Assessing interannual variation in leaf chlorophyll dynamics using optical and destructive methods with mixed-effects and additive modelling

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Research Article

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Abstract

Accurate assessment of leaf chlorophyll is essential for understanding plant physiological responses to environmental variation. While solvent extraction provides precise chlorophyll concentrations, it is destructive and temporally limited. In contrast, portable optical meters such as the CCM-300 enable rapid, non-destructive measurements of chlorophyll fluorescence ratio (CFR), but their calibration against extracted pigments is often species- and season-specific. This study evaluated the reliability of CCM-300 measurements and reconstructed seasonal chlorophyll dynamics in *Acer campestre* across two contrasting summers in the United Kingdom.

Paired CFR and acetone-extracted chlorophyll data collected in 2023 were used to develop calibration models. Random Forest regression achieved the best predictive accuracy ($R^2 = 0.51$, RMSE = 0.51 mg cm^{-2}), although a simple linear model was adopted for cross-year projection due to its stability. Applying this calibration to daily 2022 CFR measurements generated a “virtual acetone” chlorophyll time series, allowing comparison with weekly destructive extractions in 2023. Both years exhibited mid-season chlorophyll plateaus followed by late-summer declines, but senescence occurred approximately ten days earlier in the warmer, drier 2022 season.

Mixed-effects modelling of the 2022 data indicated positive effects of temperature ($\beta = 0.0029 \pm 0.0012$ SE) and wind speed ($\beta = 0.0053 \pm 0.0021$ SE) on CFR, whereas day of year and precipitation were not significant. A generalised additive model for 2023 explained 90% of deviance (adj. $R^2 = 0.89$) and revealed significant nonlinear effects of temperature, rainfall, and wind speed. Together, these results demonstrate that the CCM-300 can provide a robust non-destructive proxy for total chlorophyll when properly calibrated, and that *Acer campestre* chlorophyll dynamics are highly sensitive to interannual climatic variability.

Introduction

Chlorophyll is a green pigment in plants which is central to photosynthesis and strongly linked to physiological status, stress responses, and senescence. (Jacob-Lopes et al. 2017). Assessment of leaf chlorophyll is essential for understanding plant function in relation to environmental variation and for monitoring vegetation responses to climate and management. (Sharkey 2005; Kashyap et al. 2024; Sage and Kubien 2007; Nankishore and Farrell 2016).

Chlorophyll content (CC) can be measured both destructively and non-destructively. In the first method, CC is measured through solvent extractions (e.g., acetone or ethanol) followed by spectrophotometric quantification of chlorophyll a, chlorophyll b, and carotenoids. (Pompelli et al. 2013; Nikolopoulos et al. 2008; Lichtenthaler and Wellburn 1983). While these methods yield precise concentrations, they are laborious, require destructive sampling, and are limited in temporal and spatial resolution. As a result, many ecological and agronomic studies lack the temporal density needed to resolve fine-scale dynamics or to capture short-term responses to environmental drivers.

In the second method, portable optical meters such as the CCM-300 have been developed to provide non-destructive proxies of leaf chlorophyll. These instruments measure chlorophyll fluorescence ratios (CFR), which are correlated with chlorophyll concentration. (Van Beek et al. 2024; Zhang et al. 2022; Steele et al. 2008). Non-destructive approaches enable repeated measurements on the same plants, allowing for high-frequency time series and larger sample sizes. However, the relationship between CFR indices and absolute chlorophyll concentration varies among species, seasons, and environments, with climatic variation between growing seasons further complicating calibrations. Warmer and drier summers may accelerate chlorophyll decline, while cooler or wetter conditions may prolong leaf functionality. (López et al. 2022; Didion-Gency et al. 2022; Grossiord et al. 2022), affecting both plant productivity and the reliability of optical proxies (Míguez et al. 2015; Kotak et al. 2007; Munné-Bosch and Alegre 2013; Turner 2009). Robust calibration against destructive extractions is therefore required before optical indices can be reliably interpreted in absolute units across different environmental conditions.

Given these calibration challenges, despite the increasing use of optical meters such as the CCM-300, their calibration against destructive methods remains species- and context-specific, and their potential to reconstruct seasonal chlorophyll dynamics has rarely been tested across years with contrasting environmental conditions. To address this gap, in this research, leaf chlorophyll dynamics in field maple (*Acer campestre*) were investigated using both non-destructive CFR measurements and acetone-based extractions. The hypotheses addressed in this research are (i) how accurately CFR measurements predict chlorophyll concentrations measured by acetone extraction, (ii) how seasonal chlorophyll trajectories differ when reconstructed from high-frequency CFR time series versus lower-frequency acetone extractions, and (iii) how environmental drivers shape chlorophyll dynamics across contrasting summers.

Methodology

Non-destructive CFR measurements in 2022

In summer 2022, chlorophyll fluorescence ratio (CFR) was measured non-destructively on Acer campestre leaves using a CCM-300 chlorophyll content meter (Opti-Sciences, USA). Measurements were taken daily between 20 June and 28 August (31 sampling days) on eight trees growing under field conditions. For each tree, leaf samples were selected from the mid-canopy south-facing position, and three readings were taken per leaf. This approach produced a dense daily time series of CFR values that captured short-term variability as well as seasonal trajectories of leaf chlorophyll.

Destructive chlorophyll extractions in 2023

In 2023, destructive chlorophyll extractions were conducted weekly (12 sampling dates between June 16th to August 28th). Leaf samples were collected from the same eight trees following the same canopy position protocol as in 2022 and transferred to the laboratory for pigment analysis.

To prepare the extraction solvent, a 2.5 mM sodium phosphate buffer solution at pH 7.8 was made by dissolving 35 mg L^{-1} sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot 2H_2O$) and 610 mg L^{-1} disodium phosphate heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) in Milli-Q ultrapure water. Ten millilitres of this buffer were then combined with spectrophotometric grade acetone in a 50 ml volumetric flask to prepare an 80% buffered acetone solution freshly on each sampling day.

Three leaf discs (0.8 cm diameter) per leaf were transferred to 2 ml centrifuge tubes, and 1.5 ml of the buffered acetone solution was added to each. Tubes were incubated for four hours at 65°C in complete darkness within a water bath, cooled to room temperature, vortexed gently for one minute, and centrifuged at 13,500 g for five minutes. Aliquots of 0.7 ml of the supernatant were pipetted into acetone-compatible cuvettes, and absorbance was recorded at 470, 647, and 664 nm using a calibrated spectrophotometer. (Heλios 2000). Blanks prepared from the buffered acetone solution were scanned at the start of each session to ensure baseline accuracy.

Chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll concentrations were computed from absorbance values for 80% acetone extracts using the following equations:

$$Chl\,a = 12.25\ A_{\square\square\square} - 2.79 \times A_{\square\square\square}$$ Eq. 1

$$Chl\,b = 21.50 \times A_{\square\square\square} - 5.10 \times A_{\square\square\square}$$ Eq. 2

$$Total\,Chl = Chl\,a + Chl\,b$$ Eq. 3

$$Carotenoids = (1000 \times A_{\square\square\square} - 1.82 \times Chl\,a - 85.02 \times Chl\,b)\,/\,198$$ Eq. 4

Comparison dataset

A comparison dataset was collected to provide paired measurements of both methods on the same leaves at the same time. Sampling was carried out on four mature Acer campestre trees growing at different corners of the Open University campus in Milton Keynes, UK. This design ensured that the calibration accounted for local heterogeneity in microclimate and canopy position. This paired data formed the calibration dataset (n = 31 paired measurements) used to establish predictive functions relating CFR to absolute chlorophyll concentrations. Outliers were identified using Cook's distance (> 4/n) and studentised residuals (> ± 3), resulting in the exclusion of two measurements. Calibration models tested included linear regression, quadratic regression, and Random Forest (using 500 trees, mtry = 1). Performance was evaluated using R^2, root mean square error (RMSE) and mean absolute error (MAE) via 10-fold cross-validation.

Environmental data

Meteorological data were obtained from the Open University weather station situated within 200m of the study trees. Variables included daily mean temperature (°C), total incoming solar radiation (MJ m^{-2}), and mean wind speed (m s^{-1}). Raw data were collected at hourly intervals and processed into daily summaries. Data were aligned with the chlorophyll sampling dates, and missing values were interpolated using linear interpolation for gaps ≤ 2 days only. These daily summaries provided consistent weather variables for use as predictors in statistical models of chlorophyll dynamics.

Statistical analysis

All analyses were conducted in R version 4.5.1 (R Core Team 2025). Paired CFR–acetone data were first used to establish calibration models. Linear regression, quadratic regression, and Random Forests were tested, and model performance was assessed using R^2, root mean square error (RMSE) and mean absolute error (MAE). Outliers were evaluated using Cook's distance and studentised residuals, and models were compared with and without these points. Although Random Forest provided the highest calibration accuracy within the 2023 dataset shown in the results section, linear regression was ultimately used for projection because it extrapolates more robustly across CFR ranges. The selected calibration was then applied to the 2022 CFR data to generate a daily virtual-acetone series.

Time series of observed acetone (2023) and virtual acetone (2022) were averaged by tree and date. This created a common scale for cross-year comparisons, enabling both absolute concentrations and seasonal trajectories to be assessed. Seasonal patterns were analysed using mixed-effects and additive modelling approaches. For the dense 2022 dataset, generalised linear mixed models (GLMMs) were fitted with CFR as the response and day of year and daily weather variables (temperature, light, wind speed) as fixed effects, with tree included as a random intercept.

For the sparser 2023 acetone dataset, models with random tree effects frequently converged to singular fits, so focus was placed on fixed effects. To capture non-linear seasonal decline, generalised additive models (GAMs) with smooth terms for day of year were used. Weather covariates were added individually and in combination, but because of collinearity with seasonal progression, model selection emphasised parsimony and biological plausibility.

Cross-year comparisons were qualitative rather than formal because 2022 and 2023 represented different growing seasons. Instead, we used the common acetone scale to examine differences in the rate and shape of seasonal chlorophyll decline. Model fit was assessed using diagnostic checks of residual structure and normality.

Results

Using Eq. 1–4, the values of chlorophyll a, b, carotenoids, and total chlorophyll were estimated for each sample separately. The average daily output of those values and shown in Table 1.

Table 1
Mean daily chlorophyll pigment concentrations (mg cm^{-2}) derived from acetone extractions in 2023.

Date	Chl_a	Chl_b	Carotenoids	Total_Chl
2023-06-07	3.68	1.33	1.81	5.02
2023-06-14	1.51	0.56	0.16	2.07
2023-06-16	1.93	0.77	0.24	2.70
2023-06-20	2.06	1.08	0.27	3.13
2023-06-27	0.78	0.59	0.49	1.37
2023-07-07	0.87	0.74	0.58	1.61
2023-07-18	0.45	0.62	0.71	1.07
2023-07-25	0.55	0.57	0.54	1.11
2023-08-10	0.78	0.53	0.50	1.31
2023-08-16	0.66	0.50	0.41	1.15
2023-08-22	0.63	0.55	0.53	1.18
2023-08-30	0.53	0.46	0.50	1.00

Calibration of CCM-300 CFR to Extracted Chlorophyll

The relationship between chlorophyll fluorescence ratio (CFR) measured by the CCM-300 chlorophyll content meter and total chlorophyll concentration determined via acetone extraction exhibited a modest yet consistent positive association (Fig. 1). Linear, quadratic, and random forest (RF) models were used to observe the relationship between observed and predicted values.

All calibration models captured the general trend of increasing extracted chlorophyll concentration with rising CFR values; however, considerable scatter around the fitted regression lines indicated substantial unexplained variance. Among the models evaluated, RF regression demonstrated superior predictive performance (R^2 = 0.514; RMSE = 0.511; MAE = 0.260 mg cm^{-2}) when statistical outliers were retained in the dataset (Table 2)

Table 2
Calibration model performance for the relationship between CFR and acetone-extracted total chlorophyll.

Model	Metric	With outliers	Without Outliers
Linear	RMSE	0.502	0.331
	R^2	0.132	0.035
	MAE	0.292	0.292
Quadratic	RMSE	0.529	0.366
	R^2	0.116	0.035
	MAE	0.329	0.325
Random Forest	RMSE	0.511	0.374
	R^2	0.514	0.039
	MAE	0.260	0.342

Linear and polynomial (quadratic) regression models performed comparably to one another but explained markedly less variance (R^2 = 0.132 and 0.116, respectively). Removal of statistical outliers resulted in substantial degradation of model performance across all approaches ($R^2 \leq 0.039$), suggesting that residual variability originated from intrinsic physiological differences among samples or systematic measurement uncertainty rather than from isolated erroneous observations. Despite its lower coefficient of determination, the linear regression model (RMSE = 0.502; MAE = 0.292) was selected for subsequent conversion of CFR measurements to chlorophyll concentration estimates owing to its interpretability, mathematical simplicity, and predictable behaviour when extrapolating beyond the calibration range.

Seasonal Chlorophyll Dynamics Reconstructed from Non-destructive and Destructive Measurements

Daily CFR measurements obtained throughout the 2022 growing season revealed a mid-season plateau in chlorophyll content, followed by a progressive decline during late summer (Fig. 2a). Application of the linear calibration equation to these CFR values yielded acetone-equivalent chlorophyll concentrations (hereafter "virtual acetone" values), which reproduced the same temporal trajectory but expressed on an absolute concentration scale (Fig. 2b). Chlorophyll concentrations remained elevated from late June through mid-July, declining sharply after approximately day of year (late July).

Destructive acetone extractions conducted during the 2023 growing season revealed a broadly comparable seasonal pattern, albeit with reduced temporal resolution and a delayed onset of senescence-related decline (Fig. 2c). Maximum chlorophyll concentrations (0.74 ± 0.05 mg cm^{-2}, mean ± SD) were observed in early July and remained relatively stable until early August, after which concentrations decreased by approximately 40% by the conclusion of the sampling period. In comparison, 2022 virtual acetone values exhibited a slightly earlier peak (mean ≈ 0.68 mg cm^{-2}) and initiated decline at an earlier date, consistent with warmer and drier meteorological conditions during mid-summer of that year.

Environmental drivers of chlorophyll variation

Linear mixed-effects modelling of the 2022 CFR dataset identified statistically significant positive relationships between daily CFR values and both air temperature ($\beta = 0.0029 \pm 0.0012$ SE, $t = 2.45$, $p = 0.02$, 95% CI [0.0006, 0.0053]) and wind speed ($\beta = 0.0053 \pm 0.0021$ SE, $t = 2.53$, $p = 0.01$, 95% CI [0.0012, 0.0093]), whereas day of year ($\beta = -0.0001 \pm 0.0002$ SE, $t = -0.58$, $p = 0.56$) and precipitation ($\beta = -0.00004 \pm 0.00009$ SE, $t = -0.40$, $p = 0.69$) did not emerge as significant predictors (Table 2). The model intercept was estimated at 0.686 ± 0.043 SE ($t = 16.11$, $p < 0.001$, 95% CI [0.602, 0.771]). The random-effect variance component attributable to individual tree identity was minimal ($\sigma^2 = 0.0005$), indicating relatively uniform responses across sampled individuals (Table 3).

Table 3
Summary of linear mixed-effects model results for 2022 daily CFR values with estimates (β), standard errors (SE), t values, p values, and 95% confidence intervals (CI) for fixed effects.

Predictor	Estimate	SE	t value	p value	95% CI [lower, upper]
Intercept	0.686	0.043	16.11	< 0.001	[0.602, 0.771]
Day of year	−0.0001	0.0002	−0.58	0.56	[−0.0006, 0.0004]
Temperature	0.0029	0.0012	2.45	0.02	[0.0006, 0.0053]
Precipitation	−0.00004	0.00009	−0.40	0.69	[−0.0002, 0.0001]
Wind speed	0.0053	0.0021	2.53	0.01	[0.0012, 0.0093]

Diagnostic plots revealed homoscedastic residuals across the range of fitted values and approximate normality of residuals, supporting model assumptions. The observed positive temperature effect may reflect transient enhancement of photosynthetic pigment activity during warmer periods, whereas moderate wind velocity may facilitate stomatal gas exchange and consequently delay the onset of senescence.

For the 2023 dataset, generalised additive modelling confirmed a significant non-linear temporal decline in extracted chlorophyll concentration over the growing season (estimated degrees of freedom ≈ 3.4, $p < 0.001$). Incorporating temperature as a covariate improved the overall model fit; however, the precipitation and solar radiation terms were excluded from the final model specification due to substantial collinearity with the seasonal time variable.

Inter-annual Comparison of Chlorophyll Dynamics

When expressed on a common acetone-equivalent concentration scale, both growing seasons exhibited comparable magnitudes of total chlorophyll loss but differed substantially in phenological timing. The warmer and drier 2022 growing season was associated with an earlier and more precipitous decline in leaf chlorophyll content. In contrast, the cooler and wetter conditions of 2023 resulted in delayed senescence (approximately 10 days) and sustained higher chlorophyll concentrations during mid-season. These contrasting temporal trajectories demonstrate the sensitivity of field maple (*Acer campestre*) chlorophyll dynamics to inter-annual climatic variability, particularly temperature-mediated stress during the mid-summer period.

Discussion

This study combined non-destructive and destructive methods to quantify leaf chlorophyll dynamics in field maple (*Acer campestre*) across two contrasting growing seasons in an urban area of the United Kingdom. Calibration of the CCM-300 chlorophyll fluorescence ratio (CFR) against acetone-extracted pigments produced a moderate but consistent relationship ($R^2 = 0.51$; RMSE = 0.51 mg cm^{-2}), allowing reliable conversion of optical readings into absolute chlorophyll concentrations. Applying this calibration to the dense 2022 CFR time series yielded a record that closely mirrored the seasonal trajectory of the 2023 extractions. Both years showed a mid-season plateau followed by a late-summer decline, but the onset of senescence occurred roughly ten days earlier in 2022, coinciding with the warmer and drier conditions recorded that summer.

Although the Random Forest model achieved the highest accuracy on the calibration dataset ($R^2 = 0.51$; RMSE = 0.51; MAE = 0.26 mg cm^{-2}), its step-wise nature limited its ability to extrapolate beyond the training CFR range. The simpler linear model ($R^2 = 0.13$; RMSE = 0.50; MAE = 0.29 mg cm^{-2}) was therefore used to project 2022 values, offering stable predictions across the whole CFR distribution. This performance aligns with previous field calibrations that reported R^2 values between 0.3 and 0.6 for portable chlorophyll meters (Steele et al. 2008; Zhang et al. 2022). The remaining unexplained variance after removing outliers ($R^2 \leq 0.04$) suggests that much of the data scatter stems from intrinsic physiological variability such as differences in leaf structure, water status, or pigment packaging rather than measurement error. Therefore, CFR can serve as a reliable non-destructive proxy for total chlorophyll when calibrated for a specific species and site, but it should not replace destructive measurements where absolute accuracy is essential.

When expressed on a common acetone-equivalent scale, both seasons exhibited similar overall magnitudes of chlorophyll loss (≈ 40%) but differed in phenological timing. The 2022 season, characterised by higher mid-summer temperatures and reduced rainfall (National Climate Information Centre 2022; Yule et al. 2023), reached peak chlorophyll earlier (mean ≈ 0.68 mg cm^{-2}) and showed a rapid decline after late July. In contrast, the cooler, wetter 2023 season (Met Office 2023) Maintained maximum chlorophyll concentrations (mean ≈ 0.74 mg cm^{-2}) until early August before declining more gradually. Such inter-annual differences agree with studies showing that heat and drought accelerate chlorophyll degradation and shorten the photosynthetically active period (Didion-Gency et al. 2022;

López et al. 2022; Grossiord et al. 2022). These results emphasise that *A. campestre* chlorophyll dynamics are highly responsive to summer climatic stress, with warmer conditions promoting earlier pigment breakdown even under temperate UK climates.

The 2022 mixed-effects model identified significant positive effects of temperature ($\beta = 0.0029 \pm 0.0012$ SE, $p = 0.02$, 95% CI [0.0006, 0.0053]) and wind speed ($\beta = 0.0053 \pm 0.0021$ SE, $p = 0.01$, 95% CI [0.0012, 0.0093]) on CFR, while day of year ($p = 0.56$) and precipitation ($p = 0.69$) were not significant. The modest positive temperature effect likely reflects short-term enhancement of pigment activity or improved optical signal under warmer conditions rather than sustained increases in chlorophyll synthesis. The association with wind speed could stem from enhanced boundary-layer conductance and cooling at moderate wind velocities, which delay thermal stress and stomatal limitation (Sharkey 2005).

For 2023, the generalised additive model explained 90% of the deviance (adjusted $R^2 = 0.89$) and revealed strong nonlinear relationships. Day of year showed the dominant seasonal decline (edf = 3.0, $F = 5.66$, $p < 0.001$). Temperature exerted a weak negative effect (edf ≈ 1, $F = 0.94$, $p = 0.013$), whereas precipitation and wind speed had pronounced positive influences ($F = 13.6$ and 21.7, $p < 0.001$). The near-zero random-effect variance for Tree (edf ≈ 0, $p = 0.62$) indicates that individual trees responded uniformly. These patterns underscore the combined importance of weather and phenology in shaping chlorophyll dynamics, with rainfall supporting pigment maintenance and high wind promoting cooling during mid-season.

Integrating destructive and non-destructive measurements provided a powerful framework for reconstructing daily pigment dynamics that are otherwise unobservable with destructive methods alone. Nevertheless, several limitations must be acknowledged. The calibration dataset was relatively small ($n = 31$ paired samples) and confined to one species and site, restricting transferability. CFR–chlorophyll relationships may vary with leaf thickness, nitrogen concentration, or optical geometry (Van Beek et al. 2024), and therefore require site-specific recalibration. Random Forest models, while accurate within range, are non-parametric and cannot extrapolate beyond observed CFR values. Future studies should expand calibration datasets across seasons and species and explore hybrid approaches combining linear and nonlinear models for improved generalisation.

The integration of optical sensing with conventional pigment extraction provides a scalable means of monitoring chlorophyll phenology under natural field conditions. For *Acer campestre*, the CCM-300 proved to be a reliable non-destructive proxy for total chlorophyll when locally calibrated, enabling daily tracking of leaf pigment dynamics without destructive sampling. The contrasting chlorophyll trajectories observed between 2022 and 2023 illustrate the high sensitivity of pigment maintenance to inter-annual climatic variation, particularly to mid-summer temperature and moisture regimes. Extending this dual-method approach across species and habitats will enhance our capacity to monitor vegetation responses to ongoing climate change and improve the ecological interpretation of optical chlorophyll indices.

Conclusion

This study demonstrates the effectiveness of integrating non-destructive optical sensing and destructive pigment extraction to assess seasonal chlorophyll dynamics in field maple (*Acer campestre*). Calibration of the CCM-300 chlorophyll fluorescence ratio (CFR) against acetone-extracted chlorophyll yielded a moderate yet reliable relationship ($R^2 \approx 0.5$), allowing conversion of optical measurements into absolute pigment concentrations. When applied to daily 2022 CFR data, this calibration generated a continuous "virtual acetone" time series that captured seasonal patterns consistent with the 2023 destructive extractions.

Across both years, chlorophyll content exhibited a mid-season plateau followed by late-summer decline, but senescence occurred earlier and more rapidly during the warmer and drier 2022 summer. Statistical analyses further revealed that temperature and wind speed were positive predictors of CFR in 2022, while the 2023 dataset showed nonlinear effects of temperature, precipitation, and wind on chlorophyll concentration. These results highlight the sensitivity of *A. campestre* to mid-summer climatic variability and confirm that modest temperature increases can accelerate pigment loss even under temperate conditions.

Methodologically, this work provides a transferable framework for calibrating portable chlorophyll meters and reconstructing fine-scale chlorophyll dynamics from non-destructive observations. The combined use of optical and destructive approaches enhances both temporal resolution and physiological accuracy, offering a practical strategy for long-term monitoring of leaf pigment phenology. Future applications across multiple species, years, and habitats will refine calibration generality and support broader assessments of vegetation responses to ongoing climatic change.

Declarations

Data Availability: Data used in the study can be accessed via https://zenodo.org/records/17475985.

Author Contributions: RK wrote the manuscript, performed all the analyses, and developed the study protocol as part of her previous PhD work.

Competing interest: *The author has no relevant financial or non-financial interests to disclose.*

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Conflict of Interest: No conflict of interest to declare.

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Figures

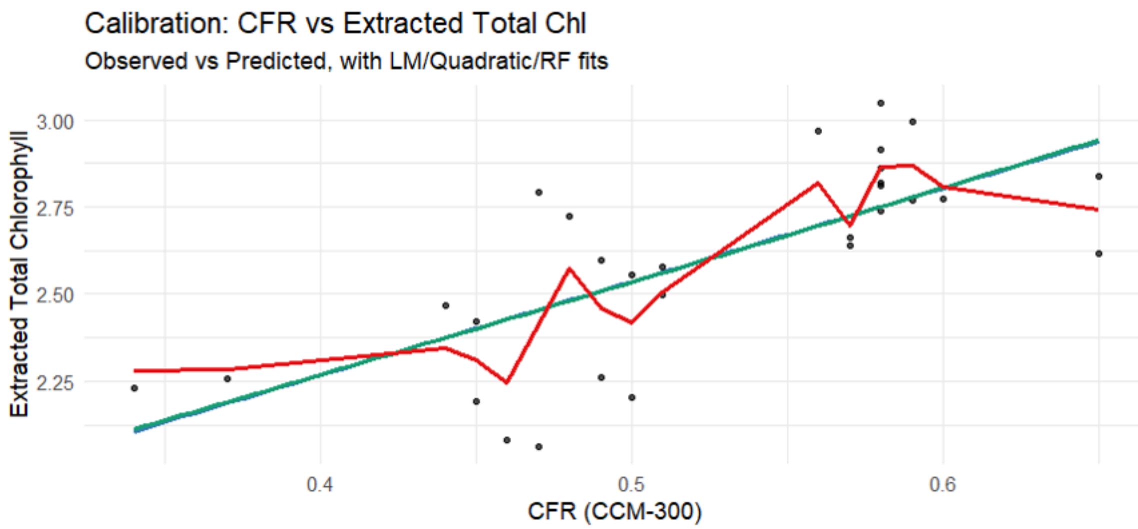

Figure 1

Calibration of CCM-300 chlorophyll fluorescence ratio (CFR) against acetone-extracted total chlorophyll concentration in Acer campestre. Observed paired data (points) with fitted models: linear (green line), quadratic (blue), and Random Forest (red).

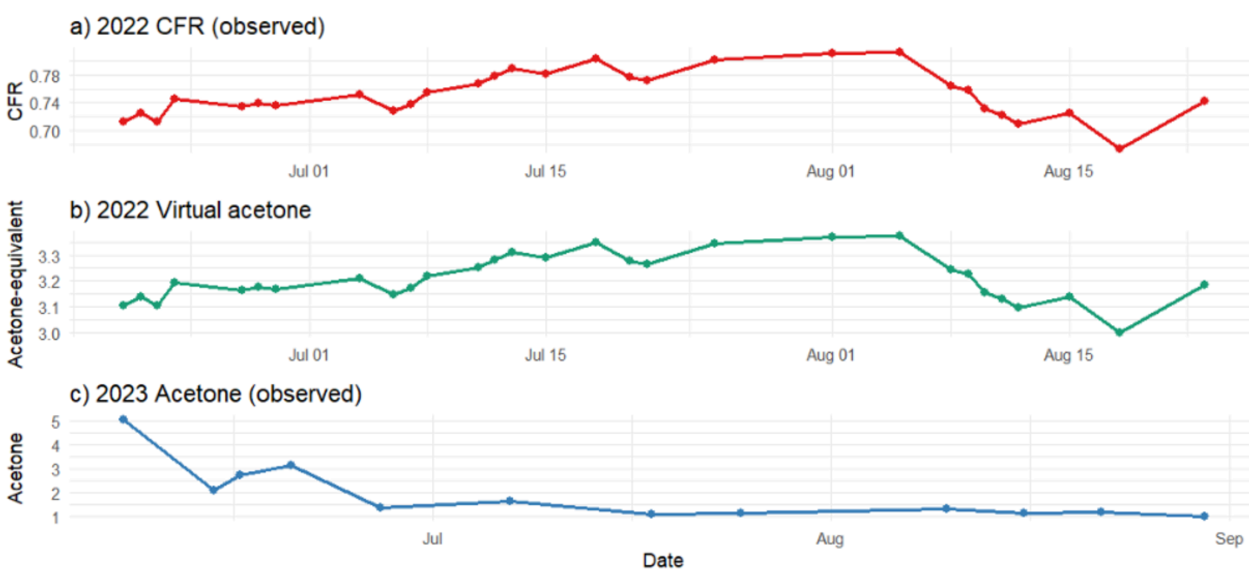

Figure 2

Seasonal chlorophyll dynamics reconstructed from 2022 non-destructive CFR measurements and 2023 acetone extractions. a) Daily CFR values (2022) showing mid-season stability and late-summer decline,

(b) “Virtual acetone” concentrations for 2022, obtained by applying the linear calibration to CFR values,
(c) Weekly acetone-extracted total chlorophyll in 2023.

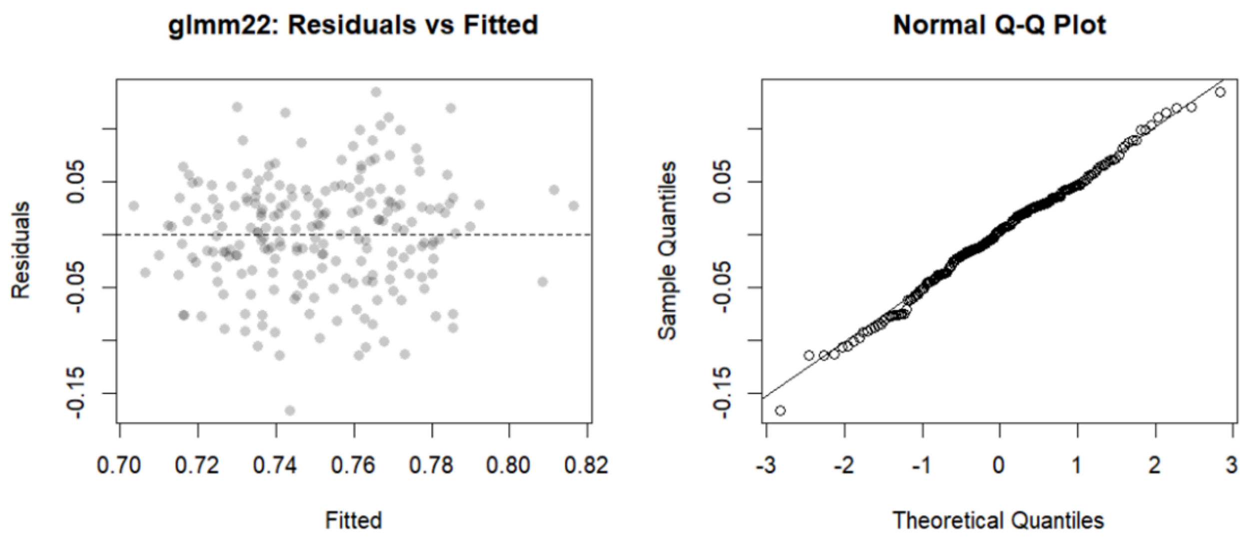

Figure 3

Observed versus predicted total chlorophyll concentrations for paired calibration samples.