Assessing the Impact of Invasive *Trapa natans* on Freshwater Microbial Communities in Collins Lake, New York

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KEYWORDS: microbial, motility, freshwater, canopy, bioindicator

OVERVIEW: Trapa natans forms dense floating canopies when introduced as an invasive macrophyte in aquatic ecosystems. This research explores how microbial density, morphotype diversity, and motility are impacted by changes in canopy cover at Collins Lake in New York. Our research employing a unified, globally normalized multi-metric index, provides baseline data for microbial community structure and functional activity that is critical for the restoration of Collins Lake. This research fills a significant knowledge gap that exists in littoral and pelagic communities of freshwater ecosystems.

SUMMARY

Trapa natans, more commonly known as water chestnut, is an introduced aquatic plant that negatively impacts freshwater systems and local microbial communities. This study examines the impacts Trapa natans have on the microbial communities and overall health of Collins Lake, NY. We predicted the aquatic and benthic zones beneath a Trapa natans canopy would have altered microbial integrity compared to a non-invaded control. This was hypothesized to occur due to changes in the outflow of gas and the buildup of organic matter caused by decreased light levels and canopy formation. Samples of water and sediments (n=40) were collected from sites with a Trapa natans canopy and from clear zone control sites. From these samples, morphotype diversity and cellular motility were analyzed to assess microbial integrity by using a Microbial Integrity Index (MII) that ranged from 0.00 to 1.00. Water chestnut canopies were found to have pronounced impacts on microbial integrity at the air-water interfaces and sediment-water interfaces, including altered cellular motility and morphotype diversity. From this study, it can be concluded that a water chestnut canopy creates a localized microbial stress that alters microbial community dynamics within the surrounding environment.

INTRODUCTION

Freshwater ecosystems are impacted by both biotic and abiotic factors and can be complex and dynamic. One such factor is the introduction of non-native invasive aquatic plants. In North American temperate freshwater bodies, an example is the water chestnut or Trapa natans. Introduced as an ornamental plant, Trapa natans forms a dense float-plane that covers and competes for space with other native plants. The effects of this dense cover include a reduction of available light to the water column, and a blockage of gas exchange at the air-water interface.

While the impacts of chestnut overgrowths on aquatic plants and fish populations have been studied, little is known about the local impacts on the microbial community. There are important functions that microorganisms carry out in the degradation of organic matter and the nutrient cycles of freshwater lakes. As plant canopies begin to form, light availability changes, thus affecting aquatic sediments. These changes may drastically affect microorganisms in both the water and the benthic sediment. Understanding how changes in microbial community density, structure and function affect the freshwater ecosystem is critical to assessing the ecosystem as a whole.

This study evaluated the impact of Trapa natans invasion on microbial communities in Collins Lake, New York. We expected that microbial integrity and morphotype diversity would change in the invaded areas when compared to the clear zone uninvaded controls. Based on preliminary data, we saw structural and functional shifts in microbial integrity across both aquatic and sediment habitats as a result of Trapa natans' invasion. For these reasons, researchers suggest that the impacts of Trapa natans in Collins Lake may help inform freshwater management in the state of New York.

RESULTS

Overall Microbial Integrity Under *Trapa natans* Canopies

The aim of this study was to evaluate the biological and functional status of aquatic microbial communities in the presence of invasive plants by combining a series of structural, numerical, and motility metrics of microbial communities into a globally normalized Microbial Integrity Index (MII) on a scale of 0 – 1. Based on microscopic examination of 40 total samples from all four groups, non-invaded clear zone controls displayed a high degree of Integrity in water (0.598 ± 0.050) and sediment (0.529 ± 0.061). In water and sediment under the dense canopy of Trapa natans, a clear shift in structure was noted, as the mean MII values were 0.365 ± 0.063 for canopy water and 0.504 ± 0.038 for canopy sediment (Figures 1 and 4). These results highlight the measurable microbial ecosystem shifts caused by the invasion of floating mat vegetation across aquatic and sediment ecosystems.

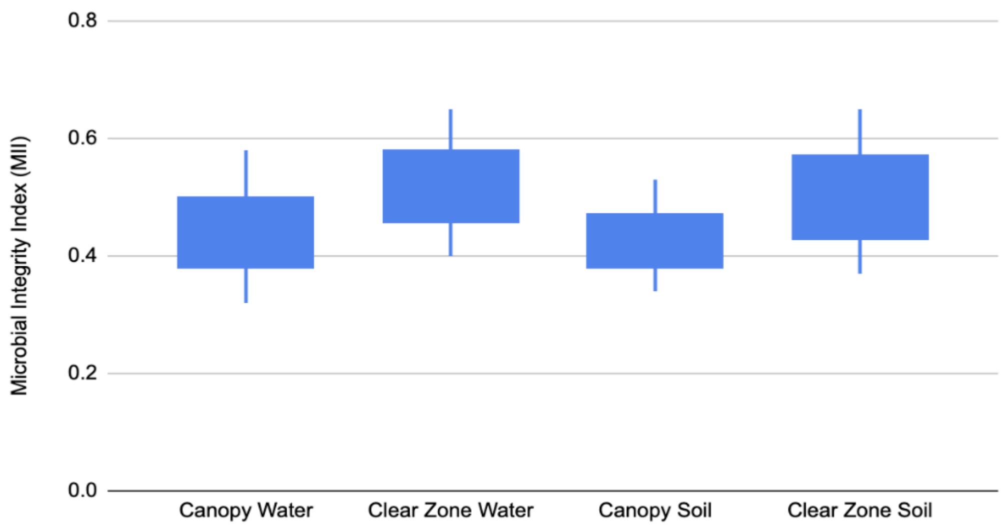

Figure 1. Composite Microbial Integrity Index (MII) across sampling zones.
Mean MII scores comparing non-invaded clear zone controls against *Trapa natans* canopy sites (n=40). Bars and error representations illustrate group distributions.

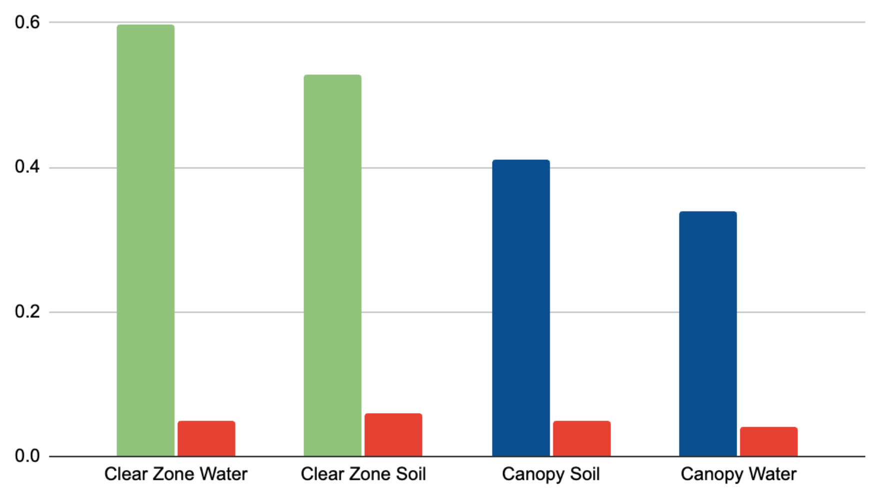

Figure 4. Final Microbial Integrity Index (MII) means comparison.
Overall MII scores across all four experimental conditions: Clear Water, Clear Soil, Canopy Water, and Canopy Soil (n=10 per group). Red bars are Standard Deviations.

Coupled Relationships of Cell Density and Cellular Motility

To characterize the impact of spatial colonization on microbial population size and metabolic activity, we analyzed continuous cell density sub-scores and active directional locomotion within sampling media. Non-invaded clear zone samples exhibited activity profiles with an active motility sub-score of 0.887 in open water and 0.606 in clear soil (Figure 2). Conversely, samples collected from Trapa natans' canopy showed changes in active motility with scores of 0.601 in canopy water and 0.103 in canopy soil (Figure 2, Figure 5). Normalized cell density sub-scores in canopy environments shifted to 0.153 in water and 0.888 in benthic soil, indicative of the accumulation of organic detritus and specific biomass in the stagnant, light-deprived canopy environment.

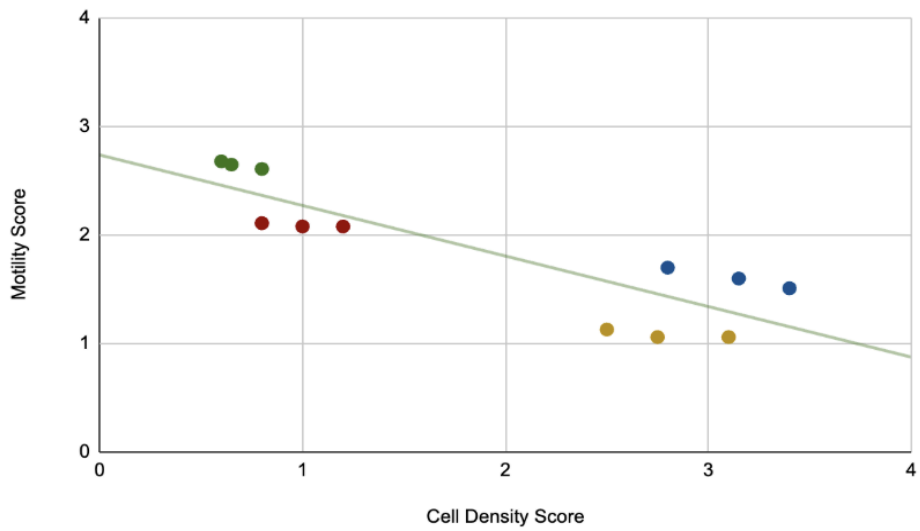

Figure 2. Bacterial cell density versus active motility.
Bivariate correlation mapping calculated cell density sub-scores against active directional motility scores across all four sampling groups, scaled continuously from 0 to 4 for bivariate spatial resolution. Color coding designates environmental conditions: **Clear Zone Water** (green), **Clear Zone Soil** (red), **Canopy Water** (blue), and **Canopy Soil** (yellow). The linear regression line highlights a strong negative relationship between increasing cell abundance and active swimming motility under canopy stress.

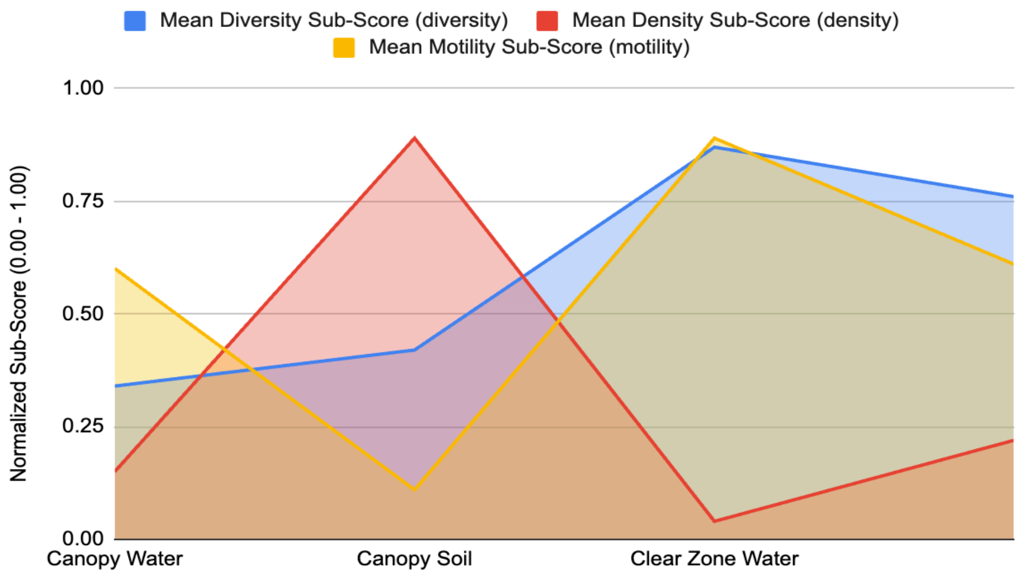

Figure 5. Sub-score breakdown of microbial metrics.
Group means (n=10) for the underlying normalized parameters—Morphotype Diversity, Cell Density, and Motility/Activity—contrasting aquatic water column and benthic soil media under canopy cover versus clear controls.

Structural Simplification of Morphotype Diversity

To study whether canopy-induced environmental stress affects community diversity, we examined the Morphotype Diversity sub-score, which evaluates distinct bacterial cell shapes in a given field of view. Clear zone water (0.865) and clear zone soil (0.790) displayed diverse microbial communities characterized by the co-occurrence of bacilli, cocci, spirilla, and flagellated micro-eukaryotes (Figure 7). Samples under *Trapa natans* exhibited a shift in morphotypic distribution, with sub-scores dropping to 0.340 in canopy water and 0.410 in canopy soil (Figure 3, Figure 5). This shift in structural complexity indicates that the dense surface canopy acts as an environmental filter, selecting for resilient bacterial geometries while suppressing overall morphotypic richness.

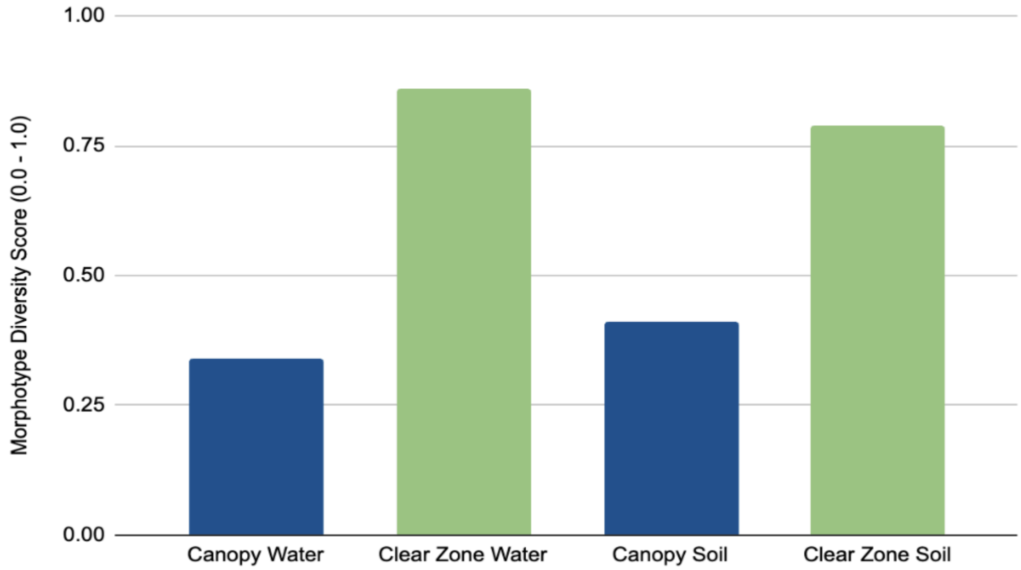

Figure 3. Bacterial morphotype diversity sub-scores.
Structural community richness evaluated across sampling conditions based on visual bacterial geometries per field of view under brightfield microscopy.

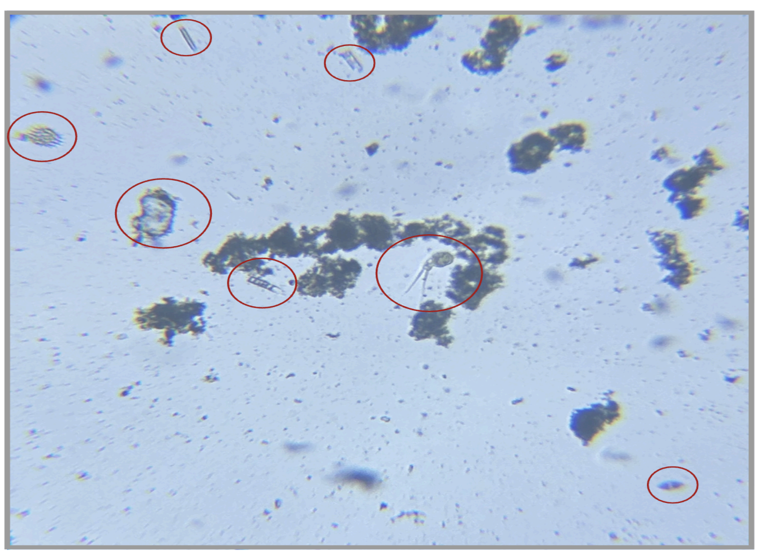

Figure 7. Microscopic evaluation of structural morphotype diversity.
Annotated brightfield micrograph (1000x oil immersion) of a wet-mount sample from the Clear Zone Water (W-CLR) control group. Red callouts highlight distinct structural bacterial geometries and flagellated micro-eukaryotes, illustrating the high baseline morphotype richness ($S_{diversity}$= 0.865) prior to canopy-induced ecological stress.

Habitat Vulnerability Across Aquatic and Benthic Media

To analyze if floating invasive canopies have more of an impact on the open water column versus the sediment layers below, sub-score profiles were assessed across different environmental media. Analyzing relative matrices indicated the aquatic column experienced a 38.9% reduction in overall MII in comparison to control samples. Shifts occurred in cellular motility (decrease by 32.2%) and morphotype diversity (decrease by 60.7%; Figure 5, Figure 6). Analysis of the soil microbial population indicated a 4.7% net change in overall MII in clear soil (0.529) versus canopy soil (0.504). There were reductions by 48.1% and 83.0% in morphotype diversity and cellular motility, respectively (Figure 6). Ultimately, these data support the conclusion that the invasive species of aquatic macrophytes Trapa natans has a significant impact on the entire system both in the aquatic and sediment microhabitats.

Metric	Clear Zone Water	Canopy Water	Water Drop (%)	Clear Zone Soil	Canopy Soil	Soil Drop (%)
Morphotype Diversity	0.865	0.34	60.70%	0.79	0.41	48.10%
Cell Density	0.041	0.153	-273.2%*	0.217	0.888	-309.0%*
Motility & Activity	0.887	0.601	32.20%	0.606	0.103	83.00%
Overall MII	0.598	0.365	38.90%	0.529	0.504	4.70%

Figure 6. Heatmap of microbial metric degradation.
Matrix comparing normalized sub-scores (0.00--1.00) and relative percentage reductions between water column and benthic soil habitats under *Trapa natans* canopy invasion relative to non-invaded controls. Color gradients denote ecosystem status from degraded (red) to healthy (green). *(Note: Negative percentage drops indicate an increase in value from the clear zone to the canopy zone).*

DISCUSSION

The findings of this study suggest that floating macrophytes of Trapa natans dramatically adjust the integrity of the localized microbial communities in Collins Lake, New York. Based on our analysis of cell density, morphotype diversity, and cellular motility assessed by the Microbial Integrity Index (MII), our data suggest that invasive floating macrophytes exert an ecological impact on both the aquatic and benthic sediment habitats.

The main reason for MII reduction in invaded zones is the suppression of cellular motility and structural morphotype richness. The clear zone controls exhibited distinct motility and balanced diversity of bacterial shapes, while canopy-covered zones showed substantial reductions in motility (32.2% in water, 83.0% in soil) and morphotypic simplification (Figures 3 and 5). We believe that this functional and structural decline is attributed to the physical microenvironment created by Trapa natans. The dense surface rosettes build an intricate barrier to sun penetration and deteriorate photosynthesis and develop water column hypoxia or anoxia. In the stagnant and anaerobic environment, it is likely that the flagellar motility is metabolically shut off, and

bacterial strains that are dormant or non-motile are selected.
Additionally, the high input of decaying plant organic matter beneath the canopy likely leads to structural homogenization, allowing saprophytic morphotypes to outcompete sensitive, aerobic bacterial species.

It is also interesting to note that beneath the canopy, the soil exhibited elevated normalized cell density sub-scores (0.888), which largely buffered the soil MII score from decreasing as much as the water column (Figure 6). Soil bacterial cell density may remain elevated in sediment due to the excessive organic shedding from plant canopy. However, since there was a 83.0% decrease in the motility of these soil samples, high cell density in benthic sediment is not a symptom of healthy ecosystems, but rather an accumulation of specialized or stressed biomass that is likely subjected to significant constraints on its metabolism.

These results may have been limited by several factors. The first of these is that wet-mount optical microscopy at fixed magnifications also limits the identification of the specific bacterial taxa to broad visual morphotypes. As a result, our diversity assessment was limited to broad visual morphotypes like bacilli, cocci, and spirilla rather than genetic species. Second, environmental parameters like dissolved oxygen, oxidation-reduction potential, temperature profiles, and the levels of light penetration were not measured in conjunction with microscopy, which also makes our proposed mechanisms of canopy-induced hypoxia and light attenuation highly speculative. Lastly, all the samples were collected from Collins Lake within a single seasonal period. Therefore, dynamics of plant growth, decay, and seasonal variations were not assessed.

These studies help explain that Trapa natans, in addition to the impact observed at the macroscopic level with respect to repositioning aquatic plants, impacts aquatic ecosystems at the microbial level. Understanding microbial shifts and their impacts on the lake ecosystem is important to local management efforts. These efforts include limnological impacts related to the efficiency of nutrient cycling and sediment degradation. Additional studies should consider the effect of canopy cover on the microbial community using 16S rRNA gene sequencing. Also, the microbial community can be examined after—and prior to—invasive plant harvesting efforts by installing continuous dissolved oxygen and nutrient sensors.

MATERIALS AND METHODS

Study Site & Environmental Sampling

Field sampling was conducted at Collins Lake, New York (42.82° N, 73.95° W) to target two contrasting canopy ecological zones: fully closed *Trapa natans* canopy (invaded zone) and open-water regions lacking floating vegetation (clear zone control) (Figure 8). Forty discrete samples were collected, equally distributing 10 samples to each of the four experimental groups: Canopy Water (W-CAN), Canopy Soil (S-CAN), Clear Zone Water (W-CLR), and Clear Zone Soil (S-CLR). A sterile fluid collection apparatus was used to collect water samples from the aquatic water column at a standardized depth of 0.5 m below the water's surface. Benthic soil and sediment samples were collected from the upper 5 cm of the lake sediment using a custom-made 5 ft PVC syringe core sediment sampler to maintain the sediment-water interface during sampling. All samples were transferred to sterile 5 mL conical polypropylene tubes (Fisher Scientific), transported in dark, insulated coolers on ice, and processed in the laboratory within 4 hours of sampling.

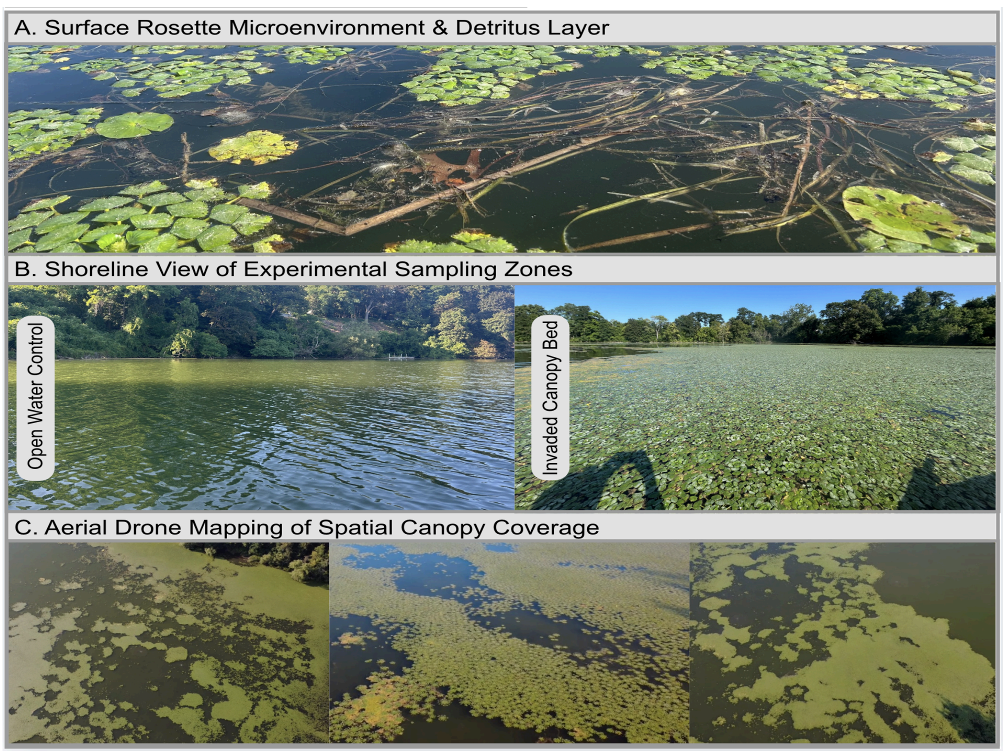

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Figure 8. Visual characterization of *Trapa natans* invasion across spatial scales at Collins Lake, NY.
(A) High-resolution surface view highlighting the dense layer of *Trapa natans* rosettes, floating stems, and accumulated organic detritus at the air-water interface.
(B) Side-by-side shoreline perspective contrasting the clear, uninvaded open-water control zone (left) against the impenetrable, fully closed surface

canopy bed (right).

(C) Multi-angle aerial photography illustrating the spatial distribution, patchiness, and overall canopy density across littoral regions of the lake.

Microscopic Evaluation & Metric Scoring

Wet mount slides began preparation as soon as samples returned to the lab. In preparation for soil samples, 1.0 g of wet sediment was homogenized in 9.0 mL of sterile physiological saline (0.9% NaCl) before slide preparation. A 20 µL sample of each preparation was placed on a standard microscope slide along with a cover slip for viewing. Samples were viewed using oil immersion at 400x and 1000x. Five random fields of view were analyzed per slide and averaged to account for the micro distribution. The following three biological parameters were measured:

1. Cell Density ($x_{density}$): Total microbial cell counts were determined for a standard grid, and a conversion to cell concentration per milliliter (cells/mL) was made.
2. Morphotype Diversity ($x_{diversity}$): Each sample preparation was assessed for the visual presence of various geometries of bacteria (e.g. small rod bacilli, spherical cocci, helical spirilla, and filamentous/colonial structures) and then summed to assess the structural pattern present.
3. Motility & Activity ($x_{motility}$): An estimation of the percentage of cells engaged in directed movement versus Brownian motion was made for a 30 second time period for each field of view.

Microbial Integrity Index (MII) Calculation & Statistical Analysis

To combine disparate units into a unified health metric across both aquatic and benthic habitats without scale distortion, raw measurement values (x) from all 40 samples (combining water and soil datasets globally) were standardized onto a unified 0.00 to 1.00 scale using global min-max normalization:

$$S = \frac{x - x_{\min}}{x_{\max} - x_{\min}}$$

where x_{min} and x_{max} represent the absolute dataset minimum and maximum values for each parameter. Health metrics across aquatic and benthic habitats are more meaningful when scales are unified. The values for raw measurements (x) for all 40 samples (combined datasets for water and soil worldwide) were transformed to a unified scale of 0.00 to 1.00 using global minimum-maximum normalization. To determine the unified global bounds (x_{min}, x_{max}), values for highly concentrated soil bacterial density and less concentrated count for plankton in water were normalised. In doing so, distortion of scale and the assurance that all composite MII scores would stay bound between 0.00 and 1.00 were both satisfied. The transformed sub-scores (S) were then integrated into the Microbial Integrity Index (MII) with (pre-assigned) ecological weights that sum to 1.00:

$$\mathrm{MII} = (S_{\mathrm{diversity}} \times 0.40) + (S_{\mathrm{density}} \times 0.35) + (S_{\mathrm{motility}} \times 0.25)$$

All processing of datasets, group mean calculations, derivations of standard deviations (±SD), and percentage drop comparisons, were done using Python (Pandas/NumPy) and MS Excel. Group metrics were reported as mean ± standard deviation.

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