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The Effects of Carbon Nanotubes and Silver Quantum Dots on Gas Exchange in Arabidopsis Thaliana

Maryam Ibrahim Subaylaa

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THE EFFECTS OF CARBON NANOTUBES AND SILVER QUANTUM DOTS

ON GAS EXCHANGE IN *ARABIDOPSIS THALIANA*

A Masters Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Biology

By

Maryam Subaylaa

May 2017

THE EFFECTS OF CARBON NANOTUBES AND QUANTUM DOTS ON GAS EXCHANGE IN *ARABIDOPSIS THALIANA*

Biology

Missouri State University, May 2017

Master of Science

Maryam Subaylaa

ABSTRACT

Engineered nanoparticles (ENPs) are increasingly being used in commercial products, and may accumulate in soils when the products are disposed. I examined the effects of two common ENPs, carbon nanotubes (CNTs) and silver quantum dots (Ag-QDs), on plant gas exchange. To do this, I grew *Arabidopsis thaliana* in soil (n=36) for 6 weeks and added a CNT suspension at increasing concentrations (10, 30, 90, 150, 190, 250 µg/ml) each week. I also grew *A. thaliana* in petri dishes (n=83) containing Murashige and Skoog (MS) medium, with a concentration of 4µg/ ml Ag-QDs or 4µg/ml CNTs. I measured carbon assimilation rates, stomata conductance, and transpiration rates, using a LI-6400XT Portable Photosynthesis System. I found that gas exchange in soil-grown *A. thaliana* was unaffected by CNTs. There were no effects on rates of photosynthesis, transpiration or stomata conductance. There was also no apparent effect on light or carbon fixation reactions. I found that gas exchange in petri dish-grown *A. thaliana* was negatively affected by Ag-QDs, and marginally affected by CNTs. There was a reduction in photosynthesis rates, but no apparent effects on stomatal conductance and transpiration rates in *A. thalian* grown with either Ag-QDs or CNTs. The negative effects of the ENPs were directly related to light and Calvin cycle reactions. My research illustrates a model system for examining ENP effects on plants, and demonstrated that if Ag-QDs are disposed of in soils, they can negatively affect plant growth.

KEYWORDS: *Arabidopsis thaliana*, carbon nanotubes, quantum dots, gas exchange, physiology

This abstract is approved as to form and content

_____
Alexander Wait
Chairperson, Advisory Committee
Missouri State University

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Approved:

_____
Dr. Alexander Wait

_____
Dr. Laszlo Kovacs

_____
Dr. Michelle Bowe

_____
Dr. Julie Masterson: Dean, Graduate College

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INTRODUCTION

Background

Engineered nanoparticles (ENPs) are increasingly being used in consumer products and electronic devices (Hegde *et al*., 2016). Because they are so useful, more and different types are rapidly being developed and manufactured. ENPs are now found in drugs, electronic devices, and many commonly used products: sunscreens, cosmetics, health and fitness, automotive, food, home and garden, clothing, footwear, and eyeglass/lens coatings (Husen & Siddiai, 2014, Zhang *et al*., 2015). Since manufacturing ENPs is increasing (Navarro *et al*., 2010; Husen & Siddiai, 2014), organisms being exposed to them in nature is probable; therefore, their potential toxicity needs to be characterized (Cañas *et al*., 2008). My research focused on how two different ENPs, single walled carbon nanotubes (SWCNTs) and silver quantum dots (Ag-QDs), affect plant gas exchange using the model plant *Arabidopsis thaliana*. There is very little research on the effects of ENPs on plant gas exchange; however, there is an emerging body of research on the effects of ENPs on other plant functions. The range of plant responses to ENPs ranges from being toxic to being beneficial. Bellow, I review the current literature.

ENPs and Seed Germination

CNTs can create changes on the surface of the seed of plants. Tiwari *et al*., (2014) found that multi-walled CNTs (MWCNTs) at a concentration of 20 mg/l penetrated maize seeds and positively affected seed germination and growth by promoting water

uptake. On the other hand, MWCNTs were toxic to the plants at concentrations higher than 20 mg/l because of nanotube aggregation. As a result, they impeded water transportation by capillary action. Relevant to this study, Srinivasan & Saraswathi (2010) found that MWCNTs enhanced seed germination and increased seed weight in tomato. Lin & Xing (2007) examined seed germination in six plant species (radish, rape, ryegrass, lettuce, corn, and cucumber) treated with five types of nanomaterials (MWCNTs, aluminum, alumina, zinc, and zinc oxide) at concentrations of 2000 mg/L, and found that MWCNTs did not negatively affect seed germination (Hu *et al.*, 2010). Nair *et al.*, 2011 reported that germination of rice seed treated with high concentrations (1 ml QDs+ 0.5 ml H_2O or 0.5 ml QDs +1 ml H_2O) of cadmium selenide quantum dots (CdSe QDs) was inhibited. However, at low concentrations (0.25 ml QDs+1.25 ml H_2O) of QDs there was no effects on the seed germination, but plant growth was reduced.

ENPs and Plant Roots

The suggested mechanisms for ENPs entering plant root cells and affecting plant growth are endocytosis and/or binding of ENPs with a carrier protein being transported into plants through water or ion channels. Previous studies have shown that CNTs can enter plant cells and move along roots (Qiaoling *et al.*, 2009; Larue *et al.*, 2012; Giraldo *et al.*, 2014). CNTs can penetrate seed coats through endocytosis mechanisms by creating pores in plant cells, as was observed in the black layer region of the maize seed (Qiaoling *et al*, 2009; Srinivasan & Saraswathi, 2010; Giraldo *et al*, 2014). Researchers have also documented that CNTs increase porosity, resulting in increased water uptake (Srinivasan & Saraswathi, 2010).

The entry of quantum dots (QDs) have also been documented in plant cells, and researchers have indicated that QDs can be targeted to nuclei by arginine-rich intracellular peptides without evidence of cytotoxicity at a concentration up to 200 nM (Liu *et al*., 2010). In another study, QDs were observed in the root hair surfaces of *Arabidopsis*, but they were not internalized and transported into root cells (Navarro *et al*., 2012). However, QD absorption into the root can be enhanced when humic acid (HA) is used in the growth media, resulting in root damage (Navarro *et al*., 2012). Al-Salim *et al*., (2011) observed fluorescence in severed stems of *A. thaliana* vessels that were exposed to QDs, illustrating that indeed the QDs can be taken up by plant roots. However, the height level of water-soluble QDs through a stem was found to depend on the type of QDs; and, QD uptake through roots did not occur in intact *Arabidopsis* plants (Al-Salim *et al*., (2011). Lin and Xing (2008) found that zinc oxide (ZnO) nanoparticles that were at a concentration of 100mg/l were internalized into *Lolium perenne* (rye-grass). The mechanism of uptake of individual ZnO nanoparticles in the root endodermis and stele was via the apoplastic route, and followed by cell-to-cell transport via plasmodesmata.

Nutrient uptake in roots has also been shown to be affected by ENPs. For example, Tiwari *et al*., (2014) reported that when MWCNTs are presented into media, Fe^{3+} is reduced to Fe^{2+}, possibly due to MWCNT ion-transient dipole (ITD) interactions. They hypothesized that after Fe^{2+} enters plant, it interacts with Ca^{2+} and a replacement action cation between the two ions occurs. Thus, Ca^{2+} left the cell wall matrix out of the seedling, and the Ca content in the root seedling was reduced. This lead to the reduction of plant dry weight and water content of the root. Ultimately, however, positive effects on plant growth via increased water and nutrient uptake was observed.

Silver nanoparticles (Ag-NPs) have also been found to affect nutrient absorption in plants. Geisler-Lee *et al*., (2014) reported that *A. thaliana* grown in soil treated with Ag-NPs had difficulty in absorbing inorganic nitrogen nutrient. Zuverza-Mena *et al*., (2016) examined the effect of Ag-NPs in *A. thaliana*. They found that the reduction of nutrient uptake was due to blockage of intracellular communication via nutrient carrier proteins. Ag-NPs also significantly reduced Ca, Mg, B, Mn, Cu, and Zn absorption in radish seedlings. The indicated effects of Ag-NPs on nutrient uptake was shown to be that Ag-NPs blocked protein channels, disrupted plant membranes, and decreased the level of expression of metal transporter genes.

ENPs in Plant Leaves

Larue *et al*., (2012) and Giraldo *et al*., (2014) reported that SWCNTs introduced into *A. thaliana* leaves passed through an extracted chloroplast via kinetic trapping by lipid exchange and increased photosynthetic activity. When the nanomaterial interacted with the chloroplast membranes, the lipid layers which cover the chloroplast envelopes wrapped around the SWCNTs and adsorbed them. After the disruption of the lipid membrane and SWCNT penetration, the lipid membrane was repaired.

QDs were found in *A. thaliana* leaves after treatments of CdSe/CdZnS QDs with different coatings (Yeonjong *et al*., 2015). QDs induced leaf stress, but the stress level was dependent on the type of coatings, and the concentration level of Cd and Se which were released from QDs, absorbed through roots, and then translocated into the plant leaves (Yeonjong *et al*., 2015). Similarly, Alimohammadi *et al.,* (2011) reported strong

fluorescent signals of SWCNT-QDs in tomato leaves. This SWCNT-QDs were absorbed through the tomato roots and transported into the leaves.

Reported Effects of CNTs on Plant Gas Exchange

There are few studies that have examined the physiological response of plants to CNTs directly. For example, some of the studies indicated the effects of CNTs on the plant growth by their influence on the water intake. Positive effects of CNTs have been reported on water uptake and chloroplast activity (Srinivasan & Saraswathi, 2010; Tiwari *et al.*, 2014), but no studies directly measured gas exchange. However, Zhu *et al.*, (2008) recommended scientists consider plants in their studies when they track carbon nanotube (CNTs) movement in the environment, since they found that CNTs accumulate in the pumpkin cells. Consistent with this recommendation, there is evidence that MWCNTs are toxic to plants at concentrations more than 20 mg/l because of the barriers that MWCNT causes when they aggregate around the cells where water is delivered. As a result, they imped the capillary action for water transportation (Tiwari *et al.*, 2014).

Ag-QDs have been reported to decrease the transfer distance of photo-generated electrons and increase the rate of electron transport, thus reducing the loss of light reflection (Lian *et al.*, 2015). In another study, titanium oxide (TiO_2) exhibited positive effects on the light- harvesting complex by increasing the absorption of peak intensity of the chloroplast and accelerating the rate of whole chain electron transport; thus, accelerate the transformation from light energy to electronic energy promoting photosynthesis in *A. thaliana* (Ze *et al.*, 2011).

SWCNTs have also been shown to promote photosynthetic activity by 49% when incorporating CNTs into chloroplasts extracted from plants and by 30% when incorporating them into leaves of living plants (Giraldo *et al*., 2014). In contrast, SWCNTs in the concentration range of 0.1-20 mg/L caused photosynthesis yield reduction by 18% in marine alga (Thakkar *et al*., 2016). Similarly, carbon nanotubes induced inhibition to the development of the algae *Chlamydomonas reinhardtii*, and also considerably decreased quantum yield in PSII due to inhibition in the total electron transport. Additionally, the chemiosmotic mechanism of photosynthetic ATP formation was inhibited after exposure to CNTs (Matorin *et al*., 2010). PSII photochemistry process and electron transport activity of *Lemna- gibba* plant were also inhibited by both nickel oxide nanoparticles and nickel (II) oxide at a concentration of 1000 µg/ml (Oukarroum *et al.,* 2015). Lin *et al*., (2009) found that photosynthesis rates in algae exposed to QDs were significantly reduced.

With the use of *Anabaena spherica*, Tang *et al*., (2013) were able to examine the toxicity of exposure to nano TiO_2 and Zn^{+2} suspension. After 96 hours of exposure to TiO_2 nanoparticles at concentrations above 10.0 mg/L, changes in the photochemical transformation of energy and the content of chlorophyll-a was caused by nanoparticle aggregation in the algae cells, indicating that the cells were light stressed. However, increasing nano TiO_2 concentration above 1.0 mg/L reduced toxicity effect in the presence of Zn^{+2}, as soluble concentration of Zn^{+2} is reduced by adsorption onto nanoTiO_2.

Genes that take part in energy pathways and electron transport systems of photosystem I and II and light harvesting complexes have been shown to be down regulated upon exposure to zinc oxide (Landa *et al*., 2015). In the same study, *A. thaliana* genes that take part in energy pathways, as well as electron transport were also down

regulated upon Fullerene soot nanoparticle exposure. Wang *et al*., (2015) reported the inhibition of photosystem structure genes as well as the expression of chlorophyll synthesis genes upon exposure of *A. thaliana* to ZnO nanoparticles (300 mg/L). In their study, chlorophyll a and b contents, the net rate of photosynthesis, intercellular CO_2 concentration, leaf stomatal conductance and transpiration rate were significantly reduced by more than 50%. Chlorophyll content and growth of *A. thaliana* was also negatively affected after treatments of cerium oxide and indium oxide nanoparticles (Ma *et al*., 2013). Furthermore, chlorophyll content of tomato seedlings was reduced by 1.5fold after exposure to single walled carbon nanotube functionalized with quantum dots at 50 µg/ml concentration (Alimohammadi *et al.,* 2011). A similar result was indicated by Lin *et al.,* (2009) who found that the chlorophyll content of *A. thaliana* T87 suspension cells decreased after treatment with MWCNTs.

Influence of Nanotubes on Plant Oxidative Stress

Nanotubes have shown to alter gene expression that is related to plant physiological process. For example, Landa *et al*., (2015) examined *A. thaiana* gene expression after treatments of zinc oxide nanoparticles, and they found that the number of genes which take part in osmotic, water, salt and oxidative stresses, along with defense and wounding pathogens were high. On the other hand, Wang *et al*., (2014) examined drought and salt stress gene response to graphene oxide nanoparticles, and the data indicated that the gene was down regulated, which explained the adverse effect on seedling development in *A. thaliana*.

The up-regulation of genes that play a role in responses to oxidative stress was reported by Shen *et al*., (2010) who noted that the injection of SWCNTs into *A. thaliana* leaves improved levels of the mRNAs coding for proteins that take part in the scavenging of H_2O_2. As reported by Landa *et al*., (2015), up regulation of most genes that take an active role in oxidative stress responses show an increase in the intensities of reactive oxygen species (ROS) in the presence of fullerene soot (FS) nanoparticles.

The increase in genes encoding enzyme activity for the protection against oxidative stress indicate that zinc oxide is able to promote the production ROS in exposed roots (Xu *et al*., 2010). In another study, the gene expression involved in producing antioxidant molecules, which protects plants from oxidative stress, were down regulated when the plant was exposed to other types of nanotubes (cerium oxide and indium oxide nanoparticles) (Ma *et al*., 2013). Landa *et al*., (2015) also observed an activation of genes that take part in responses to oxidative stress after exposing *A. thalian* with titanium dioxide. This result agrees with earlier observations that titanium dioxide increases antioxidant enzyme activity which is likely to improve defense against other sources of oxidative stresses (Tumburu *et al*., 2015).

Research Goals

Studies that have examined the effects of ENPs (engineered nanoparticles) on plant physiological processes provided some evidence that ENPs can have toxic effects. There are few studies, however, on the effects ENPs on plant photosynthesis and gas exchange. My research project aims were to identify the effects of CNTs and Ag-QDs on carbon fixation rates by examining light reaction and Calvin cycle processes in *A*.

thaliana. The data presented in this study offer new evidence on the gas exchange responses of *A. thaliana* when exposed to CNTs and Ag-QDs. The resulting information can be applied to the estimation of environmental risks related to the exposure of plants to ENPs. *A. thaliana* was chosen as the experimental plant since it is the model plant system for genetic experiments, and, it has been successfully utilized for a number of physiological investigations (Hoffman, 1965)

CNTs were selected because they are used in high quantities in nanotechnology products and have been considered prominently in literature to evaluate their effect on plants. On the other hand; in my knowledge, Ag-QD effects on plant have not been tested, although these nanoparticles are used in applications related to increasing light absorption efficiency.

METHODS

Two different experiments were designed to test the negative effects of the selected nanoparticles (Single wall Carbon Nanotube; purity>95% diameter 1.5nm, length 1-5 microns, and surface area 1020.48 M2/gram obtained from Nanolab, and Ag-QDs; diameter 18.5±3.4, surface area 29.0 m^2/g, and Ag mass concentration 0.021 mg/Ml obtained from 20 nm Pelco® Citrate NanoXact[TM] Silver); note that these nanoparticles were handled according to material safety data sheets. I examined the effects of ENPs on gas exchange in wild type *A. thaliana* Columbia-0 (Col-0) plants purchased from Lehle Seeds Company (Waltham, Massachusetts). In a soil experiment, *A. thaliana* seeds were planted in soil (18 replicates of controls and CNTs) and treated with and without CNTs. CNT concentration was increased weekly from 10, 30, 90, 150, 190, to 250μg/ ml respectively, and delivered in a 1 ml solution in each pot each week. In a second experiment (petri dishe experiment), *A. thaliana* was grown in petri dishes (three replicates of controls, CNTs (4μg/ml), and Ag-QDs (4μg/ml)) on MS (Murashige and Skoog) medium. At three different growth days (14, 22, and 30), measurements were recorded for all 10 sets of a petri dishes.

Seed and Soil Preparation and Measurement Methods for *A. thalian* Pot Experiment

I plated *A. thaliana* seeds on 500 μl of 0.08% agar poured into six microcentrifuge tubes. The tubes were covered with tin foil and kept in a refrigerator for two days. To prepare soil for planting, I filled a pot with mixed potting soil that was obtained from Sun-Gro® Horticulture (San Diego, California). I washed the soil with

water to remove fungi and other materials that might exist in the soil as described by Lehle Seeds instructions. I repeated this step two to three times, and then left soil to dry. After the cleaning process, potting soil was placed in the Arraysystem pots, and five seedlings were transferred from the gel to the soil.

I grew *A. thaliana* using Arasystem which is designed by Arasystem for *A. thaliana*. This system included tray, pots, baskets, inverted cons, and con tubes. Some advantages of this system are that it reduces the effects of plant competition and enhances plant growth. Thirty-six pots were used for planting *A. thaliana* (18 replicate pots were prepared for controls and 18 replicate treatments of CNTs). I filled baskets and I transferred 5 seeds from the gel to the soil. I covered the pots along tray with plastic and grew them on benches under photosynthetically active radiation of 150 μmol m^{-2} s^{-1} and under a cycle of 11hours light/13hours dark. After two weeks of germination, I reduced the number of seedlings in each basket to two plants. The plants were fertilized once a week from top soil after three weeks of germination. The baskets were moved around randomly to minimize the effect of confounding variables that might interact with the treatment.

Gas exchange was measured using a LI-6400XT Portable Photosynthesis System equipped with 6 cm^2 leaf chamber. The measurements were recorded at growth photosynthetically active radiation (PAR), which was 150 μmol m^{-2} s^{-1} and at saturating PAR (600 μmol m^{-2} s^{-1}). Flow rate in chamber was set to 300 μmol s^{-1} and flow speed set to slow. 6 cm^2 of leaves were placed in the cuvette chamber. Using a Decagon WP4C Dewpoint, potentiometer, I measured water potential of leaves after 43 and 91 days of growth. Leaf area was measured at 51d and 79 d using LI-3000C Portable Leaf Area

Meter. Leaf samples that I measured for water stress were different from leaf samples that I measured for leaf area. Therefore, I measured fresh and dry weight at each time I measured water stress and leaf area. For chlorophyll content, random leaves were measured non-destructively on growth days 86, 91, and 92 using a SPAD Chlorophyll Content Meter.

Seed and Medium Preparation and Measurement Methods for Petri Dishes Experiment

A. thaliana seeds (4 mg) for each petri dish were sterilized by placing them on a cone into a sterilized chamber. In a fume hood, a beaker containing bleach (100 ml) and Hydro chloric acid (HCL) (3 ml) was placed in the sterilized chamber. The sterilized chamber was kept in the fume hood for two hours to allow seeds being sterilized by the elevated chlorine gas from the beaker.

Medium was prepared for 9 plates (3 controls, 3 CNTs, and 3 Ag-QDs; Table 1). In 18 clean flasks, Agar (0.2g) was added into each of 9 flasks, and distilled water was added into other 9 flasks for a total volume of ENP up to 5ml with concentration of 4μg/ml. 5, 4.26 or 0.24 ml of water was added into each of the 3 flask for controls, CNTs, and Ag-QDs respectively. In a separate beaker, 3-Morpholinopropane-1-sulfonic acid (MOPS) buffer (0.225g) and MS salts (0.4875g) was dissolved in 135 ml of distilled water. The pH of the solution was adjusted to 7.0 by adding 100 ml of mM KOH and distilled water in amount that makes solution volume up to 180 ml. The solution (20 ml) was added to flasks containing agar. Flasks were autoclaved at 121°C for 20 minutes. After 20 minutes, agar flasks were placed in warm water bath set at 55 °C. For flasks

with water only, nanoparticle was added to each flask as described in Table 1. Flasks with unsterilized nanoparticles were supplemented with Amphotericin B and carbencillin to avoid bacterial or fungal contamination. The flasks were sonicated, and the agar containing flasks were poured into the flasks that contain the mixed nanoparticle with distilled water and held in the water in the sonicator to make sure that nanoparticles were evenly distributed within the medium. After 2 minutes, the flask composition was poured into the 9 plates and left to cool at room temperature.

Seeds were sprinkled evenly onto each of 9 plates. The plates were sealed with parafilm and then placed in a refrigerator. After 3 days, the plates were taken out of the refrigerator and the parafilm was removed from each plate. An open zip-lock bag was used to cover the plates to prevent water loss from the medium or bacteria or fungi growth in the medium. The plates finally were placed in the growth chamber (Conviron Model Adaptis A1000-AR Chamber) at 21°C, 150 μmol m^{-2} s^{-1}, short day cycle (10 hours light and 14 hours dark). Plates were rotated randomly each day within the growth chamber to avoid the difference effect associated with plate position within the chamber.

Calibration, flow rate and speed, and IRGAs were set as it is indicated in the potting soil gas exchange measurements. Block temperature was controlled to be as same as leaf temperature. For light curve measurements, data was recorded at three light level (150, 500, 0 μmol m^{-2} s^{-1} respectively) and CO_2 mixture of reference was maintained at 400 μmol CO_2 mol^{-1} air. On the other hand, CO_2 level was set at 400, 700, or 0 μmol CO_2 mol^{-1} air, and light intensity was maintained at 500 μmol m^{-2} s^{-1} for A-Ci curve measurements. Leaf area was set depending on how much of chosen Arabidopsis sample filled the space of the Licor cuvette. The selected sample including roots and leaves were

placed in the leaf chamber. Between 4 -6 cm^2 of plants were placed on filter paper and then the sample was placed in the chamber.

Light and A/Ci Response Curve Fitting Program

A curve fitting program developed by Sharkey (2016), which is available online for free with instructions for use, was used to estimate variables associated with light and Calvin cycle reactions. For light response curve, the users need to enter T leaf (leaf temperature), P_{atm} (atmospheric pressure), Rd (day respiration), ambient O_2, g_m values, A (photosynthesis rate), Ci (intracellular concentration) and light intensity. The mean values of T leaf, photosynthesis rate, intracellular CO_2 concentration, which were recorded by the Licor for each treatment in each day, were entered in this Excel sheet. Light intensity (0, 150, and 500 μmol m^{-2} s^{-1}) was assigned next to each data point. Rd was assigned as the data points measured at the lowest light intensity (PAR=0 μmol m^{-2} s^{-1}). P_{atm} =101.3kPa at 0 elevation, O_2 =21kPa, and g_m=2 μmol m^{-2} s^{-1} Pa^{-1} were kept constant for all treatments; note that it is better to indicate g_m values that were directly measured or estimated by other methods, otherwise 2 is reasonable as it is indicated in the Microsoft model instructions. After adding these values, solver finds solutions and fits the data to the assigned points. This program estimates Jmax (electron transport rate at highest light level). Buckley & Diaz-Espejo (2015) suggest reporting Jmax as J and specifying the light level which it was measured as follows: write J with an explicit annotation for light intensity such as J_{500} to avoid error associated with using an asymptotic submodel (this model underestimate the true Jmax value), $\Phi >= 0.5$ (initial slop for modeled J), and $\Theta >=$ 1(convexity factor).

For A/Ci response curves, the users enter Tleaf, P_{atm}, O_2, A, Ci as they are indicated in the light response curve. In addition to these values, limiting factors are assigned as follows: rubisco=1, RUBP regeneration=2, and TPU=3. After assigning those values, solver calculates the following: Vcmax, J, TPU, Rd, and g_m (the maximum carboxylation rate of Rubisco, rate of electron transport for the given light intensity, rate of triose phosphate use, day respiration, and mesophyll conductance, respectively).

Statistical Methods

I used ANOVA to examine the effects of CNTs and Ag-QDs on dry weight, leaf area, water potential, chlorophyll content, photosynthesis, intracellular CO_2, stomatal conductance, transpiration, and water use efficiency rate measured at PAR levels of 150 and 600 μmol m^{-2} s^{-1}. For the pot experiment, each of these variables were applied as fixed factors, but growth days was a random factor because measurements were taken randomly on different growth days. For the petri dish experiment, the variables were applied as response, while treatments (control, CNT, and Ag-QD), and growth days (14, 22, and 30 day) were applied as fixed factors. The interactions between treatments and growth days for each of the variables were also tested. Tukey's test for multiple comparison was run if *P*-value was significant (α=0.05).

RESULTS

In my results, I included the variables that appeared to have major effects on carbon assimilation rates in my tables and figures. Other variables that might not affect carbon assimilation rates were included in the appendices.

Carbon Assimilation and Intracellular Response to CNTs and Ag-QDs

The results of gas exchange measurements indicate that *A. thaliana* grown in soil was not statistically affected by CNTs. While carbon assimilation rate at growth (150 μmol m^{-2} s^{-1}) and saturating light (600 μmol m^{-2} s^{-1}) was lower by 15% and 12%, respectively, in CNT-grown plants relative to controls (Table 2), the rates were not statistically significantly different. Similarly, intracellular CO_2 concentration at PAR= 150 and 600 μmol m^{-2} s^{-1} was not statistically affected by CNTs.

Carbon assimilation rate for *A. thaliana* grown in agar and treated with Ag-QDs was significantly decreased, with a 56% reduction compared to control grown plants, when measured at PAR 150, and 500 μ mol m^{-2} s^{-1}. Carbon assimilation rate for CNT-grown *A. thaliana* was lower by 21% when measured at PAR 150 μ mol m^{-2} s^{-1} and by 23% at PAR 600 μ mol m^{-2} s^{-1}. Carbon Assimilation rate reduction was identified further by the results that were obtained from intracellular CO_2 concentration in Table 3. Intracellular CO_2 concentration was significantly higher in Ag-QD-treated plants compared to controls and CNTs; however, CNTs did not statistically affect intracellular CO_2 concentration.

Jmax, Vcmax, J, and TPU Response to CNTs and Ag-QDs

Jmax values which was calculated from light response curve supported the indicated results about carbon assimilation rates that were measured at saturated light. Plants treated with Ag-QDs had significant lower Jmax compared to control plants (p-value=0.001); however, Jmax in CNT-grown plants were not statistically different from controls. Quantum efficiency and convexity factors of Jmax were not statistically affected by these ENPs (Table 4).

The estimated parameters from A/Ci curve (J and TPU) that are associated with limited photosynthesis RUBP-regeneration and TPU were significantly low in both CNTs and Ag-QD-treated *A. thaliana*. Therefore, carboxylation rates response to partial pressure of CO_2 at 400 and 700 μ mol m^{-2} s^{-1} were obviously decreased by Ag-QDs and CNTs. However, *Arabodopsis* treated with CNTs were fairly able to fix carbon efficiently by Rubisco as was noticed from A/Ci curve; therefore, Vcmax were not statistically low for CNT treated plants. In contrast, Ag-QDs grown plants had significant low Vcmax (Table 5).

Light and A/Ci Responses Curve to CNTs and Ag-QDs

Light response curve indicated that *A. thaliana* grown in CNTs and controls had about the same compensation points, and the rate of carbon assimilation matches the rate of respiration (Figure 1). Plants grown in Ag-QDs had a significantly lower required a slightly higher light level than plant grown in controls and CNTs to reach compensation point. Quantum efficiency of photosynthesis, which is represented by the curve slope,

and the saturation points (rate of A at maximum light intensity) were marginally lower in CNT treated plants, while they were significantly low in Ag-QDs (Figure 1).

A/Ci response curve indicated that plant grown in CNTs reached compensation point at the same concentration of CO_2 as controls, but the compensation point occurred at higher supplemented rate of CO_2 in Ag-QDs treated plants than in the other treatments. The slope of the curve which represents Rubisco activity was slightly shifted to the right side for CNT-grown *A. thaliana*, while it was greatly shifted for Ag-QDs from control slops. That is Rubisco was slowly carboxylated at low CO_2 concentration in the Ag-QDs grown *A. thaliana* compared to controls, while Rubisco carboxylation was not apparently affected under treatment of CNTs. Carbon assimilation rate response to CO_2 >400 indicated that RUBP-regeneration was highly affected by Ag-QDs than CNTs. Similarly, at saturated point which represent TPU activity, Ag-QDs grown *A. thaliana* had lower carbon assimilation rate than CNTs treated plants relative to controls (Figure 2).

Stomatal Conductance, Transpiration Rate, and Water Use Efficiency Response to CNTs and Ag-QDs

My results indicated that CNTs, either in soil or agar, and Ag-QDs in agar did not affect stomatal conductance (g), and transpiration rate (E) of *A. thaliana* (Appendix A&B). Since CNTs and Ag-QDs did not cause a reduction in the rate of stomatal conductance, CO_2 were not limited by g. Then, CO_2 should be available for Calvin cycle to run the reactions in all treated *A. thaliana.* In addition, my statistical results indicated that there were effects of Ag-QDs on water use efficiency (WUE) (Appendix B). The

effect of Ag-QDs on WUE was particularly referred to carbon assimilation rates (A) response to Ag-QDs, but not g or E, since they both did not affect gas exchange.

There was an effect of time (14, 22, or 30 days of growth) on assimilation rates and TPU (Appendix C & D). However, I found no interaction between treatments and growth days, which means that photosynthesis response to the treatments is independent of number of days of growth. However, the effects of CNTs and Ag-QDs were most obvious after 22 days of growth. In contrast, on the growth day30, carbon assimilation rate was lower than on day14 and 22. In this growth day (30), the plants started flowering and most of the energy is consumed for reproductivity rather than building sugar; thus, carbon assimilation rate was very low.

Chlorophyll Content, Leaf Area, Dry Weight, and Water Potential Response to CNTs for *A. thaliana* Grown in Soil

With my results that were indicated about the effects of CNTs on carbon assimilation rates of *A. thaliana* grown in soil, I supported these results with more information about plant chlorophyll contents which were determined by measuring the greenness of *A. thaliana* leaves. CNTs did not induce negative effects on *A. thaliana* chlorophyll content, so that allowed the plant to absorb photons from light and transfer excitation energy through chlorophyll pigments without affecting processes that involved in the light reactions. More evidence about the effects of CNTs on *A. thaliana* grown in soil was provided from my results on the plant leaf area and dry weight. Leaf area and dry weight were not affected under exposure of CNTs. Therefore, specific leaf area which is indicator of leaf thickness were not affected by CNTs. There were no effects of

these nanoparticles on water potential (WP) for *A. thaliana* grown in soil and agar, so *A. thaliana* were not water stressed under exposure to CNTs and Ag-QDs. However, there was time effect on WP for *A. thaliana* grown in agar resulted in the different of plant growth speed in different growth days (Appendix E). ANOVA table in Appendix F indicated the statistical effects of CNTs on the indicated variables in Appendix E and all variables indicated in Tables 2, 3, 4, and 5, and Appendices A, B, C, D.

DISCUSSION

The effect of engineered nanoparticles on photosynthetic rates and photosynthetic reactions have been reported in only a few studies (Line *et al*., 2009; Matorin *et al*., 2010; Ze *et al*., 2011; Tang *et al*., 2013; Giraldo *et al*., 2014; Oukarroum *et al.,* 2015; Wang *et al*., 2015; Thakkar *et al*., 2016). I evaluated the effects of two engineered nanoparticles on a number of photosynthetic processes: photosynthetic rate (A), intracellular CO_2 concentration (Ci), stomata conductance (g), transpiration rates (E), and water use efficiency (WUE)). In addition, I examined a number of processes that limit photosynthetic reactions: maximum rate of electron transport at saturating light (Jmax), initial slope of J (Φ), convexity factor (Θ) and A/Ci response curve (maximum carboxylation rate (Vcmax), photosynthetic electron transport rate (J), triose phosphate use (TPU) to provide evidence about nanotube effect on the process involve in the light and dark reaction of *A. thaliana*.

I found that Ag-QDs had a greater negative effect on variables that limit photosynthetic assimilation. On the other hand, *A. thaliana* processes that involve in the light and dark reaction were less affected by CNTs.

With my data that shows less effect of CNTs on *A. thaliana* than Ag-QDs, Shen *et al*., (2010); Wang *et al*., (2014); Landa *et al*., (2015) who did analysis on gene expression level of *A. thaliana* treated with different type of nanotubes that induced plant stress reported a high amount of change in the gene expression involved in oxidative stress and defense-winding pathogens were upregulated. In addition, Nair & Chung (2014) found that Ag-NPs (0.2 and 0.5 mg/L) induced modulation of PCNA and MMR

gene expression that associated with oxidative stress in *A. thaliana* seedlings after 24 and 72 exposure of Ag-NPs. In our experiment, *A. thaliana* stress induced by Ag-QDs could be resulted in up regulation of a high number of same the indicated stress related genes.

CNTs Effect on Carbon Assimilation Rate in the Pot Experiment and Petri Dishes Experiment

Carbon assimilation rate of *A. thaliana* grown in soil and MS medium treated with CNTs was not inhibited. However, photosynthesis rate was slightly decreased compared to controls by 15% and 12% at ambient and maximum light intensity respectively for *A. thaliana* grown in soil and by 21% and 23 at ambient and maximum light intensity respectively for *A. thaliana* grown in agar (Table 2&3). A similar response of *Polyboroides radiatusand* and *Sorghum bicolor* was reported by Aslani *et al*., (2014) who indicated that plants grown in agar was more susceptible to nanotube toxicity effects than plants grown in soil. However, CNTs did significantly influence carbon assimilation in either medium.

Matorin *et al*., (2010); Oukarroum *et al.,* (2015); Thakkar *et al*., (2016) reported the negative effects of CNTs at concentrations of 20 mg/L and 1000 µg/Ml on photosynthesis and chlorophyll content of algae. In my study, there is a reduction in photosynthetic rate, but statistically, CNTs did not affect *A. thaliana.* However, my data indicated significant effect of CNTs in MS medium on J and TPU. Therefore, the reduction in photosynthetic rates are mainly due to effects on Calvin cycle reactions.

Nair & Chung (2014) also indicated that Ag-NPs with concentrations of 0.5 and 1 mg/L induced significant reduction in total chlorophyll content of *A. thaliana* grown in Hoagland's medium. Consistent with this, *A. thaliana* treated with Ag-QDs exhibited yellow color in their leaves, so that chlorophyll content basically could be negatively affected by these nanoparticles.

Carbon Assimilation Rate Response to Ag-QDs Treated *A. thaliana*

I found that Ag-QDs reduced carbon assimilation rates by 56% (Table 3). This is consistent with Lin *et al*., (2009) who reported that assimilation rates were reduced in *Anabaena spherica*. In addition, Wang *et al*., (2015) found that chlorophyll a and b contents, net rates of photosynthesis, intercellular CO_2 concentration, leaf stomatal conductance and transpiration rate were reduced by more than 50% in *A. thaliana* grown in soil containing ZnO NPs (300 mg/L) for 6 weeks. In addition, they reported that genes associated with oxidative stress and toxicity caused the reduction in chlorophyll expression and carbon assimilation. Consistent with this, my data for *A. thaliana* grown in MS medium indicated reduction of carbon assimilation rate by 56%, but intracellular CO_2 concentration is significantly increased in Ag-QDs treated plant which means that CO_2 is not captured efficiently and this supported by estimated parameters calculated from A/Ci curve, which will be discussed later. The reduction in carbon assimilation rate induced by Ag-QDs possibly due to up regulation of genes involve in oxidative stress as Wang indicated. However, my data were not identical with Wang findings for stomatal conductance, and transportation rate. In my study these two variables were not affected by both treatments Ag-QDs and CNTs in both soil and MS medium.

Ag-QDs and CNTs Effect on Light Reaction Processes

Responses of photosynthesis to light can be explained by the estimated parameter of Jmax which can be determined by the equation:

$$J = \frac{(A+R_d)(4C_c+8\Gamma_*)}{(C_c-\Gamma_*)}, \quad J = \frac{J_{max}+\phi i - \sqrt{(J_{max}+\phi i)^2-4\Theta J_{max}\phi i}}{2\Theta}$$

Jmax provides information about a theoretical maximum electron transport rate that supports $NADP^+$ reduction (Sharkey 2016). I found that Jmax was reduced by 51% (Table 4); thus, Ag-QDs probably affected electron carrier's occupation and induce inhibition in NADPH production. Yan et al., 2013, explained that electrons that are not delivered to $NADP^+$ go to the Mehler reaction and this causes an increase in reactive oxygen species and PS1 photoinhibition. However, Jmax value of CNTs is decreased by 23%, so this nanotube does not greatly affect NADP+ reduction as much as Ag-QDs appears to. Beside the effect of Ag-QDs on NADPH, Ag-QDs probably affected ATP generation which is an important reaction for producing H^+ that generate a chemismotic gradient in the grana lamella and permits ATP synthases for interaction between ADP and Pi to make up ATP (Taiz & Zeiger, 2002).

Ag-QDs and CNTs Effect on Calvin Cycle Reactions

There is no literature reporting the effects of nanotubes on Calvin cycle reactions. I found that, by using A/Ci response curves that Rubisco, RUBP- regeneration, and TPU activity were inhibited under treatment of Ag-QDs (Figure 2). The three limiting factors in CNT treated *A. thaliana* were slightly reduced compared with Ag-QDs effect.

The carbon fixation process was affected by the inhibition process of the light reaction that was discussed. The source of energy (NADPH and ATP), which is

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regenerated from the light reaction, is the component for running Calvin cycle. Due to the inhibition of producing NADPH induced by Ag-QDs, the expected response from Calvin cycle is to fix carbon inefficiently. RUBP-regeneration is the limited photosynthesis associated with electron transport rate that used to support NADP+ reduction (Sharkey, 2015). Thus, RUBP-regeneration limited photosynthesis is affected by light condition. The reduction of TPU could be related to one of the genes that were down regulated and involved in transporting carbohydrate. The decrease in this gene expression probably affected the use of TPU for exporting sugar. Therefore, TPU declined in the treated plants.

Information Supports Ag-QDs Negative Effects on Carbon Assimilation Rate

The negative effect of Ag-QDs occurs inside plant leaves. This is unsurprising since Yeonjong *et al*., (2015) found that QDs were absorbed through roots and traveled to *A. thaliana* leaves, leading to leaf stress. In addition, Alimohammadi *et al.,* (2011) detected a fluorescence of QD in tomato leaves. Ag-QDs reached the plant leaves possibly by traveling from root endoderm via apoplastic path way or plasmodesmata as how ZnO nanoparticle entered *Lolium perenne* (Lin & Xing, 2008). Endocytosis or binding QDs with protein carrier are other mechanisms to deliver QDs into plant leaves. In addition, arginine-rich intracellular delivery peptides were identified as way for QDs to travel inside plant cell (Liu *et al*., 2010).

Ag-NPs was reported to have a negative effect on absorbing nutrients by blocking intracellular communication or presence of Ag^+ ions, which were released from Ag-NPs, affecting nutrient carrier proteins function (Geisler-Lee *et al*., 2014; Zuverza-Mena *et al*.,

2016). It is possible that Ag-QDs affect nutrient uptake if they aggregate around plant cells. However, my data shows significant decrease in the photosynthesis activity that occurs in the plant leaves and agreed with other studies which detected QD fluorescence signals in the plant leaves. In addition, a gene encoding a phosphatase for preventing early leaf senescence were up regulated. The other reason of not considering the indicated negative effect of Ag-NPs on nutrient uptake is that Geisler-Lee *et al*., (2014) found that the Ag-NPs impact on nutrient uptake occurred in the late of plant growth stage, but the effect of Ag-QDs in my study appeared in early stage of plant. Over all, for my study, the main effect of Ag-QDs on *A. thaliana* appeared on carbon assimilation rate.

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Table 1. Amount and concentration prepared for each treatment

Plates	Water	Silver or CNT	Total
Control x 3	5.0 mL x 3	0	25 mL x 3
CNT x 3 at 4 μg/mL (starting concentration 135 μg/mL)	4.26 mL x 3	0.740 mL x 3	25 mL x 3
Ag-QD x 3 at 4 μg/mL (starting concentration 21 μg/mL)	0.24 mL x 3	4.76 mL x 3	25 mL x 3
Total: 9 plates	28.5 mL		225 mL

Table 2. Mean ± (SE) for carbon assimilation rate and intracellular CO_2 concentration

Variables	Control	CNT
[a] A_{amb} (μmol CO_2 m^{-2} s^{-1})	1.73 ± 0.355 a	1.47 ± 0.234 a
[b] A_{max} (μmol CO_2 m^{-2} s^{-1})	2.79 ± 0.491 a	2.43 ± 0.377 a
[c] Ci_{amb} (μmol CO_2 mol^{-1} air)	256.2 ± 9.84 a	251.4 ± 26.2 a
[d] Ci_{max} (μmol CO_2 mol^{-1} air)	178.9 ± 13.7 a	213.2 ± 16.7 a

[a] A_{amb}, ambient photosynthesis at light intensity PPFD=150 μmol m^{-2} s^{-1}; [b] A_{max}, maximum photosynthesis at PPFD=600 μmol m^{-2} s^{-1}; [c] Ci_{amb}, intracellular CO_2 concentration at PPDF= 150 μmol m^{-2} s^{-1}; [d] Ci_{max}, intracellular CO_2 concentration at PPDF=600 μmol m^{-2} s^{-1}. The indicated variables for *A. thaliana* (n=10) grown in soil are not significantly different ($P < 0.05$) between treatments (control and CNT at 250 μg/ml). Values are means ± SE, and the treatments that share the same letters (a) are not significantly different.

Table 3. Mean ± (SE) for carbon assimilation rate and intracellular CO_2 concentration

Variables	Control	CNT	Ag-QD
[a] A_{amb} (μmol CO_2 m^{-2} s^{-1})	4.54 ± 0.315 a	3.55± 0.224 a	1.96 ± 0.127 b
[b] A_{max} (μmol CO_2 m^{-2} s^{-1})	5.92 ± 0.456 a	4.52 ± 0.266 a	2.59 ± 0.171 b
[c] Ci_{amb} (μmol CO_2 mol^{-1} air)	364.00 ± 2.90 a	364.33 ± 2.42 a	376.08 ± 2.02 b
[d] Ci_{max} (μmol CO_2 mol^{-1} air)	357.70 ± 3.37 a	358.87 ± 2.52 a	371.25 ± 2.44 b

[a] A_{amb}, ambient photosynthesis at light intensity PPFD=150 μmol m^{-2} s^{-1}; [b] Amax, maximum photosynthesis at PPFD=500 μmol m^{-2} s^{-1}; [c] Ci_{amb}, intracellular CO_2 concentration at PPDF= 150 μmol m^{-2} s^{-1}; [d] Ci_{max}, intracellular CO_2 concentration at PPDF=500 μmol m^{-2} s^{-1}. The indicated variables for *A. thaliana* grown in petri dish are not significantly different ($P < 0.05$) between control and CNT at 4μg/ml and significantly different between control and Ag-QD at 4μg/ml. Values are means ± SE (n=83) and the letters (a/b/c) indicate the significant difference between treatments. The treatments that do not share the same letters are significantly different.

Table 4. Mean (±SE) for estimated parameters from light response curve

*Variables	Control	CNT	Ag-QD
Jmax (μmol m^{-2} s^{-1})	43 ± 4.38 a	33 ± 2.97 ab	21 ± 2.25 b
Φ	0.4358 ± 0.0169 a	0.3973 ± 0.0381 a	0.3373 ± 0.0461 a
Θ	0.5850 ± 0.0641 a	0.4994 ± 0.0590 a	0.3815 ± 0.0606 a

*Jmax, maximum rate of electron transport at saturating light; Φ, initial slope of J; Θ, convexity factor. *Arabidopsis* (n=30) grown in petri dish are not significantly different (P < 0.05) between control and CNT at 4μg/ml (P=0.081), and significantly different between control and Ag-QD at 4μg/ml (P=0.001). There is no significant difference between treatments in Φ and Θ. The letters (a/b) indicate the significant difference between treatments. The treatments that do not share the same letters are significantly different.

Table 5. Mean ± (SE) for Estimated Parameters from A-Ci-Curve Fitting Program

*Variables	Control	CNT	Ag-QD
Vcmax (μmol m^{-2} s^{-1})	56 ± 0.18 a	53 ± 2.79 a	45 ± 2.62 b
J (μmol m^{-2} s^{-1})	64 ± 1.96 a	53 ± 2.79 b	39 ± 2.12 c
TPU (μmol m^{-2} s^{-1})	4.4 ± 0.21 a	3.5 ± 0.19 b	2.8± 0.16 c

*Vcmax, maximum carboxylation rate; J, photosynthetic electron transport at the measured light intensity; TPU, triose phosphate use. J and TPU are significantly different ($P < 0.05$) between treatments (control, CNT at 4μg/ml and Ag-QD at 4μg/ml). Values are means ± SE (n=30) and the letters (a/b/c) indicate the significant difference between treatments. The treatments that do not share the same letters are significantly different.
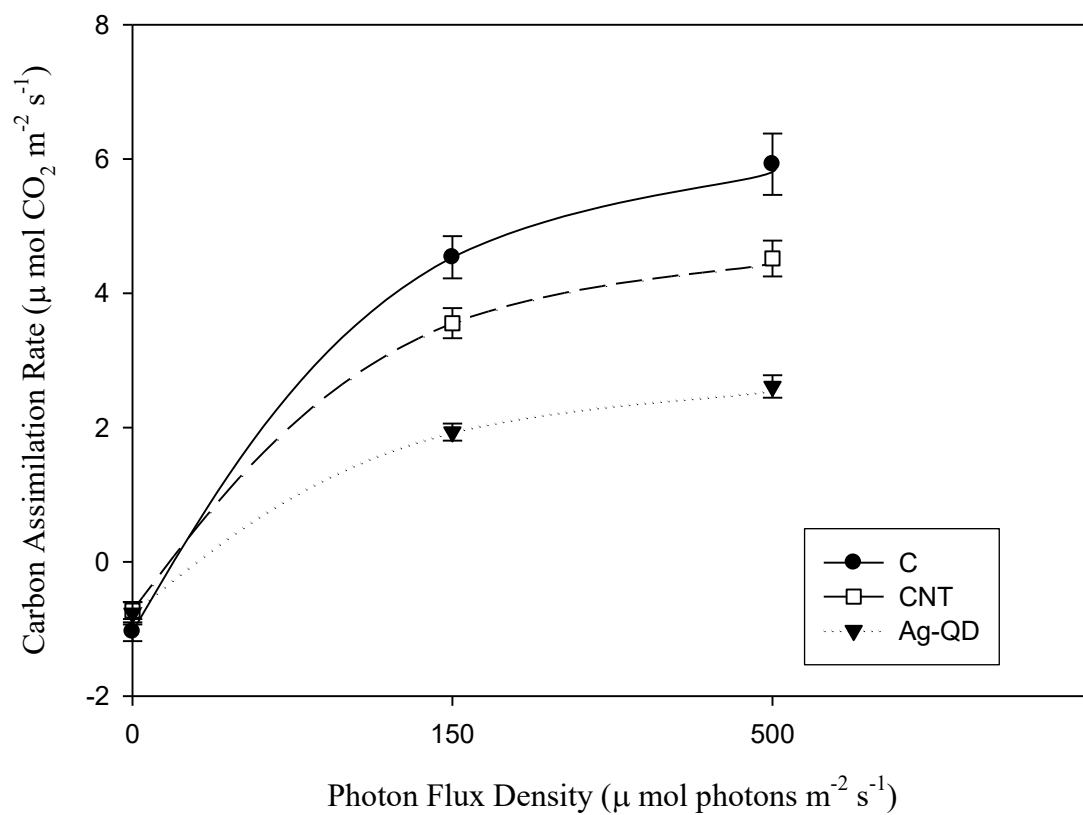
Figure 1. Mean carbon assimilation responses of *A. thaliana* (n=30) in controls (circles, and lines), CNTs (squares and dashed lines), and Ag-QDs (triangles and doted lines) in all growth days (14, 22, and 30) plotted against photon flux density. SE are shown at each symbol.
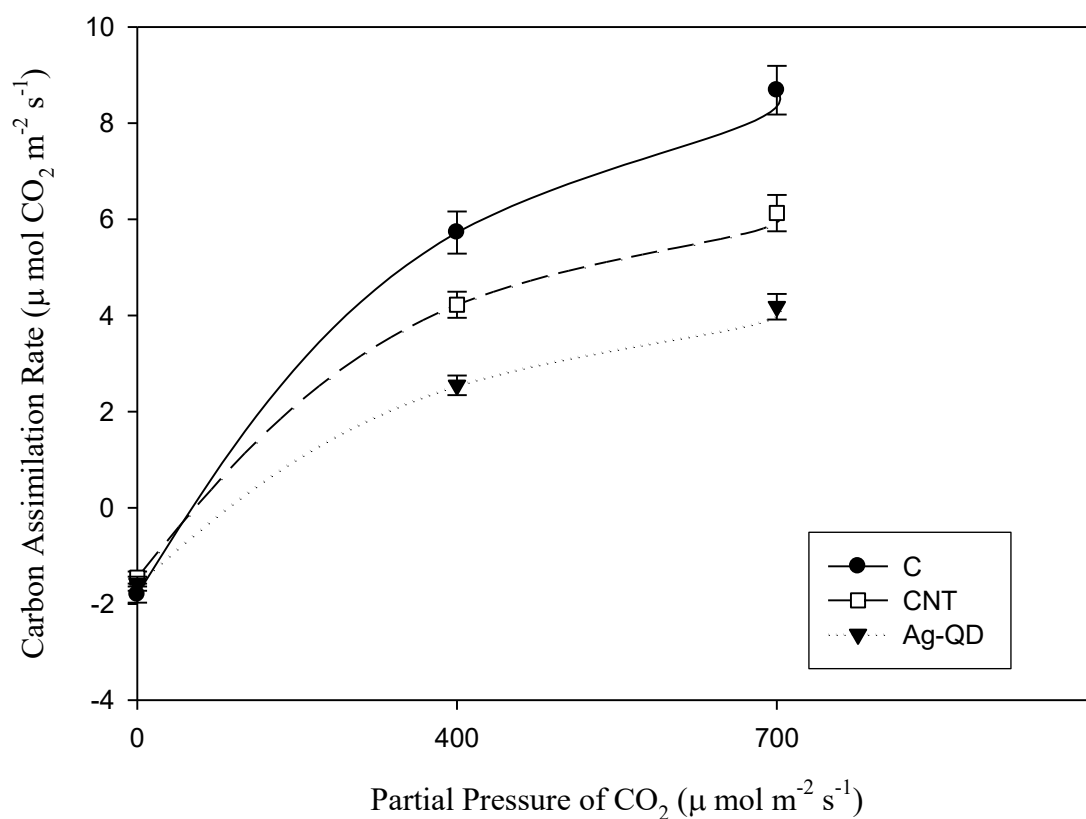
Figure 2. Carbon assimilation rate response of *A. thaliana* (n=30) in controls (circles and lines), CNTs (squares and dashed lines), and Ag-QDs (triangles and lines) in all growth days (14,22, and 30) plotted against partial pressure of CO_2 (0, 400, and 700 µ mol m^{-2} s^{-1}). SE are shown at each symbol.

Appendix A. Mean ± (SE) stomata conductance, transpiration rate, and water use efficiency for soil experiment

Variables	Control	CNT
[a] g_{amb} (mol CO^2 $m^{-2}s^{-1}$)	0.0217 ± 0.00374 a	0.0226 ± 0.00543 a
[b] g_{max} (mol CO^2 $m^{-2}s^{-1}$)	0.0246 ± 0.0059 a	0.0244 ± 0.00428 a
[c] E_{amb} (mol H_2O $m^{-2}s^{-1}$)	0.610 ± 0.101 a	0.608 ± 0.131 a
[d] E_{max} (mol H_2O $m^{-2}s^{-1}$)	0.710 ± 0.160 a	0.683 ± 0.111 a
[e] A_{amb}/g (μmol CO_2 mol^{-1} CO_2 m^{-2} s^{-1})	77.19 ± 5.93 a	76.8 ± 17.5 a
[f] A_{max}/g (μmol CO_2 mol^{-1} CO_2 m^{-2} s^{-1})	120.19 ± 9.73 a	103.5 ± 10.8 a
[g] A_{amb}/E (μmol CO_2 mol^{-1} H_2O m^{-2} s^{-1})	2.732 ± 0.232 a	2.747 ± 0.567 a
[h] A_{max}/E (μmol CO_2 mol^{-1} H_2O m^{-2} s^{-1})	4.087 ± 0.258 a	3.631 ± 0.319 a

[a] g_{amb}; stomata conductance at PAR=150 μmol m^{-2} s^{-1}, [b] g_{max}; stomata conductance at PAR= 600 μmol m^{-2} s^{-1}, [c] E_{max}; transpiration rate at PAR= 150 μmol m^{-2} s^{-1}, [d] E_{max}; transpiration rate at PAR= 600 μmol m^{-2} s^{-1}, [e] A_{amb}/g water use efficiency at PAR =150 μmol m^{-2} s^{-1}, [f] A_{max}/g; water use efficiency at PAR= 600 μmol m^{-2} s^{-1}, [g] A_{amb}/E; transpiration rate at PAR=150 μmol m^{-2} s^{-1}, [h] A_{max}/E; transpiration rate at PAR= 600 μmol m^{-2} s^{-1}. The indicated variables for *Arabidopsis* grown in soil are not significantly different ($P < 0.05$) between treatments (control, CNT). Values are means ± SE (n= 10) and the treatments that share the same letters (a) are not significantly different.

Appendix B. Mean ± (SE) stomata conductance, transpiration rate, and water use efficiency for agar experiment

Variables	Control	CNTs	Ag-QDs
[a] g_{amb} (mol CO^2 $m^{-2}s^{-1}$)	0.719 ± 0.128 a	0.613 ± 0.110 a	0.598 ± 0.139 a
[b] g_{max} (mol CO^2 $m^{-2}s^{-1}$)	0.667 ± 0.109 a	0.513 ± 0.072 a	0.709 ± 0.159 a
[c] E_{amb} (mol H_2O $m^{-2}s^{-1}$)	7.170 ± 0.530 a	5.831 ± 0.361 a	7.096 ± 0.723 a
[d] E_{max} (mol H_2O $m^{-2}s^{-1}$)	7.303 ± 0.515 a	5.821 ± 0.321 a	7.156 ± 0.696 a
[e] A_{max}/g (μmol CO_2 mol^{-1} CO_2 m^{-2} s^{-1})	14.02 ± 1.77 a	12.64 ± 1.44 a	7.96 ± 1.20 b
[f] A_{max}/E (μmol CO_2 mol^{-1} H_2O m^{-2} s^{-1})	0.88 ± 0.073 a	0.79 ± 0.055 a	0.47 ± 0.056 b

[a] g_{amb}; stomata conductance at PAR=150 μmol m^{-2} s^{-1}, [b] g_{max}; stomata conductance at PAR= 600 μmol m^{-2} s^{-1}, [c] E_{max}; transpiration rate at PAR= 150 μmol m^{-2} s^{-1}, [d] E_{max}; transpiration rate at PAR= 600 μmol m^{-2} s^{-1}.
[e] A_{max}/g; water use efficiency at PAR= 600 μmol m^{-2} s^{-1}, [f] A_{max}/E; transpiration rate at PAR= 600 μmol m^{-2} s^{-1} are not significantly different (P < 0.05) between control and CNT at 4μg/ml and significantly different between control and Ag-QD at 4μg/ml. Values are means ± SE (n= 10) and the letters (a/b) indicate the significant difference between treatments. The treatments that do not share the same letters are significantly different.

Appendix C. Mean ± (SE) for photosynthesis response to treatments for each growth day

*Variables	Growth Days	Control	CNT	`Ag-QD
A_{amb} (μmol CO_2 m^{-2} s^{-1})	14	4.30 ± 0.39 a	3.36 ± 0.36 a	1.62 ± 0.12 b
A_{max} (μmol CO_2 m^{-2} s^{-1})	14	5.31 ± 0.49 a	4.18 ± 0.42 a	2.23 ± 0.16 b
A_{amb} (μmol CO_2 m^{-2} s^{-1})	22	5.92 ± 0.65 a	4.51 ± 0.29 a	2.72 ± 0.22 b
A_{max} (μmol CO_2 m^{-2} s^{-1})	22	7.91 ± 0.95 a	5.62 ± 0.36 a	3.32 ± 0.21 b
A_{amb} (μmol CO_2 m^{-2} s^{-1})	30	3.88 ± 0.41 a	2.92 ± 0.24 ab	1.86 ± 0.26 b
A_{max} (μmol CO_2 m^{-2} s^{-1})	30	5.56 ± 0.88 a	3.90 ± 0.19 ab	2.81 ± 0.53 b

*A_{amb}, ambient photosynthesis at light intensity PPFD=150 μmol m-2 s^{-1} and A_{max}, maximum photosynthesis at PPFD=500 μmol m-2 s-1 which were measured under ambient CO2 (400 μmol CO2 mol^{-1} air) are not significantly different P < 0.05 between control and CNT and significantly different between control and Ag-QD on growth days (14, 22, and 30). the letters (a/b) indicate the significant difference between treatments. The treatments that do not share the same letters are significantly different. Sample size for each growth day (14, 22, 30) is n= (39, 35, and 18) respectively.

Appendix D. Mean ± (SE) triose phosphate use response to treatments for each growth day

*Variables	Growth Days	Control	CNT	`Ag-QD
TPU (μmol m^{-2} s^{-1})	14	4.5 ± 0.19 a	3.7 ± 0.204 ab	2.8 ± 0.26 b
TPU (μmol m^{-2} s^{-1})	22	4.8 ± 0.19 a	3.5 ± 0.15 ab	2.9 ± 0.11 b
TPU (μmol m^{-2} s^{-1})	30	3.6 ± 0.78	2.9 ± 0.72 b	2.4 ± 0.41 b

*TPU, triose phosphate use are significantly different $P < 0.05$ between treatments (control, CNT, and Ag-QD), and growth days (14, 22, and 30). The letters (a/b) indicate the significant difference between treatments. The treatments that do not share the same letters are significantly different. Sample size for each growth day (14, 22, 30) is n= (39, 35, and 18) respectively.

Appendix E. Graphs. Chlorophyll content, leaf area, dry weight, and water potential
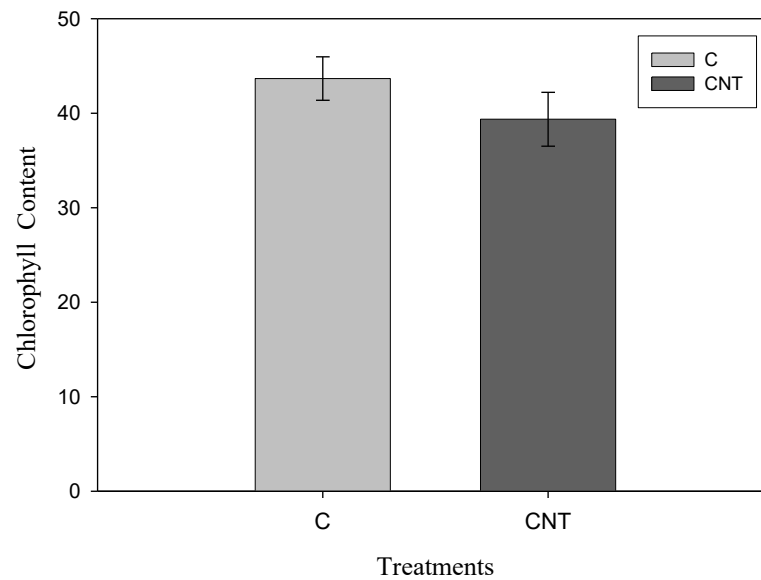
Chlorophyll content of *Arabidopsis* (n= 26) for each treatment (Control and CNT) in all growth days. The bar error is the mean of standard error for each treatment.
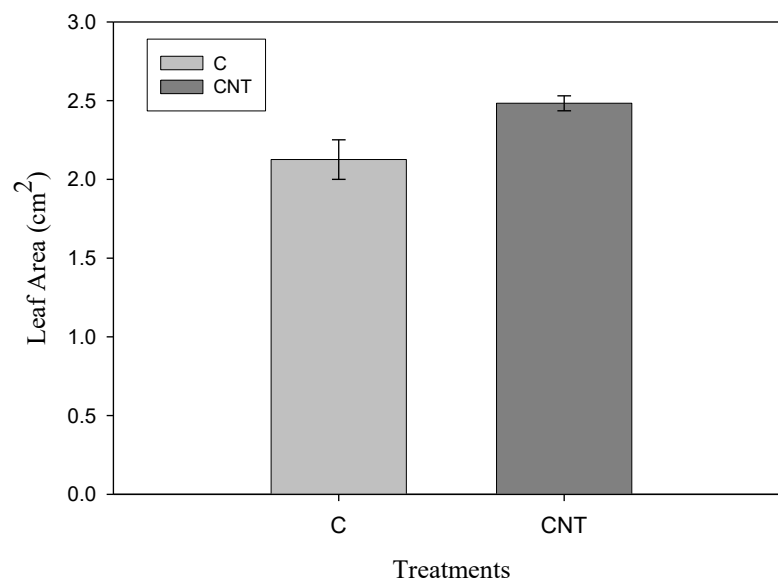
Leaf area of *Arabidopsis* (n=18) for each treatment (Control and CNT) in all growth days. The bar error is the mean of standard error for each treatment.
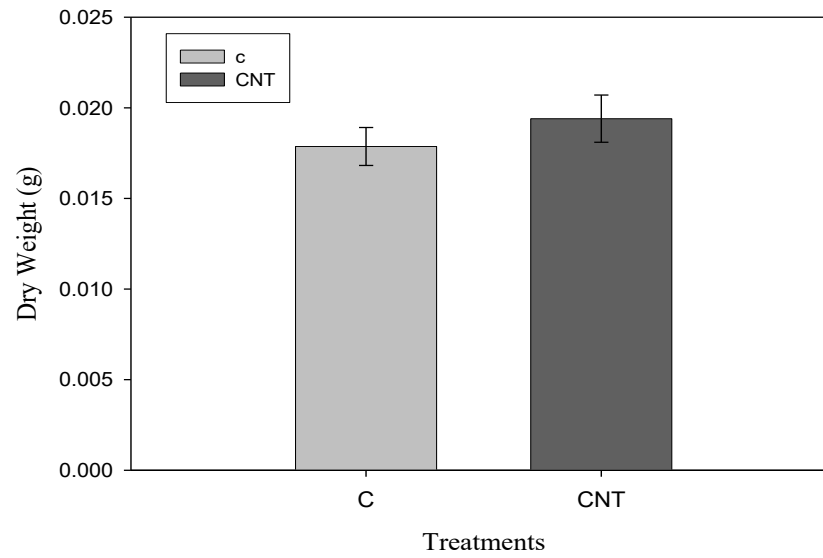
Dry weight (DW) of *Arabidopsis* (n= 18) for each treatment (Control and CNT) in all growth days. The bar error is the mean of standard error for each treatment.
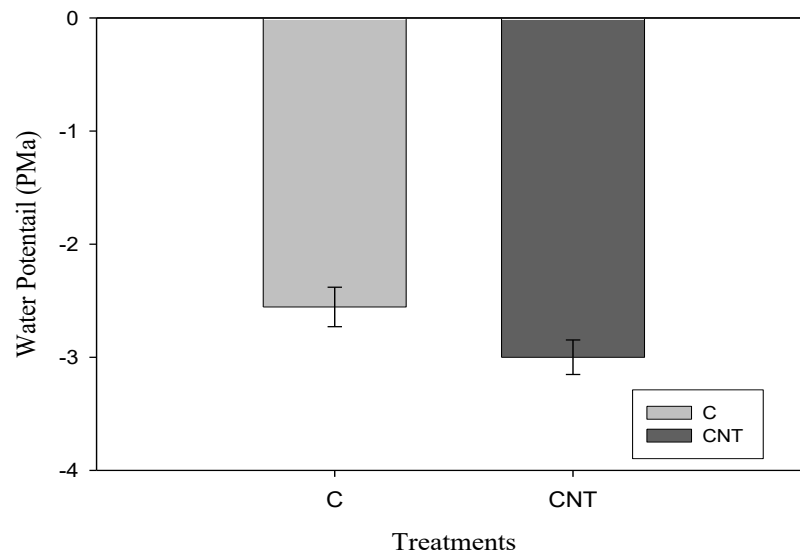
Water potential (WP) of *Arabidopsis* (n= 12) for each treatment (Control and CNT) in all growth days. The bar error is the mean of standard error for each treatment.
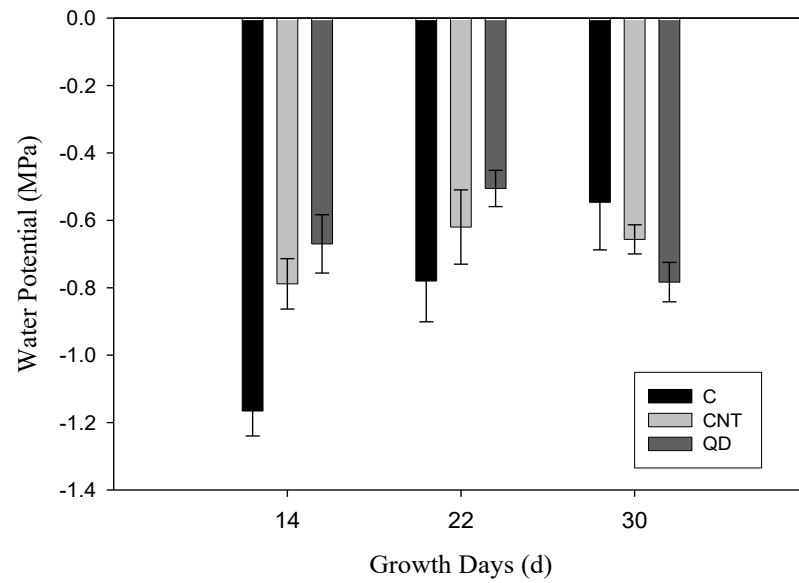
Water potential (WP) of *Arabidopsis* (n= 79) for each treatment (Control, CNT and, Ag-QD) in each growth day (14, 22, and 30). The bar error is the mean of standard error for each treatment.

Appendix F. ANOVA tables

ANOVA table for ambient CO2 assimilation rate data (Aamb) at light level (PAR=150 μmol m-2 s^{-1}). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 10). Treatments (Control and CNT) and growth days (63 and 83) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	0.12502	0.12502	0.23	0.651
Growth Days	1	0.04736	0.04736	0.09	0.780
Treatment*Growth Days	1	0.25355	0.25355	0.46	0.523
Error	6	3.31369	0.55228		
Total	9	3.77886			

ANOVA table for maximum CO2 assimilation rate data (Amax) at light level (PAR=600 μmol m-2 s^{-1}). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 9). Treatments (Control and CNT) and growth days (63 and 83) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	0.31504	0.31504	0.29	0.613
Growth Days	1	0.05256	0.05256	0.05	0.834
Treatment*Growth Days	1	0.28183	0.28183	0.26	0.632
Error	5	5.41668	1.08334		
Total	8	6.01887			

ANOVA table for intracellular CO2 rate data at light level (PAR=150 μmol m-2 s^{-1}). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 10). Treatments (Control and CNT) and growth days (63 and 83) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	369.6	369.6	0.25	0.633
Growth Days	1	3424.4	3424.4	2.35	0.176
Treatment*Growth Days	1	3448.4	3448.4	2.36	0.175
Error	6	8753.0	1458.8		
Total	9	15684.7			

ANOVA table for intracellular CO2 rate data at light level (PAR=500 µmol m-2 s^{-1}). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 9). Treatments (Control and CNT) and growth days (63 and 83) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	1848.2	1848.2	2.32	0.188
Growth Days	1	3261.0	3261.0	4.09	0.099
Treatment*Growth Days	1	357.4	357.4	0.45	0.533
Error	5	3988.9	797.8		
Total	8	10442.3			

ANOVA table for stomata conductance data at light level (PAR=150 µmol m^{-2} s^{-1}). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 10). Treatments (Control and CNT) and growth days (63 and 83) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	0.000002	0.000002	0.02	0.900
Growth Days	1	0.000000	0.000000	0.00	0.993
Treatment*Growth Days	1	0.000137	0.000137	1.12	0.330
Error	6	0.000733	0.000122		
Total	9	0.000873			

ANOVA table for stomata conductance data at light level (PAR=500 µmol m^{-2} s^{-1}). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 9). Treatments (Control and CNT) and growth days (63 and 83) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	0.000002	0.000002	0.01	0.911
Growth Days	1	0.000017	0.000017	0.12	0.744
Treatment*Growth Days	1	0.000053	0.000053	0.38	0.567
Error	5	0.000708	0.000142		
Total	8	0.000784			

ANOVA table for transpiration rate data at light level (PAR=150 μmol m^{-2} s^{-1}). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 10). Treatments (Control and CNT) and growth days (63 and 83) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	0.000032	0.000032	0.00	0.984
Growth Days	1	0.000196	0.000196	0.00	0.961
Treatment*Growth Days	1	0.087955	0.087955	1.15	0.324
Error	6	0.457284	0.076214		
Total	9	0.545443			

ANOVA table for transpiration rate data at light level (PAR=500 μmol m^{-2} s^{-1}). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 9). Treatments (Control and CNT) and growth days (63 and 83) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	0.005270	0.005270	0.05	0.826
Growth Days	1	0.012263	0.012263	0.13	0.738
Treatment*Growth Days	1	0.045582	0.045582	0.47	0.525
Error	5	0.489445	0.097889		
Total	8	0.553750			

ANOVA table for water use efficiency (Aamb/g) data at light level (PAR=150). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 10). Treatments (Control and CNT) and growth days (63 and 83) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	26.25	26.25	0.03	0.867
Growth Days	1	826.80	826.80	0.97	0.364
Treatment*Growth Days	1	862.75	862.75	1.01	0.354
Error	6	5133.28	855.55		
Total	9	6823.25			

ANOVA table for water use efficiency (Aamb/E) data at light level (PAR=150 µmol m^{-2} s^{-1}). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 10). Treatments (Control and CNT) and growth days (63 and 83) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	0.05914	0.05914	0.06	0.809
Growth Days	1	1.19838	1.19838	1.30	0.298
Treatment*Growth Days	1	0.77172	0.77172	0.84	0.396
Error	6	5.53331	0.92222		
Total	9	7.50403			

ANOVA table for water use efficiency (Amax/g) data at light level (PAR=500). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 9). Treatments (Control and CNT) and growth days (63 and 83) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	392.6	392.6	0.93	0.380
Growth Days	1	826.5	826.5	1.95	0.221
Treatment*Growth Days	1	391.3	391.3	0.92	0.380
Error	5	2116.4	423.3		
Total	8	4068.4			

ANOVA table for water use efficiency (Amax/E) data at light level (PAR=500). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 9). Treatments (Control and CNT) and growth days (63 and 83) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	0.2648	0.2648	0.95	0.375
Growth Days	1	0.9087	0.9087	3.25	0.131
Treatment*Growth Days	1	0.4037	0.4037	1.45	0.283
Error	5	1.3967	0.2793		
Total	8	3.2930			

ANOVA table for leaf area data. The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 18). Measurements for controls were taken in growth days 51 and 79, and in the 79th for CNTs. Treatments (Control and CNT) were treated as fixed factors and growth days (63 and 83) as random factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	0.00083	0.00083	0.02	0.900
Growth days	1	1.39401	1.39401	27.59	0.000
Error	15	0.75782	0.05052		
Total	17	2.66305			

ANOVA table for dry weight data. The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 18). Measurements for controls were taken in growth days 51 and 79, and in the 79th for CNTs. Treatments (Control and CNT) were treated as fixed factors and growth days (63 and 83) as random factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	0.000010	0.000010	0.47	0.502
Growth days	1	0.000014	0.000014	0.66	0.429
Error	15	0.000320	0.000021		
Total	17	0.000336			

ANOVA table for specific leaf area data. The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 18). Measurements for controls were taken in growth days 51 and 79, and in the 79th for CNTs. Treatments (Control and CNT) were treated as fixed factors and growth days (63 and 83) as random factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	2379	2379	1.94	0.184
Growth days	1	11181	11181	9.10	0.009
Error	15	18426	1228		
Total	17	29630			

ANOVA table for chlorophyll content data. The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 26). Measurements for controls and CNTs were taken in growth day 86, 91, and 92. Treatments (Control and CNT) were treated as fixed factors and growth days (86, 91, and 92) as random factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	54.91	54.91	1.19	0.386 x
Growth day	2	1532.79	766.39	16.35	0.058
Treatment*growth day	2	93.76	46.88	1.94	0.170
Error	20	483.13	24.16		
Total	25	2216.83			

ANOVA table for water potential (WP) data. The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 12). Measurements for controls and CNTs were taken in growth day 43 and 91. Treatments (Control and CNT) were treated as fixed factors and growth days (43, and 91) as random factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	0.24000	0.240000	0.93	0.359
Growth day	1	0.00889	0.008889	0.03	0.857
Error	9	2.31333	0.257037		
Total	11	2.76667			

ANOVA table for ambient CO2 assimilation rate data (Aamb) at light level (PAR=150) and in growth day 14. The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 39). Treatments (Control, CNT and, Ag-QD) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	2	48.01	24.003	18.67	0.000
Error	36	46.27	1.285		
Total	38	94.28			

ANOVA table for ambient CO2 assimilation rate data (Aamb) at light level (PAR=150). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	80.572	40.2862	28.42	0.000
Growth Days	2	17.497	8.7487	6.17	0.003
Treatment*Growth Days	4	1.703	0.4258	0.30	0.877
Error	74	104.904	1.4176		
Total	82	216.522			

ANOVA table for maximum CO2 assimilation rate data (Amax) at light level (PAR=500) and growth day 14. The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 39). Treatments (Control, CNT and, Ag-QD) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	63.17	31.586	16.58	0.000
Error	36	68.56	1.905		
Total	38	131.74			

ANOVA table for maximum CO2 assimilation rate data (Amax) at light level (PAR=500). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	138.477	69.238	25.80	0.000
Growth Days	2	27.987	13.993	5.21	0.008
Treatment*Growth Days	4	4.832	1.208	0.45	0.772
Error	74	198.596	2.684		
Total	82	385.492			

ANOVA table for intracellular CO2 data (Ci) at light level (PAR=150). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	2281.8	1140.88	6.75	0.002
Growth Days	2	939.6	469.78	2.78	0.069
Treatment*Growth Days	4	211.0	52.75	0.31	0.869
Error	74	12506.7	169.01		
Total	82	16245.5			

ANOVA table for intracellular CO2 data (Ci) at light level (PAR=150) and growth day 14. The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 39). Treatments (Control, CNT and, Ag-QD) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	1332	665.9	3.17	0.054
Error	36	7554	209.8		
Total	38	8886			

ANOVA table for intracellular CO2 data (Ci) at light level (PAR=500). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	2533.0	1266.48	5.75	0.005
Growth Days	2	1001.7	500.87	2.27	0.110
Treatment*Growth Days	4	260.2	65.06	0.30	0.880
Error	74	16307.4	220.37		
Total	82	20651.1			

ANOVA table for stomata conductance data (g) at light level (PAR=150). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	0.4057	0.2029	0.50	0.611
Growth Days	2	0.2321	0.1161	0.28	0.754
Treatment*Growth Days	4	1.6296	0.4074	1.00	0.416
Error	69	28.2284	0.4091		
Total	77	30.6189			

ANOVA table for stomata conductance data (g) at light level (PAR=500). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	0.7054	0.3527	0.98	0.381
Growth Days	2	0.8364	0.4182	1.16	0.320
Treatment*Growth Days	4	0.8302	0.2075	0.58	0.681
Error	71	25.6139	0.3608		
Total	79	27.8618			

ANOVA table for transpiration rate data (E) at light level (PAR=150). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	34.19	17.096	2.02	0.140
Growth Days	2	30.28	15.142	1.79	0.174
Treatment*Growth Days	4	22.64	5.660	0.67	0.615
Error	74	625.83	8.457		
Total	82	709.37			

ANOVA table for transpiration rate data (E) at light level (PAR=500). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	38.78	19.391	2.50	0.089
Growth Days	2	24.75	12.373	1.59	0.210
Treatment*Growth Days	4	20.09	5.023	0.65	0.631
Error	74	574.86	7.768		
Total	82	655.99			

ANOVA table for water use efficiency (Aamb/g) at light level (PAR=150). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	329.08	164.54	4.29	0.017
Growth Days	2	302.43	151.21	3.95	0.024
Treatment*Growth Days	4	71.37	17.84	0.47	0.761
Error	74	2836.40	38.33		
Total	82	3648.29			

ANOVA table for water use efficiency (Aamb/E) at light level (PAR=150). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	1.7221	0.86104	15.97	0.000
Growth Days	2	0.2987	0.14936	2.77	0.069
Treatment*Growth Days	4	0.1192	0.02981	0.55	0.698
Error	74	3.9910	0.05393		
Total	82	6.4304			

ANOVA table for water use efficiency (A max/g) at light level (PAR=500). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	413.49	206.75	3.27	0.043
Growth Days	2	202.13	101.06	1.60	0.209
Treatment*Growth Days	4	71.82	17.95	0.28	0.887
Error	74	4672.85	63.15		
Total	82	5499.86			

ANOVA table for water use efficiency (A max/E) at light level (PAR=500). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	2.0895	1.04477	9.58	0.000
Growth Days	2	0.2405	0.12024	1.10	0.337
Treatment*Growth Days	4	0.2136	0.05340	0.49	0.743
Error	74	8.0705	0.10906		
Total	82	11.0334			

ANOVA table for maximum carboxylation (Vcmax). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	548.27	274.13	10.24	0.001
Growth day	2	34.62	17.31	0.65	0.534
Treatment*growth day	4	84.50	21.12	0.79	0.545
Error	21	562.05	26.76		
Total	29	1281.12			

ANOVA table for photosynthetic electron transport rate (J). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	2605.90	1302.95	24.22	0.000
Growth day	2	255.80	127.90	2.38	0.117
Treatment*growth day	4	63.61	15.90	0.30	0.877
Error	21	1129.65	53.79		
Total	29	4595.31			

ANOVA table for triose phosphate use (TPU). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	11.1039	5.5520	19.71	0.000
Growth day	2	2.7821	1.3910	4.94	0.017
Treatment*growth day	4	0.7554	0.1888	0.67	0.620
Error	21	5.9141	0.2816		
Total	29	23.3817			

ANOVA table for maximum electron transport rate (Jmax). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 83). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	2072.9	1036.45	9.25	0.001
Growth day	2	487.9	243.94	2.18	0.138
Treatment*growth day	4	135.0	33.76	0.30	0.874
Error	21	2352.8	112.04		
Total	29	5426.7			

ANOVA table for maximum electron transport rate (Jmax). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 56). Treatments (Control and CNT) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	523.80	523.80	3.54	0.081
Growth day	2	404.53	202.26	1.37	0.287
Treatment*growth day	2	41.20	20.60	0.14	0.871
Error	14	2073.75	148.12		
Total	19	3019.10			

ANOVA table for maximum electron transport rate (Jmax). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 55). Treatments (Control and Ag-QDs) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	2072.87	2072.87	17.32	0.001
Growth day	2	430.89	215.45	1.80	0.201
Treatment*growth day	2	76.95	38.48	0.32	0.730
Error	14	1675.07	119.65		
Total	19	4626.46			

ANOVA table for water potential (WP). The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 79). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	0.3206	0.16028	1.87	0.162
Growth Days	2	1.0319	0.51597	6.01	0.004
Treatment*Growth Days	4	0.7702	0.19256	2.24	0.073
Error	70	6.0105	0.08586		
Total	78	9.5589			

ANOVA table for dry weight. The test used for ANOVA is general linear model with a significant level p-value < 0.05 and sample size (n= 63). Treatments (Control, CNT and, Ag-QD) and growth days (14, 22, and 30) were treated as fixed factors.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	0.000023	0.000012	0.04	0.962
Growth Days	2	0.000588	0.000294	0.98	0.383
Treatment*Growth Days	4	0.001055	0.000264	0.87	0.485
Error	58	0.017499	0.000302		
Total	66	0.019300			