

Resource availability reshapes clonal recovery and trait coordination across fragment configurations of *Salvinia natans*

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Abstract

Background

Clonal aquatic plants can regenerate from fragments, but how light and nutrient availability influence recovery across fragment configurations remains poorly understood. We conducted a four-week factorial experiment with the floating fern *Salvinia natans*, combining two light levels, two nutrient levels and three configurations: light fragmentation (LF; two three-node fragments), moderate fragmentation (MF; three two-node fragments) and severe fragmentation (SF; six single-node fragments). Initial total node number was standardized across configurations, with five replicate pots per treatment combination. We measured growth, biomass allocation and frond-specific metabolism, and examined trait coordination using plant trait multilayer networks (PTMNs).

Results

Under high-light, nutrient-rich conditions, MF produced more frond pairs and ramets and accumulated more dry weight than LF or SF. Under low light, dry-weight accumulation remained low and differences among configurations narrowed. High light combined with low nutrients was associated with higher carbohydrate concentrations and a greater submerged-frond to floating-frond dry-weight ratio. PTMNs showed contrasting patterns of trait coordination: LF had the greatest cross-layer connectivity, MF the strongest local clustering, and SF the highest module interlayer association. Carbohydrate traits and biomass allocation occupied central network positions, with the leading hubs differing among configurations.

Conclusions

The growth advantage of MF depended on resource availability. Fragment configurations were also associated with distinct relationships among carbohydrate status, tissue allocation and clonal growth. These findings show that recovery responses differed in both growth performance and the coordination of traits across functional layers.

Background

Fragmentation is both a consequence of physical disturbance and a major route of vegetative regeneration in clonal aquatic plants [1–4]. When a clone is broken, the same amount of plant material can be partitioned into a few large fragments or many small ones, changing not only the resources retained by each unit but also the number and arrangement of potential growth units [5–7]. Most fragmentation studies have therefore emphasized fragment size or survival, yet the ecological outcome of fragmentation should depend on the configuration of the surviving material and on the environment into which fragments are released. Light and nutrients are especially important because they determine whether fragments can convert retained reserves and newly acquired resources into renewed clonal growth [8–10]. The floating fern *Salvinia natans* is well suited to test this context dependence. Its modular architecture enables rapid vegetative regeneration, while its floating and submerged fronds differ functionally in carbon acquisition and nutrient uptake [11, 12].

Partitioning a fixed amount of clonal material creates a fundamental trade-off between fragment size and fragment number. Larger fragments retain more connected tissue and potentially larger internal reserves, which may buffer severing stress and support early regrowth, but they produce fewer independent growth units. Smaller fragments increase propagule number and the number of potential sites for clonal expansion, but each unit begins with less tissue and fewer stored resources [1–3, 6–8]. The resulting configurations also differ in growth-point arrangement, retained clonal connections, within-clone competition, and the number of cut edges. These opposing effects make an intermediate configuration a plausible compromise between resource retention and propagule multiplication. Previous studies show that responses to fragmentation vary with nutrient availability, population context, and the pattern of fragmentation [9, 13–15]. Whether an intermediate configuration has a consistent advantage across environments, however, remains unresolved.

Resource availability provides a mechanism by which the relative performance of fragment configurations may change. When both light and nutrients are abundant, fragments can rapidly convert photosynthetic carbon and mineral nutrients into new fronds, ramets, and structural biomass, allowing configurational differences to become fully expressed. Under low light, carbon acquisition may impose a common constraint that suppresses growth across configurations and compresses their performance differences [16–20]. By contrast, when light is relatively abundant but nutrients are limiting, carbon gain and nutrient-supported tissue construction may become imbalanced, altering carbon–nitrogen metabolism and biomass allocation [21–23]. Such an imbalance could favor carbon retention

rather than continued structural growth. The same fragment configuration may therefore support rapid proliferation in one resource environment but shift toward storage and allocation adjustment in another.

Post-fragmentation recovery is also a coordinated, multidimensional process. Severing connected modules disrupts physiological integration and resource sharing among ramets [24–28]. Because growth and biomass allocation are closely linked to carbon- and nitrogen-related metabolism [18, 19, 29–32], post-fragmentation recovery may also involve coordinated changes in metabolite concentrations. In *S. natans*, the functional differentiation between floating and submerged fronds makes these tissue-specific adjustments especially informative. Yet conventional analyses usually treat growth, allocation, and physiology as separate response sets. Plant trait networks instead represent traits as nodes and their statistically supported associations as edges, revealing how multiple functions covary as an integrated phenotype [33–36]. Plant trait multilayer networks (PTMNs) extend this approach by organizing traits into functional layers and quantifying associations within and among functional domains [37]. This framework allows us to ask whether fragmentation changes not only individual trait values, but also the cross-functional architecture through which growth, allocation, and metabolism are coordinated during recovery.

Here, we manipulated three composite fragment configurations of *S. natans* under contrasting light and nutrient conditions: light fragmentation (LF; two three-node fragments), moderate fragmentation (MF; three two-node fragments), and severe fragmentation (SF; six one-node fragments). All configurations began with six frond pairs, holding the initial frond-pair number constant while altering how clonal material was partitioned among fragments. We asked three questions: (1) Does resource availability change the relative growth and biomass production of the three configurations? (2) How do floating- and submerged-frond metabolic traits respond to differences in light and nutrient availability? and (3) Do fragment configurations differ in multilayer trait coordination and hub traits during regrowth? We predicted that MF would perform best under high light and high nutrient availability because it combines intermediate fragment size with multiple independent growth units; that high light combined with low nutrient supply would favor carbohydrate retention relative to dry-weight production; and that fragment configuration would alter the organization of trait coordination across growth, allocation, and metabolic functions. Because fragment size, number, retained connections, growth-point arrangement, and cut-edge number varied jointly, our treatments represent contrasting fragment configurations rather than independent manipulations of any single structural component.

Methods

Plant material and pre-cultivation

S. natans (L.) Allioni is a fast-growing clonal aquatic fern in the family Salviniaceae [38]. It is widely distributed from Asia through Europe to North Africa and has also been spread by humans to other regions [39]. The plant forms creeping stems up to approximately 20 cm long, can produce as many as 12 axillary branches, and bears small floating fronds together with finely divided submerged fronds [40]. Plants used in this study were purchased from Yingtao Aquarium, a commercial aquarium supplier in Guangzhou, Guangdong Province, China. The species was formally identified as *Salvinia natans* (L.) Allioni by Dr. Meng Zhang, Senior Engineer at the Jiangxi Academy of Environmental Sciences, China, based on morphological characteristics. Because all experimental plant material was commercially obtained and no wild plants were collected by the authors, no field collection permit or licence was required. No voucher specimen was deposited. The experimental use of the plant material complied with relevant institutional, national and international guidelines. Healthy individuals of similar size were cleaned and acclimated for 7 days in a climate chamber at $25 \pm 2^\circ\text{C}$, $60 \pm 5\%$ relative humidity, and a 12-h light/12-h dark cycle. Plants were maintained in 10% Hoagland solution, and one-third of the solution volume was replaced daily.

Experimental design

The experiment followed a fully factorial design with light availability, nutrient supply, and fragment configuration as fixed treatment factors. Midday light intensity was measured at noon on three clear days. High- and low-light treatments received 81.5% and 32.3% of natural light (599.0 ± 32.1 and $237.4 \pm 13.4 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively) and were established using black shade nets. Low nutrient supply consisted of 3% Hoagland solution containing 6.3 mg N L^{-1} and 0.93 mg P L^{-1} , whereas high nutrient supply consisted of 15% Hoagland solution containing 31.5 mg N L^{-1} and 4.65 mg P L^{-1} . These concentrations were selected to generate a clear nutrient contrast; Hoagland-based nutrient gradients have previously been used to manipulate resource availability in *S. natans* [41].

LF consisted of two fragments with three frond pairs each, MF of three fragments with two frond pairs each, and SF of six fragments with one frond pair each (Fig. 1). All experimental units therefore began with six frond pairs. The factorial design produced 12 treatment

combinations, with five replicates per combination and 60 experimental units in total. Each unit consisted of a 5-L plastic bucket (20 cm diameter × 18 cm height) containing 3 L of the assigned nutrient solution. High- and low-light treatments were established in separate shade-net areas. Within each light treatment, buckets were randomly arranged and repositioned weekly within the same light area to reduce persistent positional effects. Initial fresh weight averaged 0.630 ± 0.024 g across experimental units. The nutrient solution was replaced weekly to limit nutrient depletion and algal growth.

Growth, biomass, and physiological measurements

The experiment lasted 4 weeks. Water temperature ranged from 25.7 to 31.8°C, with a mean of 28.9°C. Frond-pair number and ramet number were recorded weekly without destructive sampling. At harvest, plants were rinsed, blotted dry, and separated into floating and submerged fronds. Because *S. natans* lacks true roots, its finely divided root-like organs were analyzed as submerged fronds. The floating-frond fraction included the floating fronds and their connecting stems. Samples were weighed fresh and then dried at 80°C to constant weight. Total dry weight (DW) was calculated as the sum of floating- and submerged-frond DW, and the submerged-frond to floating-frond dry-weight ratio (S:F DW ratio) was used as an allocation index.

Physiological analyses were conducted on dried, powdered tissue. Approximately 50 mg of each sample was extracted twice with 5 mL of 80% ethanol at 80°C for 20 min and centrifuged at 4,000 rpm for 20 min. The combined supernatant was used to quantify soluble carbohydrates (SC) using glucose as the standard [42] and free amino acids (FAA) using alanine as the standard [43]. The remaining pellet was used for starch determination.

Statistical analysis

Frond-pair number and ramet number were analyzed using generalized linear mixed models (GLMMs) fitted in glmmTMB. Light availability, nutrient supply, fragment configuration, cultivation time, and all interactions were included as fixed effects, and bucket identity was included as a random intercept to account for repeated measurements. Final DW, allocation, and physiological traits were analyzed by three-way analysis of variance (ANOVA) with light availability, nutrient supply, fragment configuration, and their interactions as fixed effects. The S:F DW ratio was log-transformed, and floating- and submerged-frond starch concentrations were transformed as $\log(x + 1)$; the other traits were analyzed without transformation. Residual normality was assessed with Shapiro-Wilk tests and variance homogeneity with Levene tests. Significant treatment interactions were followed by Tukey-adjusted comparisons using emmeans. Statistical significance was evaluated at $P < 0.05$.

Multivariate trait composition was visualized by non-metric multidimensional scaling (NMDS) of Gower distances calculated from ten traits using daisy in cluster. Marginal effects of light availability, nutrient supply, fragment configuration, and their interactions were tested by permutational multivariate analysis of variance (PERMANOVA) with adonis2 in vegan using 999 permutations. Homogeneity of multivariate dispersion among the 12 treatment combinations was assessed with betadisper in vegan using 999 permutations.

PTMNs were constructed separately for LF, MF, and SF using 20 experimental units per network (five replicates from each of the four resource environments) and MultiTraits [44], following the PTMN framework of He et al. [37]. Ten traits were assigned to four functional layers: growth, biomass and allocation, floating-frond metabolism, and submerged-frond metabolism (Table 1). Starch concentrations were transformed as $\log(x + 1)$ before Pearson correlations were calculated. Edges were retained when $|r| \geq 0.20$ and the P value adjusted for the false discovery rate (FDR) was < 0.05 , following the filtering implemented in the PTMN function. Network topology was described by interlayer edge density, average interlayer path length, average interlayer clustering coefficient, and module interlayer association. Trait importance was quantified by interlayer degree; the three highest-ranked traits in each network were designated as hub traits. All analyses were conducted in R version 4.6.0.

Table 1
Traits and functional layers used in the plant trait multilayer network

Layer	Traits	Ecological meaning
Growth	Frond-pair number; ramet number	Regenerative and clonal growth capacity
Biomass and allocation	Total DW; S:F DW ratio	Biomass accumulation and allocation
Floating-frond metabolism	Floating-frond FAA; floating-frond SC; floating-frond starch	Carbon storage and nitrogen-related metabolism in floating fronds
Submerged-frond metabolism	Submerged-frond FAA; submerged-frond SC; submerged-frond starch	Carbon storage and nitrogen-related metabolism in submerged fronds

DW, dry weight; S:F DW ratio, submerged-frond to floating-frond dry-weight ratio; FAA, free amino acids; SC, soluble carbohydrates.

Results

Resource availability determined growth differences among fragment configurations

Frond-pair number increased throughout the 4-week experiment and responded to light availability, nutrient supply, fragment configuration, and cultivation time (Fig. 2; Table 2). Significant interactions occurred between light and nutrients, light and fragment configuration, light and time, and nutrients and time. The light $\times$ nutrient $\times$ fragment configuration and light $\times$ nutrient $\times$ time interactions were also significant, indicating that both configuration rankings and temporal growth responses varied among resource environments.

Ramet production followed the same general pattern. Light availability, nutrient supply, fragment configuration, and cultivation time all had significant effects, with significant light $\times$ nutrient, light $\times$ fragment configuration, light $\times$ time, nutrient $\times$ time, and light $\times$ nutrient $\times$ fragment configuration interactions (Table 2).

Under high light with high nutrients (HH), MF produced the most frond pairs and ramets, followed by SF and LF. Under high light with low nutrients (HL), MF also produced the most frond pairs, whereas differences in ramet number were smaller. Growth declined under low light with high nutrients (LH) and low light with low nutrients (LL), and the three configurations became more similar. Under LL, SF produced relatively more ramets, but final DW remained low. Thus, the performance advantage of MF was greatest under high light.

Table 2
GLMM results for growth responses to light, nutrients, time, and fragment configuration

Effect	Frond-pair number			Ramet number		
	LR χ^2	df	Sig.	LR χ^2	df	Sig.
Light	53.803	1	***	62.263	1	***
Nutrients	6.491	1	*	12.729	1	***
Fragment configuration	28.113	2	***	16.117	2	***
Week	3296.498	3	***	260.278	3	***
Light × Nutrients	3.922	1	*	8.361	1	**
Light × Fragment configuration	6.348	2	*	12.029	2	**
Nutrients × Fragment configuration	1.192	2	n.s.	5.960	2	n.s.
Light × Week	235.869	3	***	26.048	3	***
Nutrients × Week	75.419	3	***	27.296	3	***
Fragment configuration × Week	3.435	6	n.s.	1.314	6	n.s.
Light × Nutrients × Fragment configuration	21.200	2	***	15.649	2	***
Light × Nutrients × Week	18.933	3	***	7.794	3	n.s.
Light × Fragment configuration × Week	3.858	6	n.s.	1.040	6	n.s.
Nutrients × Fragment configuration × Week	4.023	6	n.s.	1.656	6	n.s.
Light × Nutrients × Fragment configuration × Week	2.814	6	n.s.	2.491	6	n.s.

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; n.s., not significant ($P \geq 0.05$). GLMM, generalized linear mixed model; LR χ^2 , likelihood-ratio chi-square; df, degrees of freedom; Sig., significance.

Resource context shaped dry weight and biomass allocation

Total and floating-frond DW were affected by light, nutrients, fragment configuration, all two-way interactions, and the three-way interaction (Fig. 3; Table 3). DW accumulation was substantially greater under high light, with the highest values in HH-MF. MF also produced more DW than LF or SF under HL. Under LH and LL, DW remained low and differences among configurations were small, showing that the growth advantage of MF was expressed primarily under high light.

Submerged-frond DW responded strongly to light and fragment configuration. Significant light × fragment configuration, nutrient × fragment configuration, and three-way interactions showed that treatment differences varied among resource environments. The highest submerged-frond DW occurred in HL-MF, followed by HH-MF. The S:F DW ratio also varied with light, nutrients, and fragment configuration; it increased markedly under HL and reached its maximum in MF (Fig. 3; Table 3).

Floating and submerged fronds showed distinct metabolic responses

Floating-frond metabolites responded strongly to resources and fragment configuration (Fig. 4; Table 3). SC and starch were affected by all three main factors and all interactions, with their highest concentrations under HL. Within this environment, SC were highest in MF, whereas starch was highest in LF. Floating-frond FAA were generally higher under high light, especially under HH; light, nutrients, fragment configuration, light × nutrient, light × fragment configuration, and the three-way interaction were significant.

Submerged-frond metabolites displayed a different response pattern. SC were highest under HL and reached their maximum in MF. Starch also accumulated strongly under HL but generally declined from LF to MF and SF. For FAA, the nutrient main effect and all interaction terms were significant, whereas the light and fragment-configuration main effects were not. Thus, measured carbon- and nitrogen-related metabolite concentrations differed between tissues and among treatment combinations.

Table 3
ANOVA results for biomass, allocation, and metabolic traits across experimental factors

Trait	Light		Nutrients		Fragment configuration		Light × Nutrients		Light × Fragment configuration		Nutrients × Fragment configuration		Light × Nutrients × Fragment configuration	
	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.
Total DW	480.11	***	25.83	***	36.35	***	20.42	***	34.34	***	8.60	***	11.14	***
Floating-frond DW	449.89	***	44.38	***	34.34	***	37.51	***	31.98	***	11.00	***	14.00	***
Submerged-frond DW	486.24	***	1.62	n.s.	37.41	***	3.51	n.s.	36.37	***	5.53	**	7.21	**
S:F DW ratio	103.99	***	21.72	***	4.18	*	61.45	***	0.97	n.s.	3.01	n.s.	5.52	**
Floating-frond FAA	676.18	***	290.77	***	7.59	**	81.40	***	3.32	*	1.29	n.s.	10.56	***
Floating-frond SC	88.32	***	70.87	***	12.10	***	95.56	***	12.19	***	15.74	***	12.57	***
Floating-frond starch	485.21	***	124.28	***	16.17	***	145.56	***	5.53	**	9.17	***	4.22	*
Submerged-frond FAA	1.77	n.s.	5.80	*	0.46	n.s.	153.59	***	6.00	**	5.78	**	9.79	***
Submerged-frond SC	51.61	***	95.72	***	7.17	**	84.56	***	1.83	n.s.	3.58	*	3.83	*
Submerged-frond starch	61.05	***	378.68	***	6.86	**	93.52	***	5.38	**	15.96	***	1.86	n.s.

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; n.s., not significant ($P \geq 0.05$). ANOVA, analysis of variance; DW, dry weight; S:F DW ratio, submerged-frond to floating-frond dry-weight ratio; FAA, free amino acids; SC, soluble carbohydrates.

Resource environments produced distinct multivariate trait profiles

The two-dimensional NMDS ordination summarized the traits measured at harvest with low stress (0.054; Fig. 5). The light × nutrient × fragment configuration interaction was significant in PERMANOVA ($R^2 = 0.045$, $P = 0.001$). Multivariate dispersion also differed among the 12 treatment combinations (betadisper, $P = 0.001$), indicating that both centroid location and within-treatment variability contributed to the ordination pattern. High-light treatments were separated from low-light treatments, and HH and HL occupied distinct regions of the ordination. The separation among configuration centroids was visually greater under high light than under low light. Under HH, the MF centroid lay apart from LF and SF, and under HL the three configuration centroids occupied different regions. Under low light, configuration centroids were closer and overlapped more extensively. Because multivariate dispersion also differed among treatments, these patterns reflect both centroid location and within-treatment variability.

Fragment configurations showed contrasting multilayer trait-network organization

PTMN topology showed contrasting numerical patterns among fragment configurations (Figs. 6 and 7). LF contained 23 edges, including 18 interlayer edges, whereas MF and SF each contained 21 edges, including 17 interlayer edges. LF had the highest interlayer edge density (0.486) and the shortest average interlayer path length (1.649), indicating that cross-layer covariation was more broadly distributed across the network. MF had the highest average interlayer clustering coefficient (0.873), showing that trait associations were concentrated within locally connected neighborhoods. SF had the highest module interlayer association (0.838), reflecting a high proportion of interlayer connections among traits within the same modules. These patterns indicate contrasting scales of cross-layer trait covariation among the three configurations.

The traits with the highest interlayer degrees differed among configurations (Fig. 8). In LF, total DW ranked first (6), followed by floating-frond SC and starch (both 5). In MF, floating-frond starch and the S:F DW ratio had the highest values (both 5), followed by total DW and floating-frond SC (both 4). In SF, submerged-frond starch ranked first (5), followed by frond-pair number, ramet number and the S:F DW ratio (all 4).

Discussion

The effect of fragment configuration on recovery depended strongly on resource availability in *S. natans*. MF supported the greatest growth under high-light, nutrient-rich conditions, whereas low light reduced its advantage over LF and SF. The intermediate configuration therefore performed best when resource conditions supported rapid regrowth. Differences in biomass allocation and frond metabolism further showed that resource environments influenced how growth was accompanied by changes in carbohydrate status and tissue investment. PTMNs extended these comparisons by revealing distinct patterns of coordination among growth, allocation and metabolic traits across configurations. Together, these findings show that the growth advantage of MF was resource dependent, while different fragment configurations were also associated with distinct patterns of trait coordination during recovery.

The superior performance of MF under favorable resources is consistent with an intermediate configuration balancing retained material against the number of independent growth units. LF retained larger connected fragments but generated only two units, whereas SF produced six single-node fragments with less material per unit. MF combined three two-node fragments and may therefore have retained sufficient structural and physiological capacity while providing more independent sites for ramet development. Fragment size is known to influence post-fragmentation survival and regrowth in aquatic plants [1], and its effect can vary with environmental conditions [10, 45–47]. In *S. natans*, nutrient availability modifies fragment-size effects on growth [8], while recovery in fragmented *Vallisneria natans* depends on light and nutrient supply [9]. Our results extend this work by showing that, when the initial frond-pair number is held constant, an intermediate way of partitioning that material can outperform both fewer large fragments and many small fragments. The MF advantage is therefore best viewed as a property of the overall fragment configuration.

Light availability determined whether this configurational advantage could be expressed. Under high light, MF increased both frond and ramet numbers and accumulated more DW than LF or SF. Under low light, all configurations remained at comparatively low biomass and their performance converged, consistent with carbon acquisition imposing a common constraint on regrowth. Light is a primary determinant of carbon acquisition and growth in aquatic plants [16], and prolonged light limitation suppresses both morphological and physiological performance [17–20]. Comparable changes in growth and carbon-nitrogen metabolism across light regimes have been reported in submerged macrophytes [21]. An additional feature of the low-light response was that SF could still maintain relatively high ramet production under LL, even though this proliferation did not translate into comparable DW gain. Thus, the capacity to initiate new clonal units and the capacity to build structural biomass were partly decoupled under strong resource limitation, indicating that propagule production alone does not fully represent recovery.

Nutrient supply further determined whether high-light responses were expressed mainly as structural growth or as carbohydrate retention. HH supported the greatest biomass production, whereas HL was associated with higher SC and starch concentrations and a higher S:F DW ratio. When nutrient-supported tissue construction is constrained, assimilated carbon can accumulate in soluble or storage pools rather than being incorporated into new biomass [29, 48–51], consistent with shifts in source-sink balance and carbon partitioning between growth and storage [49, 50, 52, 53]. The contrasting responses of floating and submerged fronds add a functional dimension to this shift. Floating fronds mainly support photosynthetic carbon acquisition, whereas submerged fronds are more directly involved in nutrient acquisition from the water column in *S. natans* [11, 12]. The parallel increase in carbohydrate concentrations and relative allocation to submerged fronds under HL therefore suggests a coordinated adjustment in which carbon was retained while biomass allocation shifted toward water-contacting tissues. The multivariate ordination supported this broader interpretation, with configuration differences being more pronounced under high than low light.

Fragment configuration shaped trait coordination during recovery. LF showed more extensive cross-layer connections among growth, biomass allocation and metabolism, whereas MF was characterized by stronger local clustering across functional layers. In SF, the high module interlayer association reflected a high proportion of interlayer connections among traits within the same modules [37]. These contrasting architectures show that fragment configuration influenced not only individual trait values but also the relationships among plant functions. Previous studies have linked variation in aquatic plant trait networks to light and nutrient availability [34, 54–56]. Our results show that, across the same resource treatments, contrasting fragment configurations were associated with different patterns of trait coordination.

Carbohydrate metabolism and biomass allocation were central to these differences. In LF, total DW was positively associated with frond-pair number, ramet number and floating-frond carbohydrates, linking biomass accumulation and clonal growth with carbohydrate status in photosynthetic tissues (Table S6). This coupling connects our findings with the relationships between carbohydrate traits, growth and network organization reported in other aquatic macrophytes [34, 54]. In MF, floating-frond carbohydrate traits remained highly connected, while the S:F DW ratio emerged as a prominent hub. Its associations with carbohydrate traits in both frond types highlight tissue allocation as an important component of metabolic coordination, a role also identified for allocation traits in other aquatic plant networks [35, 54]. SF showed a different pattern: submerged-frond starch was positively associated with the S:F DW ratio and other carbohydrate traits but negatively associated with frond-pair number and ramet number (Table S6). These opposing associations suggest a balance between starch accumulation and clonal expansion, consistent with the role of carbohydrates in plant storage and growth [29, 30, 34]. Across configurations, carbohydrate metabolism and biomass allocation remained central components of trait coordination, but their connections to biomass accumulation and clonal growth differed.

These findings challenge a simple monotonic expectation that either larger fragments or a greater number of smaller fragments should always recover best. Instead, recovery depends on how clonal material is partitioned and on whether resource conditions support rapid conversion of retained and newly acquired resources into growth. Because aquatic clonal plants occupy heterogeneous habitats and often show substantial phenotypic plasticity [57, 58], this context dependence may influence post-disturbance persistence and spread. Free-floating macrophytes respond strongly to nutrient availability, hydraulic connectivity, and surrounding plant communities [59–61], while vegetative fragments can be transported after disturbance [2]. Fragment configuration and resource context should therefore be considered together when predicting recovery.

Conclusions

Post-fragmentation recovery in *S. natans* depended jointly on fragment configuration and resource availability. MF showed the greatest growth under favorable light and nutrient conditions, whereas low light reduced differences among configurations. Under high light with limited nutrients, recovery shifted from biomass production toward carbohydrate retention and greater relative allocation to submerged fronds. PTMN analysis further showed that fragment configurations differed in the organization of trait coordination, indicating that fragmentation influenced not only recovery performance but also how growth, allocation, and metabolism were integrated. Overall, the advantage of MF was strongly resource dependent, and different fragment configurations followed distinct functional pathways during recovery. These results highlight the importance of considering fragment configuration together with environmental conditions when evaluating the regeneration potential of clonal aquatic plants.

Abbreviations

ANOVA
analysis of variance
DW
dry weight
FAA
free amino acids
FDR
false discovery rate
GLMM
generalized linear mixed model
HH
high light with high nutrients
HL
high light with low nutrients
LF
light fragmentation
LH
low light with high nutrients
LL
low light with low nutrients

MF
moderate fragmentation
NMDS
non-metric multidimensional scaling
PERMANOVA
permutational multivariate analysis of variance
PTMN
plant trait multilayer network
SC
soluble carbohydrates
SE
standard error
SF
severe fragmentation
S:F DW ratio
submerged-frond to floating-frond dry-weight ratio.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

HS, YB, WZ, XL, PL and MZ contributed to the conceptualization and methodology of the study. HS, XL and PL conducted the investigation and prepared experimental materials and resources. YB and MZ contributed to data curation, formal analysis and visualization. HS, WZ and PL drafted the manuscript. All authors contributed to the interpretation and validation of the results, revised the manuscript critically, and approved the final version.

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Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

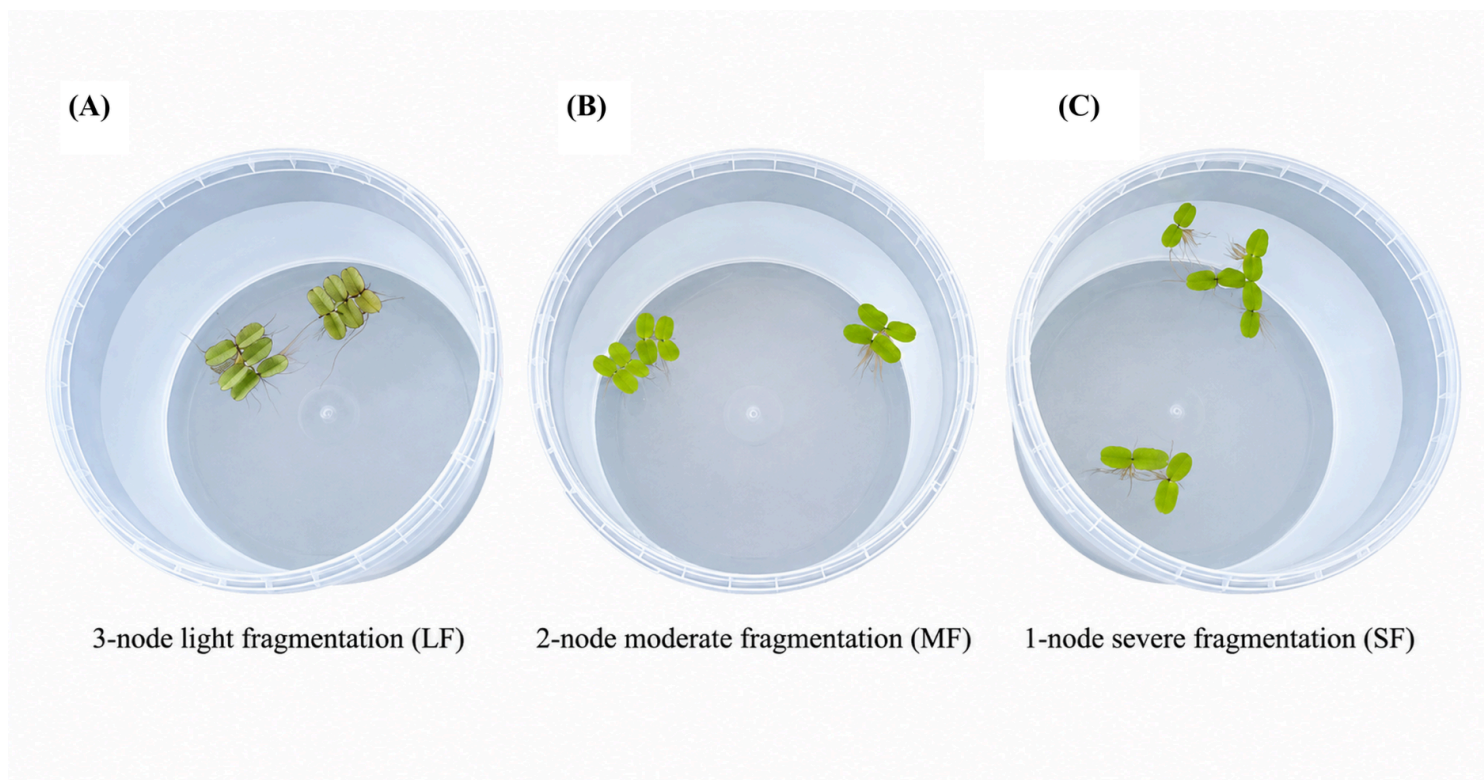


Figure 1

Experimental illustration of the three fragment configurations of *S. natans*. From left to right: light fragmentation (LF), two three-node fragments; moderate fragmentation (MF), three two-node fragments; and severe fragmentation (SF), six one-node fragments. All configurations began with six frond pairs but differed jointly in fragment size and fragment number.

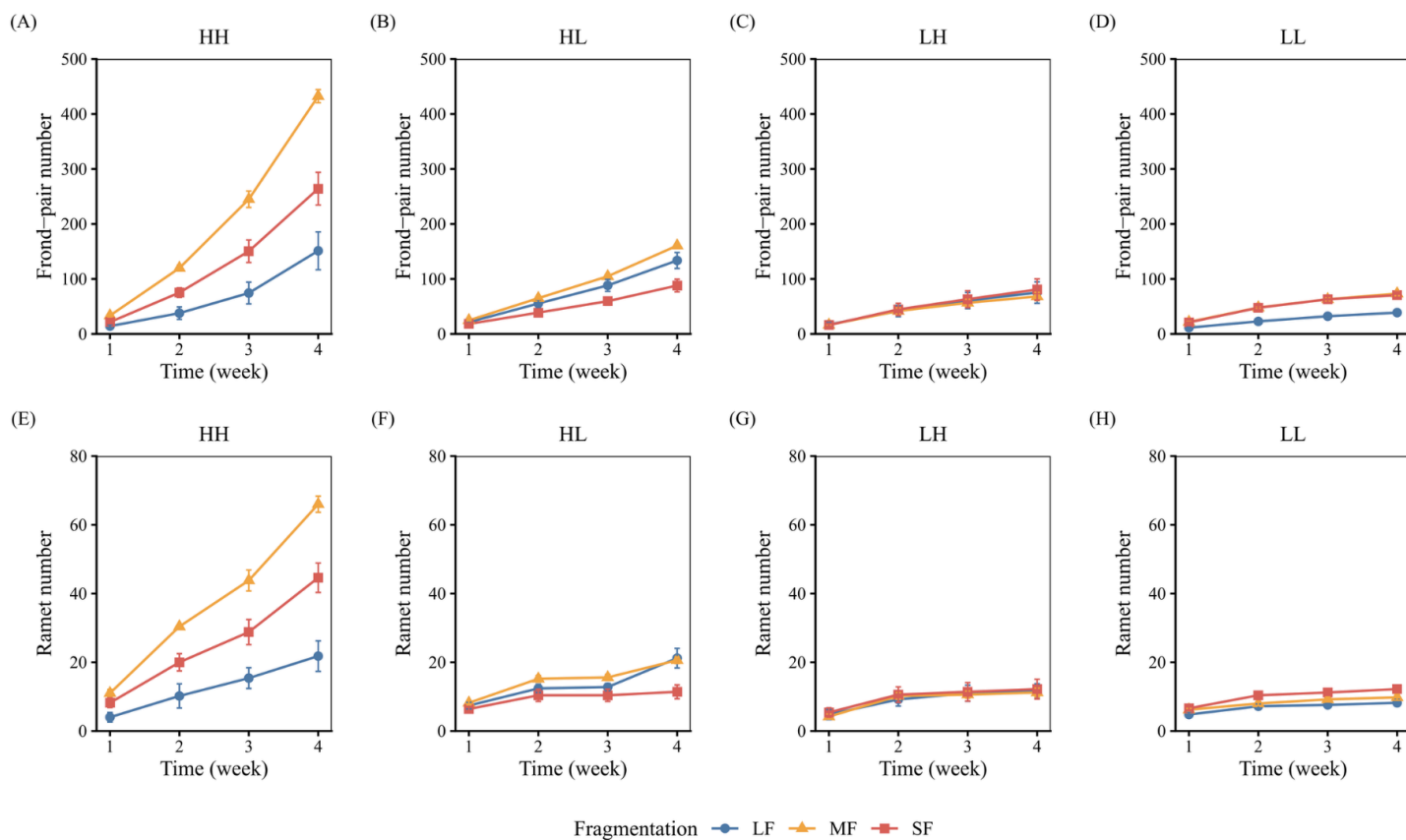


Figure 2

Growth dynamics of *S. natans* across resource environments and fragment configurations. HH, high light with high nutrients; HL, high light with low nutrients; LH, low light with high nutrients; LL, low light with low nutrients. LF, light fragmentation; MF, moderate fragmentation; SF, severe fragmentation. Values are means $\pm$ standard error (SE; $n = 5$).

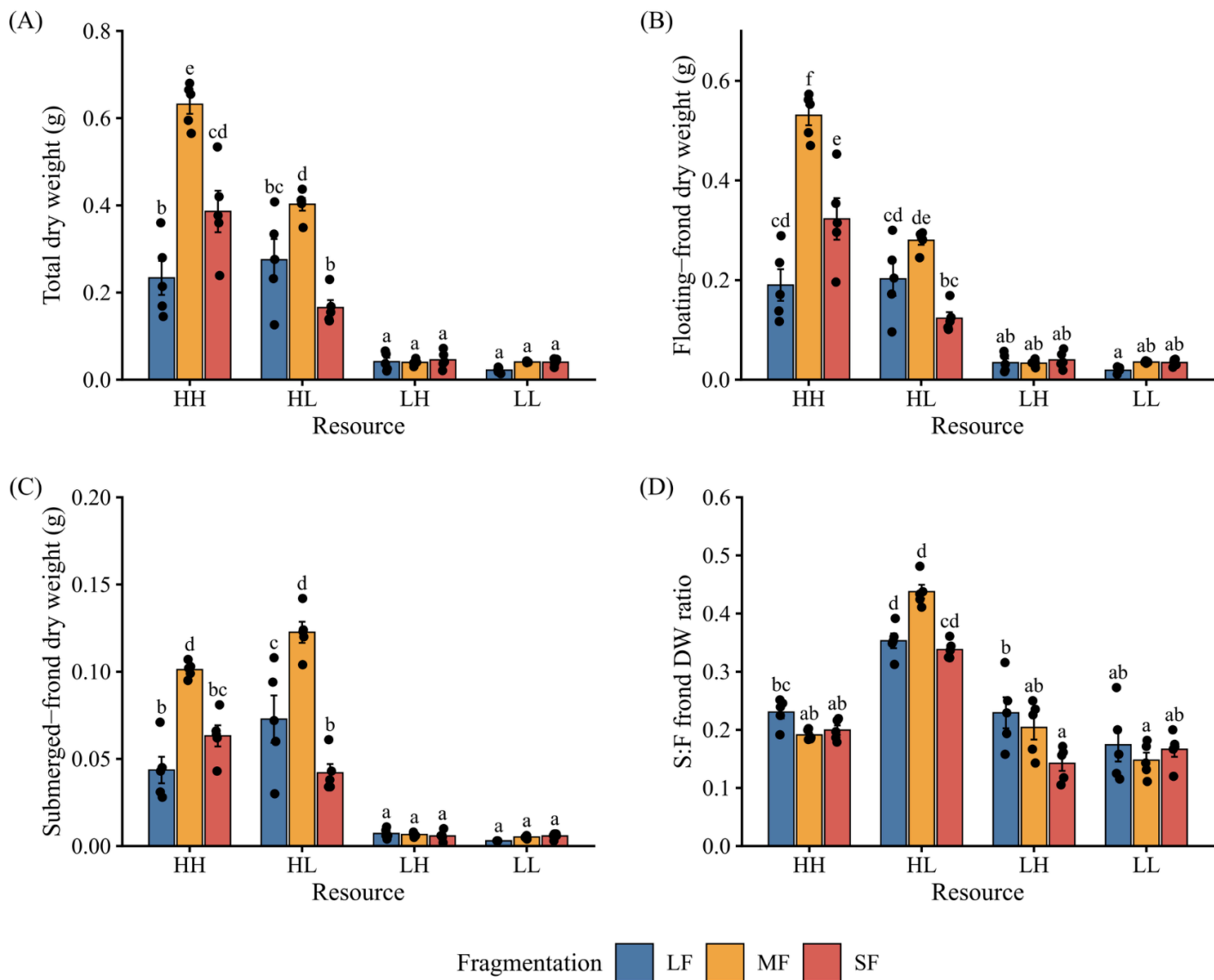


Figure 3

Biomass and allocation of *S. natans* across resource environments and fragment configurations. (A) Total dry weight, (B) floating-frond dry weight, (C) submerged-frond dry weight, and (D) submerged-frond to floating-frond dry-weight ratio. HH, high light with high nutrients; HL, high light with low nutrients; LH, low light with high nutrients; LL, low light with low nutrients. Values are means $\pm$ standard error (SE; $n = 5$). Different lowercase letters indicate significant differences among treatment combinations at $P < 0.05$. LF, light fragmentation; MF, moderate fragmentation; SF, severe fragmentation. DW, dry weight; S:F frond DW ratio, submerged-frond to floating-frond dry-weight ratio (S:F DW ratio in the text).

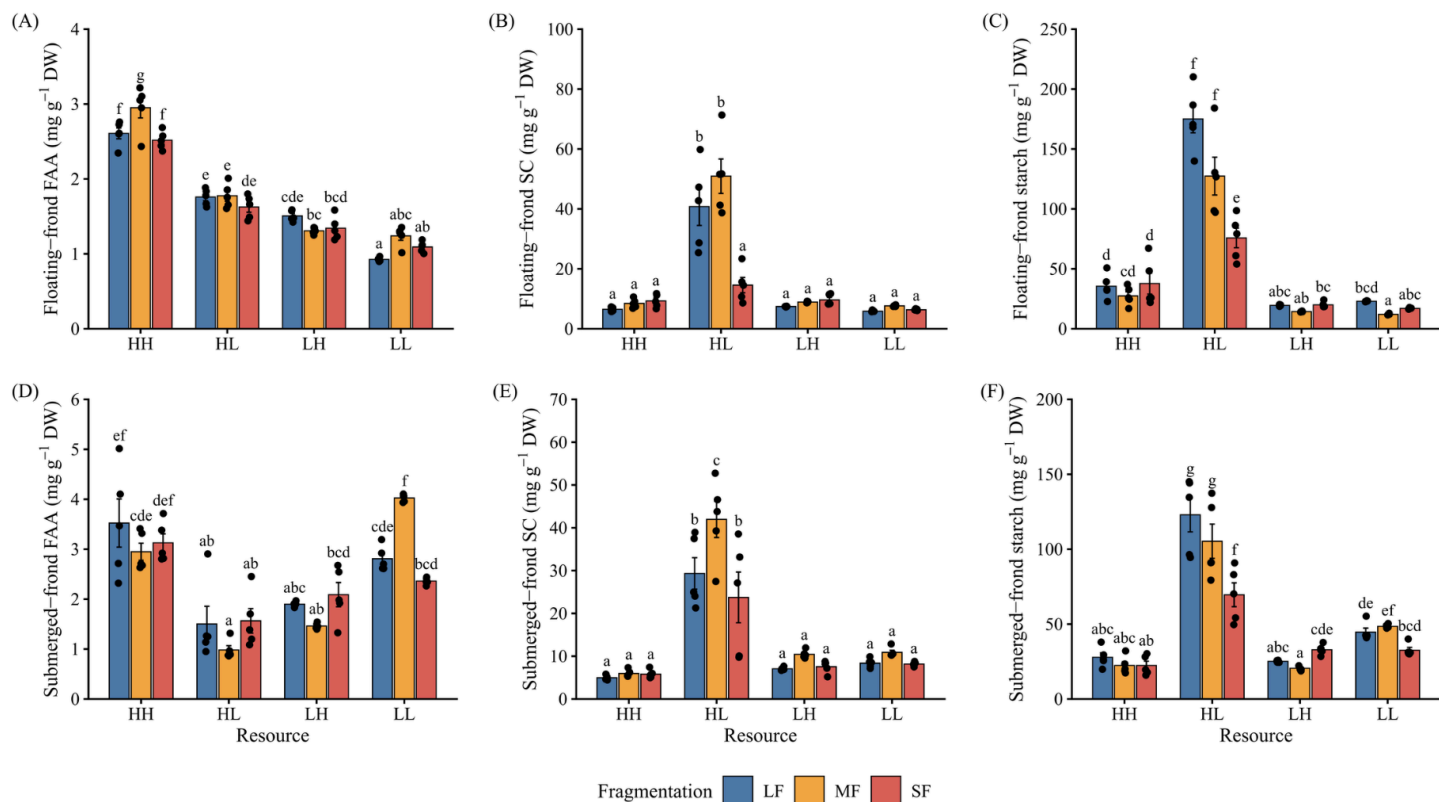


Figure 4

Tissue-specific metabolite responses across resource environments and fragment configurations. (A) Floating-frond FAA, (B) floating-frond SC, (C) floating-frond starch, (D) submerged-frond FAA, (E) submerged-frond SC, and (F) submerged-frond starch. FAA, free amino acids; SC, soluble carbohydrates. Values are means $\pm$ standard error (SE; $n = 5$). Different lowercase letters indicate significant differences among treatment combinations at $P < 0.05$. LF, light fragmentation; MF, moderate fragmentation; SF, severe fragmentation. HH, high light with high nutrients; HL, high light with low nutrients; LH, low light with high nutrients; LL, low light with low nutrients. DW, dry weight.

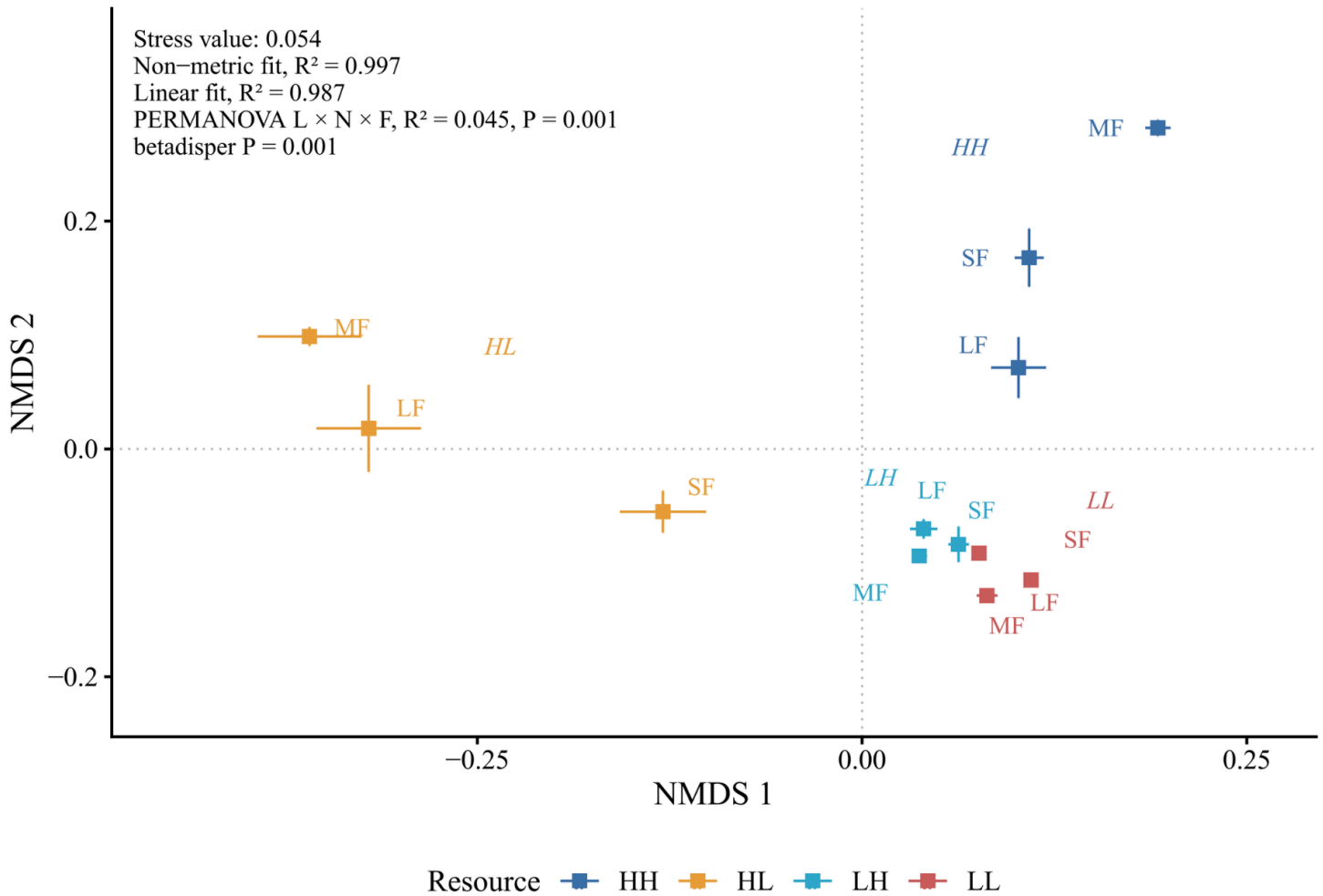


Figure 5

Non-metric multidimensional scaling (NMDS) ordination of traits across resource environments and fragment configurations. Symbols represent treatment centroids, and error bars indicate means $\pm$ standard error (SE). The two-dimensional solution had a stress value of 0.054. The light $\times$ nutrient $\times$ fragment configuration interaction was significant in permutational multivariate analysis of variance (PERMANOVA; $R^2 = 0.045$, $P = 0.001$), and multivariate dispersion differed among treatment combinations (betadisper, $P = 0.001$). LF, light fragmentation; MF, moderate fragmentation; SF, severe fragmentation. HH, high light with high nutrients; HL, high light with low nutrients; LH, low light with high nutrients; LL, low light with low nutrients.

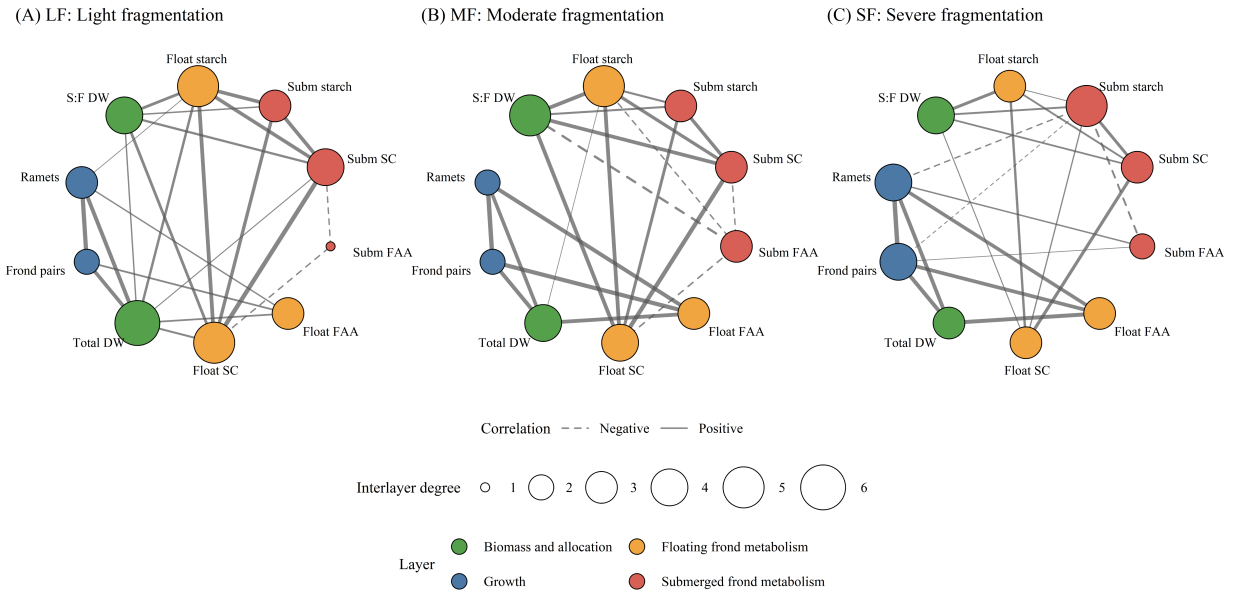


Figure 6

Plant trait multilayer networks across *S.natans* fragment configurations. (A) LF, light fragmentation; (B) MF, moderate fragmentation; and (C) SF, severe fragmentation. Traits were assigned to growth, biomass and allocation, floating-frond metabolism, and submerged-frond metabolism layers. Node colors indicate functional layers, node sizes indicate interlayer degree, solid lines indicate positive correlations, dashed lines indicate negative correlations, and line widths represent absolute correlation coefficients. Each network was constructed from 20 experimental units. DW, dry weight; S:F DW, S:F DW ratio (submerged-frond to floating-frond dry-weight ratio); FAA, free amino acids; SC, soluble carbohydrates; Float, floating fronds; Subm, submerged fronds.

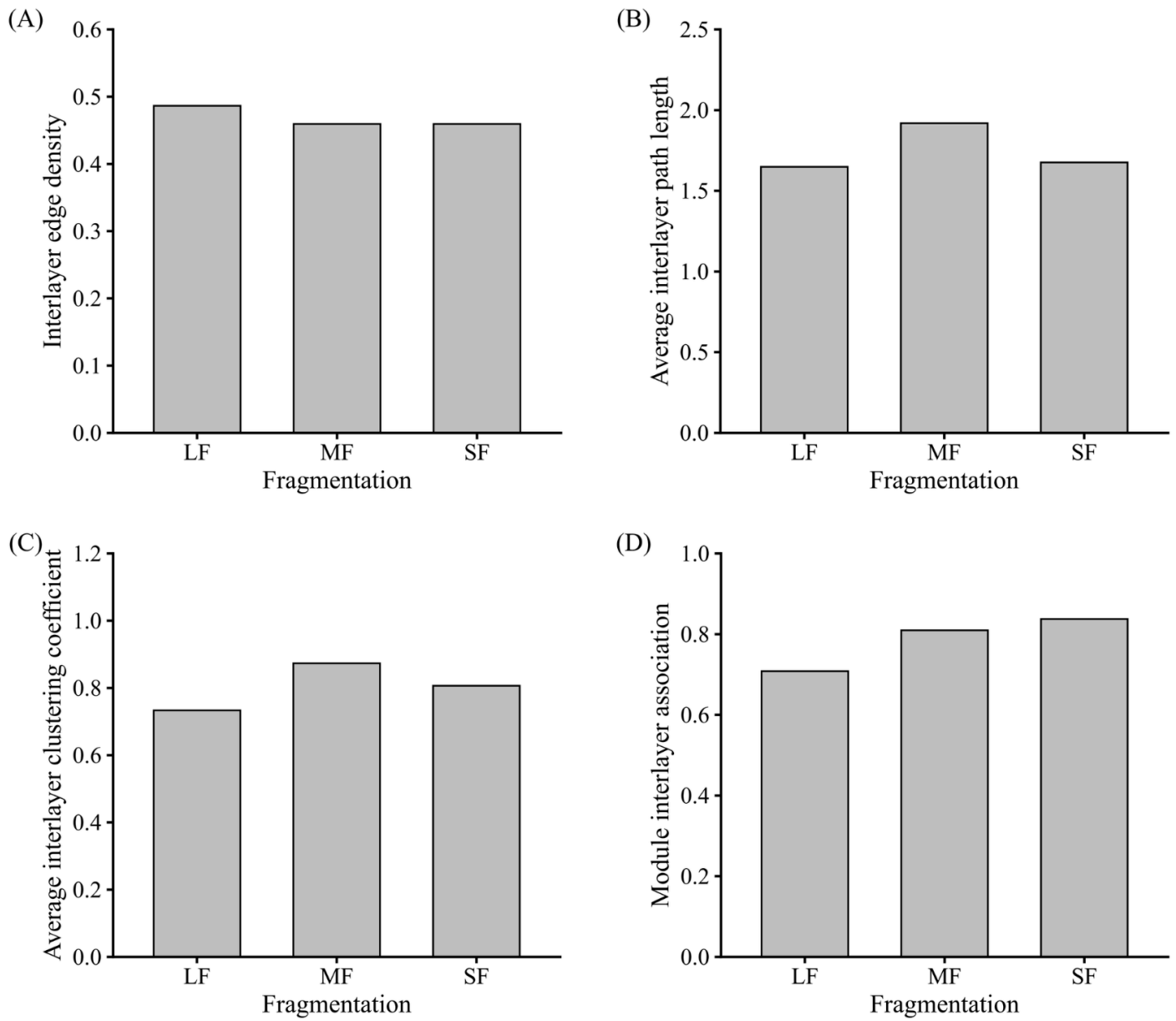


Figure 7

Network-level parameters across fragment configurations. (A) Interlayer edge density, (B) average interlayer path length, (C) average interlayer clustering coefficient, and (D) module interlayer association ($n=20$ experimental units per network). LF, light fragmentation; MF, moderate fragmentation; SF, severe fragmentation.

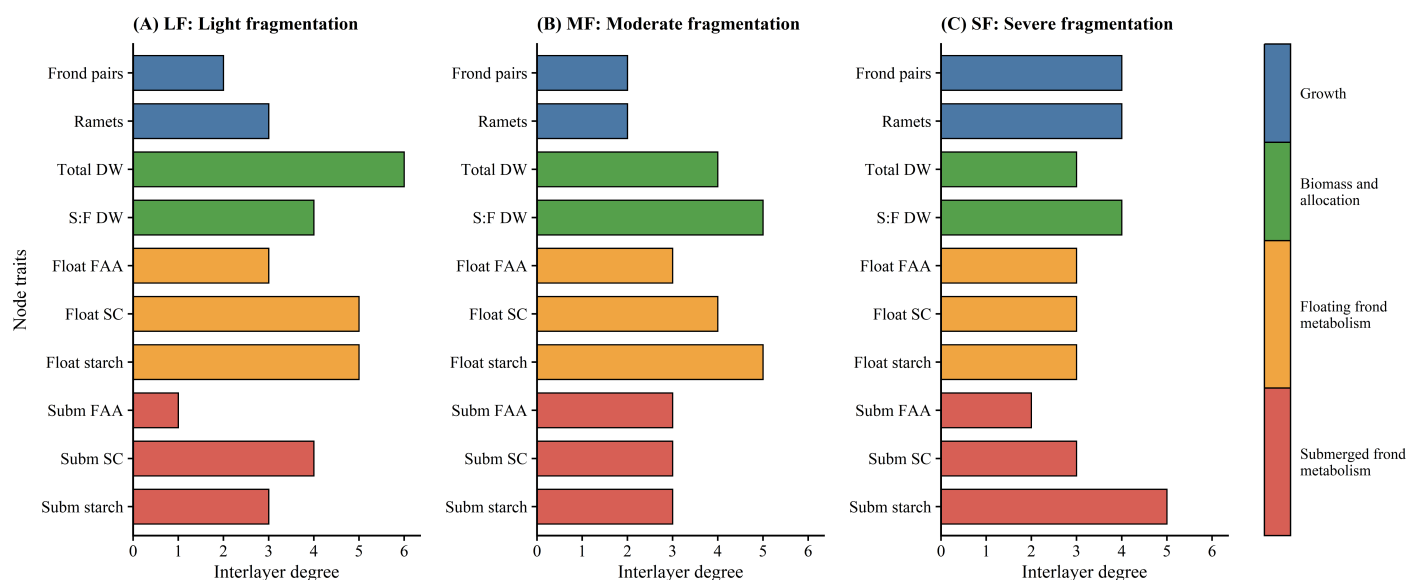


Figure 8

Trait interlayer degree across *S. natans* fragment configurations. Bar length indicates interlayer degree, and colors indicate the four functional layers. DW, dry weight; S:F DW, S:F DW ratio (submerged-frond to floating-frond dry-weight ratio); FAA, free amino acids; SC, soluble carbohydrates; Float, floating fronds; Subm, submerged fronds. LF, light fragmentation; MF, moderate fragmentation; SF, severe fragmentation.

Supplementary Files

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