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THE EFFECT OF ROOTS CONFINEMENT ON THE RELATIVE GROWTH OF ROOTS AND CANOPY OF *OPUNTIA FICUS-INDICA*

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THE EFFECT OF ROOTS CONFINEMENT ON THE RELATIVE GROWTH OF ROOTS AND CANOPY OF *Opuntia ficus-indica*

FINAL PH.D.
THESIS

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Contents

List of Figures

Abbreviation

Abbreviation	**Details**
$\delta^{13}C$	C isotopic Signature
^{12}C	Abundant Natural, stable isotope with a six protons, six neutrons and six electrons
^{13}C	Natural, stable isotope of carbon with a nucleus containing six protons and seven neutrons
C	Carbon
BVR	Plant biomass at certain rooting space volume
CAM	Crassulacean Acid Metabolism
CAM-C	The amount of accumulated carbon after panting *Opuntia ficus-indica* in C_3 soil
DVCT	Digital Vegetation Charting Technique
EA-IRMS	Elemental Analyser Isotope Ratio Mass Spectrometer
IAEA-CH-6	International Atomic & Energy Agency, $\delta^{13}C$ Cane Sugar
IA-R001	Iso-Analytical Limited $\delta^{13}C$ Wheat Flour Standard
IA-R005	Iso-Analytical Limited $\delta^{13}C$ Beet Sugar Standard
IA-R006	Iso-Analytical Limited $\delta^{13}C$ Cane Sugar Standard
N	Nitrogen
Ncd	New carbon derived
Ocd	Old Carbon derived
PA	Perchloric Acid
RGB	Red, Green, Blue
RLD	Root Length Density
SOC	Soil organic carbon
SRL	Specific Root Length
V-PDB	Vienna Pee Dee Belemnite

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Sawsan Hassan

The effect of roots confinement on the relative growth of roots and canopy of *Opuntia ficus-indica*

Summary

The influence of soil volume on roots and canopy growth performance of cactus pear (*Opuntia ficus-indica*) was studied at Palermo University. In November 2014, 1-year-old *Opuntia ficus-indica* cladodes were planted in five different volumes of soil 50, 33, 18, 9 and 5 Liters. Three replicates (plants) per pot size were dug out at 6 and 12, 18 and 24 month intervals, thus in total, there were 5 x 3 x 4= 60 experimental plots. The resulting experimental design was a completely randomized design with combinations of two factors, soil volume and month of the sampling, with three replications. Roots of each plant were washed and visually divided into three groups depending on their diameters: Fine roots less ≤ 2 mm; medium roots (2-5 mm); large roots >5 mm, the roots of each group was manually separated and measured. Roots surface area was measured using image processing VegMeasure software®. Root volumes were calculated from surface area and root length by assuming that roots are cylindrical. Root measurements were taken prior to root dry mass estimation. Cladode surface area, thickness, number of new cladodes, cladodes fresh and dry mass were measured and recorded for each plant. Roots: shoot mass, root density, root length density and specific root length were calculated. Mother cladode and roots starch content estimation was performed using the perchloric acid method while the natural signature of $\delta^{13}C$ and the roots turnover was determined depending on the portion of C in soil that was derived from the cactus pear root. Results indicated a significant effect of soil volume and sampling dates and their interaction ($P<0.01$) on: total roots length, roots surface area, dry mass, volume, specific roots length, the large roots surface area, medium roots surface area, dry mass of the large, medium roots, number second generation cladodes, canopy dry mass, total canopy surface area, carbon isotopic signature $\delta\ ^{13}C$ and carbon derived by roots per soil unite. Whereas, root density, roots length density, fine roots dry mass, total number of cladodes, mother cladodes and roots starch content and roots turnover were significantly affected by soil volume and sampling dates only. Increasing the soil volume enhanced the total roots length, surface area, roots dry mass, and

total roots volume. However, the smallest soil volume showed stable roots growth over time in terms of the total roots length, total surface area and the total roots volume, as well as the total dry mass. In contrast, soil volume restriction enhanced root density as well as the root length density and the specific root length. On the other hand, the large, medium and fine roots dry mass and surface area and length tended to increase with the soil volume. The number of the first generation cladodes was affected by the soil volume restriction. The lower number of the second generation cladodes produced in the lower soil volume, plants in the smallest soil volume stopped producing new second generation cladodes after the first sampling date. Moreover, the total number of the new cladodes increased with soil volume over time and ranged between (3-15 cladodes per plant). Linear canopy dry mass increases were observed with respect to the soil volume increase. The roots: canopy dry mass and the roots volume: canopy dry mass ratios increased with the soil volume increase, this is because of positive effect of the soil volume increase on both roots and canopy. Results showed an increase in starch accumulation in both roots and the mother cladodes along with soil volume decrease. Furthermore, there was an increasing negative $\delta^{13}C$ signature values over time as result of the contribution of cactus pear root (CAM-C) to the soil organic matter (C3-soil). The CAM-C contribution increased from 27 C (g of soil kg^{-1}) in the biggest soil volume to 57 C (g of soil kg^{-1}) in the smallest soil volume. This can be explained by the higher roots mortality in the small soil volume which increased the turnover percentage with time ranging between (10-15.4%). These results suggest that the limitation of soil availability has resulted in root and canopy growth limitation and greater root turnover.

1. Introduction

Arid and semi-arid regions cover approximately 30% of the world's continental surface (Nobel, 1994). Arid and semi-arid regions are a challenge to conventional cropping systems because of limited or erratic rainfall, poor soils and high temperatures (Le Houérou, 1996). Productivity in these areas can be increased by the cultivation of adapted crops such as *Opuntia* species, especially cactus pear (Pimienta-Barrios and Muñoz-Urias, 1995). Cactus pear or prickly pear (genus *Opuntia*) is a member of the Cactaceae family (Reyes Aguero et al. 2005) that has more than 1500 known species worldwide (Hegwood, 1990). *Opuntia* species are Crassulacean acid metabolism (CAM) plants that convert water to biomass four fold more efficiently than either C4 or C3 plants. In addition to being a drought tolerant fruit crop (Galizzi et al. 2004; Gugliuzza et al. 2000), they have multiple uses for both humans and animals (Nefzaoui and Ben Salem, 2000). They can contribute to sustainable food production, especially in countries with large arid and semi-arid lands (Felker and Inglese, 2003). *Opuntias* have developed phenological, physiological, and structural adaptations to the arid areas characterized by drought, erratic rainfall and poor soils. Cactus pear (*Opuntia ficus-indica.* (L) Mill) has gained an important place in the agricultural systems as a fruit, forage and fodder provider, particularly in subsistence agriculture where they have a comparative advantage for their capacity to grow with minimal agronomic inputs and for their resistance to drought. As a good candidate for arid and semi-arid area ecosystems, this plant could be planted in rocky areas or in areas where the soil volume is limited due to the high level of soil erosion resulting from the loss of plant cover that is associated with land degradation.

Soil volume restriction or root pruning has been reported to result in significant reduction of canopy growth of the trees (Bravdo et al. 1992; Myers, 1992), changes in the root system activities and morphology (Aphalo and Rikala, 2003). Thus, the knowledge of the effect of the root restriction on *Opuntia ficus-indica* roots and canopy behavior is required to pursue the potential of this species where soil depth or volume is limited.

This study investigated the effect of soil volume restriction on below and above ground growth of *Opuntia ficus-indica* through understanding the limit imposed by root confinement via different soil volumes and architecture on root and canopy growth. We hypothesized that the reduction of soil volume would result in a reduction of root growth, that in turn, would reduce the canopy growth. This is likely to be related to slower root turnover and starch accumulation.

2. Review of the literature

2.1. Roots and canopy growth and interaction on permanent plants

2.1.1. Root: canopy ratio

Roots and above ground canopy functionally support each other's and maintain an active balance in biomass (roots:shoots ratio) which reflects relative richness of above-ground resources (light and CO_2) compared with root-zone resources (water and nutrients) (Poorter et al. 2012a). Whole-plant growth rates and measures such as root: shoot ratio are thus an outcome of developmental stage and of environmental influences. In plants the belowground environment is often inhospitable and restrictive to tree root growth. Obstacles to a healthy root system are frequently mentioned as the primary cause for a wide range of tree growth and health problems (Hawver and Bassuk, 2006). Change in root: shoot ratio during a plant's life cycle is noticeable, but growth rates of roots and shoots continually adjust to resource availability and environmental conditions. Functional balance theory suggests that plants reallocate carbon and other nutrients among active tissues to obtain resources that most limit growth (Brouwer, 1983). Another theory suggests that plants assign resources among organs to optimize whole plant growth (Bloom et al. 1985). These theories suggest plants adapt to produce a specific root: shoot ratio but this ratio will move to balance resources limiting growth with a degree of flexibility (Shipley and Meziane, 2002). Root: shoot ratio is an indicator to show the plant reaction to growing conditions it changes with plant growth and development in addition to shifting in response to limiting resources above and below ground. Therefore, care must be taken to account for plant size and ontology, especially when evaluated on young plants (Müller et al. 2000). Along with shoot reaction to above-ground conditions effects, root biomass is influenced by below-ground conditions where low availability of nutrients and water resources usually leads to greater root: shoot ratio. Significant relationships between water and root development were observed (Masmoudi et al. 2007). Reports showed that root distribution can be significantly affected by the neighboring trees and the soil characteristics such as soil texture and depth (Fernandez et al. 1992) in addition to roots adapt to the available root zone and bloom within the potential root zone (Connor and Fereres, 2005). Root extension is also associated with the available carbohydrate amounts (Dichio et al. 2002) and the phenological growth stage. Rapid growth is also observed in spring and autumn and new shoot growth. Low carbohydrate resources results in reduced canopy growth and root length in trees and can affect the root: shoot: ratio as a result of competition between shoots, flowers, fruits and roots (Dichio et al. 2002). Reduction of root-canopy ratio implies a systematic reduction of the capacity of roots to absorb water.

2.1.2. Perennial plants roots system

Roots represent half of the plant, they supply growing plants with water and minerals, play important roles in carbohydrate storage and hormonal signaling, and physically anchor trees in the ground (Kozlowski and Pallardy, 1997). Roots are a key organ for plant adaptation to variable environments and therefore for biodiversity (Cornwell and Grubb, 2003). Root as systems are essential components of global ecosystems the Belowground Net Primary Productivity ranges from 40 to 85 % of the total NPP (Scurlock and Olson, 2002). More recently, roots have been thoughts to be one of the major sources for plant signaling, not to mention their possible role as 'brain diffuse like system' of the plant (Mancuso, 2005). They show a high extent of plasticity in terms of development in response to changes in the local environment conditions of the soil. On the other hand, the root systems of perennial plants are complex due to its functions and roles, this system is not one structure but rather involve two, and some-times three, main types of root structure: coarse woody roots (the large diameter roots), those represent the largest part of the root system biomass and serve functions of perennial organs, carbohydrate and nutrient storage during the season, these large roots serve to anchor the plant and to support lateral roots (Comas et al. 2013). Moreover, they play important role in the transportation of water and nutrients to above ground plant parts. The second root structure type is the fine roots of woody plants. These roots epitomize most of the surface area of the root system and serves as the responsible organ for water and nutrient uptake (Waisel and Eshel, 2002). However, they are limited to the terminal two root segments (first and second branch orders counting back from root tips), and have a key role in foraging for belowground resources (Guo et al. 2008). Finally, fine (or lateral) roots that relate to the capacity of the plant to absorb water and nutrients, especially in cases where there is competition for resources

(Robinson, 2001). Those roots are the most active portion of the root system as they comprise the majority of the length and surface area of these root systems in woody plants (Rewald et al. 2011). Fine roots are usually categorized into arbitrary size classes (e.g., roots 0 to 1 or 0 to 2mm in diameter) (Pregitzer et al. 2002). In general, fine roots are less than 2mm in diameter and include mycorrhizae (Zhang et al. 2008). Fine roots are an important and dynamic component of all terrestrial ecosystems, they can account for a significant portion of ecosystem net primary productivity (Pregitzer et al. 2002).

Root structure in terms of diameter distribution is also important at the ecosystem level. As they have vital role through contributing to the soil porosity through controlling the size of pores. These pores, which have specific physical and chemical properties (Read et al. 2003), are used as micro-habitats by the micro-faunas, as well as by specific microbial communities (Lavelle et al. 2004).

2.1.3. Restricted soil volume effects:

2.1.3.1. Root volume

Rooting volume can be considered as a resource by itself (McConnaughay and Bazzaz, 1991). Root restriction due to reduction in rooting volume can affect whole plant growth through chemical signals (Aiken and Smucker, 1996). Therefore, a rooting volume value can be considered as an environmental gradient. Mechanical restrictions imposed on root growth and structure by container volume is a central matter of concern in plants (Dominguez-Lerena et al. 2006; Aphalo and Rikala, 2003). Root restriction reduces growth with no effect or an increase in shoot/root biomass ratio (Clemens et al. 1999). The effect of root restriction in conifer species has been studied in several species separately (Dominguez- Lerena et al. 2006; South et al. 2005; Lamhamedi et al. 1998). Growth response to reduced rooting volume might be species-specific (Climent et al. 2008).

2.1.3.2. Plant growth habits

The plant shows characteristic and behavioral differences in growth habit under root restriction compared to the one under normal field cultivation (Zhu et al. 2006). Restricted soil volume for root growth can have limiting effects on overall plant growth and influence plant responses (Hess and De Kroon, 2007). Therefore, root restriction might be considered as one type of the physical stress for plant roots. Plants experience many physiological and morphological changes in response to reduced rooting volume, which can affect transplant quality and performance. Root restriction and container size may affect all roots and shoot growth, biomass accumulation and partitioning, photosynthesis, leaf chlorophyll content, plant water relations, nutrient uptake, respiration, flowering, and yield. Plant responses to reduced soil volume have been reported for a wide range of plant species, with some conflicting data (Poorter et al. 2012b). Root restriction can increase root mass and the amount of fibrous roots (Wang et al. 2001). The effect of the root restriction may affect shoot growth through additional metabolism processes (Ismail and Noor, 1996). For example, root restriction might alter plant water balance and consequently affect leaf growth (Peterson et al. 1991). It has also been proposed that the reduction of plant growth under root restriction may be caused by a decrease in the synthesis and translocation of growth substances from the roots (Ismail and Davies, 1998). Root restriction resulted in many physiological changes such as carbohydrate metabolism (Ronchi, et al. 2006), nutrient uptake (Yang et al. 2007), transpiration (Ray and Sinclair 1998) and hormone production (Liu and Latimer, 1995).

In general, trees are exposed to multiple stresses. To sustain their growth, trees must either use an extensive strategy and invest assimilates, which leads to an increase in biomass and length in the fine root system, or a concentrated approach, with morphological adaptations of the fine roots (Lõhmus et al. 2006). Morphological adaptations of the fine roots allow plants to survive even under severe soil conditions (Ostonen et al. 2006). However, different tree species seem to have different strategies for improving the mineral nutrition of the plant (Comas et al. 2002; Comas and Eissenstat, 2004). Increasing specific root length is one of the possible fine root morphological parameters (intensive strategy), which increases the volume of soil exploited per unit biomass invested in the fine roots.

2.2. Structure, growth and function of roots and canopy in *Opuntia ficus-indica*

2.2.1. *Opuntia ficus-indica* roots system

Roots are that part of the plant that develops and grows downward into the soil, this part is anchoring the plant and absorbing nutrient and moisture. Roots play very important roles in the pant life cycle, starting from the germination and during the whole plant life. The task becomes very significant especially in the dryland ecosystems which are considered as very fragile systems since they are susceptible to various forms of degradation and have low amounts of soil organic matter, nutrients and severe water limitations (Ferrol et al. 2004). Dry land species must have morphological and genetic characteristics that enable them to survive under such conditions. Some of this characteristics must related to the roots systems especially that these systems are subjected to prolonged droughts that are interrupted by irregular and often light rainfall (Nobel and Huang, 1992). *Opuntia* species have developed phenological, physiological and mechanical adaptations for growth and survival in arid environments where water is a vital factor for the survival of other plant species. Cactus pear (*Opuntia ficus-indica*) plants is one of *Opuntia* species that could be good candidate for such environments. These plants display Crassulacean acid metabolism (CAM), whereby these plants open their stomates and take up CO_2 at night, when temperatures are lower and humidity higher than during the daytime. This results in reduced water loss that enable them to withstand drought (Felker et al. 1997). Indeed, they are highly efficient in the use of water and take up water at very low soil-water contents (De Kock, 2001; Reynolds and Arias, 2001; Snyman, 2004a, 2005). Due to their shallow and widespread root system (Snyman, 2006), cacti are able to exploit limited rainfall to their fullest potential (Snyman, 2005).

2.2.2. *Opuntia ficus-indica* root structure

The root system of cactus pear is very complex and it exhibits different kinds of roots (Snyman, 2004a). In general, different types of roots can be classified according to their developmental origin. The root that develops from the embryonic radicle is termed a primary root or true root or tap root. Later, when the primary root reaches a certain length, lateral roots are produced. Any root formed on another root is considered a lateral root. The tap root system consists of the tap root and its branches. When a root is formed on an organ other than a root, it is termed an adventitious root, the group of adventitious roots and their branches constitute adventitious root system. According to Snyman (2004b, 2005), the root system of *O. ficus-indica* is very complex and may has four kinds of roots: (1) Primary roots: formed from a primary skeleton of barely fibrous roots, 20 to 30 cm long, which very soon increase in thickness, by secondary growth, to form a periderm. When the root skeleton is kept dry for some time and then re-watered, absorbing roots appear from hidden buds within few hours to act swiftly to the moister event (Dubrovsky et al. 1998). The ways of adventitious root development in *O. ficus-indica* has shown that the fine lateral roots on the tap root die off with age. This process stimulates cell division in the parenchyma root tissues and the formation of meristem spots with adventitious roots (Gibson and Nobel, 1986). This fine and fragile mass of roots is formed from short and branched rootlets, completely covered with root hairs. Also, different kinds of ectomycorrizae, most of them of the vesicular-arbuscular type, grow together with the short and branched rootlets. What is more, the ability to produce adventitious roots is also useful for clonal propagation of *O. ficus-indica* and other agronomic species (Le Houérou, 1996). (2) Absorbing roots: which are formed within a few hours as the lateral buds rapidly respond to the advent of moisture have been named as “rain roots” by Gibson and Nobel (1986). They develop from the hidden latent bud in the cortex of the older roots. These “rain roots” die off as soon as the soil dries (Passioura, 1988). (3) Root spurs: which according to Boke (1980) are those that develop from the most bulky mass of roots as clusters. The spur base of *O. ficus-indica* exhibits a crown of appending-like bracts and, contrary to Boke's description (1980), the roots developed from the spur in *Opuntia ficus-indica* are of two classes: short, gross and fleshy, with plenty of root hairs; and of the rest, two or three slender and long ones, similar to the absorbent root system. It is not known whether the short roots die off or mature with time. (4) Roots developing from areoles: these roots develop when the areoles are in contact with soil. At the onset of their development, they are gross and without root hairs; they have a prominent caliptra, with the epidermal cells forming bract-like appendages. The growth of the young roots is very rapid; they become slender with a cortex three to four cells thick, and are covered by many root hairs. In some of these type of cells, water deficit induces the formation of a higher number of endodermal cells with Casparian Bands closer to the root tip (De Micco and Aronne, 2012).

2.2.3. *Opuntia ficus-indica* roots function

Roots provide essential functions including the uptake of water and nutrients for plant growth, serve a role as storage organs and anchor the plants to the soil. According to their function and position within a root system cactus roots can also be categorized. Preston (1900, 1901a) described the differences between anchoring and absorbing roots in different cactus species and defined some functional differences in these root types related primarily to the thickness of the vascular cylinder (Preston, 1900, 1901). Cannon (1911) used this approach, stating that anchoring roots can be: vertically oriented, deeply penetrating, taproots; or (2) horizontally oriented, supporting roots. Generally, water storage capacity (capacitance) is relatively small in cactus roots compared to shoots (Nobel, 1996). For succulent roots, however, the capacity is greater than for nonsucculent roots, and may be comparable to that of the water-storage parenchyma in stems. Water-storage tissue in succulent roots has the ability to withstand a high degree of dehydration without irreversible damage, and may also help prevent water loss and decrease root shrinkage during drought. In addition to storing water, cactus roots frequently accumulate starch. To accommodate starch reserves, the roots of some species acquire a distinct morphology.

2.2.4. Root distribution in the soil

Opuntia ficus-indica has a shallow and fleshy root systems occurring mainly in the upper layers of the soil, where the water content is changeable (Waisel et al. 1996). Root distribution may depend on the type of soil and management (Snyman, 2005). In case of good soil environments, the tap-root develops, down to 30 cm into the soil. However, when the soil is dry, fleshy side roots develop from the tap-root to take up soil moisture deeper into the soil. Yet, in all kinds of soil, the majority of the masses of absorbing roots are found in the upper soil layers, with a maximum depth of 30 cm, but spreading laterally about 4 to 8 m away from the plant base, this shallow root distribution not only helps to absorb light rainfall, but also gives the cactus the ability to compete with other plants (Dougherty et al. 1996).

2.3. *Opuntia ficus-indica* canopy

The Cactaceae family includes approximately 130 genera and 1500 species. The *Opuntia*s are the most important due to their utility (Flores-Valdez and Osorio, 1996). Being member of CAM family, *Opuntia* has the potential to produce large quantities of biomass in water-limited condition (Felker et al. 2006). On the other hand, CAM species showed an average increase in biomass productivity of 35% in response to a doubled atmospheric CO_2 concentration (Drennan and Nobel, 2000). This indicates that there will be increase in the potential area for cultivation of CAM species along with the concentration of atmospheric CO_2 increase (Drennan and Nobel, 2000). Within the genus *Opuntia*, *Opuntia ficus-indica* is the most important species as a multiple use crop for food and feed especially in arid and semiarid lands during periods of drought and shortage of forage plants (Le Houérou, 2000; Juárez and Passera, 2002).

2.3.1. *Opuntia ficus-indica* canopy growth

Globally drylands occupy 41 percent of the earth's land surface (IUCN, 2008). These ecosystems are described as very sensitive systems since they are vulnerable to various forms of degradation (Ferrol et al. 2004). Cactus pear has great potential to improve the productivity in arid and semi-arid areas (De Kock, 2001). This plant has the capacity to still extract water, coming from the night dew that cover the upper part of soil, due to their root systems (Snyman, 2004b). These traits enable these crop to survive in areas with 200 to 300 mm rainfall (Brutsch, 1988). On the other hand, *Opuntia ficus-indica* well-defined with low root:shoot ratio (0·14 on a dry mass basis; Nobel 1988) in addition to low respiratory cost for both root growth and maintenance (Nobel et al. 1992). Due to these specifications, *Opuntia ficus-indica* may be a good candidate to contribute to the sustainable production system that will increase the efficiency and economic viability of small and medium sized farms of lower income farmers to enhance the food security of populations in these areas (Nefzaoui et al. 2014).

Opuntia ficus-indica is shrubby or tree-like plant up to 6 m high, usually with well-developed trunks. Stem segments vary and can be broadly obovate or oblong to speculate, flattened, 20-50 cm long, 20-30 cm wide, green colour, covered by a very thin waxy layer, areoles 2-5 cm apart. Glochids falling away early, spines absent or (2-7) per areole, 0.5-1.0 cm long, weak whitish. Flowers yellow, rarely orange, 6-8 cm long and 5-10 cm in diameter during anthesis. Fruit with numerous (c. 30-40) areoles, with glochids, rarely with spines, tuberculate, ovoid to oblong, 6 (-8) cm long, 3 (-5) cm in diameter, yellow, orange, pink, green or reddish.

2.3.2. *Opuntia ficus-indica* as a forage

Opuntia ficus-indica is a very productive plant, under natural conditions, it can produce 180 t ha^{-1} yr^{-1} fresh weight which is equal to 20 t dry matter ha^{-1} yr^{-1}. The high water content in cactus can help to solve the livestock watering in the dry area (Dubeux et al. 2015). Under sufficient irrigation this productivity can reach 40 t dry matter ha^{-1} yr^{-1} when the water is available (García de Cortázar and Nobel, 1991). Globally, cactus is widely used as a forage in Mexico, South Africa, Tunisia, and Brazil (Mondragon-Jacobo and Perez-Gonzalez 2000; Felker et al. 2006). Nutrient values of Cactus (*Opuntia* spp.) depends on the genetic characteristics of the species or clones, the age of the cladode, the cladode sampling location, the cladode harvesting season, and the growing conditions, such as soil fertility and climate (Nefzaoui and Ben Salem, 2001). Cladodes are high in water, carbohydrate, ash and vitamins A and C, but they are low in crude protein (CP) and fiber (Le Houérou, 1996; Batista et al. 2003). They exhibit a high palatability (Nefzaoui and Ben Salem, 2002).

2.3.3. Opuntia ficus-indica for fruit production

Opuntia ficus-indica is widely cultivated in arid and semi-arid regions worldwide with increasing importance as a fruit crop (Inglese et al. 2009). The fruit yield of cactus pear is extremely erratic and yields vary greatly due to many causes: environmental conditions, genotypes and their interactions, orchard management and practices (Potgieter, 2007). Under rain-fed conditions with 400-600 mm per year, fruit yields may range between 1-5 t ha^{-1} under traditional practice systems and up to 15-30 t ha^{-1} with intensive practice systems (Monjauze and Le Houérou, 1965). Fruit yield is expected to increase from planting until it reaches the maximum when the plant is fully mature at five year age (Potgieter, 2007). Flowers develop from areolae along the cladode crown on one-year old cladodes whereas new cladodes usually develop on older cladodes (Inglese et al. 1994). The cladodes fertility is affected by environmental conditions, plant and cladode age, and dry matter (DM) accumulation (Valdez-Cepeda et al. 2013).

2.4. Soil organic carbon (SOC) and roots turnover

Soils constitute the greatest stock of terrestrial organic carbon (Batjes, 1996). Soil organic carbon (SOC) is related to atmospheric CO_2 levels (Lal, 2004) and can be affected by land-use and management (McCulley et al. 2005). Human interference is affecting the global ecosystem which is affecting the SOC (Canadell et al. 2007). When land cover changes from one type to another, there can be changes in the plant root, soil fauna, soil microorganisms, and soil conditions which can severely alter the soil carbon stock (Lv and Liang, 2012). Thus, research on SOC dynamics is valuable to estimate the impact of these changes on SOC (Post and Kwon, 2000). The natural abundance of different isotopic forms of carbon δ^{13} is widely used to study soil carbon dynamics as affected by plants (Desjardins et al. 2006; West et al. 2010). Due to the differences in the $\delta^{13}C$ signature. This technique has been used to assess the sources of SOC and to determine the SOC turnover rate (Boutton et al. 2009; Kuzyakov and Larinova, 2005). There are many factors that affect the tree root biomass and turnover including: temperature, nutrient availability, soil acidity and water availability (Eissenstat et al. 2000; Lauenroth and Gill, 2003; Leuschner and Hertel, 2003). Among these factors, roots (mainly the fine roots) are thought to be the most important factor that contributes substantially to the global terrestrial carbon (C) cycle and that are a major reservoir of C (Vogt and Persson, 1991). Fine roots represent a significant percentage of net primary productivity (Hobbie et. 2010). The decomposition of theses roots is assumed to serve as a potential soil C source (Raich et al. 2010). Fine roots have a much shorter lifespan than coarse roots, as a consequence, their biomass varies both seasonally and due to changing environmental conditions (Cheng and Bledsoe, 2002), ensuing high annual turnover rates (Gaul et al. 2008; Eissenstat et al. 2000). Contrary, coarse roots are more important to long-term ecosystem productivity due to slow root decomposition and turnover of carbon (Raz-Yaseef et al. 2013; Langley et al. 2003). Another major factor that affect the SOC dynamics abandonment is the climate (Alberti et al. 2011) in addition to abiotic factors can also affect root growth and SOC (Brye et al. 2004; Alvarez and Lavado, 1998). Moreover, the SOC content can be changed both quantitatively and qualitatively depending on the source of the organic carbon released by different crop plants (Novara et al. 2014). Cactus pear showed high adaptation and fast biomass growth under harsh condition and could be a good option to increase the SOC in drier environments, however, the contribution of this crop to SOC was found to be low (Navara et al. 2014) which could be the result of low root: shoot ratio.

2.5. Starch and Nitrogen

Carbohydrates are the primary products of photosynthesis (Taiz and Zeiger, 2006). Some of the first carbohydrates produced by photosynthesis in perennials are the simple sugars (Pallardy, 2008). Later on these simple sugars are converted into storage forms of energy. Sucrose is the main carbohydrate used to transport sugars to other plant parts (Taiz and Zeiger, 2006). Starch is an important carbohydrate and is considered to be the major carbohydrate reserve in woody plants (Pallardy, 2008). Starch can be used as source of energy by plants for different processes such as reproduction, maintenance, storage, or growth (Lilly, 2001). Roots and stems are known to be essential for carbohydrate storage in several tree species (Kaelke and Dawson, 2005). The amount of carbohydrate stored in the roots changes seasonally, with the lowest reserves in spring after bud flush and the highest reserves late in the season or during dormancy (Landhäusser and Lieffers, 2003), mainly because carbohydrates are frequently undergoing conversion from one form to another (Pallardy, 2008) as plant can use them to produce energy (Nelson and Cox, 2005). The seasonal pattern changes of starch levels vary among species (Johansson, 1993) and different environmental condition such as temperature (Pallardy, 2008; Kaipiainen and Sofronova, 2003). Moreover, these changes seem to be affected by nutrient availability, particularly nitrogen (Adams et al. 1986), and starch accumulation seems to have negative relationship with plant N-status (Rytter and Ericsson, 1993).

3. Materials and Methods

3.1. Experimental condition

The study was conducted during the period May 2014 – August 2016 in the Agricultural Faculty of Palermo University (38° 7' 4.0800" N 13° 22' 11.2800" E, 29 m a.s.l). The climate is typical Mediterranean semi-arid with an average annual rainfall of approximately 700 mm. The dry period of the year can extend to seven months (April–October).

3.1.1. Cultivation of *Opuntia ficus-indica* cladodes

One-year-old *Opuntia ficus-indica* cladodes of the cultivar "Gialla" obtained from Palermo University (36.5 ± 0.5 cm long, 19 ± 0.2 cm wide) were cut and dried for two weeks in the shade to allow healing of the wounded areas. Five different sizes of pots, 50, 33, 18, 9 and 5 Liters, were filled with dry fine, sandy loam soil (A (< 0.002 mm) = 9.9 %, L (0.002-0.05 mm) = 13 %, S (0.05-2 mm) = 77.2 %). Field capacity was 35% and wilting point 20% (g/g). (Soil had a pH of 6.8 and contained about 80 g kg^{-1} organic matter, 10 g kg^{-1} total Nitrogen). At the end of May 2014, cladodes were planted in pots with half of their length in the soil. Plants were watered regularly throughout the season (when the temperature increases from spring through summer) to maintain soil water content and to avoid any visible sign of water stress. Four different sampling dates were used (6, 12, 18 and 24 months). For each sampling date, three planted replicates (pots) were set up, thus in total, there were 5 x 3 x 4 = 60 pots. Three control pots with only bare soil treated the same way as the planted pots (i.e. same amount of water) were assigned in order to estimate the soil organic carbon (SOC) accumulation and C isotopic signature in the bare soil in each growing period.

3.1.2. Experimental design

The experimental design was a completely randomized design in possible combinations of the two factors, soil volume or pot size and month of the sampling, with three replications.

3.2. Data Collection

For each sampling dates, three pots from each size were selected randomly in each pot size, plants were dug out carefully. The samples for the soil were collected from each pot, samples were passed through 2 mm sieve to obtain roots (2 mm diameter), dried and stored before SOC and $\delta^{13}C$ determination. To avoid root damage or loss during harvest, each plant was swamped in a water bath for 5–30 min and then was lightly shaken to release them. The root system was separated from the soil, under a gentle water jet, using a sieve to collect any root fragments detached from the system. This process was continued until all visible sand and soil particles were removed. Particles that adhered to strongly to the roots were manually removed. Roots were separated from mother cladodes areoles (where they developed from), drained of the access water then they were stored in a refrigerator at 4 °C for later measurements and analysis, the root morphology estimations were taken prior to root biomass measurements. Depending on the age, cladodes of each plant were numbered and separated. Number of the areoles that developed roots were recorded for all mother cladodes.

3.2.1. Soil samples, soil organic carbon accumulation and C isotopic signature measurements

The $\delta^{13}C$ and SOC of bulk soil, root biomass of cactus pear and soil before cactus pear planting (C_3-C soil) were measured using an EA-IRMS (elemental analyser isotope ratio mass spectrometer Carlo Erba Na 1500,model Isoprime (2006), Manchester, UK.). The reference material used for analysis was IA-R001 (Iso-Analytical Limited wheat flour standard, $\delta^{13}C$ Vienna Pee Dee Belemnite (V-PDB) = -26.43 ‰). IA-R001 is traceable to IAEA-CH-6 (International Atomic & Energy Agency, cane sugar, $\delta^{13}C$ V-PDB = -10.43 ‰). IA-R001, IA-R005 (Iso-Analytical Limited beet sugar standard, $\delta^{13}C$ V-PDB = -26.03‰), and IA-R006 (Iso-Analytical Limited cane sugar standard, $\delta^{13}C$ V-PDB = -11.64 ‰) were used as quality control for the analysis. The C isotope results are expressed in delta (δ) notation and $\delta^{13}C$ values are reported in parts per thousand (‰) relative to V-PDB standard.

Natural abundance of $\delta^{13}C$ was used to determine the portion of C in soil that was derived from the cactus pear root. This portion were calculated by the mixing equation (Gearing, 1991):

$$New\ carbon\ derived\ (Ncd) = \frac{\delta^{13}C_{new} - \delta^{13}C_{old}}{\delta^{13}C_{biomass\ new\ species} - \delta^{13}C_{old}} \qquad \text{(Eq. 1)}$$

and

$$Old\ Carbon\ derived\ (Ocd) = 1 - Ncd \qquad \text{(Eq. 2)}$$

where Ncd is the fraction of C derived from new vegetation (cactus pear), $\delta^{13}C_{new}$ is the isotope ratio of the soil sample, $\delta^{13}C_{biomass\ new\ species}$ is the isotope ratio of cactus pear, and $\delta^{13}C_{old}$ is the isotopic ratio of the soil before cactus pear plantation (C_3-C soil).

Under cactus pear the Ncd corresponds to CAM-C portion and Ocd correspond to C_3-C portion.

The root turnover was calculated for each soil volume according to the following equation:

$$Root\ turnover\ (\%) = \frac{New\ C\ derived * SOC}{Root\ weight * C_{root}} * 100 \qquad \text{(Eq. 3)}$$

where the SOC is the C content in the bulk soil for each pot (g); and C root is the concentration of C in the root biomass (g kg^{-1}) and Root weight is root dry weight (g) in each pot.

3.2.2. Roots Measurements

3.2.2.1. Roots length

Roots of each plant were divided visually into three groups depending on its diameter: fine roots less ≤ 2 mm, Medium roots (2-5 mm) large roots > 5 mm. The roots of each group were measured with a ruler to a precision of 1 mm. The whole root system was calculated as the sum of the lengths of all groups.

3.2.2.2. Roots surface area:

Roots surface area was measured using VegMeasure software®. VegMeasure is a Digital Vegetation Charting Technique (DVCT) developed based on computerised vegetation measurement program by the Department of Rangeland Ecology and Management at Oregon State University and the International Center for Agricultural Research in the Dry Area (ICARDA) (Louhaichi et al. 2010).

The three spectral reflectance bands (RGB) of the digital color camera are ratioed by the software to create meaningful classes. This technique allows customization of the images. Vertical roots images were taken using COOLPIX AW110 digital camera equipped with a 28-mm lens. The camera was mounted to a camera stand that was designed for mounting the camera for laboratory testing. The base board has a non-reflective dull black surface. The column is 760 mm in height marked with scales for height determination. A hand-operated camera armed conveniently adjusts the camera up and down horizontally. To minimize the overlapping, roots from each category were divided into small portions, and spread carefully on a black board to minimize overlaps then pictured. The dimensions of each image were 4608 × 3456 pixels and the size was about 6 Mb in JPG format. The camera lens was 35 cm from the black board surface. One pixel in the digital image represented 0.013 mm^2 at the board surface.

Estimates of yellowness (% yellow roots surface cover) were calculated from the digital camera images using supervised classification technique in VegMeasure software® (Johnson et al. 2009; Louhaichi et al. 2001). The colors from the obtained digital camera picture was interpreted by the software to create two meaningful classes. In this case manual classification of roots surface area and the black board surface area were set up for the images. After uploading all images few images were selected to set the threshold for each class. The pixels for each category in the image having the same value would be displayed with a distinct color. Statistics" button displayed values of each category and remains unclassified, by adding more colors to each category unclassified value getting lower and lower. In this study this value was 0%, this means all the colours in the original picture were added to its category. At this stage these classifications were applied to all images.

a. Original image

b. Processed image

Figure 1. Estimation of root surface area using a digital camera mounted on a monopod 35 cm above the surface: (a) original image captured with the equipment, (b) extracted image of the roots from the digital image using image processing using VegMeasure program

Figure 1 shows the original and proceed pictures for one of the root images. The output folder is containing the processed images and a summary excel file that illustrates the name of each image and the values (%) of the classification for each category. The total surface area of yellow roots from the image classification was calculated by summing the total area occupied by pixels classified as roots surface area. These values were multiplied by π "3.14" assuming the roots have cylindrical shapes. Classification accuracy was assessed using the accuracy assessment tool in VegMeasure© through computing the error matrix and the Kappa Index of Agreement. This latter is commonly used in remote sensing classification to assess the degree of success of a classification technique. The error matrix permits measurement of overall accuracy, category accuracy, producer's accuracy and user's accuracy (Congalton, 1991).

3.2.2.3. Root volume

Root volumes were calculated from surface area and root length (or) by assuming that roots are cylindrical. After finishing the roots measurements, three random subsamples from each pot roots were weighed and dried in a forced-draft oven at 75 °C for 72 h and the dry weight for each group was calculated.

For the root starch content three other samples were taken from each group in each pot dried at 50 °C for 72 h and sent to the laboratory.

3.2.3. Canopy measurements

In each sampling date cladodes of each plant in each pots were counted and numbered according to its age. Cladodes were clustered into three groups: mother cladodes, first generation cladodes and second generation cladodes. The total number of the cladodes in each group was recorded.

For each cladode in each group the following measurements were taken: cladodes surface area, cladodes thickness, cladodes fresh and dry weight

3.2.3.1. Cladodes surface area (cm^2)

The width and length of each cladode were measured. The maximum cladode width (W) was the widest point perpendicular to the half part of the cladode, and the length (L) was the distance from one end to the other end along the longest axis of the cladode.

These values were used to estimate the area of the cladode using the formula of the ellipse:

$$X = (W/2)*(L/2)*\pi \qquad \text{(Eq.3)}$$

where:

X = estimated area; W = width, minor axis; L = length, major axis; and $\pi = 3.14$

3.2.3.2. Cladodes thickness (mm)

The cladodes thickness was measured in mm with a vernier caliper.

3.2.3.3. Cladodes fresh and dry weight

The fresh weight of each cladode was taken, three subsamples of each cladodes were cut weighed and dried in a forced-draft oven at 75 °C for 72 h to estimate the dry weight. To estimate the starch content two samples were taken from each mother cladode: one from below soil and one from above soil part weighed, and dried in oven at 50 °C.

3.2.4. Calculated data

For each individual plant, the following parameters were calculated:

The root: shoot ratio was calculated (Dry weight for roots/dry weight for canopy= root/shoot ratio); root length density per soil unite (RLD) (total root length/soil volume cm L^{-1}); the specific root length (SRL) (the total root length /the root biomass cm g^{-1}); and BVR is the plant biomass at certain rooting space volume, BVR was estimated (total plant biomass: soil volume ratio; g L^{-1}) (Trubat et al. 2006; Sorgona et al. 2005; Kerstiens and Hawas, 1994).

3.2.5. Starch and nitrogen content analysis

Starch contents were measured using by the perchloric acid method. The prepared tissue was first extracted with boiling ethanol to remove interfering sugars and to gelatinize the starch granule. Next, the starch was solubilized by extracting the tissue in perchloric acid. This was accomplished in several ways, which included soaking or immersing the sample in acid (PA1), percolating acid through the sample (PA2), or percolating the sample and then precipitating starch with KI (PA3) (Hassid and Neufeld, 1964; Hoffpauir, 1956; McCready et al. 1950; Pucher et al. 1948). The solubilized starch solution was then reacted with a mixture of concentrated sulfuric acid and anthrone to hydrolyze starch to glucose and produce a color product that was quantified colorimetrically on a spectrophotometer (Yemm and Willis, 1954; Viles and Silverman, 1949).

To quantitate N-content in mother cladode and the roots tissues, samples were digested in a CEM microwave oven using H_2SO_4, H_2O_2, H_2O, and $HClO_4$. Following digestion, a sensitive colorimetric assay for ammonium is used to estimate N-content (Cataldo, 1974).

3.3. Statistical Analysis

Effects of soil volume and month of the cut were examined in terms of variability. The total variation in response, e.g. root length, was partitioned in terms of soil volume, month of cut and their interactions and presented as analysis of variance (ANOVA) table including p-values indicating significance of the main effects (soil volume and month of cut) and their interaction. The estimated mean values of these factors were obtained along with their standard errors. Furthermore, since soil volume and months of cut are quantitative factors, the relationship between response and these factor levels were examined using polynomial regressions. The two factors main-effects and interactions were partitioned into single degrees of freedom. All analysis was carried out using Genstat.

4. Results

4.1. Root measurements

Results indicate a significant effect of soil volume and sampling dates and their interaction (P<*0.01*) on total root length, root surface area, the large roots (Root with diameter > 5 mm) surface area, medium roots surface area, root dry mass, dry mass of the large and medium roots, root volume, specific root length (SRL) and BVR. Fine roots dry mass, root density and roots length density were significantly affected by soil volume and sampling dates. While, fine roots surface area was affected significantly by the soil volume only, neither by the sampling date nor by the soil volume X sampling date interaction.

Total root length

The effect of the soil volume on total root length was already noted by the end of the first six months after planting (Fig. 2). The root length segregated into two groups of slow length increase (5, 9 and 18 Liters soil volume) and high length increase (in the largest pots 33 and 50 Liters). The highest roots length was observed after 24 months of planting in the highest soil volume (18405 ± 987) while the lowest was in the smallest soil volume during the sampling time (2753 ± 978).

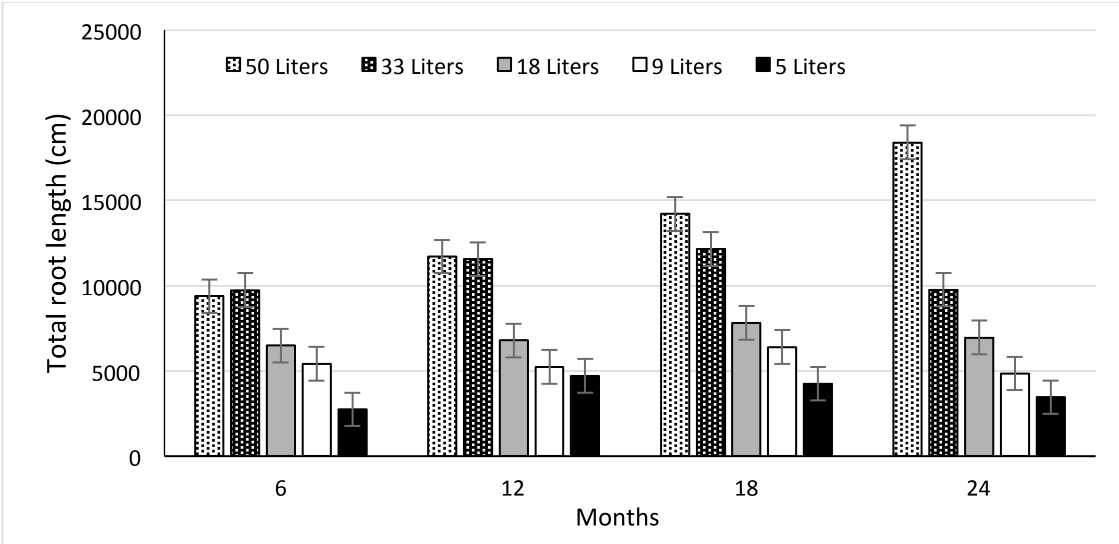

Figure 2. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on the total roots length (cm) per plant of *Opuntia ficus-indica.* Data are presented as means ± SE (*N=3 plants*)

Among the small containers, plants in 5 Liters soil volume exhibited the lowest root length values during in the four sampling dates (6, 12, 18, and 24 months). Nevertheless, the differences between the two largest soil volumes in terms of the roots length were recorded during the last two sampling dates (18 and 24 months).

Total root surface area

The roots of the plants grown in the large pots recorded higher surface area than that observed by the plants in small pots. The plants sampled after 18 and 24 months after planting had higher root surface area compared to the plants sampled after 6 and 12 months of planting (*P<0.01*). The root surface area ranged between 5639 to 757 cm^2 (90% reduction) with the highest value of plants sampled from the largest soil volume after 24 months and the lowest values of plants grown in 6 Liters soil volume and sampled after six months of panting (Fig. 3). During the first 12 months there were no significant differences between 50 and 33 Liters soil volume that had the highest values. Root surface area in lower soil volumes 18 and 9 Liters was significantly different from 12 months after planting onwards. Root surface area in the smallest soil volume was always the lowest.

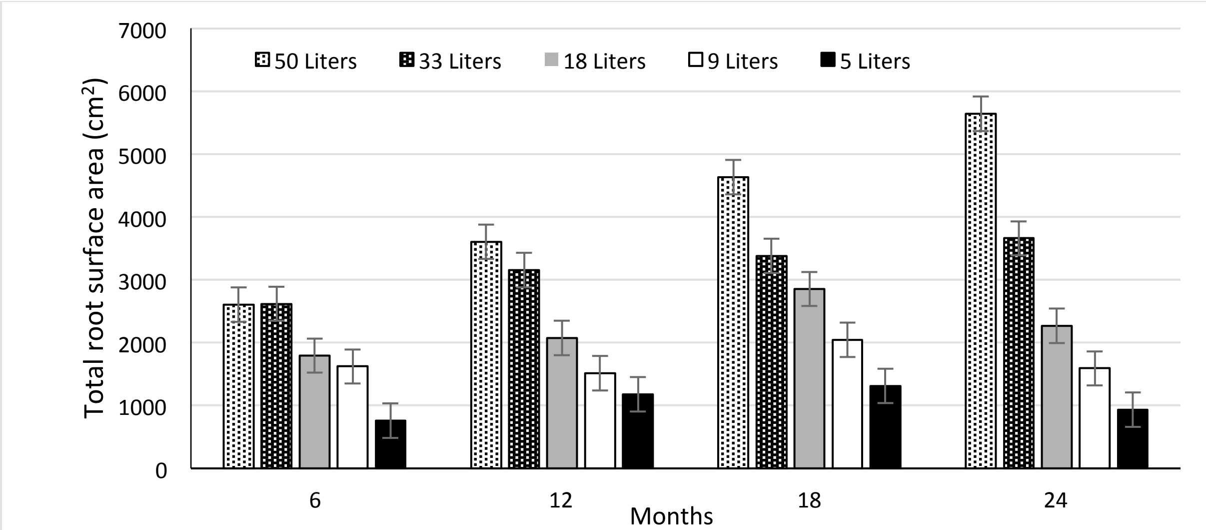

Figure 3. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on the total roots surface area (cm^2) per plant of *Opuntia ficus-indica.* Data are presented as means ± SE (*N=3 plants*)

In terms of quantitative data, root surface area in 50 Liters soil volume doubled over the time of the experiment, while only a marginal increase occurred in 33 Liters soil volume from 6 to 12 months after planting. Root surface area in 18 and 9 Liters showed a decrease during the last measurement, 24 months after planting, while root surface area in the smallest pots did not show any significant change during the experiment.

Large root surface area

Large roots surface area increased positively with soil volume over time, it was approximately constant between 6 and 12 months of sampling dates but increased significantly by the third and fourth sampling date (18 and 24 months). A significant effect of the soil volume restriction on the large roots surface area was recorded in the third and fourth sampling dates (18 and 24 months).

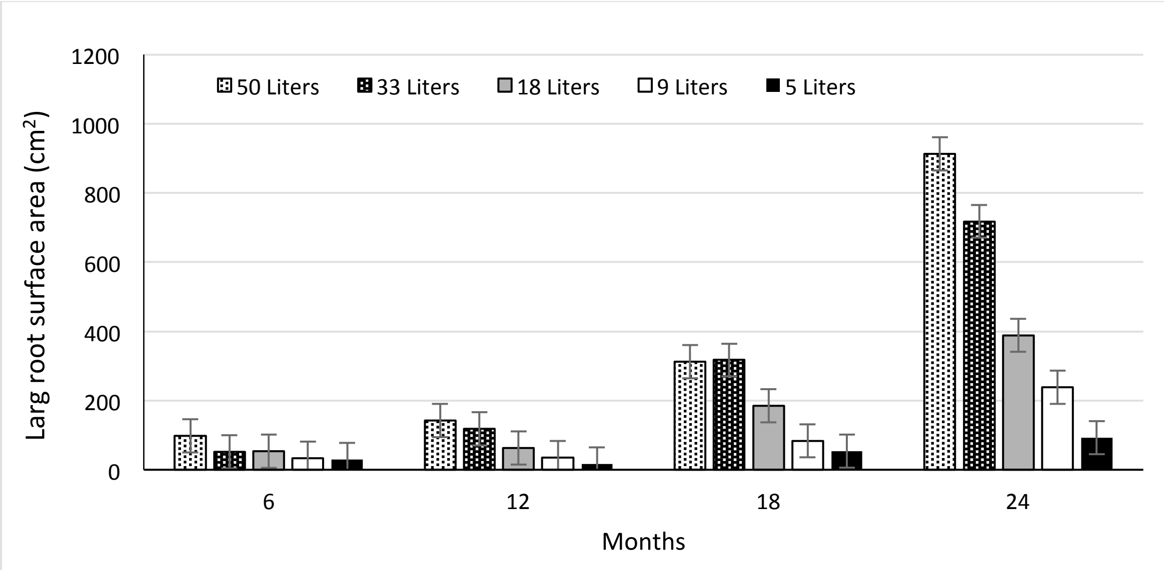

Figure 4. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on large roots surface area (cm^2) of *Opuntia ficus-indica.* Data are presented as means ± SE (*N=3 plant)*

The highest roots surface varied from (319 cm^2) in the last sampling date for the plants raised in the largest soil volume (50 Liters) to (17 cm^2) for the smallest soil volume in the second sampling date (12 months) (Fig. 4). A linear regression between the large roots surface area and large roots volume, large roots length was carried out, resulting in coefficient of determination of (0.95). This result suggest a strong relationship between large roots surface area and these two parameters.

The medium root surface area

Medium roots surface area had approximately the same trend of the large roots surface area. The significant effect of the soil volume on this parameter was shown in the four sampling dates, the largest soil volume resulted in plants having lager medium surface area in the four sampling dates. However, no significant differences were observed between the two (33 and 50 Liters) soil volume in the first sampling dates (Fig. 5). Medium roots surface area didn't differ significantly between the 6 and 12 months and between 18 and 24 months. The medium surface area of the plants sampled after 18 and 24 months of planting was greater than the ones in the plants sampled after 6 and 12 months (*$P<0.001$*). The linear regression between the medium roots surface area and medium roots volume; medium roots surface area and medium roots length showed very strong positive relationship with coefficient of determination of (0.96 and 0.94 respectively).

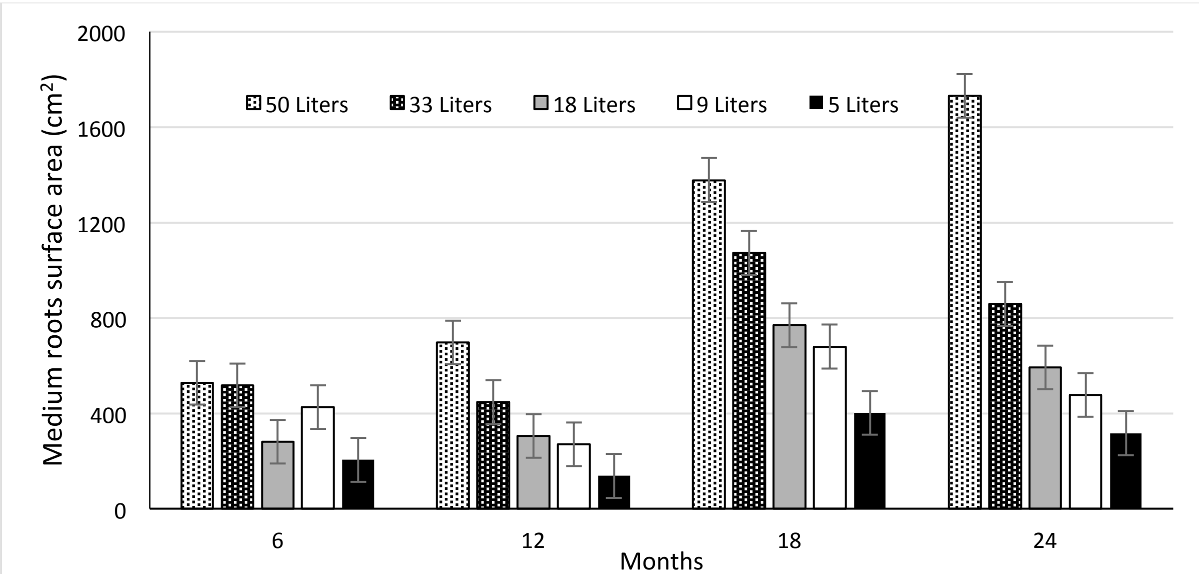

Figure 5. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on medium roots surface area (cm^2) of *Opuntia ficus-indica.* Data are presented as means ± SE (*N=3 plant)*

Fine root surface area

A significant effect of the soil volume on the fine roots surface area was observed from the first sampling date (6 months). No differences were recorded between the two largest soil volume (33 and 50 Liters) in the first two sampling dates. Nevertheless, this difference became large and significant in the third and fourth sampling date. The lowest fine roots area (522 cm^2), was recorded in the plants grown in the smallest soil volume (5 Liters) (Fig. 6). Likewise, for the large and medium roots surface area, strong and positive relationship were found between the fine roots surface area and fine roots length and fine roots volume when regression analysis was applied, the values of 0.91 and 0.94 coefficient of determination for fine roots length and fine roots volume respectively.

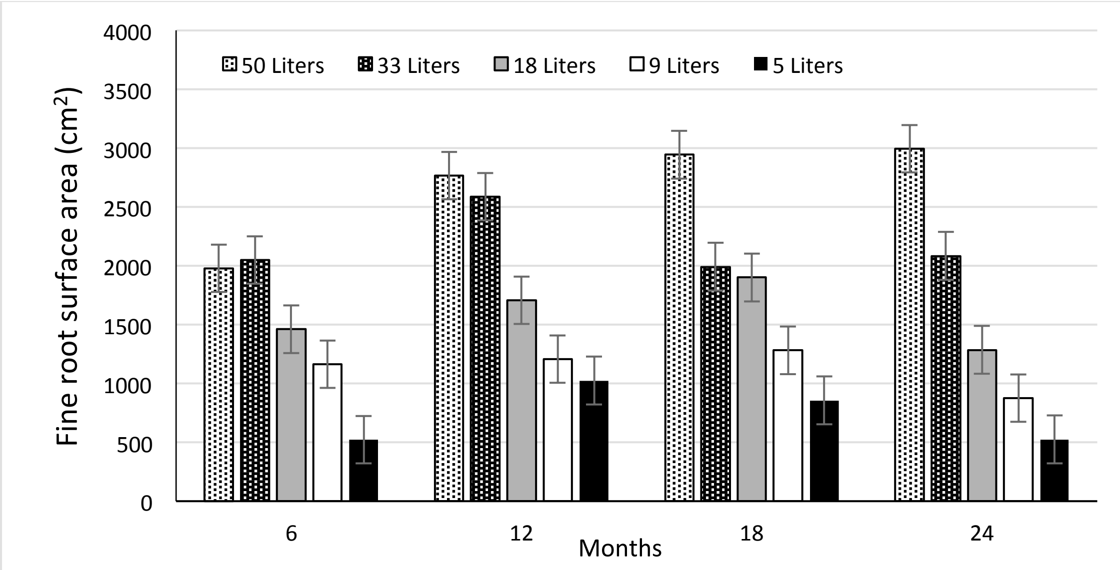

Figure 6. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on fine roots surface area (cm^2) of *Opuntia ficus-indica.* Data are presented as means ± SE (*N=3 plant)*

Total root dry mass

Final dry weight values of plants grown in 5 Liters soil volume were about 85% lower than those grown in the largest soil volume (50 liters) (Fig. 4). The total roots dry mass of the plants grown in the two largest soil volumes (50 and 33 Liters) increased with sampling dates. However, the plants grown in the (18, 9 and 6 Liters) showed relative stable dry weight over sampling dates (Fig. 7). After 24 months of planting roots of the plants grown in the 50 Liters showed the highest roots dry mass (206 g) while plants grown in 5 Liters soil volume recorded the lowest roots dry mass.

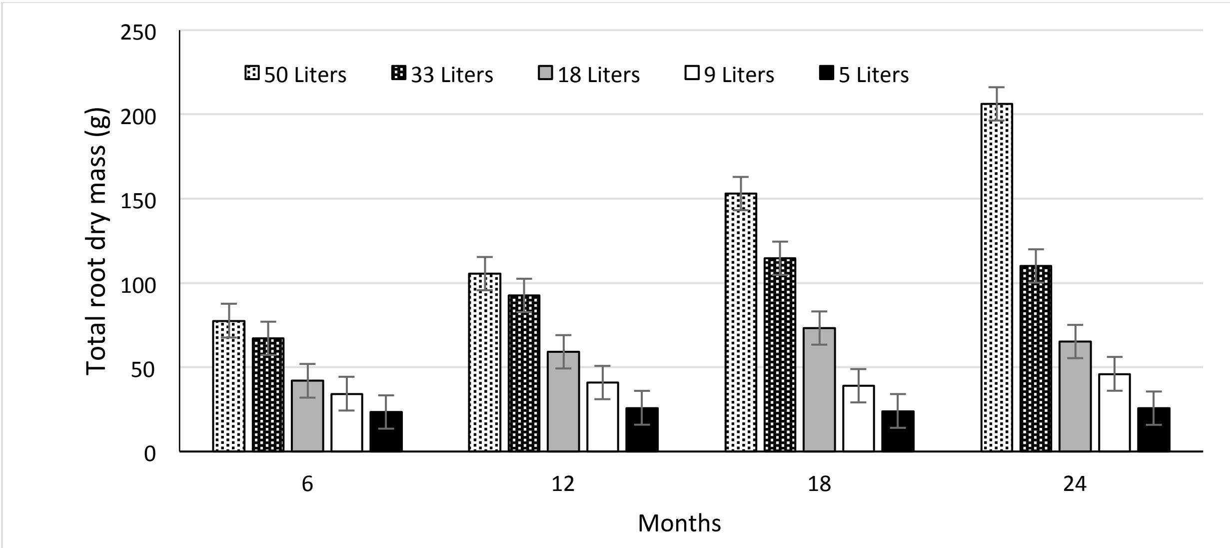

Figure 7. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on the total roots dry mass (g) per plant of *Opuntia ficus-indica.* Data are presented as means ± SE (*N=3 plants*)

Large root dry mass

A significant increase of the large roots volume related to soil volume increase was observed starting from the six-month-old plants. This increase was not significant for the 12 months' plants. This increase was clear and significant in the plants of 18 and 24 months age. The plants grown in (50 Liters) soil volume exhibited the large highest roots dry weight in the four sampling dates with values ranged between 114 g in the fourth sapling date to 11 g in the second sampling date. over time, big roots dry weight was separated into two groups of low dry weight (6 and 12 months) and high dry weight (18 and 24 months) (Fig. 8).

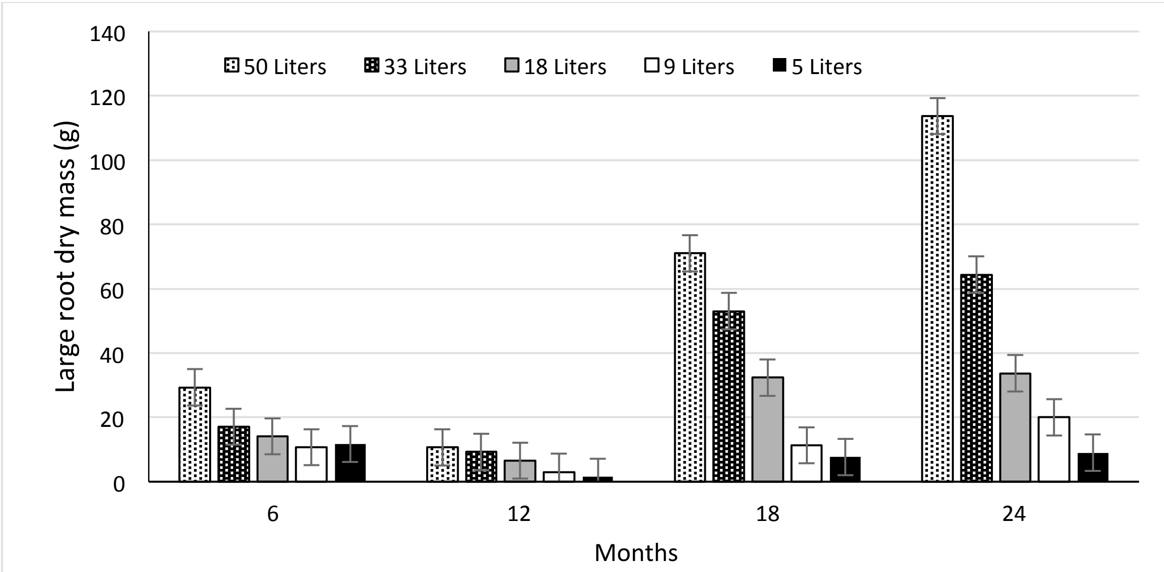

Figure 8. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on large roots dry mass (g) of *Opuntia ficus-indica*. Data are presented as means ± SE (*N=3 plant*)

Medium root dry mass

Linear and positive significant relationship between the soil volume and the medium roots mass was observed in the second, third and fourth sampling dates. This relation was not linear in the first sampling date. The plants with highest medium roots dry mass were the plants grown in the (50 and 33 Liters) (Fig. 9).

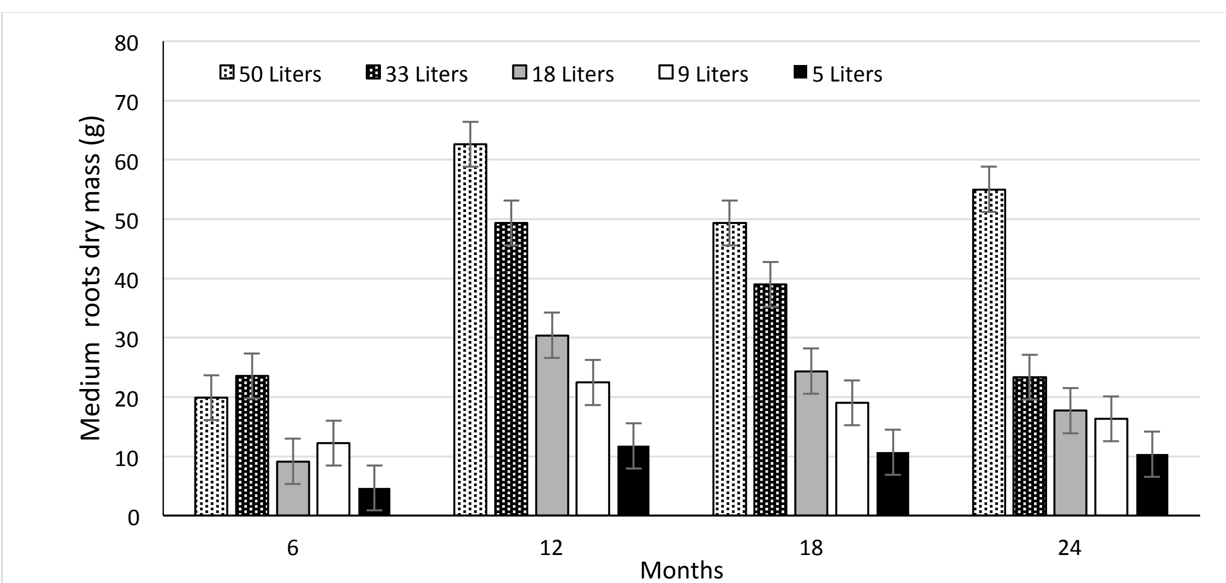

Figure 9. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on medium roots dry mass (g) of *Opuntia ficus indica*. Data are presented as means ± SE (*N=3 plant*)

In the last three sampling dates, plants grown in (50 Liters) recorded the highest mass of medium roots while the lowest was recorded for the plants raised in (5 L) soil volume. The medium roots dry mass varied between 63 g (50 L soil volume, 24 months sampling date) to 5 g (5 Liters soil volume, 6 months sampling date). Medium dry mass values increased significantly after the firs sampling date, however, this mass was decreasing during the last two sampling dates. The highest significant values medium dry weight was observed in the second sapling date (Fig. 9).

The fine root mass

Data in Figure (10) showed the linear positive and significant effect of the soil volume on the fine roots mass, during the for sampling dates with the highest fine roots mass as recorded in the largest soil volume. No significant differences were observed between the soil volume 33 and 50 Liters in the first two sampling dates. However, this difference becomes clear and significant in the last two sampling dates. The highest mass of the fine roots (38 g) was obtained from the plants placed in the largest soil volume and harvested after 24 months of the planting. No clear trend of the fine roots dry mass over time observed, the highest significant fine roots mass was recorded in the second sampling date. Similar values for the fine roots mass were observed in the first, third and fourth sampling date.

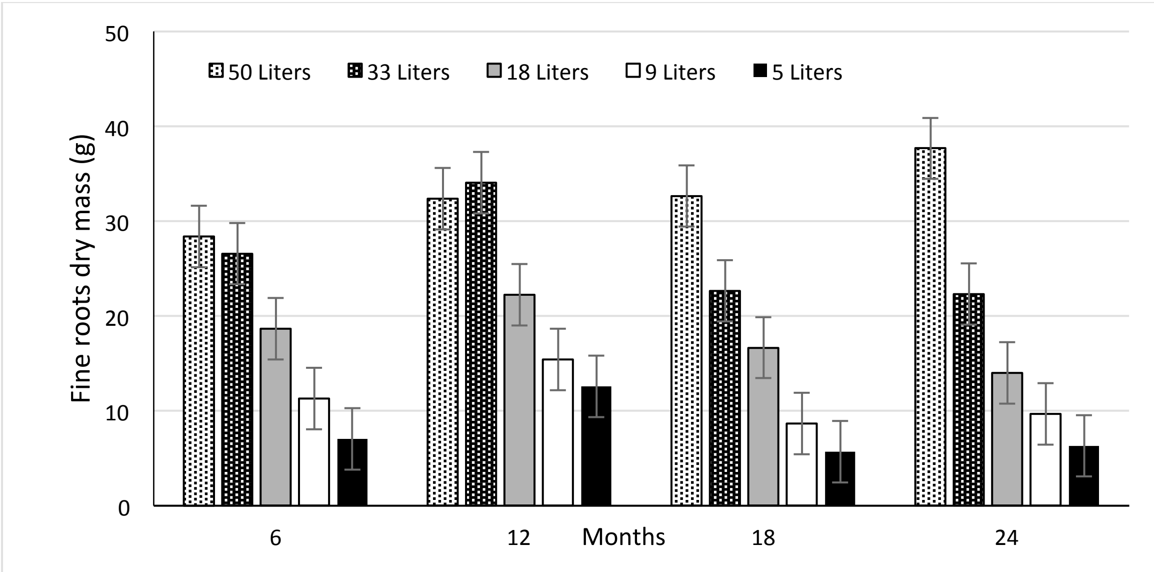

Figure 10. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on fine roots dry mass (g) of *Opuntia ficus-indica*. Data are presented as means ± SE (*N=3 plant*)

Total root volume

Significant increase of the root volume was observed with the soil volume increase during the four samplings dates (Fig. 11). The highest roots volume was recorded in the plants sampled from the largest pot size after 24 months of planting, it was (20, 46 and 56% greater than the roots volume values of the plants grown in the same pot size sampled after 6, 12 and 18 months of planting date respectively. No significant changes of roots volume over time were observed in the plants grown in the smallest soil volume (5 Liters).

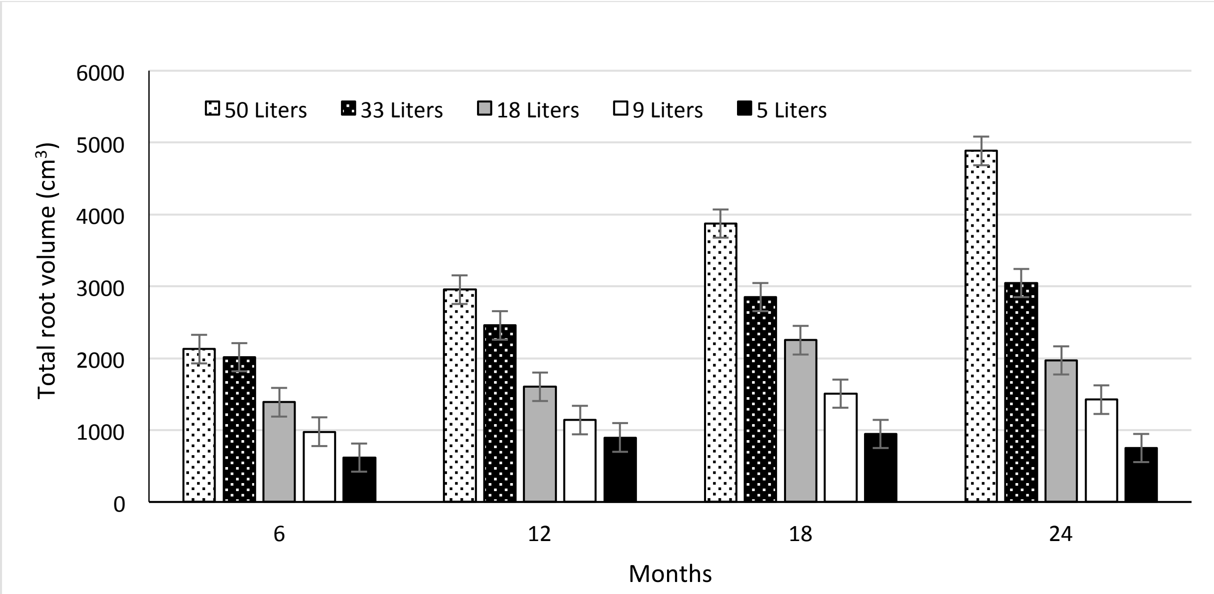

Figure 11. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on the total roots volume (cm^3) per plant of *Opuntia ficus-indica.* Data are presented as means ± SE (*N=3 plants*)

Root density

Root density values ranged between 43 cm^3/L and 167.6 cm^3/L. Among the four soil volumes, the plants grown in the smallest soli volumes (5, 9 Liters) exhibited the greatest roots density, together with a significant decrease 24 months after planting. There was an increase in root density with plant age (6, 12, 18 months). However, this increase was stopped at the last sampling date (24 months) and no significant differences were found between the root density in the third and fourth sampling date (Fig. 12).

Specific root length (SRL)

There was no clear trend for the soil volume effect in the first sampling date. Plants grown in (33, 18 and 9 Liters had similar specific roots length which were higher ($p< 0.001$) than the ones observed in the plants grown in 5 and 50 Liters. However, this trend became clearer in the following three cuts. Both soil volume and sampling date had negative effect on the specific roots length. The highest value was recorded in the plant placed in the lowest soil volume (5L) after 12 months of planting, while the highest value was recorded in the last sampling date for the plants in the largest soil volume (Fig. 13).

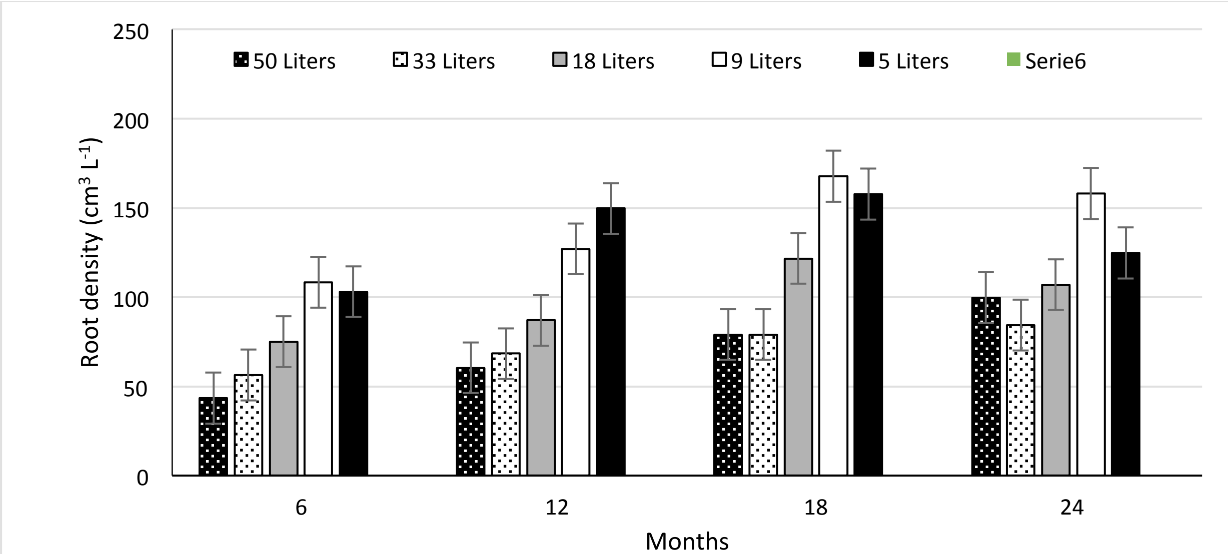

Figure 12. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on the total Root density (cm^3L^{-1}) of *Opuntia ficus-indica.* Data are presented as means ± SE (*N=3 plants*)

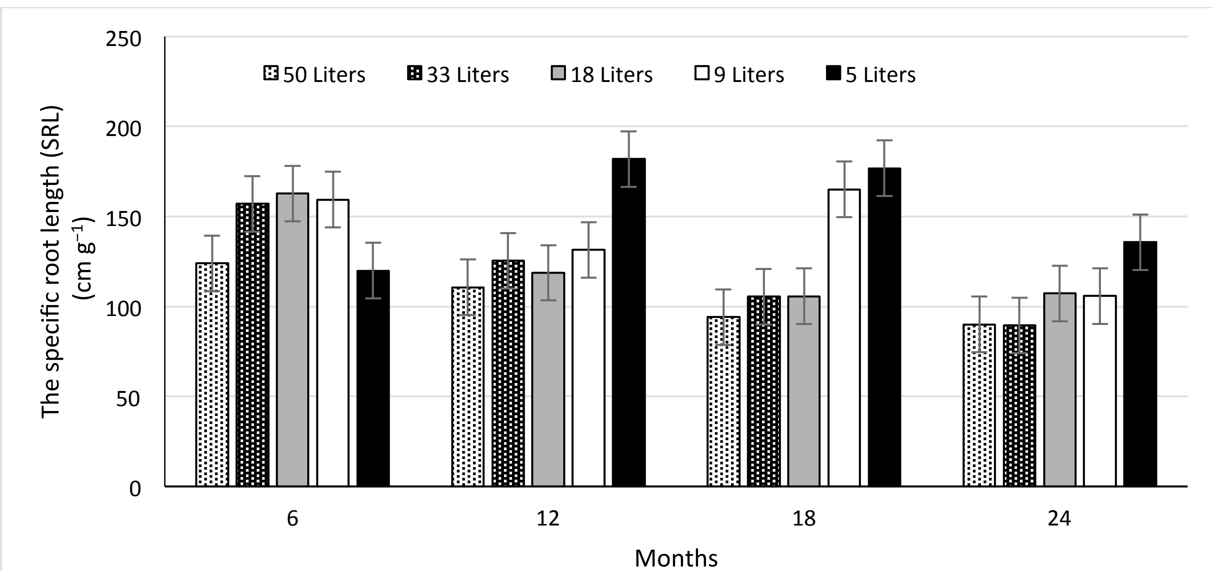

Figure 13. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on specific roots length (SRL) ($cm\ g^{-1}$) of *Opuntia ficus-indica.* Data are presented as means ± SE (*N=3 plants*)

Root length density (RLD)

Roots length density was decreased with the soil volume increase (Fig. 14). Plants grown in smaller soil volume (5 and 9 Liters) had a more extended root significantly twice or more roots length density greater than those raised in bigger soil volumes (50 and 33 Liters). No significant differences were observed among the plants sampled after (12, 18 and 24 months) which was significantly higher than the one sampled in 6 months after planting.

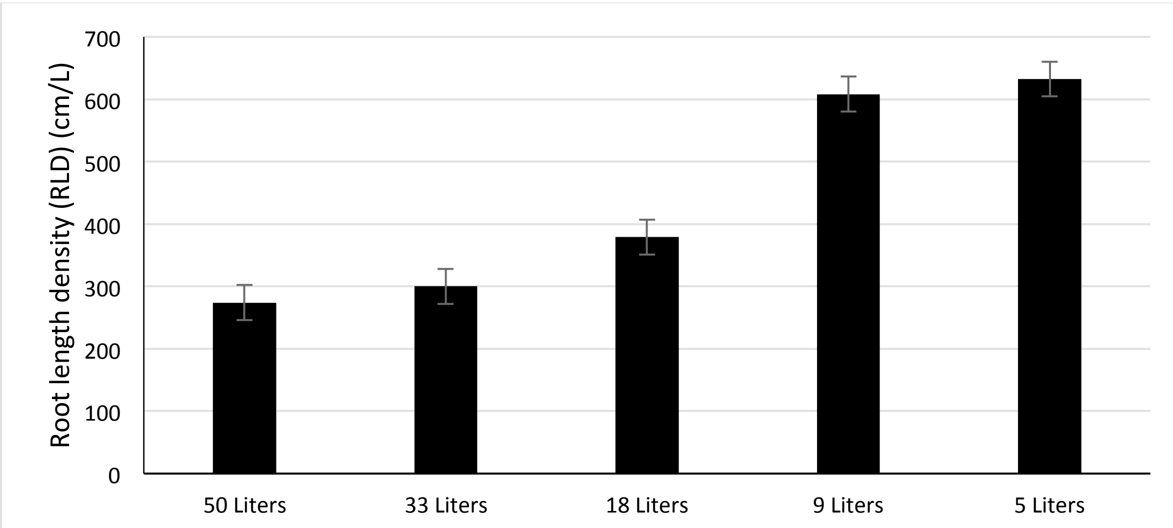

Figure 14. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) on roots length density (RLD) (cm/L) of *Opuntia ficus-indica.* Data are presented as means ± SE (*N=12 plants*)

BVR (Total Plant Biomass: Soil volume ratio) (g L^{-1})

BVR values ranged from 3.2 for the pot size 9 L in the first sampling date to 30.8 in pot size 33 L at the last sampling date (Fig. 15). Pot size effects started when BVR exceed 2 g/L. As the values of BVR for all the treatments in the four sampling dates were more than 2, this means the plant growth was affected by the pot volume. This effect was increasing over time.

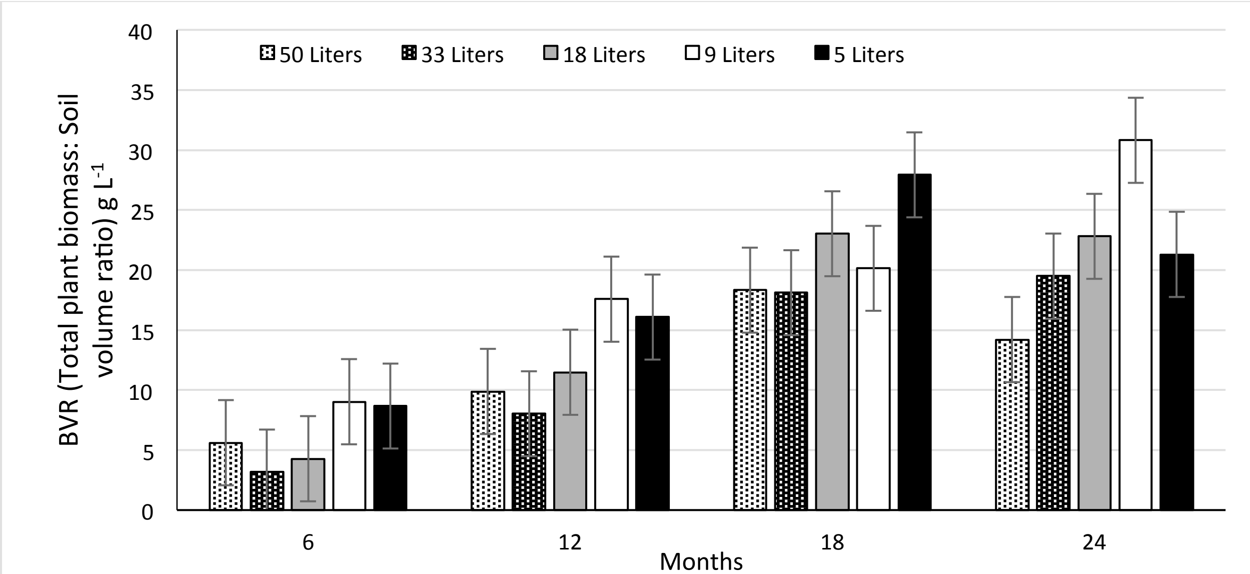

Figure 15. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on total Plant Biomass: Soil volume ratio (BVR) of *Opuntia ficus-indica.* Data are presented as means ± SE (*N=3 plant)*

4.2. Root system structure

Soil volume and sampling dates and their interaction significantly affected (*p*< *0.001*) the large roots (Root with diameter > 5 mm) surface area, medium roots surface area, dry mass of the large, medium roots. Yet, fine roots dry mass was affected by the soil volume and the sampling date only. While, fine roots surface area was affected significantly by the soil volume only, neither by the sampling date nor by the soil volume X sampling date interaction.

4.3. Canopy measurements

The soil volume, sampling dates and their interaction had significant effects on the number of second generation cladodes, dry mass of canopy and total canopy surface area. The number of the first generation cladodes was significantly affected by the soil volume. No effect of the sampling dates or the interaction of the soil and the sampling date was recorded. However, total number of cladodes was affected by the soil volume and the sampling dates but not by their interaction. Soil volume and the sampling dates X soil volume interaction showed significant effects on the total number of the areoles developed roots.

Number of the first generation and second generation cladodes

Plants placed in the largest soil volume significantly produced the highest number of the first generation cladodes in the four sampling dates. The highest value was in the second sampling date (6 cladodes) while the lowest number (2 cladodes) was for the plants raised in (18 Liters) soil volume in the second sampling date. Overall, no clear correlation was observed between the (33, 18, 9 and 5 Liters) soil volumes and number of first generation cladodes (Data is not shown).

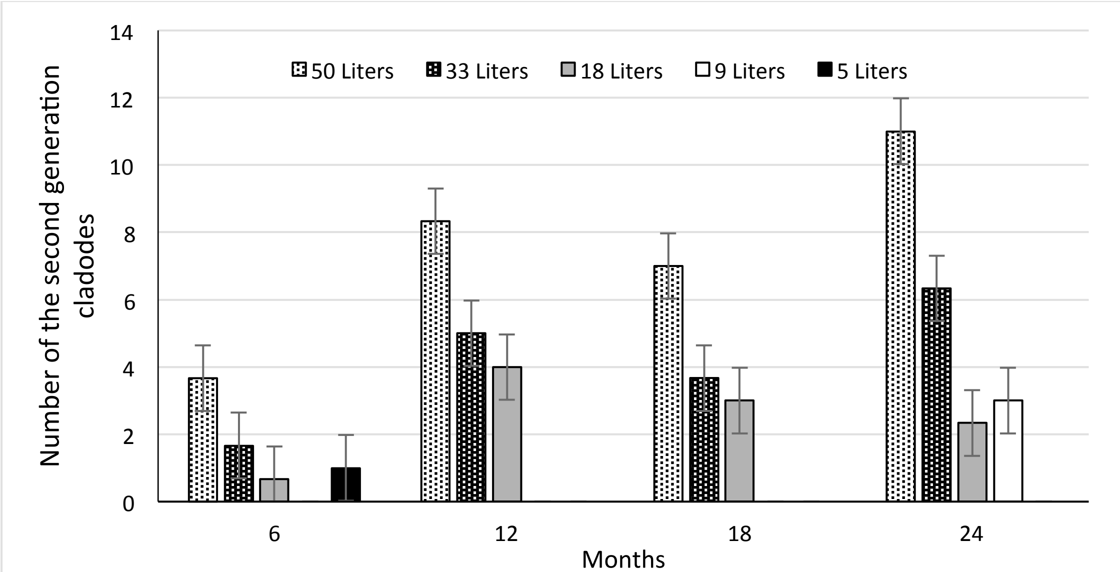

Figure 4. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on number of the second generation cladodes per plant of *Opuntia ficus-indica*. Data are presented as means ± SE (*N=3 plant*)

On the other hand, the soil volume had a clear effect on the number of the second generation cladodes. Plants placed in the smallest soil volume (5 Liters) stopped producing new second generation cladodes after the first sampling date. The highest number of the second generation cladodes was recorded in the plants of the largest size 50 Liters after 24 months of planting (55% more than 33 Liters) (Fig. 16).

Total number of the cladodes

The soil volume restriction tended to have clear effects on the total number of the new cladodes, the number ranged between 3-16 cladodes, as expected the highest number was produced by the plants raised in the highest soil volume and the lower number was in the lowest soil volume. These trends were similar with the four sampling dates (Fig. 17).

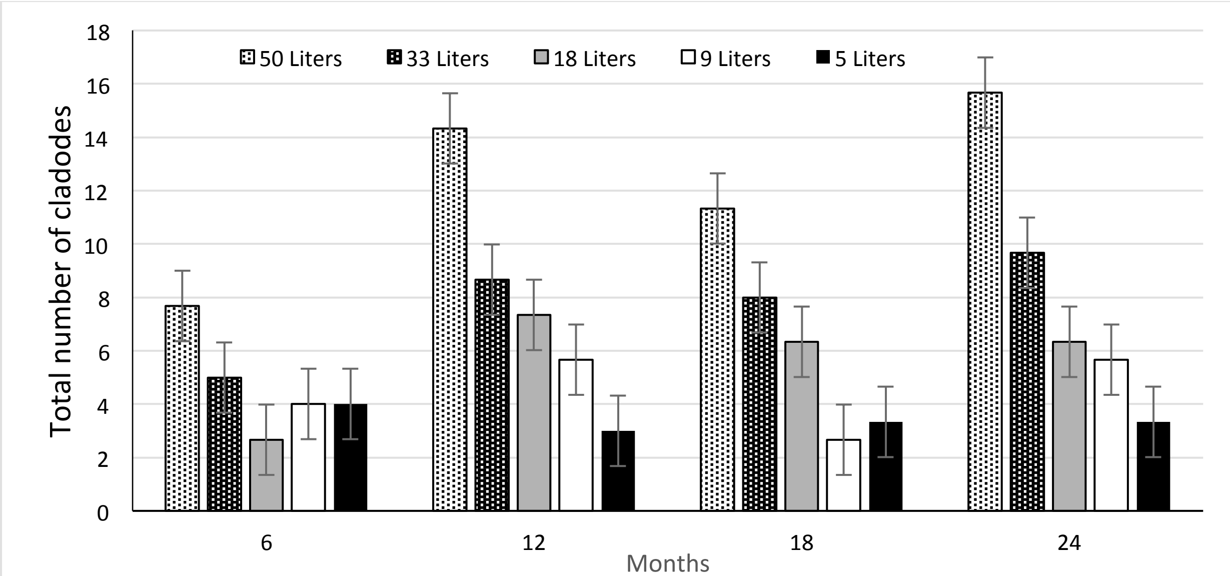

Figure 5. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on number of the total number of cladodes per plant of *Opuntia ficus-indica*. Data are presented as means ± SE (*N=3 plant*)

Dry mass of canopy

Linear and positive effect of the soil volume on canopy dry mass was observed. Plants placed in the highest soil volume produced the highest dry mass in the third sampling dates (898 g) compared to 52 g dry mass of the plants placed in 5 Liters soil volume and sampled after six months of planting. Dry canopy mass was increased over time, the highest value was recorded in the third and fourth sampling dates, nevertheless no significant differences were observed between these two sampling dates (Fig. 18).

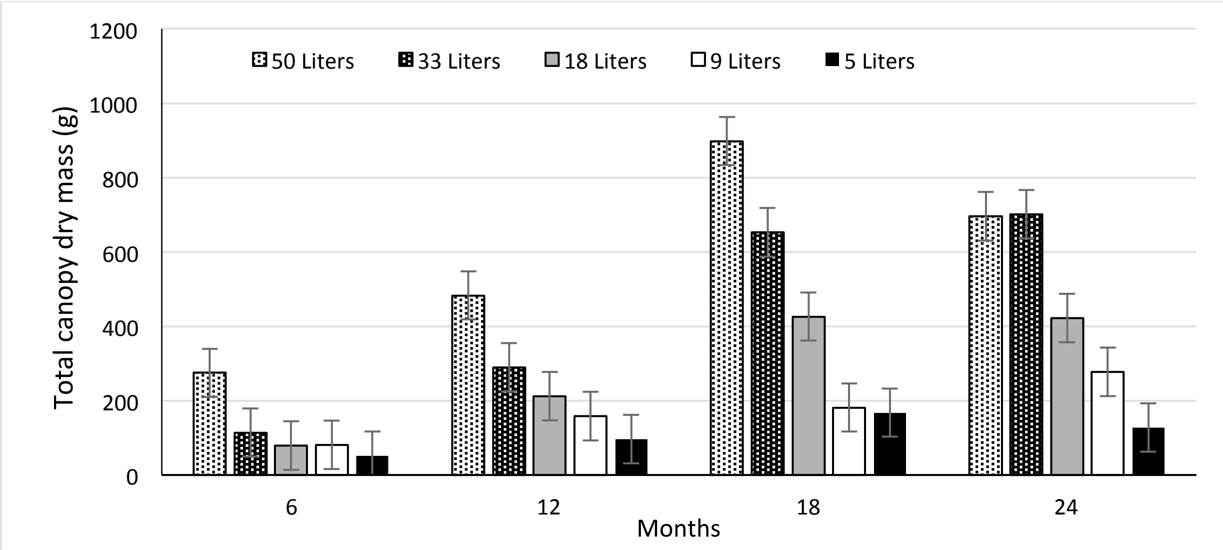

Figure 6. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on dry mass of canopy (g) of *Opuntia ficus-indica*. Data are presented as means ± SE (*N=3 plant*)

Total canopy surface area

Total canopy surface area was affected significantly by the soli volume; a linear and positive relationship was observed in the four sampling dates, the total surface area varied from 9053 cm^2 (highest soil volume in the second sampling date) to 527 cm^2 (lowest soil volume in the third sampling date) (Fig. 19). The sampling date affected the total canopy surface area significantly, however no clear trend over time was noticed. The highest value was observed in the second sampling date followed by the first sampling date, the plants sampled in the third dates produced the lowest total surface area.

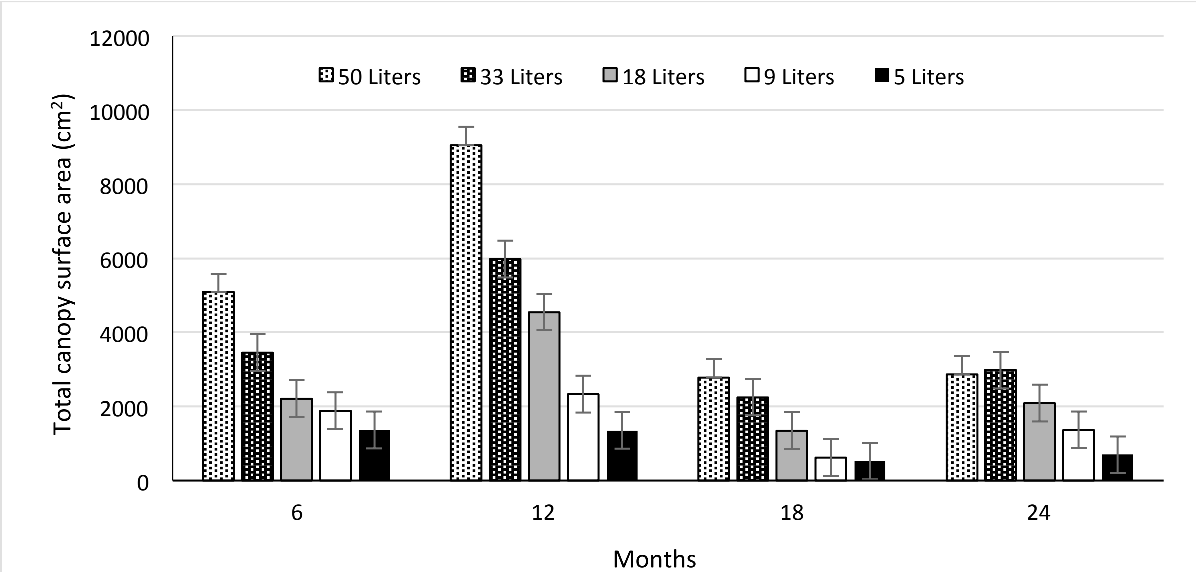

Figure 7. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on total canopy surface area (cm^2) of *Opuntia ficus-indica*. Data are presented as means ± SE (*N=3 plant*)

Number of the areoles developed roots

Soil volume and the sampling dates X soil volume interaction showed significant effect on the total number of the areoles developed roots. Plants placed in highest soil volume enabled higher number of areoles to produce roots (64, 65, 72 and 65) in the four sampling dates (6, 12, 18 and 24 months) respectively (data is not shown).

4.4. Roots: canopy ratios

Root: shoot dry mass ratio

Root mass: canopy mass ratio is another variable that give indicator about the balance extend between the roots and canopy. The results showed clear and significant impact of the soil volume on this ratio. The differences among the four sampling dates were not noticeable. Positive and linear relationship between soil volume and root: shoot ratio where observed in all the two treatment interaction in the third and fourth sampling date. The values of this ratio ranged between 7.7 to 37 % (Fig. 20).

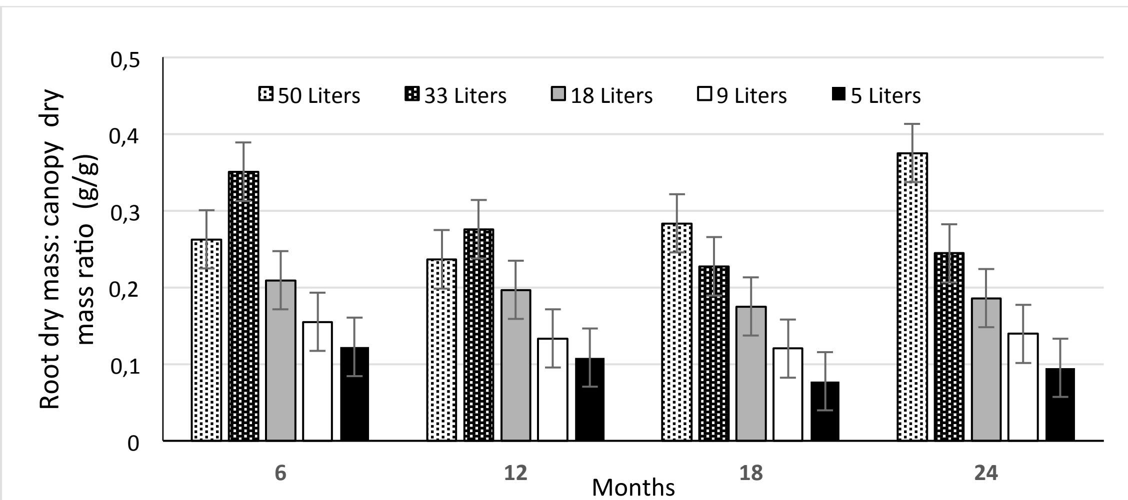

Figure 8. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and four different sampling dates (6, 12, 18 and 24 months) on Root dry mass: canopy dry mass ratio (g/g) of *Opuntia ficus-indica*. Data are presented as means ± SE (*N=3 plant*)

4.5. Relationship between Opuntia ficus- indica root and canopy variables

Positive and significant correlation between canopy mass and the fine, medium and large roots volume were recorded. The medium roots seemed to contribute the most to the canopy mass (r=0.78) followed by the biggest root (r=0. 67), the last one was the fine roots (r=0.57). The large and the medium roots area had high significant correlation to the canopy dry mass (r= 0.77; 0.72 respectively $p<0.001$). Fine, medium and large roots length correlated positively with the canopy mass (r) ranged between 0.72 for the large and medium roots length to 0.60 for the fine root length *(*$p<0.001$*)*.

***Opuntia ficus-indica* root turnover**

The $\delta^{13}C$ value of soil before cactus pear plantation was -25.6‰ and increased after the plantation due to the addition into the soil of organic matter from cactus pear through root turnover ($\delta^{13}C$ value of cactus pear root= -21‰). The $\delta^{13}C$ value of soil was significantly affected by soil volume, sampling dates and their interaction. $\delta^{13}C$ of soil ranged between -25.4‰ to -22.5‰, with the lowest value in soil sampled in the first sampling date and the highest values in soil sampled after 24 months (Fig. 21). The $\delta^{13}C$ significantly increased with the soil volume increase (*p< 0.001*).

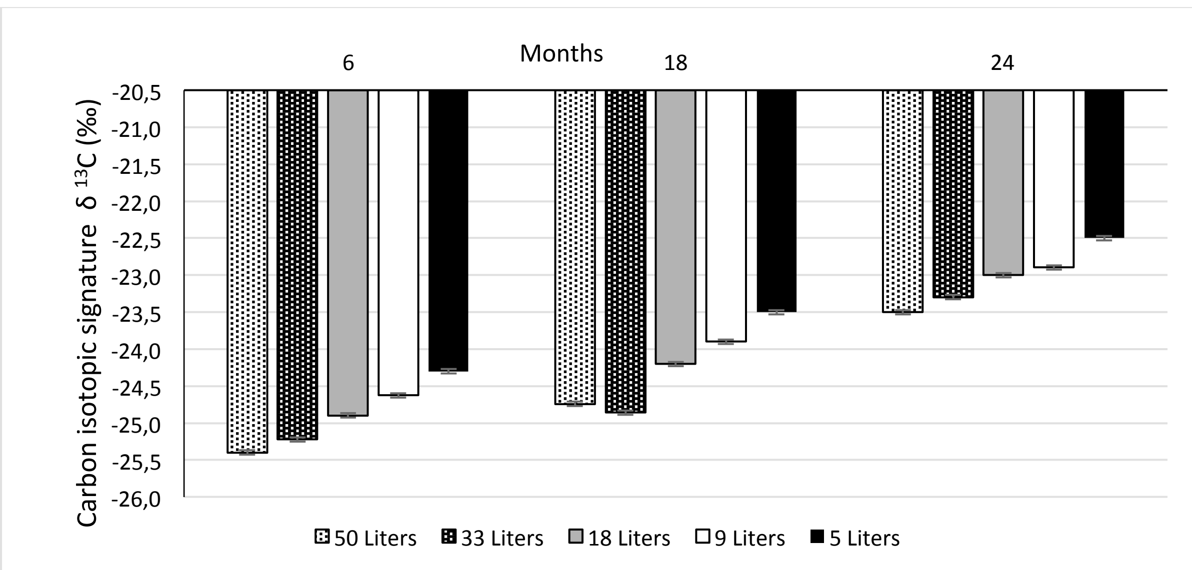

Figure 9. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) and three different sampling dates (6, 18 and 24 months) on carbon isotopic signature $\delta\ ^{13}C$ (‰) of *Opuntia ficus-indica*. Data are presented as means ± SE (*N=3 plant*)

Results of carbon derived by roots per soil unit and the root turnover were affected significantly by the soil volume and sampling dates; the highest percentage of SOC derived by root was found in the smallest soil volume, while the lowest was found in the largest soil volumes. The carbon derived (from *Opuntia ficus-indica* roots) ranged between 0.27 gC kg^{-1}soil to 0.57 g kg^{-1} soil (Fig 22).

The contribution of the carbon derived from the cactus plants to the soil increased over time, the highest value was after 24 month since planting (0.78 g C kg^{-1} soil) which was almost 4 times more comparing to the concentration derived after 6 month since plantation (Fig. 22).

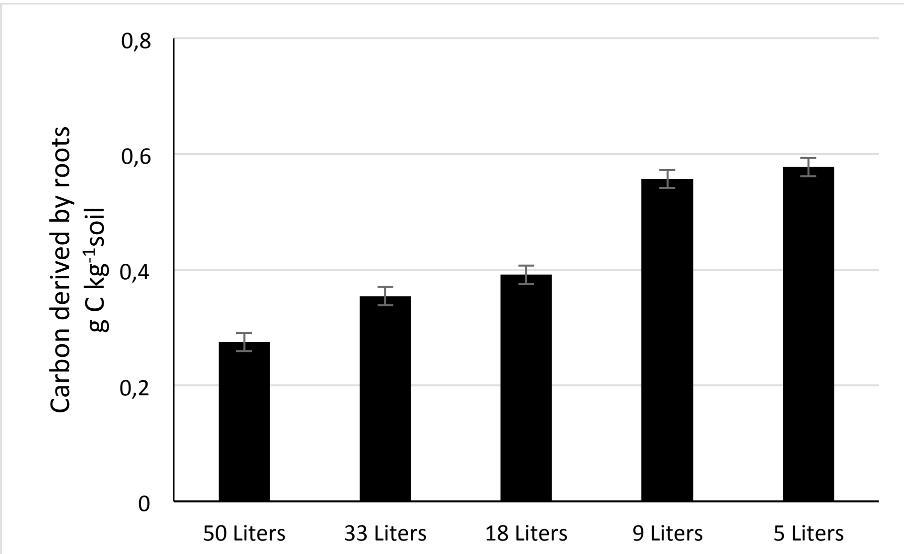

Figure 10. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) on Carbon derived by roots g C kg^{-1}soil of *Opuntia ficus-indica*. Data are presented as means ± SE (*N=3 plant*)

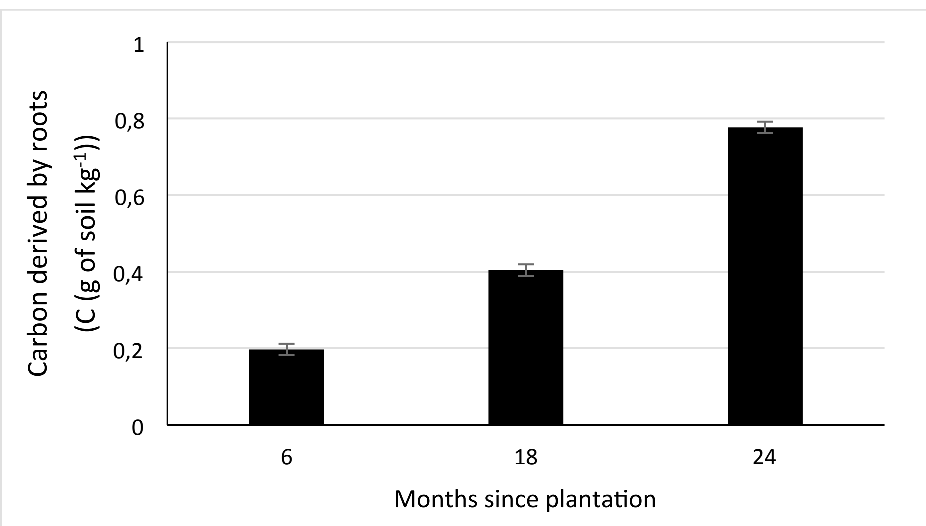

Figure 11. Effect of three different sampling dates (6, 18 and 24 months) on Carbon derived by roots (C (g of soil kg^{-1})) of *Opuntia ficus-indica*. Data are presented as means ± SE (*N=3 plant*)

Roots of the plants placed in the smallest soil volume had higher turnover rate (% per year) comparing to the ones planted in the bigger soil volume; the root turnover rate increased by 36% with a reduction of soil volume by ten times (Fig. 24). The Opuntia ficus indica roots turnover rate increased over time. The highest rate was observed after 2 years of planting (9.6%) while the lowest rate observed after 6 months of planting (2.7%) (Fig 25).

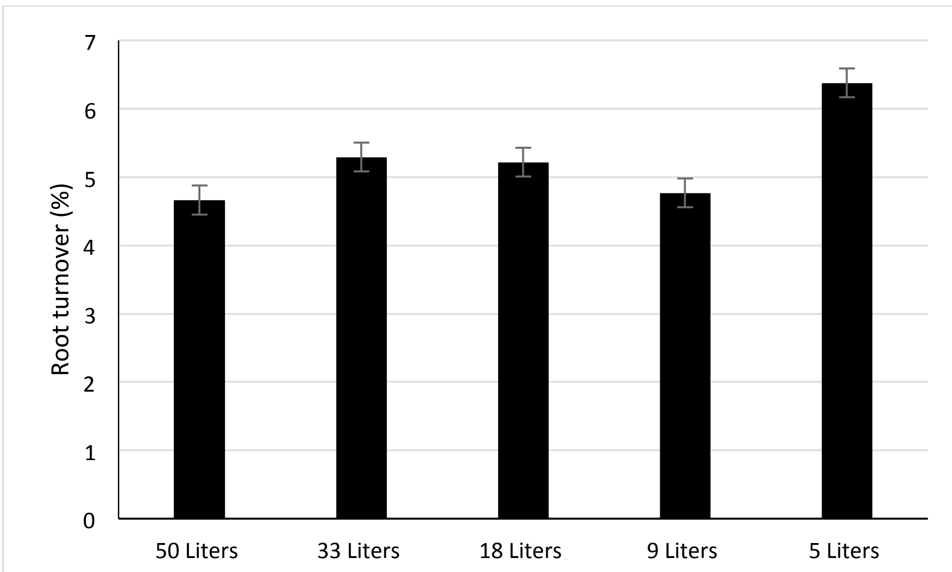

Figure 12. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) on roots turnover rate (% per year) of *Opuntia ficus-indica*. Data are presented as means ± SE (*N=3 plant*)

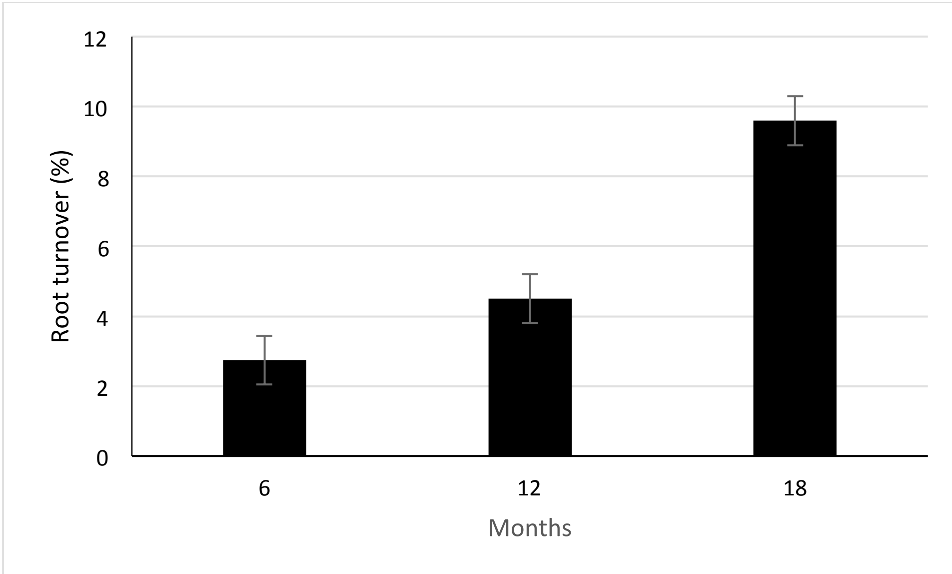

Figure 13. Effect of three different sampling dates (6, 18 and 24 months) on roots turnover rate (% per year) of *Opuntia ficus-indica*. Data are presented as means ± SE (*N=3 plant*)

4.6. Starch and Nitrogen content

Starch content in the mother cladodes

Mother cladode and roots starch content were affected significantly by the soil volume but not by the sampling date and soil volume X sampling date interaction. Soil volume restriction increased the starch contact of the mother cladode. No significant differences were found among the three smallest soil volumes (18, 9 and 5 Litres) but with the soil volumes 33 and 50 Litres which has lower starch content (Fig. 26).

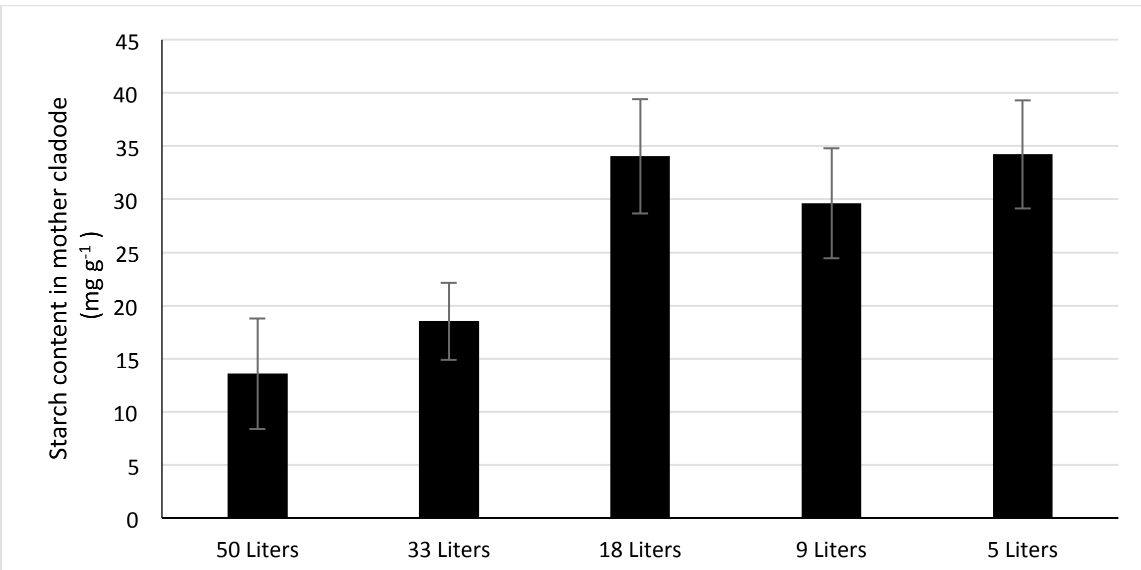

Figure 14. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) on starch content in the mother cladodes (mg g^{-1}) of *Opuntia ficus-indica.* Data are presented as means ± SE (*N=12 plants*)

Starch content in the roots

The root starch contact increased linearly with the soil volume decrease with values ranged between 7.7 to 12.7 mg in the roots of the plants grown in the smallest soil volume (5 Liters) to 7.2 mg in the plants grown in 33 Litres (Fig. 27).

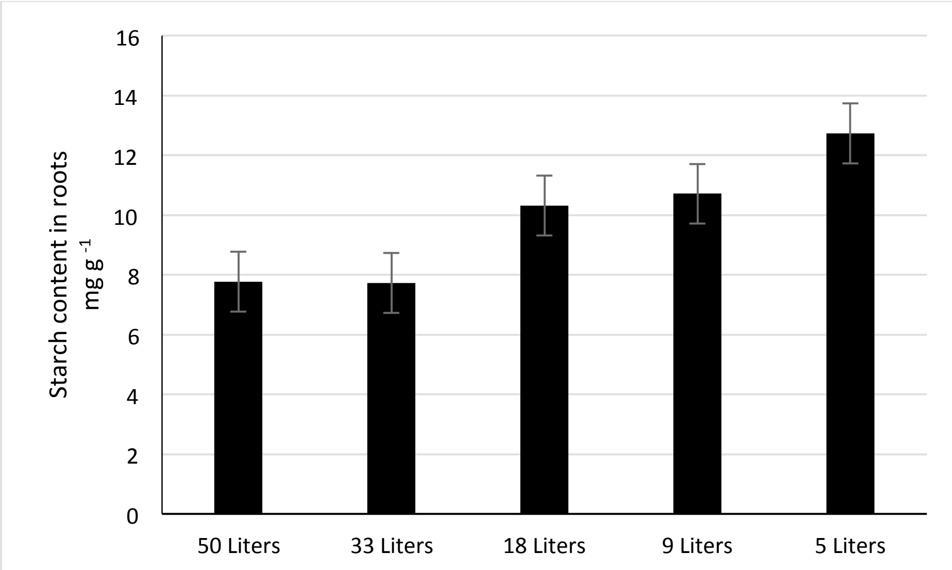

Figure 15. Effect of five different soil volumes (50, 33, 18, 9 and 5 Liters) on starch content in roots (mg g^{-1}) of *Opuntia ficus-indica.* Data are presented as means ± SE (*N=12 plants*)

Nitrogen content mother cladodes (µg/g)

There was no effect of the soil volume, sampling date and the interaction on the nitrogen content in the mother cladode. The values ranged between 121 µg/g in the mother cladode of the plants planted in 9 Liters to 148 µg/g in the mother cladode of the plants of 18 Liters.

5. Discussion

5.1. Roots measurement

Many soil volume restriction trials were conducted where plants grown in pots. Restricted soil volume had limiting effects on overall plant growth and influence plant development. Root system growth was inhibited by soil volume restriction: the total roots length; surface area, dry mass, and volume were affected and decreased due to this restriction. This is consistent with previous studies for a number of plant species growing under restriction to rooting space (Hess and De Kroon, 2007; Ronchi et al. 2006; Zhu, et al. 2006). Previous reports explained this reduction in roots system as a results of lower resources supply like nutrient acquisition (Poorter et al. 2012b); low carbohydrate resources (Dichio et al. 2012) and the soil temperature increase due to the small pot size (Xu et al. 2001). The influences of the soil volume restriction on the root growth seem to change over time. In this study, this influence became relevant staring the 6-month old plants, this effect increased over time for the large soil volume, this in agreement with Endean and Carlson (1975). Nevertheless, the smallest soil volume showed stable roots growth in terms of the total roots length; total surface area; the total roots volume, and total dry mass. This can be explained by the fact that the large soil volume has more nutrient supplies that can support this growth while it is limited in the small soil volume (Poorter et al. 2012a), especially that the differences among the different soil volumes become clearer over time. In contrast, soil volume restriction enhanced root density as well as the root length density. Generally, these two parameters have been used as potential indicators of the mineral nutrition (Majdi, 2001). These results agreed with (Inglese and Pace, 2000) who claimed significant increase of *Opuntia ficus- indica* root density with soil volume restriction increase. Under environmental stresses, trees must use an adaptive strategy in order to sustain and increase soil exploration in order to improve nutrition (Lõhmus et al. 2006). This later can be achieved through different mechanisms such as: increasing root length; increasing root branching; increasing specific root length (SRL) when compared to optimal conditions (Lynch, 2007; Gahoonia and Nielsen, 2004; Lynch and Brown, 2001). Our data showed that SRL has increased with root confinement. Usually plants with high SRL show high uptake rates of water

(Eissenstat, 1991) and nutrients (Comas et al. 2002; Reich et al. 1998) which impacted the whole plant growth in general. The findings in this work were the same as those reported by (Snyman, 2007) who stated SRL values within the ranges reported here. Root length density (RLD) is another important determinant of plant water and nutrient acquisition. RLD is a good indicator of the impact of the cultural practices on root development in the soil. In this study we observed that root length density values increased with soil volume restriction, meaning that the plant develops more roots when soil/nutrient sources is not available. BVR as (total plant biomass: rooting volume ratio; gL^{-1}), has been used only by Kerstiens and Hawes (1994). Our results showed higher BVR values than the threshold reported by Poorter et al. (2012a, b), who suggest that to prevent constraints of pot volume on plant growth, the plant biomass to soil volume ratio (BVR) at harvest should not exceed1 gL^{-1}, we notice that the effect of pot size soil/ volume is clearly noticeable in this experiment.

The large roots were essentially the main roots that developed from the areoles. Generally these roots are gross and without root hairs (Snyman, 2004b, 2005). The main roots dry mass and surface area increased with the soil volume after 18 and 24 months of planting but not in the early stages of growth. The large roots surface area actually had a strong linear relationship with large roots volume and large roots length. This illustrated that all the previous parameters follow the large roots surface area. These roots serve to anchor the plant and to support lateral roots as well as carbohydrate and nutrient storage during the season (Comas et al. 2013). The higher surface area and the dry mass values related to the soil volume increase might be linked to the large roots length increase as well as the thickness. This in turn is related to the resources availability. The large roots length values found in this study were higher than the values found by (Snyman, 2007) which is most likely due to the longer experimental period.

Fine roots are those roots that serve as the responsible organ for water and nutrient uptake (Eissenstat and Yanai, 2002). Fine roots categorized according to their size classes (diameter ≤ 2 mm in diameter) (Pregitzer et al. 2002). These roots are defined on a functional basis as the main means for resource uptake (nutrients and water). In the current work, the peak fine roots growth was found after 12 months of planting. Contrary, there was not much changes in the fine roots production after that. Under our experimental conditions, bloom (flowering) stage took place at June, which is the end of the rapid shoot growth where the maximum fine roots growth reached. Previous research has shown, the peak in root growth in trees may occur before the rapid growth of the shoot in the spring, during the vegetative termination stage in the summer, or after the shoot stops growing in the fall (Wang, 2005; Wang et al. 1997). Fine roots surface area, length and volume found to be affected by the soil volume restriction. The fine roots production was impacted by soil volume positively. On the other hand, the medium roots dry mass followed the same trend of the fine roots dry mass while the medium roots surface area followed the same large roots surface area approach, this is applicable to medium roots volume and length as strong linear relationship was found between the medium roots surface area and its length and volume. Nevertheless, our findings were in agreement with the finding of Nobel et al. (1994) regarding the effect of the soil volume on the surface area of the main and lateral roots (medium and fine roots), and with Bauhus and Messier, 1999 and Rewald et al. 2011 who claimed that the length and surface area and volume of the root systems, was affected positively with the soil volume increase. Comparing to other roots variables, fine root length appears to be a better indicator for determining root production and loss and reflects root growth characteristics more than total roots length (Johnson et al. 2001), while the thickness and dry mass of the main roots seem to be better indicator for the root system growth.

5.2. *Opuntia ficus-indica* canopy

The number of the first generation cladodes was affected by the soil volume restriction, but not by the sampling date. These results could be explained by the different root growth in relation to the pot size and with the decomposition rate of root biomass. The first generation cladodes appeared from mother cladodes at the same time for all the plants, and this happened mostly for one time. If there is any new growth, this will be produced from the areoles of the first generation cladodes. The effect of the soil volume on the number of the first generation cladodes didn't have any clear trend. However, there was with the second generation and the total cladodes number. The lower the soil volume, the lower number of the cladodes produced. The number of the cladodes of the largest soil volume was at least 30, 44, 48 and 48 %, higher than the plants in the pot size 33, 18, 9 and 5 liters respectively ranging between (3 to 15 cladodes per plant). Plants in the smallest soil volume stopped producing new second generation cladodes after the first cut. These results could be explained by the low resources availability in the small soil volume led plants to reduce their canopy growth (Berdanier and Clark, 2016). The low number of the cladodes led to low canopy surface area in the small soil volume, the surface area is a vital trait as it affects the daily net CO_2 uptake and the photosynthesis capacity (Terashima et al. 2011).

The canopy dry weight was affected by the soil volume with a linear dry mass increase with soil volume increase, this agrees with Nobel et al. (1994). The soil volume increase led to increase in roots surface area and canopy surface area as well, this could lead to greater water and nutrients uptake that will enhance the canopy growth and development (Nobel et al. 1994). The canopy dry mass ranged between (52 -898 g plant or kg h^{-1}). These values were less than the values reported previously (Snyman, 2006) and this could be related mainly to the soil volume restriction effect as in most of the previous research experiments were conducted under field condition. The root: canopy dry mass increased with the soil volume increase. This is because of positive effect of the soil volume increase on both roots and canopy. The values we obtained in this study were higher than those obtained by Nobel (1998) and Snyman (2006) findings. This could be as a result of roots density and biomass per soil unite increase as a result of nutrient supply decrease which enhance the fine roots growth (Hertel et al. 2013).

5.3. *Opuntia ficus-indica* root turnover

The restricted soil volume affects the resource availability as the larger soil volume contain more nutrients then the small one (Guillermo et al. 2013). In general, the low nutrient availability may lead to low uptake. This decrease in nutrient uptake is presumably one of the primary reasons for acceleration of root turnover due to reduced root longevity (Gaul et al. 2008; Eissenstat et al. 2000). Root density and biomass per soil unit increased with decreasing nutrient supply, this probably represents a compensatory fine root growth response to low uptake rates that lead to reduction in mean fine root age and replacement by new ones with thinner diameter and larger surface area (Hertel et al. 2013). This could be an efficient function to optimize the cost-benefit ratio of fine roots production per resource unite (Ostonen et al. 2007; Eissenstat et al. 2000; Eissenstat and Yanai, 1997). The reduction of the root longevity will increase the supply of organic carbon (g) derived by roots into the soil. As a result, the decrease in nutrient uptake is presumably one of the primary reasons for acceleration of plant root turnover due to reduced root longevity (Gaul et al. 2008; Eissenstat et al. 2000) which reflect higher plant roots turnover. This is in consistent with our results regarding the higher specific roots length that were observed in the roots of the plants raised in small soil volume reflecting thinner and longer roots which was combined with great amounts of carbon (g) derived by roots in the soil unit (kg). The results showed decrease of the negative $\delta^{13}C$ values over time which is due to the contribution of cactus pear root (CAM-C) to the soil organic matter measured in the pot (C_3 -soil) as the more negative values of $\delta^{13}C$ means lighter in mass in the soil unite (O'Leary, 1988). The Contribution % of CAM-C to total soil carbon (new carbon derived) was useful to understand the root turn over in different soil volume. This relative value provides, in fact, the contribution of CAM-C biomass to total SOC for the unit weight of soil. The CAM-C contribution increased from 0.27 C g of soil kg^{-1} in the biggest pot to 0.57 C g of soil kg^{-1} in the smallest pot. This can be explained by the higher roots mortality in the small soil volume which increase the turnover percentage per time which ranged between (10-15.4 %). This result was higher than the finding of Novara et al. (2014) which again could be explained by soil volume restriction that affects the nutrient availability and hence higher root turnover.

Drylands cover over 40% of the earth's land surface (IUCN, 2008). Land degradation occurs in all continents and will remain an important global issue for the 21st century (Nefzaoui et al, 2014). This is due to its adverse impact on agronomic productivity, the environment, and its effect on food security and the quality of life (Eswaran et al, 2001) Agronomists and soil scientists support claim that land is a non-renewable resource and some adverse effects of degradative processes on land quality are irreversible, e.g. reduction in effective rooting depth. Thus, there is a need to identify low input plant species that can flourish under limiting condition and particularly soil volume. Cactus pear is a species that has shown great potential to withstand under degraded ecosystems characterized by limited resources. Based on the findings of this study, one can report that cactus pear enhances accumulation of soil organic carbon and make better use of shallow soils. Therefore as practical management cactus pear is recommended wherever the soils are too shallow, too stony, too steep, too sandy or the climate is too dry for practical farming. Furthermore, cactus pear is a strategic option to improve rangeland and convert marginal soils to productive lands and mitigate land degradation in the arid and semi-arid areas.

5.4. Starch and Nitrogen content

The soil restriction enhanced the starch accumulation in both roots and the mother cladodes. The highest accumulation found in the smallest soli volume. Generally, *Opuntia*, does not develop new cladodes under stress (Pimienta-Barrios et al. 2002, 2003). Plants raised in the small soil volume stopped giving second generation cladodes after 12 years of planting, this seemed to have a kind of dormancy. Plants during dormancy tend to accumulate more starch (Landhäusser and Lieffers, 2003). The starch accumulation was higher in the mother cladodes comparing to the roots, this indicate the importance of the mother cladodes as a source-sink for both roots and shoots. The mother cladode nitrogen content was not affected by the soil volume.

6. Conclusion

Not many studies have been conducted on *Opuntia ficus-indica* root dynamics under soil limitation conditions. In recent years there has been increased interest in this crop for fodder and fruit in the dry area. Thus, there is a need for more such studies so that the adaptability and performance of different species and cultivars under different environmental conditions can be quantified. The common thoughts that cactus pears need low inputs to give high yields. Yet, this statement is not entirely true, despite the fact that cactus pear can survive where many other crops cannot. Therefore, the importance of appropriate inputs regarding soil volume, water, light and temperature are vital to get high yield or at least to understand the performance of this plant under any of the previous factors limitations. Main findings presented in this research study suggest that root restriction can substantially affects the roots and canopy growth of *Opuntia ficus-indica*. Soil volume restriction resulted in reduction of canopy growth and canopy dry matter accumulation of cactus pear. This reduction was associated to a lower cladode number and surface area. On the other hand, the total roots length, surface area, dry mass, and volume were inhibited by the soil volume restriction. The soil volume decrease has impacted the growth and the surface area of the main roots negatively, while an increase in lateral roots (fine) growth in the soil volume unite, specific root length, root density as well as the root length density took place. The higher SRL values would indicate high uptake rates of water and nutrients while the RLD is a good signal of the impact of the cultural practices on root development in the soil. In other word plant develops more roots when soil/nutrient sources are not available. The general conclusion can be made that the root system of this plant is not as stable as one would have expected, but is perhaps more adaptable to environmental conditions and to the stage of the plant growth also to the time plants has been grown in this restricted soil volume, this effect seems to be minor or moderate at the early stages of the plant growth but increase later. The more lateral roots and finer root system per soil volume of *Opuntia ficus- indica* seems to be a kind adaptive strategy in order to enable the plants sustain and increase the roots surface area in order to increase, improve and explore new nutritive resources. The root: canopy dry mass ratio increased with the soil volume increase but with higher values than the ones reported in the previous studies, due to roots density and biomass per soil unite increase.

In this study, we evaluated the effect of the soil volume restriction on SOC derived from the roots which reflect the plant roots turnover. Using an approach based on natural differences in $\delta^{13}C$ of plants with C3 photosynthesis. Our findings have some implications for the understanding of carbon turnover and organic matter stabilization under the soil volume restriction conditions, the results have shown that, the restricted soil volume enhanced the increasing the C stock derived from the roots from 0.27 C g of soil kg^{-1} in the biggest soil volume to 0.57 C g of soil kg^{-1} in the lowest soil volume. On the other hands, the starch accumulation in both roots and the mother cladodes was increased with the soil volume restriction increase.

To conclude, plants under stress tend to have higher proportion of roots that can compete more effectively for soil nutrients, this will affect the growth of the canopy shoots. The plants use the above ground resources to maintain and produce more roots, this end up with limited canopy growth and more starch and turnover in the roots. In this trial, the plants placed in the smallest soil volume (5 Liters) stopped producing new second generation cladodes after the first sampling date. Afterwards, all the investments were put into the roots growth and starch accumulation in both mother cladode and roots resulting in the highest percentage of the roots turnover. This result confirmed the importance of the *Opuntia ficus-indica* as a potential plant that can survive under low soil volume. This plant has the ability to balance its growth and stay alive under the harsh environments. In addition, the plant can provide reasonable organic carbon amount that improve the quality of the soil and ecosystems.

Having said that, more research is required to explore the interaction between many environmental factors effecting the cactus pear growth and behavior.

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