RESEARCH PAPER

Hormetic Growth Response and Transgenerational Morphological Recovery in *Desmonostoc persicum* Kabirnataj et al. under Gamma Irradiation Stress

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ABSTRACT

Ionizing radiation typically induces deleterious effects on biological systems; however, low-dose stimulation (hormesis) remains an underexplored phenomenon in cyanobacteria. This study investigated the morphometric plasticity and transgenerational stability of *Desmonostoc persicum* Kabirnataj et al. exposed to ^{60}Co gamma irradiation doses of 0, 0.48, 0.96, and 1.44 kGy. A multi-faceted statistical approach, including polynomial regression, Principal Component Analysis (PCA), and phenotypic instability analysis was performed. Results indicated a significant non-linear, hormetic response ($p < .05$), where the 0.48 kGy dose stimulated vegetative cell hypertrophy and heterocyst differentiation. Conversely, high doses (1.44 kGy) triggered genomic instability, evidenced by a two-fold increase in the Coefficient of Variation (CV) for cell dimensions and filament fragmentation. Crucially, transgenerational analysis revealed a reaction norm in which second-generation lineages from irradiated parents converged toward control phenotypes, suggesting that the observed alterations were physiological acclimatizations rather than permanent genetic mutations. These findings highlight the robust radio-resistance and adaptive plasticity of *D. persicum.*

Keywords: Cyanobacteria, BG110 media, Phenotypic Plasticity, Radiation.

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Introduction:

Cyanobacteria, are ancient and cosmopolitan prokaryotes, they exhibit extraordinary resilience to extreme environmental stressors, including desiccation, ultraviolet (UV) radiation, and ionizing radiation (1–4). The genus *Desmonostoc* (formerly classified under *Nostoc*) comprises filamentous, heterocystous species adept at nitrogen fixation and persistence in terrestrial ecosystems, such as arid soils (5). Elucidating the underlying radioresistance mechanisms in these organisms holds profound implications for evolutionary biology, bioremediation of radionuclide-contaminated environments, and astrobiological applications in radiation-exposed extraterrestrial habitats (6). The canonical model for biological responses to ionizing radiation-the linear no-threshold (LNT) paradigm-posits a monotonic increase in cellular damage with dose, driven by direct DNA strand breaks and indirect oxidative insults via reactive oxygen species (ROS) (7). Yet, mounting empirical evidence challenges this view, revealing hormesis: a biphasic dose-response curve wherein low doses paradoxically enhance physiological performance (i.e., growth, repair, and differentiation) before high doses elicit inhibition or toxicity. In cyanobacteria, low-dose gamma irradiation has been linked to upregulated antioxidant systems, improved photosynthetic yields, and metabolic reprogramming, contrasting with the genomic fragmentation and filament disruption observed at supralethal exposures (8,9). Despite these insights into acute responses, critical gaps persist regarding the morphological manifestations of hormesis-such as cell hypertrophy or heterocyst induction and, crucially, the transgenerational fidelity of these adaptations. Radiation induced phenotypic shifts ephemeral acclimations that dissipate across progeny, or enduring heritable modifications that confer multigenerational resilience? To address these questions, the present study quantifies the morphometric responses of *Desmonostoc persicum*-soil-isolated strain from Maharashtra, India. Graded doses of ^{60}Co gamma irradiation (0-1.44 kGy). Employing polynomial regression for dose-response modeling, principal component analysis (PCA) for multivariate trait clustering, and coefficient of variation (CV) metrics for phenotypic instability, we rigorously test the hypothesis of a hormetic trajectory in vegetative, heterocyst, and akinete dimensions. Furthermore, by tracking trait convergence across two generations, we delineate whether observed plasticity reflects transient physiological buffering or stable epigenetic or genetic legacies, thereby illuminating adaptive strategies in this radioresistant cyanobacterium.

Materials and Methods

Organism and Culture Conditions

The cyanobacterial strain *Desmonostoc persicum* (Accession No. AUR-1008) was isolated from soil samples in the Chhatrapati sambhajinagar district, Maharashtra, India. Cultures were maintained in BG11$_0$ medium at 25 ± 2 °C under a 12:12 h light: dark photoperiod with a light intensity of 40 µmol photons m^{-2} s^{-1} (10).

Gamma Irradiation and Experimental Design

Exponentially growing cultures were exposed to gamma radiation using a ^{60}Co source (Bio-Beam 600) at a dose rate of 1 Gy min^{-1} at the Department of Biophysics, Institute of Science, Chhatrapati Sambhajinagar. The experimental design included four dosage groups: control (0 kGy), low dose (0.48 kGy), medium dose (0.96 kGy), and high dose (1.44 kGy), with three biological replicates per group. Exposure times were calculated accordingly: 8 min for 0.48 kGy, 16 min for 0.96 kGy, and 24 min for 1.44 kGy. Following irradiation, 100 µL aliquots of each culture were inoculated onto BG11$_0$ agar plates and incubated at 25 ± 2 °C under the same photoperiod for 20 days to allow colony formation (Generation 1; Gen 1). To assess transgenerational effects, individual surviving colonies from Gen 1 were sub-cultured into fresh BG11$_0$ liquid medium, propagated for an additional 20 days (Generation 2; Gen 2), and analyzed for morphological recovery.

Morphometric Measurements

Morphometric observations were conducted using a calibrated Nikon Eclipse E200 light microscope at 40× magnification. For each treatment and generation, traits including vegetative cell length and width (with and without sheath), heterocyst length and width, and akinete length and width were measured across 30 randomly selected filaments per biological replicate (n = 90 measurements per trait per treatment per generation). Measurements were performed using Mosaic 2.1 image analysis software for micrometry, with reproducibility ensured by averaging 15 cells per filament.

Statistical Analysis

All analyses were conducted using R software (v4.3.1). Dose–response relationships for key traits (e.g., vegetative cell width) were modeled using second-order polynomial regression to detect hormetic trends, with model fit assessed through R^2 values and residual diagnostics. Principal Component Analysis (PCA) was performed on standardized morphometric variables using the *factoextra* package to visualize multivariate clustering and trait loadings across irradiation doses and generations (Kassambara & Mundt, 2020). Phenotypic instability was quantified using the Coefficient of Variation (CV = standard deviation/mean × 100) for cell dimensions within and across generations. Differences in trait means among treatments and generations were evaluated using one-way ANOVA followed by Tukey's Honest Significant Difference (HSD) post-hoc test, with significance declared at $\alpha = 0.05$. Non-parametric Kruskal–Wallis tests were applied when normality assumptions were violated, as determined by the Shapiro–Wilk test ($p < 0.05$).

Results

Hormetic Growth Response

Contrary to linear damage models, *D. persicum* exhibited a distinct hormetic response to gamma irradiation. Polynomial regression analysis of vegetative cell width revealed a significant quadratic trend ($R^2 = 0.72$, $p < .05$). As shown in Fig. 1, growth was stimulated at low doses, with a theoretical peak efficacy occurring at approximately 0.61 kGy. The 0.48 kGy treatment group showed significant hypertrophy compared to controls (Table 1), whereas the highest dose (1.44 kGy) resulted in reduced cell dimensions, indicative of toxicity. This biphasic pattern aligns with hormetic effects previously observed in other cyanobacteria, where chronic low-dose gamma irradiation (e.g., up to 0.5 kGy) enhances photosynthetic efficiency and biomass accumulation before declining at higher exposures (8).

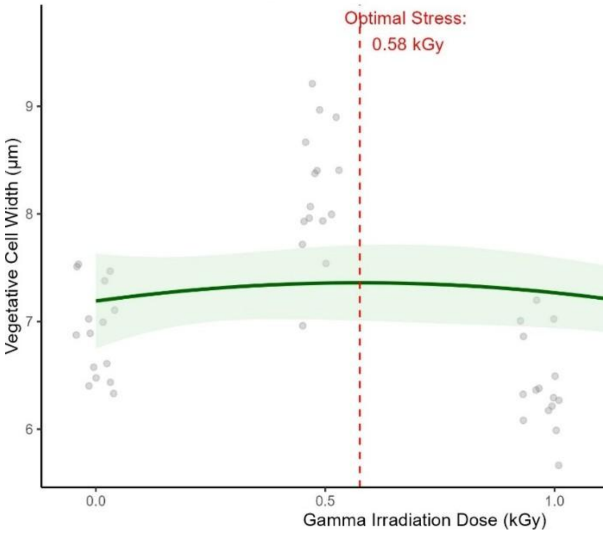
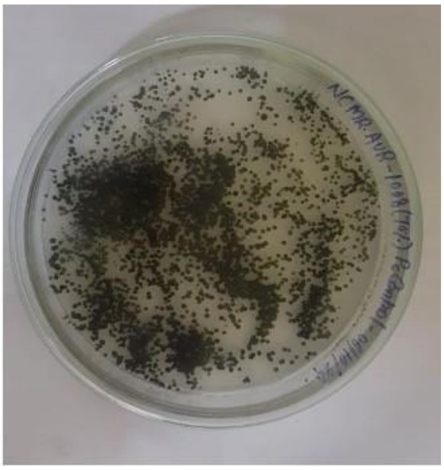


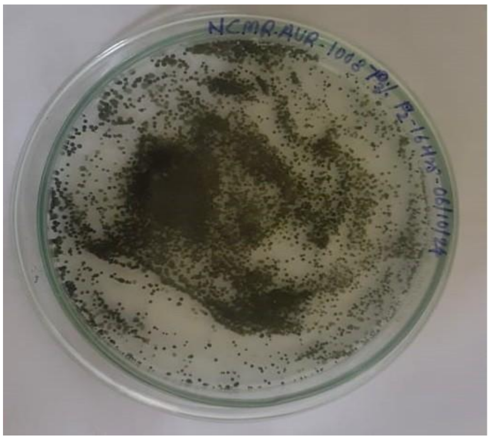
Figure 1: Hormetic Dose-Response Curve. Polynomial regression showing the non- linear response of vegetative cell width to gamma irradiation. The red dashed line indi- cates the theoretical peak stimulation dose.

Microscopic observation of colony morphology corroborated these morphometric findings, with plates treated with 0.48 kGy exhibiting denser, more intensely pigmented colonies compared to the pale, chlorotic colonies observed at 1.44 kGy (Fig. 2).

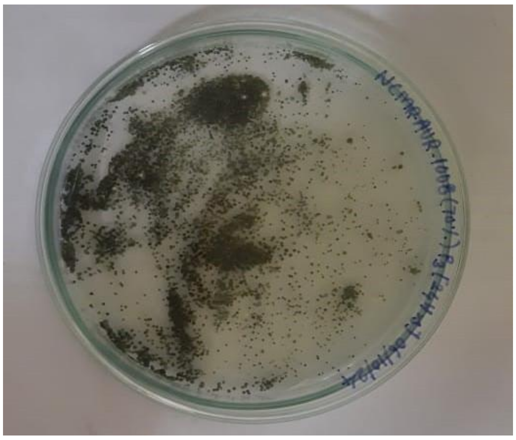


(a) Control (0 kGy)

(b) 0.48 kGy



(c) 0.96 kGy



(d) 1.44 kGy

Figure 2: Macroscopic Growth Response of *Desmonostoc persicum* in $BG11_0$ Agar. (A) Control showing standard colony morphology. (B) 0.48 kGy treatment showing enhanced biomass and intense pigmentation (Hormesis). (C) 0.96 kGy treatment showing reduced expansion. (D) 1.44 kGy treatment displaying chlorosis and sparse growth due to severe photo-oxidative stress.

Multivariate Differentiation

Principal Component Analysis (PCA) demonstrated that radiation stress induced coordinated morphological differentiation. The first two principal components explained a significant proportion of the total variance (Fig. 3). The biplot revealed that the 0.48 kGy group formed a distinct cluster, characterized by increased heterocyst and vegetative cell dimensions, clearly separating it from both the control and high- dose groups. Similar multivariate separations have been reported in irradiated filamentous cyanobacteria, where PCA highlights dose-dependent shifts in cell differentiation and other stress-responsive traits (11).

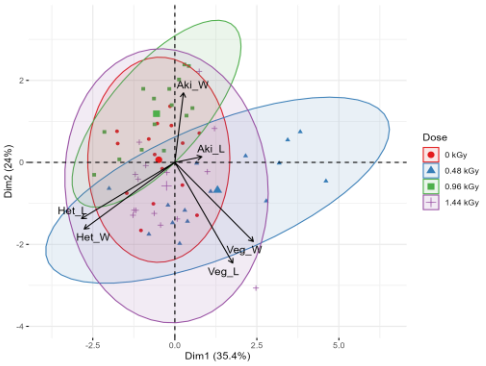


Figure 3: PCA Biplot of Morphometric Traits. Vectors represent the correlation between traits; colored ellipses represent the 95% confidence interval for each treatment group.

Genomic Instability and Phenotypic Noise

While low doses induced organized hypertrophy, high doses (1.44 kGy) resulted in a loss of developmental regulation. As illustrated in Fig. 4, the Coefficient of Variation (CV)-a proxy for phenotypic instability-doubled for vegetative cell width in the 1.44 kGy group compared to the control. This increased statistical variance was mirrored microscopically by filament fragmentation and irregular cell shapes in the high-dose samples (Fig. 5). Such elevated phenotypic noise aligns with known genomic perturbations in radiation-exposed cyanobacteria, including upregulated DNA repair pathways that become overwhelmed under acute stress, ultimately leading to fragmentation and substantial variability in filament integrity (12,13).

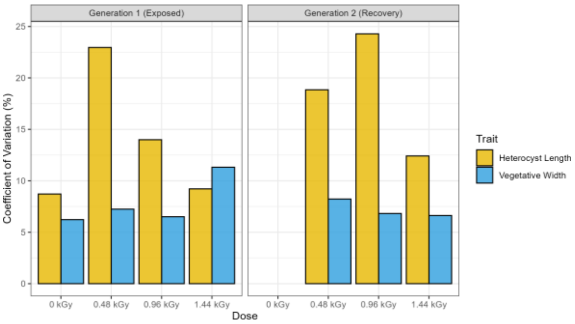
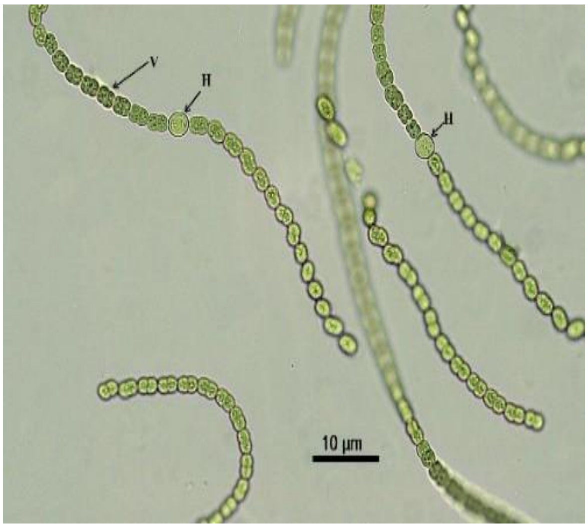
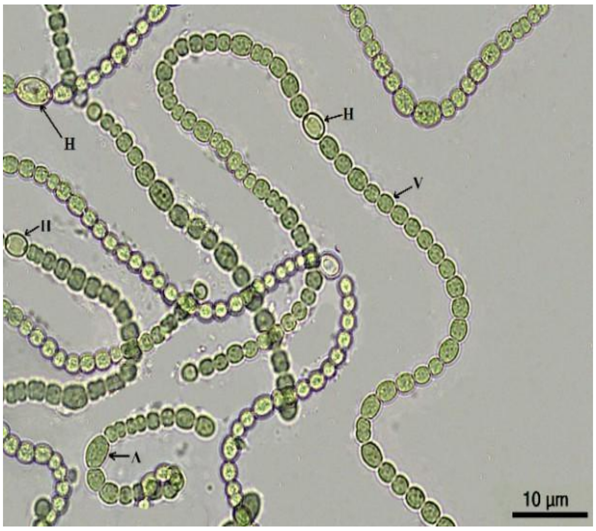


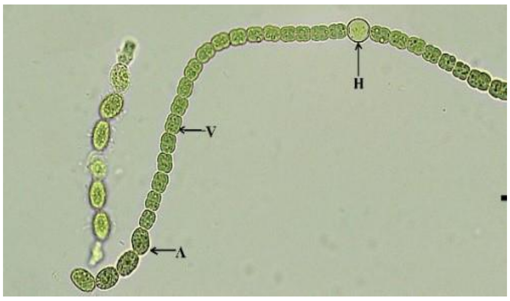
Figure 4: Phenotypic Instability Analysis. Bar plot showing the increase in Coefficient of Variation (CV%) at higher radiation doses, indicating increased developmental noise.



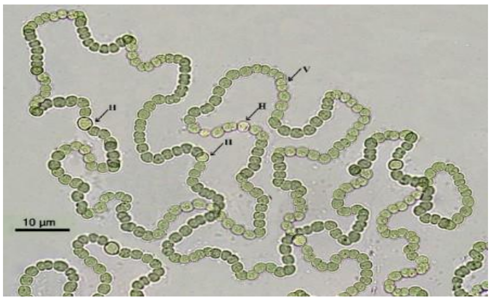
A (Gen 1)



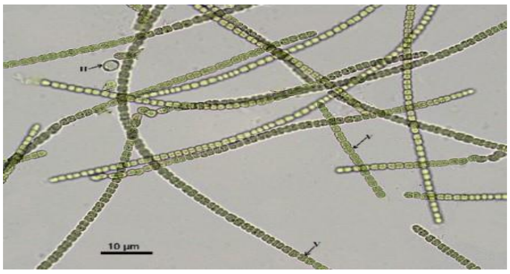
E (Gen 2)



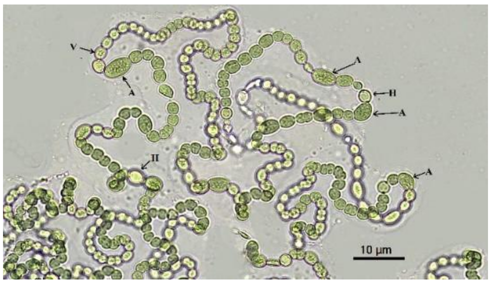
B (Gen 1)



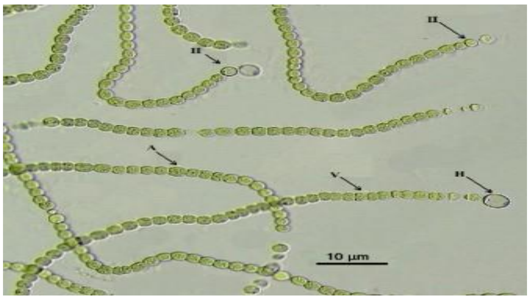
D (Gen 1)



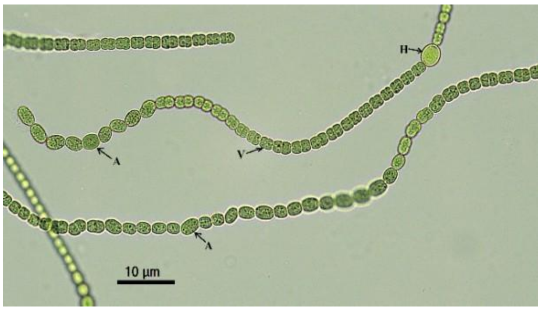
F (Gen 2)



H (Gen 2)



C (Gen 1)



G (Gen 2)

Figure 5: Microscopic morphology of *Desmonostoc persicum* filaments under gamma irradiation stress. (A) Control showing intact filamentous structure. (B) Low-dose (0.48 kGy) with enhanced pigmentation. (C) Mid-dose (0.96 kGy) displaying initial fragmentation. (D) High-dose (1.44 kGy) exhibiting severe depigmentation and lysis. (E-H) Recovery phases post-irradiation (Generation 2), showing partial regeneration and stress markers. Scale bars = 10 μm.

Transgenerational Recovery

To distinguish between genetic mutations and physiological adaptation, we analyzed the second generation (Gen 2). (14) suggested that persistent phenotypic changes across generations typically indicate genetic damage. However, our data show a robust recovery trajectory (Fig. 6). While Gen 1 displayed significant morphological divergence, Gen 2 lineages converged back toward control dimensions, suggesting that the initial stress response was a transient physiological adaptation rather than a permanent mutation. This pattern of morphotype-specific recovery post-irradiation further supports the absence of heritable genomic instability, consistent with observations in related cyanobacteria where irradiated lineages regain baseline morphology within a single generation (15).

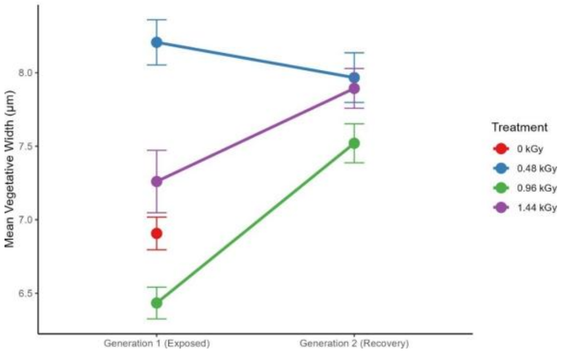


Figure 6: Transgenerational Recovery. Reaction norm plot showing the convergence of phenotypes in Generation 2, indicating recovery from radiation stress.

Discussion

The Present study provides the first integrated morphometric and transgenerational evidence of gamma radiation–induced hormesis in the cyanobacterium *Desmonostoc persicum*. It demonstrate a biphasic response that challenges the linear no-threshold (LNT) model traditionally applied to microbial systems, instead revealing a dose-dependent continuum that progresses from adaptive plasticity to genomic instability, followed by phenotypic recovery. The observed stimulation of vegetative cell hypertrophy and heterocyst differentiation at 0.48 kGy (Fig. 1) establishes a clear hormetic morphometric trajectory. This aligns with the overshoot model, wherein low-dose stressors activate overcompensatory responses in antioxidant and DNA repair systems (7). While similar biomass increases have been reported in *Limnospira indica* (8), our findings uniquely identify the specific cellular modifications—enlarged vegetative cells and enhanced differentiation of specialized cells—that underlie this response. These low-dose benefits echo gamma-induced enhancements of CO_2 fixation observed in *Arthrospira platensis* (16), highlighting potential applications of radiation hormesis for engineering robust microbial strains for carbon capture and bioremediation.

Multivariate analysis further supported that the low-dose response represents a coordinated reprogramming of cellular architecture rather than random variation. The PCA biplot (Fig. 3) demonstrated a systematic shift toward a phenotype dominated by enlarged vegetative cells and heterocysts, traits that may enhance nitrogen fixation under stress. However, this adaptive capacity exhibited a strict threshold: at 1.44 kGy, the coordinated response collapsed into phenotypic noise, with a two-fold increase in the Coefficient of Variation (Fig. 4) and clear filament fragmentation. This shift represents a phenotypic tipping point where regulatory networks, such as the LexA-mediated DNA damage response (11), likely become saturated, resulting in oxidative damage and a breakdown of developmental integrity (12). Thus, our findings provide a quantitative morphometric framework for genomic instability previously described only at molecular scales.

The most critical insight from this study is the transgenerational reversibility of radiation-induced phenotypes. The convergence of second-generation lineages toward control morphology (Fig. 6) indicates that the observed deviations are transient physiological acclimations rather than stable genetic mutations. This rapid recovery suggests the involvement of robust epigenetic reset mechanisms, similar to stress-induced DNA methylation responses documented in *Synechocystis* (17). It also parallels morphotype-specific recovery noted in *Arthrospira* (15), implying that architectural buffering contributes to resilience. Importantly, this recovery implies that although high doses introduce substantial disruptions, surviving populations retain genomic integrity sufficient to restore baseline morphology, minimizing long-term mutational risks.

Collectively, the present results position *D. persicum* not just as a radioresistant organism but as a model of dynamic phenotypic adaptation. Its ability to harness low-dose radiation for stimulatory benefits, tolerate high-dose damage, and regain normal morphology in subsequent generations highlights a remarkable adaptive repertoire. This makes it a strong candidate for bioremediation in fluctuating radionuclide-contaminated environments and suggests that low-dose cosmic radiation may even prime cyanobacterial life-support systems for space exploration. Future research should focus on identifying the molecular mechanisms such as ROS-responsive epigenetic regulation and the efficiency of DNA repair pathways that underpin this plasticity. Advancing from descriptive morphology to mechanistic understanding will allow hormesis to be leveraged as a strategic tool for ecological restoration and biotechnological innovation.

Table 1: One-way ANOVA results for morphometric traits in Generation 1

Trait	df	Sum Sq	F-value	p-value	Effect Size (η^2) G
Vegetative Width	3, 56	24.3	24.30	< .001***	0.566 (Large)
Vegetative Length	3, 56	12.1	7.70	< .001***	0.292 (Large)
Heterocyst Length	3, 56	1.37	1.37	.262	0.068 (Small)
Akinete Length	3, 56	9.21	9.21	< .001***	0.330 (Large)

Note: df = degrees of freedom (numerator, denominator); * denotes significance at p < .001. Effect sizes interpreted as Small > 0.01, Medium > 0.06, Large > 0.14.**

Conclusion

This study reveals the remarkable adaptive repertoire of *Desmonostoc persicum* under gamma irradiation, following a characteristic hormetic pattern stimulatory hypertrophy and enhanced pigmentation at low doses (0.48 kGy), contrasted with dose-dependent toxicity and phenotypic disruption at higher exposures (1.44 kGy). Multivariate analyses, including PCA and phenotypic-noise metrics, clearly delineate a threshold beyond which coordinated morphological reprogramming gives way to fragmentation and genomic instability, highlighting the finite limits of cyanobacterial resilience (11,12).

Importantly, the transgenerational perspective underscores pronounced plasticity: second-generation lineages rapidly reconverged toward control morphologies, indicating that the initial alterations were transient physiological responses rather than heritable mutations (15,17). These findings not only affirm the intrinsic radio-resistance of *D. persicum* but also illuminate its potential utility in the bioremediation of radionuclide-contaminated environments and its value as a model organism for radiation-primed microbial consortia in astrobiological research (16).

Overall, by integrating hormesis theory with empirical cyanobacterial ecology, this work shifts the narrative from perceiving ionizing radiation solely as a threat to exploring its sublethal gradients as tools for ecological restoration and biotechnological innovation. Future studies should unravel the molecular mechanisms particularly ROS-mediated regulatory and epigenomic pathways to fully harness this duality for sustainable applications.

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Ethical statements

This study did not involve humans or animals, so ethical approval was not needed.

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