Algal Research 90 (2025) 104206

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Phenotypic plasticity can be modulated by epigenetic means via DNA methylation in the microalgae *Desmodesmus armatus*

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ARTICLE INFO

Keywords:
Epigenetics
DNA methylation
Phenotypic plasticity
Photosynthesis

ABSTRACT

Understanding how organisms adapt to environmental changes is a critical question in biology, with significant implications for biodiversity conservation, ecosystem stability, and biotechnology. Phenotypic plasticity, the ability of an organism to alter its traits in response to environmental conditions, is a cornerstone of adaptive potential and ecological resilience. In this study we investigate if phenotypic plasticity is modulated through epigenetic mechanisms in *Desmodesmus armatus,* using low-dose UV-C radiation as a driver of epigenetic change. We examined how UV-C exposure influences various phenotypic traits, including growth rate and photosynthetic performance, across different environmental conditions. Our results indicate that UV-C treatment significantly changes phenotypic plasticity, particularly affecting traits associated with minimum saturating light, maximum non-photochemical quenching, growth rate, and maximum culture density. Nutrients play a crucial role in shaping the physiological responses of microalgae, influencing their growth, metabolism, and adaptive capabilities under varying environmental conditions. Our data shows that global DNA methylation levels are significantly altered using UV-C in a nutrient-dependent manner. The findings suggest that low doses of UV-C can effectively modulate the epigenome, leading to considerable reorganization of phenotypic traits. This research enhances our understanding of how epigenetic factors contribute to the adaptive capacity of the microalgae *D. armatus* and underscores the potential of epigenetic interventions in managing phenotypic outcomes in response to environmental stresses.

1. Introduction

In biology, the phenotype (P) of an organism encompasses the observable physical properties resulting from the interaction of its genetic makeup (genotype, G) with the environment (E_N); $P = G^*E_N$. This includes but is not limited to, characteristics such as morphology, physiology, biochemical properties, and behaviour. For photosynthetic organisms, including plants, algae, and certain bacteria, the phenotype additionally involves a range of traits related to their photosynthetic capabilities, such as leaf structure, pigment composition, and photosynthetic efficiency. Understanding how these phenotypic traits change in response to the environment is crucial for exploring how these organisms adapt. One critical aspect of this biological flexibility, known as phenotypic plasticity, is the extent to which a trait can change and the range of environmental changes that it can respond to. Phenotypic plasticity is trait-specific, with some traits having a wide range of possible responses while others having a limited range. Studies have shown that plasticity decreases in magnitude in environments with less predictable fluctuations [1,2]. This adaptation occurs across multiple biological levels, from DNA methylation to gene expression and cell morphology [2]. Ultimately, the magnitude of the phenotypic plasticity of an organism can either promote or deter its evolution depending on the circumstance [3]. For population growth it is generally considered that an increased level of plasticity is beneficial because it would allow that organism to survive or thrive in many environments [4]. In microalgae it has been demonstrated that fluctuating environments promote evolution of populations with high plasticity [5]. Conversely, plasticity can also be an adaptive response to fluctuating environments [6]. It can accelerate adaptation to new abiotic conditions [7] and stabilize evolution by reducing genetic changes and selective sweeps [6]. However, the concept of infinite possible phenomes, the myriad ways organisms can express their genetic material in response to environmental inputs, complicates this framework [8]. This added level of complexity underscores the necessity of standardising environmental variables in experimental designs. Additionally, integrating epigenetic factors is crucial for making definitive statements about phenotypic

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https://doi.org/10.1016/j.algal.2025.104206
Received 19 October 2024; Received in revised form 8 July 2025; Accepted 10 July 2025
Available online 11 July 2025

outcomes. Epigenetics (E_P) adds another layer of regulation by influencing how genes are expressed without altering the DNA sequence, thus, it plays a critical role in how organisms adapt to environmental changes and ultimately shapes their phenotypic plasticity; $P = G^*E_N^*E_P$.

It is essential to distinguish between phenotypic plasticity and evolutionary adaptation. Phenotypic plasticity refers to the ability of an organism to change its phenotype in response to environmental changes without genetic alterations, serving as a short-term response mechanism. In contrast, evolutionary adaptation involves genetic changes that occur over multiple generations, often influenced by the environment, which can lead to permanent changes in the species' phenotype [9]. While these concepts are related—phenotypic plasticity can influence evolutionary outcomes by affecting survival and reproduction—they operate on different scales of time and mechanism.

Two non-exclusive competing theories have been discussed and investigated to explain the mechanisms that drive formations of de novo phenotypes and, by extension, levels of phenotypic plasticity. The first theory, known as the genetic change driven phenotype, proposes that random mutations can create new genetic variants that might lead to new phenotypes or modify existing phenotypic plasticity [10]. However, the emergence of phenotypic plasticity itself is not solely dependent on these mutations; instead, mutations provide the genetic variability upon which natural selection can act [11]. This process must be accompanied by positive selection for these new or modified traits to become stable within the population. Change in phenotypic plasticity via this process is slow but results in stable changes. The second theory is the epigenetic change driven phenotype – this theory postulates that biochemical changes drive the formation of new phenotypes that arise from different combinations of existing traits [12,13]. In the initial stages, changes in phenotypic plasticity are driven by regulatory processes with no genome alterations. If the new phenotype is positively selected in the population, the genome will be changed to accommodate the phenotype. Plasticity modulation via this mechanism is fast, stabilising within a few generations, and is driven by epigenetic changes [14]. Ample evidence supports both processes as drivers of evolution and phenotype plasticity [11]. However, very little experimental evidence exists to support these theories in microalgae [5,15]. Previous research has shown that in the green algae *Desmodesmus* phenotypic plasticity can be modulated by chemical signals, fluctuating environments [16,17], and grazing pressure [18] making it a good candidate to further explore the molecular mechanisms driving changes of plasticity levels.

Epigenetics in an organism encompasses a range of modifications involving chemical markers on DNA and associated proteins, which influence the structural organization of genomic DNA within the nucleus and modify how DNA is packaged [19–21]. Such alterations include DNA methylation, modifications to histones after translation, restructuring of chromatin, and RNA-driven gene silencing [19]. These epigenetic mechanisms drive biochemical changes at a cellular level, which permit organisms to rapidly adjust to drastic shifts in environmental factors such as light levels, diurnal cycles, temperature variations, and changes in mineral and nutritional availability [22–24]. To modulate the epigenetic state of the organism, we chose to use low doses of ultraviolet light (UV-C), which is known to impact the methylation levels of the genome [25–27].

UV-C radiation has been shown to elicit an epigenetic response in *Vitis amurensis*, where DNA methylation acts as the main regulator of gene expression for stilbene synthase, the enzyme that directly catalyses the production of t-resveratrol [27]. Recent research has also demonstrated that using UV radiation in a photoperiod-dependent manner, specifically low UV exposure for 4 h with a lamp emitting a combination of 10 % UV-B (280–315 nm), 30 % UV-A (315–400 nm), and 60 % visible wavelengths (280 to 700 nm), significantly enhances cell viability, growth index, and biochemical differentiation in *Cannabis indica* cell suspension cultures. This study also highlighted the critical role of DNA hypermethylation as a protective mechanism against UV genotoxicity [25]. The epigenetic effects of UV-C radiation on *Chlamydomonas reinhardtii* was also investigated and shown to impact the secondary metabolite production and miRNA expression [26]. These studies underscore the significance of epigenetic responses in modulating cellular characteristics and gene regulation under conditions of UV-C induced stress. Building on this knowledge, we hypothesize that UV-C is able to modulate the epigenome to the extent that it will impact phenotypic plasticity. In this work, we set out to explore the extent of the impact of epigenetic alterations on phenotypic plasticity on a limited number of traits using UV-C as a driver of epigenetic change.

2. Material and methods

2.1. Culturing conditions

For all experiments, a 250–1000 mL culture of *Desmodesmus armatus* from the Climate Change Cluster culture collection (UTS-LD) was maintained at 20 °C under 200 μmol photons m^{-2} s^{-1} continuous light, measured using a light meter (LI-250A Light Sensor, LI-COR, USA) equipped with a US-SQS/L light sensor (Heinz Walz GmbH, Effeltrich Germany). The cultures were non-axenic, and were grown in a commercial MLA growth medium (Algaboost MLA, Algaboost, Australia) and aerated by pumping atmospheric air at 6 L min^{-1} using an aquarium pump (Aqua One precision 7500).

2.2. UV-C treatment

Cell culture was adjusted to an optical density (OD) of 0.32 at 750 nm, measured in a 96-well plate (Falcon 96-well, flat-bottom, Corning, NY, USA) using a microplate reader (Spark Cyto, Tecan, Männedorf, Switzerland). Multiple 10 mL aliquots were transferred into clear glass Petri dishes (90–95 mm in diameter, 6–10 mm in height). These were placed into a UV-C cross-linker (Bio-Link BLX 254, Villber, Collégien, France) sequentially and exposed to UV-C irradiation at 254 nm at 0.04 Joules (J) cm^{-2} for all experiments except the UV-C dose response where additional dosages were used: 0.08 and 0.14 J cm^{-2}.

2.3. Phenotypic plasticity assay preparation

A UV-C treated or untreated (control) algae samples (0.2 mL) was mixed with 1.8 mL fresh MLA growth media to get 50 %, 100 % or 150 % final concentration relative to the manufacturer recommendation. These samples were loaded in individual wells in 24-well plates (Falcon 24-well Clear Flat Bottom, Product Number: 353047, Corning, NY, USA), each plate with 4 biological replicates for each condition (4 samples of 50 % MLA + UV-C; 4 samples of 100 % MLA + UV-C; 4 samples of 150 % MLA + UV-C; 4 samples of 50 % MLA Control; 4 samples of 100 % MLA Control; 4 samples of 150 % MLA Control). Samples were randomly distributed in each well-plate using a unique distribution pattern for every plate in every experiment to compensate for any plate effect. Randomization was done using a random number generator in Microsoft Excel.

2.4. Phenotyping experimental setup

For each experiment, 12 plates were prepared as described in "Phenotypic plasticity assay preparation" and were placed in 6 different growth conditions: 15 °C at 50 μmol photons m^{-2} s^{-1} (μE), 15 °C at 200 μE, 20 °C at 50 μE, 20 °C at 200 μE, 28 °C at 50 μE, and 28 °C at 200 μE (Supplementary Fig. 4). The experiment was carried out in 3 incubators (Innova S44i - Stackable Incubator Shaker, Eppendorf, Germany), with an orbital agitator set at 80 rotations per minute (rpm), in continuous 24 h light. To distribute light more evenly in the plate, a reflective sheet was used to cover the bottom and the 4 sides of the plate. For reducing light to 50 μE in the same incubator, half of the well-plates were covered in neutral density filters (LEE 210 0.6ND, LEE Filters, Andover, Hampshire,

United Kingdom). Plates were maintained in culture for up to 7 days.

2.5. Spectroscopic measurements

Optical density was measured in the 24-well plate directly using a plate reader (Spark Cyto, Tecan, Männedorf, Switzerland). Values were recorded at 680 and 750 nm, which were used to calculate the growth rate (μOD750, μOD680), maximum culture density (OD750max, OD680max) and the chlorophyll per biomass (OD680/750).

2.6. Fluorometry measurements

On day 2 of the experiment, rapid light curve (RLC) measurements were performed on all plates. The measurement was done on 10-min dark-adapted samples, using a 12-step light intensity RLC with each light intensity maintained for 30 s. The light intensities were: 4, 26, 54.8, 82.9, 155.95, 230, 379, 523, 666, 873.4, 1076, 1334 μE. An inverted Closed FluorCAM (FluorCAM, PSI, Brno, Czech Republic) was used for the RLC with red actinic light for the illumination steps. Maximum relative electron transfer rate (rETRmax), rate of photosynthesis in light limiting conditions (α), and minimum saturating light (Ek) were calculated from effective quantum yield of Photosystem II values using the equation developed by Webb et al. [28] and adapted by Silsbe and Kromkampfor [29]. Maximum non-photochemical quenching (NPQmax) was selected as the maximum observed NPQ value during the RLC.

2.7. DNA methylation analysis

For the DNA methylation assay a collapsed experimental design was used, selecting a subset of environmental conditions based on observed phenotypic differences to streamline the analysis of key responses. This approach allowed us to efficiently test conditions that represent each fixed factor (UV-C, temperature, light, and nutrients) while maintaining biological relevance and experimental feasibility. Microalgae were exposed to 0.04 J cm^{-2} UV-C treatment, as described in "UV-C treatment" section, and subsequently grown in 20 mL cell culture flasks in various conditions for 7 days. Cultures were transferred after one week of inoculation to 50 mL graduated tubes (Falcon, Corning, NY, United States) which were then centrifuged for 5 min at 1000 *g* using a tabletop centrifuge (Multifuge X4R Pro, Thermo Fisher Scientific, Waltham, MA, United States). The supernatant was removed and pelleted microalgae were stored in −80 °C until DNA extraction. DNA was extracted using a commercial extraction kit (DNeasy PowerSoil Pro Kit, Qiagen, Hilden, Germany), and quantified using a spectrophotometer (NanoDrop, Thermo Fisher Scientific, Waltham, MA, United States). Percentage of methylated DNA was quantified using a global DNA methylation assay kit, which quantifies 5 Methyl Cytosine (5-mC) using a colorimetric technique (ab233486, Abcam, Cambridge, United Kingdom). Quantification was done according to instructions from the manufacturer without any modifications.

2.8. Data analysis

Environmentally standardised plasticity index (ESPI) quantifies phenotypic plasticity by measuring the degree to which traits change in response to environmental conditions. It was calculated using a quantification method from previous work [30]. This index integrates the magnitude of phenotypic changes across environmental gradients and provides a standardised measure that allows comparisons across traits and species (Supplementary Fig. 4). Two adaptations were added to the original calculation: 1) selection of the maximum and minimum mean phenotypic values was done using the highest Q-value of an ANOVA Tukey's test (OriginPro, OriginLab Corporation, Northampton, MA, USA), where all experimental conditions were tested; 2) the environment distance between the two selected conditions was calculated as the Euclidian distance of two 3 dimensional points; the 3 dimensions were light, temperature and nutrients (%MLA); each dimension coordinates were normalised before use in the formula, therefore 50 μE had a coordinate of 0, 200 μE had a coordinate of 1, 15 °C had a coordinate of 0, 20 °C had a coordinate of 0.71429, 28 °C had a coordinate of 1, 50 % MLA had a coordinate of 0, 100 % MLA had a coordinate of 0.666, and 150 % MLA had a coordinate of 1. Raw data as well as calculated ESPI values are available in supplementary file "Raw data.xlsx".

3. Results

To test our hypothesis of UV-C induced changes in phenotypic plasticity we compared various traits of Control versus UV-C treated *D. armatus* cells. After UV-C treatment these cells were grown in multiple 24-well plates with samples randomization in each plate to minimize any random effect from the plate. In each experiment we used 8 biological replicates, and we performed the entire experiment 4 separate times to assure reproducibility of the results.

The impact of UV-C on the environmentally standardised plasticity index (ESPI) was trait-specific in our experimental setup, with 5 out of 10 traits statistically significant altered (Fig. 1, Supplementary Table 1). We observed no significant impact on the growth rate measured using optical density at 680 nm, which corresponds to the absorbance peak of chlorophyll *a*, and primarily reflects the chlorophyll concentration, providing insights into photosynthetic capacity. However, it induced a statistically significant increase of growth rate (+33 % relative to Control) plasticity measured at 750 nm (Fig. 1, Supplementary Table 1), a

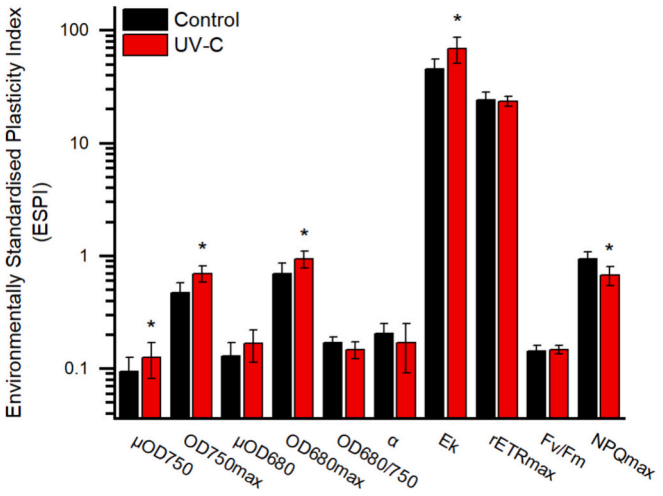

Fig. 1. UV-C induced changes of phenotypic plasticity. Environmentally Standardised Phenotypic Plasticity (ESPI) was calculated from a total of 4 experiments, each experiment with 18 tested conditions, each condition with 8 biological replicates. The growth rate was measured via the optical density of the sample at 750 nm (μOD750), the highest culture density measured at 750 nm (OD750max) and 680 nm (OD680max), minimum saturating light intensity (Ek) and maximum Non-Photochemical Quenching (NPQmax) have significantly altered plasticity, indicated with stars above the UV-C treated samples. Plasticity values were not altered for growth rate measured via optical density of the sample at 680 nm (μOD680), ratio of chlorophyll per biomass (OD680/750), rate of photosynthesis in light limiting conditions (α), relative maximum electron transfer rate (rETRmax), and maximum quantum yield of Photosystem 2 (Fv/fm). Statistical significance was determined by a pairwise *t*-test between UV-C treated (UV-C; red bars) and untreated (Control; black bars) and with a *p*-value threshold of <0.05 for a significant difference. Vertical lines with whiskers indicate the standard deviation of ESPI values, averaged across 4 independent experiments ($n = 4$). Each ESPI value was derived by identifying the two conditions with the most different phenotypic values for each trait. The phenotypic and environmental distances between these conditions were calculated using data from 8 biological replicates per condition, see Supplementary Fig. 4 for a visual representation.

wavelength largely outside the absorbance spectrum of photosynthetic pigments, thus indicating changes in cell mass or turbidity independent of chlorophyll content. Maximum culture density measured using both wavelengths (OD750max, OD680max) and minimum saturating light (Ek) were traits that had their plasticity increased after UV-C treatment with 47 %, 35 % and 51 % respectively, relative to Control (Fig. 1, Supplementary Table 1). The only trait that showed a reduction of plasticity post UV-C was NPQmax (−29 % relative to Control), whereas all other traits showed no changes (Fig. 1). To test whether there is a dose dependent response of ESPI to UV-C, we treated cells with 0.04, 0.08 and 0.14 J cm^{-2} UV-C radiation and quantified the induced plasticity in the cell populations (Supplementary Fig. 1). Results indicated that ESPI is modulated by UV-C in a dose-dependent manner, with most of the measured traits showing an increase of plasticity with increasing UV-C dosage (Supplementary Fig. 1). The only exceptions to this relationship were chlorophyll *a* per cell (OD680/750), and NPQmax which appeared to remain relatively stable regardless of UV-C dosage (Supplementary Fig. 1).

Next, we investigated the UV-C effects on trait distribution in the phenome, which delineates the trait distribution within a non-metric multidimensional scaling (nMDS) space (Fig. 2). This analysis visually represents the overall spread and central tendency of phenotypic traits under different UV-C exposure levels. We observed a slight shift in trait distribution attributable to the lowest UV-C dose treatment, suggesting a modulation of phenotypic traits beyond the observable changes in growth and NPQ previously noted (Fig. 2A). When we looked at the trait distributions to increasing UV-C dosages we could see that increasing dosages result in a greater departure from the distribution of the Control, non-UV-C treated samples (Fig. 2B). These findings highlight the complex interplay between UV-C dosage and the phenome, illustrating how UV-C induced stress can significantly influence the phenotypic landscape of *D. armatus*. The impact of UV-C on each individually measured trait was negative on almost all traits, resulting in overall lower values relative to control (Supplementary Fig. 2).

UV-C, when compared to the other three environmental factors tested in our experiment, had a more significant effect globally on individual traits relative to nutrients but lower relative to temperature and light (Fig. 3A). This is evidenced by the F-values of the Generalized Linear Mixed Model analysis of the entire dataset (Fig. 3A). Surprisingly, Ek was not significantly impacted by UV-C in this analysis, which contrasts with ESPI result where Ek was significantly altered (Fig. 3B, Fig. 1). This discrepancy may be explained by the fact that the global analysis of the phenome captures changes across the entire dataset, including incremental shifts in Ek, while ESPI focuses solely on the two most extreme phenotypes, potentially missing these broader patterns (Supplementary Fig. 2G). The second-degree interaction between the 4 factors was most significant overall between temperature and light, with the greatest effect seen on NPQmax (F-value: 311.57), and temperature and UV-C, with the most significant effect seen on α (F-value: 270.85). The third-degree interaction between the environmental factors was the most impactful on temperature with light and with UV-C, with the greatest impact on α (F-value: 22.35) (Fig. 3A), and overall non-significant for temperature with nutrients and UV-C, as well as for light with nutrients and UV-C (Fig. 3B). The fourth-degree interaction was significant only on NPQmax with a marginal effect on OD680/750 (Figure 3AB). Experimental replication was treated as a random effect and was not significant, with Z values not exceeding a deviation greater than 1.96 or lower than −1.96 relative to 0 (Supplementary Table 2).

We further investigated if UV-C significantly impacted global DNA methylation levels by quantifying the % of 5-methylcytosine (5-mC) from the total DNA. Methylation levels were significantly altered in UV-C treated samples relative to Control in 2 out of the six tested conditions. UV-C induced a significant decrease in the %5-mC levels in conditions where nutrients were in excess (150 % MLA), and caused an increase in conditions with low nutrients (50 % MLA) (Fig. 4). We further analysed which of the factors (UV-C, temperature, light intensity, nutrient levels) in our experimental setup had the largest impact on DNA methylation (Table 1). Nutrient levels, and UV-C with nutrient levels significantly changed the methylation levels (p-values <0.05), with a greater effect measured from the nutrient and UV-C interaction (F-value 22.169), relative to nutrients (F-value 16.408). The interacting effect of UV-C with the other variables did not yield significant impact on DNA methylation levels (Table 1). To link the observed changes in DNA methylation levels to ESPI we calculated 2 new ESPI values, one with all 6 conditions where methylation was quantified (+Met), and one with only the 4 conditions that did not have significant changes in methylation (−Met, Supplementary Table 3). The plasticity indexes which included samples with altered DNA methylation (+Met) in UV-C relative to Control samples showed reduced relative plasticity in all traits except μOD750 (Supplementary Table 2, red % values). When the differentially methylated samples are included in the plasticity index calculations, NPQmax and OD750max show significant differences between the Control and UV-C conditions, with p-values of 0.0405 and 0.0058, respectively (Supplementary Table 3). Three other traits (OD680max, α and Ek) lost significance due to inclusion of the two differentially methylated conditions (Supplementary Table 3).

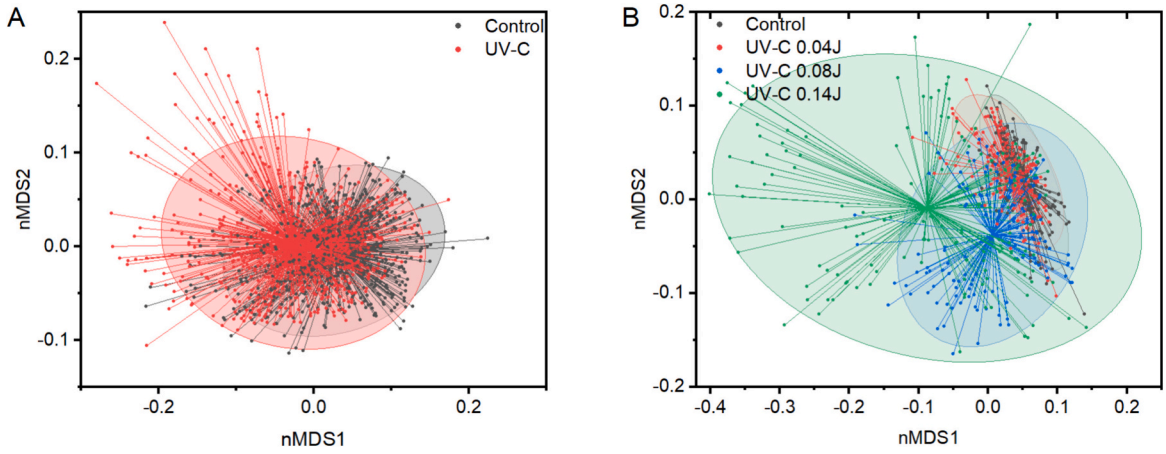

Fig. 2. Trait distribution in nMDS space as a function of UV-C dosage. Panel A illustrates all traits' overall spread and central tendency under control conditions (black dots) versus exposure to UV-C (red dots). The red ellipse delineates the 90 % confidence interval around the centroid for the UV-C exposed group. Panel B shows the distribution of traits for different UV-C dosages: 0.04 J (red dots), 0.08 J (blue dots), and 0.14 J (green dots). Each colour-coded ellipse represents the 90 % confidence interval around the centroid for the respective dosage group, demonstrating the variation in trait response at increasing UV-C levels. All 10 recorded parameters from the four experiments were analysed using nMDS.

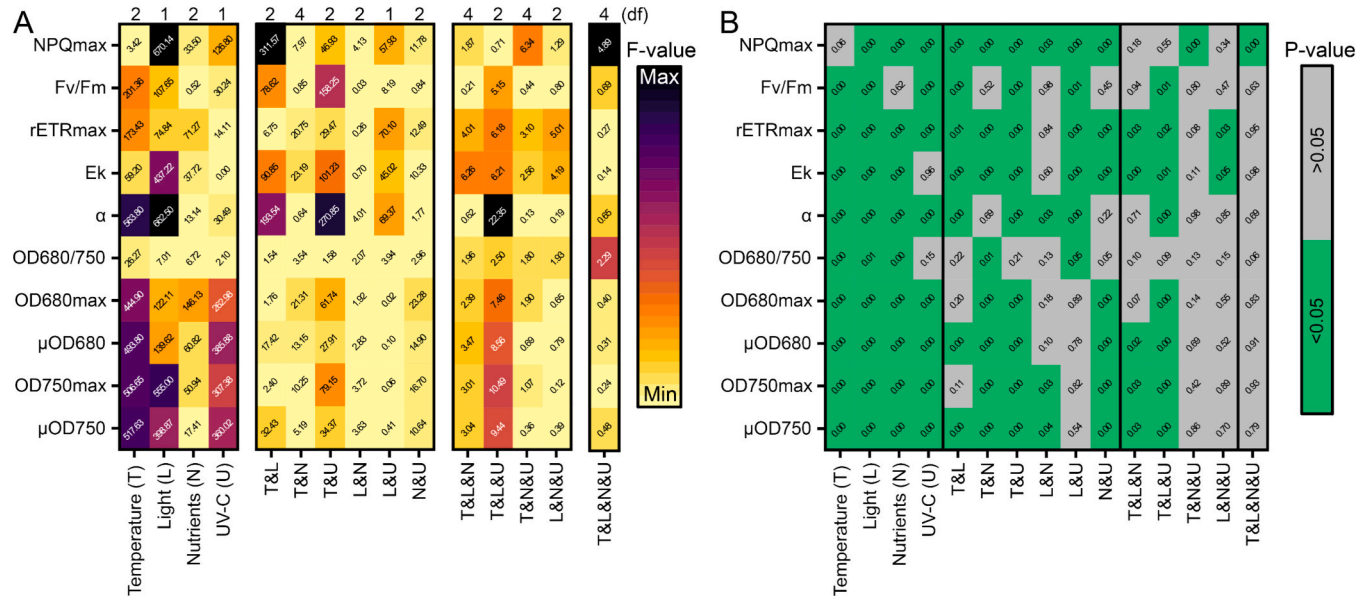

Fig. 3. Heatmaps displaying the individual and interacting impact of temperature, light, UV-C treatment, and nutrients on the measured traits. Panel A presents the F-values and panel B the *p*-values of a Generalized Linear Mixed Model analysis performed in SPSS (Version: 28.0.1.1 (14)), with Temperature (T), Light (L), Nutrients (N) and UV-C treatment (U), and their interaction as fixed factors, and all 10 traits as dependent (target) variables. Panel A is separated into 4 sections showing the derived F-values for 1) the individual 4 fixed factors (first section from left to right) showing the degree of significance of the effect of each trait; 2) second-order interaction significance; 3) third panel shows the third order interaction significance levels; 4) fourth order integration. Panel B shows the corresponding p-values for the impact of the individual factors and their interactions. Grey coloured boxes correspond to non-significant effect ($p < 0.05$) and green-coloured boxes to a significant effect ($p > 0.05$) of the fixed factor(s) (T, L, N, U) on individual traits. Degrees of freedom for each analysis is indicated on the top of Panel A. Experimental batch was treated as a random effect its variance is shown in Supplementary Table 2. Results are obtained from statistical analysis of data from 4 experimental replications, each with 8 biological replicates.

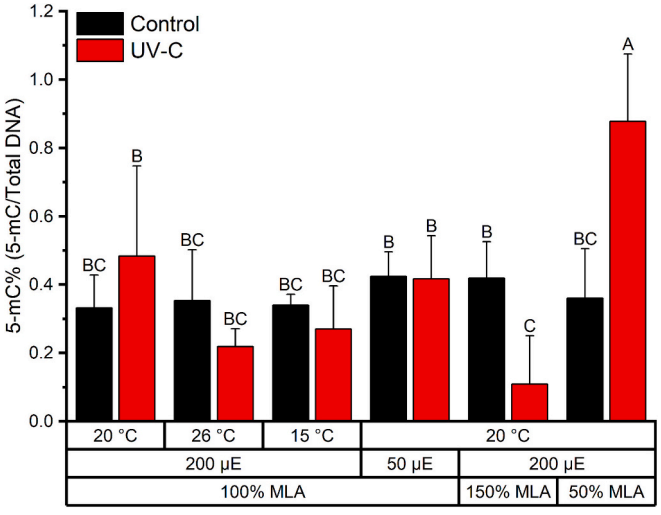

Fig. 4. DNA methylation in response to environmental change. Percentage of DNA that is methylated in response to temperature, light and nutrient availability changes. UV-C treatment had the greatest impact on methylation levels in conditions of non-optimum nutrient availability which resulted in either reduced levels in 150 % MLA, or increased levels of methylation in 50 % MLA. Data represents averages with standard deviation from 5 biological replicates, with significance levels set at <0.05, with 11 degrees of freedom. Letters indicate statistical groups determined by One-Way ANOVA with Tukey's pot-hoc analysis for mean comparison, performed in OriginPro.

Lastly, we compared how the complete phenome, i.e. the total number of conditions and traits, were changed by comparing UV-C irradiated samples to non-irradiated on a trait to trait basis (Table 2). The 18 conditions and 10 traits yielded a total of 180 comparisons which we categorised in statistically significant changed and non-significant. The 0.04 J UV-C treatment yielded 48–63 % significant changes of the phenome, the 0.08 J 61 %, and 0.14 J up to 75 % (Table 2).

4. Discussion

Phenotypic plasticity, often involving changes in trait expression that can be partially or fully reversible in response to environmental fluctuations, has been extensively studied and discussed across various scientific domains, particularly in health and environmental sciences [11,31,32]. It is a crucial feature of all living organisms, without which any small change in the environment will result in the collapse of the population or a low survival probability for the organism. Plasticity originates primarily at the DNA level, which codes for all the building blocks of the organism. DNA will store the information and provide the appropriate biochemical response to changes in the organism's environment. These responses are very complex, e.g. it has been documented that in *Tetraselmis* sp. 681 genes change their expression in response to temperature changes [33], and in *Dunaliella salina* up to 11,297 genes are differentially expressed in response to changes in light [34]. An important consideration is understanding why certain genes are differentially expressed. Typically, the synthesis of new proteins in response to environmental cues is triggered rapidly by the activation of gene transcription. This process, known as inducible gene expression, usually involves complex signal transduction pathways that orchestrate these changes [35]. On top of these processes is a complex set of additional mechanisms called epigenetic transcriptional regulators [36], which will trigger gene expression or silencing via completely independent mechanisms. Among these, DNA methylation is one of the most studied mechanisms, which has been shown to alter gene expression independent of the transcriptional signal transduction cascades [37,38].

DNA methylation and its downstream effects have been investigated in a limited number of microalgae, suggesting that epigenetic mechanisms can significantly alter physiological and adaptive responses. For instance, in *Chlamydomonas reinhardtii*, genes involved in carotenoid

Table 1
Analysis of significance and variance of methylation levels. Analysis of variance in methylation levels was performed in SPSS using a Generalized Linear Model statistical test, where methylation levels were treated as the dependent variable, and UV-C, temperature, light and nutrients as fixed factors. First four rows show results of single variable impact on DNA methylation levels, and the last 3 rows show the interacting effect of UV-C with temperature, light and nutrients. Number of replicates is 5.

Variables	Variance (F-value)	Significance (p-value)	Mean square	Degrees of freedom	Type III Sum of Squares
UV-C	0.24	0.626	0.005	1	0.005
Temperature	2.233	0.118	0.043	2	0.087
Light	0.04	0.843	0.001	1	0.001
Nutrients	16.408	0.001	0.319	2	0.638
UV * Temperature	2.904	0.064	0.056	2	0.113
UV * Light	1.623	0.209	0.032	1	0.032
UV * Nutrients	22.169	0.001	0.431	2	0.862

Table 2
UV-C induced phenome re-arrangement. This table summarizes the outcomes of pairwise statistical comparisons (Student t-test) between control and UV-C treated groups across four experiments, each involving 180 comparisons. It details the number of traits showing significant ($p < 0.05$) or non-significant ($p > 0.05$) changes due to UV-C treatment. The rightmost column indicates the percentage of the phenome significantly altered in each experiment, calculated as the proportion of traits with significant changes relative to all tested conditions.

Experiment #	UV-C dosage (J)	$p < 0.05$	$p > 0.05$	% phenome altered
1	0.04	98	82	54.44 %
2	0.04	101	79	56.11 %
3	0.04	114	66	63.33 %
4	0.04	88	92	48.89 %
4	0.08	110	70	61.11 %
4	0.14	135	45	75.00 %

metabolism are regulated by DNA methylation, ultimately modulating pigment composition and content [39]. In the halotolerant microalga *Dunaliella salina*, phenotypic plasticity under osmotic stress is linked to a complex interplay between environmental cues, gene expression, and DNA methylation [40]. Similarly, *Scenedesmus acutus* exhibits changes in methylation-driven gene expression that confer tolerance to heavy metals [41]. Although the methylated portion of the genome can be relatively small, only about 1.15 % in *Picochlorum soloecismus*, even this modest fraction has been shown to exert a pronounced impact on growth phenotypes [42].

Marine diatoms also provide compelling examples of methylation-linked regulation. In *Phaeodactylum tricornutum*, elevated CO_2 and temperature correlate with greater DNA methylation in specific gene regions, sometimes resulting in distinct expression profiles [43]. In the same species, methylation events appear to target transposable elements via cytosine methyltransferase enzymes [44], which in turn shape patterns of gene expression and silencing [45]. Indeed, *P. tricornutum* is currently one of the most extensively studied diatoms with respect to epigenetic mechanisms, as evidenced by several recent publications [46,47].

Beyond diatoms and green algae, brown algal taxa are also drawing attention for their epigenetic landscapes. In *Saccharina japonica*, for example, an integrative approach demonstrated that cytosine methylation, including in chloroplast DNA, does occur despite low overall levels [48]. Interestingly, methylation patterns in *S. japonica* can differ between distinct life-cycle stages, hinting that organellar methylation may regulate photosynthetic activity or developmental transitions in multicellular marine algae. Collectively, these studies underscore how even limited, region-specific DNA methylation can orchestrate significant downstream changes in gene expression, ultimately reshaping phenotypic traits in response to diverse environmental stresses.

In our experimental setup, we use low-dose UV-C to elicit changes in the epigenome, bypassing the transcriptional signal transduction cascades to determine if phenotypic plasticity is modulated at the epigenetic level. The underlying hypothesis is based on previous research where epigenetic effects have been linked to UV-C [27,49], and the choice of low-dose irradiation well below the documented mutagenic dosage. In our experimental setup, the resulting cell death post UV-C was ~20 %, contrasting to the 98 % cell death, which is used to induce mutagenesis [50] (Supplementary Fig. 3 A). Moreover, consistent with recent findings that UV-C dosages below 0.15 J cm^{-2} do not produce significant DNA mutation [51] our growth recovery assay for the three UV-C dosages used showed no statistical differences relative to control (Supplementary Fig. 3B). While we cannot entirely exclude the possibility of rare, random UV-induced mutations, the stochastic nature of such events makes it highly unlikely that they would reproducibly drive the consistent changes in phenotypic plasticity observed across four independent experiments, each with eight biological replicates. Nevertheless, we recognize that even low-dose UV-C may induce mutational events, and future studies employing mutation frequency assays or plating-based selection methods could help quantify their potential contribution to these phenotypic outcomes. We designed our experiments to induce epigenetic reorganization before exposing *D. armatus* cells to 18 different environmental conditions, and to measure the impact of the epigenome remodeling on the phenotypic plasticity. On a trait-to-trait basis, there was an observed negative impact of the UV-C irradiation (Supplementary Fig. 2), which resulted in overall lower values; however, only one trait saw its plasticity reduced. This observed pattern suggests that UV-C irradiation uniformly lowers trait values across the board, thus maintaining the relative differences between maximum and minimum values. Essentially, while the absolute trait levels are decreased, the range of variability or plasticity within the measured trait space remains unchanged in half and is changed in the other half of the trait set. This indicates that UV-C does significantly alter the capacity for some traits to respond to environmental variability. The ecological consequences of these findings are significant, as reductions in trait values may lead to diminished fitness under specific environmental conditions, potentially constraining the organism's ability to compete, reproduce, or survive. However, when these are accompanied by changes in plasticity it may also act as a compensatory mechanism, allowing populations to maintain adaptability and resilience in fluctuating environments, and to evolve to a greater extent [5]. Interestingly, in almost all experiments, more than 50 % of all measured traits exhibited significant changes in response to the various environmental conditions (Table 2). This observation suggests that the trait responses to the environmental stimuli have been broadly reorganized. However, it is important to clarify that while the response of individual traits have changed, the range of their responses—or their plasticity—has not necessarily been affected. This means that although the traits respond differently under altered epigenetic influences, their ability to vary or adapt across a spectrum of environmental conditions remains largely intact. In essence, the trait values themselves have shifted, but the capacity of these traits to exhibit variation under different environmental pressures has not diminished, thus maintaining their phenotypic plasticity.

Using global DNA methylation, we confirmed that UV-C induces measurable 5-mC changes genome-wide, with clear differences observed in response to nutrient availability. It was surprising to see the extent of the observed changes for nutrients, which suggests a strong epigenetic regulation of this response. This observation is consistent with findings reported by other authors on *C. reinhardtii*, where epigenetic modifications were shown to play a significant role in adaptation to environmental stresses, such as nutrient availability, through changes in DNA methylation levels [23]. Given that no statistical differences were observed in 5-mC levels for other factors, it is possible that epigenetic regulation at DNA methylation level may still have taken place but was highly specific, targeting only a small subset of genes and not detectable at the whole-genome level. Alternatively, the regulation may have occurred through non-methylation processes or not epigenetically controlled. Furthermore, it is reasonable to consider that any epigenetic changes induced by other factors were transient and not long-lasting, potentially diminishing by the seventh day following UV-C treatment. This suggests that while some epigenetic modifications were initially present, they may not have been maintained long enough to be detectable at the time of measurement.

Our findings point to a broad regulation of the phenome via one or more epigenetic processes, with a major role on phenome organization and a medium impact on plasticity size. We can confirm that low doses of UV-C induces changes in DNA methylation levels and that low doses of irradiation can be used for remodeling the DNA methylation part of the epigenome. Nonetheless, it should be noted that while UV-C is known to have an impact on DNA methylation, we cannot exclude the possibility that it also induces other epigenetic changes, such as histone acetylation or chromatin remodeling.

It is important to emphasize that while our colorimetric assay reliably detects global shifts in DNA methylation levels, it does not provide locus-specific information about methylation changes. This is a critical limitation because methylation effects on gene expression are known to vary depending on genomic context for example, methylation at promoter or enhancer regions typically represses gene transcription, whereas methylation within coding sequences or introns may influence transcript processing and splicing [19,23,37]. In previous research on *Chlamydomonas reinhardtii* and other microalgae, shifts in methylation at particular loci have been shown to correlate with stress responses and adaptive changes, highlighting the importance of examining both global and region-specific modifications [19,23].

Recent advances highlight the prevalent role of epigenetic modifications in microalgal phenotypic regulation. In *C. reinhardtii*, the DGTT3 promoter exhibits elevated DNA methylation under low-temperature and phosphate-deficiency stress, correlating with transgene silencing and altered lipid biosynthesis [52]. In the oleaginous microalga *Nannochloropsis oceanica*, genome-wide mapping of N^6-adenine methylation (6 mA) revealed its enrichment in gene bodies of lipid-metabolism genes, where 6 mA abundance positively associates with transcriptional activity and oil productivity [53]. Heavy-metal stress in *Scenedesmus acutus* induces distinct differentially methylated regions (DMRs) between Cr-tolerant and wild-type strains, notably within sulfate-assimilation genes, underpinning chromium tolerance [41]. In the model diatom *P. tricornutum*, 5-methylcytosine forms mosaic patterns across transposable elements and gene bodies, where gene-body methylation correlates with conditional gene silencing and stress resilience [45]. Moreover, long-term adaptation of *P. tricornutum* to elevated CO_2 and warming involves CHH-context methylation islands within gene bodies that cooperate with differential gene expression to rewire central carbon and amino-acid metabolism [43]. In multicellular kelps, chloroplast DNA methylation varies by life stage—gametophytes versus sporophytes—and associates with stage-specific expression of key photosynthetic genes [48], while single-base bisulfite sequencing of Saccharina japonica confirms low-level, CHH-biased 5mC whose distribution inversely correlates with transcript abundance during development [54]. These findings show a broad, multilayered epigenetic architecture by which DNA methylation shape phenotypic plasticity and adaptation in in macro and micro-algae.

It is clear that while global methylation assays capture broad epigenomic shifts, locus-specific patterns often underlie functional outcomes. Consequently, our conclusions on UV-C induced DNA methylation pertain solely to global methylation levels, without reference to specific genomic regions. Future studies employing sequencing-based methods such as bisulfite sequencing are recommended to precisely map genomic loci of methylation alterations. Further research could also explore transcriptomic and methylomic comparisons across diverse algal species, identify genomic versus epigenomic regulatory mechanisms underlying phenotypic plasticity, and assess the temporal stability of methylation changes. Additional epigenetic mechanisms, including histone modifications, non-coding RNA activity, and chromatin remodeling, should likewise be examined to provide a more complete understanding of epigenetic regulation in microalgal adaptation.

CRediT authorship contribution statement

Andrei Herdean: Writing – review & editing, Writing – original draft, Visualization, Supervision, Investigation, Formal analysis, Data curation, Conceptualization. **Massimo Bedoya:** Investigation, Formal analysis, Data curation, Conceptualization. **Mikael Kim:** Writing – review & editing, Formal analysis, Data curation. **Nature Poddar:** Writing – review & editing, Supervision. **Lilian Hoch:** Writing – review & editing, Conceptualization. **Peter J. Ralph:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Andrei Herdean reports was provided by University of Technology Sydney. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2025.104206.

Data availability

All raw data is provided in supplementary files

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