Root and crop responses of sweet pepper (*Capsicum annuum*) to increasing N fertilization

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Highlights (3 to 5 bullet points)

- Most root length in sweet pepper developed in the sand mulch and 0.10–0.20 m depth
- N deficiency increased root length density in the soil surface layers and reduced yield
- Yield was inversely correlated with root density

Abstract: Rooting is the mechanism by which roots explore soil resources to nourish and anchor the plant to the ground. In vegetable crops, nitrogen (N) application exceeds crop demand due to over fertilization, thereby contributing to N losses through nitrate (NO_3^-) leaching. To improve N fertilization, knowledge of the response of rooting behaviour and root dynamics to N fertilization will be very useful. In this study, the effect of rates of N application on rooting were assessed in two sweet pepper (*Capsicum annuum*) crops grown in an artificially layered soil, with sand mulch, in Almería (south-eastern Spain). The treatments were very deficient, conventional, and very excessive in terms of N application. Yield, crop N absorption and dry matter of the shoot part were determined. Statistically significant differences were found in shoot dry matter between the very deficient N, compared to conventional and very excessive N. Root length density decreased with increasing application of N, with significantly higher density in the very deficient N application. In relation to depth, root length density in the very deficient N was nearly double (in the 2016 crop) and triple (in the 2017 crop) than in conventional N in the sand mulch layer (0–0.10 m depth). In contrast, root length density in the very deficient N treatment was in general lower than in conventional and very excessive N application in the 0.10–0.20 m layer. In the deeper layers, 0.20–0.30 and 0.30–0.40 m, no effects of N treatments on root length density were found. In relative terms, plants subjected to very deficient N treatment allocated relatively more roots in the sand mulch layer and less roots in the 0.10–0.20 m layer than when subjected to conventional and very excessive N. Root length density was negatively correlated with shoot dry matter, crop N absorption, yield and residual soil mineral N at the end of the crops. Overall, results of the present work suggest that conventional and very excessive N application maximized the development of the shoot part and crop yield and diminished root length density, particularly in the sand mulch layer (0–0.10 m depth). A higher root length density was not sufficient under very deficient N in terms of matching dry matter and yield of the conventional N treatment.

Keywords: *Capsicum annuum*, fertilization, nitrogen management, root density, soil layer, vegetable crops.

1. Introduction

Several factors can influence the rooting of a plant, such as species, soil proprieties (physical, chemical and biological characteristics), agricultural practices, climate and competition with neighbouring roots (Herrera et al., 2007; Kristensen and Thorup- Kristensen, 2007; Primavesi, 1982) In nature, the availability of water and nutrients can be very heterogeneous in spatial and temporal terms, so root systems have to face these conditions with morphological and physiological changes (Nacry et al., 2013). Roots are able to adapt to prevailing environmental conditions and have the capacity to exploit localized rich zones or "patches" and respond to them (Hodge, 2010). Roots tend to exploit areas rich in nutrients, water and oxygen, in this way absorption is maximized at a minimum cost, destining most of those assimilated to the development of the aerial part (Gallardo et al., 1996).

It has been long known that the amount and location of soil plant available nitrogen (N) affects root distribution and crop growth (Drew, 1975; Drew et al., 1973; Franco et al., 2011; Herrera et al., 2007). In tomato (*Solanum lycopersicum*), root length distribution and the soil volume explored were larger with lower N compared to higher rates of applied N fertilizer (Lecompte et al., 2008). In wheat (*Triticum aestivum*), high rates of N fertilization inhibited root growth (Comfort et al., 1988). In maize (*Zea mays*), high nitrate (NO_3^-) availability strongly inhibited root growth (Chun et al., 2005). In other herbaceous species, such as *Hordeum vulgare* (Nacry et al., 2013) and *Arabidopsis thaliana* (Zhang and Forde, 2000), high N supply restricted root growth. Increased crop available N increased root length of *Raphanus sativus* in deeper soil (Kristensen and Thorup-Kristensen, 2007). Deep rooting was diminished by the accumulation of N in the surface layers of the soil (Svoboda and Haberle, 2006).

Root diameter is a parameter involved in the processes of absorption of water and N (Kimberly et al., 2009). This is affected by the available N concentration in soil which in turn affects crop nutrient absorption (Gong and Zhao, 2019). Fine roots, i.e. diameter of <0.002 m, are the main route of nutrient absorption from the rhizosphere (Eissenstat, 1992). Smaller diameter roots have a larger specific root surface resulting in larger soil volume being in contact with the root

(McCully, 1999). There is evidence that fine roots are especially sensitive to the N availability but with different responses (Guo et al., 2008; Noguchi et al., 2013).

At the global scale, due to a low efficiency of use of fertilizers, it is urgent to optimize N applications (Tilman et al., 2002). In vegetable crops, N is generally applied in large amounts that exceed crop demand (Gallardo et al., 2006; Thompson et al., 2007). Nitrogen losses by NO_3^- leaching is often considerable in vegetable crops, due to high fertilization rates, shallow root systems and low N recovery (Padilla et al., 2018; Thompson et al., 2007). These problems are common in many regions of the world, for example in the south of Spain (Thompson et al., 2007a), south-eastern United States (Zotarelli et al., 2009), and China (Ju et al., 2006).

It is believed that a root system that explores the deeper horizons of the soil can increase the efficiency of N absorption (Gastal and Lemaire, 2002; King et al., 2003). In this way, the importance of root growth in deeper soil is reaffirmed (Rasmussen et al., 2015). Given the mobility of NO_3^- in the soil, the location of roots may be more advantageous than high root length density to maximize crop N uptake (Herrera et al., 2007). In horticultural crops such as sweet pepper *(Capsicum annuum*), roots are concentrated in the superficial horizons of soils with silty clay loam texture (Castilla, 1986; Martinez, 1987; Padilla et al., 2017a). In lettuce (*Lactuca sativa*) without water and nutrient limitation, roots proliferated in the first 0.20 m of the soil (Gallardo et al., 1996). Deeper rooting has been observed in other studies with vegetable crops. In tomato grown in California, higher root density was found in the upper 0.40 m (Peterson et al., 2016). In muskmelon (*Cucumis melo)* grown in Yangling, China, roots were mostly distributed within the first 0.40 m of soil (Li et al., 2016).

The effects of N application on shoot growth and yield have been extensively studied in vegetable crops (Primavesi, 1982). However, studies focused on rooting patterns, in terms of root length and distribution throughout the soil profile, are scarce (Thorup-Kristensen and Kirkegaard, 2016). Such studies in soil are limited due to the difficulties in sampling roots (Mancuso, 2012). Rooting studies become difficult due to the complexity of the rhizosphere (Ryan et al., 2016). Two methods for studying root distribution and density, and their dynamics,

99 are traditional destructive soil core sampling, and periodical observations using minirhizotron
100 tubes (Hendrick and Pregitzer, 1996; Machado and Oliveira, 2002; Mancuso, 2012).

101 The objective of this work was to evaluate rooting patterns and root dynamics in response to
102 application of increasing doses of N in sweet pepper. There are more than 30,000 ha of
103 greenhouses in the area (CAPDR, 2016). The predominant cultivation system is the
104 "enarenado" soil where more than 90% of the cultivated surface is found under this system
105 (García et al., 2016). This vegetable cropping system is prone to appreciable NO_3^- losses to
106 underlying aquifers (Thompson et al., 2007). More than 8,000 ha are destined for the cultivation
107 of sweet pepper each year, being one of the main crops in the region (Valera et al., 2017). This
108 work aims to generate knowledge on the effect of N on rooting patterns and root dynamics in an
109 important vegetable crop, which will help to improve the management of N in the crop and thus
110 contribute to reducing NO_3^- leaching loss. The information generated in this work can also be
111 worthy to be included into simulation-based decision support systems.

112 ## 2. Material and methods

113 *2.1. Greenhouse crop and experimental design*

114 Two crops were grown in a greenhouse in soil subjected to three N treatments. A combination
115 of destructive root sampling and observations in minirhizotron tubes was used. The research
116 was conducted in Almeria, south-eastern Spain.

117 Two sweet pepper crops (*Capsicum annuum* 'Melchor') were grown in an artificially layered
118 soil known locally as "enarenado" (Thompson et al., 2007b) . The "enarenado" consisted of a
119 series of layers: 0.30 m layer of silty loam texture soil, imported from a building site, placed on
120 the original loam soil, a 0.02 m manure layer of manure placed over the imported silty loam
121 soil, and a 0.10 m layer of coarse-sand or fine gravel (mainly 0.002-0.005 m diameter) placed
122 over the manure layer as mulching (Padilla et al., 2017b).

123 The experimental work was carried out in the Experimental Station of the University of
124 Almería, in Retamar, Almería, SE Spain (36°51′51″N, 2°16′56″W and 92 m elevation). The

125 greenhouse structure consisted of polycarbonate walls and a trilaminate low-density
126 polyethylene (LDPE) film roof (200 μm thickness) with approximately 60% photosynthetically
127 active radiation (PAR) transmittance. It had no heating or artificial light, had passive ventilation
128 (side panels and folding roof windows) with an east-west orientation, with rows of crops aligned
129 from north to south. The cropping area was 1300 m^2. The greenhouse was organized into 24
130 plots, measuring 6 m × 6 m; 12 plots were used in the current study. Each plot contained three
131 paired lines of plants (six lines of plants in total), with 12 plants in each line with a space of 0.5
132 m between them. The separation between the two lines that formed the paired line of plants was
133 0.8 m and the separation between two paired lines was 1.2 m. One plant was placed at 0.06 m
134 and immediately adjacent to each dripper, giving a plant density of two plants m^{-2} and 72 plants
135 per replicated plot. There were border areas along the edges of the greenhouse. Drip irrigation
136 above ground was used for combined irrigation and mineral fertilizers application. The emitters
137 had a discharge rate of 3 L h^{-1}. Irrigation was scheduled to maintain soil matric potential (SMP)
138 in the root zone, at 0.22 m deep from the surface of the sand mulch, within -15 to -25 kPa; one
139 tensiometer (Irrometer, Co., Riverside, CA, USA) per plot was used to measure SMP.

140 Two cultures were used for evaluation, the first one was transplanted on July 19, 2016 with a
141 duration of 248 and the second one was transplanted on July 21, 2017 with a duration of 214
142 days.

143 In each crop, there were three treatments with different N concentrations, N1, N2 and N3. N
144 levels were defined based on local fertilization practices (Camacho and Fernandez, 2013).
145 Based on local practices, the N2 treatment was regarded as conventional. N amount applied
146 throughout the crops was obtained by multiplying N concentration in the nutrient solution by
147 irrigation water volume. There were four replications arranged in random blocks as detailed in
148 Table 1. 88% of N was applied as NO_3^-, the rest as ammonium (NH_4^+) (Table 1). Other
149 macronutrients remained constant in all treatments in the following concentrations: $H_2PO_4^-$, 2
150 mmol L^{-1}; K^+, 4 mmol L^{-1}; Ca^{+2}, 4 mmol L^{-1}; Mg^{+2}, 1.5 mmol L^{-1}; SO_4^{-2}, 2.35 mmol L^{-1}; on average for
151 the two crops (2016-2017). Crops were managed following local practice.

Table 1. Mineral N (NO_3^-–N + NH_4^+–N) in soil at the beginning of each crop, N concentration in the nutrient solution applied, mineral N applied in the nutrient solution. On average across the two crops, 92% of mineral N in soil at the beginning of each crop was in the form of NO_3^-–N, the rest as NH_4^+–N.

Crop	N treatment	Mineral N at planting (kg N ha^{-1})	N in nutrient solution (mmol L^{-1})	N amount applied (kg N ha^{-1})
2016	Very deficient (N1)	87	2.0	88
	Conventional (N2)	85	9.7	561
	Very excessive (N3)	119	17.7	1320
2017	Very deficient (N1)	34	2.0	86
	Conventional (N2)	51	9.7	519
	Very excessive (N3)	85	15.7	1198

2.2. Crop dry matter and crop N uptake

Dry matter was measured by clipping two plants per replicate plot at ground level, with a periodicity of 15 days. Dry matter was determined by dividing and fresh weighing the different organs of the plants and drying to constant weight in stoves at 65 ° C. Total yield was calculated by summing fresh weights of red fruits from each harvest.

The %N of each organ of the aerial part of the plant was determined using a N analyser (Rapid N, Elementar Analysensysteme GmbH, Langenselbold, Germany). The total absorption of N was obtained from %N and dry mass weight of each organ, as in Gallardo et al., (2020). The efficiency in the use of N of total yield was calculated by dividing total yield by crop N uptake.

2.3. Soil mineral N

Soil mineral N (NO_3^-−N + NH_4^+−N) was determined at the beginning and end of each crop. Samples were taken until 0.70 m relative to the surface of the sand mulch, at three depth intervals (0.10–0.30, 0.30–0.50, 0.50–0.70 m), the analysis procedure is detailed in Gallardo et al., (2020).

171 *2.4. Root analyses*

172 Root samples were taken on 31 January 2017 for the first crop and on 15 February 2018 for the
173 second. Soil cores were taken in two positions, at 0.10 m distant to the plant (P1) and at 0.30 m
174 distant to the plant (P2) parallel to the row of plants. Distance to a dripper was of 0.05 m at P1
175 and 0.25 m at P2. Within each position, four sampling depths were taken: sand mulch layer (0–
176 0.10 m), and soil depths of 0.10–0.20, 0.20–0.30 and 0.30–0.40 m. A manual auger with a 0.045
177 m internal diameter was used for the sand layer. For the rest of the soil layers, a 0.03 m internal
178 diameter auger was used. Roots in sand and soil samples were washed with water and stained
179 with a neutral red solution at 0.35 g L^{-1}. The staining solution was prepared with ethanol 70% to
180 preserve roots refrigerated at 4 ºC. Washed roots were scanned at 600 dpi in grey scale, details
181 for scanning can be obtained from Padilla et al., (2017a). The WinRHIZO Reg 2016 program
182 (Regents Instruments Inc., Quebec, Canada) was used for measuring length and diameter of
183 roots. Root length density (m m^{-3}) was computed using the volume of soil sampled in each
184 layer. Relative root length distribution per soil layer was calculated as the root length of a given
185 soil layer divided by root length of all layers.

186 Root length growth dynamics in two soil layers were non-destructively measured using the
187 minirhizotron technique. Two transparent minirhizotron tubes were installed, in each replicated
188 plot, in July 2016 and were left to stabilize during the 2016 crop because the installation of the
189 tubes disturbed the soil. The tubes were 0.60 m long and had 0.064 m internal diameter. In the
190 lower part, tubes were sealed with a waterproof cap; in the upper part, a removable rubber cup
191 prevented the passage of light. The part of tube that protruded above the sand surface was
192 covered with aluminium tape that prevented heating and light penetration into the tube. The
193 tubes were installed at 0.10 m of a plant, to a depth of 0.48 m, relative to the sand mulch layer.
194 Root images were taken by sliding a cylindrical and rotating scanner into the tube (CI-600, CID
195 Inc., Camas, WA, USA); for more details see Padilla et al., (2017a). Two images (0.22 x 0.19
196 m, 300 dpi) were taken per tube, the first one from the surface of the sand mulch layer to 0.22 m
197 depth (0–0.22 m, hereafter), and the second one from 0.22 to 0.44 m depth (0.22–0.44 m,

198 hereafter). The 0–0.22 m image comprised the 0.10 m of the sand mulch layer and the first 0.12
199 m of the imported soil, and the 0.22–0.44 m image comprised the remaining depth of imported
200 soil and some of the original soil. Root images were taken throughout the 2017 crop, every 43
201 days on average. In the first 90 days of the 2017 crop, root images were taken every 26 days. In
202 total, there were 14 root censuses. On each separate image, roots were digitized and analysed for
203 root length per m^2 of soil (WinRHIZO Tron 2019, Regents Instruments Inc.).

204 *2.5. Data analysis and statistics*

205 For the comparative analysis of the aerial part, analysis of variance of repeated measures (RM-
206 ANOVA) over time was used, followed by post hoc least square difference tests. For analysis of
207 root length density between the three N treatments, factorial ANOVA was used, with four
208 factors, block, N treatment, soil layer and sampling position. Differences in root length
209 dynamics were evaluated by RM-ANOVA; factors were block, N, soil layer and time. The
210 Spearman coefficient was used to evaluate the correlation between two variables (whether linear
211 or not). Statistical procedures were performed with STATISTICA 13 (TIBCO Software, Inc.,
212 Palo Alto, CA, USA). Significant differences were established at $P<0.05$.

213 ## 3. Results

214 *3.1. Shoot dry matter*

215 Significant differences in shoot dry matter were recorded between N application treatments
216 (RM-ANOVA N x Time, $p < 0.05$) (Table 2) (Figure 1).

217 **Table 2.** Results of repeated-measure analysis of variance testing the effect of N treatments on shoot dry
218 matter production dynamics of the 2016 and 2017 sweet pepper crops. Significant effects at $p<0.05$ are
219 shown in bold. df are degrees of freedom, F is the Fisher value of ANOVA and p is the probability value.

		2016 crop		**2017 crop**	
Effect	**df**	**F**	**p**	**F**	**p**
Nitrogen (N)	2	287.19	**<0.001**	127.96	**<0.001**
Block	3	0.65	0.151	0.22	0.877
Error	6				
Time (T)	7	234.78	**<0.001**	249.78	**<0.001**
T x N	14	5.47	**<0.001**	17.66	**<0.001**
Error	42				

Shoot dry matter of the very deficient N was significantly lower than in the conventional and excessive treatments. Conventional and excessive N treatments had comparable dry matter in most sampling dates (Figure 1).

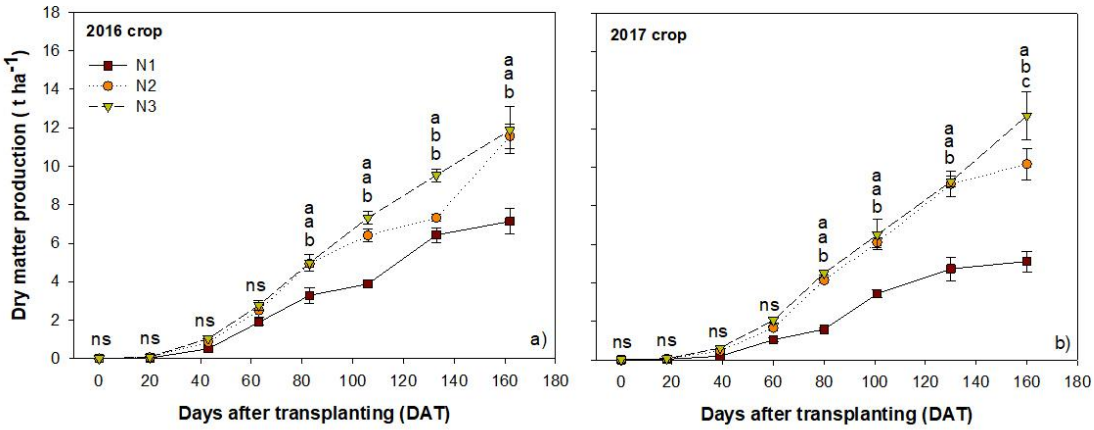

Figure 1. Shoot dry matter evolution for the three N treatments in the 2016 (a) (N1, 2.0 mmol N L^{-1}; N2, 9.7 mmol N L^{-1}; N3, 17.7 mmol N L^{-1}) and 2017 (b) (N1, 2.0 mmol N L^{-1}; N2, 9.7 mmol M L^{-1}; N3, 15.7 mmol N L^{-1}) in two sweet pepper crops. Different lower-case letters above each symbol show significant differences between N treatments for each sampling date, at $p<0.05$. Values are means ± SE.

3.2 Yield and efficiency in the use of N

Regarding the efficiency in the use of N, the very deficient N treatment was the one that had the most efficiency, followed in the order of efficiency by the conventional N treatment and well below the very excessive N treatment. Despite the high efficiency of the very deficient N treatment, total yield was lowest in both years of cultivation (Table 3).

Table 3. Total crop N uptake, total yield and nitrogen use efficiency for total yield (NUE_{Yield}) for each treatment in the 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).

Crop	Treatment	N Uptake (kg N ha^{-1})	Total yield (kg m^{-2})	NUE_{Yield} (T kg N^{-1})
2016	Very deficient (N1)	191 ± 12 **a**	67 ± 2.0 **a**	0.76 **a**
	Conventional (N2)	418 ± 21 **b**	91 ± 4.0 **b**	0.16 **b**
	Very excessive (N3)	388 ± 22 **b**	89 ± 4.0 **b**	0.06 **c**
2017	Very deficient (N1)	87 ± 5 **a**	33 ± 3.0 **a**	0.38 **a**
	Conventional (N2)	268 ± 10 **b**	60 ± 1.0 **b**	0.11 **b**
	Very excessive (N3)	341 ± 22 **c**	68 ± 1.0 **c**	0.05 **b**

3.3. Root length density

Considering the soil profile studied, there were significant differences in root length density between positions P1 and P2 (ANOVA, $p < 0.05$), being higher at P1 (i.e. at 0.10 m from the plant) in both crops (Figure 2). In the 2016 crop, the average root length density for the three treatments at P1 (17,980 m m^{-3}) more than doubled averaged root length density at P2 (8,265 m m^{-3}) (Figure 2a). In this crop, there were not differences between N treatment regardless of the sampling position. In the 2017 crop, the average root length density for the three treatments at P1 (21,008 m m^{-3}) was nearly 11 times higher than at P2 (1,932 m m^{-3}) (Figure 2b). In this crop, there were differences between N treatments at P1 (Table 4). At P1, root length density decreased with N addition, with root length density of the very deficient N treatment (26,271 m m^{-3}) being 1.6 times higher than root length density of the very excessive N treatment (15,604 m m^{-3}) (Figure 2b); conventional N had intermediate root length density values (Figure 2b).

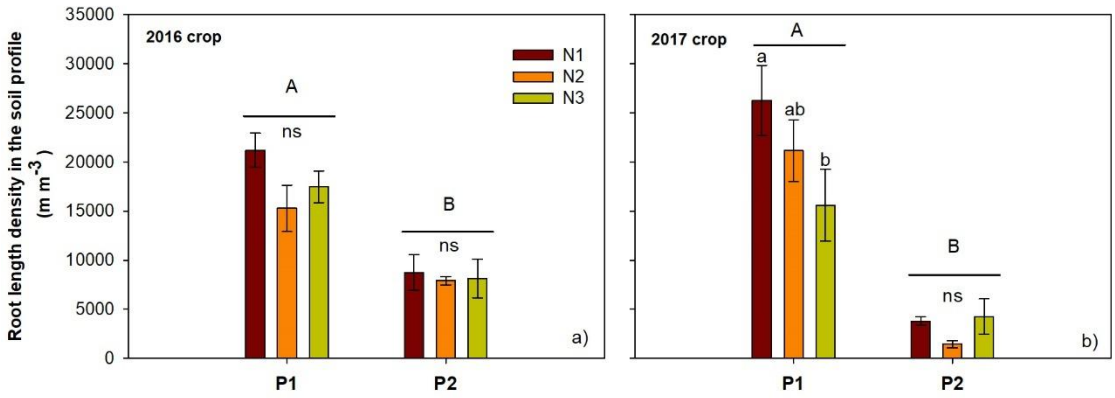

Figure 2. Root length density in the soil profile in the two sampling positions (P1, at 0.10 m distant to the plant, and P2, at 0.30 m distant to the plant, parallel to the row of plants) for the three N treatments. Plot (a) represents the 2016 crop (N1, 2.0 mmol N L^{-1}; N2, 9.7 mmol N L^{-1}; N3, 17.7 mmol N L^{-1}) and plot (b) represents the 2017 crop (N1, 2.0 mmol N L^{-1}; N2, 9.7 mmol N L^{-1}; N3, 15.7 mmol N L^{-1}). Different upper-case letters above horizontal lines show significant differences between sampling position. Different lower-case letters over bars show significant difference between treatments within each sampling position. Values are means ± SE. ns, not significant at $p<0.05$.

Focusing on individual soil layers, there were significant differences in root length density contingent on N treatments (ANOVA N x Layer, $p<0.001$) (Table 4).

Table 4. Results of analysis of variance testing the effect of N treatments, sampling position and soil layer, on root length density of two sweet pepper crops. Significant effects at $p<0.05$ are shown in bold. df are degrees of freedom, F is the Fisher value of ANOVA and p is the probability value.

		Root length density			
		2016 crop		2017 crop	
	df	F	p	F	p
Nitrogen (N)	2	0.3	0.761	1.3	0.265
Position (P)	1	39.5	**<0.001**	72.4	**<0.001**
Layer (L)	3	56.7	**<0.001**	18.2	**<0.001**
N x P	2	0.3	0.768	1.3	0.275
N x L	6	3.4	**<0.001**	4.5	**<0.001**
P x L	3	9	**<0.001**	13.3	**<0.001**
N x P x L	6	2	0.068	5.5	**<0.001**
Block	3	1.1	0.37	0.5	0.685
Error	165				

For both crops, significant differences between N application were found in the sand mulch layer and in the 0.10 – 0.20 m soil layers (Table 4; Figure 3). In the sand mulch layer, the very deficient N treatment had nearly double the root length density of the conventional N treatment in the 2016 crop (19,780 *vs.* 10,013 m m^{-3}; Figure 3a), and nearly triple that of the conventional N treatment in the 2017 crop (27,331 *vs.* 9,352 m m^{-3}; Figure 3b). Root length density of the conventional and very excessive N treatments was statistically comparable in both years. By contrast, in the 0.10 – 0.20 m soil layer, root length density was significantly lowest in the very deficient N treatment in both crops (Figure 3).

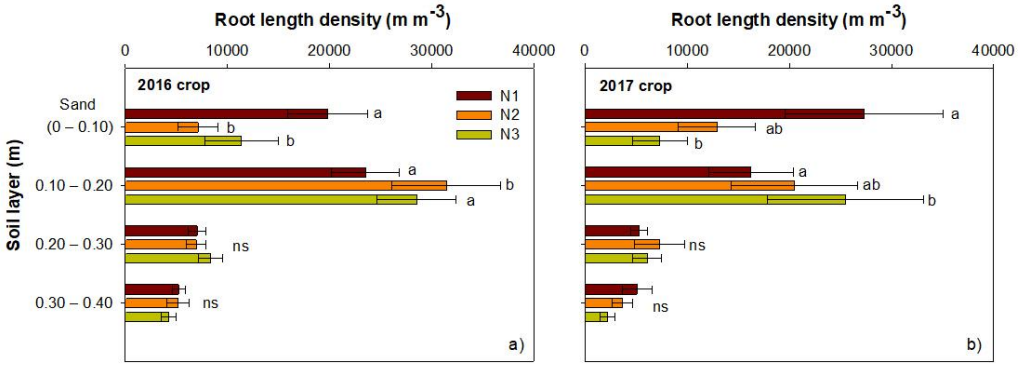

Figure 3. Root length density in each layer for the three N treatments. Panel (a) represents the 2016 crop (N1, 2.0 mmol N L^{-1}; N2, 9.7 mmol N L^{-1}; N3, 17.7 mmol N L^{-1}), and panel (b) represents the 2017 crop (N1, 2.0 mmol N L^{-1}; N2, 9.7 mmol N L^{-1}; N3, 15.7 mmol N L^{-1}). Different lower-case letters within each soil layer show significance difference between N treatments at $p<0.05$. Data have been pooled across P1 (0.10 m distant to the plant) and P2 (0.30 m distant to the plant) positions. Values are means ± SE. ns, not significant at $p>0.05$.

In both crops, there were significant differences in root length density between sampling position (i.e. P1 *vs.* P2) depending on soil layer (ANOVA Position x Layer, $p<0.001$), regardless of N treatment (Table 4). In the sand mulch and 0.10 – 0.20 m soil layers, root length density at P1 was higher than at P2, in both crops, whereas there were not significant differences between sampling positions in the rest of soil layers (i.e. 0.20 – 0.30 m and 0.30 – 0.40 m) (Figure 4).

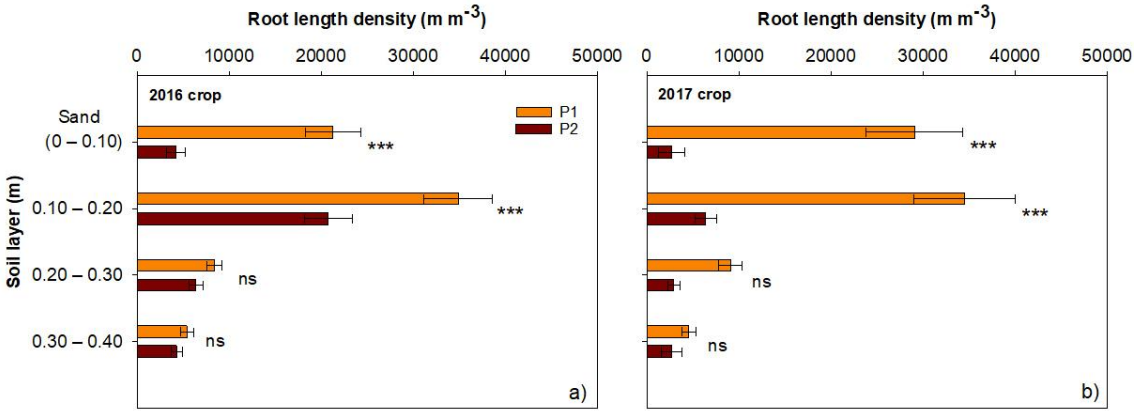

Figure 4. Root length density in the two-sampling positions (P1, at 0.10 m distant to the plant; P2, at 0.30 m distant to the plant parallel to the row of plants) per soil layer. Asterisks within each soil layer show significant differences between sampling positions. Panel (a) represents the 2016 crop (N1, 2.0 mmol N L^{-1}; N2, 9.7 mmol N L^{-1}; N3, 17.7 mmol N L^{-1}) and panel (b) represents the 2017 crop (N1, 2.0 mmol N L^{-1}; N2, 9.7 mmol N L^{-1}; N3, 15.7 mmol N L^{-1}). Data have been pooled across N1, N2 and N3 treatments. Values are means ± SE. ***, $P<0.001$; ns, not significant at $p<0.05$.

Regarding root length density per diameter class, 98% of the root length measured was of fine roots (roots <0.002 m diameter) and the remaining were coarse roots (roots >0.002 m diameter) (data not shown).

3.4. Relative root length distribution

For the relative root length distribution per soil layer, significant interactions were found between N treatments, position and soil layer (Table 5).

Table 5. Results of analysis of variance testing the effect of N treatments, sampling position and soil layer, on relative root length distribution of two sweet pepper crops. Significant effects at p<0.05 are shown in bold. df are degrees of freedom, F is the Fisher value of ANOVA and p is the probability value.

	Root percentage				
		2016 crop		**2017 crop**	
	df	**F**	**p**	**F**	**p**
Nitrogen (N)	2	0	1	0	1
Position (P)	1	0	1	0	1
Layer (L)	3	138.8	**<0.001**	32	**<0.001**
N x P	2	0	1	0	1
N x L	6	6.6	**<0.001**	3.5	**0.003**
P x L	3	13.1	**<0.001**	8.4	**<0.001**
N x P x L	6	2.1	0.053	4.4	**<0.001**
Block	3	0	1	0	1
Error	165				

In the P1 sampling position, averaged for both crops, root length in the sand mulch layer was 33% of total root length, 45% in the 0.10–0.20 m soil layer, and 14 and 7% for the 0.20–0.30 and 0.30–0.40 m soil layers, respectively (Figure 5). In P2 sampling position, the root length in the sand mulch layer was 14% of total root length, 50% in the 0.10–0.20 m soil layer, and 20 and 15% in the 0.20–0.30 and 0.30–0.40 m soil layers, respectively. These data show that relative root distribution in the sand layer notably decreased from the P1 to the P2 sampling positions (Figure 5).

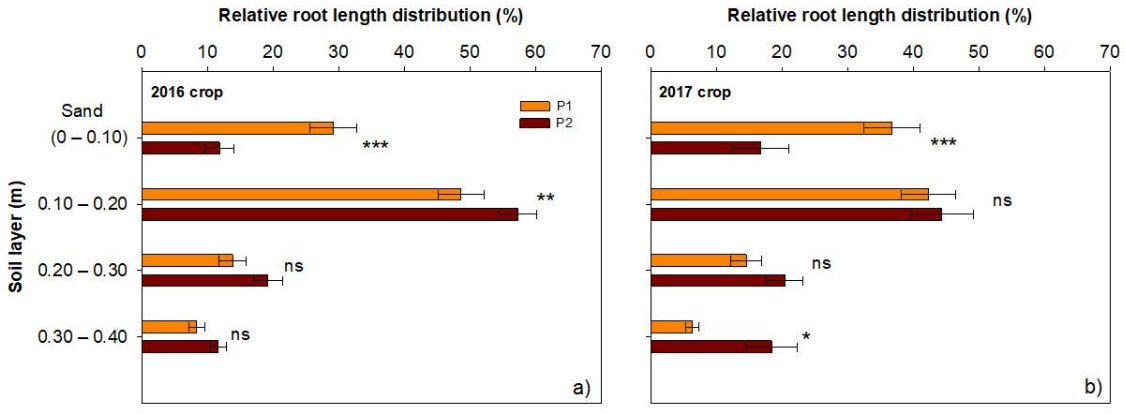

Figure 5. Relative root length distribution per soil layer and sampling position (P1, at 0.10 m distant to the plant; P2, at 0.30 m distant to the plant parallel to the row of plants). Panel (a) represents the 2016 crop and panel (b) the 2017 crop. Asterisks within each soil layer show significant differences between sampling positions. Data have been pooled across N treatments. Values are means ± SE. ***, p<0.001; **, p<0.01; *, p<0.05; ns, not significant at p>0.05.

In terms of relative root length distribution, the very deficient N treatment (N1) had significantly higher root length percentage in the sand mulch layer and 0.10–0.20 m soil layer, than the conventional and very excessive N treatments (Figure 6). These effects were consistent regardless of the year of the crop and sampling position. In contrast, there were generally no significant differences in relative root allocation between N treatments in the 0.20–0.30 and 0.30–0.40 m soil layers (Figure 7).

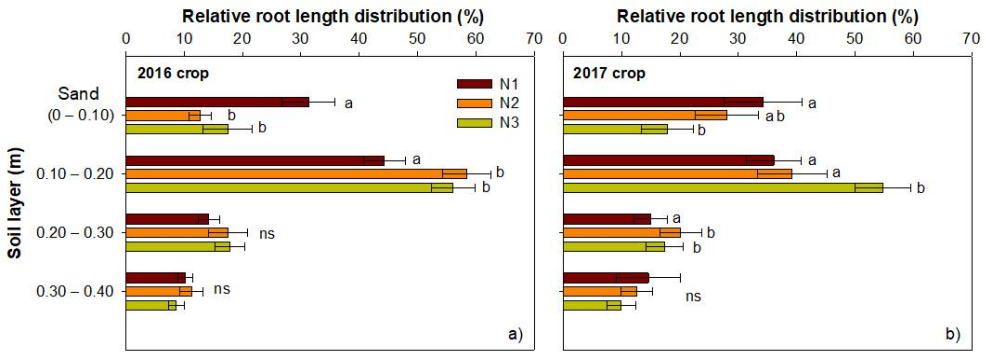

Figure 6. Relative root length distribution per soil layer for the three N treatments. Panel (a) represents the 2016 crop (N1, 2.0 mmol N L^{-1}; N2, 9.7 mmol N L^{-1}; N3, 17.7 mmol N L^{-1}) and panel (b) shows the 2017 crop (N1, 2.0 mmol N L^{-1}; N2, 9.7 mmol N L^{-1}; N3, 15.7 mmol N L^{-1}). Different lower-case letters within each soil layer show significant differences between N treatments at $p<0.05$. Data have been pooled across P1 position (0.10 m distant to the plant) and P2 position (0.30 m distant to the plant). Values are means ± SE. ns, not significant at $p>0.05$.

3.5. Average root diameter

In the two crops, there was a significant effect of sampling position on average root diameter, for all N treatments and soil layers (Table 6). The roots in the P2 sampling position were statistically finer than those in the P1 sampling position. Averaging across the crops and N treatments, roots were 0.03 mm finer in P2 than in P1.

Table 6. Results of analysis of variance testing the effect of N treatments, sampling position and soil layer, on average root diameter of two sweet pepper crops. Significant effects at $p<0.05$ are shown in bold. df are degrees of freedom, F is the Fisher value of ANOVA and p is the probability value.

		Root diameter			
		2016		**2017**	
	df	**F**	**p**	**F**	**p**
Nitrogen (N)	2	3.620	0.029	0.698	0.499

Position (P)	1	7.367	**0.007**	7.354	**0.008**
Layer (L)	3	8.117	**<0.001**	5.441	**<0.001**
N x P	2	3.009	0.052	1.898	0.154
N x L	6	2.998	**0.008**	2.772	**0.014**
P x L	3	1.574	0.198	2.101	0.103
N x P x L	6	0.567	0.756	0.582	0.744
Block	3	5.806	**<0.001**	5.846	**<0.001**
Error	165				

For the two crops, there were significant differences in average root diameter between N treatments depending on soil layer (Table 6). Differences between N treatments were significant in the sand layer in both crops, and in the 0.10–0.20 m soil layer in the 2017 crop. In those soil layers, the tendency was for coarser roots with increasing N application, with finer roots in the very deficient N treatment (N1) (Figure 7). For both years, there were no differences in average root diameter in the 0.20–0.30 and 0.30–0.40 m soil layers (Figure 7).

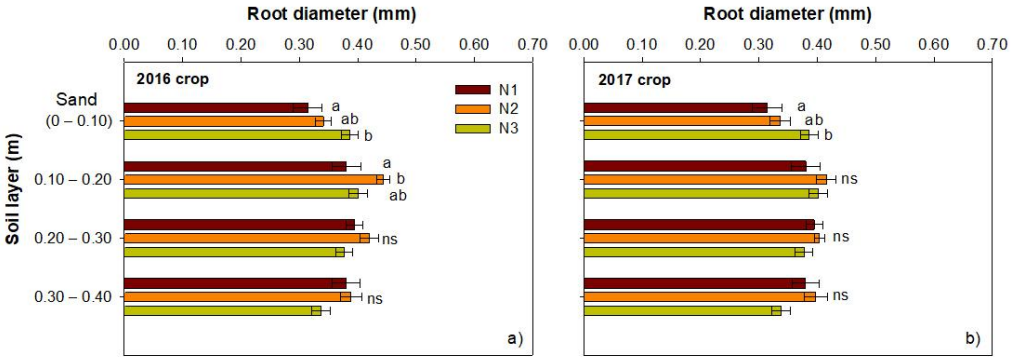

Figure 7. Average root diameter in each soil layer for the three N treatments. Panel (a) represents the 2016 crop (N1, 2.0 mmol N L^{-1}; N2, 9.7 mmol N L^{-1}; N3, 17.7 mmol N L^{-1}) and panel (b) shows the 2017 crop (N1, 2.0 mmol N L^{-1}; N2, 9.7 mmol N L^{-1}; N3, 15.7 mmol N L^{-1}). Different lower-case letters within each soil layer show significant differences between N treatments at $p<0.05$. Data have been pooled across P1 position (0.10 m distant to the plant) and P2 position (0.30 m distant to the plant). Values are means ± SE. ns, not significant at $p>0.05$.

3.6. Root dynamics in minirhizotron tubes

Root length assessed using the minirhizotron tubes in the 2017 crop was very low until 30 DAT. From this point onwards, root length grew constantly and rapidly until 100 DAT. From 100 DAT until the end of the crop, root length growth continued but with appreciably smaller

increments (Figure 8). Root length dynamics were affected by N treatment and soil layer, but these two factors did not interact significantly (RM-ANOVA, p> 0.16) (Table 7). In most of the cycle of the 2017 crop, there were no significant differences between N treatments in root length (Figure 8). The exception occurred in four sampling dates (at 75, 90, 103 and 204 DAT) when root length was significantly higher in the conventional N treatment (N2) than in the very deficient (N1) and very excessive N (N3) N treatments (Figure 8a). Soil layer was a significant effect on root length, with higher root length in the 0–0.22 m soil layer (D1) than in the 0.22–0.44 m soil layer (D2) in most of the cycle, except at the beginning of crop, at 14, 20 and 28 DAT (Figure 8b).

Table 7. Results of repeated-measure analysis of variance testing the effect of N treatment and depth of soil layer on root length dynamics, of the 2017 sweet pepper crop. Significant effects at p<0.05 are shown in bold. df are degrees of freedom, F is the Fisher value of ANOVA and p is the probability value.

Effect	df	F	p
Block	2	0.37	0.694
Nitrogen (N)	1	28.25	**<0.001**
Depth (D)	2	1.28	0.290
N x D	3	1.85	0.155
Error	37		
Time (T)	12	175.50	**<0.001**
T x N	24	2.23	**<0.001**
T x D	12	11.37	**<0.001**
T x N x D	24	0.55	0.962
Error	444		

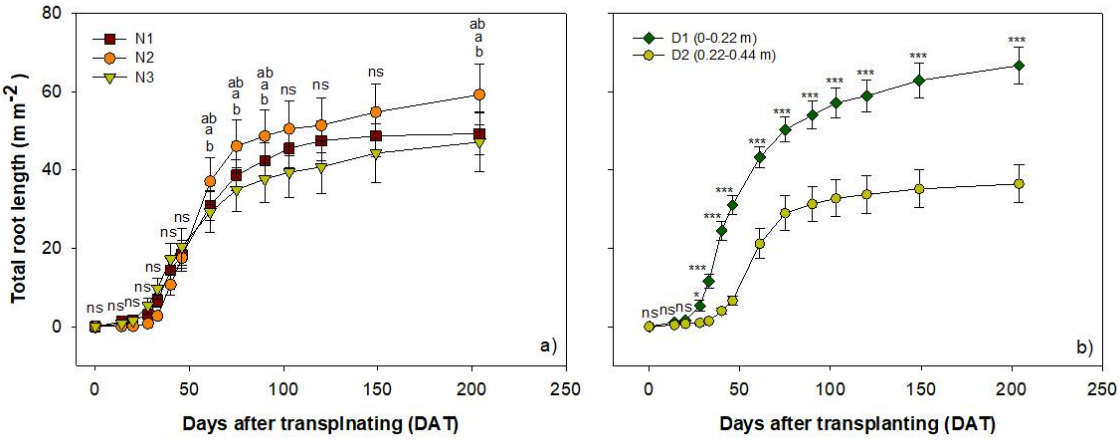

Figure 8. Root length dynamics observed through minirhizotron tubes in three N treatments in the 2017 crop. Panel (a) shows root length in each of the three N treatments (N1, 2.0 mmol N L^{-1}; N2, 9.7 mmol N

L^{-1}; N3, 15.7 mmol N L^{-1}) when pooling over the two soil layers (i.e. 0–0.22 and 0.22–0.44 m). Panel (b) shows root length in each of the two soil layers (D1, 0–0.22 m; D2, 0.22–0.44 m) when pooling over the three N treatments (i.e. N1, N2 and N3). Different lower-case letters above each symbol show significant differences between N treatments for each date, at $p<0.05$. Asterisks show significance between soil depths. Values are means ± SE. ***, $p<0.001$; **, $p<0.01$; *, $p<0.05$; ns, $p>0.05$.

Root length production rate, calculated from minirhizotron images, was higher in the first soil layer (0–0.22 m) than in the second soil layer (0.22–0.44 m) (ANOVA Depth $p<0.001$). On average across the three N treatments, the root length production rate was 1.8 times larger in the 0–0.22 m soil layer (Figure 9). There were not significant differences between N treatments (ANOVA Nitrogen, $p> 0.05$) (Table 8) in root length production rate.

Table 8. Results of analysis of variance testing the effect of N treatments on root length production rate over the 2017 sweet pepper crop. Significant effects at $p<0.05$ are shown in bold. df are degrees of freedom, F is the Fisher value of ANOVA and p is the probability value.

Effect	df	F	P
Nitrogen (N)	2	1.65	0.205
Depth (D)	1	**23.05**	**<0.001**
N x D	2	0.77	0.469
Block	3	1.06	0.375
Error	39		

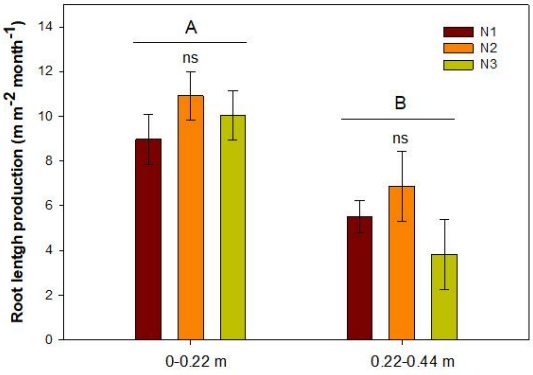

Figure 9. Root length production rate over the 2017 sweet pepper crop at two soil layers (0–0.22 m and 0.22–0.44 m) for the three N treatments (N1, 2.0 mmol N L^{-1}; N2, 9.7 mmol N L^{-1}; N3, 15.7 mmol N L^{-1}), observed in minirhizotron tubes. Different upper-case letters show significant differences between soil layers. Values are means ± SE. ns, not significant at $p>0.05$.

3.7. Correlation between variables

Root length density at the P1 sampling position had a strong and significant negative correlation with shoot dry matter production, crop N uptake and yield (r_S > 0.67, Table 8), and also a

negative correlation with residual mineral N in the soil at the end of the crops (r_S = 0.55) (Table 9). Root length growth rate calculated using minirhizotron images was not significantly correlated with any of the variables analysed (Table 9).

Table 9. Spearman correlation coefficient (rS) between two variables for the 2016 and 2017 crops. Asterisks show significance of correlation, at P<0.05 (*), P<0.01 (**) and P<0.001 (***). Absence of asterisks denotes not-significant correlations at P>0.05. Significant correlations have been shown in bold. P1 and P2 refers to sampling position (i.e. P1, at 0.10 m distant to the plant; P2, at 0.30 m distant to the plant parallel to the row of plants). D1 and D2 refers to depth of observation of minirhizotron images (i.e. D1, 0–0.22 m; D2, 0.22–0.44 m).

	Crop N_{uptake}	Yield	Residual N_{soil}	Root length $density_{P1}$	Root length $density_{P2}$	Root growth $rate_{D1}$	Root growth $rate_{D2}$
Dry matter	**0.98*** **	**0.85*** **	**0.77*** **	**-0.73*** **	0.17	0.17	-0.27
Crop N_{uptake}	-	**0.85*** **	**0.80*** **	**-0.72*** **	0.17	0.18	-0.27
Yield	**0.85*** **	-	**0.66*** **	**-0.67*** **	**0.53** **	0.08	-0.35
Residual N_{soil}	**0.80*** **	**0.66*** **	-	**-0.55** **	-0.01	0.15	-0.33
Root length $density_{P1}$	**-0.72*** **	**-0.67*** **	**-0.55** **	-	-0.36	-0.09	0.35
Root length $density_{P2}$	0.17	**0.53** **	-0.01	-0.36	-	0.00	0.27
Root growth $rate_{W1}$	0.18	0.08	0.15	-0.09	0.00	-	0.43
Root growth $rate_{W2}$	-0.27	-0.35	-0.33	0.35	0.27	0.43	-

4. Discussion

This study showed that the different N treatments, applied by fertigation, resulted in higher root length density in the very deficient N treatment, concentrating the roots in the most superficial soil layer, compared to the conventional and very excessive N treatments. This finding is consistent with studies that reported that N located near the root and high soil N concentrations reduced the extension of the roots (Drew, 1975; Drew et al., 1973; Lain et al., 1995; Primavesi, 1982). Lecompte et al., (2008) studied the distribution of roots and NO_3^- of fertigated tomato crops and concluded that the spatial distribution of roots was strongly influenced by N fertilization. In this way there is consistency that after a great initial rooting, high soil N availability caused the root system to recede, whereas low soil N availability was associated with further root extension.

After 80 DAT, the very deficient N treatment consistently had less shoot dry matter production than the other two N treatments. Belowground dry matter production was not quantified in the present study; instead, root length density was evaluated. The root length density increased, and the shoot dry matter decreased in the very deficient N treatment with respect to the conventional and very excessive N treatments. These results agree in part with the work of Lecompte et al., (2008) where a very deficient N supply significantly increased the belowground dry matter and decreased the dry matter shoot, regarding the N excessive treatments. This response of the crop to N deficiency would show that assimilates are preferentially used for root development rather than shoot development. The opposite occurs with a high N supply (Drew, 1975; Garnett et al., 2009), the development of the aerial part is increased and the development of the roots is decreased. In the present research high N supply maximized shoot biomass growth and reduced belowground length growth, which confirms the literature.

The proliferation of roots in response to localized soil N is not contradictory to the inhibition of root growth at excessive N applications. According to Zhang and Forde, (2000), suppression of root growth is a systemic inhibitory response to shoot accumulation of NO_3^-, while proliferation of roots in a localized nutrient-rich patch is a stimulatory effect triggered by NO_3^- concentration in the rhizosphere.

Root length distribution in the artificially stratified “enarenado” soil showed that 30% of the root length was located in the sand mulch layer (0–0.10 m depth), and 48% in the 0.10–0.20 m soil layer. Below 0.20 m, the length of roots was very low. Castilla, (1986) reported similar shallow rooting of fertigated tomato in an “enarenado” soil. Approximately 25% of the roots were in the sand layer (Castilla, 1986). The rooting patterns reported in the present work are shallower than those of Castilla, (1986); these differences may be due to different rooting behaviour of tomato and sweet pepper, soil type and site history.

In this study in "enarenado" soil, most of the roots developed in the sand layer and the upper layer of the soil, which coincided with Castilla, (1986). One of the possible explanations could be due to the constant supply of water and nutrients applied by the fertigation system. This

450 combined system favours the concentration of roots in the upper layers of soil, where water
451 nutrients are applied and concentrated (Machado and Oliveira, 2003; Oliveira and Calado, 1996;
452 Peterson et al., 2016).

453 In tomato crop in soil under fertigation system (Oliveira and Calado, 1996), the largest
454 proportion of roots was found in the top 0.40 m of the soil and thereafter rapidly decreased with
455 depth. There was a high concentration of roots in the 0.30 to 0.40 m layer, which was attributed
456 to a compacted soil horizon immediately below 0.40 m which impeded deeper root penetration.

457 The results of the present study did not show increased root growth in the deep layers in
458 response to N fertilization, concurring with Rasmussen et al. (2015). Several reports agree that
459 N fertilization seems to affect the root density more than rooting depth (Thorup-Kristensen and
460 Van Den Boogaard, 1999; Mahgoub et al., 2017).

461 In the present study, root length was concentrated near the emitter (i.e., at the P1 sampling
462 position) where the water and nutrients are applied. This is consistent with Padilla et al., (2017a)
463 in an "enarenado" soil, where the root density was higher at the sampling position near the base
464 of the plant.

465 In the P1 sampling position, there were correlations among the variables studied. Root length
466 density was negatively correlated with dry matter, yield, crop N uptake, and residual N mineral
467 in soil. This means that root length density decreases with higher residual soil mineral N (Drew,
468 1975; Lain et al., 1995). These results further indicate that a higher root length density may not
469 be sufficient to achieve higher dry matter production or yield. Rather, it may demonstrate
470 compensatory growth of roots when the N supply is low (Lecompte et al., 2008). In the
471 cropping system in which this work was conducted, with high frequency drip
472 irrigation/fertigation, higher root length density does not compensate for a low N supply.

473 Regarding the efficiency in the absorption of N, the very deficient N was the one with the
474 highest efficiency. As the application of N increased, the efficiency in the use decreased, this
475 coincides with several works (Candido et al., 2009; Rodríguez et al., 2020; Yasuor et al., 2013).

In any case, the increase in the efficiency of N use of the very deficient N treatment was not able to compensate for lower N application and was the one with the lowest yield in both years of study (Rodríguez et al., 2020).

Regarding the study of root dynamics in minirhizotron tubes, higher root length was registered in 0–0.22 m of the "enarenado" soil. This coincided with the soil core sampling where higher root length density was observed in the sand mulch layer (0–0.10 m depth) and the 0.10–0.20 m soil layer. Concentration of roots close to the drip emitter and the less compacted upper soil are likely explanations cause for more favourable root growth in the 0–0.20 m of "enarenado" soil (Padilla, et al., 2017a).

The analysis of root length through minirhizotron images was less sensitive to detect differences between N treatments than destructive soil core sampling. Using the minirhizotron, there were not differences in the analysed layers (i.e., 0–0.22 and 0.22–0.44 cm depth), whereas destructive root sampling found larger root length density in the very deficient N treatment. This lack of co-coincidence may in part be explained by differences in sampling depths. The first 0.22 m of soil was scanned in a single minirhizotron image, thereby integrating the 0–0.10 m layer of the sand mulch and the 0.10–0.20 m of imported soil. Destructive root samples, in this study, showed that the effect of N on root length density in the sand mulch layer was the opposite to that in the 0.10–0.20 m soil layer below the sand mulch. Future work with minirhizotron images in "enarenado" soil should aim to separate results of root length between the sand mulch layer and the imported soil layer, as was done with soil core samples in the present study.

Conclusions

In the present study, water and nutrients were applied by drip emitters near the plant, and roots of sweet pepper were mostly located near the drip emitter. In this artificially stratified "enarenado" soil, nearly 80% of the roots was distributed in the sand mulch layer (0–0.10 m depth) and the 0.10–0.20 m soil layer. Root distribution below 0.20 m of soil was very low, most likely due to high-frequency fertigation. The results of the present work suggest that

conventional and very excessive N application maximized the shoot biomass growth and crop yield but resulted in reduced root length density particularly in the sand mulch layer; the opposite occurred under very deficient N application, in addition to a reduced efficiency in the use of N. These findings suggest that a higher root length density, and a high efficiency of the use of N per se was not sufficient to compensate for the low amount of N applied in order to achieve high dry matter production and yield.

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References

Camacho, F., Fernandez, E., 2013. Manual práctico de Fertirrigacón en riego por goteo, Segunda. ed. Madrid, España.

Candido, V., Miccolis, V., Rivelli, A.R., 2009. Yield traits and water and nitrogen use efficiencies of bell pepper grown in plastic-greenhouse. Ital. J. Agron. 4, 91–100. https://doi.org/10.4081/ija.2009.3.91

CAPDR, 2016. Cartografia invernaderos en el litoral de Andalucía. Cons. Agric. Pesca y Desarro. Rural 20.

527 Castilla, N., 1986. Tomato Root Development on Sand Mulch, Plastic Greenhouse in Almeria
528 (Spain).

529 Chun, L., Mi, G., Li, J., Chen, F., Fusuo, Z., 2005. Genetic analysis of maize root chara stress
530 Liang. Plant Soil 276, 369–382. https://doi.org/10.1007/sl1104-005-5876-2

531 Comfort, S.D., Malzer, G.L., Busch, R.H., 1988. Nitrogen Fertilization of Spring Wheat
532 Genotypes: Influence on Root Growth and Soil Water Depletion. Agron. J. 80, 114–120.

533 Drew, M., 1975. Comparison of the effects of a localized supply of phosphate, Nitrate,
534 ammonium, and potassium on the growth of the seminal root system, and the shoot in
535 barley 479–490.

536 Drew, M.C., Saker, L.R., Ashley, T.W., 1973. Nutrient supply and the growth of the seminal
537 root system in barley: I. The effect of nitrate concentration on the growth of axes and
538 laterals. J. Exp. Bot. 24, 1189–1202. https://doi.org/10.1093/jxb/24.6.1189

539 Eissenstat, D., 1992. Costs and benefits of constructing roots of small diameter. J. Plant Nutr.
540 15, 763–782. https://doi.org/2 DOI 10.1080/01904169209364361

541 Fereres, E., Goldhamer, D.A., Parsons, L.R., 2003. Irrigation water management of horticultural
542 crops. HortScience 38, 1036–1042. https://doi.org/10.21273/hortsci.38.5.1036

543 Franco, J.A., Bañón, S., Vicente, M.J., Mirallales, J., Martinez-Sánchez, J.J., 2011. Root
544 development in horticultural plants grown under abiotic stress conditions – a review. J.
545 Hortic. Sci. Biotechnol. 86, 543–556.

546 Gallardo, M., Jackson, L.E., Thompson, R.B., 1996. Shoot and root physiological responses to
547 localized zones of soil moisture in cultivated and wild lettuce (Lactuca spp.). Plant, Cell
548 Environ. 19, 1169–1178. https://doi.org/10.1111/j.1365-3040.1996.tb00432.x

549 Gallardo, M., Padilla, F.M., Peña-Fleitas, M.T., de Souza, R., Rodríguez, A., Thompson, R.B.,
550 2020. Crop response of greenhouse soil-grown cucumber to total available N in a Nitrate
551 Vulnerable Zone. Eur. J. Agron. 114, 125993. https://doi.org/10.1016/j.eja.2019.125993

552 Gallardo, M., Thompson, R.B., Lopez-Toral, J.R., Fernandez, M.D., Granados, R., 2006. Effect
553 of Applied N Concentration in a Fertigated Vegetable Crop on Soil Solution Nitrate and
554 Nitrate Leaching Loss. Acta Hortic. 80.

555 García, M., Céspedes, A., Pérez, P., Lorenzo, P., 2016. El sistema de producción hortícola
556 protegido de la provincia de Almería, IFAPA España.

557 Garnett, T., Conn, V., Kaiser, B.N., 2009. Root based approaches to improving nitrogen use
558 efficiency in plants. Plant, Cell Environ. 32, 1272–1283. https://doi.org/10.1111/j.1365-
559 3040.2009.02011.x

560 Gastal, F., Lemaire, G., 2002. N uptake and distribution in crops: An agronomical and
561 ecophysiological perspective. J. Exp. Bot. 53, 789–799.
562 https://doi.org/10.1093/jexbot/53.370.789

563 Gong, L., Zhao, J., 2019. The response of fine root morphological and physiological traits to
564 added nitrogen in Schrenk's spruce (Picea schrenkiana) of the Tianshan mountains, China.
565 PeerJ 2019, 1–20. https://doi.org/10.7717/peerj.8194

566 Guo, D., Mitchell, R.J., Withington, J.M., Fan, P.P., Hendricks, J.J., 2008. Endogenous and
567 exogenous controls of root life span, mortality and nitrogen flux in a longleaf pine forest:
568 Root branch order predominates. J. Ecol. 96, 737–745. https://doi.org/10.1111/j.1365-
569 2745.2008.01385.x

570 Hendrick, R.L., Pregitzer, K.S., 1996. Applications of minirhizotrons to understand root
571 function in forests and other natural ecosystems. Plant Soil 185, 293–304.
572 https://doi.org/10.1007/BF02257535

573 Herrera, J.M., Stamp, P., Liedgens, M., 2007. Interannual variability in root growth of spring
574 wheat (Triticum aestivum L.) at low and high nitrogen supply. Eur. J. Agron. 26, 317–326.
575 https://doi.org/10.1016/j.eja.2006.11.003

576 Hodge, A., 2010. Roots: The Acquisition of Water and Nutrients from the Heterogeneous Soil

577 Environment. https://doi.org/10.1007/978-3-642-02167-1_12

578 Ju, X.T., Kou, C.L., Zhang, F.S., Christie, P., 2006. Nitrogen balance and groundwater nitrate
579 contamination: Comparison among three intensive cropping systems on the North China
580 Plain. Environ. Pollut. 143, 117–125.

581 Kimberly, D., Bonifas, J., Lindquist, L., 2009. Effects of Nitrogen Supply on the Root
582 Morphology of Corn and Velvetleaf. J. Plant Nutr. 1371–1382.
583 https://doi.org/10.1080/0110.1080/01 904160903007893

584 King, J., Gay, A., Sylvester-Bradley, R., Bingham, I., Foulkes, J., Gregory, P., Robinson, D.,
585 2003. Modelling cereal root systems for water and nitrogen capture: Towards an economic
586 optimum. Ann. Bot. 91, 383–390. https://doi.org/10.1093/aob/mcg033

587 Kristensen, H., Thorup- Kristensen, K., 2007. Effects of vertical distribution of soil inorganic
588 nitrogen on root growth and subsequent nitrogen uptake by field vegetable crops. Soil Use
589 Manag. https://doi.org/https://doi.org/10.1111/j.1475-2743.2007.00105.x

590 Kristensen, H.L., Thorup-Kristensen, K., 2007. Effects of vertical distribution of soil inorganic
591 nitrogen on root growth and subsequent nitrogen uptake by field vegetable crops. Soil Use
592 Manag. 23, 338–347. https://doi.org/10.1111/j.1475-2743.2007.00105.x

593 Lain, P., Ourry, A., Boucaud, J., 1995. Shoot control of nitrate uptake rates by roots of Brassica
594 napus L.:Effects of localized. Planta 196, 77–83.

595 Lecompte, F., Bressoud, F., Pares, L., De Bruyne, F., 2008. Root and nitrate distribution as
596 related to the critical plant N status of a fertigated tomato crop. J. Hortic. Sci. Biotechnol.
597 83, 223–231. https://doi.org/10.1080/14620316.2008.11512373

598 Li, Y., Niu, W., Xu, J., Wang, J., Zhang, M., lv, W., 2016. Root morphology of greenhouse
599 produced muskmelon under sub-surface drip irrigation with supplemental soil aeration.
600 Sci. Hortic. (Amsterdam). 201, 287–294. https://doi.org/10.1016/j.scienta.2016.02.018

601 Machado, R.M.A., Oliveira, M.D.R.G., 2003. Comparison of tomato root distributions by

minirhizotron and destructive sampling. Plant Soil 255, 375–385. https://doi.org/10.1023/A:1026198919074

Machado, R.M.A., Oliveira, M.D.R.G., 2002. Tomato root distribution, yield and fruit quality under different subsurface drip irrigation regimes and depths. Irrig. Sci. 24, 15–24. https://doi.org/10.1007/s00271-005-0002-z

Mahgoub, N.A., Ibrahim, A.M., Ali, O.M., 2017. Effect of different irrigation systems on root growth of maize and cowpea plants in sandy soil. Eurasian J. Soil Sci. 6, 374–379. https://doi.org/10.18393/ejss.319952

Mancuso, S., 2012. Measuring roots: An updated approach, Measuring Roots: An Updated Approach. https://doi.org/10.1007/978-3-642-22067-8

Martinez, R., 1987. Comportamiento del riego bajo enarenado en invernadero. Balances de salinidad y fertilizantes, en cultivo de pimiento y judia.

McCully, M., 1999. ROOTS IN SOIL: Unearthing the Complexities of Roots and Their Rhizospheres. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50, 695–718. https://doi.org/10.1146/annurev.arplant.50.1.695

Nacry, P., Bouguyon, E., Gojon, A., 2013. Nitrogen acquisition by roots: Physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. Plant Soil 370, 1–29. https://doi.org/10.1007/s11104-013-1645-9

Noguchi, K., Nagakura, J., Kaneko, S., 2013. Biomass and morphology of fine roots of sugi (Cryptomeria japonica) after 3 years of nitrogen fertilization. Front. Plant Sci. 4, 1–7. https://doi.org/10.3389/fpls.2013.00347

Oliveira, M., Calado, A., 1996. Tomato root distribution under dripirrigation. Am. Soc. Hortic. Sci. 644–648.

Padilla, F.M., Gallardo, M., Manzano-Agugliaro, F., 2018. Global trends in nitrate leaching research in the 1960–2017 period. Sci. Total Environ. 643, 400–413.

https://doi.org/10.1016/j.scitotenv.2018.06.215

Padilla, F. M., Peña-Fleitas, M., Fernández, M., del Moral, F., Thompson, R., Gallardo, M., 2017. Responses of soil properties, crop yield and root growth to improved irrigation and N fertilization, soil tillage and compost addition in a pepper crop. Sci. Hortic. (Amsterdam). 225, 422–430. https://doi.org/10.1016/j.scienta.2017.07.035

Padilla, F.M., Peña-fleitas, M.T., Fernández, M.D., Thompson, R.B., Gallardo, M., 2017a. Responses of soil properties , crop yield and root growth to improved irrigation and N fertilization , soil tillage and compost addition in a pepper crop. Sci. Hortic. (Amsterdam). 225, 422–430. https://doi.org/10.1016/j.scienta.2017.07.035

Padilla, F.M., Peña-Fleitas, M.T., Gallardo, M., Giménez, C., Thompson, R.B., 2017b. Derivation of sufficiency values of a chlorophyll meter to estimate cucumber nitrogen status and yield. Comput. Electron. Agric. 141, 54–64. https://doi.org/10.1016/j.compag.2017.07.005

Peterson, C.A., Soares, T., Torbert, E., 2016. Drip Irrigation Effect on Soil Function , Root Systems and Productivity in Organic Tomato and Corn, in: Organic Agriculture Research Symposium.

Primavesi, A., 1982. MANEJO ECOLOGICO DEL SUELO, Quinta. ed. El Ateneo, Sao Paulo, Brasil.

Rasmussen, I.S., Dresbøll, D.B., Thorup-Kristensen, K., 2015. Winter wheat cultivars and nitrogen (N) fertilization-Effects on root growth, N uptake efficiency and N use efficiency. Eur. J. Agron. 68, 38–49. https://doi.org/10.1016/j.eja.2015.04.003

Rodríguez, A., Peña-Fleitas, M.T., Gallardo, M., de Souza, R., Padilla, F.M., Thompson, R.B., 2020. Sweet pepper and nitrogen supply in greenhouse production : Critical nitrogen curve , agronomic responses and risk of nitrogen loss. Eur. J. Agron. 117, 126046. https://doi.org/10.1016/j.eja.2020.126046

652 Ryan, P.R., Delhaize, E., Watt, M., Richardson, A.E., 2016. Plant roots: Understanding
653 structure and function in an ocean of complexity. Ann. Bot. 118, 555–559.
654 https://doi.org/10.1093/aob/mcw192

655 Svoboda, P., Haberle, J., 2006. The effect of nitrogen fertilization on root distribution of winter
656 wheat. Plant, Soil Environ. 52, 308–313. https://doi.org/10.17221/3446-pse

657 Thompson, R.B., Martínez-Gaitan, C., Gallardo, M., Giménez, C., Fernández, M.D., 2007a.
658 Identification of irrigation and N management practices that contribute to nitrate leaching
659 loss from an intensive vegetable production system by use of a comprehensive survey.
660 Agric. Water Manag. 89, 261–274. https://doi.org/10.1016/J.AGWAT.2007.01.013

661 Thompson, R.B., Martínez-Gaitan, C., Gallardo, M., Giménez, C., Fernández, M.D., 2007b.
662 Identification of irrigation and N management practices that contribute to nitrate leaching
663 loss from an intensive vegetable production system by use of a comprehensive survey.
664 Agric. Water Manag. 89, 261–274. https://doi.org/10.1016/J.AGWAT.2007.01.013

665 Thorup-Kristensen, K., Kirkegaard, J., 2016. Root system-based limits to agricultural
666 productivity and efficiency: The farming systems context. Ann. Bot. 118, 573–592.
667 https://doi.org/10.1093/aob/mcw122

668 Thorup-Kristensen, K., Van Den Boogaard, R., 1999. Vertical and horizontal development of
669 the root system of carrots following green manure. Plant Soil 212, 145–153.
670 https://doi.org/10.1023/A:1004646522369

671 Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. 2 Tilman. Nature 418,
672 671–677. https://doi.org/https://doi.org/10.1038/nature01014

673 Valera, D.L., Belmonte, L.J., Molina-Aiz, F.D., López, A., Camacho, F., 2017. The greenhouses
674 of Almería, Spain: Technological analysis and profitability. Acta Hortic. 1170, 219–226.
675 https://doi.org/10.17660/ActaHortic.2017.1170.25

676 Yasuor, H., Ben-Gal, A., Yermiyahu, U., Beit-Yannai, E., Cohen, S., 2013. Nitrogen

677 management of greenhouse pepper production: Agronomic, nutritional, and environmental
678 implications. HortScience 48, 1241–1249. https://doi.org/10.21273/hortsci.48.10.1241

679 Zhang, H.M., Forde, B.G., 2000. Regulation of Arabidopsis root development by nitrate
680 availability Hanma. J. Exp. Bot. 51, 51–59.

681 Zotarelli, L., Dukes, M.D., Scholberg, J.M.S., Muñoz-Carpena, R., Icerman, J., 2009. Tomato
682 nitrogen accumulation and fertilizer use efficiency on a sandy soil, as affected by nitrogen
683 rate and irrigation scheduling. Agric. Water Manag. 96, 1247–1258.
684 https://doi.org/10.1016/j.agwat.2009.03.019

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***Declaration of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Rafael Grasso: Data Curation, Investigation, Writing - Original Draft, Writing- Reviewing and Editing; Romina de Souza: Data Curation, Investigation, Writing- Reviewing and Editing; M. Teresa Peña-Fleitas: Data Curation, Investigation; Marisa Gallardo: Funding acquisition, Methodology, Investigation, Writing- Reviewing and Editing; Rodney B. Thompson: Funding acquisition, Methodology, Investigation, Writing- Reviewing and Editing; Francisco M. Padilla: Funding acquisition, Methodology, Investigation, Writing- Reviewing and Editing