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Comparison of imaging methods for RGR determination and early stress detection in *Lemna minuta* grown on buffalo wastewater

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Cultivating duckweed (Lemnaceae) on livestock wastewater represents a leading strategy for the circular bioeconomy, particularly relevant in Campania (Italy), where about 80% of the national buffalo herd is concentrated and where sustainable wastewater management is a priority challenge. Several image analysis techniques are now available to calculate the relative growth rate (RGR) of these macrophytes, ranging from classic RGB imaging to more sophisticated chlorophyll fluorimetry-based approaches, yet a systematic comparison of their reliability is still lacking, particularly under physiological stress. In this work, *Lemna minuta* (clone LER035) was grown on ozonated buffalo wastewater at different total ammoniacal nitrogen (TAN) concentrations, and RGR was calculated using three imaging methods – RGB camera, light-adapted PAM fluorimetry, and dark-adapted PAM fluorimetry – to compare their agreement. Among the treatments showing a positive RGR, the treatment based on centrifuged digestate at low nitrogen concentration (50 mg L⁻¹ TAN, DC_low) was the only one to outperform the control (0.343 vs. 0.307 day⁻¹). Overall, light-adapted PAM and RGB showed a high, robust correlation ($\rho = 0.988$), confirmed when separately analyzing the positive-RGR ($\rho = 0.980$) and negative-RGR ($\rho = 0.916$) subgroups. Under stress, however, the two methods were not numerically interchangeable, with divergence ranging from negligible in healthy treatments to marked (+0.141 on average) in stressed ones. The RGB method therefore remains valid for RGR determination; however, PAM technology detects stress well ahead of RGB, identifying growth decline approximately 4 days earlier in 81% of stressed replicates and capturing impaired photosynthetic efficiency before visible tissue disintegration.

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References

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