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Utilizing Vegetation Indices Derived from VNIR-SWIR Hyperspectral Data to Characterize Growth, Maturation, and Senescence in Wheat and Barley

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Abstract

Cereal crops, including wheat and barley, are essential for global food security, but their productivity is strongly affected by nitrogen availability and water limitation. This study investigated the phenotypic responses of two commercially significant spring wheat cultivars, Videodur (DU) and Sensas (SW), and two spring barley cultivars, Tiroler Imperial (SG1) and Amidala (SG2), exposed to two nitrogen regimes, low nitrogen at 25 kg N/ha (N25) and high nitrogen at 130 kg N/ha (N130), under drought and well-watered conditions. Plants were monitored from the late vegetative stage through maturity under controlled multivariable climatic conditions similar to field settings. A high-throughput phenotyping workflow was applied, combining precision watering, RGB imaging, infrared thermography, and VNIR–SWIR hyperspectral imaging to quantify plant growth, projected digital biomass, plant temperature, water use efficiency, and spectral vegetation indices associated with pigment dynamics, water status, maturation, and senescence. The results revealed cultivar-specific responses to combined nitrogen and drought stress. Under drought conditions, the high nitrogen treatment (N130) increased plant temperature (T_{plant}) for barley (cv. SG1) and wheat (cv. SW) compared to N25, thereby accelerating early maturation. However, the decline in chlorophyll was not uniformly faster across all cultivars tested. The DU cultivar exhibited superior chlorophyll absorption and reflectance, indicating better drought adaptation compared to other tested species. The high nitrogen treatment (N130) reduced water use efficiency (WUE) in the SW and SG2 cultivars compared to N25, implying that these cultivars used more water. Enhanced nitrogen did not consistently improve water use efficiency but did accelerate the growth cycle. SG2 was particularly sensitive to drought, showing declines in vegetation indices, except for the Water Content Index, highlighting the need for precise water and nitrogen management. Overall, the integration of hyperspectral, thermal, RGB, and water use measurements enabled the identification of trait signatures linked to drought adaptation, nitrogen response, maturation, and senescence. These findings provide practical insights for optimizing nitrogen and irrigation management and for supporting breeding strategies aimed at improving cereal crop resilience under climate-change-associated stress conditions.



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1. Introduction

Nitrogen is one of the key nutrients and largest agricultural input for the global food production system [1]. Farmers often over-apply nitrogen to ensure crop yield [2]. However, excessive nitrogen use can cause senescence, increase lodging and disease risk, and raise production costs [3]. Recent efforts to estimate nitrogen use efficiency (NUE) have focused on constructing nitrogen budgets and indicators based on farm-level inputs and outputs. Finding robust field data on nitrogen fertilizer efficiency is challenging, yet essential for identifying areas with the greatest potential for improvement and monitoring progress of crop performance [4].

Cereal crops, including wheat and barley, play a crucial role in global food security. However, their productivity is often limited by environmental stressors such as nitrogen deficiency and water scarcity. Predicting how plants will react to the combined effects of various stresses is challenging [5]. Understanding how different cultivars respond to these stressors is essential for developing resilient and high-yielding varieties [6]. High-throughput phenotyping workflow combining precision irrigation, RGB imaging, infrared thermography, and VNIR–SWIR spectroscopy is being used to quantify growth, photosynthesis, and stress-related traits [7–9]. This integrated setup enabled consistent measurement of plant structure, temperature, and spectral signals under controlled yet variable conditions, facilitating trait comparisons across genotypes and treatments [10,11].

Water use efficiency (WUE), a critical parameter in crop physiology, is defined as the ratio of biomass or grain yield produced to the amount of water consumed through transpiration and evaporation. It serves as a key indicator of a plant's ability to utilize water resources effectively, particularly under water-limited conditions. Lakhiar et al. [12] highlighted the importance of precision watering techniques in evaluating water use efficiency and drought tolerance in wheat cultivars. Such approaches not only optimize water usage but also enhance the selection of genotypes with superior performance under water stress.

In addition to water management, advancements in hyperspectral imaging have provided new opportunities for crop phenotyping. Wiegmann et al. [13] demonstrated the utility of visible-near infrared (VNIR) and short-wave infrared (SWIR) spectroscopy in predicting grain yield and nutrient content in barley under varying nitrogen levels. These findings underscore the potential of VNIR-SWIR hyperspectral data to assess critical growth stages, including maturation and senescence, in cereal crops such as wheat and barley. By integrating vegetation indices derived from hyperspectral data with phenotypic traits, researchers can develop robust models to predict crop performance under diverse environmental conditions.

Vegetation indices such as NDVI, PRI, OSAVI, PSRI, SIPI, and MCARI_1 are crucial tools in remote sensing for assessing plant health and stress [14]. The Normalized Difference Vegetation Index (NDVI) measures vegetation greenness and is widely used to monitor plant health and biomass. The Photochemical Reflectance Index (PRI) provides insights into photosynthetic efficiency and light use, making it valuable for understanding plant stress responses. The Optimized Soil-Adjusted Vegetation Index (OSAVI) improves NDVI by reducing soil background effects, enhancing its accuracy in sparse vegetation areas [15]. The Plant Senescence Reflectance Index (PSRI) is sensitive to changes in leaf pigments, degradation of chlorophyll and the accumulation of carotenoids, indicating plant senescence and stress [16]. The Structure Insensitive Pigment Index (SIPI) helps in assessing the concentration of carotenoids relative to chlorophyll, providing information on plant health and stress [17]. Lastly, the Modified Chlorophyll Absorption in Reflectance Index (MCARI_1) is designed to minimize the influence of non-photosynthetic elements, offering a more accurate measure of chlorophyll content [18]. The water content index (WCI) in

cereal crops can be significantly affected by drought conditions and nitrogen utilization. During the vegetative stage, plants have high water content, often around 80–90%, due to active growth and high-water requirements for cell expansion and metabolic processes [19]. As plants transition to the reproductive stage, water content decreases, focusing on grain development, which requires less water. At maturity, barley and wheat water content drops significantly to about 10–15%, ensuring the grains are dry enough for harvesting and storage.

In terms of crop yield, Normalized Difference Vegetation Index (NDVI) values from the late vegetative to maturity stages are particularly important. During these stages, NDVI helps monitor the photosynthetic activity and biomass of crops, which are strong indicators of potential yield [20]. Higher NDVI values generally correlate with healthier, more productive crops, while lower values may indicate stress or poor growth conditions. Typically, NDVI values above 0.6 indicate dense, healthy vegetation or crops with a high leaf area index. The Structure Insensitive Pigment Index (SIPI) maximizes sensitivity to the bulk carotenoids-to-chlorophyll ratio while minimizing the impact of variable canopy structure. It is particularly useful in areas with high variability in canopy structure or leaf area index.

These indices collectively enable a comprehensive analysis of vegetation health, particularly in linking nitrogen utilization and drought stress [21]. Monitoring these indices helps to better understand how plants manage nitrogen under varying water availability, ultimately aiding in the development of strategies to improve crop resilience and productivity under stress conditions. This approach aids in selecting better-performing lines for yield, disease resistance, and stress tolerance, while also uncovering the genetic basis of complex traits to accelerate crop improvement programs [10].

Despite the increasing use of hyperspectral vegetation indices for monitoring crop stress, there remains a need to clarify how contrasting wheat and barley cultivars differ in their physiological and spectral responses to the combined effects of nitrogen availability and drought during the critical transition from late vegetative growth to maturity. Therefore, this study aimed to use high-throughput phenotyping, including VNIR–SWIR hyperspectral imaging, infrared thermography, RGB-based biomass estimation, and water-use measurements, to compare cultivar-specific responses of two wheat cultivars, Videodur (DU) and Sensas (SW), and two barley cultivars, Tiroler Imperial (SG1) and Amidala (SG2), under low nitrogen, high nitrogen, and drought conditions. We hypothesized that nitrogen supply modifies drought responses in a cultivar-specific manner, resulting in distinct spectral, thermal, senescence, and water-use-efficiency signatures, and that these integrated trait responses can distinguish relatively drought-adapted cultivars from drought-sensitive ones.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Seed grains of Durum Videodur (DU), spring wheat Sensas (SW) and spring barley varieties, Tiroler Imperial (SG1) and Amidala (SG2), were obtained from AGES GmbH, Spargelfeldstrasse, Vienna, Austria. Germination rates for Videodur were found to be 89% and 96% for Sensas, Tiroler Imperial and Amidala, respectively. Seven 50 L bags of Plagron ProMix substrate were placed on a tarpaulin and mixed homogeneously using a shovel. Each pot was filled with 1.7 kg of substrate, equivalent to about 8 L, compressed by hand to 4 L. Afterward, 20 grains were sown per pot, with 10 grains arranged in a circle on the outer edge, 8 grains in a circle on the inside, and 2 grains in the center of the pot. Twelve pots of each variety were sown and ten plants were singled out per pot (1 plant in the center, 4 plants in a circle around it, 5 plants in a circle outside the edge of the pot). The

pots (4 genotypes $\times$ 2 N rates $\times$ 2 water levels $\times$ 3 replicates = 48 pots) are as shown in Table S1.

In this study, two nitrogen treatments were applied: nitrogen-limited (N25) at 25 kg N/ha and nitrogen-sufficient (N130) at 130 kg N/ha. For the N25 treatment, each pot received 6.072 g of Ensin and 1.052 g of DC-45, while the N130 treatment involved 30.245 g of Ensin and 1.052 g of DC-45 per pot. Ensin contains two nitrification inhibitors: 1,2,4-triazole (TZ) and dicyandiamide (DCD). It is manufactured by Duslo a.s. in Šal'a, Slovakia, and SKW Piesteritz in Wittenberg, Germany. DC-45 is another nitrification inhibitor used in fertilizers to slow the conversion of ammonia to nitrate, enhancing nitrogen use efficiency and reducing nitrogen losses from the soil. It is supplied by Agrana Beteiligungs-AG, Vienna, Austria. The concentration of DC-45 was kept constant across N25 and N130 treatments because DC-45 was not intended to serve as an additional treatment factor. It was rather included at a uniform background rate to maintain comparable nitrification-inhibitor conditions across nitrogen regimes. This allowed the experimental comparison to focus on the effects of contrasting nitrogen supply and drought stress. Ensin contains the nitrification inhibitors 1,2,4-triazole (TZ) and dicyandiamide (DCD), while DC-45 is also used in fertilizers to slow the conversion of ammonia to nitrate, thereby improving nitrogen use efficiency and reducing nitrogen losses from the soil.

Micronutrients were administered by mixing 25 L of water with 25 caps (~10 milliliter/cap) of micronutrient fertilizer, which was evenly distributed using a watering can. Irrigation was managed with two drippers per pot to ensure even distribution. The greenhouse climate was controlled with a maximum temperature of 18 °C during the day and 15 °C at night.

Additional information on plant material: The plant material used in this study includes a spring durum wheat variety (Videodur), a summer soft wheat variety (Sensas), and two spring barley varieties (Tiroler Imperial and Amidala). The wheat varieties belong to different species, making this study more of a species comparison than a variety comparison. The barley variety Tiroler Imperial is over 73 years old, raising questions about the heterogeneity of the accession. To address this, detailed information on the propagation and degree of heterogeneity of the accession is provided to clarify this aspect. References are available on the European Search Catalog for Plant Genetic Resources (EURISCO) and Germplasm Resources Information Network (GRIN) (<https://npgsweb.ars-grin.gov/gringlobal/accessiondetail?id=1169427>) (accessed on 16 April 2026).

2.2. Digital Imaging at VBCF

During the early reproductive to late maturity stage, spanning three weeks, plants were transferred to the PHENOPlant facility at the Vienna BioCenter Core Facility (VBCF), where high-throughput imaging and environmental measurements were performed under controlled multivariable climatic conditions using the PSI PlantScreen™ Modular System, Photon Systems Instruments spol. s r.o., Drásov, Czech Republic.

- Hyperspectral imaging in the visible–near-infrared (VNIR) and short-wave infrared (SWIR) regions was performed using two line-scanning hyperspectral cameras mounted adjacent to each other on a movable gantry of the phenotyping system [22]. Illumination for both cameras was provided by a halogen lamp mounted adjacent to the cameras to ensure homogeneous illumination during scanning. VNIR images were acquired with a line-scanning hyperspectral camera covering 350–900 nm at a spectral resolution of 0.8 nm, and SWIR images were acquired with a second line-scanning hyperspectral camera covering 900–1700 nm at a spectral resolution of 2 nm. Images were acquired at four photosynthetic photon flux density (PPFD) setpoints: 0, 150, 500,

and 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. VNIR–SWIR hyperspectral curves were integrated and normalized, and the spectral curves used for the reported analyses were taken from the 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ measurements. Reflectance was recorded against the instrument calibration reference. The four PPFD levels represented increasing actinic illumination, and the 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ level was used for all analyses because it approximated peak daytime photosynthetic conditions and provided the most intense and direct illumination condition for capturing physiological processes. Each spectrum was additionally normalized within its own sensor range, with VNIR and SWIR normalized separately, so that genotype comparisons and vegetation indices described in Section 2.4 reflected differences in spectral shape rather than absolute reflectance magnitude or residual illumination differences between scans.

- RGB imaging unit consisted of a light-isolated imaging box equipped with a turning table for precise angle positioning and two RGB cameras mounted on robotic arms for side-view and top-view imaging [8,22]. Each camera was supplemented with LED-based lighting to provide homogeneous illumination of the imaged plants. RGB images were acquired once per day from side views at 0° and 180° (RGB1) and from the top view (RGB2). Images were captured using a GigE uEye UI-5580SE-C 5 Megapixels QXGA camera with a 1/2" CMOS sensor (IDS, Obersulm, Germany) at a resolution of 2560 × 1920 pixels. The imaged area was 800 × 800 mm for the top-view position and 1205 × 1005 mm for the side-view position. Image-derived projected plant areas from the side-view and top-view RGB images were used to estimate digital biomass.
- Soil moisture content (SC) was monitored through automated watering based on weight [8,11].
- Thermal images were recorded in wheat and barley cultivars from side-view rotations at 0° and 180° under both nitrogen regimes, with and without drought, as described in Paul et al. [9,11] and Ahmad et al. [22]. Images were acquired using a FLIR A615 thermal camera, FLIR Systems Inc., Boston, MA, USA, equipped with a 45° lens. The camera resolution was 640 × 710 pixels, with a thermal sensitivity of <50 mK, and the imaged area was 1205 × 1005 mm. Thermal images were acquired in line-scan mode, with 710 pixels per line and a scanning speed of 50 Hz, and measurements were performed in darkness to minimize interference from external illumination. Plant temperature (T_{plant}) was computed using pixelwise differences relative to ambient probes and the background at IR800, following the established measurement routine. Specifically, plant pixels were segmented from the IR800 background and the mean canopy temperature was referenced to the ambient air temperature recorded by the chamber probes ($T_{\text{plant}} = \text{mean canopy temperature} - \text{ambient probe temperature}$), computed per image and averaged over the 0° and 180° side views. Transpiration was not measured directly in this study; it is inferred from T_{plant} together with the gravimetric pot water balance.

2.3. Abiotic Stress and Multivariate Climate

For each variety in the two-factorial experiment, 12 pots were used: six were nitrogen-limited (25 kg N/ha) and six were nitrogen-sufficient (130 kg N/ha). Within each nitrogen level, half of the pots were subjected to drought stress and half served as controls, resulting in three replicates per treatment. Phenotyping days were split between two microclimatic conditions (temperature and light) in the high-throughput growth environment at the Plant Sciences Facility, VBCF, as shown in Figure 1.

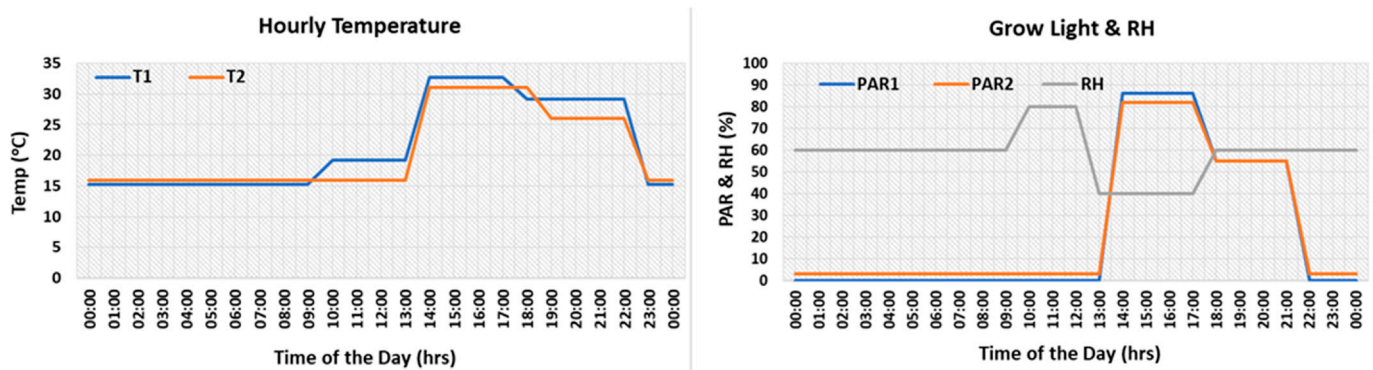


Figure 1. Microclimatic conditions set at HTP phenotyping measurement days in Plant Sciences Facility, VBCF. T1 and T2 represent the temperature ($^{\circ}\text{C}$); PAR1 and PAR2 represent the percentage of grow light PPFD measured in $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, set for the first half and last days of phenotyping respectively. RH represents the relative humidity (%) maintained in the growth room throughout the days of phenotyping.

2.4. Vegetation Indices

Hyperspectral indices were computed at $800 \mu\text{moles photons m}^{-2} \text{s}^{-1}$ to capture peak daytime physiology and are interpreted in relation to cultivar-specific trajectories during phenotyping. A set of vegetation indices from the VNIR–SWIR spectra was used to track pigment dynamics, canopy structure effects, senescence, and water status.

- NDVI (The Normalized Difference Vegetation Index) is a widely used indicator in agriculture for predicting green biomass, nitrogen content and assessing growth [20]. NDVI is calculated using the formula:

$$\text{NDVI} = (\text{r}800 - \text{r}670) / (\text{r}800 + \text{r}670) \quad (1)$$

where the normalized difference between NIR (around 800 nm) and red reflectance (around 670 nm) was used to quantify canopy greenness and biomass trends across treatments.

$$\text{NDVI}_2 = (\text{r}400 - \text{r}670) / (\text{r}400 + \text{r}670) \quad (2)$$

NDVI₂ is more responsive to alterations in leaf pigments (carotenoids and chlorophyll) that manifest in the blue–red domain.

- MCARI₁ (Modified Chlorophyll Absorption in Reflectance Index) estimates chlorophyll while reducing the influence of non-photosynthetic background [18].

$$\text{MCARI}_1 = 1.2 * (2.5 * (\text{r}800 - \text{r}670) - 1.3 * (\text{r}800 - \text{r}550)) \quad (3)$$

- OSAVI (Optimized Soil Adjusted Vegetation Index) minimized soil brightness effects with an adjusted NIR–red contrast [15,23].

$$\text{OSAVI} = [(1 + y) * (\text{r}800 - \text{r}670)] / (\text{r}800 - \text{r}670) + y \quad (4)$$

where (y) is a soil adjustment factor = 0.16.

PRI (Photochemical Reflectance Index) monitors rapid photosynthetic light-use responses via a carotenoid-sensitive reflectance ratio [24].

$$\text{PRI} = (\text{r}531 - \text{r}570) / (\text{r}531 + \text{r}570) \quad (5)$$

- PSRI (Plant Senescence Reflectance Index) detects the onset of plant senescence and grain maturation by assessing changes in carotenoid pigments relative to chlorophyll, making it useful for monitoring plant stress and the senescence phase [25–27].

$$\text{PSRI} = (\text{r680} - \text{r500}) / \text{r750} \quad (6)$$

- SIPI (Structure Insensitive Pigment Index) is used as a spectral proxy for pigment composition by maximizing sensitivity to the carotenoid-to-chlorophyll ratio while reducing confounding effects from the variable canopy structure [16–18,28].

$$\text{SIPI} = (\text{r790} - \text{r450}) / (\text{r790} + \text{r650}) \quad (7)$$

- WCI (Water Content Index) estimates relative leaf water status by leveraging strong water absorption in the SWIR near 1440 nm; higher ratio values indicate lower water content, and lower ratios indicate higher water content [19,26].

$$\text{Water Content Index} = \text{r1440} / \text{r960} \quad (8)$$

where r1440 is the reflectance at 1440 nm and r960 is the reflectance at 960 nm

2.5. Statistical Analyses

All statistical analyses were performed using IBM SPSS Statistics software (IBM SPSS Statistics for Windows, Version 27.0. Released 2020. Armonk, NY, USA) and Python software (Jupyter Notebook 6.5.4, Anaconda Navigator). We analyzed three independent plant pots per species, time point, nitrogen status and drought stress. Each of these plant pots consisted of 10 plant seedlings. For each analysis, z-scores were calculated to identify potential outliers within each species. In specific vegetation indices, lines used are the treatment means and the lighter overlaid points are the individual pot replicate measurements; treatment 25/130 denotes 25/130 kg N ha⁻¹, and drought_stress 0 = well-watered control (solid lines) versus 1 = drought (dashed lines). In this study, no data points exceeded the threshold of ± 3 standard deviations from the mean of the dataset for each measurement of indices, plant temperature, etc.

A linear mixed-effects model (LMM) was used to assess the effects of nitrogen treatment, drought stress, and time on the response variable for each of the four genotypes (DU, SW, SG1, SG2). LMMs account for multiple sources of variability by incorporating both fixed effects and random effects. The fixed effects included nitrogen treatment (N25 or N130), drought stress (binary: presence/absence), and time (days of phenotyping). The random effect accounted for variability among replicates, recognizing that each plant pot (tray_ID) could have a unique trajectory over time, differing in either slope or intercept. The primary model used was:

$$\text{Variable_in_question} \sim \text{days_phenotyping} + \text{treatment} + \text{drought_stress} + (\text{days_phenotyping} | \text{tray_id})$$

featuring a random slope. Alternative models incorporating random intercepts, random slopes, and interactions were explored. Specifically,

- days_phenotyping + treatment + drought_stress + (days_phenotyping | tray_id)
- days_phenotyping + treatment + (1 | tray_id)
- days_phenotyping * treatment * drought_stress + (days_phenotyping | tray_id)
- days_phenotyping * treatment * drought_stress + (1 | tray_id)
- days_phenotyping + treatment * drought_stress + (days_phenotyping | tray_id), were tested.

However, the primary model provided the best fit in most cases based on the lowest Akaike Information Criterion (AIC). For instance, for MCARI_1 in SG1, we selected the formula $\text{MCARI} \sim \text{days_phenotyping} + \text{treatment} * \text{drought_stress} + (\text{days_phenotyping} | \text{tray_id})$ because it had a lower AIC. Additionally, for Tplant in SG2, we also used an interaction term: $\text{Tplant} \sim \text{days phenotyping} + \text{treatment} * \text{drought stress} + (\text{days phenotyping} | \text{tray_id})$. Model selection followed an explicit, AIC-based rule: for each species $\times$ index $\times$ period, the candidate models were fitted and the lowest-AIC converged model was selected, retaining the more parsimonious additive model where competing models were within $\Delta\text{AIC} < 2$. The model selected for each trait, together with its marginal and conditional R^2 [29,30] and residual diagnostics, is reported in the Supplementary Materials. All linear mixed models were fitted with the lme4 package [31] via the pymr4 interface [32], and R^2 values were computed with the MuMIn package [30]. Candidate fits that failed to converge or returned a singular fit were excluded from model selection (12.6% of all candidate fits, predominantly the random-slope specifications); where a random-slope model did not converge, the corresponding random-intercept model was selected instead (Supplementary Table S2). For two combinations—Tplant at $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in SW and SG2—the tray-level variance was estimated as zero (a singular fit even with a random intercept), so no random-effects structure was supported and these were fitted as ordinary linear models. The design was balanced at the treatment level (three replicate trays per species $\times$ nitrogen $\times$ drought combination), but owing to measurement-scheduling gaps and to plant senescence toward the end of the experiment, 117 of the 1344 day $\times$ light-level combinations were not measured and 52 were under-replicated. In particular, the final day (day 20) is absent from three of the four light levels, as most plants had senesced and died by the end of the phenotyping period. The linear mixed models accommodate this as unbalanced data.

To separate the drought signal from the dominant phenology and time-of-day variance, a within-day diurnal spectral index was computed as the Euclidean norm of the evening-minus-morning change in normalized VNIR–SWIR reflectance for each genotype $\times$ water regime $\times$ day with both a morning (10–13 h) and an evening (18–20 h) scan (141 day-pairs). As a within-plant difference, this index cancels plant-identity and position effects. It was analyzed with a linear mixed model (diurnal index $\sim$ genotype $\times$ drought + (1 | day)), and the genotype $\times$ drought interaction was tested by a likelihood-ratio test (Supplementary Figure S5).

To assess treatment effects on relative growth rate (RGR) and water use efficiency (WUE), a two-way ANOVA followed by Tukey's post hoc test was conducted. WUE was quantified as digital biomass per unit of water used, expressed as $\text{pixels} \times 1 \times 10^{10}$ per gram [9].

Digital biomass was computed as $\text{digital biomass} = \sqrt{(\text{area_RGB1}^2 \times \text{area_RGB2}^2)}$, with area_RGB1 and area_RGB2 being the projected plant areas (pixels) from the multi-angle side view (RGB1) and the top view (RGB2), following Paul et al. [9,11] and Klukas et al. [33]; the water used per pot was the gravimetric loss recorded by the weighing station (weight after watering – weight). The image-derived digital biomass proxy correlates with destructively harvested biomass in cereals [34]. Treatment effects were analyzed with two-way ANOVA followed by Tukey's HSD to compare nitrogen and drought combinations across genotypes. This metric serves as an indicator of how effectively plants convert water into biomass under stress, complementing thermal and spectral readouts.

Finally, principal component analysis (PCA) was applied after standardizing the dataset to explore relationships between variables in a reduced-dimensional space. The PCA biplot visualizes variable contributions to each principal component, with arrow lengths indicating their respective influence.

3. Results

Nitrogen plays a crucial role in helping plants manage water stress more effectively, leading to relatively higher water content even under severe drought conditions [35]. As a key component of chlorophyll, it boosts photosynthesis and activates stress response pathways, improving water use efficiency [36]. Under severe drought, plants activate various stress response mechanisms, and while overall water content decreases, the relative increase in water content with nitrogen utilization is significant.

3.1. Hyperspectral Indices and Trait Detection

VNIR-SWIR hyperspectral imaging combined with vegetation indices helps assess plant health, growth, and stress responses for improved crop management [21]. For instance, NDVI was used as a proxy for correlating biomass and relative growth rate (RGR), indicating its importance in monitoring plant growth under varying nitrogen and water conditions [37]. PSRI values provide insights into the senescence process, with higher values indicating increased senescence and lower values suggesting healthier, non-senescent states.

3.2. Spectral Signatures for Abiotic Stress Effects

VNIR-SWIR stands for Visible and Near-Infrared (400 to 1000 nm) to Short-Wave Infrared (1000 to 2500 nm). Transient curves in this study typically refer to the time-dependent changes in the spectral properties of vegetation indices and the dynamic processes of plant physiology in relation to drought and nitrogen utilization [10]. As the experiments were conducted under controlled artificial lighting, imaging data with the most intense and direct illumination ($800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ simulating midday peak sunlight at 15:15 h) were considered for explaining the variability in physiological traits. Vegetation indices are mainly derived from reflectance data within the Red and NIR bands.

Visual inspection of the values revealed notable differences in reflectance observed between genotypes experiencing drought and those well-watered. Carotenoids absorb light in the blue region, with peaks typically around 450–480 nm. They also contribute to the reflectance in the green-yellow region. Spectral properties of vegetation change considerably during crop stress, as the pigment chlorophyll rapidly decays and loses its absorption properties, increasing reflectance in the green to reddish part (530–670 nm) of the visible region [38]. Increased reflectance in the green region (550 nm) can indicate nitrogen deficiency, as chlorophyll absorption decreases. The crop spectral reflectance increased more rapidly in the infrared region and the slope of the red-edge became steeper, especially in the treatments under full irrigation, where a shift to longer wavelengths and an increase in the amplitude of the red-edge peak were observed. The values of spectral reflectance for non-stressed plants were higher in the range of (710–950 nm) compared to the plants under stress (700–740 nm), indicating reduced chlorophyll content.

During the phenotyping period at the maturation stage, carotenoid absorption peaks were predominantly observed in wheat cultivars DU and SW. In barley cultivars, particularly SG2, followed by SG1, reflectance was dominantly observed in the green-yellow region (Figure 2). Post-irrigation recovery did not result in significant changes in the spectral signature peaks on Day 20, compared to Day 19 of phenotyping. This indicates that the spectral characteristics remained stable during the recovery phase. This section presents descriptive statistics to provide an initial overview of differences between genotypes. A more in-depth analysis of the various indices will be performed in subsequent sections. In the post-drought-recovery panel (D20, one day after re-watering), the spectral signatures remained broadly similar to those of the final drought day (D19), with no marked spectral recovery evident within one day of re-watering (Figure 2).

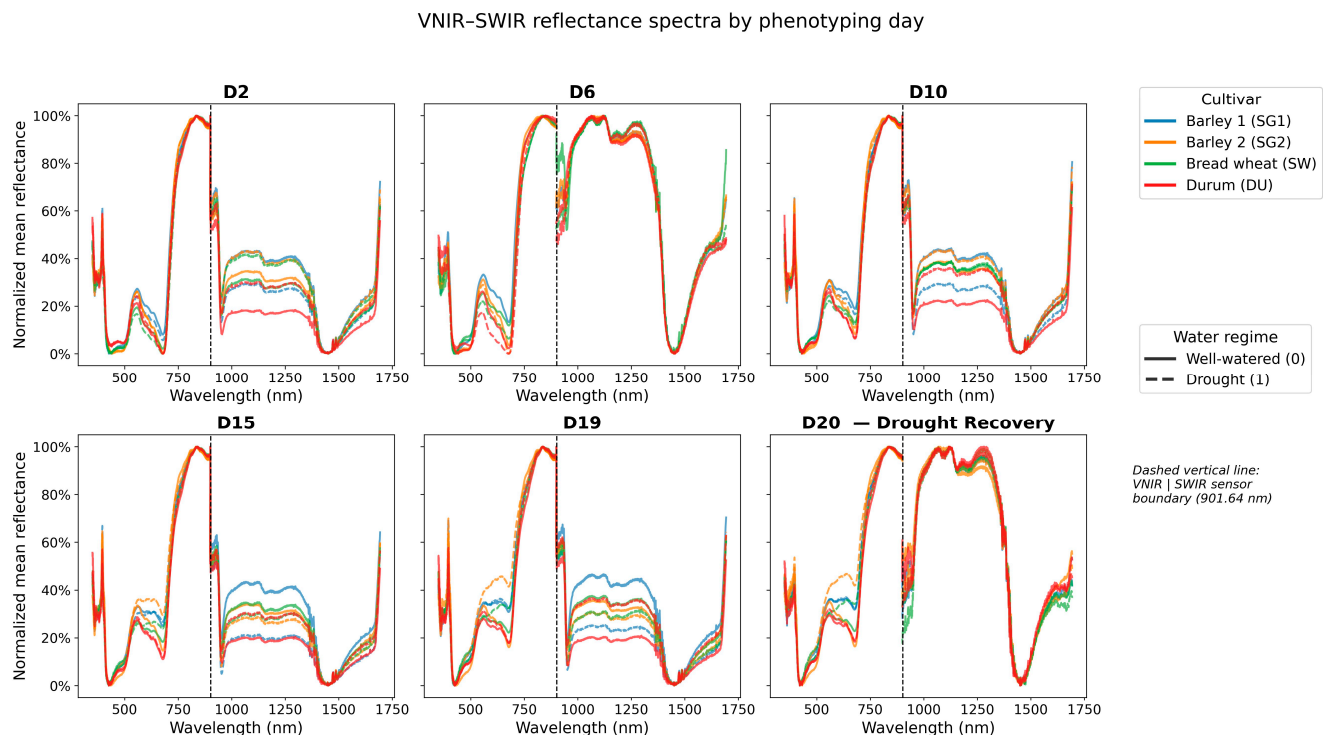


Figure 2. VNIR-SWIR (Visible-Near Infrared to Short-Wave Infrared) wavelength (nm) curves for different crops under drought stress in the days of phenotyping from late vegetative to maturity stage. Curves are prepared from the average of three biological replicates per treatment. Each pot replicate consists of 10 plant seedlings. D2, D5, D10, D15 and D19 are days of phenotyping (VNIR) hyperspectral imaging measurements along with drought and D20 is one day post-drought recovery. Line color denotes the cultivar and line style the water regime (solid = well-watered control, dashed = drought). Spectra are the 15:15 measurement ($800 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, peak-daytime illumination), except D20—the drought-recovery day after re-watering—for which only the 19:15 (evening) scan was available. Each spectrum was min–max normalized to 0–1 separately for each sensor and curve; to avoid anchoring this normalization to the noisy VNIR | SWIR detector seam, the 900–960 nm seam region (shown as a gray band) was excluded before normalization.

3.3. Water Content Index (WCI)

Nitrogen regulates water content even under drought conditions. When plants face drought, they initiate stress responses to cope with water scarcity. Sufficient nitrogen enhances the effectiveness of these stress responses, resulting in relatively higher water content compared to plants without adequate nitrogen.

To gauge the significance, a linear mixed model (LMM) was used. N130 treatment was found to have a significant, small drop in WCI (estimate = -0.002) in DU. This suggests that excessive nitrogen application may reduce water retention in some cultivars, possibly due to increased transpiration or metabolic demands. These metabolic demands could include enhanced growth and development, increased protein synthesis, and higher photosynthetic activity, all of which require substantial water and energy resources. While drought had no effect on N25 treatments in DU, SW and SG1, it did have an increasing effect with SG2 (estimate = 0.127 , $p = 0.014$), indicating that under drought conditions, SG2 increased its water content, likely due to adaptive water conservation mechanisms. In all four crops, WCI significantly increased over time, but only by a minor amount. (Figure 3). The small but significant increase in WCI over time suggests some degree of adaptation or water uptake over the observation period.

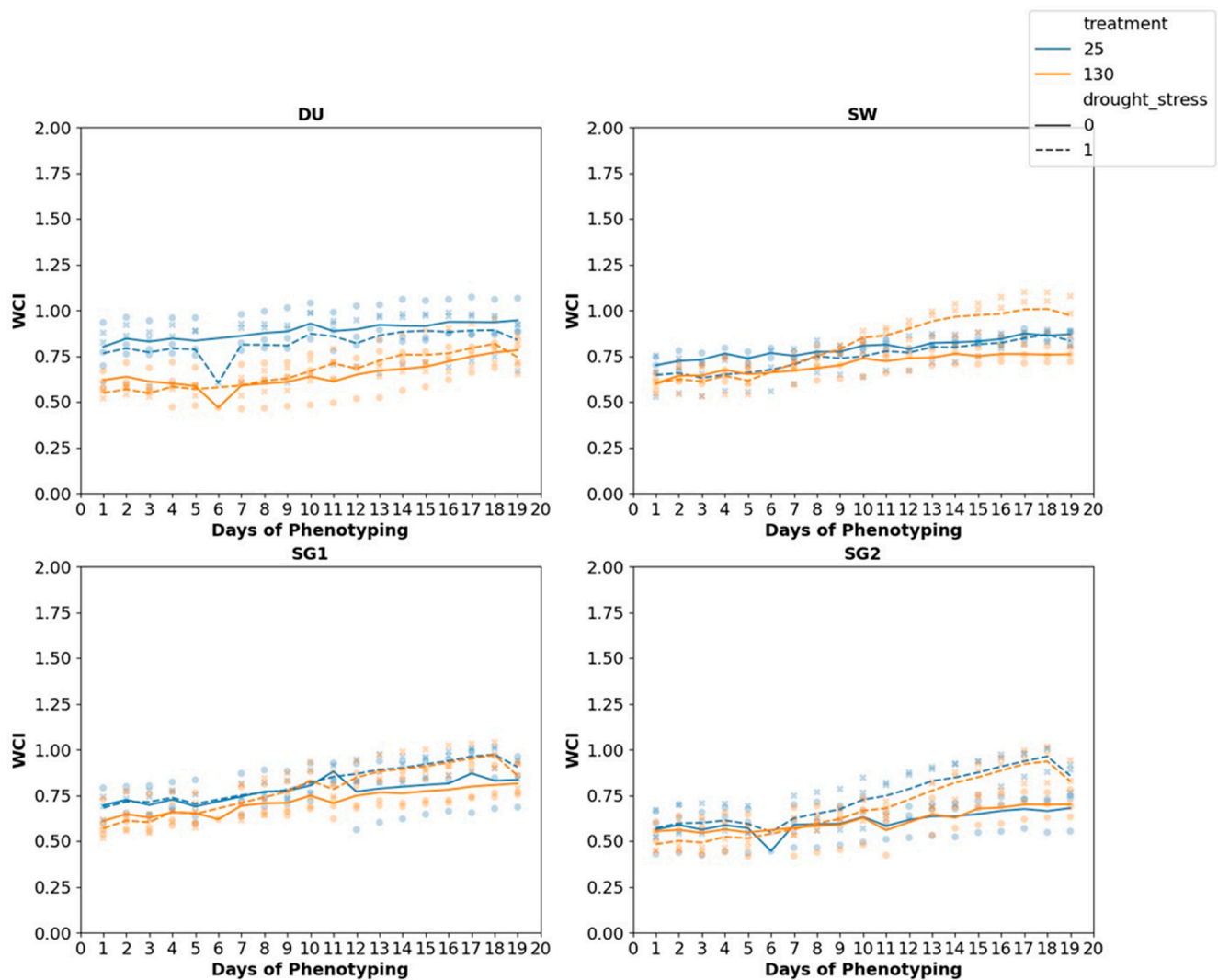


Figure 3. Water content index (WCI) parameter calculated for different crops under drought stress in the days of phenotyping from the late vegetative to maturity stage. Curves are prepared from the average of three biological replicates per treatment. Each pot replicate consists of 10 plant seedlings. According to the linear mixed model, DU was significant in treatment ($p = 0.004$); SG2 was significant in drought stress ($p = 0.035$). All cultivars were significant in days of phenotyping ($p = 0.000$). Lines represent treatment means, and lighter overlaid points represent individual pot replicate measurements. Light blue indicates the N25 treatment (25 kg N ha^{-1}), and orange indicates the N130 treatment (130 kg N ha^{-1}). Solid lines represent well-watered controls, whereas dashed lines represent drought stress.

3.4. Normalized Difference Vegetation Index (NDVI)

NDVI decreased from the late vegetative stage to maturity stage under drought in barley and wheat cultivars at N130, indicating a decline in photosynthetic activity, plant greenness, biomass and overall vegetation health as the phenotyping period progresses. The decline in NDVI is comparatively lower in N25, while the well-watered controls of N130 and N25 showed more or less the senescence dynamics of growth in NDVI for all cultivars (Figure 4). N130 had a significant but small increase in NDVI in DU (estimate = 0.001). Drought stress had a significant negative impact on NDVI in both treatments of SG2 (estimate = -0.153 , $p = 0.001$).

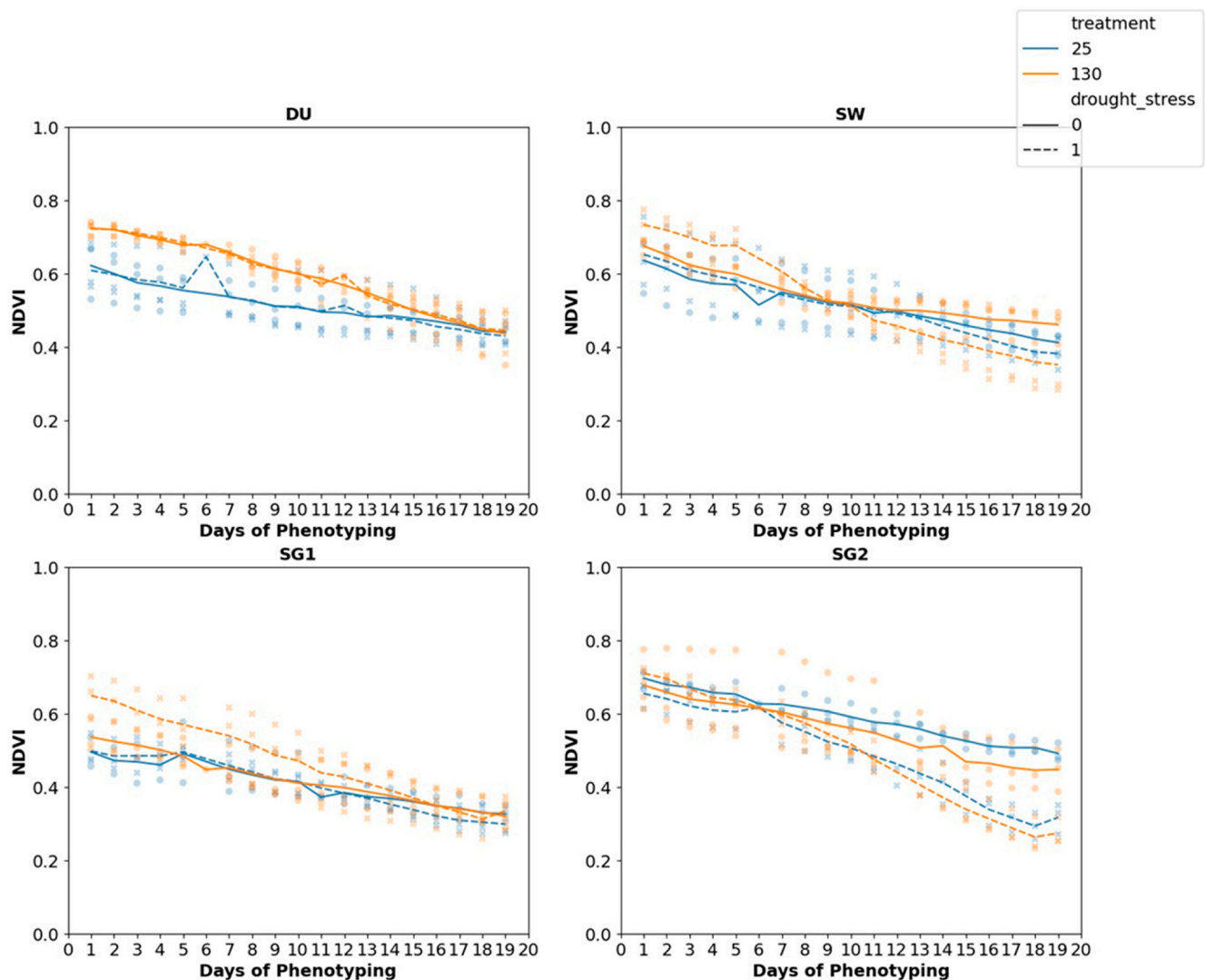


Figure 4. NDVI dynamics of wheat and barley cultivars under nitrogen and drought treatments during the phenotyping period. Values represent the average of three biological replicates per treatment. Each pot replicate consists of 10 plant seedlings. According to the linear mixed model, DU was significant in treatment ($p = 0.021$); SG2 was highly significant in drought stress ($p = 0.000$). All cultivars were significant in days of phenotyping ($p = 0.000$). Lines represent treatment means, and lighter overlaid points represent individual pot replicate measurements. Light blue indicates the N25 treatment (25 kg N ha⁻¹), and orange indicates the N130 treatment (130 kg N ha⁻¹). Solid lines represent well-watered controls, whereas dashed lines represent drought stress.

3.5. Structure Insensitive Pigment Index (SIPI)

Drought and nitrogen stress can alter the carotenoid-to-chlorophyll ratio, affecting SIPI values. SIPI values decline significantly over time across all cultivars, suggesting a gradual reduction in the carotenoid-to-chlorophyll ratio, indicating plant stress and potential senescence. Even though the SIPI index showed higher values in N130 treatment vegetative stages of DU, SW and SG1 cultivars, there is a decline towards the end of the phenotyping period (Figure 5). Drought stress had a significantly negative effect on SIPI values of cv. SG2 (estimate = -0.154 , $p = 0.000$) cv.

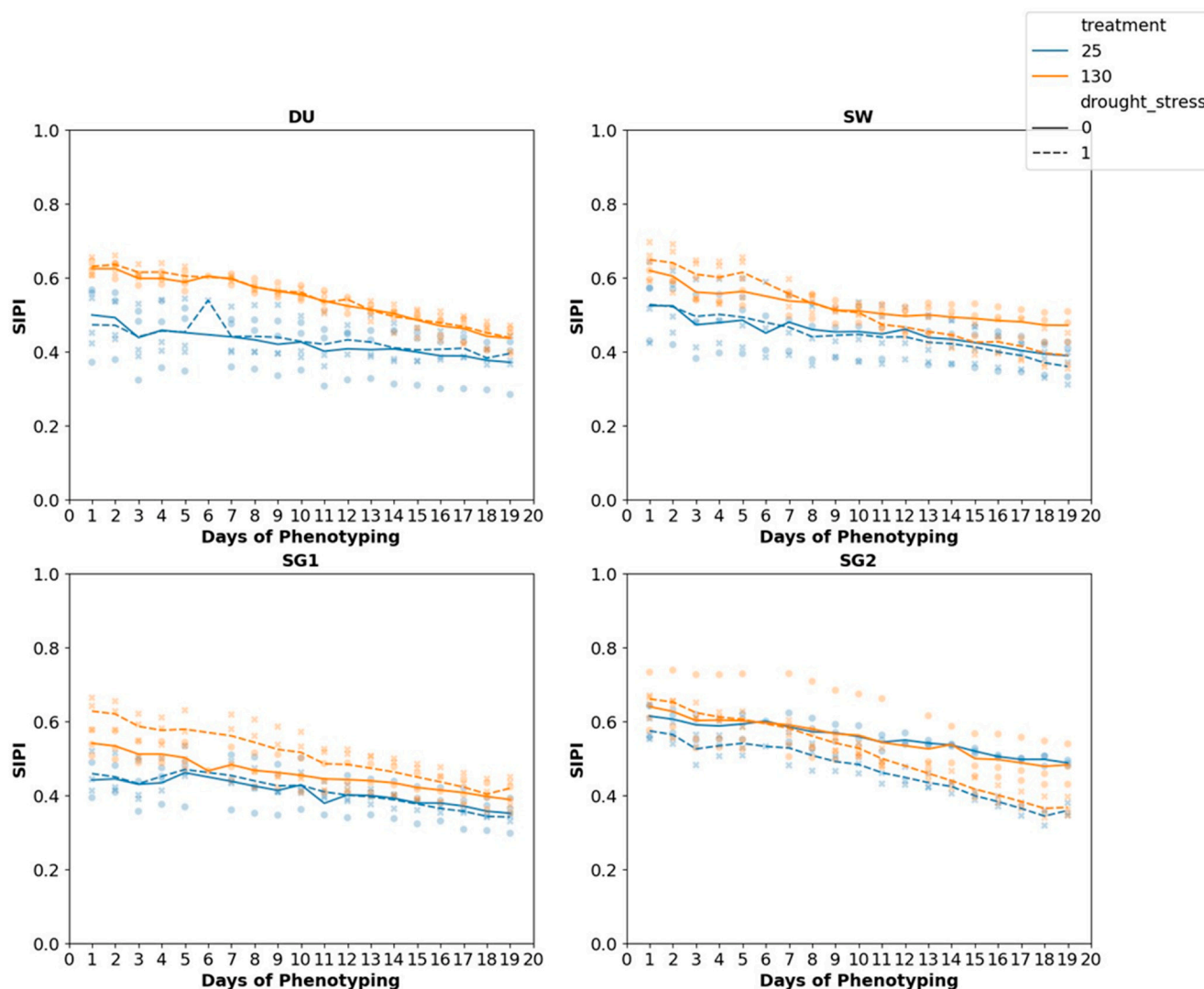


Figure 5. Comparison of SIPI (Structure Insensitive Pigment Index) for different crops following nitrogen and drought stress treatments measured over the phenotyping period using VNIR. Values represent the average of three biological replicates per treatment. Each pot replicate consists of 10 plant seedlings. According to the linear mixed model, DU was significant in treatment ($p = 0.01$); SG2 was significant in drought stress ($p = 0.000$). All cultivars were significant in days of phenotyping ($p = 0.000$). Lines represent treatment means, and lighter overlaid points represent individual pot replicate measurements. Light blue indicates the N25 treatment (25 kg N ha^{-1}), and orange indicates the N130 treatment (130 kg N ha^{-1}). Solid lines represent well-watered controls, whereas dashed lines represent drought stress.

3.6. Modified Chlorophyll Absorption in Reflectance Index 1 (MCARI₁)

During the vegetative to maturity growth period, the MCARI₁ value decreased significantly over time in all cultivars. Under drought conditions with N130 treatment, SG1, SG2, and SW exhibited sharp declines in MCARI₁ value of 80%, 127%, and 120%, respectively, while the DU cultivar showed a more moderate decline of 40% (Figure 6). Interestingly, nitrogen treatment had a slight positive effect (estimate = 0.01) that was statistically significant in a mixed model for DU, SW and SG1, but not for SG2 cv. For SG1, the approach was selected based on its lower AIC value, indicating that higher nitrogen levels contributed to better chlorophyll content and plant health in these cultivars. The change in MCARI₁ index value on well-watered N130 was 50% for barley cultivars. On the contrary, moderate declines in MCARI₁ values were observed in wheat cultivars, with

DU showing a 43% decline and SW a 30% decline. The N25 treatment showed a moderate MCARI_1 index value across all crops with and without drought effect, with a slight decline observed for SG2 under drought. All in all, SG2 was negatively impacted by drought stress (estimate = -0.181), showing a considerable reduction in MCARI_1 value, which indicates that this cultivar is highly sensitive to water stress. In contrast, drought stress did not have a significant impact on the other cultivars, suggesting that these cultivars exhibit some level of resilience to water limitations.

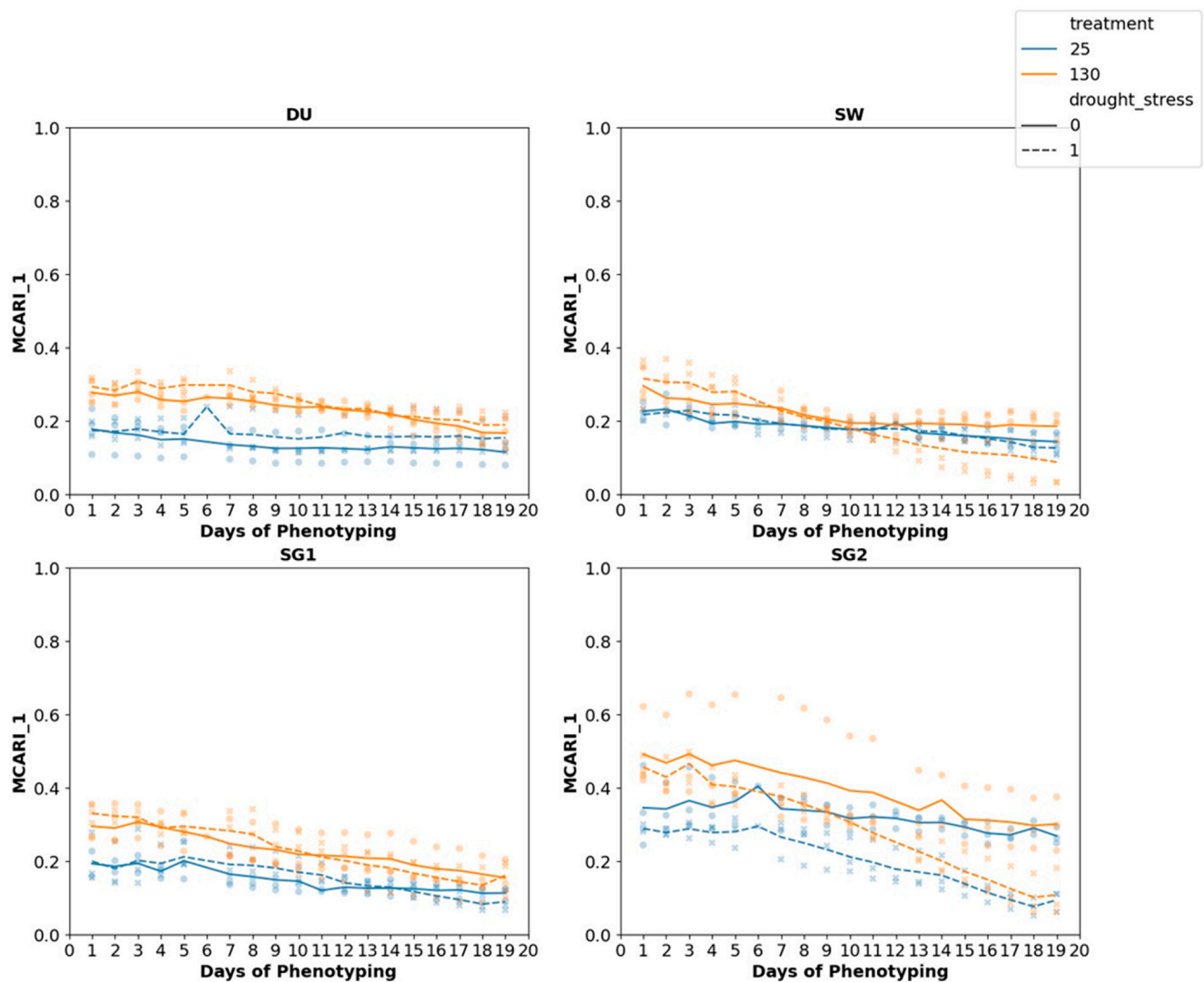


Figure 6. Comparison of MCARI_1 (Modified Chlorophyll Absorption Ratio Index 1) for different crops following nitrogen and drought stress treatments measured over the phenotyping period using VNIR. Values represent the average of three biological replicates per treatment. Each pot replicate consists of 10 plant seedlings. According to the linear mixed model, SG1, SW and DU were significant in treatment ($p = 0.049$, $p = 0.023$ and $p = 0.000$, respectively); SG2 was significant in drought stress ($p = 0.000$). Lines represent treatment means, and lighter overlaid points represent individual pot replicate measurements. Light blue indicates the N25 treatment (25 kg N ha^{-1}), and orange indicates the N130 treatment (130 kg N ha^{-1}). Solid lines represent well-watered controls, whereas dashed lines represent drought stress. All cultivars were significant in days of phenotyping ($p = 0.000$ (SG2, SG1), 0.002 (SW), 0.001 (DU)). For SG1, this formula fitted the data better: $\text{MCARI} \sim \text{days phenotyping} + \text{treatment} * \text{drought stress} + (\text{days phenotyping} | \text{trayID})$.

3.7. Optimized Soil-Adjusted Vegetation Index (OSAVI)

The mixed model analysis of the OSAVI across different cultivars (DU, SW, SG1, and SG2) over the phenotyping period reveals that OSAVI values decline significantly over time, with the rate of decline varying between cultivars. In this study, all cultivars in the N130 treatment had OSAVI values between 0.5 and 0.6, whereas those in the N25 treatment had values between 0.4 and 0.5 during the vegetative stage. Towards the maturity stage, OSAVI declined to 0.4 in N130 and to 0.3 in N25 irrespective of drought, while SG2 showed a decline in both treatments in comparison to well-watered controls (Figure 7). In SG1, SW and DU, treatment N130 had a positive effect on OSAVI values, indicating that enhanced nitrogen levels contributed to improved vegetation index values. Enhanced nitrogen application can be considered a viable strategy to sustain plant health in these cultivars. However, since drought stress did not have a significant impact on these cultivars, they may have mechanisms to cope with limited water availability, making them suitable for cultivation in drought-prone areas. In SG2, nitrogen treatment had no significant impact but, on the other hand, drought stress had a significant negative effect (estimate = -0.157). SG2 is therefore highly sensitive to drought stress, indicating that high water availability is crucial for its health and growth.

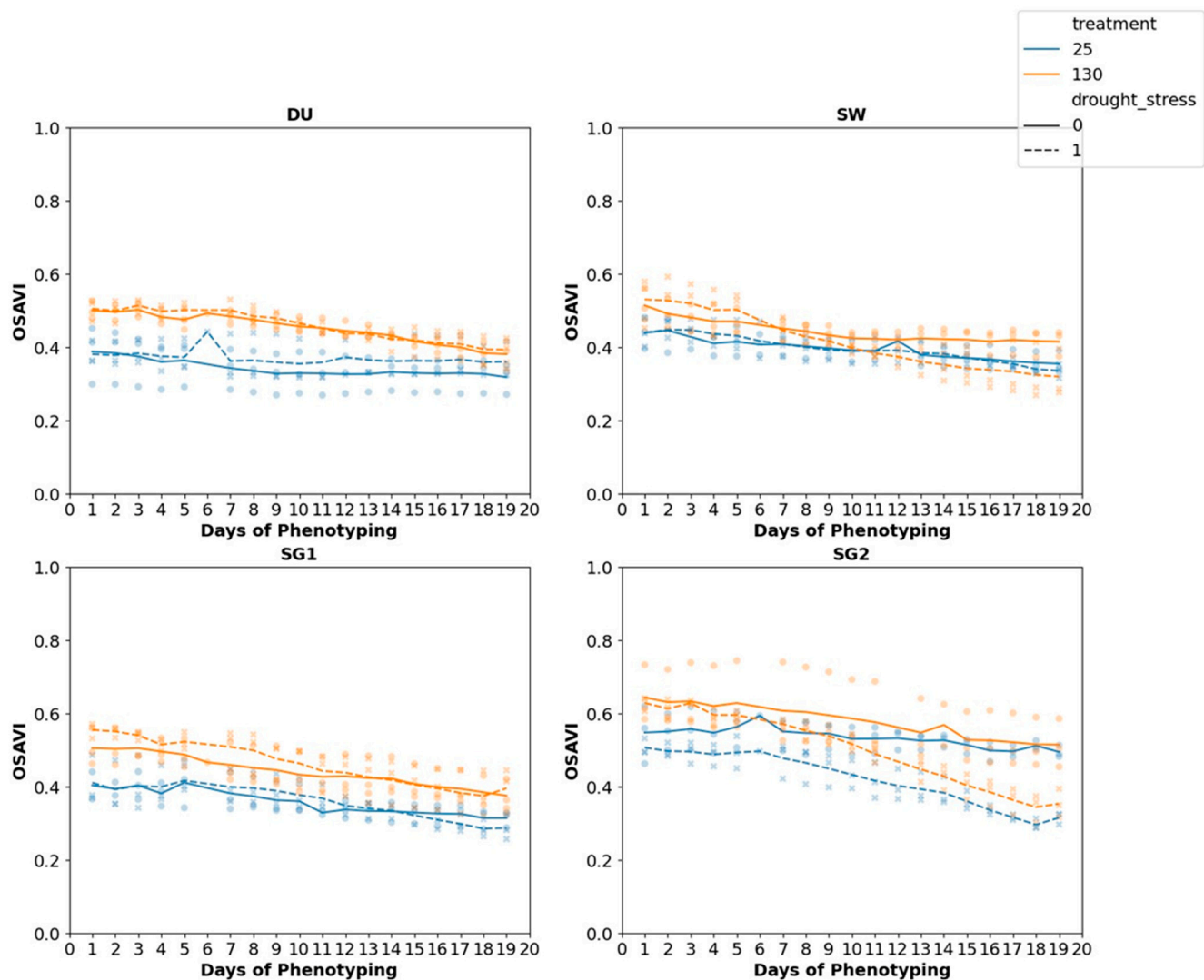


Figure 7. Comparison of OSAVI (Optimized Soil-Adjusted Vegetation Index) for different crops following nitrogen and drought stress treatments measured over the phenotyping period using VNIR.

Values represent the average of three biological replicates per treatment. Each pot replicate consists of 10 plant seedlings. According to the linear mixed model, SG1, SW and DU were significant in treatment ($p = 0.003$, $p = 0.041$ and $p = 0.001$, respectively); SG2 was significant in drought stress ($p = 0.000$). All cultivars were significant in days of phenotyping ($p = 0.001$ (DU, SW), $p = 0.000$ (SG1, SG2)). Lines represent treatment means, and lighter overlaid points represent individual pot replicate measurements. Light blue indicates the N25 treatment (25 kg N ha⁻¹), and orange indicates the N130 treatment (130 kg N ha⁻¹). Solid lines represent well-watered controls, whereas dashed lines represent drought stress.

3.8. Plant Senescence Reflectance Index (PSRI)

The PSRI values exhibit mild responses over time across all cultivars, indicating a gradual increase in plant senescence as phenotyping progresses (Figure 8). Treatment with N130 resulted in higher PSRI values across all cultivars, indicating accelerated senescence compared to N25, particularly during the late maturity stages.

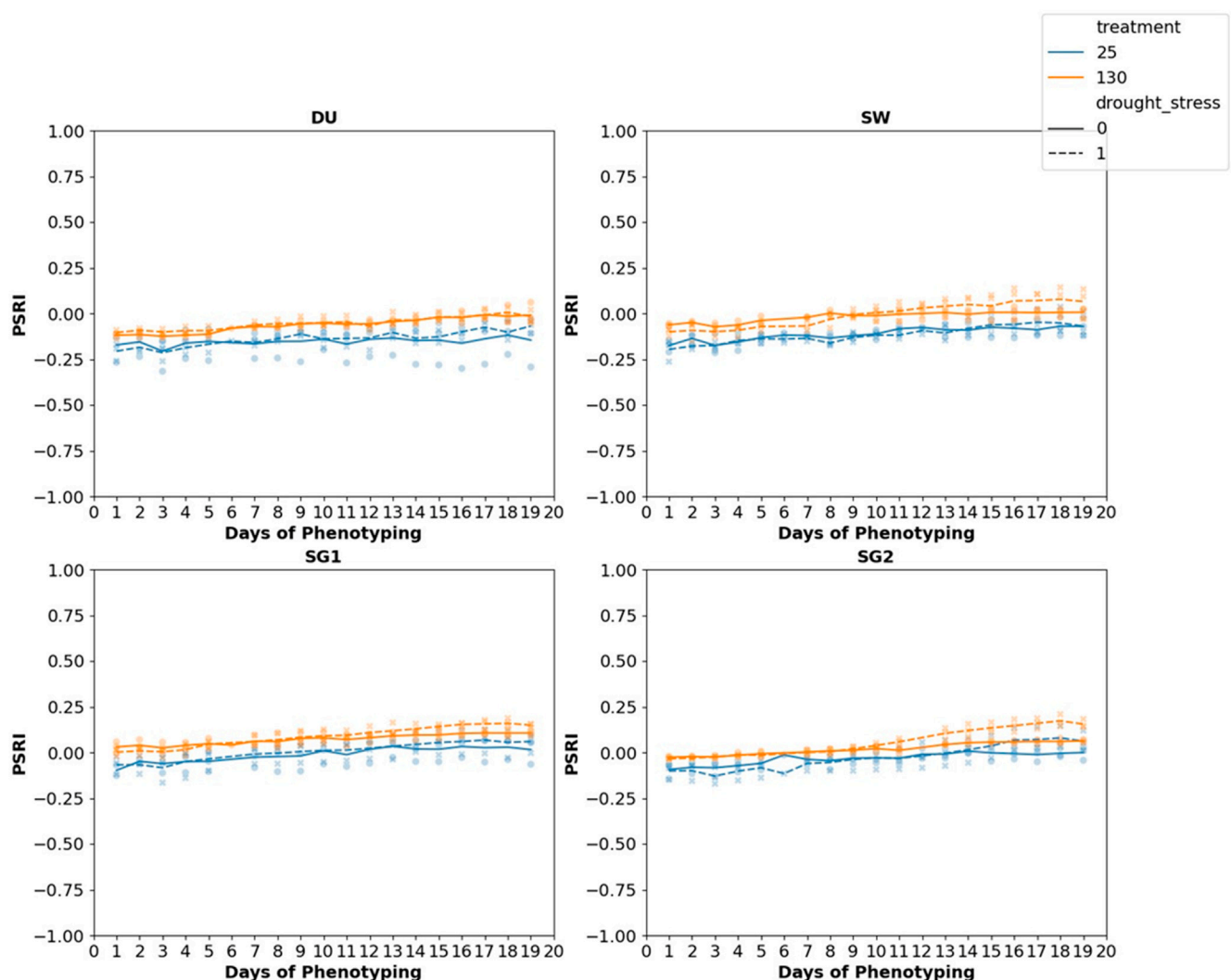


Figure 8. Comparison of PSRI (Plant Senescence Reflectance Index) for different crops following nitrogen and drought stress treatments measured over the phenotyping period using VNIR. Values represent the average of three biological replicates per treatment. Each pot replicate consists of 10 plant seedlings. According to the linear mixed model, SG1, SG2 and DU were significant in treatment ($p = 0.005$, $p = 0.004$ and $p = 0.014$, respectively). SW was significant in both treatment

($p = 0.000$) and drought stress ($p = 0.006$). All cultivars were significant in days of phenotyping ($p = 0.000$). Lines represent treatment means, and lighter overlaid points represent individual pot replicate measurements. Light blue indicates the N25 treatment (25 kg N ha^{-1}), and orange indicates the N130 treatment (130 kg N ha^{-1}). Solid lines represent well-watered controls, whereas dashed lines represent drought stress.

3.9. Crop Temperature and Transpiration

Tplant (temperature of crop plant) values show potential accumulation of heat or reduced transpiration during the phenotyping period [9,39]. The increase was most pronounced in SG2, suggesting higher sensitivity to heat accumulation, according to the formula that included an interaction term between treatment and drought stress [11]. Nitrogen treatment had a modest effect on crop temperature across most conditions for SG1 and SW, where high nitrogen levels resulted in increased Tplant values. This suggests that excess nitrogen could contribute to higher transpiration demands and reduced cooling capacity (estimate = 0.024 and estimate = 0.031, respectively). However, higher leaf T can also be due to decreased transpiration rather than higher transpiration demand. Moreover, other plant factors, such as leaf angle (light interception) and leaf reflectance, might have also contributed to higher leaf T. Transpiration was not measured directly; the values above are inferred from canopy temperature (Tplant), an indirect proxy (see Section 2.2).

3.10. Water Use Efficiency (WUE)

Water use efficiency (WUE) is a critical physiological trait that reflects the efficiency of water utilization in producing biomass [9]. Enhanced WUE values suggest better adaptation to drought and potentially enhanced grain yield [39]. WUE, calculated as the ratio of digital biomass pixels {px (1×10^{10})} produced per gram of water utilized, is used here as a relative indicator of water productivity under drought conditions. The analysis revealed significant differences in WUE under drought stress for all crops except SW, as determined by Tukey's HSD pairwise test, indicating that the efficiency in converting water into biomass was lower under drought. In combination with the nitrogen treatment, all cultivars, except SG1, showed a significant effect, indicating that the WUE was negatively impacted when both stressors were applied simultaneously. Nitrogen treatment had a highly significant effect on WUE, with higher nitrogen levels (N130) leading to lower water use efficiency (Figure 9). This WUE is a relative, image-based proxy (digital biomass per unit water), not a gravimetric dry-matter or leaf-level (intrinsic) WUE; it is sensitive to canopy architecture and projected-area saturation, and the water term reflects whole-pot water loss (transpiration plus soil evaporation). Absolute values should therefore not be compared directly with conventional field WUE.

For SW, the application of nitrogen had a notable impact on growth and development, leading to a measurable increase in WUE, as indicated by the significant results from the ANOVA and Tukey post hoc pairwise test, but this increase was not reflected by biomass measurement under drought conditions; SW exhibited higher chlorophyll absorption in the visible region and higher reflectance in the NIR region, suggesting better adaptation to drought stress and potentially higher WUE. Similarly, SG1 and SG2 cultivars showed increased chlorophyll absorption in the green region and lower reflectance in the NIR region under drought, indicating better drought efficiency and potentially lower WUE. In contrast, while SW also showed increased chlorophyll absorption in the green region under drought, it did not exhibit the same level of NIR reflectance as DU, suggesting a moderate WUE under drought conditions.

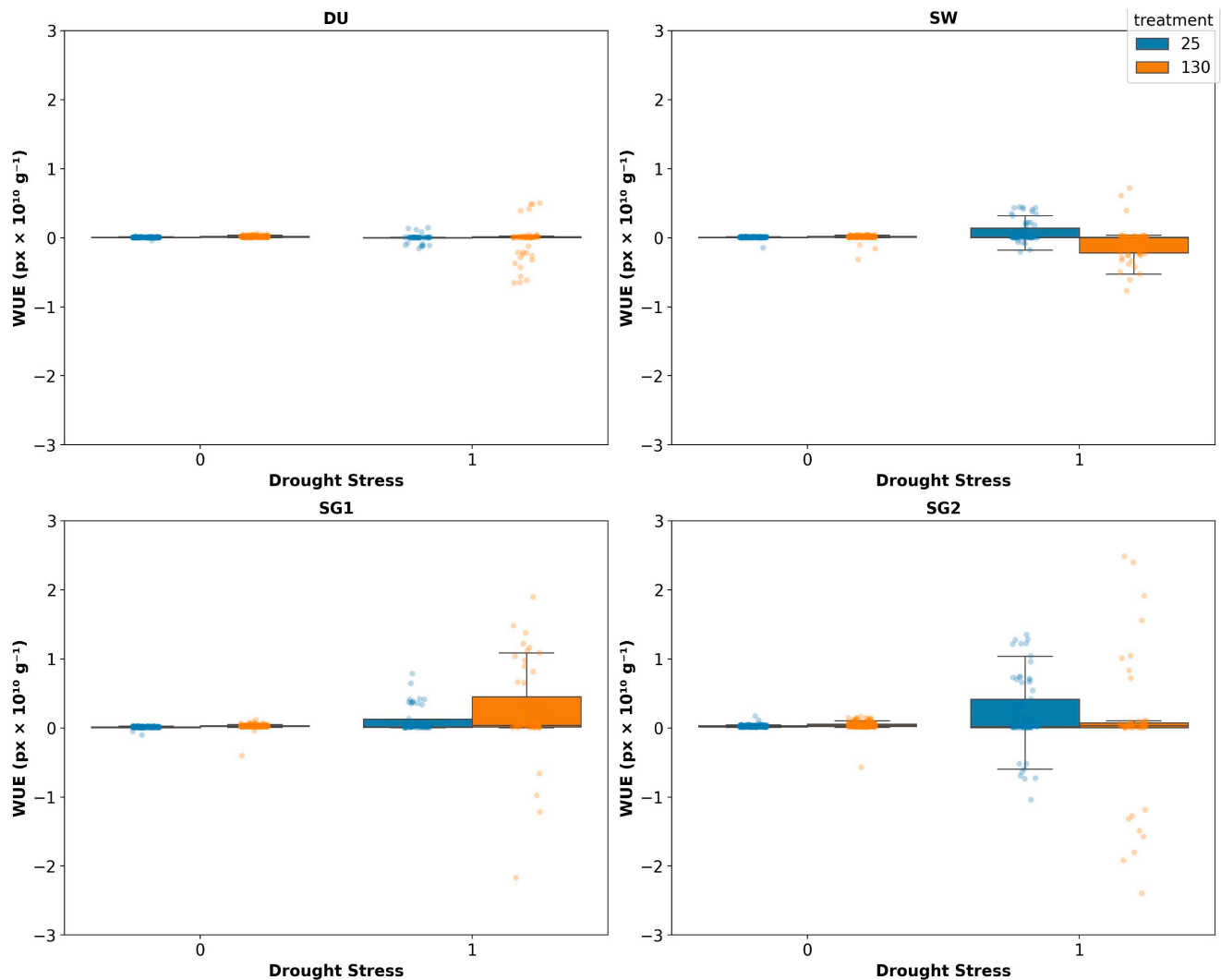


Figure 9. Box plot of water use efficiency (WUE) calculated as the ratio of digital biomass pixels produced per gram of water utilized (px (1×10^{10})/g) for different crops under nitrogen and drought treatments from the late vegetative to maturity stage. The boxes show the WUE distribution, and the overlaid points represent individual pot replicate values (px $\times 1 \times 10^{10}$ per g). Each pot replicate consisted of 10 plant seedlings. The data was analyzed by two-way ANOVA with a Tukey's post hoc test. The following were significant—DU: 25N, drought_stress 0 vs. 130N, drought_stress 1; 25N drought_stress 1 vs. 130N, drought_stress 1; and 130N, drought_stress 0 vs. 130N, drought_stress 1. For SW: 25N, drought_stress 0 vs. 25N, drought_stress 1; 25N, drought_stress 0 vs. 130N, drought_stress 1; 25N, drought_stress 1 vs. 130N, drought_stress 0; 25N, drought_stress 1 vs. 130N, drought_stress 1; and 130N, drought_stress 0 vs. 130N, drought_stress 1. For SG2: 25N, drought_stress 0 vs. 25N, drought_stress 1; 25N, drought_stress 1 vs. 130N, drought_stress 0; and 25N, drought_stress 1 vs. 130N, drought_stress 1.

3.11. Principal Component Analysis (PCA)

Principal component analysis (PCA) helps visualize the relationships between different physiological traits and treatments. PCA plots of SG1, SG2, SW and DU visualize the relationships between different vegetation indices in a reduced dimensionality space (Figure 10). When the points are colored according to their treatment, we can see that the two nitrogen treatments cluster together nicely. The black arrows represent the vectors of different variables. Their direction and length show how strongly each variable contributes to the principal components. N130 treatment in SW, SG1 and SG2 is highly correlated in

PC1, with the exception of DU and SG1 that moved towards PC2. WCI showed a shift towards PC1, while PRI showed more shift towards PC2.

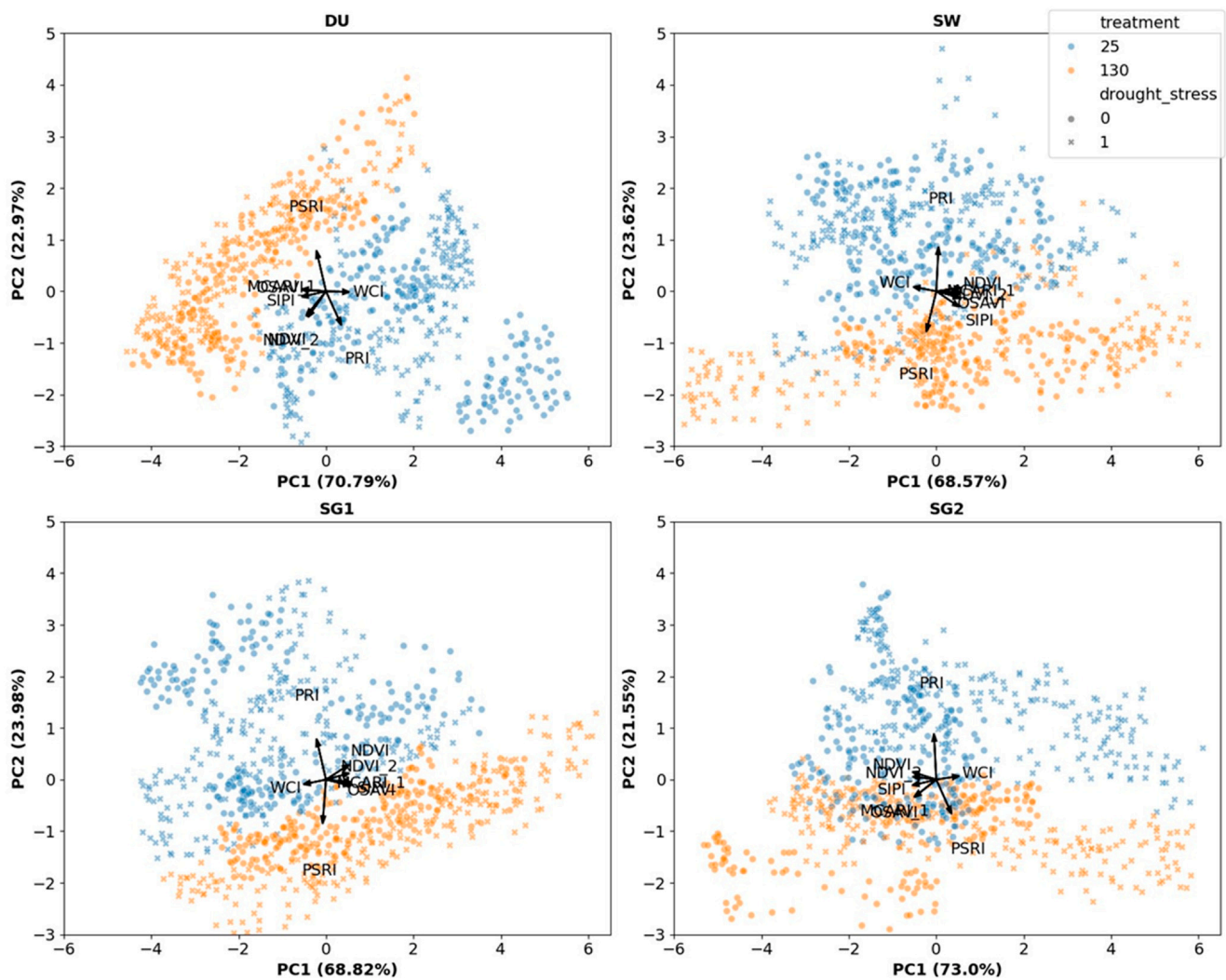


Figure 10. Principal component analysis (PCA) for vegetation indices in different crops following nitrogen and drought stress treatments measured over the phenotyping period using VNIR. Values represent the average of three biological replicates per treatment. Each pot replicate consists of 10 plant seedlings. Abbreviations of parameters are NDVI—Normalized Difference Vegetation Index; PRI—Photochemical Reflectance Index; OSAVI—Optimized Soil Adjusted Vegetation Index; PSRI—Plant Senescence Reflectance Index; SIPI—Structure Insensitive Pigment Index; MCARI_1—Modified Chlorophyll Absorption in Reflectance Index and WCI—Water Content Index.

4. Discussion

The results from the high-throughput phenotyping study reveal significant insights into the responses of wheat and barley cultivars to nitrogen and drought stress [14]. The application of VNIR-SWIR hyperspectral imaging and various vegetation indices, such as NDVI, PSRI, and WCI, enabled the identification of key physiological traits (like growth patterns and senescence) and biochemical traits (such as photosynthetic pigments and WUE) in plants under multivariable climatic conditions. It helps in understanding how plants adapt and respond to different environmental factors. By analyzing the interplay between biomass, WUE, spectral indices, and temperature, several key insights emerge [40,41]. In this study, nitrogen-related differences in WCI, vegetation indices, Tplant, and WUE suggest cultivar-specific changes in plant water status, senescence, and stress responses

under drought. The observed cultivar-specific spectral and thermal responses are interpreted as indicators of plant stress status and performance under combined nitrogen and drought treatments.

4.1. Trade-Offs and Cultivar-Specific Responses

There is a trade-off between yield and stress tolerance. SG2, while producing the highest biomass under optimal conditions, is highly sensitive to drought, as indicated by its declining NDVI, rising temperature, and reduced WUE. DU, on the other hand, shows steady WUE and lower biomass, making it more suitable for environments with limited water availability. DU and SW exhibit physiological traits (lower temperature, stable WUE, and slower spectral declines) that make them resilient to drought. SG1 and SG2 require precise water and nutrient management to avoid stress-induced yield loss.

4.2. Nitrogen Effects and Senescence Dynamics

Enhanced nitrogen levels (N130) did not consistently improve WUE. Nitrogen treatment had a highly significant effect on WUE ($p < 0.001$), with N130 leading to lower water use efficiency in SW, and had no significant effect on SG1, SG2 and DU cultivars. The PCA analysis further highlighted the strong correlation between nitrogen treatments and vegetation indices, with N130 treatments clustering together and showing enhanced PSRI and Tplant compared to N25 treatments. Additionally, the mixed-effects model and ANOVA with Tukey's post hoc tests confirmed the significant impact of nitrogen and drought stress on relative growth rates and WUE, underscoring the importance of optimizing nitrogen application to improve crop yield and productivity amidst climate change.

4.3. Spectral Physiology Under Stress

Under drought conditions, wheat cultivar DU exhibited enhanced chlorophyll absorption in the visible region and greater reflectance in the near-infrared (NIR) region compared to SW and the barley cultivars. This indicates that DU may have a better adaptation mechanism to drought stress. Barley cultivars SG1 and SG2, along with wheat cultivar SW, showed increased chlorophyll absorption in the green region under drought conditions. However, SG1 exhibited lower reflectance in the NIR region, suggesting better drought efficiency (Figure 2). Water stress often results in increased reflectance in the visible region and decreased reflectance in the NIR region (700 to 1300 nm) due to changes in leaf structure and water content.

4.4. Water Status and Thermal Regulation

The water content index (WCI) increased in barley cultivars under drought conditions at both N25 and N130. SW under drought showed a significant increase in WCI at N130 during maturity (Figure 3). DU and SG2 showed no significant nitrogen effect, suggesting their ability to maintain thermal regulation regardless of nutrient levels (Figure S1). According to the linear mixed model for WCI, DU was significant in treatment ($p = 0.004$), SG2 was significant in drought stress ($p = 0.035$), and all cultivars were significant in days of phenotyping ($p = 0.000$). DU and SW are more drought-resistant and showed minimal temperature changes, indicating more efficient water use and transpiration control mechanisms. Under drought, high nitrogen (N130) increased plant temperature (Tplant) for barley (SG1) and wheat (SW) compared to N25, thereby accelerating early maturation. Consistent with this, drought changed the within-day spectral dynamics in a genotype-specific manner (Figure 11; genotype $\times$ drought interaction $p < 0.0001$): durum suppressed its diurnal spectral swing under drought, consistent with stomatal closure and conservative water use, whereas the barley cultivars amplified it, with the same contrast appearing in the SWIR water-absorption bands and thus indicating a leaf-water-content basis.

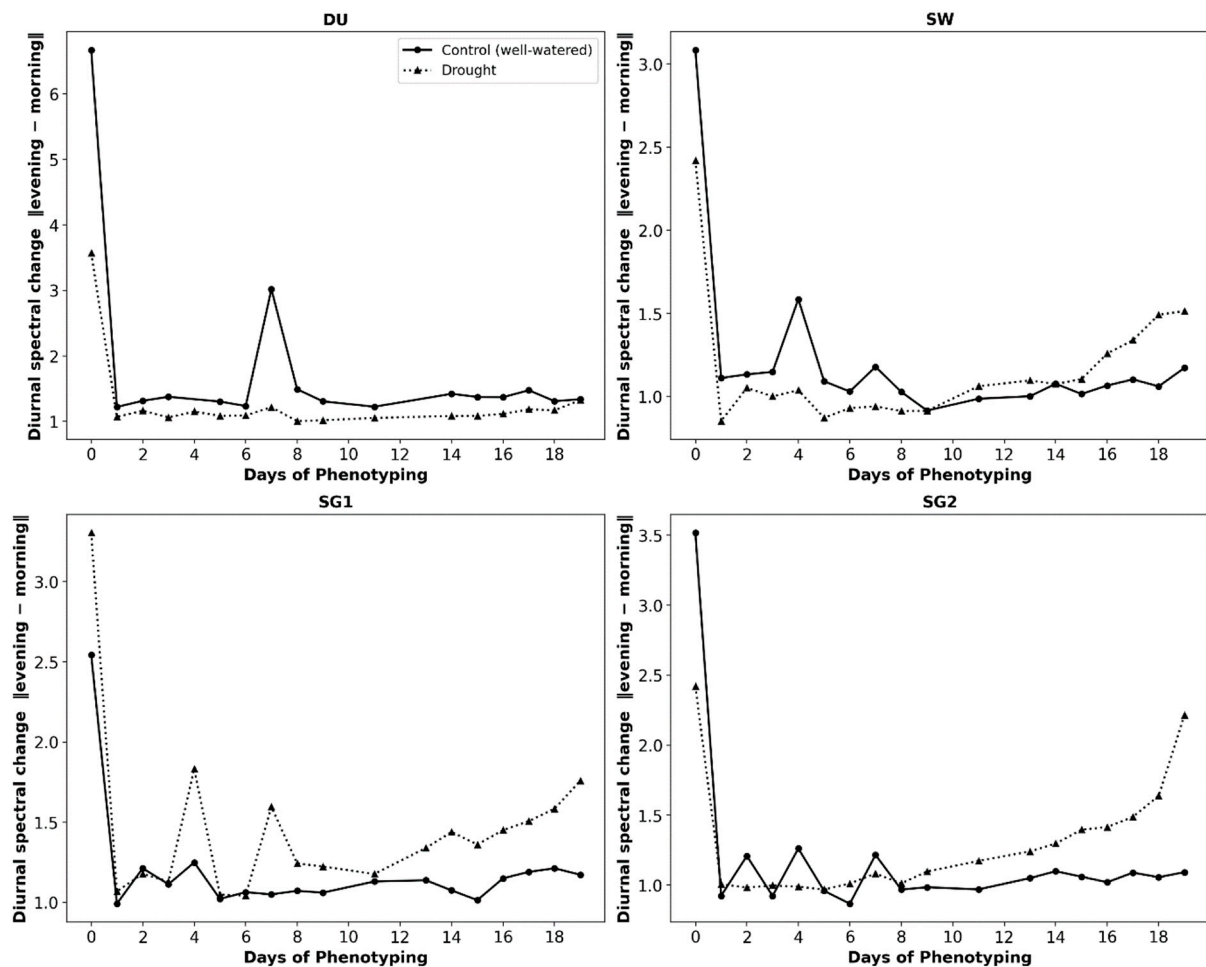


Figure 11. Diurnal spectral change ($\| \text{evening} - \text{morning} \|$) of the normalized VNIR–SWIR reflectance) over the phenotyping period for each genotype (DU, SW, SG1, SG2); solid line = well-watered control, dotted line = drought. Drought altered the diurnal spectral swing in a genotype-dependent manner (genotype $\times$ drought interaction $\chi^2 = 21.6$, $p < 0.0001$; linear mixed model with day as a random block): durum (DU) suppressed its daily swing under drought, whereas the barley cultivars (SG1, SG2) amplified it. The same contrast appears in the SWIR water-absorption bands (970–1450 nm), indicating a leaf-water-content basis.

4.5. Practical Implications

DU exhibited superior chlorophyll absorption and reflectance, indicating better drought adaptation compared to other species tested. Enhanced nitrogen levels (N130) reduced water use efficiency (WUE) in SW and SG2 compared to N25, implying greater water use without consistent efficiency gains.

The analysis revealed significant differences in WUE under drought stress for all crops except SW, and nitrogen treatment had a highly significant effect, with higher nitrogen (N130) leading to lower WUE (Figure 9). SG2 was particularly sensitive to drought, showing declines in all vegetation indices except WCI, highlighting the need for precise water and nitrogen management to avoid stress-induced yield loss.

5. Conclusions

The use of high-throughput VNIR–SWIR hyperspectral imaging has been pivotal in identifying key trait signatures in drought-tolerant wheat and barley cultivars. This technology enables the characterization of physiological and biochemical traits related to growth, maturation and senescence during critical growth periods under multivariable climatic

conditions. The application of vegetation indices derived from spectral data has proven effective in pinpointing specific traits crucial for crop performance. These indices help in understanding how plants manage nitrogen and water under varying conditions, thereby aiding in the development of resilient agricultural practices. By controlling senescence and optimizing crop performance, the findings can inform breeding programs dedicated to enhancing productivity amidst climate change. The study found that enhanced nitrogen supply (N130) increases plant temperature, fastens the growth cycle and leads to early maturation. Even though N130 treatment accelerates senescence, it reduces crop yield loss by significantly impacting crop development under stress conditions. These findings are crucial for understanding how crops can sustain performance under fluctuating environmental conditions. The study underscores the importance of integrating advanced imaging technologies and vegetation indices in crop management strategies to enhance resilience and productivity in the face of climate change.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriengineering8080329/s1>, Figure S1: Crop plant temperature under nitrogen and drought treatments; Figure S2: Digital biomass dynamics during phenotyping; Figure S3: Relative growth rates under nitrogen and water treatments; Figure S4: Linear mixed-model residual diagnostics; Figure S5: Water-use efficiency under nitrogen and drought treatments; Table S1: Randomised experimental design layout; Table S2: Per-trait linear mixed-model selection and fit.

Author Contributions: Conceptualization, K.P.; methodology, K.P., V.P. and P.R.; investigation, P.R.; data curation, formal analysis, K.P., V.P. and H.-P.K.; K.P., writing—original draft preparation, K.P.; writing—review and editing, K.P. and H.-P.K.; visualization, K.P.; Funding Acquisition, H.-P.K. and P.R.; project administration, H.-P.K. All authors have read and agreed to the published version of the manuscript.

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