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Elevated CO_2 and nitrogen form alter physiological, morphological and biochemical characteristics of *Raphanus sativus* var. *sativus*

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1. Introduction, Knowledge, Objectives

Future production of vegetable crops will be affected by climatic change and the associated predicted increase of atmospheric CO_2 concentration. Considering different climate scenarios, which are based on emission projections and technical developments, it is likely to have double the atmospheric CO_2 concentration for the end of the 21st century compared to today's ambient concentration (IPCC, 2014). Plants respond to elevated CO_2 (eCO_2) concentrations differently. The effect of eCO_2 on vegetable crops has been investigated in a number of studies (Bloom, 2015; Poorter, 1993; Choi et al., 2010; Idso and Kimball, 1989). Elevated CO_2 stimulated plant growth about 39 % in average; in C_3 plants the effect is stronger than in C_4 and CAM plants (Poorter, 1993). The reported higher growth rate may be a result of the rising photosynthetic efficiency (Bloom, 2015; Poorter, 1993). However, eCO_2 was also shown to have no or only little effect on the photosynthetic capacity of numerous plants, including *Raphanus sativus* (Choi et al., 2010). Thus, there may be other growth factors altering the eCO_2-effect. Beside climate parameters, nutrient supply plays a major role in plant development.

Nitrogen constitutes a limiting factor for plant productivity in most terrestrial ecosystems and usually is the nutrient element with the highest effect on crop yields (Bloom, 2015). The nitrogen availability and the nitrogen form are determining the plant production. The cation ammonium (NH_4^+) is quickly absorbed by microorganisms and transformed into the anion nitrate (NO_3^-) through nitrification. Moreover, NH_4^+ is adsorbed to soil colloids. Unlike NH_4^+, NO_3^- is highly mobile within the soil and hence directly plant available. Therefore, the nitrogen uptake by plants is predominantly taking place in the form of nitrate. However, the interaction between elevated CO_2 and the nitrogen form and its effect on plant development and physiological aspects such as water use efficiency is still widely unknown.

To best of our knowledge, nothing is known about these effects on small radish as an important vegetable crop in Europe (Behr, 2018). Based on the interaction, changes in morphological and physiological characteristics as well as in the nutritional compounds may arise. The fertilization effect may be altered with climate change dependent on the nitrogen form. For growers, knowledge about these changes is crucial to react with other fertilization strategies in the future. In case of NO_3^- fertilization this might lead to environmental and quality problems, for example due to higher NO_3^- contents in plant tissues and increased nitrate leaching to the groundwater bodies.

Thus, the objective of this study was to elucidate the interactive effects of eCO_2 and nitrogen fertilization on physiological, morphological and biochemical characteristics of a vegetable crop. By using the economically and nutritionally relevant crop *Raphanus sativus* var. *sativus*, findings will contribute to decision-making on farm level and of consumers. The study will reply to the following hypotheses:

- Fresh weight, dry weight and the size of radish tuber increase with elevated CO_2 and ammonium fertilization.
- The photosynthetic rate is higher under elevated CO_2 and ammonium fertilization.
- The water use efficiency will be improved under elevated CO_2.

2. Material and Methods

Potted plants of small radish (*Raphanus sativus* var. *sativus,* 'Celesta', Enza Zaden, Germany) were cultivated in pots in controlled atmosphere. The experiment was carried out in four climate chambers (Fitotron®, type: HGC 0714, Weiss Technik, Germany) with constant climate conditions (20/14 °C day/night temperature, 65 % rel. humidity, 1000 µmol/m²s light intensity with 8 h exposure time). The main testing factor was the CO_2 concentration with two factor ranges, ambient CO_2 (400 ppm; aCO_2) and elevated CO_2 (1000 ppm; eCO_2). The sub testing factor was the nitrogen form with two factor ranges, nitrate fertilization (NO_3^-) and ammonium fertilization (NH_4^+). The four treatments combinations with 22 plants each (Table 1) were arranged in a quadruple repeated nested anova design. The plants were directly seeded in unfertilized soil in pot liners 10. Fertilization based on demand (Feller et al., 2011) and 4.2 mgN/pot were applied two times during the experiment. The nutrient ratio was 1:0.6:2.7 (N:P:K). Plants were irrigated daily, if the water holding capacity fell below 80 %. The water supply was calculated to fill up to 90% water holding capacity. In order to avoid systematic errors by plant location in the chamber, the plants in each climate chamber were randomized in the course of each irrigation date. The experiment lasted four weeks. Assimilation rate was measured by means of gas exchange with an infra-red gas analyser (GFS 3000, Walz, Germany) using following conditions for leaf stabilization in the cuvette: leaf temperature of 20 °C, saturating quantum lux density of 1000 µmol/m²s and CO_2 concentration in the cuvette of 400 ppm or 1000 ppm. At harvest date the fresh weight (FM), dry weight (DM), tuber diameter to calculate the tuber volume (under hypothesis of a sphere) and the water use efficiency (WUE, calculated from the quotient of the fresh weight and water supply) were measured.

Table 1: Plan of treatment combinations with the factors CO_2 and nitrogen form.

Variants	CO_2 (ppm)	Nitrogen form	Acronym	No. of plants
1	400	NH_4^+	a.NH4	22
2	400	NO_3^-	a.NO3	22
3	1000	NH_4^+	e.NH4	22
4	1000	NO_3^-	e.NO3	22

The statistical analysis was carried out with R-statistic (version 3.3.3) using a nested anova design.

3. Results

The FM of plants fertilized with ammonium was higher than of nitrate-fertilized plants. This effect was enhanced through eCO_2 (Figure 1). Elevated CO_2 led to a significant higher WUE (Figure 1), whereby plants developed higher biomass with the same water supply. Furthermore, a fertilizer effect was observed: WUE at aCO_2 as well as at eCO_2 differed significantly between the fertilizer variants. Consequently, there is an interaction between both factors.

Considering the DM (Table 2), there was no fertilizer effect at aCO_2. The NH_4^+ treatment showed higher DM under eCO_2 compared to NO_3^- treatments. The tuber volume was not affected by nitrogen form; at eCO_2 radish plants got higher tuber sizes (Table 2).

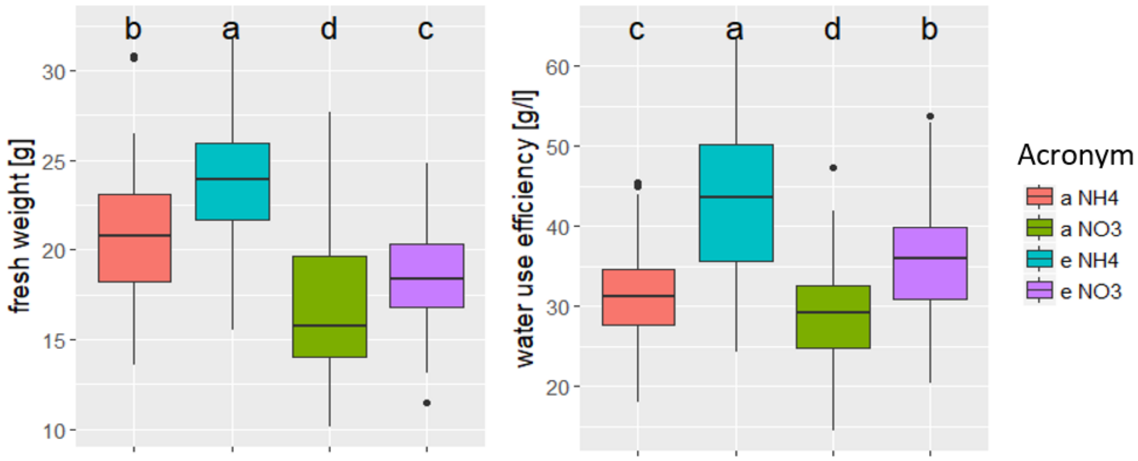

Figure 1. Boxplots of fresh weight (left) and WUE (right). Kruskal-Wallis test, α=5 %, p-values for FM and WUE 0.000.

Table 2. Means of plant growth parameters: dry weight (DM) and volume tuber. Significant differences between the variants are marked with different letters (Kruskal-Wallis, α=5 %)

variant	DM [g]	groups	p-value	variant	volume tuber [cm³]	groups	p-value
a.NH4	2,96	c	0.01254	a.NH4	20,04	bc	$3.33E^{-10}$
a.NO3	2,81	c		a.NO3	18,67	c	
e.NH4	3,86	a		e.NH4	25,03	a	
e.NO3	3,38	b		e.NO3	21,43	bc	

The assimilation rate showed no interaction between the CO_2 concentrations and the nitrogen (p-value=0.4515). In detail, the variants had the following no significant assimilation rates: a.NH4: 29.16 µmol/m²s; a.NO3: 28.3 µmol/m²s; e.NH4: 34.07 µmol/m²s and e.NH4:34.07 µmol/m²s. Only the factor CO_2 exhibit a significance (p-value=0.0071). The mean assimilation rate at eCO_2 was 32.84 µmol/m²s and accounted for 28.73 µmol/m²s at aCO_2.

4. Discussion

Until now, little is known about the combination and possible interactions of the nitrogen form in the fertilizer and the CO_2 concentration of the atmosphere. In this experiment, an interaction between the factors CO_2 and nitrogen form was confirmed for the vegetable crop *Raphanus sativus* var. *sativus,* 'Celesta'.

The hypothesis, that the fresh weight, dry weight and the size of the radish tuber is higher under eCO_2 and fertilization with ammonium was approved. Under aCO_2, the fresh weight of the plants averaged higher, if they were fertilized with ammonium. This effect was enhanced with eCO_2. Ammonium as a cation is able to attach to soil colloids and first must be desorbed to be available for the plant. Usually NH_4^+ dissolved in the soil solution is rapidly oxidized to NO_3^- by soil microorganisms, which is readily taken up by the plants. This might be one reason for the observation that there was a slight long-term effect for the NH_4^+-treatment in comparison to the NO_3^--treatment: nitrogen was available over a longer period for the ammonium-fertilized plants entailing an improved biomass production. This may also be an explanation for the higher tuber size in the NH_4^+-treatment. The growth of the tuber started approximately at the second date of fertilization (14 d after begin of experiment). In the first 14 days of the experiment, the plants may have used the nutrient primarily to develop the leaf mass. After 14 days the main sink apparently changed to the tuber. This change of main sinks was also reported by Tegeder et al. (2018). Thus, the directly available nitrate, after the first fertilization date, was primarily used for leaf production. The nutrients from the second fertilization date, the nitrate and the nitrified ammonium, were apparently completely used for the development of the tuber.

The enhanced biomass production under eCO_2 conditions as described in literature by Choi et al. 2010 and Idso & Kimball 1989 was confirmed in this experiment indicated as fresh, dry weight and tuber volume. Bloom (2015) showed that with eCO_2 the photosynthetic activity is getting higher based on a reduction of the rubisco activity and the photorespiration. This effect was approved by the gas exchange measurement, while Choi et al. (2010) could not asses a change in the photosynthetic activity. There might be physiological differences between the radish cultivars used in reported and our study. Whereas, Poorter (1993) reported an average higher plant development of 37% based on the higher photosynthetic activity for i.a. *Raphanus sativus*. Accordingly, the hypothesis that the photosynthetic rate is higher under eCO_2 and fertilization with ammonium could partly be confirmed, since there was no significant fertilization effect.

The third hypothesis stated that the WUE will be improved at elevated CO_2 and could be confirmed. This effect was also reported by Bowes (1991) who attributed the observation of an increased WUE under eCO_2 to the decrease of stomatal conductance and transpiration rate. In our case the stomatal conductance and transpiration rate were not evaluated but the higher WUE was confirmed. This is also an explanation for the higher biomass production. Moreover, the nitrogen form also showed a significant effect on the WUE. Even at aCO_2 the ammonium-fed plants had a higher WUE. Additional, eCO_2 increased the WUE in combination with ammonium-based fertilization. Hence, the NH_4^+-treatments produced higher biomasses at the same water availability. In case of NO_3^- fertilization and reduced WUE this might lead to environmental and quality problems, for example due to higher NO_3^- contents in plant tissues and higher nitrate leaching to the groundwater.

All parameters showed an advantage of the ammonium based fertilization as compared with a nitrate based fertilization for cultivation of small radish with elevated CO_2-concentrations in the future. The fertilization effect for small radish will change with climate change depending on the nitrogen form. For growers, this knowledge is crucial to react with other fertilizer strategies in the future.

5. Conclusions

This experiment revealed that a fertilization strategy with ammonium is more advantageous under eCO_2 in contrast to fertilization with nitrate for the cultivation of small radish. Furthermore, the WUE is improved which may enable irrigation with reduced water quantities. It was demonstrated that the interaction between eCO_2 and the nitrogen form had an influence on the growth of small radish as a primarily quality attribute. To understand and assess the product small radish under climate change conditions, further qualitative attributes, e.g. contents of nutrients and photopigments will be analyzed.

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