

Cross-scale assessment of pigment differences between healthy and bark-beetle-damaged Norway spruce stands using UAV and airborne hyperspectral imagery

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Abstract: Chlorophyll and carotenoid content provide information on forest canopy condition and may reveal biochemical differences associated with disturbance. However, it remains uncertain whether these differences can be consistently detected from hyperspectral observations acquired at different spatial scales. We assessed pigment differences between two contrasting Norway spruce [*Picea abies* (L.) Karst.] reference stands: one without visible crown damage and one disturbed following bark beetle infestation in Krkonoše National Park, Czechia. The study combined 54 biochemical needle samples from 18 trees and simultaneous UAV Headwall Nano-Hyperspec® and airborne CASI-1500 imagery. A hybrid framework based on PROSAIL simulations and Random Forest regression was used to retrieve chlorophyll and carotenoid content. After averaging the three within-tree image-derived estimates to obtain one crown-level value per tree, linear models based on 18 independent crown-level observations showed higher retrieved chlorophyll and carotenoid content at Tetřeví Boudy (healthy) than at Rejdiště (infested). UAV retrieval achieved $R^2 = 0.96$ and $RMSE$ (root mean square error) = $2.64 \mu\text{g}\cdot\text{cm}^{-2}$ for chlorophyll and $R^2 = 0.80$ and $RMSE = 0.9 \mu\text{g}\cdot\text{cm}^{-2}$ for carotenoids. Airborne retrievals reached $R^2 = 0.93$ and $RMSE = 3.76 \mu\text{g}\cdot\text{cm}^{-2}$ for chlorophyll and $R^2 = 0.76$ and $RMSE = 0.95 \mu\text{g}\cdot\text{cm}^{-2}$ for carotenoids. Chlorophyll showed stronger cross-scale correlation than carotenoids ($r = 0.98$ and 0.89 , respectively), while the main spatial patterns and differences between stands were preserved at both scales. The results provide proof of concept that physiologically meaningful pigment differences between contrasting Norway spruce stands can be captured using UAV and airborne hyperspectral data. Because only two stands and one acquisition date were analysed, although the single-stand-per-condition design precludes attribution of the contrast to bark beetle damage, and broader validation across stands, disturbance stages and seasons is required.

Keywords: carotenoids; chlorophyll; forest health; imaging spectroscopy; PROSAIL

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Photosynthetic pigments are closely linked to the physiological condition of forest foliage. Chlorophyll controls light absorption for photosynthesis, while carotenoids support light harvesting and protect the photosynthetic apparatus against excess radiation and oxidative stress (Nisar et al. 2015; Swapnil et al. 2021). Changes in pigment content may therefore accompany declining photosynthetic capacity and stress-related deterioration before complete crown discolouration or foliage loss becomes visible. In Norway spruce, bark beetle attack can alter foliar biochemical properties, including chlorophyll content, before or during the development of visible symptoms (Abdullah et al. 2018). This physiological sensitivity is particularly relevant because drought and bark beetle outbreaks have caused extensive decline of Norway spruce forests across Central Europe, creating an urgent need for timely and spatially explicit forest-health information (Dudík et al. 2021; Bijou et al. 2023, 2025; Huo et al. 2026).

Conventional pigment analysis based on solvent extraction and spectrophotometric or chromatographic determination is destructive, time-consuming and spatially limited (Shen et al. 2020; Chen et al. 2025). Imaging spectroscopy offers a non-destructive means of extending biochemical measurements from sampled foliage to tree crowns and forest stands. Its narrow spectral bands capture subtle variation in absorption and reflectance related to chlorophyll and carotenoids, enabling spatially continuous assessment of canopy biochemical properties (Moorthy et al. 2008; Sonobe et al. 2021; Dou et al. 2025).

Plant traits can be retrieved from hyperspectral data using empirical, machine-learning, physically based, or hybrid approaches (Verrelst et al. 2015). Radiative transfer models such as PROSAIL describe interactions between radiation, foliage and canopy structure, but their inversion is ill-posed and sensitive to model parameterisation (Verrelst et al. 2019). Hybrid approaches combine physically based simulations with machine-learning algorithms and can therefore provide both mechanistic consistency and flexible nonlinear retrieval (Guo et al. 2024).

Previous studies have demonstrated the potential of radiative transfer modelling and imaging spectroscopy for estimating forest chlorophyll. Schlerf et al. (2010) retrieved chlorophyll in Norway spruce using imaging spectroscopy,

Darvishzadeh et al. (2019) used INFORM inversion to map chlorophyll in spruce stands, and Ali et al. (2021) retrieved canopy chlorophyll time series as a proxy for bark beetle infestation. Abdullah et al. (2018) further showed that early bark beetle attack affects both foliar biochemical properties and spectral reflectance. Carotenoid retrieval has received less attention because its spectral signal is weaker and more easily affected by canopy structure, illumination and spectral mixing. Moreover, most studies have examined a single platform or observational scale, limiting understanding of whether physiologically meaningful differences remain detectable when fine-resolution crown observations are aggregated to the stand scale.

This scale issue is important for forest health assessment. Unmanned Aerial Vehicle (UAV) hyper-spectral imagery can resolve individual crowns and within-crown variation, whereas airborne systems provide broader coverage but integrate foliage, woody elements, shadows and background within coarser pixels. Recent forest studies have demonstrated the value of UAV observations for tracking bark-beetle-related changes at individual-tree and stand levels (Östersund et al. 2024; Huo et al. 2026). A retrieval framework intended for forest monitoring should therefore not only estimate pigments accurately but also preserve biologically meaningful contrasts between forest conditions across observational scales.

Norway spruce [*Picea abies* (L.) Karst.] remains an ecologically and economically important tree species in Central European forests (Remeš et al. 2020; Bosela et al. 2025). Its vulnerability to drought and bark beetle disturbance has increased the need for methods that can characterise forest physiological condition before or during visible canopy decline (Bijou et al. 2025; Huo et al. 2026). Chlorophyll and carotenoid content provide complementary information: chlorophyll is closely linked to photosynthetic capacity, whereas carotenoids contribute to photoprotection and stress response. Their combined spatial assessment may therefore improve the interpretation of biochemical differences between undamaged and damaged stands.

The present study provides a cross-scale assessment of two Norway spruce reference stands of contrasting condition: a structurally homogeneous stand without visible crown damage and

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a structurally disturbed stand affected by bark beetle during the preceding five years. Simultaneous UAV and airborne hyperspectral acquisitions allowed us to evaluate whether biochemical differences between the stands were detectable at both crown and stand scales. Because each condition is represented by a single stand, condition is fully confounded with site, and the stand comparison is therefore observational; it quantifies a site-specific difference between two reference stands and does not isolate an effect of bark beetle disturbance history. The study likewise does not aim to establish an operational monitoring system; it evaluates whether a unified physiologically informed retrieval framework can preserve physiologically meaningful pigment patterns across observational scales.

The study addressed three objectives:

- (i) To quantify the difference in retrieved chlorophyll and carotenoid content between two structurally contrasting Norway spruce reference stands, one without visible crown damage and one disturbed following bark beetle infestation;
- (ii) To assess whether these biochemical differences and their main spatial patterns are consistently captured by UAV and airborne hyperspectral observations;
- (iii) To evaluate the performance of a hybrid PROSAIL–Random Forest framework for pigment retrieval at the two observational scales.

As the design includes one stand per condition, Objective (i) is descriptive: disturbance history, canopy structure and site conditions are inherently confounded, and the quantified pigment differences therefore characterise the contrast between these two specific stands rather than an effect attributable to bark beetle infestation.

MATERIAL AND METHODS

Study workflow

Figure 1 summarises the study workflow. Biochemical needle measurements and simultaneously acquired UAV and airborne hyperspectral imagery were combined within a hybrid PROSAIL–Random Forest framework. PROSAIL simulations were used to generate synthetic reflectance–trait relationships, and separate Random Forest models were applied to the spectral data from each platform to retrieve chlorophyll

and carotenoid content. Retrievals were validated against laboratory measurements, differences between the two stands were evaluated using crown-level statistical analysis, and crown-level UAV and airborne estimates were compared to assess cross-scale consistency.

Study area

The study area is located in Krkonoše National Park (KRNAP), Czechia (Figure 2). Established in 1963, KRNAP protects mountain ecosystems in which forests cover approximately 79% of the area and are dominated by Norway spruce. The study comprised two 30 × 30 m plots with contrasting canopy condition. Tetřeví Boudy represented a homogeneous, undamaged Norway spruce stand, whereas Rejdiště had been severely damaged by bark beetle during the preceding five years. The Tetřeví Boudy plot contained 61 mature Norway spruce trees and the Rejdiště plot contained 47. Damage classes were assigned according to the five-grade classification based on crown defoliation described by Entcheva et al. (1996). Tetřeví Boudy represented the comparatively healthy reference stand, characterised by approximately 30% crown defoliation, the absence of visible needle colour changes, and damage class DC2. In contrast, Rejdiště represented the severely damaged stand, with approximately 54% crown defoliation, visible needle colour changes, and damage class DC3. Ten living trees were selected at Tetřeví Boudy and eight living trees at Rejdiště to represent the range of canopy conditions and spatial positions within each plot (Figure 2C, D). To capture within-tree physiological variability, a tree climber collected needles from sun-exposed branches in the upper crown of each tree for three age classes (1, 2 and 4 years). In total, 30 samples were collected from 10 trees at Tetřeví Boudy and 24 samples from 8 trees at Rejdiště, giving 54 biochemical samples from 18 trees, with three needle-age samples per tree. Samples were transported to the laboratory for pigment extraction and analysis.

Stand and site characteristics of both plots are summarised in Table 1 and Table 2 for the coordinates and mean ± standard deviation of biochemical trait values for different needle ages. Both stands were surveyed on the same date and under identical short-term meteorological conditions, but a difference in longer-term water availability between the sites cannot be excluded.

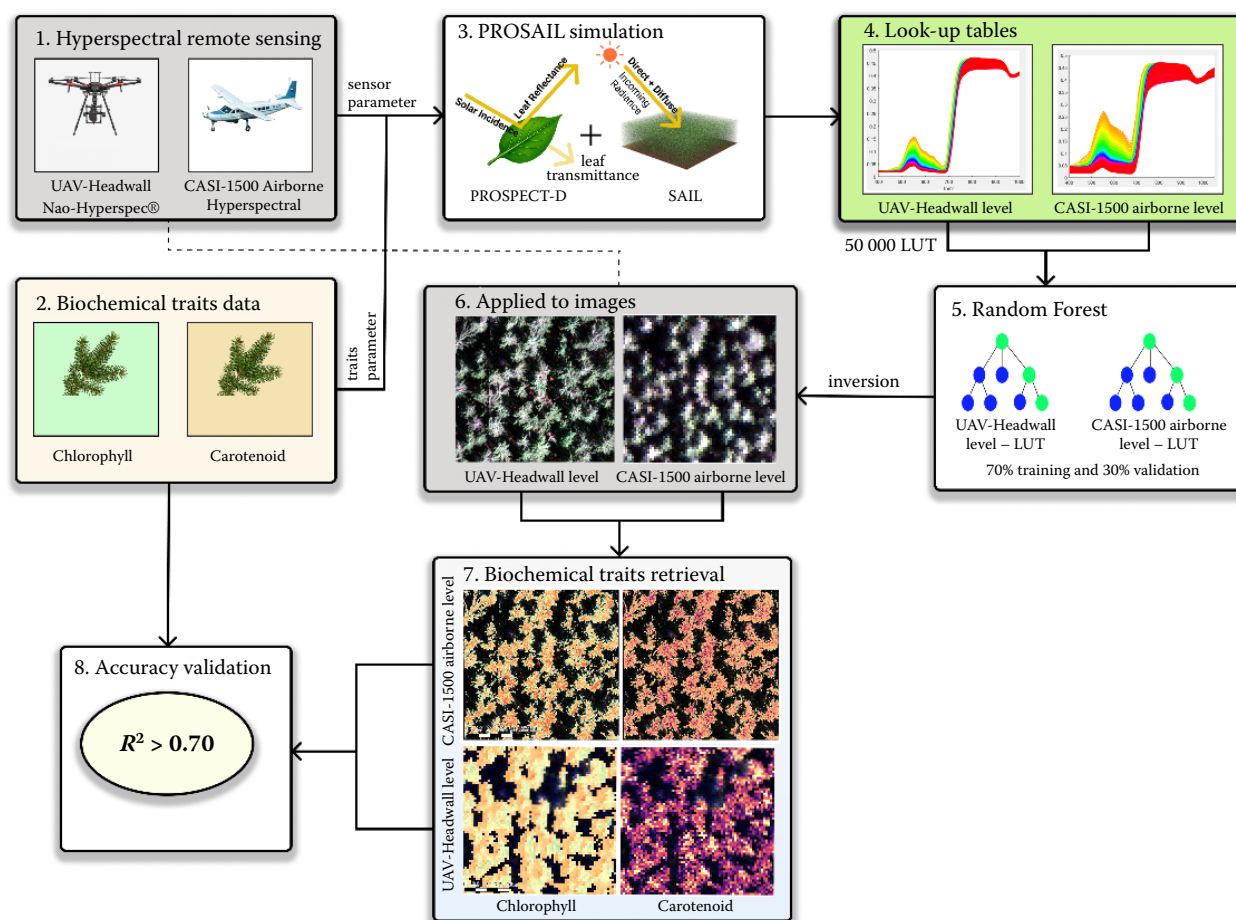


Figure 1. Study workflow for chlorophyll and carotenoid retrieval and comparison between undamaged and bark-beetle-damaged Norway spruce stands using UAV and airborne hyperspectral data

Biochemical trait measurements

Needle samples were collected on 30 July 2024 and analysed for chlorophyll and carotenoid content. Needles were cut into 2–3 mm segments, sealed in plastic vials and stored at -20°C before analysis. Pigments were extracted in darkness at 4°C for seven days using N-dimethylformamide (Porra et al. 1989). Absorbance of the extracts was measured at 480 nm, 647 nm, 664 nm and 750 nm using a spectrophotometer. Pigment concentrations were calculated according to Wellburn (1994) and expressed per unit specific needle area. Needle projection area was determined by scanning fresh needles using an EPSON Perfection V600 Photo scanner (Seiko Epson Corporation, Japan) at 800 dpi and analysing the resulting binary images in ImageJ. The projected needle area was converted to hemi-surface area using the correction factors of Homolová et al. (2013), and the resulting needle area was used to express pigment concentrations on an area basis ($\mu\text{g}\cdot\text{cm}^{-2}$).

Each biochemical sample was spatially linked to its corresponding sampling position within the tree crown. Sampling positions were identified in the orthorectified UAV imagery using field records and visual interpretation of the high-resolution imagery. A 0.5×0.5 m region of interest (ROI) was centred on each sampling position. At the approximately 5 cm UAV spatial resolution, each ROI contained approximately 100 pixels before filtering. Background and strongly shaded pixels were excluded, and reflectance values of the remaining valid pixels were averaged band-by-band to produce one representative UAV spectrum for each biochemical sample. The same georeferenced sampling locations were transferred to the co-registered CASI-1500 (ITRES Research Limited, Canada) imagery at 0.5 m spatial resolution. The corresponding CASI pixel was extracted; where an ROI intersected more than one pixel, an area-weighted mean of intersecting valid pixels was used. This

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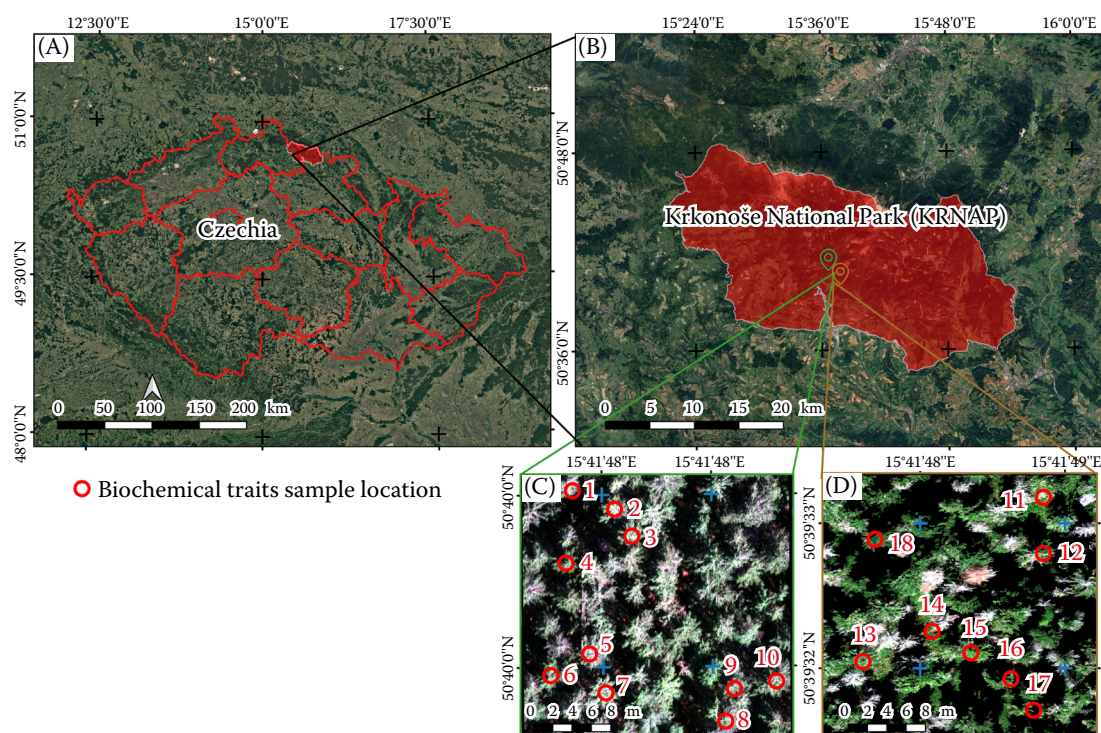


Figure 2. Study area in (A) Czechia and (B) Krkonoše National Park, with the (C) Tetřeví Boudy and the (D) Rejdiště stands. Red points and numbers – trees sampled for biochemical pigment analysis; coordinates in panels (A), (B), (C), and (D) are geographic coordinates in WGS 84; background imagery in panels (C) and (D) consists of UAV RGB orthomosaics acquired on 30 July 2024.

Source of basemap in panels (A) and (B): Esri World Imagery basemap images.

procedure resulted in 54 spatially matched biochemical–spectral observations for each sensor platform, with three observations nested within each of the 18 trees.

Hyperspectral data

UAV hyperspectral data. A dual-platform UAV campaign was conducted on 30 July 2024 to coincide with biochemical sampling and the airborne campaign. Hyperspectral image cubes covering 400–1 000 nm (269 bands; approximately 0.05 m spatial resolution) were acquired with a Headwall Nano-Hyperspec® sensor (Headwall Photonic, USA) mounted on a DJI Matrice 600 Pro (SZ DJI Technology, China). Multispectral and RGB imagery was acquired simultaneously using a DJI Phantom 4 Multispectral RTK (SZ DJI Technology, China) and used to generate the digital elevation model required for orthorectification. The hyperspectral data were processed in Headwall SpectralView (Version 3.1.0, 2020) following radiometric calibration, reflectance conversion and geometric correction (Červená et al. 2020).

A 3 × 3 m portable reference target (Type 822, Group 8 Technology, Inc., Provo, USA) comprising panels with nominal reflectance values of 56%, 32% and 11% was placed within the flight area. Mean panel radiance was related to the corresponding calibrated reflectance curves to convert image radiance to reflectance (Angel et al. 2019). Hyperspectral flight lines were geometrically corrected using synchronised GNSS/IMU (Global Navigation Satellite System/Inertial Measurement Unit) data and orthorectified with a high-resolution elevation model derived from the Phantom 4 RTK point cloud. RGB and multispectral orthomosaics and elevation models were generated in Agisoft Metashape Professional (Version 1.7.2., 2021). Orthorectified hyperspectral flight lines were aligned in ENVI (Version 5.3, 2016) using ground control points and the RGB orthomosaic as a spatial reference. A Savitzky–Golay filter with a window length of 3 and polynomial order of 2 was applied to reduce spectral noise.

Airborne hyperspectral data. Airborne hyperspectral data were acquired on 30 July 2024 using

Table 1. Stand and site characteristics of the two Norway spruce study plots in Krkonoše National Park

Characteristic	Tetřeví Boudy	Rejdiště
Coordinates	50°39'55.47"N, 15°41'47"E	50°39'32.61"N, 15°41'51"E
Elevation (m a.s.l.)	1 025	1 000
Mean annual air temperature (°C)	4.5	4.5
Mean annual precipitation (mm)	1 100	1 100
Stand origin (natural regeneration/planted)	planted unmanaged	planted unmanaged
Mean stand age (years)	60–70	70–90
Number of mature Norway spruce trees in the 30 × 30 m plot	61	47
Live green canopy cover (%)	93	70
Mean DBH (cm)	35–45	35–55
Understory and ground vegetation	moderately dense low understory dominated by herbs and low shrubs, with mosses and sparse spruce regeneration	dense herbaceous ground vegetation dominated by grasses, with low shrubs and scattered spruce regeneration, some taller young spruce trees
Year bark beetle infestation first recorded	not applicable	2019
Dead or defoliated trees at time of acquisition (%)	0	25 (trees infested in 2024 were in yellow and red damage phases based on visual assessment at the time of sample collection)
Trees sampled for biochemical analysis	10	8
Biochemical samples (3 needle-age classes per tree)	30	24

DBH – diameter at breast height; the two plots are located approximately 0.9 km apart

the Flying Laboratory of Imaging Systems operated by the Global Change Research Institute (Hanuš et al. 2023). The CASI-1500 sensor (ITRES Research Limited, Canada), mounted on a Cessna 208B Grand Caravan (Cessna Aircraft Company, USA), recorded 48 bands between 400 and 1 000 nm. Data were acquired under clear-sky, near-nadir conditions and

processed to a 0.5 m spatial-resolution mosaic. Radiometric, geometric and atmospheric corrections followed (Hanuš et al. 2023), with additional vicarious calibration based on ground reference targets. Bands below 450 nm with low signal-to-noise ratios and bands strongly affected by water-vapour absorption were excluded from the analysis.

Table 2. Laboratory-measure pigment content by site and needle-age class

Site	Needle ages (years)	Chlorophyll ($\mu\text{g}\cdot\text{cm}^{-2}$), mean $\pm$ SD	Carotenoids ($\mu\text{g}\cdot\text{cm}^{-2}$), mean $\pm$ SD
Tetřeví Boudy	1	25.34 $\pm$ 3.67	3.43 $\pm$ 0.28
Tetřeví Boudy	2	45.25 $\pm$ 5.93	5.86 $\pm$ 0.50
Tetřeví Boudy	4	59.10 $\pm$ 11.82	8.41 $\pm$ 1.42
Rejdiště	1	30.58 $\pm$ 5.81	2.76 $\pm$ 0.54
Rejdiště	2	40.42 $\pm$ 11.89	4.91 $\pm$ 1.17
Rejdiště	4	40.02 $\pm$ 14.94	5.76 $\pm$ 1.36

SD – standard deviation

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Canopy classification and background masking

Prior to pigment retrieval, hyperspectral imagery was classified to separate sunlit green spruce canopy from non-target background components, including branches, understory vegetation, soil, and shadows. Object-Based Image Analysis (OBIA) was performed in ENVI (Version 5.5, 2020) using the Feature Extraction workflow. Image segmentation was conducted using all hyperspectral bands together with spatial information, with a scale level of 30 and a merge level of 85. The scale level controlled the size and spectral–spatial homogeneity of the resulting image objects, whereas the merge level controlled the aggregation of adjacent segments with similar properties.

Five land-cover classes were defined: (i) sunlit green spruce canopy, (ii) branches and woody material, (iii) understory vegetation, (iv) soil/background, and (v) shadow. Fifty representative samples were selected for each class by visual interpretation of the hyperspectral and corresponding high-resolution imagery. For each class, 30 samples were randomly assigned to classifier training and 20 samples to independent validation. Classification features included spectral statistics (mean, standard deviation, minimum, and maximum reflectance), texture measures (range, mean, variance, and entropy), and spatial characteristics (area, length, compactness, convexity, and solidity). The segmented objects were classified using a Support Vector Machine (SVM) with a radial basis function kernel.

Classification performance was evaluated using the independent validation samples. Only objects classified as sunlit green spruce canopy were retained for subsequent PROSAIL–Random Forest

inversion and pigment mapping; pixels/objects classified as branches, understory vegetation, soil/background, or shadow were excluded. This masking procedure ensured that chlorophyll and carotenoid retrievals were restricted to illuminated spruce foliage and minimised contamination from non-foliage background components.

PROSAIL (PROSPECT-5 and 4SAIL)

Chlorophyll and carotenoid content were retrieved using a hybrid inversion of a PROSAIL (Jacquemoud et al. 2009) look-up table (LUT). PROSAIL combines the PROSPECT leaf optical model with the SAIL canopy reflectance model. We used PROSPECT-5, which explicitly includes carotenoids as an input parameter (Feret et al. 2008), together with 4SAIL. The parameter ranges used to generate the LUT are summarised in Table 3 and were selected from field information and published values relevant to spruce canopies (Zhao et al. 2024). The LUT contained 50 000 parameter combinations, providing a compromise between representation of the parameter space and computational demand. Because the Headwall Nano-Hyperspec and CASI-1500 sensors differ substantially in spectral sampling and bandwidth, separate sensor configurations were defined in ARTMO (Version 3.30, 2022) for the two platforms. For each sensor, the spectral configuration was specified at the individual-band level using the minimum wavelength, maximum wavelength, band-centre wavelength, and full width at half maximum (*FWHM*). The Headwall Nano-Hyperspec configuration comprised 269 relatively narrow bands across approximately 400–1000 nm, with an *FWHM* of approximately 2.24 nm for the bands

Table 3. Parameter setting for PROSPECT-5 and 4SAIL used to generate the look-up table (LUT)

Model	Parameter	Unit	Value/range
PROSPECT-5	leaf structure	–	1.0–2.5
	chlorophyll a + b	$\mu\text{g}\cdot\text{cm}^{-2}$	5–85
	carotenoid	$\mu\text{g}\cdot\text{cm}^{-2}$	1–11
	equivalent water thickness	cm	0.001–0.030
	dry matter content	$\text{g}\cdot\text{cm}^{-2}$	0.00–0.03
	leaf area index	$\text{m}^2\cdot\text{m}^{-2}$	1–8
4SAIL	average leaf angle	°	10–80
	solar zenith angle	°	40–70
	soil coefficient	–	0–1

Parameter ranges were defined based on field information and published values relevant to spruce canopies (Zhao et al. 2024)

shown in the ARTMO sensor definition. In contrast, the CASI-1500 configuration comprised 48 broader bands across approximately 400–1000 nm, with an *FWHM* of approximately 14.24–14.25 nm for the bands shown in the ARTMO configuration. PROSAIL simulations were generated separately for the Headwall Nano-Hyperspec and CASI-1500 configurations defined in ARTMO. The resulting sensor-specific simulated datasets were subsequently used independently for model training, ensuring that the simulated spectral inputs corresponded to the spectral dimensionality and band definitions of the respective sensor datasets.

Random Forest

A Random Forest (RF) algorithm was employed to invert the PROSAIL look-up table (LUT) for the retrieval of chlorophyll and carotenoid. RF is a non-parametric ensemble learning algorithm that has been widely applied for estimating vegetation biochemical and biophysical properties from hyperspectral data because of its ability to model complex nonlinear relationships without requiring assumptions about data distributions (Breiman 2001). In combination with PROSAIL, RF efficiently learns the relationship between simulated hyperspectral reflectance and the corresponding biochemical traits contained in the LUT while remaining robust to multicollinearity, spectral noise, and the high dimensionality of hyperspectral datasets. These characteristics make RF particularly suitable for LUT inversion and hyperspectral retrieval of vegetation traits.

RF is based on the bootstrap aggregation (bagging) strategy, in which multiple decision trees are independently constructed from bootstrap samples of the training dataset. At each node, the optimal split is selected from a randomly chosen subset of predictor variables, reducing the correlation among individual trees and improving model generalisation. The final prediction is obtained by averaging the outputs of all trees in the ensemble, resulting in stable and accurate estimates of leaf biochemical traits (LBTs). Separate Random Forest models were trained for each sensor and pigment combination. Thus, four models were developed: UAV–chlorophyll, UAV–carotenoids, CASI-1500–chlorophyll, and CASI-1500–carotenoids. Each model was trained using the PROSAIL simulations generated for the corresponding ARTMO sensor configuration. For the inversion process, the

50 000 PROSAIL LUT simulations were randomly divided into 70% for model training and 30% for internal validation. The RF models were trained exclusively using the synthetic PROSAIL-LUT dataset, with simulated hyperspectral reflectance values serving as predictor variables and chlorophyll or carotenoid as response variables. The measured LBT data were not used during model training. Instead, the 54 LBT samples collected from 18 Norway spruce trees were reserved exclusively for independent validation of the retrieval performance using the UAV and airborne hyperspectral imagery. All modelling procedures were implemented using the ARTMO software and its Machine Learning Regression Algorithms (MLRA) toolbox (Rivera et al. 2015). The RF models were constructed using 500 decision trees, a configuration commonly adopted to achieve stable prediction accuracy while maintaining computational efficiency. At each split, the number of predictor variables considered for node partitioning was set to the square root of the total number of predictor variables ($max_features = \sqrt{p}$), where p represents the total number of spectral predictor variables. The minimum number of samples required to define a terminal node was set to one ($min_samples_leaf = 1$), allowing each decision tree to grow to its maximum depth. Unless otherwise specified, the remaining hyperparameters followed the default implementation of the ARTMO MLRA toolbox. The trained RF models were subsequently applied to the UAV-based Headwall Nano-Hyperspec® and airborne CASI-1500 hyperspectral datasets to retrieve spatially explicit chlorophyll and carotenoid distributions.

Accuracy validation

To evaluate the accuracy of chlorophyll and carotenoid retrieval from the PROSAIL LUT trained with the RF algorithm using UAV and airborne hyperspectral data, each biochemical observation was paired with the corresponding hyperspectral spectrum extracted from its sampling location. The validation dataset consisted of 54 paired LBT–spectral observations representing three needle-age classes (1-, 2-, and 4-year-old needles) sampled from 18 Norway spruce trees. Because these biochemical observations were nested within trees and canopy reflectance cannot fully resolve individual needle-age classes, the 54 paired observations should not be interpreted as statistically independent tree samples. Rather, they represent repeated within-tree

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measurements associated with the corresponding crown spectra. Consequently, the reported validation statistics describe the agreement between retrieved canopy reflectance and the associated biochemical measurements, while acknowledging the presence of within-tree pseudoreplication. Retrieval performance was assessed using the coefficient of determination (R^2) and the root mean square error ($RMSE$). The R^2 and $RMSE$ were calculated using Equations (1–2). Values of R^2 closer to 1 indicate better retrieval performance, whereas lower $RMSE$ values indicate smaller prediction errors.

$$R^2 = 1 - \frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2} \quad (1)$$

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (y_i - \hat{y}_i)^2} \quad (2)$$

where:

- n – number of observations;
- i – index of the individual observation (1– n);
- y – observed value; observed and predicted values are denoted by the corresponding symbols, the bar indicates the mean observed value.

Statistical comparison between stands

To evaluate whether the retrieved chlorophyll and carotenoid differed between the two reference stands while avoiding pseudoreplication from repeated within-tree observations, the three image-derived pigment estimates associated with the sampled positions within each tree were averaged to obtain one representative crown-level value per tree. This resulted in 18 crown-level observations for each pigment and sensor platform, comprising 10 trees at Tetřeví Boudy and 8 trees at Rejdiště.

Separate linear models (LMs) were fitted for chlorophyll and carotenoids for each sensor platform, with *Site* (Tetřeví Boudy vs Rejdiště) included as the explanatory factor. The model was formulated as demonstrated by Equation (3):

$$Y_{ij} = \beta_0 + \beta_1 \text{Site}_i + \varepsilon_{ij} \quad (3)$$

where:

- Y_{ij} – the retrieved chlorophyll or carotenoid content for the j -th tree at the i -th site;
- β_0 – the intercept;

- β_1 – difference between the two reference stands;
- ε_{ij} – residual error, assumed to be normally distributed, see Equation (4):

$$\varepsilon_{ij} \sim N(0, \sigma^2) \quad (4)$$

where:

- N – normal (Gaussian) distribution;
- σ – standard deviation of the residual errors.

The site coefficient was used to estimate the undamaged-minus-damaged difference in crown level pigment content. Estimated differences, 95% confidence intervals (CI), and corresponding P -values were obtained from the fitted models. Statistical significance was assessed at the 0.05 level.

RESULTS

Pigment retrieval accuracy and differences between stands. The hybrid PROSAIL–Random Forest framework retrieved chlorophyll more accurately than carotenoids from both hyperspectral platforms (Figure 3). UAV retrievals achieved $R^2 = 0.96$ and $RMSE = 2.64 \mu\text{g}\cdot\text{cm}^{-2}$ for chlorophyll and $R^2 = 0.80$ and $RMSE = 0.9 \mu\text{g}\cdot\text{cm}^{-2}$ for carotenoids. CASI-1500 retrievals achieved $R^2 = 0.93$ and $RMSE = 3.76 \mu\text{g}\cdot\text{cm}^{-2}$ for chlorophyll and $R^2 = 0.76$ and $RMSE = 0.95 \mu\text{g}\cdot\text{cm}^{-2}$ for carotenoids. Site-specific chlorophyll results were $R^2 = 0.98$ and $RMSE = 1.99 \mu\text{g}\cdot\text{cm}^{-2}$ at Tetřeví Boudy and $R^2 = 0.89$ and $RMSE = 3.28 \mu\text{g}\cdot\text{cm}^{-2}$ at Rejdiště for the UAV data; the corresponding CASI-1500 values were $R^2 = 0.94$ and $RMSE = 3.63 \mu\text{g}\cdot\text{cm}^{-2}$ and $R^2 = 0.81$ and $RMSE = 3.91 \mu\text{g}\cdot\text{cm}^{-2}$. For carotenoids, UAV retrievals reached $R^2 = 0.82$ and $RMSE = 1.07 \mu\text{g}\cdot\text{cm}^{-2}$ at Tetřeví Boudy and $R^2 = 0.77$ and $RMSE = 0.63 \mu\text{g}\cdot\text{cm}^{-2}$ at Rejdiště. CASI-1500 values were $R^2 = 0.82$ and $RMSE = 1.01 \mu\text{g}\cdot\text{cm}^{-2}$ at Tetřeví Boudy and $R^2 = 0.52$ and $RMSE = 0.88 \mu\text{g}\cdot\text{cm}^{-2}$ at Rejdiště. Thus, UAV data provided higher accuracy for chlorophyll, whereas carotenoid performance was broadly comparable between platforms. Observations generally followed the 1:1 line, although deviations increased toward the upper ends of the pigment ranges.

Spatial pigment patterns and stand-level differences. Retrieved pigment maps showed clear individual-tree and within-crown variability and consistent differences between the two stands (Figures 4 and 5). UAV-derived chlorophyll ranged

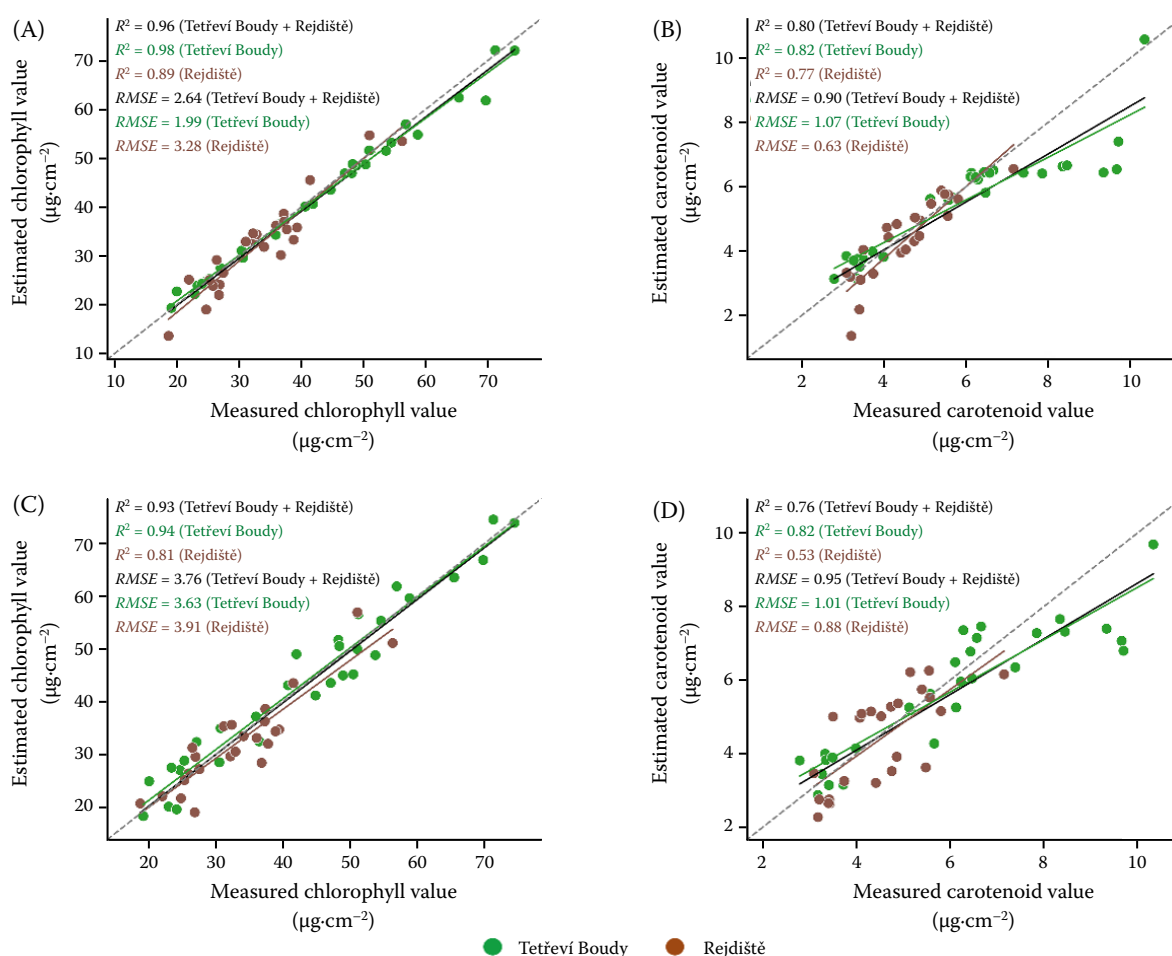


Figure 3. Validation of (A, C) chlorophyll and (B, D) carotenoid retrievals against biochemical measurements for UAV Headwall Nano-Hyperspec® (A, B) and airborne CASI-1500 data (C, D)

R^2 – coefficient of determination; RMSE – root mean square error

from $17.42 \mu\text{g}\cdot\text{cm}^{-2}$ to $77.349 \mu\text{g}\cdot\text{cm}^{-2}$ at Tetřeví Boudy (mean $32.73 \mu\text{g}\cdot\text{cm}^{-2}$) and from $6.26 \mu\text{g}\cdot\text{cm}^{-2}$ to $64.51 \mu\text{g}\cdot\text{cm}^{-2}$ at Rejdiště (mean $29.83 \mu\text{g}\cdot\text{cm}^{-2}$). UAV-derived carotenoids ranged from $1.45 \mu\text{g}\cdot\text{cm}^{-2}$ to $10.77 \mu\text{g}\cdot\text{cm}^{-2}$ at Tetřeví Boudy (mean $6.21 \mu\text{g}\cdot\text{cm}^{-2}$) and from $0.20 \mu\text{g}\cdot\text{cm}^{-2}$ to $7.82 \mu\text{g}\cdot\text{cm}^{-2}$ at Rejdiště (mean $4.52 \mu\text{g}\cdot\text{cm}^{-2}$). CASI-1500 chlorophyll ranged from $17.26 \mu\text{g}\cdot\text{cm}^{-2}$ to $74.36 \mu\text{g}\cdot\text{cm}^{-2}$ at Tetřeví Boudy (mean $33.09 \mu\text{g}\cdot\text{cm}^{-2}$) and from $3.56 \mu\text{g}\cdot\text{cm}^{-2}$ to $59.26 \mu\text{g}\cdot\text{cm}^{-2}$ at Rejdiště (mean $26.01 \mu\text{g}\cdot\text{cm}^{-2}$). Corresponding carotenoid values ranged from $0.73 \mu\text{g}\cdot\text{cm}^{-2}$ to $8.21 \mu\text{g}\cdot\text{cm}^{-2}$ (mean $3.48 \mu\text{g}\cdot\text{cm}^{-2}$) and from $0.19 \mu\text{g}\cdot\text{cm}^{-2}$ to $7.32 \mu\text{g}\cdot\text{cm}^{-2}$ (mean $2.94 \mu\text{g}\cdot\text{cm}^{-2}$), respectively. Despite its coarser spatial resolution, CASI-1500 preserved the main stand-level spatial patterns observed in the UAV maps, but with smoother transitions.

The visually undamaged Tetřeví Boudy stand consistently exhibited higher retrieved crown-level

pigment content than the severely damaged Rejdiště stand. Following aggregation of the three within-tree observations, stand differences were evaluated using one mean crown-level estimate per sampled tree ($n = 18$; 10 at Tetřeví Boudy and 8 at Rejdiště). For UAV retrievals, the estimated between-stand difference (undamaged minus damaged) was $9.5 \mu\text{g}\cdot\text{cm}^{-2}$ for chlorophyll (95% CI 4.2–14.8, $P = 0.002$) and $1.05 \mu\text{g}\cdot\text{cm}^{-2}$ for carotenoids (95% CI 0.55–1.52, $P < 0.001$). CASI-1500 retrievals showed corresponding differences of $10.71 \mu\text{g}\cdot\text{cm}^{-2}$ for chlorophyll (95% CI 4.92–16.54, $P = 0.001$) and $1.15 \mu\text{g}\cdot\text{cm}^{-2}$ for carotenoids (95% CI 0.58–1.62, $P < 0.001$). These results indicate a consistent site-level pigment contrast between two reference stands at both observational scales.

Cross-scale consistency of UAV and airborne retrievals. To assess cross-scale consistency while avoiding pseudoreplication, the three im-

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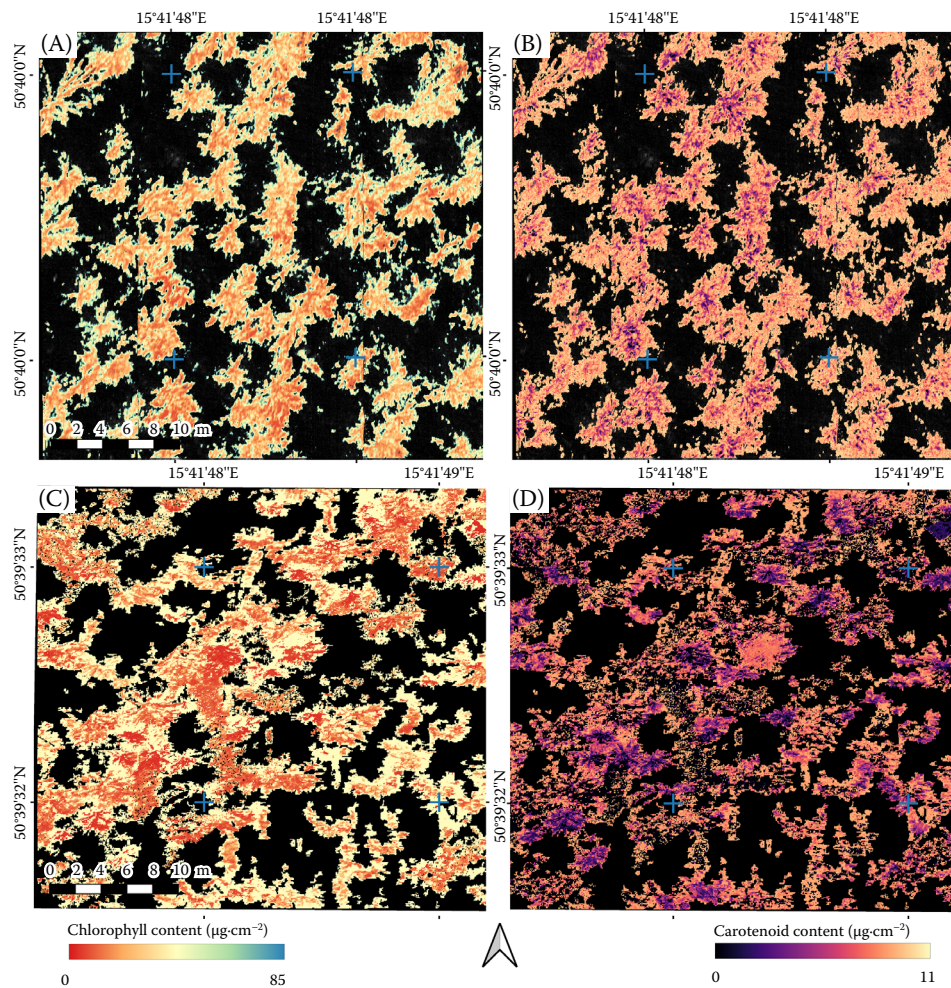


Figure 4. Spatial distribution of leaf biochemical traits (LBTs) retrieved from UAV-based Headwall Nano-Hyperspec® data using the PROSAIL-RF model: (A, C) chlorophyll and (B, D) carotenoid content for the Tetřeví Boudy and Rejdiště study sites, respectively

age-derived estimates associated with each sampled tree were averaged to one crown-level value per tree and platform, giving 18 paired observations (10 trees at Tetřeví Boudy and 8 at Rejdiště; Figure 6). Pearson correlation was used to describe the agreement in relative ranking of trees between platforms; it is not a pixel-by-pixel comparison of the continuous maps and does not measure numerical agreement. Pearson correlations were used to evaluate preservation of relative spatial patterns; they should not be interpreted as complete measures of numerical agreement between platforms.

For chlorophyll, aggregated crown-level UAV estimates were strongly correlated with CASI-1500 estimates ($r = 0.98$ for the combined dataset; $r = 0.96$ at Tetřeví Boudy and $r = 0.98$ at Rejdiště). Carotenoid estimates also remained strongly correlated across platforms, but the relationship

was weaker than for chlorophyll ($r = 0.89$ overall; $r = 0.59$ at Tetřeví Boudy and $r = 0.88$ at Rejdiště). Overall, the crown-level comparison indicated that the main variation in retrieved pigment content was consistently represented by both UAV and airborne hyperspectral observations.

Summary of key findings. Three findings are central to the study. First, crown-level retrievals showed significantly higher retrieved chlorophyll and carotenoid content in the undamaged Tetřeví Boudy stand than in the severely damaged Rejdiště stand. Second, this site-level biochemical contrast and the main spatial patterns were detected by both UAV and airborne hyperspectral observations. Third, chlorophyll retrieval showed stronger cross-scale consistency than carotenoid retrieval, while UAV data provided higher chlorophyll accuracy and carotenoid accuracy was broadly comparable between platforms.

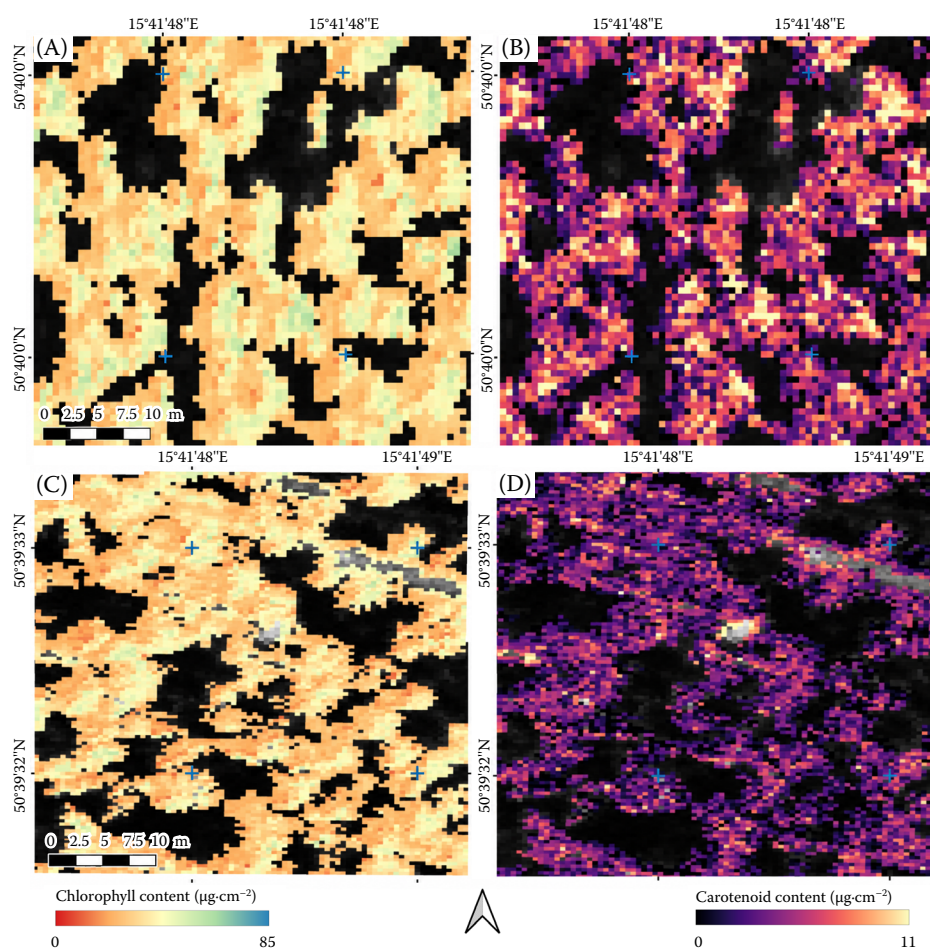


Figure 5. Spatial distribution of leaf biochemical traits (LBTs) retrieved from CASI airborne hyperspectral data using the PROSAIL-RF model: (A, C) chlorophyll and (B, D) carotenoid content for the Tetřeví Boudy and Rejdiště study sites, respectively

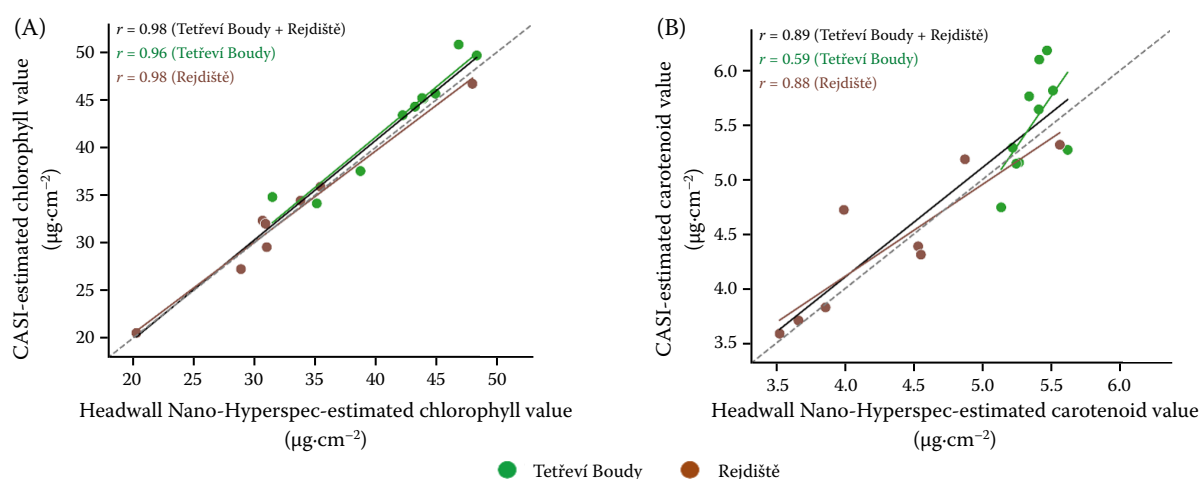


Figure 6. Crown-level comparison of CASI-1500 and Headwall Nano-Hyperspec® estimates of (A) chlorophyll and (B) carotenoid content

Each point represents the mean of the three sampling positions within one tree ($n = 18$; 10 at Tetřeví Boudy and 8 at Rejdiště); Pearson correlation coefficients are shown for the combined dataset and for each site; the dashed line represents the 1:1 line

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DISCUSSION

Pigment differences between the two reference stands. The primary observational result was the consistently higher crown-level retrieved chlorophyll and carotenoid content in the undamaged Tetřeví Boudy stand than in the severely damaged Rejdiště stand. Crown-level statistical comparisons based on one aggregated estimate per sampled tree showed significant differences between the two reference stands for both pigments, and the same direction of difference was observed independently in the UAV and airborne datasets. Lower chlorophyll content in the damaged stand is consistent with the reduction in foliar biochemical content reported for bark-beetle-attacked Norway spruce and with declining photosynthetic capacity in stressed conifer foliage (Abdullah et al. 2018; Zarco-Tejada et al. 2019). The lower carotenoid content may indicate changes in photoprotective pigment pools during prolonged canopy deterioration, although this interpretation requires direct physiological validation because carotenoid responses depend on pigment composition, stress stage and environmental conditions (Nisar et al. 2015; Swapnil et al. 2021). The lower pigment content retrieved for Rejdiště is consistent with reduced biochemical vitality in a structurally disturbed canopy. Because the study compared only one undamaged and one damaged stand, however, the observed contrast represents a site-specific association and cannot be attributed exclusively to bark beetle damage. Other differences in stand structure, environmental conditions and disturbance history may also have contributed.

The comparable direction and magnitude of the stand differences across platforms indicate that the biochemical contrast was sufficiently strong to remain detectable after spatial aggregation. These results should nevertheless be interpreted as a comparison between two contrasting stands rather than as a replicated test of bark beetle effects. Health status was confounded with site, and the stands may also have differed in canopy structure, stand density, microclimate, topography and disturbance history. The study therefore demonstrates an association between stand condition and pigment content, not that bark beetle disturbance alone caused the observed differences. Replication across multiple undamaged stands, disturbance stages and environmental settings will be required to establish the generality of this relationship.

The pigment maps displayed substantial individual-tree and within-crown variability. Because both sensors observed the canopy predominantly from nadir, these patterns should not be interpreted as direct vertical biochemical gradients. They reflect the combined influence of foliage biochemistry, needle-age composition, crown geometry, illumination, shadows, woody elements, lichens and retrieval uncertainty (Verrelst et al. 2010; Homolová et al. 2013). The smoother CASI-1500 maps are consistent with spatial averaging within larger pixels, especially in the structurally heterogeneous damaged stand.

Cross-scale performance of the hybrid retrieval framework. The hybrid PROSAIL–Random Forest framework retrieved chlorophyll with high accuracy from both platforms and produced satisfactory carotenoid estimates. UAV data achieved higher chlorophyll accuracy, probably because their finer spatial resolution reduced spectral mixing and provided a more detailed representation of individual crowns and within-crown variability. This interpretation is consistent with previous work showing that forest chlorophyll retrieval is influenced by crown structure, woody elements and the spatial correspondence between biochemical samples and canopy spectra (Schlerf et al. 2010; Verrelst et al. 2010; Homolová et al. 2013). Carotenoid performance was broadly comparable between UAV and CASI-1500 data, although its lower R^2 values indicate greater retrieval difficulty than for chlorophyll. This is consistent with the weaker and less distinct spectral expression of carotenoids in canopy reflectance.

The cross-scale comparison showed strong consistency between UAV and airborne pigment retrievals, indicating that the main spatial patterns were preserved across the two observational scales. This agreement suggests that, despite substantial differences in spatial resolution, both platforms captured similar variation in canopy pigment status across the study sites. Nevertheless, differences in spatial resolution, canopy structure, and spectral mixing may contribute to variability between sensor-derived estimates, particularly within the structurally heterogeneous in these two stands. Correlation primarily reflects the preservation of relative spatial patterns and does not demonstrate complete numerical agreement or direct transferability between sensors. The results therefore support cross-scale consistency of the

retrieved pigment patterns, while indicating that UAV and airborne estimates should not be considered directly interchangeable.

The study adds to previous work that has usually evaluated biochemical retrieval at a single observational scale (Hernández-Clemente et al. 2012; Hoeppepner et al. 2020; Meng et al. 2025). UAV observations can provide detailed crown-level information and support field sampling, method development and local validation, whereas airborne observations extend coverage to larger forest areas. Recent bark beetle studies have likewise shown that UAV data can resolve changes in individual-tree health and that multi-temporal, cross-site observations are important because the timing and expression of decline vary among stands (Östersund et al. 2024; Bijou et al. 2025; Huo et al. 2026). The complementary use of UAV and airborne observations is therefore promising for forest health assessment, provided that scale-dependent uncertainty and sensor-specific effects are explicitly considered.

Methodological limitations and implications for forest monitoring. The validation design has important limitations. Three needle-age classes were sampled from each of 18 trees, while canopy spectra integrate the complete sunlit crown and cannot resolve individual needle-age classes. The 54 observations are therefore repeated biochemical measurements nested within trees rather than 54 independent tree samples. The reported validation statistics describe correspondence between canopy retrievals and associated biochemical measurements, but may overstate the effective sample size. Future studies should add tree-level validation based on crown-level pigment summaries and include a larger number of independent trees and stands.

PROSAIL inversion is also sensitive to parameterisation and to simplifications of forest canopy structure. Norway spruce crowns contain clumped foliage, branches, gaps and shadows that are only partly represented by a turbid-medium canopy model (Verrelst et al. 2010, 2019). Spatial aggregation further reduces fine-scale information and increases spectral mixing. Future studies should test whether LiDAR-derived structural variables improve the representation of crown architecture, as demonstrated in combined hyperspectral–LiDAR trait estimation (Meng et al. 2025), and should include explicit uncertainty analysis. Re-

peated acquisitions across phenological and disturbance stages are also needed because the timing and spectral expression of bark beetle decline can vary markedly within and among stands (Östersund et al. 2024; Huo et al. 2026).

Despite these constraints, the study demonstrates that pigment retrieval can provide physiologically interpretable information on contrasting forest conditions. The value for forest monitoring lies not in replacing field assessment, but in extending biochemical observations from sampled trees to spatially continuous crown and stand maps. A combined strategy in which UAV surveys support detailed local assessment and airborne or future satellite imaging spectroscopy provides broader coverage could help characterise spatial variation in forest physiological condition. Operational use will require replicated validation, standardised acquisition and calibration procedures, and explicit assessment of uncertainty. The results address the three study objectives: they quantify significant site-level pigment differences between the two reference stands, show that the direction and principal spatial pattern of this contrast were captured by both platforms, and demonstrate that the hybrid PROSAIL–Random Forest framework retrieved both pigments at UAV and airborne scales, although cross-scale consistency was stronger for chlorophyll than for carotenoids.

CONCLUSION

In response to the first objective, the two Norway spruce stands differed in retrieved chlorophyll and carotenoid content, with higher values observed at Tetřeví Boudy than at Rejdiště. Given the absence of stand-level replication, this contrast represents a site-specific observational association between stand condition and canopy pigment status, while not establishing a causal role of bark beetle disturbance.

Regarding the second objective, the direction of the biochemical contrast and the principal spatial patterns were captured by both UAV Headwall Nano-Hyperspec® and airborne CASI-1500 observations. Chlorophyll showed stronger cross-scale correlation than carotenoids ($r = 0.96$ and 0.88 , respectively), indicating that chlorophyll patterns were more stable across the two observational scales, whereas carotenoid retrieval was more sensitive to aggregation and canopy heterogeneity.

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For the third objective, the hybrid PROSAIL–Random Forest framework retrieved chlorophyll with high accuracy from both platforms, with better performance for the UAV data, while carotenoid accuracy was broadly comparable between platforms. The results support the complementary use of UAV and airborne imaging spectroscopy for spatial assessment of forest pigment status, but correlation alone does not demonstrate unrestricted numerical interchangeability between sensors.

The conclusions are limited by the comparison of only one undamaged and one damaged stand, one acquisition date, and repeated needle-age measurements within 18 trees (54 samples). The observed stand contrast should therefore be interpreted as a proof of concept and a site-specific association rather than a general causal effect of bark beetle disturbance. Detailed information on stand age, tree size, phenotypes, infestation year, and water status was unavailable, and these factors may have contributed to the observed differences between the stands. Validation across additional stands, disturbance stages, seasons and environmental conditions, together with tree-level sensitivity analysis, agreement metrics and explicit uncertainty assessment, is required before operational application. Within these limits, the study shows that hyperspectral pigment retrieval can extend destructive biochemical measurements to spatially continuous canopy maps and preserve the principal difference between undamaged and severely damaged Norway spruce stands across two observational scales.

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