vegetable crops, relatively limited dilution was also observed in cucumber (Padilla et al., 2016), but not in tomato (Padilla et al., 2015). Lemaire et al., (2007) and Seginer, (2004) described the effect of low density on N dilution.

4.1.2. NNI*i* – maximum relative yield and luxury N uptake

The linear-plateau analysis of the relationship between relative yield and NNI*i* established a critical NNI*i* value of 0.86 for maximum relative yield. Below this value, relative yield decreased and, above it, there was no additional increase in relative yield. This NNI*i* identified situations of deficient and non-deficient N nutrition; it can be used to evaluate the N status of greenhouse-grown sweet pepper crops in SE Spain and probably throughout the Mediterranean Basin.

For the N3 (conventional N management), N4 (excessive N supply) and the N5 (very excessive N supply) treatments, NNI*i* values were close to one, and were very similar. This indicated that very little luxury N uptake occurred with a high N supply. This was supported by the generally strong relationship between crop N uptake and critical crop N uptake values throughout the three crops (Fig. 3). Under conditions of excessive N supply, NNI values appreciably greater than 1.0 have been reported for crops such as tomato (maximium NNI =

1.30) (Padilla et al., 2015), and in potato (maximum NNI = 1.40-1.53) (Bélanger et al., 2001; Abdallah et al., 2016). However, in other crops, excessive N supply did not result in NNI values appreciably greater than 1.0, for example in cucumber (Padilla et al., 2016).

4.2. Response of sweet pepper to total available N

The highest yields, of up to 90 t ha^{-1}, were obtained in the 2016 crop. For an equivalent N supply, yields were lower in the 2014 and 2017 crops, with respective maximum yields of 53 and 69 t ha^{-1}. The durations of the crops and the transplanting dates were presumably influential contributing factors. The 2014 and 2017 crops were, respectively, 78 and 34 days shorter than the 2016 crop. The transplanting date of the 2014 crop was notably later than that of the 2016 and 2017 crops.

In indeterminate crops such as sweet pepper, the duration of the crop can appreciably affect yield. A comparatively late planting in the summer, as in the 2014 crop, further reduces yield because of reduced growth during the shortened summer-autumn growing period. The yields in the three crops of the present study were within the range of commercial greenhouse production in Almeria, where average marketable yield is 68 t ha^{-1} (MAPAMA, 2019).

4.2.1. Recommended N supply

The minimum TAN for maximum relative yield (i.e. the optimal TAN value) of sweet pepper, determined in this study using linear-plateau regression analysis, was 425 kg N ha^{-1} (Fig. 4). A similar value of 463 kg N ha^{-1} was obtained by relating the optimal NNI*i* value of 0.86 (Fig. 3) to TAN (Fig. 5). Using these results, the suggested minimum total N supply (as TAN) to maximize the production of sweet pepper crops in greenhouse production in Almeria is 430 kg N ha^{-1}.

The greenhouse production areas in Almeria have been declared Nitrate Vulnerable Zones (NVZ; BOJA, 2015) in accordance with the EU Nitrates Directive (Anonymous, 1991). Recommended N supply for greenhouse-grown sweet pepper crops are for mineral fertilizer N

(García-Serrano et al., 2010), and not for TAN. However, these current limits and recommendations for mineral fertilizer N do not consider N supplied by the soil (mineralization of organic N, residual mineral N from previous crops). These additional amounts supplied by soil can be considerable. Where mineral N fertilizer rates follow the recommendations and/or are within the NVZ limit, the additional N supplied by the soil may result in appreciable N loss. To be most effective, N fertilizer recommendations and limits should also consider the soil N supply.

In commercial greenhouse vegetable production in SE Spain, N is generally applied on the basis of concentration in the nutrient solution which is applied in each irrigation (Thompson et al., 2007; 2017b). Consequently, local growers and advisors are more familiar with N concentrations than with quantities or rates of N. For this reason, the suggested optimal TAN value was converted to a value of N concentration, by dividing 430 kg N ha^{-1} by the irrigation volume applied to the N3 treatments (conventional management), with a respective concentration of 8.4 mmol L^{-1}. The optimal N concentrations, considered as the total N supply, were 6.2 mmol L^{-1} for the 2014 crop, 8.7 mmol L^{-1} for the 2016 crop and 8.5 mmol L^{-1} for the 2017 crop. The 2014 value can be considered as being excessively low because of the short and late growing season in 2014. A typical local recommended N concentration for mineral N fertilizer for commercial greenhouse production of pepper in SE Spain is 12 mmol L^{-1} (Fernández and Camacho, 2008).

The N concentration supplied to crops would be higher than this recommended value if the soil N supply was included. Gallardo et al. (2006) reported that applied N concentrations from mineral fertilizer of 7–9 mmol N L^{-1} maintained yield and decreased NO_3^- leaching when compared to conventional management of 10–12 mmol N L^{-1} in soil-grown greenhouse pepper. The results of the present study and of Gallardo et al. (2006) suggest there is appreciable potential to reduce the N concentrations routinely applied to pepper crops in greenhouses in SE Spain, particularly if the soil N supply is considered.

Tools are required to assist vegetable growers to reduce N application with minimal risk of yield reductions. The VegSyst-DSS decision support system prepares site and crop-specific N fertilizer plans for this cropping system and recommends the applied N concentration after considering both soil mineral N at planting and N mineralized from organic material (Gallardo et al., 2014, 2016; Giménez et al., 2019). Using the VegSyst-DSS in combination with petiole sap analysis and soil solution monitoring, the average applied mineral N fertilizer was reduced by 38%, without yield reduction (Magán et al., 2019). Granados et al. (2013) reported that the use of a prescriptive-corrective management system for both N and irrigation, of greenhouse-grown sweet pepper, reduced applied fertilizer N by 35% without affecting yield.

4.2.2. Nitrogen Use Efficiency

In the present study, NUE indices all declined with increasing N supply; similar results for pepper were reported by Van Eerd. (2007), Candido et al. (2009), and Yasuor et al. (2013). The relationships of both $N_{ut}E_{Yield}$ and $N_{ut}E_{DMP}$ with crop N uptake demonstrated that the efficiency of pepper to use absorbed N to produce fruit and dry matter was reduced with increasing crop N uptake, and was constant above crop N uptake values of approximately 260 kg N ha^{-1}. These data demonstrate that reductions in NUE_{Yield} and NUE_{DMP} with increasing N supply are initially influenced by reductions in $N_{upt}E$ and in $N_{ut}E_{Yield}$ or $N_{ut}E_{DMP}$. However, above crop N uptake values of approximately 260 kg N ha^{-1}, the reductions in NUE_{Yield} and NUE_{DMP} are only due to reduction in $N_{upt}E$. The relatively larger contribution of $N_{upt}E$ compared to $N_{ut}E$ in NUE_{Yield} and NUE_{DMP} with increasing N supply was also reported by Caviglia et al. (2014). Generally, $N_{ut}E$, NUE_{Yield} and NUE_{DMP} are calculated only in relation to mineral fertilizer N, and not TAN as in the present study.

In a thorough review of these parameters in potato, Milroy et al. (2019) considered that initial soil mineral N and mineralized N appreciably affected the variability in values of $N_{upt}E$, NUE_{Yield} and NUE_{DMP} reported for mineral N fertilizer. This suggests that using TAN as done in the present study, rather than just fertilizer N for calculating these indices, is likely to appreciably reduce the variability reported for a given species in NUE indices.

4.2.3. Potential for NO_3^- leaching loss to the environment

The potential NO_3^- leaching loss increased exponentially as the N supply exceeded the optimal TAN value. This is consistent with considerable NO_3^- leaching loss from this intensive vegetable production system (Pulido-Bosch, 2005; Peña-Fleitas et al., 2013; Thompson et al., 2013; Domínguez, 2014) and the very excessive N supply in commercial production (Thompson et al., 2007; Jadoski et al., 2013). Reducing the total amounts of N supplied to crops will contribute to a substantial reduction in the NO_3^- leaching loss associated with this system.

The meta-analysis of Quemada et al. (2013) indicated that the combination of adequate consideration of TAN with good irrigation management reduced NO_3^- leaching losses by 40–80% without reducing total yield. Combined improved irrigation and N management have resulted in large reductions in NO_3^- leaching loss compared to conventional management in pepper crops grown in greenhouses in Almeria, without yield reduction (Granados et al., 2013; Magán et al., 2019).

5. Conclusions

A critical N curve of $\%Nc = 4.71 \times DMP^{-0.22}$ was developed for sweet pepper. This CNC has appreciably less N dilution, with increasing DMP, than the general C3 CNC of Lemaire and Gastal, (1997). An NNI*i* value of 0.86 was associated with maximum relative yield; the associated amount of total available N (TAN, i.e. the sum of the soil N at planting, N mineralized from organic material in soil, and mineral N fertilizer) was 463 kg N ha^{-1}. Linear-plateau analysis of relative yield versus TAN suggested that 425 kg N ha^{-1} is the minimum amount of TAN required for maximum yield of sweet pepper crops with an autumn-winter growing cycle, grown in soil, under SE Spain greenhouse conditions. This value was associated with a $N_{upt}E$ of 0.63 kg kg^{-1} and with a potential NO_3^- leaching loss of 125 kg N ha^{-1}. A TAN value of 430 kg N ha^{-1} was recommended for sweet pepper in these conditions. With increasing TAN above the recommended value, $N_{upt}E$ progressively declined and the potential NO_3^- leaching loss increased considerably. The CNC for sweet pepper developed in this work will be very useful for future work developing and evaluating improved N management practices for sweet pepper. The use of TAN rather than just fertilizer N is a more comprehensive approach, which will result in N recommendations that optimize production, reduce N fertilizer use, and reduce N losses to the environment.

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