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Additional Information

Remote sensing devices as key methods in the advanced turfgrass phenotyping under different water regimes

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Abstract: Drought stress is one of the main environmental stresses affecting turfgrass growing and natural grasslands development. Traditional methods for turfgrass drought phenotyping in field are time-consuming and labor-intensive. However, remote sensing techniques emerge as effective, rapid and easy approaches to optimize turfgrass selection under water stress. Remote sensing approaches are considered as important strategies to select species of turfgrass tolerable to drought allowing green space sustainability and environment protection in regions with water limitation. Here we evaluated differences between six mixtures of C₃-C₄ turfgrass grown under two water regimes (limited and high irrigation). The performance of turf species was achieved using the green area (GA) vegetation index calculated from RGB (red green, blue) images obtained by ground camera and drone imagery, the normalized difference vegetation index (NDVI), the plant canopy temperature (CT) and soil moisture content (SM). Both vegetation (GA and NDVI) and water status (CT and SM) indices presented a significant difference in turfgrass growth under the two water regimes. Differences among turfgrass species were detected under limited and high irrigation using the vegetation indices. Both NDVI and GA allowed clear separation between drought-tolerant and susceptible turfgrass, as well as the identification of the mixtures with a rapid green regeneration after a period of limited irrigation. Moreover, the canopy temperature also discriminated between turfgrass species but only under limited irrigation, while soil moisture values did not differentiate between species. Furthermore, the regression and conceptual model using remote sensing parameters revealed the most adequate criteria to detect turfgrass variability under each growing condition. This study also highlights the usefulness of green area vegetation index derived from drone imagery. GA obtained by drone images in this study explained turfgrass variability better than that derived from ground RGB images or the NDVI.

Keywords: Remote sensing, NDVI, RGB images, canopy temperature, water deficit, turfgrass.

43 1. Introduction

44 Drought is one of the main environmental stresses incited high risk in grasslands
45 development and natural green areas sustainability. The high level of water deficit in many
46 urban areas and the high consumption of water by turf makes the selection of drought-tolerant
47 species a fundamental criterion for planning urban green space in smart cities. Turfgrass
48 phenotyping to drought tolerance is an important strategy to select species more adequate to
49 regions with water limitations allowing then to the preservation of green spaces and
50 environment. In this context, the selection of turfgrass varieties with superior drought
51 resistance and low water use emerges as an effective way to decrease the water requirements
52 of natural turfgrass areas (Saunders, 2009). Therefore, turfgrass varieties with lower water
53 needs and that can remain acceptable visually quality under dry conditions would be the
54 species of choice in urban green spaces (Jansen van vuuren, 1997). However, traditional
55 methods used to phenotype selection in field is time-consuming and labor-intensive. Araus
56 and Cairns. (2014) have reported that limitations in field phenotyping restrict our ability to
57 dissect the genetics of quantitative traits, particularly those related to stress tolerance.
58 Likewise, breeders of turfgrass reported that the implicit heavy time- and labor demands of
59 field phenotyping hinder the collection of more comprehensive data during early crop
60 selection stages (Zhang et al., 2019).

61 In this context, remote sensing technology has revealed as an alternative approach, in
62 recent decades, for the selection of drought-tolerant varieties in a short time and without the
63 need for hard labor. Low-cost phenotyping methods through remote sensing are becoming
64 more widely used for estimating various plant traits, including chlorophyll content, nitrogen
65 concentration and biomass (Yousfi et al., 2019; Marin et al., 2020; Saberioon et al., 2014;
66 Thoele and Ehlert, 2010) and can provide a valuable information on plants adaptation to
67 abiotic stress like water scarcity and extreme temperatures (Araus and Cairns, 2014). Remote
68 sensing techniques used in filed like the hand-held point sensors such as spectroradiometers
69 (Deery et al., 2014; Yousfi et al., 2019), thermal sensors (Amani et al., 1996), imagers (Jones
70 et al., 2009; Marin et al., 2020) and drones (Deery et al., 2014) can provide relevant
71 information on plant phenotypes (Montes et al., 2011) and a rapid approximation of plant
72 biochemical and biophysical criteria for large areas in field trials (Li et al., 2014).
73 Furthermore, the non-invasive remote sensing methods such as digital image analysis and
74 spectral reflectance for quantifying turfgrass cover and quality (Montes et al., 2011;
75 Richardson et al., 2001; Jiang and Carrow, 2007) are considerate as major strategies to
76 achieve grassland sustainability and less water consumption.

77 Vegetation indices derived from remote sensing approaches are the criteria widely
78 employed in field phenotyping platforms. One of the most well-known indices is the
79 normalized difference vegetation index (NDVI), derived from optical remote sensing. The
80 NDVI has been used widely for the estimation of plant biomass (Hansen et al., 2003; Babar
81 et al., 2006) and for turfgrass management (Marin et al., 2020; Carrow et al., 2010; Murphy
82 et al., 2014). This index is based on the concept of a relationship between the absorption of
83 visible light and strong reflectance of near-infrared light by chlorophyll (Viña et al., 2011).
84 NDVI is correlated positively with turfgrass quality (Caturegli et al., 2016) and can be
85 affected by differences in species and environments (Caturegli et al., 2015). In addition,

86 information derived from digital RGB (red, green, blue) images can also inform on canopy
87 vegetation (Yousfi et al., 2019; Casadesus et al., 2007). Digital image analysis has been
88 successfully used to assess turfgrass color and the percentage of green cover (Marin et al.,
89 2020; Karcher et al., 2003; Richardson et al., 2001), as well as detect weeds in turfgrass plots
90 (Parra et al., 2020). The color information from RGB images can be frequently utilized to
91 estimate leaf chlorophyll content and nitrogen concentration (Amani et al., 1996; Li et al.,
92 2015), plant biomass (Amani et al., 1996; Montes et al., 2011; yousfi et al., 2016) and plant
93 height (Bendig et al., 2014; Schirrmann et al., 2016).

94 Furthermore, canopy temperature measurements taken using infrared thermometers
95 sensor are also commonly used for the detection of water stress-induced stomatal closure and
96 as a guide for genotypic performance under drought (Idso et al., 1981). Canopy temperature
97 is a relative measure of plant transpiration associated with water uptake from the soil
98 (Reynolds et al., 2007). Given that a major role of transpiration is leaf cooling, canopy
99 temperature provides an indicator of the degree to which transpiration cools leaves under a
100 demanding environmental load (Araus et al., 2008).

101 In this study, we examined the performance of different mixtures of C₃-C₄ turfgrass under
102 limited and higher irrigation using remote sensing parameters. The choice of C₃-C₄ mixtures
103 permit us a large wide range of environmental conditions since C₃ species are typical for
104 cooler temperature regions and the C₄ grasses are adapted to persist in warmer environments,
105 and both species differ in the photosynthetic system for the uptake of carbon dioxide. Green
106 biomass was estimated by NDVI (measured with a portable spectroradiometer with an active
107 sensor) and by the green area vegetation index (GA) derived from ground and aerial (using a
108 drone) digital pictures. Additionally, water status indices were determined by measuring the
109 canopy temperature by infrared thermometry and the soil water content by moisture sensors.
110 The main objective of this study is to select the turfgrass more tolerant to water limitation
111 and those with a rapid green regeneration after a period of stress, using remote sensing
112 methods. Moreover, we also evaluated the efficacy of the vegetation indices obtained by
113 ground and aerial imagery, alone or in combination with canopy temperature, to track
114 turfgrass variability under the two irrigation regimes. To the best of our knowledge, this study
115 is the first to propose a conceptual model relating turfgrass species variability with
116 differences in vegetation indices, canopy temperature and soil moisture under high and
117 limited irrigation. Understanding the relationships between vegetation indices and water
118 status parameters in turfgrass may help to design efficient breeding strategies to select those
119 species most suitable for a given environmental condition (especially drought), thereby
120 contributing to grassland sustainability.

121

122 **2. Materials and Methods**

123 *2.1. Plant material and growing conditions*

124 Field trials were conducted during 2019 at the Madrid Institute for Rural, Agrarian and
125 Food Research and Development (IMIDRA) in Alcalá de Henares. This site is characterized
126 by loamy sand fertile soils and a continental climate. Six C₃-C₄ turfgrass mixtures were
127 studied. To this end, we mixed seeds from *Festuca arundinacea* and *Poa pratensis* (both C₃)

128 with those from three C₄ turfs, *Cynodon dactylon*, *Buchloe dactyloides* and *Zoysia japonica*,
 129 in a with 75:25 ratio of C₃ to C₄. The description and characteristics of the turfgrass species
 130 used are provided in Table 1. Irrigation was applied with sprinklers in blocks connected by
 131 valves and controlled by the Rain Bird irrigation system (ESP-LXME Model). Two different
 132 irrigation regimes were assayed, limited irrigation (50 % of container capacity) and high
 133 irrigation (100% of container capacity). Soil humidity (Fig. 2) was controlled by sensors
 134 (Plantae station, Plantae, Spain) placed in the experimental plots and in the root active zone
 135 (at a depth of 10 cm). Turfgrass seeds were planted on 4 April 2019 in a total of 36 plots (six
 136 turfgrass mixtures, three replicates per mixture and two water regimes), each measuring 3 m
 137 × 1.5 m (Fig. 2). Water deficit was imposed for two months (after plant germination) by
 138 decreasing the amount of water applied. Afterward, irrigation was then increased to reach a
 139 high soil moisture content (Fig. 1) and was maintained for the following two months in order
 140 to evaluate the degree of green regeneration of each mixture. Measurements were taken firstly
 141 in the two months of limited irrigation (two days of measurement in each month), and
 142 subsequently in the two month of high irrigation (also we have measured twice in each month
 143 of high irrigation treatment).

144 **Table 1.** Description of C₃ and C₄ turfgrass used in this study

Description	
C₃ Species	
<i>Festuca arundinacea</i>	Highly resistant to heat and drought due to its extensive root system, adapted to a wide range of climatic conditions.
<i>Poa pratensis</i>	Vigorous root system that gives high density. Adaptable to various soils, climates, and typically used in mixtures. Excellent tolerance to salinity and shade and relatively resistant to heat and drought.
C₄ species	
<i>Cynodon dactylon</i>	It is the most important C ₄ grass species of warm climates. Resistant to long periods of drought, adapts to all kinds of soils and with strong stolons that confer high coverage capacity.
<i>Buchloe dactyloides</i>	Species of warm climates. It adapts to all types of soil, preferring alkaline substrates. Resistant to drought and aridity. Poor adaptation to shade.
<i>Zoysia japonica</i>	Species of warm climates showing some tolerance to cold. It prefers the sun but can tolerate a little shade. Tolerates heat and drought. Powerful root system allowing it to resist drought better than other plants.

145 Source: Dalmau Seeds: www.semillasdalmau.com and Zulueta seeds: www.zulueta.com.

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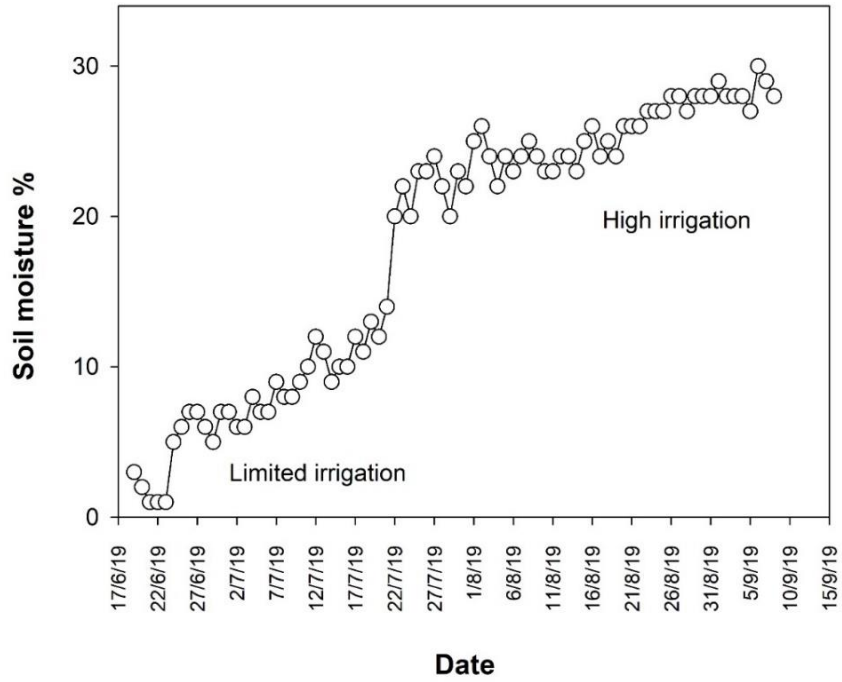


Figure 1. Daily soil moisture measurements collected by the Plantae Sensor.

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Figure 2. Drone image of turfgrass plots used in the field trial.

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156 2.2. *NDVI*

157 A portable spectroradiometer (GreenSeeker handheld crop sensor, Trimble, USA) was
158 used to measure the NDVI (Normalized Difference Vegetation Index). This index is
159 calculated by the following equation: $(\text{NIR}-\text{R})/(\text{NIR}+\text{R})$, where R is the reflectance in the
160 red band and NIR is the reflectance in the near-infrared band. A constant distance of 50–60
161 cm above and vertical to the canopy was maintained between the sensor and the plots.
162

163 2.3. *Ground and aerial RGB images*

164 RGB (red, green, blue) images were taken in each plot about 80 cm above the plant
165 canopy in a zenithal plane using a SONY DSC-W120 camera. RGB aerial images were taken
166 on the same day using a BEBOP drone (Parrot, Paris, France) equipped with an RGB camera.
167 The BreedPix 0.2 free-access software established for digital image processing was used for
168 image analysis (Casadesús et al., 2005). This software rapidly provides digital values on the
169 basis of different color properties and measures the green area (GA; portion of pixels with
170 $60 < \text{Hue} < 120$ from the total number of pixels) by capturing differences in biomass. Two
171 GA indices were analyzed, $\text{GA}_{\text{ground RGB}}$ (calculated from the ground image) and $\text{GA}_{\text{aerial RGB}}$
172 (calculated from aerial image).

173 2.4 *Canopy temperature measurements*

174 Canopy temperature (CT) was measured on the same day as the vegetation indices, using
175 an infrared thermometer (Fluke 561 sensor, China). Measurements were taken approximately
176 1 m above the plants and with the sun behind the user of the device. Three measurements
177 were taken in each plot and the average was taken as a plot data.

178 2.5 *Plot moisture content*

179 Soil moisture (SM) was measured on the same day as the remotely sensed traits using a
180 Field Scout Time Domain Reflectometry sensor 350 (TDR 350, Spectrum Technologies, Inc
181 USA). Measurements were taken with the 7cm TDR rods, and at three sites in each plot.
182 Average of the three measurement was taken as a data of each plot.

183 2.6. *Statistical analysis*

184 Data were subjected to factorial analyses of variance (ANOVA) to test the effects of irrigation
185 on turfgrass growth. A bivariate correlation procedure was used to analyze the relationships
186 between NDVI, $\text{GA}_{\text{ground RGB}}$, $\text{GA}_{\text{aerial RGB}}$, and CT. Moreover, we performed a multiple linear
187 regression analysis (stepwise) to analyze turfgrass variability under different growing
188 conditions. Statistical analysis was done using IBM SPSS Statistics 24 (SPSS Inc., Chicago,
189 IL, USA). Sigma-Plot 11.0 for Windows (Systat Software Inc., Point Richmond, CA, USA)
190 was used to establish the figures. Finally, we performed path analyses (Li, 1975) to quantify
191 the relative contributions of the direct and indirect effects of remote sensing traits on turfgrass
192 variability. This methodology offers the possibility of building associations between
193 variables on prior knowledge. A path analysis determines simple correlations between
194 independent factors (in this case CT and SM), and regresses them on each intermediary
195 (NDVI, $\text{GA}_{\text{ground RGB}}$, $\text{GA}_{\text{aerial RGB}}$) to obtain direct effects in the form of partial regression
196 coefficients (i.e. path coefficients) involving traits that displayed turfgrass growing variation.
197 This model helps to understand differences in growing between the turf species (examined
198 in this study) under the two irrigation regimes tested. A model with a comparative fit index

199 (CFI) with values > 0.9 was taken as indicative of good fit (Arbuckle, 1997). Data were
 200 analyzed using the Amos Graphics package (IBM SPSS Amos, USA).
 201

202 3. Results

203 3.1 Effect of irrigation on NDVI and green area

204 Irrigation significantly affected the turfgrass NDVI and GA (Table 2). Low values for the
 205 three vegetation indices (NDVI, and GA calculated from both ground and aerial RGB
 206 images) were observed under limited irrigation. In addition, soil moisture (SM) content under
 207 high irrigation was double that under limited irrigation, whereas the canopy temperature (CT)
 208 of plants increased with the decrease of irrigation (Table 2).

209 **Table 2.** Effect of irrigation on NDVI (Normalized Difference Vegetation Index), GA_{ground RGB}
 210 (Green Area calculated from ground RGB images), GA_{aerial RGB} (Green Area calculated from
 211 drone RGB images), CT (canopy temperature) and SM (soil moisture) and the corresponding
 212 ANOVA. Values presented are the means of the 72 measurements in each irrigation regime (6
 213 turfgrass mixtures, three replicate per mixtures and four date of measurements in each
 214 treatment). Significance levels: ***p < 0.001.

	Limited irrigation	High irrigation	Level of significance
NDVI	0.65	0.80	0.000***
GA_{ground RGB}	0.49	0.78	0.000***
GA_{aerial RGB}	0.50	0.79	0.000***
CT	20.00	13.70	0.000***
SM	24.05	45.02	0.000***

215

216 3.2 Classification of turfgrass species on the basis of vegetation and water status indices

217

218 Turfgrass species showed a significant difference on compartment under the two irrigation
 219 regimes (Table 3). All the vegetation indices measured (NDVI, GA_{ground RGB}, GA_{aerial RGB})
 220 discriminated significantly (P < 0.000) between Festuca and Poa turfgrass mixtures under
 221 both water stress and high irrigation (Table 3). The mixtures of Festuca with the three C₄
 222 turfgrass showed higher vegetation indices under limited irrigation compared to Poa-C₄
 223 mixtures. Conversely, Poa-C₄ mixtures showed better vegetation indices than Festuca-C₄
 224 mixtures under the high irrigation. Moreover, canopy temperature (CT) also allowed
 225 significant discrimination between turfgrass species but only under the water stress regime
 226 (Table 3) with lower values of canopy temperature observed in Festuca mixtures under
 227 limited irrigation. However, for the soil moisture (TDR measures), the species effect was not
 228 significant under both water regime (Table 3).

229

230

231 **Table 3.** Means values of NDVI, $GA_{\text{ground RGB}}$, $GA_{\text{aerial RGB}}$, CT and SM of Festuca and Poa
 232 mixtures with C₄ turf. Significance levels of difference among species under limited and high
 233 irrigation are presented. Species values are the means of the 36 measurements (3 turfgrass
 234 mixtures, three replicate per mixtures and four date of measurements in each treatment).
 235 Significance levels: ns, not significant; ns, not significant; *** $p < 0.001$. Abbreviation of
 236 variable as in Table 2. Significance levels.

237

	Limited irrigation			High irrigation		
	Mixtures			Mixtures		
	Festuca-C ₄	Poa-C ₄	Significance	Festuca-C ₄	Poa-C ₄	Significance
NDVI	0.69	0.58	0.000***	0.77	0.80	0.000***
$GA_{\text{ground RGB}}$	0.59	0.40	0.000***	0.75	0.81	0.000***
$GA_{\text{aerial RGB}}$	0.57	0.44	0.000***	0.76	0.83	0.001***
CT	18.33	20.51	0.000***	13.33	14.20	0.065 ^{ns}
SM	23.50	24.5	0.179 ^{ns}	44.78	45.27	0.095 ^{ns}

238

239 Furthermore, comparison intra mixtures showed that under limited irrigation Festuca
 240 mixed with Cynodon, Zoysia and Buchloe did not present any difference between them on
 241 vegetation indices, nonetheless CT was lower in Festuca-Cynodon mixture. Whereas under
 242 high irrigation Festuca-Zoysia and Festuca-Buchloe (with higher NDVI, $GA_{\text{ground RGB}}$ and
 243 $GA_{\text{aerial RGB}}$) slightly outperformed Festuca-Cynodon (Fig. 3A, B, C). With respect to the Poa
 244 mixtures, significant differences were observed under both water regimes (Fig. 3). In this
 245 regard, under limited irrigation, Poa-Cynodon showed higher vegetation indices and lower
 246 CT (Fig. 3A, B, C, D), while under the high irrigation regime Poa-Zoysia and Poa-Buchloe
 247 showed better growing parameters than Poa-Cynodon. Whereas, SM did show any
 248 differences intra mixtures (Fig 3E).

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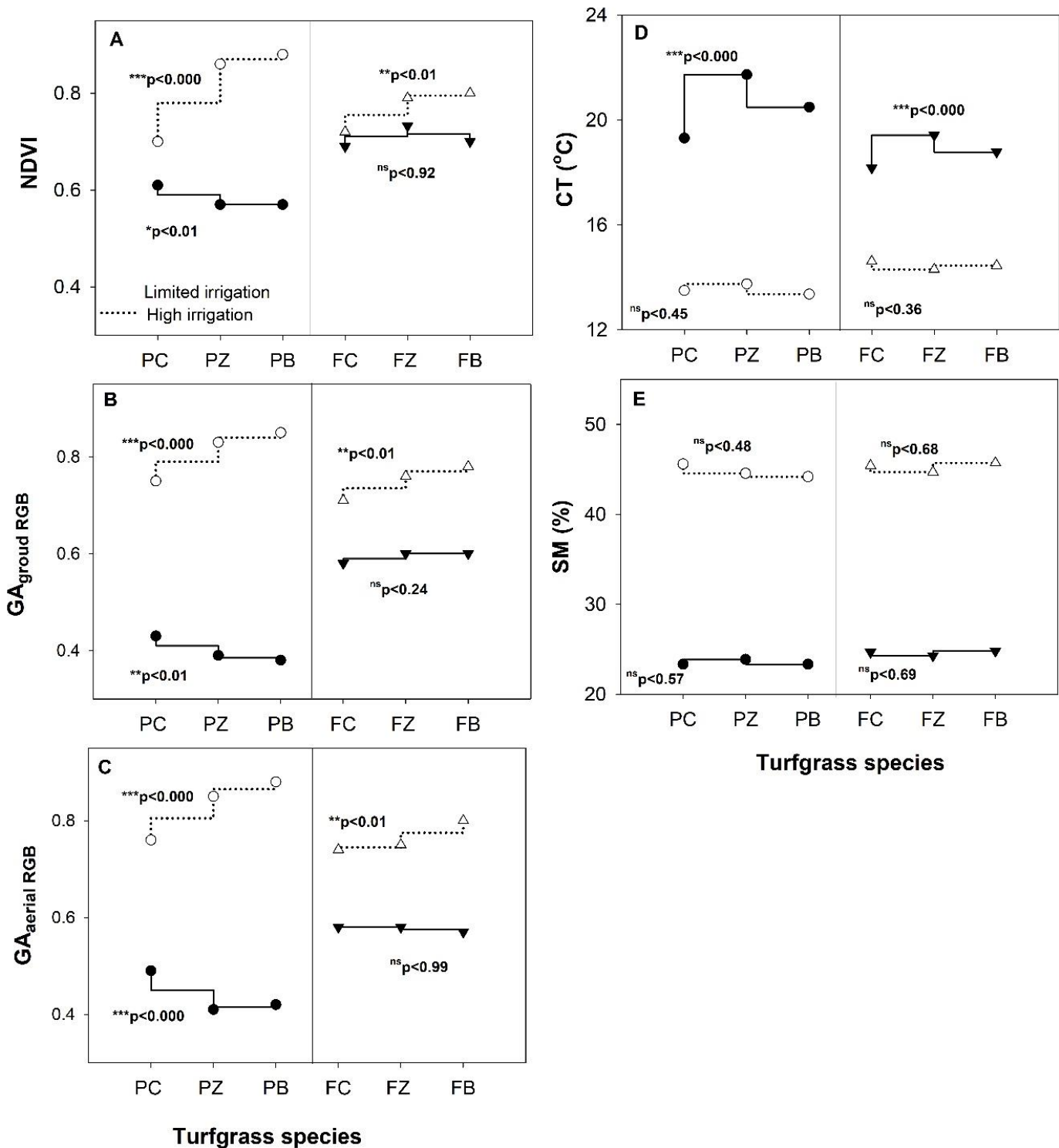
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Figure 3. NDVI, GA_{ground} RGB, GA_{aerial} RGB, CT and SM values of each turfgrass mixture under limited and high irrigation. Abbreviation of variable as explained in Table 1. Turf mixtures are: PC, Poa-Cynodon; PZ, Poa-Zoysia; PB, Poa-Buchloe; FC, Festuca-Cynodon; FZ, Festuca-Ziysia; and FB, Festuca-Buchloe.

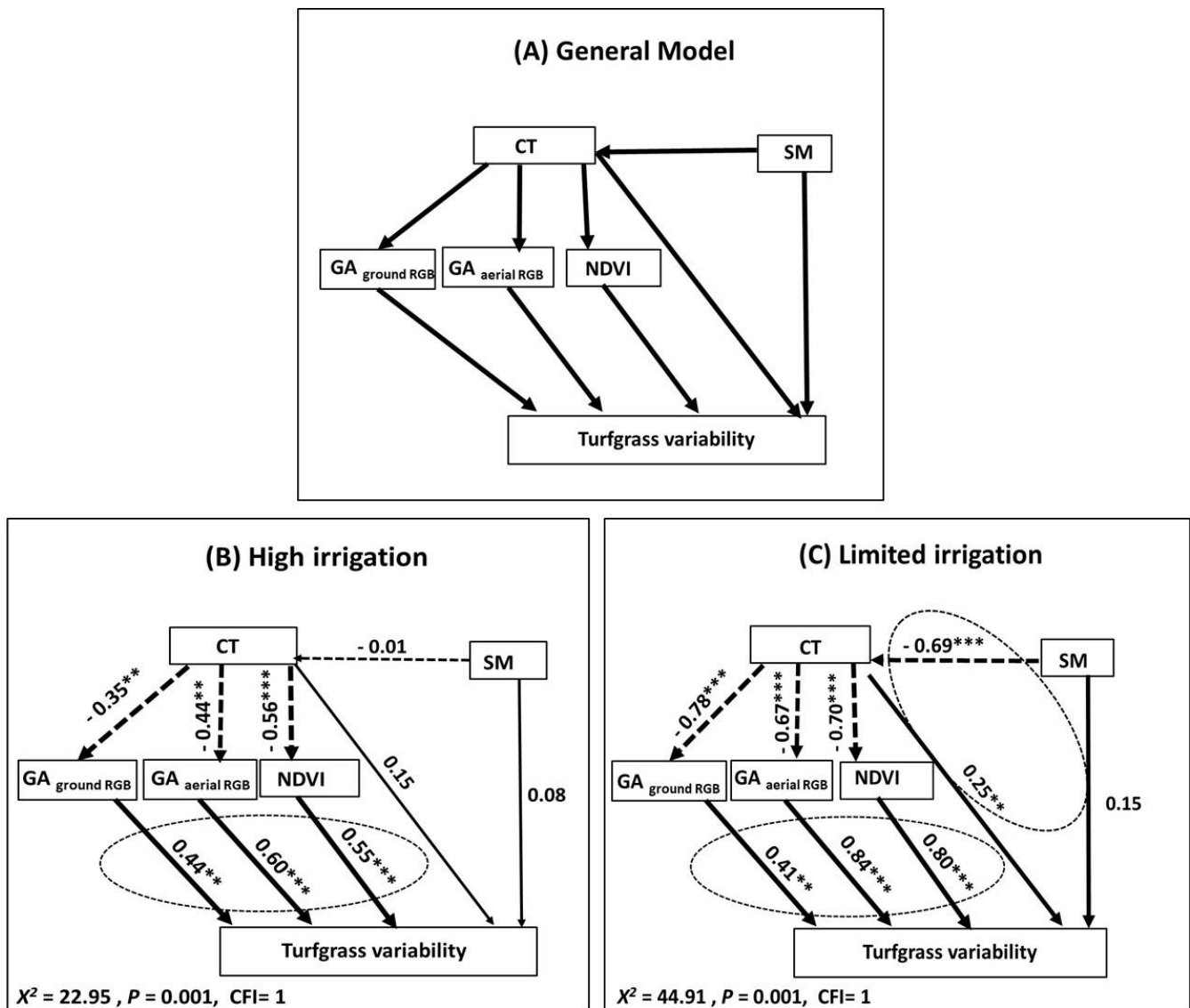
264 3.3 Remote sensing trait and turfgrass variability

265 A multiple linear regression (stepwise) explaining turfgrass species variability as a
 266 function of traits related to NDVI, $GA_{ground\ RGB}$ and $GA_{aerial\ RGB}$, CT and SM under limited and
 267 high irrigation (Table 4) was performed. The $GA_{aerial\ RGB}$ was chosen as the first explanatory
 268 variable of turfgrass variability under high irrigation and with a strong R^2 (0.85).
 269 Additionally, $GA_{ground\ RGB}$ and NDVI were the two other variables chosen by the model.
 270 However, under limited irrigation, the first variable chosen was the NDVI, with ($R^2= 0.41$),
 271 followed by $GA_{aerial\ RGB}$ and $GA_{ground\ RGB}$. In contrast to high irrigation, under limited
 272 irrigation the CT contributed (even less than the vegetation indices) to explaining the model.

273 **Table 4.** Multiple linear regressions (stepwise) explaining turfgrass variability using the
 274 NDVI, $GA_{aerial\ RGB}$, $GA_{ground\ RGB}$, CT and SM as independent variables. Abbreviation of
 275 variables as in Table 2. Significance levels: *** $p < 0.001$.

Growing Conditions	Variable Chosen	R^2
High irrigation	$GA_{aerial\ RGB}$,	0.85
	$GA_{aerial\ RGB}$, $GA_{ground\ RGB}$	0.91
	NDVI, $GA_{aerial\ RGB}$, $GA_{ground\ RGB}$	0.95***
Limited irrigation	NDVI	0.41
	NDVI, $GA_{aerial\ RGB}$	0.83
	NDVI, $GA_{aerial\ RGB}$, $GA_{ground\ RGB}$	0.94
	NDVI, $GA_{aerial\ RGB}$, $GA_{ground\ RGB}$, CT	0.96***

286
 287 In addition, a conceptual model based on a path analysis was proposed (Fig. 4A) for the
 288 data of both water regimes (comparative fit index (CFI)>0.9 and $P>0.05$; as the objective was
 289 to develop models that fit the data well). Under high irrigation, neither CT nor SM had any
 290 direct significant effect on the variability of C₃-C₄ turfgrass mixtures. However, the canopy
 291 temperature (CT) had a significant negative effect on the three vegetation indices (NDVI,
 292 $GA_{aerial\ RGB}$, $GA_{ground\ RGB}$), which in turn were associated significantly with turfgrass
 293 variability (Fig. 4B). Under limited irrigation, the effects of CT and SM on turfgrass
 294 variability were considerable but with a lower coefficient. Nevertheless, these two parameters
 295 (related to plant and soil water status) indirectly affected turfgrass discrimination. Thus, SM
 296 was negatively related to CT, which in turn was strongly and negatively related to the three
 297 vegetation indices. Finally, the vegetation indices showed a high and significant effect on
 298 turfgrass variability under limited irrigation (Fig. 4C), with Higher correlation coefficient of
 299 $GA_{aerial\ RGB}$, $GA_{ground\ RGB}$ than NDVI.

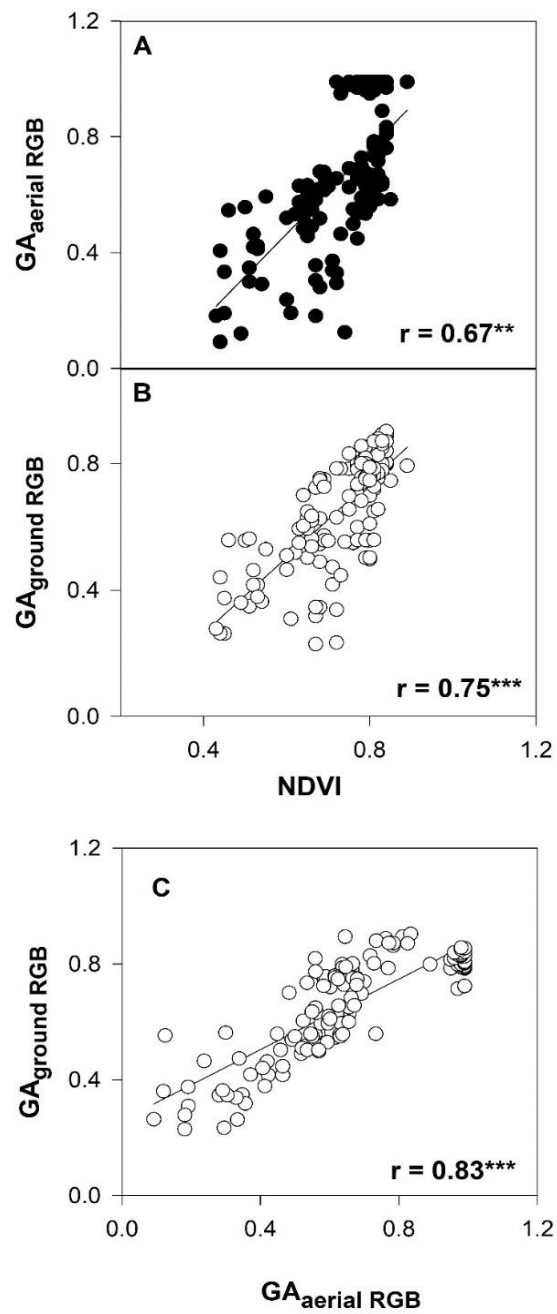


301 **Figure 4.** Path analyses of turfgrass species variability under different water regimes.
 302 Parameters included in the model are: NDVI, GA_{ground RGB}, GA_{aerial RGB}, CT and SM.
 303 Abbreviation of variables as in Table 2. CFIs with values > 0.9 are taken as indicative of
 304 good fit. Significance levels: ** $p < 0.01$ and *** $p < 0.001$.

305

306 3.4 Relationships between ground and aerial measurements

307 NDVI was correlated positively, and with the same pattern, with the GA obtained by aerial
 308 and ground RGB when all turfgrass species, water regimes and replicates are combined (Fig.
 309 5A, B). Moreover, the GA_{ground RGB} was also strongly correlated ($r = 0.83^{***}$) with GA_{aerial}
 310 RGB. (Fig. 5C). Likewise, the three vegetation indices were strongly and negatively correlated
 311 with the canopy temperature and with the same pattern (Fig. 6A, B, C).



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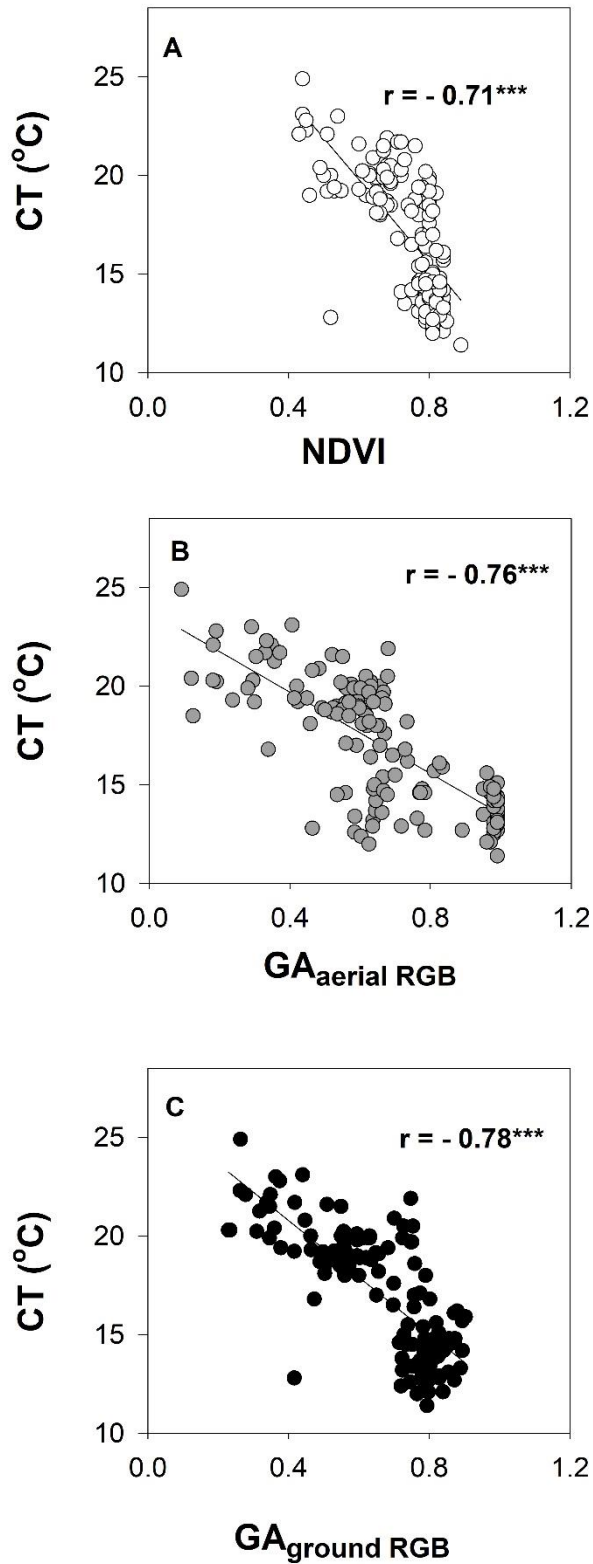
315 **Figure 5.** Correlation coefficients of the relationships between the different

316 vegetation indices (NDVI, $GA_{\text{aerial RGB}}$ and GA_{ground}). Water regime and turfgrass

317 species were analyzed together. Significance levels: $**p < 0.010$; $***p < 0.000$.

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Figure 6. Correlation coefficients of the relationships between the different vegetation indices (NDVI, $GA_{\text{aerial RGB}}$ and GA_{ground}) and the canopy temperature (CT). Water regime and turfgrass species were analyzed together. Significance levels: $**p < 0.010$; $***p < 0.000$. Abbreviations of variables as in Table 2.

326 4. Discussion

327 4.1 Potential of NDVI and RGB imagery to screen differences in turfgrass species

328 Vegetation indices based on RGB images, and the NDVI have demonstrated high-
329 throughput for the precise prediction of several traits that are valuable for breeders and
330 agronomists (Vergara-Díaz et al., 2016). In this study, NDVI and both $GA_{\text{ground RGB}}$ and
331 $GA_{\text{aerial RGB}}$ of the turfgrass plots were significantly lower under limited irrigation than high
332 irrigation. In this context, water-stressed canopies have been reported to have a lower spectral
333 reflectance in the NIR wavebands than unstressed ones (Fan et al., 2020). The leaves of plants
334 growing under water stress reflect significantly less NIR and greater red irradiance.
335 Consequently, the NDVI and GA values are lower under stress conditions. Similar changes
336 in the reflectance at the visible and NIR wavebands caused by irrigation were observed in
337 other turf studies on Fescue (Fenstermaker-Shaulis et al., 1997), Lolium (Baghzouz et al.,
338 2006) and Cynodon grass (Baghzouz et al., 2007). The changes in the reflectance values of
339 turfgrass observed in this study informs on the growing status of plants under limited and
340 higher irrigation and can indicate perfectly of the turf performance and quality under water
341 stress conditions. In accordance, Vergara-Díaz et al. (2016) have reported that vegetation
342 indices based on RGB images, and the NDVI have demonstrated high-throughput for the
343 precise prediction of several traits that are valuable for breeders and agronomists (Vergara-
344 Díaz et al., 2016). Moreover, Richardson et al. (2001) informed that vegetation indices can
345 be used to evaluate turf quality, color, dry matter, chlorophyll, carotenoids and nitrogen
346 content. Other studies also demonstrated the utility of the NDVI and other plant stress
347 indicators based on spectral reflectance for assessing turfgrass performance (Montes et al.,
348 2011; Trenholm et al., 2000, Marin et al., 2020) and has been used to measure drought stress
349 (Bell et al., 2002; Trenholm et al., 1999). Our results demonstrate the usefulness of NDVI
350 and GA measurements from ground and aerial images to distinguish between turfgrass
351 species under limited and higher. Accordingly, in a previous-study, we also highlighted the
352 utility of NDVI and GA to differentiate between C₃-C₄ turfgrass under water deficit (Marin
353 et al., 2020). In addition, other authors also showed that vegetation indices are useful to
354 discriminate between turfgrass cultivars grown under the same conditions and maintained
355 under identical agronomical practices (Caturegli et al., 2014).

356 Moreover, here we have also demonstrated the greater growth of Festuca mixture with
357 the three C₄ turf under water stress compared to the Poa mixtures. In this context, the
358 metabolism and architecture of roots in Festuca species are key for the development of
359 tolerance to water deficit (Perlikowski et al., 2020). Likewise, it has been described that
360 Festuca species adapt to drought stress through alterations in leaf and root morphology
361 (Wang et al., 2008).

362 Furthermore, the data obtained from the optical remote sensing techniques in this study,
363 showed an interesting pattern in the spectral characteristics of both C₃-C₄ mixtures.
364 Vegetation indices exhibited that Festuca mixture with Cynodon, Buchloe and Zoysia
365 presented a similar pattern of growth under limited irrigation, while under high irrigation
366 Fescue-Buchloe and Fescue-Zoysia outperformed Festuca-Cynodon. Nevertheless, the
367 NDVI, $GA_{\text{aerial RGB}}$ and $GA_{\text{ground RGB}}$ of Poa with the three C₄ species revealed significant
368 differences under the two water regimes. Under limited irrigation, Poa-Cynodon was found

369 to have better vegetation indices, while under high irrigation Poa-Buchloe and Poa- Zoysia
370 outperformed Poa-Cynodon, with Poa-Buchloe showing the best growth under this condition.
371 According to this, several types of Bermuda grass have a deep and large root systems, a
372 morphological trait that allows them to reach available water at greater depth under stress
373 conditions (Carrow, 1996). On the basis of our results, we can suggest that mixtures of
374 Festuca with Cynodon, Buchloe or Zoysia have a similar tolerance to water deficit and would
375 be similar suitable for regions with limited water availability. Whereas, in the case of Pao
376 mixtures, Poa-Cynodon would be the mixture of choice for drought regions. Furthermore,
377 under climates with higher precipitation and no water deficiency, the Festuca-Buchloe and
378 Festuca-Zoysia mixtures can offer better turf quality. Likewise, the Poa-Zoysia and Poa-
379 Buchloe mixtures would be the most suitable in such climates due to their high quality under
380 optimal growing conditions. Additionally, we also observed that after a period of growth
381 under limited irrigation followed by high irrigation, the C₃ mixture with Buchloe provided
382 the highest vegetation indices indicating better green biomass regeneration after growing
383 under water deficit. Accordingly, the most characteristic response of drought-dormant
384 Buffalo grass (*Buchloe dactyloides*) is its rapid ability to regenerate growth after a period of
385 water stress followed by water availability (Shantz, 1911).

386

387 4.2 Turfgrass canopy temperature and water status

388 The canopy temperature (also referred to as leaf temperature) has been widely employed for
389 estimating plant water stress (Blum et al., 1982) and for providing a relative measure of plant
390 transpiration (Araus et al., 2008). In this study, the canopy temperature (CT) of the six
391 turfgrass mixtures, measured by an infrared thermometer, was lower under limited irrigation
392 than high irrigation. Under water deficit, the decreased in water uptake caused the stomata to
393 close, thereby reducing transpiration and increasing leaf temperature (Blonquist et al., 2009).
394 However, a lower CT indicates a greater capacity for transpiration and for taking up water
395 from the soil, and therefore a better plant water status (Blum et al., 1982). Also, and consistent
396 with the findings of other studies (Lopes and Reynolds, 2012) we observed that the CT was
397 strongly related to the vegetation indices. In this regard, lower CTs are strongly associated
398 with higher green biomass, and these two parameters can help to identify turf species with
399 the greatest tolerance to drought.

400 Our results also reveal that, under limited irrigation, the mixture of Festuca with the three
401 C₄ turf showed a lower CT than the Poa mixtures. In this context, *Festuca arundinacea* is
402 water use efficiency than other turfgrass species (Horst et al., 1997). Furthermore, in response
403 to water stress, Festuca spp. undergoes an osmotic adjustment that maintains sufficient turgor
404 pressure in the growing zone to ensure leaf elongation (Wang et al., 2008). In contrast to the
405 vegetation indices, the CT of the three Festuca mixtures under limited irrigation showed a
406 clear difference between mixtures, with Festuca-Buchloe exhibiting the lowest CT. It has
407 been reported that Buchloe grass requires low levels of water for survival and that under
408 semi-arid conditions it needs less irrigation to maintain good turf quality than tall Festuca or
409 and Zoysia grass (Hicks et al., 1984). Also, lower evapotranspiration rates in *Buchloe*
410 *dactyloides* indicate that this species uses less water than selected ornamentals (Horst et al.,
411 1997). However, our comparison between Poa mixtures revealed that Poa-Cynodon showed

412 better performance under water deficit. In this context, the thick leaf cuticles and smaller
413 stomatal openings (Zhou et al., 2009) as well as the reduced leaf surface, stoma density, and
414 lower water transpiration rate (Mukhtar et al., 2013) of *Cynodon dactylon* confer better
415 tolerance to water stress compared to the other turfgrass species.

416

417 *4.3 Soil moisture*

418 Soil moisture (SM) values measured by the TDR 350 did not show any differences
419 between the turfgrass species under limited or high irrigation (Table 3, Fig. 4D).
420 Nevertheless, the analyze combining all SM data of the six turfgrass mixtures evaluated in
421 this study demonstrated difference between turfgrass growth under the two irrigation regimes
422 (Table 2). In this context, such sensors can indicate when the soil profile is full of water or
423 dry and therefore healthy turf is maintained by avoiding plant stress caused by soil that is too
424 dry or too waterlogged (Bremer and Ham, 2003). Accordingly, moisture sensors are among
425 the most used devices to manage crop irrigation schemes and have been reported to improve
426 irrigation efficacy in lawns (Parra et al., 2019). We propose SM as a useful criterion for
427 managing turf irrigation, but not for distinguishing between turfgrass mixtures. Moreover,
428 the feasibility of SM sensing devices has also been addressed for the irrigation management
429 of Bermuda grass (Osborne et al., 2002). We considerate the SM data obtained by the soil
430 sensors in this study as indirect measures to evaluate the development of turfgrass species.
431 Osborne et al. 2002 reported that as SM decreases, plants show a decrease in tissue moisture
432 content, which in turn influences their reflectance properties.

433

434 *4.4 Relationship between ground and aerial optical remote sensing trait*

435 The two groups of vegetation indices (NDVI and ground and aerial RGB images) showed
436 high and positive correlations between them (Fig. 7). This result is in accordance with
437 previous research (Adamsen et al., 1999; Lukina et al., 1999, Montes et al., 2011) and
438 confirms that these indices inform about biomass status in a comparable manner. Likewise,
439 there was a high association between $GA_{\text{aerial RGB}}$ and $GA_{\text{ground RGB}}$ images. However, GA
440 obtained by drone images is better than GA of ground images with respect to explaining the
441 variability in turfgrass growth (Fig. 4D, Fig. 5, Table 4) and was less labor-intensive and with
442 less time-consuming. The use of spectral vegetation indices derived from UAV imagery is
443 emerging as a rapid and cost-efficient approach for plant phenotyping (Angelos et al., 2017).
444 In present, many low cost drones with an integrated simple RGB camera are now available
445 to take images of large turfgrass surfaces in a short time. In this context, Zhang et al. (2019)
446 reported that unmanned aerial vehicle (UAV) imagery is considered a powerful tool for
447 turfgrass breeders allowing them to evaluate crop performance and greatly increase the
448 efficacy of data collection in relatively large trials (advantages in field of view, spatial and
449 temporal resolution). Furthermore, the high correlation found between the NDVI and the
450 $GA_{\text{aerial RGB}}$ index in this study allows us to confirm the reliability UAV-based measurements
451 for selecting drought-tolerant species in large areas. The GreenSeeker hand-held sensor, a
452 low-cost practical device, has proved useful for detecting turfgrass stress for small areas,
453 (Caturegli et al., 2015). However, proximal sensors would need to be complemented with
454 special cameras on board UAVs to monitor the entire surface of large areas Caturegli et al.,
455 2015).

456

457 4.5 Comparative model using remote sensing methods for turfgrass breeding

458 Results of the lineal regression presented here showed the usefulness of the vegetation
459 indices derived from RGB imagery to explain the differences between turfgrass species under
460 the high irrigation. Of note, the GA vegetation index derived from aerial imagery was the
461 most important parameter chosen by the model and with a higher impact ($r^2 = 0.85$).
462 Moreover, NDVI made a negligible contribution to explaining variability under high
463 irrigation, due to the problem of saturation. In this context, a saturated NDVI in higher
464 periods of turfgrass activity informs less than RGB indices, which are characterized by less
465 evident saturation (Montes et al., 2011). However, under limited irrigation, NDVI was the
466 first criterion chosen by the regression model. Other authors indicated that the NDVI is an
467 index based on the strong contrast between the near infrared and the red band reflectance of
468 a vegetation canopy and this difference becomes extensive as canopy vegetation cover
469 increases (Amani et al., 1996). This would explain why the NDVI is a more appropriate
470 approach to study stress conditions in sparse canopies or during the early senescence of plants
471 (Casadesus et al., 2007; Aparicio et al., 2000). However, our study combined both NDVI and
472 vegetation indices derived from RGB images for collecting more and complete information
473 to discriminate between turfgrass species under the two irrigation regimes. Accordingly, data
474 fusion in remote sensing can facilitate estimations of biomass, possibly resolving saturation
475 difficulties observed with VIS-NIR sensor data under higher density vegetation (Tilly et al.,
476 2015).

477
478 Likewise, the path model performed in the present study confirms the stepwise result and
479 reveals the high capacity of vegetation indices (NDVI and both $GA_{\text{aerial RGB}}$ and $GA_{\text{ground RGB}}$)
480 to explain turfgrass variability, specially the GA index derived from drone images under high
481 irrigation, where the water status parameters (CT and SM) have no direct effect on turfgrass
482 species variability. Moreover, under limited irrigation, vegetation indices are still more
483 important criterion to explain variability of turf. In this case (water stress conditions), CT
484 contribute to explaining the model (although to a lower extent than the vegetation indices)
485 and have a direct effect on turf species variability. To the best of our knowledge, this is the
486 first study to report on a model combining remote sensing techniques to explain variability
487 in the growth of different turfgrass species under different water regimes. The results of this
488 study are expected to help breeders to select appropriate criteria for turfgrass phenotyping
489 under distinct environmental conditions.

491 5. Conclusion

492 This study demonstrates the efficiency of vegetation indices to reveal variability in the
493 performance of turfgrass species under different growing conditions. Our results confirm the
494 usefulness of the vegetation indices for phenotyping most drought-tolerant turfgrass mixtures
495 in terms of retaining greener biomass during periods of limited irrigation. Although the NDVI
496 is extensively used to monitor changes in growth under different conditions, here we have
497 shown that the GA vegetation index derived from aerial images provides a better evaluation
498 of turfgrass mixtures in a short time and with high efficiency. Furthermore, the path analysis

499 model developed confirms the importance of the traits assessed by remote sensing for
500 establishing variability in turfgrass performance. The path analysis and regression model
501 revealed the most suitable criteria to be used in certain environmental conditions for
502 discriminating between turfgrass species. Therefore, optimized turfgrass breeding strategies
503 can allow the precise and rapid (in the case of drones) phenotyping of a large numbers of turf
504 mixtures to identify those with greatest tolerant to water stress, contributing in this way in
505 the preservation of water resources, the grasslands sustainability and the environment
506 protection. In future work, we propose to add thermography (thermal imagery) to the model
507 for detecting water stress in the same turfgrass mixtures used in this study. We believe that a
508 strategy combining aerial RGB and thermal imagery may potentially enhance turfgrass
509 phenotyping and help to rapidly identify those mixtures most tolerant to drought conditions
510 or those with rapid green regeneration after a period of water stress.

511

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515

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