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# Assessing Water Stress in Maize Using UAV Thermal Infrared Sensing

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# Abstract

Crop water stress (CWS) caused by inadequate soil moisture content (SMC) is a major factor affecting agricultural productivity. Remote sensing technologies, such as thermal infrared (TIR) cameras on Unmanned Aerial Vehicles (UAVs), offer a potential solution for monitoring CWS. In this study, the relationships between canopy temperature ( $T_c$ ) obtained from UAV-based TIR data and ground measurements of SMC, plant water content (PWC), and dried leaves weight (DLW) were investigated to support the use of TIR for CWS detection. Two different soil-pixel exclusion methods using the Otsu algorithm and Normalized Difference Vegetation Index (NDVI) thresholding were compared, and the influence of cover crops on UAV-ground measurement relationships was assessed. The study focused on maize at three different growth stages after six years of winter cover crop use, hypothesizing cover crops impact these relationships. The findings revealed a correlation between  $T_c$  and PWC and DLW ( $r=-0.45^*/-0.67^{***}$ , and  $-0.19^{**}/-0.58^{***}$ , respectively), suggesting  $T_c$  could serve as a proxy for crop water status. However,  $T_c$  was not effective in estimating SMC. In this relation, the cover crop represented by the grass species "oat" may have led to unfavourable conditions for maize that resulted in lower PWC and biomass than other treatments ( $p < 0.05$ ). Furthermore, the performance of the Otsu algorithm varied depending on the maize growth stage, which affected its applicability. Overall, the study highlighted the challenges and opportunities associated with thermal imaging for CWS monitoring, emphasising the importance of data interpretation. Further research is needed to explore the relationship between  $T_c$  and SMC with the inclusion of NDVI as a normalization factor and to better understand the impact of long-term cover crops on the SMC in no-tillage conditions.

Keywords: remote sensing, canopy temperature, thermography, UAV, crop water stress, maize, soil moisture content, cover crops.



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List of Abbreviations:

CWS = Crop Water Stress  
DFW = Dried Fruit Weight  
DLW = Dried Leaves Weight  
DN = Digital Number  
ExG index = Excess Green index  
Gs = stomatal conductance  
LST= land surface temperature  
NDVI = Normalised Difference Vegetation Index  
PSF = Plant-Soil Feedback  
PWC = Percentage of Water in the leaves  
RGB = Red Green Blue wavelengths  
sdT<sub>c</sub> = standard deviation of canopy temperature  
SNR = Signal to Noise Ratio  
SOC = Soil Organic Carbon  
SOM = Soil Organic Matter  
SMC = Soil Water or Soil Moisture Content  
SWD = soil water deficit  
T<sub>c</sub> = canopy temperature  
TIR = thermal infrared  
TVDI = temperature vegetation dryness index  
UAV = Unmanned Aerial Vehicles



# 1 Introduction

## 1.1 Context and background

Crop water stress, which is defined as water deficiency in the soil for crop use, represents a problem for farmers since plant growth may be undermined in the long term, leading to overall decreased productivity (Gerhards et al., 2019). Therefore, techniques for water stress monitoring are of the utmost importance. Currently, techniques for the detection of water stress in crops rely on direct measurements of the soil water content or assessment of physiological and biophysical changes occurring in crops (Ekinzog et al., 2022).

In recent years, with advancements in technology, imagery of plant or canopy temperature ( $T_c$ ) using UAV-mounted thermal infrared (TIR) cameras started being explored due to its ability to offer a more complete picture of the spatial variability of crop water status compared to ground measurements (Messina and Modica, 2020). With TIR data collected by Unmanned Aerial Vehicles (UAVs), the workload in the field can be minimized. However, issues linked to the limitations of TIR cameras are introduced and need to be tackled with procedures that correct for the sensor's intrinsic characteristics and ambient environmental conditions (Kelly et al., 2019). Given that, protocols known to derive accurate temperature data have been developed and methods for  $T_c$  extraction have been proposed (Jones and Sirault, 2014; Kelly et al., 2019).

Nonetheless, using thermal imaging for monitoring purposes is further complicated by the fact that  $T_c$  is not only influenced by soil water content. Another potential way for controlling  $T_c$  is through biomass growth, as increased foliage leads to higher evapotranspiration and lower  $T_c$  (Ekinzog et al., 2022). For example, as biomass grows, a confounding effect in the relation between soil water content and  $T_c$  is introduced. On the other hand, the isolated influence of canopy structural parameters, which are for example represented by the biomass allocation to leaves and stems, can be seen later in the phenological stages, when at physiological maturity the canopy is not transpiring anymore (Anderegg et al., 2021). The link between  $T_c$  and soil water content, which reflects plant water status, was established long ago. Since then, TIR images have been helping to drive irrigation strategies aimed at decreasing  $T_c$  through an increase in soil water content via irrigation (Ekinzog et al., 2022; Gerhards et al., 2019). However, sustainable agricultural practices claim that water-use efficiency can be not only achieved with punctual water inputs but also by encouraging mechanisms for water retention and decreased loss. One of the mechanisms is cover crop use. The purpose behind the cultivation of cover crops is to benefit from their influence on soil properties, which the following plants can take advantage of (Blanco-Canqui and Ruis, 2020). This concept is called plant-soil feedback (PSF). Cover crops were found to affect the soil structure through improved water infiltration and absorption, as well as soil fertility by improving nutrient retention and cycling (Blanco-Canqui and Ruis, 2020; Daryanto et al., 2018; Koudahe et al., 2022).

To add complexity, these two benefits of PSF need different duration of cover crop use to become explicit; additionally, different cover crops are reported to impact them differently (Blanco-Canqui and Ruis, 2020; Hunter et al., 2021).

## 1.2 Problem definition

The UAV-derived  $T_c$  serves for crop water stress monitoring, and water in the soil is the interest around which the study revolves, as it can be targeted efficiently and timely to reduce  $T_c$  (Zhang et al., 2019). A significant correlation between soil moisture in the first 40 cm depth and Crop Water Stress Index (CWSI) is reported in the study by Ru et al. (2020). As  $T_c$ , CWSI has a strong relationship with the plant water status indicators, but differently from  $T_c$ , CWSI requires an artificial setup with an irrigation scheme, which is often difficult to set

at the field level. Therefore, the choice of  $T_c$  lies in its simplicity and the fact Zhang et al. (2019) reported that it is an effective indicator of water stress in maize that it is better able to reflect the status of soil water content at shallow root zone depths (20 cm), typical of this crop. Because of its importance, the effect of soil water content on  $T_c$  measured with UAV must be evaluated. Moreover, it has to be verified if the relation between soil water content and  $T_c$  is maintained during the growing season. Existing literature focused on examining the relation on a single acquisition day at different soil depths for exploring the water uptake zone for maize (Zhang et al., 2019). While having this relationship confirmed for different crop stages with soil water content sampled at 20 cm would mean that remote sensing techniques with their advantages over ground measurements could gather information needed on the water in the soil, and  $T_c$  could be used as a proxy for plant status (Messina and Modica, 2020). However, given the confounding effect of biomass and soil water content on  $T_c$ , it becomes important to distinguish which influences the  $T_c$  the most at the field level and to separate the two during the crop growth to see if the strength of the relationship is maintained over the growing season. This research represents a novelty in the sense that existing literature has focused on determining the causative relation between crop water stress and the consequently reduced biomass growth rather than the influence of biomass on  $T_c$  (Li et al., 2019; Song et al., 2019). Furthermore, some studies investigated other canopy aerodynamic characteristics such as roughness and crop height (Gerhards et al., 2019; Prashar and Jones, 2014), but so far no research focused on the influence of biomass on the canopy energy balance.

Biomass accumulation and soil water content, and consequently  $T_c$  may be affected by the PSF after cover crops. The existing literature about cover crops suggests that their short-term use associated with tillage repeated for a few years has the potential to increase macropores' presence (Haruna et al., 2023), which however is not reported to bring benefits in terms of soil hydraulic properties (Hunter et al., 2021). The effect on soil water content for long-term studies on the cover crop is only documented in the case of no-tillage practices. For prolonged cover crop use and no-tillage, Araya et al. (2022) report an improvement in soil structure accompanied by increased water retention. Nonetheless, little is known about whether tillage's positive effects on macropores may persist over time when combined with a cover crop grown in succession for several years. Therefore, a study on the long-term use of cover crops of different types associated with ploughing becomes relevant. Assessment of cover crop performances when compared to the control (fallow) could highlight a difference in soil water content, besides the expected biomass variations already reported in the literature due to the fertilizing properties of cover crops (Fageria et al., 2005). Cover crops have to be studied individually by measuring the soil water content and biomass of the following cash crop. The use of  $T_c$  could help evaluate the effects of different cover crops and shed light on important benefits on soil hydraulic properties due to their long-term use, however, it is unclear if (and how) the use of different cover crops affects the relationship between canopy temperature and soil water content.

For the achievement of thermal observations with the temporal and spatial scales more suitable to the study, UAV is generally deployed because of its flexibility in timing and flight height (Delavarpour et al., 2021). However, as it occurs with satellite or airborne images, further steps for obtaining  $T_c$  are to be undertaken and these depend on the method used for  $T_c$  extraction. The choice of the method used for removing soil from the pixels still constitutes a determinant passage that affects the overall thermal map and the results (Jones and Sirault, 2014). Besides a routinely used method based on NDVI, more sophisticated approaches such as the Otsu algorithm have been studied and shown promising results (Jones and Sirault, 2014; Zhang et al., 2019). Zhang et al. (2019) demonstrated that  $T_c$  extracted by the Otsu method is highly correlated with the ground-truth measurements with  $R^2$  of 0.94 ( $n = 15$ ) and RMSE of  $0.7^\circ\text{C}$ , and therefore representing the  $T_c$  with high accuracy. Decisions on which methods to use need to be drawn according to the achievable accuracy in removing soil pixels.

### 1.3 Overall research aim and research questions

The main aim of this thesis is to assess whether UAV thermal data can be used to obtain information about soil water content which is a proxy of crop water status and to determine to what extent biomass and soil water content derived from ground measurements are responsible for  $T_c$  changes obtained from the thermal camera on a UAV. Secondly, the effects of cover crops on this relationship will be investigated. To address this aim, three research questions will be answered. The first research question evaluates the performance of the background soil removal methods. The second and the third research questions focus on exploring the relation between UAV-derived  $T_c$  with soil water content and structural parameter as biomass at the field and plot level respectively during the growing season of maize.

To execute the above-mentioned aim, the following research questions are set up:

RQ1: Which soil background removal method performs best at different maize growth stages?

RQ2: What is the relationship between  $T_c$ , biomass, and soil water content and how can the ground measurements of the last two help to interpret the thermal data of maize crops?

RQ3: How do cover crops influence the interrelation of soil water content, maize  $T_c$  and biomass?

## 2 Material and Methods

### 2.1 Study site and Experimental Design

This study was conducted in a 1.21 ha research field (Latitude 51.995123 Longitude 5.660100), located 500 meters north of the Wageningen University and Research campus, the Netherlands, during the summer of 2022 (Figure 1 A). The study field was divided into five blocks with eight plots each (Figure 1 B). Within a block, each plot, whose dimensions were 10 m x 6 m, was randomly assigned one of the eight selected cover crop treatments. The treatments consisted of different species of cover crops, which have been grown in the same location every winter since 2016: oat (*Avena strigosa*, abbreviated as “O” in the text), radish (*Raphanus sativus* – “R”) and vetch (*Vicia sativa* – “V”), bi- or tri-species mixtures (“VO”, “VR”, “OR” and “VOR” with mixing ratios of 50:50 and 33:33:33); one control plot was the fallow treatment (“F” in the text).

A



B

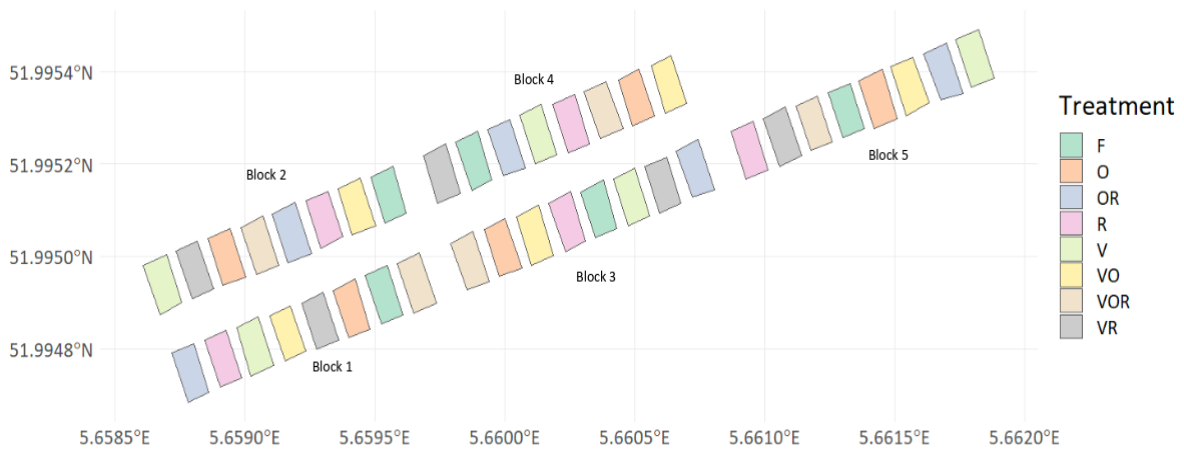


Figure 1 A Location of the study area with zoom into the field level (RGB orthomosaic derived from DJI Matrice 210 (RTK) on 09/08/2022) and Figure 1 B distribution of cover crop types in the blocks. Abbreviations: oat (*Avena strigosa* – “O”), radish (*Raphanus sativus* – “R”) and vetch (*Vicia sativa* – “V”), bi- or tri-species (“VO”, “VR”, “OR” and “VOR”); fallow (“F”).

After cover crops, the cash crop of the year was grown. Although soil texture was reported to be of one type only and defined as sandy (B2) according to the Dutch system of soil classification (Heinen et al., 2022), soil sampling in November 2021 (Appendix I) demonstrated that there is a soil organic matter (SOM) gradient

along the length of the field which must be taken into account for soil water content considerations. More specifically there is an increase of SOM from blocks 1-2 to block 5 (Appendix I).

In 2022, the cash crop was maize (*Zea mays*), which was sown on April 14<sup>th</sup>, 2022 in 7 parallel rows following east–west row direction. The maize plants emerged on May 13<sup>th</sup> and were harvested on August 25<sup>th</sup>, 2022 after a 135-day life span. To eliminate interference from nutritional stress and weeds, fertilizers, and herbicides were applied according to the local cultivation practices. As part of the weed management strategy and cover crop termination, ploughing of cover crop biomass was performed before maize sowing. The availability of water for plants is described by wilting point and field capacity. For this textural classification, wilting point and field capacity were obtained from the water retention curve (not displayed) and reported to be around 3 and 13%, respectively. Irrigation was not provided and, in the summer of 2022, the study area had several rainfalls which occurred before June 27<sup>th</sup>, which is the first day of data collection. In August, precipitation events were scarce and only two light rain occurrences were documented two days before the last data collection (Appendix II).

## 2.2 Field and UAV Data Collection

### 2.2.1 Field data

During the growing season, 3 field campaigns and UAV flights took place on the days reported in Table 1. The choice of dates was dictated by the need to analyse maize throughout the growing season. These field campaigns correspond to the V12 stage considered to be the active growth and leaves/cob development stage, the R2 stage in which the kernel develops, and the R5 stage which is the final critical production stage of physiological maturity (grain filling). Biomass measurements occurred 1-5 days before or after the UAV flights. Biomass measurements include dried leaves weight (DLW) and water content in the leaves expressed as a percentage (PWC). Besides leaf measurements, samples of maize cob were collected (dried fruit weight or DFW) and used as a measure of maize production.

In each plot, 3 sampling sites were selected for data collection of biomass weights and moisture (leaves and stem) and the average values of these three readings were used to represent the sampling plot. Analysis of the samples was conducted in the laboratories of Wageningen University, on the same day of biomass collection. Weights before and after sample drying at 105°C for 24 h were taken and moisture content in the plants' components were calculated using the following formula (1):




$$\text{Moisture content (\%)} = (W2 - W3) / (W2 - W1) \times 100 \quad (1)$$

where, W1 corresponds to the weight of the container with the lid, while W2 and W3 are the weights of the container with lid plus sample before and after drying, respectively.

Additionally, the soil water content (SMC, in Siemens) in the centre of each plot was measured every fifteen minutes using the traditional gravimetric method with TMS-4 dataloggers (Wild J., 2019). The gravimetric measurements are later converted into volumetric water content. The deployed sensor was located in the ground at the effective rooting depth (0–12 cm).

Maize is expected not to be affected by rapid changes in external conditions, such as water in the soil, due to its isohydric nature (Ihuoma and Madramootoo, 2017). Because of this, an average of the SMC readings before and during the flight on the day of the UAV campaign was considered and used in the analysis (for timing refer to Table 1).

Table 1 Summary of UAV, ground measurements and captured growth stages of maize (from vegetative phases to physiological maturity) according to Nleya et al. (2019).

|   |                    |  | Field data                 |   | UAV drone flight                             |
|---|--------------------|--|----------------------------|---|--|
|   | Growth stage       | Stage description  | Biomass                    | SMC   | Thermal and multispectral                    |
|    | Vegetative<br>V12  | Tassel develops rapidly. Lateral shoots and cob development.   | June<br>22 <sup>nd</sup>   | June<br>27 <sup>th</sup> –<br>00:00-<br>12.45   | June<br>27 <sup>th</sup> - 12:30-<br>12:50   |
|   | Reproductive<br>R2 | Kernel development. Silking stage                              | August<br>10 <sup>th</sup> | August<br>9 <sup>th</sup> –<br>00:00-<br>16:30  | August<br>9 <sup>th</sup> - 16:08-<br>16:36  |
|  | Reproductive<br>R5 | Physiological maturity and drying of kernels. End of mass gain | August<br>22 <sup>nd</sup> | August<br>23 <sup>rd</sup> –<br>00:00-<br>17:45 | August<br>23 <sup>rd</sup> - 17:22-<br>17:50 |

### 2.2.2 UAV Thermal and RGB Imaging Systems

In this study, a quad-rotor UAV remote sensing system (DJI Matrice 210 (RTK)) equipped with a FLIR Tau 2 thermal camera (FLIR Systems, Wilsonville, OR, USA) and an Hiphen Airphen multispectral camera (Hiphen, Agricultural Imaging Solutions, Avignon, France) was used. The main technical parameters are shown in Table 2. Flight planning was conducted with DJIFlightPlanner software (DJI, 2022), which allows the user to generate a route of waypoints from the desired take-off point.

Table 2 Main parameters of UAV thermal and RGB image acquisition system.

| Camera   | Parameter                  | Value   |
|--|----------------------------|---|
| UAV thermal image acquisition system (FLIR Tau 2 19 mm)          | Imager resolution          | 640 x 512 pixels  |
|  | Lens focal length          | 19 mm   |
|  | Spectral bands             | 7.5–13.5 $\mu\text{m}$  |
|  | Lens field of view         | 32°x 26°  |
|  | Accuracy                   | -25 to +100°C   |
|  | Thermal sensitivity (NETD) | <50mK   |
|  | Weight (camera + lens)     | < 70 g  |
| Multispectral image acquisition system (Hiphen Airphen, 6 bands) | Imager resolution          | 1280 x 960 pixels   |
|  | Band range                 | 6 spectral bands among [450/530/570/675/710/730/750/850 nm] (FWHM=10nm) |
|  | Lens field of view         | 8mm - 33°x26° / 4.2mm - 60°x46°   |
|  | Image format               | TIFF  |

### 2.2.3 Acquisition and Pre-treatment of UAV Thermal and RGB Images

The accuracy of temperature measurements is influenced by ambient environmental conditions such as relative humidity, air temperature, the temperature of the surrounding objects, and the distance between the FLIR Tau 2 camera and the target. In this case, the meteorological effect was minimized by flying in absence of rain, snow, and dust (Messina and Modica, 2020). However, the passage of clouds could not be avoided during certain flights (June 27<sup>th</sup> and August 23<sup>rd</sup>), and because of technical issues, all flights except the first were accomplished late in the afternoon, therefore the shadowing effect is always present to a certain degree (Table 1). A trade-off with the battery duration was obtained by flying at an altitude of 20 m, which allowed to cover the area of interest in 20 minutes with 80% front and side overlap between images. Before every flight, a 20 minutes stabilization on the ground was adopted for reducing the systematic error and camera digital number (DN) shift due to wind and ambient temperature (Kelly et al., 2019). Additionally, the camera was set to perform non-uniformity correction every 20 seconds throughout the flight to account for possible detector drifts. Besides the thermal data acquired with FLIR Tau 2 facing downward vertically, multispectral images were captured with Hiphen Airphen provided of 6 bands (specifics in Table 2). The colour balancing feature with Agisoft Metashape (AGISOFT, 2023) that aims to normalize pixel values based on a comparison of common areas was used at the moment of orthomosaic creation. Colour calibration was done using the tie points as the source data. After images were acquired, mosaic processing was performed using Agisoft Metashape software. Thermal and RGB orthomosaics were geo-referenced using from five to seven ground control points whose coordinates were measured using an RTK differential GNSS device (Topcon HIPER V) with precision within 1 cm.

### 2.3 Canopy Temperature Extraction Method

For this study, two methods for extraction of vegetation pixels were investigated, based on thresholding of the NDVI and Otsu algorithm. For NDVI calculation (2), multispectral images were used and after several trials



with different thresholds for vegetation, an arbitrary value set to 0.45 was adopted (Nandibewoor et al., 2015).

$$NDVI = (NIR - RED) / (NIR + RED) \quad (2)$$

For the Otsu algorithm, RGB images were further processed following the steps shown in Figure 2. Following this method, georeferenced optical image orthomosaics were converted into greyscale imagery using equation (3). The resulting single-band orthomosaic underwent a back- and foreground separation based on the frequency distribution of pixels. Otsu algorithm segments the image making the variance on each of the classes of a greyscale image minimal (Otsu, 1979).

$$GREYSCALE \ INTENSITY = 0.2989 * RED + 0.587 * GREEN + 0.1140 * BLUE \quad (3)$$

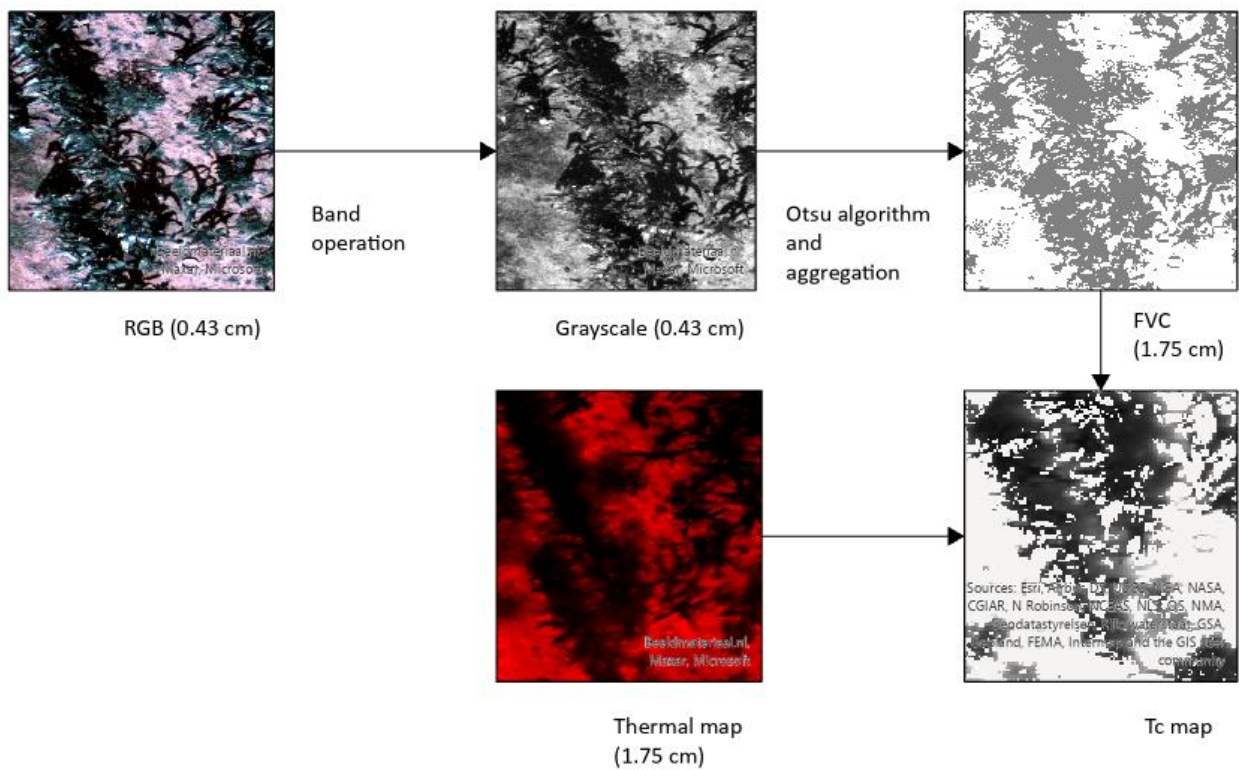


Figure 2 The main steps of the co-registration approach Otsu method proposed in this study using both unmanned aerial vehicle (UAV) thermal and red–green–blue (RGB) remote sensing imagery. FVC (fractional vegetation cover) and greyscale were derived from optical image.

The segmented orthomosaic was then used to mask the georeferenced thermal image orthomosaic for having a resulting T<sub>c</sub> map, after resampling from 0.43 to 1.75 cm by using the nearest-neighbour interpolation algorithm (Figure 2). The entire process of the RGRI-Otsu method was implemented in R programming language (RStudio, 2022) and ArcGIS Pro desktop (Redlands, 2022). Similarly, the resulting NDVI-based mask from the same day was overlaid with the thermal data. The resultant T<sub>c</sub> maps for each of the three UAV flights were then subjected to an accuracy assessment. Accuracy assessment was done by comparing the results of the two adopted methods to the RGB orthomosaics. With random sampling, 500 thermal pixels were selected and assessed based on the visual interpretation of the RGB image. Paired pixels were counted as True Positive, predicted canopy pixels in the T<sub>c</sub> map that were not paired with vegetation in RGB as False Positive,



and canopy in RGB without a pair in  $T_c$  were assigned False Negative. Based on this information, the producer's accuracy for soil is calculated as the number of soil pixels in RGB classified accurately as such divided by the total number of reference sites for that class. As a further step for ensuring  $T_c$  was reflecting only vegetation, median  $T_c$  was used in the following analysis.

## 2.4 Statistical Analysis

### 2.4.1 Multilinear regression and correlation

After the accuracy assessment, the method with the highest producer's accuracy for soil pixels for each day was selected and used for statistical analysis. The relationships between ground measurements and maize median  $T_c$  for each field campaign-UAV flight were explored using multilinear regression. Specifically, linear regression models were used with the coefficient of determination ( $R^2$ ) and *p-value* calculated for comparisons. The regressions were implemented by using R programming language and `lm()` function (Fox J., 2019). DFW is not considered in multilinear regression since other components of maize plants such as maize fruit and stems are not determinant for plant transpiration, therefore DFW is assumed to be a variable with no effect on  $T_c$ .

### 2.4.2 Analysis of Variance and Post-Hoc Test

Two-way analysis of variance (ANOVA) was used to investigate the effect of varying cover crops and the effect of SOM gradient over the field on the median  $T_c$ . ANOVA was applied using R programming language. Tukey's Honestly Significant Difference (Tukey's HSD) post-hoc test was used to perform pair-wise comparisons between cover crop treatments. Homoscedasticity and normality were checked with Levene and Shapiro test, respectively. Finally, the *p-value* and the coefficient of determination ( $R^2$ ) were obtained to explore the significant relationships. During this study, a level of 5% was considered significant (*p-value* < 0.05).

### 3 Results

#### 3.1 Removal of Soil Background Pixels in the Crop Research Plots

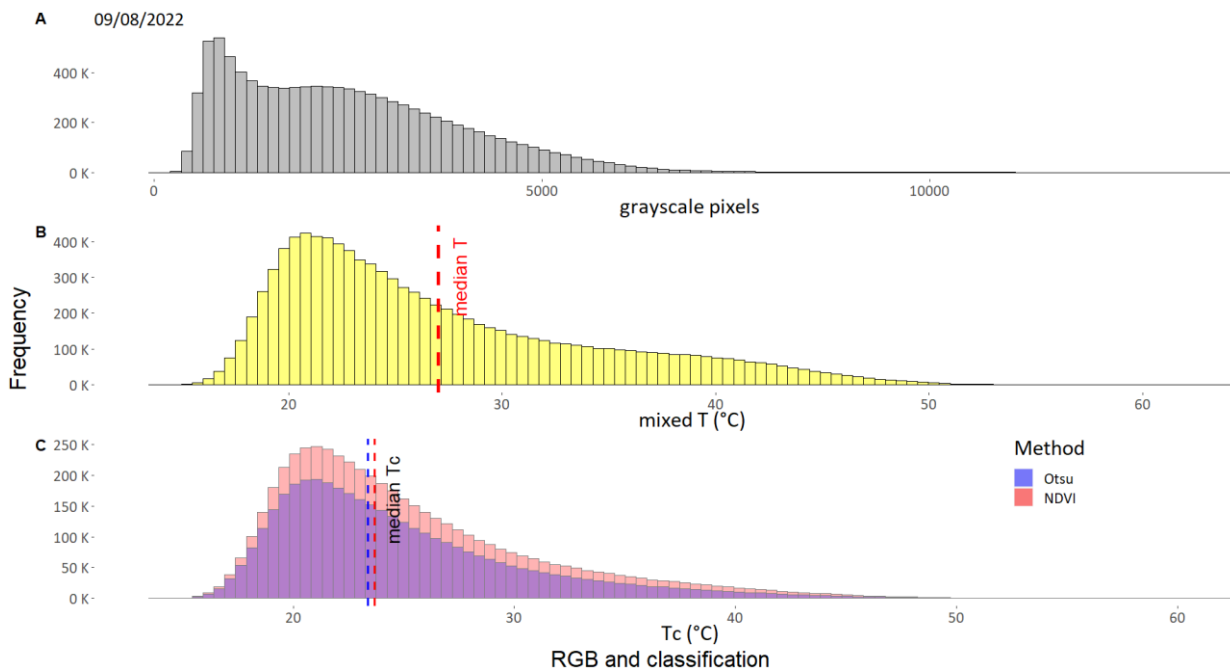
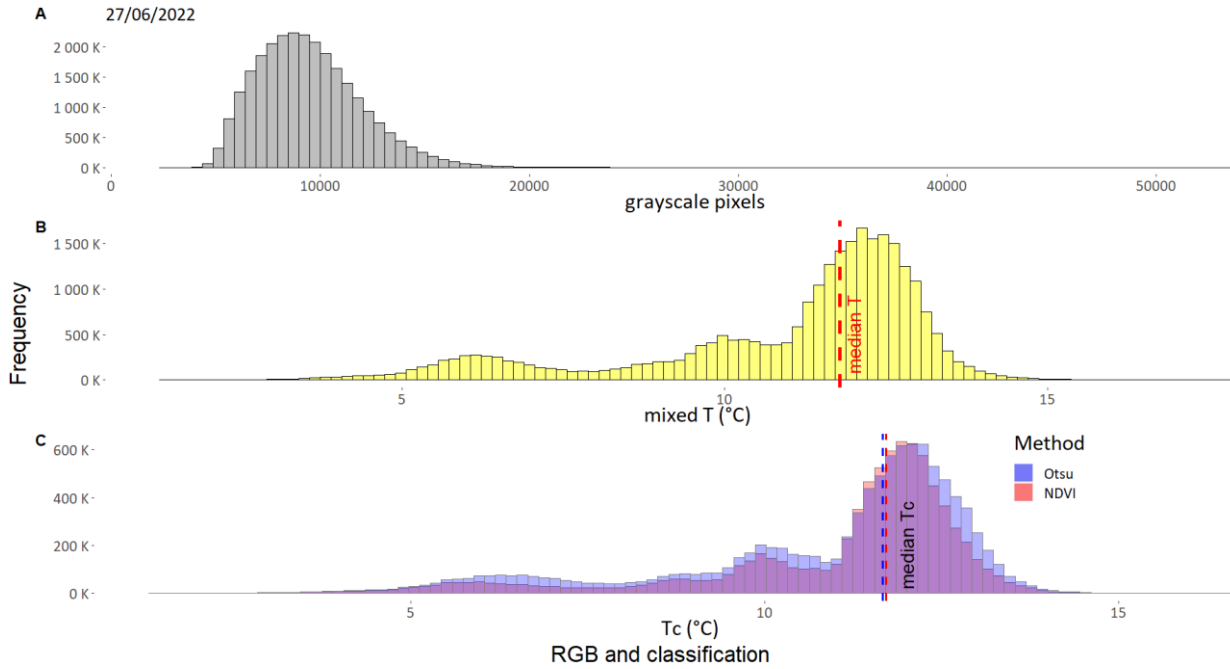
On the 9<sup>th</sup> of August, NDVI selection results in an accurate classification of vegetation (0.98) at the expense of lower producer’s accuracy for soil compared to Otsu (0.55 and 0.87, respectively) (Table 3). On the same day, the Otsu method identified more soil pixels correctly than NDVI and have a comparable high producer’s accuracy for vegetation (0.86) (Table 3). Therefore, the  $T_c$  map derived from Otsu on the 9<sup>th</sup> of August offers a clearer picture of maize temperature, which is less contaminated by soil readings. Whilst the Otsu method performed at its best on the 9<sup>th</sup> of August 2022 (331 soil pixels correctly classified), its performance declined to 57 pixels on the 23<sup>rd</sup> of August 2022 (Table 3). Unlike Otsu separation, the NDVI method with a fixed 0.45 threshold results in an overall low but stable performance on the two dates (85 and 82 soil pixels correctly classified), which makes it a better option for extracting  $T_c$  on 23<sup>rd</sup> August 2022. Furthermore, the NDVI method performed better on the 27<sup>th</sup> of June. In this case, the NDVI producer’s accuracy for soil is 0.96 against 0.63 reported for the Otsu method (Table 3).

Table 3 Results of accuracy assessments performed on 500 random points.

| Dates                            | Class      | Otsu method         |                             | NDVI method         |                             |
|----------------------------------|------------|---------------------|-----------------------------|---------------------|-----------------------------|
|                                  |            | Producer’s accuracy | Pixels correctly classified | Producer’s accuracy | Pixels correctly classified |
| <b>27<sup>th</sup> of June</b>   | Vegetation | 0.80                | 231                         | 0.83                | 238                         |
|                                  | Soil       | 0.63                | 135                         | <b>0.96</b>         | 207                         |
| <b>9<sup>th</sup> of August</b>  | Vegetation | 0.86                | 301                         | 0.98                | 342                         |
|                                  | Soil       | <b>0.87</b>         | 134                         | 0.55                | 85                          |
| <b>23<sup>rd</sup> of August</b> | Vegetation | 0.87                | 331                         | 0.89                | 341                         |
|                                  | Soil       | 0.47                | 57                          | <b>0.67</b>         | 82                          |

Figure 3 shows the frequency distributions of greyscale pixels (A) and thermal pixels (B and C). A greyscale image is the monochromatic representation of RGB in which only pixel intensity information counts. The greyscale image is computed before the Otsu algorithm and the pixel pattern distribution dictates the success of Otsu separation. The thermal pixels are displayed before (B) and after (C) use of Otsu and NDVI methods on data acquired on the three days with UAV flight campaigns. From the comparison of Figure 3 B-C and Figure 4 B on the 9<sup>th</sup> of August, the two methods show potential for removing the pixels with higher temperature values corresponding to soil or other non-vegetative components present in the field. Both methods give a skewed right distribution of pixels representing maize canopy temperature. However, they differ in the frequency of pixels that are classified as vegetation, with the NDVI method considering more pixels as a canopy on the 9<sup>th</sup> of August. Unlike the 9<sup>th</sup> of August, on 23<sup>rd</sup> August 2022, the overall temperature distribution within field plots, which has its maximum value at 41.6°C, does not present the two typical peaks associated with left- and right-shifted vegetation and soil temperatures. The resulting  $T_c$  maps show the same range of temperatures of the overall field temperature and do not significantly differ from each other. Similar patterns to (B) can be seen in the pixels frequency distribution of the greyscale images (A) for the 9<sup>th</sup> and 23<sup>rd</sup> of August. However, this does not apply on the 27<sup>th</sup> of June, as a different distribution is found for greyscale

and thermal pixels. In (A), unimodality is reported, while in (B) thermal pixels are spread over a narrow and low-temperature range (2-17° C). The thermal pixel distribution in this range has three peaks and the highest peak is at around 13° C. The distribution is maintained after the application of NDVI and Otsu separations.



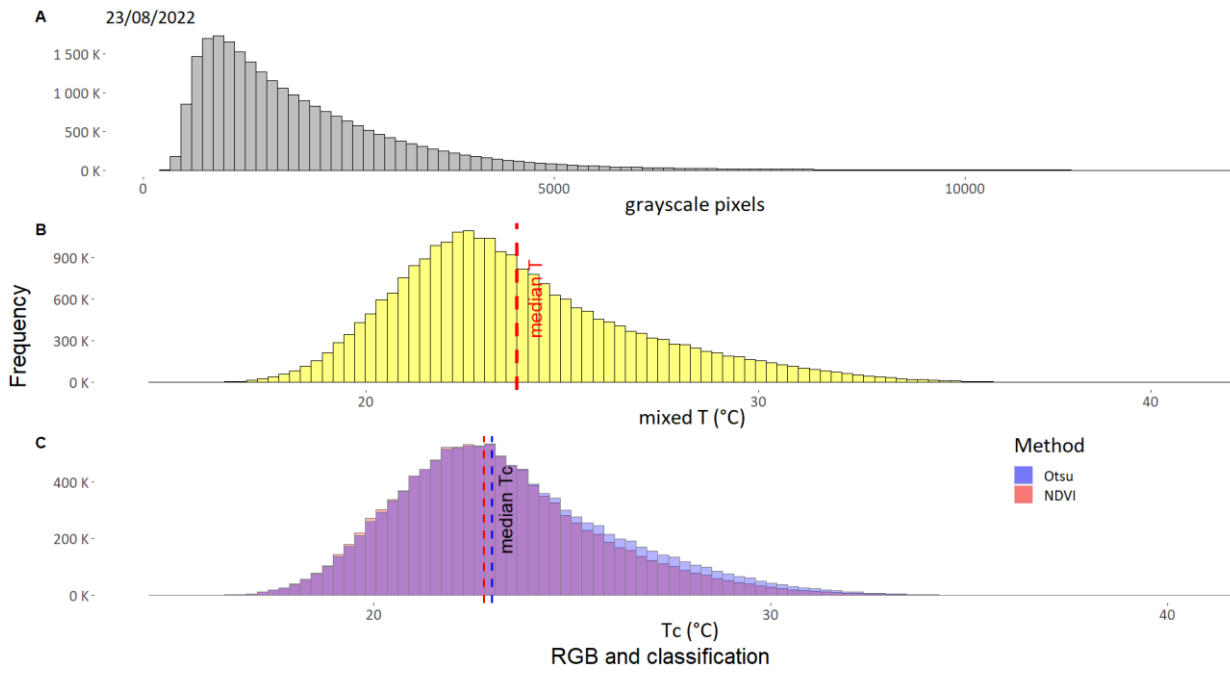


Figure 3 Grayscale pixels distribution (A) and pixel-based temperatures before soil removal (B) and after (C). Median values for mixed temperature and  $T_c$  in B and C respectively are shown as dashed line.

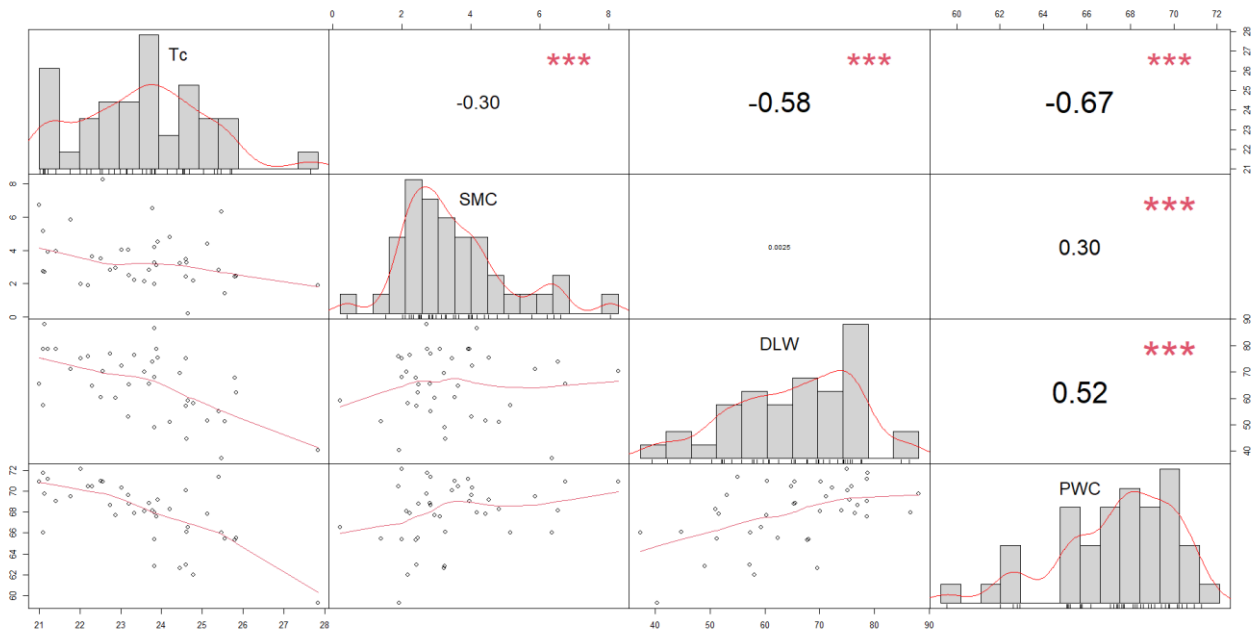


Figure 4 Zoomed RGB and NDVI-Otsu segmentation. The chosen selected method on each day is underlined (vegetation in green, soil in pink-brown. Block 3 "OR" is shown).

### 3.2 Relationship between UAV-based and ground-based measurements

From the results of correlation analysis which are shown in Figure 5,  $T_c$  negatively correlates with SMC and both biomass measurements (PWC and DLW) on all investigated days. However, the strength of this correlation varied. On the 9<sup>th</sup> of August, the relationship between  $T_c$  and PWC is the strongest (correlation coefficient=-0.67 \*\*\*), followed by DLW in the second place and SMC (correlation coefficient=-0.58 \*\*\* and -0.30 \*\*\*, respectively). These relations become overall weaker on the 23<sup>rd</sup> of August and the order changed. Similarly to the 9<sup>th</sup> of August, on the 23<sup>rd</sup>, a strong correlation between  $T_c$  and PWC is also highlighted (correlation coefficient=-0.40 \*\*\*). However, on this date SMC is reported to influence  $T_c$  more than DLW (correlation coefficient=-0.35 \*\*\* and -0.19 \*\*, respectively). Furthermore, the correlation plot shows a positive relation between SMC and PWC which was taken one day after, on the 10<sup>th</sup> of August (Figure 5 A). On the 23<sup>rd</sup> of August, the relation subsides and is not significant (Figure 5 B).

A - Reproductive phase R2 - 9/08/2022



B - Reproductive phase R5 - 23/08/2022

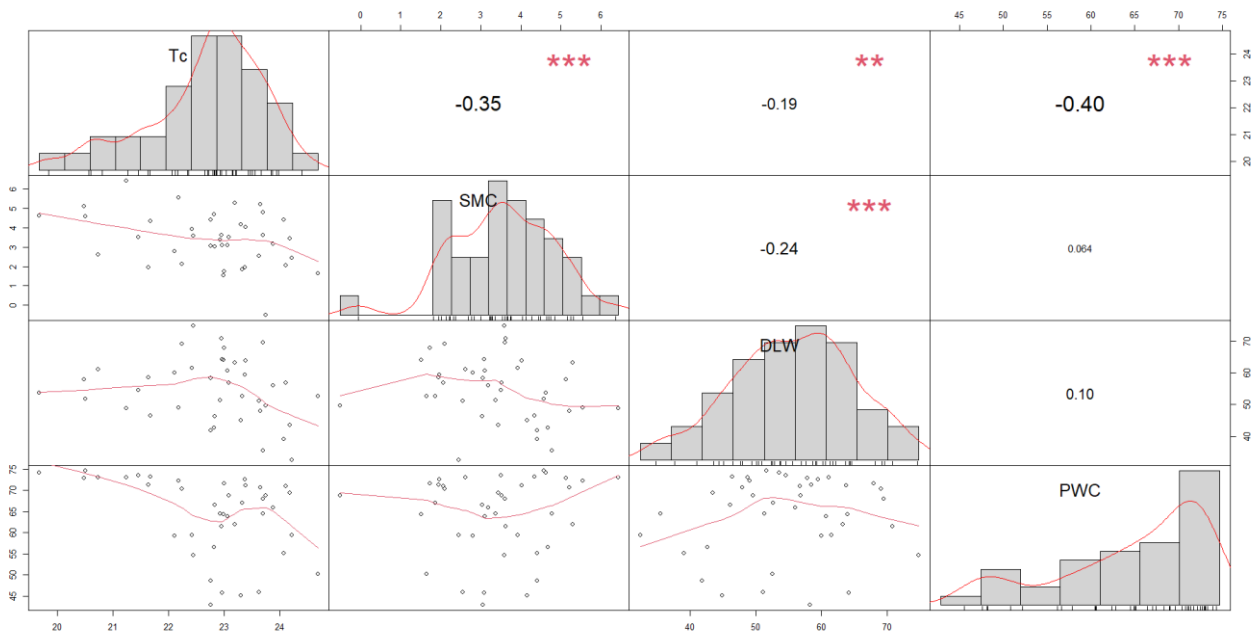


Figure 5 A and B correlation plots display the correlations on the 9<sup>th</sup> and 23<sup>rd</sup> of August, respectively. On the diagonal, the distribution of each variable is shown. Below the diagonal and above it, scatterplot with fitted lines and correlation with significance level are reported, respectively. p-values of 0.001, 0.01, 0.05, 0.1, 1 correspond to “\*\*\*”, “\*\*”, “\*”, “.”, “”.

On the 27<sup>th</sup> of June, linear regression and correlation analysis were conducted twice, with all the blocks and with the exclusion of blocks 2 and 4, which from a visual inspection present non-uniform distribution of temperature (Figure 6). When blocks 2 and 4 are considered, the correlations with  $T_c$  are weak and insignificant either between biomass measurements which were taken five days before and the SMC sampled on the day of the flight (not displayed). Nonetheless, when the readings from the two blocks are excluded from analysis, PWC and DLW become able to influence  $T_c$  to a certain degree (correlation



coefficient=-0.49 \* and -0.45 \*, respectively) (Appendix III). However, in this scenario, there is no significant correlation between SMC and  $T_c$ .

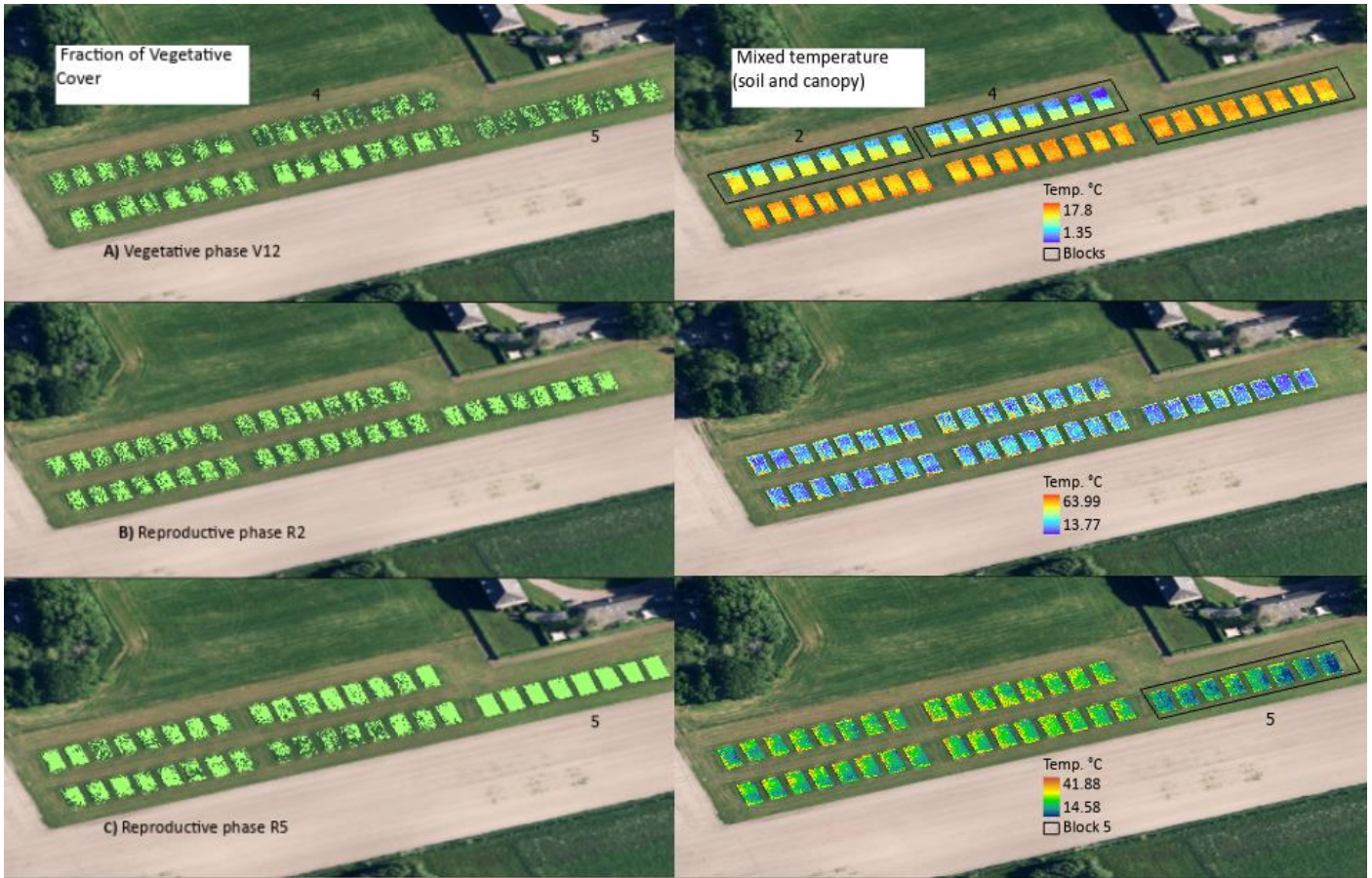


Figure 6 Canopy cover in green after application of the selected soil-pixel exclusion method (left) and thermal orthomosaic (right).

Results from linear regression are presented in Table 4, which illustrates the relationships between selected  $T_c$ -predictor variables and median  $T_c$ . Overall, on the 9<sup>th</sup> of August, there was a significant negative correlation between  $T_c$  and PWC, DLW, and SMC ( $p < 0.001$ ) with an adjusted  $R^2$  of 0.50 (Table 4). When the relationships are taken individually, the largest  $R^2$  of 0.44 is found for median  $T_c$  and PWC, while  $R^2$  of 0.33 and 0.09 for DLW and SMC, respectively (results not displayed). Interestingly, 4 out of 8 measurements from block 3 present lower PWC than the rest of the samples for the 9<sup>th</sup> of August. In particular, maize after fallow treatment has less than 60% PWC (Figure 7 B). For the range of PWC within which most of the measurements are, 4 out of 8 samples from block 5 have the lowest  $T_c$  reported for the 9<sup>th</sup> of August. Similarly to the 9<sup>th</sup> of August, a significant negative correlation between  $T_c$  and PWC, DLW, and SMC is reported for the 23<sup>rd</sup> of August, which however results in  $R^2$  of 0.26 ( $p < 0.001$ ). In the relationship between PWC, SMC, and DLW with  $T_c$ , changes of PWC show to be slightly better linearly related with variations of maize temperature (Figure 7 C). Also in this case, block 5 presents the lowest  $T_c$  reported for that day (Figure 7 C). On the 27<sup>th</sup> of June, PWC, SMC, and DLW do not impact the  $T_c$ , unless blocks 2 and 4 are excluded from the analysis (Table 4). After this step is performed, PWC and DLW become relevant in influencing  $T_c$  ( $R^2=0.44$ ,  $p < 0.001$ ) and PWC has the strongest influence (Figure 7 A). From one day to the other, a progressive shift of the PWC range to lower values is documented. However, for some measurements, such as from block 5, the PWC on the 23<sup>rd</sup> of August continues to be similar to the initial values measured on the 27<sup>th</sup> of June (Figure 7 A, B, C).

Table 4 Results of multilinear regression of PWC, dried leaves weight and SMC on median  $T_c$  derived with NDVI/Otsu algorithm.  $p$  value: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 '' 1.

| Dates                      | $R^2$ and $p$  |
|----------------------------|--|
| 27 <sup>th</sup> of June   | 0.03 / 0.44 ***<br>(with and without<br>block 2 and 4) |
| 9 <sup>th</sup> of August  | 0.50 ***   |
| 23 <sup>rd</sup> of August | 0.26 ***   |

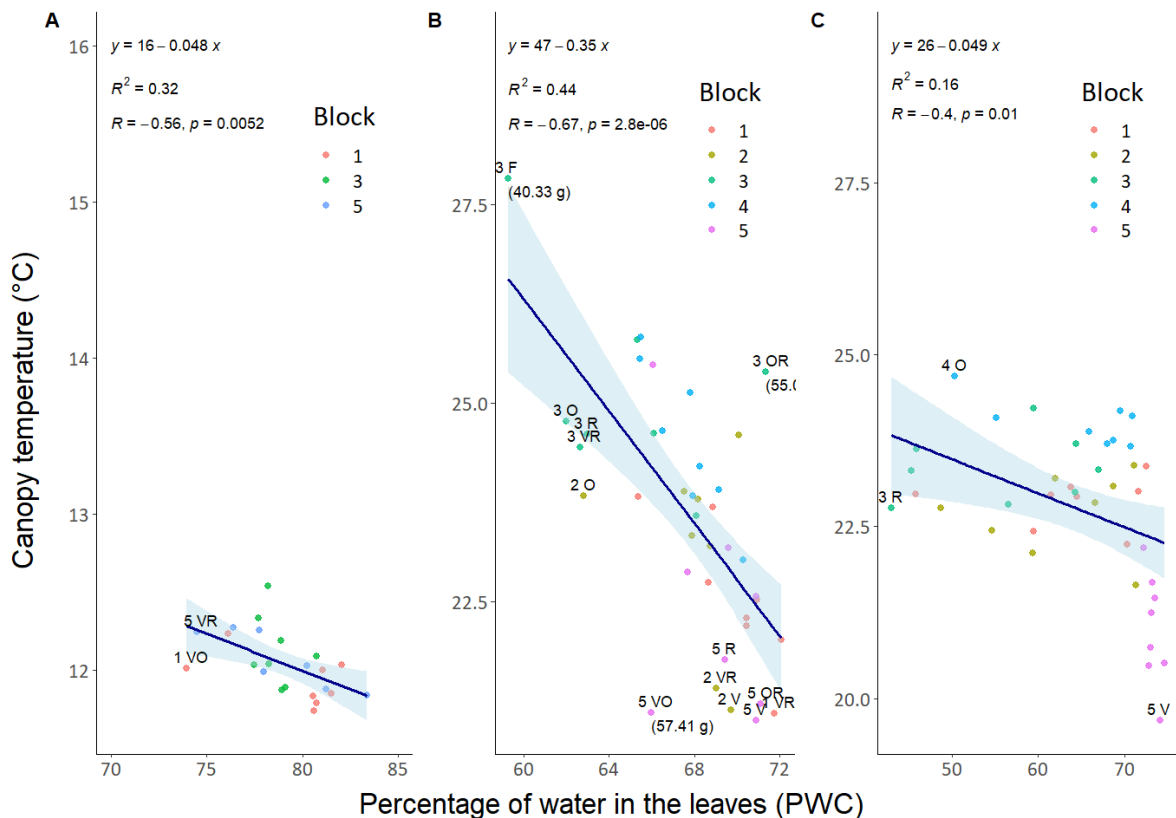


Figure 7 Pearson's correlation between median  $T_c$  and PWC for June 27<sup>th</sup>, August 9<sup>th</sup> and 23<sup>rd</sup> respectively (A, B and C). Block subdivision is highlighted in colours and treatment types are specified with their labels.  $p$  value: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 '' 1.

### 3.3 Cover crop influence on the canopy temperature – soil water content relationship

Results from two-way Anova confirm an influence of blocks for all factors on the 9<sup>th</sup> and 23<sup>rd</sup> of August (not displayed). For the two dates, treatment effect given by cover crops is generally present for all factors, except for SMC (Figure 8 and 9). In addition, on August 23<sup>rd</sup>, cover crop use does not show to influence median  $T_c$  (Figure 9). On the 27<sup>th</sup> of June, treatments influence SMC (Appendix IV) while block presence is reported to have a significant impact only on  $T_c$  and DLW (not displayed). Differences between blocks can be also visually assessed in Figure 6. On the 27<sup>th</sup> of June, block 5 and 4 present canopy gaps and larger areas of exposed soil (visible in Figure 6, on the top left), while the thermal map shows a gradient of lower to higher temperatures within blocks 2 and 4 moving from field border to inner aisle (Figure 6, on the top right). On the 23<sup>rd</sup> of August,



the vegetation in most blocks is yellow (Appendix V) with the exception of block 5 where green vegetation cover is present and accompanied by lower  $T_c$  (Figure 6, on bottom left and right).

On the 27<sup>th</sup> of June, SMC is measured to be around field capacity values (10-13%) (Appendix II), and a small but significant difference is found between treatments (Appendix IV). Vetch-oat (“VO”) results in more soil water in comparison to vetch-oat-radish (“VOR”) and radish alone (“R”). On the 9<sup>th</sup> and 23<sup>rd</sup> of August, SMC readings suggest that the field is close to wilting capacity (Appendix II) and as stated beforehand SMC values do not differ significantly between the plots previously cultivated with different cover crops (Figure 8 and 9). However, a pattern is found between  $T_c$ , PWC, DLW, and DFW for certain crops. For example, on the 9<sup>th</sup> of August for control (fallow “F”), a higher maize  $T_c$  than vetch (“V”) and vetch-radish (“VR”) is associated with reduced dried leaves and fruit weight in comparison to vetch-radish (Figure 8 C, D and E) (significant difference ( $p < 0.05$ ) between treatments is indicated with the letter “a” and “b”). At the same time, a lower  $T_c$  for maize grown after vetch relates with a significantly higher PWC than that found in maize after oat (“O”) (Figure 8 A and B). The latter one, when mixed with radish, does not differ from vetch alone in terms of PWC (Figure 8 A). A positive correlation between moisture in the leaves and DLW/DFW is found for oat-radish and oat, respectively (Figure 8 A, D, and E).

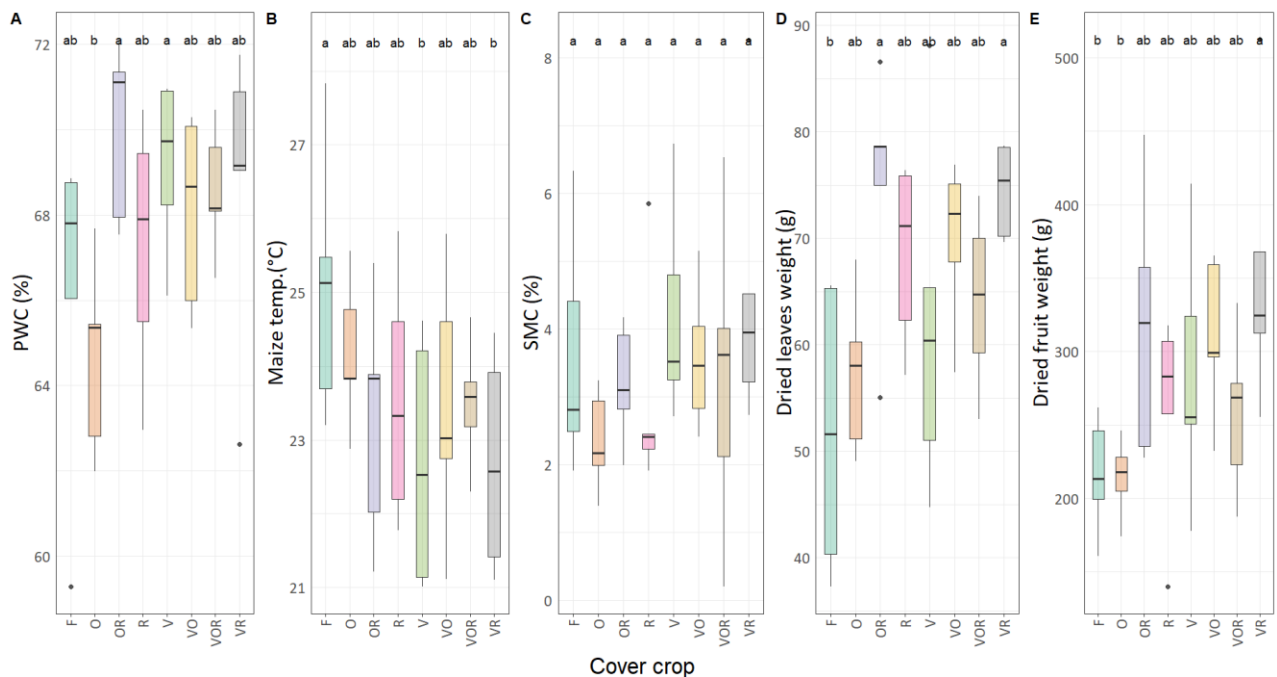


Figure 8 The distributions of leaves moisture, maize  $T_c$ , soil moisture content, dried leaves weight and dried fruit weight of maize after 8 cover crops treatment for the 9<sup>th</sup> of August. Lowercase letters indicate significant differences between treatments and a different letter indicate significant differences between treatments ( $p < 0.05$ ).

On the 23<sup>rd</sup> of August, vetch and oat-radish maintain the significantly higher PWC found on the 9<sup>th</sup> of August, which is accompanied in this case by higher DFW for the oat-radish combination (Figure 9 A and E). As it was reported on the 9<sup>th</sup>, on the 23<sup>rd</sup> of August oat alone was associated with lower PWC and DFW in maize. For

the first time on the 23<sup>rd</sup> of August, the combination of the 3 cover crops (“VOR”) led to a significant increase in PWC and DFW (Figure 9 A and E).

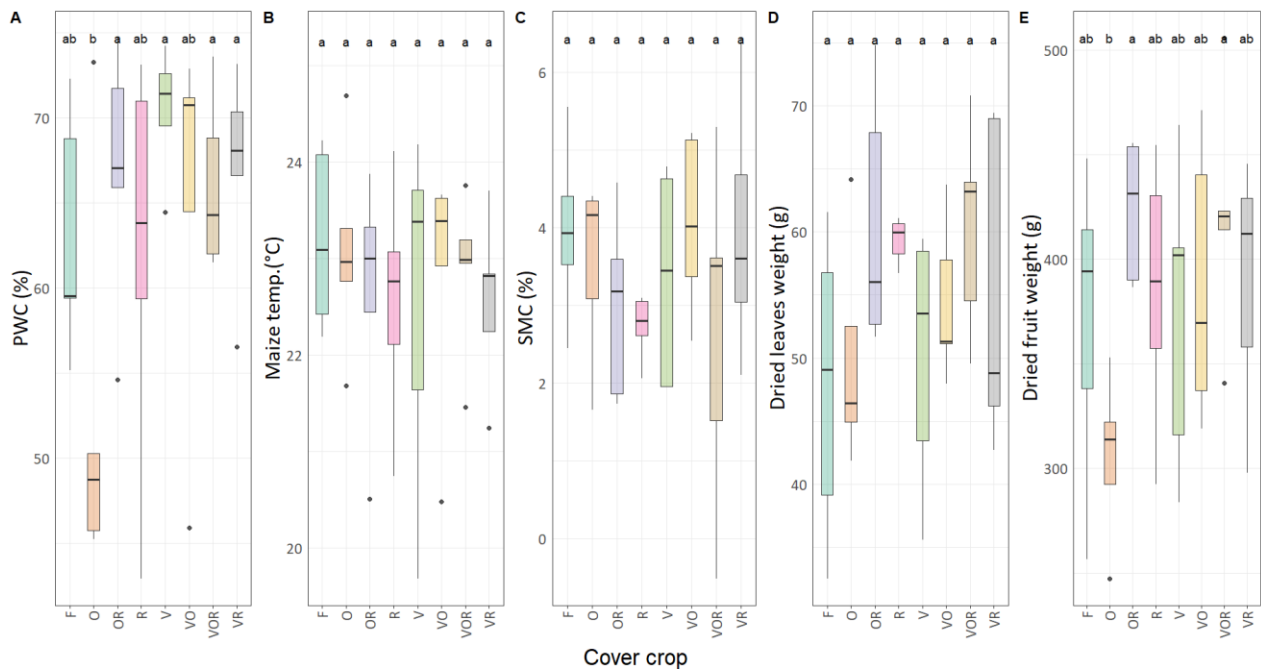


Figure 9 The distributions of leaves moisture, maize  $T_c$ , soil moisture content, dried leaves weight and dried fruit weight of maize after 8 cover crops treatment for the 23<sup>rd</sup> of August. Lowercase letters indicate significant differences between treatments and a different letter indicate significant differences between treatments ( $p < 0.05$ ).

## 4 Discussion

### 4.1 Soil pixels removal - NDVI and Otsu algorithm accuracy for soil exclusion and vegetation selection

In this paragraph, the effectiveness of the Otsu algorithm in excluding soil pixels is examined by analysing how different factors, such as maize size and colour, affect its performance at various stages of maize growth. Subsequently, the accuracy of NDVI is discussed in relation to these findings.

In general, Otsu is frequently employed for image segmentation because of its straightforward algorithm and high level of automation. However, Otsu performs well in segmenting images with bimodal variance between classes, which may not always be present (Song et al., 2022). On August 9<sup>th</sup>, the bimodal variance was observed in the greyscale image obtained from RGB, allowing for the application of Otsu. In contrast, its absence on other dates hindered its effectiveness (Goh et al., 2018). Goh et al. (2018) also found that image parameters such as intensity level between vegetation class and background, vegetation size, vegetation position, and noise can impact the performance of Otsu thresholding. This was evident in the imagery from August 23<sup>rd</sup>, where vegetation intensity was very dim and close to background intensity (Appendix V), resulting in confusion between yellow leaves and bright soil. Additionally, the image was noisy and contained regions with different intensities, which may have affected the segmentation due to Otsu using the intensity of the entire image, potentially leading to local colour imbalances.

On the other hand, unlike on August 23<sup>rd</sup>, Otsu's accuracy on June 27<sup>th</sup> may have been limited by the inverted size of the foreground and background. In this case, the background represented by soil was predominant over maize as the crop was in the starting phase V12, which was especially noticeable in blocks 5 and 4 where vegetation growth appeared stunted and scarce (Figure 6, top left). Furthermore, sunlit leaves, which appeared brighter, were seemingly assigned to the background class, increasing the frequency of greyscale pixels for this class (Figure 3).

Although Otsu thresholding may not be effective in extracting soil at the beginning and end of maize growth, it performs reasonably well in detecting vegetation on the three dates. The producer's accuracy for vegetation, which ranges between 80% to 87%, remains high throughout the summer, including the early growth stages, as reported by García-Martínez et al. (2020). Similarly to this thesis, García-Martínez et al. (2020) found vegetation cover estimations with errors of less than 5% in the early stages of maize development. During the crop's growth, García-Martínez et al. (2020) observed that applying Otsu after using the Excess Green index (ExGI) resulted in errors ranging from 2.2% to 17.8% in estimating the vegetation cover fraction. In the case of a complex photo background with soil and weeds, Meyer and Camargo Neto (2008) reported an accuracy of approximately 50% for ExG and Otsu in detecting vegetation, while accuracy reached 88% in the case of a simple background with bare soil. Song et al. (2022) also found that the accuracy of ExGI plus Otsu was approximately 50%, which is substantially lower than the accuracy found in this study for all three days. García-Martínez et al. (2020), Meyer and Camargo Neto (2008), and Song et al. (2022) use the Excess Green index on which Otsu thresholding is applied, instead of the more common greyscale intensity image obtained from RGB. The ExGI index uses the contrast between the green, red, and blue portions of the spectrum to differentiate between vegetation and soil (Camargo Neto and Meyer, 2005; Vidović et al., 2016). Studies have shown that it performs better than other indices that rely only on RGB to distinguish vegetation (M. Woebbecke et al., 1995; Vidović et al., 2016). Specifically, the ExGI index is more effective in identifying weeds from non-vegetation backgrounds at a significance level of 0.05, albeit with higher computation requirements than other methods (M. Woebbecke et al., 1995). From comparison of results using ExGI (García-Martínez et al., 2020; Meyer and Camargo Neto, 2008; Song et al., 2022) and this thesis, the variant ExGI index is likely to have similar accuracy in estimating vegetation cover compared to Otsu applied to greyscale orthomosaics. This is true when weeds are not the focus of the analysis. However, if the study requires an accurate analysis of maize by excluding weeds along the plot borders, the ExGI index may be more appropriate than greyscale intensity.

Despite the high accuracy of Otsu on greyscale in selecting vegetation, NDVI results in even higher accuracy, ranging between 83% to 98% for the three days. On June 27<sup>th</sup>, sunlit leaves tended to be misclassified as soil by Otsu algorithm, resulting in a reduced level of plant identification accuracy for Otsu compared to NDVI. For the other two days, shadows were present as the UAV campaigns were conducted in the afternoon. In the absence of complementary techniques like Hue Saturation Intensity, NDVI tends to classify more shaded pixels as vegetation (Cai et al., 2010) and darker green pixels with lower temperatures are considered as canopy in our ground truth.

Since the initial stages, both Otsu and NDVI exhibited effective performance in vegetation selection. However, on August 23<sup>rd</sup>, NDVI could have achieved improved vegetation detection if the threshold value was set lower than 0.45. This is because in the later stages, some vegetation turns yellow, and accurate classification requires lower threshold values for NDVI. Generally, the histogram representation of thermal pixels reflects the typical temperature differences between soil and vegetation (Jones and Sirault, 2014), suggesting that TIR data could be used to fine-tune the NDVI threshold. As a recommendation for future research, incorporating initial temperature representation could optimize the separation process.

Additionally, incorporating thermographic information after applying the selected methods could provide an overview of the effectiveness of the separation process.

## 4.2 Relationships between ground measurements and thermal maps on field level

As hypothesized, this study shows that ground measurements of crop water stress are related to  $T_c$ . Correlation analysis results reported in Figure 5 indicate that on the 9<sup>th</sup> and 23<sup>rd</sup> of August,  $T_c$  negatively correlated with SMC, PWC, and DLW, suggesting that lower soil water content increases  $T_c$  through stomata closure. Similar effects were observed with the presence of water in the leaves (PWC) and the addition of vegetative layers represented by DLW. In the latter case, the total transpiration of a maize plant results from the sum of transpiration occurring in each leaf, indicating an additive effect. The choice to use DLW instead of the fresh weight of the leaves is because PWC already accounts for water content, and a measure of leaf weight alone or growth was needed. Acorsi and Gimenez (2021) used fresh biomass along with grain yield. Pearson's correlation analysis between these plant variables and  $T_c$  showed negative correlation values of 0.56 and 0.45, respectively, which are similar to values reported in previous studies on maize by Zia-Khan et al. (2012). In contrast, on the 27<sup>th</sup> of June, the correlations between biomass variables and  $T_c$  were weak and insignificant (correlation coefficient below 0.1) due to the passage of a cloud, which rendered 16 of 40 measurements unusable. The deviation of several point measurements in a small dataset may cause a divergence from the true relationship, which may not be observed for this reason. However, when diverging readings from blocks 2 and 4 were removed from the analysis (Table 4 and Figure 7), variations in PWC and DLW became significantly linearly related to  $T_c$  also on this day. The relationships may not be strong due to the 5 days between ground measurements and the UAV campaign. However, due to the isohydric nature of maize, it is expected that PWC may not have changed considerably within the five days. Maize maintains a stable leaf water status over a wide range of evaporative demands or soil water supplies (Ihuoma and Madramootoo, 2017; Tardieu and Simonneau, 1998), and therefore, a sudden change in soil water is not reflected in fast water uptake. In the opposite situation, maize steadily maintains leaf water content to a certain extent, as reported in this case. Regarding the combination of DLW- $T_c$  on the 27<sup>th</sup> of June, the significant negative relationship found can be attributed to the voluminous and highly transpiring biomass that is typical of the V12 stage of maize growth (Nleya et al., 2019). However, substantial changes may have occurred between the days of biomass sampling and thermal data collection, which could impact the strength of the relationship. Therefore, the relationship between DLW and  $T_c$  could have been stronger if DLW measurements were taken on the same day as the UAV flight.

### 4.2.1 Soil water content- $T_c$

Among the relationship found, correlation analysis confirms a weak negative correlation between  $T_c$  and SMC on the 9<sup>th</sup> of August, which becomes stronger on the 23<sup>rd</sup> of August, as shown in Figure 5. On the 23<sup>rd</sup> of August, it is possible that DLW did not increase any longer, and the current biomass could not be highly transpiring, resulting in a lower impact of PWC on  $T_c$ . At this point of maize growth, SMC could not be obscured anymore and play a role in  $T_c$  changes. However, SMC still explains little of the observed  $T_c$  variations. The finding that SMC has little influence on  $T_c$  on the 9<sup>th</sup> and 23<sup>rd</sup> of August contradicts the results of a study by González-Dugo et al. (2006), in which the standard deviation of canopy temperature ( $sdT_c$ ) was compared to CWSI, a well-known parameter used for irrigation scheduling and  $sdT_c$  was found to reflect SMC ( $R^2=0.77$ ). Han et al. (2016) and González-Dugo et al. (2006) suggest that  $sdT_c$  could be used as an indicator of water stress due to its significant relationship with soil water deficit (SWD). However, in this thesis, median  $T_c$  was adopted instead of  $sdT_c$  to ensure that only vegetative pixels were used. Nevertheless, no evidence of sensitivity to water deficit was found for median  $T_c$  in this study, which contrasts with previous research on

sd $T_c$  as previously described and studies using mean  $T_c$ . For example, Acorsi and Gimenez (2021) found an  $R^2$  of 0.42 between the mean  $T_c$  and soil water status, which substantially increased to  $R^2=0.88$  when soil physical attributes were introduced. According to the variable importance classification,  $T_c$  ranked second among the 11 attributes included in the model for predicting water content (Acorsi and Gimenez, 2021; DeJonge et al., 2020). In Zhang et al. (2019), the use of different irrigation systems, resulting in a wider range of soil water contents, led to even higher  $R^2$  values (between 0.40 and 0.53) for linear regression models between SMC at 0-0.2 m soil depth and  $T_c$ . In this thesis, there was no artificial irrigation, and the SMC observations were distributed over a narrow range of values, which may have unfavourably resulted in lower correlation values for the multilinear regression models.

Little effect of SMC on  $T_c$  at later stages may also be due to variations in water uptake in the soil profile. Although maize typically relies on water from shallow soil depths taken up by lateral roots (Ahmed et al., 2015), de Lara et al. (2019) found that maize yield has a significant relationship with deeper SMC readings (90 to 150 cm) later during the growing season. Similar trends were detected by Hupet and Vanclooster (2002), whose research demonstrated that well-developed maize crops source water from deeper soil layers (50 to 100 cm). In our study, the SMC sensor is fixed at a shallow depth, and may not be able to detect changes in water demand by deeper roots occurring at later stages. The water that potentially influences  $T_c$  on the 9<sup>th</sup> and 23<sup>rd</sup> of August may be located at deeper depths that are not considered by the SMC sensor. This fact may partially explain the absence of a significant relationship between SMC and  $T_c$  on the 9<sup>th</sup> and the weaker significant relationship between  $T_c$  and SMC on the 23<sup>rd</sup> of August. However, previous studies have indicated that around 40% of soil water extraction for maize still occurs in the first 25 cm depth, justifying the experimental setup (Zhang et al., 2019). As highlighted by Zhang et al. (2019), water uptake from superficial soil layers is seen in vegetative phases, and they reported significant correlations between  $T_c$  and SMC measured at depths of 10, 20, and 30 cm, and no significant relationships at depths of 45, 60, and 90 cm. Additionally, the insignificant and weakly significant  $T_c$ -SMC relationship on the 9<sup>th</sup> and 23<sup>rd</sup> of August, respectively, may also be influenced by the fact that on both days, the observed SMC values were close to the wilting point. Wilting point was likely due to the dry season and the low water holding capacity of sandy soil. Consequently, there was little water in the soil for plant uptake.

Based on the results of this study, thermography using median  $T_c$  cannot be considered a reliable proxy for SMC during the growing season of maize. However, recent research has shown the potential of RGB alone in assessing soil moisture. For instance, Lu et al. (2020) demonstrated that surface soil moisture (0-10 cm) in a mixed soil-vegetation setting can be estimated using the brightness of UAV visible images. While the application of RGB brightness is a novel concept, optical vegetation indices are a well-documented technique for SMC estimation. However, unlike thermography, vegetation indices capture past changes that occurred gradually in leaf pigments and do not provide real-time information. In their study, Wang et al. (2018) combined the advantages of vegetation indices and thermal information to estimate root-zone SMC in different soil-vegetation conditions, achieving accurate results ( $R^2=0.58-0.69$  and RMSDs around  $0.025 \text{ m}^3 \cdot \text{m}^{-3}$ ) using the "temperature-vegetation triangle approach".

#### 4.2.2 Biomass- $T_c$

As crop growth progresses, there is a documented shift in the range of PWC towards lower values, from 70-85% on June 27<sup>th</sup> to 60-75% and 40-75% on the other two days, respectively. This suggests that maize leaves are maturing and approaching senescence. However, measurements from block 5 show that PWC on August 23<sup>rd</sup> remains similar to the initial values measured on June 27<sup>th</sup>, as mentioned in 0. This could be attributed to the later establishment of maize in block 5. Notably, at the time of harvest, NDVI of the entire block appears to have higher values, indicating good overall vegetation coverage and noticeable photosynthetic

activity, as evidenced by the lower  $T_c$  for that block (Figure 6, bottom right). Photosynthetic activity is closely related to SMC and PWC. In fact, increased soil water availability generally leads to increased stomatal conductance, allowing for transpiration and consequent cooling, as well as  $CO_2$  assimilation for photosynthesis (Rossini et al., 2015). Results by Feng and Zhou (2018) indicate that maize PWC and SMC significantly affect the net photosynthetic rate in all developmental stages, which in turn is closely related to fruit yield and biomass growth (Rossini et al., 2015; Zhou et al., 2021), consistent with findings by Junior et al. (2021). Junior et al. (2021) reported that at 30% irrigation compared to 150% setting, only half of the stomatal conductance was observed, resulting in limited cooling of leaves. In this study, the SMC values found on August 9<sup>th</sup> and 23<sup>rd</sup> (maximum of 8%) correspond to the 30% irrigation condition in Junior et al. (2021), while the range of SMC values found on June 27<sup>th</sup> (around 10-14% SMC) can be compared to the 150% irrigation condition. Junior et al. (2021) also found that the highest net  $CO_2$  assimilation rates, stomatal conductance, and transpiration rates were observed under the highest soil water availability conditions. Similarly to the limited water availability situation described by Junior et al. (2021), the reported  $T_c$  on August 9<sup>th</sup> and 23<sup>rd</sup> is likely a result of stomatal closure, indicating possible water stress in the crops. On August 23<sup>rd</sup>, the absence of bimodality in thermal pixels, as shown in Figure 3, suggests that the maize may no longer be actively transpiring or photosynthesizing, as its temperature is similar to that of the soil. Furthermore, the observed curled yellow leaves on August 23<sup>rd</sup> may also indicate dryness, as the PWC on that day does not strongly affect  $T_c$ . In contrast, on June 27<sup>th</sup>, when the soil is reported to be at field capacity, the canopy may still be actively transpiring and cooling down through stomata action. Furthermore, in V12 stage, high foliage leads to increased surface conductance, resulting in higher evapotranspiration, energy dissipation, and lower  $T_c$ , as reported by Ekinzog et al. (2022). Tall and dense canopies also attenuate radiation and reduce  $T_c$ , according to Westreenen et al. (2020). Through these mechanisms, biomass affects  $T_c$  in all stages, except during maturity when the DLW remains constant.

Besides the findings about SMC and leaves transpiration, Junior et al. (2021) indicated that the physiological indicators considered (stomatal conductance, transpiration, and net  $CO_2$  assimilation rate), as well as NDVI, correlate with CWSI, suggesting that NDVI could be used as an alternative to assess maize water stress ( $R^2 = 0.763$ ;  $p < 0.001$ ). Similarly, in Appendix VI, NDVI is shown to have a strong correlation with median  $T_c$ , indicating that it could be used not only to exclude soil pixels but also as a support for assessing maize water stress in conjunction with  $T_c$ . Recent methodologies have investigated integration of vegetation and temperature indices. Among these approaches, the temperature vegetation dryness index (TVDI), which is derived from land surface temperature (LST) and the NDVI, has gained widespread popularity to estimate soil moisture and vegetation water content (Wang et al., 2011).

In this study, we found that  $T_c$  can be used as a proxy for crop water status (PWC), but it is also influenced by DLW during growth. Incorporating NDVI as a normalization factor for  $T_c$  can offer advantages in assessing crop water stress, as it enables real-time monitoring of photosynthetic activity changes with higher temporal resolution compared to structural parameters such as DLW. Furthermore, previous reports by Wang et al. (2011) and Wang et al. (2018) have demonstrated NDVI's capability in estimating both SMC and PWC, indicating its potential to enhance the model when used with  $T_c$ .

Moreover, besides adding NDVI, the removal of SMC from the model may lead to an improvement, as it provides minimal or negligible information. Another rationale for excluding SMC is the interactive effect between PWC and SMC on August 9<sup>th</sup> (Figure 5 B). However, multicollinearity was not detected, as evidenced by VIF values close to 1 (Appendix VII).

## 4.3 Cover crop influence on the canopy temperature – soil water content relationship

### 4.3.1 Block effect

The role of soil organic matter (SOM) in influencing crop growth and water retention is explored in this paragraph through an analysis of its impact on various factors at different stages of maize growth, revealing the significance of SOM differences between blocks and their effect on crop establishment, development, and water retention. The prior knowledge about an existing SOM gradient in the field provides some explanation for our findings (Appendix I). The difference in SOM between blocks is found to be responsible for a significant difference for all the considered factors on all dates, except for SMC, DFW, and PWC on the 27<sup>th</sup> of June. In the beginning, on the 27<sup>th</sup> of June, maize may be facing equal conditions in all blocks, which start diverging with crop growth, kernel formation, and changing climatic factors due to SOM differences. In fact, on the 27<sup>th</sup> of June, the young thriving maize, which presents comparably high PWC in all blocks, is in the initial phase of cob development. Cob constitutes a fixed part of the DFW which is added to a variable kernel weight forming in the following phases. Besides, SMC in the starting phase ranges around higher values (field capacity) for all blocks and the SOM gradient effect in retaining water may not play a role. SOM's abilities to increase water retention in coarse sandy soils may start to be effective in more limited SMC conditions which are reported in August. According to Lal (2020), a small increase of SOM along the field leads to an increase in water holding capacity and stored water. The higher stored water in block 5 may be responsible for later maize establishment but at the same time may be the reason for healthier and transpiring vegetation observed in late August. As Tian et al. (2022) describe, with improved water content there is often lower soil temperature which leads to retarded germination rate and crop growth.

### 4.3.2 Treatment effect

*(Expected) effect on SMC by treatments through soil structure and SOM addition mechanisms*

The contribution of SOM within the field appears to have a greater impact on SMC compared to the expected SOM contribution from cover crop treatments. This is because variations in organic matter levels between blocks led to the observed effect on SMC, while treatments (except for "VO" on June 27<sup>th</sup>) did not show a significant influence. One documented mechanism by which cover crops are expected to contribute to changes in SMC is the addition of biomass, which is decomposed into organic compounds upon cover crop termination (Dabney et al., 2001; Koudahe et al., 2022). However, results from this study and Acharya et al. (2022) suggest that 6 and 5 years of repeated cover crop use may not have a significant impact on SOM storage.

Another mechanism by which cover crops could impact SMC is through the varying rooting system, which leads to different degrees of soil structure improvement (Koudahe et al., 2022). The use of cover crops in non-irrigated systems can be motivated by the potential for higher SMC after cover crop utilization, which is particularly desirable during the initial phase of cash crop growth. However, for reasons that are not identified, only on June 27<sup>th</sup>, the "VO" treatment contributed significantly higher SMC compared to "VOR" and "R". The treatment effect on SMC found on June 27<sup>th</sup> does not persist and remains limited to the initial phase.

The finding of no significant effect of cover crops on SMC or finding it only on one date suggests that there may be factors hindering this relationship, such as tillage. Araya et al. (2022) reported that soils under no-till and cover cropping systems improved soil structure in terms of pore size distribution, while changes in hydraulic conductivity under these systems led to increased infiltration rate and water retention. Tillage for terminating the cover crop and incorporating it may have compacted the soil, reducing pore size distribution and disturbing the soil structure improvement operated by cover crop roots. Soil structure is also conditioned by soil aggregates, and aggregates form and stabilize more easily in the presence of increasing levels of SOM

(Celik, 2005). Tillage may alter aggregate formation through the mechanical destruction of organic matter, which becomes more exposed to chemical and biological decomposition. Tian et al. (2022) indicate that no-tillage could be the solution for enhancing SOM, especially in sandy soil where soil aggregation is more difficult to obtain (Ayoubi et al., 2020). Restovich et al. (2022), in their no-till setup, demonstrated that the presence of SOM-enriched soil after 5-year cover crops, accompanied by stable soil aggregation due to a higher proportion of macropores and mesopores over 300  $\mu\text{m}$ .

As previously mentioned, the higher SMC in the "VO" treatment during vegetative development on June 27<sup>th</sup> does not seem to bring any advantages over the other treatments. Even though higher water provision during the most demanding phase would be expected to favour maize growth in the following phases (de Lara et al., 2019), the "VO" treatment does not show any clear benefits in terms of maize growth and transpiration

#### *Effect on biomass by treatments*

The effect of some treatments on PWC and biomass growth can be seen on the 9<sup>th</sup> and 23<sup>rd</sup> of August. These effects are reflected in different maize  $T_c$  on the first day. Treatment "V" maintains a higher PWC over the two days which is accompanied by a significantly lower  $T_c$  on the 9<sup>th</sup>. When vetch is combined with radish to form "VR", there is an additional fertilizing effect seen as an increase of DLW and DFW, which however is not relevant for the final yield on the 23<sup>rd</sup> of August.

In general, legumes such as vetch result in a larger contribution of soil inorganic N compared to non-legume residues due to fast decomposition and nitrogen (N) release (Blanco-Canqui and Ruis, 2020; Koudahe et al., 2022; Piva et al., 2021). Furthermore, maize can benefit from additional N availability given by atmospheric N fixation during legume growth (Piva et al., 2021; Thapa et al., 2022). Elhakeem et al. (2023) showed how pure radish or in mixtures could increase N availability for the following crop by reducing N leaching up to 70% through a rapid uptake in autumn. Subsequently, residues from radish were reported to mineralize quickly, resulting in an increase of soil mineral N in spring at the time of maize sowing (70–110% more than fallow).

If "V" and "VR" show to contribute positively to maize, "O" is not helping the crop physiology and in addition, this treatment may have a more deteriorating effect on maize than fallow. Fallow, with an accompanying rise in  $T_c$  during growth, may result in delayed growth as observed on the 9<sup>th</sup> day. Despite this initial delay, the growth of maize after fallow is comparable to most of the treatments on the 23<sup>rd</sup> of August. Similarly, "O" treatment starts showing lower PWC associated with reduced DFW on the 9<sup>th</sup>, and this effect persists till the end, in accordance with Piva et al. (2021). Thapa et al. (2022) reported that oat, a grass cover crop with a high C:N ratio resulting in a lower mineralization rate, ultimately provided higher levels of SOC and total N compared to other cover crops. In this case, slower mineralization may be mismatched with maize growth and cash crop may not be benefitting from any of the positive effects of oat on the soil. Nitrogen and water are the major requirements for maize (Rafique, 2020), and oat treatment on the 9<sup>th</sup> and 23<sup>rd</sup> of August may be lacking both in different degrees. Since plant response to water stress is expressed by a variety of physiological changes such as leaf water content, but also biophysical changes as biomass and yield (de Lara et al., 2019; Khatun et al., 2021), it may be possible that maize after oat experiences water stress recognizable by low PWC and DFW. From the moment crop water stress occurs on the 9<sup>th</sup> and 23<sup>rd</sup> of August, which are the later stages between flowering to the grain filling, yield formation rather than biomass growth is affected. However, with the association of "O" with "VR", the negative effects of oat may be averted. On the 23<sup>rd</sup> of August, this treatment is the only one which gives maize an advantage in terms of higher PWC and fruit yield. As Thapa et al. (2022) suggest, a mixture of legume, grass species, and brassica ("VOR") could complement each other and provide all necessary and positive aspects for improving soil biological, chemical, and physical



properties (Dabney et al., 2001; Elhakeem et al., 2023). Despite the variability in crop yield responses, the results of this treatment suggest that cover cropping has no significant effect on SMC and SOM levels, which aligns with the findings of Acharya et al. (2022) who observed similar conclusions after a 5-year study of limited-irrigation cover crop use. Furthermore, Pavinato et al. (2017) emphasized in a shorter 3-year experiment that cover crops were unable to influence maize yield, highlighting the importance of longer-term studies to accurately assess the effects of cover cropping on maize aboveground production.

## 5 Conclusions

This study investigated the correlation between canopy temperature ( $T_c$ ) obtained from thermal infrared (TIR) sensors on unmanned aerial vehicles (UAVs) and ground-based measurements used in crop water stress assessment, such as dried leaves weight (DLW), plant water content (PWC), and soil moisture content (SMC). Additionally, the study examined the influence of cover crops on the relationship between  $T_c$  and ground-based measurements, and compared the effectiveness of the Otsu algorithm and NDVI in removing soil pixels from TIR data.

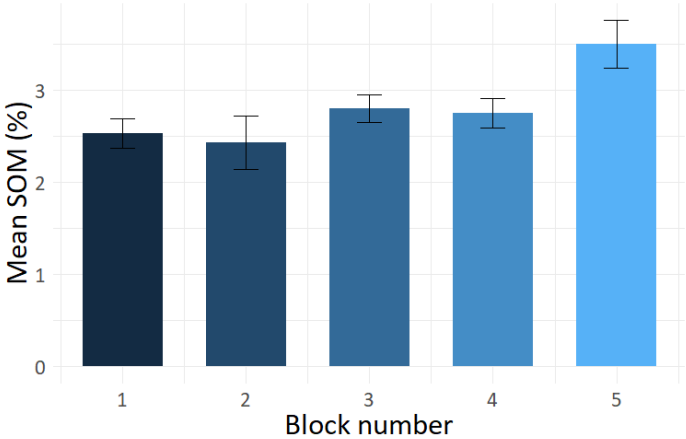
The results revealed that the effectiveness of the Otsu algorithm in removing soil pixels varied depending on the date of analysis, as it was influenced by environmental conditions and the growth stage of maize. Specifically, Otsu performed better than NDVI in classifying soil when vegetation was established but not yet ripened, while NDVI showed higher performance in the early and maturity phases. After employing the most precise soil removal technique on each day,  $T_c$  was determined to be an indicator of crop water status (PWC). In addition, the influence of DLW on the  $T_c$ -PWC relationship was found and should be considered, particularly during stages of active crop growth, when DLW varies substantially, and the additive effect of multiple transpiring leaves becomes evident. Interestingly,  $T_c$  was assessed to be a non-significant predictor of 0-12 cm SMC in this model.

The repeated cover crop use and tillage did not result in any improvement on SMC retention compared to the control group. However, differences in SMC and  $T_c$  were observed with an unrelated soil organic matter (SOM) gradient in the field. Plant-soil feedback was evident with pure grass species, and the negative effect of oats was expressed through physiological and biophysical changes in maize (decrease of biomass and PWC, while higher  $T_c$ ), likely linked to nitrogen deficiency and water stress.

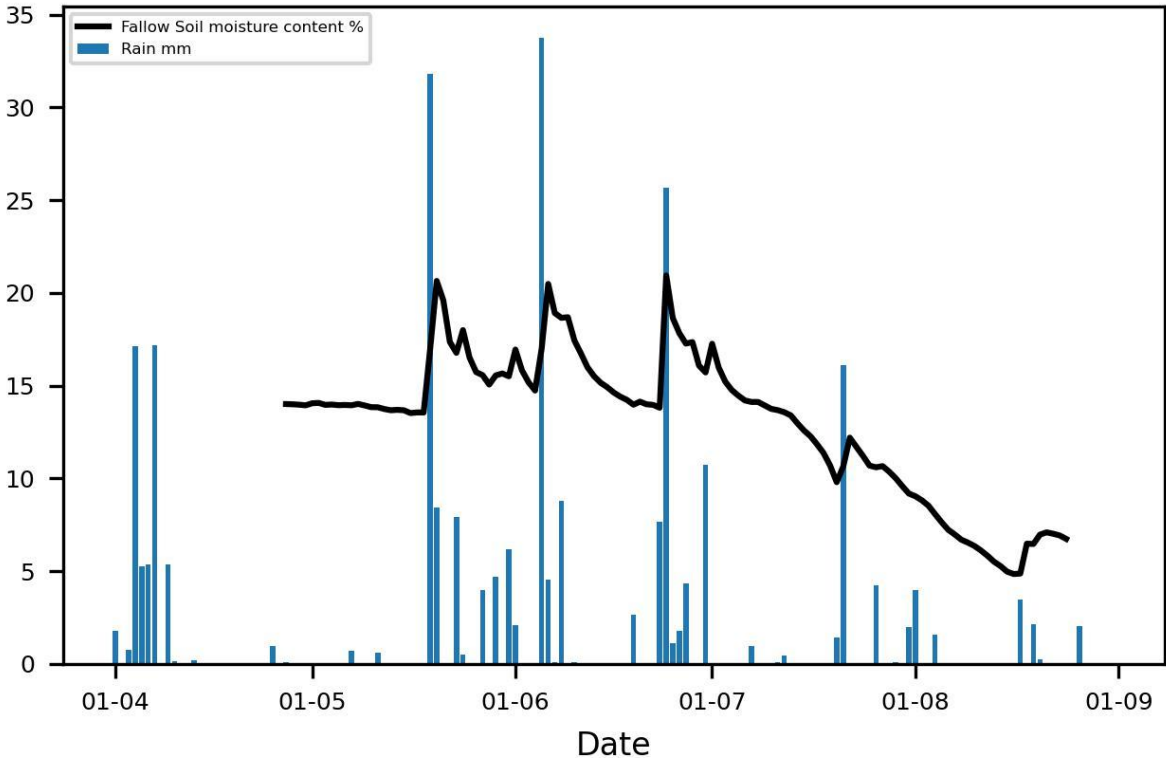
In conclusion,  $T_c$  derived from TIR sensors on UAVs has the potential to serve as an alternative to PWC for evaluating the water status of maize plants, highlighting the promising role of remote sensing as a valuable tool for detecting water stress in crops. However, further research is needed to explore the use of NDVI as a normalization factor for  $T_c$  for better understanding of the impact of long-term cover crops on the soil water content in no-tillage conditions.

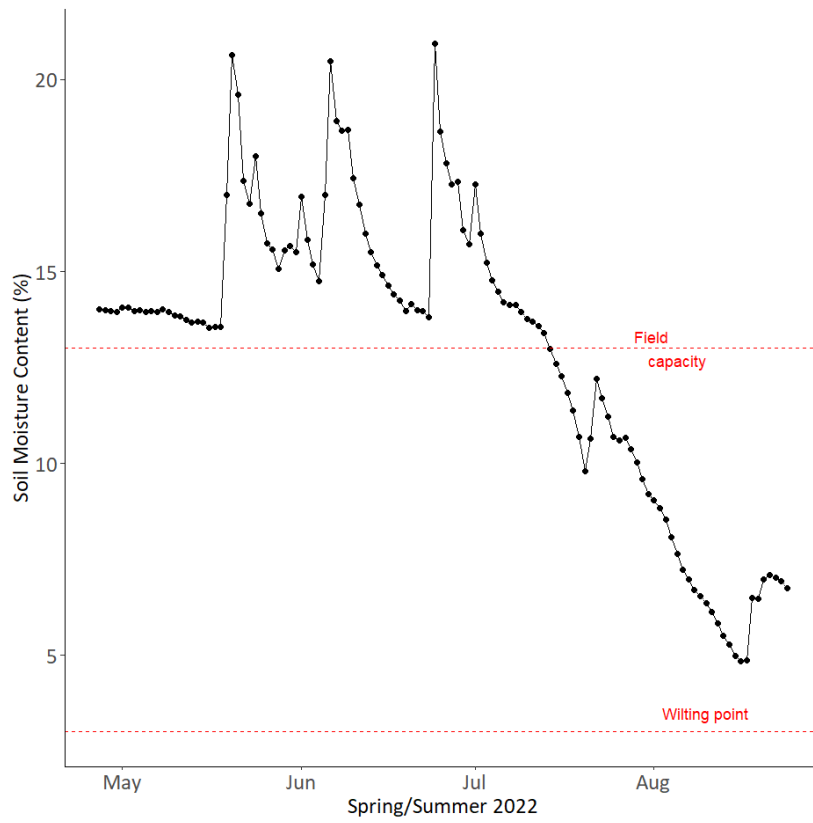
# Appendix

APPENDIX I - SOM readings in November 2021, during cover crop growth.

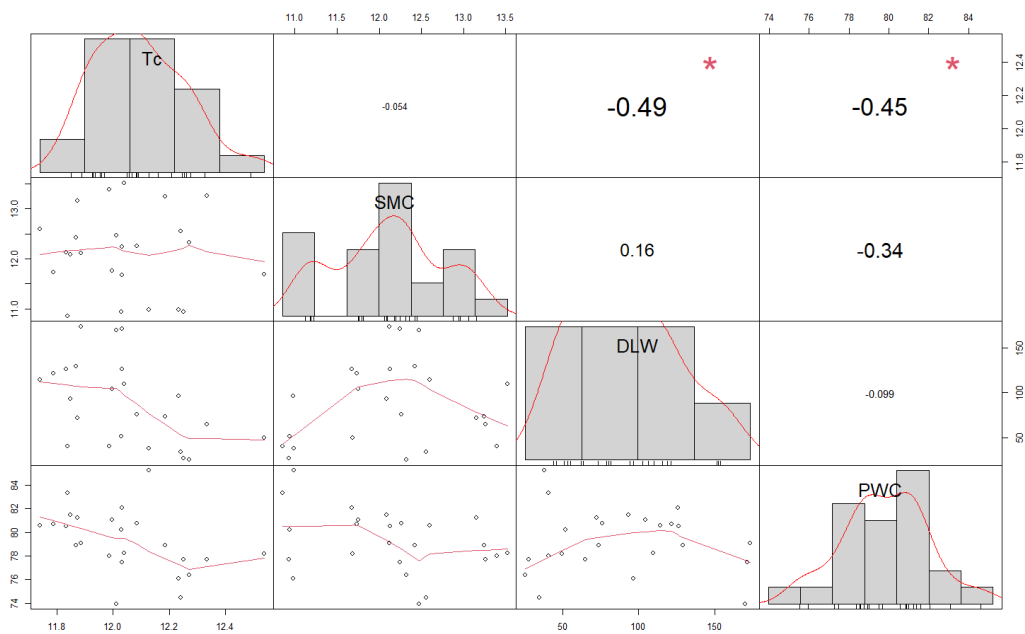


APPENDIX II - Rain (mm) from Nergena weather station and SMC readings during summer 2022.

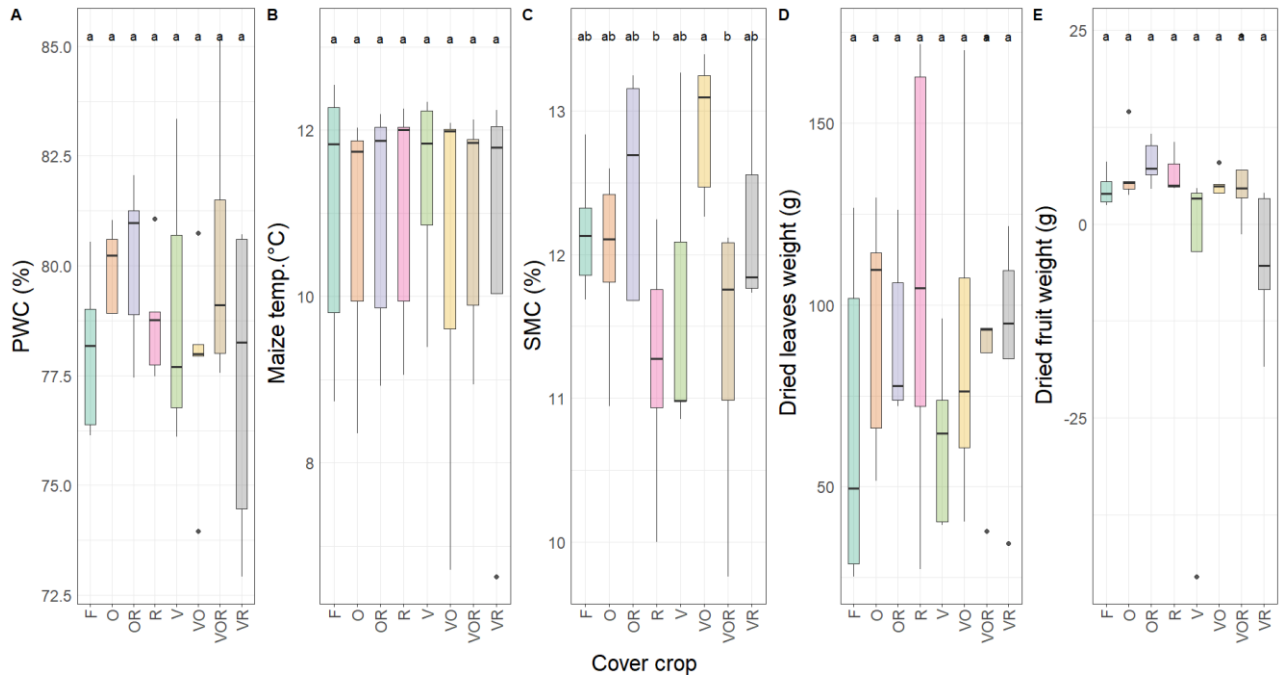




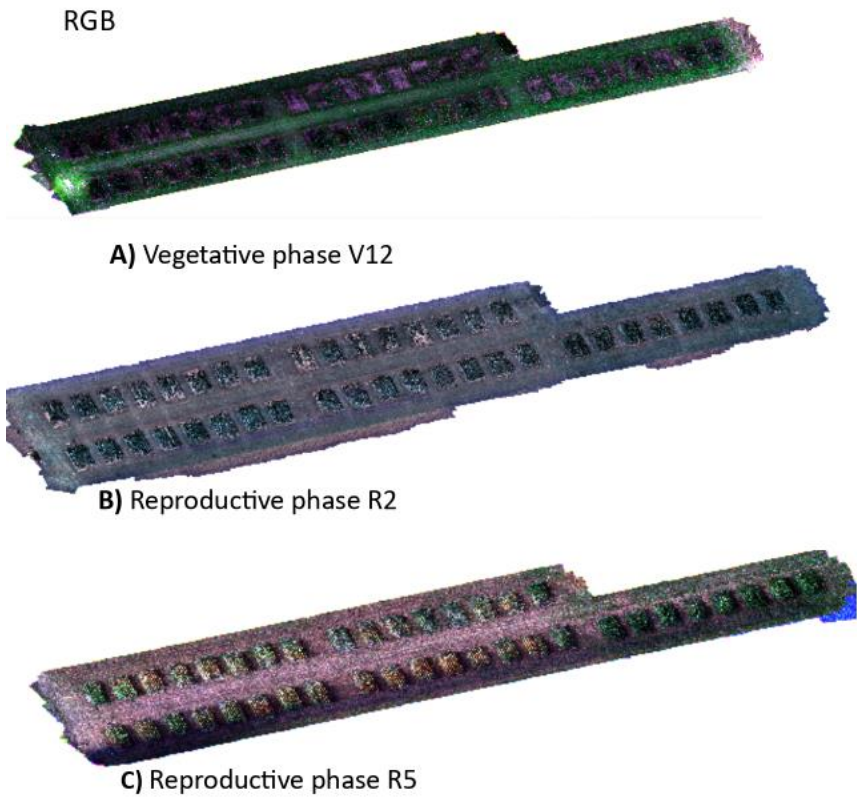
**APPENDIX III - Results of correlation analysis for 27<sup>th</sup> of June.**



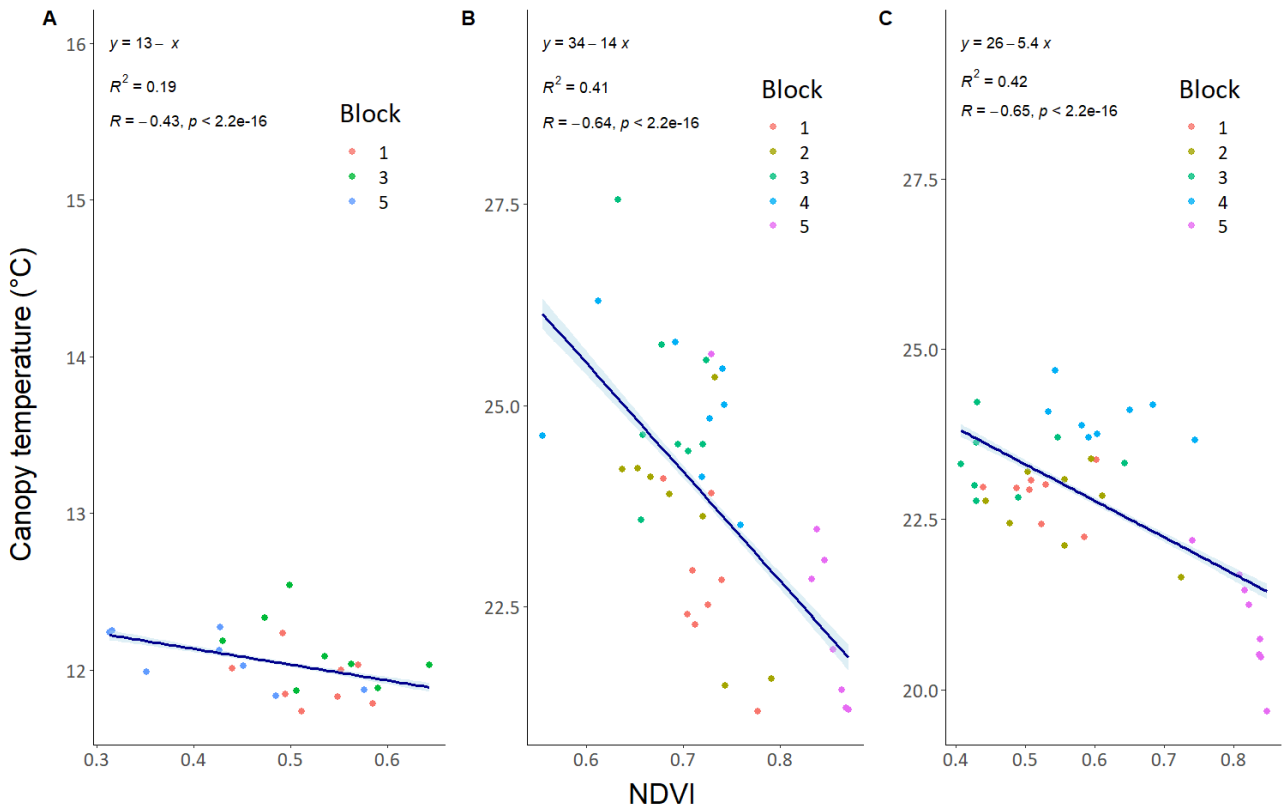
**APPENDIX IV** - The distributions of leaves moisture, maize  $T_c$ , soil moisture content, dried leaves weight and dried fruit weight of maize after 8 cover crops treatment for the 27<sup>th</sup> of June. Lowercase letters indicate significant differences between treatments and a different letter indicate significant differences between treatments ( $p < 0.05$ ).



**APPENDIX V** - RGB orthomosaics for 27<sup>th</sup> of June, 9<sup>th</sup> and 23<sup>rd</sup> of August highlight the different illumination levels and noise.



APPENDIX VI - Significant relation between median NDVI and median  $T_c$  on 27<sup>th</sup> of June, 9<sup>th</sup> and 23<sup>rd</sup> of August.



APPENDIX VII - Results from test for multicollinearity (VIF) in multilinear regression.

|     | PWC  | DLW  | SMC  |
|-----|------|------|------|
| VIF | 1.54 | 1.40 | 1.13 |

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## Annex

Zip file containing:

- ArcGIS project, 4 R scripts (one for each day and an additional one for extra visualisations), excel tables, shapefiles and rasters used;  
Pictures of field campaigns;
- EndNote library with references and downloaded papers in “Literature” folder;
- AgiSoft Methashape procedure used for generating orthomosaics;
- Report and proposal (Word, PDF) in “submission\_presentations” folder,
- Midterm & Final presentation (PPTX) in “submission\_presentations” folder.