

Article

Intraspecific Drought Stress Responses Detected Using Remotely Sensed Vegetation Indices in Sitka Spruce (*Picea sitchensis*)

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Highlights

What are the main findings?

- Hyperspectral vegetation indices detected intraspecific variation in drought response among Sitka spruce clones, revealing contrasting physiological response trajectories.
- Pigment and red-edge indices were sensitive to drought, providing early and sustained signals of stress, whereas NDVI showed little response under the experimental conditions.

What are the implications of the main findings?

- Proximal hyperspectral sensing enables rapid, non-destructive screening of drought resilience, supporting its potential application into forest breeding programmes for climate adaptation.
- Pigment and red-edge indices showed greater sensitivity to drought than NDVI, highlighting their value for the early detection of physiological stress in forest systems.

Abstract

UK forestry faces increasing drought risk under climate change, raising concerns about the resilience of Sitka spruce, the UK's dominant commercial conifer. This study assessed whether hyperspectral vegetation indices can detect intraspecific drought responses to support resilience screening. An eight-week controlled drought experiment was conducted on 60 grafted Sitka spruce trees representing six clonal groups from the UK breeding population. Needle-level hyperspectral reflectance was used to derive vegetation indices associated with chlorophyll status, photoprotective pigments, and water content, alongside chlorophyll fluorescence (F_v/F_m). Pigment-based and red-edge indices showed the strongest sensitivity to drought, with ARI1 increasing by up to 1.2 under drought relative to controls, while water-related indices captured later-stage hydraulic decline. Clonal variation was detected in the timing and magnitude of pigment regulation, water loss, and photosynthetic impairment, indicating contrasting drought-response trajectories among clones. These results demonstrate that hyperspectral approaches enable rapid, non-destructive detection of physiologically meaningful drought responses and can support the identification of drought-resilient genotypes for climate-adaptive forest management.

Keywords: hyperspectral; forestry; photoprotection; spectroscopy; abiotic stressors



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1. Introduction

Climate change is increasing drought frequency across Europe, reducing forest productivity [1]. Increasingly dry summers pose challenges to UK commercial forestry [2,3]. The long-lived nature of trees makes forests vulnerable to extreme weather events, creating high maladaptation costs [4]. Selecting drought-resilient planting material is therefore central to climate-adaptive forest management.

Sitka spruce (*Picea sitchensis* (Bong.) Carr) is the dominant plantation species in the UK and, together with Ireland, covers over one million hectares [5]. It is widely used for replanting and afforestation but, relative to other commercial conifers, can be susceptible to drought [4,6].

Recent drought events have impacted Sitka spruce. During the extreme European drought of 2003, widespread damage and mortality were recorded across north-eastern Scotland [7,8]. In the warm, dry spring and summer of 2018, photosynthesis in a mature plantation decreased by 5%, and mortality of seedlings increased following reforestation [1]. Summers similar to 2018 are projected to become typical in the UK by 2050 [9], raising concerns about the long-term climatic suitability of Sitka spruce across parts of the UK.

Intraspecific drought response is critical for understanding species resilience under climate change. The Sitka spruce breeding programme, established in 1963, has successfully improved timber volume and quality [10]. Due to its high productivity, Sitka spruce is still likely to be favoured across much of the UK in the near future [11]. Integrating drought tolerance screening into breeding programmes may provide a scalable pathway to enhance forest resilience without requiring species replacement if well-validated high-throughput methods are developed [12].

Recent advances in hyperspectral remote sensing have improved the detection of drought-induced physiological stress in forest vegetation. Leaf-level hyperspectral measurements have been successfully used to estimate needle water content in conifer species and identify spectral regions associated with drought-related changes in foliar water status [13]. Similarly, hyperspectral imaging studies have demonstrated that pigment-sensitive metrics, including photochemical reflectance and red-edge indices, respond to drought-induced changes in chlorophyll and carotenoid dynamics in *Scots pine* [14]. Beyond controlled experiments, spectral indices such as NDWI, CIRE and CCI have also shown strong relationships with long-term tree water deficit measurements in temperate forests, highlighting their potential for operational drought monitoring [15]. However, most studies have focused on species-level drought detection, physiological monitoring, or comparisons among species. Although previous studies have shown that drought reduces growth, water status and gas exchange in Sitka spruce and that responses can vary among genetic families [16,17], the application of hyperspectral vegetation indices (VIs) to characterise intraspecific drought variation, particularly at the clonal level within commercially important forestry species, remains comparatively limited.

This study investigates the application of hyperspectral VI to quantify variation among Sitka spruce clones in their response to controlled progressive drought. Specifically, we aim to (i) identify VIs responsive to progressive drought stress, (ii) determine whether clonal differences in drought response can be detected spectrally, and (iii) evaluate whether pigment and water-related indices capture contrasting drought-response strategies among clones. These findings have implications for the development of rapid, non-destructive drought resilience screening tools within forest tree breeding programmes.

2. Materials and Methods

2.1. Study Location and Plant Material

The experiment was conducted in a polytunnel located at the Forest Research Northern Research Station, Scotland, UK (55°51'46.8972''N, 3°12'4.68''W; 185 m a.s.l.), during the summer of 2022, allowing for exclusion of environmental precipitation and control of plant water inputs. A temperature-operated fan increased air flow and cooled the environment.

Sitka spruce plants were taken from the Forest Research clonal archive (Roslin, UK). Sixty trees were used, consisting of ten ramets (copies produced by grafting onto rootstock) from six clones. Ramets originated from scions collected from mature (90–100 years old) grafts of original breeding population trees, selected in plantations, growing in west Scotland and southwest England. The selected clonal groups formed part of commercially relevant UK Sitka spruce breeding material and were chosen to sample variation within the breeding population rather than to comprehensively represent the species' full genetic diversity. Scions were grafted to rootstock (derived from unimproved wild-stand seed from Haida Gwaii, British Columbia) in 2015 and planted in increasingly large pots, up to 9.5 L at the time of the experiment, in 'nursery stock general' soil (ICL, Suffolk, UK) with slow-release fertiliser (120 N, 60 P, and 199 K mg L⁻¹). Ramets were randomly assigned to control or drought treatments, ensuring equal representation of genotypes within treatments and moved to the polytunnel in late May 2022.

2.2. Spectra Collection

Needle reflectance spectra were collected weekly throughout the experiment (7 June–24 August 2022) using a field spectroradiometer (ASD FieldSpec 4 Standard Res; Analytik Ltd., Cambridge, UK) sampling wavelengths from 350 to 2500 nm (3–10 nm spectral resolution). Non-destructive sampling was required to maintain full canopies and avoid stress associated with repeated defoliation (Figure 1).

Needles were arranged to form a flat, continuous surface over the ASD contact probe using low-reflectance gloves to minimise external light contamination and reduce overlap between needles. The ASD contact probe provides a fixed illumination and viewing geometry, reducing variability associated with measurement angle and ambient illumination. The probe was repositioned between measurements to minimise orientation and shadow effects associated with regular needle arrangement and reduce orientation bias [18]. Five measurements (25 rapidly collected spectra) were averaged to generate a single representative spectrum for each sample. Due to the regular needle arrangement, predominantly upward-facing needle surfaces were sampled.

Overwintered needles from the previous year's growth (2021), collected from the upper 25% of the canopy profile, were used for spectral measurements. Current-year needles were excluded because of high spectral variability during needle expansion [19]. Needle age was determined by counting internodes backwards from the current year's growth [20]. A white reference calibration was performed every 15 min using a 99% Spectralon panel, and sampling equipment was routinely inspected and cleaned to avoid contamination.



Figure 1. Needle-level hyperspectral measurements acquired from Sitka spruce (*Picea sitchensis*) using an ASD FieldSpec 4 spectroradiometer fitted with a contact probe. Reflectance measurements were collected repeatedly throughout the drought experiment to monitor temporal changes in physiological status under control and drought treatments.

2.3. Spectra Processing

Spectra were interpolated to 1 nm and converted to absolute reflectance (R_{abs}) by

$$R_{abs} = \frac{R_{sample}}{DN_{WR}} \times R_{WR}, \quad (1)$$

where R_{sample} are the raw spectra, DN_{WR} is the white reference taken in the field and R_{WR} is a lab-calibrated reflectance value (NERC field spectroscopy facility, Edinburgh, UK).

Vegetation indices (VIs) provide physiologically interpretable metrics and reduce spectral dimensionality. They were selected from the literature based on their physiological interpretability and demonstrated sensitivity to drought-related variation in chlorophyll status (Greenness VI, (VIg)), photoprotective pigments (Pigment VI (VIp)), and foliar water content (Water Content VI (VIw)) across forest and vegetation studies. The objective of the present study was to evaluate the performance of established hyperspectral indices for detecting intraspecific drought responses in Sitka spruce, rather than to develop a Sitka spruce-specific drought index. The VIs used in this study, together with their wavelengths, physiological interpretation and references, are provided in Table S1.

2.4. Soil Moisture and Watering Regime

Soil moisture was monitored using ThetaProbe ML2x sensors (Delta-T Devices Ltd., Cambridge, UK). Continuous measurements were recorded every 30 min using a CR1000x datalogger (Campbell Scientific, Shepshed, UK). One tree per genotype per treatment was equipped with an in situ probe. Manual measurements were also taken two to three times per week using an ML2x sensor connected to an HH2 metre across all individual plants.

Three readings per pot were averaged to estimate soil moisture. A soil-specific calibration was applied following manufacturer guidance.

Prior to treatment initiation, field capacity (FC) was determined for each pot by saturating soil and allowing drainage for two hours. Control plants were maintained between 80 and 100% FC throughout the experiment. Drought treatments were imposed progressively, with soil moisture declining during the first two weeks to approximately 20–25% FC before being maintained near this threshold through weekly watering to 20% FC followed by progressive drying between watering events. During the final three weeks of the experiment, drought-treated plants were left unwatered. This produced stable high soil moisture in control plants and repeated drying cycles of increasing intensity in the drought treatment as the experiment progressed (Figure S2).

2.5. Chlorophyll Fluorescence (F_v/F_m)

Maximal photochemical efficiency of photosystem II (F_v/F_m) was measured with a Pulse-Amplitude-Modulation (PAM) chlorophyll fluorometer (MINI-PAM-II, H. Walz, Effeltrich, Germany). F_v/F_m was calculated as

$$F_v/F_m = \frac{F_m - F_o}{F_m} \quad (2)$$

where F_o is the minimum fluorescence and F_m is the maximum fluorescence in the dark-adapted state. Needles were dark acclimated for 30 min using a leaf clip (DLC-8). F_o is taken using a low-intensity measuring light. F_m is taken immediately after a 0.8 s saturating pulse.

2.6. Statistical Analysis

To assess the effects of drought and clonal group on VI, a Bayesian quadratic regression model was fitted for each VI using a Gaussian likelihood implemented in the R package brms. Bayesian modelling was selected because it enables direct estimation of parameter uncertainty, performs robustly for hierarchical repeated-measures data with moderate sample sizes, and accommodates non-linear temporal responses within a probabilistic framework. Clonal group was modelled as a fixed effect because the objective was to quantify differences among the six selected clones rather than estimate variance within a larger population of clones. Fixed effects included treatment (control or drought), clone, and time since drought initiation (in days), together with their interactions. A second order polynomial term for time was included to capture non-linear temporal responses. Time was standardised (mean = 0, SD = 1) prior to model fitting, and the clonal group was fitted using zero-to-sum contrasts. Random intercepts and time slopes were included for individual trees to account for repeated measurements and within-clone variation among ramets.

For each VI, the intercept was assigned a weakly informative normal prior centred on the expected magnitude of that index based on its known numerical range and exploratory inspection of the data. Fixed-effect coefficients were assigned zero-centred weakly informative priors, reflecting the expectation that treatment, clone and temporal effects would be modest relative to overall index magnitude while allowing substantial deviation if supported by the data. Random-effect standard deviations were assigned weakly informative priors on the positive scale. A full list of model priors is presented in Table S2. Four Markov chain Monte Carlo chains run for 1000 warm-up and 3000 post-warm-up samples per chain. Checks of chain convergence and posterior predictive ability were performed on all models. Model convergence was assessed using trace plots, $\hat{R}$ statistics and effective sample sizes. All fitted models exhibited satisfactory convergence, with $\hat{R}$ values of 1.00 for all monitored parameters. Minimum bulk and tail effective sample sizes across all fitted models were 1179 and 1215, respectively.

Although the number of ramets per clone was modest, each individual was measured repeatedly throughout the eight-week experiment, resulting in a longitudinal dataset that substantially increased the amount of information available relative to a single end-point assessment. The Bayesian hierarchical framework was selected because it is well suited to repeated-measures data and moderate sample sizes, while explicitly propagating uncertainty through posterior distributions and credible intervals. The hierarchical model accounted for repeated observations through tree-level random effects, allowing uncertainty associated with individual variation to be propagated into posterior estimates. Consequently, inference was based on the full temporal dataset rather than individual sampling dates in isolation.

To compare the temporal sensitivity of VIs to drought stress, a response lag analysis was performed using posterior predictions from the fitted Bayesian models. For each VI, posterior mean trajectories were generated for drought and control treatments and averaged across clonal groups. The posterior treatment difference (drought – control) was then calculated for each day of the experiment. Response lag was defined as the first day after drought initiation at which the 95% credible interval of the treatment difference excluded zero for two consecutive prediction points. Maximum treatment divergence was defined as the largest absolute posterior mean treatment difference observed during the experimental period. Response lag and maximum divergence metrics were used to compare the relative timing and magnitude of vegetation index responses to drought.

Clone-specific deviations from the mean drought response were evaluated using posterior treatment-by-clone interaction estimates. Differences were inferred when 95% credible intervals excluded zero.

To explore relationships among VIs, a supplementary Spearman rank correlation analysis was conducted using mean drought-treatment values for each sampling date. Correlations were calculated among temporal trajectories of all vegetation indices and visualised using a correlation matrix.

Fv/Fm was analysed using linear mixed-effects models to account for repeated measurements on individual trees. Time since drought initiation was treated as a continuous variable and modelled using natural splines ($df = 3$), with spline complexity determined using Akaike's Information Criterion (AIC) to accommodate non-linear temporal responses. Models with spline degrees of freedom ranging from 1 to 5 were evaluated, and $df = 3$ was selected because it provided the best balance between model fit and complexity according to AIC. Fixed effects included time, treatment, clone, and their interactions, and individual ramet identity was included as a random intercept to account for within-tree dependence.

3. Results

3.1. Temporal Trends

Over the experimental period, most VIs exhibited clear linear temporal trends independent of watering treatment (Figure 2 and Figures S3–S5). Evidence for non-linear temporal effects was strongest for ARI1, while ARI2 showed weaker support. No evidence for change over time was found for CIgr and WI. No consistent evidence of clone-specific temporal responses independent of treatment was observed (Figures S3–S5).

3.2. Vegetation Indices

No consistent drought response was detected in NDVI, with posterior estimates centred near zero (Figure 2 and Figure S1). In contrast, red-edge indices exhibited consistent declines under drought. Maximum treatment differences reached 0.22 (95% CI: 0.03–0.41) for CIre and 0.05 (95% CI: 0.02–0.08) for NDVIre by day 57. Declines accelerated over time, resulting in progressive separation between droughted and control plants (Figure 2).

All stress-pigment vegetation indices (VIp) exhibited strong drought responses, with posterior treatment contrasts clearly separated from zero (Figure 2 and Figure S2). Divergence between treatments was evident early and increased over time. By the midpoint of the experiment, ARI1 and ARI2 had diverged by 0.48 (95% CI: 0.34–0.62) and 0.33 (95% CI: 0.25–0.42), respectively, under drought relative to controls, while PRI differed by 0.015 (95% CI: 0.01–0.02). PRI and CCI displayed predominantly linear temporal responses, whereas ARI1 and ARI2 showed evidence of non-linear (decelerating) dynamics (Figure 2, Figures S2 and S5). ARI1 and ARI2 exhibited the shortest response lags (4–5 days), followed by NDWI1, WI and PRI (11–12 days). Chlorophyll-related indices generally responded later, with NDVIre and CIre diverging after 41 and 51 days, respectively. No sustained divergence was detected for NDVI or CIgr (Table 1).

Table 1. Temporal response characteristics of vegetation indices. Response lag was defined as the first day after drought initiation at which the posterior treatment difference (drought – control) was credibly different from zero (95% credible interval excluding zero). Maximum drought-control difference corresponds to the largest absolute posterior mean difference observed during the experiment.

Vegetation Index	Response Lag (Days)	Maximum Treatment Difference
ARI1	4	0.627
ARI2	5	0.470
NDWI1	11	0.016
WI	11	−0.014
PRI	12	−0.031
CCI	24	−0.034
NDVIre	41	−0.054
MSI	43	0.036
NDWI2	44	−0.037
CIre	51	−0.218
WI/NDVI	52	−0.025
CIgr	Not detected	–
NDVI	Not detected	–
SRWI	Present from onset *	−0.053

* SRWI exhibited a treatment difference from the onset of the drought period and therefore a response lag could not be estimated.

All water-related vegetation indices (VIw) diverged between droughted and control plants (Figure 2 and Figure S3). Treatment responses were generally quadratic, characterised by initial increases during the first half of the experiment followed by marked declines as drought progressed (Figure 2 and Figure S6). MSI exhibited the inverse pattern due to its formulation.

NDWI2 and SRWI showed the strongest drought responses, decreasing relative to controls, whereas MSI increased under drought. In contrast, NDWI1, WI, and WI/NDVI displayed weaker overall treatment effects, with credible intervals for linear treatment terms overlapping zero (Figure S5).

Correlation analysis of drought-treatment trajectories revealed strong associations among indices targeting similar physiological processes (Figure S12). Anthocyanin indices (ARI1 and ARI2), red-edge/chlorophyll indices (CIre, NDVIre and CCI) and water-related indices (MSI, NDWI1, NDWI2 and SRWI) exhibited strong within-group correlations, supporting the physiological coherence of these index classes.

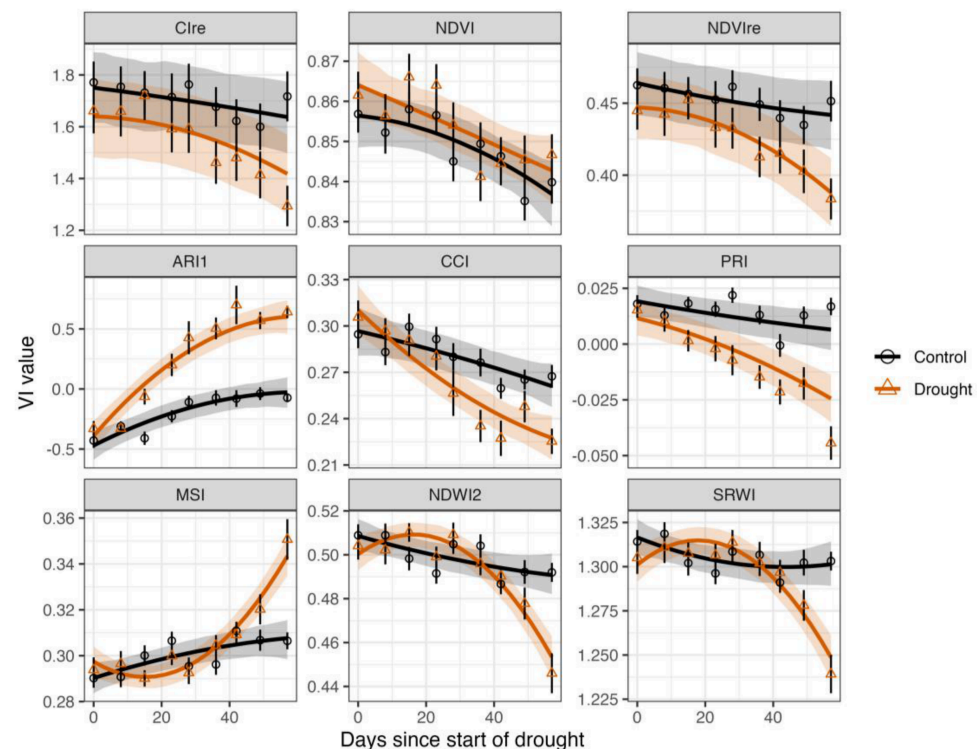


Figure 2. Selected Sitka spruce vegetation indices (VIs) responses over time. Points are average VI values ($\pm$ SE). Solid lines and shaded bands represent modelled posterior means and 95% credible intervals. Treatment groups are identified in the legend. Divergence between treatments over time indicates progressive drought effects. Full model outputs for all vegetation indices are provided in the Supplementary Material.

3.3. Clone Effects

Intraspecific variation in drought response was detected across pigment- and water-related vegetation indices, although the magnitude of clonal divergence varied among indices. Clone-specific differences were assessed using posterior clone-effect estimates and were considered credible when the associated 95% credible intervals excluded zero (Figures S2–S5).

Clone 6 exhibited the strongest and most consistent divergence in pigment-related vegetation indices relative to the mean clonal response. Under drought, Clone 6 showed pronounced increases in ARI1 and ARI2 during the early stages of the experiment, while PRI exhibited a stronger decline relative to the average clonal trajectory (Figure 3). By the midpoint of the experiment, ARI1 and ARI2 had diverged by 1.2 (95% CI: 0.86–1.52) and 0.83 (95% CI: 0.61–1.05), respectively, under drought relative to controls, while PRI differed by 0.05 (95% CI: 0.03–0.07). Other clones generally followed the mean pigment-response pattern, although more modest statistical deviations in response magnitude and temporal dynamics were detected in the supplementary analyses (Figures S2 and S8).

Variation among clones was also detected in water-related vegetation indices, although the magnitude and consistency of these differences varied across indices. Clone 2 showed the largest statistical deviation from the mean drought response across MSI, NDWI1, NDWI2, SRWI and WI, primarily during the later stages of the experiment as drought intensified. These responses were characterised by stronger divergence from controls and altered temporal response patterns relative to the mean clonal trajectory (Figure 3 and Figure S9). Clone 1 exhibited comparatively smaller departures from the mean response, while Clones 3 and 4 showed some differences in response shape across selected VIw, although these effects were less consistent among indices.

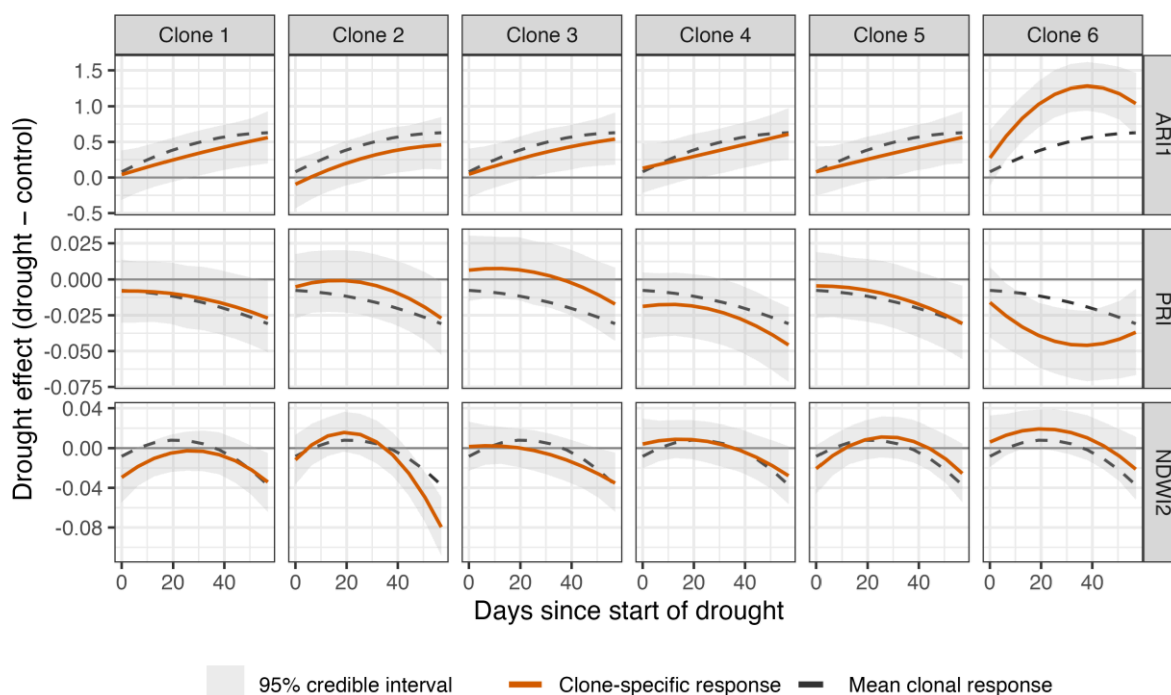


Figure 3. Modelled clonal drought response for selected vegetation indices (VIs). Drought effect is the modelled difference between the control and drought group. Coloured lines and shaded bands represent posterior means and 95% credible intervals for each clone. Dashed lines represent the mean clonal response. The horizontal line indicates zero effect. Full model outputs for all vegetation indices are provided in the Supplementary Material.

Overall, the strongest clone-specific responses were associated with Clone 6 for pigment-related indices and Clone 2 for water-related indices, whereas the remaining clones largely followed the average drought-response trajectory with comparatively modest deviations.

3.4. Chlorophyll Fluorescence (F_v/F_m) Response

F_v/F_m exhibited non-linear temporal dynamics over the course of the experiment. Under control conditions, F_v/F_m remained relatively stable across clones, whereas droughted plants showed progressive reductions in photosynthetic efficiency (Figure 4). A significant time $\times$ treatment $\times$ clone interaction indicated that drought-induced trajectories differed among clones.

Divergence between control and drought treatments occurred earliest in Clone 6 (approximately day 23), followed by Clone 4 (around day 30). In contrast, Clones 2 and 3 did not exhibit significant treatment separation until later in the experiment (day 49), while Clones 1 and 5 showed significant reductions only at the final sampling date (day 56). The magnitude of late-stage decline was greatest in Clones 2 and 4, consistent with pronounced reductions in photosystem II efficiency under sustained drought. Clone 1 displayed the smallest overall change in F_v/F_m throughout the experimental period.

In several clones, transient mid-experiment reductions in F_v/F_m were followed by partial recovery prior to further decline, indicating short-term physiological adjustments potentially exacerbated by fluctuations in polytunnel conditions.

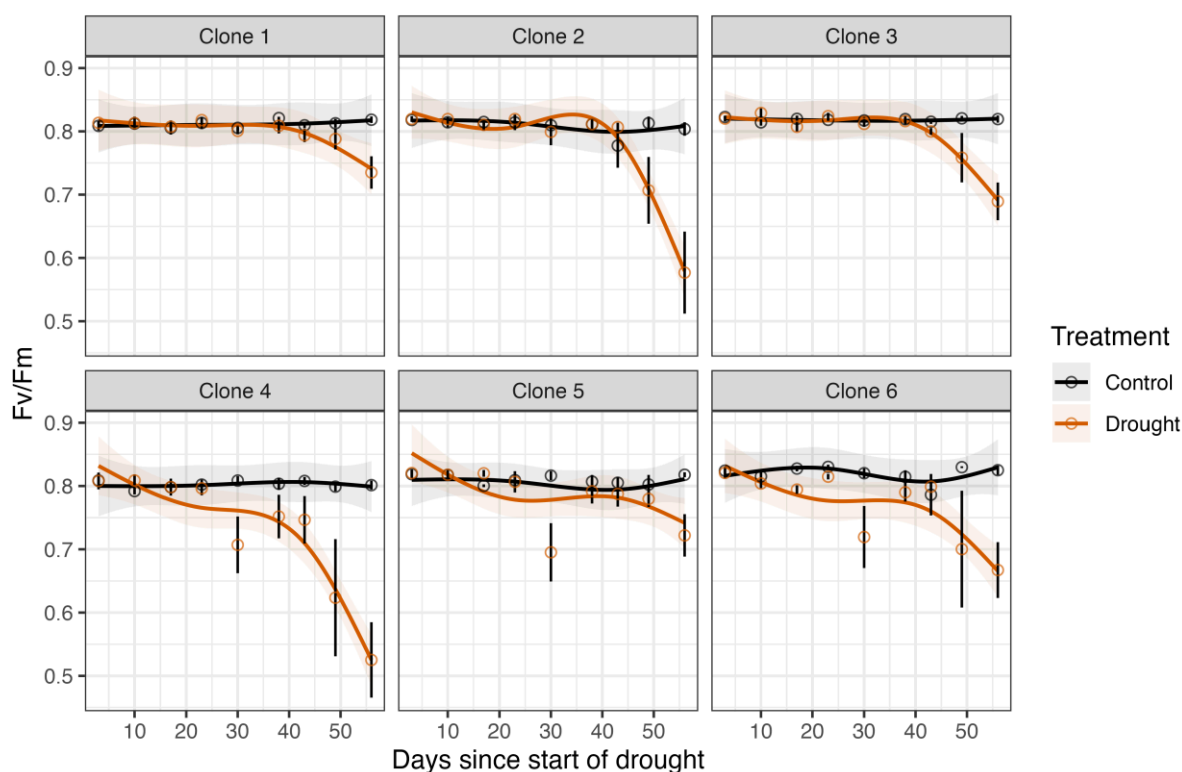


Figure 4. Clonal maximum photochemical efficiency (F_v/F_m) response over time. Points are average measurements ($\pm$ SE). Lines and shading are the modelled response and 95% confidence interval. Treatment groups are identified in the legend.

4. Discussion

4.1. Chlorophyll and Greenness Response

The combination of hyperspectral VIs provides a biophysical picture of drought response in Sitka spruce. Drought responses were detected in all VIs except NDVI. Chlorophyll-associated indices (VIg) decreased or remained stable under drought, pigment indices (VIp) indicated higher photoprotective pigment expression, and water content indices (VIw) captured foliar water loss in the latter half of the drought.

NDVI, the most widely used VI, was insensitive to drought stress in the present study. NDVI is sensitive to green leaf area and canopy structure but can saturate in evergreen canopies [21]. Although some defoliation was observed in this study—an emergency response in conifers—the needle-level measurements do not capture canopy parameters such as leaf area index (LAI) [22]. However, this alone may not fully explain the limited NDVI response. NDVI is also known to exhibit saturation effects in dense evergreen foliage, where chlorophyll absorption is already high and red reflectance is strongly constrained. Under such conditions, further physiological changes may result in only minor changes in NDVI despite measurable alterations in pigment composition and water status. The greater sensitivity observed for red-edge indices is therefore consistent with previous studies reporting limitations of NDVI for detecting subtle physiological stress in conifer species. In contrast, red-edge indices (CI_{re} and NDVI_{re}) declined steadily under drought. The red-edge region (approximately 680–750 nm) represents a transition zone between strong chlorophyll absorption and high near-infrared reflectance. As chlorophyll concentration decreases, the red-edge shifts toward shorter wavelengths, reducing saturation effects and increasing sensitivity to subtle pigment variation [23–25]. The stronger response of CI_{re} and NDVI_{re} therefore likely reflects enhanced sensitivity to needle-level chlorophyll decline.

The drought response of CIgr (which uses green light reflectance) was weaker than the red-edge indices. Green light penetrates more deeply into leaf tissue and is absorbed less efficiently than red wavelengths [26]. Chloroplasts in the upper cell layers experience the greatest excess light and photoinhibition. Chloroplasts contributing to green reflectance may therefore experience reduced excess energy relative to those in upper cell layers, potentially limiting the magnitude of drought-induced change detected by CIgr [27].

4.2. Photoprotective Pigment Dynamics

PRI and CCI declined under drought stress and are closely associated with carotenoid dynamics, specifically the xanthophyll cycle. Xanthophyll pigments change the distribution of absorbed light at 531 nm in response to changes in energy dissipation [21,28]. During stress, excess absorbed energy is dissipated as heat via the de-epoxidation of violaxanthin to zeaxanthin, while the reverse reaction occurs under non-saturating light conditions [29,30]. The observed decline in PRI and CCI is therefore consistent with enhanced non-photochemical quenching under drought, a response widely reported across conifer species [31–35].

The drought response of the two anthocyanin-based VIs was among clearest of all the selected VIs. Separation between the control and drought group was evident after two weeks and increased for the remainder of the experiment. Increased ARI1 and ARI2 indicate elevated anthocyanin concentrations, reflecting enhanced photoprotection as plants acclimate to stressful conditions. The anthocyanin drought response was rapid and sustained, suggesting that regulation of these pigments is important in Sitka spruce across mild to severe drought stress. Pigment-based responses preceded detectable declines in *Fv/Fm* in several clones, indicating that photoprotective adjustments were initiated before measurable impairment of photosystem II efficiency.

There are uncertainties surrounding the exact role of anthocyanins under drought stress [36–38]. However, substantial evidence supports a light screening function, whereby photoinhibition is reduced through absorption of high-energy photons and shielding light-saturated photosynthetic machinery [37,39,40]. Antioxidant roles have also been proposed, although this is under debate [36,41].

4.3. Foliar Water Dynamics

All water-related indices (VIw) exhibited a similar pattern during the drought treatment. Initially, VIw decreased relative to the control, then sharply diverged from days 30 to 40. These indices are correlated with foliar water content and water potential in conifers [42]. The rapid shift observed in the latter stages of the experiment suggests substantial needle water loss once plants were no longer able to maintain hydraulic function.

The initial increase in water-related indices relative to controls was unexpected. Declines in needle water content in conifers under drought can be relatively slow [43]. One possible explanation is that, during the early stages of drought, stomatal regulation reduced transpiration sufficiently to maintain needle water status despite declining soil moisture availability. Similar behaviour has been observed in pre-dawn water potential measurements, where reduced transpiration allows temporary maintenance of plant water status [44]. However, no direct measurements of stomatal conductance, leaf water potential or relative water content were collected in this study, and this interpretation should therefore be regarded as a hypothesis rather than a confirmed physiological mechanism. Beyond a certain level of drought stress [45], the rapid divergence of water-related indices may indicate progressive hydraulic impairment and needle desiccation.

This interpretation is broadly consistent with previous drought studies in Sitka spruce, which reported declines in stomatal conductance, transpiration and branch water potential

under water limitation, indicating that regulation of water loss is an important component of drought response in this species [16].

These findings suggest that VIw are sensitive indicators of severe water limitation but may be less reliable during early or moderate stress. Further work combining spectral indices with direct measurements of needle water content and water potential would help clarify the temporal dynamics of hydraulic decline in Sitka spruce.

WI/NDVI displayed the weakest drought response of all VIw, likely reflecting the inclusion of NDVI in its formulation, which was insensitive to drought under the present experimental conditions.

4.4. Maximal Photosynthetic Capacity and Clonal Variation

Declines in Fv/Fm under drought occurred non-linearly and varied among clones, with divergence between treatments emerging at different stages of the experiment. Early separation was detected in Clones 4 and 6, whereas Clones 2 and 3 only exhibited significant divergence during the later stages of drought, and Clones 1 and 5 showed treatment differences only at the final sampling date. These contrasting temporal patterns suggest differences in the thresholds at which photosystem II efficiency becomes impaired under sustained water limitation.

In some clones, transient mid-experiment reductions in Fv/Fm were followed by partial recovery prior to further decline, indicating short-term physiological adjustment potentially influenced by fluctuations in polytunnel environmental conditions, as previously recorded in Sitka spruce [46]. Such temporary reductions are consistent with reversible photoinhibition rather than irreversible photodamage [47].

The delayed but severe collapse observed in Clones 2 and 4 may reflect hydraulic failure following prolonged drought, whereas the relative stability of Clone 1 suggests greater drought avoidance or more effective maintenance of photochemical function under stress.

4.5. Clonal Differences in Drought Strategy

Evidence of clonal variation in drought response was detected across pigment- and water-related vegetation indices, although the magnitude of divergence varied among indices and clones. Clone 6 exhibited the strongest pigment-related divergence relative to the mean clonal response and was among the first to display separation in Fv/Fm . In contrast, Clone 2 showed comparatively modest early pigment responses but stronger late-stage divergence in water-related indices as drought intensified.

Drought avoidance and drought tolerance represent distinct physiological strategies for coping with water limitation. Drought avoidance involves mechanisms that minimise water loss or maintain tissue hydration, including stomatal regulation and maintenance of plant water status. In contrast, drought tolerance refers to the capacity to maintain physiological function despite declining water availability through mechanisms such as photoprotection, osmotic adjustment and antioxidative defence. Because direct measurements of stomatal conductance, water potential and hydraulic function were not collected in the present study, these terms are used as conceptual frameworks for interpreting spectral responses rather than as definitive physiological classifications.

Together, Clones 2 and 6 represented the clearest examples of contrasting drought-response trajectories within the study population. The more extreme VIw response in Clone 2 may indicate a reduced capacity to maintain water status during prolonged drought. Maintenance of leaf water content and hydraulic integrity through stomatal regulation represents a primary drought avoidance strategy, as photosynthesis and growth are constrained to conserve water [48]. However, a plant's capacity to avoid drought is limited by drought duration and intensity.

In contrast, pigment regulation under drought is more characteristic of tolerance strategies, whereby plants acclimate to sustained stress while attempting to maintain metabolic function [49]. Enhanced energy dissipation, osmotic adjustment and antioxidative protection all contribute to maintaining photosynthetic function under sub-optimal conditions. Clone 6 rapidly upregulated anthocyanins and exhibited strong xanthophyll cycle responses, consistent with an earlier activation of photoprotective mechanisms.

Clone 1 appeared least affected by the experimental drought. Treatment differences in Fv/Fm were only detectable by the final week and were relatively small in magnitude. Relatively modest changes in V_{Iw} were also detected in Clone 1, suggesting lower drought susceptibility and greater physiological stability under declining water availability.

The clonal differences observed in the present study are broadly consistent with previous drought studies in Sitka spruce. Grant et al. [17] reported significant variation in growth and biomass production among full-sibling Sitka spruce families subjected to restricted water availability, indicating that drought response is at least partly under genetic control. Similarly, Grant and O'Reilly [16] observed reductions in water potential, stomatal conductance and transpiration under drought, although evidence for strong family-level physiological differentiation was limited. The present study extends these findings by demonstrating that hyperspectral vegetation indices can detect subtle variation in pigment- and water-related responses among clones throughout the progression of drought stress. Differences between studies likely reflect variation in plant age, drought severity, experimental duration and the physiological traits assessed.

Intraspecific drought responses are widespread in conifers [50–53]. However, evaluation of this variation using remotely sensed spectral indices is rare [54,55]. Vegetation indices have been successfully applied to identify drought-tolerant varieties in agricultural crops [56,57]; however, despite increasing use of hyperspectral methods in forest monitoring, their application to assessing intraspecific drought tolerance in conifers remains limited. Recent hyperspectral studies have similarly demonstrated sensitivity of pigment- and water-related indices to drought-induced physiological variation in conifers, supporting the broader applicability of these approaches for forest drought monitoring. However, few studies have evaluated whether such indices can distinguish variation among genotypes within a commercially important forestry species.

Collectively, these patterns suggest contrasting drought-response trajectories among selected clones. Clone 6 exhibited early and sustained photoprotective pigment regulation consistent with a stronger pigment-based stress response, whereas Clone 2 showed greater late-stage divergence in water-related indices indicative of hydraulic decline under sustained drought. Clone 1 maintained comparatively stable physiological responses throughout the experiment. Although many clone-specific differences were modest, these results indicate measurable intraspecific variation in drought-response mechanisms within Sitka spruce.

4.6. Seasonal and Temporal Trends

Temporal trends were detected across most vegetation indices irrespective of drought treatment, reflecting seasonal adjustment in needle biochemistry. Evergreen conifers regulate photosynthetic capacity primarily through pigment pool adjustments rather than canopy senescence [21]. Seasonal declines in chlorophyll and concurrent increases in carotenoid and xanthophyll pools can elevate visible reflectance as summer progresses [21,58], consistent with patterns observed in both treatments during the present study.

Subtle seasonal changes in needle water content may also influence SWIR reflectance [59]. A slight increase over the season in the SWIR recorded by Qi et al. [59]

is comparable to the spectra collected here. These temporal trends were evident in both control and drought treatments, demonstrating that seasonal adjustment was distinct from drought-specific responses.

4.7. Implications for Breeding and Management

The present analysis demonstrates that hyperspectral vegetation indices can detect physiologically meaningful drought responses in Sitka spruce and differentiate clonal repose patterns under sustained water limitation. This could facilitate non-destructive screening of drought resilience and highlights the potential for integrating hyperspectral phenotyping into forest breeding programmes aimed at climate adaptation. Pigment-related indices were particularly sensitive to progressive stress, while water-related indices captured late-stage hydraulic decline. These spectral responses aligned with divergence in Fv/Fm , indicating that reflectance-based metrics tracked underlying changes in photochemical efficiency.

In practical breeding applications, hyperspectral measurements could be used to rapidly screen large numbers of candidate genotypes during controlled drought trials. Early-responding indices such as ARI1, ARI2 and PRI may enable identification of contrasting drought-response trajectories before visible symptoms develop. Because measurements are rapid and non-destructive, repeated assessments throughout drought progression are feasible and could complement conventional assessments of growth, survival and productivity within breeding programmes. Such approaches may help prioritise candidate genotypes for more detailed physiological and field-based evaluation, although further validation across larger populations, multiple provinces and operational forest environments will be required before implementation in routine breeding programmes.

Among VIp, PRI tracked xanthophyll cycle dynamics consistently, reinforcing its value as a marker of photoprotection under stress [32–35]. Anthocyanin-based indices detected early stress responses and show clear separation between control and drought treatments. Because anthocyanins are less directly linked to photosynthetic efficiency than carotenoids, they may provide a more stress-specific signal in evergreen conifers, where seasonal canopy senescence does not confound reflectance patterns.

4.8. Limitations

Although Fv/Fm provided a useful indicator of drought-induced changes in photosystem II efficiency, inclusion of additional fluorescence parameters such as NPQ or qE would allow more detailed assessment of photoprotective energy dissipation and reversible photoinhibition dynamics. Direct measurements of pigment concentrations, needle water content, stomatal conductance and plant water potential were not collected and would provide valuable validation of the physiological interpretations inferred from spectral indices.

The present study was conducted on potted, grafted Sitka spruce ramets under controlled polytunnel conditions using needle-level spectral measurements. Consequently, the observed relationships may differ in mature trees growing within heterogeneous forest environments. The physiological responses observed may also have been influenced by factors specific to the experimental material, including long-term cultivation in pots and pre-existing differences in vigour among clones. While several of the VIs investigated are routinely derived from UAV and satellite hyperspectral imagery, additional research is required to determine whether the clone-specific responses observed at the needle scale remain detectable at canopy and stand scales. Furthermore, genetic differences identified under controlled conditions may be influenced by environmental variation, competition and soil heterogeneity in operational forests. Validation across larger field trials and mixed

stands will therefore be necessary before these relationships can be applied to operational drought monitoring or breeding programmes.

5. Conclusions

Hyperspectral vegetation indices identified clear pigment- and water-related responses to progressive drought stress in Sitka spruce, several of which varied among clones. Pigment-related indices, particularly ARI1, ARI2 and PRI, responded rapidly to drought and revealed contrasting patterns of photoprotective regulation among clones. Water-related indices exhibited a more complex temporal response, with transient early increases followed by pronounced divergence as drought intensified. Together with chlorophyll fluorescence measurements, these responses indicate measurable intraspecific variation in drought-associated physiological responses within the study population.

The combined use of pigment-, water- and fluorescence-based indicators provided complementary insights into drought progression and clone-specific responses. These findings demonstrate the potential of hyperspectral approaches for non-destructive assessment of drought stress and physiological variation in Sitka spruce under controlled conditions. However, further research is required to determine whether the observed relationships are maintained in mature trees and under operational forest conditions, and to evaluate their transferability to canopy-scale remote sensing platforms. Such work could help assess the potential of hyperspectral methods as tools for supporting future drought resilience screening and forest monitoring applications.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/rs18142280/s1>, Figure S1: Experimental set up; Figure S2: Soil moisture as a percentage of field capacity across the experimental period; Figure S3: Key determinants of Sitka spruce greenness vegetation index variation; Figure S4: Key determinants of Sitka spruce pigment vegetation index variation; Figure S5: Key determinants of Sitka spruce water content vegetation index variation; Figure S6: Sitka spruce greenness vegetation index responses over time; Figure S7: Sitka spruce pigment vegetation index responses over time; Figure S8: Sitka spruce water content vegetation index responses over time; Figure S9: Modelled clonal drought responses for greenness vegetation indices; Figure S10: Modelled clonal drought responses for pigment vegetation indices; Figure S11: Modelled clonal drought responses for water content vegetation indices; Figure S12: Spearman correlation matrix among mean vegetation index trajectories during drought progression. Table S1. Vegetation indices used in this study, including formulae, physiological interpretation and literature references [21,24,60–69]. Table S2. Prior distributions used for Bayesian hierarchical models fitted to each vegetation index.

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Abbreviations

<i>Fv/Fm</i>	Maximal photochemical efficiency of photosystem II
VI	vegetation index
VIg	greenness vegetation index
VIp	pigment vegetation index
VIw	water content vegetation index
FC	field capacity
AIC	Akaike's Information Criterion

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