

Dual Role of Plasma-Activated Water in Hydroponic Lettuce: Nitrogen Supplementation and Growth Enhancement via ROS Signaling

Adriana Mišúthová¹, Zuzana Lukačová², Ramin Mehrabifard¹, Zdenko Machala¹

¹Division of Environmental Physics, Comenius University Bratislava, Slovakia

²Department of Plant Physiology, Comenius University Bratislava, Slovakia

Email: adriana.misuthova@fmph.uniba.sk



Background

The growing global population highlights the urgent need for sustainable food production. Modern agriculture heavily relies on commercial fertilizers, with nitrogen (N) being a critical nutrient for plant growth and development [1]. Cold atmospheric plasma has emerged as a promising alternative technology, generating reactive oxygen and nitrogen species (RONS) that dissolve in water to form plasma-activated water (PAW). Due to their prolonged bioavailability, these species induce complex chemical changes and can act as signaling molecules in plant metabolism, while also enhancing nutrient availability, particularly N. As a result, PAW represents a sustainable agricultural approach with the potential to improve nutrient uptake and plant performance, especially in N-deficient soils where limited N availability constrains growth. Furthermore, its application, such as in seed priming, has demonstrated significant potential to enhance the crop yield [2, 3].

Objective

This study investigates the **effects of PAW** generated by a **multi-transient spark discharge** system on the growth and physiological responses of **hydroponically cultivated lettuce** (*Lactuca sativa* L.) in N-supplemented and N-deprived conditions, with a particular focus on evaluating PAW as a sustainable source of nitrogen for plant nutrition.

Conclusion

Experiment A: Standard nutrient conditions

- PAW seed **priming** resulted in a significant **increase in plant growth**.
- The application of **PAW**, especially in combination with **priming**, led to **higher concentrations of chlorophylls and secondary metabolites**.
- Plants treated with **prim PAW** were rated as the **most palatable** in sensory evaluation.

Experiment B: Nitrogen-deprived conditions

- PAW** was able to partially **supplement nitrogen** under N-deprived conditions.
- Its application also **increased the concentration of chlorophylls and secondary metabolites**.

PAW enhances the plant growth, metabolic activity, and sensory quality, and also partially compensates nitrogen deficiency, highlighting its **potential as a sustainable technology in agriculture**.

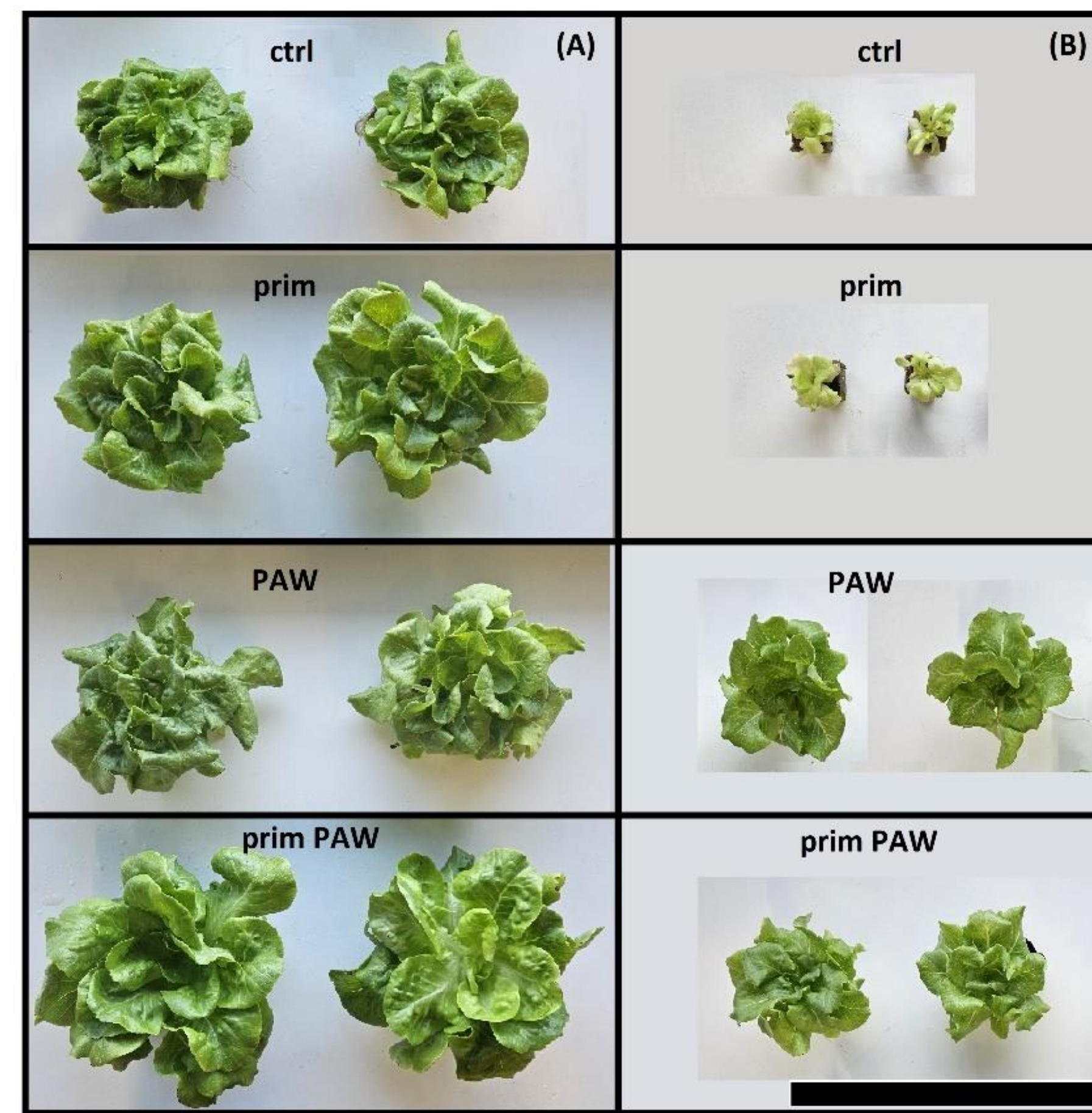
Acknowledgement

The research was supported by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project 09I03-03-V03-00033 EnvAdvice, and the Slovak Research and Development Agency APVV-22-0247.

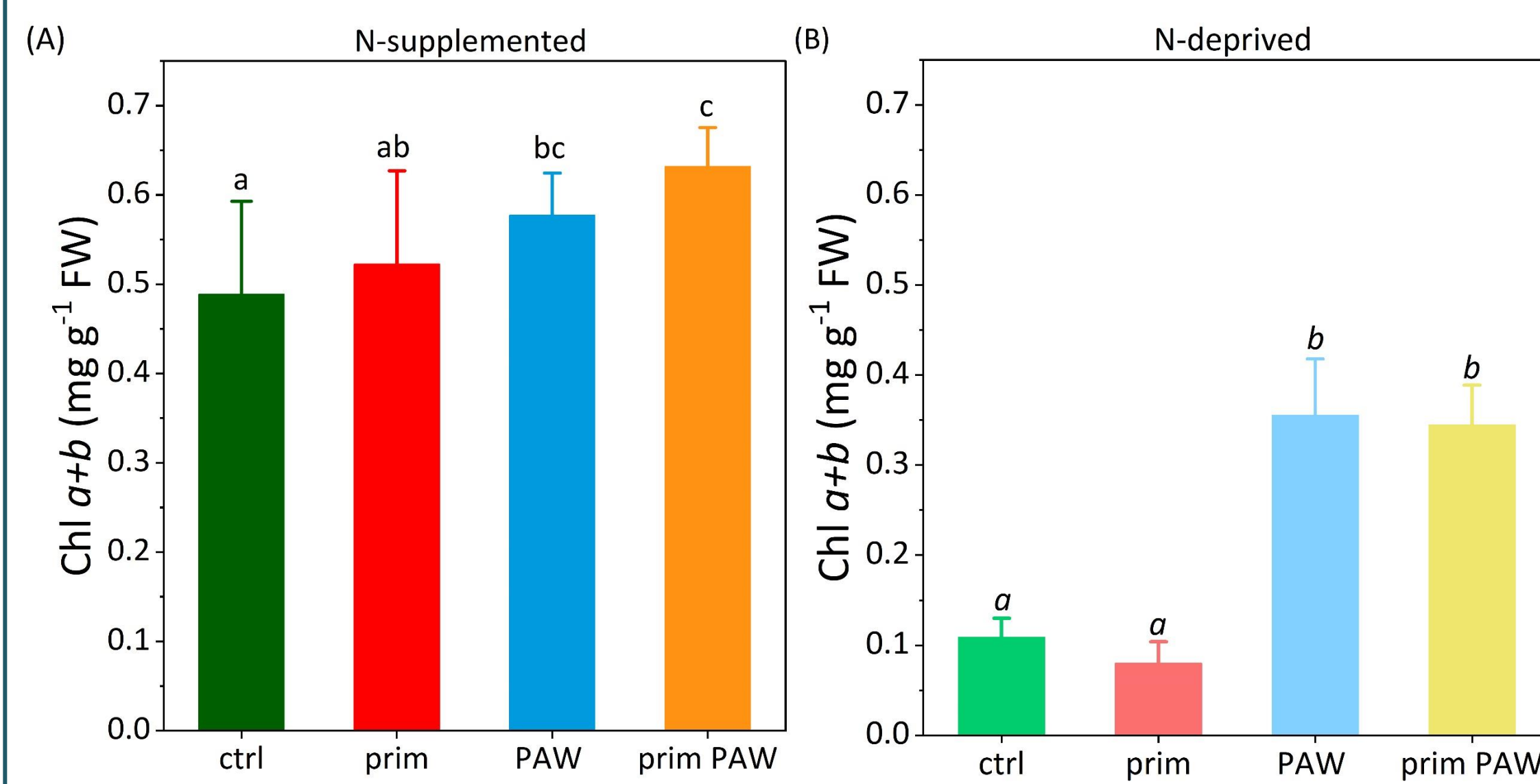
References

- [1] A. Sharma, R. Chetani IJESRT 5, (2017), 2, 677–680.
- [2] R. Thirumdas, A. Kothakota, U. Annapure, K. Siliveru, R. Blundell, R. Gatt, V.P. Valdramidis, Trends. Food. Sci. Technol. 77, (2018), 21–31.
- [3] G.B.N. Yemeli, M. Janda, Z. Machala. Plasma Chem. Plasma Process. 42, (2022) 69, 1143–1168.

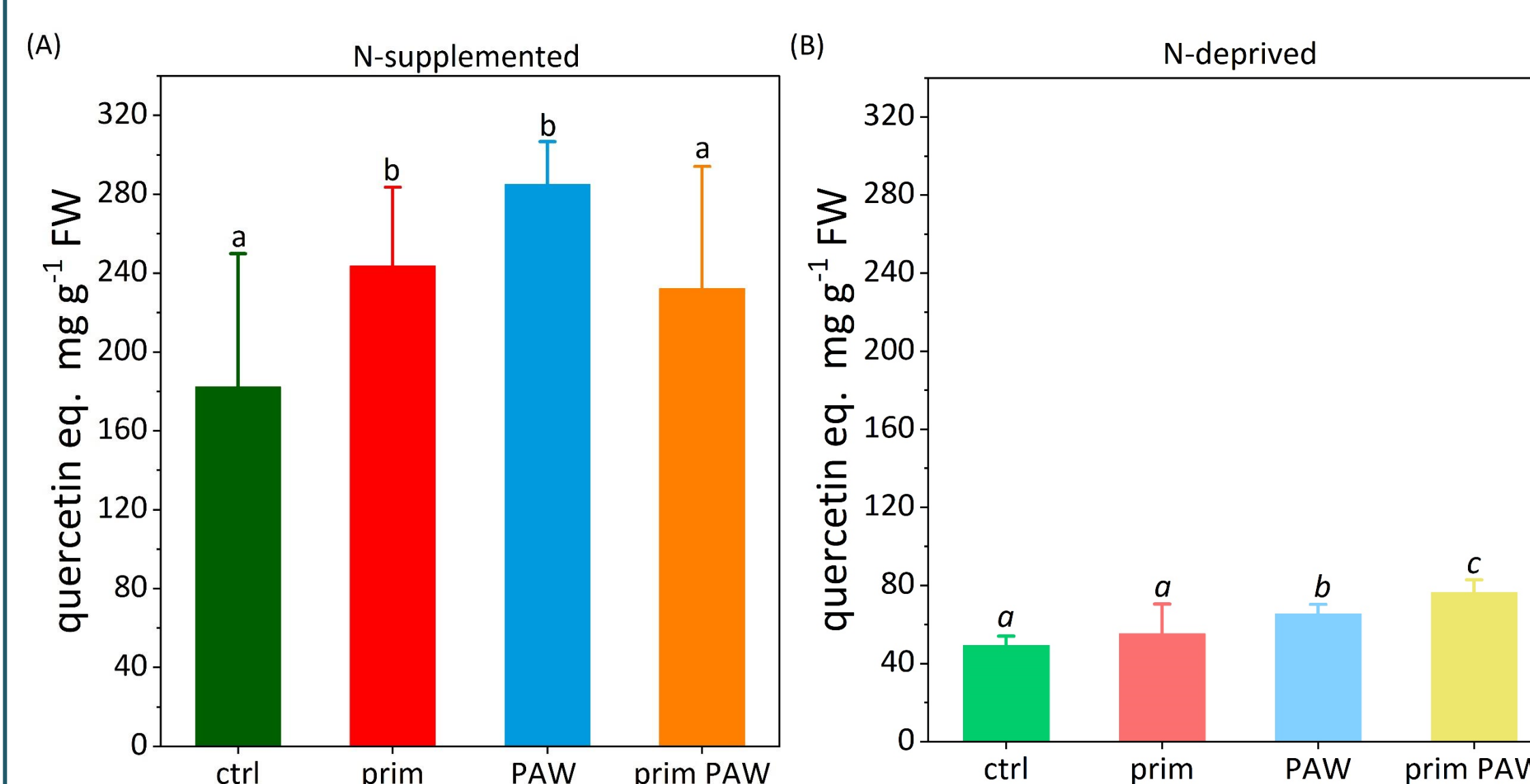
Results



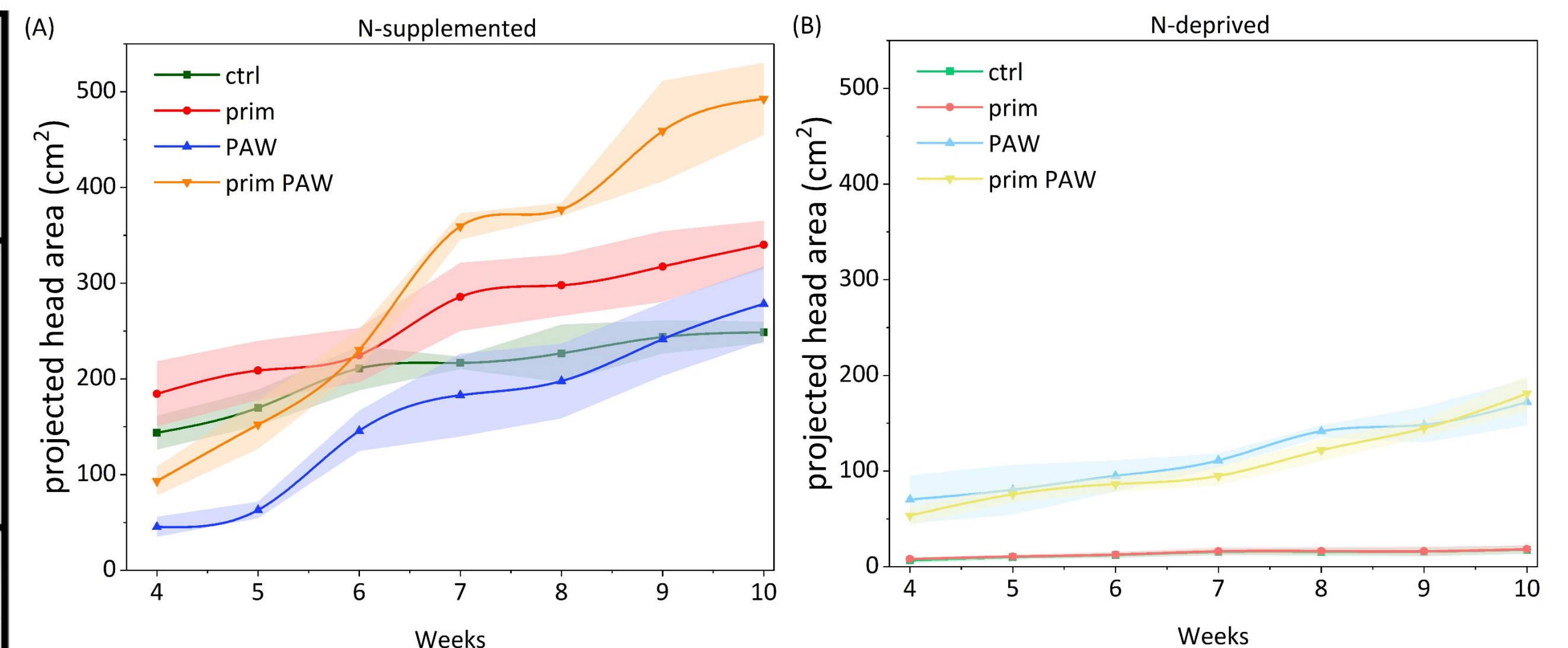
Overall appearance of the lettuce plants after 10 weeks of cultivation in the half-strength Hoagland solution in N-supplemented (A) and N-deprived (B) conditions. Scale bar = 30 cm.



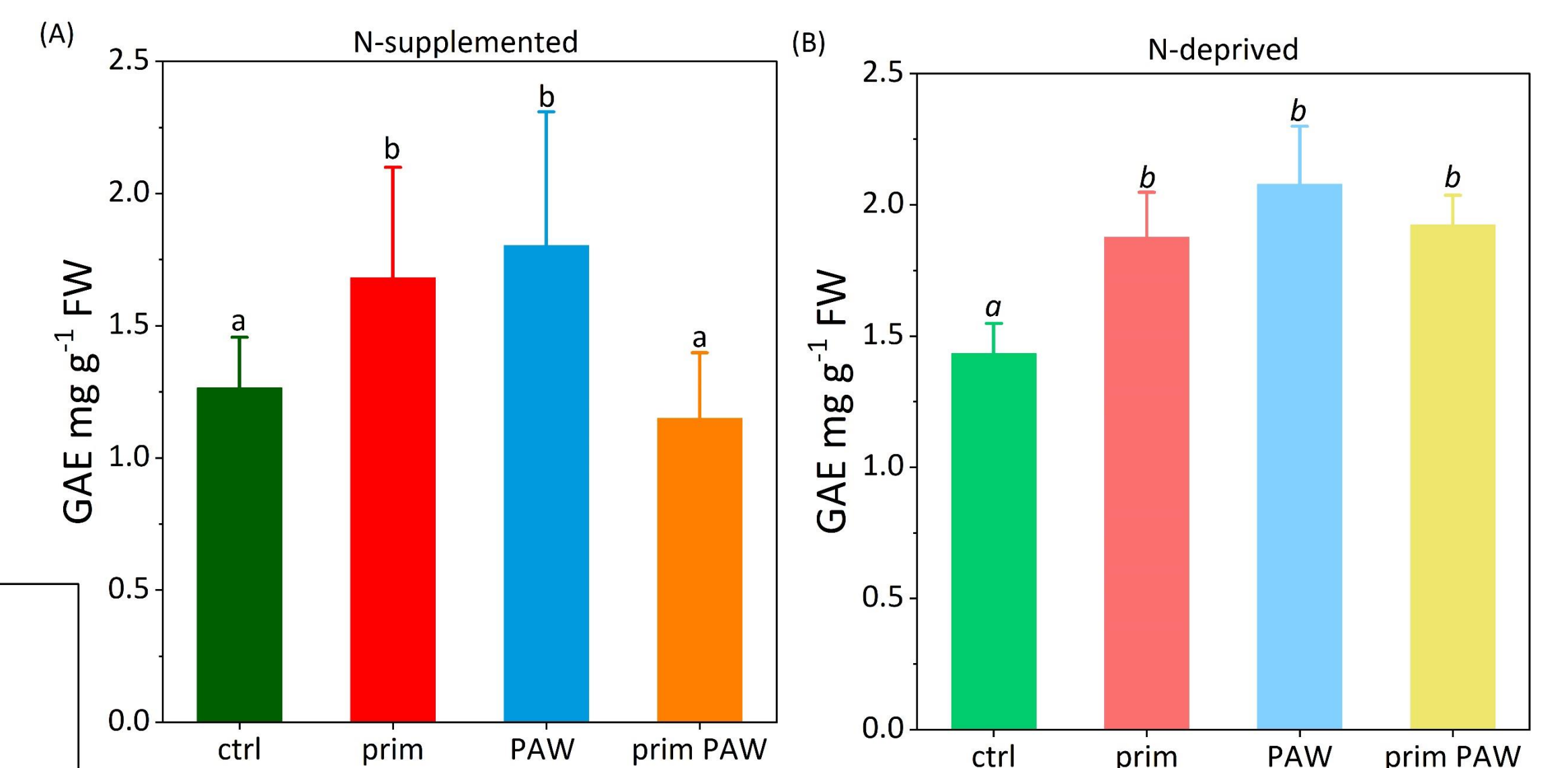
Total chlorophyll (chl a+b) of lettuce cultivated in the half-strength Hoagland solution under N-supplemented (A) and N-deprived (B) conditions.



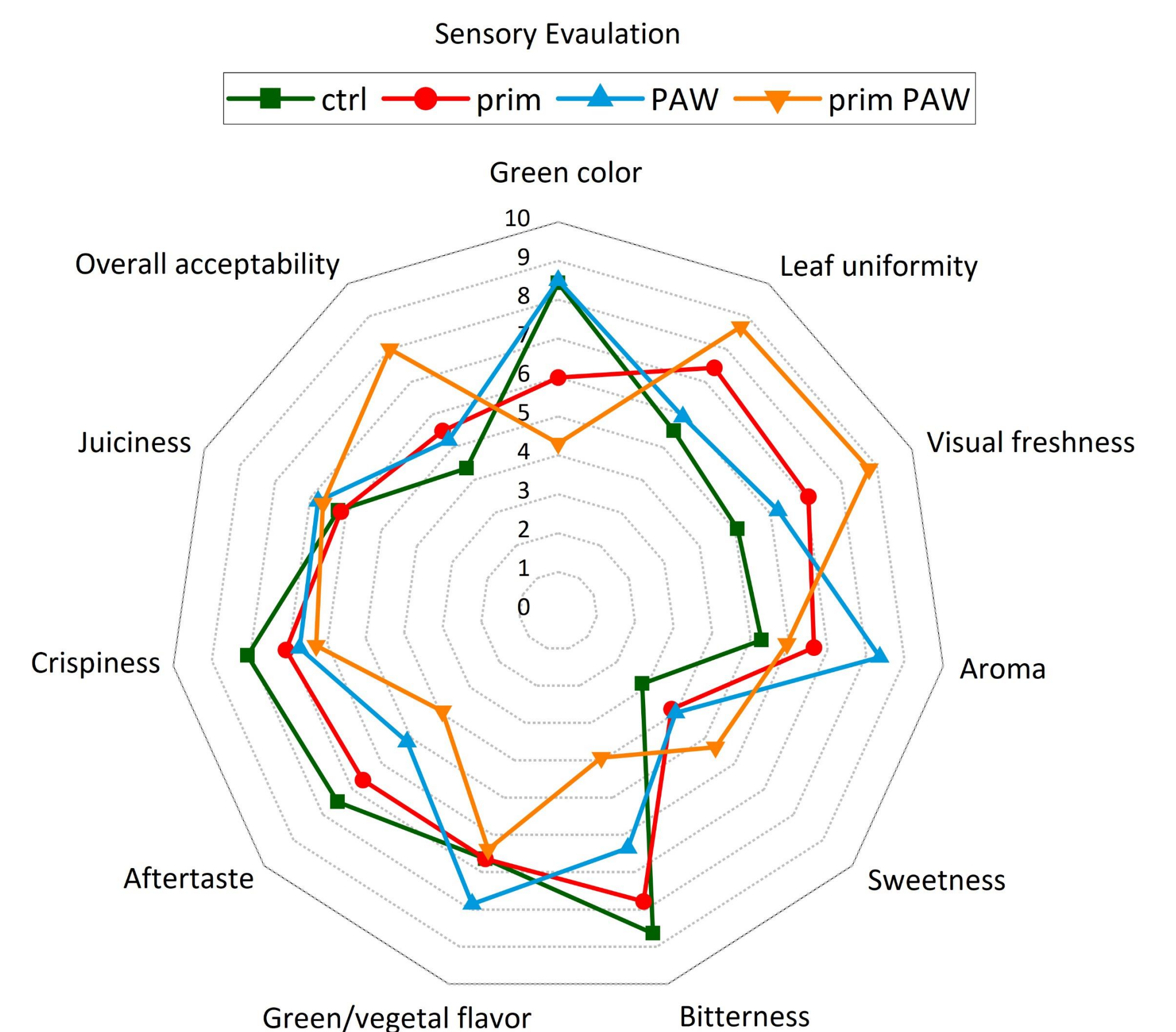
Equivalents of quercetin in lettuce cultivated in the half-strength Hoagland solution under N-supplemented (A) and N-deprived (B) conditions.



Projected lettuce head area during the 10 weeks of cultivation in the half-strength Hoagland solution in N-supplemented (A) and N-deprived (B) conditions.



Equivalents of soluble phenolics of lettuce cultivated in the half-strength Hoagland solution under N-supplemented (A) and N-deprived (B) conditions.



Sensory evaluation of lettuce after 10 weeks of cultivation in the half-strength Hoagland solution under N-supplemented conditions.

Material & Methods

Plant material

Lettuce plants (*Lactuca sativa* L.), cultivar Matilda, were cultivated for 10 weeks in hydroponic system. The plants were grown under controlled conditions of cultivation chamber (25/23°C, 16 h photoperiod, 250 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ PAR).

Experiment A treatments: Standard nutrient conditions

- Control (**ctrl**) - $\frac{1}{2}$ Hoagland solution
- Seed priming (**prim**) - seeds soaked in PAW for 1.5 h and grown in $\frac{1}{2}$ Hoagland solution
- Plasma-activated water (**PAW**) - PAW supplemented with $\frac{1}{2}$ Hoagland solution
- Primed seeds and Plasma-activated water (**prim PAW**) - seeds soaked in PAW for 1.5 h and grown in PAW supplemented with $\frac{1}{2}$ Hoagland solution

Experiment B treatments: Nitrogen-deprived conditions

- Control (**ctrl**) - $\frac{1}{2}$ Nitrogen-deprived Hoagland solution
- Seed priming (**prim**) - seeds soaked in PAW for 1.5 h and grown in $\frac{1}{2}$ Nitrogen-deprived Hoagland solution
- Plasma-activated water (**PAW**) - PAW supplemented with $\frac{1}{2}$ Nitrogen-deprived Hoagland solution
- Primed seeds and Plasma-activated water (**prim PAW**) - seeds soaked in PAW for 1.5 h and grown in PAW supplemented with $\frac{1}{2}$ Nitrogen-deprived Hoagland solution

The pH of the solutions was adjusted to 6.2. Solutions were changed every 7 days.

Determination of the lettuce head

At the two-rosette stage (week 4), plants were photographed weekly and analyzed in ImageJ.

Concentration of chlorophylls

Chlorophylls and carotenoids concentration was determined spectrophotometrically according to Lichtenthaler.

Total soluble phenolics content

Total content of soluble phenolics was determined spectrophotometrically according to Ainsworth and Gillespie.

Sensory testing

After 10 weeks, 15 participants (age 23–71) evaluated the taste, visual appearance, and aroma. The study was approved by the institutional ethics committee, and an informed consent was obtained from all participants.

Statistical analysis

Statistical analysis was performed using Statgraphics Centurion and Microsoft Excel. Differences among individual treatments were evaluated based on statistical analyses (multifactor ANOVA, LSD test) at a significance level of $P \leq 0.05$.

Preparation of PAW

