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Short-term effects of elevated temperature and CO_2 on *Pisum sativum*

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Abstract The concentration of CO_2 is predicted to further increase till the end of this century and the changes in global temperature are also expected. Due to the fact that increasing CO_2 and temperature will change the agricultural environment we investigated photosynthesis, plant productivity and organic carbon (C org.) accumulation of pea (*Pisum sativum* L.) under increasing levels of air temperature and atmospheric CO_2. A closed growth chamber experiment was performed in a controlled environment at ambient [21 °C/400 ppm] and elevated [25 °C/800 ppm] temperature and CO_2 conditions.

The results showed that after 4 weeks of treatment aboveground (49 %, $p<0.05$) and belowground (6 %, $p>0.05$) biomass of pea increased, also significantly increased photosynthesis (54 %) and leaf area (37 %) under elevated climate [25 °C/800 ppm] conditions compared to the conditions of [21 °C/400 ppm]. Either it was estimated that organic carbon partitioning in leaves and stems increased ($p>0.05$) under elevated climate conditions [25 °C/800 ppm], but decreased in roots ($p<0.05$).

Our results suggest that the effects of increasing levels of air temperature and atmospheric CO_2 were greater for photosynthesis, leaf area and aboveground biomass of pea. Also results demonstrated a promising potential in biomass C accumulation.

Keywords: climate change, photosynthesis, organic carbon, closed chamber experiment, *Pisum sativum*

1. Introduction

It is expected that CO_2 value could increase to an atmospheric concentration of between 750 and 1300 ppm for the end of the century and temperature is predicted to increase between 1.8 and 6.0 °C (IPCC, 2014).

Elevated CO_2 concentrations directly influence soil-plant systems via enhancing plant growth (Butterly, 2015). Elevated global temperature in the future will impact ecology and agriculture and may prove to be a major factor limiting crop production (Kurek *et al*., 2007; Perez *et al*., 2009; Qin *et al*., 2008). Climatic changes due to temperature, water availability variation, and increase in CO_2 will also affect the carbon–nitrogen balance in the plants and eventually how seeds set and grow (Vadez *et al*., 2012).

Since global increases in temperature and CO_2 may have interactive effects on photosynthesis, many studies have examined the effects of elevated CO_2 and increased growth temperature (typically 3–5 ◦ C) on photosynthesis (Morison and Lawlor 1999). Only a few studies have examined the interactions between elevated CO_2 and higher mean growth temperature on plant carbon accumulation. According to Dyson (2008) “If we can control what the plants do with carbon, the fate of the carbon in the atmosphere is in our hands”. That is why one of the aim of our research is to analyze carbon partitioning in legumes plants. Legumes provide important sources of oil, fiber, and protein-rich food and feed while supplying nitrogen (N) to agro-ecosystems via their unique ability to fix atmospheric N_2 in symbiosis with the soil bacteria rhizobia, increasing soil carbon content, and stimulating the productivity of the crops that follow (Jensen *et al*., 2011)

2. Methods

The experiment was conducted in a closed controlled environment plant growth chambers located at Vytautas Magnus University in 2016. Seeds of pea (*Pisum sativum* L., cv. Pinochis) (15 individuals per pot) were planted in 3 L plastic pots (diameter 21 cm, height 10.6 cm) containing a growth substrate composed of a mixture of field soil (the soil was taken from ASU Training Farm, Kaunas District), perlite and fine sand (5:3:2, by volume). A nutrient supply corresponding to 120 kg N ha^{-1} was used until the beginning of treatment. Additional fertilization with a complex nutrient (NPK 12-11-18 + microelements) solution, increasing the N level until 180 kg N ha^{-1}, was applied two weeks after the treatments.

Elevated CO_2 and temperature (day/night temperature of 25/18 °C and 800 ppm of CO_2) treatment was applied when the seedlings of pea were germinated and lasted for 4 weeks. Until that time all plants were grown in the control chamber under conditions of current climate – an average day/night temperature of 21/14 °C and 400 μmol mol^{-1} of CO_2. The following stable conditions were maintained in both chambers: a photoperiod of 14 h, relative air humidity of 60–70%, and 200 µmol m–2 s–1 photon flux density of photosynthetically active radiation. The chamber was 2.5 m high, 2 m wide and 2 m long. The pots in the chamber were watered sufficiently and regularly. All treatments were run in three replicates.

Gas exchange was measured with portable photosynthesis system LI-6400 (LI-COR, USA) with randomly selected youngest fully expanded leaves at the end of experiment. The photosynthetic rate determined at the corresponding growth CO_2 concentrations (A_{growth}, µmol CO_2 m^{-2} s^{-1}), stomatal conductance ($g_{s(growth)}$, mmol H_2O m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}) were recorded automatically for approximately 5 minutes.

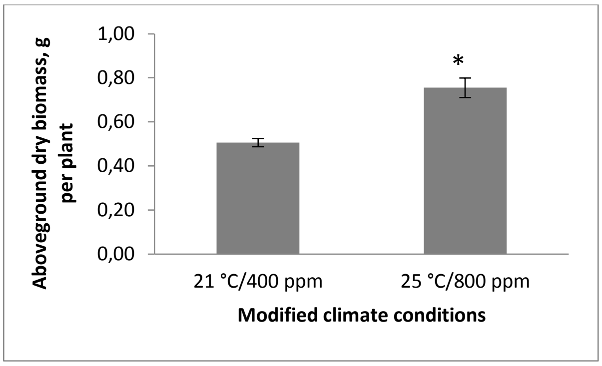
The measurement of leaf area was carried out on the last day of treatment. The leaf areas of all leaves per plant of five plants per treatment were measured with a scanner (CanoScan 4400F, Canon, USA) and then the leaf area of all leaves per plant was determined by GIMP 2.8 software.

For determination of dry weight, plants from each pot were harvested and the samples of leaves, stems and roots were dried in an electric oven at 70 °C until a constant dry weight was obtained. Biomasses of the samples were calculated into dry biomass per plant. Measurements of carbon accumulation were carried out at a 28-day period after the treatment. A subsample of plant roots was dried in an electric air-forced oven at 70 °C until a constant dry weight was obtained (at least 72 hours). Soil samples were also taken at a 28-day period after the treatment. The samples were air dried at room temperature and sieved through 2 mm mesh on purpose to remove all visible roots and plant remains. The dried samples of shoots, roots and soil were ground to a fine powder with a mill (Retsch HM400, Germany). Organic carbon content was measured with a Shimadzu TOC-V solid sample module SSM-5000A in the laboratory of Vytautas Magnus University.

The independent-samples t-test was applied to estimate the difference between reference and treatment values and p value < 0.05 was the threshold for significance. All analyses were performed by STATISTICA and the results were expressed as the mean values and their standard errors (±SE).

3. Results and discussion

The results showed that after 4 weeks of treatment aboveground biomass of pea (Figure 1) significantly ($p<0.05$) increased 49 % under elevated climate conditions [25 °C/800 ppm] compared to ambient climate [21 °C/400 ppm] still there was only a small increase in belowground biomass of pea (Figure 2) (6 %, $p>0.05$) under elevated conditions, compared to ambient.



Note. * − statistically significant difference at $p<0.05$, as compared to the reference treatment [21ºC/400 ppm]

Figure 1. Dry aboveground biomass of pea at different climate conditions (mean ± SE)

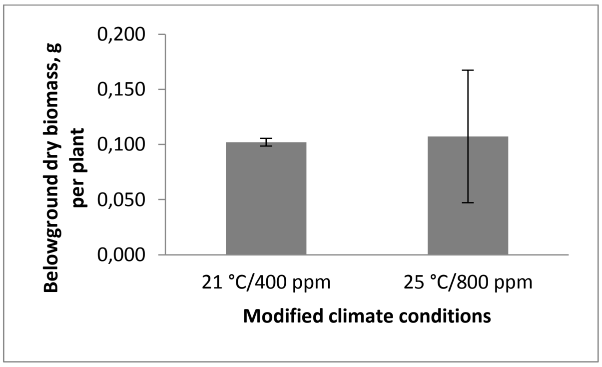
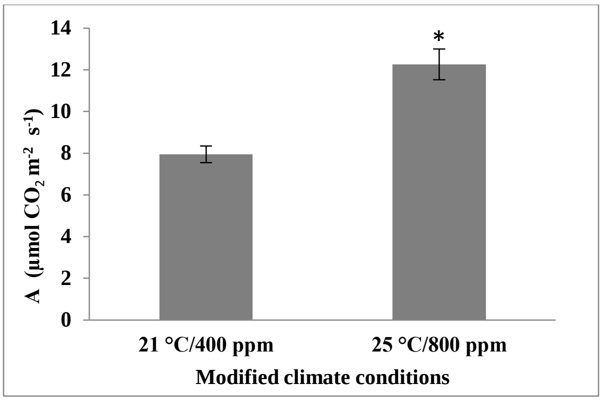


Figure 2. Dry belowground biomass of pea at different climate conditions (mean ± SE)

According to some authors (Erice *et al*., 2006; Irigoyen *et al*., 2014) plants usually grew more when exposed to a combination of elevated CO_2 concentrations and elevated temperature than under current CO_2 and temperature conditions. However Juknys (2011) investigated, that increasing biomass of pea under elevated both CO_2 ant temperature were positive but not statistically significant. The change of aboveground biomass in our treatment was bigger compared with other authors results. For example, Butterly (2015) found that elevated CO_2 increased shoot biomass of field pea by 36 %.

Increasing levels of air temperature and atmospheric CO_2 significantly increased photosynthesis (54 %) (Figure 3). According to Irigoyen (2014) when the atmospheric CO_2 concentration rises suddenly (or in a temporal window up to a few days) from 400 to 700 µmol mol^{-1}, the photosynthetic C fixation of C_3 plants increases. Either an increase in photosynthesis by 25 – 75 % has been detected in many experimental studies on the impact of doubled CO_2 concentration on C3 crops (Urban, 2003; Kirschbaum, 2004).



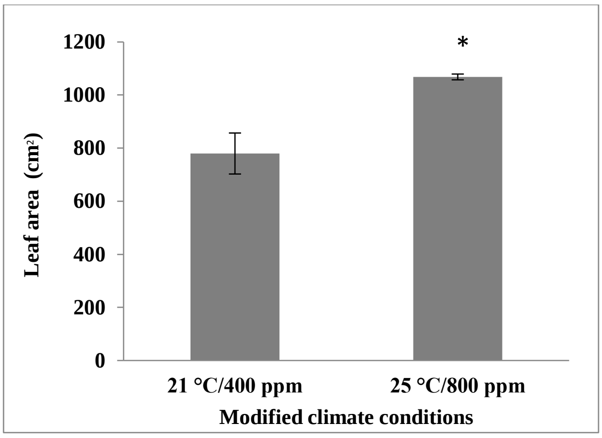
Note. * − statistically significant difference at $p<0.05$, as compared to the reference treatment [21ºC/400 ppm]

Figure 3. The rate of photosynthesis under different climate conditions

The effect of changing climate on transpiration and stomata conductance was not statistically significant ($p>0.05$) with a change accordingly to 2 % and -4 %.

Reduced stomatal conductance in a higher CO_2 environment will maintain plant water relations, but may have implications for heat stress as leaf temperature rises with reduced transpiration (Vadez *et al*., 2012).

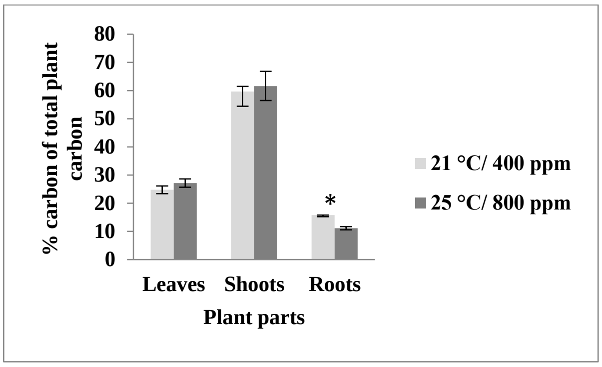
It is said, that a prevailing response of crops to rising CO_2 is an increase in leaf area (Srinivasan *et al*., 2016). According to our results, leaf area significantly increased 37 % under elevated climate conditions [25 °C/800 ppm] compared to ambient climate [21 °C/400 ppm] (Figure 4). For example, model simulations showed that soybean crops grown under current and elevated (550 ppm) CO_2 overinvest in leaves, and this is predicted to decrease productivity and seed yield 8% and 10%, respectively (Srinivasan *et al*., 2016).



Note. * − statistically significant difference at $p<0.05$, as compared to the reference treatment [21ºC/400 ppm]

Figure 4. Leaf area under different climate conditions (mean ± SE)

Organic carbon partitioning in leaves and stems increased ($p>0.05$) under elevated climate conditions [25 °C/800 ppm], but decreased in roots ($p<0.05$) (Figure 5).



Note. * − statistically significant difference at $p<0.05$, as compared to the reference treatment [21ºC/400 ppm]

Figure 5. % carbon of total plant carbon in plant parts under different climate conditions

Decreasing carbon in roots can be explained as nodules are competing for carbon use with the roots (Voisin *et al.* 2003a). As such, nodulation can limit root growth (according to our results, there was only 6 % increase of pea roots biomass (Figure 2) ($p>0.05$) under elevated conditions, compared to ambient). When net photosynthesis rate per plant increased, net sink strength also increased, suggesting that the C supply from photosynthesis was limiting. It has been shown by our treatment and other authors (Voisin *et al*., 2003b) that the percentage of C respired by belowground parts decreased when net photosynthesis rate increased. This suggests that the amount of C respired (for synthesis, maintenance of structures and/or symbiotic N_2 fixation or root mineral N assimilation activities) was decreasing the C supply available for investment in biomass of the root system (Warembourg, 1983) (Voisin *et al*., 2003b).

4. Conclusion

The results presented here demonstrated that, elevated temperature and CO_2 conditions positively affected dry aboveground and belowground biomass of pea; photosynthesis and leaf area also increased under [25 °C/800 ppm] climate conditions. Although organic carbon partitioning in leaves and stems increased under elevated climate conditions [25 °C/800 ppm], but decreased in roots because of nodules were competing for carbon use with the roots.

Further researches are needed to investigate the alteration of C/N ratio in legumes under elevated climate conditions

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