Departamento de Agronomía
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# Effect of N on fruit production and quality of greenhouse horticultural crops, and practices to improve the use of nutrients

Ph.D. Dissertation

Almería Septiembre, 2021

# Efecto del N sobre la producción y calidad de cultivos hortícolas en invernadero, y prácticas para mejorar el uso de nutrientes

Tesis Doctoral

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Ph.D. Dissertation

Rafael Gillson Grasso Rodríguez

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# Programa de Doctorado en Agricultura Protegida

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## Scientific contributions during the predoctoral stage

### Published JCR articles included in the doctoral thesis

Grasso, R., de Souza, R., Peña-Fleitas, M. T., Gallardo, M., Thompson, R. B., & Padilla, F. M. (2020). Root and crop responses of sweet pepper (*Capsicum annuum*) to increasing N fertilization. *Scientia Horticulturae* 273: 109645. https://doi.org/10.1016/j.scienta.2020.109645

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### Other published JCR articles not included in the doctoral thesis

de Souza, R., Grasso, R., Peña-Fleitas, M.T., Gallardo, M., Thompson, R.B., & Padilla, F.M. (2020). Effect of cultivar on chlorophyll meter and canopy reflectance measurements in cucumber. *Sensors 20*(2): 509. https://doi.org/10.3390/s20020509

de Souza, R., Peña-Fleitas, M.T., Thompson, R.B., Gallardo, M., Grasso, R., & Padilla, F.M. (2019). The use of chlorophyll meters to assess crop N status and derivation of sufficiency values for sweet pepper. *Sensors* 19(13): 2949. https://doi.org/10.3390/s19132949

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**Proceedings and conference communications**

Attended the EUVRIN Workshop held in Bleiswijk (The Netherlands), From September 13$^{th}$ to September 14$^{th}$, 2018, and presented the oral presentation "Rapid analysis systems to determine the concentration of nitrate in nutrient solution and soil solution of fertigated crops"

FERTINNOWA conference: Sharing fertigation best practices across Europe at Almeria, Spain on 3-5 October 2018. Oral presentation. de Souza, R., Grasso, R., Peña-Fleitas, M.T., Gallardo, M., Thompson, R.B., Padilla, F.M. 2019. Efecto del cultivar en las medidas de medidores de clorofila e índices de vegetación en pepino., in: II Congreso Jóvenes Investigadores Ciencias Agroalimentarias de la Universidad de Almería. 17 October 2019. Almería, Spain pp. 75–78.

La Universidad de Almería certifica que la comunicación en forma de póster titulada: Sistemas de análisis rápidos para determinar la [NO3-] Y [K+] en solución nutritiva y solución del suelo y cuyos autores son: R. Grasso, M.T. Peña-Fleitas T, F.M. Padilla, F, R.B. Thompson fue presentada en el II Congreso de Jóvenes Investigadores en Ciencias Agroalimentarias, celebrado el 17 de octubre de 2019 en la Universidad de Almería. En Almería, a 17 de octubre de 2019.

XII Jornadas ceiA3 del Grupo de Sustratos de la SECH Universidad de Almería 11 y 12 de diciembre de 2019, presentación oral. Evaluación de dos equipos de análisis rápido para la determinación de la concentración de nitrato y potasio en solución nutritiva y en solución de suelo. R. Grasso, M.T. Peña-Fleitas, F.M. Padilla, R.B. Thompson.

# 1. Summary

## 1.1. Summary

Intensive greenhouse vegetable crops are commonly associated with appreciable nitrate leaching. This situation may be due to the large amount of nitrogen fertilizer applications and the limited monitoring measures and management practices to improve the N management. Taking this problem into account, the general objective of this thesis was to evaluate the effect of different doses of N and management practices to optimize the use of this nutrient in greenhouse vegetable crops. Three manuscripts were proposed, the first one focused on the effect of increasing doses of N on the rooting of two pepper crops (*Capsicum annuum*) grown in soil. Yield, crop N uptake and the dry matter of the shoot were evaluated. The second manuscript dealt with the effect of tillage on sweet pepper (*Capsicum annuum*) grown in a greenhouse with soil. Soil parameters such as matric potential and penetration resistance, climatic variables, N and water consumption, N leaching and dry matter production were evaluated. In the last manuscript, the effect of N dose on fruit quality parameters and cultivar effect on muskmelon (*Cucumis melo*) and sweet pepper (*Capsicum annuum*) crops were addressed. In addition to internal quality parameters of the fruits, morphometric and commercial performance were measured. The main findings of the first manuscript were that the conventional and very excessive application of N maximized the biomass production of the shoot and crop yield, and decreased root length density in sweet pepper crop. In the second manuscript, tillage did not prove to be a practice that alone could increase dry matter or crop yields. On the other hand, this practice caused a significant decrease in irrigation, applied N, drainage, and nitrate leaching. With respect to the last manuscript, fruit responded to very poor N fertilization with the pulp being more orange in muskmelon and the skin being more reddish in sweet pepper. In contrast, ºBrix increased with very poor N fertilization in

muskmelon but ºBrix decreased with poor N fertilization in sweet pepper. In the succession of all three manuscripts, the various effects of N and management measures were demonstrated. It is concluded that application of N fertilizers above the level considered optimal generated losses of fertilizers, did not increase yields, delayed harvests and decreased the quality of the fruits. In this way, achieving a precise application of N and proposing management practices will be a necessary alternative to increase the efficiency of N.

## 1.2. Resumen

La producción intensiva de hortalizas bajo invernadero se asocia comúnmente con una apreciable contaminación con nitratos de los acuíferos subyacentes. Esta situación puede deberse a la gran cantidad de fertilizantes nitrogenados aportados y a las escasas medidas de monitoreo y prácticas de manejo para evitar el lavado de este nutriente. Tomando en cuenta esta problemática, en esta tesis se planteó como objetivo general evaluar el efecto de diferentes dosis de N y prácticas de manejo para optimizar el uso de este nutriente en cultivos hortícolas bajo invernadero. Se plantearon tres manuscritos, el primero se enfocó en el efecto de dosis crecientes de N sobre el enraizamiento de dos cultivos de pimiento (*Capsicum annuum*) cultivados en suelo "enarenado". Se evaluó el rendimiento, la absorción de N del cultivo y la materia seca de la parte aérea. El siguiente manuscrito trató del efecto del laboreo en pimiento (*Capsicum annuum*) cultivado en invernadero con suelo "enarenado". En este manuscrito se evaluaron parámetros a nivel de suelo, como potencial matricial y resistencia a la penetración, variables climáticas, consumo de nitrógeno, consumo de agua, lixiviación de N y producción de materia seca. En el último manuscrito se abordó el efecto de distintas dosis de N sobre parámetros de calidad y efecto del cultivar en melón (*Cucumis melo*) y pimiento (*Capsicum annuum*). Se midieron parámetros de calidad interna de los frutos, morfométricos y rendimiento comercial. Como resultados del primer manuscrito, se encontró que el efecto del N sobre el desarrollo de las raíces sugiere que la aplicación convencional y muy excesiva de N maximizó el desarrollo de la parte aérea y el rendimiento del cultivo y disminuyó la densidad radicular en pimiento. En el segundo capítulo, el laboreo no demostró ser una práctica que por sí sola aumente la producción de materia seca o el rendimiento del cultivo. En cambio, esta práctica sí provocó una disminución significativa del riego, el N

aplicado, el drenaje y la lixiviación de N. Respecto al último manuscrito, en respuesta a una fertilización con N muy deficiente, la pulpa fue más anaranjada en el melón y la piel más rojiza en el pimiento. En contraste, los ºBrix con fertilización muy deficiente se incrementaron para el cultivo del melón y decayeron para el cultivo de pimiento. En el conjunto de los artículos elaborados han quedado demostrado los diversos efectos del N y medidas de manejo sobre melón y pimiento. Se llega a la conclusión de que las aplicaciones de N por encima del nivel considerado óptimo generan pérdidas de fertilizantes, no aumentan los rendimientos, retrasan las cosechas y disminuyen la calidad de los frutos. De esta manera, realizar una aplicación precisa de N y proponer medidas de manejo durante el ciclo de cultivo, son alternativas necesarias para aumentar la eficiencia del uso de N.

# 2. Introduction

Nitrogen (N) is one of the most important nutrients for plant growth, being classified as a main macronutrient (Azcón-Bieto and Talón, 2003). The most important functions in the plant is the manufacture of amino acids, proteins and other assimilates necessary for growth and the production of dry matter (Burns et al., 2010). Its importance is such that after water, N is the most limiting nutrient in plant growth; this conditions the management practices given by farmers and advisors (Azcón-Bieto and Talón, 2003). In intensive production of vegetables, the cost of fertilizers is low compared to the rest of the production costs (Valera et al., 2014) and as a result, vegetable farmers do not feel strong economic pressure to reduce N fertilizer application (Sonneveld and Voogt, 2009). These situations together with the misconception that the more N the higher the yield, is a possible explanation for excess N applications in many areas of the world (Gallardo et al., 2006; R.B. Thompson et al., 2007b; Zotarelli et al., 2009). Excess N has several consequences such as groundwater contamination, delayed harvest, poor fruit quality, and increased susceptibility to diseases (Gianquinto et al., 2011).

Soil N is present in four main forms: (a) organic matter, such as plant material, amino sugars, and humus; (b) soil organisms and microorganisms; (c) ammonium ions ($NH_4^+$) retained by clay minerals and organic matter, and (d) forms of mineral N in the soil solution, including nitrate ($NO_3^-$) and low concentrations of nitrite ($NO_2^-$) (Cameron et al., 2013). Losses due to leaching within production systems are basically due to $NO_3^-$ that washes out of the soil profile and is dragged to groundwater being a worldwide phenomenon (Padilla et al., 2018). The $NO_3^-$ can reach rivers and lakes causing serious eutrophication problems (Cameron et al., 2013).

For this reason, N management practices in vegetable crops in Europe are under increasing scrutiny for their potentially adverse environmental effects (Hartz, 2003). The implementation of the EU Nitrates Directive has imposed a requirement to restrict N application on EU farms to reduce nitrate losses to water (Burns et al., 2010). It has been found that over fertilization of vegetable crops is a widespread problem (Hartz, 2003).

In particular in vegetable crops, there is information on the management of N for various crops, seeking a dynamic management that allows the administration of fertilizers when their use is crucial for the growth and yield of the plants (Gianquinto et al., 2011). There is technical capacity in these systems since high frequency fertigation systems are used, which could supply N in the irrigation water in small times and doses (Farneselli-2015). Despite the accumulation of scientific information on N management, it has not been enough to reverse the current situation of increasing water bodies contamination (Lecompte et al., 2008).

Several reports agree that crop yield increases as the concentration of N applied increases and decreases when the crop's N requirements are not met (Lima e Silva et al., 2007). In general, in vegetable crops the application of N exceeds the crop demand, which has various consequences such as reduced N uptake efficiency, direct effects on crops, and contamination of water bodies with leached nitrate ($NO_3^-$) (Rodríguez et al., 2020; Thompson et al., 2017). Indeed, it is estimated that only 30-40% of the N applied is absorbed by crops (Tilman et al., 2002a). N application in vegetable crops are commonly based on the experience of technical advisors and producers, in most cases without proper diagnosing and monitoring systems of crop N status that would allow adjustments of the N applied (Thompson et al., 2009). Intensive vegetable crops are grown in relatively small-sized greenhouses, so the cost of N fertilizers is not significant even when applied in excess (Locascio et al., 1997). This problem is generalized in several regions of the world, such as the southeast of Spain (SE) (Thompson et al., 2007b), the southeast of United States (Zotarelli et al., 2009), and China (Ju et al., 2006). It is thus necessary to deepen the study of the various effects of increasing N fertilization on crops and the environment.

In the particular case of Almeria (south-eastern Spain), specifically some areas of Campo de Dalías, Bajo Andarax and Campo de Níjar, N management in the form of $NO_3^-$ is a serious problem registered in those areas being declared as nitrate vulnerable zones to contamination by nitrates of agricultural origin (Rodrigo et al., 2007). In this area there is a high concentration of greenhouses with more than 30,000 ha (Thompson et al., 2009; Valera et al., 2014). The management of nutrients in greenhouse horticultural production systems in Almería is based on the experience of advisors and producers (Thompson et al., 2007b). Although this system has the technical capacity with fertigation systems for precise N and irrigation management, the greatest problem is not being considered (Thompson et al., 2007b).

In this way, it is pertinent to continue deepening best N management practices at the farm level, whereby the [$NO_3^-$] applied to the crops at each given moment of the crop cycle is adjusted, and improve cultural management practices that help minimize the impacts generated by the misuse of N fertilizers (Parks et al., 2012).

One such field where our understanding needs to be improved is on the effects of increasing N fertilization on root growth and dynamics in vegetable crops. There are several factors that influence the root system, such as N availability, cultivar, soil properties (physical, chemical and biological characteristics), agricultural practices (e.g. tillage), climate or competition with others roots (Primavesi, 1982). In terms of N availability, there is evidence that the root system has the ability to adapt to available mineral nutrients in the soil profile during the growing cycle (Nacry et al., 2013; Hodge, 2010). This can be interpreted as a resource saving strategy to allocate the least amount of nutrients to the root system (Gallardo et al., 1996).

Several reports agree that N fertilization affects the quantity and distribution of roots in the soil profile (Drew, 1975; Drew et al., 1973; Franco et al., 2011; Herrera et al., 2007). In several crops, the common tendency is to reduce the root system in response to the high application of N, this behavior is differential in each stage of the crop cycle (Comfort et al., 1988; Lecompte et al., 2008). However, at low N fertilization, the root system tends to increase and explore more soil volume (Lecompte et al., 2008). The spatial location of the N fertilization application also had an effect on the root responses; deep location of N caused an extension and deeper rooting of the crops (Kristensen and Thorup-Kristensen, 2007). By contrast, shallow location of N (i.e. application in the surface layer), led to concentration of the roots in the most superficial soil layers (Svoboda and Haberle, 2006).

Root diameter is another important trait involved in the acquisition of mineral nutrients that can be affected by N fertilization (Kimberly et al., 2009; Gong and Zhao, 2019). Roots of small diameter tend to be more efficient in nutrient absorption but there is no consistent evidence on the response of root diameter to N availability (Guo et al., 2008; Noguchi et al., 2013).

Two of the strategies to increase N uptake efficiency are to increase the root length density (Herrera et al., 2007) and to develop deeper root systems that are capable of capturing N from deep soil layers (Rasmussen et al., 2015). However, in vegetable crops grown in greenhouses in south-east Spain, the tendency for root systems is to concentrate in the first 0.20-0.25 m of soil layers (Castilla, 1986; Raya Martinez, 1987a; Padilla et al., 2017b). In tomato crops grown in open fields, the depth of the root system was similarly concentrated in shallow soil layers, particularly within the first 0.40 m depth (Peterson et al., 2016).

In general, studies on the effect of N fertilization on growth of the aerial part of the plant and yield are very abundant, but much less is known on the effect of root development in soil (Primavesi, 1982; Thorup-Kristensen and Kirkegaard, 2016). This lack of knowledge is likely caused by the inherent difficulties in studying roots in soil (Mancuso, 2012). However, minirhizotron tubes can be used to monitor root growth throughout the crop cycle without destroying the root system (Hendrick and Pregitzer, 1996; Machado and Oliveira, 2002; Mancuso, 2012).

The first manuscript of this thesis aims to improve our knowledge on the effect of increasing N fertilization on crop growth and yield, focusing particularly on root dynamics. The study was conducted in sweet pepper (*Capsicum annuum*) grown in soil in a greenhouse in Almería (south-east Spain). Sweet pepper is one of the most important crops in Almería, with an annual area of 8,174 ha dedicated to its cultivation (Reche, 2010).

In Almeria vegetable crops are grown in “enarenado” soil, which is an artificial soil formation technique by superposition of different layers or horizons, generally three layers (Jiménez and Lao, 2002). It is composed of the following layers from the base of the original soil, a first layer of glen soil of about 30-40cm is placed, above it a layer of manure of about 5-10cm and finally a layer of coarse sand of about 10-15cm (Valera et al., 2014). The main objective is to reduce the evaporation of water from the soil, its use being indicated in areas of saline soils and in marginal lands (Jiménez and Lao, 2002).

Tillage is a management practice conducted to alleviate soil compaction that could increase the use of soil N and water by crops. Tillage would improve the exploration of the soil by roots and therefore would increase the uptake of water and nutrients (Wortmann et al., 2008; Quincke et al., 2007).

Soil structure is one of the main factors affecting the general development of roots (Passioura, 1991). A good soil structure is characterized by containing an adequate proportion of pores with water and air that optimizes the plant nutrition and the rooting process (Primavesi, 1982). Soil degradation processes such as erosion and compaction cause a degradation of soil structure, generating serious problems in several crops at the global scale (Batey, 2009; Hamza and Anderson, 2005).

Soil compaction is one of the principal problems of modern agriculture. Commonly, it is mainly associated with the traffic of heavy machinery and of the operators, especially in intensive crops (Abu-Hamdeh, 2003; Iler and Stevenson, 1991). Soil compaction has various effects on the soil environment, such as decreasing the total soil porosity and consequently affecting the supply of water and air to the roots (Iler and Stevenson, 1991; Tracy et al., 2013). Another important problem underlying soil compaction is the difficulty of mechanical penetration of the roots in the soil, which limits roots to the most superficial soil layers (Kadžienž et al., 2011; Batey, 2009; Thorup-

Kristensen, 2011) and restricts the absorption of nutrients and water to the soil surface layers (Franco et al., 2011b; Gregory et al., 2013). Soil compaction negative effects are aggravated in soils with a high moisture content and low organic matter content (Hamza and Anderson, 2005; Batey, 2009). Organic matter acts as a buffer for compaction processes, being one of the main agents responsible for the stability of soil aggregates (Primavesi, 1982). Therefore, it is necessary to promote management practices that tend to facilitate the exploration of the soil by the roots.

Tillage has proven to be effective for alleviating soil compaction processes under certain conditions (Wortmann et al., 2008; Quincke et al., 2007). Several studies have reported a reduction in soil bulk density and decreased soil penetration resistance after tillage (Erbach et al., 1992). Consequently, the supply of available water and oxygen for the root has been shown to be increased (Lampurlanés et al., 2001; Mu et al., 2016).

In vegetable crops grown in greenhouses in the Almeria province (south-east Spain), cultural practices with tractors have decreased because the spatial distribution of the crops within greenhouses makes it difficult for machinery to move (Valera et al., 2014). However, there is a great transit of personnel in cultural practices such as transplantation, pruning, phytosanitary applications and multiple fruit harvests (López-Gálvez and Naredo, 1996). The intensive use of soils, the absence of tillage, added to the high moisture content, exacerbate the compaction problem (Padilla et al., 2017).

In several studies with vegetable crops such as pepper and tomato in an artificially-stratified soil in SE Spain, it has been shown that most of the roots are found in the most superficial layers of the soil, but a consensus was not reached regarding the causes of this restriction (Castilla, 1986; Raya Martinez, 1987a; Padilla et al., 2017c) considering that the potential rooting depth of tomato other horticultural crops such as melon growing in soil without restrictions reached a depth of at least 40cm (Peterson et al., 2016; Li et al., 2016). Mechanical restrictions to deep rooting may exist in greenhouse-grown crops (Castilla, 1986). In addition, water and fertilizers applied by drip fertigation in low amounts but at high frequency may concentrate roots in the wet bulb (Padilla et al., 2017) (Thorup-Kristensen, 2011). However, there is currently little scientific work that evaluated the effect of tillage on root development in "enarenado" soils. The second manuscript of this thesis deals with this issue.

Studying the impact of N fertilization and the cultivar effects of the fruit quality is a relevant aspect for vegetable crops. The fresh fruit markets have increased the demands regarding organoleptic quality and safety. In turn, these requirements must cope with an increase in yields, in order to maintain or increase the profit margin of farmers.

To a large extent, the commercial success of the fresh fruit market is due to advances in fertilization management and the development of new cultivars adapted to different growing areas (Crisosto et al., 1995). In the particular case of N, it has been shown to have marked effects on fruits; N deficiency leads to small fruits with a bad taste and reduced productivity (Crisosto et al., 1995). Excessive N levels led to delayed fruit maturity, low percentage of red coloration and decreases fruit size compared to optimal levels of N in fruit trees (Daane et al., 1995). In apple crops, it has been shown that an excess of N led to an undesirable decrease in fruit firmness (Sams, 1999). Another very important factor is the effect of the cultivar. In tomato cultivation, it was shown that this effect contributed to explain the differences in internal quality of the fruits compared to the effect of N (Kaniszewski et al., 2019; Tilahun et al., 2018). Sams (1999) found in an extensive review of fruit trees and vegetables that the quality of the fruits was affected both by fertilization and by genetic factors associated with each cultivar, and that there may be interactions depending on each species and levels of fertilization.

In general, several reports have dealt with the effect of N, both in excess and with a deficient supply, on the internal and morphometric quality parameters of fruits. There is consensus that deficient N causes small fruits and low yield, whereas N excess delays fruit ripening and fruit growth is delayed (Lima et al., 2007; Locascio et al., 1997). In the case of muskmelon cultivation, there are studies reporting increases in °Brix with increasing values of N fertilization in the 0-300 kg N $ha^{-1}$ range (Monteiro et al., 2003; De Faria et al., 2000a). In general, applications of almost 400 kg of N $ha^{-1}$ resulted in fruits with a larger seed cavity and thicker skin pulp than applications of 0 to 150 kg of N $ha^{-1}$ in melon (Castellanos et al., 2012; Monteiro et al., 2003). However, in sweet pepper, increasing N applications of 108, 498, 880 and 1343 kg N $ha^{-1}$ did not affect the physical or chemical quality of the fruit, including the sugar content and acidity (Yasuor et al., 2013). Local recommendation of N fertilization for greenhouse crops is of 175 kg N $ha^{-1}$, for muskmelon with an expected yield of 40,000-50,000 kg $ha^{-1}$, and 350-450 kg N $ha^{-1}$, for sweet pepper with an expected yield of 50,000-60,000 kg $ha^{-1}$ (Reche, 2010, 2008).

Several reports have evaluated the effect of cultivar on yield, internal fruit quality and morphometrical parameters of the fruit (Warner et al., 2004; Ketelaere et al., 2004; Lado et al., 2012). In tomato crops, there were differences between cultivars in fruit quality parameters such as dry matter content, soluble solids content, firmness, colour or acidity (Warner et al., 2004; Kaniszewski et al., 2019). In strawberry, colour, firmness, soluble solids content and acidity were all affected by cultivar identity (Lado et al., 2012).

In sweet pepper, the cultivar effect on fruit quality involved differences in beta carotenes, ascorbic acid, total flavonoids and soluble phenols (Szafirowska and Elkner, 2008).

The third manuscript of this thesis addresses the differences between cultivars in internal and external fruit quality parameters and yield, and the differential responses to increasing N fertilization in muskmelon and sweet pepper grown in soil in greenhouse. Due to the importance of the sweet pepper (*Capsicum annuum*) - muskmelon (*Cucumis melo*) rotation in south-east Spain (Valera et al., 2014), these cultures were taken as a model for the present study.

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# 3. Objectives

The first objective of the thesis was to improve our knowledge on the effect of N on rooting patterns and root dynamics in an important vegetable cropping system, which will provide insights to improve the crop N management and thus contribute to reducing $NO_3^-$ leaching loss. This objective was addressed in the first manuscript of the thesis. The research was conducted with sweet pepper in a cultivation system called “enarenado” soil, in the Almeria province, where more than 90% of the cultivated surface is found under this system (García et al., 2016). In addition, sweet pepper cultivation area is close to 8000 ha per year (Valera et al., 2017) and there are increasing problems of water pollution due to loss of $NO_3^-$ (Thompson et al., 2007).

The second manuscript of the thesis continues with the cultivation of sweet pepper, deepening into the rooting processes in the “enarenado” soil. The objective of this manuscript was to evaluate the effect of tillage in “enarenado” soils. Soil properties (i.e., bulk density, resistance to penetration and soil matric potential) were measured, together with crop variables such as dry matter production, crop yield and crop N consumption, irrigation, N fertilizer applied and N leaching. Rooting pattern was characterized by destructive root length density sampling, relative root length distribution and root observation through minirhizotron tubes.

Finally, the objectives of the third manuscript of the thesis were to evaluate the response of parameters of internal and external fruit quality and yield to increasing N fertilization in three cultivars of muskmelon and sweet pepper grown in soil in greenhouse. We hypothesized that both fruit quality and yield would increase with N application up to a point where no improvement in fruit quality and yield would be detected.  This third objective of the thesis was relevant to improve our knowledge on the effects of increasing N application on fruit quality and yield, because of the importance of sweet pepper and muskmelon crops in the study area, the high application of N rates in commercial farms and the diversity of cultivars, together with the increasing demands of high quality fruits in commercial farming

# 4. Manuscript One. Root and crop responses of sweet pepper (*Capsicum annuum*) to increasing N fertilization

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### 4.1. 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.

## 4.2. 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., 2002b). 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 (R.B. 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, are traditional destructive soil core sampling, and periodical observations using minirhizotron tubes (Hendrick and Pregitzer, 1996; Machado and Oliveira, 2002; Mancuso, 2012).

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

## 4.3. Material and methods

### *4.3.1. Greenhouse crop and experimental design*

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

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

The experimental work was carried out in the Experimental Station of the University of Almería, in Retamar, Almería, SE Spain (36°51′51″N, 2°16′56″W and 92 m elevation). The greenhouse structure consisted of polycarbonate walls and a trilaminate low-density polyethylene (LDPE) film roof (200 μm thickness) with approximately 60% photosynthetically active radiation (PAR) transmittance. It had no heating or artificial light, had passive ventilation (side panels and folding roof windows) with an east-west orientation, with rows of crops aligned from north to south. The cropping area was 1300 $m^2$. The greenhouse was organized into 24 plots, measuring 6 m × 6 m; 12 plots were used in the current study. Each plot contained three paired lines of plants (six lines of plants in total), with 12 plants in each line with a space of 0.5 m between them. The separation between the two lines that formed the paired line of plants was 0.8 m and the separation between two paired lines was 1.2 m. One plant was placed at 0.06 m and immediately adjacent to each dripper, giving a plant density of two plants $m^{-2}$ and 72 plants per replicated plot. There were border areas along the edges of the greenhouse. Drip irrigation above ground was used for combined irrigation and mineral fertilizers application. The emitters had a discharge rate of 3 L $h^{-1}$. Irrigation was scheduled to maintain soil matric potential (SMP) in the root zone, at 0.22 m deep from the surface of the sand mulch, within -15 to -25 kPa; one tensiometer (Irrometer, Co., Riverside, CA, USA) per plot was used to measure SMP.

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

In each crop, there were three treatments with different N concentrations, N1, N2 and N3. N levels were defined based on local fertilization practices (Camacho and Fernandez, 2013). Based on local practices, the N2 treatment was regarded as conventional. N amount applied throughout the crops was obtained by multiplying N concentration in the nutrient solution by irrigation water volume. There were four replications arranged in random blocks as detailed in Table 1. 88% of N was applied as $NO_3^-$, the rest as ammonium ($NH_4^+$) (Table 1). Other macronutrients remained constant in all treatments in the following concentrations: $H_2PO_4^-$, 2 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 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 |

### *4.3.2. 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.

### *4.3.3. 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).

### *4.3.4. Root analyses*

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

Root length growth dynamics in two soil layers were non-destructively measured using the minirhizotron technique. Two transparent minirhizotron tubes were installed, in each replicated plot, in July 2016 and were left to stabilize during the 2016 crop because the installation of the tubes disturbed the soil. The tubes were 0.60 m long and had 0.064 m internal diameter. In the lower part, tubes were sealed with a waterproof cap; in the upper part, a removable rubber cup prevented the passage of light. The part of tube that protruded above the sand surface was covered with aluminium tape that prevented heating and light penetration into the tube. The tubes were installed at 0.10 m of a plant, to a depth of 0.48 m, relative to the sand mulch layer. Root images were taken by sliding a cylindrical and rotating scanner into the tube (CI-600, CID Inc., Camas, WA, USA); for more details see Padilla et al., (2017a). Two images (0.22 x 0.19 m, 300 dpi) were taken per tube, the first one from the surface of the sand mulch layer to 0.22 m depth (0–0.22 m, hereafter), and the second one from 0.22 to 0.44 m depth (0.22–0.44 m, hereafter). The 0–0.22 m image comprised the 0.10 m of the sand mulch layer and the first 0.12 m of the imported soil, and the 0.22–0.44 m image comprised the remaining depth of imported soil and some of the original soil. Root images were taken throughout the 2017 crop, every 43 days on average. In the first 90 days of the 2017 crop, root images were taken every 26 days. In total, there were 14 root censuses. On each separate image, roots were digitized and analysed for root length per $m^2$ of soil (WinRHIZO Tron 2019, Regents Instruments Inc.).

*4.3.5. Data analysis and statistics*

For the comparative analysis of the aerial part, analysis of variance of repeated measures (RM-ANOVA) over time was used, followed by post hoc least square difference tests. For analysis of root length density between the three N treatments, factorial ANOVA was used, with four factors, block, N treatment, soil layer and sampling position. Differences in root length dynamics were evaluated by RM-ANOVA; factors were block, N, soil layer and time. The Spearman coefficient was used to evaluate the correlation between two variables (whether linear or not). Statistical procedures were performed with

STATISTICA 13 (TIBCO Software, Inc., Palo Alto, CA, USA). Significant differences were established at P<0.05.

## 4.4. Results

### *4.4.1. Shoot dry matter*

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

**Table 2.** Results of repeated-measure analysis of variance testing the effect of N treatments on shoot dry matter production dynamics of the 2016 and 2017 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.

| | | **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).

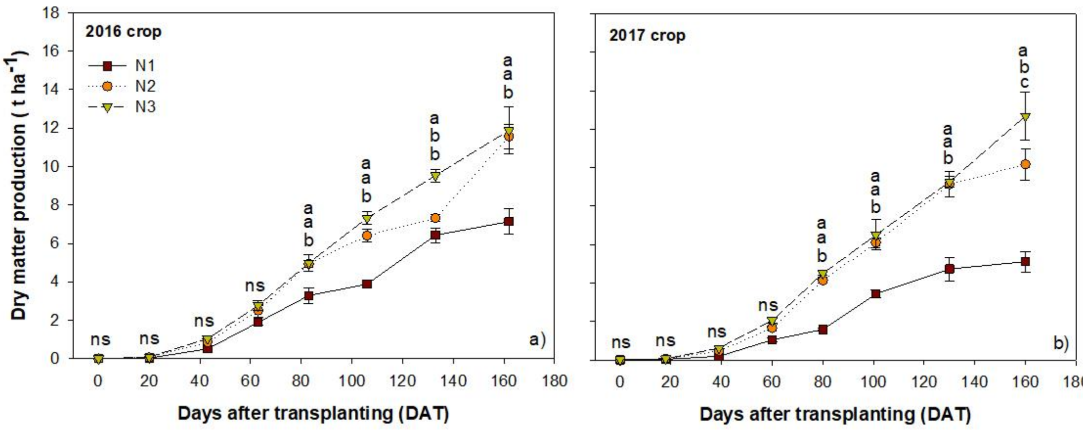


**Figure 1.** Shoot dry matter evolution for the three N treatments in the 2016 (a) (N1, 2.0 mmol N L$^{-1}$; N2, 9.7 mmol N L$^{-1}$; N3, 17.7 mmol N L$^{-1}$) and 2017 (b) (N1, 2.0 mmol N L$^{-1}$; N2, 9.7 mmol M L$^{-1}$; N3, 15.7 mmol N L$^{-1}$) in two sweet pepper crops. Different lower-case letters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

### *4.4.2. 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** |

### 4.4.3. 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).

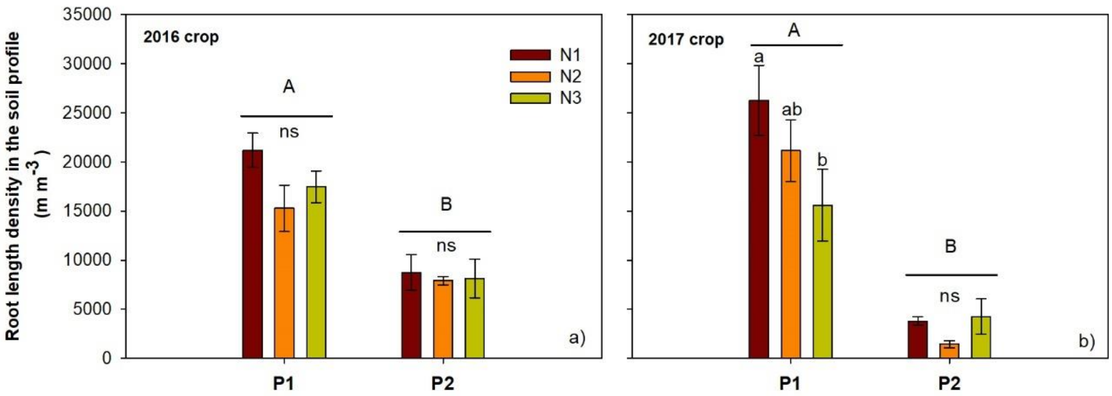


**Figure 2.** Root length density in the soil profile in the two sampling positions (P1, at 0.10 m distant to the plant, and P2, at 0.30 m distant to the plant, parallel to the row of plants) for the three N treatments. Plot (a) represents the 2016 crop (N1, 2.0 mmol N $L^{-1}$; N2, 9.7 mmol N $L^{-1}$; 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).

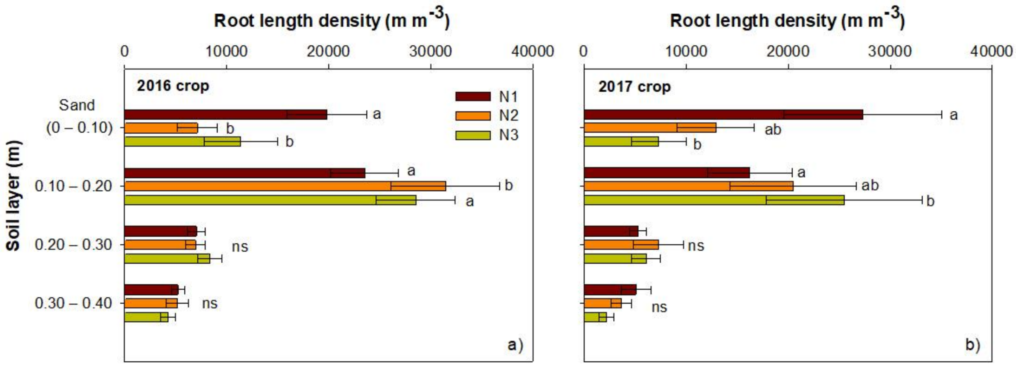


**Figure 3.** Root length density in each layer for the three N treatments. Panel (a) represents the 2016 crop (N1, 2.0 mmol N $L^{-1}$; N2, 9.7 mmol N $L^{-1}$; N3, 17.7 mmol N $L^{-1}$), and panel (b) represents the 2017 crop (N1, 2.0 mmol N $L^{-1}$; N2, 9.7 mmol N $L^{-1}$; N3, 15.7 mmol N $L^{-1}$). 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).

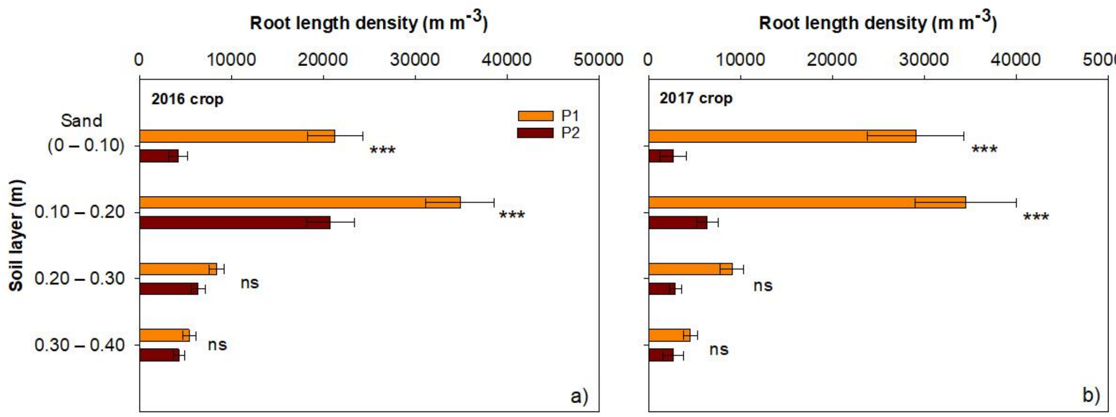


**Figure 4.** Root length density in the two-sampling positions (P1, at 0.10 m distant to the plant; P2, at 0.30 m distant to the plant parallel to the row of plants) per soil layer. Asterisks within each soil layer show significant differences between sampling positions. Panel (a) represents the 2016 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).

### *4.4.4. 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).

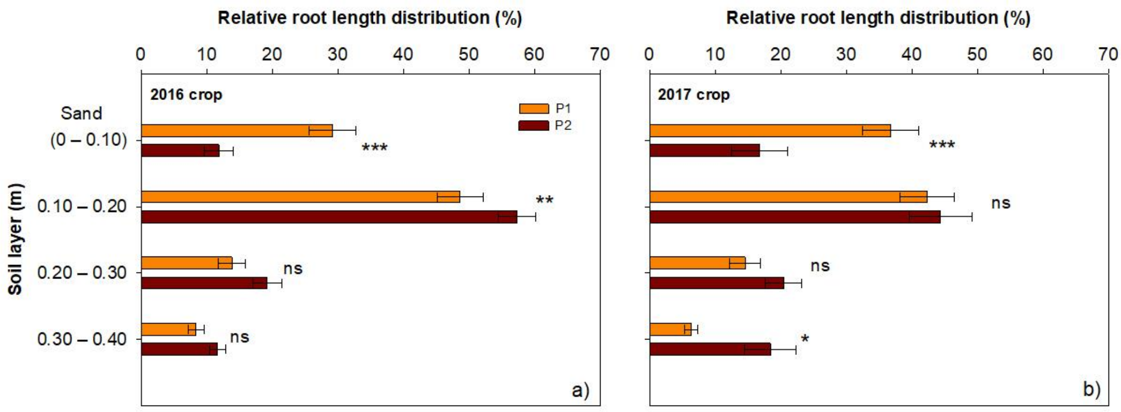


**Figure 5**. Relative root length distribution per soil layer and sampling position (P1, at 0.10 m distant to the plant; P2, at 0.30 m distant to the plant parallel to the row of plants). Panel (a) represents the 2016 crop and panel (b) the 2017 crop. Asterisks within each soil layer show significant 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).

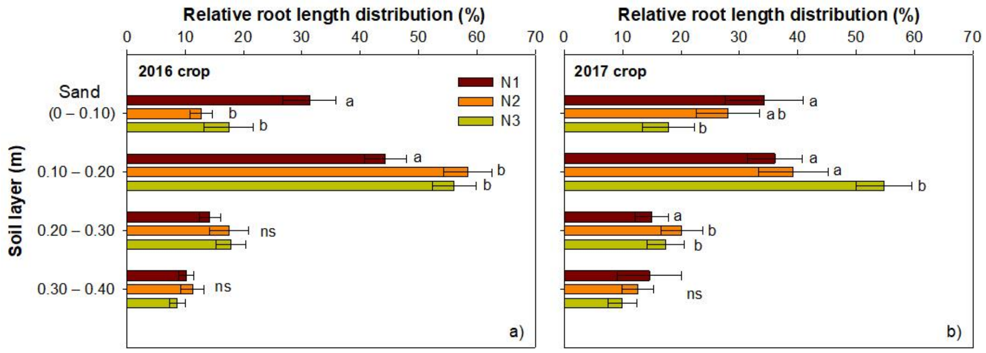


**Figure 6.** Relative root length distribution per soil layer for the three N treatments. Panel (a) represents the 2016 crop (N1, 2.0 mmol N $L^{-1}$; N2, 9.7 mmol N $L^{-1}$; N3, 17.7 mmol N $L^{-1}$) and panel (b) shows the 2017 crop (N1, 2.0 mmol N $L^{-1}$; N2, 9.7 mmol N $L^{-1}$; N3, 15.7 mmol 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.

### *4.4.5. 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).

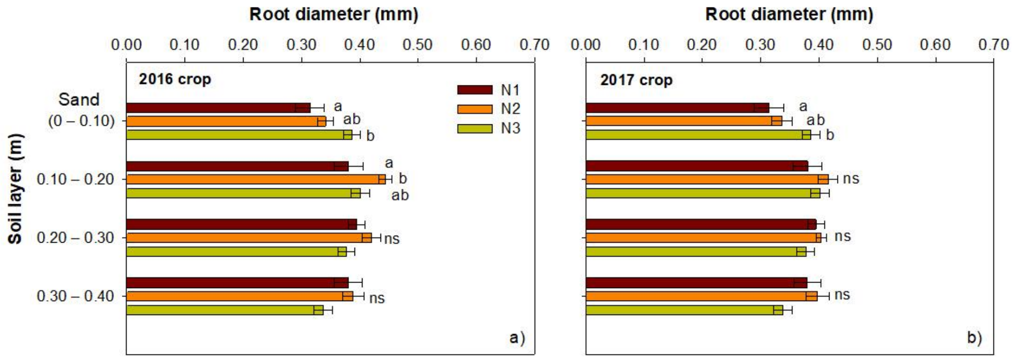


**Figure 7.** Average root diameter in each soil layer for the three N treatments. Panel (a) represents the 2016 crop (N1, 2.0 mmol N $L^{-1}$; N2, 9.7 mmol N $L^{-1}$; N3, 17.7 mmol N $L^{-1}$) and panel (b) shows the 2017 crop (N1, 2.0 mmol N $L^{-1}$; N2, 9.7 mmol N $L^{-1}$; N3, 15.7 mmol N $L^{-1}$). 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 $p>0.05$.

### *4.4.6. 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 | | |

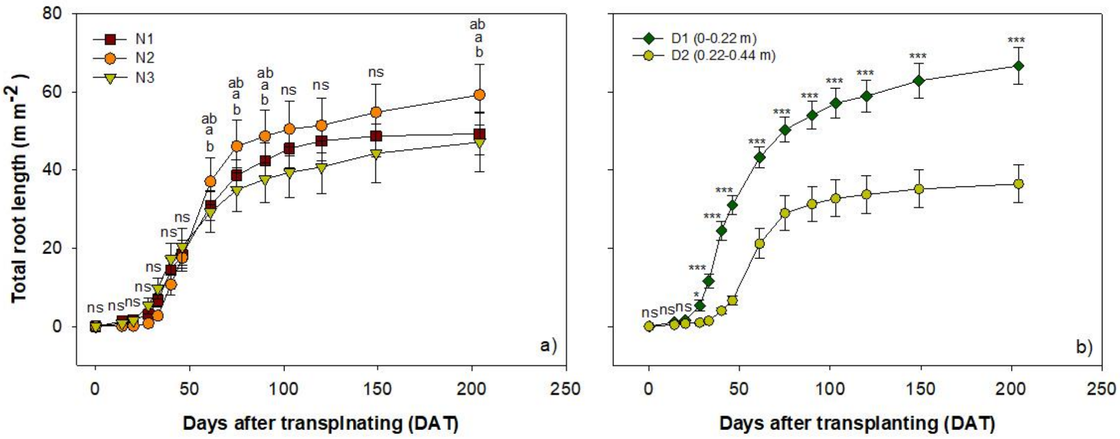


**Figure 8**. Root length dynamics observed through minirhizotron tubes in three N treatments in the 2017 crop. Panel (a) shows root length in each of the three N treatments (N1, 2.0 mmol N $L^{-1}$; N2, 9.7 mmol N $L^{-1}$; N3, 15.7 mmol N $L^{-1}$) when pooling over the two soil layers (i.e. 0–0.22 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 | | |

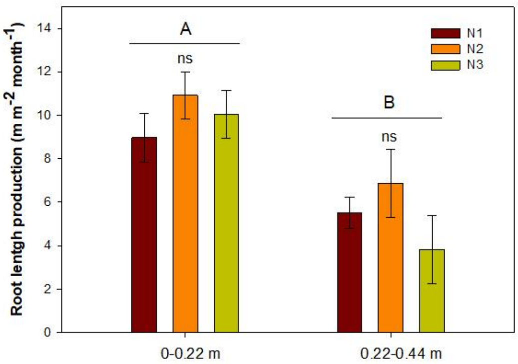


**Figure 9.** Root length production rate over the 2017 sweet pepper crop at two soil layers (0–0.22 m and 0.22–0.44 m) for the three N treatments (N1, 2.0 mmol N $L^{-1}$; N2, 9.7 mmol N $L^{-1}$; N3, 15.7 mmol N $L^{-1}$), observed in minirhizotron tubes. Different upper-case letters show significant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

### *4.4.7. 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 | - |

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

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

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

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

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

Regarding the efficiency in the absorption of N, the very deficient N was the one with the highest efficiency. As the application of N increased, the efficiency in the use decreased, this 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.

### 4.6. 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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Curation, Investigation. Francisco M. Padilla: Funding acquisition, Methodology, Investigation, Writing - Review & Editing.

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# 5. Manuscript Two: Tillage effects on soil properties, crop responses and root density of sweet pepper (*Capsicum annuum*)

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## 5.1. Abstract

Aim of study: Soil compaction causes an increase in bulk density, resistance to penetration, low diffusion of oxygen and water in the soil. Tillage is one of the techniques to alleviate compaction. The objective of this work was to evaluate the effects of tillage on sweet pepper grown in greenhouse soil. Area of study: The experimental work was conducted in a plastic greenhouse at the Experimental Station of the University of Almeria (SE Spain, 36° 51’ N, 2° 16’ W and 92 m elevation). Material and methods: The soil was ploughed with a single pass with ripper to 15 cm depth and with rotavator to 10 cm depth. The control treatment was soil untilled. Crop dry matter production and root length growth and density of sweet pepper were evaluated, in addition to soil characteristics such as bulk density, resistance to penetration and soil matric potential.

Main results: Tillage reduced soil bulk density from 1.70 to 1.60 kg $L^{-1}$ in the 10-40 cm of soil depth. There was a notable reduction in irrigation (12%), total N applied (13%), drainage (91%) and N leaching (95%) in the tillage treatment. However, tillage did not improve significantly crop dry matter production and yield. The absence of tillage effect is possible due to a slight reduction in the bulk density of the soil. Research highlights: The tillage treatment produced a notable reduction in irrigation, total N applied, drainage and N leaching when compared to the control.

## 5.2. Introduction

Soil structure is one of the main factors affecting crop growth over the long term (Passioura, 1991). Good soil structure consists of soil aggregation associated with high porosity, rapid infiltration rates, water retention capacity and increased air circulation which all facilitate root penetration and proliferation (Primavesi, 1982). A major problem of modern agriculture is the loss of soil aggregation, with compaction being one of the most important causes (Hamza & Anderson, 2005; Batey, 2009). Compaction alters the overall structure of soil pores, reduces pore number and size, and increases soil bulk density and resistance to soil penetration (Iler & Stevenson, 1991; Abu-Hamdeh, 2003).The effect of soil compaction on crop growth depends on soil texture. In general, soil bulk density values that limit root growth are 1.40–1.45 kg $L^{-1}$ in silty to clay textured soil, and 1.65–1.75 kg $L^{-1}$ in sandy soils (Daddow & Warrington, 1983).

The most frequent causes of soil compaction are use of heavy machinery, intensive cultivation, no crop rotation, and inadequate soil management (Hamza & Anderson, 2005; Batey, 2009). Soil compaction is accentuated in soils with low organic matter content and in soils with high moisture content (Hamza & Anderson, 2005). Organic matter loss can cause soil disaggregation, increasing susceptibility to compaction (Cochrane & Aylmore, 1994).

Soil tillage is a management practice that can alleviate soil compaction in diverse agricultural systems (Abu-Hamdeh, 2003; Quincke *et al*., 2007; Wortmann *et al*., 2008). Erbach *et al*. (1992) reported that different forms of tillage reduced differentially soil bulk density and resistance to penetration within the depth of tilled soil. The most effective tillage practices increase availability of soil water to crops due to increased infiltration

(Lampurlanés *et al*., 2001). In crop rotations of wheat and maize, deep tillage increased yield due to a reduction of soil compaction and increased soil water holding capacity (Mu *et al*., 2016). Soil management practices that facilitate deep rooting are likely to improve the efficient use of N and water, thereby decreasing the likelihood of nitrate ($NO_3^-$) leaching (Thorup-Kristensen, 2011).

The compaction processes in soil-grown crops in greenhouse have become widespread worldwide. Indeed, higher soil bulk density values have been reported for greenhouse soils than in bare soils (Liang *et al*., 2013). In recent years, mechanization in greenhouse crops has increased notably, which resulted in an increase in soil compaction (Erdem *et al*., 2006). This has led to increased soil bulk density, with values above 1.60 kg $L^{-1}$, whereas root growth is thought to be restricted above 1.75 kg $L^{-1}$ (Primavesi, 1982). Serious compaction and soil degradation have been detected in greenhouse crops due to continuous cultivation, such as in cucumber crops (Liang *et al*., 2013).

In the area of Almería, in south eastern Spain, the system of cultivation in “enarenado” soil is used, which consists of covering the soil with a layer of siliceous sand that maintains humidity (Valera *et al*., 2014). A drip irrigation system is used with fertigation by tapes, in order to apply the dissolved fertilizers in the irrigation water, and the soil is kept constantly humid (Thompson *et al*., 2007a). Within greenhouses, most cultural practices such as transplanting, pruning, harvesting, and the application of plant protection products are carried out manually by farm workers (Valera *et al*., 2014; Padilla *et al*., 2017). Due to the presence of a sand mulch, tillage is hardly carried out, since the sand mulch layer must be removed prior to tillage and be replaced (Valera *et al*., 2014). The low frequency of tillage accentuates soil compaction processes in SE vegetable crops (Castilla, 1986; Martinez, 1987; Padilla *et al*., 2017).

The objective of this work was to assess the effects of tillage on soil properties, crop responses and root length growth and density of sweet pepper (Capsicum annuum L.) grown in soil in a greenhouse in SE Spain. We measured soil properties (bulk density, resistance to penetration, soil matric potential), crop responses (dry matter production, crop yield, nitrogen N uptake) and rooting pattern (root length density, relative root length distribution, root observation through minirhizotron tubes).

## 5.3. Material and methods

### *5.3.1. Experimental details*

The work was carried out in a plastic greenhouse located at the Experimental Farm of the University of Almería (Almería, Spain, 36° 51’ N, 2° 16’ W; 92 m elevation). The area of the greenhouse dedicated to the crop was approximately 1,300 $m^2$. The greenhouse was similar to commercial greenhouses of the Almería region.

Two sweet pepper (cv. Melchor) cropping cycles were grown in soil with a summer-winter cycle, from 18 July 2016 to 24 March 2017 (248 days from transplant to end; hereafter the 2016-17 crop), and from 21 July 2017 to 20 February 2018 (214 days from transplant to end; hereafter the 2017-18 crop).

The soil was an artificial layered “enarenado” soil, which is typical of the region (Valera-Martínez *et al*., 2016). The “enarenado” soil consists of a 30 cm layer of silty clay loam soil, imported from a quarry, placed over the original sandy loam soil and a 10 cm layer of course river sand placed on the imported silty clay loam soil as a mulch (Francisco M. Padilla et al., 2017c).

There were eight plots of 6 × 6 m, with four plots (*i.e.*, replications) per treatment in a fully randomized block design. Polyethylene film sheets buried to 30 cm depth in the borders of the plots prevented water movement between plots. Five-week-old seedlings were planted 6-8 cm from each dripper; the plant density was 2 plants/m$^2$.

Drip irrigation and fertigation were used. Drippers with a flow rate of 3 L h$^{-1}$ were installed every 50 cm in drip lines, arranged in paired lines with an 80 cm separation. There was a 120 cm spacing between the paired driplines. There were 2 emitters/m$^2$. Fertigation with complete nutrient solutions, applying all macro and micronutrients commenced at 9 and 10 days after transplant for the 2016-17 and 2017-18 crops, respectively. The N concentrations applied was the same for the two cycles, 9.7 mmol L$^{-1}$. All cultural practices were consistent with local crop management.

Climatic conditions were recorded inside the greenhouse throughout both crops. Data were stored in a datalogger.

*5.3.2. Tillage treatments*

There was a tillage treatment and a control with no tillage (*i.e.*, conventional soil management). For the tillage treatment, the gravel mulch was removed, and the soil was ploughed with a single pass with ripper to 15 cm depth, followed by a single pass with rotavator to 10 cm depth. A small tractor pulled the tillage implements. Following cultivation, the gravel was replaced on the surface of the cultivated soil and was evenly spread to form a 10 cm thick mulch layer. Tillage was conducted at the end of June 2016, before the commencement of the 2016-17 crop. No tillage was conducted before the 2017-18 crop. There were four replicated plots of the tillage and no tillage treatments.

In both treatments, irrigation volumes and frequency were modified to maintain soil matric potential between -15 and -25 kPa. Tensiometers (Irrometer Co., Riverside, CA, USA) were installed at 25 cm depth (relative to the surface of the gravel mulch layer) in each plot to measure soil matric potential. The range of -15 to -25 kPa range avoided crop water stress (Thompson *et al*., 2007b). The intended applied N concentration of fertigation of both treatments was of 10 mmol $L^{-1}$; 92% of applied mineral N was in the form of $NO_3^-$, the rest as ammonium ($NH_4^+$)

Irrigation volumes were measured with water meters. Nutrient solution samples were collected in both treatments, two times per week, to determine the $NO_3^-$ and $NH_4^+$ concentrations (SAN++, Skalar Analytical B.V., Breda, The Netherlands). Drainage was collected from free-draining lysimeters (Gallardo *et al*., 2014). Drainage was collected from free-draining lysimeters, the soil of the lysimeter reproduced the “enarenado” soil, their detailed description is in Gallardo *et al*. (2020).

*5.3.3. Soil parameters measurements*

Soil bulk density was determined one month after soil cultivation, on 27 July 2016. Soil coring rings (5.3 cm internal diameter, 4 cm wall height, Eijkelkamp Soil and Water, Giesbeek, The Netherlands) were used for determination at 15.5-19.5 and 30.5-34.5 cm soil depths. All soil depth values are relative to the surface of gravel mulch. One determination was conducted in each replicated plot. Each sample was taken 8 cm from a plant perpendicular to the line of plants.

Soil penetration resistance (kPa) was measured with a compaction meter (FieldScout SC 900, Spectrum Technologies, Inc., Aurora, IL, USA). Measurements were recorded automatically in a data logger for every 2.5 cm of soil depth, from 10 to 25 cm. The sampling point was at 8 cm from the plant perpendicular to the line of plants. In each of the two pepper crops, measurements were conducted at the beginning and at the end of the crop.

Mineral N ($NO_3^-$–N and $NH_4^+$–N) content of the soil was determined immediately before and at the end of each of the two crops. Sampling in each plot was located at 5 and 60 cm from the plant perpendicular to the line of plants. Soil mineral N was calculated as: 0.65 × position 5 cm + 0.35 × position 60 cm (Soto *et al*., 2015). The soil was sampled in each position to a depth of 70 cm in three depth intervals (10–30, 30–50, 50–70 cm). Soil mineral N content was determined following extraction with potassium chloride (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 analyser (Model SAN++, Skalar Analytical B.V., Breda, The Netherlands).

### *5.3.4. Crop dry matter production and yield*

Crop dry matter production and yield was determined from eight plants, in an area of 4 $m^2$, in each plot. Plants were removed at ground level and the fresh weight material of all leaf, stem and fruit was determined. Dry weight was obtained after oven-drying subsamples at 65 °C. Mass of pruned material was determined as described above. Subsamples of leaf, stem and fruit were individually ground and sequentially in a knife mill and ball mill. Total N content (%) was determined (Rapid N, Elementar Analysensysteme GmbH, Hanau, Germany). The mass of N in leaf, stem and fruit was calculated from %N and mass of dry matter of that component. Crop N uptake (kg $ha^{-1}$) was the sum of N in leaf, stem and fruit.

### *5.3.5. Root analyses*

Towards the end of both crops, on 31 January 2017 and on 15 February 2018, soil cores were taken at 10 cm from the plant (P1) and at 30 cm from the plant (P2), perpendicular to the line of plants. In each position, the gravel mulch layer and 10-20, 20-30 and 30-40 cm depth layers were sampled; depth is expressed relative to the surface of the gravel mulch. A soil auger of 4.5 cm internal diameter was used for the gravel mulch layer and an auger of 3 cm internal diameter for the 10-20, 20-30 and 30-40 cm soil layers. Two replicate cores per sampling position were collected in each of four replicated plots of each of the two tillage treatments.

Roots of each gravel and soil sample were washed and were dyed with neutral red. Washed roots were scanned (Epson Perfection V800, Seiko Epson Corporation, Nagano, Japan) at 400 dpi in grey scale. The WinRHIZO Reg 2016 software (Regents Instruments Inc., Quebec, Canada) was used to measure root length in each sample. Relative root length distribution in each soil layer, relative to the whole soil profile sampled, was calculated as the percentage of root length in each soil layer divided by the total root length in the whole soil profile.

Transparent minirhizotron tubes were installed to non-destructively monitor root dynamics of sweet pepper throughout the 2017-18 crop. Installation of tubes occurred one year before, in July 2016, to allow for soil stabilization. The minirhizotron tubes were installed vertically to a depth of 48 cm from the surface of the gravel mulch, and at a distance of 10 cm from the plant in the direction of the line of plants. The tubes were of polymethyl methacrylate and were 60 cm long with 6.4 cm internal diameter. PVC caps were glued in the bottom of the tubes. The part of the tube that protruded above the gravel mulch layer was wrapped in aluminium tape to prevent light penetration. Two images (22 × 19 cm, 300 dpi) were taken per tube, the first one from the surface of the gravel mulch to a depth of 22 cm (0-22 cm, hereafter), and the second one from 22 to 44 cm depth (22-44 cm, hereafter). The 0-22 cm image comprised the 10 cm of the gravel mulch and the first 12 cm of the imported soil; the 22-44 cm image comprised the rest of imported soil and some of the original soil. Tube images were scanned (CI-600 Root Scanner, CID Inc., Camas, WA, USA) at 300 dpi and roots were digitized and analysed for root length per square meter of soil using the WinRHIZO Tron 2019 software (Regents Instruments Inc.). During the 207-2018 crop, tube images were taken every 43 days, on average; however, during the first 90 days of the crop, it was every 26 days on average.

*5.3.6. Data analysis*

In each of the two crops, differences in measured parameters between the tillage and no tillage treatments were tested by factorial analysis of variance (ANOVA) and pairwise LSD tests. Significant differences were established at $p<0.05$. Factors of ANOVA included block, tillage treatment and soil layer. Root length dynamics of minirhizotron tubes were evaluated by repeated-measure analysis of variance (RM-ANOVA). The ANOVA analysis were performed with the STATISTICA 13 software (TIBCO Software Inc., Palo Alto, CA, USA).

## 5.4. Results

*5.4.1. Soil parameters measurements*

Soil bulk density was slightly reduced in the tillage treatment (averaged value of 1.60±0.03 kg $L^{-1}$) when compared to the no tillage treatment (averaged value of 1.70±0.03 kg $L^{-1}$). The relative reductions in the tillage treatment were of 7.3 and 5.5%, for the 15.5-19.5 cm and30.5-34.5 cm depth soil layers, respectively.

In both crops, soil penetration resistance (kPa) was reduced in the tillage treatment throughout the depth of soil that was measured (*i.e.*, 15.5-19.5-25 cm), both at transplanting and at the end of the crop (Fig. 1). The reduction of penetration resistance in the tillage treatment was larger in the first crop (2016-2017), being 52% at transplanting and 36% at the end of the crop, considering the 15.5-19.5 cm depth. Soil penetration resistance was also reduced in the tillage treatment during the second crop (Fig. 1).

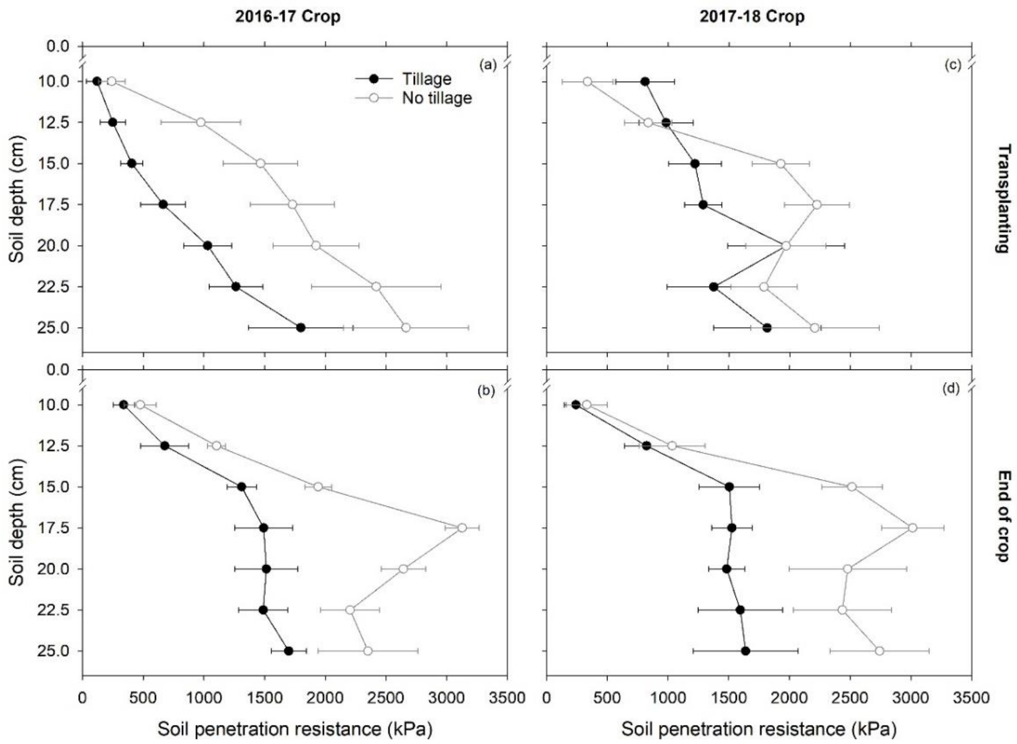


**Figure 1.** Soil penetration resistance in the two treatments (tillage and no tillage), after transplanting (a and c) and at the end of the crop (b and d), of two sweet pepper crops grown in soil in a greenhouse. Panels (a) and (b) show data of the 2016-17 crop, and panels (c) and (d) show data of the 2017-18 crop. Values are means ± SE.

### *5.4.2. Cropping details*

Climatic conditions of both crops were very similar in terms of air temperature, relative air humidity and the integral of solar radiation. There were notable differences in soil matric potential between crops, with the 2016-17 crop having less negative soil matric potential values (*i.e.*, wetter soils) than the 2017-18 crop (Fig. 2). Soil matric potential in the tillage and no tillage treatments was very similar (Fig. 2); the averaged soil matric potential of the no tillage treatment was only 2.6 and 1.6 kPa more negative (*i.e.*, drier) than that of the tillage treatment, for the 2016-17 and 2017-18 crops, respectively.

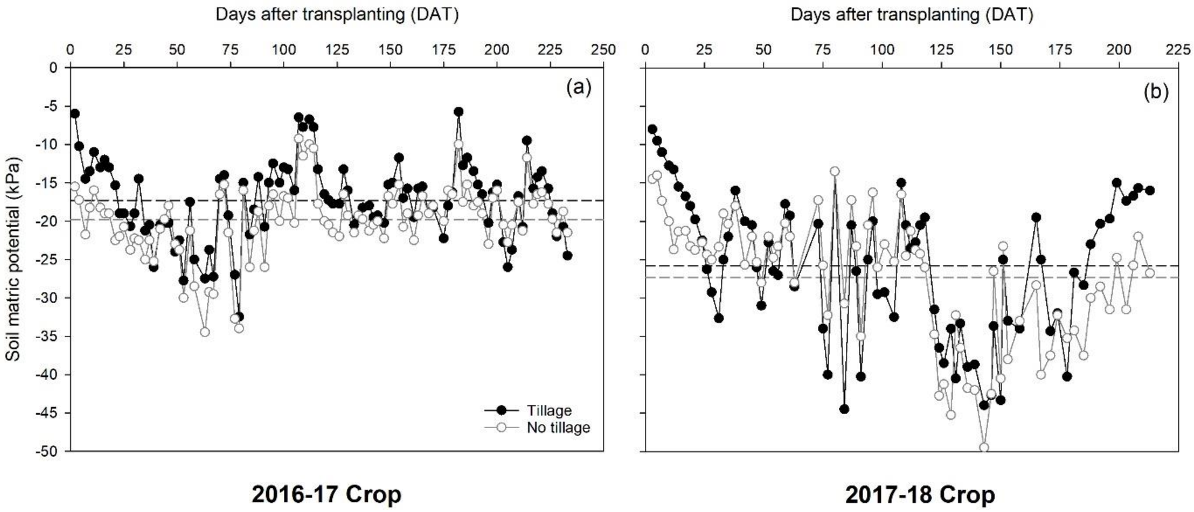


**Figure 2.** Daily soil matric potential in the two treatments (tillage and no tillage) during two sweet pepper crops grown in soil in a greenhouse. Panel (a) shows data of the 2016-17 crop, and panel (b) shows data of the 2017-18 crop. Values are means of four replications per treatment. Horizontal dotted lines represent the average over the entire crop cycle.

Total N applied and irrigation were notably reduced in the tillage treatment in both crops (Table 1). The averaged reductions in the tillage treatment were of 12% for irrigation, and 12.5% for total N applied (Table 1; Fig. 3). There were large reductions in drainage and N leaching in the tillage treatments, being 91% for drainage, and 95% for N leaching (Table 1; Fig. 3).

**Table 1.** Crop details and significance of t-tests between the two treatments (tillage and no tillage), of two sweet pepper cropping cycles conducted in soil in a greenhouse. Within each crop and variable, different lower-case letters indicate significant differences between treatments at $p$<0.05. Values are means ± SE.

| **Crops** | **Treatment** | **N initial‡ (kg N ha$^{-1}$)** | **[N] nutrient solution (mmol L$^{-1}$)** | **Irrigation (mm)** | **N applied (kg N ha$^{-1}$)** | **Drainage (mm)** | **N leached (kg N ha$^{-1}$)** | **N residual‡ (kg N ha$^{-1}$)** |
|---|---|---|---|---|---|---|---|---|
| 2016-17 | Tillage | 84±12a | 9.7 | 413 | 538 | 14±5a | 27±11a | 192±42a |
| | No tillage | 54±4a | 9.6 | 502 | 647 | 112±14b | 72±8b | 312±59a |
| 2017-18 | Tillage | 49±8a | 9.7 | 383 | 519 | 3±0a | 0±0a | 113±18a |
| | No tillage | 36±9b | 9.9 | 407 | 561 | 60±1b | 18±3b | 169±15b |

‡10-70 cm soil depth

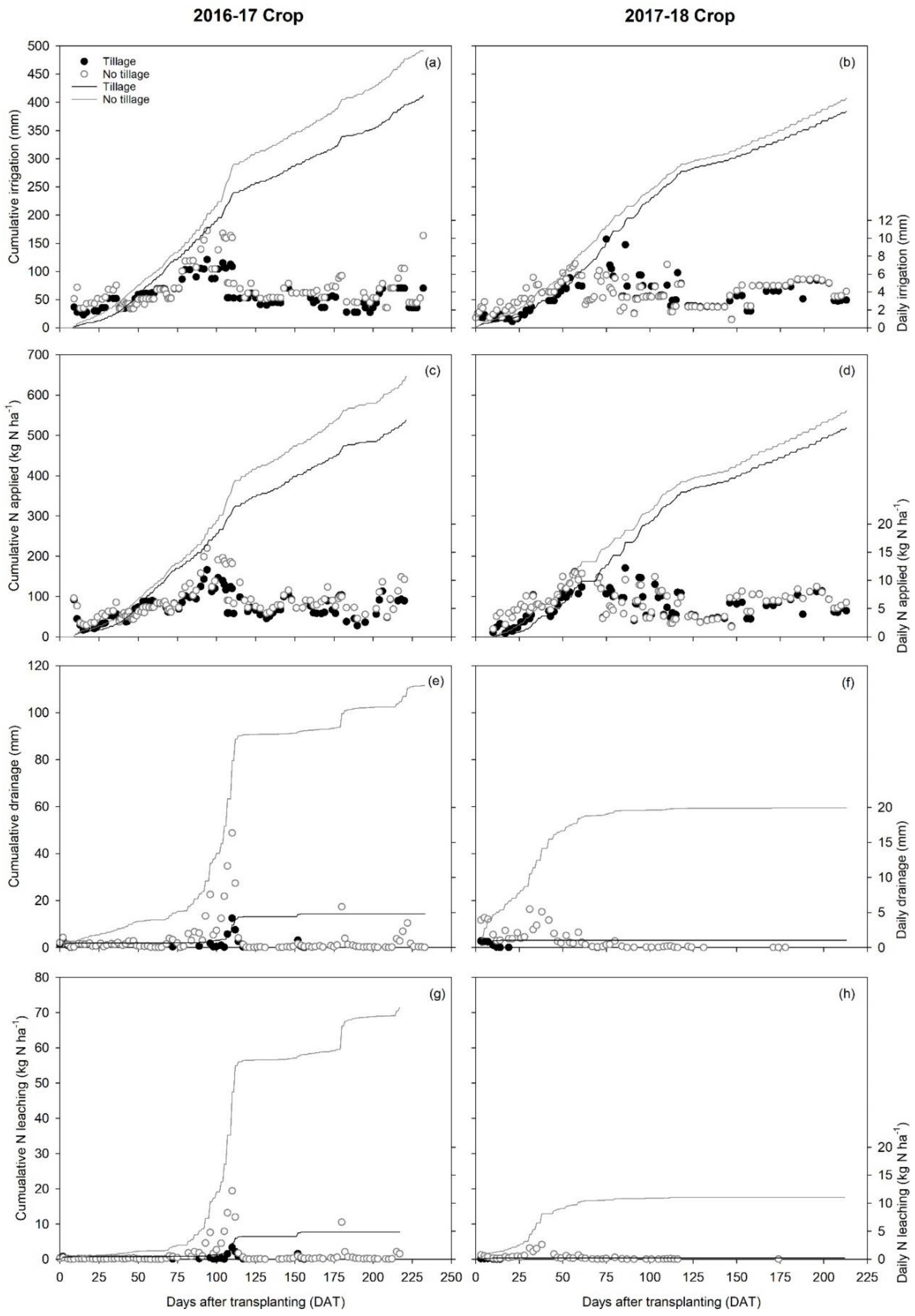


**Figure 3**. Irrigation, N applied, drainage and N leaching in the two treatments (tillage and no tillage) during two sweet pepper crops grown in soil in a greenhouse. Lines show cumulative values (left axis) and dots show daily values (right axis).

Irrigation and the amount of N applied through fertigation was lower in the 2017-18 crop (Table 1; Fig. 4). Drainage and N leaching was lower in the 2017-18 crop (Table 1; Fig. 4). Regarding the comparison between tillage treatments, N concentration in nutrient solution of fertigation (Table 1) in the tillage and no tillage treatments were maintained in very similar values for both treatments.

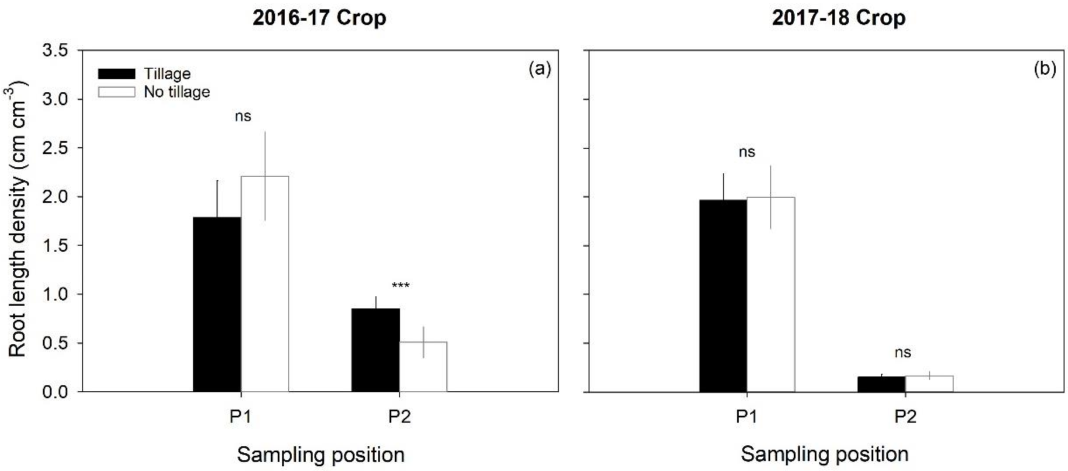


**Figure 4.** Root length density in the whole soil profile (0-40 cm), at 10 cm (P1) and 30 cm (P2) from the plant, in the tillage and no tillage treatments, of two sweet pepper crops grown in soil in a greenhouse. Panel (a) shows data of the 2016-17 crop, and panel (b) shows data of the 2017-18 crop. Values are means ± SE; ns, $p$<0.05; ***, $p$<0.001.

### *5.4.3. Crop dry matter and yield*

There was no significant effect of tillage on crop dry matter and yield in any of the two crops (Table 2). Crop N uptake was significantly higher (13%) in the tillage treatment compared to the no tillage treatment in the 2016-17 crop; there were no significant differences in the 2017-18 crop.

| Crops | Treatment | Dry matter (t ha$^{-1}$) | Yield (t ha$^{-1}$) | N uptake (kg N ha$^{-1}$) |
|---|---|---|---|---|
| 2016-17 | Tillage | 15.3±0.7a | 91.5±4.2a | 418.8±21.7a |
| | No tillage | 13.7±0.8a | 89.4±4.2a | 365.1±24.3b |
| 2017-18 | Tillage | 10.6±0.1a | 61.0±1.7a | 274.5±11.3a |
| | No tillage | 11.2±0.3a | 62.0±5.5a | 289.5±2.9a |

**Table 2.** Values of aboveground dry matter, fresh fruit yield and crop N uptake, and results of t-tests between the two treatments (tillage and no tillage), of two sweet pepper crops grown in soil in a greenhouse. Within each crop and variable, different lower-case letters indicate significant differences between treatments at $p<0.05$. Values are means ± SE.

### *5.4.4. Root analyses*

Tillage had a significant effect on root length density in the first crop (2016-17 crop) but not in the second crop (2017-18 crop) (Table 3). The effect of tillage on root length density in the 2016-17 crop was dependent on the sampling position as revealed by the significant Tillage × Position interaction but was independent of layer (Table 3). Tillage significantly increased root length density in the P2 sampling position (*i.e.*, at 30 cm from the plant), but not in the P1 sampling position (at 10 cm from the plant), regardless of the soil layer (Fig. 4).

Tillage did not have significant effects on relative root length distribution, in either crop (Table 3). There was a marginal Tillage × Layer interaction in the 2016-17 crop ($p=0.069$) whereby tillage decreased root length in the gravel mulch layer and increased root length in the rest of soil layers (Fig. 5).

**Table 3.** Results of analysis of variance (ANOVA) testing the effect of tillage, sampling position and soil layer, on root length density and relative root length distribution, of two sweet pepper crops grown in soil in a greenhouse.

| Crop | Effect | df | Root length density | | Relative root length distribution | |
|---|---|---|---|---|---|---|
| | | | F-value | *p*-value | F-value | *p*-value |
| 2016-17 | Block | 3 | 2.05 | 0.114 | 0.02 | 0.996 |
| | Tillage (T) | 1 | 41.40 | <0.001 | 0.44 | 0.510 |
| | Position (P) | 1 | 66.53 | <0.001 | 0.23 | 0.631 |
| | Layer (L) | 3 | 38.90 | <0.001 | 80.04 | <0.001 |
| | T × P | 1 | 11.84 | <0.001 | 0.00 | 0.999 |
| | T × L | 3 | 1.97 | 0.126 | 2.46 | 0.069 |
| | P × L | 3 | 3.16 | 0.017 | 6.59 | <0.001 |
| | T × P × L | 3 | 2.05 | 0.114 | 0.02 | 0.996 |
| | Error | 81 | | | | |
| 2017-18 | Block | 3 | 0.94 | 0.425 | 0.17 | 0.914 |
| | Tillage (T) | 1 | 0.09 | 0.768 | 0.39 | 0.535 |
| | Position (P) | 1 | 275.31 | <0.001 | 1.72 | 0.192 |
| | Layer (L) | 3 | 25.56 | <0.001 | 19.52 | <0.001 |
| | T × P | 1 | 0.03 | 0.871 | 0.09 | 0.764 |
| | T × L | 3 | 0.54 | 0.654 | 1.23 | 0.305 |
| | P × L | 3 | 16.21 | <0.001 | 4.46 | 0.006 |
| | T × P × L | 3 | 0.34 | 0.797 | 0.73 | 0.538 |
| | Error | 100 | | | | |

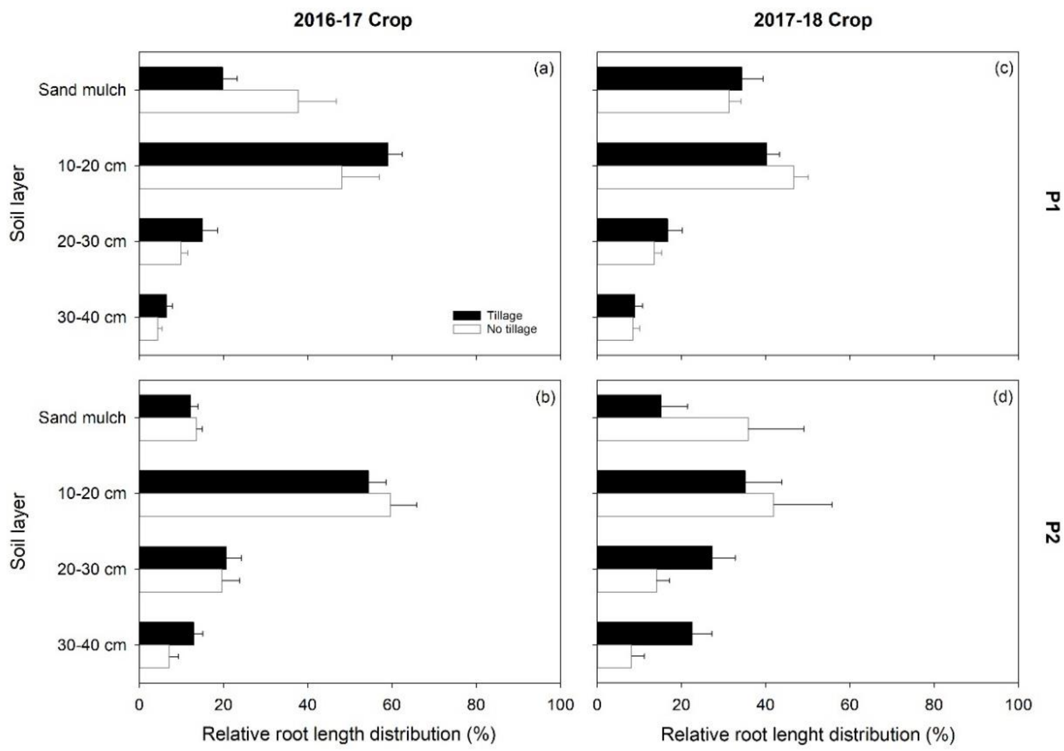


**Figure 5.** Relative root length distribution (%) in the two treatments (tillage and no tillage), at two sampling positions, P1 (at 10 cm from the plant) and P2 (at 30 cm from the plant), of two sweet pepper crops grown in soil in a greenhouse. Panels (a) and (b) shows data of the 2016-17 crop, and panels (c) and (d) shows data of the 2017-18 crop. Values are means ± SE.

Tillage had no significant effect on root length dynamics observed throughout the crop in minirhizotron images in the 2017-18 crop (Table 4; Fig. 6). Root length growth rate in the 0-22 cm soil layer was estimated at 96±6 m $m^{-2}$ $year^{-1}$ in the tillage treatment and 104±16 m $m^{-2}$ $year^{-1}$ in the no tillage treatment ($p$=0.680). In the 22-44 cm soil layer, root length growth rate was estimated at 52±7 m $m^{-2}$ $year^{-1}$ in the tillage treatment and 73±14 m $m^{-2}$ $year^{-1}$ in the no tillage treatment ($p$=0.314).

**Table 4.** Results of repeated-measure analysis of variance (ANOVA) testing the effect of tillage and soil layer, on root length dynamics observed in minirhizotron tubes, of the 2017-18 sweet pepper grown in soil in a greenhouse.

| Effect | df | F-value | *p*-value |
|---|---|---|---|
| Block (B) | 3 | 1.19 | 0.344 |
| Tillage (T) | 1 | 0.02 | 0.885 |
| Layer (L) | 1 | 48.94 | <0.001 |
| T × L | 1 | 0.91 | 0.355 |
| Error | 16 | | |
| Date (D) | 12 | 277.15 | <0.001 |
| D × T | 12 | 1.34 | <0.197 |
| D × L | 12 | 8.57 | <0.001 |
| D × T × L | 12 | 1.14 | 0.328 |
| Error | 192 | | |

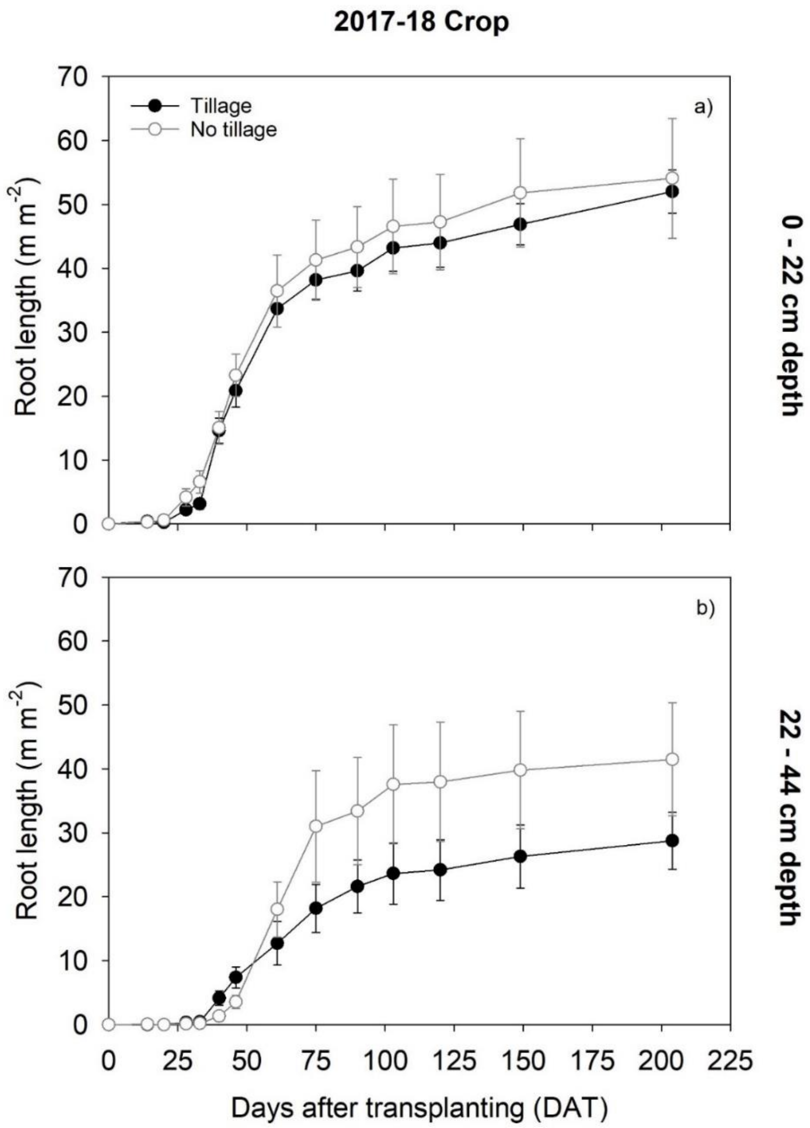


**Figure 6.** Root length dynamics observed through minirhizotron tubes in the two treatments (tillage and no tillage), at two soil layers, 0-22 and 22-44 cm depth of a 2017-18 sweet pepper crop grown in soil in a greenhouse. Values are means ± SE.

### 5.5. Discussion

Several studies have shown reduced soil compaction after tillage in greenhouse-grown vegetable crops, such as Erdem *et al*. (2006) and Padilla *et al*. (2017) in sweet pepper, and Castilla (1986) in tomato. In the present study, it is possible that reduction of soil compaction due to tillage was less than expected because of movement of machinery during tillage, which is a cause of soil compaction, the effect of which is accentuated in moist soils (Batey, 2009). In the confined space of this experimental greenhouse, manoeuvring the tractor and tractor-mounted equipment was not straightforward. It is also possible that ploughing with a single pass with ripper to 15 cm depth, followed by a single pass with rotavator to 10 cm depth, were not the most adequate procedures.

There was a notable reduction in soil penetration resistance in the tillage treatment. This effect was greatest at the beginning of the first crop (2016-17 crop) and was progressively diluted until the end of the second crop (2017-18 crop). The constant passage of personnel for cultural practices and manually pushed carts may have increased soil penetration resistance during the first crop, in addition to a soil that is constantly kept close to field capacity (Francisco M. Padilla et al., 2017c). These conditions of high soil moisture may have causes the soil compaction processes to be accelerated (García *et al*., 2016). This is coincident with Erdem *et al*. (2006) in a sweet pepper crop grown in a greenhouse: soil penetration resistance increased during the first 40 days of the cycle due to irrigation management and mechanical weed control.

This study succeeded in maintaining very similar soil water status in the root zone in both treatments. The averaged soil matric potential recorded in the two treatments, during the two crops, was -22.6 kPa; this value is sufficient enough to prevent water stress in sweet pepper as the leaf water potential threshold values for water stress, for sweet pepper in greenhouse-grown crops, was determined at -58 kPa (Thompson *et al*., 2007b). Similar soil matric potentials in the tillage and no tillage treatments were achieved by adjusting irrigation volumes to maintain the soil matric potential, at 25 cm depth relative to the gravel mulch, within the range of -15 and -25 kPa. As a result of this irrigation management, irrigation volume and consequently total N applied through fertigation was reduced 12 and 12.5% in the tillage treatment, respectively. It is possible that tillage increased the rate of water infiltration into the soil, which would result in increased soil water retention (Hamza & Anderson, 2005), which is consistent with results of Wang *et al*. (2015) where increased soil water content was found in response to tillage in greenhouse-grown pepper crops. On the contrary, in the treatment without tillage, the irrigation volume had to be greater to maintain the same soil matric potential (Quincke *et al*., 2007).

The present study found that tillage notably reduced drainage (91%) and consequently N leaching (95%) compared to the no tillage treatment, suggesting that tillage increased soil water infiltration. There is consensus in literature that reduced soil bulk density after tillage generally increases infiltration rates and nutrient leaching losses (Hamblin, 1986; Passioura, 1991; Hamza and Anderson, 2005; Quincke et al., 2007). In the treatment with tillage, the irrigation volume was also lower, in this way it is possible that the washing of N was also lower (Thompson *et al*., 2007b). In contrast, Pareja-Sánchez *et al*. (2017) reported that soil water infiltration was greatly reduced under tillage compared to no tillage in maize crops. The underlying mechanism seemed to be in the destruction of soil structure and formation of a tillage pan, resulting in lower soil water infiltration rates (Pareja-Sánchez *et al*., 2017).

Tillage had no significant effects on crop dry matter production and crop yield in either crop, but crop N uptake was increased during the first crop (2016-17 crop). Considering that N application was consistently lower in the tillage treatment throughout the crop, this result suggests more efficient N use in the tillage treatment. Although, tillage did not result in more crop growth and yield, it apparently increased N use efficiency. To increase yield of vegetable crops in this system, it may be necessary to find the optimal combination with other cropping factors. For instance, Padilla *et al*. (2017) reported that sweet pepper yield decreased in a tillage treatment with addition of compost compared to a conventional management with no tillage. The explanation was that compost addition increased salinity (Padilla *et al*., 2017). In other horticultural crops such as cabbage, growth and yield were similar with conventional tillage and reduced tillage (Mochizuki *et al*., 2007).

Despite tillage may not increase yield, crop development could be affected (Jones & Popham, 1997; Unger & Jones, 1998). In compacted soils, tillage can break crusts but this may not be enough to improve physical soil properties (Primavesi, 1982). Another possible explanation proposed by Wang *et al*. (2015) for the no effect of tillage on yield is that there may be other factors, such as irrigation, that condition yield more than tillage itself. In extreme cases, soil resistance can limit root growth and reduce crop yield regardless of soil moisture status (Whalley *et al*., 2008).

Tillage had little effects on root length density and relative root length distribution in both crops. The only effect detected was higher root length density at 30 cm from the plant (*i.e.*, at the P2 sampling position) in the first crop, which would indicate that tillage favoured horizontal root extension. Root penetration in the soil profile has been shown to decrease when the soil bulk density exceeds 1.6 kg $L^{-1}$, but this effect is dependent on soil moisture (Primavesi, 1982). In the present study, tillage reduced soil bulk density from 1.70 to 1.60 kg $L^{-1}$, which could still be excessive for root penetration.

In contrast to the present study, previous work in “enarenado” soils in Almería greenhouses reported that tillage decreased root density in the gravel mulch layer (0-10 cm depth) and increased root density in the 10-20 cm soil layer (Castilla, 1986; Martinez, 1987; Padilla *et al*., 2017). It is possible that in the present study that the reduction in soil penetration resistance, achieved with tillage, was not sufficient to enhance root penetration. Indeed, values of 1700 kPa of soil penetration resistance registered in the present study in the tillage treatment, at 25 cm soil depth, were notably higher than the values of soil penetration resistance of 1300 kPa reported by Erdem *et al*. (2006) at 20 cm soil depth, also with a sweet pepper crop.

Root production, estimated from minirhizotron images, were consistent with results of destructive root analysis, with no significant differences between tillage treatments. However, it is worth highlighting that there was a trend towards higher root length in the no tillage treatment in the 22-44 cm soil layer. This treatment received a greater amount of water and nutrients to reach the target soil water potential and had larger drainage volumes and more $NO_3^-$ leaching than the tillage treatment. It is reasonable to expect that more N was located in the deeper soil layers of this treatment, and that the tendency for higher root proliferation in this layer was a response to location of N at depth (Kristensen & Thorup-Kristensen, 2007). It is possible that in this way that the interaction of tillage with other factors such as fertilization and irrigation affected the dynamics of root growth.

Overall, this study has shown that tillage did not enhance crop dry matter production or yield of either of the two cropping seasons. However, irrigation, N applied, drainage and N leaching were notably reduced with tillage, most likely due to increase infiltration capacity. In addition, tillage increased crop N uptake, increasing N use efficiency. The absence of effects of tillage on root length density and relative root length distribution, together with the slight reduction in soil bulk density and high values of soil penetration resistance, suggest that soil compaction was little affected by tillage. In "enarenado" soils, tillage is problematic because the layer of gravel mulch must be removed prior to tilling. Results of the present study suggest that the tillage applied in the experimental greenhouse is not justified in terms of improved crop growth, yield and root production.

## 5.6. Acknowledgements

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# 6. Manuscript Three: Cultivar effects on fruit quality and yield of muskmelon and sweet pepper in response to increasing N levels applied by fertigation

Rafael Grasso, M. Teresa Peña-Fleitas, Romina de Souza, Alejandra Rodriguez, Rodney B. Thompson, Marisa Gallardo and Francisco M. Padilla

## 6.1. Abstract

Fruit quality and yield are the parameters that define most the profitability of vegetable farms. The muskmelon and sweet pepper crops are one of the most profitable rotations of greenhouse crops in Almería, south-eastern Spain. There is evidence that fruit quality may be affected by nitrogen (N) fertilization and cultivar, but these responses may act differently. In this study, the effect of different doses of N applied by fertigation, very deficient N (N1, 2 mmol $L^{-1}$), deficient N (N2, 8 mmol $L^{-1}$) and conventional N (N3, 13 mmol $L^{-1}$), and differences between three cultivars for each of the two species, were addressed in greenhouse crops grown in soil in Amería. In addition to fruit yield, fruit quality parameters such as fruit firmness, colour, ºBrix, pH, acidity and morphometric variables, were evaluated. For most of the parameters evaluated in both crops, there were no significant interactions between N and cultivar, indicating that effects of N and cultivar, whenever significant, occurred individually and consistently regardless of the level of the other factor. For fruit firmness in both crops, the N treatment did not affect this parameter. In general, in response to lower N fertilization (N1 treatment), the pulp was more orange in muskmelon and the skin was more reddish in the sweet pepper, than in the N2 and N3 treatments. There were differences in coloration between muskmelon and sweet pepper cultivars. Regarding ºBrix, N1 caused an 11% increase in ºBrix values for muskmelon and a 4% decrease for sweet pepper. In both crops, the highest yield was obtained in the N2 treatment. However, the best fruit quality attributes were not always achieved by N2 treatment in muskmelon. In this case, to increase orange pulp colour and ºBrix in fruits, lower N fertilizations rates are needed at the cost of decreasing fruit yield.

**Keywords**: *Capsicum annuum*; Colorimeter; *Cucumis melo;* ºBrix; fertilization; nitrogen.

## 6.2. Introduction

There is a high concentration of plastic greenhouses in south-eastern (SE) Spain, that are used for vegetable production. More than 30,000 hectares are located in Almeria province (Valera et al., 2014). A variety of vegetable crops are grown, mostly for export to northern European countries (Castilla and Hernandez, 2005). In Almeria province, two of the most important greenhouse-grown crops are sweet pepper and muskmelon, with annual cropped areas in 2012 of 8,406 and 3,740 ha, respectively (Valera et al., 2014). The sequence of autumn-winter grown sweet pepper and spring grown muskmelon is a common rotation in this system (Valera et al., 2014).

The high concentration of greenhouses in SE Spain is associated with intensive use of resources, such as water and fertilisers. In general, in commercial greenhouse vegetable production in this region, management of irrigation and fertilisers is based on the experience of producers and technical advisors (Thompson et al., 2007b). Local nitrogen (N) fertiliser recommendations are 175 kg N $ha^{-1}$ for muskmelon for an expected yield of 40-50 t $ha^{-1}$, and 350-450 kg N $ha^{-1}$ for sweet pepper for an expected yield of 50-60 t $ha^{-1}$ (Reche, 2010, 2008). Commonly, in this vegetable production system, N is applied in excess, which is associated with nitrate ($NO_3^-$) leaching loss which negatively impacts the local environment (Gallardo et al., 2006; Thompson et al., 2020a, 2020b, 2007a).

Crop yield increases asymptotically with N fertiliser application; the rate of N fertiliser required to maximize yield within a given system is uncertain for various reasons (Thompson et al., 2017). For this reason, N fertiliser is commonly applied in excess to ensure that N does not limit yield (Locascio et al., 1997; Warner et al., 2004). An insufficient N supply results in low yield and small fruits, whereas excessive N can delay fruit ripening (Crisosto et al., 1995; Daane et al., 1995), is an unnecessary cost, and is commonly associated with negative environmental impacts. In muskmelon and sweet pepper crops, the reported effects of increasing N on fruit quality are varied. In muskmelon, increasing N application either had no effect on °Brix (Monteiro et al., 2003; Purquerio et al., 2005), or increased °Brix (De Faria et al., 2000a). Applications of nearly 400 kg N $ha^{-1}$ resulted in muskmelon fruits with a larger seed cavity, thicker skin and firmer flesh, than applications of 0–150 kg N $ha^{-1}$ (Castellanos et al., 2012; Monteiro et al., 2003).

In sweet pepper, increasing N applications up to 1,300 kg N $ha^{-1}$ did not affect either physical or chemical fruit quality, including sugar content and acidity (Yasuor et al., 2013). However, Xiang et al. (2018) reported that 225 kg N $ha^{-1}$ improved soluble solids content, vitamin C content and soluble solids content, compared to 0, 100 and 300 kg N $ha^{-1}$. In general, the reported effects of increasing N application on fruit quality of muskmelon and sweet pepper are inconsistent.

There is appreciable research reporting the effects of cultivar on various parameters of fruit quality and yield of vegetable crops as tomato and strawberry (Warner et al., 2004; Ketelaere et al., 2004; Lado et al., 2012). Much of the research has focussed on tomato, and has reported effect of cultivar on the red colour of fruit, firmness, soluble solids and commercial yield (Warner et al., 2004; Kaniszewski et al., 2019; Ketelaere et al., 2004; Sams, 1999). In muskmelon crops, significant differences were found between cultivars in fruit morphometric parameters, such as pulp percentage, seed percentage and shape, and yield (Alenazi et al., 2015). Regarding parameters of internal fruit quality, differences between cultivars of muskmelon have also been reported in total soluble solids content, titratable acidity and beta carotenes (Botía et al., 2005; Sharma et al., 2014). In the case of sweet pepper, differences between cultivars were also found in fruit quality compounds such as beta carotene, ascorbic acid, total flavonoids and soluble phenols (Szafirowska and Elkner, 2008).

The high concentration of greenhouses in SE Spain and the overuse of N fertiliser, in this system, have caused substantial aquifer contamination with $NO_3^-$. In accordance with the European Union legislation (EEC, 1997), most of the greenhouse cropping areas of SE Spain have been declared as Nitrate Vulnerable Zones (Junta de Andalucia, 2008) which requires implementation of improved crop management practices to reduce $NO_3^-$ contamination of water bodies (Padilla et al., 2018; Thompson et al., 2020a, 2007a). An additional and important consideration is that consumers are increasingly demanding high product quality and environmental friendly production of fruit and vegetables (Thompson et al., 2020a; Valera et al., 2014). Given the importance of muskmelon and sweet pepper, and the N management issue in the greenhouse system of SE Spain, information is required of cultivar and N effects on fruit quality of these two crops. The objectives of this work were to evaluate the responses of parameters of internal and external fruit quality, and of yield, to increasing N fertilisation in three cultivars of both muskmelon and sweet pepper grown in a greenhouse in soil.

## 6.3. Materials and Methods

### *6.3.1. Crops and experimental site*

One crop of muskmelon (*Cucumis melo* L.) and another of sweet pepper (*Capsicum annuum* L.) were grown in soil in a greenhouse in Almeria (SE Spain, 36° 51’ N latitude, 2° 16’ W longitude; 92 m above sea level). Growing conditions were very similar to commercial greenhouses in the area. In the greenhouse, the cropped area was approximately 1,300 $m^2$. The soil was artificially stratified at greenhouse construction; it consisted of a layer of silty loam soil (30 cm thickness), placed over the naturally-occurring soil; a layer of coarse sand (10 mm thickness) acted as mulch. This type of soil is the typical of the region and is locally known as “enarenado” (Valera et al., 2016).

Drip irrigation and fertigation were used. Drippers with a flow rate of 3 L $h^{-1}$ were positioned every 50 cm in drip lines, arranged in paired lines with a separation of 80 cm between the two paired drip lines. There was 120 cm between adjacent paired lines. There were two emitters per $m^{-2}$. Seedlings were planted 6–8 cm from each dripper, perpendicular to the drip line. Twelve 12 x 6 m plots were used. Polyethylene film sheets buried to 30 cm depth in the borders of the plots prevented water movement between plots.

All cultural practices were conducted according to local crop management. Climatic conditions were recorded inside the greenhouse throughout both crops. Data were stored in a datalogger. The application of the N treatments, through fertigation, commenced 9 and 6 days after transplanting (DAT), for the muskmelon and sweet pepper crops, respectively. Complete nutrient solutions were applied for macro and micronutrients.

*6.3.2. Experimental design*

The muskmelon crop was transplanted on 27 February and harvested on 11 June 2020 (105 days). Three cantaloupe-type muskmelon cultivars were evaluated: TEZAC (Seminis, Inc., Bayer AG, Leverkusen, Germany), MAGIAR (Nunhems, BASF SE, Ludwigshafen, Germany) and JACOBO (Semillas Fitó, Barcelona, Spain). The sweet pepper crop was transplanted on 22 July 2020 and harvested on 28 January 2021 (190 days). Three cultivars of sweet pepper were used: MELCHOR (Zeraim Iberica, Syngenta Crop Protection AG, Basel, Switzerland), MACHADO (Hazera Seeds Ltd., Limagrain

Group, Saint Beauzire, France) and CLX PLRJ 731 (HM.CLAUSE SAS, La Motte, Portes-lès-Valence, France). In both crops, the three cultivars were planted in each experimental plot; two paired lines of plants (i.e., four lines) were planted with each cultivar, in each plot. The position of the paired lines of each cultivar in each plot was randomized.

In both crops, three treatments with different N concentration were applied in the nutrition solution by fertigation. Intended N concentrations for both crops were 2, 8 and 14 mmol N $L^{-1}$, for very deficient N (N1), deficient N (N2), and conventional N application (N3), respectively, according to local practices (Camacho and Fernandez, 2013). Concentration of other macro and micronutrients were applied at the same non-limiting concentration in all three treatments: $HPO_4^-$, 2 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 $L^{-1}$. Irrigation volume was adjusted to maintain the soil matric potential in the range -15 to -25 kPa at 10 cm depth from the surface of the imported loam soil. For this, one tensiometer (Irrometer, Co., Riverside, CA, USA) was installed in the same cultivar in each replicate plot of each crop.

The treatments are described in terms of applied N concentration (mmol $L^{-1}$) and amount of mineral N applied ($NO_3^-$–N + $NH_4^+$–N; kg N $ha^{-1}$) in Table 1. For all treatments, N was applied mostly as $NO_3^-$ (93% in muskmelon and 88% in sweet pepper), the rest as ammonium ($NH_4^+$).

**Table 1.** Mineral N ($NO_3^-$–N + $NH_4^+$–N) in soil (0-60 cm depth) at the beginning of each crop, N concentration in the nutrient solution applied and mineral N amount applied in fertigation in the two crops.

| Crop | N treatment | Mineral N at planting (kg N ha$^{-1}$) | N concentration in nutrient solution (mmol L$^{-1}$) | N amount applied (kg N ha$^{-1}$) |
|---|---|---|---|---|
| Muskmelon | Very deficient (N1) | 86 | 2.7 | 61 |
| | Deficient (N2) | 96 | 8.3 | 302 |
| | Conventional (N3) | 65 | 14.0 | 582 |
| Sweet pepper | Very deficient (N1) | 30 | 2.0 | 65 |
| | Deficient (N2) | 17 | 7.6 | 425 |
| | Conventional (N3) | 41 | 13.0 | 700 |

*6.3.3. Fruit yield evaluation*

Total yield was calculated by summing the fresh weight of mature fruit in muskmelon and of red fruits in sweet pepper that were collected throughout the crops, from eight marked plants, of each cultivar, in each plot. In the muskmelon crop, there were two fruit harvests at 96 and 104 DAT. In the sweet pepper crops, there were six harvests between 98 and 187 DAT; fruit quality was evaluated in the fourth and sixth harvests conducted at 100 and 120 DAT, respectively. For both crops, marketable and non-marketable fruit were quantified, and average individual fresh fruit weight and number were recorded. All non-marketable fruit were categorized by criteria. For muskmelon, the criteria were cracking, sunstroke, malformation and <500 g fresh weight, according to Visconti et al., (2019). For sweet pepper, the criteria were malformation, diseases, and discoloration according to del Amor et al., (2009). In muskmelon and sweet pepper, equatorial and polar diameters of the fruit were measured in 10 randomly selected fruits in each cultivar and replicate plot. In the muskmelon crop, both diameters were measured in the first harvest at 96 DAT; in sweet pepper, both diameters were measured in two harvests at 100 and 120 DAT

*6.3.4. Fruit quality evaluation*

Fruit quality parameters were evaluated in muskmelon in the first harvest (96 DAT), and in sweet pepper, in the 100 and 120 DAT harvests. In both crops, four fruits were randomly selected per cultivar and plot. The fruits were immediately taken to the laboratory where internal colour measurements were made of the flesh in muskmelon, and external colour measurements were made on the skin in sweet pepper. Colour measurements were made using a chroma meter (Minolta CR-400, Konica Minolta, Osaka, Japan) for determination of lightness (L*), red/green (a*) and blue/yellow (b*) coordinates. For muskmelon, the fruits were cut into halves, and colour was measured in one half of the fruit in three points equidistant from the equatorial zone. In sweet pepper, colour was measured on the external skin in three points equidistant from the equatorial zone. Pitch angle (h) and the chroma (C*) were calculated as h = arctan (a* / b*), and C* = sqrt $((a^*)^2 + (b^*)^2)$. The colour index (IC*) was calculated as IC*= (a* x 1000) / (L*x b*).

Firmness of fruit was determined in the interior of one half of the muskmelon fruit, and on the external skin of sweet pepper, in three equidistant points per fruit using a hand-held penetrometer (PCE-PTR 200N, PCE Ibérica S.L., Albacete, Spain).

To determine ºBrix, titratable acidity and pH, measurements were made in the juice of muskmelon pulp and of whole fruits of sweet pepper. In both crops, individual fruit samples were processed per cultivar and plot. In muskmelon, the skin and seeds were removed; in sweet pepper, the peduncle and the seeds were removed. The juice was obtained using a domestic kitchen blender. ºBrix was determined with a digital refractometer (PAL-1, ATAGO CO., LTD, Tokyo, Japan). pH was determined with a hand-held pHmeter (LAQUA PC110-K, Horiba, Ltd., Kyoto, Japan). Titratable acidity for both crops was determined using the following procedure: 10 mL of fruit juice was mixed with 50 mL of distilled water and a few drops of phenolphthalein indicator. The solution was titrated with 0.1 M NaOH until the indicator turned from clear to pink (Domene and Segura, 2014).

### *6.3.5. Data analysis*

Differences in measured parameters between N treatments and cultivars were evaluated by factorial analysis of variance (ANOVA), followed by pairwise LSD posthoc tests when the N x Cultivar interaction, or the main effects, were significant at $p<0.05$. Plot was included as a block factor in ANOVA. If needed, variables were transformed to meet ANOVA assumptions. Statistical analysis was performed with STATISTICA 13 (TIBCO Software, Inc., Palo Alto, CA, USA).

## 6.4. Results

The Nitrogen x Cultivar interaction was not statistically significant in the following parameters evaluated: fruit firmness, fruit colour, ºBrix, acidity, morphometric parameters, yield, and percentage of fruit discard, for muskmelon, and fruit firmness, fruit colour (L*, b*, h, IC*), ºBrix, pH, and acidity, in sweet pepper (Tables 1-5). Therefore, effects of N treatments and cultivars were described as main effects in all parameters, analysing either the effect of the N or the cultivar in isolation.

### 6.4.1. *N effects on fruit quality and yield*

There were not significant differences in fruit firmness between N treatments, both for muskmelon and sweet pepper crops (Table 2; Figure 1).

**Table 2.** Results of factorial analysis of variance (ANOVA) testing the effect of three N treatments and three different cultivars on external fruit firmness, in muskmelon and sweet pepper.

| | | **Muskmelon** | | **Sweet pepper** | |
|---|---|---|---|---|---|
| Effect | df | F | P | F | P |
| Block (B) | 3 | 11.78 | <0.001 | 0.28 | 0.840 |
| Nitrogen (N) | 2 | 0.77 | 0.474 | 0.52 | 0.603 |
| Cultivar (C) | 2 | 12.00 | <0.001 | 47.15 | <0.001 |
| N x C | 4 | 1.23 | 0.323 | 0.13 | 0.970 |
| Error | 24 | | | | |

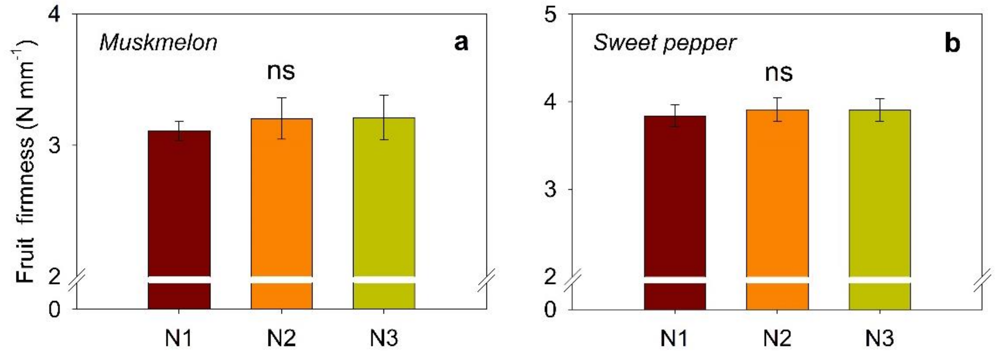


**Figure 1.** Fruit firmness (N $mm^{-1}$) for the different N treatments, for muskmelon (a) and sweet pepper (b) crops. Values have been averaged across the three cultivars in each species. In muskmelon, fruit measurements are internal, in sweet pepper, measurements are external. ns: not significant differences between N treatments, at p<0.05. Values are means ± SE.

In muskmelon, significant differences were found between N treatments in L* (lightness) and b* (yellowness) coordinates, and in h (pitch angle), C* (chroma) and IC* (colour index), but not in a* (red colour) coordinate (Table 3). For L* and b* coordinates and h and C*, values were significantly lower in N1 than in N2 and N3 (Figure 2a, c, d). In the case of IC*, N1 and N2 treatments were statistically higher than the N3 treatment (Figure 2f). In sweet pepper, significant differences were found between N treatments in

L*, a*, b*, h, C* and IC* (Table 3). For L* and b* coordinates and h, values were significantly lower in N1 than in N2 and N3 (Figure 2g, k, h). In the case of IC*, N1 treatment was statistically higher than N2 and N3 treatments (Figure 2l). For a* and C* coordinates, differences between nitrogen levels depended on the cultivar (Figure 2h, k).

**Table 3.** Results of factorial analysis of variance (ANOVA) testing the effect of three N treatments and three different cultivars on internal fruit colour coordinates L* (lightness), a* (red colour), b* (yellowness), and on calculated colour parameters of h (pitch angle), C* (chroma) and IC* (colour index), in muskmelon and sweet pepper. Significance is shown as: ns, p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001.

| | | **Muskmelon** | | | | | | **Sweet pepper** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | F-value | | | | | | F-value | | | | | |
| Effect | df | L* | a* | b* | h | C* | IC* | L* | a* | b* | h | C* | IC* |
| Block (B) | 3 | 3.65* | 1.80ns | 4.01* | 0.70ns | 3.10* | 1.45ns | 1.24ns | 1.54ns | 0.83ns | 1.47ns | 1.76ns | 1.06ns |
| Nitrogen (N) | 2 | 3.91* | 0.26ns | 12.82*** | 5.96** | 9.12*** | 8.59** | 13.85*** | 0.26ns | 18.67*** | 6.54** | 3.13ns | 15.86*** |
| Cultivar (C) | 2 | 11.77*** | 142.80*** | 123.70*** | 304.66*** | 79.87*** | 284.87*** | 10.99*** | 15.43*** | 15.12*** | 2.47ns | 21.77*** | 3.70* |
| N x C | 4 | 2.16ns | 2.49ns | 1.94ns | 0.93ns | 1.64ns | 1.30ns | 0.78ns | 6.19*** | 2.21ns | 2.07ns | 6.07** | 1.61ns |
| Error | 24 | | | | | | | | | | | | |

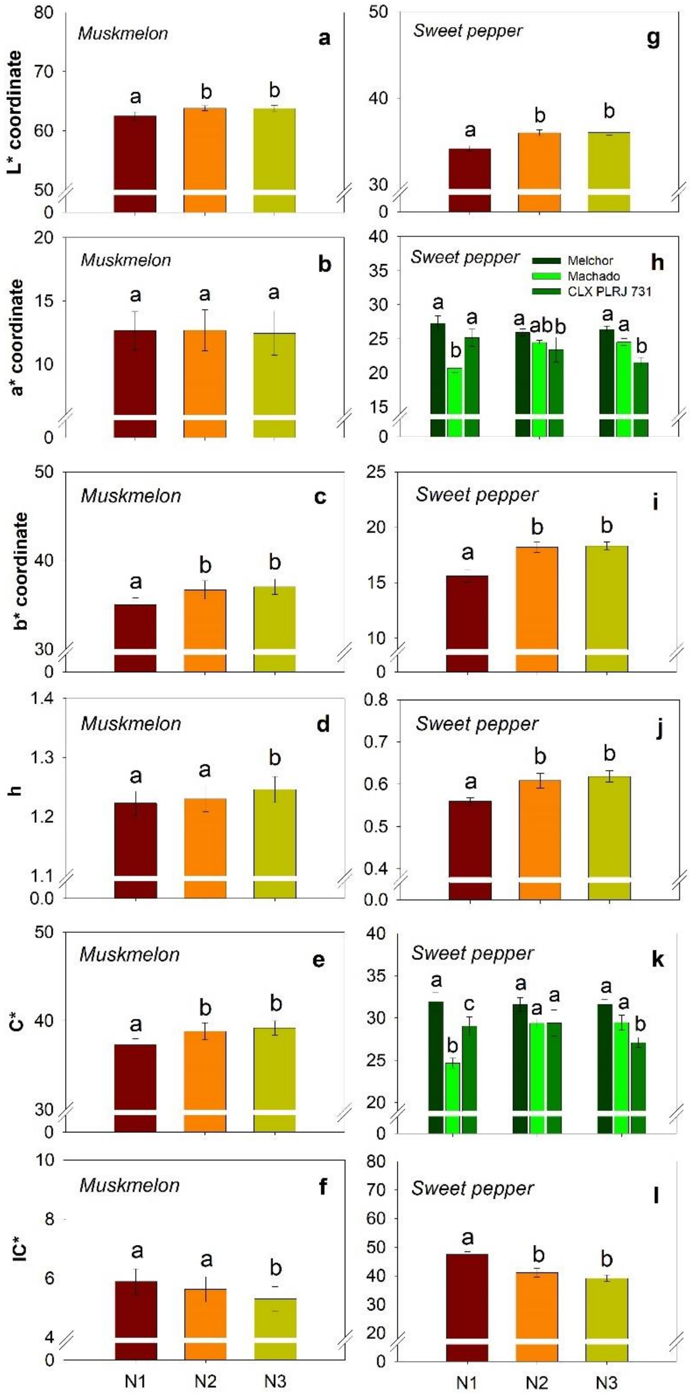


**Figure 2.** Fruit colour coordinates L* (lightness; a, g), a* (red colour; b, h) and b* (yellowness; c, i) and parameters of internal fruit colour h (pitch angle; d, j), C* (chroma; e, k) and IC* (colour index; f, l), for the different N treatments, for muskmelon crop (a-f), and for sweet pepper (g-l). Fruit measurements are internal. Values have been averaged across the three cultivars except in panels h and k. Different lower-case letters above each column show significant differences between N treatments, at $p<0.05$, except in panels h and k that show differences between cultivars within each N treatment. Values are means ± SE.

Regarding the parameters of internal fruit quality in muskmelon and sweet pepper, significant differences were found between N treatments in all three °Brix, pH and acidity (Table 4; Figure 3). In muskmelon, the °Brix was statistically 12% higher in N1 treatment compared to N2 and N3 treatments (Figure 3a); however, pH and acidity were 2% and 15% lower in N1 treatment than in N2 and N3 treatments, respectively (Figure 3b, c). In sweet pepper, the °Brix was statistically 4 % lower in N1 treatment compared to N2 and N3 treatments (Figure 3d). pH was 2% lower in N1 treatment than in N2 and N3 treatments (Figure 3e) and acidity was 18% and 6% higher in N2 than in N1 and N3, respectively (Figure 3f).

**Table 4.** Results of factorial analysis of variance (ANOVA) testing the effect of three N treatments and three cultivars on internal fruit quality parameters (°Brix, pH and acidity), in muskmelon and sweet pepper. Significant effects are shown as: ns, p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001.

| | | **Muskmelon** | | | **Sweet pepper** | | |
|---|---|---|---|---|---|---|---|
| | | F-value | | | F-value | | |
| Effect | df | °Brix | pH | Acidity | °Brix | pH | Acidity |
| Block (B) | 3 | 4.11* | 1.86ns | 1.47ns | 3.56* | 0.30ns | 0.60ns |
| Nitrogen (N) | 2 | 11.79*** | 8.08** | 4.80* | 6.68*** | 4.10*** | 5.30*** |
| Cultivar (C) | 2 | 58.58*** | 57.14*** | 7.12** | 63.79*** | 20.90*** | 2.12ns |
| N x C | 4 | 0.67ns | 3.00* | 1.40ns | 0.80ns | 2.10ns | 0.36ns |
| Error | 24 | | | | | | |

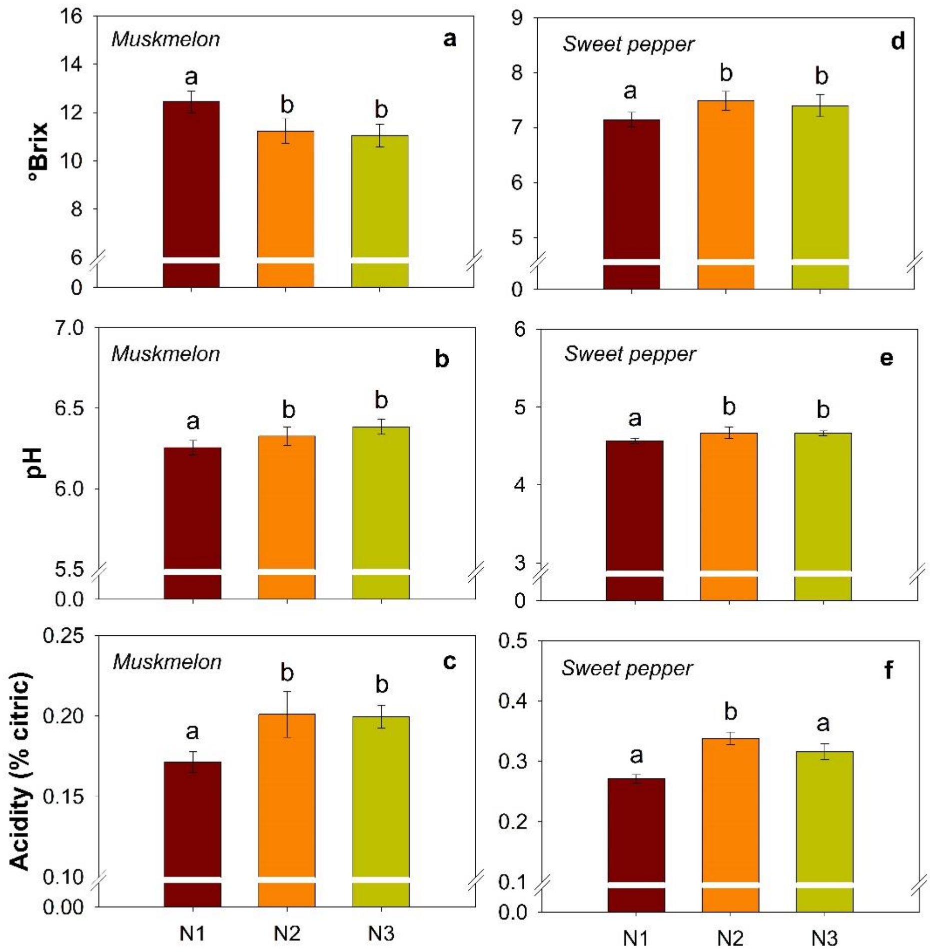


**Figure 3**. Internal fruit quality parameters (°Brix (a, d), pH (b, e), and acidity (% citric acid) (c, f)) at three different N treatments in muskmelon and sweet pepper. Values have been averaged across three cultivars. Different lower-case letters above each column show significant differences between N treatments, at $p<0.05$. Values are means ± SE.

Regarding the effect of N treatments on morphometric parameters of muskmelon, there were significant differences, between N treatments, in fruit weight and equatorial and polar diameters (Table 5; Figure 4). For these three parameters, the N1 treatment had statistically lower values than the N2 and N3 treatments, which were not statistically different from one another (Figure 4a–c). The N1 treatment had muskmelon fruits with 13% lower weight than the N2 and N3 treatments. Regarding the effect of N treatments on morphometric parameters of sweet pepper fruit, there were significant differences between N treatments only for polar diameter (Table 5; Figure 4); the polar diameter in the N2 treatments was 9% lower than in N1 and N3 treatments (Figure 4f).

**Table 5.** Results of factorial analysis of variance (ANOVA) testing the effect of three N treatments and three different cultivars on fruit weight and equatorial and polar fruit diameters, in muskmelon and sweet pepper. Significant effects are shown as: ns, p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001.

| | | **Muskmelon** | | | **Sweet pepper** | | |
|---|---|---|---|---|---|---|---|
| | | F-value | | | F-value | | |
| Effect | df | Fruit weight | Equatorial diameter | Polar diameter | Fruit weight | Equatorial diameter | Polar diameter |
| Block (B) | 3 | 0.98ns | 0.46ns | 1.70ns | 0.99ns | 0.78ns | 0.53ns |
| Nitrogen (N) | 2 | 8.56** | 8.44** | 9.57** | 1.13ns | 0.33ns | 3.48* |
| Cultivar (C) | 2 | 1.11ns | 2.36ns | 1.18ns | 86.64*** | 10.13*** | 120.26*** |
| N x C | 4 | 0.45ns | 0.25ns | 1.15ns | 0.46ns | 0.13ns | 1.15ns |
| Error | 24 | | | | | | |

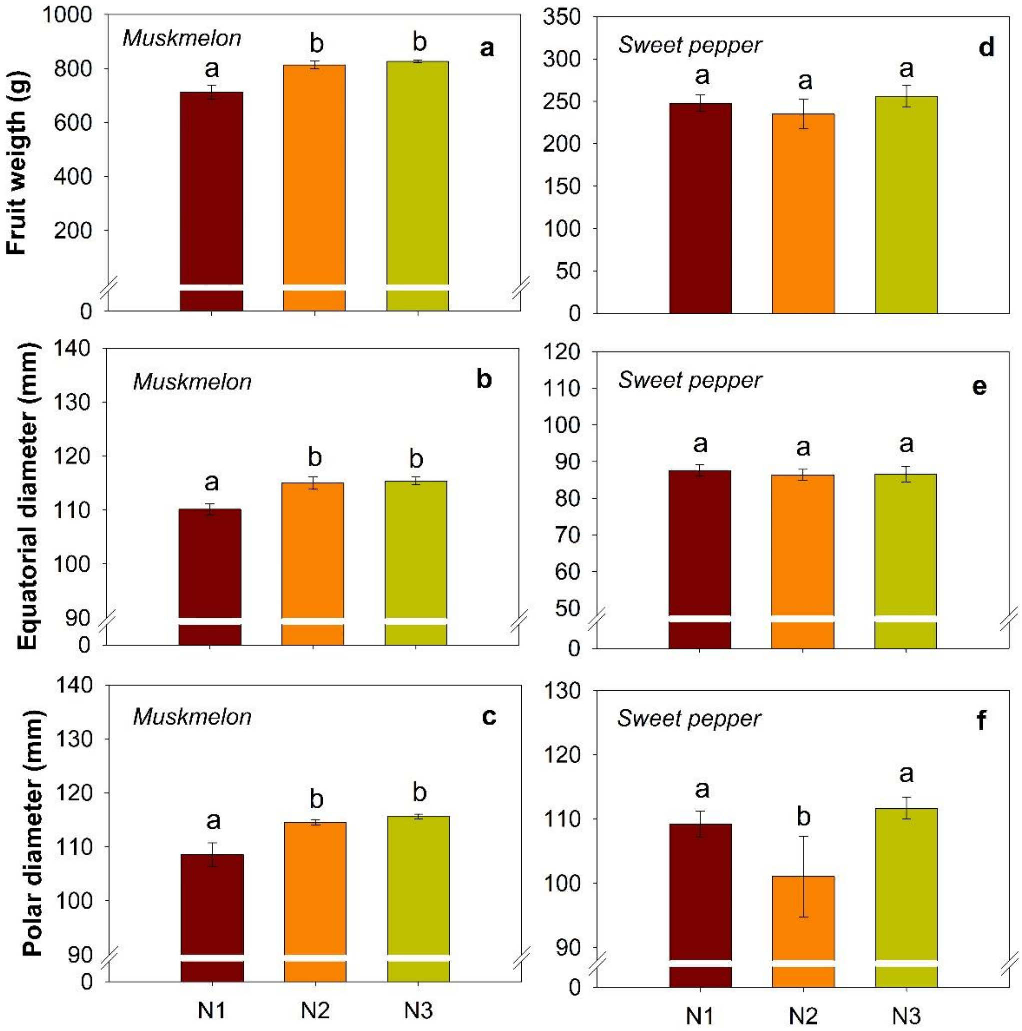


**Figure 4**. Fruit weight (a, d) and equatorial (b, e) and polar (c, f) fruit diameters at different N treatments in muskmelon and sweet pepper. Values have been averaged across three cultivars. Different lower-case letters above each column show significant differences between N treatments, at $p<0.05$. Values are means ± SE.

Regarding total yield in muskmelon and sweet pepper, there were significant differences between N treatments in total yield and in the percentage of non-marketable fruit in both crops (Table 6; Figure 5). For muskmelon, total yield was significantly 40% less than in the N1 compared to the N2 and N3 treatments, which were not significantly different (Figure 5a). The percentage of non-marketable fruit was significantly 40%

higher in the N1 treatment than in N2 and N3 treatments, which were not significantly different (Figure 5b). For sweet pepper, total yield and the percentage of non-marketable fruit differed between N treatments (Table 6, Figure 5c, d). Total yield was statistically lower in the N1 than in the N2 and N3 treatments, whereas the percentage of non-marketable fruit was 38% higher in the N1 than in the N2 and N3 treatments (Figure 5c, d).

**Table 6.** Results of factorial analysis of variance (ANOVA) testing the effect of three N treatments and three cultivars on total yield and percentage discard fruit, in muskmelon and sweet pepper. Significant effects are shown as: ns, p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001.

| | | **Muskmelon** | | **Sweet pepper** | |
|---|---|---|---|---|---|
| | | F-value | | F-value | |
| | df | Total yield | % Discard fruit | Total yield | % Discard fruit |
| Block (B) | 3 | 3.77* | 2.80ns | 0.59ns | 54.54** |
| Nitrogen (N) | 2 | 21.88* | 21.38* | 139.49*** | 10.16ns |
| Cultivar (C) | 2 | 65.57* | 6.44** | 2.37ns | 0.27ns |
| N x C | 4 | 0.85ns | 1.26ns | 4.84*** | 29.53*** |
| Error | 24 | | | | |

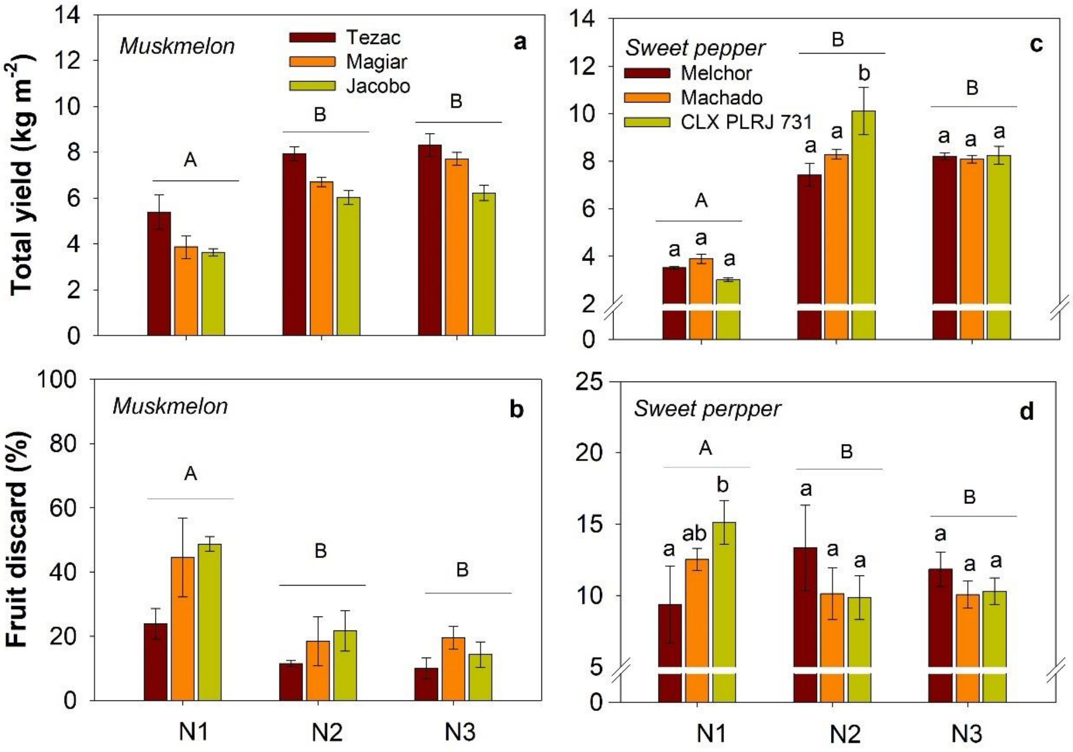


**Figure 5**. Total yield (a, c) and percentage discard fruit (b, d) for the different N treatments, for muskmelon and sweet pepper. In muskmelon, values have been averaged across the three cultivars. Different lower-case letters above each column show significant differences between N treatments, and upper-case letters show significant differences between cultivars within each N treatment, at $p<0.05$. Values are means ± SE.

### *6.4.2. Cultivar effects on fruit quality and yield*

There were significant differences in internal fruit firmness between cultivars both in muskmelon and sweet pepper (Table 2); the muskmelon cultivar TEZAC had significantly lower firmness values than cultivars MAGIAR and JACOBO (Figure 6a). For sweet pepper, the MACHADO cultivar had 20 and 6% higher firmness than the CLX PLRJ 731 and MELCHOR cultivars, respectively (Figure 6b).

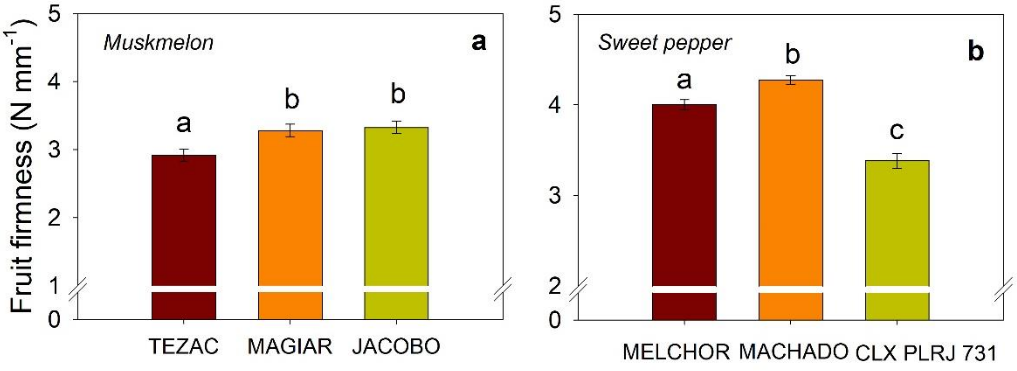


**Figure 6.** Fruit firmness (N $mm^{-1}$), for three different cultivars, in muskmelon (a) and sweet pepper (b). In muskmelon, fruit measurements are internal, in sweet pepper, measurements are external. Values have been averaged across three N treatments. Different lower-case letters above each column show significant differences between cultivars, at $p<0.05$. Values are means ± SE.

Significant differences were found in fruit colour coordinates and colour parameters between cultivars in both muskmelon and sweet pepper (Table 3; Figure 7). In muskmelon, cultivar MAGIAR had significantly lower L* and b* coordinates and h (Figure 7a, c, d), and higher a* coordinate and IC* (Figure 7b, f), than TEZAC and JACOBO. In sweet pepper, the cultivar MELCHOR had significantly higher L* and b* coordinates than MACHADO and CLX PLRJ 731 (Figure 7f, h).

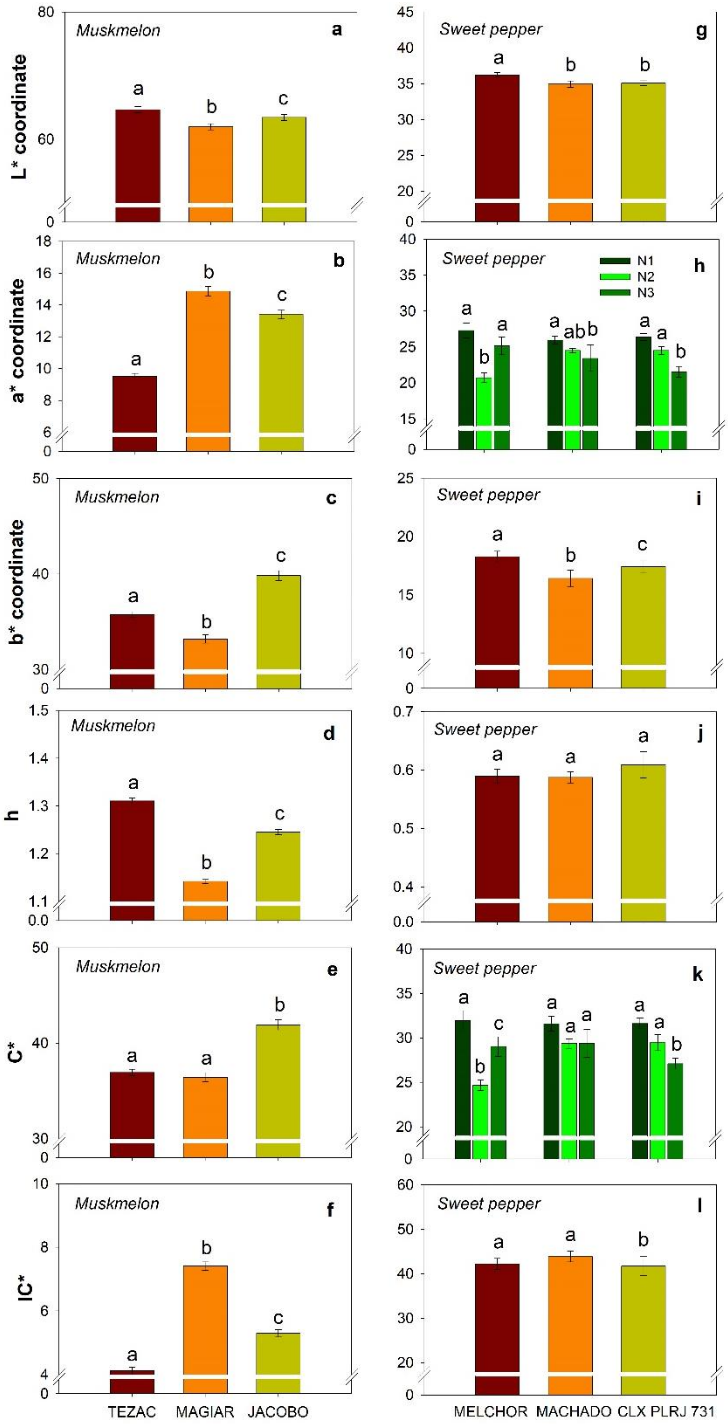


**Figure 7.** Fruit colour coordinates L* (lightness; a, g), a* (red colour; b, h) and b* (yellowness; c, i) and parameters of internal fruit colour h (pitch angle; d, j), C* (chroma; e, k) and IC* (colour index; f, l), for the three different cultivars, in muskmelon (a-f) and sweet pepper (g-l). Fruit measurements are internal. Values have been averaged across three N treatments except in panels h and k. Different lower-case letters above each column show significant differences between N treatments, at $p<0.05$. Values are means ± SE.

Regarding the parameters of internal fruit quality in muskmelon and sweet pepper, there were significant differences between cultivars in both crops (Table 5; Figure 8). For muskmelon, ºBrix and pH increased from TEZAC to MAGIAR to JACOBO (Figure 8a, b). The cultivar JACOBO had 14 and 25% more ºBrix than MAGIAR and TEZAC, respectively. Cultivars MAGIAR and JACOBO presented higher acidity values than the cultivar TEZAC (Figure 8c). For sweet pepper, ºBrix and acidity values in the cultivar CLX PLRJ 731 were significantly lower than in MELCHOR and MACHADO. By contrast, pH was significantly higher in CLX PLRJ 731 than in MELCHOR and MACHADO (Figure 8d-f).

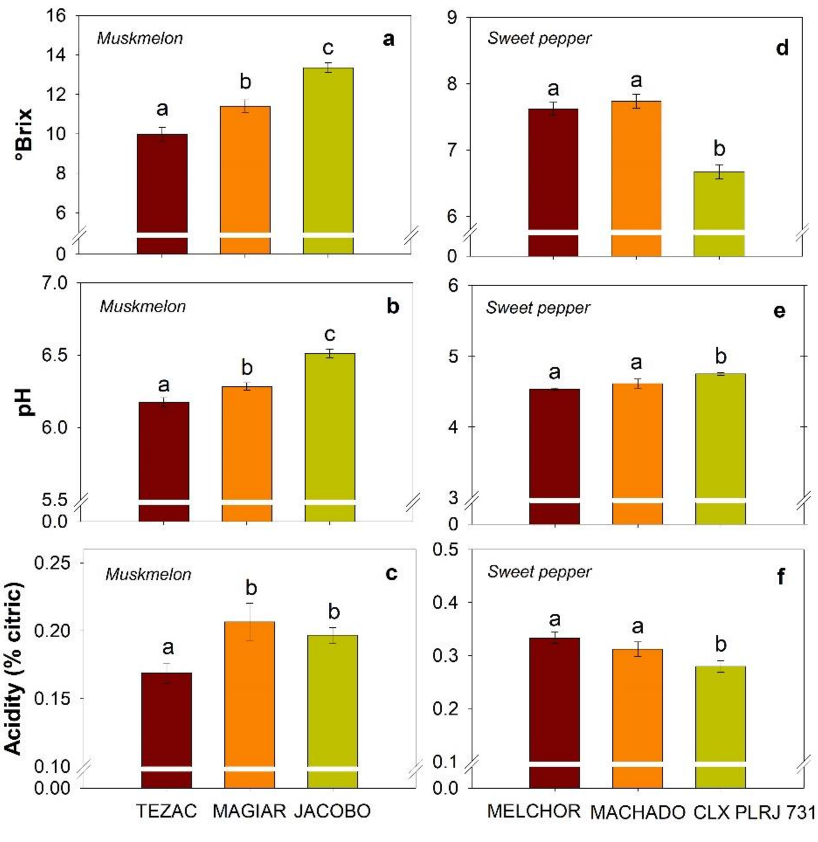


**Figure 8**. Internal fruit quality parameters (°Brix (a, d), pH (b, e), and acidity (% citric acid) (c, f)) for three different cultivars of muskmelon (a-c) and sweet pepper (d-f). Values have been averaged across three N treatments. Different lower-case letters above each column show significant differences between cultivars, at $p<0.05$. Values are means ± SE.

Regarding the morphometric variables of muskmelon fruit, there were no significant differences in fruit weight, equatorial and polar fruit diameter between cultivars (Figure 9). In sweet pepper, there were significant differences in the morphometric variables of fruit weight, equatorial and polar diameter of the fruit, between cultivars (Tables 5, 6; Figure 9). The cultivar CLX PLRJ 731 had on average 5% less fruit weight than MELCHOR and MACHADO (Figure 9d). For equatorial and polar diameter, the CLX PLRJ 731 cultivar had significantly higher values than MELCHOR and MACHADO (Figure 9d).

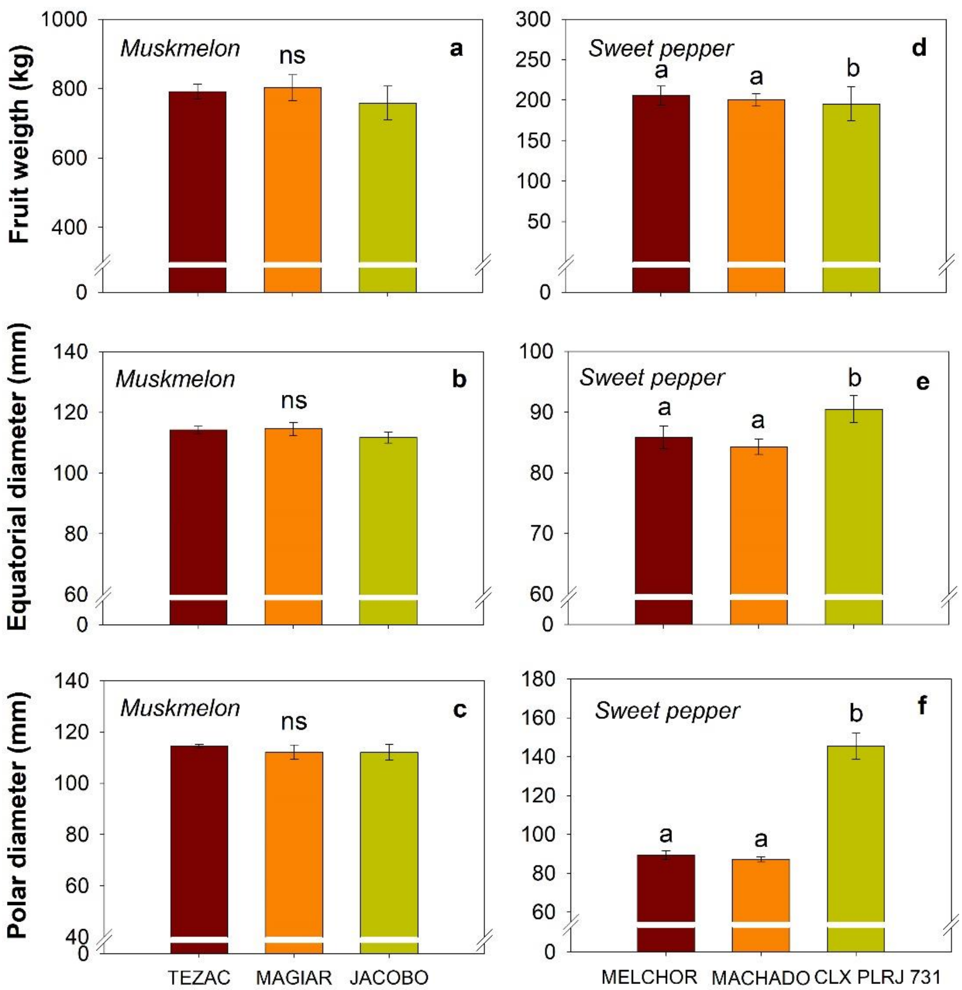


**Figure 9**. Fruit weight (a, d) and equatorial (b, e) and polar (c, f) fruit diameters for three different cultivars of muskmelon (a-c) and sweet pepper (d-f). Values have been averaged across three N treatments. Different

lower-case letters above each column show significant differences between cultivars, at $p<0.05$. Values are means ± SE.

## 6.5. Discussion

### *6.5.1. N effects on fruit quality and yield*

The absence of significant effects on fruit firmness of increasing N addition in muskmelon was consistent with the results of Ferrante et al. (2008) with netted melon (*Cucumis melo* var. *reticulatus* cv. Prodigio) with increasing N from 0 to 165 kg N $ha^{-1}$. In the present work, the maximum N application of 580 kg N $ha^{-1}$ did not affect fruit firmness. In contrast, Lima e Silva et al. (2007) with a hybrid of yellow melon (cv. Gold Mine) in open field conditions reported that increasing N from 0 to 150 kg N $ha^{-1}$ decreased fruit firmness. It is possible that differences in literature regarding muskmelon are due to cultivar effects and/or to differences in environmental conditions. For sweet pepper, fruit firmness was not affected by increasing N application from 65 to 700 kg N $ha^{-1}$, which is consistent with reports by Contreras et al. (2013) and del Amor et al. (2008). The results of the present study and of the literature suggest that fruit firmness is not influenced by amount of N fertiliser in sweet pepper. In general, the available results suggest that the same applies to muskmelon; however, the available reports ae not unanimous.

Regarding internal fruit colour parameters in muskmelon, an intense orange flesh colour is one of the quality attributes most valued by consumers (Krarup et al., 2016). The red colour (a*) was not affected by N treatments, but lightness (L*) and yellowness (b*) increased from the N1 to the N2 treatment. This finding partly agrees with findings of Ferrante et al. (2008) who reported significant differences only in lightness (L*) of muskmelon fruit with N applications ranging from 0 to 165 kg N ha$^{-1}$. The differences in lightness and yellowness can be associated with changes in the contents of pigments such as carotenoids and chlorophylls (Flores et al., 2004). The lack of differences in red colour (a*) between N treatments, leads to the conclusion that N application does not affect this important quality attribute in muskmelon, under the conditions of the present study.

For sweet pepper, the rate of applied N affected external fruit colour, the fruit of the N1 treatment had reduced lightness (L*) and more yellowish colour (b*). In contrast, del Amor et al. (2008) reported that a moderately N deficient treatment of 7 mmol L$^{-1}$ had no effect on these parameters. In the present work, the very deficient N treatment had a concentration of only 2 mmol N L$^{-1}$; it is possible that this very low N concentration caused the responses on L* and b* parameters. There are reports that confirm that N deficiency can affect the colour of sweet pepper fruit (Fageria, 2001).

A decrease in °Brix was found in response to increasing N fertilisation in muskmelon; with a maximum value of 12.5 °Brix being obtained with a N application of 61 kg N ha$^{-1}$ (i.e., the N1 treatment). Considering that the minimum reference value for commercialization is 10 ºBrix (Ferrante et al., 2008), a °Brix value of 12.5 would be very acceptable. It is noteworthy that the ºBrix values of muskmelon fruit in the N2 and N3 treatments were above the threshold value of 10º, indicating that higher N fertilisation also produced fruit with acceptable quality for the commercial market. Other studies reported no response of ºBrix to increasing N application, from 0 to165 kg N ha$^{-1}$ (Ferrante et al., 2008; Lima e Silva et al., 2007), and from 0 to 300 kg N ha$^{-1}$ (Monteiro et al., 2014). It is possible that the lack of effect of increasing N addition on ºBrix in the literature was caused by an insufficient range of N application, unlike in the present study with applications of 302 (N2 treatment) and 582 kg N $ha^{-1}$ (N3 treatment). Overall, it can be concluded that lower N application resulted in sweeter muskmelon fruit.

For sweet pepper, increasing N fertilisation from N1 to N2 and N3 led to a slight increase in ºBrix to an average value of 7.5 ºBrix in the N2 and N3 treatments. Taking into account that the reference values for red peppers are in the range 7–8 ºBrix (Contreras et al., 2013; Niklis et al., 2002), our results indicate that N2 and N3 treatments led to peppers with acceptable marketable quality in terms of ºBrix. The values obtained in the N2 and N3 treatments agree with Contreras et al. (2013) of 8 ºBrix with 9.8 mmol N $L^{-1}$ applied by fertigation throughout the crop. The lower ºBrix values found with low N fertilisation (N1 treatment), in the present study, were likely caused by a slight delay in the ripening of the fruit, since ºBrix increases with the ripening processes of the sweet pepper fruit (Niklis et al., 2002).

Total yield increased from N1 to N2 and N3 in muskmelon, but there were no differences between N2 and N3 treatments. This suggests that a N application of 302 kg N $ha^{-1}$, corresponding to an application of 8 mmol N $L^{-1}$, was sufficient to achieve a maximum yield of 6.8 kg $m^2$ in muskmelon. De Faria et al. (2000) reported that N applications of 130–180 kg N $ha^{-1}$ were associated with relatively low yields that averaged 3.8 kg $m^2$.

The percentages of non-marketable muskmelon fruit in the present study were also informative, with significantly lower percentages in treatments N2 and N3, indicating that low N application contributed to more fruit with external flaws. The main reasons for muskmelon fruit from treatment N1 being non-marketable were fruit malformation and fruit weight of <500g. These observations coincided with several reports with muskmelon where low N application increased percentages of non-marketable fruit (Buwalda and Freeman, 1986; Ferrante et al., 2008; Pérez et al., 2004).

For sweet pepper, as with muskmelon, fruit yield also increased from the N1 to the N2 and N3 treatments between which there was no significant difference. A maximum total yield of 8.5 kg $m^{-2}$ was obtained in the N2 treatment with a N application of 425 kg N $ha^{-1}$, corresponding to a concentration of 8.4 mmol N $L^{-1}$. This is consistent with the results of Grasso et al. (2020) and Rodríguez et al. (2020) where maximum yield of sweet pepper of 7.5 and 7.0 kg $m^{-2}$ were obtained with N applications of 519 kg N $ha^{-1}$ (9.7 mmol N $L^{-1}$) and 530 kg N $ha^{-1}$ (10 mmol N $L^{-1}$), respectively. The N1 treatment had the highest percentage of non-marketable fruit , similar to the results of Grasso et al. (2020).

*6.5.2. Cultivar effects on fruit quality and yield*

There were differences between muskmelon cultivars in internal fruit firmness; TEZAC had the lowest firmness while MAGIAR y JACOBO had higher and similar firmness. Fruit firmness is affected by temperature during fruit ripening, and cultivars can differ in their sensitivity to temperature (Jiménez-Esparza et al., 2017). For sweet pepper, MACHADO had the highest external fruit firmness, reaching a maximum value of 4.3 N $mm^{-1}$. This value is appreciably less than the values reported by del Amor et al. (2009) for various sweet pepper cultivars that averaged 6.2 N $mm^{-1}$. Higher fruit firmness is likely to be beneficial for transporting fruit (Mitchell et al., 2007). This is very relevant for vegetable production in SE Spain, where much of the production is exported to northern Europe (Reche, 2008; 2010).

There was a cultivar effect in muskmelon for L*, a* and b* colour coordinates, and for parameters h, C* and IC*. In red colour (a*), MAGIAR was the cultivar with highest value, which generated an intense orange pulp colour which is very relevant to consumer preference (a*>15) is sought after by consumers (Krarup et al., 2016). a* values ≤10 are associated with a pale orange colour that is undesirable to consumers (Krarup et al., 2016). Consumers would likely be more attracted to fruit of MAGIAR because of its more intense orange colour. In the case of sweet pepper, there were differences between cultivars for lightness (L*) and yellowness (b*). MELCHOR had the highest luminosity value and the highest yellowness; these two colour coordinates also differed between cultivars in other sweet pepper cultivars (del Amor et al., 2009). For IC*, the cultivars with higher values were MELCHOR and MACHADO. It is possible that this similarity between cultivars is due to an increase in the red colour coordinate (a*) that indicates more reddish colour (Niklis et al., 2002; Soltani et al., 2011). Increasing the a* values in the MELCHOR and MACHADO cultivars indicates a greater reddish colour, which is a characteristic more desired by consumers (del Amor et al., 2009).

Within the three muskmelon cultivars, there were differences in ºBrix values, with JACOBO having the highest values, with an average value of 13.5 °Brix between the three N treatments. In a study that evaluated fruit quality of a large number of "Galia" type melon cultivars grown in a greenhouse (Mitchell et al., 2007), cultivars with more than 10 °Brix were considered to have adequate quality. It is important to note that the JACOBO cultivar in the current study exceeded this target value by more than 3 ºBrix units. In addition, the cultivar MAGIAR, evaluated in this study, had ºBrix values higher than 10 (i.e., 11.4 °Brix), but the cultivar TEZAC barely reached this threshold value (i.e., 10.0 °Brix). For sweet pepper, MACHADO and MELCHOR cultivars had the highest ºBrix values of 7.6 and 7.7, respectively. These values were slightly lower than values of 8 ºBrix reported by Contreras et al. (2013) and Eissa et al. (2007) in various sweet pepper cultivars. For CLX PLRJ 731, an average value of 6.7 ºBrix was determined for the three N treatments. Taking into account that the target ºBrix values for red peppers are 7–8 ºBrix (Contreras et al., 2013; Niklis et al., 2002), MACHADO and MELCHOR had acceptable values, but CLX PLRJ 731 was below the acceptable range.

Cultivars evaluated in the present study also differed in total yield. The muskmelon cultivar TEZAC presented the highest marketable yield. Kaur et al., (2017) found differences in yields between melon cultivars, attributed to the number of fruits per vine and the weight of the fruit. However, in the present study, there were no differences between cultivars in mean fruit weight and the diameters of the fruits. It is more likely that differences in yield between cultivars were due to fruit number. For sweet pepper, the yield of CLX PLRJ 731 was higher than of MELCHOR and MACHADO. It is possible that these differences were due to the number of fruits per plant, since the percentage of non-marketable fruit did not differ between cultivars, and the mean fruit weight of MELCHOR and MACHADO cultivars was higher

## 7.6. Conclusions

For most of the parameters evaluated in muskmelon and sweet pepper crops, there were no significant interactions between N treatments and cultivars, indicating that effects of N and cultivar, whenever significant, occurred individually and consistently regardless of the level of the other factor. For fruit firmness in both crops, the N supply did not affect this parameter. In the N1 treatment, the pulp was more orange in muskmelon, and the skin was more reddish in sweet pepper, compared to the N2 and N3 treatments. Of the muskmelon cultivars, MAGIAR had a more orange pulp, whereas for sweet pepper, the cultivars MELCHOR and MACHADO had a more intense red colour. The N1 treatment had 11% more ºBrix for melon, and 4% less in sweet pepper, compared to N2 and N3 treatments. The muskmelon cultivar JACOBO showed 20% higher ºBrix values than the other two cultivars, and the MELCHOR and MACHADO sweet pepper cultivars had 13% higher ºBrix than CLX PLRJ 731. In both crops, the highest yield was obtained in the N2 treatment. However, the best fruit quality attributes were not always achieved by N2 treatment in muskmelon. In this case, to increase the orange pulp colour and ºBrix in fruits, lower N fertilisation rates are required with the cost of decreasing fruit yield.

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# 7. General discussion of the thesis

The first study of the thesis focused on the effect of increasing the dose of N on root development and the accumulation of aerial biomass of sweet pepper crop. This study has shown that very deficient N caused a compensatory growth of the roots in sweet pepper crops, mostly in the most superficial layers of the soil. Compensatory growth is interpreted when the crop uses the reserves to increase root development in response to a deficient N (Drew, 1975; Drew et al., 1973; Lain et al., 1995; Primavesi, 1982). Otherwise, at excessive N, the development of the aerial part is maximized (Drew, 1975; Garnett et al., 2009). Indeed, in the very deficient N treatment, there was a marked decrease in aboveground biomass production. These findings are consistent with reports of other vegetable crops such as tomato, where increased root systems was detected as N fertilization decreased (Lecompte et al., 2008). Compensatory growth could be an evolutionary response of the plant in response to a changing environment of N, where the control signals for the development of the root could be related to two control points, one located in the root and another in the aerial part of the plant (Zhang and Forde, 2000).

This work has also shown that sweet pepper grown in soil in greenhouse is very shallow. Approximately 80% of the roots was in the most superficial layers of the soil, in the first 0.20 m, regardless of the N application, being N deficient, conventional or very excessive N applications. The study of root dynamics with minirhizotron tubes confirmed these results and the greatest root length was found in the first 0-0.20m of enarenado soil. One of the possible explanations of the shallow-rooting of the crops is the application of high frequency fertigation (Primavesi, 1982). This is consistent with other studies on "enarenado" soil crops where the highest concentration of roots was found in the first soil layers (Padilla, et al., 2017a; Castilla, 1986). In addition, based on other works such as Padilla et al. (2017a) the question that arises is that there might be other factors, in addition to N, such as soil compaction that may be restricting the root system of the pepper to the most superficial layers in enarenado soil. In this way, it was proposed to study the effect of tillage to alleviate this effect on the development of the roots, growth of the crop and use of N by the roots of sweet pepper crops.

Tillage is a way to facilitate deeper rooting of vegetable crops (Abu-Hamdeh, 2003; Quincke et al., 2007; Wortmann et al., 2008). However, tillage of the imported soil layer did not increase root length density and rooting pattern consistently in two sweet pepper crops, neither when evaluated through destructive measurements nor when addressed non-destructively through minirhizotron tubes. Tillage reduced soil bulk density and penetration resistance, concurrent with previous studies in greenhouse soil (Padilla et al., 2017; Erdem et al., 2006), but it is probable that the frequent transit of operators for cultural practices, added to soil moisture near to field capacity during the entire cultivation period, caused an accelerated compaction of the tillaged soils (Padilla et al., 2017; García et al., 2016). Tillage was also meant to improve crop performance in terms of growth and fruit production, but tillage did not lead to an increase in dry matter production and fruit yield in the sweet pepper crops evaluated in this thesis. The only beneficial effects of tillage detected in this thesis appeared in terms of reduced irrigation and applied N, drainage, and N leaching. One of the possible explanations was that tillage increased retention of soil water and consequently reduced infiltration (Hamza and Anderson, 2005; Wang et al., 2015), drainage and N leaching. These results are beneficial for farmers, in terms of reducing the use of inputs (water and N fertilizers), and for the environment, in terms of reducing nitrate ($NO_3^-$) leaching.

In terms of N application effects on crop dry matter production, yield and fruit quality, the results of this thesis suggest that the conventional 10 mmol N $L^{-1}$ concentration and highly excessive 16 mmol $L^{-1}$ maximized shoot dry matter production and crop yield in sweet pepper. In the case of very deficient N application, of 2 mmol N $L^{-1}$, root length density was maximized but this was not enough to compensate for the loss of yield.

The rotation of sweet pepper and melon in the Almería area is one of the most profitable in this system, for this reason these two crops were studied (Valera et al., 2014). The study of the effect of N and cultivar on the yield and quality parameters of the fruit in muskmelon and sweet pepper was included in chapter 3. For most of the parameters evaluated, there were not significant interactions between N treatments and cultivars, indicating that effects of N and cultivar, whenever significant, occurred individually and consistently regardless of the level of the other factor. Regarding the effects of N within the parameters studied, it was found that increasing N doses did not have an effect on fruit firmness, this is consistent with other studies with lower doses of N (Ferrante et al., 2008). In the case of the internal colour parameters of the fruit for muskmelon and sweet pepper , the luminosity coordinates (L *) and yellow (b *) increased with the N doses, but the red coordinate (a*) was unaffected, which is consistent with Fageria, (2001) and Ferrante et al., (2008). Regarding total soluble solids, deficient N caused increased °Brix, the opposite case occurred in sweet pepper where N deficient showed a decrease in ºBrix. The N2 white 8 mmol $L^{-1}$treatment had the maximum yield and the lowest fruit discard with the minimum dose of N in both, muskmelon and sweet pepper.

Regarding the effect of the cultivar, fruit firmness was very different depending on the cultivar, coincident with Mitchell et al. (2007). Regarding the evaluated colour parameters, there was also a differential behaviour between the cultivars, with the cultivar MAGIAR presenting an intense orange colour. For sweet pepper, the MELCHOR and MACHADO cultivars presented the most intense red coloration and the most luminous characters accepted by consumers (del Amor et al., 2009). Regarding total soluble solids, there were also differences between cultivars; for muskmelon in the cultivar JACOBO and sweet pepper MELCHOR and MACHADO registered the highest value. A significant effect of cultivar on yield was also observed; for de muskmelon the cultivar TEZAC and SENSEI in N2 was the one that registered the highest yield, in addition to having the lowest percentage discard fruit.

In pepper crops, it is concluded that the conventional and very excessive N doses maximized the growth of the biomass of the shoots, but the root density decreased in the most superficial layers of the soil. The opposite happens with deficient N, where the development of the root is maximized, and the aerial part is minimized. In addition to the effect of N on root concentration, it is possible that other factors such as soil compaction will affect root development. In the study on the effect of tillage on the development of the root in the pepper crop, tillage did not improve the production of aerial dry matter or the density of the roots. However, irrigation, applied N, drainage and leaching were markedly reduced in tillage treatment, probably due to increased water infiltration into the soil and the formation of a tillage tray. In any case, it was shown that tillage by itself is not a practice that improves the soil structure. It would be interesting to delve into other techniques that can complement the work, such as the incorporation of organic materials that help to improve the structure of the soil, improving the yield of pepper crops.

In the study of the effect of N and cultivars for muskmelon and sweet pepper crops, in most of the parameters evaluated, an effect of the interaction between factors was not seen. Regarding the N levels, the N2 treatment was the one that presented the highest attributes in morphometric parameters and the highest yields for both crops. But in muskmelon, the °Brix, which was superior in the treatment deficient N1; the opposite occurred in sweet pepper crop with the N2 treatment with the highest ºBrix. Regarding the other main effects of cultivar, of the three cultivars evaluated for the muskmelon and sweet pepper, there was no cultivar that stands out for all the evaluated parameters. Regarding fruit firmness, in muskmelon MAGIAR and JACOBO showed similar values and for sweet pepper MACHADO was higher, which could be a remarkable attribute when transporting the fruit. Regarding another important variable such as the colour of the fruit, the cultivar MAGIAR for muskmelon and MELCHOR and MACHADO for sweet pepper stood out from the rest, presenting an intense orange colour that is highly accepted by consumers. Regarding the content of soluble solids, the JACOBO for muskmelon presented the highest 13 °Brix and MELCHOR and MACHADO for sweet pepper cultivars were the higher with 7.5 ºBrix on average. In terms of total yield, for muskmelon TEZAC records the highest values and JACOBO the lowest values. For sweet pepper the difference between the cultivars depends on the level of N. The highest yield was obtained in N2 where the SENSEI cultivar reached the highest.

Final considerations, which is for the greenhouse crops evaluated, it is possible to obtain high yields and quality of fruits by adjusting the dose of N. This yield benefits for farmers since the use of inputs is reduced, in addition to significantly reducing losses nitrogen leaching.

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