

Project Attribution

Impossible Aquariums Initiative

This report is part of the Impossible Aquariums Initiative, an independent educational and research effort founded by Vital Barreto Conte (Victor) to advance a scientifically grounded understanding of aquarium ecology. The initiative combines home-lab experimentation, open-source data sharing, and the development of accessible tools for hobbyists seeking to create balanced, sustainable aquatic ecosystems.

The SUBEX-2025-05-01 experiment represents one of the initiative's cornerstone projects, serving both as an educational resource and as preliminary groundwork for the refinement of the SLESS (Symbiotic Litho-Ecological Substrate System) methodology. By documenting each stage transparently—from design to unexpected outcomes—my project aims to encourage other aquarists to apply empirical reasoning, reproducibility, and ecological awareness in their own setups.

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The SUBEX Experiment

Project: Substrate Comparison Experiment (SUBEX)

Experiment Code: SUBEX-2025-05-01

Researcher: Vital Barreto Conte, Victor.

Location: Garage Home Lab, Orlando, FL

Date of Experiment: May 1, 2025 - October 1, 2025

Date of Report: October 11, 2025

Author's Note

This document presents a detailed account of a home-lab experiment born from simple curiosity: to test the marketing claims of popular aquarium substrates under controlled conditions. By design, it is a pilot study rather than a formal academic paper. The goal was to follow a disciplined process, gather meaningful data, and present the findings with full transparency.

During the course of the experiment, I faced several real-world challenges. A gap in data collection became unavoidable due to personal and professional obligations. In addition, the two inert-sand control tanks experienced significant evaporation caused by the high summer heat of a Florida garage. Although I made every effort to maintain consistent water levels using a single source of distilled water, the competing demands of work, study, and daily life occasionally interfered.

I chose to complete and present this report not in spite of these imperfections, but because of them. The unexpected results from the neglected controls—and the lessons learned from the process itself—proved as valuable as the planned outcomes. My hope is that this record, with both its successes and its limitations, will serve as a useful and trustworthy resource for fellow hobbyists. It documents what occurred, what was learned, and how future experiments might be improved.

Abstract

The choice of substrate is one of the most critical factors in establishing a successful planted aquarium, yet hobbyist decisions are often influenced more by marketing claims than by empirical evidence. This study systematically evaluated five widely used commercial substrates—Aqueon Plant & Shrimp, sifted organic compost, UNS Controsoil, Seachem Flourite, and Fluval Stratum—against two inert sand controls, all tested in identical 2.5-gallon aquariums. Performance was assessed over five months by tracking initial water chemistry, long-term plant health, root development, and the diversity of the emerging microfaunal community.

The results revealed a distinct trade-off between vegetative and ecological performance. Seachem Flourite, an inert clay gravel, supported the most vigorous plant growth, producing extensive root systems and healthy foliage. In contrast, Fluval Stratum, a volcanic-ash-based soil, promoted a significantly richer and more abundant micro-ecosystem. During the initial establishment phase, Aqueon Plant & Shrimp provided the most stable environment for sensitive species, exhibiting minimal “melting.” Unexpectedly, the heavy root-feeding plant *Cryptocoryne hudsoni* developed its most extensive root system in the nutrient-poor inert sand control—a likely survival adaptation driven by the decomposition of less resilient neighboring plants.

Overall, the findings indicate that no single substrate is universally superior. The optimal choice depends on the aquarist’s specific goal: maximizing plant biomass or fostering a self-sustaining and diverse aquatic ecosystem.

1. Introduction

1.1 Background

The foundation of every successful planted aquarium lies in its substrate. It fulfills several critical functions: providing physical anchorage for root systems, serving as a primary site for beneficial microbial colonization, and often acting as a long-term reservoir and delivery medium for essential plant nutrients. Modern aquarium hobbyists are presented with an extensive range of commercial substrates, each marketed with claims of superior plant growth or improved water quality. Despite the substrate’s central ecological role, selection is frequently guided by anecdotal experience rather than controlled, comparative evidence.

1.2 Purpose of the Study

This study was undertaken to address that gap by offering an evidence-based comparison of five commonly used commercial substrates and a standard inert sand control. The primary objective was to move beyond marketing claims and empirically evaluate how each substrate performs under identical, carefully controlled conditions. Given the exploratory nature of these questions, the experiment was structured as a pilot study—not only to generate preliminary data

on substrate performance but also to establish and refine a repeatable experimental protocol suitable for future, more rigorous investigations..

1.3 Hypothesis

Based on prevailing hobbyist assumptions, it was hypothesized that the nutrient-rich aquatic soils—UNS Controsoil and Fluval Stratum—would outperform the other substrates in both long-term plant growth and the establishment of a diverse microfaunal community. Conversely, the chemically inert substrates—Seachem Flourite and the inert sand controls—were expected to yield comparatively limited results.

2. Literature Review & Background

A review of scientific and expert hobbyist literature reveals a complex interplay between a substrate's physical properties, its chemical composition, and the resulting ecological outcomes.

2.1 Substrate Classification and Geochemistry

Aquarium substrates are broadly classified by their chemical reactivity and nutrient content.

- **Inert Substrates**, like the quartz sand used in this study's controls, are geochemically stable. Some processed inert substrates, like the clay-based **Seachem Flourite**, are engineered to have a high Cation Exchange Capacity (CEC), which allows them to adsorb nutrients from the water column and make them available to plant roots.
- **Active substrates**, such as the baked soil granules of **Fluval Stratum** and **UNS Controsoil**, are nutrient-rich and chemically active. Composed of volcanic ash or andosols, they contain humic acids that chelate micronutrients and create a buffering system that often lowers and stabilizes pH.

2.2 The Role of Organic Matter and the Benthic Zone

The organic compost tank in this experiment is a simplistic application of some principles from the Walstad Method concept of dirtied tanks. This approach uses a layer of terrestrial topsoil where microbial decomposition provides a slow, steady release of CO₂ and essential nutrients directly to the plant roots. However, the success is critically dependent on the integrity of an inert cap to prevent rapid leaching of organic compounds and a subsequent collapse of water quality.

2.3 Substrate's Influence on Flora and Fauna

The physical structure of a substrate—its grain size and porosity—creates a unique habitat for both beneficial bacteria and a diverse community of microfauna (the "aufwuchs"), including protozoa, rotifers, and crustaceans. These organisms are a critical part of the tank's food web. While some existing literature extensively covers the impact of substrates on plant growth, there is a comparative lack of detailed information on how specific commercial substrates foster these communities. This study, with its focus on microscopic analysis, aims to contribute empirical data to this under-documented aspect of substrate science.

3. Materials and Methods

3.1 Experimental Units

The experiment was conducted using seven identical glass aquariums, each with a total volume of 2.5 gallons ($12.25 \times 8.25 \times 6.25$ inches). All tanks were positioned on a shared metal shelving unit enclosed within a mesh screen inside a garage home lab in Orlando, Florida, to maintain uniform ambient temperature and light exposure. To prevent external contamination and the escape of fauna, each aquarium was equipped with an additional custom-made mesh lid. **Figure 1. Experimental Setup.** The five primary experimental mesh-capped aquariums are positioned on the laboratory metal rack, enclosed by a mesh-screen cage for protection.



Aeration: A central air pump (Tetra 40 Gallons, “Tetra Whisper Air Pump”) supplied aeration to all seven tanks. The airline was divided using a stainless-steel manifold with individual flow-control valves, each calibrated to produce an equalized bubble rate per aquarium, ensuring uniform water circulation and gas exchange.

Figure 2: Aeration Manifold. Six-valve stainless-steel manifold used to equalize airflow from the Tetra Whisper air pump to each aquarium.



Brand: ST.MARY TINO 4.2 ★★★★★ (279)
Grow Light Strip waterproof Grow Lights for Indoor Plants Full Spectrum plant grow lights indoor with Auto ON/Off Timer White Red 120 LEDs Sunlike Grow Lamp for plant light for indoor plants (2)



Lighting: Illumination was provided by a ST.MARY TINO “Grow Light Strip White Red 120 LEDs” fixture suspended above the tanks. The light array delivered a balanced spectrum of white and red LEDs and was controlled by a timer that maintained a consistent 9-hour photoperiod each day. **Figure 3: LED Lighting Fixture.** ST.MARY TINO LED strips used to provide a consistent 9-hour photoperiod with a full spectrum of white and red light.

Temperature: The aquariums were housed in the gated garage from May to October 2025. The space lacked active ventilation or air-conditioning, and ambient temperatures occasionally reached 28 °C (82 °F).

Despite this, the thermal mass of the aquariums and their placement on the metal rack kept the water temperature stable at approximately 25 °C (± 1 °C) throughout the experiment. Temperature measurements were taken using identical submersible mercury thermometers (AquaBlue brand), each with an accuracy of ± 1 °C within the tested range.. **Figure 4: Glass Mercury Thermometers.** AquaBlue brand thermometers used to monitor water temperature in each aquarium.



3.2 Substrate Treatments

A total of seven substrate conditions were established, with one condition per aquarium. Manufacturer preparation and direction protocols (e.g., rinsing, layering) were followed for all commercial products to ensure consistency.

Four Commercial Substrates.

- I. Aqueon Plant & Shrimp Substrate
- II. UNS Controsoil
- III. Seachem Flourite Black
- IV. Fluval Plant & Shrimp Stratum

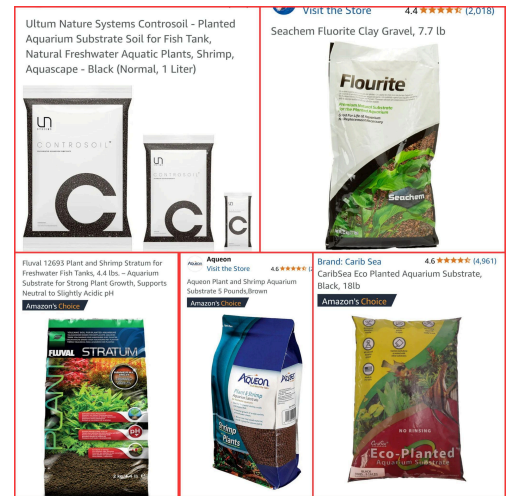


Figure 5: Commercial Substrates Tested. Original packaging of the four commercial substrates used in the experiment: UNS Controsoil, Seachem Flourite, Fluval Stratum, and Aqueon Plant & Shrimp Substrate.

One Organic Substrate

- I. R&M Organics Premium Organic Compost, sifted to remove large particulates. This layer was capped with a 5 cm mid-layer of coarse inert sand (Aqua Natural Diamond Black Quartz, Day 1), followed by the addition of a 1 cm fine inert-sand cap (Quikrete Play Sand) later in the experiment (Day 23)..



Figure 6: Organic Compost. R&M Organics Premium Organic Compost used for the DIY soil-based treatment (Subex 2).

Aqua Natural Diamond Black Quartz inert sand was used for both controls.

- I. **Inert Sand Control (+)** — Inert quartz sand that received the standard microbial inoculation.
- II. **Inert Sand Control (–)** — Inert quartz sand with no microbial inoculation, serving as a sterile baseline.



Figure 7: Play Sand. Quikrete Play Sand used as the fine-sand cap for the organic-compost tank.

Figure 8: Inert Quartz Sand. Aqua Natural Diamond Black Quartz sand is used as the primary medium for the control tanks and as the coarse mid-layer in the organic-compost tank.

3.3 Water Preparation

The initial water for all aquariums consisted of a standardized 1:1 mixture of conditioned municipal tap water and distilled water. This blend was formulated to establish a baseline with moderate mineral content—approximately GH 4–5 °dGH and KH 3 °dKH—thereby enhancing the sensitivity of subsequent measurements to substrate-induced chemical shifts. Seachem Prime® was used as the dechlorinating agent for all preparations. Complete water changes were performed on Day 1 and Day 25 using the same standardized 1:1 blend. To compensate for evaporation during the remainder of the experiment, only distilled water was added to maintain consistent solute concentration.

3.4 Flora and Fauna Selection

Initial Planting (May 10)

Each aquarium was planted with an identical cohort consisting of two *Cryptocoryne hudsoni*, five *Sagittaria subulata*, and two *Bacopa caroliniana*.



Figure 9: Initial Plant Batches. The three primary plant species as received from the supplier (Aquascaping Supply Store): *Bacopa* (top left), *Cryptocoryne* (top right), and *Sagittaria* (bottom).



Figure 10: *Sagittaria subulata* Preparation. Individual stems separated from rock wool plugs for accurate counting and biomass allocation.

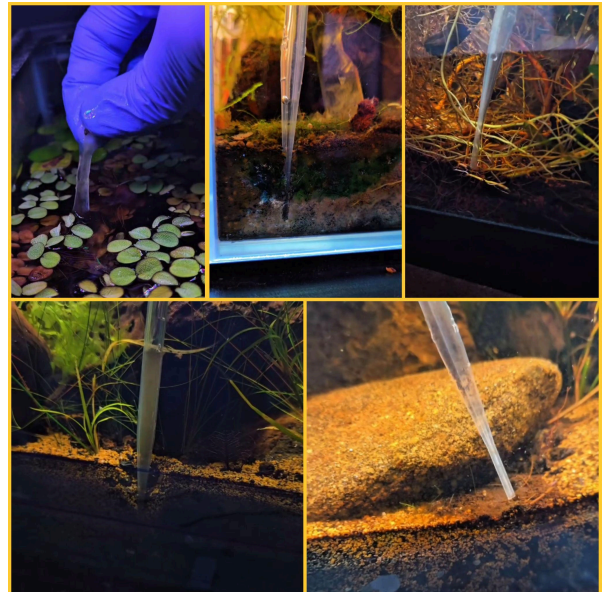


Figure 11: *Cryptocoryne hudsoni* Preparation. Individual plantlets separated and paired by similar size to ensure consistent starting conditions in each aquarium.

Microbial and Faunal Inoculation: Except for the sterile control, each aquarium was inoculated with 25 mL of a standardized bacterial blend derived from three established aquariums. The samples were passed through a 250- μ m mesh to isolate a broad, general community of microorganisms while excluding larger organisms that could occur sporadically and confound results.

In addition, a small starter culture of two scuds (*Gammarus* sp.), two green hydras (*Hydra viridissima*), and eight ostracods (seed shrimp) was added to each inoculated tank. The combined source samples were placed in a 250-mL glass beaker, stirred, and the sedimented material was collected and microscopically analyzed to confirm microbial activity before use.

Figure 12: Microbial Inoculant Collection. Samples for the bacterial blend were collected from multiple established aquariums to ensure a diverse starting biome.



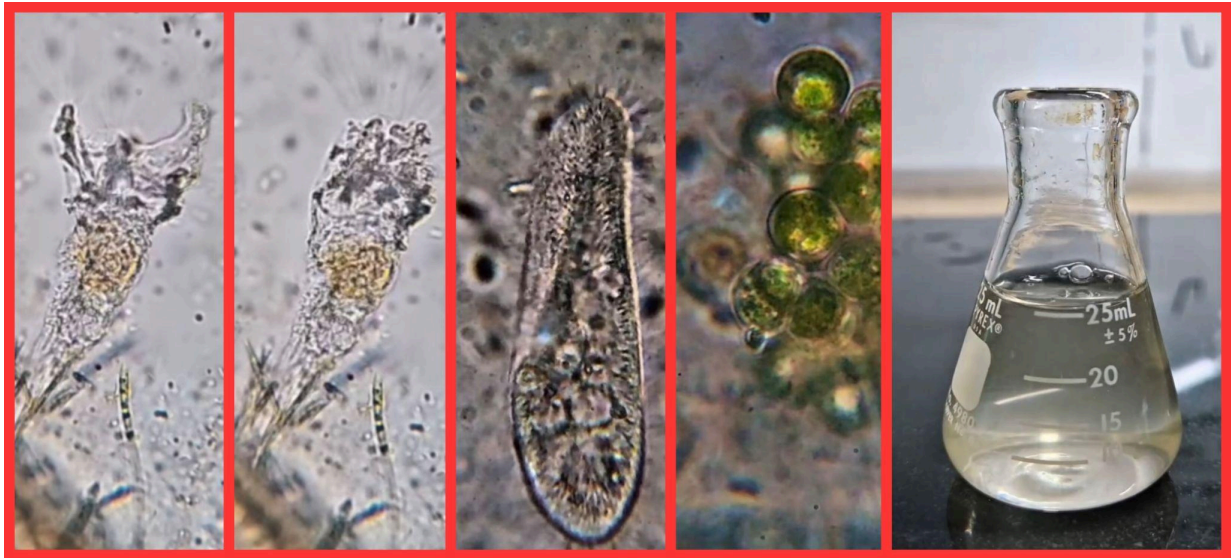


Figure 13: Microbial Blend Analysis and Final Product. Collage showing representative life forms observed in the inoculant, including rotifers (far left), a ciliate (center), and algae (right). The 25-mL Erlenmeyer flask (far right) contains the standardized inoculation dose for each aquarium.

Second Planting (May 23): To increase biological diversity, each aquarium was supplemented with approximately 1.5 g of flame moss, two clumps of the floating fern *Salvinia minima*, one *Rotala sp.*, and one unidentified plant species (UNK).



Figure 14: Second Planting Preparation. From left to right: *Rotala rotundifolia* stems, stems of the unidentified (UNK) species, and weighed portions of flame moss.



Figure 15: *Salvinia minima* Preparation. Clumps of the floating fern separated by size to ensure equal distribution among aquariums.

3.5 Data Collection and Instrumentation

Water Chemistry: Water parameters—including nitrate, nitrite, and ammonia ($\text{NH}_3/\text{NH}_4^+$, NO_2^- , NO_3^-)—were measured twice using both API and Seachem liquid test kits. General hardness (GH) and carbonate hardness (KH) were measured using API kits exclusively. Water pH was monitored using three complementary methods: an API liquid test kit, GE Healthcare Life Sciences Whatman® pH test strips, and a MEXYBE 2025 digital pH/TDS meter (4-in-1 model). Total Dissolved Solids (TDS) were measured with the same digital instrument to ensure internal consistency across reading - TDS meter (MEXYBE 2025 all-new 4 in 1 tds meter digital water tester).

Photographic Documentation: All photographs and videos were captured using smartphone cameras (Samsung Galaxy S25+ and Samsung Galaxy S21). The devices were chosen for their consistent color calibration and focus capability in both macro and standard lighting conditions.

Microscopic Analysis: Substrate and water samples were examined using an AmScope B490B compound microscope. Observations were conducted under both brightfield and darkfield illumination, with magnifications ranging from 40× to 2000×. Microscopic inspection was used to identify and record microbial activity, biofilm development, and substrate-surface colonization.

Substrate Integrity Analysis: At the conclusion of the experiment, substrate samples from each aquarium were photographed and examined microscopically to assess granule stability, particle breakdown, and structural changes relative to unused, brand-new samples. These observations provided a visual comparison of material degradation across substrate types.

Table 1: Water Parameter Fluctuations (First 17 Days). Graphs showing the pronounced spike in nitrogenous compounds (NO_2^- , NO_3^- , NH_4^+) and Total Dissolved Solids (TDS) in the Organic Compost treatment relative to the stability of the commercial substrates.

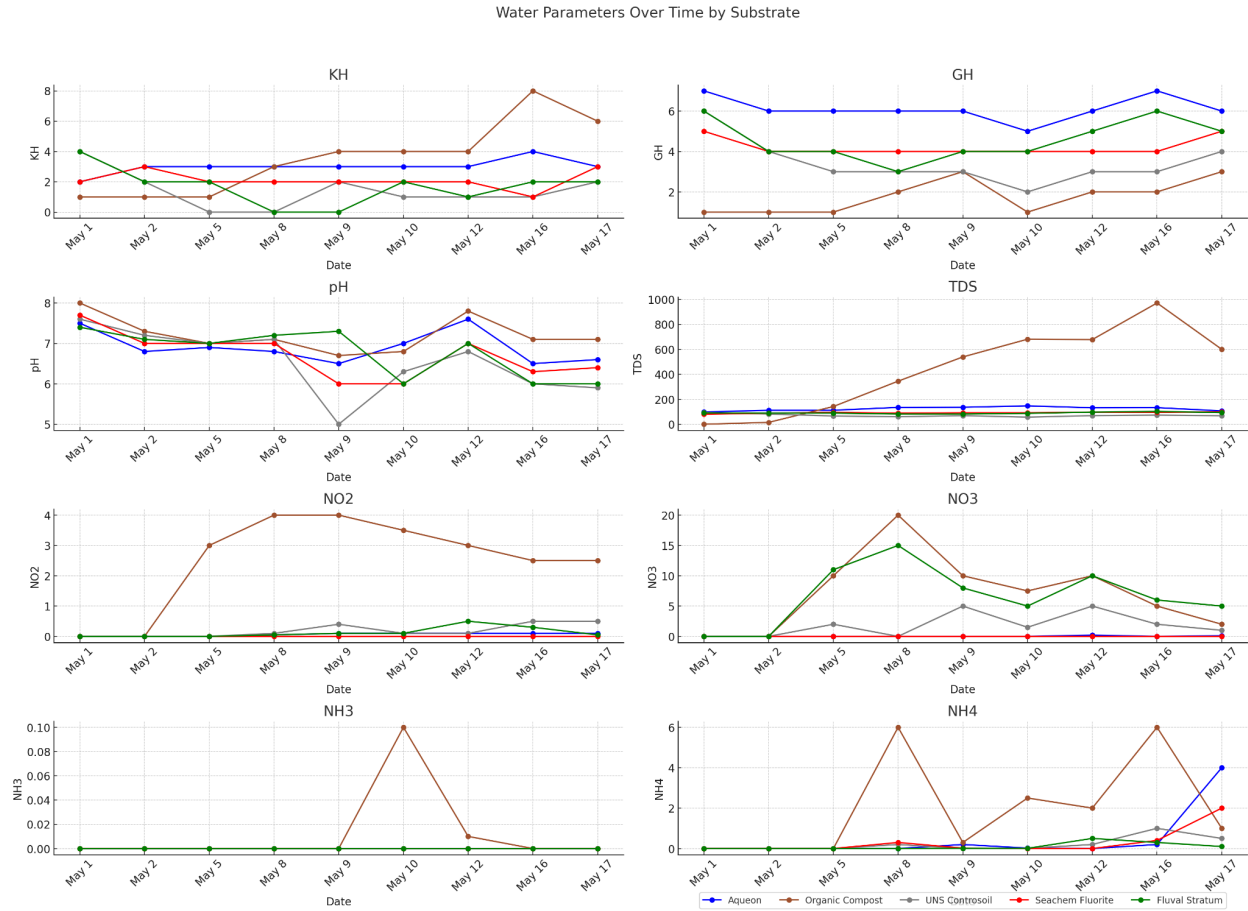


Figure 16: Water Parameters Over Time by Substrate. Graphs highlighting the significant increase in nitrogen compounds and TDS in the Organic Compost tank compared with the relative stability of the commercial substrates.

4.1.2 Initial Plant Acclimation - Initial Planting

Plant responses varied markedly among substrates during the first week. Within 168 hours (7 days) of the initial planting, *Cryptocoryne hudsoni* and *Sagittaria subulata* in the Aqueon Plant & Shrimp Substrate (Subex 1) were the only plants maintaining structure and coloration. In contrast, plants in the UNS Controsoil, Seachem Flourite, and Fluval Stratum aquariums exhibited visible stress, including fading color and tissue “melting.” Plants in the Organic Compost tank declined rapidly. The stem-feeding *Bacopa caroliniana* remained largely unaffected by substrate type across all treatments.

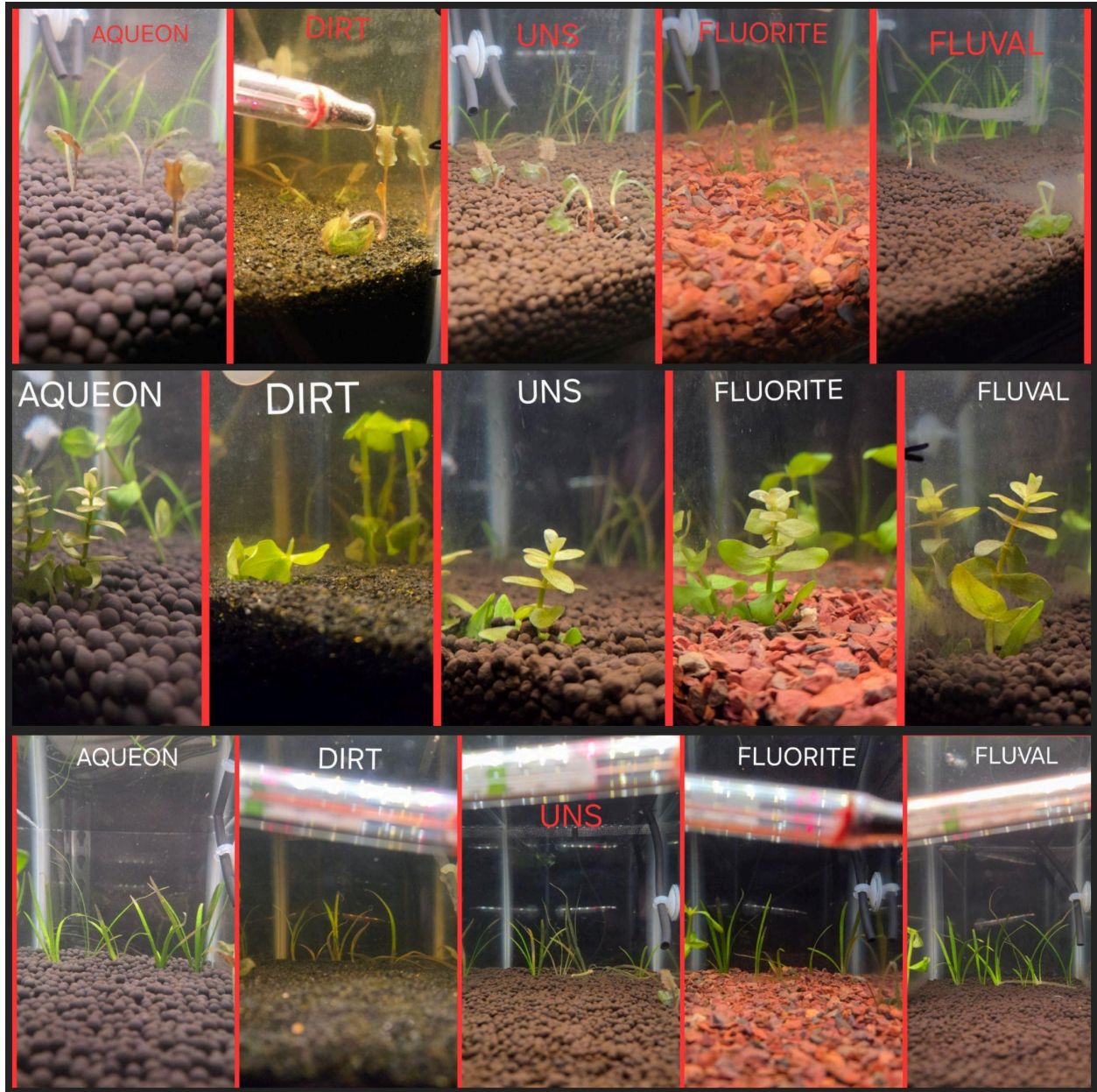


Figure 17: Comparative Plant Health on Day 17. Visual comparison of *Cryptocoryne* (top), *Bacopa* (middle), and *Sagittaria* (bottom) across the five primary treatments. Note the plant stability in the Aqueon tank versus the melting observed in others.

4.1.3 Early Microfauna Development

By May 20, distinct microfaunal communities had become visible to the naked eye in each tank, indicating the onset of ecological establishment.

The UNS Controsoil aquarium exhibited the greatest variety of microscopic organisms and crustaceans such as scuds.

The Organic Compost tank contained the highest density of microorganisms but lacked larger crustaceans.

The Seachem Flourite aquarium was characterized by numerous worms (annelids) and decaying plant matter, corresponding with the melting of vegetation. No scuds were observed in this tank.

The Fluval Stratum aquarium, however, showed increased numbers of ostracods and scuds, suggesting more advanced microfaunal colonization.

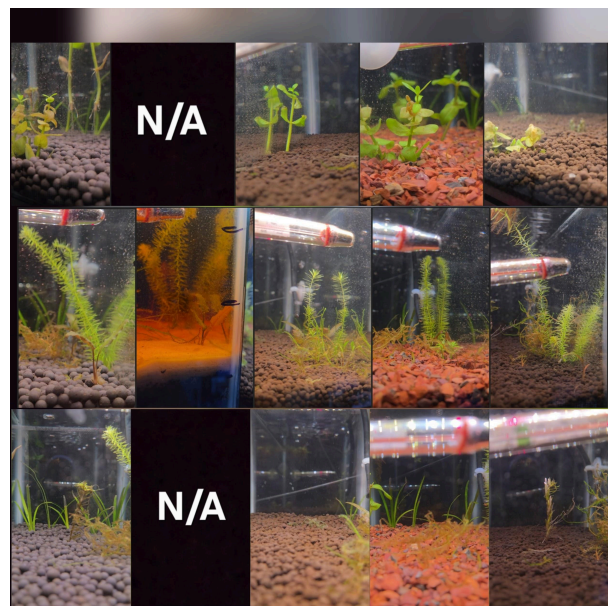
4.2 Data Gap Acknowledgment

It is important to note that quantitative water-parameter logging was discontinued after May 17, 2025. A significant data gap occurred between June 11 and September 9, 2025, during which no quantitative or qualitative measurements were recorded. These omissions were due to time and resource constraints and should be considered when interpreting subsequent results.

4.2.1 June Plant Qualitative Analysis

A qualitative observation session was conducted on June 11, 2025, assessing *Bacopa caroliniana*, the unidentified (UNK) species, *Sagittaria subulata*, and *Rotala sp.* across all substrate treatments. N/A designations were applied where plants were entirely absent—most notably in Subex 2 (Organic Compost).

The UNK species from the second planting exhibited vigorous growth in all aquariums, with the longest and most developed individuals occurring in the Aqueon, Organic Compost, and Fluval Stratum tanks. In contrast, individuals grown in UNS Controsoil and Seachem Flourite demonstrated new shoot formation but lower, more compact growth. **Figure 18. Comparative Plant Health on Day 42. Visual comparison of *Bacopa* (top), the UNK species (middle), and *Sagittaria* (bottom). Panels marked N/A denote plant absence in the Organic Compost tank.**



Bacopa caroliniana showed varied adaptation across substrates:

- I. Subex 1 (Aqueon): moderate adaptation with minimal vertical development.
- II. Subex 3 (UNS Controsoil): stagnation with limited new growth.
- III. Subex 4 (Seachem Flourite): clear and consistent development.
- IV. Subex 5 (Fluval Stratum): noticeable decay and tissue loss.

Sagittaria subulata had disappeared from Subex 2 and Subex 5, with a single underdeveloped individual surviving in Subex 3. Individuals in Subex 1 and Subex 4 retained structure and displayed limited upward growth. Moss patches were present in all aquariums, while floater species were not assessed during this phase.

4.3 Final Comparative Analysis: High-Level Overview

The final assessment revealed a clear divergence in the performance of the tested substrates. The following subsections describe the state of each aquarium at the conclusion of the experiment, based on observations and data collected between September 23 and October 1, 2025.

4.3.1 Visual Development of Aquariums Over Time

The five primary experimental aquariums showed distinct visual development throughout the five-month study. The images document the progressive decline of the Organic Compost tank and the steady increase in plant biomass within the Seachem Flourite and Fluval Stratum tanks. Subex 3 (UNS Controsoil) and Subex 4 (Seachem Flourite) consistently maintained clear water, while Subex 2 (Organic Compost) became dark and required frequent full water changes due to rapid turbidity increase. Subex 1 (Aqueon) and Subex 5 (Fluval Stratum) showed minor algae buildup on glass, which slightly reduced water clarity without affecting overall health.



Figure 19a: May 1, 2025 – Horizontal shot of all commercial-substrate and organic-compost aquariums on Day 1.



Figure 19b: May 23, 2025 – Horizontal shot of all aquariums on Day 23.



Figure 19c: June 11, 2025 – Horizontal shot of all aquariums on Day 42.



Figure 19d: September 25, 2025 – Horizontal shot of all aquariums on Day 145.

College Summary – Visual Development of Aquariums Over Time. Time-lapse comparison (top to bottom: Day 1, Day 23, Day 42, Day 145) showing the progressive failure of the Organic Compost tank and the increase in plant biomass within the Flourite and Fluval Stratum tanks.

4.3.2 Comparative Plant Growth and Root Health

Two representative species were collected from the commercial-substrate series (*Cryptocoryne hudsoni* and *Bacopa caroliniana*) for metric and morphological comparison.

Control and organic treatments are discussed qualitatively due to limited survivorship.

Cryptocoryne hudsoni

- I. Aqueon (Subex 1): Two plants survived with short, thin roots and very small leaves. Root systems were shallow and sparse, indicating limited nutrient uptake and poor anchoring.
- II. Organic Compost (Subex 2): One surviving plant exhibited short but sturdy roots radiating in a compact “palm-like” pattern at the stem base, suggesting stress adaptation to anaerobic or unstable substrate conditions.
- III. UNS Controsoil (Subex 3): The single specimen displayed extensive root development, with multiple lateral root shoots and abundant foliage of large size. This tank produced the greatest root mass relative to plant number.
- IV. Seachem Flourite (Subex 4): Both plants survived and showed vibrant coloration, upright leaf posture, and multiple new leaf shoots per stem. Root systems were well branched, moderately long, and evenly distributed.
- V. Fluval Stratum (Subex 5): Plants developed very long, slender roots darkened by substrate pigmentation. Leaves were long, undulated, and numerous, reflecting robust growth under slightly softer substrate conditions.



Figure 20: Final Comparative Analysis of *Cryptocoryne hudsoni*. Side-by-side comparison of extracted plants from the five commercial substrates.

Bacopa caroliniana

Only three substrates supported surviving *Bacopa* individuals.

- I. UNS Controsoil (Subex 3): One short, linear stem with thin leaves and minimal root branching, indicating low nutrient availability or compacted structure.
- II. Seachem Flourite (Subex 4): Both specimens thrived, forming tall stems with expanded leaf spacing and multiple root offshoots. Growth rate was highest among all *Bacopa* samples.
- III. Fluval Stratum (Subex 5): One plant survived, mirroring the vigor of the Seachem sample but with darker root coloration attributed to substrate staining.



Figure 21: Final Comparative Analysis of *Bacopa caroliniana*. Comparison of surviving plants showing morphological differences in stem elongation, leaf density, and root branching.

Comparative Interpretation

Across species, Seachem Flourite yielded the most balanced vegetative and root development, while Fluval Stratum promoted the longest but thinnest roots, emphasizing elongation over branching. UNS Controsoil favored dense, exploratory root systems but showed reduced overall shoot height. Organic Compost favored localized, stress-resilient root morphology at the cost of leaf production. Aqueon, though stable, produced minimal root and foliage growth under the same conditions.

4.3.3 Comparative Substrate Integrity

Substrate longevity was evaluated through visual and microscopic examination at the conclusion of the experiment. Results revealed distinct differences in physical stability among materials (Figures 22–28).

The inert substrates—Seachem Flourite (Figure 27) and Aqueon (Figure 24)—showed no measurable breakdown, retaining their original shape and surface texture. In contrast, the soil-based substrates displayed visible degradation.

Fluval Stratum (Figure 28) exhibited moderate surface wear, with granules becoming more rounded and porous, indicating slow erosion. UNS Controsoil (Figure 26) experienced the most significant structural degradation, marked by extensive cracking and fracturing. This mechanical breakdown likely correlates with the chemical fluctuations and biological trends observed in those systems.

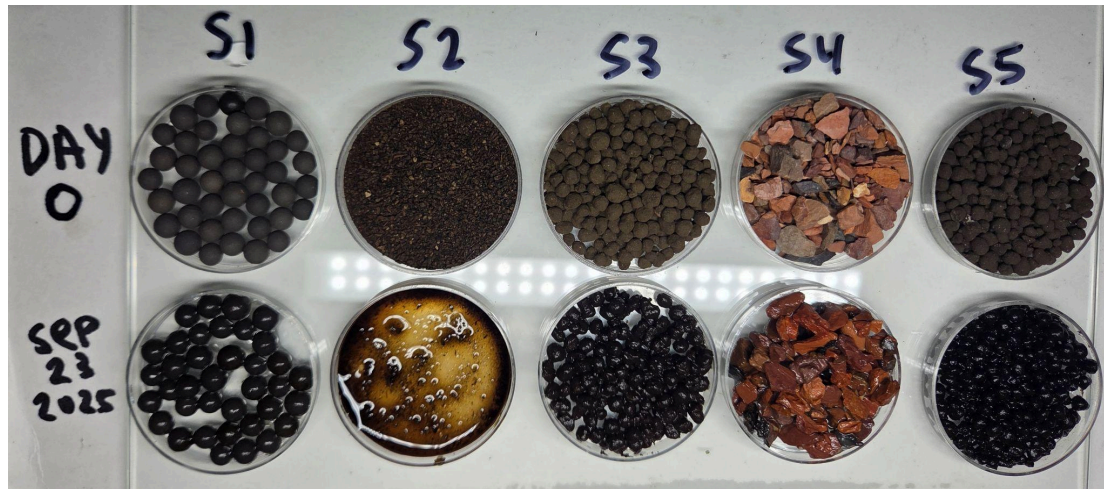


Figure 22: Final Substrate Appearance in Situ (Day 148). Close-up views of each substrate bed. Note the layering and compaction in the Organic Compost tank (second from left). Lower samples are freshly wet, photographed immediately after removal.



Figure 23: Comparative Substrate Analysis (Day 0 vs. Day 145). Top row: Unused samples of each substrate. Bottom row: Samples after 145 days. Note the leaching in the Organic Compost (S2) and the darkening of soil-based substrates (S3 and S5).

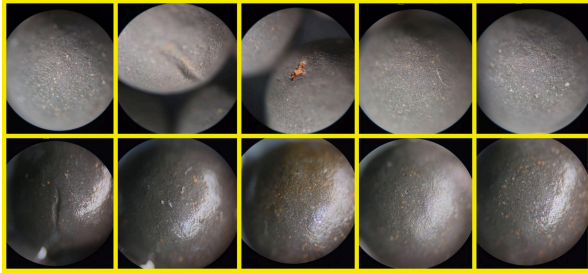


Figure 24: Microscopic Analysis of Aqueon Granules. Comparison of unused (top) and used (bottom) granules showing no structural change, aside from thin biofilm and algal growth on the used sample. (Brightfield, 100× magnification).

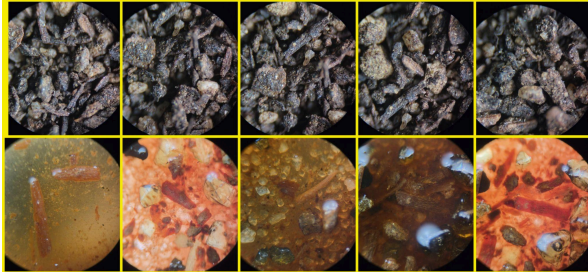


Figure 25: Microscopic Analysis of Organic Compost. Comparison of unused (top) and used (bottom) material. The used sample shows a heterogeneous mix of sand, fine organic sediment, and decomposed plant matter. (Brightfield, 40×–100× magnification).

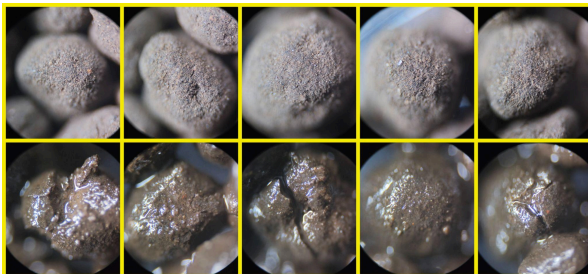


Figure 26: Microscopic Analysis of UNS Controsoil Granules. Used granules display extensive cracking, fracturing, and physical degradation. (Brightfield, 100× magnification).



Figure 27: Microscopic Analysis of Seachem Flourite. Comparison of unused (top/middle) and used (bottom) granules. No visible structural alteration; particles remained intact. (Brightfield, 100× magnification).

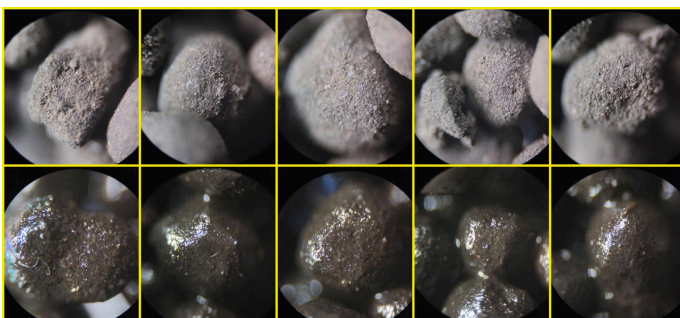


Figure 28: Microscopic Analysis of Fluval Stratum Granules. Comparison of unused (top) and used (bottom) granules showing rounded, wrinkled surfaces consistent with moderate erosion. (Brightfield, 100× magnification).

4.4 Detailed Final State Analysis of Individual Aquariums

4.4.1 Top Performer (Plant Growth): Subex 4 - Seachem Flourite

The Seachem Flourite aquarium was the unambiguous top performer in terms of plant health, coloration, and overall biomass. The final state of the tank displayed vigorous growth across all species and exceptional long-term water clarity. The water surface remained clear of excessive floater proliferation, suggesting a balanced nutrient dynamic dominated by root-based uptake.

Microscopic analysis confirmed complete preservation of substrate integrity, with no signs of cracking or erosion. The baked clay granules remained structurally intact throughout the experiment. The combination of high mineral stability and the substrate's intrinsic trace-element content—particularly iron and other micronutrients—likely sustained continuous root metabolism and leaf pigmentation even in low-nutrient conditions. This stability and mineral accessibility indicate that Flourite functions as an ideal long-term anchoring medium capable of delivering nutrients efficiently without releasing excessive solubles into the water column or altering overall chemistry.



Figure 29: Final State of Seachem Flourite Aquarium (Side View). Demonstrates robust plant growth across multiple species and excellent water clarity.



Figure 30: Final State of Seachem Flourite Aquarium (Top View). A healthy but controlled population of *Salvinia minima* is visible.

4.4.2 Top Performer (Microscopic Diversity): Subex 3 - UNS Controsoil

While the UNS Controsoil aquarium did not match the macroscopic plant vigor of Seachem Flourite, it excelled ecologically by supporting the richest and most varied community of microorganisms. Microscopic surveys revealed dense populations of rotifers, ostracods, annelid worms, ciliates, gastrotrichs, and red aquatic mites (Hydrachnidia), demonstrating a highly active benthic microecosystem. This exceptional microbial diversity was accompanied by the most pronounced substrate degradation observed among all treatments. The granules exhibited widespread cracking and fracturing after prolonged submersion, increasing their surface area and releasing fine particulates into the surrounding medium. This structural breakdown likely enhanced microbial colonization and nutrient availability through localized leaching but simultaneously reduced oxygen diffusion at the sediment interface, limiting the establishment of strong plant roots.

Thus, UNS Controsoil represented a biologically dynamic but mechanically unstable environment—highly favorable for microfaunal growth, yet suboptimal for sustained macrophyte health.

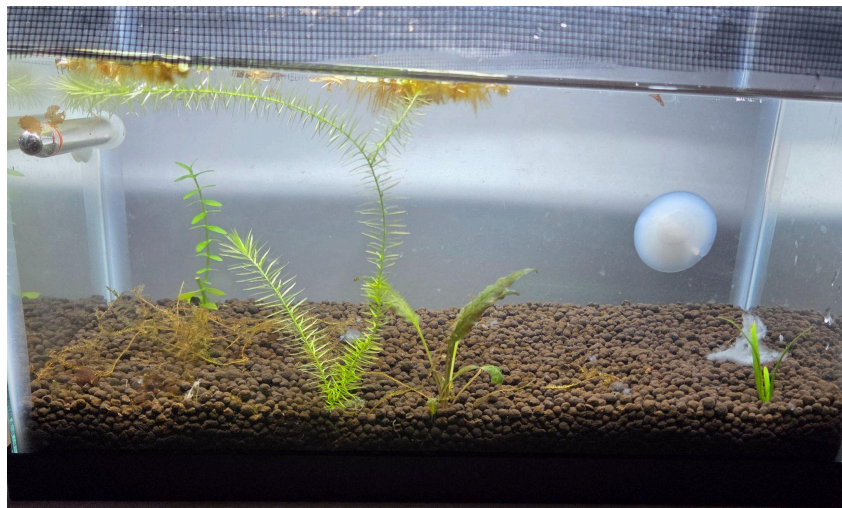


Figure 31: Final State of UNS Controsoil Aquarium (Side View). Shows surviving but underdeveloped plant life with clear water.



Figure 32: Final State of UNS Controsoil Aquarium (Top View). A sparse population of *Salvinia minima* is visible.

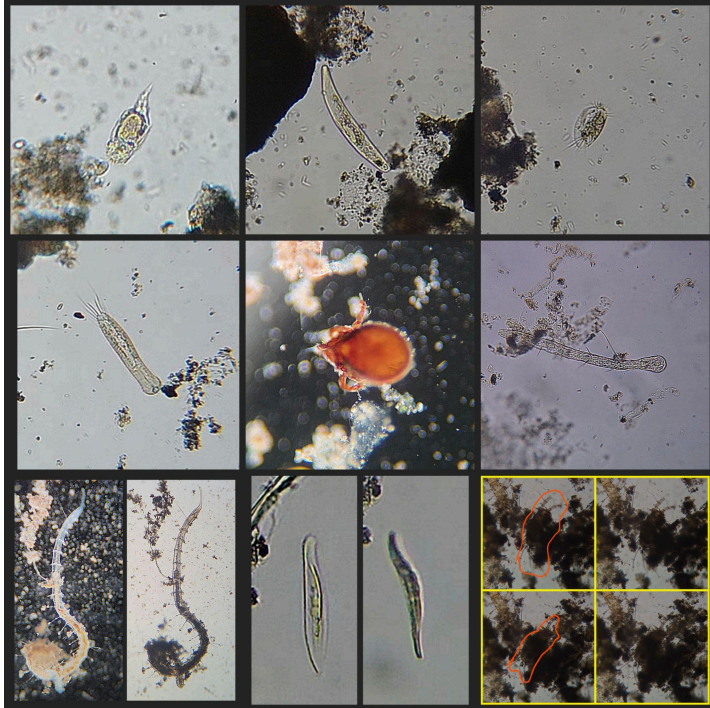


Figure 33: Microscopic Diversity in UNS Controsoil (Collage A). A collage showcasing the high density and variety of microorganisms found in the substrate, including rotifers, a gastrotrich, a red water mite (*Hydrachnidia*), annelid worms, and various ciliates. (Brightfield and Darkfield, 100x-400x magnification).

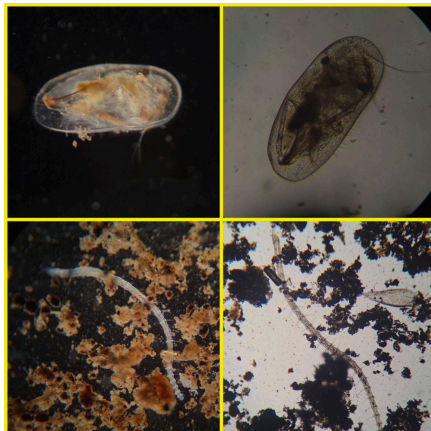


Figure 34: Microscopic Diversity in UNS Controsoil (Collage B). Further examples of the robust micro-ecosystem, including Ostracods (top) and different species of aquatic annelid worms (bottom). (Brightfield and Darkfield, 100x magnification).

4.4.3 Top Performer (Ecosystem Complexity): Subex 5 - Fluval Stratum

The Fluval Stratum tank hosted the most complex and functionally diverse ecosystem observed. Plant growth was moderate to strong, with pronounced vertical elongation and widespread root anchorage. The water surface was nearly covered by *Salvinia minima*, reflecting elevated nutrient concentrations in the water column. Beneath this canopy, a thriving macrofaunal and microfaunal community developed, including numerous live scuds (*Gammarus* sp.), annelid worms, and dense colonies of the sessile ciliate *Vorticella* attached to plant tissues. The coexistence of these taxa indicates a detritivore-driven system maintained by steady nutrient turnover. The porous volcanic structure of Fluval Stratum likely supported this trophic complexity by providing microhabitats and mild nutrient leaching that fueled the detrital food web. However, it also contributed to moderate granule rounding and erosion, suggesting gradual mineral weathering.

Overall, this substrate produced a self-sustaining microecosystem characterized by balance between plant, microbial, and invertebrate populations—a hallmark of a mature aquatic biotope.



Figure 35: Final State of Fluval Stratum Aquarium (Side View). Shows healthy plants with significant vertical growth.



Figure 36: Final State of Fluval Stratum Aquarium (Top View). The water surface is almost entirely covered by a dense colony of *Salvinia minima*.



Figure 37: *Vorticella* Colonies in Fluval Stratum. A close-up photograph showing clusters of the sessile ciliate *Vorticella* (indicated by red arrows) attached to a developing plant stem.

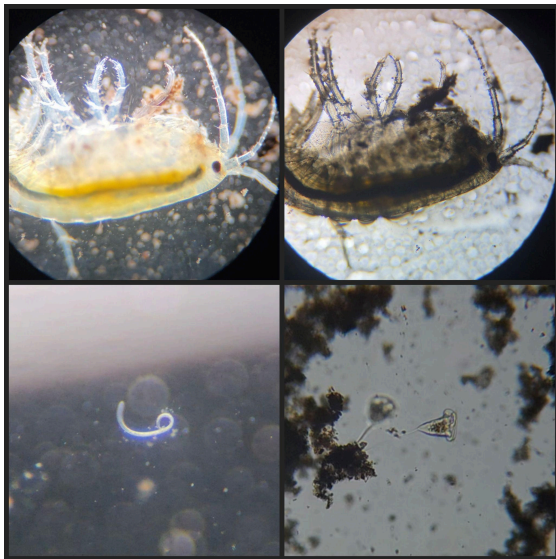


Figure 38: Key Microfauna of Fluval Stratum. Top row: A Scud (*Gammarus* sp.) viewed under Darkfield (left) and Brightfield (right) illumination (100x TM). Bottom row: An annelid worm under Darkfield (left, 200x TM) and two attached *Vorticella* organisms (right, 200x TM).



Figure 39: Macroscopic View of a Scud. A close-up photograph of a live scud on the substrate surface in the Fluval Stratum aquarium.

4.4.4 Key Finding (Root Adaptation): Inert Sand Controls (+/-)

The most scientifically significant result of the experiment came from the compromised control tanks. Prior to their late-stage neglect, both the inoculated (Figure 40) and uninoculated (Figure 41) tanks showed widespread plant failure, with one major exception: the *Cryptocoryne hudsoni*. Upon final analysis, the *Cryptocoryne hudsoni* from the inoculated control (+) tank displayed the most extensive and complex root system of the entire experiment (Figure 42), surpassing even the top-performing commercial substrates. The plant from the uninoculated control (-) showed a similar, though less pronounced, adaptive response (Figure 43).

This powerful adaptive growth is direct evidence of a classic survival response. In an environment devoid of substrate-bound nutrients, and where the only nutrient source was the slow decomposition of the other failing plants, the *Cryptocoryne hudsoni* allocated its energy to root development to maximize its scavenging ability. The slightly superior root growth in the inoculated (+) control suggests that the presence of a microbial community, however small, may have aided in the mineralization of this decaying matter, making nutrients more readily available. This finding demonstrates that for certain hardy root-feeders, environmental stress can trigger a powerful adaptive growth strategy focused on building a foundational root system rather than foliage.



Figure 40: Final State of Inert Sand Control (+). Inoculated control showing surviving *Cryptocoryne* and water loss by evaporation.



Figure 41: Final State of Inert Sand Control (-). The uninoculated control tank, also showing surviving *Cryptocoryne* and low water level.



Figure 42: *Cryptocoryne* Analysis from Inoculated Control (+).
The extracted plants show limited foliage but a remarkably complex and widespread root system.

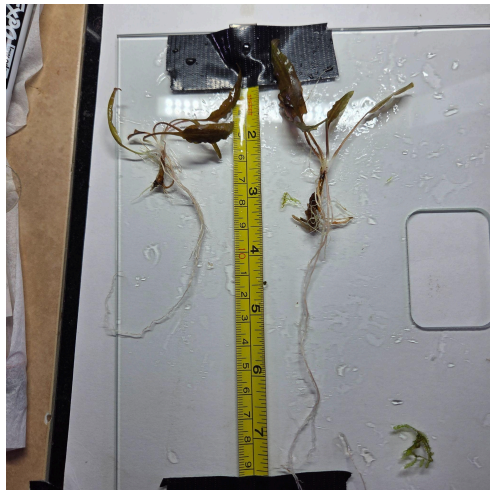


Figure 43: *Cryptocoryne* Analysis from Uninoculated Control (-).
The extracted plants show a similarly adaptive, though less extensive, root structure compared to the inoculated control.

4.4.5 Analysis of Remaining Substrates

- **Organic Compost (Failure Analysis):** The Organic Compost tank (Subex 2) served as a clear case study in ecological collapse resulting from structural failure within a DIY substrate system. Evidence from the glass–substrate interface confirmed a breach of the sand cap during planting (Figure 47), allowing anaerobic organic matter to rise and oxidize at the surface. This breach triggered rapid nutrient leaching, resulting in opaque, tanned water that remained resistant to clarification even after complete water changes (Figure 44). A thick, iridescent surface biofilm developed (Figure 46), and biological observations revealed near-total die-off of macrofauna and higher plants, leaving only a few *Salvinia minima* floaters (Figure 45). Microscopic examination confirmed the collapse: the sediment was littered with the shells and remains of ostracods and amoebae (Figure 48), while only resilient bdelloid rotifers persisted within anoxic debris (Figure 49a). This event demonstrates the sensitivity of soil-based substrates to mechanical disturbance. Even small breaches in stratification can induce redox inversion, nutrient shock, and rapid system destabilization—underscoring the critical importance of structural integrity in soil-capped aquatic designs.

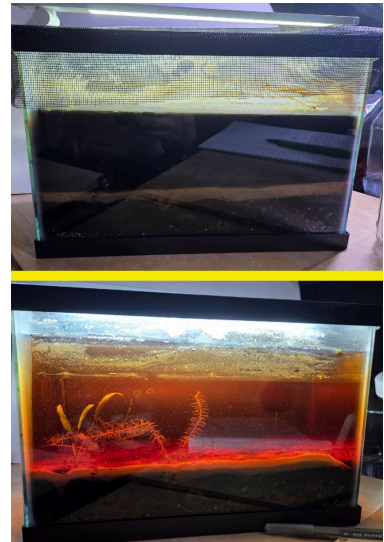


Figure 44: Water Clarity in Organic Compost Tank. A comparison showing the opaque, dark water before a major water change (top) and the clearer, but still heavily tanned, water after (bottom).

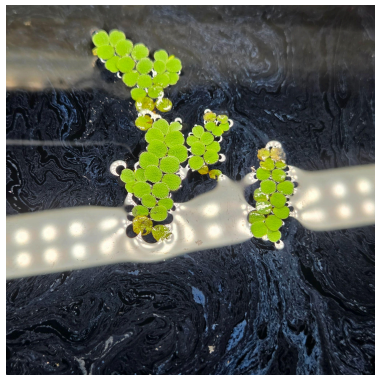


Figure 45: Surviving Floaters in Organic Compost. The few remaining clumps of *Salvinia minima* floating amidst an oily surface film.

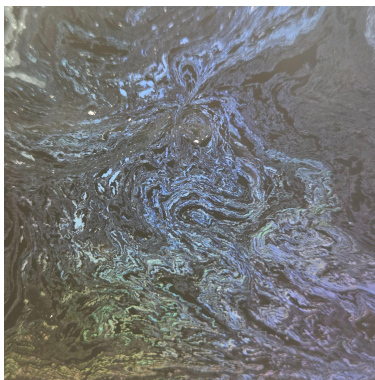


Figure 46: Oily Surface Biofilm in Organic Compost. A close-up of the thick, iridescent biofilm that consistently formed on the water's surface.

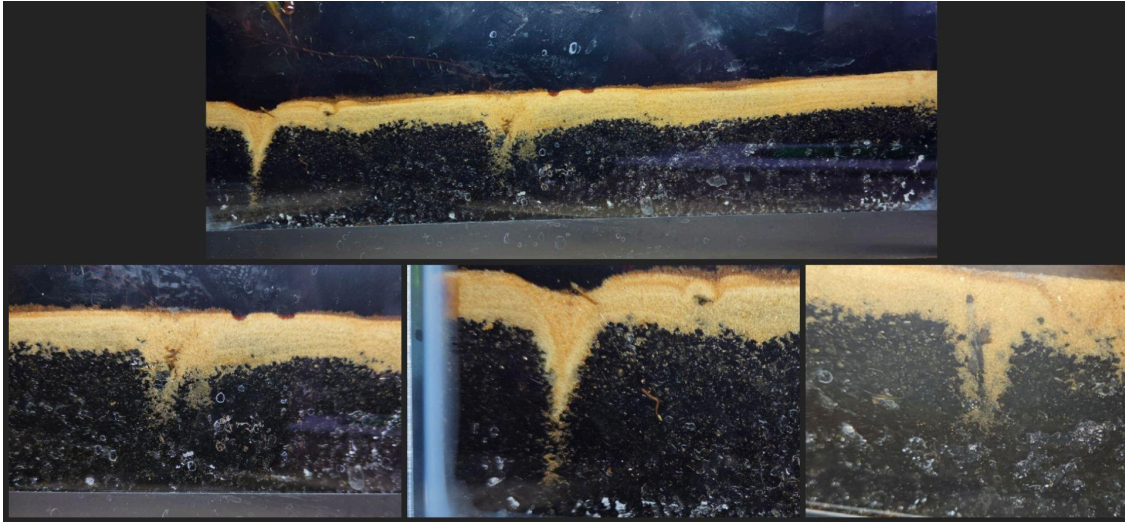


Figure 47: Evidence of Sand Cap Puncture. A collage showing multiple points where the fine sand cap was breached, allowing the underlying coarse sand and compost to leak upwards.

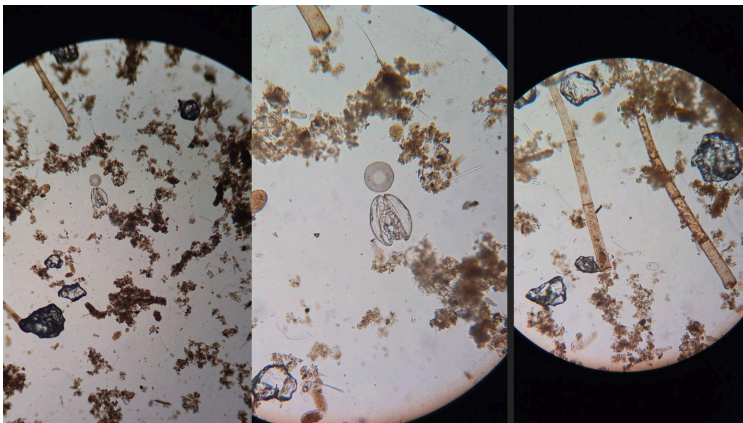


Figure 48: Microscopic Evidence of Ecosystem Collapse. Samples from the compost tank showing a landscape of decay, littered with the shells and carcasses of Ostracods and Testate Amoebae. (Brightfield, 100x-200x TM).

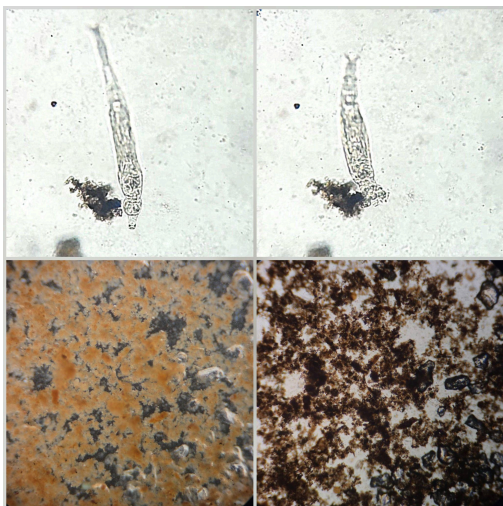


Figure 49a: Surviving Microfauna in Organic Compost. Abundant bdelloid rotifers (top row, 200x TM) were the primary survivors found amidst the anoxic detritus (bottom row, 100x TM).

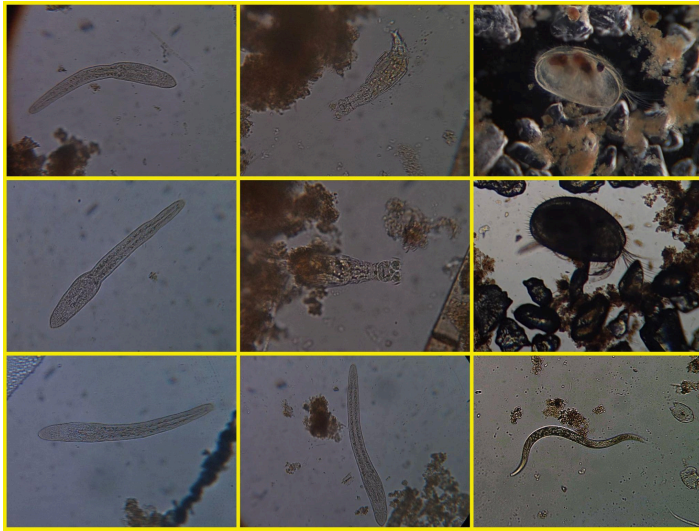


Figure 49b: Collection of more microorganisms found.

- Aqueon:** The Aqueon substrate (Subex 1) provided long-term stability but minimal biological productivity. Initially, it supported steady plant establishment, yet growth stagnated over time, and the final state showed only moderate foliage with localized algae on the glass (Figure 50). Microscopic observations revealed a simple, low-diversity community dominated by ostracods, rotifers, and annelid worms (Figures 51–52). The substrate granules themselves remained completely unchanged structurally (Figure 24), confirming Aqueon's inert nature. While this stability prevented chemical fluctuations, it also limited nutrient cycling and microbial enrichment. The absence of mineral exchange or organic decomposition likely restricted available nutrients for both flora and fauna, resulting in a stable but biologically underdeveloped ecosystem.

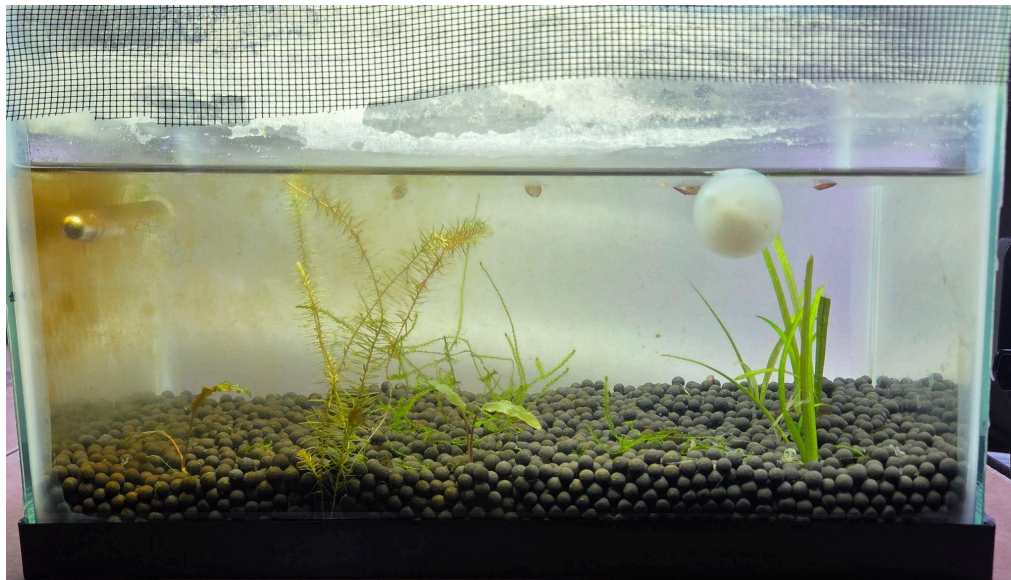


Figure 50: Final State of Aqueon Aquarium (Side View). Shows limited but stable plant growth with visible algae on the glass.



Figure 51 & 52: Microscopic Analysis of Aqueon Substrate. These images show the microfauna community, which was less diverse than other substrates, primarily consisting of common ostracods, rotifers, and annelid worms.

5. Discussion

An objective analysis of the collected data—from the initial water-chemistry records to the final photographic evidence—allows several key deductions regarding the performance of the tested aquarium substrates. The following interpretations are drawn directly from experimental observations rather than personal bias.

The most significant finding is that early-stage stability does not predict long-term plant performance. The Aqueon substrate, which provided the most stable conditions during the first 42 days, was ultimately surpassed in plant biomass by substrates that induced greater initial stress. This suggests that the early “melting” phase represents a temporary acclimation period that can precede more vigorous growth once equilibrium is achieved.

The results also reveal a clear trade-off between nutrient availability and ecosystem stability. The catastrophic failure of the organic-compost tank demonstrates that an uncontrolled release of nutrients is more damaging to plant health than an initial nutrient deficit. Conversely, the success of the inert yet high-CEC Seachem Flourite indicates that a substrate’s capacity to regulate nutrient delivery to roots is more critical than its raw nutrient content.

Moreover, the findings highlight a distinct dichotomy between substrates that favor plant growth and those that favor ecological complexity. Visual evidence shows that Seachem Flourite created an ideal environment for vigorous vegetation but supported a sparse microfaunal community, whereas Fluval Stratum yielded more modest plant biomass while fostering a rich, multi-layered food web.

Finally, the data from the inert-sand controls offer an important insight into plant adaptation strategies. The observation that *Cryptocoryne hudsoni* in these nutrient-poor tanks developed the most extensive root systems provides direct evidence of a survival response. In an environment where the only available nutrients originated from decomposing plant matter, the species redirected energy toward root expansion to maximize nutrient scavenging.

6. Conclusion

For aquarium hobbyists, substrate selection should be guided by the system's primary objective. Based on the outcomes of this experiment, Seachem Flourite appears most suitable for aquariums focused on maximizing plant growth and visual health. In contrast, Fluval Stratum is better suited to those seeking to cultivate a biologically diverse ecosystem that supports shrimp, fry, and other sensitive fauna. This experiment reinforces that there is no single "best" substrate—only a best choice for a defined purpose. Ultimately, it is through independent, evidence-based testing such as this that the aquarium community can move beyond marketing claims and make informed decisions that suit their own unique aquatic ecosystems.

7. Limitations of the Study

As a pilot project conducted in a home-lab environment, this study was subject to several limitations that must be acknowledged.

- I. **Data Gap:** The most significant limitation was the three-month hiatus in quantitative data collection between June 11 and September 9. This interruption reduced the temporal resolution of the dataset and limited the ability to track gradual chemical and biological changes throughout the experiment.
- II. **Compromised Control Group:** The two inert-sand control aquariums were partially compromised due to delayed setup and evaporation driven by the high ambient temperatures of the non-climate-controlled garage environment. These factors introduced environmental stress that complicated direct comparison with the primary test groups.
- III. **Lack of Replication:** Each substrate was represented by a single aquarium without replication. Consequently, results should be interpreted as detailed case observations rather than statistically representative findings.
- IV. **Despite these constraints,** the consistency of the trends observed across parameters and taxa suggests that the overall conclusions remain valid within the intended exploratory scope of this study.

8. References

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Project Attribution

Impossible Aquariums Initiative

This report is part of the Impossible Aquariums Initiative, an independent educational and research effort founded by Vital Barreto Conte (Victor) to advance a scientifically grounded understanding of aquarium ecology. The initiative combines home-lab experimentation, open-source data sharing, and the development of accessible tools for hobbyists seeking to create balanced, sustainable aquatic ecosystems.

The SUBEX-2025-05-01 experiment represents one of the initiative's cornerstone projects, serving both as an educational resource and as preliminary groundwork for the refinement of the SLESS (Symbiotic Litho-Ecological Substrate System) methodology. By documenting each stage transparently—from design to unexpected outcomes—my project aims to encourage other aquarists to apply empirical reasoning, reproducibility, and ecological awareness in their own setups.

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