

1 Multi temporal multispectral UAV remote sensing allows for yield 2 assessment across European wheat varieties already in tillering 3 stage

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10 **Abstract**

11 High throughput field phenotyping techniques employing multispectral cameras allow to extract a
12 variety of variables and features to predict yield and yield related traits, but little is known about which
13 types of multispectral features may allow to forecast yield potential in the early growth phase. In this
14 study, we hypothesized that the best features for predicting yield in an early stage might be different
15 from the best predictors for the late growth stages. Based on a variety testing trial of 19 European
16 wheat varieties in 2021, multispectral images were taken on 19 dates ranging from tillering to harvest
17 by an unmanned aerial vehicle measuring reflectance in five bands, including visible bands, Red-edge
18 and the near-infrared (NIR). Orthomosaic images were created, and then the single band reflectances,
19 vegetation indices (VI) and texture features (TF) based on a gray level correlation matrix (GLCM)
20 were extracted. We evaluated the performance of these three types of features for yield prediction and
21 classification at different growth stages by, i) using features on each of the measurement dates, ii)
22 smoothing features across the 19 dates, and iii) combining features across the directly adjacent dates,
23 in combination with the random forest models. Our results showed that, for most features,
24 measurements at the flowering stage showed the best performance and the Red reflectance was able to
25 predict yield with a RMSE of 47.4 g m⁻² ($R^2 = 0.63$), the best VI was NDRE predicting yield with a
26 RMSE of 47.9 g m⁻² ($R^2 = 0.63$), the best TF was contrast predicting yield with a RMSE of 57.2 g m⁻²
27 ($R^2 = 0.46$) at the booting stage. Combining dates improved yield prediction in all dates and made the
28 prediction errors more stable across dates. Rather than the Red-edge band, visible bands especially the
29 Red band enabled to distinguish between the high- and low-yielding varieties already in the tillering
30 stage, with a total accuracy of 76.7%. The study confirms our hypothesis and further implies that, in
31 the early stages, the visible bands may be more effective than Red-edge bands in assessing the yield
32 potential in a range of testing varieties.

33

34 **Keywords:** wheat variety testing, yield prediction, UAV remote sensing, image texture features,
35 machine learning, phenology

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39 **1 Introduction**

40 Improving crop yields in the face of climate change is a significant challenge for plant breeding (Ray
41 et al., 2013). To achieve efficient phenotyping for breeding, it is essential to quickly and accurately
42 identify high-yielding genotypes from a large pool of genotypes (Araus & Cairns, 2014). While
43 traditional phenotyping for variety selection is highly dependent on breeder's eyes and experiences,
44 high-throughput phenotyping (HTP) emerged in recent years as a more standardizable approach of
45 characterizing plant structure and function and assessing their interactions with the environment by
46 employing various technologies such as imaging, remote sensing, and artificial intelligence (Hund et
47 al., 2019; Watt et al., 2020). HTP-generated high-dimensional phenotypic data embrace the spectral
48 (frequency), spatial, and temporal domains, leading to challenges of analyzing the high dimensional
49 data before they can aid in identifying the genotypes (Bowman et al., 2015; Prey et al., 2022). In
50 particular, field-based HTP including the phenotypic data handling and analysis remain the most
51 significant challenge and requires further development to be fully effective (Kirchgessner et al., 2017;
52 Walter et al., 2015).

53 Field-based HTP techniques are expected to be fast, cost-effective, and non-destructive (Cabrera-
54 Bosquet et al., 2012). Unmanned aerial systems (UAS-) based remote sensing techniques are
55 increasingly used for HTP of plant traits and yield. Among these sensing techniques, canopy spectral
56 reflectance is highly promising and has been successfully utilized to estimate a diverse range of traits
57 in wheat such as leaf area index (Bukowiecki et al., 2020; Zhang et al., 2021), biomass (Yue et al.,
58 2019), leaf nitrogen and chlorophyll in wheat (Pan et al., 2023) to more general traits such as grain
59 yield and quality (Duan et al., 2017; Prey et al., 2020; Vatter et al., 2022). There are several sensors
60 available for measuring yield under field conditions, such as RGB cameras (Fernandez-Gallego et al.,
61 2019), as well as thermal sensors (Elsayed et al., 2017) and active sensors such as LiDAR (Li et al.,
62 2022). The use of multispectral cameras mounted on unmanned aerial systems (UAS) has proven to be
63 a practical, easy-to-use, and cost-effective approach (Araus et al., 2022).

64 Multispectral images allow for extracting a variety of features that can be used to predict yield in
65 wheat. Generally, these features can be grouped three categories. Firstly, single band reflectance in
66 specific wavelengths can be directly extracted from multispectral data. Secondly, the reflectance of
67 single bands can be combined to calculate vegetation indices (VIs), which are often more sensitive to
68 specific traits and less affected by environmental conditions during measurement (Tucker, 1979).
69 However, both single band reflectance and VIs may suffer from saturation, particularly for closed
70 canopies (Rischbeck et al., 2016). Thirdly, texture features (TFs) can be extracted to describe the
71 distribution of pixels within a region of interest (ROI). Although TFs can be extracted from any
72 reflectance and VI raster, they often perform less effectively than single band reflectance or VIs in
73 predicting yield in wheat (J. Li et al., 2019). Also, TFs are frequently used in combination with VIs to
74 predict plant traits, e.g., leaf area and biomass in wheat (Zhang et al., 2021). While canopy height
75 often follows a clear temporal dynamics, multispectral features show different dynamics, depending
76 on their sensitivity to a given trait or canopy properties. Also, research indicates that TFs depend
77 heavily on the phenological stage and are therefore their dynamics might interesting to be studied
78 (Culbert et al., 2009). Yet, only limited research focuses on the dynamics of different features in
79 phenotyping, especially the TFs. Among the TF algorithms, one of the mostly used is based on the
80 grey level co-occurrence matrices (GLCMs). In order to calculate GLCMs different parameters such
81 as the level of quantization, the size of the moving window, the moving distance and direction
82 (Haralick et al., 1973). They can be calculated on all available images or rasters of individual bands
83 and VIs. Further, TFs are highly dependent on the GSD and the size of the observed object; and
84 therefore on the camera and the flight height. Zhang et al. (2021) found the best performing TFs for
85 yield prediction were based on the RED as well as the NIR bands. In contrast, Zheng et al. (2019)
86 found that GLCM-based TFs were poorly correlated to above ground biomass (AGB) in rice and
87 calculated normalized difference texture indices that showed a higher correlation with AGB. Further,
88 the phenology has a big influence on the relationship between AGB and the normalized difference

89 texture features (S. Li et al., 2019). Despite that combining feature (e.g., TFs, VIs) and models often
90 improve the prediction, the more reliable performance of yield prediction has been reported in only a
91 few growth stages from the booting to early grain filling (Bowman et al., 2015; Vatter et al., 2022).

92 Accurately predicting yield using canopy multi-/hyperspectral reflectance further requires careful
93 consideration of the phenological stage of the crop. The anthesis stage or the grain filling stage are
94 often identified as the most suitable stages for yield prediction in wheat (Bowman et al., 2015; Duan et
95 al., 2017; Hassan et al., 2019). In contrast, canopy spectral-based yield prediction has been often
96 reported with a lower accuracy (Prey et al., 2022). Multispectral cameras mounted on UAS enable
97 breeders and researchers to assess the aforementioned spectral and texture features at a high temporal
98 frequency and precision. Within a proper time-window, using a time series for yield prediction allows
99 for the extraction of dynamic canopy traits that could potentially be useful for yield prediction. For
100 instance, Pinter et al. (1981) suggested summing measurement dates after heading to improve yield
101 prediction in wheat and barley. Raun et al. (2001) suggested to take two spectral measurements after
102 dormancy. Time series are further often used to extract information for canopy height, and Taniguchi
103 et al. (2022) used a time-series canopy height model to predict several yield-related traits after the
104 heading stage. During the stem elongation stage in wheat, Kronenberg et al. (2020) used laser
105 scanning to capture the time series of plant height development and identified quantitative trait loci
106 that accounted for the variability in height dynamic.

107 Collectively, despite these successes, little is known about which types of multispectral features may
108 allow us to forecast yield potential in the early growth phase. Therefore, this study aims (1) to identify
109 the best performing multispectral traits for yield prediction and classification in wheat (2) to research,
110 if yield types can be classified in relatively early stages and finally (3) to investigate, how traits
111 measured at different time points can be combined to predict yield more accurately.

112 2 Methods

113 2.1 Study site and Environmental monitoring

114 A trial consisting of 19 diverse European winter wheat elite varieties (*Triticum aestivum*). was grown
115 in plots with a size of 10 m x 1.85 m. The plots were placed in a randomized complete block design
116 with four replicates, resulting in 76 plots totally. The trial took place at the research station of the
117 Technical University of Munich in Dürnast, Freising (48.40630° N, 11.69535° E). The soil at this
118 location can be characterized by a homogeneous Cambisol with 20.8 % clay, 61.5 % silt and 16.6 %
119 sand. All plots were fertilized by applying 180 kg N ha⁻¹ in three equal splits at BBCH 25, 32 and 65.
120 Plant protection was carried out according to local practice. Sowing took place on the 10.11.2020 and
121 all plots were harvested at full maturity on the 03.08.2021. Precipitation during this period was 1020
122 mm, the average temperature was 8.2 °C. Climate data was collected from a weather station (Station id
123 5404) and operated by the Climate Data Center of the German Weather Service. The temperature was
124 aggregated to phenologically meaningful growing degree-days (GDD):

$$Tmean_d = \frac{\sum \frac{maxT_{d,h} + minT_{d,h}}{2} - baseT}{24}$$
$$GDD = \sum_{d=1}^n Tmean_d$$

125 where $Tmean_d$ is the mean temperature for day d after sowing, $maxT_{d,h}$ and $minT_{d,h}$ are hourly
126 maximum and minimum temperatures for day d and $baseT$ is the base temperature, which was set to 0
127 °C.

128 2.2 Grain yield, Phenology assessment and Leaf area index measurements

129 The entire plots were harvested using a combined harvester. The water content of the grains was
130 determined by weighing the grains after harvest, drying them at 65 °C until constant weight was
131 reached and weighing them again. The final yield was normalized to a moisture content of 14 %. The
132 three varieties with the lowest average yield were classified as low yielding and the three varieties
133 with the highest average yield as high yielding. The phenology of each plot was visually rated using
134 the BBCH scale (Meier et al., 2009) on a plot level. Leaf area index (LAI) was measured using a Licor
135 2000 leaf area meter (LI-COR Biosciences Lincoln, U.S.A.) with a 45° view cap to minimize operator
136 influence. Three measurements were taken at the top of the canopy and four measurements were taken
137 under the canopy at three different locations per plot, which were then averaged.

138 2.3 Multispectral image acquisition and processing

139 Spectral measurements were acquired using a Phantom 4 Multispectral RTK (DJI, Shenzhen, China).
140 The UAV captures reflectance in wavelengths of 450, 560, 668, 717 and 840 nm and measures the
141 incoming sunlight by a sensor on top of the UAV. Flight height was set to 10 m AGL resulting in a
142 ground sampling distance of 0.7 cm. Overlap in both directions was set to 90 %, the UAV stopped for
143 each image acquisition. Before and after each flight, images of a panel with a known reflectance were
144 taken. Flights were carried out twice per week during heading and flowering stages and once per week
145 at other stages. First flight was carried out on the 25.03.2021 and the last flight on the 20.07.2021,
146 which resulted in 19 flights totally. Images were taken around the solar noon and under sunny
147 conditions, if possible.

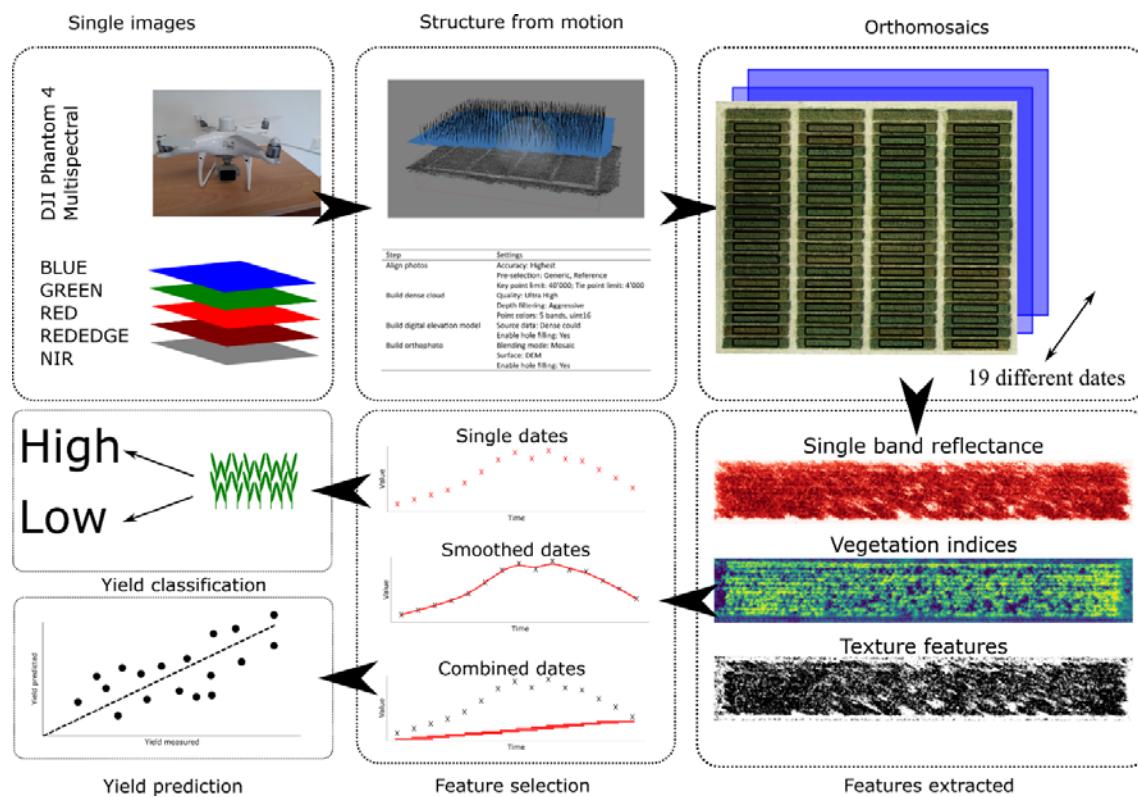


Figure 1: Workflow applied.

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149 The images from each flight were mosaicked using the Agisoft Metashape Professional 1.8.4 (Agisoft,
150 St. Petersburg, Russia) structure-from-motion software. The images were radiometrically calibrated
151 using the reflectance panels on the ground and the incident light sensor on the UAV, with a uniform
152 set of processing parameters used for all flight dates (Figure 1). The point cloud was georeferenced
153 using the real-time kinetic global positioning system (RTK-GPS) integrated into the UAS, with the
154 RTK correction signal provided by SAPOS (Deutsche Landesvermessung). Reflectance of individual

155 bands was extracted by calculating the median of a specific region of interest (ROI) representing a plot
156 using a custom Python 3.7 script (Python Software Foundation, <https://www.python.org/>).

157 2.3.1 Selection and calculation of spectral indices

158 To compare our approach across a range of vegetation indices (VIs), we classified them into five main
159 groups based on their calculation method and selected a representative VI for each group. The five
160 groups were differential-type, simple-ratio type, normalized differential type, three-band type, and
161 combination of two spectral indices type (Table 1). We calculated the indices using a custom Python
162 3.7 script (Python Software Foundation, <https://www.python.org/>) and computed the median value for
163 each index over the regions of interest (ROIs) corresponding to the plots.

164 *Table 1: Vegetation indices (VIs) calculated.*

Index type	Index	Formula	Reference
Difference	DVI	$840 - 668$	(Shibayama et al., 1999)
Ratio	RVI	$\frac{840}{668}$	(Shibayama et al., 1999)
Normalized	NDRE	$\frac{840 - 717}{840 + 717}$	(Barnes et al., 2000)
Three Band	MCARI	$((717 - 668) - 0.2 * (717 - 560)) * \left(\frac{717}{668}\right)$	(Daughtry et al., 2000)
Combination of indices	CCII	$\frac{TCARI}{OSAVI}$	
	TCARI	$3 * [(717 - 668) - 0.2 * (717 - 550) * \left(\frac{717}{670}\right)]$	(Haboudane et al., 2002)
	OSAVI	$(1 + 1.16) * \frac{(840 - 668)}{840 + 717 + 0.16}$	

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166 2.3.2 Selection and calculation of texture features

167 To generate a manageable number of TFs, we focused on calculating TFs for the RED reflectance
168 band only as it had the best performance for yield prediction when using single bands. A 5 x 5 kernel
169 size was used to calculate the GLCM features over the entire raster. This small kernel size was chosen
170 because wheat leaf sizes are relatively small compared to our GSD. A quantization level of 32 was
171 used, with the lowest level corresponding to the first percentile of the respective raster and the highest
172 level corresponding to the 99th percentile. This ensured that we could still capture the variation in our
173 image. GLCMs were constructed with a moving distance of 1 pixel and moving directions of 0°, 45°
174 and 90° to eliminate possible effects of direction. The Contrast, Correlation, Dissimilarity, Energy, and
175 Homogeneity features were extracted from each GLCM (Haralick et al., 1973) and saved as the center
176 pixel in a raster. From these rasters, the final value per plot was extracted by averaging all values
177 within the ROI. All calculations were performed using a custom Python 3.7 script (Python Software
178 Foundation, <https://www.python.org/>). The extracted features are listed in (Table 2).

179 Table 2: Calculation of grey correlation matrix features according to Haralick *et al.* (1973).

Texture feature calculated on RED raster	Formula	Explanation
Contrast	$\sum_{i,j=0}^{N-1} P_{ij}(i-j)^2$	Amount of local variation in pixel values
Correlation	$\sum_{i,j=0}^{N-1} P_{ij} \frac{(i-\mu)(j-\mu)}{\sigma^2}$	Linear dependency of grey level values in the GLCM
Dissimilarity	$\sum_{i,j=0}^{N-1} P_{i,j} i-j $	Local roughness of the pixel values
Energy	$\sum_{i,j=0}^{N-1} (P_{ij})^2$	Local steadiness of the gray levels
Homogeneity	$\sum_{i,j=0}^{N-1} \frac{P_{ij}}{1 + (i-j)^2}$	Homogeneity of the pixel values

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181 **2.3.3 Temporal processing of the extracted features**

182 Three temporal feature selection strategies were evaluated (Figure 1). The first strategy involved
183 selecting data from individual dates, resulting in one feature per observation. The second strategy
184 involved smoothing the values per plot using splines, implemented in the statgenHTP package, with
185 the default settings (Millet *et al.*, 2022). Summed GDD from harvest were used as the time axis.
186 Finally, features were selected using a moving time window with a width of 3. For each recorded date,
187 the model included features from the current date and the previous as well as the following date,
188 resulting in a total of three features per observation. This strategy is referred to as the moving window
189 model.

190 **2.4 Yield prediction model and yield potential classification model**

191 To predict yield on a plot level and classify yield performance groups, we employed Random Forest
192 (RF) machine learning models in R 4.2 (R Core Team, 2021). We optimized the number of trees per
193 forest to 500 and used the R package *caret* (Kuhn, 2008). The number of trees per forest was set to
194 500 and the number of features per node was optimized by minimizing the root mean square error for
195 the regression models and the accuracy for the classification models.

196 **2.5 Statistical analysis**

197 Pearson correlation coefficient between yield and spectral features was calculated using measurements
198 taken on the 25.06.2021. At this date, most varieties were in the mid to end flowering and the
199 correlation of VIs and yield was maximal for most VIs. The performances of the regression RF models
200 were assessed by the coefficient of determination (R^2) as well as the root mean square error (RMSE)
201 using a 10-fold cross validation that was repeated 3 times and averaged:

202

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

203

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (x_i - y_i)^2}{n}}$$

204

205 Where x_i and y_i represent the observed and the predicted yield, \bar{x}_i and \bar{y}_i represent the mean of the

206 observed and the predicted yield, respectively. n represents the number of samples. The performances
207 of the classification RF models were assessed by the accuracy of the prediction using a 10-fold cross
208 validation that was repeated 3 times and averaged:

$$Accuracy = \frac{\text{True positive} + \text{True negative}}{\text{Total number of classified objects}}$$

209 **3 Results**

210 **3.1 Yield LAI and phenology**

211 Substantial grain yield (GY) variation was observed between experimental plots (Table 3). The highest
212 yield was observed in the variety *RGT-Reform* (411.4 g m⁻²), the highest yield in the variety *Skyfall*
213 (642.7 g m⁻²). The high yielding varieties showed a significantly higher LAI during the stem
214 elongation, the booting and at the late grain filling stage than the low yielding varieties (Figure 2).
215 Phenology showed only few significant differences between the high and low yield groups, namely at
216 the stem elongation and the flowering stage ($p < 0.05$). Still, it can be observed that the high yielding
217 varieties were generally advanced in their phenology (Figure 2).

218 **3.2 Correlations between grain yield, the UAV based reflectance, vegetative
219 indices and texture features**

220 During booting, most extracted features show a high correlation with each other as well as with yield
221 (Figure 2). The GREEN and RED reflectance showed an equally high correlation with yield ($r = -0.75$)
222 at the booting stage, which was similar for RED at the early grain filling stage but significantly lower
223 for the GREEN reflectance ($r = -0.42$). Among single band reflectance, the REDEdge region
224 expressed the lowest correlation with yield ($r = -0.33$) and was generally low correlated to other
225 features.

226 Among VIs, the NDRE showed the highest correlation with yield during the booting stage ($r = 0.79$),
227 followed by the RVI ($r = 0.73$), the CCII was negatively correlated to yield ($r = -0.67$). The correlations
228 of VIs to yield do not change significantly at the early grain filling stage. TFs showed a moderate
229 correlation with yield, and the DISSIMILARITY and ENERGY were found to be best two,
230 respectively, with r values of -0.69 and 0.65 at the booting stage. Correlation of the TFs to yield
231 changed drastically at the early grain filling stage, when the highest correlated feature
232 CORRELATION showed an R of -0.34. The feature ENERGY was not correlated to yield at this stage
233 anymore. Generally, VIs were the feature type that showed the highest correlation to yield at the
234 booting as well as at the early grain filling stage.

Table 3: Grain yield and phenology of the single varieties. Values represent the mean of the four replicates; the values in brackets represent the standard deviation of the four replicates.

Variety	Grain Yield	Stem Elongation	Booting	Heading	Flowering	Early Grain Filling	Late Grain Filling
Absalon	532,0 (32,8)	635,0 (27,7)	917,5 (37,3)	1046,5 (23,6)	1153,3 (38,7)	1397,0 (59,2)	1653,3 (79,2)
Aurelius	541,9 (56,4)	609,3 (3,5)	963,5 (52,6)	1069,3 (17,6)	1109,0 (0,0)	1368,8 (62,5)	1673,3 (63,9)
Axioma	473,0 (41,8)	607,5 (4,0)	950,3 (31,7)	1042,8 (23,8)	1121,5 (11,2)	1350,0 (51,8)	1636,5 (57,7)
Bernstein	522,3 (33,7)	626,8 (16,6)	981,5 (6,9)	1091,0 (30,2)	1201,0 (70,6)	1434,8 (27,3)	1684,8 (93,6)
Bologna	490,7 (33,3)	625,5 (16,7)	926,8 (61,1)	1038,5 (30,0)	1109,0 (0,0)	1363,0 (69,7)	1651,5 (64,4)
CH-Nara	559,4 (30,2)	635,0 (27,7)	921,3 (16,7)	1042,0 (16,2)	1137,0 (30,9)	1356,3 (47,8)	1601,5 (23,7)
Chevignon	598,5 (63,5)	615,5 (30,6)	911,8 (17,7)	1050,8 (25,4)	1144,8 (21,1)	1358,3 (45,9)	1579,3 (13,0)
Costello	478,6 (38,8)	635,5 (22,3)	959,0 (31,2)	1103,0 (38,0)	1207,8 (67,8)	1411,8 (39,7)	1739,8 (2,5)
Dagmar	617,1 (45,4)	611,0 (0,0)	969,0 (41,6)	1052,0 (22,3)	1114,0 (16,0)	1345,3 (53,3)	1653,5 (74,5)
Elixer	540,4 (76,2)	643,0 (27,7)	953,0 (43,2)	1118,3 (34,7)	1219,5 (87,4)	1444,0 (20,8)	1742,0 (5,8)
Hyvento	576,2 (51,1)	641,3 (21,0)	956,0 (34,8)	1121,8 (6,0)	1181,8 (41,7)	1418,5 (23,7)	1756,0 (119,6)
Julie	544,2 (95,9)	635,0 (27,7)	915,3 (21,8)	1057,3 (15,8)	1142,8 (16,8)	1347,3 (44,6)	1648,5 (65,7)
Julius	443,3 (14,6)	639,0 (17,3)	962,0 (26,9)	1092,0 (40,1)	1201,3 (65,4)	1442,8 (28,5)	1782,0 (62,2)
Montalbano	584,1 (68,8)	612,8 (3,5)	954,0 (29,5)	1104,0 (14,6)	1192,0 (24,0)	1428,0 (18,9)	1720,5 (78,5)
Mv Nador	552,4 (45,0)	643,0 (27,7)	962,3 (62,6)	1084,3 (40,4)	1157,3 (76,5)	1343,8 (47,7)	1624,5 (74,4)
Nogal	504,2 (81,6)	633,3 (29,9)	972,8 (51,7)	1058,8 (27,3)	1084,0 (7,7)	1281,5 (26,4)	1602,0 (16,9)
RGT-Reform	411,4 (60,2)	629,0 (20,8)	941,0 (14,3)	1105,0 (9,9)	1179,0 (48,3)	1426,8 (18,9)	1641,3 (69,7)
Rumor	543,9 (15,5)	623,0 (24,0)	926,8 (22,5)	1045,3 (14,5)	1124,8 (23,2)	1403,8 (10,5)	1635,5 (67,6)
Skyfall	642,7 (49,6)	658,7 (82,6)	909,0 (41,2)	1067,8 (34,2)	1157,5 (32,8)	1424,8 (21,0)	1642,8 (84,7)
All	535,5 (73,8)	628,5 (26,4)	945,1 (39,3)	1073,2 (35,8)	1154,6 (54,3)	1386,6 (56,6)	1666,8 (80,1)

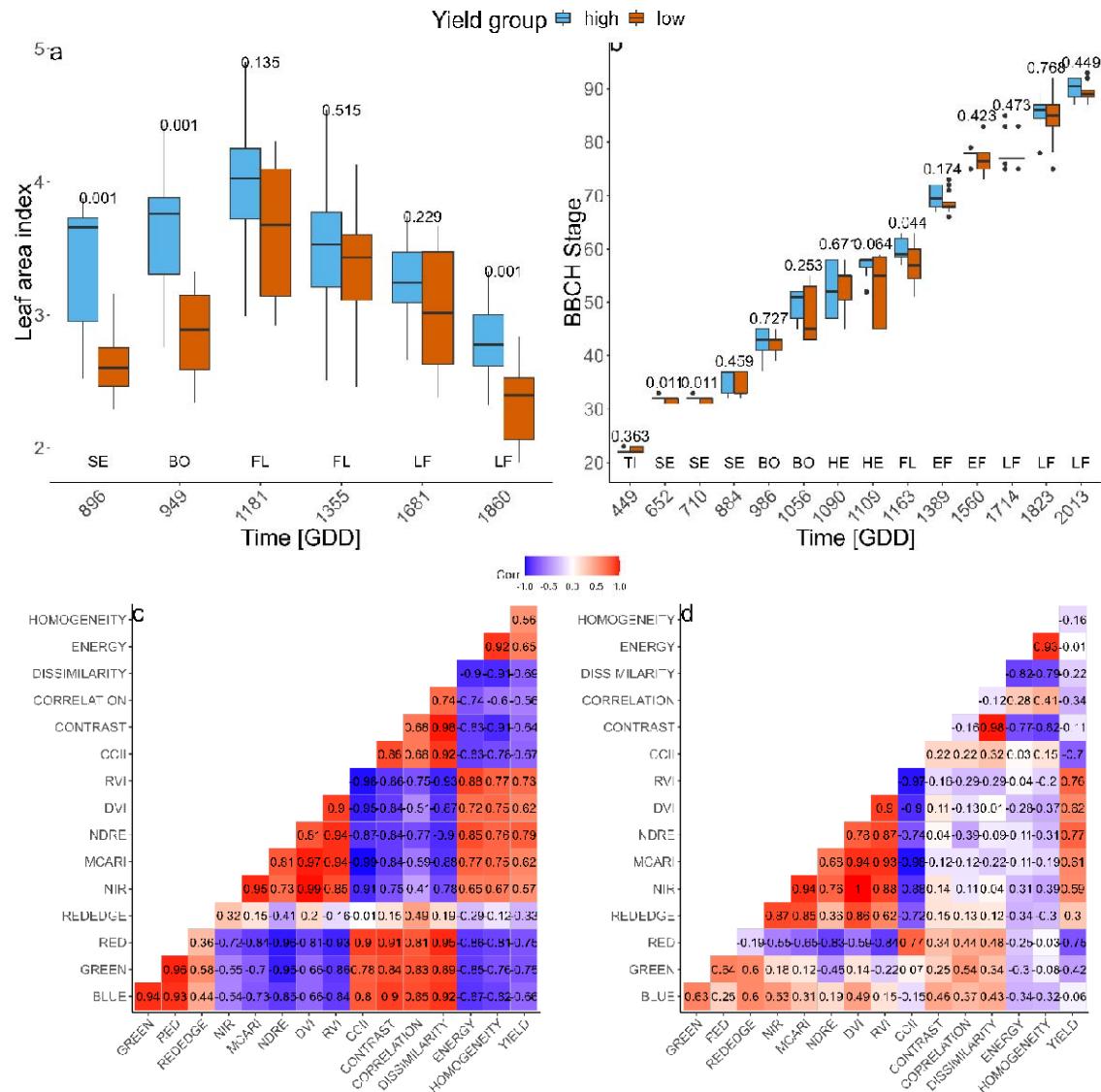


Figure 2: Leaf area index (a) and phenology (b) at different growth stages for the two high and low yield groups. Numbers above the boxplot pairs show the p-value of a t-test. Correlation of yield and indices on a single date at 949 GDD after sowing at the booting stage (c) and 1389 GDD after sowing at the beginning of the early grain filling stage (d). The numbers display the Pearson correlation coefficients.

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3.3 Time series of UAV based reflectance, vegetative indices, texture features

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Reflectance of the BLUE, GREEN and the RED band decreased with plant growth during tillering and stem elongation stages and increase with senescence, yielding a minimal reflectance around booting and flowering stages (Figure 3). The reflectance of the REDEDGE band was characterized by an increase in the first four measurement dates during tillering, a decrease during stem elongation, an increase at flowering and again a decrease with early grain filling stage. The reflectance of the NIR band increases with time to a maximum at late flowering stage and decreases again until full maturity. Significant differences were found for all reflectance bands, except REDEDGE, from the tillering to the early grain filling stage.

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All VIs showed significant differences between yield groups for all dates, except the first two and the last dates. DVI, RVI and NDRE even show significant differences between groups for all dates, except the first one (Figure 3).

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The TFs CONTRAST, CORRELATION and DISSIMILARITY decreased during the stem elongation stage, reached a minimum around flowering stage and increase afterwards (Figure 3). ENERGY and

251 HOMOGENEITY showed an increase until heading stage and a steady decreased from then.
 252 Significant differences in the TFs between yield groups were mainly found from the tillering to the
 253 flowering stages but not later (Figure 3).

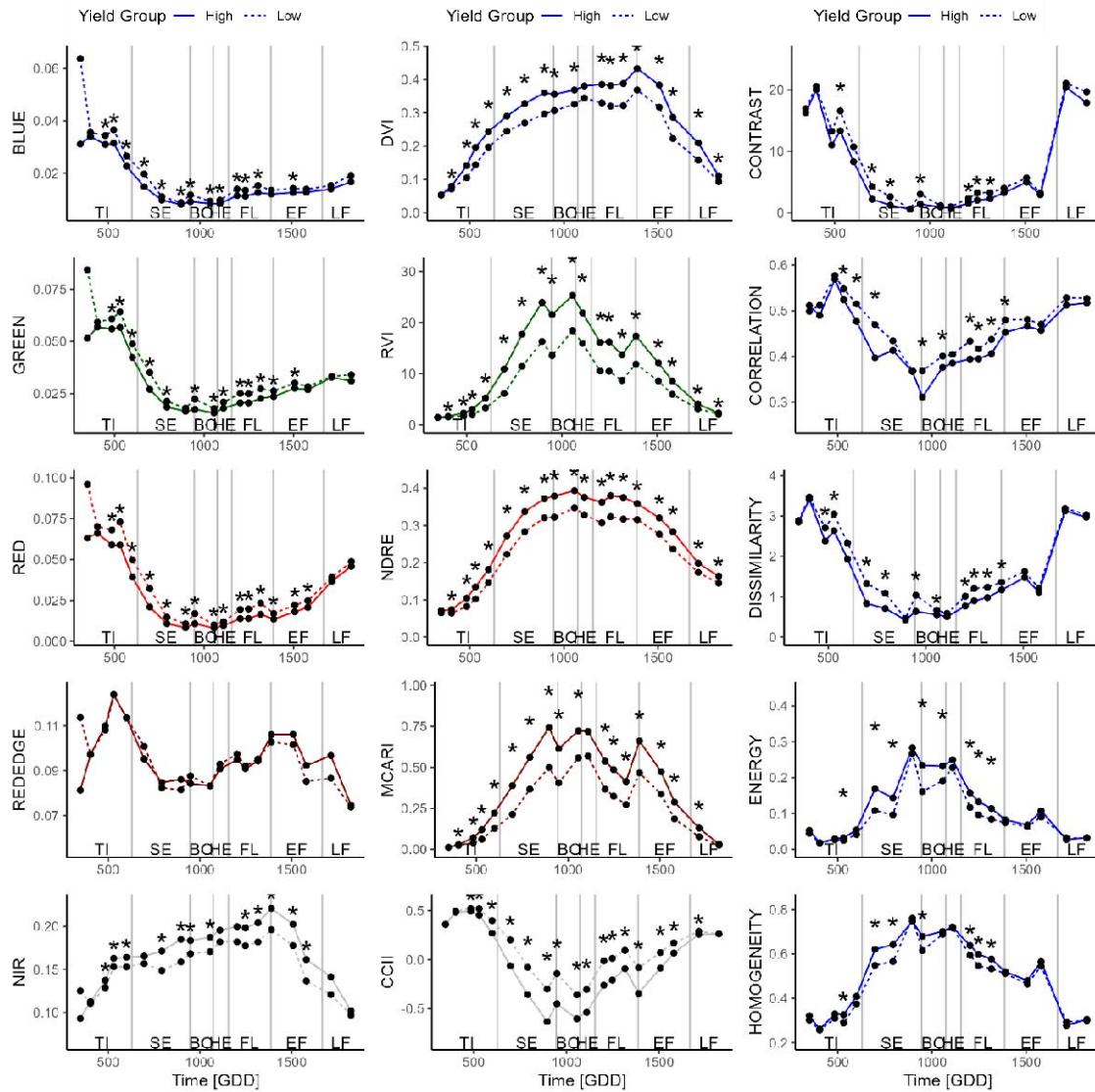


Figure 3: Dynamics of single band reflectances (left), vegetation indices (middle) and texture features (right) for different dates. The solid line shows the high yield group, the dashed line the mean value for the low yield group. The asterisks display significant differences after a t-test ($p < 0.05$) in the respective values and dates between the two yield groups.

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255 3.4 RF regression model for yield prediction using individual flights and time 256 series of UAV traits

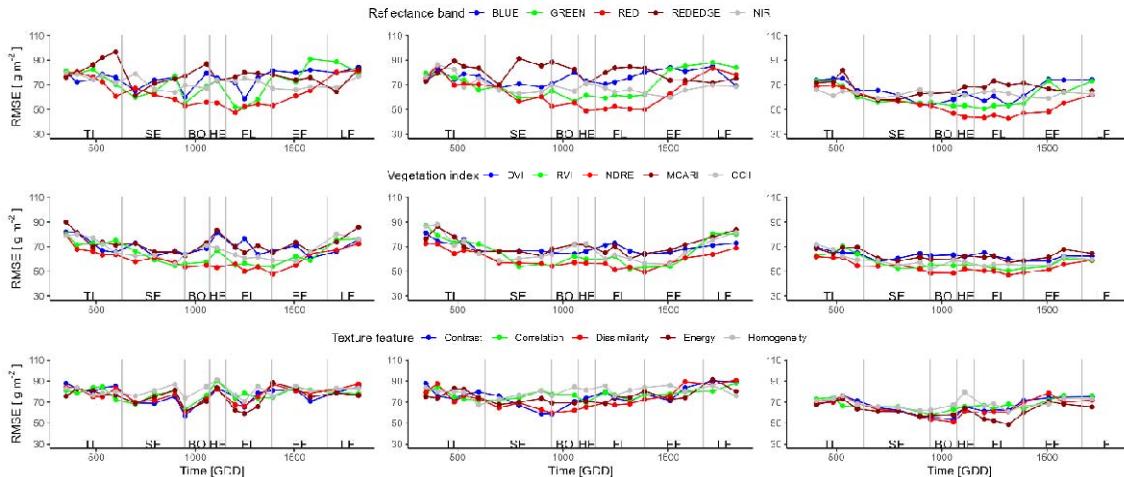
257 Significant effects on yield prediction model performance were found among the features chosen as
 258 well as the time points selected (Figure 4, Table 1).

259 Based on reflectance measurements on a single date, the RED band showed the best performance in
 260 yield, with an RMSE of 47.4 g m^{-2} when taking the measurement at flowering stage, followed by the
 261 NDRE predicting yield with a RMSE of 47.9 g m^{-2} at the early grain filling stage. The performance of
 262 the prediction fluctuates significantly for single dates especially for single bands such as GREEN and
 263 BLUE, much less fluctuation can be observed for VIs. The best TF on a single day is CONTRAST
 264 predicting yield with an RMSE of 57.2 g m^{-2} at the booting stage. The performances of prediction
 265 models using TFs single dates are changing not only with phenology but also show big differences

266 between two adjoining flights. For all TFs are affected in a similar way of single flights with eg. the
267 measurement between the booting and the heading stage showed a better performance in yield
268 prediction compared to the two adjacent dates (Figure 4).

269 Predicting yield from single dates that were previously smoothed on a plot level, was generally worse
270 than predictions from non-smoothed single dates (Table 4). The RED band predicted yield with an
271 RMSE of 49.0 g m⁻² at heading stage. The best performing date for a single band often changed
272 substantially after smoothing. Optimal time point for yield prediction using the NIR band was at stem
273 elongation if the data is not smoothed and at early grain filling if the data is smoothed. Prediction of
274 yield using the NIR band is slightly more accurate when using the smoothed data (RMSE of 59.9 g m⁻²)
275 compared to using the original data (RMSE of 63.9 g m⁻²). The performance or optimal time points
276 of VIs for yield prediction do not change significantly when smoothing the data. Smoothing of TFs
277 improves or worsens the prediction of yield. It improves the prediction slightly for HOMOGENEITY
278 but also worsens the prediction for ENERGY from an RMSE of 59.3 g m⁻² to 67.7 g m⁻². The
279 fluctuations between dates became much less compared to non-smoothed single dates, especially in
280 the highly fluctuating TFs.

281 Based on reflectance measurements across several dates, using each of the individual bands improved
282 the yield prediction. The RED band, which already showed the best performance for single date yields
283 an RMSE of 42.7 g m⁻² when selecting three dates around flowering. The prediction of yield using VIs
284 also improved for all bands but to less extent as for the single reflectance bands. The prediction using
285 NDRE improved slightly to an RMSE of 47.0 g m⁻². TFs were the feature class that improved most
286 when using several time points for yield prediction and all features were better able to prediction yield
287 compared to single dates. A substantial improvement was found in the ENERGY feature that was able
288 to predict yield with an RMSE of 48.7 g m⁻² when using measurements around flowering stage (Table
289 4). Using a time window lead to predictions that were much less fluctuating compared to
290 measurements on single flights (Figure 4). Predictions using the RED band show am underestimation
291 of yields higher than 630 g m⁻² (Figure 6).



292 *Figure 4: Root mean square errors of yield prediction modes built using single band reflectances (top), vegetation
293 indices (middle) and texture features (bottom). The models were built using measurements from single dates (left), smoothing
294 features on single plots (middle) and choosing a window of three adjacent time points (right).*

293 *Table 4: Display of the time points yielding the lowest RMSE for yield prediction for all reflectance bands, vegetation indices*
 294 *and texture features. Values in bold highlight the best model for a given spectral and temporal feature type combination.*

Raster	R ²	RMSE	Date	PS	VI	R ²	RMSE	Date	PS	TF	R ²	RMSE	Date	PS
Single time points														
BLUE	0.45	58.8	1248	FL	DVI	0.38	60.8	1580	EF	CONTRAST	0.46	57.2	949	BO
GREEN	0.57	51.8	1248	FL	RVI	0.52	54.1	1389	EF	CORRELATION	0.37	63.1	949	BO
RED	0.63	47.4	1202	FL	NDRE	0.63	47.9	1389	EF	DISSIMILARITY	0.42	60.0	949	BO
REDEdge	0.39	61.3	697	SE	MCARI	0.38	62.6	949	BO	ENERGY	0.43	59.3	1248	FL
NIR	0.36	63.9	896	SE	CCII	0.48	56.1	896	SE	HOMOGENEITY	0.28	70.4	1248	FL
Smoothed single time points														
BLUE	0.37	67.4	696	SE	DVI	0.30	63.7	1388	EF	CONTRAST	0.41	58.7	949	BO
GREEN	0.48	56.6	1055	BO	RVI	0.50	52.3	1317	FL	CORRELATION	0.21	69.8	1110	HE
RED	0.58	49.0	1110	HE	NDRE	0.57	49.6	1388	EF	DISSIMILARITY	0.42	59.9	949	BO
REDEdge	0.32	69.9	696	SE	MCARI	0.37	60.2	1317	FL	ENERGY	0.26	67.7	696	SE
NIR	0.41	59.9	1509	EF	CCII	0.45	56.0	1509	EF	HOMOGENEITY	0.29	67.2	600	TI
Moving time window														
BLUE	0.54	53.3	1316	FL	DVI	0.43	58.1	697	SE	CONTRAST	0.51	53.2	1056	BO
GREEN	0.58	50.7	1202	FL	RVI	0.56	50.6	1316	FL	CORRELATION	0.41	57.6	949	BO
RED	0.69	42.7	1316	FL	NDRE	0.64	47.0	1316	FL	DISSIMILARITY	0.54	51.1	1056	BO
REDEdge	0.42	57.8	697	SE	MCARI	0.44	57.2	1316	FL	ENERGY	0.60	48.7	1316	FL
NIR	0.42	59.1	697	SE	CCII	0.56	51.7	949	BO	HOMOGENEITY	0.36	61.1	1316	FL

295

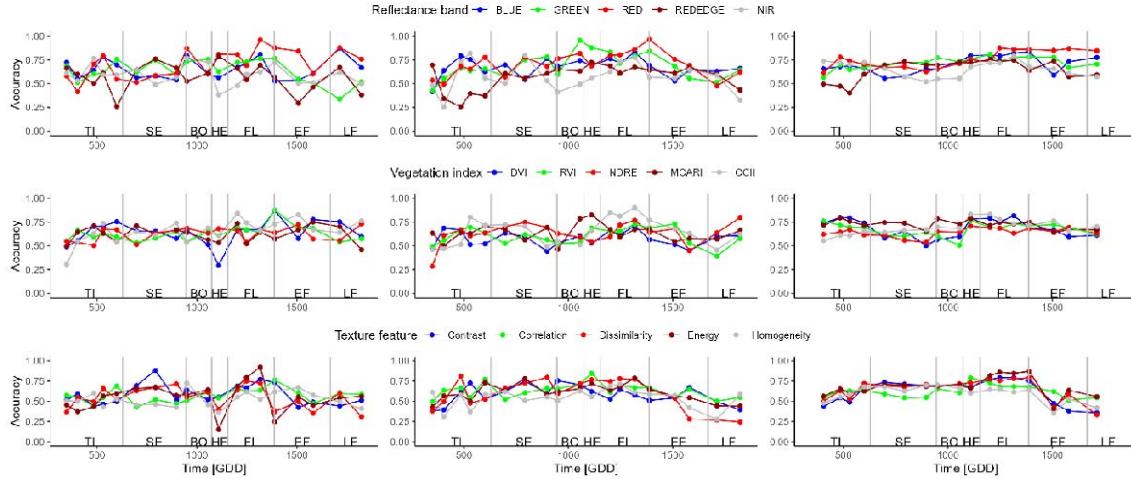
296 **3.5 RF classification model for classifying the low and high yielding varieties 297 using individual flights and time series of UAV traits**

298 Classification of yield groups using single bands was best with the RED band resulting in an accuracy
 299 of 0.962 followed by the BLUE band with an accuracy of 0.873, both at the flowering stage. The NIR
 300 band performed best during the booting stage. Classification using VIs was lower than accuracy for
 301 single bands and the highest accuracy was found for the RVI VI (0.875). All VIs show their best
 302 performance at the heading stage or later. The TFs showed a similar performance for yield type
 303 classification as the VIs. The best performing TF was CONTRAST with an accuracy of 0.898 when
 304 being measured during stem elongation stage. Fluctuations in accuracy between subsequent flights
 305 was relatively high for models built using single band reflectance as well as models using TFs on
 306 single dates (Figure 5).

307 Smoothing reflectances did not change improve the accuracy for yield type classification for the RED
 308 band, yielding an accuracy of 0.963 on time point later at the early grain filling stage (Table 5). The
 309 accuracy of the GREEN improved substantially to 0.955 at the booting stage and the NIR band
 310 increase in accuracy as well, while a date on the tillering stage was found to be most suitable.
 311 Accuracies of the DVI and RVI VIs dropped whereas the accuracies increase slightly for the other VIs
 312 after smoothing. Eg. the accuracy of the CCII increased to 0.902 and was achieved during flowering
 313 stage. The best performing TF ENERGY decreased when smoothing to 0.787. CORRELATION
 314 however was better able to classify yield types with an accuracy of 0.845. DISSIMILARITY showed
 315 the best performance at the tillering stage after smoothing. Smoothing changed the fluctuations in
 316 accuracy between single flights slightly for the VIs and to a bigger extent for the single band
 317 reflectances and mainly for the TFs (Figure 5).

318 The moving time window did not improve yield type classification but worsened for the RED band
 319 yielding an accuracy of 0.875 and did not change the accuracies of the other single bands significantly
 320 (Table 5). A similar result can be found for the moving window when applied to the VIs where the
 321 RVI yielded an accuracy of 0.765 at the flowering stage. A moving time window improved the
 322 classification for all VIs except DVI. However, these changes were only small for all VIs. The best VI
 323 for yield prediction using a moving window is the RVI with an accuracy of 0.856. Predictions by TFs
 324 worsen when using several time points as features compared to single dates. Using a time window, the

325 TF ENERGY and DISSIMILARITY show almost similar accuracies of 0.803 and 0.801, respectively.
 326 While the accuracies were not significantly improved by using a time window for prediction, the
 327 fluctuations between dates were reduced significantly and thus the performances of the predictions
 328 became much more stable among dates (Figure 5).



329
 330 *Figure 5: Accuracy prediction models built using single band reflectances (top), vegetation indices (middle)
 331 and texture features (bottom). The models were built using measurements from single dates (left),
 332 cumulating features from the beginning (middle) and choosing a window of three time points (right).*

333
 334 *Table 5: Display of the classification accuracy of different yield groups using predictors for all reflectance bands, vegetation
 335 indices and texture features at all dates.*

Raster	Accuracy	Date	PS	VI	Accuracy	Date	PS	TF	Accuracy	Date	PS
Single time points											
BLUE	0.873	1714	LF	DVI	0.868	1389	EF	CONTRAST	0.882	792	SE
GREEN	0.765	1389	EF	RVI	0.875	1389	EF	CORRELATION	0.763	1389	EF
RED	0.962	1316	FL	NDRE	0.732	1823	LF	DISSIMILARITY	0.752	1248	FL
REDEdge	0.790	1109	HE	MCARI	0.752	1580	EF	ENERGY	0.923	1316	FL
NIR	0.768	949	BO	CCII	0.843	1202	FL	HOMOGENEITY	0.727	949	BO
Smoothed single time points											
BLUE	0.832	1316	FL	DVI	0.712	1316	FL	CONTRAST	0.755	949	BO
GREEN	0.955	1055	BO	RVI	0.732	1509	EF	CORRELATION	0.845	1109	HE
RED	0.963	1389	EF	NDRE	0.798	1822	LF	DISSIMILARITY	0.813	484	TI
REDEdge	0.725	1109	HE	MCARI	0.825	1109	HE	ENERGY	0.787	1317	FL
NIR	0.822	530	TI	CCII	0.902	1316	FL	HOMOGENEITY	0.713	1247	FL
Moving time window											
BLUE	0.837	1389	EF	DVI	0.817	1316	FL	CONTRAST	0.793	1316	FL
GREEN	0.808	1202	FL	RVI	0.765	1109	HE	CORRELATION	0.788	1109	HE
RED	0.875	1248	FL	NDRE	0.708	1109	HE	DISSIMILARITY	0.790	1389	EF
REDEdge	0.753	1202	FL	MCARI	0.795	484	TI	ENERGY	0.870	1389	EF
NIR	0.793	1316	FL	CCII	0.833	1202	FL	HOMOGENEITY	0.706	949	BO

336

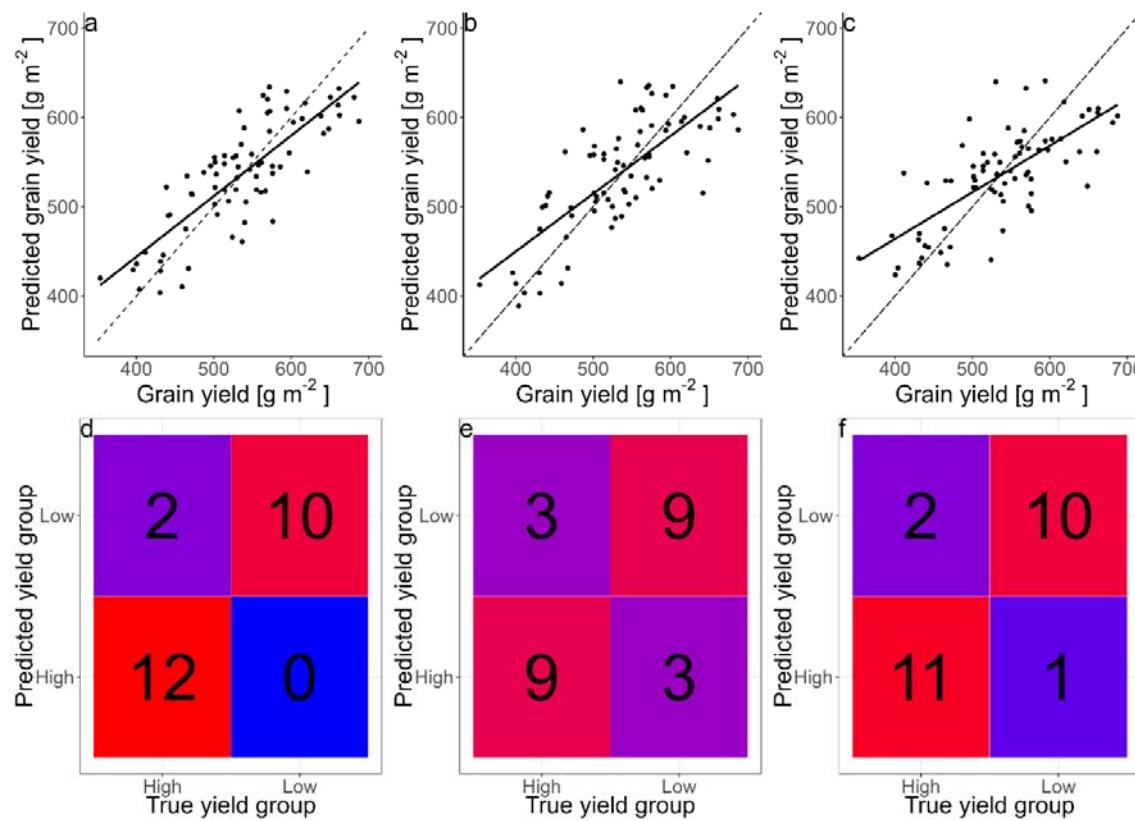


Figure 6: Yield prediction based on the red band (a), the NDRE index (b) and the energy TF (c) from moving window features at the best performing dates (Table 4). Confusion matrices of yield type predictions based on data of the best performing single dates of the RED band (d), the CCII VI (e) and the energy TF (f).

337

338

4 Discussion

339

4.1 Dynamic responses of individual bands

340

The red edge bands have been widely studied for assessing crop performance and yield in various crops, including wheat (Horler et al., 1983). Canopy reflectance in the red edge wavelength range is influenced by two optical properties of canopies: chlorophyll absorption in the red region and multiple scattering effects on the near-infrared (NIR). Although red edge reflectance has been commonly used for yield prediction in previous studies, our findings suggest that this may not be a reliable indicator of yield in our specific case.

346

The inter-variety variability in canopy reflectance in the red-edge region might be large and have overweight the subtle differences in canopy reflectance associated with yield, making the red edge reflectance less useful for yield prediction. In addition, the correlation of reflectance in the red edge region with yield is known to change quickly with the exact wavelength measured in the red edge region (Pavuluri et al., 2015). In contrast, visible bands (Blue, Green and Red) can be more sensitive to yield-related variations in chlorophyll, biomass accumulation during the tillering until the stem elongation stage until the beginning of the booting stage since they are known to be correlated to both, chlorophyll concentration and LAI (Daughtry et al., 2000). Accordingly, our results showed that the RED, GREEN, and BLUE bands were among the most effective spectral features for yield prediction, exhibiting significant differences between high and low yielding varieties at almost all measurement dates. These visible bands are highly sensitive to chlorophyll, with their reflectance decreasing during the transition from the stem elongation to the beginning of the booting stage, and increasing again from heading until harvest due to senescence and chlorophyll degradation. Therefore, our findings suggest that the flowering stage in which the chlorophyll content reaches the peak is crucial.

359

360

361 The NIR region is known to be sensitive to leaf area and especially ground cover (Korobov & Railyan,
362 1993), making it a useful band for predicting biomass and yield. Our results indicated that the NIR
363 band performed best during the stem elongation stage for yield prediction and at the booting stage
364 when there were significant differences in leaf area index (LAI) between the two yield groups. This
365 aligns with the findings by Korobov and Railyan (1993), who reported a higher correlation of NIR
366 reflectance with dry matter and ground cover during booting stage compared to later stages. Thus,
367 normalizing the difference of the NIR and the REDEDGE reflectance in form of the NDRE index,
368 showed a good performance for chlorophyll estimation (Barnes et al., 2000). Usually, VIs containing
369 information from the red edge region of the spectrum are considered being more sensitive to
370 chlorophyll absorption in dense canopies (Nguy-Robertson et al., 2012). It is expected that combining
371 the highly LAI-sensitive NIR band with the red edge band containing more information about leaf
372 pigments in the canopy and therefore improves the performance of our yield prediction model at the
373 flowering to early grain filling stages.

374 **4.2 The influence of growth stage for yield prediction and classification**

375 The performance of yield prediction and classification depends highly on the growth stages of the
376 crop. Our study found that the flowering stage and early grain filling stage are the most suitable for
377 predicting yield in winter wheat. This is consistent with the findings of other studies (Hassan et al.,
378 2019; Prey et al., 2022; Prey et al., 2020; Wang et al., 2022). It is often argued that yield cannot be
379 measured directly using remote sensing approaches, given the fact that yield formation in wheat
380 involves several components, including ear density, kernel number per ear, and grain weight (Satorre
381 & Slafer, 1999). Furthermore, these components form at different stages and are therefore not present
382 at every phenological stage. Still, remotely sensed information and variations in canopy images and
383 reflectance are often closely related to these yield components, such as vegetative biomass and
384 chlorophyll content (Wu et al., 2008). Therefore, when these traits usually reach their peak levels
385 around the flowering stage, i.e., the crop transitions from vegetative to generative growth, their
386 associated variations in canopy reflectance also indicate the variability in yield

387 Early differences in biomass and LAI dynamics between genotypes are well-documented (Grieder et
388 al., 2015; Pang et al., 2014), and these differences can be useful for predicting yield and classifying
389 varieties in early growth stages. While our study found that yield prediction at the tillering stage
390 feasible, it remains to be challenging for excluding the poorly-performing varieties. From tillering to
391 harvest, wheat is known to compensate for e.g. a low stand count by altering the number of yield
392 components (Holen et al., 2001) complicating predictions at this stage. This might be the reason, that
393 our classification models were more performant in detecting the high yielding varieties. It can be
394 argued that our approach has a limitation that we cannot be certain whether the algorithm is sensitive
395 to canopy traits that are yield-dependent or to variety-specific traits that do not influence yield
396 performance. Therefore, the validation of our models in other varieties or environments will be
397 critical. After flowering, the performance of our models then decreased with the onset of senescence.
398 While yield formation does continue during senescence (Anderegg et al., 2020; Spano et al., 2003),
399 our results showed that this stage is less correlated with grain yield than the earlier stages. Further, no
400 significant differences in phenology were detected between the yield groups, suggesting that the yield
401 differences between the high- and low yield groups are not likely due to their senescence dynamics.
402 Unlike in our study employing an identical/moderate fertilization rate, even under high N conditions,
403 differences in the onset of senescence were not found to be significantly correlated to grain yield per
404 unit crop N uptake at harvest (Gaju et al., 2011) making classification at this time point difficult.
405 Nevertheless, our study confirms that the tillering stage is an already promising time for variety
406 classification, as evidenced by the strong performance of our classification models at this stage.

407 **4.3 Comparison of variable- and feature types for yield prediction and 408 classification**

409 Our study found that single-band reflectance, such as the RED band, were as effective as or even more
410 effective than vegetation indices (VIs) for predicting yield. The RED band is known to be related to

411 leaf area index (LAI), although this relationship is often nonlinear (Hinzman et al., 1984) and therefore
412 requires non-linear methods such as RF to perform well for yield prediction. Pavuluri et al. (2015)
413 found a saturation of RED reflectance when predicting yield, which can also be found in our
414 prediction models. In contrast, VIs typically show good linear correlations with grain yield, with
415 NDVI being widely used for yield prediction (Hassan et al., 2019). Many VIs have been screened by
416 Prey et al. (2020) and few have been showing a consistent performance over the years, which makes a
417 general selection difficult. Further, VIs narrow down the information that is accessible and Vatter et al.
418 (2022) found good performances for yield prediction when using 11 wavebands from a multispectral
419 camera that were fed to a deep learning model. Machine learning approaches based on reflectance
420 were found to not improve the accuracy yield prediction but showed great potential in predicting grain
421 protein (Zhou et al., 2021). Machine learning models, however, often require a big amount of training
422 data, which can be challenging to gather.

423 TFs are complex in their calculation and they offer a variety of possible ways of calculation, possible
424 combination with underlying rasters and ways to be calculated. Detailed information on how TFs are
425 calculated is often lacking (Wang et al., 2021; Zhang et al., 2021; Zheng et al., 2019). Therefore, TFs
426 still have to be examined in detail and their parameters optimized under different experimental
427 conditions and scenarios of sensing data collection. We calculated TFs in a standardized way, but still
428 found a high variability between dates. TFs are further known to be highly dependent on the GSD and
429 therefore the flight height (Zheng et al., 2019). Therefore, smoothing benefitted the yield prediction by
430 making the performance more stable but not better. A novel approach is presented by Herrero-Huerta
431 et al. (2020) who calculated so-called canopy roughness directly on the point cloud from the structure
432 from motion processing and showed its correlation to biomass. TFs are further often used in
433 combination because there might be additional information (Liu et al., 2022; Wang et al., 2021),
434 especially in the later stage, when they are not strongly correlated to single band reflectances and VIs
435 anymore as indicate by our results.

436 **4.4 Limitations and outlook**

437 The red-edge position and its shape is often used estimate the stress status of field crops (Boochs et al.,
438 1990; Guyot et al., 1988). However, it is obvious that the dynamics (time series) of the Red-edge band
439 is difficult to interpret compared to the visible bands. During the early stages of tillering, the red-edge
440 reflectance increased, possibly due to an increase of ground cover, whereas later it decreased again,
441 when the canopy height increased during the SE stage. At the beginning of heading another increase in
442 the Red-edge reflectance can be observed, accompanied with the increase of reflectance in the visible
443 bands. However, in contrast to other bands, the Red-edge reflectance decreases with the onset of
444 senescence at the early grain filling stage, possibly due to a reduction in chlorophyll and shrinking
445 canopy structure (Wang et al., 2022). However, fluctuation also occurs during the mentioned stable
446 period. These fluctuations can be of various origins. For instance, the appearance of the canopy might
447 change significantly due to the emergence of the spikes. Although this study was unable to exploit the
448 entire shape of the red-edge reflectance, due to limitations in our multispectral camera having one
449 band in the red edge region, future work should further advance the understanding of the dynamics of
450 red-edge reflectance and responsible canopy characteristics.

451 Also, features should in addition to their performance for yield prediction be assessed regarding their
452 heritability (h^2) since breeders are interested in knowing the genetic variation underlying a trait or in
453 our case a spectral feature. Generally, this study shows that a trait time series followed by smoothing
454 and a moving window allow for more stable predictions when also not better predictions.

455 **5 Conclusions**

456 Most spectral and texture features derived from the canopy multispectral images were related to
457 variations in yield, but they- delivered the best predictions of yield only between the booting and end
458 of the flowering stage. However, in earlier stages, the visible (Red, Green, Blue) bands can accurately
459 predict yields and distinguish between the low- and high-yielding genotypes. Single-band reflectance,

460 particularly the red band, is a reliable predictor of yield. Combining additional bands, such as the red-
461 edge and NIR, into VIs like NDRE, improves performance significantly, but it limits the machine
462 learning algorithm's ability to build a strong model. Texture features generally performed poorly for
463 yield prediction, and their performances were inconsistent across dates in this study, suggesting that
464 further research is still needed to better understand the applicability of different texture features for
465 yield- and traits predictions and methods to optimize texture feature extraction. Smoothing or
466 combining data across a time series can enhance the performance of yield prediction and classification
467 models, particularly in the early growth stages. Future studies should combine different feature types
468 to leverage complementary information captured by different types of multispectral features and
469 variables.

470 **Author contributions**

471 KY designed the experiment. MC managed the UAV flights for aerial imagery, analyzed the data, and
472 conducted the ground-based field measurements with supervision from KY. MC and KY wrote and
473 revised the manuscript. Both authors contributed to the article and approved the submitted version.

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