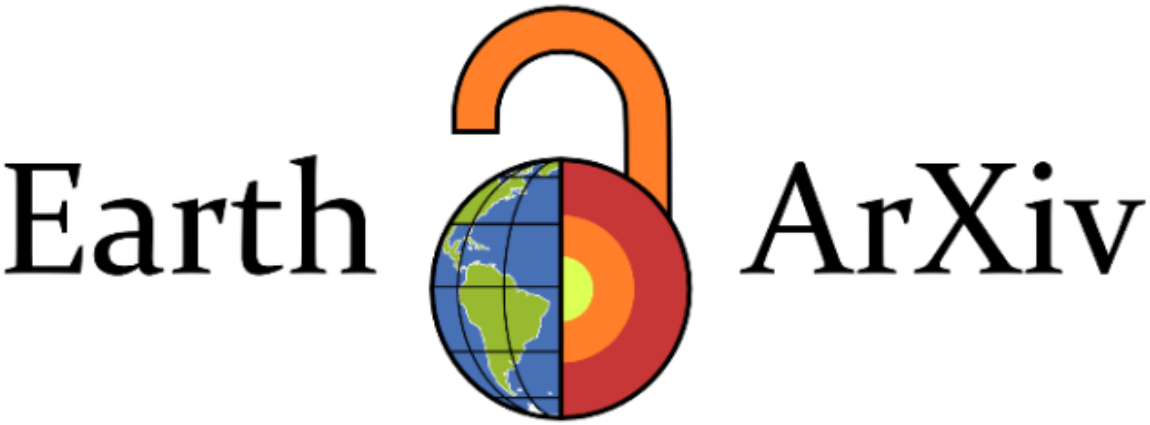

*phytoclass*Shiny: a Guided Workflow for Phytoplankton Pigment Chemotaxonomy

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Running Head: *phytoclass*Shiny for Chemotaxonomy

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Abstract

Estimating phytoplankton community structure using diagnostic pigment biomarkers relies on chemotaxonomic tools like CHEMTAX and the mathematical improvements of the *phytoclass* R package. However, *phytoclass* requires programming experience that limits its accessibility to non-coders. We present *phytoclass*Shiny, a free, open-source, offline R Shiny application that guides users through a linear, seven-step workflow - from high-performance liquid chromatography (HPLC) data import and quality control to simulated annealing optimisation and export - without requiring programming skills. Every session's parameters, data-handling decisions, and results are recorded in a shareable configuration file and audit log, addressing current reporting gaps and supporting full reproducibility. We validated *phytoclass*Shiny's phytoplankton community estimates against the native *phytoclass* package using a benchmark Southern Ocean dataset. Estimates agreed closely across seven of eight phytoplankton groups ($R^2 \geq 0.97$), with one group showing marginally lower agreement (Pelagophytes, $R^2 = 0.90$). This minor variation was attributed to a disclosed difference in benchmark parameters rather than by any alteration of phytoclass's underlying mathematics. Repeated tests using a fixed random seed reproduced results exactly. *phytoclass*Shiny therefore removes the programming burden of *phytoclass* analysis without any cost in accuracy. However, like all chemotaxonomic methods, successful analysis still relies on the user's foundational taxonomic and ecological knowledge of their study system. *phytoclass*Shiny broadens access to pigment-based chemotaxonomy for researchers without programming experience, and, when used deliberately, can help newcomers build the very foundational experience that emergent *phytoclass* method requires.

Introduction

Diagnostic pigment biomarkers can be used to quantify phytoplankton community structure through various chemotaxonomic methods (Mackey et al., 1996; Jeffrey et al., 2011; Roy et al., 2011). However, accurately estimating community structure remains challenging as different algal groups often share pigments, and different environmental conditions alter pigment:chlorophyll-a ratios on which such methods rely. While tools like CHEMTAX and the recently developed *phytoclass* package can mathematically resolve these overlaps, their reliance on manual adjustments and programming skills creates a barrier to entry. The adoption of advanced scientific methods is often hindered by the need for specialised skills (Kasprzak et al., 2020). Specifically, the need for programming skills can limit novel method access and increase the risk of inconsistent or incorrect use thereof. To address this barrier, interactive applications built with frameworks such as R Shiny (Chang et al., 2012) provide user-friendly dashboards for complex R codes.

Technical complexity has historically limited the accessibility of phytoplankton chemotaxonomy. The legacy program CHEMTAX (Mackey et al., 1996) requires expert a priori knowledge of in situ phytoplankton species to construct a biologically appropriate reference matrix (Higgins et al., 2011); its successor *phytoclass* (Hayward et al., 2023) requires similar taxonomic knowledge and additionally demands programming experience to implement its improved optimisation approach. phytoclass estimates phytoplankton communities from high-performance liquid chromatography (HPLC) pigment data via simulated annealing, complemented and validated against a refactored CHEMTAX function in R. This package was designed to find the true global minimum of the solution space through iterative randomisation, removing the sensitivity to initial conditions (a fundamental limitation of CHEMTAX), and providing a more accurate phytoplankton community estimate as a result.

phytoclass resolves the mathematical limitations of CHEMTAX, but its command-line parameters and performance metrics are not routinely reported (Devred et al., 2025; Fukai et al., 2025; Schlenker and Pinckney, 2025; Sun et al., 2025; Xu et al., 2025). Furthermore, the recommended pigment data pre-clustering and final optimisation matrices appear only in studies involving the package's original developers (Hayward et al., 2024; Hwang et al., 2025). These challenges highlight the need for a tool that guides users through the *phytoclass* algorithm's setup, staging, and analysis, ensuring appropriate and reproducible implementation. Such guiding tools support the wider use of complex ecological methods (Nicolosi Gelis et al., 2022; Camarena-Orozco et al., 2024) that can evolve to meet a community's needs, as demonstrated by the Shiny application WALLACE, a GUI for modelling species niches and distributions (Kass et al., 2018, 2023).

*phytoclass*Shiny (Quinlan, 2025) was developed to address technical barriers for non-coders, bridge the implementation gap for the emergent *phytoclass* method, and facilitate guided, verifiable, and replicable phytoplankton community estimation. It is an open-source, free-to-use, offline, R-based graphical user interface (GUI) that makes *phytoclass* analysis easier and more convenient for non-coders. Recently, a similar online application emerged that also implemented *phytoclass* within a GUI (Hayward et al., 2026). This valuable application offers robust, granular customisations that benefit advanced users and those transitioning directly from legacy chemotaxonomic methods. *phytoclass*Shiny complements these efforts by safety-railing common user errors: pigment datasets are semi-automatically parsed, quality-

controlled, filtered, and prepared for *phytoclass* analysis, following best-practice guidelines (Hayward et al., 2023).

Here, we describe how *phytoclass*Shiny's stepwise, guided workflow makes phytoplankton pigment chemotaxonomy accessible to non-programmers. We then assess whether the application preserves the accuracy and reproducibility of the native *phytoclass* algorithm, validated against a benchmark dataset using published analysis variables, and tested for internal consistency under two fixed-seed randomisation scenarios.

Method

A linear seven-step graphical interface guides users from initial parameter setup through batch loading, standardisation, and quality-control filtering of HPLC pigment data. After the setup (Steps 0-1), data import, cleaning and column mapping (Step 1-4) - and following an optional but encouraged sample grouping (Step 5) - the staged and grouped pigment data is processed using the *phytoclass* simulated annealing algorithm (Steps 6). The results of this are used to report absolute and relative phytoplankton community estimates alongside optimised pigment ratio tables (Step 7). The computational accuracy and reliability of *phytoclass*Shiny's pigment-to-phytoplankton framework are validated against a benchmark Southern Ocean HPLC pigment dataset (Viljoen and Fietz, 2025) from a winter study (Viljoen et al., 2025) to measure agreement with the original *phytoclass* package and tested under two different fixed-seed scenarios to confirm internal consistency and the influence of various fixed seed values.

Each analysis conducted followed the same setup configuration unless reported otherwise: Iterations (Niter) = 500, Cooling Step Size = 0.009, Fixed Random Seed enabled, Seed Value = 131234, Custom Min/Max Bounds enabled, MinMax Profile = MinMax_Archive_v2.0.0.xlsx, and all data cleaning toggles enabled (i.e., remove duplicate samples, change blank cells to 0, change negative numbers to 0, and remove empty samples). No data filters were applied. For the random seed scenarios (same-seed vs different seeds), values of 56789 (Baseline A, repeated for B and C) and 5678910 (Baseline D, repeated for E and F) were used respectively.

Application Development

*phytoclass*Shiny is a free, open-source, locally hosted (offline) application built in the R programming environment (R Core Team, 2021). The graphical user interface (available in either RStudio or a browser) is rendered using the shiny and bslib packages. Data handling and cleaning use the tidyverse suite; clustering uses the vegan and cluster packages; and the core pigment-to-phytoplankton estimates are calculated by the *phytoclass* package (Hayward et al., 2023). The application functions as a coordinating wrapper: user inputs (e.g., defining parameter values) and actions (e.g., pressing buttons). Each stage feeds into and triggers established, unmodified functions running in the background.

Design & Reproducibility

*phytoclass*Shiny enforces a linear, modular workflow, working within the limitation of the single-threaded R programming language that it is built on. Each subsequent stage is locked until its predecessor has been successfully completed to ensure that the application processes one instruction at a time and avoids conflicting logic. Thus, if an upstream input is changed (e.g., a data cleaning rule is toggled in Step 1), every downstream step (Steps 2–7) must be re-run. This prevents silent mismatches between the settings a user sees and interacts with, and how their data is handled in the background. This safety-railing prevents common user errors and ensures reproducibility.

Every session's parameters, user-selected toggles, and defined inputs are saved to a session-specific configuration file that can be reloaded and shared to ensure reproducibility. Additionally, every data-handling process happening in the background (e.g., Step 3 column-mapping choices and samples excluded during Step 4 quality control) is recorded in an audit log and saved alongside each session's results. Applying a fixed random seed ensures these results can be reproduced across repeated runs and

on different devices, as demonstrated in the validation and assessment (Results and Discussion) below. Together, these features allow a complete analysis, and every choice behind it, to be both reproduced and verified by the original user or a subsequent reviewer. The following three sections describe what happens at each point in the workflow and why. The application overview schematic (Fig. 1) detailing the application stages (Setup, Data Staging, and Analysis). Their respective steps are detailed in the schematic overview diagrams (Supplementary Material, Figs. S1-S8) and accompanying screenshots (Supplementary Material, Figs. S9-S15) of the in-app session resolving the validation results (Supplementary Material, Figs. S16-S18).

Application Setup (Steps 0–1)

The launcher automatically downloads and configures the packages required by the application in a one-time pre-configuration (Step 0: Automatic Setup; see Fig. S1). The launcher script downloads all required R packages from CRAN (requiring an internet connection) into a sandboxed library within the application folder and creates the profile and project files needed to tell the application to use this sandboxed library instead of the default system library. This process records the status and version of each required package, and confirms the application has the necessary folder access permissions; a detailed launch log is saved for troubleshooting purposes. Once downloaded and set up, the application folder is fully self-contained and transferable, requiring only that R be installed on the device it is run on (no internet connection is required to run it thereafter). Using the launcher after setting up the application workspace will instead launch the application itself.

The same launcher then confirms the workspace and starts the application (Step 1: Setup; see Fig. S2 and S9). By default, the application loads a template configuration (config_template.YAML) and requires the user to confirm, via the "Check Matrix Files" button, that the two starting pigment matrices, Fm_Pro (Prochlorococcus-inclusive) and Fm_NoPro (-exclusive), are present. From here, users set the parameters that govern later steps: *phytoclass* settings (iterations, cooling step, min-max table, and random seed), data quality-control rules (duplicate, empty-cell, negative-value, and zero-sum checks), and any environmental bounds (location, date, depth) to apply during filtering. These choices can be saved to a session-specific configuration file (config_session.YAML). If saved, any changes made during a session are reloaded automatically in future runs, with the option to reset to the original template.

Data Staging (Steps 2–5)

Staging ensures the user's raw pigment data is appropriately structured for, and understood by, the functions that process it. The application does this in four steps: importing (Step 2), mapping (Step 3), filtering and cleaning (Step 4), and optional grouping (Step 5); see Fig. 1 for general application overview.

Users import individual or multiple HPLC pigment files as standard Excel spreadsheet workbooks (.xls or .xlsx) via a file browser (Step 2; see Figs. S3 and S10). This is a deliberate choice, since non-programmers are more likely familiar with spreadsheet formats, meaning they can access and verify the output of each step themselves. This may require converting plain-text datasets (e.g., .csv) into spreadsheet format before upload. Each pigment dataset file requires that its data be present on the first sheet, arranged in the conventional samples-as-rows, pigments-and-metadata-as-columns structure; other

sheets are ignored. On import, the application standardises formatting automatically: invalid header characters are replaced, non-numeric values (e.g., "<LOD") are coerced to NA, and inconsistent date formats are parsed into explicit year/month/day fields, with each sample assigned a unique identifier for traceability.

The application then semi-automatically maps each file's column headers to the names it requires (Step 3; see Figs. S4 and S11), using the stored session alias list. Any column required by the application is checked for in the user's data using the application's expected header text; if that exact text is either absent or present under a different name, the required variable is flagged as missing. Interacting with this warning launches a pop-up wizard that allows users to manually match their column headings to the variables the application requires. This mapping can be applied to all other loaded files at once, and saved as an alias for future sessions. This step also cross-checks the mapped pigments against those required by the Fm matrices or any enabled filters. The application will provide a warning to the user before analysis if a dataset lacks a pigment needed to resolve a specific phytoplankton group, and will block attempts to filter if the toggled filter's parameter is missing. These prevent missing pigments from being interpreted as a true biological absence, and ensures filtering is only applied correctly.

Filtering (Step 4; see Figs. S5 and S12) applies the quality-control rules set during Setup (Step 1) to a working copy of the data; the original file is never edited. Missing or negative pigment values are set to zero, duplicate and zero-sum samples are flagged, and any samples outside the defined location, date, or depth bounds are excluded, leaving a quality-controlled master dataset. The table in this step reports the per-dataset count of samples loaded into the application, samples failing each enabled quality-control or filtering check, and the final number of samples passed to the following step.

Finally, users choose whether to group samples by source file or by pigment concentration similarity (Step 5; see Figs. S6 and S13). Analysing similar samples together improves the precision of the simulated annealing step that follows (Hayward et al., 2023). We opted to implement clustering directly in the application as a pre-analysis step, rather than using *phytoclass*'s built-in clustering function. This was done to keep the same linear, modular sequence as every other stage, while also allowing step-specific computational demands to be handled separately (only an issue when handling large or multiple datasets). Samples can be normalised to total chlorophyll-a and transformed (log or Box-Cox) to limit the influence of extreme bloom events, then clustered by Ward's hierarchical method or k-means; the number of clusters is detected automatically but can be adjusted using the accompanying PCA, dendrogram, silhouette, and elbow plots. We recommend at least 12 samples per group, or 20 where higher measurement uncertainty is expected (Hayward et al., 2023). Once confirmed, the resulting group (by file or by cluster) becomes the sample matrix passed to the analysis stage.

Analysis (Steps 6–7)

Analysis (Step 6; see Figs. S7 and S14) runs the core *phytoclass* simulated annealing algorithm iteratively on each grouped pigment sample matrix (Sm); see Fig. 1 for overview. Before each run, the application checks for divinyl-chlorophyll-a (a marker unique to Prochlorococcus) to automatically select the appropriate starting matrix (Fm_Pro or Fm_NoPro), which is then passed, along with the group's pigment data, to the corresponding simulated annealing function. By default, this runs for 500 iterations with a cooling step of 0.009, matching the settings recommended by Hayward et al. (2023). A progress tracker

displays elapsed and estimated remaining time, based on prior runs on the user's device; multiple runs on the same device result in more accurate runtime estimates, as the session configuration file's record improves.

Each run returns the group's phytoplankton biomass estimates, the optimised pigment-ratio matrix, the root-mean-square error (RMSE) for that run, and the matrix's condition number, reported together in a results table alongside a success or failure flag for every sample group (file or cluster). A run is flagged for review if its RMSE exceeds 0.1, the threshold above which Hayward et al. (2023) recommend increasing the iteration limit or step size, or re-clustering the data to include greater variance among grouped pigment samples.

Finally, visualisations of community composition are presented (Step 7; see Figs. S8 and S15) as stacked-area and bar-plot, accompanied by the relevant optimised Fm matrix for that group. All results are exported as a single, timestamped, zipped file containing the session's parameters, biomass estimates, optimised ratio matrix, and full quality control and filtering log, so that every analysis remains fully documented and reproducible.

Results

We conducted three sets of tests. The first compared the results of *phytoclassShiny* with those of *phytoclass* on a benchmark Southern Ocean data set, without knowing the min-max table used previously with *phytoclass* (Viljoen et al. 2025). These tests gave strong, but not perfect, correlation. The second test used a fixed-seed to assess the internal reproducibility of *phytoclass*Shiny and gave perfect correlation. The third test compared two different fixed seed values in three full application sessions for each seed. These further demonstrated that fixed-seed runs reproduce the exact same decimal results, whereas changing the fixed seed value does alter the phytoplankton group estimates to some minor extent.

We first assessed *phytoclass*Shiny's performance in guiding a complete analysis of the benchmark dataset, then evaluated whether the resulting community estimates and computational behavior matched those of the native *phytoclass* package (Fig. S16). We also tested the repeatability for the same dataset and setup conditions, under both fixed (Fig. S17) and different random seeds (Fig. S18).

*phytoclass*Shiny completed a full pigment-to-phytoplankton analysis of the benchmark dataset in under one minute. The column-mapping utility (Step 3) resolved missing connections between the input dataset's column headers and the application's required variables (Chl_c1, Per, X19but, and X19hex). Automated quality control confirmed that all 45 samples passed the predefined cleaning rules, with none flagged for exclusion. The simulated annealing module completed with a 'Success' status, returning a mean RMSE of 0.009 and a mean condition number of 1040.61 - well within the recommended threshold (Hayward et al., 2023). These diagnostics indicate that the optimisation converged cleanly and that the starting pigment matrix was well-conditioned for inversion; whether this convergence also reflects an accurate community estimate is addressed below.

Comparing these estimates against the benchmark *phytoclass* output revealed strong, linear agreement (Fig. S16). For five of the eight groups evaluated (Cryptophytes, Dinoflagellates, Prasinophytes, Synechococcus, and Prochlorococcus), the fit was perfect ($R^2 = 1.00$); Haptophytes and Diatoms had a near-perfect fit ($R^2 = 0.97$ and $R^2 = 0.97$ respectively); whereas Pelagophytes showed a marginally lower fit ($R^2 = 0.90$). The optimised pigment-ratio table (F_Opt) underlying this agreement (Table I) shows the pigment:Tchla ratio resolved for each phytoplankton group configured to contain that pigment (Fm value = 1) and within each groups expected pigment ratio range (min-max table).

Repeatability testing showed that *phytoclass*Shiny reproduces results exactly under a fixed random seed. Across six independent, consecutive sessions (A, B, C vs D, E, F), the same dataset was analysed using two fixed-seed scenarios (Seed 56789/Baseline A and Seed 5678910/Baseline D). Replicate-minus-baseline residuals were uniformly zero across the full biomass range for every phytoplankton group (Fig. S17), and RMSE, condition number, and R^2 all matched to the decimal place within each seed group ($R^2 = 1.00$; Fig. S18). Pairwise comparison both within and between the two different fixed-seed groups showed further exact replication within groups and a minor degree of variation between the two groups ($R^2 = 0.93$; Fig. S18). This variability is expected due to the stochastic nature of simulated annealing function which uses randomness in its search for a solution, and partitions shared pigments differently (Table I).

Discussion

The results from the various testing scenarios indicate that *phytoclass*Shiny reproduces the native *phytoclass* package's estimates with high fidelity, and that any deviation from a perfect match is mechanistic rather than arbitrary. Phytoplankton groups assigned to at least one exclusive diagnostic pigment in the starting reference matrix resolved with near-perfect linearity: this includes divinyl-chlorophyll-a for Prochlorococcus, alloxanthin for Cryptophytes, peridinin for Dinoflagellates, prasinoxanthin for Prasinophytes, and 19'-hexanoyloxyfucoxanthin for Haptophytes. Groups lacking a strictly unique biomarker and relying on overlapping pigment indicators instead, such as Pelagophytes and Diatoms sharing fucoxanthin and chlorophyll c3 with Haptophytes, showed localised scatter and slightly weaker regression slopes (0.88 and 0.96, respectively). The optimised output matrix (Table I) shows that while the simulated annealing algorithm strictly enforces the zero-bound rules of the starting matrix, it must actively partition the variance of these shared pigments across multiple groups within their min-max ranges, which introduces minor deviations during iteration.

This mechanistic explanation accounts for the pattern of agreement across groups, but not the reason why some groups fall short of an exact match. *phytoclass*Shiny functions as a coordinating wrapper around the native *phytoclass* functions, calling them directly rather than reimplementing their mathematics; identical inputs should therefore return identical output. The repeatability results above confirm this directly: under matched inputs and a fixed seed, *phytoclass*Shiny and *phytoclass* agree exactly to the reported precision. The benchmark comparison, in contrast, could not fully match one input: the exact min-max reference table used in the original published analysis was not publicly available. The default table distributed with *phytoclass* (version 2.0) was substituted instead. This inconsistency is deliberately preserved. The difference between a near-perfect and perfect match with the benchmark results is caused by a single difference in an unreported setup parameter, rather than to any alteration of *phytoclass*'s underlying mathematics by the application itself, highlighting the necessity for detailed reporting of all analysis parameters (iterations, step size, seed, and min-max table) to ensure reproducibility.

These findings demonstrate the accessibility and reproducibility case made throughout this paper, with one important caveat. CHEMTAX and *phytoclass* both require the user to already know which phytoplankton groups are ecologically plausible for their study system, since this knowledge underpins the reference matrix each method optimises against; *phytoclass*'s advance lies in how it searches that matrix's solution space, not in removing this taxonomic requirement. *phytoclass*Shiny inherits this same dependency but removes the starting barrier and programming skills required to use CHEMTAX and *phytoclass*, semi-automating the data handling, quality control, and the optimisation procedure itself. The results reported here indicate that this benefit to non-programmers does not come at the cost of accuracy: the parts of the method *phytoclass*Shiny automates sit alongside, rather than in place of, the ecological and taxonomic judgement that the method has always required.

This distinction matters beyond *phytoclass*Shiny itself. Automated tools across ecology are easing the computational burden of analysis. These advances do not remove the expert judgement underpinning them but rather relocate where that judgement is needed. Calbert (2026) makes this point in the context of automated plankton classification and AI-assisted synthesis: while automation can benefit observational scale, a tool built on uncertainty will likely amplify uncertainty instead of resolving it. For

*phytoclass*Shiny, expertise is still required at both ends of the workflow: in assessing whether the reference matrix groups and pigments (Fm) are appropriate to the sampled environment, and in judging whether the resulting community estimates are ecologically plausible for it. A user unfamiliar with which phytoplankton groups are likely to occur in their system, or with the limits of pigment-based resolution among taxa sharing biomarkers, cannot outsource that judgement to the application.

We therefore position *phytoclass*Shiny as a tool that eases the mathematical and programming barriers non-programmers face, while bridging the implementation gap that the *phytoclass* method faces. Importantly, *phytoclass*Shiny does not substitute for expertise, but spotlights the decisions for the user to intentionally engage with, select, review, or acknowledge: which columns were automatically matched vs those mapped manually (Step 3), which cleaning rules or filters were enabled and how many samples passed or failed (Step 4), if and how their clustering solution may negatively influence the simulated annealing solution (Step 5), and which reference matrix a group was resolved against with excluded pigments or phytoplankton classes clearly indicated (Step 6). For each group, the convergence status, RMSE, and condition number are reported for review (Steps 6-7), with conditional warnings if these run metrics exceed thresholds. These safety railings place the information required to make informed decisions in front of the user where and when appropriate. When used deliberately, the applications guided, transparent workflow supports the development of expertise, offering newcomers a structured route into the logic of pigment chemotaxonomy as they build their regional and taxonomic intuition required for the appropriate use of this method.

*phytoclass*Shiny's beginner-oriented design distinguishes it from the more advanced, browser-hosted GUI recently developed by Hayward et al. (2026), which offers greater customisation likely to suit coding-proficient users, those transitioning from CHEMTAX, and those familiar with *phytoclass*. The present validation was not designed as a head-to-head comparison against these tools, and no such comparison should be inferred from it. Rather, we establish that *phytoclass*Shiny's guided approach carries no accuracy cost relative to the native package, supporting its intended role as a complementary entry point for non-specialists.

These findings should be read within the scope of the validation performed. The benchmark comparison draws on a single dataset from one region and season, and the min-max table substitution discussed above - although deliberate - means this was not a fully controlled comparison against the original analysis. Importantly, the repeatability comparisons account for the difference a single change in setup parameter introduces in the phytoplankton estimates, highlighting the necessity for a tool that encourages reporting all analysis parameters. Performance on larger datasets and other ocean regions has been tested internally; however freshwater systems remain untested, as does direct comparison against other *phytoclass* GUIs. Future validation extending to these cases would strengthen confidence in the application's general reliability.

Conclusions

The new *phytoclass*Shiny application offers a guided tool for newcomers to pigment chemotaxonomy, and facilitates guided, verifiable, and replicable phytoplankton community estimation. Phytoplankton chemotaxonomy has always required taxonomic and ecological knowledge of the study system - a foundation that is shared between CHEMTAX, *phytoclass*, *phytoclass*Shiny, and all other chemotaxonomic methods. What has differed between them is the additional burden each place on the user beyond this foundation. CHEMTAX demands iterative, expert-guided manual adjustment during optimisation, and *phytoclass* demands programming experience to implement its improved algorithm. *phytoclass*Shiny removes this latter, method-specific burden, guiding non-programmers through data preparation, quality control, and simulated annealing analysis via a linear, seven-step interface. The validation reported here shows that this accessibility carries no accuracy cost with *phytoclass*Shiny reproducing the native package's community estimates with high fidelity ($R^2 \geq 0.97$ for seven of eight groups). The difference in results was traced to a single disclosed difference in benchmark dataset parameters rather than to the application itself. Under two starting seed scenarios, *phytoclass*Shiny reproduced its own results exactly when randomisation was fixed and showed only minor variation when two different seeds' results were compared.

This reproducibility addresses the reporting gap raised at the outset of this paper. By recording every setting, seed, and data-handling decision in a standardised, shareable configuration file and audit log, *phytoclass*Shiny captures the parameters and performance metrics often unreported in published analyses. The benefit is evidenced in our own validation attempt where a missing min-max table can alter the phytoplankton estimates. It further mitigates the risks that long-term aquatic monitoring programmes face when adopting new computational methods (Jones et al., 2025): because the application runs offline, requires no ongoing maintenance, and preserves a complete record of past sessions, it lowers the continuity costs that typically accompany a shift to a new method.

Alongside the more advanced, customisable GUI recently developed for *phytoclass* (Hayward et al., 2026), *phytoclass*Shiny occupies a validated niche as a guided tool for non-specialists. Used deliberately, it may also help newcomers build the taxonomic and regional familiarity that phytoplankton chemotaxonomy requires, rather than substitute for it. In broadening access to pigment-based chemotaxonomy without displacing the tools built for more experienced users, it extends access to an emergent method for researchers who have so far been least able to use it.

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Data Archiving

The *phytoclass*Shiny application developed, used, and updated throughout the duration of this study, including its complete source code and reference material, is openly available under a GNU General Public License v3.0 (GPL-3.0) in the Zenodo repository at https://zenodo.org/records/21967832 (Quinlan, 2025). The benchmark HPLC phytoplankton pigment concentration data can be found in a separate Zenodo repository at https://doi.org/10.5281/zenodo.14610498 (Viljoen and Fietz, 2025).

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Table and Figure Legends

Fig. 1

Fig. 1: Dependency map for phytoclassShiny. Columns track the app's sequence from pre-launch (Step 0) through Setup (Step 1), Data Staging (Steps 2–5), and Pigment Analysis (Steps 6–7); a step's tab is locked until its predecessor completes, as shown in the "Unlocks after Step N" badges. Rows are the seven categories of data and configuration the app carries through that sequence: pigment data files, QC rules, spatial/temporal filters, reference matrices, phytoclass parameters, clustering parameters, and session configuration. Each row's cells show where that item is set and, via the dotted connectors, which later step uses it (e.g., an enabled spatial/temporal filter both adds its column as a required field in Map Variables and is applied during Filter & Clean; the reference matrices are validated in Setup and selected per group in Run Analysis). Three buttons sit outside the step sequence and are available at any point: Save (writes toggles and parameters to the session configuration file), Download Report (bundles every completed step's results, gated section-by-section by which steps have actually run), and Exit.

Table I

Table I: Final optimised pigment ratio matrix (Fm_Opt) determined by the phytoclassShiny application's wrapped simulated annealing optimization process on the benchmark dataset. Rows (italics) represent the target phytoplankton functional groups, and columns (bold) represent the diagnostic biomarker pigments. Unlike the binary starting matrices, values here denote the final, calculated decimal ratios (pigment-to-chlorophyll-a) that yield the lowest Root Mean Square Error (RMSE) for the community structure. This optimised matrix demonstrates phytoclass' adherence to boundary constraints (maintaining all zero-bounds) while partitioning the weight of shared pigments (e.g., fucoxanthin and chlorophyll c3 - shaded in yellow) across functional groups with overlapping pigments and no unique biomarker pigments.

Table I Footnote:

Abbreviations: Peri, peridinin; X19but, 19'-butanoyloxyfucoxanthin; Fuco, fucoxanthin; Neox, neoxanthin; Pras, prasinoxanthin; Viol, violaxanthin; X19hex, 19'-hexanoyloxyfucoxanthin; Allo, alloxanthin; Zea, zeaxanthin; Lut, lutein; Chl_c3, chlorophyll c3; Chl_b, chlorophyll b; Dvchla, divinyl chlorophyll a; Tchla, total chlorophyll a. Suffixes -1 and -2 denote alternative diagnostic-pigment variants for a functional group within phytoclass's default F matrix.

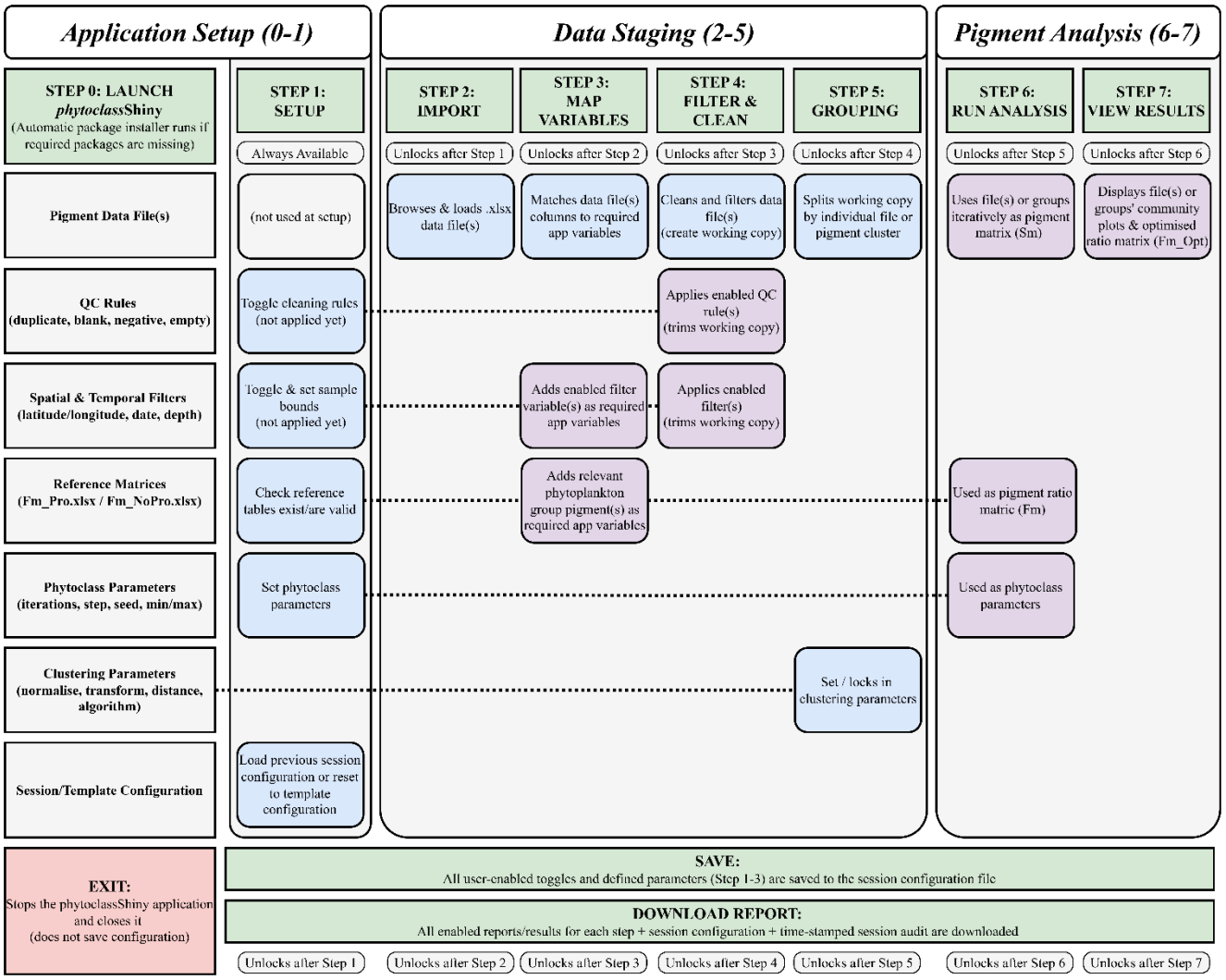
Tables

Table I

Class	Peri	X19but	Fuco	Neox	Pras	Viol	X19hex	Allo	Zea	Lut	Chl_c3	Chl_b	Dvchla	Tchla
Pelagophytes	0	1.41	0.39	0	0	0	0	0	0	0	0.15	0	0	1
Diatoms-2	0	0	0.33	0	0	0	0	0	0	0	0.12	0	0	1
Haptophytes	0	0.22	0.03	0	0	0	0.51	0	0	0	0.2	0	0	1
Cryptophytes	0	0	0	0	0	0	0	0.21	0	0	0	0	0	1
Dinoflagellates-1	0.34	0	0	0	0	0	0	0	0	0	0	0	0	1
Prasinophytes	0	0	0	0.05	0.14	0.01	0	0	0.02	0.03	0	0.8	0	1
Synechococcus	0	0	0	0	0	0	0	0	0.11	0	0	0	0	1
Prochlorococcus	0	0	0	0	0	0	0	0	0.32	0	0	0	1	0

Figures

Fig. 1



Supplementary Material

Table of Contents

Table of Figures

Application

Schematic Overview Diagrams

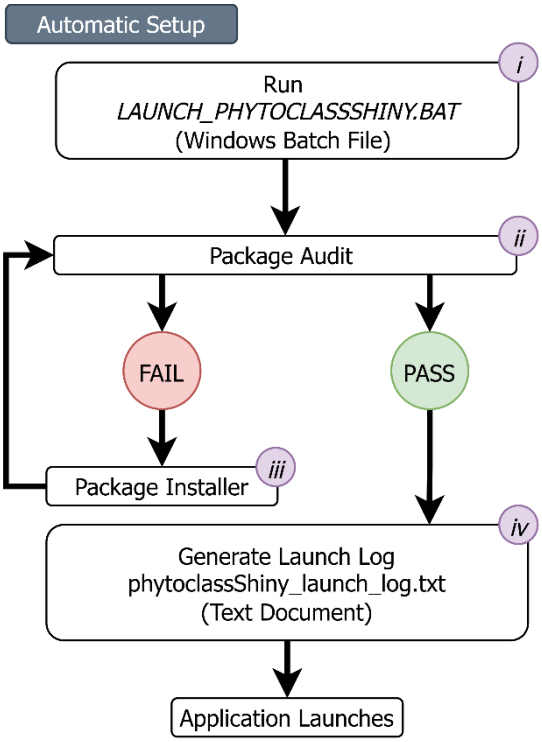


Fig. S 1: Schematic overview of Step 0 (AutomaticSetup) of the phytoclassShiny application packages and workspace required to launch the application. launching the Windows batch file triggers a scan for the required packages and their versions; any missing or outdated packages are installed and the audit re-run until it passes, at which point a launch log is written and the application starts.

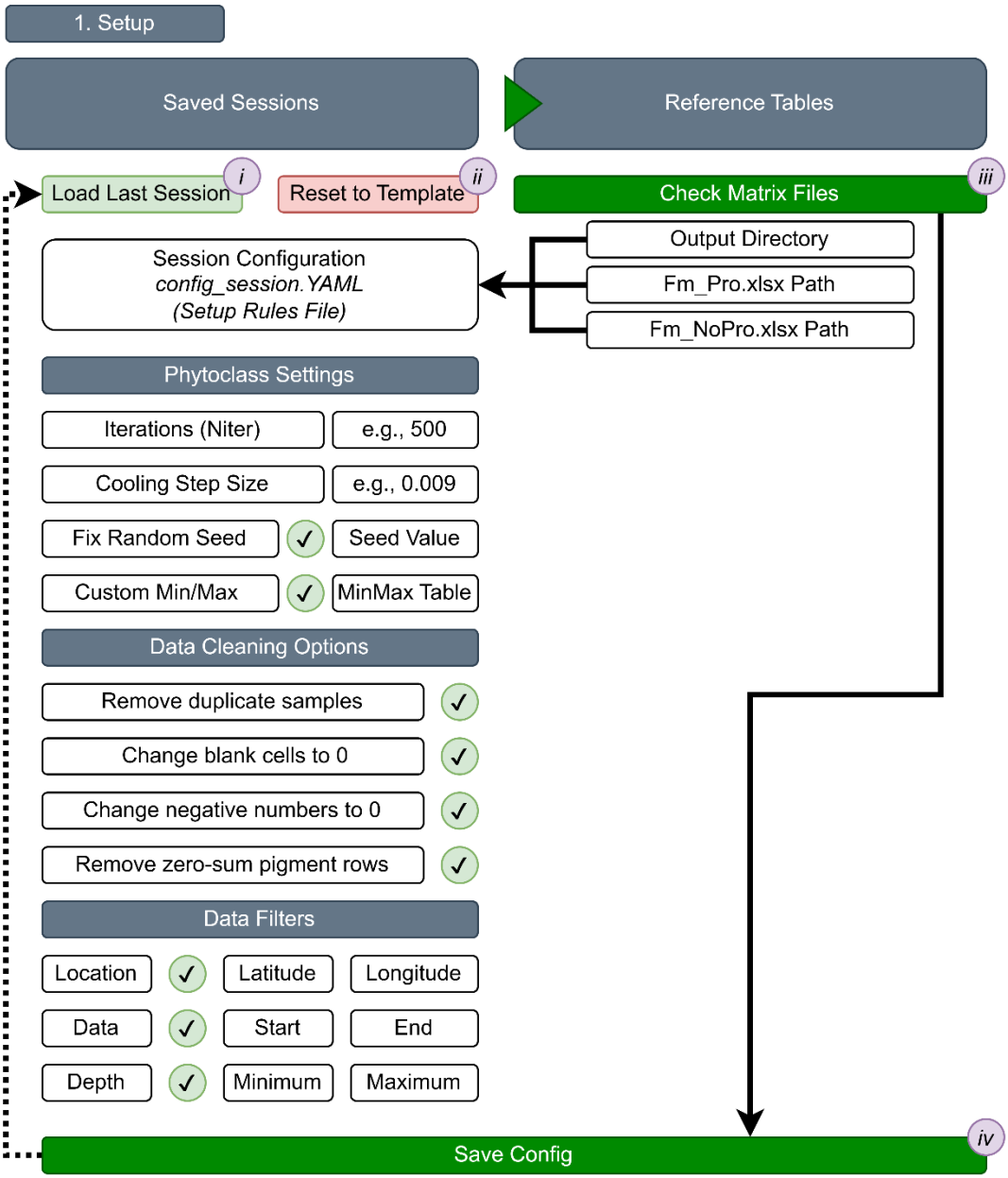


Fig. S 2: Schematic overview of Step 1 (Setup) of the phytoclassShiny workflow. Users load a previous session or reset to the default template parameters (i, ii), and confirm the Fm_Pro and Fm_NoPro reference matrices are present via "Check Matrix Files" (iii); simulated annealing parameters, data-cleaning rules, and optional location, date, and depth filters are configured here and saved to the session configuration file via "Save Config" (iv). For corresponding in-app screenshot, see Fig. S 9.

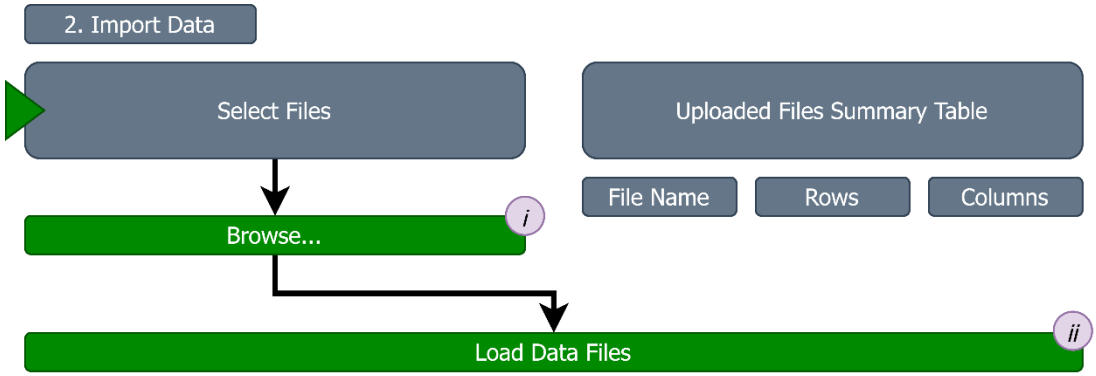


Fig. S 3: Schematic overview of Step 2 (Import Data) of the phytoclassShiny workflow. Raw pigment files are selected (i) and loaded (ii), producing a per-file summary of row and column counts. For corresponding in-app screenshot, see Fig. S 10.

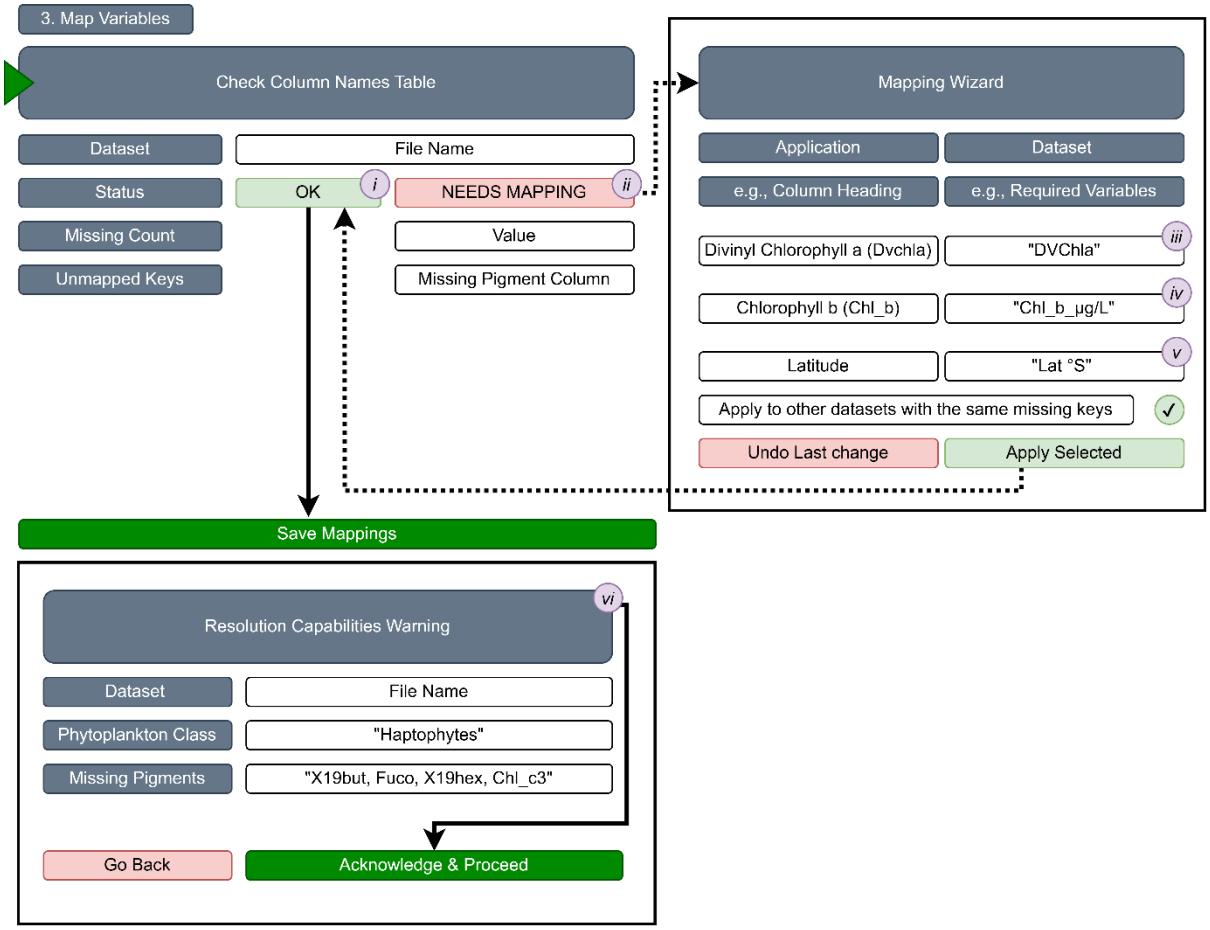


Fig. S 4: Schematic overview of Step 3 (Map Variables) of the phytoclassShiny workflow. each dataset's mapping status is reviewed in a summary table (i, OK; ii, NEEDS MAPPING); selecting a flagged dataset opens the mapping wizard, where required variables are matched to source columns (iii–v) and mappings can be propagated to other datasets sharing the same gaps. Saving mappings triggers a Resolution Capabilities Warning (vi) if any phytoplankton class cannot be fully resolved from the pigments available, which the user can acknowledge or address by returning to the wizard. For corresponding in-app screenshot, see Fig. S 11.

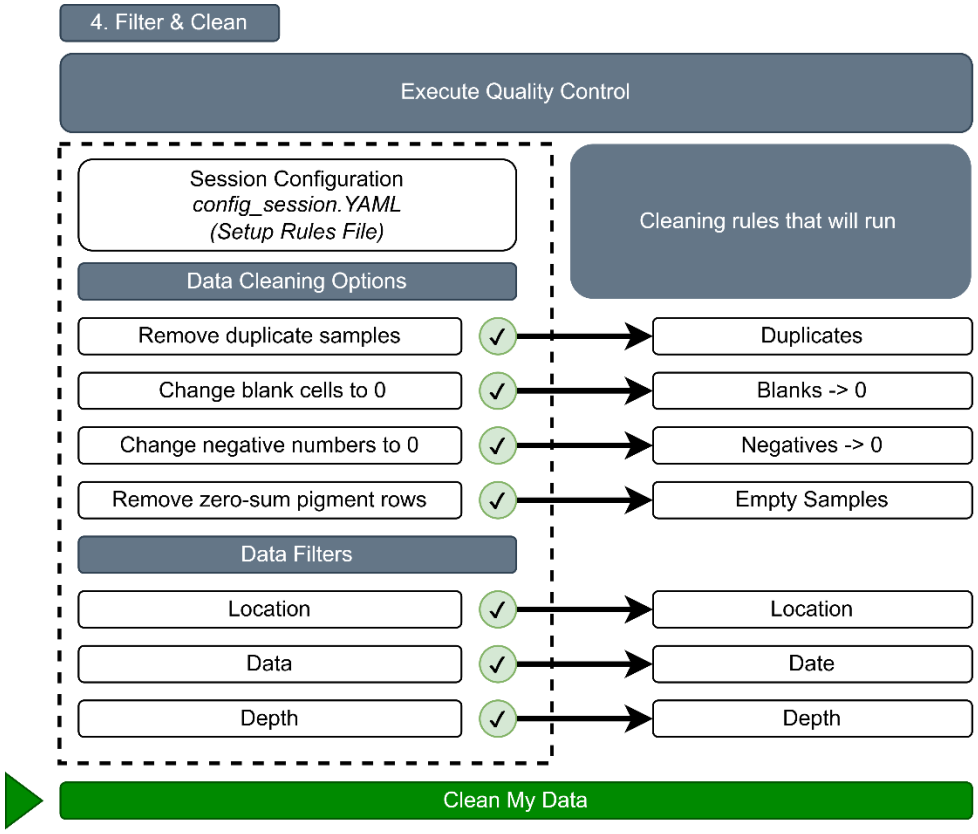


Fig. S 5: Schematic overview of Step 4 (Filter & Clean) of the phytoclassShiny workflow. The active cleaning rules and filters configured in Step 1 are applied to a working copy of the data. For corresponding in-app screenshot, see Fig. S 12.

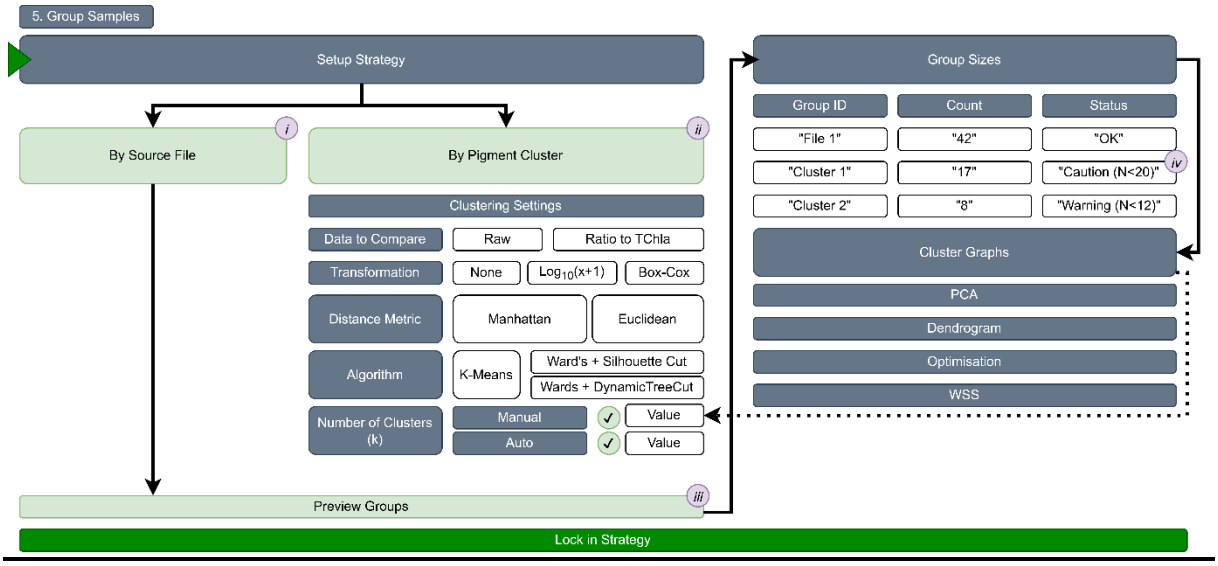


Fig. S 6: Schematic overview of Step 5 (Group Samples) of the phytoclassShiny workflow. Users group samples by source file (i) or by pigment cluster (ii), configuring normalisation, transformation, distance metric, clustering algorithm, and the number of clusters before previewing the resulting group sizes and diagnostic plots (iii); group-size warnings (iv) and the accompanying PCA, dendrogram, and optimisation plots inform whether the number of clusters should be adjusted before the strategy is locked in. For corresponding in-app screenshot, see Fig. S 13.

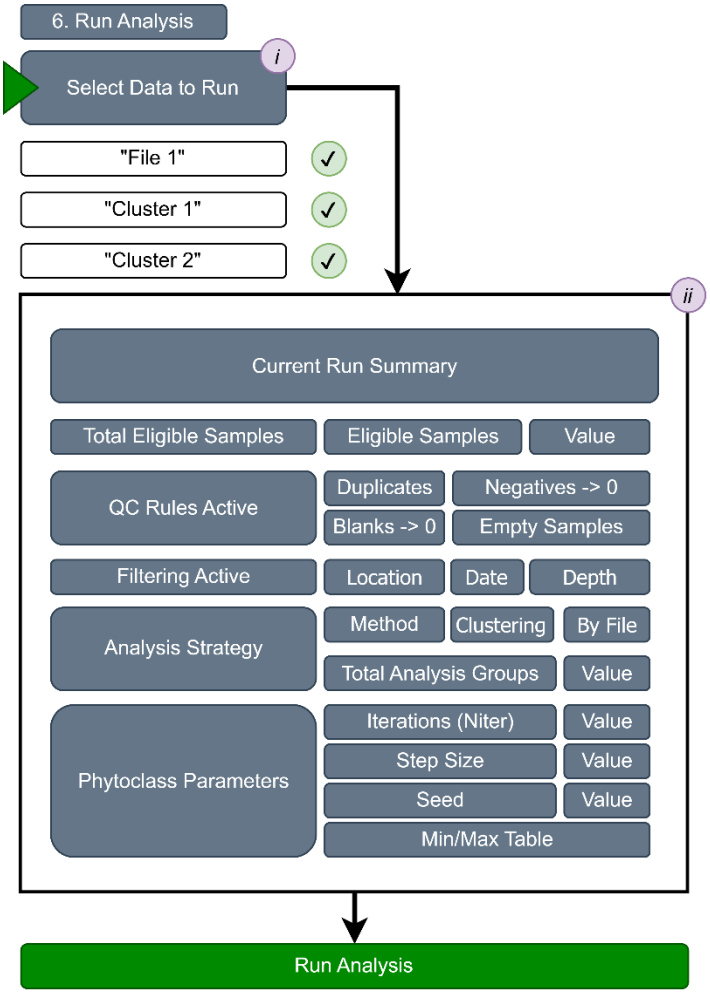


Fig. S 7: Schematic overview of Step 6 (Run Analysis) of the phytoclassShiny workflow. Users select which sample groups to process (i); a run summary (ii) reports the eligible sample count, active quality-control and filtering rules, the chosen grouping strategy, and the simulated annealing parameters that will be applied, before the analysis is run. For corresponding in-app screenshot, see Fig. S 14.

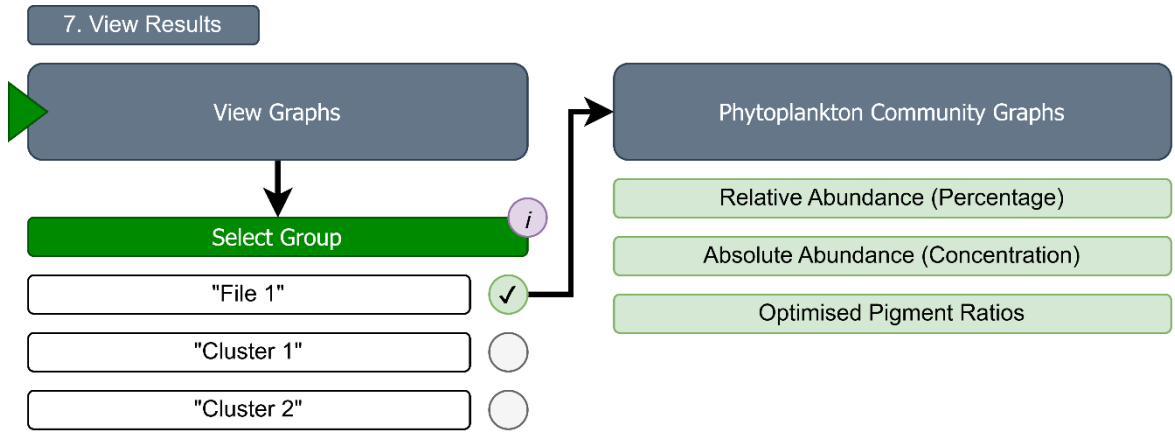


Fig. S 8: Schematic overview of Step 7 (View Results) of the phytoclassShiny workflow. Users select a processed group (i) to view its relative abundance, absolute abundance, and optimised pigment ratio outputs. For corresponding in-app screenshot, see Fig. S 15

Screenshots

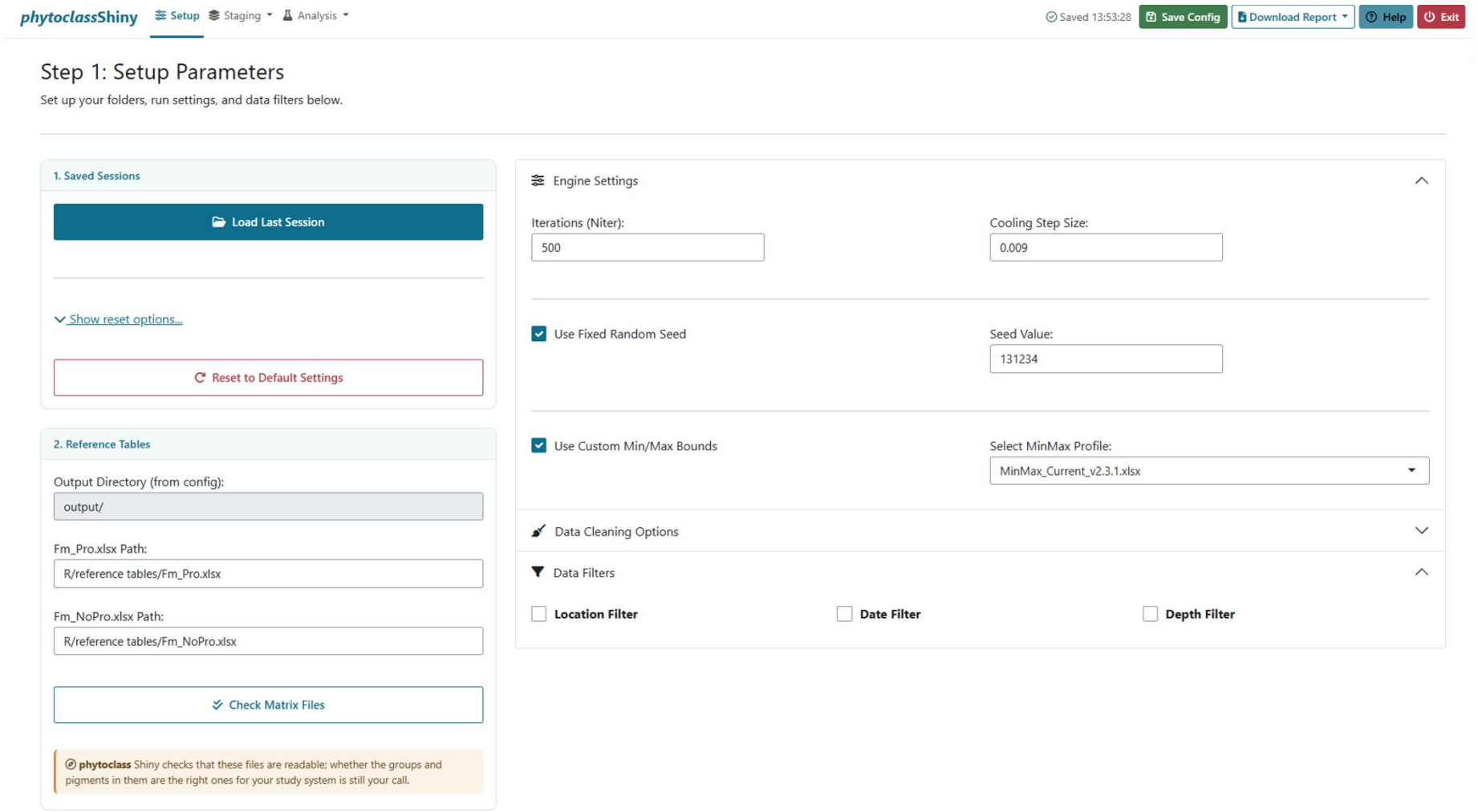


Fig. S 9: Step 1 (Setup Parameters). Phytoclass settings, reference matrix paths, data cleaning toggles, and data filters are configured here; a fixed random seed and custom Min/Max profile are enabled in this example. For corresponding schematic overview, see Fig. S 2.

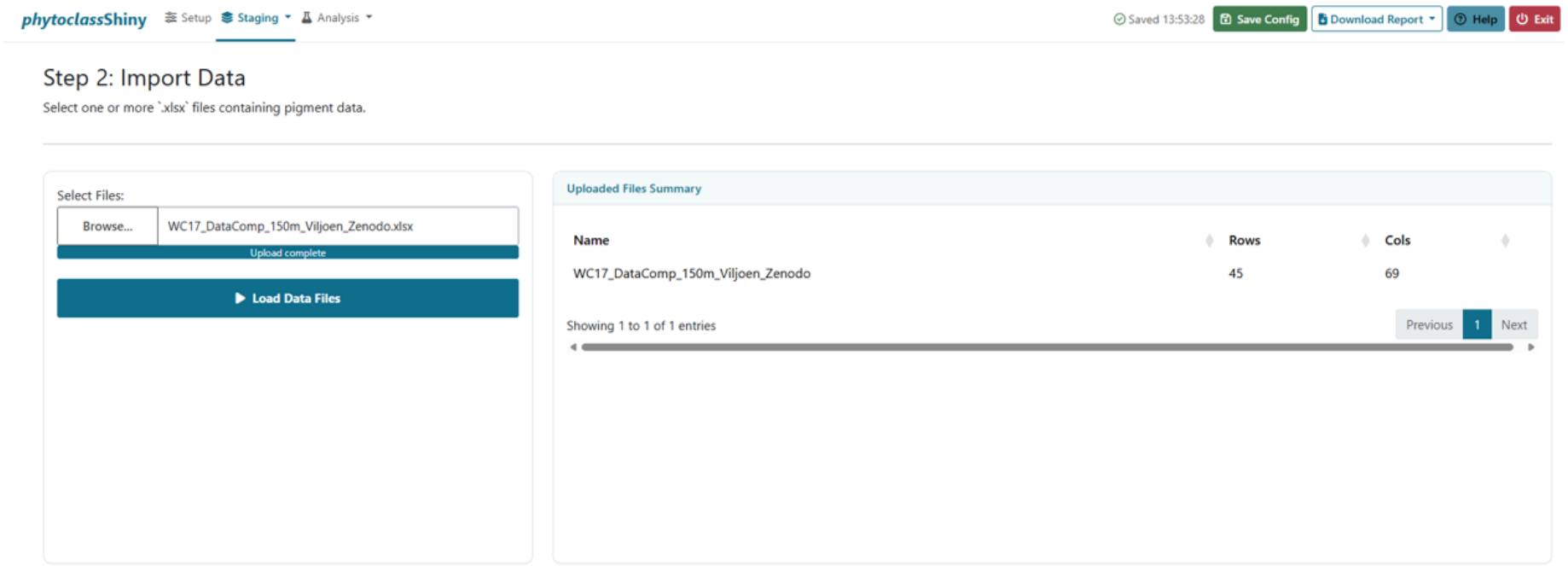


Fig. S 10: Step 2 (Import Data). A single HPLC pigment file (45 samples, 69 columns) has been selected using the file browser and loaded into the application. For corresponding schematic overview, see Fig. S 3.

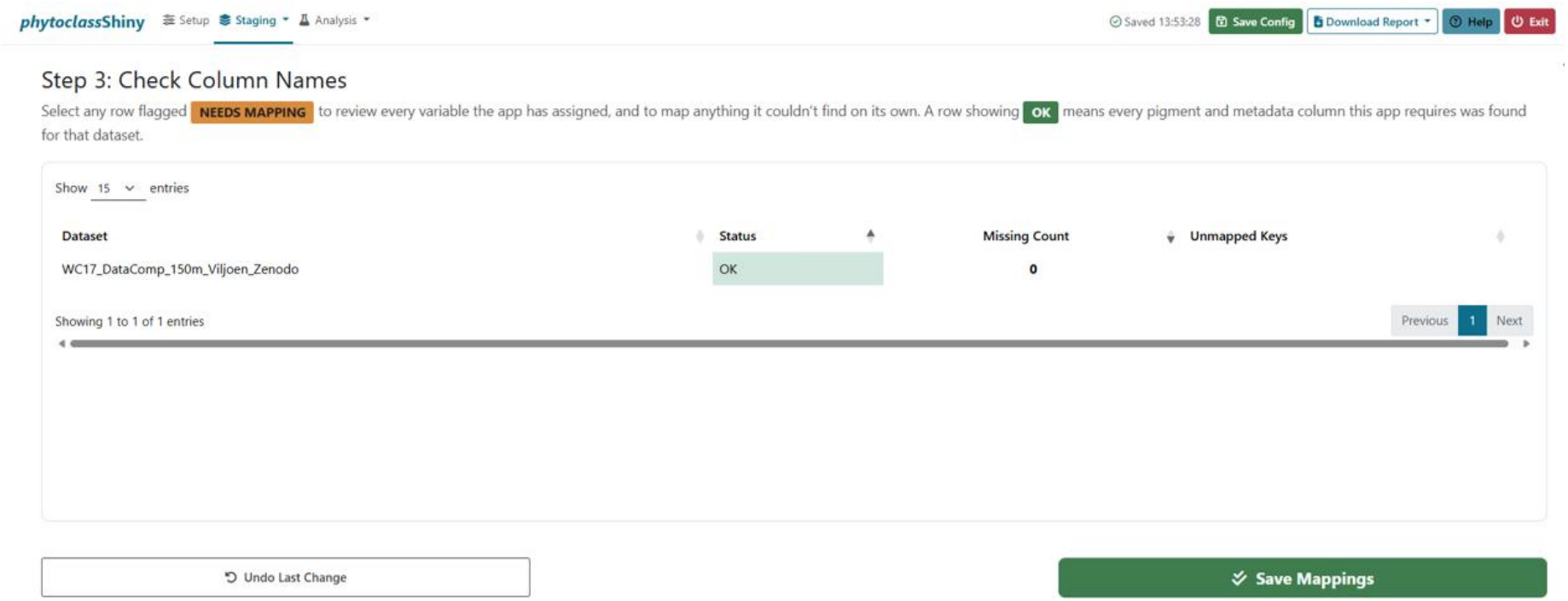


Fig. S 11: Step 3 (Map Column Names). The uploaded dataset shows an "OK" mapping status, indicating every required variable was matched automatically with no manual mapping needed. For corresponding schematic overview, see Fig. S 4.

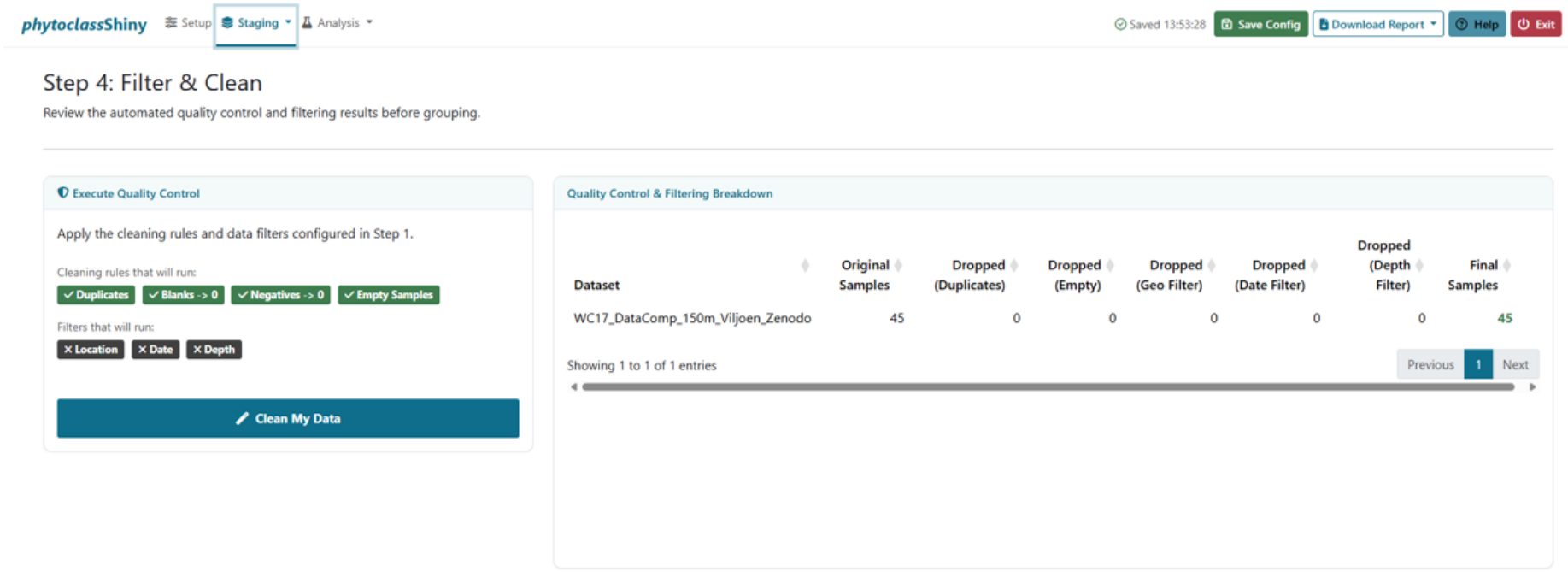


Fig. S 12: Step 4 (Filter & Clean). Quality-control results for the uploaded dataset: all 45 samples passed cleaning with none dropped. For corresponding schematic overview, see Fig. S 5.

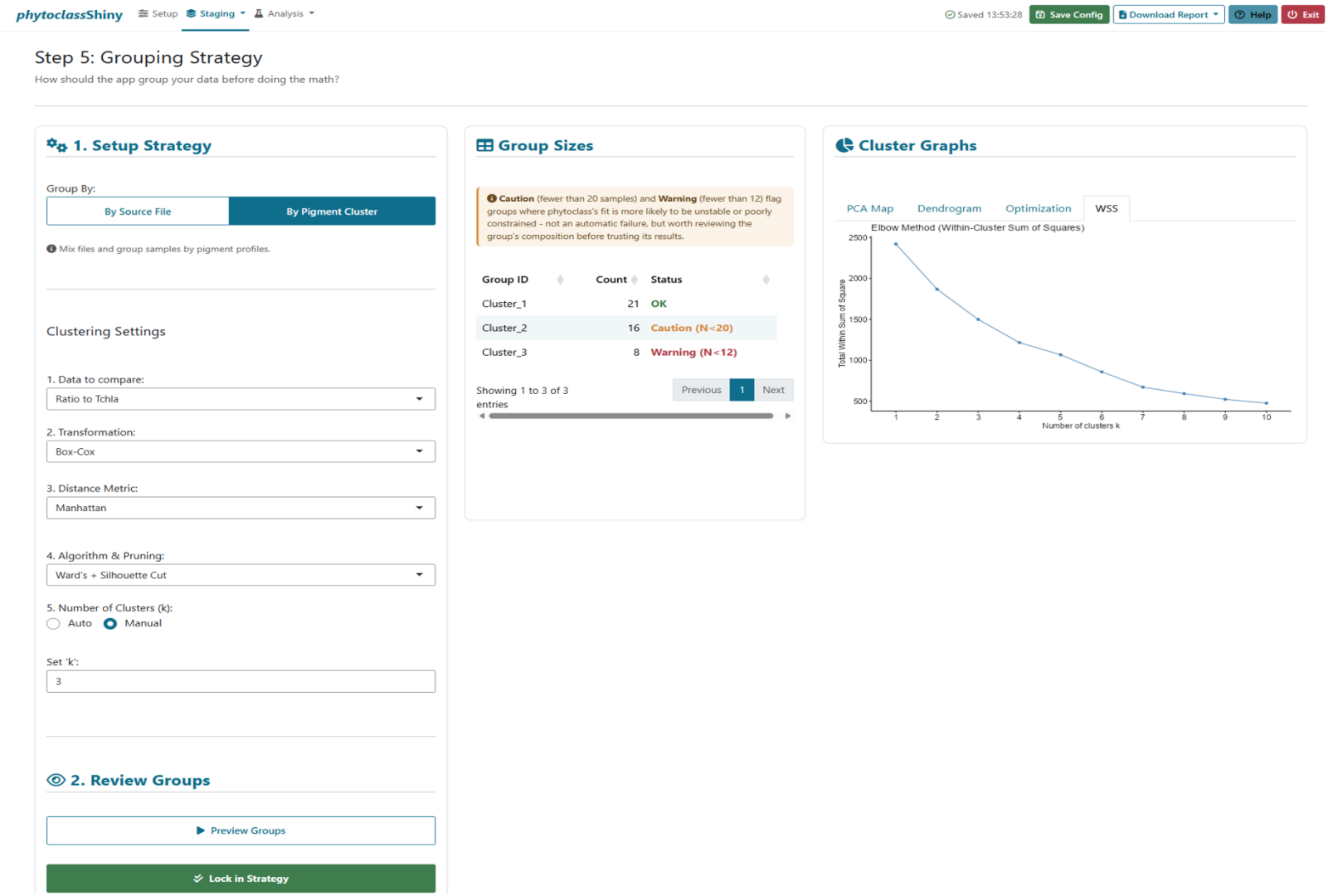


Fig. S 13: Step 5 (Grouping Strategy). Samples grouped by pigment cluster into three groups; Cluster_3 is flagged "Warning (N<12)" and Cluster_2 "Caution (N<20)" based on sample size, with the elbow plot shown alongside. For corresponding schematic overview, see Fig. S 6.

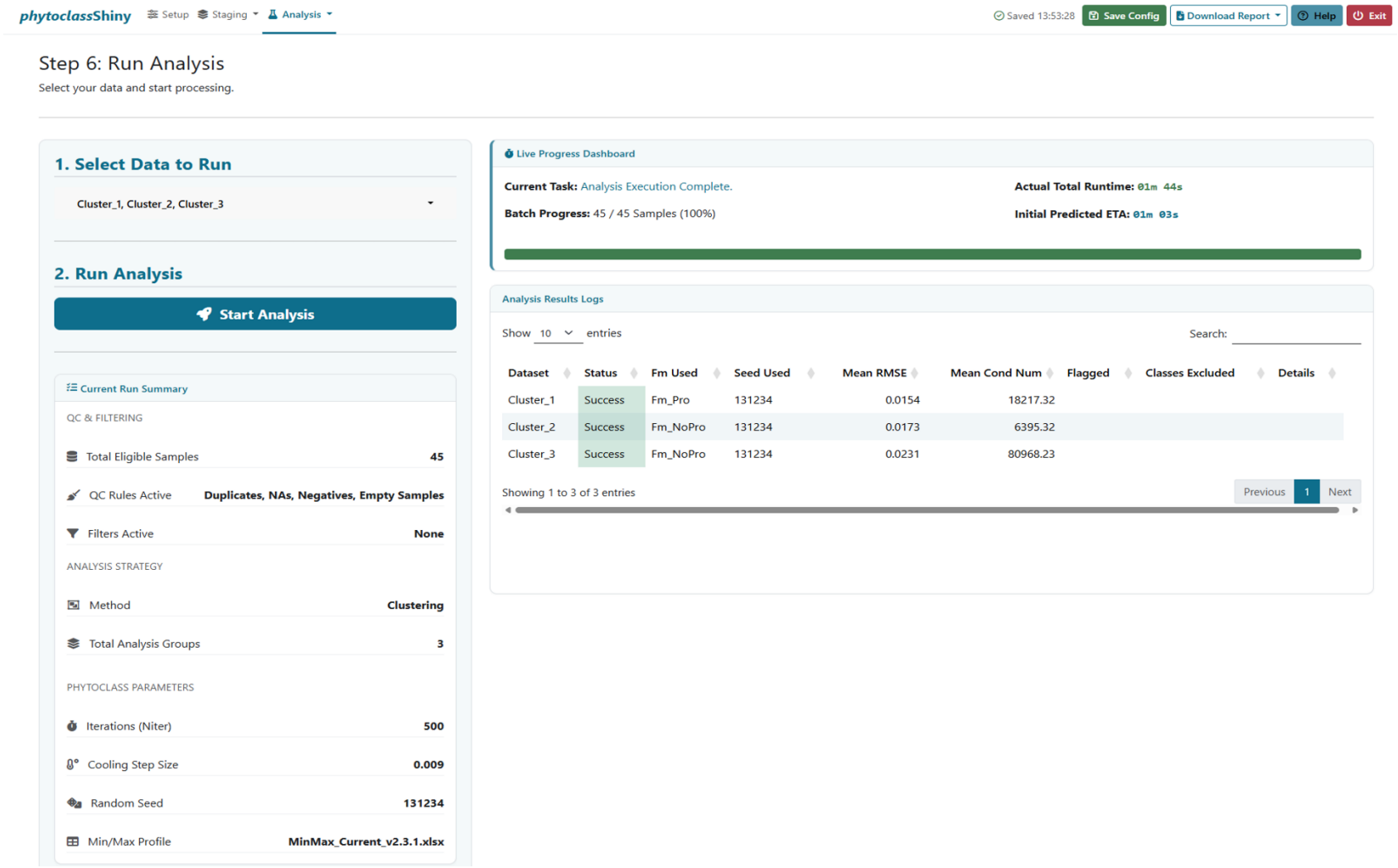


Fig. S 14: Step 6 (Run Analysis). Completed analysis run for all three clusters, showing the automatically selected reference matrix (Fm_Pro or Fm_NoPro), fit metrics, and the "Classes Excluded" column reporting none excluded in this run. For corresponding schematic overview, see Fig. S 7.

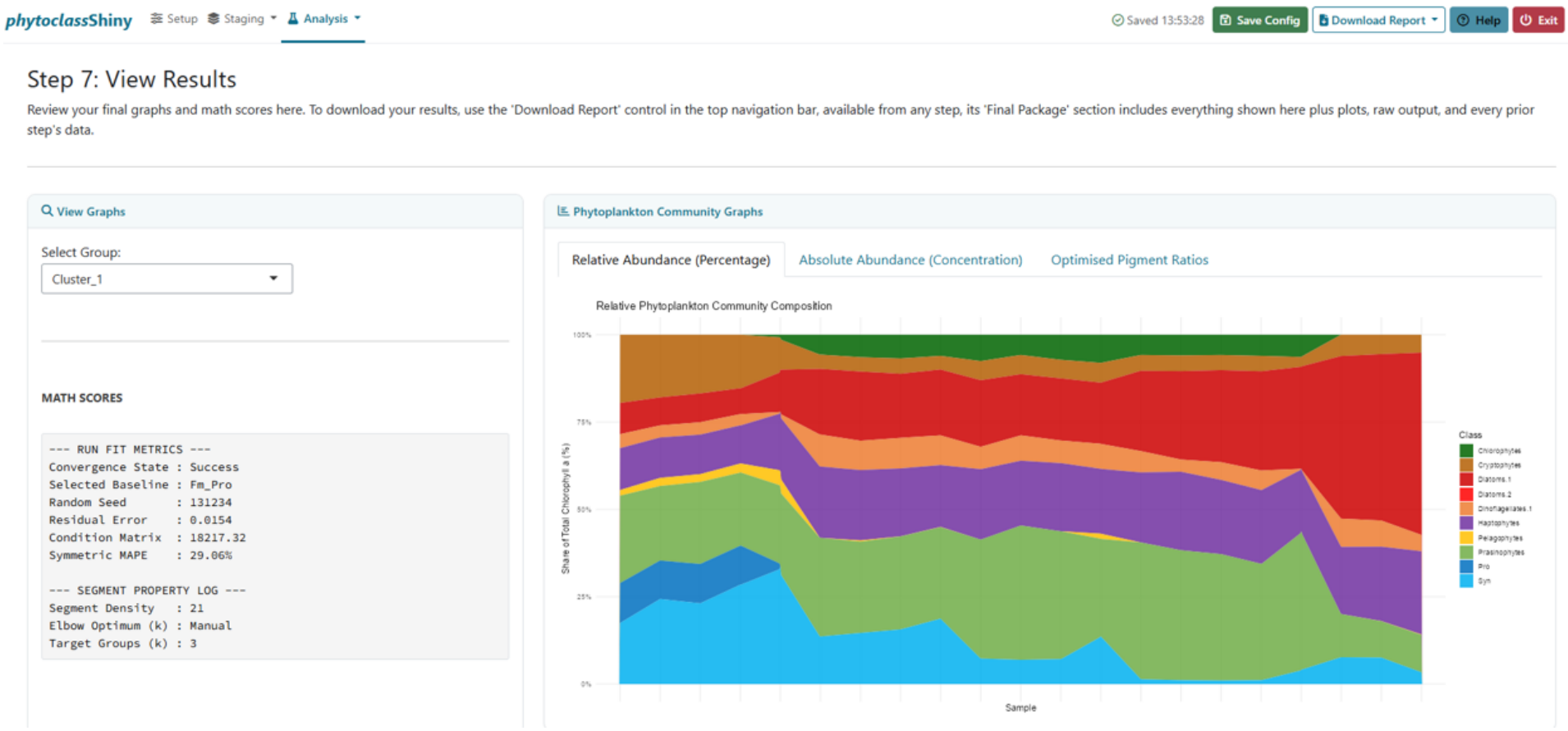


Fig. S 15: Step 7 (View Results). Relative community composition for Cluster_1, alongside its run fit metrics (RMSE, condition number, sMAPE) and clustering summary. For corresponding schematic overview, see

Fig. S 8.

Guided Workflow

Step 0: Application Setup and Launch

*phytoclass*Shiny was downloaded from GitHub, unzipped, and launched using the application launcher (.BAT file). The launcher detected a first-time run: although .Rprofile and *phytoclass*Shiny.Rproj were already present, the required R packages were missing. This triggered the package installer, which automatically created a system/app_packages folder and sourced all required packages (876 MB) from the official CRAN repository into this sandboxed environment. The launch log confirmed that *phytoclass*Shiny had the necessary read/write permissions at its install location (C:/Users/Name/Desktop/*phytoclass*Shiny-main/) and that every required package downloaded successfully and matched the expected version. The application window opened automatically once installation finished, was closed, and was relaunched via the same launcher; this second launch passed the environment check without triggering any further package downloads.

Step 1: Setup Configuration

Unless stated otherwise, every analysis in this study used the same setup configuration: Iterations (Niter): 500; Cooling Step Size: 0.009; Fixed Random Seed enabled, Seed Value: 131234; Custom Min/Max Bounds enabled, MinMax Profile: MinMax_Archive_v2.0.0.xlsx; and all four data-cleaning toggles enabled (remove duplicate samples, change blank cells to 0, change negative numbers to 0, remove empty samples). No data filters were applied. These settings were saved to the session configuration file, which was verified using the Download Report function with the "Setup Information (Step 1)" option enabled.

Step 2: Data Preparation and Import

The validation dataset (WC17_HPLC_pigments_Viljoen_Zenodo.xlsx) was downloaded, and its pigment-data sheet ("HPLC pigments Winter 2017") was reordered to be the first sheet in the workbook file. Visual inspection confirmed the pigment data met the app's required structure of samples as rows and pigments/metadata as columns (n = 46). Sample 8_Pig-2 (row 43) was then removed to match the validation setup (n = 45). The Fm_Pro.xlsx and Fm_NoPro.xlsx reference matrices were also edited at this stage, deleting the Chlorophytes and Diatoms-1 group rows so that these groups would not be resolved, again to match the validation setup. This prepared file was then selected within the app via the Step 2 file browser.

Step 3: Pigment Column mapping

On import, the app flagged four pigments as unmapped: Chlorophyll c1/c2 (Chl_c1), Peridinin (Per), 19′-Butanoyloxyfucoxanthin (X19but), and 19′-Hexanoyloxyfucoxanthin (X19hex). Selecting the dataset (flagged "NEEDS MAPPING") opened the mapping wizard, where each pigment was located via the searchable column-name dropdown and mapped to its corresponding column: Chlorophyll_c1.c2, Peridinin, X19.Butanoyloxyfucoxanthin, and X19.Hexanoyloxyfucoxanthin, respectively. The remaining columns had been mapped automatically; these were reviewed and confirmed: Divinyl Chlorophyll a (Dvchla), Chlorophyll b (Chl_b), Chlorophyll c3 (Chl_c3), Fucoxanthin (Fuco), Prasinoxanthin (Pra),

Violaxanthin (Viol), Alloxanthin (Allo), Zeaxanthin (Zea), Lutein (Lut), Neoxanthin (Neox), and Total Chlorophyll a (Tchla).

Five of these automatically mapped pigments (Divinyl Chlorophyll a, Prasinoxanthin, Violaxanthin, Zeaxanthin, and Neoxanthin) showed "LOD" (below limit of detection) in the mapping wizard's column preview, which displays only the first three sample rows. Because the app's empty-pigment warning triggers only when a pigment reads zero across every sample in the full dataset, no warning appeared here. This absence of a warning implied that valid, non-zero values existed elsewhere in the dataset, which manual inspection of the full file subsequently confirmed.

Once all aliases were applied, the file status updated to "OK" and the mapping was saved. To avoid repeating this step in future runs, the newly confirmed column names were also saved to the session configuration file, updating the alias list for each matched pigment.

Step 4: Data Cleaning

All four data-cleaning toggles were confirmed enabled, and no filters were applied. No samples were flagged or removed at this step.

Step 5: Grouping

The dataset was grouped "By Source File." The working copy contained more samples than both the caution ($n < 20$) and warning ($n < 12$) thresholds, so the grouping was locked in without issue and passed to Step 6 for analysis.

Step 6: Analysis

Before running, the analysis summary confirmed the expected sample count, the active QC rules, that no filters were enabled, grouping by source file, a single analysis group, and the *phytoclass* parameters listed above. Based on the template run-speed benchmark, the app predicted a runtime of 00m 23s; the analysis actually completed in 00m 42s, updating the session's processing-speed estimator accordingly. Subsequent runs of the same configuration and dataset on the same computer then predicted 00m 42s and completed in exactly 00m 42s. Every analysis in this study, start to finish, completed in under one minute. Chl_c1 exhibited zero variance across samples in the analysed file and was therefore excluded (not implemented) by *phytoclass*.

Validation

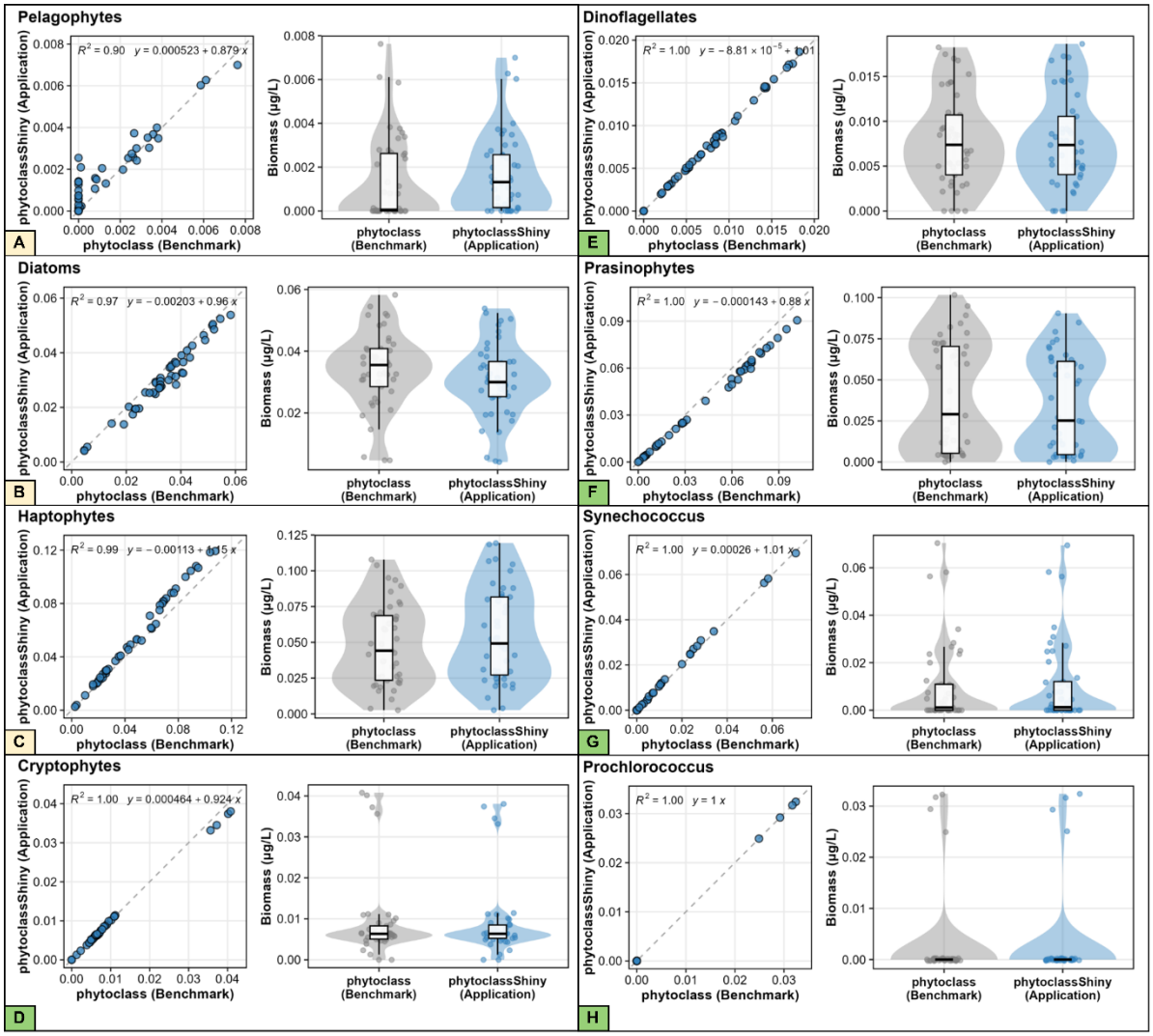


Fig. S 16: Validation of phytoclassShiny against the original phytoclass R package across the eight phytoplankton functional arranged by frames A-H (A, Pelagophytes; B, Diatoms; C, Haptophytes; D, Cryptophytes; E, Dinoflagellates; F, Prasinophytes; G, Synechococcus; H, Prochlorococcus). In each panel, the left plot compares per-sample biomass (µg/L) for each group estimated by phytoclassShiny ("Application," y-axis) against the benchmark dataset ("Benchmark," x-axis); the dashed line marks 1:1 agreement, the fitted regression line and its equation and R^2 are given inset. The benchmark dataset used the same samples and setup running the phytoclass package natively, however the benchmark min-max table was not reported. The right plot overlays a violin, boxplot, and individual sample points for each method's biomass (TChla proxy) distribution. Across all eight groups R^2 ranges from 0.90-0.99 (Pelagophytes, Diatoms, Haptophytes) with the remainder all 1.00 (Cryptophytes, Dinoflagellates, Prasinophytes, Synechococcus, and Prochlorococcus). Regression slopes close to 1 and intercepts close to 0 indicate phytoclassShiny reproduces phytoclass's simulated-annealing output without meaningful loss of accuracy, even without all the exact benchmark parameters available.

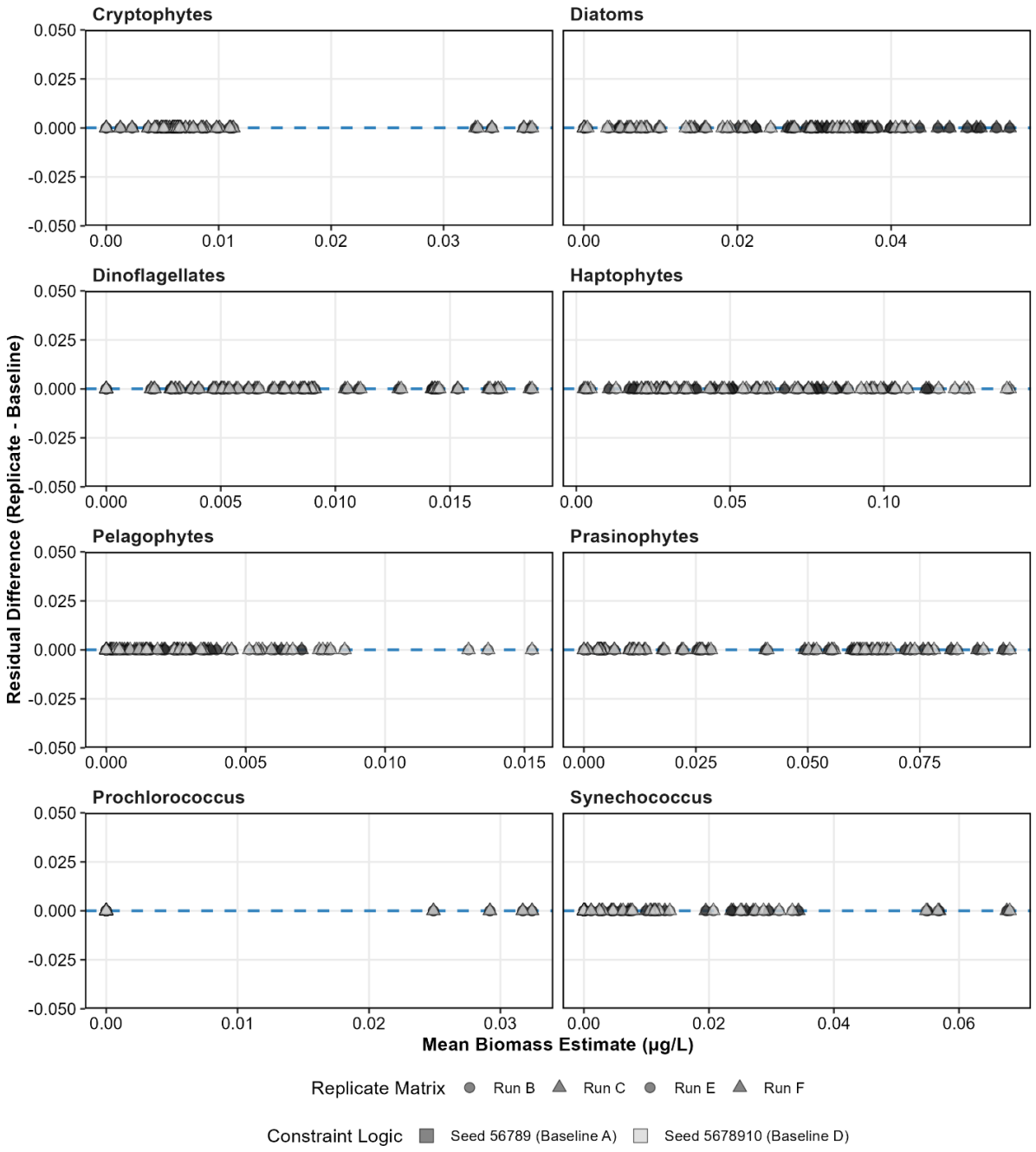


Fig. S 17: Bland-Altman agreement analysis of simulated annealing replicates. To verify internal consistency, the application was tested using two independent fixed random seeds across restarted sessions. The x-axis displays the mean absolute biomass estimate (µg/L) between both fixed-seed baseline runs (Session A and D) compared to its respective replicates (For A - sessions B/C; for D - sessions E/F). The y-axis plots the residual difference between each fixed-seed run. Shapes denote the specific replicate matrix, while grayscale shades differentiate between the two independent seed groups. The alignment of all data points along the dashed blue line (y = 0.000) confirms zero variance between independent sessions when a fixed seed is used, proving complete reproducibility.

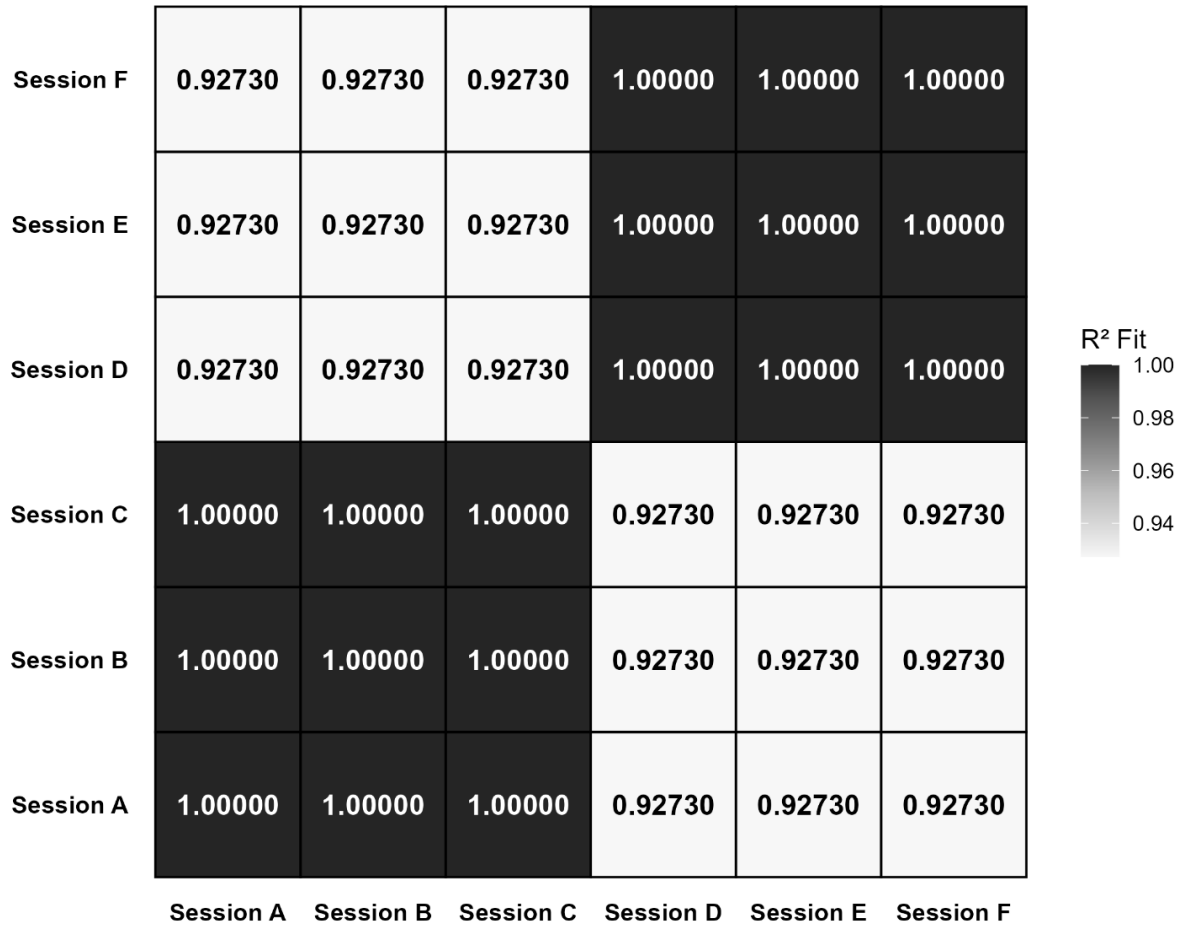


Fig. S 18: Consistency of the application's phytoplankton community estimates across multiple independent runs with a fixed seed (56789) for three sessions (A, B, C), and a different seed (5678910) for another three session (D, E, F). This correlation plot compares phytoplankton community estimates generated across these six sessions. The heatmap visualises the coefficient of determination (R^2) between every possible pair of runs. Black quadrants (R^2 = 1.00000) indicate exact matches, while white quadrants (R^2 = 0.92730) demonstrate variation that occurs when different fixed-seed values are used. The consistent and complete match among blocks within both fixed-seed groups shows that phytoclassShiny reproduces results exactly when a fixed random seed is applied. The minor difference between fixed-seed groups' estimates indicates that using a random seed causes some variation in results, which may limit reproducibility.